

# 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

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](http://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.

## 1.3 LEGISLATION COVERING WILDLIFE CRIME

5

**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](http://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 estimates 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 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.

### 1.3 LEGISLATION COVERING WILDLIFE CRIME

7

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](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](http://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](http://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 ( $1 \text{ ng} = 1 \times 10^{-9} \text{ g}$ ) 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 ( $10^{-15} \text{ g}$ ) 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<sup>1</sup>. 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<sup>®</sup> 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.

<sup>1</sup> It should be noted that the Leverhulme Trust (UK) funded research assisting the authors.

### 1.5.3 Performing DNA typing in wildlife investigations

Forensic science analyses are performed typically only if instructed by an agency that is tasked with enforcing legislation. The enforcement varies between countries where for instance the US National Fish & Wildlife Service can perform this role in the United States. At a federal level in Australia, this falls under the Department of the Sustainability, Environment, Water, Population and Communities; with much devolved to State level. In Australia an Operations Environment Forensic Support Group has been developed to coordinate activities relevant to wildlife crime. The Department of Environment, Food and Rural Affairs in the United Kingdom is the government ministry under which legislation relevant to wildlife crime is prepared; here PAW, whose membership is drawn from many stakeholders, assists and advises the government. Cooperation and sharing of resources and information can only be of assistance and in this regard the Association of South-East Asian Nations (ASEAN), in 2005, developed a Wildlife Enforcement Network. ASEAN includes the following countries: Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand and Vietnam.

The increase in legislation relevant to alleged crimes against wildlife requires that some forensic science organisation can undertake any analysis deemed necessary. Much forensic science is relevant to crimes against people or property, where the analysis of samples from alleged wildlife crime incidents does not fit easily or naturally. In the United States the establishment of a dedicated laboratory, now called the US Fish & Wildlife Service Forensics Laboratory, based in Ashland, Oregon, provided expertise to assist with such investigations. It remains the case that world-wide many forensic DNA investigations of alleged wildlife crime are either conducted by operational forensic science laboratories whose primary focus is human identification, or by universities where there is a strong background in molecular genetics but who only assist with forensic cases as and when requested. There is expertise in Australia at the museums, such as the Australian Museum in Sydney ([www.australianmuseum.net.au](http://www.australianmuseum.net.au)) and at universities such as Murdoch University ([www.wildlifeforensics.com.au](http://www.wildlifeforensics.com.au)) and Flinders University ([www.flinders.edu.au/people/adrian.linacre](http://www.flinders.edu.au/people/adrian.linacre)). A member of the Australian Museum, Murdoch and Flinders Universities and a representative from the National Institute of Forensic Science, are on the Operations Environment Forensic Support Group, with the aim of coordinating best practice wildlife forensic science. Trent University in Ontario, Canada, houses the Wildlife Forensic DNA Laboratory within the Natural Resources DNA Profiling and Forensic Centre (NRDPFC [web.nrdpfc.ca](http://web.nrdpfc.ca)) and provides a complete DNA typing service. Established in 1982 the Wildlife Institute of India ([www.wii.gov.in](http://www.wii.gov.in)) runs a number of projects relevant to endangered and

protected species in the subcontinent. The majority of operational DNA forensic laboratories have a remit of human identification. These laboratories have standard operating procedures for each step as part of their general quality management system. New methods, such as the processes of species identification, are not easily introduced into operational work. Additionally, the majority of forensic laboratories have backlogs in their casework and are under severe financial constraints. The introduction and validation of new techniques, such as those required for wildlife investigation, are time consuming and expensive, and are therefore prohibitive. The result is that devoting time and resources to wildlife investigations has a low priority in these laboratories. The follow on is that cases that are requested for examination are either not accepted by the laboratory or this type of work is contracted out to academics within universities who have access to the appropriate equipment.

Forensic science within universities falls under two types: either a facility with the capability of performing forensic science investigations and who undertake this type of analysis routinely; or a research focused laboratory that can assist on a one-off or case by case basis. Those laboratories that specialise in forensic science and forensic science research can accommodate casework and are in a position to provide the correct controls to ensure their casework conforms to forensic quality standards. Those university laboratories that do not specialise in forensic science are generally contacted in rare circumstances when police and prosecutors are presented with an unusual form of evidence. Usually this leads the police to seek out assistance from scientists who may be able to: (i) identify the evidence (i.e. species) and (ii) individualise the evidence. There can be issues with the quality of any work conducted, regardless of the laboratory that undertakes forensic wildlife work. It would be assumed that work conducted in an operational laboratory, whether human identification or wildlife work, would meet the same rigorous standards. Other laboratories would have to ensure that: proper anti-contamination procedures were in place prior to undertaking any examination; the tests performed were validated prior to use; the appropriate control samples were processed; and the report is written in the appropriate format. For a more detailed discussion of this topic see Ogden (2010).

## 1.6 Accreditation and certification

Quality management is a crucial aspect of mainstream forensic science laboratories. It is a requirement, for instance, for all members of the European Network of Forensic Science Institutes (ENFSI) that laboratories are accredited to ISO 17025. This international standard covers analytical laboratories and any laboratory that meets these standards has demonstrated a high

standard in quality management. The process of gaining ISO 17025 accreditation is financially costly and is rarely gained by university-based organisations, and while there is no doubt that many will work to a minimum of 'Good Laboratory Practice', work conducted in non-accredited laboratories may face greater challenge in the courtroom.

The International Laboratory Accreditation Cooperation (ILAC) works to oversee evaluation of analytical laboratories to those standards developed by the International Organisation for Standardisation (ISO – actually comes from the Greek word for equal). In 1999 a standard for analytical testing was developed with an added aspect of competence. The determination of whether a laboratory meets ISO 17025 is performed at a national level: in the United States for instance by the American Society of Crime Laboratory Directors (ASCLAD); in the United Kingdom by the United Kingdom Accreditation Service (UKAS); and in Australia by the National Association of Testing Authorities (NATA). All developed countries have their own testing authority, or will use another country's testing authority, in the process of accreditation.

There are two main sections in ISO 17025: (i) Management Requirements and (ii) Technical Requirements. Management Requirements describe the operation and effectiveness of the quality management system within the laboratory. The Technical Requirements include factors which determine the maintenance of equipment, the calibration of equipment and factors that might affect the robustness and reliability of the tests performed in laboratory. It is not possible in one chapter to detail all the standards in the ISO 17025 documentation; rather the broad areas are listed below:

- Descriptors of personnel such as the competence/training level to perform specific tasks;
- Training records underpinning the above descriptors;
- Recording of the maintenance and performance of equipment;
- Calibration of equipment;
- Use of appropriate reference materials;
- Internal validation and verification of procedures performed by the laboratory;
- Checking and confirmation of data obtained in an analysis;
- Record of proficiency tests;
- Review of case files and statements prior to submitting to a court; and
- Methods of secure storage of samples/items to minimise contamination.

Accreditation to ISO 17025 does include a competence aspect but is aimed primarily at the quality of the laboratory performance rather than the competence of the staff. There are few means of certification for forensic scientists let alone for those involved in wildlife forensic science. The SWFS have established proficiency tests and a process towards certification of practitioners in

the United States. Proficiency tests are one way to establish competence to perform a test, although declared tests where the laboratory personnel are aware of the testing procedure are less effective than undeclared tests. Training leading to a qualification demonstrates competence or knowledge at the time of taking the test, but does not indicate maintained competence over years. It is necessary to maintain competence or knowledge depending on the role of the person involved in the forensic process. An example in this regard is a witness professing expertise and therefore an ability to give opinion evidence given that they have a PhD; but if the doctorate was awarded 25 years previously, does this still make them an expert? Current and relevant expertise would need to be demonstrated, which may include maintenance of expertise through proficiency tests, prolific publication of relevant research papers and/or presenting at international conferences.

There are requirements laid down by the UK Law Commission (The Law Commission, 2011) with regard to the expert witness and these are relevant internationally.

**Assistance:** an expert's opinion: is admissible to furnish the court with . . . Information which is likely to be outside the experience and knowledge of a judge or jury. If on the proven facts a judge or jury can form their own conclusions without help, then the opinion of an expert is not necessary.

**Expertise:** this knowledge or competence can be gained through recent and relevant professional qualifications or, competence through proficiency tests; however, a definition given is: a person may be qualified to give expert evidence by virtue of study, training, experience or any other appropriate means.

**Impartiality:** in the adversarial criminal justice system an expert may be instructed by the prosecution or defence, but ultimately the expert must be impartial and work for the court. This is defined by: for an expert to be qualified to give evidence as an expert, he or she must be able to provide an objective, unbiased opinion on matter to which his or her evidence relates.

**Evidential Reliability:** described as being 'whether the subject matter of the (expert's) opinion forms part of a body of knowledge or experience which is sufficiently organised to be accepted as a reliable body of knowledge or experience'.

In most jurisdictions it is the judge that is gatekeeper of acceptance of expertise although credentials of an expert may be challenged in the court.

The aspect of impartiality is further underpinned by a Code of Ethics. Most societies or organisations have a Code of Ethics to which all members should adhere. Such a code is being developed by the SWFS specific to members and developed for those involved with wildlife forensic science investigations.

The UK Forensic Science Society, the Australia New Zealand Forensic Science Society, and the ENFSI have such a Code; this may be documented in a statement provided for the court. In the UK there is the disclosure of Experts Evidence that sets out clearly the role and obligation of the expert witness. These standards outlining disclosure are provided in Box 1.2 and a paragraph stating that the expert is bound by these standards is commonly provided in any statement by an expert witness. Not only should adherence to the appropriate ethical codes be stated but also it is important that they are actually read and understood.

***Box 1.2 The duties of an expert witness in the UK.  
(adapted from the web site of the Crown Prosecution Service)***

**Discharging your obligations**

There are three key obligations arising for you, as an expert, as the investigation progresses. Your understanding of these obligations and your delivery of them is the key to you adequately fulfilling your disclosure obligations. The relevant steps are to **retain**, to **record** and to **reveal**.

***Retain***

**What to retain** You should retain everything, including physical, written and electronically captured material, until otherwise instructed and the investigator has indicated the appropriate action to take.

**How long to retain** The period of time for which materials are required to be retained will vary from case to case and will depend on a number of factors. Examples include the nature of the offence; the stage and status of any legal proceedings; whether the case is of special interest. It must also be remembered that the retention requirement may alter as a result of a change of circumstances during the course of the investigation.

You should, therefore, obtain advice from the investigator for the retention period that applies to this particular investigation and always before contemplating destruction of any material.

***Record***

**When to record** The requirement for you to commence making records begins at the time you receive instructions and continues for the whole of the time you are involved.

Circumstances may exist, however, where practitioners should commence making records, in accordance with this guidance, prior to any instructions from the police. Examples of this would be:

- where as a pathologist the outcome of a ‘routine’ post-mortem suggests to you that death has been caused under suspicious circumstances;
- as a medical practitioner you find injuries that are not consistent with the alleged cause;
- as a fire scene examiner you believe a fire to have been started deliberately.

In all these examples the criminal investigation will start after the practitioner’s original involvement but the results of the previous examinations will almost certainly be material to any investigation and subsequent prosecution. The list is not intended to be exhaustive.

**If you have any doubts, start recording.**

**What to record** You should keep records of all the work you have carried out and any findings you make in relation to the investigation. The guidance provided below reflects best practice and your records, as a minimum, should contain information relating to the collection and movement of items, including:

- the date on which you take or receive material (physical items and information) and the date of subsequent movement of the material to another party;
- from who or where and to whom or where material is moved;
- the means by which you receive or pass material from/to another party;
- the examination of materials;
- your notes, and those of any assistant, should be signed, dated, attributable to the individual and produced contemporaneously, whenever practicable;
- the notes should be sufficiently detailed and expressed in such a manner that another expert in your field can follow the nature of the work undertaken, any assumptions made and the inferences you have drawn from the work;
- verbal and other communications;
- you should keep your own notes of all meetings you attend;
- you should keep your own notes of telephone conversations and it is important that points of agreement, or disagreement and agreed actions are recorded;

- you should ensure that a record of all emails and other electronic transmissions (such as images), sent or received, is kept;
- you should keep clear notes of any witness accounts or explanations that you have been provided with, or any other information received.

**How to record** The media you use for making your records should be capable of meeting all the requirements given above, be durable and provide a reliable means of retrieval.

Your notes, in whatever form, should also be structured in a manner that facilitates review, while complying with any necessary security requirements. Any updates, alterations or comments should be clear. It is important that your notes are clear and comprehensive. This will allow another person who may subsequently review them to have a full understanding of the position at any given time.

### **Reveal**

**What to reveal** You are required to reveal everything you have recorded.

It is a necessary and important part of your disclosure obligations to make the Prosecution Team aware of all the material you have in your possession in relation to the investigation. This will then enable them to make informed decisions as to what material is relevant, and then what material satisfies the disclosure test.

**How to reveal** There are two ways in which you will reveal material to the Prosecution Team.

**The Report** Your report(s) should contain information relating to the following:

- details of your qualifications, experience or accreditation relevant to the work performed;
- the range and extent of your expertise;
- details of any information upon which you have relied in arriving at your opinion;
- details of any statements of fact upon which you have relied in reaching your opinion;
- clarification of which of the facts are within your own knowledge;
- information relating to who has carried out measurements, examinations, tests etc and if under your supervision;
- your opinion(s) and a justification for these;
- where you have provided qualified opinions details of the qualifications;
- a summary of all your conclusions.

**Statements** In addition to all of the above you may be required to make a formal statement. The statement should contain all of the above and the following:

- the declaration which confirms that you understand your duty to the court in respect of disclosure;
- an acknowledgement that you will inform all parties and, where appropriate, the court, in the event that your view changes on any material issue.

**When compiling your report/statement you should ensure that due regard is given to any information that points away from, as well as towards, the defendant(s).**

**You must not give expert opinion beyond your area of expertise.**

**The Index of Unused Material** In order to reveal material to the Prosecution Team, it is necessary that you to complete an index of unused material, (the Index) describing all the unused material in your possession. All the material not identified in your report/statement should be placed on the Index. ([www.cps.gov.uk/legal/d\\_to\\_g/disclosure\\_manual/annex\\_k\\_disclosure\\_manual/](http://www.cps.gov.uk/legal/d_to_g/disclosure_manual/annex_k_disclosure_manual/)).

## 1.7 Standardisation and validation

The methods used in wildlife testing need to be of the same standard and meet the same acceptance in court as those used in human identification, although this is not always the case or possible. Since the first reported use of human DNA for forensic science in 1985, there have been huge amounts of time, resources, funding and research that have gone into human DNA testing to ensure that it is accurate, reliable, robust and reproducible. As the same levels of resources are not available for wildlife work and a new set of tests would need to be developed for each different species of interest, there are currently no non-human tests with the same levels of discrimination as are available for human DNA work.

Prior to 2010 there was a single publication recommending standardisation of methods used in the forensic investigation of wildlife crime (Budowle *et al.*, 2005). Two societies that oversee aspects of wildlife forensic genetics are the ISFG, with a remit particular in human identification, and ISAG, whose remit is primarily in animal genetics. The main focus of the ISFG is that of human genetics having developed from the International Society for Forensic Haemogenetics. While the ISAG is focused on animal species, it is concerned primarily with domesticated species and with species with commercial value. The growing, although still a niche area, of wildlife forensic science does not fall under either of these categories. The ISFG, in discussion

with the ISAG, instigated a commission to examine standards and procedures relevant to forensic practice using non-human DNA; this commission was to up-date the earlier study by Budowle *et al.* (2005). As a result of the ISFG Commission, 13 recommendations were published (Linacre *et al.*, 2010) starting at the scene, through species identification and linkage assignment to the reporting of results. Reference where appropriate will be made to these recommendations in this book but are provided in Box 1.3.

***Box 1.3 The 13 recommendations reported by the International Society for Forensic Genetics Commission on non-human (animal) DNA in forensic genetic investigations***

**Recommendation #1**

The same procedures to ensure integrity and traceability of the items should be employed in the collection and examination of animal samples as undertaken for any other forensic investigation.

**Recommendation #2**

Validation studies from non-domesticated species should use voucher specimens where possible. If this is not possible then a justification needs to be made for the sample type used.

**Recommendation #3**

The choice of locus/loci used in species identification, such as, but not restricted to, the mitochondrial genes *cyt b*, COI, and the D-loop region, needs to be justified based on the ability to identify the unknown species among those that are close genetic relatives.

**Recommendation #4**

The nucleotide sequence and map showing the location of the primers used in species testing needs to be provided or referenced to a previously published article.

**Recommendation #5**

Intraspecies and interspecies studies should be provided for any novel primer set used in species identification. The process undertaken to

validate the test should be provided, including, but not exclusively, studies on sensitivity, specificity, reproducibility and mixed samples.

**Recommendation #6**

Primers used to amplify polymorphic DNA should be tested to ensure specificity and reproducibility and should be published in the public domain.

**Recommendation #7**

If repeat-based polymorphic loci are used for individualisation, tetrameric short tandem repeat systems should be used preferentially.

**Recommendation #8**

Sequenced allelic ladders are essential for the accurate designation of alleles and should be used in all STR typing. The number of repeats should be the basis of reporting of results rather than using only the size based on the number of base pairs of any samples tested.

**Recommendation #9**

In relationship testing, the mutation probabilities of the STR alleles should be estimated if encountered, or at least the probability of a mutational event occurring should be considered when there is genetic inconsistency at a single or few loci while all other loci show genetic consistency.

**Recommendation #10**

Relevant population and forensic genetic parameters including allele frequencies should be estimated.

**Recommendation #11**

A kinship factor should be determined and applied in any calculation. The type of kinship factor applied should be stated clearly and justification should be made for the factor incorporated.

**Recommendation #12**

A comprehensive casefile should be maintained. A likelihood ratio approach is the recommended way to evaluate the weight of the evidence, considering more than one proposition.

**Recommendation #13**

Accreditation should be sought if DNA testing of non-human animal DNA for a particular purpose is to become routine.

More recently the SWFS has been established with conferences planned for every three years. Membership is predominantly based in North America at the time of publication of this book although much of the work of the Society will be relevant to wildlife forensic science performed anywhere in the world. Research in non-human uses of DNA, and in particular in regard to wildlife DNA, is very much international.

The science behind forensic testing came under scrutiny in the United States through the publication of 'Strengthening Forensic Science in the United States: A Path Forward' by the US National Academy of Sciences (National Research Council, 2009). While DNA was held up as the 'Gold Standard', one recommendation (Recommendation 7 of the NAS Report) called for mandatory laboratory accreditation and personal certification. This in turn led to the establishment of a Scientific Working Group for Wildlife Forensic Sciences (SWGWILD), which has established proficiency tests and is setting standards within forensic wildlife laboratories. It should be noted that SWGWILD and the whole area of forensic wildlife covers areas outside of DNA typing. Testing for isotopes, profiling the chemical signature of samples and the whole field of morphology (including the skill set of microscopy) can be part of forensic wildlife investigations. Specifically when it comes to aspects of DNA typing (such as linking a sample to an individual organism), the ideal situation is to set common international standards, be it for wildlife or human forensic testing, as the standards, methodologies and scope of future technologies are pertinent to any jurisdiction. In this regard societies with an international membership are well-placed to develop such standards.

It is necessary to show the court that the processes used in wildlife forensic science are reliable, robust and reproducible. These three tenets form the process of validation of a technique to show that the test is fit for the purpose for which it is intended. Robust, reliable and reproducible are in many ways interrelated but also distinct; robust being a method that gives a result almost all of the time and does not need to be repeated; reliable describes a method where the result obtained is the one expected and it is accurate or precise; and reproducible refers to a method that can be repeated and the same result obtained.

Related to the processes of reliability, robustness and reproducibility are the three S's; sensitivity, specificity and stability. The limit of detection addresses the question of the sensitivity of a technique and can often be used to separate background data from real data. Specificity testing shows that the test works on the substrate for which it is developed and should not work

on any substrate not specified as part of the test. Stability experiments are designed to show how environmental factors affect the data obtained, often over a defined time period.

Tests designed to show the specificity, sensitivity and stability of the method can then be part of a study to illustrate the robustness, reliability and reproducibility. The rate of obtaining a false positive result needs to be shown, that is a result that gives the ‘correct’ result even though it should not do so. False negatives are the opposite, where a sample that should give the correct result gives an incorrect result. As pointed out earlier, if a research test has a false positive of 1 in 100 (or 99% accuracy) then 1 in 100 times a sample from a crime scene and a sample from a reference material may ‘match’ even though they should not do so. This error rate may be acceptable in a research context but is unacceptable in human identification and should be unacceptable in wildlife forensic science. If an error rate is unavoidable due to the nature of the test then this needs to be stated clearly if the method is ever used in the criminal justice system. The steps to validation start frequently with internal trials and if successful become available for external testing, using casework samples and blind testing and potentially publication in an international peer-reviewed journal.

The whole process of validation sounds tedious but is a crucial part of any forensic science laboratory. The steps in human identification have a comprehensive history of validation that are available at [www.cstl.nist.gov/strbase/validation.htm](http://www.cstl.nist.gov/strbase/validation.htm) and the FBI released a publication detailing the steps towards validation as a guideline to laboratories conducting human identification in the United States (Scientific Working Group on DNA Analysis Methods (SWGDM), 2004). There are published examples where a research process developed for wildlife forensic science has then been validated using much of the processes described above (Tobe and Linacre, 2008). These validation type studies are near to the end of the process of taking a research tool to the courtroom. If employed by an operational forensic science laboratory, elements of quality assurance and quality control over the process are required. International accreditation is the ultimate recognition of the quality of the work through a standard such as ISO 17025 and other national accreditation bodies. While noting the importance of accreditation through IOS 17025, it is also noted that the cost to small laboratories may be prohibitive and unrealistic – hence the importance of competence testing or certification of the laboratory staff.

## **1.8 Collection of evidential material, continuity of evidence and transportation to the laboratory**

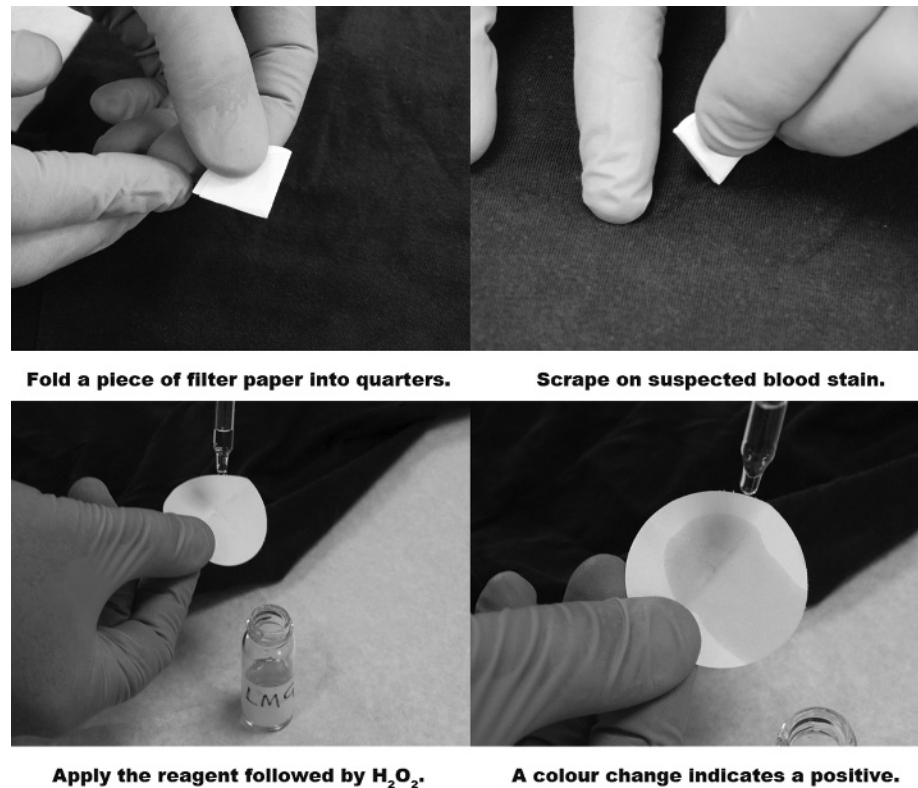
Regardless of whether a suspected wildlife crime or an alleged crime against a person or property, the same methods should be adhered to; this is

recommendation 1 of the ISFG Commission on non-human DNA (Linacre *et al.*, 2010).

The general mantra of crime scene examination is ‘Control, Record, Collect’. This essentially gives the order of the processes to be conducted. First, the scene should be *controlled*, this being the process of cordoning off the scene and establishing a log of anyone who has cause to enter the perimeter of the scene. *Record* is self-evident, but any scene requires comprehensive recording using ideally a range of media such as video, photography and sketches. It is essential that the scene is recorded to ensure that it is possible to identify the context of any item retrieved. *Collect* is the last stage, as normally the material to be collected is unlikely to disappear; that is unless weather factors such as rain, which are a real issue for outdoor scenes as can be the case in many wildlife crime scenes.

Any evidence, or other items collected, must be packaged properly. The importance of packaging is to ensure that the item is not compromised or contaminated between collection and receipt into the forensic science laboratory. The different types of material encountered in wildlife forensic science may have different ideal packaging types. Regardless of the packaging, the aim is to ensure that nothing is lost from the item and nothing new enters the package. The list below is not exclusive but covers the main types of material common in wildlife forensic science. In all instances it is best to record where, when and by whom the sample was collected. This initiates the process of continuity of evidence and also provides a record of the context of the item (see below).

- *Blood*: it is unlikely that liquid blood will be encountered but if it is then it is best if the blood is allowed to dry on a piece of filter paper. FTA paper is the best option as this paper not only dries the blood but protects it from degradation. If there is dried blood on clothing then the clothing should be packaged in a clean paper bag. The dried blood stain is best examined *in situ* in the forensic science laboratory and should not be removed from the clothing at the scene. The same is true for any portable object. The interpretation of the shape and position of the bloodstain may be important and hence these aspects should be recorded prior to removal of the blood from the item. Paper is used for packaging as this is permeable to air and will prevent the accumulation of condensed water inside the bag; this might happen if the item is placed in a sealed plastic bag. A presumptive test for the presence of blood is shown in Figure 1.2. The process is straightforward and routine in forensic science laboratories: a piece of filter paper is folded to make a corner; this corner is rubbed against the stain to be tested; the filter paper is opened out and a drop of blood testing liquid (Leuchomalachite Green in this example or Kastle–Mayer); this is followed by a drop of hydrogen peroxide; if haemoglobin is present a colour change will occur in a few seconds. Hemastix are simple filter-based



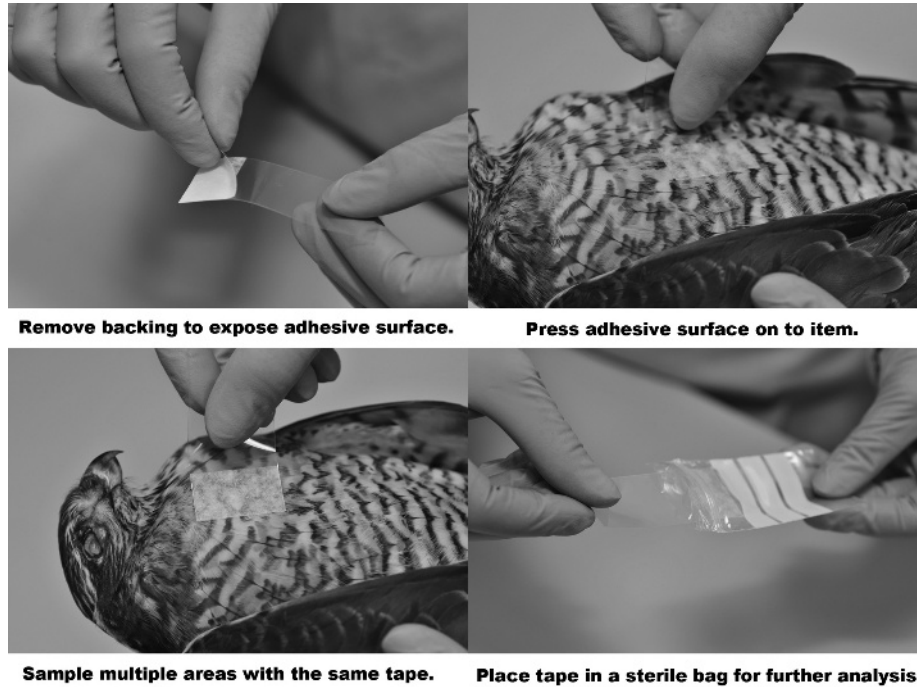
**Figure 1.2** An example of the LMG (Leucomalachite Green) presumptive test for blood. A piece of filter paper is folded into a quarter and scraped against a suspected stain. The LMG reagent and hydrogen peroxide ( $H_2O_2$ ) are added. A colour change indicates the presence of blood, although some other substances can also cause a reaction. LMG reacts with blood from any species and is not human specific. (For colour details please see colour plate section.) © S. S. Tobe, with permission.

alternatives to these fluid-based presumptive tests. The process is also very simple whereby a filter strip is placed in gentle contact with a moistened stain or swab and within a few seconds a colour change occurs if haemoglobin is present.

- *Hair/Fur/Feathers/Scales*: removal of hair/fur/feathers/scales is best performed with sterile tweezers. The samples are then placed in an envelope, sealed, and labelled appropriately before transporting to the laboratory. If there are too many hairs to remove individually, then tape-lifting might be the better option. Tape-lifting entails the examiner placing a strip of adhesive tape on the item and flattening gently for a few seconds, then removal of the tape and then placing on an adjacent part of the item if necessary. The tape may be placed four to five times to collect much loosely adhering material. The sticky side of the tape is then covered with a sheet of film, or

## 1.8 COLLECTION OF EVIDENTIAL MATERIAL

27

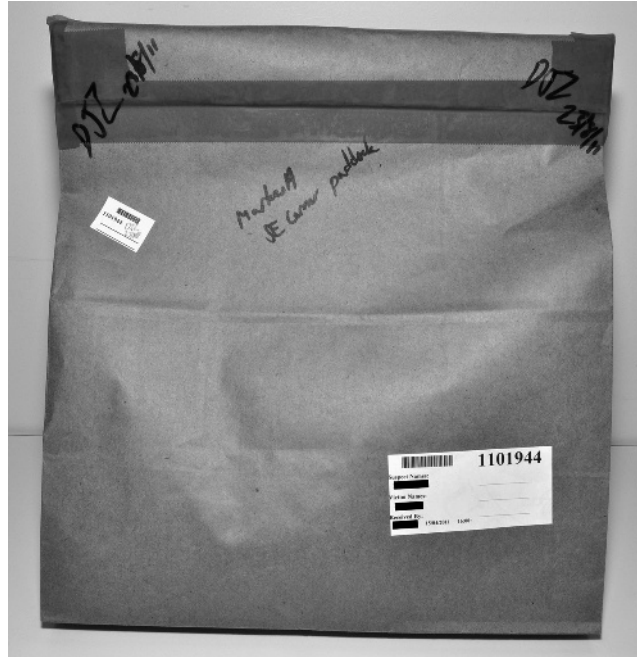


**Figure 1.3** An example of a tape lift. In this instance a raptor is being taped for DNA using a specialised mini-tape designed to collect DNA, although other material is also trapped by the adhesive surface. Larger tapes are used to collect fibres, hairs, feathers and other materials using a similar process. © S. S. Tobe, with permission.

placed in a sterile bag, to protect it and it is transferred to the laboratory for microscopic examination. An example is shown in Figure 1.3.

- *Bone/shells/teeth*: after initial photography and recording of the item, the preferred means of packaging depends on the size of the item. It should be placed in either a paper sack, for the same reasons as blood, or a cardboard box with ventilation to allow air to enter the box.
- *Pollen/trace botanical*: the removal of trace material or botanical material is similar to that of hairs and furs. In the case of pollen and other tiny traces of material it is important to ensure that any packaging, such as an envelope, is intact and does not lead to loss of material during transport, which could also result in contamination of other items.

Continuity of evidence is the process by which an item can be traced from the time it is collected through each stage of the analysis. Any person who has cause to handle/examine the item should sign and date the label associated with the item. The type of label will vary depending on the jurisdiction but typically the label should have space for the following information:



**Figure 1.4** An example of packaging illustrating that paper is used most commonly for items on which it is suspected that there may be biological materials. Note that the package is sealed with a signature across the seal. A barcode is placed on the package to indicate the case name and a unique identifier.

a description of the item to be packaged inside; the time and date it was collected; the item number or other unique identifier; the case number (if known); and the agency to whom the collector belongs. An example of the type of package is shown in Figure 1.4.

It should be stressed that the mishandling of evidence, poor packaging, or gaps in the continuity of evidence may lead to the item being ruled inadmissible in court. All the results of any subsequent analyses will also be ruled inadmissible as the item could have been compromised prior to examination. The above processes of labelling and continuity of evidence systems are standard in countries with highly developed forensic systems. There is a real problem with trying to maintain these standards in countries that are underdeveloped and very poorly resourced.

Once received into the forensic science laboratory, a barcode may be placed on the item as part of a Laboratory Information Management System (LIMS). Such systems allow all items examined in the laboratory to be traced and are used commonly in laboratories that have gained accreditation to ISO17025 standard. If there is no LIMS system in operation, and for small laboratories this is the norm, comprehensive note taking within a casefile is the alternative.

## 1.9 Note taking and maintenance of a casefile

It is essential that a casefile is maintained that contains all documents generated during the analyses. Every step in the handling and processing of samples needs to be recorded and these notes should be made contemporaneously. It should be noted that casefiles may vary in size from only a few pages, if the case is simple and requires little analysis, or may extend to many lever-arch files if the case is protracted and requires much examination. Casefiles should be indexed and every page numbered – note taking and recording is a laborious but necessary aspect of forensic investigation.

Most casefiles will have near the front a submission form detailing the allegation. This is crucial, the importance of which is detailed in the next section, as the allegation sets in motion the appropriate and most relevant methods to be performed. A record of each following step of the examination process will follow subsequently in the casefile. These notes include telephone conversations and emails – such communications (particularly telephone conversations) should contain a note of the date, time and a short minute of what was said. All meetings with investigators or legal counsel need similarly to be recorded with a minute of who was present, along with the date and time, and include the reason and outcome of the meeting.

Casefiles relevant to a DNA examination may include a record of the items submitted, when, where and by whom. Examination notes may also include a record of the size, mass and general description of the item. An example of this type of examination form is shown in Figure 1.5. Digital photographs are now a huge advantage to those examiners unable to sketch (from personal experience), however a simple diagram showing dimensions is essential. Any sample removed from an item needs to be recorded and given its own unique identifier. DNA extraction methods, which are detailed in Chapter 2, will have their own standard operating procedures (SOPs); SOPs provide a step-by-step procedure that needs to be adhered to. A copy of the SOP with information related to the particular sample being examined should be placed in the casefile along with a date and note of the operator. An example of a PCR set-up form is shown in Figure 1.6. Printouts of all DNA data, such as DNA electropherograms will be included in the casefile; this must include all controls (such as positive and negative controls) and comparisons to allelic ladders. Nothing can be hidden or ignored. Drafts of statements and any annotations made during a review step should be kept along with copies of the final statement.

Casefiles serve a number of purposes. First, they are a complete record such that any expert should be able to repeat the steps detailed in the casefile to verify that the steps were appropriate and that the data obtained were that expected given the notes in the casefile. Such comprehensive recording may be essential if an expert requires access to the notes. Further, the final statement to be given to the court may be written days or weeks after the examination was undertaken and hence the casefile is an aide-memoire to

GENERAL EXAMINATION FORM			PAGE no:	
Item Description:	Manufacturer	Case number		
Relating To:	Size	Item Number		
	Composition			
Marked Labelled		Packaging & Seal		
Hairs	Blood	Semen	Damage	
Left in situ	Positive	Pollight	No Damage	
Recovered	Negative	AP Test	Damage Present	
Root		PSA		
General Condition				
Examined By		Location	Date	
<p>Printed 30/08/2011 Authorised by AMTL</p> <p style="text-align: right;">Q:QA/FUSA Proform/Form1</p>				

**Figure 1.5** An example of a general examination form used for the recording of the examination of items.

ensure that any subsequent statement is accurate in every detail. It may not be possible to re-examine items and hence the statement must be made based on the notes taken at the time of the examination and now in the casefile. There is an adage that *'if you did not write it down you did not do it'*; this states eloquently the importance of recording everything in a casefile.

## 1.10 Case assessment and initial testing

The processes that are involved in human identification for forensic purposes have evolved to take account of developments in DNA analysis. There are many similarities in the approach to evaluating non-human DNA, although there are a few instances that are peculiar to wildlife crime.

At the outset of the case there must be an allegation, otherwise there would not be a need for the involvement of a forensic science laboratory. Given

## 1.10 CASE ASSESSMENT AND INITIAL TESTING

31

**DNA Testing Laboratory DNA Amplification Form**

Case Number: 4751/08

Operator: A Linacre Date: 11/01/2012

Sample ID	CB1	WG1a	Wg2a	Du1	-ve			
Extraction tube ID	CB1	WG1a	Wg2a	Du1	-ve			
Extraction Method	Q1103	Q1103	Q1103	Q1103	Q1103			
Final volume in DNA extract	100µL	100µL	100µL	100µL	100µL			

Amplification Procedure: SGM+

Kit Lot No: 876A9214

Expiry Date: 12/08/2012

Extraction tube ID	CB1	WG1a	WG2a	Du1	-ve			
PCR tube ID	CB1	WG1a	WG2a	Du1	-ve	PCR +ve		
Amount used in PCR	5µL	5µL	5µL	1µL	10µL	10µL		
Amount of water added	5µL	5µL	5µL	9µL	-	-		
Final Volume	25µL	25µL	25µL	25µL	25µL	25µL		

Witnessed by: S Tobe

Multimix volumes:

Powerplex-16		
# of samples	1	#10
Rxn. Mix µL	2.5	25
Primer µL	2.5	25
H <sub>2</sub> O µL		
Taq µL	0.8	8
Template		
Total	25	250

Signed: A Linacre

**Figure 1.6** An example of a DNA amplification form completed as a record of the samples analysed by PCR.

the allegation the question asked is ‘*what would I expect if the allegation were true?*’. This sets out the type of examination that should be performed to address this allegation. For instance, if a shipment of medical supplies is intercepted, the allegation is that there is reason to believe that they contain CITES listed species, the simple thought process is *if* the allegation is true then DNA from CITES listed species should be present. The alternative is

obviously that no biological material from such species is present. Given that the laboratory has a test for the presence of a range of CITES listed species, a positive result for DNA from a CITES listed species would support the allegation and be the expected result if the allegation was true. If a negative result is obtained then this would be the expected result **if** there was no DNA from such a species, or there was DNA but at levels below the sensitivity of the test. The use of positive controls will determine that the test should give a result at known concentrations of DNA from the species in question. A negative control is essential as this will determine if there is any chance that the result was obtained as a result of gross contamination. In such an allegation there is little to be gained from using a test to assign the DNA to a particular individual or population. This book will outline a range of DNA-based tests that address particular questions, however the key point is that the appropriate analytical test should be used to address the allegation.

### 1.11 Scope of book

There have been previous books on non-human DNA typing (Coyle, 2008) wildlife forensic science (Linacre, 2009) and most recently 'Wildlife Forensics, Methods and Applications' (Huffman and Wallace, 2012). These three books are multi-authored and contain one or more chapters relevant to DNA typing. The aim of this book is to concentrate on the use of DNA typing in wildlife crime and to take the reader through DNA typing with little or no requirement of knowledge of molecular genetics. No previous text book has explained in detail how DNA typing data are used in forensic wildlife investigations and the steps involved in using software to evaluate these data. The endpoint is that the reader should be able to follow through the steps and produce their own alignments and develop their own tests following proper guidelines and validation procedures. Case examples, including some of those in which the authors have been involved in their investigation, will be used to illustrate how DNA profiling can be applied to wildlife crime.

Wildlife crime encompasses many species but this book will focus on vertebrate species with an emphasis on mammalian and avian species. It should be appreciated that there is much that can be done using other vertebrate and invertebrate species, botanical and microbial DNA. Comment will be made where appropriate to these other and varied areas of DNA typing, although the scope of this book will be on wildlife crime and in particular cases involving mammalian and avian species.

### Useful websites

Australian Museum (Sydney): <http://www.australianmuseum.net.au>

Australian Wildlife Forensic Services: [www.wildlifeforensics.com.au](http://www.wildlifeforensics.com.au)

CITES: [www.cites.org](http://www.cites.org)

## REFERENCES

33

- European Union Environment Directorate: [http://ec.europa.eu/environment/cites/home\\_en.htm](http://ec.europa.eu/environment/cites/home_en.htm)
- International Society for Animal Genetics: [www.isag.us](http://www.isag.us)
- International Society for Forensic Genetics: [www.isfg.org](http://www.isfg.org)
- International Union for Conservation of Nature: [www.iun.org](http://www.iun.org)
- Natural Resources DNA Profiling and Forensic Centre (Canada): <http://web.nrdpfc.ca/>
- Partnership Against Wildlife Crime (UK): <http://www.defra.gov.uk/paw/>
- Society for Wildlife Forensic Science: [www.wildlifeformenscience.org](http://www.wildlifeformenscience.org)
- South-East Asian Nations Wildlife Enforcement Network: [www.asean-wen.org](http://www.asean-wen.org)
- STR Base: [www.cstl.nist.gov/strbase/validation.htm](http://www.cstl.nist.gov/strbase/validation.htm)
- TRAFFIC: [www.traffic.org](http://www.traffic.org)
- U.S. National Fish and Wildlife Forensics Laboratory in Oregon: [www.labs.fws.gov](http://www.labs.fws.gov)
- Wildlife Institute of India: [www.wii.gov.in](http://www.wii.gov.in)
- World Wide Fund for Nature: [www.wwf.org](http://www.wwf.org)

## References

1970. Conservation of Seals Act 1970. *1970 Chapter 30*. United Kingdom.
1992. Protection of Badgers Act 1992 *1992 Chapter 51*. United Kingdom.
- Alacs, E. & Georges, A. 2008. Wildlife across our borders: a review of the illegal trade in Australia. *Australian Journal of Forensic Sciences*, 40, 147–160.
- Alacs, E., A. Georges, A., Fitzsimmons, N.N. & Robertson, J. 2010. DNA detective: a review of molecular approaches to wildlife forensics. *Forensic Science, Medicine, and Pathology*, 6, 180–194.
- American Pet Products Association (APPA) 2010. *2009/2010 APPA National Pet Owners Survey*, American Pet Products Association.
- Azad, D. & Bhadauria, R. 2008. Role of plant science in solving the crime. *Advances in Plant Sciences*, 21, 369.
- Barton, N. & Jones, J.S. 1983. Mitochondrial-DNA – new clues about evolution. *Nature*, 306, 317–318.
- Berger, B., Eichmann, C. & Parson, W. 2008. Forensic canine STR analysis. In: Coyle, H. (ed.) *Nonhuman DNA typing*. Boca Raton: CRC Press.
- Budowle, B., Garofano, P., Hellman, A., et al. 2005. Recommendations for animal DNA forensic and identity testing. *International Journal of Legal Medicine*, 119, 295–302.
- Burke, T. & Bruford, M.W. 1987. DNA fingerprinting in birds. *Nature*, 327, 149–152.
- Chapman, D.D., Abercrombie, D.L., Douady, C.J., Pikitch, E.K., Stanhopen, M.J. & Shivji, M.S. 2003. A streamlined, bi-organelle, multiplex PCR approach to species identification: Application to global conservation and trade monitoring of the great white shark, *Carcharodon carcharias*. *Conservation Genetics*, 4, 415–425.
- Christy, B. 2010. *Wildlife Smuggling: Why Does Wildlife Crime Reporting Suck?* [Online]. Available: [http://www.huffingtonpost.com/2010/01/04/wildlife-smuggling-why-do\\_n\\_410269.html](http://www.huffingtonpost.com/2010/01/04/wildlife-smuggling-why-do_n_410269.html) [Accessed 24 October 2012].

- Courchamp, F., Angulo, E., Rivalan, P., *et al.* 2006. Rarity value and species extinction: The anthropogenic Allee effect. *Plos Biology*, 4, 2405–2410.
- Coyle, H. (ed.) 2008. *Nonhuman DNA Typing*, Boca Raton: CRC Press.
- Craft, K.J., Owens, J.D. & Ashley, M.V. 2007. Application of plant DNA markers in forensic botany: Genetic comparison of *Quercus* evidence leaves to crime scene trees using microsatellites. *Forensic Science International*, 165, 64–70.
- Eurlings, M.C.M., Van Beek, H.H. & Gravendeel, B. 2010. Polymorphic microsatellites for forensic identification of agarwood (*Aquilaria crassna*). *Forensic Science International*, 197, 30–34.
- Finkeldey, R., Leinemann, L. & Gailing, O. 2010. Molecular genetic tools to infer the origin of forest plants and wood. *Applied Microbiology and Biotechnology*, 85, 1251–1258.
- Gigliano, G.S. 1999. Preliminary data on the usefulness of Internal Transcribed Spacer I (ITS1) sequence in *Cannabis sativa* L. identification. *Journal of Forensic Sciences*, 44, 475–477.
- Goncalves, M.P., Panjer, M., Greenberg, T.S. & Magrath, W.B. Justice for Forests – Improving Criminal Justice Efforts, World Bank Study (2012), [http://siteresources.worldbank.org/EXTFINANCIALSECTOR/Resources/Illegal\\_Loading.pdf](http://siteresources.worldbank.org/EXTFINANCIALSECTOR/Resources/Illegal_Loading.pdf) [Accessed 24 October 2012].
- Halverson, J.L. & Basten, C. 2005. Forensic DNA identification of animal-derived trace evidence: Tools for linking victims and suspects. *Croatian Medical Journal*, 46, 598–605.
- Howard, C., Gilmore, S., Robertson, J. & Peakall, R. 2008. Developmental validation of a *Cannabis sativa* STR multiplex system for forensic analysis. *Journal of Forensic Sciences*, 53, 1061–1067.
- Howard, C., Gilmore, S., Robertson, J. & Peakall, R. 2009. A *Cannabis sativa* STR genotype database for Australian seizures: forensic applications and limitations. *Journal of Forensic Sciences*, 54, 556–563.
- Hsieh, H.-M., Huang, L.-H., Tsai, L.-C., Kuo, Y.-C., Meng, H.-H., Linacre, A. & Lee, J. C.-I. 2003a. Species identification of rhinoceros horns using the cytochrome *b* gene. *Forensic Science International*, 136, 1–11.
- Hsieh, H.M., Hou, R.J., Tsai, L.C., *et al.* I. 2003b. A highly polymorphic STR locus in *Cannabis sativa*. *Forensic Science International*, 131, 53–58.
- Hsieh, H. M., Huang, L. H., Tsai, L. C., *et al.* 2006. Species identification of *Kachuga tecta* using the cytochrome *b* gene. *Journal of Forensic Sciences*, 51, 52–56.
- Hsieh, H.-M., Lee, J. C.-I., Wu, J.-H., *et al.* 2011. Establishing the pangolin mitochondrial D-loop sequences from the confiscated scales. *Forensic Science International-Genetics*, 5, 303–307.
- Huffman, J. & Wallace, J. (eds.) 2012. *Wildlife Forensics: methods and applications*, Chichester, UK: John Wiley & Sons.
- International Rhino Foundation. [www.rhinos-irf.org/black/](http://www.rhinos-irf.org/black/) [Online]. [Accessed May 2010].
- Jeffreys, A.J., Wilson, V. & Thein, S.L. 1985a. Hypervariable minisatellite regions in human DNA. *Nature*, 314, 67–73.
- Jeffreys, A.J., Wilson, V. & Thein, S.L. 1985b. Individual-specific fingerprints of human DNA. *Nature*, 316, 76–79.

## REFERENCES

35

- Lander, E.S. 1989. DNA fingerprinting on trial. *Nature*, 339, 501–505.
- Lee, J., Hsieh, H.M., Huang, L.H., *et al.* 2009. Ivory identification by DNA profiling of cytochrome b gene. *International Journal of Legal Medicine*, 123, 117–121.
- Lee, J.C.I., Tsai, L.C., Yang, C.Y., *et al.* 2006. DNA profiling of shahtoosh. *Electrophoresis*, 27, 3359–3362.
- Li, Y.M., Gao, Z.X., Li, X.H., Wang, S. & Niemela, J. 2000. Illegal wildlife trade in the Himalayan region of China. *Biodiversity and Conservation*, 9, 901–918.
- Li, R. Buongiorno, Turner, J., Zhu, D., J.A.S. & Prestemon, J. 2008. Long-term effects of eliminating illegal logging on the world forest industries, trade and inventory. *Forest Policy and Economics*, 10, 480–490.
- Linacre, A. (ed.) 2009. *Forensic Science in Wildlife Investigations*, Boca Raton: CRC Press.
- Linacre, A., Gusmão, L., Hecht, W., *et al.* 2010. ISFG: Recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. *Forensic Science International Genetics*, 5(5), 501–505.
- Lo, C.F., Lin, Y.R., Chang, H.C. & Lin, J.H. 2006. Identification of turtle shell and its preparations by PCR-DNA sequencing method. *Journal of Food and Drug Analysis*, 14, 153–158.
- Martin, E. & Martin, C. 2010. Russia's mammoth ivory industry expands: what effect on elephants? *Pachyderm*, 47, 26–35.
- Mendoza, M.A., Mills, D.K., Lata, H., Chandra, S., Elsohly, M.A. & Almirall, J.R. 2009. Genetic individualization of *Cannabis sativa* by a short tandem repeat multiplex system. *Analytical and Bioanalytical Chemistry*, 393, 719–726.
- Menotti-Raymond, M., David, V. & O'Brien, S. 1997. Pet cat hair implicates murder suspect. *Nature*, 386, 774.
- Moore, M.K., Bemiss, J.A., Rice, S. M., Quattro, J.M. & Woodley, C.M. 2003. Use of restriction fragment length polymorphisms to identify sea turtle eggs and cooked meats to species. *Conservation Genetics*, 4, 95–103.
- National Research Council of the National Academies 2009 *Strengthening Forensic Science in the United States: A Path Forward*. Washington DC: National Academies Press.
- Ogden, R. 2010. Forensic science, genetics and wildlife biology: getting the right mix for a wildlife DNA forensics lab. *Forensic Science Medicine and Pathology*, 6, 172–179.
- Pääbo, S., Gifford, J.A. & Wilson, A.C. 1988. Mitochondrial DNA sequences from a 7000-year old brain. *Nucleic Acids Research*, 16, 9775–9787.
- Parson, W., Pegoraro, K., Niederstatter, H., Foger, M. & Steinlechner, M. 2000. Species identification by means of the cytochrome b gene. *International Journal of Legal Medicine*, 114, 23–28.
- Patton, S.M. 1990. DNA Fingerprinting: The Castro Case. *Harvard Journal of Law and Technology*, 3, 223–240.
- Scientific Working Group on DNA Analysis Methods (SWGDM) 2004. Revised Validation Guidelines. Forensic Science Communications. [Online] Available from [http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2004/standards/2004\\_03\\_standards02.htm/](http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2004/standards/2004_03_standards02.htm/) (accessed 31 October 2012).

- Sellar, J.M. 2009. Illegal trade and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). In: Linacre, A.M.T. (ed.) *Wildlife Forensic Investigation*. London: Taylor and Francis.
- Stambuk, S., Sutlovic, D., Bakaric, P., Petricevic, S. & Andelinovic, S. 2007. Forensic botany: Potential usefulness of microsatellite-based genotyping of croatian olive (*Olea europaea* L.) in forensic casework. *Croatian Medical Journal*, 48, 556–562.
- The Law Commission 2011. Expert Evidence in Criminal Proceedings in England and Wales. London: The Stationery Office.
- Tobe, S. & Linacre, A. 2007. Species identification of human and deer from mixed biological material. *Forensic Science International*, 169, 278–279.
- Tobe, S.S. & Linacre, A. 2011. A new assay for identifying endangered species in Traditional East Asian Medicine. *Forensic Science International: Genetics Supplement Series*, 3, e232–e233.
- Tobe, S.S. & Linacre, A.M.T. 2008. A multiplex assay to identify 18 European mammal species from mixtures using the mitochondrial cytochrome *b* gene. *Electrophoresis*, 29, 340–347.
- Tobe, S.S., Govan, J. & Welch, L.A. 2011. Recovery of human DNA profiles from poached deer remains: A feasibility study. *Science & Justice*, 51, 190–195.
- Tsai, L.C., Hsieh, H.M., Wang, J.C., Huang, L.H., Linacre, A. & Lee, J.C.I. 2006a. Cannabis seed identification by chloroplast and nuclear DNA. *Forensic Science International*, 158, 250–251.
- Tsai, L.C., Yu, Y.C., Hsieh, H.M., Wang, J.C., Linacre, A. & Lee, J.C.I. 2006b. Species identification using sequences of the trnL intron and the trnL-trnF IGS of chloroplast genome among popular plants in Taiwan. *Forensic Science International*, 164, 193–200.
- Wetton, J.H., Higgs, J.E., Spriggs, A.C., Roney, C.A., Tsang, C.S.F. & Foster, A.P. 2003. Mitochondrial profiling of dog hairs. *Forensic Science International*, 133, 235–241.
- Wilson, M.R., Stoneking, M., Holland, M.M., Dizinno, J.A. & Budowle, B. 1993. Guidelines for the use of mitochondrial-DNA sequencing in forensic-science. *Crime Laboratory Digest*, 20, 68–77.
- World Wide Fund for Nature. [www.worldwildlife.org/species/finder/tigers/index.html](http://www.worldwildlife.org/species/finder/tigers/index.html) [Online]. [Accessed 5 May 2010].
- World Wide Fund for Nature. Illegal logging. [wwf.panda.org/about\\_our\\_earth/about\\_forests/deforestation/forest\\_illegal\\_logging](http://wwf.panda.org/about_our_earth/about_forests/deforestation/forest_illegal_logging) [Online] [Accessed 24 October 2012].
- Zhang, L., Hua, N. & Sun, S. 2008. Wildlife trade, consumption and conservation awareness in southwest China. *Biodiversity and Conservation*, 17(6), 1493–1516.