Chapter

Forensic Analysis in Wildlife Crime Cases: Microscopy, DNA Profiling and Isotope Analysis

Vivek Sahajpal, Sudhanshu Mishra and Deepika Bhandari

Abstract

Illegal wildlife trade is one of the biggest threats to the environment and biodiversity. The growing volume of illegal trade in wildlife jeopardizes all the conservation efforts across the globe. Many species have become extinct due to the illegal wildlife trade and many have reached the verge of extinction. According to some estimates, the monetary values of the illegal wildlife trade are estimated to be several billion US dollars. To deal with wildlife crime cases, it becomes imperative to have a sound knowledge of the techniques required in the analysis of wildlife crime exhibits. In this chapter, we have outlined the three frequently used techniques in wildlife forensics viz. microscopy, DNA and isotope analysis for addressing the problems of species and individual identification, and additionally identification of the geographical origin of a wildlife sample. The basic essentials of these techniques have been discussed in this chapter.

Keywords: wildlife, crime, forensics, microscopy, isotope analysis, DNA

1. Introduction

Mankind has been exploiting wildlife since times immemorial for basic needs, but the recent commercialization of wildlife trade has decimated some of the species to the verge of extinction [1]. Illegal wildlife trade is one of the biggest threats to the environment and biodiversity. The growing volume of illegal trade in wildlife jeopardizes all the conservation efforts across the globe. Many species have become extinct due to the illegal wildlife trade and many have reached the verge of extinction. According to some estimates, the monetary values of illegal wildlife trade are estimated at around 53 billion USD, and it is globally the third largest illegal trade after illegal trade in narcotics and firearms [2, 3]. Further, some of the wildlife crime and trade have also been linked with other organized forms like funding of terrorist activities, according to the United States Senate Foreign Relations Committee 2009. Studies have revealed that exploitation of wildlife by hunting for trade and pet collection is the second greatest drivers, surpassed only by habitat destruction for the decline in the population of many endangered species, and is impacting mammals (33%), birds (30%) and amphibians (6%) [4]. This has raised a global concern to check illegal trade for conserving wildlife for the future generations of the world and to maintain the delicate ecological balance of the nature.

In this scenario, it becomes the need of the hour to develop wildlife forensics with the changing paradigms of wildlife crime. Some of the important techniques that have made a strong impact in the field of wildlife forensics are microscopy, DNA analysis and elemental analysis, especially the study of isotopes. The aspects of these techniques are discussed in the proceeding sections.

2. Microscopy in wildlife forensics

The spectrum of types of physical evidence in wildlife forensics is very wide and so are the techniques. Microscopy is one of the most useful tools in wildlife forensics, especially while dealing with hair evidence. Hair is one of the important physical evidence found in wildlife crime cases about mammals. Mammals form one of the largest groups of poached species and a large number of wildlife crime cases require identification of species from hair. The history of examination of hair for species identification can be traced back to the nineteenth century, but the first significant contributions in this field were the works of Hausman [5–7] in America. Numerous studies related to species characterization from hair reported in the first half of the last century [5–10].

Microscopic hair characteristics have also been widely used in biological sciences for studying food habits, prey, predator relationships and mammals inhabiting a den or a tree [11–13]. In 1938, Mathiak produced a key to the identification of hairs of mammals of Southern Michigan [11]. In the same year, Williams produced a key to the identification of hairs of moles and shrews [14].

A key for the identification of Californian mammals from hair characteristics was published by Mayer [12]. The entire key was based on a consideration of dorsal guard hairs that had been taken from one small area of the pelage. In all, around 392 species and subspecies were considered. Thoroughly descriptive guides on microscopic hair characteristics of some important mammalian species in certain geographic regions have been worked out by several prominent workers. Guide on hair structure of some selected mammals of Ontario was provided by Adorjan and Kolenosky [15]. Similarly, a guide for species identification from the hair of some selected mammals of Australia was provided by Brunner and Coman [16]. Moore et al. [17] provided a guide for the identification of hair of some mammals of North America (Wyoming). Later guides on species identification from hair were provided by Appleyard [18] and Teerink [19]. Statistical evaluation of quantifiable hair characteristics was also reported. Sato et al. performed a statistical comparison of dog and cat guard hair using numerical morphology [20]. They were able to distinguish between species based on discriminant function analysis. Similarly, Sahajpal et al. used discriminant function analysis to characterize hair from four mongoose species of India, based on the banding pattern of the hair [21]. Sahajpal et al. further reported the guard hair characteristics of four Indian bear species and bovid species listed under Schedule I of Wildlife (Protection) Act 1972 of India [22, 23].

Scanning electron microscopy (SEM) also finds great use in the study of surface morphology of hair and has also been used by several investigators. Rollins and Phan et al. used SEM for the studies of scale patterns in the wool hairs of Ibex, Cashmere/Pashmina and Shahtoosh/Tibetan antelope wool [24, 25]. They were able to show the usefulness of scale patterns of wool fibers for species characterization. A scanning electron microscopy (SEM) study on the cuticular pattern of guard hair of Tibetan antelope (*Pantholops hodgsonii*) was reported by Bahuguna and Mukerjee [26].

It is apparent from the aforesaid facts that examination of hair can provide valuable information on species identification in wildlife forensics. For species characterization of hair, the following aspects are necessary to understand.

2.1 Hair profile

The general shape or profile of the hair has pertinent value in species identification from hair. The hair can be divided into root and shaft. Most of the mammal species have guard hair that flattens toward the distal (away from the skin) end. This flattened region is often referred to as the shield.

2.2 Cuticle

The outer layer of the mammalian hair is made up of scales and is called the cuticle. The layer is very thin and almost transparent. This layer can be considered analogous to the paint on the surface of a pencil. There are three parameters for describing the cuticle:

1. Shape of scale margin

2. Distance between external margins of scales

3. Scale pattern

There are further subclassifications that are beyond the scope of this chapter.

2.3 Cortex

The thick solid layer under the cuticle is called the cortex of the hair. The thickness of the cortex varies across species, and for simple understanding, it can be considered as analogous to the wooden part of the pencil. The cortex is made up of dead cornified cells, packed on to a rigid and homogenous hyaline mass [8]. The pigments that impart a color to the hair are present in the cortex region. Though the cortical region does not have much importance in species identification, the pigment granules present in the cortex do find use in species characterization from hair.

2.4 Medulla

The innermost core of the hair is called the medulla. It can be considered analogous to the graphite lead of the pencil. Medullae have been classified into four basic groups, unbroken, broken, ladder and miscellaneous, based on the general shape, arrangement of cells and air spaces [16, 27]. These four major groups can be further divided into more descriptive categories that cannot be covered in the current chapter.

2.5 Hair cross-section

As discussed in the Section 2.1, hair shows a significant variation in shape across its length. This variation gets very clearly revealed by the outline of their cross-sections. A cross-section of hair shall essentially be circular if the hair has a cylindrical shape. However, for complex shapes, the cross-sections are of very distinct shapes. The cross-section shape and their dimensions are of significance in species identification. For calculating the ratio of medulla and cortex concerning hair thickness, cross-sections are best suited. Cross-sections from the widest portion of the shield are most informative for species identification [19].

2.6 Some important indices

The microscopic hair examination also made use of certain indices that have a significant value in species characterization. The indices find valuable use in statistical analysis. Three indices are commonly used and they are defined as follows:

a. Scale Index: Ratio of the free proximo-distal length of the scale to the diameter of the hair shaft

b. Scale Count Index: Number of scales per unit (1 mm) length of the hair shaft

c. Medullary Index: Ratio of the medullary thickness to the hair thickness (diameter)

2.7 Methods to study hair characteristics

2.7.1 Preliminary examination

Hair samples need to be initially examined for their color, texture, thickness, etc. before microscopic examination. The thickness can be measured in microns using a oculometer on a light microscope.

2.7.2 Examination of cuticle

2.7.2.1 Light microscopy–based method

The almost transparent and very thin layer of the cuticle cannot be appreciated under a transmitted light microscope by using a whole mount of hair. Only the cortex and medulla are visible in the whole mount. Therefore, to view the cuticular structure of hair, a "cast" of hair has to be made and viewed under a microscope. The suitable method is to prepare a cast of the hair. In the case of the hair "scale cast" method, a hair is placed on the surface of a suitable material such that the surface structure of the hair gets reproduced as a three-dimensional cast. This cast can be viewed under a light microscope to observe the cuticular structure of the hair. About 10–20% solution of gelatin in distilled water or 50% solution of polyvinyl acetate in distilled water is used for the preparation of scale casts [28]. A fine and uniform film of the casting media is made on a clean microscopic glass slide with the help of a glass rod or a flat brush in a single stroke along the length of the slide surface. The slide is then placed on a horizontal surface and hair samples are placed one by one on the slide with the help of tweezers. The casting media is allowed to dry for about 20–30 minutes and the hair are removed by plucking gently, leaving behind the three-dimensional cast of the hair surface, which can be used to study the scale patterns, margins and shapes. The cast is viewed under the microscope at a magnification of 100× to 400× depending upon the thickness of the hair. **Figure 1** depicts the scale pattern of the Indian Bison (*Bos gaurus*) under microscope 400× magnification.

2.7.2.2 SEM-based method

Scanning electron microscopy (SEM) is also a recommended method to study the cuticle of hair as it offers resolution much higher than light microscopy. In this method, the hair samples are initially coated with a thin film of gold or palladium under a very low pressure (10–6 Torr) to make the surface conducting. These hair samples coated with a very fine film of gold or palladium are then viewed under

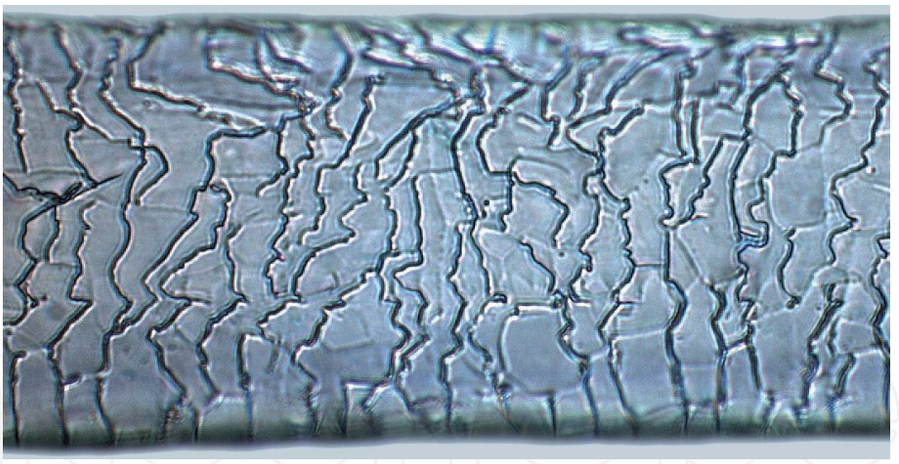

Figure 1.
The cuticle of Indian bison (Bos gaurus) under microscope 400× magnification.

an electron microscope, with the help of an electron beam. The method also has added advantage of studying the elemental profile of the hair if the SEM is coupled with energy dispersive X-ray analysis (SEM-EDXA) or wavelength dispersive X-ray analysis (SEM-WDXA).

2.7.3 Examination of medulla

Medulla can be visualized in the whole mount of hair. However, it is not usually possible to observe the fine structural details of the medulla because of the air filled in vacuoles of the medulla. Hence, it appears as a dark central region when viewed under a microscope. For a proper appreciation of the fine structure of the medulla, the air vacuoles need to be infiltrated with a solvent like xylene. To achieve this, the hair samples are cut into small pieces (0.5 cm to 1.0 cm in length) with a razor blade and immersed in xylene (preferably overnight). These hairpieces after overnight treatment with xylene can be mounted directly on a glass slide in DPX or Canada balsam and viewed under a light microscope. **Figure 2** depicts the Medulla of Serow (*Capricornis sumatraensis*) under microscope 400× magnification. From **Figure 2**, the extent of infiltration by xylene can be clearly appreciated, as medulla

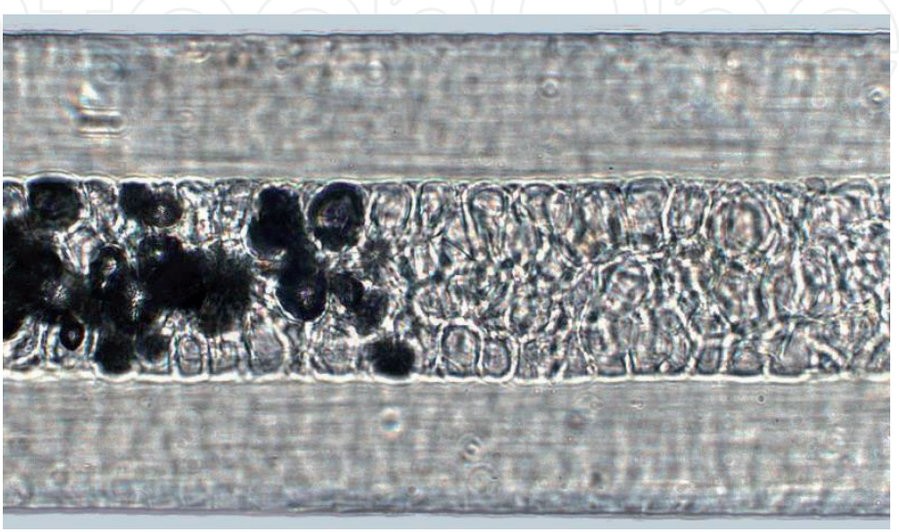

Figure 2.
Medulla of Serow (Capricornis sumatraensis) under microscope 400× magnification (note black globules represent the area where xylene has not infiltrated).

in that part is clearly visible, whereas in the part where xylene has not infiltrated, the medulla shows dark globules. The dark globules can be cleared by increasing the time of keeping the hair cuttings under xylene.

The following four observations can be made:

1. The medulla type

2. Medulla pattern

3. Medulla thickness in microns (using oculometer)

4. Medullary index

2.7.4 Study of cross-sections

The cross-sections of hair can be prepared by using a microtome. In case of nonavailability of microtome, a simple yet reliable method may be used [21]. This method requires a straw pipe, mounting wax and a razor blade for preparing cross-sections. Few hairs were inserted into a straw pipe, keeping them as straight as possible. Maintaining the vertical position of the hair, molten wax is slowly and carefully sucked into the straw. Once the molten wax rises past the hair samples, the straw pipe is constricted to prevent the molten wax from running down. The wax is allowed to solidify and the straw pipe is cut open to remove the wax stubs with embedded hair. These stubs with hair embedded in a vertical plane can be used for cutting fine cross-sections with a razor blade. The cut cross-sections are placed in a microscopic glass slide and a drop of xylene is added to remove the wax. These can be viewed under a light microscope at a magnification of 100× to 1000× depending upon the thickness of the hair. **Figure 3** depicts the cross-section of the hair takin (*Budorcas taxicolor*) under microscope 400× magnification.

The following parameters may be observed:

1. Cross-section outline

2. Medulla outline and configuration

3. Pigment distribution in the cortex

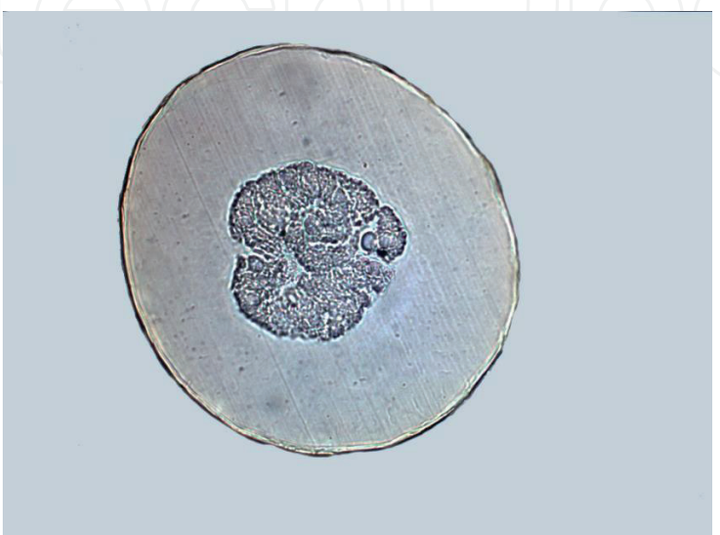

Figure 3.
Cross-section of hair of takin (Budorcas taxicolor) under microscope 400× magnification.

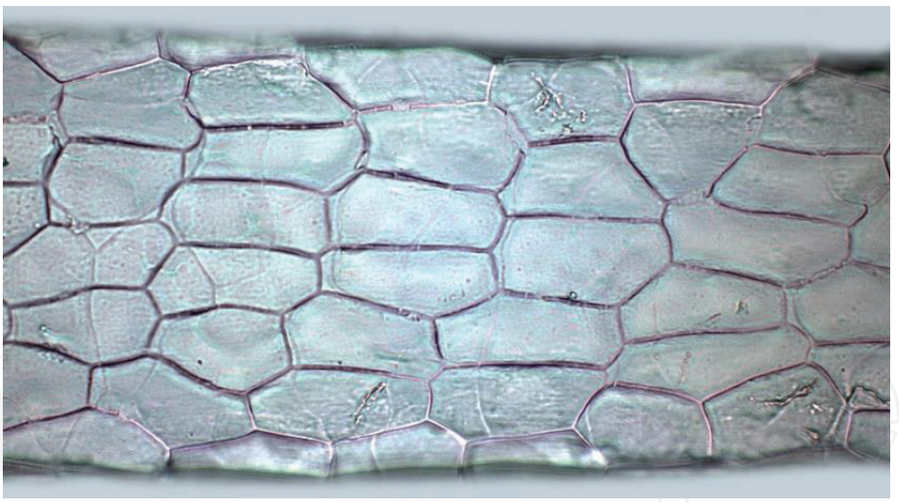

Figure 4.
The cuticle of Tibetan antelope (Pantholops hodgsonii) under microscope 400× magnification. Note the honeycomb-like structure of the scales forensic.

Examination of hair by considering these parameters usually helps to narrow down up to genus and species level. In some species like Tibetan antelope (*Pantholops hodgsonii*), mere examination of the cuticle can help in species characterization due to the unique scale pattern of the cuticle (**Figure 4**). The scales of the cuticle of the hair of Tibetan antelope (*Pantholops hodgsonii*) have a honeycomb-like shape that can be clearly appreciated in **Figure 4**. However, for most of the species, examination of all the parameters is necessary to reach a reliable result.

Sometimes, hair samples may not be in good shape, or may not be in an appropriate number, or may not be available at all. In such cases, it is worthwhile to use DNA-based techniques for species identification. The DNA-based methods are discussed in the next section.

3. DNA analysis in wildlife forensics

In the past 2–3 decades, conservation genetics has evolved as an important tool to resolve problems faced in species conservation. It has wide applications in molecular ecology, population genetics, molecular phylogenetics, taxonomy and phylogeography [29].

A recognized field of conservation genetics, now drawing growing attention, is the advancement of analytical methods to offer strong DNA-based evidence to support conservation law enforcement, which is commonly known as "wildlife DNA forensics." Wildlife forensics is related to the identification of confiscated material to ascertain the species, individual identity or relationship, and source population of the sample. However, wildlife forensics has its challenges. Despite the implementation of national and international laws to protect degrading habitat, protect biological species diversity and secure long-term survival of species, DNA forensics has become a main probing tool to curb wildlife crime [30].

In the past decades, molecular techniques have evolved rapidly allowing forensic researchers to extract genomic DNA from small remains or quantity of biological samples left at the scene of a crime and to establish a connection with the wildlife species and the offender. Forensic scientists have utilized this methodology to monitor the illegal trade of ivory [31–33] and to detect the source population of whale meat confiscated from Japanese markets [34] and Bengal tiger body parts [35]. Wildlife DNA forensics has been proven to be powerful especially in remote

wild areas and the marine environment where poaching of protected or threatened species is tough to detect [36]. This portion of the chapter introduces different methods used in wildlife DNA forensics.

3.1 Methods used in forensic genetic identification

3.1.1 Species identification

The genetic-based analysis is commonly used in wildlife forensics to identify the species from a confiscated item. Species identification is useful in illegal poaching cases to examine the trace amount of evidence from the possession of a suspect or scene of crime [37]. It has also been proven useful in detecting species from shark fins [38, 39], products generally used in wildlife trades such as traditional Chinese medicines (TCMs [40–42], hair [43] decorative items such as ivory idol [44] and burnt samples [45] where morphological identification is not possible or reliable.

Species identification is based on genetic markers that exhibit variation in DNA sequence among species, but are highly conserved or similar within a species [30]. Mitochondrial DNA (mtDNA) is generally preferred as a genetic marker over nuclear DNA (nDNA) for species identification as it is easier to extract from highly degraded and processed tissues or samples. This is because of the presence of multiple copies of mtDNA per cell compared to a single copy of nuclear DNA [46, 47]. Besides, universal mtDNA primers can be utilized to amplify the informative sequence of mtDNA across taxa that are less time-consuming in method development [48]. Polymerase chain reaction (PCR) is especially used for gene amplification [49].

In animals, mitochondrial cytochrome b (Cyt b) and cytochrome oxidase 1 (CO1) genes are commonly used as universal mtDNA markers for species identification [50–58]. The Cyt b gene is a useful mtDNA marker for the identification of several vertebrate species from illegal trade items including seals [59], snakes [60], tigers [32, 56, 61–64], sharks [52], turtles [64] and birds [65].

Sequencing of a fragment (600 bp) of the CO1 gene is highly informative and has been recommended as the inexpensive, fast and efficient approach to characterize species. Researchers around the world are making efforts to utilize the CO1 gene to catalog the entire vertebrate biodiversity on earth (www.barcodinglife.org) [47]. Furthermore, pyrosequencing is another method of DNA sequencing based on "sequencing by synthesis" that facilitates further rapid screening of DNA samples compared to methods used in conventional DNA sequencing [61]. Pyrosequencing can sequence only short fragments (50–500 bp) of DNA, which can restrict its use in DNA forensics unless we target very informative and highly variable regions of a gene [61]. Pyrosequencing has been used to identify twenty-eight European mammal species using very short fragments of 12S rRNA (17–18 bases) and 16S rRNA (15–25 bases) gene regions of mtDNA [66].

However, DNA nucleotide sequencing is a key method followed by comparing sequenced DNA fragments with reference DNA sequences of different species. The similarity or sequence homology between the unknown and reference sequences facilitates to ascertain the species of origin. Moreover, the International Society for Forensic Genetics (ISFG) has approved and validated the use of the DNA sequencing method [67, 68] and validated this method as a method for application in the detection of forensic casework [69]. Furthermore, the important advantage of DNA sequencing is that universal PCR primers can be used to amplify the DNA from unknown or random forensic case samples [56].

Species of unknown samples is assigned by analyzing and calculating the sequence homology with the reference DNA sequences [50, 70] available on DDBJ/EMBL-EBI/NCBI database collaboration (The International Nucleotide Sequence Database

Collaboration, www.insdc.org) and the Barcode of Life Data system (BOLD, boldsystems.org), which is the cloud-based data storage and analysis platform and the part of the CBOL (Consortium for the Barcoding of Life, www.barcodinglife.com).

Another method for identifying species is the construction of a phylogenetic tree. Such tree analysis helps understand the evolutionary relationship between unknown and the reference DNA sequences [56, 71, 72]. Phylogenetic trees allow identifying the reference species as likely source species if it is located closest to the unknown sample. Trees can be constructed using different methods such as neighbor-joining, maximum likelihood, Bayesian and maximum parsimony [73] and in wildlife forensics, there is no consensus over which method to use [74, 75].

Although methods that target single nucleotide polymorphisms (SNPs) other than whole DNA sequences confine their capacity to detect species, it enables researchers to analyze samples that contain multiple species, opposite to DNA sequencing using universal primers [30]. Identification of endangered species from traditional Chinese medicines that may contain plant and animal products has been successfully performed using allele-specific PCR primers and probes [63, 76, 77]. DNA sequencing–based species identification and SNP typing–based ability to examine mixed DNA of multiple species can be mixed [30]. Species-specific primers are used to sequence target species from mixed-species samples like TCMs. This tool has been used to ascertain the body parts of shark [39] and bear bile in TCMs [42].

3.1.2 Identification of the geographic origin

The great concern to wildlife conservation is to ascertain the geographic origin of confiscated items to curtail illegal poaching within the country's boundary and cross-border trafficking of wildlife derivates. In addition to species identification, it is necessary to trace the source population of individual forensic samples to implement wildlife protection laws and CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) regulations. Given this, genetic studies have been widely conducted to infer the source of origin of the poached items and can be used to identify marine stocks harvested illegally. However, few published studies are using these methods in forensic investigations.

Ascertaining geographic origin or source population is based on the ability to assign an unknown or a confiscated sample to its population of origin, counting on the availability of population genetic data from several areas and requiring an adequate genetic differentiation of the source population from other populations. Despite these restrictions, a large number of recent conservation researchers are now emphasizing the urgent need for enforcement methods to efficiently ascertain the geographic origin of samples [30].

In divergent populations, phylogeographic analyses can determine the geographic distribution of the genealogical lineages in which unique haplotypes of mtDNA are correlated with large geographic areas [30, 71]. Generally, the D-loop or hypervariable control region of mtDNA is applied as a genetic marker in ascertaining geographic origin based on haplotypes (individual sequence types of the control region) related to specific populations [30]. This method has been successfully applied to identify the large geographic origins of the Chinese sika deer (*Cervus nippon*) [78] and four species of seahorse (*Hippocampus ingens, H. trimaculatus, H. barbouri* and *H. spinosissimus*) from forensic samples [79].

Furthermore, populations with a subtle genetic variation or with insufficient variation in mtDNA can be identified using population assignment methods by employing nuclear genetic markers that exhibit differences among regions more efficiently than phylogeographic analyses. Population assignment methods are useful in assigning individuals to their source population after testing with all

populations within a large geographic area or landscape complex [35]. Therefore, a strategy to first test individuals with mtDNA haplotypes to identify the wide geographic origin and to second detect a particular source population in its large geographic area where other populations exist can be followed [35].

The frequency of the alleles at hypervariable nuclear DNA (nDNA) genetic marker observed in a natural population can be utilized to characterize population genetic structure and to estimate the probability of an individual or a sample belonging to its putative population of origin. Similarly, a forensic sample is assigned to its probable source population or geographic area [30, 35, 80–83]. The most commonly used hypervariable nDNA genetic markers for population assignment are microsatellites (**Box 1**) and AFLPs (**Box 1**) [47].

1. DNA sequencing

DNA sequencing detects each nucleotide base within a target region of a specific genetic DNA marker. For species identification, DNA sequencing of a fragment (nearly 500 bases) is most commonly utilized to offer species-specific DNA sequence. DNA sequencing facilitates the development of single nucleotide polymorphisms (SNPs), Indels and microsatellites with specific regions of DNA sequence variation.

2. SNP typing

Generally, single bp variations in the DNA sequence at a genetic marker causes differences among species, termed as single nucleotide polymorphisms (SNPs). SNP typing, also known as genotyping, investigates the specific regions with variation in the DNA sequence. SNP typing facilitates cheaper and faster tests that do not need long segments of high- or good-quality DNA but provides less information compared to conventional DNA sequencing. Three most commonly used SNP typing methods in forensics are given below:

2.1 PCR-RFLP

PCR-RFLP (Restriction Fragment Length Polymorphism) utilizes restriction endonuclease enzymes that recognize specific cleavage sites to cut DNA. The resulting nicked fragments are analyzed using agarose gel electrophoresis.

2.2 Allele-specific PCR

PCR primers, employed in the amplification of genetic markers, can be designed for highly conserved DNA regions or fragments (universal primers) or areas where highly variable DNA sequences occur between any species or populations (allele-specific primer).

2.3 Allele-specific probes

In this approach, a combination of universal primers and a specific probe that attaches to a specific variant of DNA sequence is used. Such probes allow detecting the base situated at SNP site.

3. Microsatellite DNA genotyping

Microsatellites or short tandem repeats (STRs) or simple sequence repeats (SSRs) are tandem stretches of 1-6 bp long-short nucleotide sequence motifs (e.g., ATATATAT) that occur randomly and are widely distributed in all eukaryotic genomes [84–86]. Variations in the number of repeat units lead to the difference in the size of both DNA fragments (alleles) that can be resolved and visualized on gel electrophoresis [86, 87]. These polymorphic loci are generally used in genetics and forensics studies. These are codominant markers.

4. AFLP (Amplified Fragment Length Polymorphism)

AFLPs are dominant DNA markers in which an allele is present or absent in an individual. AFLP locus cannot determine the heterozygosity of any individual. Therefore, in contrast to the codominant microsatellite DNA markers, AFLPs have less resolving power to assign an individual to its population of origin [88]; generally, at least 50 AFLP loci and 8 microsatellite loci are recommended to conduct population assignment tests [89]. Furthermore, in comparison to microsatellites, high-quality DNA requirement and greater genotyping errors of AFLPs have proven them to be less versatile [88, 90].

Box 1.
Commonly used techniques in wildlife DNA forensic [30].

Generally, a panel of highly polymorphic microsatellite loci is first selected and used to generate the genetic profile of a test sample or forensic specimen [91, 92]. This profile is assigned to a particular population by matching and comparing its observed alleles with the observed allele frequency in the population. There are several analytical methods available to perform assignment tests [83] and freely available statistical software packages [93]. Population assignment tests are highly

meticulous when the genetic database of all candidate populations has been developed, population or species boundaries are distinct, sampling is random and all population represents Hardy–Weinberg equilibrium (random mating, no inbreeding, the balance between mutation and genetic drift). Conversely, these assumptions are not feasible for several populations, for instance, when population boundaries are not evident or the genetic variations between populations are minimal or low, and populations are small [94]. Where populations are genetically widely distributed, stable isotopes (nongenetic substitutes) may be more appropriate to ascertain the origin of the samples [95].

3.1.3 Individual identification and parentage

For the last three decades, individual identification of forensic specimens based on a unique DNA profile has revolutionized human forensic studies [30]. This technique can be employed to detect the number of individuals used in the commercial market or trade, even from highly processed or powdered products [47]. Baker et al. [96] used a partial fragment of the mtDNA control region (464 bp) and 8 microsatellite loci to identify the minimum number of individual North Pacific minke whales (*Balaenoptera acutorostrata* spp.) from 12 markets.

Highly polymorphic SNP or microsatellite markers are used to generate a DNA profile with a series of gene variants or alleles (**Box 1**). The inclusion of more number of loci or markers reduces the chance that two different individuals will have the same DNA profile. Samples are identified to be from the same or different individuals based on the same or different DNA profiles, respectively. It is important to calculate the probability of identity that two individuals may share the same DNA profile [30].

In Canada, a database of DNA profiles has been established and is commonly used to support forensic investigations of the poaching cases of black bear (*Ursus americanus*), caribou (*Rangifer tarandus*), moose (*Alces alces*), elk (*Cervus canadensis*), white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) (www.forensicdna.ca/dnadatabases.html). Similarly, DNA profiling has been used to generate strong evidence by matching the blood left on the suspect's knife to the carcass of a wild boar in northern Italy [97].

Furthermore, the ability to validate familial relatedness is also a major application of wildlife DNA forensics. In a forensic investigation, the focus of establishing levels of relatedness lies predominantly on the discrimination of wild-caught animals from captive-bred [30]. Genetic or DNA markers are inherited from both parents from one generation to the next that allows using DNA profiles to validate parent–offspring relationships. Microsatellite loci–based DNA profile database is used in Australia and Europe to authenticate captive bird breeding, whereas parentage DNA analysis is applied to verify caviar of captive sturgeon (*S. Rastorguev pers.* comm.) [30].

4. Study of isotopes in wildlife forensics

Apart from species identification from unknown wildlife sample, quite often it also becomes imperative to identify the geographic origin of the sample. In addition to the DNA-based methods for ascertaining the possible geographical origin of samples, the study of the elemental profiles of samples is also a reliable means for predicting the geographic origin of the samples. This also becomes important when a particular species is protected in one area and not in another, and further when animals from the wild are captured and traded as captive-bred [98]. Among the elemental analysis techniques, a comparison of the ratios of different isotopes using methods such as inductively coupled plasma mass spectrometry (ICP-MS) and

isotope ratio mass spectrometry (IRMS) is an established method for predicting the geographic origin of wildlife samples. Variations in the concentration of elements and ratios of the isotopes have been used widely to ascertain the geographical origins of the African Rhinoceros horn [99–101]. Further, Amin et al. used mass spectrometry to study carbon and nitrogen isotopes and laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) to measure the relative abundance of isotopes of various elements to ascertain the geographic origin of African Rhinoceros horns [102]. Recently, Alexander et al. used stable isotope analyses to monitor illegally traded African gray parrots [103].

4.1 What are isotopes?

Isotopes are atoms of the same element that have a different number of neutrons in their nucleus; hence, they have the same atomic number but their atomic mass is different. Isotopes can be two types, that is, radioactive isotopes or stable isotopes.

Radioactive isotopes have an unstable nucleus that tends to attain a stable form by emitting radiation. Hence, these isotopes are called radioactive isotopes. The process is also radioactive decay, and during the process, particles and photons are emitted. Carbon-14 (14c), which is widely used in the dating of archeological samples and is a good example of a radioactive isotope. It has a nucleus with six protons and eight neutrons. With time, it decays into the nonradioactive nitrogen-14.

Stable isotopes have stable nuclei and hence do not exhibit radioactivity; that is, they do not undergo radioactive decay. Any element that has isotopes will have a lighter isotope (with a lesser number of neutrons) and a heavier isotope (with a higher number of neutrons). Further, the relative abundance of these isotopes with respect to each other varies significantly with geographical location. Hence, for an unknown sample, if the relative abundance of isotopes of a particular element is determined, it becomes possible to predict the geographical origin of a sample, based on the relative abundance of the isotopes. The elements and isotopes thereof enter the food chain and hence get incorporated into the tissues of living organisms. Further, the elements along with their isotopes remain in the remains of the organisms. As species are distributed according to ecozones and geography, their elemental profile including the relative abundance of stable isotopes is bound to vary with the geographical origins. This is used in wildlife forensics for predicting the geographical origins of wildlife samples with analysis of stable isotopes and elemental profiles. The isotopes most commonly used in forensic science for this purpose are generally H, C, N and O [98, 104].

4.2 Techniques used for isotope studies in wildlife forensics

4.2.1 ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) is a highly sensitive technique of elemental analysis with a capability to detect metals and nonmetals and very low concentrations. Further, it has the capability of detecting isotopes of an element in a given sample.

4.2.2 IRMS

Isotope-ratio mass spectrometry (IRMS) is a specialization form of mass spectrometry, in which mass spectrometric methods are used to determine the relative abundance of isotopes in a sample.

4.2.3 LA-ICP-MS

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a highly sensitive elemental and isotopic analysis method in which analysis can be directly performed on solid samples. It used a process called laser ablation in which a laser beam is focused on the surface to generate fine particles. These particles are then transported to an ICP-MS for digestion and ionization and subsequent detection of elements and isotopes.

Elaboration of these techniques shall be beyond the scope of this chapter. The usefulness of the technique has been demonstrated by several workers in this field and the technology has great potential in identifying the geographic origin of wildlife samples.

As discussed earlier, the spectrum of wildlife forensics is very wide, and to address different queries of investigation, the use of different techniques may be required. Some of these techniques, even though readily available, still may require standardization with respect to wildlife crime samples. The matters get further complicated when the evidence material is very limited; hence, it becomes imperative to have a precise idea of what technique(s) should be employed for the purpose. In this chapter, we have covered some important techniques that may be useful for dealing with wildlife crime cases. However, to get a more refined and working knowledge of the techniques, referring to detailed texts is advised.

Author details

Vivek Sahajpal[1*], Sudhanshu Mishra[2] and Deepika Bhandari[1,3]

1 Directorate of Forensics Services, Junga, Shimla, Himachal Pradesh, India

2 Sichuan Agricultural University, Chengdu, China

3 Institute of Forensics Science, University of Mumbai, Mumbai, Maharashtra, India

*Address all correspondence to: viveksahajpal@gmail.com

References

[1] Mukherjee SK. Some thoughts on wildlife trade. Cheetal. 1996;**2**:30-33

[2] Agnew DJ, Pearce J, Pramod G, Peatman T, Watson R, Beddington JR, et al. Estimating the worldwide extent of illegal fishing. PLoS One. 2009;**4**(2): e4570

[3] Europole. The EU Organised Crime Threat Assessment. European Police Office; 2011. pp. 1-60

[4] Zedan H. 2004 IUCN Red List of Threatened Species: A Global Species Assessment. Iucn; 2004

[5] Hausman LA. Structural characteristics of the hair of mammals. The American Naturalist. 1920;**54**(635): 496-523

[6] Hausman LA. Further studies of the relationships of the structural characters of mammalian hair. The American Naturalist. 1924;**58**(659):544-557

[7] Hausman LA. Recent studies of hair structure relationships. The Scientific Monthly. 1930;**30**(3):258-277

[8] Hausman LA. The cortical fusi of mammalian hair shafts. The American Naturalist. 1932;**66**(706):461-470

[9] Hausman LA. Applied microscopy of hair. The Scientific Monthly. 1944;**59**(3): 195-202

[10] Hardy JI, Plitt TM. An improved method for revealing the surface structure of fur fibers. 1940;(7):1-10

[11] Mathiak HA. A key to hairs of the mammals of southern Michigan. The Journal of Wildlife Management. 1938;**2**(4):251-268

[12] Mayer WV. The hair of California mammals with keys to the dorsal guard hairs of California mammals. The American Midland Naturalist. 1952; **48**(2):480-512

[13] Joslin P. The Asiatic lion: a study of ecology and behavior. Partial fulfillment of the requirements for the degree of Doctor of Science in the Department of Forestry and Natural Resources, University of Edinburgh, UK. 1973: 1-249

[14] Williams CS. Aids to the identification of mole and shrew hairs with general comments on hair structure and hair determination. The Journal of Wildlife Management. 1938;**2**(4):239-250

[15] Adorjan AS, Kolenosky GB. A manual for the identification of hairs of selected Ontario mammals. 1969

[16] Brunner H, Coman BJ. The Identification of Mammalian Hair. Inkata Press; 1974

[17] Moore TD, Spence LE, Dugnolle CE. Identification of the Dorsal Guard Hairs of Some Mammals of Wyoming. Wyoming Game and Fish Department; 1974

[18] Appleyard HM. Guide to the identification of animal fibres. Ed. 2. 1978

[19] Teerink BJ. Hair of West-European Mammals. Cambridge, UK: Cambridge University Press; 1991

[20] Sato H, Matsuda H, Kubota S, Kawano K. Statistical comparison of dog and cat guard hairs using numerical morphology. Forensic Science International. 2006;**158**(2-3):94-103

[21] Sahajpal V, Goyal SP, Raza R, Jayapal R. Identification of mongoose (genus: Herpestes) species from hair through band pattern studies using discriminate functional analysis (DFA)

and microscopic examination. Science & Justice. 2009;**49**(3):205-209

[22] Sahajpal V, Goyal SP, Jayapal R, Yoganand K, Thakar MK. Hair characteristics of four Indian bear species. Science & Justice. 2008;**48**(1): 8-15

[23] Sahajpal V, Goyal SP, Thakar MK, Jayapal R. Microscopic hair characteristics of a few bovid species listed under schedule-I of wildlife (protection) act 1972 of India. Forensic Science International. 2009;**189**(1-3): 34-45

[24] Rollins CK, Hall DM. Using light and scanning electron microscopic methods to differentiate ibex goat and Tibetan antelope fibers. Textile Research Journal. 1999;**69**(11): 856-860

[25] Phan KH, Wortmann G, Wortmann FJ. Microscopic characteristics of shahtoosh and its differentiation from cashmere/pashmina. In: Int. Wool Text. Org. Conference, Aachen 2000

[26] Bahuguna A, Mukherjee SK. Use of SEM to recognise Tibetan antelope (Chiru) hair and blending in wool products. Science & Justice. 2000; **40**(3):177-182

[27] Wildman AB, Microscopy of Animal Textile Fibres. Wool Industries Research Association; 1954

[28] Sahajpal V, Goyal SP. Microscopy in wildlife investigations. In: Linacre A, editor. Forensic Science in Wildlife Investigations. CRC Press; 2009

[29] Frankham R, Ballou SE, Briscoe DA, Ballou JD. Introduction to Conservation Genetics. Cambridge University Press. 2002

[30] Ogden R, Dawnay N, McEwing R. Wildlife DNA forensics—Bridging the gap between conservation genetics and law enforcement. Endangered Species Research. 2009;**9**(3):179-195

[31] Comstock KE, Ostrander EA, Wasser SK. Amplifying nuclear and mitochondrial DNA from African elephant ivory: A tool for monitoring the ivory trade. Conservation Biology. 2003;**17**(6):1840-1843

[32] Wasser SK, Shedlock AM, Comstock K, Ostrander EA, Mutayoba B, Stephens M. Assigning African elephant DNA to geographic region of origin: Applications to the ivory trade. Proceedings of the National Academy of Sciences. 2004;**101**(41):14847-14852

[33] Wasser SK, Joseph Clark WI, Drori O, Stephen Kisamo EM, Mailand C, Mutayoba B, et al. Combating the illegal trade in African elephant ivory with DNA forensics. Conservation Biology. 2008;**22**(4):1065-1071

[34] Cipriano F, Palumbi SR. Genetic tracking of a protected whale. Nature. 1999;**397**(6717):307-308

[35] Mishra S. Genotyping and molecular tracing of sex-ration in tigers of central India: Implication in forensics [Ph.D. Thesis]. Banasthali University; 2014. pp. 1-263

[36] Avise JC. Conservation genetics in the marine realm. Journal of Heredity. 1998;**89**(5):377-382

[37] Gupta SK, Verma SK, Singh L. Molecular insight into a wildlife crime: The case of a peafowl slaughter. Forensic Science International. 2005;**154**(2-3): 214-217

[38] Wong EH, Shivji MS, Hanner RH. Identifying sharks with DNA barcodes: Assessing the utility of a nucleotide diagnostic approach. Molecular Ecology Resources. 2009;**9**:243-256

[39] Chapman DD, Abercrombie DL, Douady CJ, Pikitch EK, Stanhopen MJ,

Shivji MS. 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. 2003;**4**(4):415-425

[40] Hsieh SC, Lin IH, Tseng WL, Lee CH, Wang JD. Prescription profile of potentially aristolochic acid containing Chinese herbal products: An analysis of National Health Insurance data in Taiwan between 1997 and 2003. Chinese Medicine. 2008;**3**(1):1-6

[41] Wetton JH, Tsang CS, Roney CA, Spriggs AC. An extremely sensitive species-specific ARMs PCR test for the presence of tiger bone DNA. Forensic Science International. 2004;**140**(1): 139-145

[42] Peppin L, McEwing R, Carvalho GR, Ogden R. A DNA-based approach for the forensic identification of Asiatic black bear (*Ursus thibetanus*) in a traditional Asian medicine. Journal of Forensic Sciences. 2008;**53**(6):1358-1362

[43] Sahajpal V, Goyal SP. Identification of a forensic case using microscopy and forensically informative nucleotide sequencing (FINS): A case study of small Indian civet (*Viverricula indica*). Science & Justice. 2010;**50**(2):94-97

[44] Gupta SK, Thangaraj K, Singh L. Identification of the source of ivory idol by DNA analysis. Journal of Forensic Sciences. 2011;**56**(5):1343-1345

[45] Sharma V, Gupta SK. Molecular identification of victim species and its sex from the ash: A case of burning alive leopard (*Panthera pardus*). International Journal of Legal Medicine. 2018;**132**(4): 1075-1078

[46] Randi E. Mitochondrial DNA. In: Baker A, editor. Molecular Methods in Ecology. Wiley Blackwell; 2000

[47] Alacs EA, Georges A, FitzSimmons NN, Robertson J. DNA detective: A review of molecular approaches to wildlife forensics. Forensic Science, Medicine, and Pathology. 2010;**6**(3):180-194

[48] Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, et al. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences. 1989;**86**(16):6196-6200

[49] Mullis KB, Ferré F, Richard A. Gibbs, editors. The polymerase chain reaction. Birkhäuser Verlag AG, Basel, Switzerland; 1994.

[50] Parson W, Pegoraro K, Nieder stätter H, Föger M, Steinlechner M. Species identification by means of the cytochrome b gene. International Journal of Legal Medicine. 2000;**114**(1):23-28

[51] Hedmark E, Ellegren H. Microsatellite genotyping of DNA isolated from claws left on tanned carnivore hides. International Journal of Legal Medicine. 2005;**119**(6):370-373

[52] Kumar R, Singh PJ, Nagpure NS, Kushwaha B, Srivastava SK, Lakra WS. A non-invasive technique for rapid extraction of DNA from fish scales. Indian Journal of Experimental Biology. 2007; 45: 992-997

[53] Alacs E, Alpers D, Paul J, Dillon M, Spencer PB. Identifying the presence of quokkas (*Setonix brachyurus*) and other macropods using cytochrome b analyses from faeces. Wildlife Research. 2003;**30**(1):41-47

[54] Dawnay N, Ogden R, McEwing R, Carvalho GR, Thorpe RS. Validation of the barcoding gene COI for use in forensic genetic species identification. Forensic Science International. 2007;**173**(1):1-6

[55] Hsieh HM, Chiang HL, Tsai LC, Lai SY, Huang NE, Linacre A, et al. Cytochrome b gene for species identification of the conservation animals. Forensic Science International. 2001; **122**(1):7-18

[56] Verma SK, Singh L. Novel universal primers establish identity of an enormous number of animal species for forensic application. Molecular Ecology Notes. 2003;**3**(1):28-31

[57] Hebert PD, Cywinska A, Ball SL, Dewaard JR. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences. 2003;**270**(1512): 313-321

[58] Hebert PD, Ratnasingham S, De Waard JR. Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London. Series B: Biological Sciences. 2003;**270**(suppl_1):S96-S99

[59] Malik S, Wilson PJ, Smith RJ, Lavigne DM, White BN. Pinniped penises in trade: A molecular-genetic investigation. Conservation Biology. 1997;**11**(6):1365-1374

[60] Yau FC, Wong KL, Shaw PC, But PP, Wang J. Authentication of snakes used in Chinese medicine by sequence characterized amplified region (SCAR). Biodiversity and Conservation. 2002;**11**(9):1653-1662

[61] Ronaghi M, Pettersson B, Uhlén M, Nyrén P. PCR-introduced loop structure as primer in DNA sequencing. BioTechniques. 1998;**25**(5):876-884

[62] Prado M, Franco CM, Fente CA, Cepeda A, Vázquez BI, Barros-Velázquez J. Comparison of extraction methods for the recovery, amplification and species-specific analysis of DNA from bone and bone meals. Electrophoresis. 2002;**23**(7-8): 1005-1012

[63] Wan QH, Fang SG. Application of species-specific polymerase chain reaction in the forensic identification of tiger species. Forensic Science International. 2003;**131**(1):75-78

[64] Moore MK, Bemiss JA, Rice SM, Quattro JM, Woodley CM. Use of restriction fragment length polymorphisms to identify sea turtle eggs and cooked meats to species. Conservation Genetics. 2003;**4**(1): 95-103

[65] Lee JC, Tsai LC, Huang MT, Jhuang JA, Yao CT, Chin SC, et al. A novel strategy for avian species identification by cytochrome b gene. Electrophoresis. 2008;**29**(11):2413-2418

[66] Karlsson AO, Holmlund G. Identification of mammal species using species-specific DNA pyrosequencing. Forensic Science International. 2007; **173**(1):16-20

[67] Bär W, Brinkmann B, Budowle B, Carracedo A, Gill P, Holland M, et al. Guidelines for mitochondrial DNA typing. Vox Sanguinis. 2000;**79**(2): 121-125

[68] Carracedo A, Bär W, Holland M, Tully G, Wilson M. Dna commission of the international society for forensic genetics: Guidelines for mitochondrial DNA typing. Forensic Science International. 2000;**110**(2):79-85

[69] Wilson MR, DiZinno JA, Polanskey D, Replogle J, Budowle B. Validation of mitochondrial DNA sequencing for forensic casework analysis. International Journal of Legal Medicine. 1995;**108**(2):68-74

[70] Branicki W, Kupiec T, Pawlowski R. Validation of cytochrome b sequence analysis as a method of species

identification. Journal of Forensic Science. 2003;**48**(1):1-5

[71] Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, et al. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics. 1987;**18**(1):489-522

[72] Roman J, Bowen BW. The mock turtle syndrome: Genetic identification of turtle meat purchased in the South-Eastern United States of America. Animal Conservation. 2000;**3**(1):61-65

[73] Holder M, Lewis PO. Phylogeny estimation: Traditional and Bayesian approaches. Nature Reviews Genetics. 2003;**4**(4):275-284

[74] Terol J, Mascarell R, Fernandez-Pedrosa V, Pérez-Alonso M. Statistical validation of the identification of tuna species: Bootstrap analysis of mitochondrial DNA sequences. Journal of Agricultural and Food Chemistry. 2002;**50**(5):963-969

[75] Wong KL, Wang J, But PP, Shaw PC. Application of cytochrome b DNA sequences for the authentication of endangered snake species. Forensic Science International. 2004;**139**(1):49-55

[76] Wetton JH, Parkin DT. A suite of falcon single-locus minisatellite probes: A powerful alternative to DNA fingerprinting. Molecular Ecology. 1997;**6**(2):119-128

[77] Imaizumi K, Akutsu T, Miyasaka S, Yoshino M. Development of species identification tests targeting the 16S ribosomal RNA coding region in mitochondrial DNA. International Journal of Legal Medicine. 2007;**121**(3): 184-191

[78] Wu H, Wan QH, Fang SG, Zhang SY. Application of mitochondrial DNA sequence analysis in the forensic identification of Chinese sika deer subspecies. Forensic Science International. 2005;**148**(2-3):101-105

[79] Sanders JG, Cribbs JE, Fienberg HG, Hulburd GC, Katz LS, Palumbi SR. The tip of the tail: Molecular identification of seahorses for sale in apothecary shops and curio stores in California. Conservation Genetics. 2008;**9**(1):65-71

[80] Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics. 1999;**153**(4): 1989-2000

[81] DeYoung RW, Demarais S, Honeycutt RL, Gonzales RA, Gee KL, Anderson JD. Evaluation of a DNA microsatellite panel useful for genetic exclusion studies in white-tailed deer. Wildlife Society Bulletin. 2003;**1**:220-232

[82] Gómez-Díaz E, González-Solís J. Geographic assignment of seabirds to their origin: Combining morphologic, genetic, and biogeochemical analyses. Ecological Applications. 2007;**17**(5): 1484-1498

[83] Manel S, Gaggiotti OE, Waples RS. Assignment methods: Matching biological questions with appropriate techniques. Trends in Ecology & Evolution. 2005;**20**(3):136-142

[84] Tautz D, Renz M. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research. 1984;**12**(10):4127-4138

[85] Kashi Y, Tikochinsky Y, Genislav E, Lraqi F, Nave A, Beckmann JS, et al. Large restriction fragments containing poly-TG are highly polymorphic in a variety of vertebrates. Nucleic Acids Research. 1990;**18**(5):1129-1132

[86] Weber JL. Informativeness of human (dC-dA) n·(dG-dT) n polymorphisms. Genomics. 1990;**7**(4):524-530

[87] Litt M, Luty JA. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. American Journal of Human Genetics. 1989;**44**(3):397

[88] Bensch S, Åkesson M. Ten years of AFLP in ecology and evolution: Why so few animals? Molecular Ecology. 2005;**14**(10):2899-2914

[89] Campbell D, Duchesne P, Bernatchez L. AFLP utility for population assignment studies: Analytical investigation and empirical comparison with microsatellites. Molecular Ecology. 2003;**12**(7):1979-1991

[90] Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P. How to track and assess genotyping errors in population genetics studies. Molecular Ecology. 2004;**13**(11): 3261-3273

[91] Mishra S, Sharma R, Singh SK, Munjal AK, Goyal SP. A comparative study of the use of tiger-specific and heterologous microsatellite markers for population genetic studies of the Bengal tiger (*Panthera tigris tigris*). African Journal of Biotechnology. 2014;**13**(8): 936-943

[92] Mishra S, Singh SK, Munjal AK, Aspi J, Goyal SP. Panel of polymorphic heterologous microsatellite loci to genotype critically endangered Bengal tiger: A pilot study. Springerplus. 2014;**3**(1):4

[93] Hauser L, Seamons TR, Dauer M, Naish KA, Quinn TP. An empirical verification of population assignment methods by marking and parentage data: Hatchery and wild steelhead (Oncorhynchus mykiss) in Forks Creek, Washington, USA. Molecular Ecology. 2006;**15**(11):3157-3173

[94] Maudet C, Miller C, Bassano B, Breitenmoser-Würsten C, Gauthier D, Obexer-Ruff G, et al. Microsatellite DNA and recent statistical methods in wildlife conservation management: Applications in Alpine ibex [Capra ibex (ibex)]. Molecular Ecology. 2002;**11**(3):421-436

[95] Benson S, Lennard C, Maynard P, Roux C. Forensic applications of isotope ratio mass spectrometry—A review. Forensic Science International. 2006; **157**(1):1-22

[96] Baker CS, Cooke JG, Lavery S, Dalebout ML, Ma YU, Funahashi N, et al. Estimating the number of whales entering trade using DNA profiling and capture-recapture analysis of market products. Molecular Ecology. 2007; **16**(13):2617-2626

[97] Lorenzini R. DNA forensics and the poaching of wildlife in Italy: A case study. Forensic Science International. 2005;**153**(2-3):218-221

[98] Tobe SS. Determining the Geographic Origin of Animal Samples. Boca Raton, FL: CRC Press; 2009

[99] Hall-Martin AJ, Van Der Merwe NJ, Lee-Thorp JA, Armstrong RA, Mehl CH, Struben S, et al. Determination of species and geographic origin of rhinoceros horn by isotopic analysis and its possible application to trade control. In: Ryder OA, editor. Proceedings of an International Conference: Rhinoceros Biology and Conservation. San Diego, California: Zoological Society of San Diego; 1993. pp. 123-135

[100] Hart RJ, Tredoux M, Damarupurshad A. The Characterisation of Rhino Horn and Elephant Ivory Using the Technique of Neuron Activation Analysis. South Africa: Final Report on a Project Undertaken on Behalf of the Department of Environmental Affairs; 1994

[101] Emslie RH, Brooks PM, Lee-Thorp JA, Jolles A, Smith W,

Vermaas N. Development of a continental African Rhino horn fingerprinting database and statistical models to determine the probable species and source of rhino horn, AfRSG Rhino Horn Fingerprinting for Security Project 9F0084. 1. Unpublished Report to WWF. 2001

[102] Amin R, Bramer M, Emslie R. Intelligent data analysis for conservation: Experiments with rhino horn fingerprint identification. In: Applications and Innovations in Intelligent Systems X. London: Springer; 2003. pp. 207-222

[103] Alexander J, Downs CT, Butler M, Woodborne S, Symes CT. Stable isotope analyses as a forensic tool to monitor illegally traded African grey parrots. Animal Conservation. 2019;**22**(2): 134-143

[104] Bowen GJ, Wassenaar LI, Hobson KA. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia. 2005;**143**(3):337-348