CHAPTER 1 INTRODUCTION

1.1 General Introduction

Many animal species have recently become extinct due to number reasons including habitat loss, hunting, the introduction of the invasive alien species and environmental pollution [1-11] . In the recent past many avian species have become extinct or threatened with extinction, with the primary cause of dramatic number loss due to human activities [8 , 10 , 12-16] . It is estimated that the extinctions per million species per year (E/MSY) of avian species since the 1500s is about 26 E/MSY [17].

In 1690, one of the most famous cases of avian extinction due to man is that of the dodo (*Raphus cucullatus*) [18-20]. This flightless avian species, which was native to the island of Mauritius in the Indian Ocean, was killed by humans for their meat and became prey to animals which had been introduced to the island. These two combinations led to the extinction of the species in a relatively few years [3, 20, 21]. A further example of an avian species that became extinct due to man's activity was the passenger pigeon (*Ectopistes migratorius*). This was the most abundant avian species in North America prior to persecution by man [8, 22-25]. The last member of the species died in the Cincinnati Zoological Gardens in 1914 [22, 25].

1.2 Types of wildlife crime

Wildlife crimes occurs both at an international and national level. At the international level is the trade in endangered species where protected species are traded across international boundaries; examples being African elephant ivory, traditional Chinese medicine (TCM), bird species, reptiles, amphibians, fish and invertebrates [26-33]. The second level is any crimes against wildlife or protected species afforded protection at a national level. This includes cruelty case, illegal poaching, or fishing, indiscriminate poisoning, disturbing protected habitats, or the collecting or selling or protected animals or their derivatives [34-39]. The relatively recent increase in the decline of the numbers of animal species and extinctions highlights the need not only for legal protection but the enforcement of any relevant legislation.

1.3 Legislation covering wildlife protection

1.3.1 International legislation

Since 1973, the trade in endangered wildlife species has been monitored by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES); an international agreement between governments for controlling the trade in endangered species [40-42] . Within CITES there are three Appendices, Appendix I, II and III that provide different levels of protection of trade. Appendix I (black list) lists the fauna and flora that are most threatened with extinction unless immediate protection is given [43, 44].

International trade in these species is prohibited by signatures to CITES, however there are some exceptions for non-commercial purpose such as for scientific research.

Appendix II (grey list) contains a lists of species that are not threatened with extinction now but they will become highly endangered and risk extinction if they are not protected [44]. Trading in these species is not allowed without permits or certificates. Appendix III contains a list of species that were requested from the parties to cooperate with other countries to control trading on those species listed. Trading of species is restricted in this Appendix III without permits or certificates [45].

In addition, there are a number of other organizations relevant to international wildlife trade such as United Nations Environment Programme (UNEP), which is concerned with the international environmental issues; the Food and Agriculture Organization of the United Nations (FAO), which deals with biodiversity conservation and agricultural production rely on national laws of each country [46]; World Trade Organization (WTO) which controls international trade including the wildlife trade [47]; Office International des Epizooties (OIE), which regulates animal health and disease states within wildlife populations [48]; the World Conservation Union (IUCN) [49], which acts to conserve biodiversity; and Non-Governmental Organizations (NGOs), that provide financial and technical support for conservation projects [50].

1.3.2 National legislation

Many endangered species are protected by the laws operating in each country. In Australia, there are many pieces of legislations designed to protect endangered species, such as the Environment Protection and Biodiversity Conservation Act 1999, the Endangered Species Protection Act 1993, Australian Wildlife Protection Act 1998 and Natural Heritage Trust of Australia Act 1997. Additionally, each State has their own laws to protected endangered species such as the Threatened Species Protection Act 1995 in Tasmania, the Wildlife Conservation Act 1950 in Western Australia, the Flora and Fauna Guarantee Act (1988) in Victoria, the National Parks and Wildlife Act 1972 in South Australia, the Threatened Species Conservation Act 1995 in New South Wales, the Nature Conservation Act 1980 in the Australian Capital Territory, the Nature Conservation Act in Queensland, the Territory Parks and Wildlife Conservation Act 2000 and the Environment Assessment Act in Northern Territory.

In the U.S., potential transgressions of the Endangered Species Act (1973) are investigated by the U.S. Department of Fish and Wildlife [51]. In the UK, the main legislation relevant to protection of the countryside is the Wildlife and Countryside Act 1981 [52, 53]. Part 1 section 1 to 8 of this Act is concerned with the protection of avian species. Under this Act the killing, injuring or taking of any wild bird or damaging their nest or their eggs is prohibited; this legislation was introduced by the UK parliament in an effort protect species and habitat recognised as under threat of extinction.

It is also illegal to disturb the birds at the nest or their dependent young. This Act was aimed in part in the protection of species such as: osprey (*Pandion haliaetus*), barn owl (*Tyto alba*), snowy owl (*Bubo scandiacus*), golden eagle (*Aquila chrysaetos*), white-tailed eagle (*Haliaeetus albicilla*) and gyr falcon (*Falco rusticolus*). In Britain, wild birds have also been protected by Wild Birds Protection Acts so that smuggling of wild bird or their eggs are prohibited. Another UK Act is the Hunting Act 2004; this protects wild mammals from being hunted with dogs [54].

These countries illustrate national protection; however there are many other countries that aim to protected native and wild species. For example in Thailand there is the Wild Animal Reservation and Protection Act (WARPA); the National Park Act; the National Forest Reserve Act that bans logging within in natural forests; and the Forest Plantation Act and Enhancement and Conservation of National Environmental Quality Act [55]. Within the European Union (EU), there are the national laws for each EU Member State with regard to wildlife trade in the EU. In India the Wildlife Protection Act 1972 and The Wildlife (Protection) Amendment Act, 2002, was introduced to protect native species and habitat [56-58].

1.4 Background to Forensic Science

The history of forensic science dates from early uses of fingerprints by the ancient Chinese culture for individual identification and the use of a bloody palm print in a murder case heard by a Roman Court in 1100 A.D.

The development of forensic biology is impossible to trace as it emerged from medical sciences, however the development of microscopy allowed biological material to be examined and the use of antibodies indicated the potential for individual identity. Human identification was to change in 1985 with the advent of DNA profiling, which led to the reduction in the use of antibody based tests and other protein based methods for human identification. DNA profiling is now used routinely for linking the samples from the crime scene to a suspect [59, 60]. The original method, termed DNA fingerprinting, was introduced and first used in England by Prof. Sir Alec Jeffreys in 1985; ultimately leading to the development of DNA in forensic investigations [61-64]. The original DNA fingerprinting method, based on restriction fragment length polymorphism (RFLP), was used to resolve a paternity dispute, but was soon applied in forensic casework in the UK [63-65]. This technique was performed by cutting the entire genome with restriction enzyme to generate the variable lengths of DNA fragments. Between some of the two restriction sites were highly polymorphic loci called minisatellites. These minisatellites differed between individuals due to the number of repeats at each locus but were consistent between different body fluids for any individual. The original RFLP method used Southern Blotting where the enzymatically cleaved DNA was separated by gel electrophoresis and then the DNA bands were transferred to a membrane, a single-stranded probe using a sequence to the core repeat of the minisatellites was used to bind to the complementary sequence on the membrane and the position of the probe was visualized by x-ray film originally as shown in Figure 1.1a and 1.1b [61], and later by chemiluminescence.

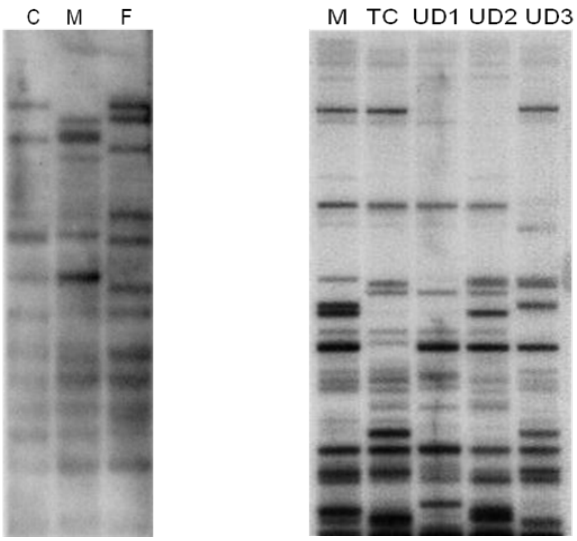

Figure 1.1a: shows the first ever DNA fingerprint on the left, produced in Alec Jeffreys laboratory on the 10th September 1984. It shows the banding pattern from a mother, child, and father using a multilocus probe. The image on the right shows the multilocus fingerprint from a mother, a tested child (TC), as the UK Immigration Officials did not believe that the boy was the biological son of the mother, and three of the mother's undisputed children (UD 1-3): the results demonstrated that the tested child was indeed the biological son of the woman and was therefore allowed to stay in the UK. The image on the left was supplied by Adrian Linacre and that on the right from reference [66].

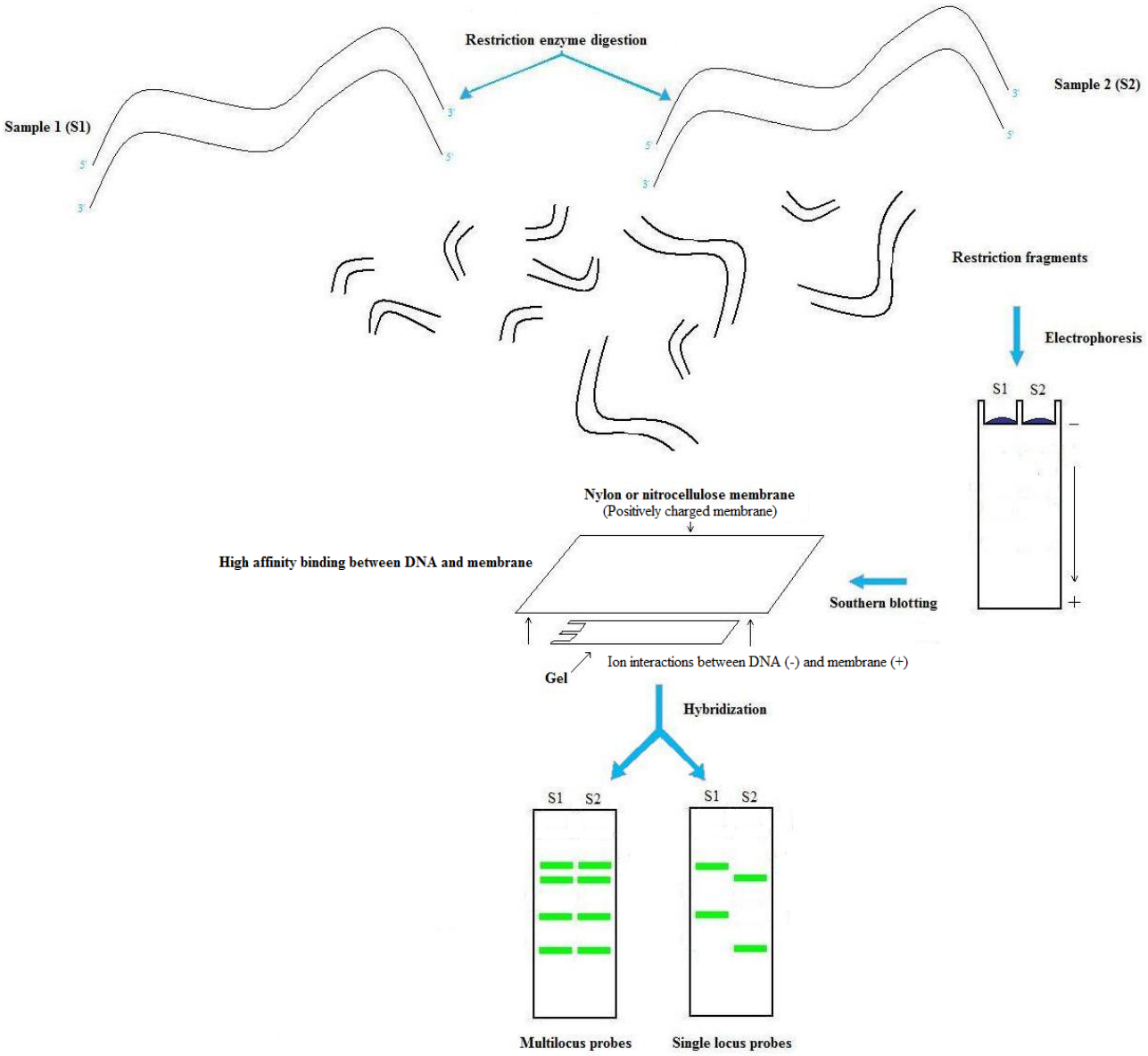

Figure 1.1b: Schematic representation of RFLP analysis; genomic DNA from sample 1 (S1) and sample 2 (S2) were cut by restriction enzymes which generated different length of restriction products. They were separated on agarose gel and transferred to membrane. Visualization was performed using hybridization with labelled probes then exposed to x-ray film. There are 2 types of RFLP profiling; Multi-locus (MLPs) profiling (using multi-locus probes) and Single locus (SLPs) profiling (using single locus probes).

The first tests used probes that would bind to more than one minisatellite, producing a range of bands for each sample. This range of bands produced a near unique pattern, leading to the term 'DNA fingerprinting'. Within two years of the first use of DNA fingerprinting the probes used were modified to bind specifically to one locus, thus producing one or two bands for each minisatellite tested [67]. Although RFLP using either multi-locus or single locus probes could distinguish unrelated individuals with a high degree of probability [60, 63], it was replaced by PCR-based techniques. RFLP techniques were time consuming by comparison, cannot be applied to degraded DNA as it required up to 1 µg of high quality DNA [68, 69]. Further, if there was a partial digestion of the DNA in the first step of the RFLP testing then there can be a problem with reproducibility.

The original PCR process used two primers and requires prior knowledge of the DNA sequence in order to synthesis the primers [70-73]. There were two methods developed that required no prior sequence knowledge and could work on the entire genome. Random Amplification of Polymorphic DNA (RAPD) uses a single short primer (about 10 nucleotides) of a random sequence to amplify from the template DNA such that a range of short amplicons will be produced [74], as shown in Figure 1.2. The premise behind RAPD is that the band pattern produced should be specific to the DNA tested [74] . RAPD was used successfully in forensic science to link botanical samples (such as the use of RAPD profiling for linking the seed pods from the suspect's truck and a tree at the crime scene [75-77]) and linking cannabis samples [78-84] . Amplified Fragment Length Polymorphism (AFLP) was a second test that requires no sequence information

and combines the idea of RAPD and RFLP. AFLP starts by cutting genomic DNA with restriction enzyme to produce overhangs to the restriction products. Synthetic adapters can be ligated to the over hangs of the restriction fragments and these adapters can now serves as the template for primers in a PCR to amplify the restriction fragments thus producing a DNA fingerprint [85], as shown in Figure 1.3. AFLP has been used in forensic science in the linkage of cannabis samples [86-89]. Many of the advantages and disadvantages of RAPD and AFLP are similar [85]. An application of PCR technique which is combined with the RFLP technique is called polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis [90]. In this process, PCR amplifications are performed using primers designed to the locus of interest, then the single base change within the PCR products can be detected using restriction enzymes to generate RFLP patterns; the PCR-RFLP profiles can be separated on gels to detect any differences between the samples [90]. This method has been applied for the identification of many species including bird species [91] and also in forensic investigation as well as the origin of meat [92] and species identification of larvae useful in forensic entomology for estimation of the time of death [93]. A summary of the DNA based *t*echniques which have been used in forensic laboratories is listed in Table 1.1.

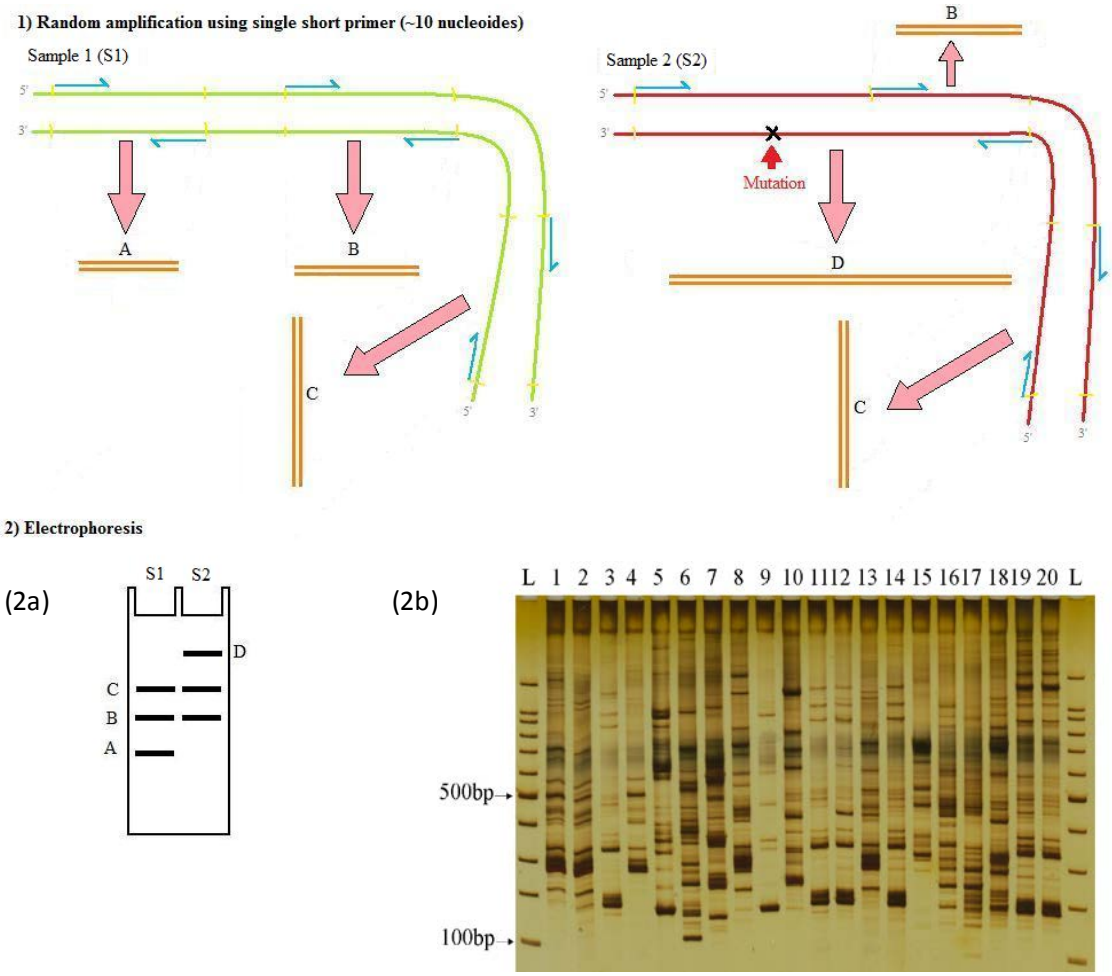

Figure 1.2: Schematic representation of RAPD analysis; genomic DNA from sample 1 (S1) and sample 2 (S2) were amplified using arbitrary single short primers. The primers are illustrated as blue arrows. These are typically 10 nucleotides in length. The different lengths of amplicons were separated by agarose gel electrophoresis (2a). The example species identification using RAPD profiling shows in (2b), the figure was taken from James Lee's thesis [66]. Lane L is 100 bp ladder. Lane 1 to 10 are from genus *Paneolus* which 1-4 are from the same species and lane 11-20 are from genus *Psilocybe* which 11 and 12 are from the same species and lane 19 and 20 are from the same species. The result shows that the sample from the same species shows very similar RAPD profile but none of them show 100% match therefore impossible to reproduce the same RAPD profile from this technique even the sample was taken from 1 individual.

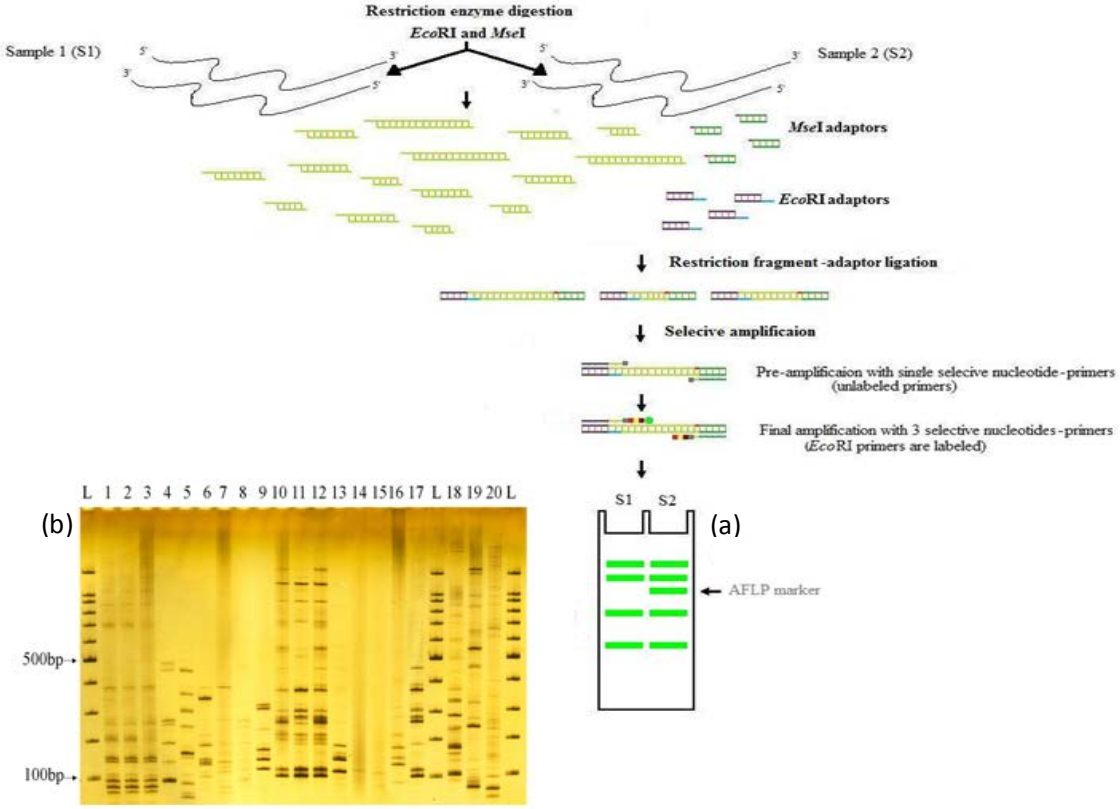

Figure 1.3: Schematic representation of AFLP analysis; genomic DNA from sample 1 (S1) and sample 2 (S2) were doubled-digested with *Eco*RI and *Mse*I. These generated different length of restriction products with 'sticky ends' generated by the enzymes to which adapters could be ligated; with these adapters being of a known sequence and can act as a template in PCR. Pre-amplification, single nucleotide selective amplification was performed using one base extending into the unknown sequence of interest; this reduces the number of PCR products produced. Example species identification using AFLP profiling shows in (b), the figure was taken from James Lee's thesis [66]. Lane M is 100 bp ladder. Lanes 1 to 17 are unknown samples. Lane 1-3 are from the same species, 10-12 are from the same species and 18-20 are reference samples from *Volvariella volvacea, Agaricus bisporus and Lentinus edodes*, respectively. The result shows that the sample from the same species shows very similar AFLP profile but none of them show 100% match therefore impossible to reproduce the same AFLP profile from this technique even the sample was taken from 1 individual.

Table 1.1: List of techniques which have been used in the species testing. (+) shows advantages of the technique and (-) shows disadvantages of the technique.

Techniques	Advantages (+) and disadvantages (-)
1. Microscopic hair analysis	+ Cheap + Fast and easy to perform + Can be automated [94], [95] - Colour is subjective [94] - Difficult to interpret [96] - Experience needed [96]
2. Antigen-antibody reactions	+ Species identification possible + High sensitivity and specificity - Species-specific probes needed [97] - Cross reactivity between closely related species [98-101] - Requires enough specimen for protein extraction [102] - Different antibody needed for each species [99] - Proteins can be degraded under casework conditions [99, 103]
3. RAPD	+ Species identification possible + Cheap + Fast + DNA sequence information is not needed [104] - Reproducibility is a problem - Cannot distinguish mixed DNA sample [105] - Variation of the same species occurs between different samples [106, 107] - DNA fingerprinting relies on quality and quantity of the sample [106, 108] - PCR condition and efficiency of primers affect band patterns [108, 109]
4. RFLP	+ Species identification possible + DNA sequence information is not needed - Requires large amounts of DNA template and *high-quality DNA* - Cannot distinguish mixed DNA sample [110, 111] - Expensive [112] - Time consuming [112] - Variation of restriction sites of the same species [111, 113] - Cannot distinguish closely related species [111, 114]
5. AFLP	+ Species identification possible + DNA sequence information is not needed - The problem and limitation reproducibility [110] - Mixtures of two or more species cannot be identified [110] - Cannot distinguish closely related species
6. SNP	+ Species identification possible + Can separate complex mixtures [115] + Possibility to add additional species as needed + No need sequencing - DNA sequence information is not needed for primer design - Can identify only species which primers have been designed for

Techniques	Advantages (+) and disadvantages (-)
7. Real-Time PCR	+ Species identification possible + High specificity + High sensitivity + Reproducibility + Fast - Can detect only a small number of species at one time - Expensive
8. Sequencing	+ Species identification possible + High specificity + High sensitivity + Reproducibility + Can analyse a wide range of species using universal primer - DNA sequence information is not needed for primer design - Cannot separate mixtures - Contamination from using universal primers - Expensive

A wide variety of biological samples are frequently encountered in the investigation of alleged wildlife crimes. These sample types may include: fish, plants (such as seed, leaves or their products) that are under legal protection; corals and pearls [32]; hides and furs [116]; skin [116-118]; ivory and horn [26, 27, 32, 118-120]; meat [34, 116]; saliva and blood [116]; faeces, urine and tissue [121]; egg [122]; feather [116]; bones [116]; teeth [118] and carcasses [124]. A challenge for wildlife forensic scientists is to identify and distinguish a species when morphology is insufficient, such as in Traditional East Asian Medicine (TEAM) as this may contain a mixture of many species [125, 126]; TEAM has been shown to contain many protected mammalian species [127-129]. Egg samples, feather and skeletal remains of a bird have been analysed previously as part of a forensic investigation [91, 122, 123]. In some of the cases listed above, identification of the species may be possible if there is sufficient morphology present. In the absence of morphology that can be used in species identification, then it may be necessary to resort to molecular genetics methods.

1.5 Avian taxonomy

Carl Linnaeus, the father of taxonomy, developed the Latin multinomial system. The classification of organisms were categorized into Kingdom, Class, Order, Genus, and Species and were identified using their genus and species names [130-132]. These are the broad classifications, although numerous additional separations have been proposed to divide taxonomic groups into up to 16 different divisions. The Table 1.2 shown taxonomy of a range of species and a couple Orders represent avian species.

Table 1.2: The taxonomy of a range of species.

Kingdom	Animalia					
Phylum	Chordata					
Class	Mammalia	Aves				
Order	Primates	Galliformes			Anseriformes	Passeriformes
Family	Hominidae	Phasianidae			Anatidae	Fringillidae
Genus	Homo	Gallus	Meleagris	Coturnix	Anas	Carpodacus
Species	*H.sapiens*	*G.gallus*	*M.gallopavo*	*C.coturnix*	*A.platyrhynchos*	*C.mexicanus*
	Human	Chicken	Turkey	Quail	Duck	Finch

Avian taxonomy has gone through a number of changes recently. Birds were classified into the class Aves. There are 34 Orders recognised, which 25 are extant, two have recently become extinct and 7 orders of birds recognised only by fossil records [133]. At present, there are about 9,000 different species of bird currently living on Earth [134]. The classification of avian species has been grouped together under the principle of that organisms should be classified according to their evolutionary relationships. In 1867, the skull was first used for

determining common inherited features of avian species [135]. The morphological traits has often used for avian classification. Most recently, the classification has been changed to molecular methods.

The DNA hybridization was the first DNA based taxonomy [136-138]. Later DNA hybridization was replaced by DNA sequencing of particular gene loci. The DNA sequences have been used for reconstructing a tree of life for showing the evolutionary relationships of all living organisms. This approach is one of the driving forces behind the recent changes in taxonomic classifications. Phylogenetic tree reconstruction has been used for predicting the tree of life and examining recent taxonomic changes or possible changes in the future. The Neornithes (modern birds) have been identified into two types based on the differences of the skulls. These include the Paleognaths (old jaw), which are Ostrich, Rheas, Cassowaries, Emus, Kiwis, Tinamous, and the Neognaths (new jaw) which are the rest of the living birds. The avian taxonomy shown in Figure 1.4 is based on previous studies [139, 140].

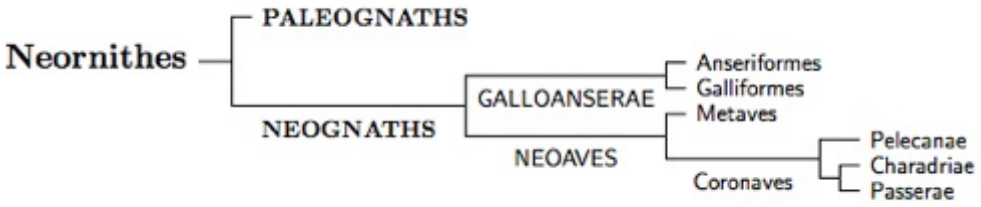

Figure 1.4 Avian taxonomy [139, 140]

The Neognaths separates the Order Anseriformes (waterfowl birds) and Galliformes (gallinaceous birds) from the other birds (Neoaves). The Galloanserae divide into the Anseriformes and Galliformes. In contrast, the Anseriformes were placed after the Pelecaniformes with the Phoenicopteriformes, Ciconiiformes. Metaves include pigeons and doves as well as sandgrouse, kagu, sunbittern, grebes, flamingos, mesites, tropicbirds, nightjars and relatives, swifts, and hummingbirds [141]. The Metaves group is a large grouping in which some species species show a high degree of relatedness, such as grebes and flamingos, whereas some species might be placed in erroneous taxons. Most currently phylogenetic trees are not clear results for the Metaves group, especially, the studies based on mitochondrial DNA [142-146]. For example, tropicbirds were grouped with accipiters, and kagu were grouped with woodpeckers and passerines [145]. Nevertheless, the Metaves group contains avian species birds that are more closely related to each other than to the rest of Neoaves. Coronaves consist of waterbirds (Pelecanae), shorebirds (Charadriae), and land birds (Passerae).

1.6 Speciation and Species concept

1.6.1 Speciation

Speciation can occur in a number of ways, either by allopatric, sympatric or parapatric methods [147-149]. Allopatric speciation occurs most commonly when an ancestral population becomes separated such as by geography, thus limiting the potential for gene flow between the two isolated populations [148]. One of the best known examples of this speciation is that of Darwin's finches (genus Geospizina) [150]. There are three different groups of Darwin's finches which are

classified based upon their ecological niche and their behaviour; these are the ground-finches (*Geospiza* sp.), tree finches (*Camarhynchus* sp.) and warbler finches (*Certhidea olivacea*) [151]; these birds are found on the Galapagos Islands and nowhere else. The different species of finch within the Galapagos archipelago have evolved to have different beak structures adapted for the feeding on the seeds of particular trees that grow on each island [150, 152-154]. Sympatric speciation occurs when there is no gene flow between populations as a result of the migration of populations; here an overlapping area between populations decreased to a point of reducing gene flow [149]. Parapatric speciation is a version of sympatric speciation where the environment is so large that gene flow among the members of the ancestral species is limited resulting in genetic drift among different sub-populations [155].

1.6.2 Species concept

The species concepts were introduced and can be categorised into 3 major groups as; the Typological Species Concept (TSC) ; the Biological Species Concept (BSC); and the Evolutionary Species Concept (ESC) [156].

Typological Species Concepts (TSC)

TSC categorize organisms based on sharing traits as the four basic characteristics; sharing in the same essence, separate from all others by a sharp discontinuity, constant through time and severe limitations to the possible variation of each species [130]. The members of each group share in the same fixed essence. The problem of these concepts when the observation of the vast amount of variation

between similar organisms [130]. The predominance of essentialist is using morphology for distinguishing between organisms therefore the criterion is simple and requires no need technical skill [130, 157].

Biological Species Concept (BSC)

No one definition is suitable however one of the best definitions is in term of the BSC by the population-geneticist Dobzhansky [158-160] which was amended by Ernst Mayr, where a species is defined as a group of organisms capable of interbreeding and producing fertile offspring [152, 158, 159, 161-164]. For example two frog species were grouped into different species because they can mate but cannot produce viable hybrid [156]. Exceptional cases can be found in zoos such as tiger and lion which can produce fertile offspring called ligers and tigons. This is an artificial and man-made instance hence modifying the BSC to "a group of organisms capable of interbreeding ***in nature*** and producing fertile offspring" would eliminate the lion/tiger anomaly. It should be noted that both lion and tiger will not mate in nature because they are naturally ecologically and geographically isolated (African lions prefer open area while Asian tigers prefer forest) and behavioural isolation (lions are social animals, living as a group while tigers are isolated animals, living as a couple only for mating). Anomalies to the BSC would still include some plants where cross pollination can occur naturally and successfully produce inter-specific hybrid plants [165, 166]. Species maintain their reproductive isolation through various isolation mechanisms [157]. BSC is the most widely accepted species concept which based on reproductive isolation.

Evolutionary species concept (ESC)

Among palaeontologists, ESC is the most popular concept. In 1967, George Gaylord (mammalian palaeontologist) stated that "a species is a series of ancestor-descendent populations passing through time and space independent of other populations, each of which possesses its own evolutionary tendencies and historical fate" [167], therefore evolutionary species defined as a group of organisms that shares a common ancestor which maintains its integrity with respect to other lineages through time and space.

Legal definitions

The legislations of many counties including Australia, lists certain species as being protected. In order to investigate any alleged transgression of the legislation, it is a requirement to identify the unknown sample(s) to determine if it is a protected species. The test employed must be species specific, be validated to the extent that is meets general acceptance, and withstand any challenge in a Court. This can be an issue if there is not broad agreement on the taxonomic classification of particular species. While in many cases this definition is adequate, more precise or differing measures are often used, such as based on similarity of DNA or morphology. The presence of specific locally adapted traits may further subdivide species into subspecies. Legal definitions of species are defined based on the best available scientific and commercial data [157]. The listing decisions are not always based on scientific criteria [130, 157, 168]. In case of subspecies was defined as "an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of the

species and differing taxonomically from other populations of the species"[157]. Later, Mayr clarified this definition, that a subspecies must share a unique geographic range, phenotypic characters, and unique natural history [169]. Another term that must be clarified is hybrid. Hybridization is defined as "the crossing of individuals belonging to two unlike natural populations that have secondarily come into contact" [157].

1.7 Avian mitochondrial DNA (mtDNA)

The mitochondria carry out essential metabolic functions, notably with respect to cellular energy production and respiration [170-173]. Mitochondrion are found in almost all eukaryotic cells [174], generally rod-shaped bacterium-like organelles [175], have outer and inner membranes which are structurally and functionally distinct and are at a high copy number in the cell . There are hundreds to thousands of mitochondria in most mammalian cells [176]. mtDNA is a circular double-stranded molecule, in the most dilute animal sample contains about 12,000 copies of mtDNA per mL (20.4 pg) [177].

Avian mtDNA in various species contains different length from 16.6 - 16.8 kb [178]. The two double strands are distinguished as a heavy strand (H) which is guanine rich and the light strand (L) which is cytosine rich [179]. The coding region of mtDNA encodes 37 genes, 13 of the enzymes in oxidative phosphorylation, two ribosomal RNA genes (rRNA) and 22 of transfer RNA genes (tRNA) [180]. The 22 tRNA genes on the mtDNA are relatively small (less than 100 bp) and exhibit little variation. Greater variation occurs in the 13 genes

involved in the oxidative phosphorylation process and the two rRNA genes. This greater variation is due to the function of the transcribed molecule and the length of the molecule. The non-coding region, which is called the displacement loop (D-loop), is the major control site for mtDNA expression because it contains the origins of replication and the elements of initiation for the leading strand of replication [181]. To illustrate the organization of loci of mitochondrial genome, the gene arrangement of the 13 loci from chicken (*G. gallus*), junglefowl (*G. varius*), guineafowl (*Numida meleagris*), human (*Homo sapiens*), sheep (*Ovis aries*), Northern native cat (*Dasyurus hallucatus*), grasshopper (*Gomphocerippus rufus*), frog (*Xenopus laevis*) and snake (*Crotalus horridus*) is shown in Table 1.3.

At the protein level, the lengths of mitochondrial amino acid sequences of these species that were obtained from the DNA database, for the 13 loci including the ND family, COI, COII, COIII, ATP6, ATP8 and cyt *b*, vary as shown in Table 1.4.

Table 1.3: Gene arrangement of the 13 loci including the ND family, COI, COII, COIII, ATP6, ATP8 and cyt *b* of chicken (*G. gallus*), junglefowl (*G. varius*), guineafowl (*Numida meleagris*), human (*Homo sapiens*), sheep (*Ovis aries*), Northern native cat (*Dasyurus hallucatus*), grasshopper (*Gomphocerippus rufus*), frog (*Xenopus laevis*) and snake (*Crotalus horridus*) obtained from GenBank. The check mark symbol (✓) indicates the same gene arrangement based on human mitochondrial loci (the first column from the left). The arrangements of the loci that different from the human mtDNA are indicated in blue.

The arrangement of the 13 loci based upon human mtDNA	Snake	Frog	Northern native cat	Sheep	Green junglefowl	Chicken	Helmeted guineafowl	Grasshopper
1.ND1	✓	✓	✓	✓	✓	✓	✓	✓
2.ND2	✓	✓	✓	✓	✓	✓	✓	✓
3.COI	✓	✓	✓	✓	✓	✓	✓	✓
4.COII	✓	✓	✓	✓	✓	✓	✓	✓
5.ATP synthase8	✓	✓	✓	✓	✓	✓	✓	✓
6.ATP synthase6	✓	✓	✓	✓	✓	✓	✓	✓
7.COIII	✓	✓	✓	✓	✓	✓	✓	✓
8.ND3	✓	✓	✓	✓	✓	✓	✓	✓
9.ND4L	✓	✓	✓	✓	✓	✓	✓	ND5
10.ND4	✓	✓	✓	✓	✓	✓	✓	✓
11.ND5	✓	✓	✓	✓	✓	✓	✓	ND4L
12.ND6	✓	✓	✓	✓	cyt *b*	cyt *b*	cyt *b*	✓
13.Cyt*b*	✓	✓	✓	✓	ND6	ND6	ND6	✓

Table 1.4: The length of mitochondrial amino acid sequences of the 13 loci including the ND family, COI, COII, COIII, ATP6, ATP8 and cyt *b* of chicken (*G. gallus*), junglefowl (*G. varius*), guineafowl (*Numida meleagris*), human (*Homo sapiens*), sheep (*Ovis aries*), Northern native cat (*Dasyurus hallucatus*), grasshopper (*Gomphocerippus rufus*), frog (*Xenopus laevis*) and snake (*Crotalus horridus*) obtained from the DNA database. The mammalian species are highlighted in green and the avian species are highlighted in pink.

Species	Amino acid length from mt genes												
	CoI	CoII	CoIII	Cyt*b*	ND1	ND2	ND3	ND4L	ND4	ND5	ND6	ATPsynthase6	ATPsynthase8
chicken	516	227	261	380	324	346	116	98	459	605	173	227	54
junglefowl	516	227	261	380	324	346	116	98	459	605	173	227	54
guineafowl	516	227	261	380	320	346	116	98	459	604	173	227	54
human	513	227	261	380	318	347	115	98	459	603	174	226	68
sheep	514	227	261	379	318	347	115	98	459	606	175	226	66
Northern native cat	513	228	261	381	318	347	116	98	459	605	166	226	69
grasshopper	513	227	265	379	314	342	117	97	444	572	173	225	53
frog	518	228	260	379	323	345	114	98	461	604	170	226	55
snake	533	228	260	358	318	345	111	96	406	590	173	226	54

There is no evidence to date that avian males pass on mtDNA through their spermatozoa rather all through the egg's cytoplasm therefore mtDNA is entirely derived from the mother [182, 183]. There are exceptions to this rule as demonstrated by mussels of the genus *Mytilus* which have biparental inheritance of mitochondria [184]. mtDNA mutations occur frequently, due to the lack of the error reading enzyme in mitochondria leading to mtDNA loci having on average a 10-20 times greater variability compared to their nuclear counter parts [185].

1.7.1 Application of mtDNA in forensic science

mtDNA has been used in particular cases where the amount of nuclear DNA is too low or highly degraded [186] such as forensic anthropology and missing person identification [186, 187]. Nevertheless, mtDNA is less discriminating than nuclear DNA because no recombination, so each member of a maternal line should have the same sequence of mtDNA, hence in forensic science for human identification mtDNA typing has a niche role [187, 188]. Although mtDNA has a relatively low power of discrimination for individual identification, the highly variable regions (HV1 and HV2) can differ between individuals if there have been two or more mutations.

The cytochrome b gene (cyt *b*) has been used in species identification of vertebrate species due to the relatively little intra-species variation but the presence of inter-species variation [99, 189, 190]. The cyt *b* gene is 1,140 bp long [189] and encodes a protein of 380 amino acids in length [191].

There is very little variation in the size of the protein although the sequence can vary, leading to the genetic variation required for species testing. The protein spans across the membrane of the mitochondria between eight and nine times [192, 193]. The amino acids that span the membrane are highly conserved and largely invariant between species. The amino acids that are outside of the membrane spanning area are expected to be less conserved and exhibit greater polymorphism between species [193]. Much of the discussion on evolutionary studies using genetic testing, however, comes from the more extensive studies on mammalian DNA [194-203]. For molecular phylogenetics and evolution studies, the conserved regions of mtDNA are ideal for designing universal primers. The COI gene is the most conserved of the three coding genes of cytochrome oxidase complex. The NADH dehydrogenase I (ND1) is the most conserved of the seven coding genes of NADH dehydrogenase family of proteins; cyt *b* is more conserved than ND1 but less than COI [204]. All mtDNA loci of human and avian are shown in Figure 1.5. The avian ND2 gene is located between the ND1 and COI genes at the position of the ND2 gene is between 5250-6290 bp in the chicken (*Gallus gallus*) mtDNA while the ND2 gene in human mtDNA is between 4469-5510 bp. The ND2 gene coding sequence of avian species is about 1042 bp in length.

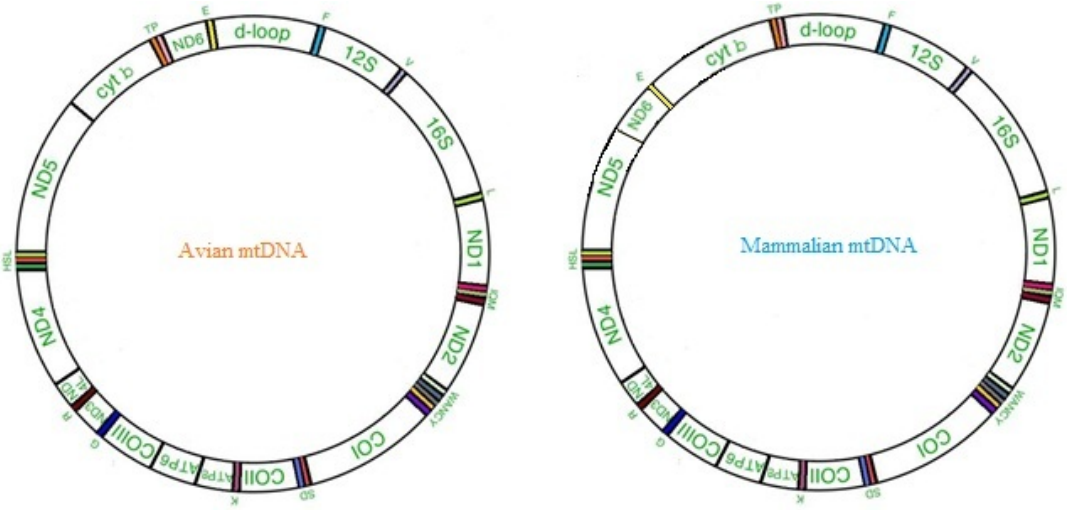

Figure 1.5: Organization of the mammalian and avian mitochondrial genomes. The D loop is normally viewed at the top with the numbered bases going clockwise. The order of the 37 genes is similar between mammalian and avian mtDNA but note that cyt *b* and ND 6 are transversed.

1.7.2 Avian mtDNA studies

Most studies of avian mtDNA are on systematic relationships between species, closely related species and subspecies [205-216]. To date few of genes other than cyt *b* and COI have been used in taxonomic and forensic studies yet there is much potential for their use in avian species identification. Avian species have been less well studied yet the analysis while a comparison of avian DNA is often required in a forensic science investigation such as bird strikes to airplanes [217, 218]. Genetic distance between inter- species and also between intra-species of avian is lower between than other vertebrate for both for mtDNA [206, 210] and for nuclear DNA [219].

1.8 Generation of Genetic Variation

Alteration to the genetic complement can either be a single base change or a whole chromosome alteration. One major type of genetic variation is a mutation. Mutational events at the DNA level can either be as a result of one or more incorrect nucleotide being added randomly during DNA replication *in vivo* or induced by external factors such as the alteration of the DNA due to environmental factors such as genotoxins, pollutants, chemical and radiation exposure [220]. On a larger scale, the gene rearrangements including translocations, deletions, inversions, insertions, and duplications leads to genetic variation. Variation leads to polymorphisms, literally mean many forms. Single-nucleotide polymorphisms (SNPs) are the most common type of polymorphism approximately occurring every 1,000 bases [221]. Single variations at the DNA level are common and lead to the assumption that every individual's DNA is unique.

Over 98 % of nuclear DNA in humans is non-coding [222] therefore most mutations occurs in these regions by chance, on the other hand most mutations in mtDNA occur within the genes as approximately 92 % of the mitochondrial genome are related to coding function [223]. Single base changes within a coding region have ranging effects depending on which base is altered. For instance the amino acid proline is encoded by CCA, CCC, CCG and CCT [224, 225]. A mutation at the third base of the codon will not affect the resulting amino acid and is called a silent, or synonymous mutation [226, 227].

The way the genetic code works means that the third base is the most redundant of the three bases of a codon. There are two amino acids (Leucine and Serine) where the first base of the codon can alter leading to a silent mutation [228]. The second base of a codon cannot mutate without alteration to the encoded amino acid and is called non-silent, or non-synonymous mutation [229]. The vertebrate mitochondrial genetic code is shown in Table 1.5.

Table 1.5: The genetic codes for vertebrate mtDNA . The base in the left is the first base of the codon, the base along the top of the table is the second base and that at the right is the third base. In a table of the genetic code U is used to indicate uracil rather than T (Thymine). The different of the vertebrate mitochondrial genetic codons from the standard codons are indicated in red.

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Trp UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Met AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Stop AGG Stop	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

The twenty naturally occurring amino acids fall into four distinct categories; acid, basic, polar and non-polar as shown in Table 1.6. Depending upon the function of the protein it may be that alteration from one amino acid to another within the same group has little effect on the protein's activity. Alteration of the amino acid between the different groups of proteins has much more of an effect, unless the amino acid is within a part of the protein that plays little part in the activity of the protein. Within gene sequences an insertion or a deletion (termed an indel) will cause a frame shift resulting in a completely different set of amino acids being made; this is unless the insertion or deletion is of three bases.

Table 1.6: The classification of 20 amino acids .

Non-Polar	Polar	Basic	Acidic
Glycine Alanine Valine Leucine Isoleucine Proline Methionine Phenylalanine Tryptophan	Serine Threonine Asparagine Glutamine Cysteine Tyrosine	Lysine Arginine Histidine	Aspartic Acid Glutamic Acid

1.9 Molecular clocks

In the past, deciphering the evolution history of organisms was based on fossil records [230], but the use of genetic and protein comparisons has added a new dimension [231]. Comparison of nuclear or amino acid data obtained from databases indicates that changes occur at predictable rates [230, 232]. Assuming a consistency in the rate of change of amino acids and DNA sequences over time allows their use as molecular clocks.

This allows the estimation of the divergence in time between species, especially in some species which have a few, or no, fossil records [230-232]. This approach has been used widely in evolutionary studies [233]. Fossil record can provide an estimate of age using radioactive dating. A genetic approach can be used to estimate the time of divergence from a common ancestor and for allowing a phylogenetic reconstruction depicting genetic distance between species [230]. A molecular clock of each species is based upon generation times, the rate of mutation, body size and metabolic rate [232]. For example, the evolution rate of rodents is faster than human and other mammals over time. The divergence rate has been used for evolutional studies in birds with a mitochondrial substitution rate of 2 % per million years. This has been used for estimating the time of divergence from genetic distance between avian species [234].

The nucleotide substitution rate within mtDNA is five to ten times higher than within nuclear DNA [235, 236] primarily due to the lack of an error reading DNA polymerase within the mitochondria. Substitution rates within the mtDNA genome of bird and reptile species are fast compared to the other animal species, snake and lizard species are relatively slow in comparison to turtle and bird species [237]. Non-synonymous substitution rates of the ATP8 gene in bird and reptile species are the fastest mitochondrial loci and the slowest is the COIII locus [237].

The evolutionary rates of the non-coding control region of mtDNA is the fastest evolving region on the mitochondrial gene whereas the protein-coding genes and the tRNA genes are moderate evolving loci [238]; and the rRNA genes are the slowest evolving loci due to different selection pressures based on their function [239-241].

1.10 Mitochondrial DNA Barcoding genes

Barcodes are DNA sequences that provide sufficient variation for distinguishing species [242, 243]. mtDNA is routinely used for non-human forensic cases. In the investigation of alleged wildlife crimes, DNA Barcodes has been used for species identification to investigate the illegal trade in protected species [244] and also animal tissues in some murder cases [244, 245]. The most commonly used mtDNA loci that have been used in the identification of species and taxonomic studies are cyt *b* and the Barcoding DNA region which is approximately 648 bp region positioned near the 5' terminus of the COI gene [242, 243, 246-262].

The selection of the Barcode loci relies on a) the possibility for universal DNA primer design for DNA amplification of all species tested and b) sufficient variation in the amplified DNA to discriminate between closely related species. For fungi and microorganism, the identification has relied heavily on analysis of the rDNA. The 18S rDNA and 28S rDNA, the nuclear rRNA genes were selected because they are highly conserved and can differentiate the higher taxonomic levels such as Families and Genera. The internal-transcribed spacer (ITS) has been used for distinguishing between these fungal species [263].

One reason for the application of mitochondrial genes in taxonomic studies include the relatively fast evolutionary rate [264]. Additionally, the slowly evolving loci such as rRNA gene, do not differ among closely related species, but they can be used for associating distantly relative species [243].

1.11 Phylogenetic tree

A phylogenetic, or evolutionary, tree is a diagram which is composed of branches and nodes to illustrate evolutionary relationships between groups of species. Phylogenetic trees can be classified as rooted-trees and unrooted trees as shown in Figure 1.6.

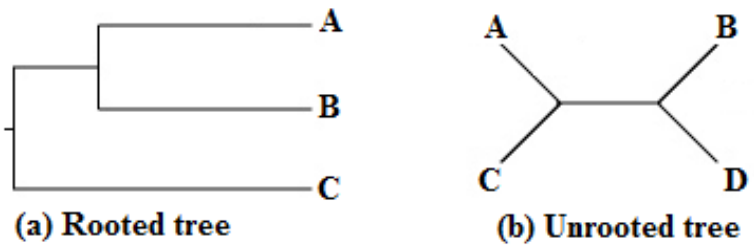

Figure 1.6: Showing rooted tree (a) and unrooted tree (b).

The phylogenetic trees are composed of several parts including root, internal node, external node, branch, branch length, and external node or leaf as shown in Figure 1.7.

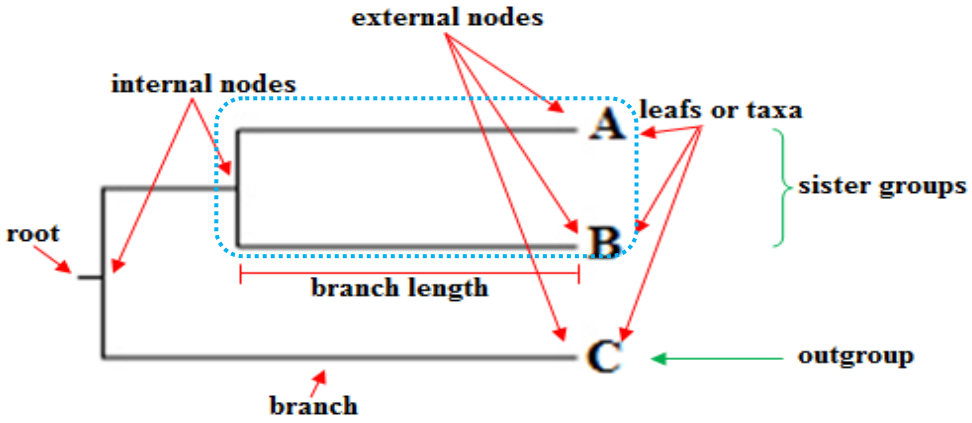

Figure 1.7: Showing parts of a phylogenetic tree. The red arrows indicate the parts of the tree. Taxon A and B are sister groups. Taxon C is the outgroup of taxon A and B. The blue box is a clade. Root is a common ancestor of all taxa on the tree. A clade is a group of taxa and their common ancestor. Internal nodes or hypothetical taxonomic units (HTUs) represent the hypothetical ancestor. External nodes or operational taxonomic units (OTUs) are the set of data under the comparison study. Branch represents the relationship between the taxa. Branch length shows the number of changes within the branch. Sister groups represent closest relatives. Outgroup represents a taxon outside the group of study.

Phylogenetics is the determination of the rate and pattern of change occurring in DNA or protein sequences in order to reconstruct the evolutionary relationships between organisms. Most online programs also generate trees from a list of examples of the online tools and the software packages for multiple sequence alignment and phylogenetic tree reconstruction is shown in Table 1.7.

Table 1.7: A list of examples of the online tools and the software packages for multiple sequence alignment and phylogenetic tree reconstruction. Multiple alignments can be performed directly on the website of the online programs on the left column while the software packages from the column on the right have to be downloaded and installed on the local computer first.

Online programs and server	Software packages
MAFFT at: - **EBI** - **GenomeNet** - **The MPI Bioinformatics Toolkit** - **SIB** - **Pasteur** - **WABI** - **DDBJ** **MUSCLE at:** - **EBI** **ClustalW at:** - **EBI** - **Pasteur** - **EMBL** - **DDBJ** - **SIB** - **GenomeNet** **T-COFFEE at:** - **EBI** - **SIB** **Kalign at:** - **EBI**	**MAFFT** **MUSCLE** **ClustalW** **MEGA** **T-COFFEE** **M-COFFEE** **MrBayes** **PHYML** **PAML** **PHYLIP** **PAUP**

There are many methods for calculating phylogenetic trees from DNA and amino acid sequences. Statistical methods can be classified into distance methods and character methods. The distance methods compute every pair of sequences in the multiple alignments and calculate the number of differences to generate the right tree step-by-step; these methods also called stepwise clustering method. The distance methods include unweighted pair-group method using arithmetic averages (UPGMA), neighbor-joining (NJ) and minimum-evolution (ME) [265].

The character methods use a strategy to find the best tree by examining all of the possible trees and then select the best; these methods are also called an exhaustive-search method. The character methods calculate evolutionary relationships from a number of changes of each character at each position which related to a possibility or state of the character; amino acids have 20 possible amino acids per position or DNA molecules have four possible bases per position. The maximum-parsimony (MP), the maximum-likelihood (ML) and the Bayesian inference (BI) methods belong to the character methods [265].

The evolutionary models that have been using with the methods above for computing the evolutionary relationships are listed below:

(a) p-distance – using the proportion of the differences between sequences.

(b) Maximum Composite Likelihood [266] – calculating the evolutionary distances between all pair of the sequence data with and without taking rate variation and substitution between the sequences into account.

(c) Jukes-Cantor [267] – using the substitution rate parameter.

(d) Tajima-Nei [268] – taking unequal rates of substitution between nucleotide pairs into account.

(e) Tamura-Nei [269] – taking the base composition and the transition/ transversion rate bias into account.

(f) Kimura 2-parameter [270] – calculating the branch lengths at two different rates based upon the probability of transition and transversion mutations.

(g) Tamura 3-parameter [271] – taking the differences in transitional and transversional rates and the G+C-content biases into account.

(h) Hasegawa-Kishino-Yano [272] – using a molecular clock to calculate the divergence dates between the sequence data.

(i) LogDet [273] – using determinants (real numbers) of the observed divergence matrices.

(j) General Time Reversible [274] – calculating the differences of base composition of the two sequences to estimate the time of divergence and substitution rates between the observed data.

1.12 The Aim of this Study

Many avian species are protected by national legislation but most molecular studies have focussed on applications relevant to mammalian species using the cyt *b* and COI loci. Other mitochondrial loci have not been studied extensively in avian species identification yet. The aim of this study is to examine all 37 of mitochondrial loci to find the most effective segment of the gene that can identify closely related avian species and reconstructing the phylogenies. These will be accomplished by first looking at entire mtDNA, then the complete genes individually and finally sections of each gene. The fraction of the gene that performs the optimally at species identification and phylogenetic tree reconstruction will be used in intra-species variation.

Chapter 1 References

1. Blackburn, T.M., P. Cassey, and R.P. Duncan, *Extinction in island endemic birds reconsidered.* Ecography, 2004. **27**(1): p. 124-128.

2. Brooks, T.M., et al., *Habitat loss and extinction in the hotspots of biodiversity.* Conservation Biology, 2002. **16**(4): p. 909-923.

3. Burney, D.A. and T.F. Flannery, *Fifty millennia of catastrophic extinctions after human contact.* Trends in Ecology & Evolution, 2005. **20**(7): p. 395-401.

4. Cardillo, M. and A. Lister, *Evolutionary biology: Death in the slow lane.* Nature, 2002. **419**(6906): p. 440-441.

5. Franco, A.M.A., et al., *Impacts of climate warming and habitat loss on extinctions at species' low-latitude range boundaries.* Global Change Biology, 2006. **12**(8): p. 1545-1553.

6. Genovesi, P., *Eradications of invasive alien species in Europe: a review.* Biological Invasions, 2005. **7**(1): p. 127-133.

7. Gurevitch, J. and D.K. Padilla, *Are invasive species a major cause of extinctions?* Trends in Ecology & Evolution, 2004. **19**(9): p. 470-474.

8. Halliday, T.R., *Extinction of the Passenger Pigeon Ectopistes-Migratorius and Its Relevance to Contemporary Conservation.* Biological Conservation, 1980. **17**(2): p. 157-162.

9. Mona, S., et al., *Population dynamic of the extinct European aurochs: genetic evidence of a north-south differentiation pattern and no evidence of post-glacial expansion.* Bmc Evolutionary Biology. **10**.

10. Pearson, D.L., et al., *Testing Hypotheses of Bird Extinctions at Rio Palenque, Ecuador, with Informal Species Lists.* Conservation Biology. **24**(2): p. 500-510.

11. Vredenburg, V.T., et al., *Concordant molecular and phenotypic data delineate new taxonomy and conservation priorities for the endangered mountain yellow-legged frog.* Journal of Zoology, 2007. **271**(4): p. 361-374.

12. Collen, B., A. Purvis, and G.M. Mace, *Biodiversity Research: When is a species really extinct? Testing extinction inference from a sighting record to inform conservation assessment.* Diversity and Distributions, 2010. **16**(5): p. 755-764.

13. Mooers, A.O. and R.A. Atkins, *Indonesia's threatened birds: over 500 million years of evolutionary heritage at risk.* Animal Conservation, 2003. **6**: p. 183-188.

14. Brooks, T.M., S.L. Pimm, and J.O. Oyugi, *Time lag between deforestation and bird extinction in tropical forest fragments.* Conservation Biology, 1999. **13**(5): p. 1140-1150.

15. Rando, J.C. and J.A. Alcover, *On the extinction of the Dune Shearwater (Puffinus holeae) from the Canary Islands.* Journal of Ornithology. **151**(2): p. 365-369.

16. Saether, B.E., et al., *Time to extinction of bird populations.* Ecology, 2005. **86**(3): p. 693-700.

17. Pimm, S., et al., *Human impacts on the rates of recent, present, and future bird extinctions.* Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(29): p. 10941-10946.

18. Pimm, S.L., *The dodo went extinct (and other ecological myths).* Annals of the Missouri Botanical Garden, 2002. **89**(2): p. 190-198.

19. Olson, S.L., *Lost land of the dodo.* Science, 2008. **321**(5891): p. 913-914.

20. Roberts, D.L. and A.R. Solow, *Flightless birds - When did the dodo become extinct?* Nature, 2003. **426**(6964): p. 245-245.

21. Janoo, A., *Discovery of isolated dodo bones [Raphus cucullatus (L.), Aves, Columbiformes] from Mauritius cave shelters highlights human predation, with a comment on the status of the family Raphidae Wetmore, 1930.* Annales de PalÃ©ontologie, 2005. **91**(2): p. 167-180.

22. Conrad, J.M., *Open access and extinction of the Passender Pigeon in North America.* Natural Resource Modeling, 2005. **18**(4): p. 501-519.

23. Jackson, J.A. and B.J.S. Jackson, *Extinction: the Passenger Pigeon, Last Hopes, Letting Go.* Wilson Journal of Ornithology, 2007. **119**(4): p. 767-772.

24. Hanski, I., *The world that became ruined - Our cognitive incapacity to perceive large-scale and long-term changes is a major obstacle to rational environmental policies.* Embo Reports, 2008. **9**: p. S34-S36.

25. Johnson, K.P., et al., *The flight of the Passenger Pigeon: Phylogenetics and biogeographic history of an extinct species.* Molecular Phylogenetics and Evolution. **57**(1): p. 455-458.

26. Stiles, D., *The ivory trade and elephant conservation.* Environmental Conservation, 2004. **31**(4): p. 309-321.

27. Wasser, S.K., et al., *Combating the illegal trade in African elephant ivory with DNA forensics*. Conservation Biology, 2008. **22**(4): p. 1065-1071.

28. Brooks, M., *African Rhino Specialist Group report*. Pachyderm, 2009(46): p. 7-13.

29. Rosen, G.E. and K.F. Smith, *Summarizing the Evidence on the International Trade in Illegal Wildlife*. Ecohealth. **7**(1): p. 24-32.

30. Gomez, A. and A.A. Aguirre, *Infectious Diseases and the Illegal Wildlife Trade*, in *Animal Biodiversity and Emerging Diseases: Prediction and Prevention*. 2008. p. 16-19.

31. Wetton, J.H., et al., *An extremely sensitive species-specific ARMs PCR test for the presence of tiger bone DNA*. Forensic science international, 2004. **140**(1): p. 139-45.

32. Li, Y.M., et al., *Illegal wildlife trade in the Himalayan region of China*. Biodiversity and Conservation, 2000. **9**(7): p. 901-918.

33. Martin, E., *The present-day Egyptian ivory trade*. Oryx, 2000. **34**(2): p. 101-108.

34. Gupta, S.K., S.K. Verma, and L. Singh, *Molecular insight into a wildlife crime: the case of a peafowl slaughter*. Forensic Science International, 2005. **154**(2-3): p. 214-217.

35. Rao, M., et al., *Hunting, Livelihoods and Declining Wildlife in the Hponkanrazi Wildlife Sanctuary, North Myanmar*. Environmental Management. **46**(2): p. 143-153.

36. Graham, N.A.J., M.D. Spalding, and C.R.C. Sheppard, *Reef shark declines in remote atolls highlight the need for multi-faceted conservation action*. Aquatic Conservation-Marine and Freshwater Ecosystems. **20**(5): p. 543-548.

37. Jachmann, H., *Illegal wildlife use and protected area management in Ghana*. Biological Conservation, 2008. **141**(7): p. 1906-1918.

38. Vandenbroucke, V., et al., *Animal poisonings in Belgium: a review of the past decade*. Vlaams Diergeneeskundig Tijdschrift. **79**(4): p. 259-268.

39. Van den berg, T., *The role of the legal and illegal trade of live birds and avian products in the spread of avian influenza*. Revue Scientifique Et Technique-Office International Des Epizooties, 2009. **28**(1): p. 93-111.

40. Berney, J., *4th Meeting of the Conference of the Parties to the Convention on International-Trade in Endangered Species of Wild Fauna and Flora (Cites), Held in Gaborone, Botswana, from 19 to 30 April 1983.* Environmental Conservation, 1983. **10**(2): p. 177-178.

41. D., O., *The convention on international trade in endangered species (Cites, 1973): implications of recent developments in international and EC environmental law.* Journal of Environmental Law, 1998. **10**(2): p. 291-314.

42. *The convention on international trade in endangered species of wild fauna and flora.* 16th Annual Conference Mid-Atlantic States Association of Avian Veterinarians, Proceedings, 1995: p. 237-241.

43. Welsch, H., *Cites - Trade in Appendix-I Species.* Environmental Policy and Law, 1984. **13**(3-4): p. 100-105.

44. Smith, M.J., et al., *Assessing the impacts of international trade on CITES-listed species: Current practices and opportunities for scientific research.* Biological Conservation, 2011. **144**(1): p. 82-91.

45. *Cites Appendix III Export Permit need not be Challenged by Importing State Authority.* Journal of Environmental Law, 2003. **15**(3): p. 341-371.

46. Cooper, M.E. and A.M. Rosser, *International regulation of wildlife trade: relevant legislation and organisations.* Revue Scientifique Et Technique De L Office International Des Epizooties, 2002. **21**(1): p. 103-123.

47. Neugartner, I., *WTO - System and functioning of the World Trade Organization.* Journal of International Economic Law, 2001. **4**(4): p. 808-809.

48. Vallat, B., J. Pinto, and A. Schudel, *International organisations and their role in helping to protect the worldwide community against natural and intentional biological disasters.* Revue Scientifique Et Technique-Office International Des Epizooties, 2006. **25**(1): p. 163-172.

49. Miller, K.R., *IUCN - the World Conservation Union.* Environmental Conservation, 1986. **13**(4): p. 287-289.

50. Eden, T., et al., *Twinning: Starting, Supporting, and Sustaining-the Role of Non-Governmental Organizations (Ngos).* Pediatric Blood & Cancer. **57**(5): p. 837-837.

51. Scott, J.M., et al., *Conservation-reliant species and the future of conservation.* Conservation Letters. **3**(2): p. 91-97.

52. Cox, G., P. Lowe, and M. Winter, *Land-Use Conflict after the Wildlife and Countryside Act 1981 - the Role of the Farming and Wildlife Advisory Group.* Journal of Rural Studies, 1985. **1**(2): p. 173-183.

53. Benson, J.F. and K.G. Willis, *Conservation Costs, Agricultural Intensification and the Wildlife and Countryside Act 1981 - a Case-Study and Simulation on Skipwith Common, North Yorkshire, England.* Biological Conservation, 1988. **44**(3): p. 157-178.

54. *The Hunting Act 2004.* Criminal Law Review, 2005: p. 171-172.

55. Nijman, V. and C.R. Shepherd, *The Role of Thailand in the International Trade in CITES-Listed Live Reptiles and Amphibians.* PLoS ONE, 2011. **6**(3): p. e17825 EP -.

56. Kunte, K., *The Wildlife (Protection) Act and conservation prioritization of butterflies of the Western Ghats, southwestern India.* Current Science, 2008. **94**(6): p. 729-735.

57. Singaravelan, N., G. Marimuthu, and P.A. Racey, *Do fruit bats deserve to be listed as vermin in the Indian Wildlife (Protection) & Amended Acts? A critical review.* Oryx, 2009. **43**(4): p. 608-613.

58. Sahajpal, V., et al., *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): p. 34-45.

59. Foreman, L.A., et al., *Interpreting DNA evidence: A review.* International Statistical Review, 2003. **71**(3): p. 473-495.

60. Schneider, P.M., *Basic issues in forensic DNA typing.* Forensic Science International, 1997. **88**(1): p. 17-22.

61. Jeffreys, A.J., V. Wilson, and S.L. Thein, *Individual-Specific Fingerprints of Human DNA.* Nature, 1985. **316**(6023): p. 76-79.

62. Lynch, M., *God's signature: DNA profiling, the new gold standard in forensic science.* Endeavour, 2003. **27**(2): p. 93-97.

63. Primorac, D. and M.S. Schanfield, *Application of forensic DNA testing in the legal system.* Croatian Medical Journal, 2000. **41**(1): p. 32-46.

64. Schanfield, M., *Forensic science: Taking giant steps forward.* Croatian Medical Journal, 2001. **42**(3): p. 219-220.

65. Brookes, A.J., *The essence of SNPs.* Gene, 1999. **234**(2): p. 177-186.

66. Lee, J.C.-I., *Towards a Molecular Approach for the Identification of Fungal Taxa that Contain Psilocybin.* PhD Thesis, University of Strathclyde, 2001.

67. Evett, I.W., et al., *A Guide to Interpreting Single Locus Profiles of DNA Mixtures in Forensic Cases.* Journal of the Forensic Science Society, 1991. **31**(1): p. 41-47.

68. Ross, A.M. and H.W.J. Harding, *DNA Typing and Forensic-Science.* Forensic Science International, 1989. **41**(3): p. 197-203.

69. Hochmeister, M.N., *DNA Technology in Forensic Applications.* Molecular Aspects of Medicine, 1995. **16**(4): p. 315-&.

70. Mullis, K. and F. Faloona, *Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction.* Methods in enzymology, 1987. **155**: p. 335-350.

71. Bartlett, J.M. and D. Stirling, *A Short History of the Polymerase Chain Reaction* 2003 p. 3-6.

72. Saiki, R.K., et al., *Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA-Polymerase.* Science, 1988. **239**(4839): p. 487-491.

73. Mc Donald, J.H., *Making PCR: A story of biotechnology.* Current Anthropology, 2000. **41**(4): p. 694-695.

74. Lee, J.C.I. and J.G. Chang, *Random Amplified Polymorphic DNA-Polymerase Chain-Reaction (Rapd Pcr) Fingerprints in Forensic Species Identification.* Forensic Science International, 1994. **67**(2): p. 103-107.

75. Craft, K.J., J.D. Owens, and M.V. Ashley, *Application of plant DNA markers in forensic botany: Genetic comparison of Quercus evidence leaves to crime scene trees using microsatellites.* Forensic Science International, 2007. **165**(1): p. 64-70.

76. Mestel, R., *Murder Trial Features Trees Genetic Fingerprint.* New Scientist, 1993. **138**(1875): p. 6-6.

77. Yoon, C.K., *Forensic-Science - Botanical Witness for the Prosecution.* Science, 1993. **260**(5110): p. 894-895.

78. Faeti, V., G. Mandolino, and P. Ranalli, *Genetic diversity of Cannabis sativa germplasm based on RAPD markers.* Plant Breeding, 1996. **115**(5): p. 367-370.

79. Linacre, A. and J. Thorpe, *Detection and identification of cannabis by DNA.* Forensic Science International, 1998. **91**(1): p. 71-76.

80. Gillan, R., et al., *Comparison of Cannabis-Sativa by Random Amplification of Polymorphic DNA (Rapd) and Hplc of Cannabinoids - a Preliminary-Study.* Science & Justice, 1995. **35**(3): p. 169-177.

81. Jagadish, V., J. Robertson, and A. Gibbs, *RAPD analysis distinguishes Cannabis sativa samples from different sources.* Forensic Science International, 1996. **79**(2): p. 113-121.

82. Congiu, L., et al., *The use of random amplified polymorphic DNA (RAPD) markers to identify strawberry varieties: a forensic application.* Molecular Ecology, 2000. **9**(2): p. 229-232.

83. Pinarkara, E., et al., *RAPD analysis of seized marijuana (Cannabis sativa L.) in Turkey.* Electronic Journal of Biotechnology, 2009. **12**(1).

84. Forapani, S., et al., *Comparison of hemp varieties using random amplified polymorphic DNA markers.* Crop Science, 2001. **41**(6): p. 1682-1689.

85. Vos, P., et al., *AFLP - a New Technique for DNA-Fingerprinting.* Nucleic Acids Research, 1995. **23**(21): p. 4407-4414.

86. Datwyler, S.L. and G.D. Weiblen, *Genetic variation in hemp and marijuana (Cannabis sativa L.) according to amplified fragment length polymorphisms.* Journal of Forensic Sciences, 2006. **51**(2): p. 371-375.

87. Coyle, H.M., et al., *A simple DNA extraction method for marijuana samples used in amplified fragment length polymorphism (AFLP) analysis.* Journal of Forensic Sciences, 2003. **48**(2): p. 343-347.

88. Peil, A., et al., *Sex-linked AFLP markers indicate a pseudoautosomal region in hemp (Cannabis sativa L.).* Theoretical and Applied Genetics, 2003. **107**(1): p. 102-109.

89. Flachowsky, H., et al., *Application of AFLP for the detection of sex-specific markers in hemp.* Plant Breeding, 2001. **120**(4): p. 305-309.

90. Butler, J.M. and D.J. Reeder, *Detection of DNA Polymorphisms Using PCR-RFLP and Capillary Electrophoresis Capillary Electrophoresis of Nucleic Acids*, K.R. Mitchelson and J. Cheng, Editors. 2001, Humana Press. p. 49-56.

91. Rudnick, J.A., et al., *Species identification of birds through genetic analysis of naturally shed feathers.* Molecular Ecology Notes, 2007. **7**(5): p. 757-762.

92. Verkaar, E.L.C., et al., *Differentiation of cattle species in beef by PCR-RFLP of mitochondrial and satellite DNA.* Meat Science, 2002. **60**(4): p. 365-369.

93. Schroeder, H., et al., *Use of PCR-RFLP for differentiation of calliphorid larvae (Diptera, Calliphoridae) on human corpses.* Forensic Science International, 2003. **132**(1): p. 76-81.

94. Verma, M.S., et al., *Hair-MAP: a prototype automated system for forensic hair comparison and analysis.* Forensic Science International, 2002. **129**(3): p. 168-186.

95. Moyo, T., S. Bangay, and G. Foster, *The identification of mammalian species through the classification of hair patterns using image pattern recognition*, in *Proceedings of the 4th international conference on Computer graphics, virtual reality, visualisation and interaction in Africa*. 2006, ACM: Cape Town, South Africa. p. 177-181.

96. Moore, J.E., *A key for the identification of animal hairs.* Journal of the Forensic Science Society, 1988. **28**(5-6): p. 335-339.

97. Saferstein, R. and P. Hall, *Chapter 7: Identification and Grouping of Bloodstains,*. Forensic Science Handbook. Inc. New Jersey, 1982: p. 267-296.

98. Prakash, P.S., et al., *Mitochondrial 12S rRNA sequence analysis in wildlife forensics.* Current Science, 2000. **78**(10): p. 1239-1241.

99. Parson, W., et al., *Species identification by means of the cytochrome b gene.* International Journal of Legal Medicine, 2000. **114**(1): p. 23-28.

100. Kang'ethe, E.K., J.M. Gathuma, and K.J. Lindqvist, *Identification of the species of origin of fresh, cooked and canned meat and meat products using antisera to thermostable muscle antigens by Ouchterlony's double diffusion test.* Journal of the Science of Food and Agriculture, 1986. **37**(2): p. 157-164.

101. Chikuni, K., et al., *Species identification of cooked meats by DNA hybridization assay.* Meat Science, 1990. **27**(2): p. 119-128.

102. Balitzki-Korte, B., et al., *Species identification by means of pyrosequencing the mitochondrial 12S rRNA gene.* International Journal of Legal Medicine, 2005. **119**(5): p. 291-294.

103. Abdel-Rahman, S.M. and M.M.M. Ahmed, *Rapid and sensitive identification of buffalo's, cattle's and sheep's milk using species-specific PCR and PCR-RFLP techniques.* Food Control, 2007. **18**(10): p. 1246-1249.

104. Shankaranarayanan, P., et al., *Genetic variation in Asiatic lions and Indian tigers.* Electrophoresis, 1997. **18**(9): p. 1693-1700.

105. Guha, S. and V.K. Kashyap, *Molecular identification of lizard by RAPD & FINS of mitochondrial 16S rRNA gene.* Legal Medicine, 2006. **8**(1): p. 5-10.

106. Lee, J.C.-I. and J.-G. Chang, *Random amplified polymorphic DNA polymerase chain reaction (RAPD PCR) fingerprints in forensic species identification.* Forensic Science International, 1994. **67**(2): p. 103-107.

107. Calvo, J.H., R. Osta, and P. Zaragoza, *Species-specific amplification for detection of bovine, ovine and caprine cheese.* Milchwissenschaft, 2002. **57**(8): p. 444-446.

108. Black, W.C., *Insect PCR with arbitrary primers: approach with care.* Molecular Biology, 1993. **2**(1): p. 1-6.

109. Welsh, J. and M. McClelland, *Fingerprinting genomes using PCR with arbitrary primers.* Nucleic Acids Research, 1990. **18**(24): p. 7213-7218.

110. Bottero, M.T., et al., *A multiplex polymerase chain reaction for the identification of cows', goats' and sheep's milk in dairy products.* International Dairy Journal, 2003. **13**(4): p. 277-282.

111. Zehner, R., S. Zimmermann, and D. Mebs, *RFLP and sequence analysis of the cytochrome b gene of selected animals and man: methodology and forensic application.* International Journal of Legal Medicine, 1998. **111**(6): p. 323-327.

112. Verma, S.K., et al., *Was elusive carnivore a panther? DNA typing of faeces reveals the mystery.* Forensic Science International, 2003. **137**(1): p. 16-20.

113. Wolf, C., J. Rentsch, and P. Hubner, *PCR-RFLP analysis of mitochondrial DNA: a reliable method for species identification.* Journal of Agricultural and Food Chemistry, 1999. **47**(4): p. 1350-1355.

114. Meyer, R., et al., *Polymerase chain reaction - restriction fragment length polymorphism analysis: a simple method for species identification in food.* Journal of AOAC International, 1995. **78**(6): p. 1542-1551.

115. Black, W.C., *PCR with arbitrary primers: approach with care.* Insect Molecular Biology, 1993. **2**(1): p. 1-6.

116. Stroud, R.K., *Wildlife forensics and the veterinary practitioner.* Seminars in Avian and Exotic Pet Medicine, 1998. **7**(4): p. 182-192.

117. Alacs, E. and A. Georges, *Wildlife across our borders: a review of the illegal trade in Australia.* Australian Journal of Forensic Sciences, 2008. **40**(2): p. 147-160.

118. Ogden R, Dawnay N, and M. R, *Wildlife DNA forensics, 2014; bridging the gap between conservation genetics and law enforcement.* Endangered Species Research, 2009. **9**(3): p. 179-195.

119. Reeve, R., *Wildlife trade, sanctions and compliance: lessons from the CITES regime.* International Affairs, 2006. **82**(5): p. 881-897.

120. Vigne, L., E. Martin, and B. Okita-Ouma, *Increased demand for rhino horn in Yemen threatens eastern Africa's rhinos.* Pachyderm, 2007(43): p. 73-86.

121. Fumagalli, L., C.J. Cabrita, and V. Castella., *Simultaneous identification of multiple mammalian species from mixed forensic samples based on mtDNA control region length polymorphism.* Forensic Science International, Genetics Supplement Series, 2009. **2**(1): p. 302-303.

122. Forbes, N.A., *Clinical examination of the avian forensic case.* Seminars in Avian and Exotic Pet Medicine, 1998. **7**(4): p. 193-200.

123. Schmaltz, G., et al., *Non-destructive sampling of maternal DNA from the external shell of bird eggs*, in *Conservation Genetics*. 2006, Springer Netherlands. p. 543-549.

124. Cooper JE, Cooper ME, and Budgen P, *Wildlife crime scene investigation: techniques, tools and technology.* Endangered Species Research, 2009. **9**(3): p. 229-238.

125. Van, N.D.N. and N. Tap, *An overview of the use of plants and animals in traditional medicine systems in Viet Nam.* TRAFFIC Southeast Asia, Greater Mekong Programme, Ha Noi, Viet Nam, 2008: p. 1-96.

126. Still, J., *Use of animal products in traditional Chinese medicine: Environmental impact and health hazards.* Complementary Therapies in Medicine, 2003. **11**(2): p. 118-122.

127. Debbie, N.g. and E.A. Burgess, *Against The Grain: Trade in Musk Deer Products In Singapore And Malaysia.* TRAFFIC Southeast Asia, 2004: p. 1-42.

128. TRAFFIC, *"What's Driving the Wildlife Trade? A Review of Expert Opinion on Economic and Social Drivers of the Wildlife Trade and Trade Control Efforts in Cambodia, Indonesia, Lao PDR and Vietnam" East Asia and Pacific Region Sustainable Development Discussion Papers.* East Asia and Pacific Region Sustainable Development Department, World Bank, Washington, DC, 2008: p. 1-120.

129. Williamson, F.D. and P. Marcus, *Proceeding of thr Third International Symposium on the Trade in Bear Parts 26-28 October, 1999, Seoul, Republic of Korea.* TRAFFIC Southeast Asia, Hong Kong, 2001: p. 1-194.

130. Mayr E, *The growth of biological thought. Diversity, evolution and inheritance*. 1982: Belknap-Harvard.

131. Johnson, R.N., *Conservation Genetics and Wildlife Forensics of Birds*, in *Wildlife Forensics*. 2011, John Wiley & Sons, Ltd. p. 293-325.

132. Kevin D. Hill, *The Endangered Species Act: What Do We Mean by Species?, 20 B.C.*

133. Wetmore, A., *A classification for the birds of the world.* Smithsonian Misc. Collns, 1960. **139**(2): p. 1-37.

134. Kotpal, R.L., *Modern Text Book of Zoology: Vertebrates*. 2010: Global Media Publications.

135. Huxley, T.H., *On the classification of birds; and on the taxonomic value of the modifications of certain of the cranial bones observable in that class.* Proceedings of the Zoological Society of London, 1867: p. 415-472.

136. Sibley, C.G. and J.E. Ahlquist, *Phylogeny and Classification of Birds.* Yale University Press.New Haven, CT, 1990.

137. Sibley, C.G. and B.L. Monroe, *Distribution and Taxonomy of the Birds of the World.* Yale University Press, New Haven, CT, 1990.

138. Sibley, C.G., J.E. Ahlquist., and B.L. Monroe, *A classification of the living birds of the world based on DNA-DNA hybridization studies.* Auk, 1988. **105**: p. 409-423.

139. Harshman, J., et al., *Phylogenomic evidence for multiple losses of flight in ratite birds.* Proc. Natl. Acad. Sci, 2008. **105**: p. 13462-13467.

140. Hackett, S.J., et al., *A phylogenetic study of birds reveals their evolutionary history.* Science, 2008. **320**: p. 1763-1767.

141. Fain, M.G. and P. Houde, *Parallel radiations in the primary clades of birds.* Evolution, 2004. **58**(11): p. 2558-2573.

142. Gibb, G.C., et al., *Mitochondrial genomes and avian phylogeny: complex characters and resolvability without explosive radiations.* Molecular Biology Evolution, 2007. **24**: p. 269-280.

143. Slack, K.E., et al., *Resolving the root of the avian mitogenic tree by breaking up long branches.* Mol. Phylogenet. Evol., 2007. **42**: p. 1-13.

144. Brown, J.W., et al., *Strong mitochondrial DNA support for a Cretaceous origin of modern avian lineages.* BMC Biol, 2008. **6**: p. 6.

145. Morgan-Richards, M., et al., *Bird evolution: testing the metaves clade with six new mitochondrial genomes.* BMC Evol. Biol, 2008. **8**: p. 20.

146. Pratt, H.D., *A New Genus for the Hawai`i Creeper, with Comments on Generic Limits among Insectivorous Hawaiian Honeycreepers.* Elepaio, 2009. **69**: p. 47-50.

147. Butlin, R.K., J. Galindo, and J.W. Grahame, *Sympatric, parapatric or allopatric: the most important way to classify speciation?* Philosophical Transactions of the Royal Society B-Biological Sciences, 2008. **363**(1506): p. 2997-3007.

148. Turelli, M., N.H. Barton, and J.A. Coyne, *Theory and speciation.* Trends in Ecology & Evolution, 2001. **16**(7): p. 330-343.

149. Kohn, L.M., *Mechanisms of Fungal Speciation.* Annual Review of Phytopathology, 2005. **43**(1): p. 279-308.

150. Grant, P.R. and B.R. Grant, *The secondary contact phase of allopatric speciation in Darwin's finches.* Proceedings of the National Academy of Sciences of the United States of America, 2009. **106**(48): p. 20141-20148.

151. Freeland, J.R. and P.T. Boag, *Phylogenetics of Darwin's finches: Paraphyly in the tree finches, and two divergent lineages in the Warbler Finch.* Auk, 1999. **116**(3): p. 577-588.

152. Albert, A.Y.K. and D. Schluter, *Selection and the origin of species.* Current Biology, 2005. **15**(8): p. R283-R288.

153. Ritchie, M.G., *Sexual selection and speciation.* Annual Review of Ecology Evolution and Systematics, 2007. **38**: p. 79-102.

154. Vincek, V., et al., *How large was the founding population of Darwin's finches?* Proceedings of the Royal Society of London. Series B: Biological Sciences, 1997. **264**(1378): p. 111-118.

155. Gavrilets, S., H. Li, and M.D. Vose, *Patterns of parapatric speciation.* Evolution, 2000. **54**(4): p. 1126-1134.

156. Boggs, C.L., J.S. Neil, and B.B. Paul, *Species and Speciation, in International Encyclopedia of the Social & Behavioral Sciences.* Pergamon: Oxford, 2001: p. 14855-14861.

157. Mayr E, *Populations, species, and evolution: an abridgment of animal species and evolution.* 1970. **13**.

158. McOuat, G.R., *Species, rules and meaning: The politics of language and the ends of definitions in 19th century natural history.* Studies In History and Philosophy of Science Part A, 1996. **27**(4): p. 473-519.

159. González-Forero, M., *Removing ambiguity from the biological species concept*. Journal of Theoretical Biology, 2009. **256**(1): p. 76-80.

160. Dobzhansky, T., *A Critique of the Species Concept in Biology*. Philosophy of Science, 1935. **2**(3): p. 344-355.

161. Noor, M.A.F., *Is the biological species concept showing its age?* Trends in Ecology & Evolution, 2002. **17**(4): p. 153-154.

162. Baker, J.M., *Adaptive speciation: the role of natural selection in mechanisms of geographic and non-geographic speciation.* Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences, 2005. **36**(2): p. 303-326.

163. Hey, J., W.M. Fitch, and F.J. Ayala, *Systematics and the origin of species: An introduction.* Proceedings of the National Academy of Sciences of the United States of America, 2005. **102**(Suppl 1): p. 6515-6519.

164. Mayr E, *Systematics and the origin of species, from the viewpoint of a zoologist*. 1999: Harvard University Press.

165. Abbott, R.J., *Plant invasions, interspecific hybridization and the evolution of new plant taxa.* Trends in Ecology & Evolution, 1992. **7**(12): p. 401-405.

166. Kato, J. and M. Mii, *Production of Interspecific Hybrid Plants in Primula*, in *Plant Cell Culture Protocols*. 2005. p. 253-262.

167. Simpson, G.G., *The Meaning of Evolution.* New Haven: Yale University Press, 1967.

168. (1982)., E.M., *The growth of biological thought: Diversity, evolution, and inheritance: By . Cambridge, MA: Harvard University Press.* Ethology and Sociobiology, 1982. **5146**(1): p. 63-64.

169. Ernst Mayr. and J. O'Brien, *Recognizing Endangered Species and Subspecies.* SCI, 1991. **251**: p. 1187-1188.

170. Alberts B, J.A., et al., *Chapter 14: Energy Conversion: Mitochondria and Chloroplasts.* Molecular Biology of the Cell, 4th edition, 2002.

171. Pon, L.A. and E.A. Schon, *Mitochondria.* Academic Press, 2007.

172. Koehler, C.M. and M.F. Bauer, *Mitochondrial function and biogenesis.* Springer, 2004.

173. Schaffer, S.W. and M.S. Suleiman, *Mitochondria: the dynamic organelle.* Springer, 2007.

174. Gray, M.W., *Mitochondria Encyclopedia of Genetics*, S. Brenner and J.H. Miller, Editors. 2001, Academic Press: New York. p. 1215-1217.

175. Keyhani, E., *Mitochondria are generally rod-shaped bacterium-like organelles*. Journal of Cell Science, 1980. **46**: p. 289-297.

176. Chan, D.C., *Mitochondria: Dynamic Organelles in Disease Aging, and Development*. Cell, 2006. **125**(7): p. 1241-1252.

177. Tobe, S.S. and p.-. A. Linacre, *Quantification of trace amounts of human and non-human mitochondrial DNA (mtDNA) using SYBR Green and real time PCR*. Forensic Science International: Genetics Supplement Series, 2008. **1**(1): p. 71-73.

178. Zink, R.M. and D.L. Dittmann, *Evolution of brown towhees : Mitochondrial DNA evidence*. Evolution, 1991. **93**(1): p. 98-105.

179. Lehtonen, M., *Mitochondrial DNA sequence variation in patients with sensorineural heating impairment and in the Finnish population.* Biocenter Oulu, Oulun yliopisto, 2002.

180. Taanman, J.W., *The mitochondrial genome: structure, transcription, translation and replication*. Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1999. **1410**(2): p. 103-123.

181. Fernández-Silva, P., J.A. Enriquez, and J. Montoya, *Replication and transcription of mammalian mitochondrial DNA*. Experimental Physiology, 2003. **88**: p. 41-56.

182. Jansen, R.P.S. and G.J. Burton, *Mitochondrial dysfunction in reproduction*. Mitochondrial Medicine - Developing the Scientific Basis to Medical Management of Mitochondrial Disease, 2004. **4**(5-6): p. 577-600.

183. Manfredi, G., et al., *The fate of human sperm-derived mtDNA in somatic cells*. American Journal of Human Genetics, 1997. **61**(4): p. 953-960.

184. Zouros, E., et al., *An unusual type of mitochondrial DNA inheritance in the blue mussel Mytilus*. Proceedings of the National Academy of Sciences of the United States of America, 1994. **91**(16): p. 7463-7467.

185. Firth, H.V., J.A. Hurst, and J.G. Hall, *Oxford desk reference: clinical genetics*. Oxford University Press, 2005: p. 708p.

186. Butler, J.M. and B.C. Levin, *Forensic applications of mitochondrial DNA*. Trends in Biotechnology, 1998. **16**(4): p. 158-162.

187. Butler, J.M., *Chapter 10: Mitochondrial DNA Analysis, in Forensic DNA Typing: Biology, Technology and Genetics of STR Markers*. Elsevier Academic Press, London, 2005: p. 241-298.

188. Zhang, D.-X. and G.M. Hewitt, *Nuclear integrations: challenges for mitochondrial DNA markers*. Trends in Ecology & Evolution, 1996. **11**(6): p. 247-251.

189. Hsieh, H.-M., et al., *Cytochrome b gene for species identification of the conservation animals*. Forensic Science International, Genetics Supplement Series, 2001. **122**(1): p. 7-18.

190. Christidis Leslie , et al., *Forensic Identification of Aviation Bird Strikes in Australia*. Australian Transport Safety Bureau, 2006.

191. Linacre, A., *Application of mitochondrial DNA technologies in wildlife investigations - species identification*. Forensic Science Review, 2006. **18**(1): p. 1-8.

192. Jagow, G., T.A. Link, and T. Ohnishi, *Organization and function of cytochrome b and ubiquinone in the cristae membrane of beef heart mitochondria*, in *Journal of Bioenergetics and Biomembranes*. 1986, Springer New York. p. 157-179.

193. Howell, N. and K. Gilbert, *Mutational analysis of the mouse mitochondrial cytochrome b gene*. Journal of Molecular Biology, 1988. **203**(3): p. 607-617.

194. Moyo, T., S. Bangay, and G. Foster, *The identification of mammalian species through the classification of hair patterns using image pattern recognition*. Proceedings of the 4th international conference on Computer graphics, virtual reality, visualisation and interaction in Africa, ACM Press: Cape Town, South Africa, 2006: p. 177-181.

195. Gregory, W.K., *The orders of mammals. Part I: Typical stages in the history of the ordinal classification of mammals*. Bulletin of the American Museum of Natural History, 1910. **27**: p. 1-104.

196. Gregory, W.K., *The orders of mammals. Part II: Genetic relations of the mammalian orders: with a discussion of the origin of the Mammalia and of the problem of the auditory ossicles*. Bulletin of the American Museum of Natural History, 1910. **27**: p. 105-524.

197. Simpson, G.G., *A new classification of mammals*. Bulletin of the American Museum of Natural History, 1931. **59**: p. 259-293.

198. Wetton, J.H., et al., *Generation of a Species-Specific DNA Sequence Library of British Mammals*. A Study by the Forensic Science Service for The Joint Nature Conservation Committee and The Environment and Heritage Service, Northern Ireland, 2002. **37**.

199. Irwin, D., T. Kocher, and A. Wilson, *Evolution of the cytochrome b gene of mammals*. Journal of Molecular Evolution, 1991. **32**(2): p. 128-144.

200. Martiniakova, M., et al., *Differences among species in compact bone tissue microstructure of mammalian skeleton: use of a discriminant function analysis for species identification.* Journal of Forensic Sciences, 2006. **51**(6): p. 1235-1239.

201. Sbisa, E., et al., *Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications.* Gene, 1997. **205**(1-2): p. 125-140.

202. An, J., et al., *A molecular genetic approach for species identification of mammals and sex determination of birds in a forensic case of poaching from South Korea.* Forensic Science International Affairs, 2007. **167**(1): p. 59-61.

203. Karlsson, A.O. and G. Holmlund, *Identification of mammal species using species specific DNA pyrosequencing.* Forensic Science International, 2007. **173**(1): p. 16-20.

204. Mas-Coma, S. and M.D. Bargues, *Populations, hybrids and the systematic concepts of species and subspecies in Chagas disease triatomine vectors inferred from nuclear ribosomal and mitochondrial DNA.* Acta Tropica. **110**(2-3): p. 112-136.

205. Kessler LG. and J. Avise, *Systematic relationships among waterfowl (Anatidae) inferred from restriction endonuclease analysis of rnitochondrial DNA.* Systematic Zoology, 1984. **33**: p. 370-380.

206. Kessler LG. and J. Avise, *A comparative description of mitochondrial DNA differentiation in selected avian and other vertebrate genera.* Molecular Biology and Evolution, 1985. **2**(709-1): p. 25.

207. Mack AL., et al., *Mitochondrial DNA: a source of genetic markers for studies of similar passerine bird species.* Auk, 1986: p. 676-681.

208. Ovenden fR., AG Makinlay, and C. RH, *Systematics and mitochondrial genome evolution of Australian rosellas (Aves: Platycercidae).* Molecular Biology and Evolution, 1982. **4**: p. 526-543.

209. Edwards SV and W. AC, *Phyiogenetically informative length polymorphism and sequence variability in mitochondrial DNA of Australian songbirds (Pomato stopus).* Genetics, 1990. **126**: p. 695.

210. Zink RM. and J. Avise, *Patterns of mitochondrial DNA and allozyme evolution in the avian genus Ammodramus.* 1990.

211. Avise C and Z. RM, *Molecular genetic divergence between avian sibling species: king and clapper rails, long-billed and short-billed dowitchers, boat-tailed and great-tailed grackles, and tufted and black-crested titmice.* Auk, 1988. **105**: p. 516-528.

212. Shields GF and W. AC, *Subspecies of the Canada Goose (Branta amadensis) have distinct mitochondrial DNAs*. Evolution, 1987a: p. 662-666.

213. Avise C and N. WS, *Molecular genetic relationships of the extinct Dusky Seaside Sparrow*. Science, 1989. **243**: p. 646-648.

214. Shields GF., *Analysis of mitochondrial DNA of Pacific Black Brant (Branta bernicla nigricans)*. Auk, 1990. **107**.

215. Van Wagner CE. and A. Baker, *Association between mitochondrial DNA and morphological evolution in Canada Geese*. Journal of Molecular Evolution, 1990.

216. Fleischer RC., Rothstein SI, and M. LS, *Mitochondrial DNA variation indicates gene flow across a zone of known secondary contact between two subspecies of the brown-headed cowbird*. Condor, 1991. **93**: p. 185-189.

217. Dove, C.J., et al., *Using DNA Barcodes to Identify Bird Species Involved in Birdstrikes*. Journal of Wildlife Management, 2008. **72**(5): p. 1231-1236.

218. Carla J. Dove, Nor Faridah Dahlan, and M. Heacker, *Forensic bird-strike identification techniques used in an accident investigation at Wiley Post Airport, Oklahoma*. Human–Wildlife Conflicts, 2009. **3**(2): p. 179–185.

219. Avise JC. and C. Aquadro, *A comparative summary of genetic distances in the vertebrate*. Evolutionary Biology, 1982. **15**: p. 151-185.

220. Verhofstad, N., et al., *New methods for assessing male germ line mutations in humans and genetic risks in their offspring*. Mutagenesis, 2008. **23**(4): p. 241-7.

221. Anthony J, B., *The essence of SNPs*. Gene, 1999. **234**(2): p. 177-186.

222. Asthana, S., et al., *Widely distributed noncoding purifying selection in the human genome*. Proceedings of the National Academy of Sciences, 2007. **104**(30): p. 12410-12415.

223. Denver, D.R., K. Morris, and W.K. Thomas, *Phylogenetics in Caenorhabditis elegans: An Analysis of Divergence and Outcrossing*. Molecular Biology and Evolution, 2003. **20**(3): p. 393-400.

224. Linacre, A., *Genetic polymorphisms*. Forensic Science in Wildlife Investigations, 2009: p. 72.

225. Wanner, R., et al., *Stabilization of the genome of the mismatch repair deficient Mycobacterium tuberculosis by context-dependent codon choice.* BMC Genomics, 2008. **9**: p. 249.

226. Hoy, M.A., *DNA, Gene Structure, and DNA Replication.* Insect molecular genetics: an introduction to principles and applications, 2003: p. 24.

227. Lemey, P., et al., *Basic concepts of molecular evolution.* The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis Testing. Published by Cambridge University Press. Cambridge University Press, 2009(2): p. 1-10.

228. Dale, J. and M.V. Schantz, *Single Nucleotide Polymorphisms.* From genes to genomes: concepts and applications of DNA technology, 2007: p. 288.

229. Wink, M., *Which molecular variations should be detected?* An Introduction to Molecular Biotechnology: Malecular Fundamentals, Methods and Applications in Modern Biotechnology, 2006: p. 463.

230. Li, W.-H. and K.D. Makova, *Molecular Clocks.* Encyclopedia of Genetics (Academic Press), 2001.

231. Bromham, L., *Molecular clocks and explosive radiations.* Journal of Molecular Evolution, 2003. **57**: p. S13-S20.

232. Blair Hedges, S. and S. Kumar, *Genomic clocks and evolutionary timescales.* Trends in Genetics, 2003. **19**(4): p. 200-206.

233. Weir, J.T. and D. Schluter, *Calibrating the avian molecular clock.* Molecular Ecology, 2008. **17**(10): p. 2321-2328.

234. Ho, S.Y.W., *Calibrating molecular estimates of substitution rates and divergence times in birds.* Journal of Avian Biology, 2007. **38**(4): p. 409-414.

235. Aquadro, C.F. and B.D. Greenberg, *Human mitochondrial DNA variation and evolution: analysis of nucleotide sequences from seven individuals.* Genetics, 1983. **103**(2): p. 287-312.

236. Upholt, W.B. and I.B. Dawid, *Mapping of mitochondrial DNA of individual sheep and goats: Rapid evolution in the D loop region.* Cell, 1977. **11**(3): p. 571-583.

237. Eo, S.H. and J.A. DeWoody, *Evolutionary rates of mitochondrial genomes correspond to diversification rates and to contemporary species richness in birds and reptiles.* Proceedings of the Royal Society B-Biological Sciences, 2010. **277**(1700): p. 3587-3592.

238. Wenink, P.W., A.J. Baker, and M.G. Tilanus, *Mitochondrial control-region sequences in two shorebird species, the turnstone and the dunlin, and their utility in population genetic studies.* Molecular Biology and Evolution, 1994. **11**(1): p. 22-31.

239. Gu, Z., et al., *Elevated evolutionary rates in the laboratory strain of Saccharomyces cerevisiae.* Proceedings of the National Academy of Sciences of the United States of America, 2005. **102**(4): p. 1092-1097.

240. Li, W.L.S. and A.G. Rodrigo, *Covariation of Branch Lengths in Phylogenies of Functionally Related Genes.* PLoS ONE, 2009. **4**(12): p. e8487.

241. Perry, J. and A. Ashworth, *Evolutionary rate of a gene affected by chromosomal position.* Current Biology, 1999. **9**(17): p. 987-S3.

242. Hebert, P.D.N., et al., *Biological identifications through DNA barcodes.* Proceedings of the Royal Society of London. Series B: Biological Sciences, 2003. **270**(1512): p. 313-321.

243. Hebert, P.D.N., et al., *Identification of Birds through DNA Barcodes.* PLoS Biol, 2004. **2**(10): p. e312.

244. Dawnay, N., et al., *Validation of the barcoding gene COI for use in forensic genetic species identification.* Forensic Science International, Genetics Supplement Series, 2007. **173**(1): p. 1-6.

245. Savolainen, P. and J. Lundeberg, *Forensic evidence based on mtDNA from dog and wolf hairs.* Journal of Forensic Sciences, 1999. **44**(1): p. 77-81.

246. Tavares, E. and A. Baker, *Single mitochondrial gene barcodes reliably identify sister-species in diverse clades of birds.* Bmc Evolutionary Biology, 2008. **8**(1): p. 81.

247. Zhou, H., et al., *Molecular phylogeny of Nearctic species of Rhynchelmis (Annelida).* Zoologica Scripta, 2010. **39**(4): p. 378-393.

248. Meusnier, I., et al., *A universal DNA mini-barcode for biodiversity analysis.* BMC Genomics, 2008. **9**(1): p. 214.

249. Hajibabaei, M., et al., *A minimalist barcode can identify a specimen whose DNA is degraded.* Molecular Ecology Notes, 2006. **6**: p. 959 - 964.

250. Rasmussen, R.S., M.T. Morrissey, and P.D.N. Hebert, *DNA Barcoding of Commercially Important Salmon and Trout Species (Oncorhynchus and Salmo) from North America.* Journal of Agricultural and Food Chemistry, 2009. **57**(18): p. 8379-8385.

251. An, J., et al., *A molecular genetic approach for species identification of mammals and sex determination of birds in a forensic case of poaching from South Korea.* Forensic Science International, 2007. **167**(1): p. 59-61.

252. Lee, J.C.I., et al., *A novel strategy for avian species identification by cytochrome b gene.* Electrophoresis, 2008. **29**(11): p. 2413-2418.

253. Linacre, A. and S. Tobe, *An overview to the investigative approach to species testing in wildlife forensic science.* Investigative Genetics, 2011. **2**(1): p. 2.

254. Smith, M.A., N.A. Poyarkov, and P.D.N. Hebert, *DNA Barcoding: CO1 DNA barcoding amphibians: take the chance, meet the challenge.* Molecular Ecology Resources, 2008. **8**(2): p. 235-246.

255. Holmes, B.H., D. Steinke, and R.D. Ward, *Identification of shark and ray fins using DNA barcoding.* Fisheries Research, 2009. **95**(2-3): p. 280-288.

256. Park, H.Y., et al., *New DNA barcodes for identification of Korean birds.* Genes & Genomics, 2011. **33**(2): p. 91-95.

257. Lohman, D., D. Prawiradilaga, and R. Meier, *Improved COI barcoding primers for Southeast Asian perching birds (Aves: Passeriformes).* Molec Ecol Res, 2009. **9**(1): p. 37-40.

258. Wong, E.H.K., M.S. Shivji, and R.H. Hanner, *Identifying sharks with DNA barcodes: assessing the utility of a nucleotide diagnostic approach.* Molecular Ecology Resources, 2009. **9**: p. 243-256.

259. Huang, J., et al., *Identifying earthworms through DNA barcodes.* Pedobiologia, 2007. **51**(4): p. 301-309.

260. Chang, C.-H., R. Rougerie, and J.-H. Chen, *Identifying earthworms through DNA barcodes: Pitfalls and promise.* Pedobiologia, 2009. **52**(3): p. 171-180.

261. Hebert, P.D.N., et al., *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**: p. S96-S99.

262. Wilson-Wilde, L., et al., *Current issues in species identification for forensic science and the validity of using the cytochrome oxidase I (COI) gene.* Forensic Science Medicine and Pathology, 2010. **6**(3): p. 233-241.

263. Vialle, A., et al., *Evaluation of mitochondrial genes as DNA barcode for Basidiomycota.* Molecular Ecology Resources, 2009. **9**(s1): p. 99-113.

264. Brown, W., M. George, and A. Wilson, *Rapid evolution of animal mitochondrial-DNA*. Proc Natl Acad Sci USA, 1979. **76**(4): p. 1967 - 1971.

265. Hall, B., *Phylogenetic Trees Made Easy: A How-to Manual*. Third Edition: Sinauer Associates, Inc, 2007.

266. Tamura, K., et al., *MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0*. Molecular Biology and Evolution, 2007. **24**(8): p. 1596-1599.

267. Jukes, T.H. and C.R. Cantor, *Evolution of Protein Molecules*. Evolution of Protein Molecules, ed. H.N. Munro, Academy Press, 1969.

268. Tajima, F. and M. Nei, *Estimation of evolutionary distance between nucleotide sequences*. Molecular Biology and Evolution, 1984. **1**(3): p. 269-285.

269. Tamura, K. and M. Nei, *Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees*. Molecular Biology and Evolution, 1993. **10**(3): p. 512-526.

270. Kimura, M., *A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences*. Journal of Molecular Evolution, 1980. **16**(2): p. 111 - 120.

271. Tamura, K., *Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases*. Molecular Biology and Evolution, 1992. **9**(4): p. 678-687.

272. Hasegawa, M., H. Kishino, and T.-a. Yano, *Dating of the human-ape splitting by a molecular clock of mitochondrial DNA*. Journal of Molecular Evolution, 1985. **22**(2): p. 160-174.

273. Lockhart, P.J., et al., *Recovering eolutionary trees under a more realistic model of sequence evolution*. Molecular Biology and Evolution, 1994. **11**(4): p. 605-612.

274. Tavaré, S., *Some Probabilistic and Statistical Problems in the Analysis of DNA Sequences*. American Mathematical Society: Lectures on Mathematics in the Life Sciences, 1986. **17**: p. 57-86.