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The Role of Wild Life DNA Forensics in Identification of Endangered Species

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Abstract: *Wildlife Forensic science is science that is applied to legal questions involving wildlife crimes. Wildlife forensics contains the molecular biology includes identification by DNA profiling and sequencing. Illegal hunting of wildlife is a serious worldwide concern for wildlife controlling. The ornamental body part and other traditional uses of the body part of wildlife are the major challenge in control of wildlife poaching. There are a developing range of modern DNA approaches that can be used in wildlife crime investigations. DNA profiling is one of the most effective protocols so far in dealing wildlife crime cases, which is the basis for the DNA wildlife forensics. The DNA profiling composed of some basic steps including the DNA extraction, PCR amplification, DNA sequencing and sequence comparison. The genes located on mitochondrial DNA (mtDNA) are the source of most effective molecular marker used in wildlife forensics. Current effort describes the use for DNA profiling for the identification of different wild life species. It includes the types of different techniques and methodology for extraction of DNA from wild animals. Once DNA is recovered from the sample, a number of analytical methods may be applied to determine: - Species of origin, Gender, Single species or multiple species content in mixed sample, Individual identity, Parts matching. The limiting factor in wildlife forensics is lack of strong data base for wildlife. There is a serious need to build the data base for the all range of the wild species. This contains the need of the research and development for strengthening gene bank of the endangered species.*

Key-Words: *- Wildlife Forensic, DNA, DNA profiling, crime etc.*

INTRODUCTION

Like human forensic, wildlife forensic has the same task, to relate suspect, victim and crime scene in concert in a triangular manner with the physical evidence recovered from the crime **[1].** Illegal hunting of wildlife is a serious worldwide concern for wildlife management. The ornamental body part and other traditional uses of the body part of wildlife are the major challenge in control of wildlife poaching. Moreover, the effective identification of the species from available biological material is a key parameter in fixing the accurate wildlife offence. Hence, wildlife forensics is a vital branch of forensics, which deals with the identification of the species from biological remnant. This science has evolved very rapidly and has enormous scope in biodiversity conservation. When intact morphological feature are available in the seizer, the examination of the morphological character of the sized biological material is one of the rapid mode of the delivering the report **[2-8].**A forensic crime investigation, set off with the basic steps, recognition, collection and preservation of biological evidences, is most important, but unfortunately it's getting less concern during handling and this is the keyhole of an investigation failure. Proper handling of evidences provides important investigation lead to forensic scientist to disprove an alibi or a connection of a suspect or victim to a crime scene **[9, 10].** Wildlife forensic science is a wide-ranging discipline compared to human identification and takes many guises depending on the nature of the allegation. A key difference is that in alleged crimes against wildlife there can often be no 'victim' to provide information regarding the investigation. Additionally, the list of species encountered in wildlife forensic science is extensive in contrast to the single species analysed in human identification. Wildlife forensic science is no longer the realm of the 'wellmeaning conservation geneticist'. Despite being a relatively new sub-discipline of forensic science, analyses of evidence handled in wildlife crime cases may be presented in court in just the same way as more traditional forensic evidence; it therefore should be treated as such. From a quality control and quality assurance perspective, a complicating factor is that most accredited forensic laboratories and associated scientists do not handle non-human samples. This is due to the particular complexities of wildlife crime analysis techniques

requiring a completely different expertise and skill set to those possessed by scientists in traditional forensic laboratories **[11]**.

Wildlife DNA Forensics

The field of conservation genetics has developed over the past 20 years to support the application of molecular genetic analysis to problems and questions encountered in species conservation. Research is primarily undertaken by academic scientists and has led to new approaches for the collection, analysis and interpretation of biological samples in addition to generating information relevant to the management of target populations. The breadth of possible applications requires conservation geneticists to draw on a wide range of established biological subjects including population genetics, molecular ecology, molecular phylogenetics, phylogeography and taxonomy. Their principal objective is to synthesize this knowledge and develop best practice solutions to specific challenges faced in conservation **[12].** Technological advancement in human forensic provides a backbone for wildlife investigation, but their progress rate of advancements has been more gradual because lack of proper attention for many years makes it an isolated field**[13].** Rather wildlife crime investigation is often lots more complicated as compared to others investigative science. There are a number of reasons like circumstances under which animal can be killed (legally or illegally), lack of proper species specific definition and lots more complication hindered for the fight against wildlife crime**[14].**

DNA source

From 90's, DNA typing after getting good recognition in biological world contributes and constitutes a major advancement to forensic examinations. DNA analysis getting popularity, especially in the field of forensic science because of its remarkable sensitivity and power of discrimination. It is to be noted and considered in practice that the crime scene samples may contain less amount of DNA**[15].**

The quality and quantity of DNA that can be isolated from commonly encountered biological sources frequently varies and depending upon different environmental conditions**[16].** Tissue, blood and semen are the best source to obtain a DNA profile, whereas other biological fluid like gastric fluid, fecal matter, vomit, bone and hair, etc. have less percentage to isolate DNA and comparatively difficult to generate a genetic profile **[17].**

Many different sources of biological evidences related to wildlife crime are reported and submitted to wildlife forensic laboratories for examination. Most common biological sources are reported for DNA recoveries are as follows in **table-1.**

Evidence sources	Possible location	DNA source	References
Weapon\ knife, tools, sticks	Handle\body	Blood\tissue	18,19
Clothing\Carpet	Surface	Hair\saliva\semen\ sweat\urine etc.	20
Traditional East Asian medicines	Medicines (tiger bone juice or rhino horn pills etc.)	Tissue	21-23
Artifacts or handicraft (Made by Hair or feathers)	Museum shops\costume	Feather\hair\shell etc.	24-27
Bite-marks	Skin\clothing	blood\saliva	28-30
Ivory idol\artifacts	Seals\hankos\chops	Bone fragments	31-33
Leather goods	Shops\museum\handbag\purses \shoes\ boots	Tissue	34-36
Horn\bones	Medicinal Products\Meusiums	Tissue	37,38
Wool\fibers\fur\ Shahtoosh shawls)	Ready-made Shawls\Carpets	Hair	39,40
Claws left on tanned hides	Museum\ illegal smuggling	Pulp of Claws\ Tissue\Hair	41,42

DNA Typing

DNA typing is one of the most effective protocols so far in dealing wildlife crime cases, which is the basis for the DNA wildlife forensics. The DNA typing composed of some basic steps including the DNA extraction, PCR amplification, DNA sequencing and sequence comparison. The genes located on mitochondrial DNA (mtDNA) are the source of most effective molecular marker used in wildlife forensics. The mtDNA inherited from mother to progeny and do not participate in crossing over. Hence, it is one of the pure forms of

the DNA and has various conserve regions on its gene, which are the basis for selection of conserved of universal primers **[43-45].** The use of universal primers minimise the effort of researcher, since it can be applied for PCR amplification to the DNA of all species without prior information of the victim species. The DNA sequence generated from the victim species can be compared with suitable data base to get the accurate identity of the source. When the specific gender is preferred in poaching as in cases of Asian elephant, *Elephasmaximus*, the gender specific markers are useful in identification of the gender of the decomposed or altered carcass in determining the cause of the death of individual **[46].**

Success in DNA Typing

The DNA typing has successfully applied in dealing the wildlife crime cases. It was helpful in identification of the trace biological material collected from wooden chopping block. In this case accused tried to mislead the cope by providing the samples of domestic chicken (*Gallus gallus*), however; the crime was established by comparison of the DNA sequences generated from the biological remnant collected from chopping wood **[47].** The victim species was identified as the Schedule-I protected animals the Pea fowl (*Pavocristatus*). In another case of wildlife crime, two idols were analysed for identification of the source of the origin. The identification was confirmed by species specific markers developed for elephant. The comparison of DNA sequence confirmed that idols were derived from Asian elephant. Hence, species specific markers are also has its significance in wildlife forensics **[48].**

Application of Individual Matching in Wildlife Forensics

Individual profiling and matching is one of the worldwide common protocols in forensics. Individual profiling is done based on widely deployed microsatellite markers, where a panel of polymorphic markers are used in generating the individual's profile. The individual profile can be matched with the biological relatives. It has enormous potential in wildlife forensics too. When a tiger (*Pantheratigris*) was poached from Zoo Park the same has been proven an asset in forensic investigation. The tiger was poached in the year 2000 and the remnant (claw) was found with accused in year 2005. There was no biological sample was made available from the deceased tiger. The genetic profile was generated from the claw along with the known biological relativesof the deceased tiger. The genetic matching confirmed that the clawbelongs to the missing individual of the alleged family [49].In another case of suspected wildfire crime, the individual matching has helped to wildlife manager in taking the proper decision. An elephant tusk was found in a reserve forest, which was collected [50].

DISCUSSION

DNA typing of non-human DNA is a fast developing area of research and professional practice. The application of DNA typing in wildlife forensic science is one of these prime uses of DNA typing and is gaining increasing profile. The use of DNA profiling in wildlife forensic science falls into following areas:

Species Identification
Gender Identification
Individual Identification
Population Identification
Parentage Analysis
Expert Testimony and Consultation

DNA is used to identify and confirm the species of origin of items of evidence suspected of being involved in illegal commercialization or illegal trade. Species identification is performed using DNA markers, which are conserved within a species but variable between species. By comparing the sequences obtained at these markers with those of known controls we can determine, with up to 99 percent confidence, the species of origin. We can also resolve individual species present in mixed meat products such has sausages to determine if specific species are being illegally processed. By examining X and Y chromosome specific DNA from evidence lacking gender-specific morphological characteristics then we can establish an animal's gender. This process is routinely used to determine the gender of an animal using materials such as blood, hair or muscle as well as several other types of biological materials. Short tandem repeat (STR) analysis is carried out for purposes of individual identification, assignment of population of origin and parentage determination. STRs are stretches of short repeated DNA sequences that are highly polymorphic. These differences can be detected and thus used to establish the individual identity of an animal.

DNA from evidence items is interrogated using a species specific array of STRs. If two evidence items exhibit the same pattern of STRs, then the probability of the samples originating from the same individual is calculated based on the most frequent alleles present in a DNA database from the species of interest. Population identification has proven important in the prosecution of cases where an animal is being claimed as originating from one geographic region but is suspected to have been taken from another, where hunting may not have been permitted. Parentage analysis is performed to determine if captive bred animals are the legal

offspring of registered breeding pairs or if animals have been illegally obtained from wild stocks. Other cases that have been handled by the lab requested that we determine confirm a sibling relationship between a number of animals thought to be from the same litter.

Conclusion

The use of DNA forensics to support conservation began over 20 year ago and has gradually developed alongside human forensic genetic techniques, conservation genetic applications and strengthening wildlife legislation. The current popularity of the field reflects the increasing availability of DNA analysis, but also highlights the alarming extent to which illegal activity is threatening endangered species. Forensic genetic methods are now used to address questions relating to the identification of species, populations, geographic origin, family relatedness and individual identity, offering a large number of possible investigative tools to enforcement officers worldwide. Wildlife DNA forensics is heavily dependent on conservation genetic research for the development of novel techniques and production of reference datasets; however, it is vital that a distinction is made between applied conservation genetics and any extension to forensic genetic investigation. The credibility of any forensic application relies on being able to demonstrate evidential security and the validity of the laboratory technique, data analysis and interpretation of results. These issues must be addressed during the transfer from research approaches to forensic tools. The increasing availability of genomic data is set to rapidly expand our potential to develop and apply wildlife DNA forensic techniques. However, in order to ensure that future research is successfully converted into practical applications for law enforcement, wildlife forensic practitioners must aim to meet the same rigorous quality standards achieved in human DNA forensics. With this in mind, the following recommendations are made for conducting wildlife DNA forensic science. To successfully apply wildlife DNA forensic techniques it is recommended that:

(1) Scientists consider the need to act impartially,

(2) Genetic identification methods are validated prior to forensic use

(3) Genetic analysis is performed in a quality assured environment that controls sample integrity, test performance, data interpretation and evidence presentation.

To further develop the field of wildlife DNA forensics we call for:

(1) Greater coordination and exchange of validated population data, reference samples and protocols

(2) Establishment of a network of accredited wildlife DNA forensic laboratories

(3) Directed research to address specific conservation law enforcement needs.

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