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Popular Article

DNA fingerprinting and wildlife forensics

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Abstract

Illegal wildlife trading has been identified as a global threat to society's security and the sustainability of biodiversity. It welcomed the tight laws governing wildlife protection. In the last 20 years, forensic science has advanced quickly, thanks to successfully developing branch of "wildlife forensics". Enforcement of legislation pertaining to wildlife conservation is increasingly aided by investigations into wildlife crimes. The latter of two approaches—morphological study and molecular analysis—was shown to be the most successful approach for addressing such problems. The primers created for conserved regions on the genes of the mitochondrial genome have a wide range of applications in dealing with DNA forensics for animals.

Keywords: DNA fingerprinting, mitochondrial genome, primers, wildlife

Introduction

The stealing, trading, exploiting, or possession of the world's wild flora and fauna in violation of national and international regulations is referred to as wildlife crime. These crimes are committed all around the world and present a significant difficulty for law enforcement. Transporting wildlife as part of illicit international trade may have a serious impact on other factors, such as the spread of illness and the introduction of invasive species. The livelihoods and resources of the populace are also impacted by the illicit trade in plant and animal products. Also, it poses a threat to the dwindling numbers of endangered species. The delicate balance of nature in ecosystems may be disturbed by selective harvesting of wild species.

Falsifying wildlife items for financial benefit is a criminal activity that is outlawed by law, along with the unlawful trading of endangered species, wildlife poaching, and cruelty to wild and domesticated animals. Common examples of wildlife crime include the poisoning and persecution of animals, the trading in endangered species' products, like tiger skins for Oriental or Chinese medicine, and the poaching of protected species, like rhinos for their horns. So, a key driver of illicit hunting, a serious problem for wildlife management, is desire for the many parts and products produced from wildlife. When animal products are marketed on common trade platforms or through online marketing, it becomes more difficult. The biggest obstacles to combating wildlife crime are ornamental, superstitious, and other traditional uses of wildlife body parts. The most efficient way to correct the precise wildlife infraction is to identify the species from the available biological material. Wildlife forensics, which deals with identifying the species from available biological traces, has developed into a crucial subfield of forensic research. In the last ten years, wildlife forensics has expanded quickly and offers a huge potential for biodiversity conservation. When the morphological feature is present in the material that was taken, the analysis of the morphological traits of the biological material that was taken is the fastest way to offer the results. In order to verify the source of the origin of unidentified case samples, morphology compares the morphological characteristics of hair and the morphological characteristics of bones to recognised reference specimens. As a result, morphological characterization is somewhat constrained when analysing an unknown sample without access to reference material that is still intact from the source origin (Gupta 2018).

Hair morphology

Primary guard hairs, secondary guard hairs, and fur hairs, often known as underfur and overhair, are the different types of hairs. Of all the hair types, guard hairs are the thickest, straightest, and biggest. Shield hairs are another name for it, and there are two categories: primary and secondary. The basal region is thinner and more flexible than the medial and distal sections in both primary and secondary guard hairs. The primary guard hairs are a tiny bit longer than the secondary guard hairs. The species-specific characteristic is present in the bigger or principal guard hairs, which are particularly helpful in identifying a species. Guard hairs are superior to under hairs in length, softness, and quality. Their lengthwise thickness is nearly constant. They are not chosen for identifying species since they have limited diagnostic value. For investigation, they required high-resolution microscopy, such as SEM. Hair on one side is longer than the other. They primarily have a circular cross section and aren't used to identify species. Vibrissae are mostly sensory, stiff, and big. They may also be referred to as sinus hairs, tactile hairs, or whiskers. They are the widest at the base and gradually narrow as they approach the tip. Certain mammals, including domestic and wild pigs, have bristle hairs. These hard, thick, and uniformly sized hairs have bristles along their whole length. They can be recognized by the presence of bifurcated or trifurcated tips and by the presence of a thin medulla.

Hair characteristics for species identification

The biological sciences have made extensive use of morphological characteristics such the microscopic hair character for dietary preferences, prey-predator relationships, and the identification of mammals living in dens or trees. Also, it has been applied to cases involving wildlife offences involving furry hides, Tibetan antelope-wool shawls, and paintbrushes manufactured from mongoose and cat hair. Since more than a century ago, many people have utilized hair to identify species. The hairs of Indian animal species have also been the subject of numerous research. As a result, it became clear that we needed a quick and reliable way to identify species from hair. A method for identifying species on the basis of a single characteristic of a hair could not be created due to the variation of the cuticular pattern over the length of a hair and among hairs from different body sections. As a result, a comprehensive strategy that incorporates all the traits as well as the external characteristics was established for species identification. There is no fast cut to reaching a conclusion while writing an inquiry report; microscopic analysis must be used as the foundation. When a veterinary expert from a veterinary medical college determined the species based on the readily apparent physical trait, it caused identification issues in one instance. DNA typing was used to find the solution.

Infrastructure setup

It is possible to examine hair under a microscope to determine the species. A decent compound microscope with a 100X to 400X magnification range is the main piece of equipment needed. The microscope can also be used to examine underfur if 1000X magnification is added to it. A digital/CCD camera and a computer with a color printer must be mounted on the microscope. It is essential that the computer have a decent graphics card capacity because hair comparison requires comparing a lot of photographs. To prepare a hair slide, you also need a microscope, microscopic slides, cover slips, tweezers, glassware (Petri dishes, beakers, glass rods), gelatin, and methylene blue.

DNA wildlife forensics

DNA forensics, also known as molecular forensics or DNA wildlife forensics, is a critical component of wildlife crime investigation. DNA extraction, PCR amplification, DNA sequencing, and sequence verification are only a few of the fundamental procedures that make up DNA typing. The various mitochondrial DNA (mtDNA) regions form the basis of effective molecular markers for phylogenetics and wildlife forensics. With only 37 genes and only roughly 16,600 base pairs, the mtDNA can be regarded as the smallest chromosome. It comes from the mother in the vast majority of species. It serves as a cornerstone of phylogenetics, a method used by biologists to determine the evolutionary history of different species. The selection of conserved primers was based on the fact that, unlike nuclear DNA, mtDNA is passed down from mother to child and is not involved in the phenomenon of DNA crossing over, making it one of the purest forms of DNA. Additionally, conserved primers can be used in PCR amplification from the template DNA of the vast majority of species without knowing the identity of the victim animal. Hence, using universal primers reduces the researcher's work. 12S rDNA, 16S rDNA, cytochrome b, and control region (CR) are the most extensively used mtDNA markers in descending order of similarity; as a result, the 12S rRNA gene is largely conserved and the CR is very varied. To determine the precise, identify of the source species, the DNA sequence derived from the case property can be compared with the appropriate database. In cases involving Asian elephants, *Elephas maximus*, the gender-specific markers are also helpful in determining the sex of the decomposing carcass in order to determine the individual's cause of death. If the primary suspect species is likely to be known, species-specific primers may occasionally be employed for the confirmation of the species.

Application of DNA Typing in Wildlife Forensics

The routine use of DNA typing in wildlife crime investigation has been successful. It turns out to be an incredibly sensitive process because there are several mitochondria in a single cell, making it simple to amplify from little amounts of biological samples that have degraded. This procedure helped to clearly identify the undetectable biological samples that were taken from the wooden cutting board. Also, the suspect made an effort to deceive the investigative team by deliberately introducing samples of legal domestic chicken (*Gallus gallus*) into the crime scene.

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Analysis of the DNA sequences acquired from the minute biological byproduct of wood-chopping could be used to prove who committed the crime. A peafowl (*Pavo cristatus*), which is listed as a Schedule-I protected species in the Wildlife (Protection) Act, 1973 of India, was the biological remnant, according to DNA testing.

The Himalayan black bear (*Selenarctos thibetanus*) and American Beaver's processed and dried internal organs have also been utilized to identify the species (*Castor canadensis*). Throughout the market, a number of phoney wild items, like tiger claws and skin, were also traded. By employing DNA typing, such bogus objects can be easily distinguished from the genuine ones. DNA analysis is used to identify the species of deer from the antlers. However, in the current situation, it has been difficult to identify the species from tanned skin products, including snake skin, which can be assessed by morphological traits such cuticular features, etc.

Application of Wildlife DNA Fingerprinting in Dealing Wildlife Offense

Microsatellite markers are non-coding repetitive DNA segments also referred to as short tandem repeats (STR) or simple sequence repeats (SSR). It is a brief motif that repeats 2–6 nucleotides in the eukaryotic and prokaryotic genomes. In kinship, demographic, and other research including marker-assisted selection and fingerprinting, it is a codominant marker. Microsatellites experience a higher rate of mutation than other neutral areas of the genome, which is the primary cause of their variability. During the recombination process at the meiotic stage, mutation is also absorbed into the STR region. Because of repeating sequences, the distribution of genomic microsatellites is linked to recombination sites. The use of microsatellite analysis for forensic research gains popularity around the middle of the 1990s. It is frequently used for genetic profiling, also known as DNA fingerprinting of people. Shorter repeats commonly experience selective allele amplification, PCR artefacts, and PCR stutter. Longer repeats, on the other hand, experience degradation and have trouble amplifying during PCR. Tri- to pentanucleotide (3-5 nucleotide) repeats are the most appropriate microsatellites for forensic examination because they provide error-free data and can withstand deterioration (Gupta 2018).

A person's or a deceased person's DNA fingerprint can be compared to that of their biological relations. This means that it has enormous potential for dealing with animal forensics. It has been used for human assassination since it was first introduced to forensic science. It was subsequently developed for the investigation into wildlife crimes.

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Scope

Dealing with wildlife forensics is severely constrained by the absence of a reliable wildlife database. By research and development to strengthen DNA databases for endangered species, it can be supplemented by the creation of a trustworthy database for the entire spectrum of wild species. For such research and development, known biological samples from a known carcass of wildlife from the reserve forest must be used; as a result, forest officials, including the patrolling party, must make an attempt to regularly collect the known biological samples. This section has been broken into two parts, namely, what not to do and what needs to be done, due to the frequent errors made by enforcement authorities.

What not to do

The most crucial steps in forensic investigation are biological sample collection, preservation, and transmission. Enforcement agencies unknowingly make certain common mistakes as a result of improper sample collecting and storage expertise. These agencies make contact with a local veterinary officer for the preservation and packing of detained biological samples because the sample that was seized is probably from an animal species. It has frequently been noted that veterinary professionals use formaldehyde/formalin to preserve such biological material. Since it has been determined that samples maintained in formalin or formaldehyde cannot be used to extract DNA with a 400–500 bp PCR amplifiable quality, formalin preservation of samples should be avoided. Several DNA forensic labs won't take any biological samples that have been formaldehyde-preserved or exposed to it.

What needs to be done

When a crime is discovered, it would be challenging to gather all the necessary materials for the sample collection. Hence, a sample collection kit can be kept on hand for handling crime scenes and collecting biological evidence in order to prevent last-minute mistakes.

Conclusion

Illegal wildlife trading has been identified as a global threat to society's security and the sustainability of biodiversity. It invited stringent rules protecting wildlife. Wildlife forensics, an emerging discipline of forensic science, has flourished over the past two decades. Enforcement of legislation pertaining to wildlife conservation is increasingly aided by investigations into wildlife crimes. The latter of the two approaches—morphological study and molecular analysis—has been shown to be the most successful approach for addressing these problems. In the context of dealing with wildlife DNA forensics, conserved primers play a key role. These primers were created using a variety of conserved areas on mitochondrial genome genes. As a result, using morphological analysis, DNA typing, and genetic profiling is now necessary to cope with the rising number of animal offenders.

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