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Poaching Forensics: Animal Victims in the Courtroom

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Abstract

Poaching and the international trade in wildlife are escalating problems driven by poverty and greed and coordinated by increasingly sophisticated criminal networks. Biodiversity loss, caused by habitat change, is exacerbated by poaching, and species globally are facing extinction. Forensic evidence underpins human and animal criminal investigations and is critical in criminal prosecution and conviction. The application of forensic tools, particularly forensic genetics, to animal case work continues to advance, providing the systems to confront the challenges of wildlife investigations. This article discusses some of these tools, their development, and implementations, as well as recent advances. Examples of cases are provided in which forensic evidence played a key role in obtaining convictions, thus laying the foundation for the future application of techniques to disrupt the criminal networks and safeguard biodiversity through species protection.

1. INTRODUCTION

1.1. Poaching and the International Wildlife Trade

Poaching and the trade in animal parts and derivatives, with the cruelty and callousness that often accompany these activities, are crimes not only against animals but against the moral foundation of humanity. Poaching, or the illegal killing or theft of wildlife, is defined as the commissioning of a criminal act in contravention of local wildlife legislation (**https://www.traffic.org/**). Poaching has been a problem since the colonial era (1) and, although considered a means of subsistence in some cases, has become a sophisticated criminal activity linked to organized crime (2) and the funding of terrorist groups and civil conflicts (3). The international wildlife trade (IWT) is a lucrative, yet relatively low-risk, transnational criminal activity. The rate of detection of trafficked animal products remains low compared to the numbers of animals being poached, and the sentences of perpetrators along the value chain, if convicted, have been relatively light in many jurisdictions (4). To tackle the problem, IWT must be recognized as a serious threat, not only to biodiversity but also to the economies of source countries, the moral integrity of society, and citizen safety.

1.2. The Extent and Escalation of the Problem

IWT is estimated to be worth between $7 billion and $23 billion annually (5). The international movement of wildlife products follows diverse and often unpredictable routes, usually starting in the source country with illegal harvesting of products, traversing one or several transit countries, and ending in the consumer countries. IWT includes a vast number of species and commodities, including live animals for the pet trade; exotic meat for consumption; and parts for inclusion in traditional remedies and to produce trinkets, ornaments, and fashion accessories. Various criminal players participate in the IWT value chain at different levels, with the poacher at the bottom of the chain, agents in the middle that transfer the product to traffickers and distributors, and users at the top. Disrupting this chain requires targeting each link, but removing the kingpins at the top is essential to achieve longer-term impact (4, 6, 7).

Charismatic species are being lost at an alarming rate. Only approximately 2,000–3,000 tigers remain in the wild, and African lion numbers have declined by 43% between 1993 and 2014, with only approximately 23,000–39,000 individuals left in the wild (8). The estimated number of white rhinoceros was 21,316 in 2012 and decreased to 18,064 in 2017, indicating an overall declining population trend (9). Rhinoceros poaching increased to a maximum of 1,215 rhinoceros poached in South Africa in 2014 but dropped to below 400 in 2020, purportedly due to COVID-19 restrictions impacting the movement of criminals and criminal goods during widespread lockdowns in the country (10). Following the lifting of restrictions, poaching again increased to approximately 451 animals in 2021 and has continued to increase, with 75 animals being poached in 93 days from the historic founder populations in the province of KwaZulu-Natal (11). White and black rhinoceros numbers have decreased in the country's iconic Kruger National Park to below 3,000 and 300 animals, respectively, a 67% decrease in the white rhino population since 2011, due mainly to poaching and exacerbated by severe recent droughts (12). Poaching for their ivory and habitat loss have resulted in a decline in the population of African forest elephants (*Loxodonta cyclotis*) of more than 80% across their range in the past 93 years. This species is now listed separately on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species as Critically Endangered, and African savanna elephants (*Loxodonta africana*) are listed as Endangered (13).

The World Wildlife Fund for Nature Living Planet Index showed a 68% average decline in species of monitored mammals, birds, fish, reptiles, and amphibians between 1970 and 2016. Since

the industrial revolution, human activity has driven biodiversity loss through land usage change; industrial and agricultural activity; climate change; loss of habitat due to human encroachment; and overexploitation of natural resources, including animal species (14, 15).

The UN Office on Drugs and Crime World Wildlife Seizure database (World WISE) features data from 180,000 seizures from 149 countries and including more than 6,000 species, drawn mainly from the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) illegal trade reports, emphasizing the extent of IWT and the enormous diversity of species involved (16).

1.3. Drivers of Poaching

Corruption and the lack of political will to accept IWT as a serious threat are two of the main enablers of poaching in source countries. Corruption is driven in turn by greed, and the inadequate compensation for government and law-enforcement officials may exacerbate the problem (17). A report by TRAFFIC details cases involving wildlife that include indicators of potential corruption, highlighting the extent and perverse nature of the problem (18). Poverty and the displacement of communities by protected areas exacerbate these problems (19). Disenfranchised communities struggling with marauding wildlife, put in a situation in which others reap all the benefits from land and wild animal ownership, will understandably lead to conflict, and without solutions that include community engagement and benefit sharing, poaching will be impossible to control (20). Lunstrum & Givá (21) state that poaching is fundamentally a problem of global economic inequality. Affluence at one end of the IWT hierarchy provides the significant rewards that drive poachers, especially from poor local communities, through a criminal chain that connects both ends of the economic spectrum; therefore, addressing both drivers, poverty and wealth, requires a complex integrative approach. Inadequate law enforcement, including a scarcity of skilled scientific support services, further compounds the problem (6, 22).

The possession, sharing, and consumption of exotic, high-value items, particularly animal species, provide a sense of respectability or deference for the person consuming or sharing the product in many Asian countries, particularly China (23). Considering that the consumption of rare animals is linked to identity and status in many Asian cultures and that the user demographics include young, affluent professionals (24), a change in consumer behavior will be difficult to achieve, very slow at least, and that demand will outstrip supply in many species, especially in sensitive species like the pangolin (25).

IWT includes skins and pelts sourced for the fashion industry, bushmeat, the exotic pet industry, and traditional Chinese medicine (TCM). TCM, which has evolved over thousands of years, is another significant driver of poaching and includes herbal treatments that often contain ingredients from various animal species, wine made with animal bones, powdered horn and scales, and bile from bears that are used to treat ailments ranging from fever, infections, general malaise, reproductive disorders, and various other problems (26).

Ivory, like other rare and exotic animal derivatives such as rhino horn, gives owners a sense of status and privilege and is valued as gifts and bribes. Ivory carving has historically been prized as a fine art form. In China, a booming economy created a market for fine art collection, including ivory art (27). As species decline due to overexploitation to supply IWT and their products become rarer, the value increases, accelerating the drive toward extinction (28).

To supply animal products sought after as status symbols, exotic foods, and TCM, the controlled breeding and harvesting of wild animals has been advanced as a means of supplying the market, decreasing prices, and thus lowering the incentive to poach animals in the wild (29). Tiger and lion bone are used in the production of wine and cake or glue consumed for medicinal

purposes, and teeth and claws are used to produce trinkets and curios (8). In contrast to the numbers of tigers and lions in the wild, the numbers of captive-bred tigers have increased in Southeast Asia and China, and in South Africa captive lion breeding has proliferated to supply a captive lion hunting market that produces lion bones for legal export as a by-product but also increasingly as a primary product for legal export (30). Lion bone exports from South Africa have increased from approximately 314 skeletons per year in 2008–2011 to more than 1,000 skeletons per year in 2013–2015, peaking at 1,700 in 2016 before CITES introduced a limited quota of 800 in 2017 (31). On July 8, 2022, the South African Department of Forestry, Fisheries and the Environment published a draft white paper on conservation and the sustainable use of South Africa's biodiversity, significantly changing the country's approach to biodiversity management, including the importance of benefit sharing by local communities and recognizing the welfare and well-being of wild animals (32). This document followed the government announcement to stop the captive breeding of lions and no longer issue permits to export the bones. Coals et al. (8) conducted the first quantitative study to assess consumer preferences in China and Vietnam for lion versus tiger and wild versus farmed bone wine products to determine if the commercial supply of wild animal products would be acceptable substitutes for the wild product. The study found that most respondents in both China and Vietnam preferred tiger over lion bone wine, and there was no overriding preference for wild or farmed sources in either China or Vietnam. Approximately 8,000–10,000 predators are held in approximately 300 breeding facilities in South Africa. The sudden legislative change concerning lion breeding in the country may result in an excess of captive-bred animals, resulting in welfare concerns due to the cost of keeping them without producing an income and a paucity of available habitat to release them. The health and welfare of captive animals are often neglected in favor of profit, and these animals then suffer serious welfare abuses (**https://bloodlions.org/**). Compliance by registered breeders can be monitored through individual identification and parentage testing of all registered stock to ensure that the traded animals are descended from captive breeding stock and not supplemented by wild animals (31).

2. THE EVOLUTION OF FORENSIC EVIDENCE IN ANIMAL CASEWORK

2.1. Forensic Evidence in Animal Cases

Wildlife forensics is a critical tool to aid law enforcement in monitoring and policing of national and international agreements regulating the wildlife trade. DNA evidence is particularly valuable in cases where material is processed, degraded, and altered to be morphologically unidentifiable (33). Almost 40 years after it was first discovered, DNA analysis still provides the key evidence in both civil and criminal cases, including animal cases (34). Developments in animal forensics have followed those in human forensics closely, and in some cases the unique requirements of animal cases have inspired novel techniques and solutions. Samples from animals and animal crime scenes cover a huge number of possibilities, for example, snake venom (35), bear bile (36), molted feathers (37), fish scales (38), porcupine quills (39), historic eggs (40), and rhinoceros horn (41), and forensic laboratories must adapt and validate their techniques accordingly.

2.2. Crime Scene Investigation and Sampling

Prosecutorial and investigative requirements in different countries and jurisdictions often dictate the depth of investigation and sampling methodology required in IWT cases. Crime scene investigation (CSI) is not a new discipline, and processes have been established in human CSI to perform these investigations effectively and without compromising evidence. Wildlife crime scenes, although subject to the same principles, may present with unique challenges and a vast

number of potential victims and sources of evidence. The involvement of rangers, game wardens, conservation professionals, and veterinarians may require adaptation of techniques and training, because the handling of the scene and sampling procedures provide the foundation for successful prosecutions (42).

The conviction and sentencing of a notorious rhinoceros poaching gang in the Eastern Cape province of South Africa provided several precedents in terms of forensic evidence. Three poachers were convicted and sentenced to 25 years of imprisonment on charges relating to 10 rhinoceros poaching incidents in the area. DNA profiling linked a bloody saw to a horn found in the possession of the poachers and to a rhinoceros called Campbell, killed on a private farm in 2016. This was the first rhinoceros poaching case in which cell phone tracking evidence was used to place the poachers close to the crime scenes. The gang used a unique method of poaching, darting the animal using a dart gun and a high dose of tranquilizers. This case was also the first in which dart gun ballistics were presented as evidence. Chemical and physical analysis linked a yellow paint chip found at the scene of the poaching to the bloodied saw. Expert veterinary testimony relating to the animal's suffering and highlighting the disregard for welfare and inherent cruelty of rhinoceros poaching was also presented (43, 44).

Like human crime scene sampling, methods to collect samples from animal crime scenes must be established prior to implementation in the field. A diverse array of animal crime scenes are possible; in cases such as rhinoceros and elephant poaching, the animal carcass is left at the scene, whereas the whole carcass may be removed in the case of tiger and lion poaching. Lions poached for their claws and teeth in South Africa are often poisoned, and CSI and necropsy must include tissue for DNA matching to recovered parts and stomach content to confirm the presence and identity of the poison. Crime scene sampling must always follow chain-of-custody techniques to maintain sample integrity before analysis. Sampling strategies have been described and published for elephant ivory (45) and rhinoceros horn (46).

2.3. The Value of Training in Effective Prosecution of Animal Cases

The success of forensic scientists developing systems to support prosecution and investigation of wildlife and animal crime must in turn be supported by legislators developing frameworks and effective legal tools with which to enforce laws and improve prosecution. Training of prosecutors and magistrates in wildlife forensic techniques and their application to prosecuting wildlife crime are essential to ensure that results are effectively and correctly introduced into court proceedings and well understood. In some instances, dedicated courts and prosecutors are required to deal with increased poaching and case loads (47).

Researchers, enforcement authorities, and those that apply the technology in the field must collaborate to ensure that new techniques or old techniques with new applications in wildlife crime are transferred from the scientist to the field to be useful. One example is the technique to lift finger marks from pangolin scales using gelatin lifters (48). Techniques being established in human crime investigative processes could be used in wildlife crime investigations in novel ways. To do this, the technique must first be evaluated in the system into which it is being incorporated, and benefits and disadvantages or modifications must be considered. Understanding of the environment, especially the nonhuman environment, includes knowledge of local conditions, current workflows, local authorities responsible for the application of the technique, and appreciation of the constraints.

2.4. Standards in Animal Forensic Testing Focusing on DNA Forensics

The discovery of unique heritable patterns in DNA by Alec Jeffreys at the University of Leicester in the 1980s undoubtably changed forensic investigation profoundly. Since the first inclusion of

this evidence in a human case in 1987, advances in the technology and standards developed around its use have snowballed (49). DNA evidence has assisted in both the conviction and exoneration of suspects in courts all over the world and has undergone intense scrutiny under different legal systems. The inclusion of DNA evidence in animal cases must and will continue to be examined, and must therefore be subject to the same standards that underpin its use in human cases (50–52). The plethora of standards and guidelines published since the first application of DNA forensics to animal cases is available for practitioners to implement in their own laboratories. These standard operating procedures describe the handling of reagents, validation of test and analysis methods, calibration of equipment, the use of positive and negative controls, data acceptability criteria, and the handling of difficult samples.

Forensic evidence must be guided by knowledge of the history, ecology, physiology, and behavior of the specific species under investigation. Species that have undergone recent bottlenecks or have been isolated for long periods, resulting in limited genetic variability, may require more extensive markers or additional marker systems to be included in their profiling tests, as well as larger representative databases to provide sufficient information to support statistical match probabilities. An example of this is the African white rhinoceros, whose numbers plummeted to approximately 50–100 animals in the early 1900s (53). The current population has very low genetic variability, and this impacts the match probability statistics in this species. Extensive sampling that includes representation of most populations in which these animals remain has, therefore, greatly benefitted forensic data applications in white rhinoceros poaching cases (54).

DNA evidence currently includes two main types: nuclear DNA (nDNA) markers and mitochondrial DNA (mtDNA). The sequence obtained from mtDNA testing must be aligned with a reference sequence, preferably from a verified voucher specimen. Guidelines have been established for mtDNA typing in forensic cases regardless of the locus used (50). The use of core nDNA marker sets, allelic ladders, shared control samples, standardized nomenclature systems, and proficiency testing underpins the sharing of nDNA data between laboratories testing either human or animal DNA. Human forensic DNA laboratories benefit from the availability of test kits validated by the manufacturers that provide a commercial standard for data sharing. Testing kits are not available for wildlife forensic testing currently, and these tests must be developed and validated in house, although some multiplex tests have been proposed and published for selected animal species (55, 56). Because the short tandem repeat (STR) markers selected for testing are rarely the same in different laboratories, and interlaboratory comparison tests are available only for a few, mainly domestic animal species (**https://www.isag.us/**), data sharing between animal testing laboratories is currently limited.

2.5. Animal DNA Evidence in Court

Expert witnesses, including forensic scientists, must present their evidence and supporting information in an unbiased manner, because their role is purely to assist the court. Evidence included in a written report must always be worded carefully and presented relative to representative data available for that species; for example, DNA match evidence should indicate the relative rarity, or lack thereof, of a profile or sequence in a population (46).

The legal system used in a specific state, country, or region defines the framework wherein evidence can be admissible, and this framework applies to both human and animal cases. Scientists and laboratories providing evidence in legal proceedings related to animal cases must be familiar with the local standards as well as seminal cases in their jurisdictions. The *Daubert* and *Frye* standards of admissibility of expert testimony are applied currently in the United States (57). The standards and guidelines relating to DNA evidence are updated regularly as new methods and

updated approaches become available and legal scrutiny identifies deficiencies and limitations of test methods.

Identifying the species of origin of a confiscated item as a CITES-listed species protected under local legislation is prerequisite to effective prosecution. Smart et al. (6) estimated that more than one-third of IWT crimes are not prosecuted because the species of origin could not be identified properly. The identification of the species of rhinoceros from which a seized horn is recovered supports aggravation in sentencing based on whether the horn originates from a white or black rhinoceros, and thus the level of threat to species survival (54).

Domestic animal cases, although not poaching cases per se, are relevant to the discussion on poaching cases, because the outcomes and rulings in these cases have set the precedents for future wildlife-related cases and highlight the limitations of evidence so that these issues can be addressed. Two early cases involving domestic cats provide important findings in terms of setting up reference population data sets, validating tests, and presenting evidence in court.

Animal hair, particularly that of domestic pets, has played a significant role in the conviction of offenders in human criminal cases due to the ease with which these hairs adhere to clothing and can be transferred. The hair of a domestic cat named Snowball found on a suspect's jacket linked him to the victim in a homicide case in Prince Edward Island, Canada (58). The case set a precedent for the introduction of STR genotyping of pet animal hairs in forensic cases and also introduced important principles of STR validation and establishment of a background database for species in animal forensic DNA analysis (59).

When only shed hair is available, it is often only possible to obtain mtDNA. The murder trial of the *State of Missouri v. Henry L. Polk, Jr.* (60) was the first legal proceeding in which cat mtDNA analysis was introduced into evidence and provided valuable precedents in terms of the requirements for admissibility of this evidence in animal-related cases. In this case, a single hair, identified morphologically to be cat hair, was obtained from the victim's clothing. STR profiling could not be done on the sample, but mtDNA sequencing of the control region was successful. The sequence matched that of two of the four cats at the suspect's residence. The mtDNA profile (mitotype) was also unique in a database of 180 cats. However, the database was considered too small to reliably support the court's inclusion of the evidence. The inclusion of a larger data set from other laboratories, and STR verification that the cats with the unique mitotype in the household were related and shared the specific mitotype in their maternal lineage, was sufficient to convict the suspect of first-degree murder. This case emphasizes two important considerations when using forensic DNA evidence: the value of mtDNA typing when STR typing is not possible and the importance of larger combined data sets to support DNA matching, although the acceptable size of such data sets had not been determined prior to the case (61).

In September 2021, Yunhua Lin was sentenced to 14 years in prison for dealing in rhinoceros horn in Malawi. Lin was the head of a trafficking syndicate that had been active in Malawi for more than a decade. During a search of Mr. Lin's commercial and residential properties in Lilongwe, ivory, pangolin scales, and 103 pieces of rhinoceros horn were discovered. DNA profiling tests linked the pieces to five individual rhinoceros submitted for profiling to the RhODIS® (Rhino DNA Index System) program previously. One of these rhinos was subsequently poached in the Liwonde National Park in Malawi in 2017. The poacher who killed the Liwonde rhinoceros confirmed the DNA evidence by testifying that he had sold the animal's horn to Mr. Lin. High Court Judge in the case Justice Violet Chipao highlighted, as aggravating circumstances, that the traffickers needed longer sentences than poachers, because they encouraged poaching, and that the recovered pieces of rhino horn came from not one but five different rhinoceros (62). This case highlights the value of regional databases such as RhODIS® in supporting forensic investigation of IWT and inclusion of samples from live animals in the database.

The successful prosecution, conviction, and sentencing of suspects in South African and other rhinoceros-range state courts, including Namibia, eSwatini, Kenya, and Malawi, confirm the utility of the RhODIS® approach to DNA forensics in cases involving rhinoceros poaching and the illegal rhinoceros horn trade, and several successful prosecutions have set an international legal precedent using this approach (54).

The RhoDIS-India program of the Indian Ministry of Environment, Forests and Climate Change was instituted following the framework of the RhODIS® in South Africa. It has successfully used a panel of 14 dinucleotide STR markers optimized for DNA profiling of the Indian greater one-horned rhinoceros (*Rhinoceros unicornis*) to link horn samples with two rhino carcasses from West Bengal and Assam for submission as scientific evidence in legal proceedings. This expansion of the RhODIS® program underpins the value of the approach for other species and emphasizes the importance of collaboration between stakeholders including scientists, law enforcement, and government authorities to effectively tackle wildlife crime (63).

2.6. The Application of DNA Evidence in Animal Forensics

Human cases have familiarized courts with the purpose, methodology, and value of DNA, and this has simplified the inclusion of DNA evidence in animal cases. However, the chain-of-custody procedures, quality-assurance processes, and technical questions are the same whether human or animal DNA evidence is presented, and animal DNA evidence is therefore subject to similar legal scrutiny, often more so due to the novelty of its use, particularly in wildlife cases.

DNA extraction techniques have been developed for commercial use to provide DNA of sufficient quantity and quality to identify the species of origin even from highly degraded samples. Several companies produce commercial DNA extraction kits that have been validated for use in many sample types, and these can be applied following the manufacturer's instructions or modified in the case of unique animal products. These modifications are often required, because animal testing laboratories are faced with unique, particularly challenging animal materials, such as shark fins, fish scales, cooked and dried meat, claws, eggshells, ivory, rhinoceros horn, scales, shells, and bear bile.

A notable distinction between human and animal DNA forensics is the significant role that species identification plays in the latter. To monitor the trade in species, authorities must first identify the species of origin of items being traded. Poaching forensics has, therefore, relied heavily on species identification tools, including morphological identification and molecular techniques (31). IWT most often includes products that have been altered in some way, e.g., processed, cooked, powdered, carved, dried, or tanned, making morphological identification impossible. Highly skilled taxonomists are rarely available in every country through and to which illegal products are shipped. Molecular technology for species identification is, however, available in most countries and is well suited to identifying processed products, and large, publicly available reference databases such as GenBank (**https://www.ncbi.nlm.nih.gov/genbank/**) and the Barcode of Life (**https://www.boldsystems.org/**) provide a means of identifying most traded species.

Wasser et al. (64) applied a smoothing method to estimate geographically specific allele frequencies to infer the geographic origin of DNA samples collected from seized African elephant tusks. They demonstrated that the method could assign 50% of samples to within 500 km and 80% to within 932 km of their actual place of origin, with accuracy varying by region. Ishida et al. (65) produced another tool to assist in assigning elephant ivory to geographic origin using mitochondrial sequence data (66) (**https://www.loxodontalocalizer.org/**). Although these data do not contribute directly to conviction, they provide invaluable intelligence to investigators along the value chain and focus attention on areas in which anti-poaching measures and law enforcement

should be increased and funding directed to reduce the pressure on local populations. Gaubert et al. (67) reported on the use of a series of informative mtDNA markers of varying lengths and variation in nuclear genes in all species of pangolins that provide a resource to trace pangolin products in the illegal trade chain, and four species of seahorse found in traditional medicine products in curio shops in California were assigned to broad geographic origins (68). Baker et al. (69) used a 464-bp fragment of the mtDNA control region and 8 STRs to develop a DNA test to identify meat from individual North Pacific minke whales sold in 12 markets in South Korea from 1999 to 2003. The official by-catch reported in this period was 485 whales, but the researchers found and estimated 827 individuals present in the market product. This suggested that there was a rampant illegal trade of North Pacific minke whales in South Korea during the period, as well as obvious underreporting.

Various groups have selected different areas of mtDNA to approach species identification. The International Barcode of Life initiative (**https://ibol.org/**), led by the Consortium for the Barcode of Life (**http://www.boldsystems.org/**) project, selected the cytochrome *c* oxidase subunit 1 gene as the mitochondrial target for universal species identification using a 658-bp fragment (70). Taniguchi et al. (71) recently developed a vertebrate-specific real-time quantitative polymerase chain reaction (PCR) test targeting the 16S rRNA region that can determine the ratio of a specific vertebrate species in a sample to the total vertebrate mtDNA in the sample in mixtures. In animal cases, the D-loop of the mtDNA is used to identify individual dogs when nDNA is insufficient, for example, in cases where only a single hair or hair shafts without roots are available. The presence of limited animal hair that lacks roots has been used as evidence in human crime cases in which the animal hair was transferred from the suspect to the victim during the commission of the crime. In these cases, mtDNA D-loop sequence is less discriminatory than nDNA but can still be used effectively either as exclusionary evidence or as evidence of inclusion when sufficient and appropriate reference samples are available (72).

The selection of genes for species identification in different studies is based on various criteria depending on the availability of reference material and the application. The hypervariable regions 1 and 2 from the control region are used frequently in human forensics, whereas the cytochrome *c* oxidase subunit 1 and cytochrome *b* genes and the two ribosomal subunits 12S and 16S are frequently used in animal species identification. This difference reflects the purpose of the tests: Human mtDNA tests are used for individual identification when the sample lacks sufficient nDNA for STR-based individual identification testing, and in animal tests the intention is mainly to identify an item's species of origin (6). mtDNA testing is well established and commonly used in forensic laboratories worldwide, and incorrect assignment usually results from misidentification of the sequences in the reference database (73). In very degraded or highly processed material, mtDNA may be the only DNA available for analysis because it occurs in much higher copy numbers per cell (up to 1,000-fold) than nDNA, and it is therefore particularly valuable for identifying leather, hair shafts, ivory, bone, quills, scales, and horn (6).

Next-generation sequencing (NGS) provides valuable insight into sequence variation and is gaining importance in understanding variation in STR markers applied across species for individual identification, especially those used in kinship analysis. Inclusion of NGS in evaluating animal forensic markers is currently lagging that in human forensics. The identification of mutations in several human paternity investigations (74) also supports the application of NGS to examine sequence variation and mutational patterns in STR loci used in animal forensic data sets. One limiting factor has been the availability and cost of the relevant technology; funding of animal forensic research lags well behind funding for human forensic research and is likely the main contributor to the lack of extensive data on animal marker systems. As the cost of NGS technology drops, STR sequencing, which can identify length-based genetic variation as well as sequence-based variation

in the repeat and flanking regions of existing STR markers, may provide an alternative, or initially an addition, to capillary electrophoresis (CE)-based STR typing in forensic genetics. Similar to the establishment of STR-based population databases, sequence-based DNA profiling must be performed on population-wide reference samples to establish statistically sufficient data for sample comparison and kinship analysis in forensic cases (75).

The future of forensic DNA testing, particularly related to marker type selection, is often debated, and some believe that as NGS becomes cheaper and more readily available, sequence-based methods will replace STR-based systems. However, an alternative, and perhaps more practical, approach would be to include the different types of polymorphisms in the array of tests available. In some cases, one data type will be more appropriate than another, and in other cases, all the data can contribute to solving a particular case. Combining a set of autosomal STRs that relate to or overlap with standard sets used in the national or international databases, a panel of Y chromosomal markers, selected mtDNA sequence, and even phenotypic single-nucleotide polymorphisms (SNPs) (see text below) could add valuable information in animal forensic cases (49).

Traditional species identification methods do not work when samples are mixed, because the universal primers amplify the most abundant and intact DNA or alternatively provide a mixed sequence that cannot be interpreted. Samples that contain mixtures of species include those found in TCM, and these are best analyzed using specific PCR primers and either a CE or real-time PCR approach (76–78). NGS applications for species identification of TCM and food product analysis are evolving rapidly, for example, to identify potential allergens, species excluded for cultural reasons, or the inclusion of endangered species (79–81). Environmental DNA metabarcoding was used as a novel, noninvasive tool to monitor the trade in endangered fish species in Hong Kong markets (82).

mtDNA is exclusively maternally transmitted, and the species origin of a sample originating from a hybrid animal will reflect only the maternal species. Y chromosome–specific markers similarly will create the same error but assign the individual to the paternal species. This can create loopholes in cases where the local legislation does not protect hybrids, such as the US Endangered Species Act. A variety of methods have been used to overcome this problem, most commonly the simultaneous use of mtDNA sequences and nDNA testing to verify parentage. Use of both types of markers is thus recommended in cases where hybridization is a concern. In such cases, the results should be qualified by stating that they are compatible with the sample originating from the species identified or from a cross between a female of the identified species and another undetermined and reproductively compatible species (83).

Currently, human forensic identification is mainly STR based. When STR loci were initially used in 1994, four loci were used, and this expanded to include various commercially available test kits with up to 24 loci. Human profiling uses tetranucleotide markers that are generally located on different chromosomes and not genetically linked. A vast number of studies have been done on the individual markers in terms of sequence differences, mutation rates, and characteristics in different human population groups. In contrast, animal testing, particularly domestic animal testing, often uses dinucleotide markers, and comparatively little work has been done on the behavior of these markers in different animal populations.

Increased levels of inbreeding, and thus sharing of alleles that are identical by descent, occur frequently in animal populations compared to in human populations, and this must be accounted for in match probability calculations with the use of a kinship factor when significant population substructuring is present. In many human populations, the kinship factor, an estimate of F_{ST} or theta (θ), is between 0.01 and 0.03. In rare and threatened species, in which a significant amount of inbreeding and population substructuring is assumed, the use of a conservatively high θ value is

advocated. Menotti-Raymond et al. (59) suggested a θ value of 0.05 in domestic cats, and Harper et al. (54) suggested a value of 0.1 in African rhinoceros.

Forensic DNA phenotyping is a novel application of DNA forensics that is becoming increasingly valuable in human investigations to narrow a suspect pool or identify unknown persons. SNPs are used to predict the geographic ancestry and phenotypic characteristics of a donor and have become possible due to the rapid expansion of human genomic information (84). The application of phenotypic characterization is much more limited in animal forensics but has been explored using SNPplexes in cats (85). Inclusion of these data in the forensic tool kit has again highlighted that developing technologies must be validated, standardized, and translated from the research setting to the field application before they can be fully used and commonly applied (86).

A sample can be assigned to a geographic area or population when the potential source populations have been sampled extensively and the population boundaries are well defined. However, in species in which population boundaries are blurred and where genetic divergence between populations is low, assignment becomes unrealistic. In the latter case, clustering methods that determine the number of groups defined by genotypes rather than boundaries can be used to assign individuals to a species cluster to narrow the source range. An individual is typically assigned to a population or group using software such as STRUCTURE (**https://web.stanford.edu/group/pritchardlab/structure.html**). In 2016, Chinese traffickers were sentenced to 14 years in prison by a Namibian court following the matching of rhinoceros horns to a carcass of a poached *Diceros bicornis bicornis* animal in the Etosha National Park in Namibia. STRUCTURE analysis of the DNA profiling data assigned the recovered black rhinoceros horns to the *D. bicornis bicornis* subspecies that occurs mainly in Namibia and the Northern Cape province of South Africa. The information provided a useful investigative lead that led Namibian authorities to discover carcasses of poached rhinoceros in the Etosha National Park, among which a matching carcass was subsequently found (87).

2.7. The Role of Reference Databases in DNA Forensics

Examples of reference databases or registers include the Norwegian minke whale DNA register, which has effectively identified by-catch specimens from Norwegian markets to reference samples in the register (88). RhODIS®, which includes all rhinoceros horns in stockpiles in South Africa and Namibia, all captive-breeding live white rhinoceros in South Africa, and a vast number of white and black rhinoceros in both countries, is another example of such a register that is used to trace unknown and seized horns to their origin (46).

STR genotype matching must be supported statistically by allele-frequency data derived from a database of genotypes that are correctly called and complete and consist of unrelated individuals sourced from representative populations. Data sets must be evaluated, preferably by an external, standardized quality-control program, before being made available publicly. Unevaluated data sets could potentially have several errors that could invalidate the allele-frequency and match probability results. Human haploid marker databases including mtDNA and Y-chromosomal STRs have been evaluated using centralized, independent quality-control tools to avoid the publication of erroneous data. Leading forensic genetic journals that require quality control of mtDNA and Y-STR population data prior to the submission of research papers support these efforts. Some of the publicly available STR databases that can be used to calculate STR profile match probabilities are available for human populations. Allele frequencies per marker and population, but not the STR profiles, are available to download.

Similar public STR databases are not available for animal forensic interrogation. Noteworthy here is that the marker selection in different wildlife testing laboratories is usually not fully

compatible, and standardized reading and analysis of STR data are not done except in domestic animals, which generally reduces the value of such databases for sharing. The conversion of current and future CE-based STR data to sequences using NGS would avoid these issues and simplify data compatibility between laboratories (89). However, replacing existing STR databases will be extremely expensive and will require retesting of samples that may no longer be available, and although current animal STR databases are relatively small, the cost of this will also be prohibitive. A system that includes both CE- and sequence-based profiling that allows for backward compatibility will be required and is likely the future of forensic databases once the cost of NGS is comparable (90).

In 1992, the members of the European DNA Profiling Group selected STRs as the preferred marker to obtain genotypes for forensic case work in humans. This preceded the establishment of human STR databases to support the linking of suspects to crime scene evidence and provide population-based allele-frequency information. In 1995, the United Kingdom set up the first national DNA database, which would hold both personal DNA profiles and results obtained from crime scenes (90). The US Federal Bureau of Investigation established a national DNA database for North America in 1998 called the Combined DNA Index System (CODIS) (91).

Pérez-Espona (33) emphasizes the value of collaboration between forensic geneticists and research-focused biobanks to provide a source of vouchered reference samples to support forensic DNA investigation. Research laboratories are often approached to provide testing and analysis in animal forensic cases; however, these laboratories and researchers may not implement the stringent quality-assurance procedures required for forensic case work or have the relevant expertise. Research laboratories approached for this work must therefore familiarize themselves with the requirements for forensic testing and chain-of-custody procedures. Particularly, research biobanks that hold valuable and rare specimens must ensure that their sampling, handling, and storage processes follow forensic conditions so that their voucher specimens can contribute to such investigations. Cooperation between research laboratories, biobanking facilities, forensic laboratories, and law enforcement authorities, as well as CITES, could play a crucial role in reducing IWT (33).

Noninvasive sampling is used extensively to assess population numbers for small and elusive species that are difficult to track and sample. It has also been used to populate background databases in species such as elephants and those species with small numbers where the risks associated with capture are too great. Noninvasive sampling usually involves the collection of fecal samples. Fecal samples can, however, prove difficult to analyze due to the limited amount of available DNA, potential contamination, and sample degradation. A database that supports forensic analysis must include error-free genotypes, and this has proven difficult to achieve with fecal material (92).

3. GLOBAL TRADE IN POACHED WILDLIFE AND DERIVATIVES

3.1. International Bodies that Control and Monitor Legal and Illegal Trade

CITES is an international treaty between countries meant to ensure that trade in a species does not threaten its survival (16). CITES lists species on three appendices according to the level of threat that species face. Once a country becomes a party to CITES, it is legally bound to the Convention and is required to implement legislation that includes a permitting system to trade in a listed species. Species that are protected under CITES through local legislation must be identifiable to successfully prosecute offenders trading in these species. The definition of species, subspecies, geographical grouping, hybrids, introgression, cryptic species, and reliable identification methods is critical to support prosecutions. Delayed issuing of CITES permits and local political

unwillingness to support the rapid movement of samples for both forensic and research purposes, which is required to develop the test methods and analyze evidence material, are currently critical hindrances to the rapid investigative follow-up and rapid and successful prosecutions required to combat IWT effectively.

Other organizations involved in combatting wildlife crime include Interpol, an international police organization that liaises with local police forces in different countries, and the International Consortium on Combating Wildlife Crime, a partnership between CITES, Interpol, the UN Office on Drugs and Crime, the World Bank, and the World Customs Organization. These organizations support local wildlife crime law enforcement agencies through training, capacity building, exchange of skills and information, and the development of tools to assist local authorities to combat poaching and IWT. Enhancing technology to assist with surveillance and law enforcement to fully understand the scope of IWT and the links to organized crime will require including fields such as criminology, financial intelligence, and economics.

3.2. Extrinsic Factors Affecting Poaching and the Illegal Trade in Wildlife

Economic aspects affecting IWT include an understanding of the trade chain and consumer and market dynamics, as well as how these are influenced by legal trade and the level to which this must be regulated. Studies to broaden the understanding of all the related fields that influence IWT and to implement tools to monitor, investigate, prosecute, and hopefully eliminate the illegal trafficking of species are often constrained by funding limitations compared to similar investigations related to human crime (93).

The illegal markets are aided by fluid trade dynamics and adaptability (16). Trade in pangolins and rhinoceros derivatives provides an example of this. Similar to rhinoceros, Asian pangolin species have become increasingly rare, causing illegal traders to move to Africa to supply both pangolins and rhinoceros horn (67). Criminal networks have long been active in Africa and have established routes to source and move ivory and other illegal products; rhinoceros horn and pangolins have thus simply been added to the repertoire.

Improving local legislation and implementing deterrent penalties are fundamental to effecting change in combatting poaching and IWT and are driven by several factors, including greater support for tackling wildlife crime by the authorities; a more strategic, intelligence-led investigative approach; strong prosecutorial support enabled by training and increased international pressure; and reinforcement of the value of collaborative effort. The timely amendment of wildlife legislation in Malawi in 2017 prior to the arrest of Yunhua Lin, for example, helped to secure a meaningful sentence in this case (62).

4. THE FUTURE OF FORENSICS IN ANIMAL CRIME

Expanding genomic information on increasing numbers of species (94) is providing more marker systems and innovative tools, at decreasing prices, to the forensic investigative repertoire. These efforts are crucial in improving DNA forensics in animal case work and understanding of the physiology and care of animals involved in IWT. The sequencing of Chinese and Malayan pangolin genomes in 2016 (95) revealed a lack of the *IFNE* (Interferon epsilon) gene that contributes to mucosal immunity in these animals and a reduced number of heat shock protein gene family members, suggesting that these animals are more susceptible to stress-induced immunosuppression. This is evidenced by the difficulty of maintaining these animals in captivity and makes them extremely vulnerable to trafficking-induced infection and stress (96), providing veterinarians, rehabilitation centers, and enforcement officials with knowledge to assist with treatment and rehabilitation of recovered live specimens (95, 97). This example highlights the urgent need to expand

species knowledge in fields such as ecology, behavior, veterinary care, reproduction, and especially genetics to understand and negate the impact of poaching through improved forensic tools and conservation approaches.

Humans share DNA markers across all populations, and although differences exist in allele-frequency estimates between population groups, STR data are comparable. Animal species, however, differ greatly in their STR typing systems, and these systems must be developed, validated, and standardized for a vast number of species. Standardized STR testing systems are valuable tools to support IWT investigation and prosecution; however, several highly trafficked species occur in limited numbers across small ranges. Setting up these test systems, including test validation, laboratories that operate under stringent forensically compatible processes, the skills and expertise to support these systems, sharing of control material, and training of personnel to perform the testing, is extremely costly and difficult. The protection of species is fundamentally the responsibility of the authorities in whose range those species reside in terms of providing law enforcement and legislative support. Prosecutions related to animal poaching occur mainly within these ranges; thus, every effort should be made to improve the availability of forensic tools and skills to support prosecutions and database systems within these areas on a country or regional level.

A global, standardized system for range-specific species is a noble but impractical idea that could perhaps be better served by improving systems to notify range countries or regions in which a collaborative data and technology center for one or several species operates and simplify the sending of test samples to these centers. Such centers should be supported in terms of funding and skills development, but perhaps most importantly by simplifying and expediting the movement of samples from seizures to these centers and providing a centralized reporting platform that informs all relevant authorities directly of the results of the testing and any matches found on the databases. A platform such as CITES may be more helpful to support the expeditious movement of samples to these centers and provide the reporting platform that links relevant organizations.

Legislative measures, including implementing comprehensive animal protection laws, increasing deterrent penalties, training the judiciary, and establishing dedicated environmental courts, will improve the prosecution of IWT crimes. This can be achieved only by strong political will in all countries involved in IWT and by recognizing the serious threat poaching and IWT pose, not only to biodiversity, and thus the future of the planet, but also to the disintegration of the moral fiber of society by rampant criminal activity. Global-scale interdisciplinary collaboration will be one of the most effective ways to overcome the complex scientific and legal challenges posed by IWT.

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Errata

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