Forensic Science in Support of Wildlife Conservation Efforts — Morphological and Chemical Approaches (Global Trends) —

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1042-7201/23-01/Jan. 2011/29–35/$7.00 •

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REFERENCE: Bell LS: Forensic science in support of wildlife conservation efforts — Morphological and chemical approaches (global trends); *Forensic Sci Rev* 23:29; 2011.

ABSTRACT: Wildlife forensics is an emerging field and involves a number of players including the international community itself, national police agencies, nongovernmental organizations (NGOs), and forensic and scientific experts. Collectively, these players attempt to prevent or prosecute crimes that specifically target wildlife, and deal with what is becoming an increasingly burgeoning global problem. The number and type of methods that are used to answer questions related to wildlife crimes and to recover evidence for prosecutions are numerous. This paper outlines the key questions that concern crimes perpetrated against wildlife and provides a context for those methods that fall generally under the headings of morphological and chemical. A commentary is provided on the relative success of these methods and how they relate either directly or indirectly to evidence gathering and to one another. It is clear that a considerable forensic toolkit exists, and more could be achieved with further developments of newer methods.

KEY WORDS: Animal derivatives, identification, manner-of-death, microscopy, pathology, peptides, poaching, provenience, stable isotopes, toxicology, wildlife forensics, wildlife trade.

INTRODUCTION

Wildlife forensics is an emerging field and one that is driven by the need not only to monitor the health and well-being of wildlife populations, but importantly, to prosecute crimes that specifically target wildlife. This effort involves a number of different players drawn from the international community, national police agencies, NGOs and forensic specialists. Today one of the single biggest global challenges is the trafficking of animals and their derivatives, the cost of which is estimated at US $10–20 billion per year, roughly 5% of the international drug trade [29]. Agencies such as the INTERPOL Wildlife Crime Working Group (www.interpol.int/public/EnvironmentalCrime/Wildlife) and TRAFFIC (Wildlife Trade Monitoring Network [www.traffic.org]), act as collaborative forums for countries to co-ordinate responses to these criminal trafficking networks. The task is sizable, and it is estimated that 40% of organized crime in Brazil, for example, is associated with wildlife; furthermore, other countries such as China support, albeit as a black market economy, the sale of CITES-listed species [29]. In fact, Southeast Asia is considered one of the main global hubs for this illegal trade [25]. What is apparent is that these criminal networks are cohesive, extensive, and well-organized, utilizing myriad methods to procure and sell protected species around the world. In 2008, as a form of corporate activism against this formidable and lucrative trade, eBay prohibited the sale of animals and ivory via its online marketplace. In terms of the collection of evidence to substantiate prosecution, forensic and scientific specialists are enlisted to undertake identification of animals and their derivatives, and to identify cause and manner of death.

This paper provides an overview of the approaches taken by forensic and scientific specialists relevant to wildlife forensic investigations, and flags new methods of interest.

I. THE KEY QUESTIONS

Forensic science has been described as the application of science to the law. This definition is useful because it helps us to understand that forensic science is not a science in itself, but is rather many branches of science brought to bear on a specific problem. It is then an extreme example of "applied" science. Depending on what that problem is, the branch or branches of science brought to bear, or combinations thereof, are almost limitless. In terms of wildlife crime, the forensic problems tend to coalesce around three main questions:

1. What is this?
2. Where did it come from?
3. How and when did it die?

One of the newest and most powerful methods to impact human and animal forensics is DNA identification. DNA identification provides a "positive" identification method that is accepted by the courts, and is today one of the most potent forms of forensic evidence. The main

application of DNA identification is obviously to question 1, but in some instances it will inform question 2. DNA is briefly flagged here, as it is treated elsewhere in this issue in some detail, and is mentioned only to situate its importance vis-à-vis other methods.

II. MORPHOLOGICAL METHODS

The morphological methods used in wildlife forensics are utilized primarily for identification and to ascertain cause and manner of death. These methods are based on anatomical identification of tissues or body elements and require a considerable understanding of comparative anatomy at the macroscopic and microscopic scale. Generalists such as osteologists or veterinarians provide identifications; more detailed histomorphological or histopathological identifications will be provided by a range of specialists, depending on the tissue type.

A. Osteology

Gross osteology uses the unique appearance or morphology of bony elements of the skeleton to make identifications. In human forensics the first question is always, is this human? This is a simple question and usually speedily made by invoking exclusion—i.e., it either is human or it is not; and if not, then there is no reason to pursue identification further. However, animal identifications are far more difficult to make because there are so many possibilities and inter- and intraspecies variants. Where whole or partial bony elements survive, the bony joints, overall size and shape, and ligamentous insertion sites provide diagnostic identifiers (characteristic traits) of species and are ranked collectively [22]. It thus becomes possible to identify a single bony element depending on its diagnostic attributes. This approach is also used to estimate minimum numbers of individuals present by using a single skeletal element such as, say, right proximal femur. This is important where many body parts are collected together for shipment and an estimation of animal count is required. When dentition is present, the chance of identification vastly increases, since dentition is unique to most species [18]. There are some species, however, that cannot be separated using anatomical gross morphological methods, and differentiation between sheep and goat is a case in point. These two animals, although clearly distinct, appear identical using osteological and dental morphological identification. This is another reason why DNA identification has such primacy, since it can make the call on these types of problems.

B. Microscopy

Where fragmentary material is recovered, microscopic identification is possible with animal material and a sizable literature exists. A useful review by Hillier and Bell [17] outlines the different microscopic morphologies associated with animals, and how they differ from one another as well as from humans. The main limitation with this approach is that although it is possible to operate the exclusion principle to narrow to a grouping of possibilities, it is unlikely to provide a definite solution to the problem of identity. For example, horses, bears, and cows share a common bony arrangement in long bones known as plexiform bone that other groups of animals (including humans) do not have. Other bony microscopic compartments known as osteonal systems are known to differ in size and volumetric frequency between species, but there is also a frustrating overlap between species, since these metrics are expressed as characteristic ranges. A further limitation is access to reference data to validate identifications for wildlife species; and where such data does exist, it is dispersed in scientific papers spread over 150 years, often reported in multiple languages. Where exotic wildlife species may be of interest, then there may be no comparative data at all. The exact same observation can be made for tooth microstructure. Dental tissue type can be easily identified to enamel or dentine using this method either by light or electron microscopy, but libraries of comparative material either do not exist for exotic species, or are not easily accessible.

One success using microscopic identification has been its application to the problem of identifying traded ivory. The principal source for ivory is the elephant, either Asian or African—or alternatively, from the extinct Pleistocene mammoth. Although trade in Asian and African elephant ivory is prohibited (with occasional internationally permitted sales contradicting this statement), trade in mammoth ivory is unrestricted, and its exploitation has existed for centuries. Most mammoth ivory originates from Siberian deposits. The main problem with ivory is that the ivory of all three species appears identical once reduced to raw blocks or derivatives. However, ivory, which is a form of dentine, has a unique microstructural characteristic known as the Schreger Pattern, and the angles subtended by this pattern are measurable and distinct. It has been demonstrated that the mean angles of the Schreger Pattern are different among all three species, with no overlap, making it a viable method for identification [12,26].

Other microscopic methods have addressed the species identification of hair. A recent study of giraffe and elephant tail hair demonstrated that it is possible to differentiate the two types of hair using cross-sectional micromorphology [30]. This investigation focused on a particular problem of verification, where traded goods in Asia and Africa were made from CITES-protected elephant derivatives. Other microscopic imaging methods for hair identification have used either light or scanning electron microscopy in secondary electron mode, where the external incremental growth pattern can be associated with specific species. The limiting factor again is the nuance between species and the lack of accessible comparators for identification—e.g., "An atlas of mammalian hair" (2004) is available only in Russian [9].

C. Necropsy

Necropsy is the animal equivalent of the human autopsy and is performed by a trained veterinarian, with a subspeciality in pathology. It is included in the morphological methods section since it is classically anatomical in nature, and relies heavily on visual observation, radiography, and microscopy [10]. Other tests may be performed, such as toxicology and histopathology, but it remains very much a visual hands-on investigation. For wildlife crimes, the necropsy is important where determinations of cause and manner of death are required. In British Columbia, Canada, black bears are poached by traffickers for their gall bladders, and their carcasses or parts are subsequently illegally exported to China to supply the traditional medicine industry. Using this example, a necropsy can determine what organs have been removed and also how that animal was killed. Depending on the condition of the body a time-of-death estimate might also be made. This type of information is immensely important to police investigators and to international organizations attempting to localize traffickers and their methods. It is also important forensic evidence for the court.

Necropsy is then the mainstay investigation in wildlife forensics beyond questions concerning determinations of identity. There are many examples of wildlife cases in the scientific literature. Not all are directly concerned with poaching; some help document deliberate poisoning, animal abuse, animal health, or suspicious death [7,8].

Collectively, the morphological methods outlined above represent an important part of the arsenal of forensic techniques available to help answer key questions by those agencies charged with documenting and investigating crimes perpetrated against wildlife.

III. CHEMICAL METHODS

A. Toxicology

Forensic toxicology usually involves two separate efforts: first, to identify chemical compounds that have been synthesized from animals illegally; and second, to identify poisoning, either deliberate or accidental. Analyses usually involve thin layer chromatography (TLC) or high-performance liquid chromatography (HPLC) and are undertaken by specialist chemists. Samples are taken either during necropsy or from confiscated organs/tissues or other derivatives.

One of the most well-documented wildlife crimes is the slaughter of bears for their body parts, particularly the gall bladders. The harvesting of bile from bear gall bladders is an ancient practice in China, and the bile is considered a potent healing ingredient in traditional Chinese medicine. This potency has driven a black market trade in bear gallbladders from all over the world, and the value of these organs has jumped significantly: in 1970 a kilo of gall bladder cost US $200; today gall bladders can trade as high as US $50,000 [13]. Not all trade has been illegal; China had until recently a large number of bear farms, where bile was extracted daily from living bears. These farms have been closed down, but some farms remain. The source of bear gall bladders is then potentially from any country that has bears and the organs are obtained by poaching, or where bear farms may operate undetected. This trade is international.

The central question when examining gall bladders or bile is what species is this and where did it come from? The bear bile acids ursodeoxycholic acid (UDCA), chenodeoxycholic acid (CDCA), and cholic acid (CA) are the active ingredients considered medicinally potent. UDCA content in bile varies between species but is high in bears [15]. New world bears, particularly black bears, have the highest UDCA content at approximately 40% [15]. UDCA concentration is elevated in blood plasma during hibernation in black bears, and the role of UDCA is associated with inhibiting apoptosis during this time as a protective mechanism [27]. The fact that concentration varies between different types of bears and also varies seasonally, especially during hibernation, adds another layer of complexity where concentration is used as a method of identification.

B. Poisoning

Where a poisoning incident has taken place and samples are taken in a well-documented time-frame, it is often possible to identify the poisoning agent. Validation and verification are possible due to extensive chemical reference libraries. However, the physiology of different species and the degradation metabolites can make identification more difficult. Vultures are under significant threat in different parts of the world and deliberate carbofuran poisoning of vultures in Kenya was detected as toxic residues in beak, feet, muscle, and soil [24]. Carbofuran is known to be highly toxic to birds and is banned as an agricultural pesticide. But in Kenya it is legally used for certain agricultural practices, plus, in many countries, there is a general lack of regulation. This poison is administered by baiting/lacing a carcass to deliberately attract and kill secondary predators such as vultures. This method has been documented and can cause mass death of vultures from one carcass alone [24]. In Spain, a monitoring study revealed that targeted poisoning caused a large number of vulture deaths across the country, and the compounds found to be most used were aldicarb (38.6%), carbofuran (31.3%), and strychnine (16.6%) [16]. The main motivation for this type of wildlife crime is a desire to reduce/irradicate avian predator populations who are deemed in competition with land owners and farmers.

C. Stable Isotopes

An interesting new area is stable light isotope tracking of human and mammal remains in geographic space. This type of work is also known as biogeolocation, biosurveillence, and isoscapes. The initial interest in this field was focused on reconstructing past climate temperatures [21], where fossil mammal bone and teeth were ulilized as proxy indicators of climate temperature. Archaeologists adapted this temperature relationship to answer questions concerning geographical movement of past human populations [3,4]. Forensic science has a direct application for this type of work, since knowledge of where someone lived either months or years prior to death can help narrow a missing-person search. The potential to expand this kind of work into wildlife forensics is obvious [1,5,19] since it can address the question, "Where did this come from?" and the jurisdictional implications that go with that.

The main geolocator tool is $\partial^{18}O$, which may be recovered as a biomarker from any tissue, but archaeological and forensic work has focused on tooth enamel, hair, and fingernail as source tissues, largely because these are more resistant to decomposition. The oxygen signal comes mainly from drinking-water intake during the mammal's lifetime and becomes fixed in animal tissue as it is formed. Drinking water is derived from meteoric waters that change via fractionation of $^{18}O/^{16}O$ as a weather system travels across a landscape. With each rain-out event, the oxygen values expressed as $\partial^{18}O$ change as a ratio to one another. These precipitation values have been mapped globally over a period of decades (GNIP database [www-naweb.iaea.org]), and provide an important comparator resource for making spatial statements based on these bioaccumulated mammal proxies possible. However, this is not yet a court-ready technique, and much remains to be determined regarding species thermophysiology of uptake and the contribution of water bound in foods. Also there are a number of environmental factors known to affect the directional fractionation of $^{18}O/^{16}O$, including the origin of a weather system itself, orography, temperature, humidity, and continental warming. These are all added complexities, but some forensic work has been done on human material where either an exclusionary provenience has been indicated or this type of information has been useful to assist identification [2,11,14,23].

Other stable isotopes, often referred to as the "dietary" isotopes, have been used to address wildlife forensic questions. The approach is similar to that described above, in that a geographical relationship is established using the variation in uptake values that are derived trophically via the food chain. This signal is stored in body tissues at the point of their formation and will continue to reside, since it is stable, until the tissue itself is remodeled out, or until the animal dies. Careful sub-sampling of body tissues allows for temporal measurements, since body tissues are turning over at different rates and different residence times; e.g., tooth enamel forms during childhood, whereas bone stores information representing the previous 18 years' worth of life [3]. Specifically, two studies addressed the problem of traded ivory in Africa using a combination of C, N, and Sr stable isotopes [20,28]. One study looked directly at ivory samples sourced across Africa and found that $^{87}Sr/^{86}Sr$ values in particular varied geographically [28]. Since Sr values come from bedrock and soil values, which in turn enter into plants and so into the food chain, the use of Sr as a geolocator tool was suggested to be highly useful to provenience African ivory. This study also found some variation in the carbon and nitrogen values, and these were equated to behavioral choices related to food. The Koch et al. (1996) study looked at African elephant bone and molars and found a similar relationship to that seen in ivory [20]. Depending on the temporal sampling of the tissues, a level of complexity

was noted and pointed to elephant ranging into different areas, where the Sr values change or became mixed. Such changes make interpretation more difficult, and knowledge of Sr values has not been mapped sufficiently in many areas in Africa, or for that matter, globally. Add to this modern-day fertilizing practices that contaminate soil, and thus alter the underlying Sr soil values, making the use of Sr as a geolocator tool less applicable than earlier studies had hoped.

D. Genus-Specific Peptide Markers

An emergent archaeological method developed for the identification of different mammal residues in potsherds or recovered as bone fragments, could also be applied to wildlife questions concerning identity [6]. This new study focused on a number of collagen type I peptide markers (type I collagen is the major protein present in the organic portion bone and teeth), and mapped how these markers differ between species. The results indicate that this is a viable method for separating species at the genus level, and managed to successfully separate the difficult question mentioned earlier: the differentiation of sheep and goat. The authors make the point that this method might be used as an alternative to DNA identification, where DNA might be contaminated; or, as a method to monitor food authenticity. Certainly, were these peptide markers expanded for more mammals, this method would make a valuable addition to solving problems concerning identity.

CONCLUSION

This paper has sought to contextualize a number of morphological and chemical methods that are used in wildlife forensics. Some are well established and provide the mainstay for many investigations. Others are more experimental, but hold promise for the future. DNA, not dealt with here, really will push forward the whole effort in this area of evidence gathering, but is certainly no panacea. Rather, new and old scientific techniques sit together as a toolkit for application and development in wildlife forensics.

REFERENCES

1. Aggarwal J, Habicht-Mauche J, Juarez C: Application of heavy stable isotopes in forensic isotope geochemistry: A review; *Appl Geochem* 23:2658; 2008.
2. Bell LS, Lee-Thorp JA, Dobney K: Mapping human movement using stable oxygen isotopic ratio mass spectrometry: potential application to forensic science demonstrated by a modern horse-human study; *Can Soc Forensic Sci J* 39:47; 2006.
3. Bell LS, Lee-Thorp JA, Elkerton A: Sailing against the wind: Reply to Millard and Schroeder: 'True British sailors': A comment on the origin of the men of the Mary Rose; *J Archaeol Sci* 37:683; 2010.
4. Bell LS, Lee-Thorp JA, Elkerton A: The sinking of the Mary Rose warship: a medieval mystery solved? *J Archaeol Sci* 36:166; 2009.
5. Bowen GJ, Wassenaar LI, Hobson KA: Global application of stable hydrogen and oxygen isotopes; *Oecologia* 143:337; 2005
6. Buckley M, Collins M, Thomas-Oats J, Wilson JC: Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; *Rapid Comm Mass Spectrom* 23:3843; 2009.
7. Byard RW, Tomo I, Kemper CM, Gibbs SE, Bossley M, Machado A, Hill M: Unusual causes of fatal upper aerodigestive tract obstruction in wild bottlenose dolphins (*Tursiops aduncus*); *Forensic Sci Med Pathol* 6:207; 2010.
8. Carapetis E, Machado A, Byard RW: Lethal consequences of ingested foreign material in seabirds; *Forensic Sci Med Pathol* 6:242; 2010.
9. Chernova OF, Tselikova TN: *An Atlas of Mammalian Hair: Fine Structure of Overhair and Hair Using Scanning Electron Microscopy*; KMK Scientific Press: Moscow, Russia; 2004.
10. Cooper JE, Cooper ME: Forensic veterinary medicine: A rapidly evolving discipline; *Forensic Sci Med Pathol* 4:75; 2008.
11. Ehleringer JR, Bower GJ, Chesson LA, West AG, Podlesak DW, Cerling TE: Hydrogen and oxygen isotope ratios in human hair related to geography; *Proceedings of the National Academy of Sciences of the United States of America* 105:2788; 2008.
12. Espinosa EO, Mann MJ: The History and Significance of the Schreger Pattern in Proboscidean Ivory Characterization; *The American Institute for Conservation of Historic & Artistic Works* 32:241; 1993.
13. Feng Y, Siu K, Wang N: Bear bile: dilemma of traditional medicinal use and animal protection; *J Ethnobiol Ethnomed* 5:1; 2009.
14. Fraser I, Meier-Augenstein W, Kalin RM: The role of stable isotopes in human identification: a longitudinal study into the variability of isotopic signals in human hair and nails; *Rapid Commun Mass Spectrom* 20:1109; 2006.
15. Hagey LR, Crombie DL, Espinosa E, Garey MC, Igim H, Hofmann AF: Ursodeoxycholic acid in the Ursidai: bilary bile acids of bears, pandas and related carnivores; *J Lipid Res* 34:1911; 1993.
16. Hernandez M, Margalida A: Poison-related mortality effects in the endangered Egyptian vulture (*Neophron percnopterus*) population in Spain; *Eur J Wildl Res* 55:415; 2009.
17. Hillier ML, Bell LS: Differentiating human bone from animal bone: a review of histological methods; *J Forensic Sci* 52:249; 2007.
18. Hillson S: *Teeth*; Cambridge University Press: Cambridge, U.K.; 2005.
19. Hobson KA: Tracing origins and migration of wildlife using stable isotopes: a review; *Oecologia* 120:314; 1999.
20. Koch PL, Heisinger J, Moss C, Carlson RW, Fogel ML, Behrensmeyer AK: Isotopic tracking of change in diet and habitat use in African elephants; *Science* 267:1340; 1995.

21. Longinelli A: Oxygen isotopes in mammal bone phosphate: a new tool for paleohydrological and paleoclimatological research? *Geochim Cosmochim Acta* 48:385; 1984.
22. Lyman RL: *Vertebrate Taphonomy*; Cambridge University Press: Cambridge, U.K.; 2001.
23. Meier-Augenstein W, Fraser I: Forensic isotope analysis leads to identification of a multilated murder victim; *Sci Justice* 48:153; 2008.
24. Otieno PO, Lalah JO, Virani M, Jondiko IO, Schramm KW: Carbofuran and its toxic metabolites provide forensic evidence for furadan exposure in vultures (*Gyps africanus*) in Kenya; *Bull Environ Contam Toxicol* 84:536; 2010.
25. Rosen GE, Smith KF: Summarizing the evidence of the international trade in illegal wildlife; *Ecohealth* 7:24; 2010.
26. Singh RR, Goyal SP, Khanna PP, Mukherjee PK, Sukumar R: Using morphometric and analytical techniques to characterize elephant ivory; *Forensic Sci Int* 162:144; 2006.
27. Solá S, Garshelis DL, Amaral JD, Noyce KV, Coy PL, Steer CJ, Iaizzo PA, Rodrigues CMP: Plasma levels of ursodeoxycholic acid in black bears, *Ursus americanus*: Seasonal changes; *Comp Biochem Physiol, Part C* 143:204; 2006.
28. van der Merwe NJ, Lee-Thorp JA, Thackeray JF, Hall-Martin A, Kruger FJ, Bell RHV, Lindeque M: Source-area determination of elephant ivory by isotopic analysis; *Nature* 346:744; 1990.
29. Wilson-Wilde L: Wildlife crime: A global problem; *Forensic Sci Med Pathol* 6:221; 2010.
30. Yates BC, Espinosa EO, Baker BW: Forensic species identification of elephant (Elephantidae) and giraffe (Giraffidae) tail hair using light microscopy; *Forensic Sci Med Pathol* 6:165; 2010.

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