WILDLIFE CSI

FORENSICS HELP IN THE BATTLE AGAINST POACHING

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Crime Scene Investigation – methods are well known to TV audiences around the world from the many programmes that have sprung up in recent times. In fact the CSI franchise is watched in 200 countries with an audience of some 280 billion. But ask any CSI practitioner and you will be told that what is shown is nowhere near real life especially the application of forensic science.

In the last few years the actual CSI methods have started to become used to provide evidence of wildlife crime. So what is forensic science and how can it be applied in CSI Wildlife?

Forensic science is the application of physical science to evaluate evidence for consideration by the law. In effect, this involves using scientific procedures to examine, identify and compare the items of evidence related to a wildlife crime scene and then link the evidence with a suspect (often a poacher) and the victim (usually a carcass).

The wildlife forensic scientist is posed with a series of questions mainly – what species is it? Where did it come from? Which individual did it come from? Was it captive bred?

TOP and MIDDLE RIGHT: sheep and goat cannot be separated using anatomical gross morphological methods.

BOTTOM LEFT: Zebra bones after lion predation.

PHOTOS BY: FELIX PATTON

The most widely quoted forensic technique is DNA analysis but it is only one of many. When it comes to identifying the species of a carcass, especially if it has been well eaten, bones and teeth are important. The technique of physical examination is known as gross osteology and is unique in many species. Surprisingly though looking obviously different, the sheep and the goat cannot be separated using anatomical gross morphological methods as their bones and teeth appear identical. A new forensic method that solves the problem of differentiating sheep and goat involves peptide markers, particularly type 1 collagen, which is the major protein present in the organic portion of bone and teeth. These markers have been shown to differ between species so can be used to separate them, including the sheep from the goat.

Where only fragments of wildlife material are available, identification may be possible using a microscope. A good example of how this is being used with wildlife is in the illegal trade in ivory. Elephants, African and Asian, are the modern source of ivory trade is illegal although ivory from the long

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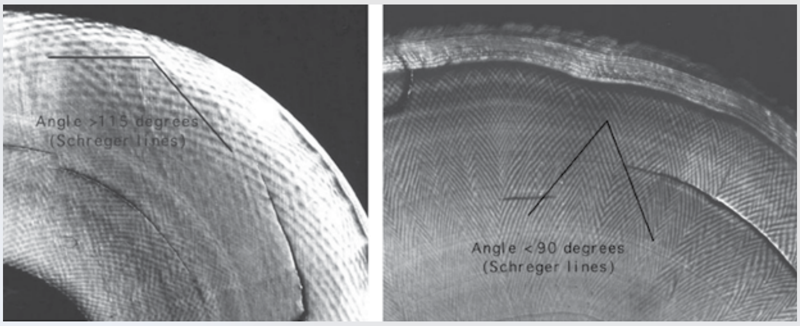

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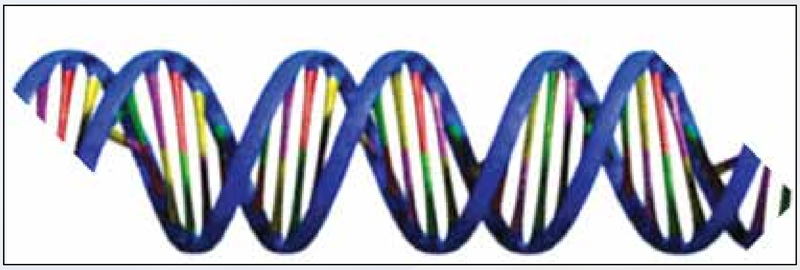

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TOP: Schreger patterns of elephant and mammoth ivory.

MIDDLE: DNA profile

BELOW: poisoned vulture.

extinct mammoth can be traded. The problem is that the ivory of all three species, when reduced to raw blocks or derivatives, appears identical. However, when viewed under a microscope, each of the three species has a unique microstructural characteristic known as the Schreger Pattern, which can be measured and so can be used for identification.

Species can also be identified by the microscopic evaluation of hair. It has recently been found that, by using cross sectional micromorphology, giraffe and elephant hair can be differentiated.

Poachers may poison wildlife. Vultures in Kenya are under serious threat from this method with carcasses laced with the pesticide Carbofuran. If samples are taken within a suitable timeframe, residues can be detected in the beak, feet and muscles of the dead bird and from soil. Carbofuran and other poisoning agents can be identified and validated and verified due to the availability of extensive chemical libraries.

To answer the question – where did it come from? – Stable isotope analysis is being used. Some of the stable isotopes of carbon, nitrogen, sulphur and hydrogen, occur naturally in animal tissues, and can be used as geomarkers in tracking nutritional origin and migration. The approach is based upon the fact that animals, which migrate or roam, carry with them isotopic signatures, which can be related back to local food webs and geographic locations.

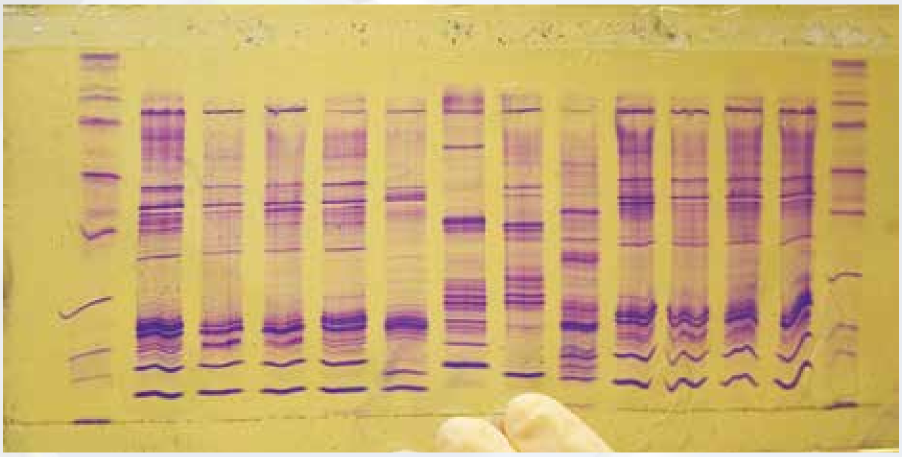
The isotope signatures can be obtained from feather, hair and nail reflecting the diet at the time they were synthesized, muscle tissue reflecting the dietary intake over previous weeks and bone collagen and tooth enamel reflecting the diet over the lifetime of the animal. Stable isotope analysis has been successfully applied in tracking the migration or movement of insects, birds, terrestrial and marine mammals.

DNA is made up of molecules called nucleotides. Each nucleotide contains a phosphate group, a sugar group and a nitrogen base of which there are four types – A (adenine), T (thymine), G (guanine) and C (cytosine). The order of the nitrogen bases in a DNA sequence forms genes.

When morphological characters are absent or where the evidence sample is limited, forensic analysis turns to genetic characters. DNA analysis is especially useful with trace evidence (blood, body fluids) partial organisms (gut piles, crafted items, bones, antlers, horn) degraded or processed tissues (cooked meats, fish filets, timber, Traditional Chinese Medicines).

For genetically identifying species, DNA markers that show variation among species are isolated and analysed. The principal method of analysis uses DNA nucleotide sequencing. The result is compared with reference sequence data from different species. The level of similarity between test and reference sequences enables the species of origin to be inferred.

Where it is necessary to demonstrate that a horn, tusk, bone or skin has originated from a specific individual, where stolen animals need to be identified and to authenticate legally traded wildlife products, DNA profiling techniques can provide key evidence to wildlife crime investigations.



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DNA profile

Fig 1. DNA Nucleotide Sequencing Illustration

Ref 1	T	A	T	T	C	A	T	A	C	A	T	A	C	G	A	C
Ref 2	T	A	T	T	C	T	T	A	T	A	T	A	C	G	A	C
Ref 3	T	A	C	T	C	T	T	A	C	A	T	A	C	G	A	C
sample	T	A	T	T	C	T	T	A	T	A	T	A	C	G	A	C

Four DNA sequences of 16 bases in length
The three reference sequences differ
The sample sequence matches Ref 2

DNA profiling works by targeting genetic markers that are highly variable within species and are therefore likely to show differences among individuals. The greater the number of markers used, the less likely it is that another individual has the same profile.

If two samples produce different DNA profiles, the possibility that they originate from the same individual can be excluded. If two samples share the same profile, it suggests that they may come from the same individual but could this be by chance? The number and variability of markers in the profile affect the probability of this, how common the alleles (alternative forms of a gene) are in the species (their frequency), and how closely related individuals are in the population where the samples were taken. To evaluate these factors requires a representative sample of DNA profiles from the population.

The development of individual profiling techniques for wildlife DNA forensic investigation is currently limited by the need to generate reference data. Compiling wildlife DNA registers in which legally traded or acquired specimens can be individually recognized through a DNA profile is important to provide a method of ensuring that illegally obtained wildlife cannot be laundered into a legitimate supply chain.

Genetic markers are inherited from one generation to the next. This enables DNA profiles to be used to verify parent–offspring relationships. The alleles present in the DNA profile of an individual must also be present in its parents, one allele per marker in each parent. If alleles are observed that do not correspond to those in the suggested parental profiles, then the possibility of the individual being their offspring can be excluded. However, there is the rare possibility of a mutation (where one allele changes to another) occurring within a generation so it is possible to make a mistake. FIGURE 2

CSI Wildlife and the associated DNA forensics is a highly specialist area with its own distinct set of challenges, situated between wildlife conservation research and applied forensic science. The discipline has developed rapidly due to the extensive research and application of human forensics. However every technique must achieve judicial approval if it is to be used to combat crime. It is important to understand how the judiciary view forensics.

The forensic scientist must objectively evaluate the evidence under both the prosecution and defence hypotheses relating to the allegation and describe the relative likelihood of the observed evidence under each scenario. For example, in the case of a DNA profile match between blood on the machete of a poacher and a poached rhino carcass, the prosecution hypothesis will state that the profiles are identical because the samples come from the same individual, while the defence hypothesis will state that the profiles are identical by chance.

The forensic analyst's job is to calculate the (statistical) probability of observing the evidence under each hypothesis, given the available circumstantial information. Findings are presented in terms of the likelihood of the evidence, not an evaluation of which hypothesis is correct; that is the role of the judge or jury.

Forensic analysis provides information on or evaluates hypotheses about the evidence available. It is driven by the questions asked by the investigators. Individual cases generate individual questions but in wildlife law enforcement most forensic enquiries require answers to the four main questions mentioned before: What species is it? Where did it come from? Which individual did it come from? Was it captive bred?

All processes of data production and interpretation may be subject to challenge. Lawyers and scientists searching for potential sources of doubt in the evidence scrutinize all sample collection and transfer, laboratory and statistical analyses, the accuracy of the reference database and the presentation of findings.

In a future edition of SWARA, a second article will show how it has been applied in practice.