A summary of current forensic tests available in the investigation of wildlife crime

Remember - general techniques used to investigate other types of crime can also be used for wildlife crime such as fingerprint analysis, fibre analysis, footwear and tyre marks, questioned documents and handwriting analysis, digital forensics, human DNA analysis, firearms analysis, soil and chemical analysis.

Further details can be found in the ‘Wildlife Crime: a guide to the use of forensic and specialist techniques in the investigation of wildlife crime’ which can be downloaded here: (http://www.defra.gov.uk/paw/files/wildlifecrime-forensics.pdf)

Members of the PAW Forensic Working Group can provide advice to law enforcement on the use of forensics in wildlife crime. Please contact the FWG co-ordinator, Stephanie Pendry, in the first instance (contact details can be found at the end).

Forensic methods specific to wildlife crime

**Taxonomy**: is the branch of biology that deals with the structure of animals and plants and is useful for identification of species.

**Stable isotope analysis**: based on measuring natural variation in the chemical elements present in biological samples to establish the geographical origin or the age of a sample.

**Radiocarbon dating**: used for aging samples based on levels of carbon isotopes following the start of atmospheric nuclear bomb tests. The technique can be applied to discriminate specimens that were alive before and after the 1947 convention cut off.

**Wildlife DNA forensics**: DNA is the genetic material present in all fauna and flora. DNA can be found in all biological samples including timber products, hair, fur, feathers, bones, blood, ivory, horn, saliva, faeces, nails, claws, teeth etc. This DNA evidence can then be used in several applications, including molecular species identification, parentage testing and individual identification.

**Toxicology analysis (including pesticides)**: Where wildlife poisoning is suspected, chemical analysis of the victim and any bait material can establish which pesticide, if any, has been used.

Specific applications for wildlife crime investigation

1. **Species identification**

   For many wildlife crime investigations it is necessary to identify the species involved to determine whether a crime has taken place. Two methods can be employed to identify a species;

   a. **Physical inspection** - From a whole specimen, or even only parts of an animal or plant it may be possible for specialists in taxonomy to identify the species present from its morphological characteristics.

   b. **DNA analysis** - Where the evidence cannot be confidently identified by physical inspection, DNA can be analysed to identify the species present. Specific regions of DNA that show variation among species but are generally conserved within species are targeted. These regions of DNA are sequenced from the specimen, and compared
to a validated reference database of known species. The level of similarity between the specimen and reference sequences enables the species of origin to be inferred.

2. Identification of geographic origin
This may be important for species which are protected by varying legislation across their range, for example ivory from African Elephants is listed under CITES Appendix I everywhere except in Botswana, Namibia, South Africa and Zimbabwe where it is listed under CITES Appendix II. Again, two possible methods can be employed.

a. Stable isotope analysis - Stable isotope analysis is based on measuring natural variation in the chemical elements present in biological samples. Many common elements, such as hydrogen, oxygen and carbon occur in different forms, known as isotopes. The presence or relative abundance of different isotopes allows isotope profiles to be generated for individual samples, which can then be compared to each other, or to reference data. Profiles from different environments will vary due to a number of physical, geological and biological factors. These factors may correspond to different geographic localities and therefore these profiles can be used to infer the geographical origin of a sample. Stable isotope analysis has been used in the UK to examine hydrogen isotope levels in Bramblings to determine if levels are consistent with UK captive-bred individuals or individuals taken from the wild in their Scandinavian home range.

b. DNA analysis – If populations of a species are sufficiently distinct from one another, it may be possible to use genetic analysis assign a sample to its population of origin. In order to carry out this analysis, genetic information from all likely source populations must be available. Genetic population assignment is now being used to identify the origins of a wide range of illegally traded species, including fish and tigers.

3. Aging samples
In some wildlife crime investigations it is necessary to know the age of a sample. For example, if a rhino horn was collected prior to 1947, then it pre-dates laws prohibiting trade in rhino horn. In order to determine whether the rhino horn was legally collected prior to 1947, a form of stable isotope analysis known as radio carbon dating can be employed. During the early part of the 1950's atmospheric nuclear weapons testing became common and had the effect of artificially increasing the amounts of different isotopes of the element carbon, particularly the normally rare carbon 14 (14C) which had doubled in abundance by 1965. As such, rhino horn that pre-dates this period, will be expected to have a lower ratio of 14C than more modern specimens.

4. Animal sexing
Where the open and closed hunting season vary between males and females (e.g. deer) it is often necessary to know the gender of a specimen to determine whether an animal was legally killed. If a carcass has been prepared for sale, morphological differences between males and females are often no longer present (e.g. antlers or genetalia). DNA analysis can, however, determine the gender of the specimen.
5. **Parentage analysis (captive breeding verification)**

The patterns of inheritance from parent to offspring allow DNA profiles to be used to verify family relationships. The genetic variants present in the DNA profile of an individual must be represented in its putative parents. If genetic variants are observed in an individual that do not match those found in the putative parents, then the possibility of the individual being their offspring can be excluded. This method of parentage analysis has been used successfully on many occasions to challenge the captive breeding claims of people illegally laundering wild taken birds of prey. Tests are available for a number of birds of prey and other species.

6. **Individual identification**

Perhaps the most powerful DNA analysis we can perform is individual DNA profiling. This technique allows investigators to link trace evidence from a suspect to a specific incident. For example, if a dead hare is found following a coursing incident, there may be traces of dog saliva on its body. The DNA from this dog saliva can then be analysed, and compared to DNA from dogs believed to have been involved the coursing. If the DNA profile from the saliva on the hare matches a particular dog, this provides strong evidence that the dog was involved in the crime. DNA profiling works by targeting genetic markers that are highly variable within species and are therefore likely to show differences among individuals. 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 and it is then necessary to calculate the probability that two individuals have the same profile by chance. For evidence from animal DNA profiling to be used in court, a very high level of accuracy is required. In order to achieve this, there is a large amount of validation required for any species before the DNA profiling tool can be used for forensic analysis. This tool is currently available in the UK for dogs, badgers and several bird of prey species.

7. **Toxicology analysis**

A number of pesticides may be involved in the illegal poisoning of wildlife. This may be the deliberate abuse of a product or misuse of the product, through carelessness or failure to comply with a safe code of practice. The practise of placing illegal poison baits in the open to target birds of prey, foxes, corvids and badgers continues around the UK. These methods are indiscriminate and may result in the death of other non-target species. A relatively small number of highly toxic products have been persistently used to illegally poison wildlife. These products may be decanted from original containers into unmarked containers. Pesticides in liquid form may be injected into a bait by use of a syringe. The government Wildlife Incident Investigation Scheme (WIIS) investigates the deaths of wildlife potentially caused by pesticide poisoning, in addition to deaths of companion pets (dogs/cats) and beneficial insects (honeybees/bumblebees). Analytical chemistry is used to detect the presence of pesticide residues in a variety of animal tissues including gut contents, vomit, faeces, blood, urine, liver, kidney and lung, as well as in poisoned bait. Pesticide residues are extracted from the tissues and analysed to determine the type of chemical, typically groups such of compounds such as organochlorines, organophosphates, carbamates, pyrethroids, anticoagulant and other rodenticides, or individual compounds such as strychnine, paraquat, cyanide and phosphine are found to be the cause. Pesticide analysis is a proven method of identifying deaths caused by
poisoning or identifying pesticides held by suspects or finding traces in syringes, vehicles or other relevant items. More information on WIIS can be found at: http://www.pesticides.gov.uk/environment.asp?id=58

8. Forensic Veterinary Pathology
This technique aims to determine whether the death or injury of an animal was caused by human activity or due to natural influences. A forensic clinical examination of a specimen is carried out by a suitably qualified and experienced forensic practitioner. A thorough examination by the practitioner will determine the condition of the specimen, any trauma or abnormal findings, life history data (age/sex etc.) and any rings/microchips/collars or tattoos. Radiographs are a useful method to determine internal trauma such as broken bones or bullets. Samples (blood/tissue) may be sent for further analysis. The practitioner may also visit the crime scene to assist in the collection of important evidence such as feathers, faeces, pellets, egg shells etc. Forensic veterinary pathology is particularly useful in cases where illegal killings have involved shooting, snaring, trapping, starvation, poisoning, drowning, hare coursing and badger baiting.

9. Taxidermy
Taxidermy involves preparing, preserving and mounting the skins of deceased animals to replicate their lifelike state. It may be possible to determine the likely cause of death even once the specimen has been mounted. An examination by an experienced taxidermist can reveal external traumas such as bullet wounds or feathers damaged by the passage of shot. Some of the larger bones and the skull may be left inside a prepared taxidermy specimen to help support the internal structure. Consequently, radiographs can be used in conjunction to determine internal traumas. However, as all of the tissue and organs have been removed this does limit the causes of death that taxidermy can uncover. A taxidermist should be able to estimate the age of a specimen. Materials used in modern taxidermy such as enamel and acrylic eyes, nylon thread and foam bodies can all be used to estimate a date. An examination of the case and style of taxidermy can also provide useful information. It is important to age a specimen as some legislation is only applicable during certain time periods. Professional and experienced taxidermists can be sourced through the Guild of Taxidermists. If specimens are found in a taxidermist’s freezer and are thought to be from illegal killings, a post mortem and/or radiography can be carried out to determine the cause of death. Birds of prey with full crops may also be indicative that the bird has been poisoned.

Professional taxidermists typically charge around £30 an hour. Radiographs cost around £20 per plate (typically 1-3 plates per specimen) plus the cost of a veterinary surgeon or pathologist preparing a report (typically £70 an hour)

10. Soil analysis
Soil evidence at a crime scene can be used to link a suspect with an offence and is normally collected by the Scenes of Crime Officer. Soil is comprised of a mixture of organic, mineral and/or synthetic components and is considered as trace evidence. As the ratios of these components can vary over a very small area it is possible to profile the characteristics of the soil at the crime scene and compare this with a soil sample found on a person’s clothes, shoes, tools or vehicle. This is done by comparing the soil colour, particle size and shape, mineralogical
composition and biological components. Reflected light microscopes can be used to compare the particle size and shape and x-ray diffraction can be used to compare mineralogical and biological composition. Soil analysis can be useful in cases where small amounts of soil have been found on a spade thought to be involved in a badger digging incident or in the tread of a shoe of a suspected egg collector where the raided nest location is known.

11. Pollen analysis
Pollen grains are abundant in almost all environments, are very durable and may persist on surfaces and in soils for many years. The pollen produced by flowering plants and conifers, along with the spores produced by ferns are microscopic and not visually obvious trace evidence at a crime scene. However, by examining the morphology of a tiny pollen grain it is possible to identify the genus and often the species of the plant. Conducting analysis on multiple pollen grains allows for the vegetation composition of an area to be determined. The composition of pollen grains at a crime scene can then be compared with a sample taken from a suspect’s clothes, shoes, hair or car. The time of year that the crime took place can also be derived as some pollen is only released during certain seasons. Pollen analysis works best when the crime scene is small such as the placement of an illegal snare or destruction of a bird of prey’s nest. In both these cases pollen from the surrounding soil can be compared with pollen found on the suspect.

DNA techniques available for specific CITES enforcement issues
The following methods are available for immediate application. Other methods can be developed with varying levels of R&D required. If you require a test that isn’t here please ask!

Timber. Species identification from timber is usually possible using genetic analysis. Identification of treated wood products depends on the ability to recover DNA from the sample. DNA extraction methods are continually improving for this application. A reliable test has recently been developed for the rapid identification of ramin.

Caviar. The majority of sturgeon species can be identified, although some species pairs are indistinguishable and hybrids cannot be easily verified. Work is currently underway in some countries to authenticate farmed caviar using DNA profiling.

Traditional Medicines. Components of traditional medicines are difficult to identify and require species specific tests. Tests exist for species such as tiger, rhino and bear, however in highly processed samples, negative results may occur, and these can be difficult to interpret.

Ivory/horn. Species identification and individual matching is possible using genetic analysis. Note that samples can also be aged with respect to being pre- or post- 1947, using radiocarbon dating (non-genetic technique).

Bird parentage (parrots, falcons etc). DNA profiling methods are available for excluding parentage in some species (peregrines, merlins, goshawks, golden eagles, gyr and saker falcons). Parentage identification is more limited, as it requires population data for each target species.
The Forensic Analysis Fund (FAF)

The Forensic Analysis Fund (FAF) offers financial support to police and customs officers seeking to carry out forensic analysis during a wildlife crime investigation. WWF, TRACE, RSPB and Defra have all contributed to the scheme which can match funding by up to 50% of the total cost of the analysis. The fund has been established to encourage the use of forensic techniques to help solve wildlife crimes and comes under the banner of the Partnership for Action against Wildlife Crime (PAW). Many wildlife cases which make use of forensic analysis would otherwise have failed to reach prosecution stage. Founded in 2008, the scheme has already provided money to help support a number of cases including:

- DNA sampling of rhino horn to establish which species and individual the horn belonged to
- Handwriting analysis of falsified birds’ egg data cards
- Radio-carbon dating to determine the age of elephant ivory being sold on eBay
- Taxidermy examination of mounted birds of prey to ascertain cause of death

If you are interested in applying for funding or require any further information please contact Stephanie Pendry at TRAFFIC (stephanie.pendry@traffic.org).

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