Information Bulletin

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NAWEG

DNA Analysis in Wildlife Forensics

INTRODUCTION

DNA forensic analysis examines evidence containing genetic material found at crime scenes in an attempt to link the suspect to the victim and the crime scene. In wildlife crimes, its use now allows analysts to answer a greater range of questions than before. For example, it can help determine not only whether samples collected during an investigation were of the same species but also whether they originated from the same animal.

The purpose of this pamphlet is to give an overview of the use of DNA analysis in wildlife forensics. It is not meant to be a complete "how to" manual. The Commission for Environmental Cooperation and the North American Wildlife Enforcement Group (NAWEG) offer this brief introduction in the hope that it will contribute to controlling wildlife crimes throughout all three nations of North America.

A number of topics are covered in this bulletin:

- Background information on DNA and DNA analysis, including the range of questions that can be answered by it.
- Information on evidence collection and handling and on how to ship samples to avoid contamination.
- Information on legal requirements for prosecution.

DNA BACKGROUND

DNA exists in the cells of living organisms as long, double-stranded molecules. Each strand has a backbone along which are arranged sequences of compounds called nucleotides. The DNA molecule can be compared to a railroad track; the rails form the backbone, and the ties represent the different nucleotides. There are only four different nucleotides but their linear sequence is genetically important—determining all hereditary characteristics of the individual, from its species and sex, down to small traits such as eye color.

Most types of DNA analysis currently in use involve a process called PCR (polymerase chain reaction). PCR "amplifies," or copies, specific short regions of DNA. Like photocopying, PCR produces many copies of a specific DNA region, yielding sufficient DNA for analysis and increasing the sensitivity of the analysis. This means that smaller samples, and even partially-degraded ones, can be analyzed using PCR—a particularly useful attribute in forensic situations where sample quality is not always optimal.

WHAT DNA ANALYSIS CAN BE USED FOR

1. **Species identification:** To identify the species of a sample, the DNA sequence along specific stretches of DNA is determined and compared to known sequences. This technique is used for samples that have been cooked, are partially degraded, or that lack enough material to be tested by cheaper methods. One drawback is that DNA sequencing is more labor intensive and expensive than other techniques in widespread use, such as protein analysis.
2. **Gender determination:** The gender (sex) of the animal from which a sample has originated can easily be determined. Specific regions of DNA are amplified using PCR and then separated for examination. In the most common system used, the presence of two DNA bands means the animal was male, one band means it was female. This test is rapid and can be done using relatively inexpensive equipment.

NAWEG and the Commission for Environmental Cooperation

When Canada, Mexico, and the United States strengthened economic ties through the North American Free Trade Agreement (NAFTA), they also pledged to undertake a new environmental partnership. In 1993, the three nations signed the North American Agreement on Environmental Cooperation (NAAEC) and created the Commission for Environmental Cooperation (CEC). Among the objectives of the NAAEC is the building of regional cooperation for the conservation, protection and enhancement of the environment. The NAAEC also commits the Parties to the effective enforcement of their respective environmental laws, including those for protection of wild flora and fauna.

Responding to these commitments, the CEC, in 1995, established an Enforcement Cooperation Program to provide a forum for regional cooperation, and to exchange expertise, build enforcement capacity and explore alternative approaches to effective enforcement. The Council of the CEC constituted a North American Working Group on Environmental Enforcement and Compliance Cooperation (EWG) to serve as this forum for regional cooperation. The NAWEG participates as a member of the EWG; it provides guidance in identifying priorities for regional cooperation in work to protect wildlife on the continent.

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3. **Individual identity:** Determining identity using DNA is known variously as genotyping, DNA fingerprinting, DNA typing, and DNA profiling. Groups of genetic markers (loci) on the DNA chains are amplified using PCR to establish genotypes ('DNA fingerprints') for each sample. These are then compared to determine if two or more samples originated from the same animal. This is the most common use of DNA analysis; however, the equipment needs are similar to those for DNA sequencing and are expensive. Calculation of the possibility that any matches occurred by chance requires a statistical analysis comparing the case samples with a genetic database. This database is constructed by DNA typing of samples in the same way as for individual identity.

   DNA typing systems are available for most of the species that wildlife officers commonly come in contact with, including mammals, birds, and fish. Systems for other species are constantly being developed in many universities for research purposes. These can be adapted for forensic applications, if necessary.

4. **Minimum number of animals:** If an officer is confronted with a large number of items, it is often useful to know how many different animals are involved. In this situation, genotypes are determined for each sample. For example, if after analysis, three different genotypes are found, this means at least three different animals are represented by the collection of samples.

5. **Parent-offspring relationships:** DNA analysis can be used to establish whether a given sample is from the parent of another sample. For example, additional charges may be laid if it can be shown that a dead bear cub found in the vicinity of a dead sow was the offspring of the sow. Again, genotypes from each sample are obtained and compared. An offspring's genotype must share certain characteristics with its parent. If these are not present, then the two animals do not have a parent-offspring relationship. If they are, then this type of relationship cannot be excluded. Establishment of a parent-offspring relationship requires a statistical analysis comparing the case samples to the population they came from. This kind of analysis depends on the availability of greater numbers of genetic markers for genotyping than is usually required for individual identity analysis (see number 3 above) and consequently is more difficult.

6. **Population identification:** Different populations of the same species of animal can be genetically distinct and these differences can be exploited to determine the geographic origin of an individual. This type of analysis might be useful in investigations where there is no known kill site. In order to establish what populations are like genetically, it is necessary to determine the genotypes of animals from those populations. These databases are constructed by DNA typing of samples in the same way as for individual identity. After this has been established, a statistical analysis can be done to determine which population the case samples are more likely to have come from. At present, however, the limited number of existing genetic databases restricts this kind of analysis to a few species in a small number of areas.

Statistical analyses require databases from different species and varying populations within species. The collection of DNA samples by wildlife officers and field biologists will help laboratories build the databases needed to make the techniques described in paragraphs 3, 5 and 6 more widely available.

LIMITING CONSIDERATIONS IN USING DNA ANALYSIS

1. DNA analysis cannot be used to determine the age of a sample.
2. It cannot be used if no DNA can be extracted.
3. DNA analysis can be costly and should be used only when necessary. For example, if it is possible to show that two samples originated from the same animal using a physical match of body parts, then DNA analysis should not be used.
4. A major drawback to DNA analysis is the cost of the equipment required to do the analyses. Local labs may find the cost of establishing DNA capability in-house to be prohibitive and this may lead to establishing regional centers of expertise. One problem with the 'regional lab' approach is the cost of bringing expert witnesses to testify at trials in the affected jurisdiction. This may require new funding initiatives or arrangements between jurisdictions.

EVIDENCE COLLECTION AND SUBMISSION

It is vital that sample integrity and evidence continuity be carefully safeguarded if DNA analysis is to be considered legally valid. Evidence collection and handling procedures must be designed to ensure this. If an

evidentiary sample is collected, submitted for analysis or handled in any way that allows it to become degraded or contaminated, accurate analysis may be impossible. There are a number of ways this can happen:

1. **Cross contamination of samples during collection.** To avoid any risk of contamination, knives used to cut samples from a carcass must be cleaned between samples.
2. **Degradation of samples due to improper storage and shipment techniques.** Once evidence is collected, it must be stored under conditions that prevent further degradation. Fresh tissue and especially wet blood samples are prone to decay if not kept frozen or at least cold. If cooling the sample is difficult under field conditions, then it may be possible to dry the sample. DNA is stable when dry and can easily be isolated from dried tissue or blood. Sample holders are available to handle wet specimens. It is also possible to store tissue in solutions of ethanol or DMSO/saturated salt at ambient temperatures.
3. **Cross contamination of samples during shipment of evidence.** Forensic samples are often shipped in plastic bags. Although this is acceptable for wet items as long as they are kept frozen or cold, samples with sharp edges such as knives, pieces of bone, and rocks, may pierce the packaging during transfer. Even pieces of tissue, which do not appear 'sharp' when fresh, are very hard when frozen and can wear holes in packing material if allowed to bang against other packages during shipment. Bags that appear intact when the contents are frozen can be surprisingly leaky by the time they arrive at their destination. Dry items shipped in paper containers such as bags or envelopes are subject to the same problems of piercing and mixing.

CONSIDERATIONS FOR PROPER SHIPPING

Evidence must be properly sealed and shipped in appropriate containers. If the items need to be kept cold, insulated containers such as coolers, with ice packs, must be used. A cardboard box containing the evidence items wrapped in newspaper is not suitable. Make sure that whatever is used as the source of cold cannot leak and damage the evidence. Often evidence is shipped to labs via a courier service. Even though these services 'guarantee' shipment within a certain time period, it is wise to prepare for a period longer than expected.

It is not necessary to send it all. DNA analysis does not require large sample sizes and if the officer retains part of the evidence, this can be used in the event of loss or damage to the original shipment. In addition, evidence is available if the defense counsel desires to have it tested by an independent lab.

Proper documents must accompany the evidence. This applies to evidence crossing international borders that is subject to inspection by customs officials as well as evidence involving species whose movement is restricted by international agreements or other laws. Take all possible steps to avoid loss of evidence continuity. Have all necessary permits in advance.

Officers should contact the lab prior to shipping any evidence. Lab personnel will be able to discuss the lab's ability to do the required analysis, predict the time it will take, estimate costs and give advice on the best method of shipment. Labs should also be available to answer questions regarding the collection of evidence. Agree on the method of shipment and confirm the approximate arrival date. This can enable the receiving lab to trace lost shipments before it is too late.

LEGAL REQUIREMENTS FOR PROSECUTION

Evidence submitted for DNA testing is subject to the same requirements as all physical evidence. This includes chain of custody (evidence continuity) and evidence preservation. Failure to maintain a proper chain of custody will likely result in inadmissible evidence. If evidence is not collected and maintained in a proper manner, it is possible that it will not be usable for forensic analysis. Evidence handling procedures, at all stages of an investigation may be questioned in court. The mere use of DNA analysis does not replace proper investigative technique and will not save an investigation if proper procedures are not carried out.

Wildlife officers and laboratories share responsibility in getting proper evidence to court. It is the officer's responsibility to ensure that evidence collection and other portions of the investigation are handled properly. It is the forensic lab's responsibility to have properly-trained lab personnel performing the analyses, since it is essential that analysts be able to qualify as expert witnesses should the case go to court. Forensic labs must also handle and store evidence properly, maintaining the chain of custody once the evidence is received by the lab.

These labs have records of how analytical procedures are performed. These procedures are standardized within the lab. Wildlife forensic labs are beginning to standardize techniques between labs performing the same types of analyses. Some labs have received accreditation from outside agencies. Others are contemplating such a move; however, even in absence of formal accreditation, labs can take steps to ensure that the work done is able to withstand the scrutiny of the courts.

The legal requirements for the presentation of evidence in court may vary in different jurisdictions. Changes in existing laws relating to evidence and case law interpreting these laws, may affect how investigations are carried out, what evidence can be collected and how evidence is presented in court. Officers must be made aware of any new technical legal requirements.

CONCLUSION

DNA analysis, and DNA typing in particular, has a wide acceptance in the scientific community. DNA typing is already being used as a tool to answer many questions unrelated to forensics. And evidence from DNA testing has been used successfully for some time in court, for human criminal cases. It is playing an increasing role in the investigation and prosecution of wildlife crimes and should be exploited by officials involved in this area.

For more information, contact the following agency in your jurisdiction:

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