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## DNA TESTING IN ANIMAL FORENSICS

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**Abstract:** The use of short tandem repeats (STRs) for the identification of animals has developed alongside similar STR applications for humans. Population studies, kinship analysis, paternity testing, and unique identification have been applied to humans and many other animals. The field of forensic science has adapted this information extensively in the prosecution of suspected criminals with great success. The power of human DNA testing is demonstrated routinely by the convictions and exonerations of individuals. We discuss how the use of nonhuman DNA testing is beginning to find a place in the prosecution of individuals. Animal evidence can be an important element in a case when used to establish an association between a crime scene and a suspect or in crimes involving a specific animal. Although the testing of animal evidence may be routine, its use in court is far less common. As the presentation of animal evidence in court increases, appropriate standards and guidelines must be applied to assure the admissibility of the DNA testing results in court. This requires rigorous validation of precision and accuracy, allele heritability and independence, accurate sampling, evidence handling, and appropriate statistical evaluation of the results before the criminal courts will accept and apply the power of DNA testing in cases involving animals.

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**Key words:** admissibility, animal forensics, animal identification, databases, legal cases, short tandem repeats.

Crime fighting in the new millennium has required law enforcement officials to employ highly sophisticated applications to process evidence from a wide range of samples and situations. The development of the biometric identification techniques that are used today arose out of the need for methods to uniquely identify individuals from biological samples left behind at crime scenes. When compared with biological samples from suspects, these data must show that either the suspect cannot be excluded as a contributor or that the suspect is exonerated. We describe the current technology and highlight similarities and differences in the use of DNA evidence in human and wildlife forensics to support law enforcement efforts to apprehend and prosecute individuals.

### HUMAN FORENSICS

The development of DNA genotyping is arguably the most significant advancement in the history of Forensic Science (Jobling and Gill 2004, Butler 2005). Human DNA profiling systems have quickly evolved since the first methods were described just 20 years ago (Jeffreys 1985). The system of multilocus probe (MLP) analysis detected a number of highly polymorphic loci simultaneously that resulted in a DNA fingerprint, or genotype, allowing an individual to be uniquely identified (Jeffreys 1985b). The strength of the evidence was demonstrated by the statistical treatment for

a match probability based on allelic distributions, which could be offered in court to support the technology (Jeffreys 1985c).

The initial typing methods required isolating relatively intact genomic DNA. Digestion with restriction enzymes produced distinct fragments, which were separated by size through gel electrophoresis. The pattern of fragments was visualized through hybridization with a labeled probe for a specific sequence of the DNA that was repeated numerous times throughout the genome. The restriction fragment pattern produced in this manner was found to be unique for any given individual. Sequence differences between individuals occurring at the restriction sites yielded an altered fragment pattern referred to as a restriction fragment length polymorphism (RFLP).

Further development of the RFLP technology identified specific minisatellite loci that were found to be a major source of genetic variation, providing an even greater power of discrimination between individuals. These single-locus probe (SLP) genotype profiles began to replace the original MLPs around 1990. One drawback to the use of SLPs, however, was the difficulty in obtaining accurate length-measurements of typical minisatellites (0.5–30 kilobases). To overcome this problem, a binning system was adopted to conservatively evaluate DNA evidence and allow comparisons between samples. However, the arbitrary nature of the size determinations and subsequent data interpretation proved problematic

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and led to scientific and legal challenges when employed in court.

By 1994, a new, more accurate system of DNA genotyping based on microsatellites or short tandem repeats (STRs) began replacing RFLP analysis (Edwards et al. 1991, 1992). Typically, STRs are composed of short nucleotide repeat units, 2 to 6 bases in length, and, like the minisatellites, are shared through direct genetic inheritance. Although less polymorphic than minisatellites, similar powers of discrimination could be achieved by analyzing more genetic loci. The STRs have several important advantages over the previous DNA typing systems. The most significant of these is that STR sequences are short enough (c.a. 100–400 bases) to be amplified using the polymerase chain reaction (PCR). Therefore, STR analysis can be completed with relatively minute quantities (0.1 nanogram) of partially degraded DNA while RFLP analysis of minisatellites requires a minimum of 50 ngs of intact DNA (Budowle et al. 2000). Accurate size determinations of the amplified fragments to a resolution of <1 base pair is achieved by using the same techniques used to sequence DNA. With the use of a combination of fluorescent-labeled primers emitting light at 4 or 5 different wavelengths, it is not uncommon to see a multiplex of 10 to 16 loci amplified in a single PCR reaction (e.g., Identifier, Applied Biosystems Inc., Foster City, California, USA; PowerPlex16, Promega Corporation, Madison, Wisconsin, USA). By 1997, 13 core STR loci were defined and validated by the Federal Bureau of Investigation (FBI) for human identification in the US legal system. In 1998, the FBI launched the national COmbined DNA Index System (CODIS) to database the DNA profiles of convicted criminals. The database of DNA profiles from convicted offenders or DNA profiles generated during investigations of other criminal cases can be searched for a match to DNA evidence from a recent crime scene. This system has been used successfully to identify a suspect in criminal cases where no other physical evidence was available.

## NONHUMAN FORENSICS

Nonhuman evidence collected at crime scenes can provide valuable clues during investigations that otherwise might have ended without results. The development of identification systems for animals has closely paralleled those used for human identification. It was noted during the development of human identity markers that repetitive DNA components and simple sequence repeats were found in all eukaryotic organisms (Tautz and

Renz 1984, Tautz 1989). These nonhuman sources were found to have hypervariability and genetic inheritance patterns similar to the human systems that allowed for unique identification and parentage analysis. Tautz (1989) reported on the variability of repeats in *Drosophila melanogaster* and pilot whales (*Globicephala melaena*). Polymorphic simple repeat sequences have also been confirmed in insects (Blanchetot 1991) and plant genomes (Weising et al. 1989, Lagercrantz et al. 1993). Subsequently, DNA genotyping has been used in population genetics studies or for individual identification in a vast array of organisms from birds (Burke and Bruford 1987), green sea turtles (*Chelonia mydas*; 2004), avocados (*Persea americana*), and recently marijuana (*Cannabis sativa*; Coyle et al. 2003, Gilmore et al. 2003).

Certain domesticated animals with high social value to humans have been of special interest for forensic analysis because of their potential association during human criminal activity. In 1987, Alec Jeffreys extended his pioneering work in human DNA genotyping to dogs and cats by identifying dispersed autosomal polymorphic fragments made up of simple repeat sequences (Jeffreys and Morton 1987). The characterization of microsatellite markers for the identification of the common house cat (*Felis catus*) in 1995 (Menotti-Raymond and O'Brien 1995) led to the first reported case of animal DNA genotyping data to be used as evidence in a U.S. court proceeding. During a murder investigation on Prince Edward Island, Canada, a leather jacket spotted with the victim's blood was found at the crime scene. The police had no evidence linking the key suspect (the estranged common-law husband) to the leather jacket. However, numerous strands of nonhuman white hair were found on the jacket. The DNA genotyping determined that the white hairs not only originated from a cat but were from a specific cat that lived with the suspect. The evidence provided the link between the suspect, the jacket, and the murder victim and helped in the conviction of the suspect.

Researchers are now characterizing whole genomes of several domesticated animal species. These include genome mapping and sequencing projects for dogs (Kirkness et al. 2003, Parker et al. 2004, Switonski et al. 2004) and cats (Menotti-Raymond et al. 2003). Information about the bovine and many other animal genome projects can be found online (<http://www.ncbi.nih.gov/Genomes/>). In addition, STR loci suitable for population studies, parentage analysis, registry databases, and unique

identification in forensics have now been reported for many animals, including dogs, cats, horses, cattle, elk and deer (Blackett and Keim 1992, Lang et al. 1996) bear and moose (Guglich et al. 1994) and alpacas (Poetsch et al. 2001). These studies provide the basis for direct comparisons between human genome characteristics and other animals. The documented similarities between human and animal genome characteristics allow animal forensics to share broad acceptance of the principles that are the basis for accepting human genetic data in court. However, there are some challenges to the broad application of DNA evidence in animal forensic cases.

## ADMISSABILITY

### Legal Standards

Trial courts have assessed the scientific soundness of STR genotyping in humans to assure that presented evidence is based on methods and reasoning that are capable of reaching the correct conclusion. Scientific soundness and general acceptance are addressed in the Frye standard (Frye v. United States, 54 App. D.C. 46, 293 Fed. 1013, 1923), which states that scientific evidence must be, "sufficiently established to have gained general acceptance in the field to which it belongs." Generally speaking, the rule is that to be admissible as scientific evidence, the technology must be at such a stage of advancement as to be demonstrable, not experimental. An easy benchmark is peer-reviewed scientific publications of designed, controlled studies to determine specific characteristics of the system. These can help define the scope of an application as well as its limitations.

More recently, the federal courts have adopted the Daubert standard (Daubert v. Merrell Dow Pharmaceuticals Inc., 509 U.S. 579, 113 S. Ct. 2786, 125 L.Ed. 2d 469, 1993). This standard requires that scientific evidence have sufficient scientific validity and reliability so as to be admitted as scientific knowledge. In contrast to the Frye standard, the Daubert standard calls for an independent judicial review and assessment of reliability of the methods used. In addition to the general acceptance principle, the Daubert standard requires that the techniques used to produce evidence have been through a thorough statistical evaluation. Error rates and procedures must be fully described to establish reliability. However, the current standards for admissibility of evidence are set at the state level. Some states follow the Frye standard, some follow the Daubert standard, and

some have developed their own standards of admissibility.

Despite having been defined in human cases, these legal standards are equally important in cases involving animal forensic evidence. An example of the developing nature of admissibility of animal forensic evidence is found in the case of *Washington v. Tuilefano and Lealuaialii* (1998). The defendants were convicted of murder based in part on the DNA identification of dog blood and hair on their clothing and shoes. An appeal was partially based on arguments that the test results should be considered inadmissible because the testing methods were not scientifically accepted. In the review, the Court of Appeals Division I in Washington stated, "Based on this information, we are not convinced that forensic canine DNA identification is a theory that has received general acceptance in the scientific community, or that reliable techniques or experiments exist to identify individual canines for forensic purposes." (Denver District Attorney, 2003) The appeals court stated that because the testing lab "has not yet published sufficient data to show that its DNA markers and associated probability estimates are reliable, we would suggest that other courts tread lightly in these waters and closely examine canine DNA results before accepting them at trial." (Denver District Attorney 2003) The lack of a Frye standard hearing during the trial was critical in the final analysis. Subsequent to this ruling, a scientific publication identifying the STR loci used for the dog identification in this particular case was published to specifically address the concerns of the Washington Court (et al. 2004). Other studies of additional canine microsatellite markers have also been published (Altet et al. 2001, Ichikawa et al. 2001). These peer-reviewed, scientific publications now provide the necessary data to meet the Frye and Daubert standard requirements.

### Establishing Exclusion Probabilities

In forensic terms, DNA identification is an exclusionary test. Therefore, the initial interpretation of the genetic analysis can be quite simple. If the genetic profile of the evidence does not match that from the suspect (within the interpretation guidelines for the analysis), the suspect is excluded and the investigation moves on to another suspect or set of evidence. However, if the genetic profiles are exactly the same, then the suspect cannot be excluded and the investigation builds its case around this suspect. The question that must then be answered is, "What is the probability that

the evidence and the suspect share the same genetic profile by chance?"

In the prosecution of human criminal cases, it is not uncommon to estimate the likelihood that the suspect (rather than some other person) is the source of the evidence left at the crime scene using statistical methods that have been determined to be scientifically and mathematically valid (Collins and Morton 1994, Evett and Weir 1998). In another approach, statistical methods consider the allele frequencies within a population to calculate a random match probability or a probability of exclusion. In human cases, the allele frequencies from 13 to 15 independent STR markers can result in a random match probability on the order of 1 in  $10^{12}$  to  $10^{18}$  (exclusion probabilities >99.999%). In animal cases, the necessary population allele frequencies either are not available or may be of limited use. For instance, several studies have been published (Anderson et al. 2002, Bonnet et al. 2002, DeYoung et al. 2003) describing statistical analysis for genetic diversity and variability within and between numerous wild populations of cervids. Although these data would meet the criteria suggested for the use of allele frequencies in the calculation of match probabilities, there remains the problem of determining the statistical significance of the match probability if the evidence does not come from the specific populations represented in the database.

Statistical support is further complicated in cases involving animals from very small populations (i.e., endangered species) or from populations of highly inbred animals (i.e., in natural or human-controlled breeding programs). Nonrandom mating results in allelic disequilibrium and reduced genetic variability. To address this problem, the forensic community is assembling DNA databases from various breeds of cats, dogs, deer, cattle, and even populations of African elephants (New Scientist 2004). These databases will provide the basis for statistical evaluation of the significance of the evidence when a match links a suspect to a crime.

### Collection and Processing of Evidence

Once in court, few challenges regarding the admissibility of animal DNA evidence in court cases actually focus on the science involved in the testing or the interpretation of data. Challenges are more likely to come from perceived collection or reporting errors. The DNA evidence is worthless without proper logistical support. Evidentiary procedures and DNA collection methods for animal samples are identical to those involving human

DNA evidence. Detailed guidelines for processing human crime scenes that were written and approved by the Technical Working Group on Crime Scene Investigation provide excellent guidance for the processing of a crime scene where animals are the main focus. (National Criminal Justice Reference Service 2000).

Each investigation should begin with an assessment of the scene and focus on preserving the integrity of the evidence. When collecting the evidence, contamination control and preventing cross-contamination at the scene is essential. The handling of physical evidence is one of the most important factors of an investigation. Samples must be collected, inventoried, preserved, transported, and submitted for testing without compromising the evidence. By following the guidelines for processing human crime scenes, errors that might result in inadmissibility can be prevented.

Other challenges often raised during the presentation of DNA evidence in court involve errors in data analysis and interpretation and errors arising during the handling and processing of samples. Disputes over the analysis and interpretation of data can be addressed because the evidence can usually be re-analyzed using different methods. Due to the complexity involved in the processing of evidence, the laboratory could introduce errors that would result in reporting errors. This is the case regardless of the animal involved in the investigation. The issues of frequencies and likelihood of error presented by Koehler in 1996 have been debated for some time (Koehler 1996). Human errors introduced during the processing of evidence are the most serious and difficult to assess. Mislabeling tubes, cross-contamination, or including the wrong data in a case are all possible human errors that would result in reporting errors. In general, human errors are more likely to occur when many samples are being processed at the same time. It is critical, therefore, that the quality control systems and standard operating procedures written by each laboratory address and attempt to minimize human error.

Quality assurance guidelines for general laboratory practices for human DNA analysis were developed to respond to admissibility challenges. The first set of guidelines was published by the American Society of Crime Lab Directors (American Society of Crime Laboratory Directors 1987). Additional guidelines have been written by the National Research Council's Committee on DNA Forensic Science (National Research Council 1992), with an update published in 1996 (National Research

Council 1996). An industry-wide standard was written by The DNA Advisory Board and adopted in October 1998 (DNA Advisory Board 2000).

### Additional Challenges in Animal Forensics

The aforementioned quality assurance standards and guidelines were developed to secure the highest accuracy and reliability of the tests performed for human identification. Laboratories performing DNA analysis for animal forensics have largely adopted these same standards and guidelines whenever possible. However, animal forensics poses some unique challenges. Whereas human testing deals with a single species, nonhuman testing deals with many species, some of which are more closely related than others. The type of sample and collection methods can also differ greatly. In general, DNA for human identification is derived from blood, semen, or epithelial cells. In addition to these sources, samples for animal identification may come from more unique starting material, including taxidermy trophies, scat, processed products (e.g., tanned hides or meat products), and possibly as components of traditional Chinese medicines (e.g., rhinoceros horn, bear gall bladders, or tiger bones). In order to extract and preserve DNA from these varied sources, animal forensic labs modify existing procedures or develop new procedures for isolating DNA.

Once the biological samples have been properly collected and the DNA isolated, the unique identification of the animal would optimally require STR probes or primers specific to each species. However, the development of markers for individual species is time-consuming and costly. As a result, many STR identification procedures have taken advantage of the finding that STR markers developed in one species can be used effectively and are informative in closely related species (e.g., bovine markers work for some cervids) due to extensive DNA sequence similarity. Although these STR homologues can be very effective, they have limitations in that they may be prone to an increased rate of null or nonamplifying alleles or result in poor repeatability due to imperfect primer sequence pairing between species.

Currently, most animal STR identification systems have been developed using short dinucleotide repeat sequences that are abundant in animal genomes. Due to the close spacing of the individual alleles, the nature of the PCR reaction often produces artifacts, commonly referred to as stutter peaks or bands. These artifacts interfere with and complicate the interpretation of the data. For

this reason, human STR systems now utilize repeats of 4 or 5 nucleotides that produce much less stutter and provide better separation between alleles, making the data interpretation less subjective.

An added complication in interpreting data for animal systems using dinucleotide repeats comes from random repeat-unit mutations that can occur in the germ-line cells. For instance, germ-line mutations can involve insertions or deletions of 1 or more repeat units or of only a single nucleotide. These types of genetic mutations are rare, but without detailed investigations, the mutation rate remains undefined and potentially affects likelihood-ratio analysis of match probabilities and other statistical treatment of data. Therefore, data interpretation guidelines must be developed for each animal STR system. These guidelines should address the potential for inconsistencies due to genetic mutations and PCR reaction artifacts.

For human analysis, the American Association of Blood Banks (AABB) has gathered data on human mutation frequencies from more than 340,000 paternity cases reported through 2003. Unfortunately, detailed data for the various nonhuman animal species is largely lacking, with the possible exception of some domestic livestock breeds. In animal populations where large-scale analyses are not feasible, the only recourse is to test population data at each STR marker for departures from Hardy-Weinburg equilibrium, a standard measure of random allele distribution. Markers that exhibit frequent disequilibrium should not be used, as this may be indicative of problems with either the detection or inheritance of STR loci. Additional improvements could be realized if tetra- and penta-nucleotide repeats can be isolated, characterized, and validated in animal systems.

Some problems associated with the interpretation and repeatability of dinucleotide STR data would be eliminated or minimized if allelic ladders were available. An allelic ladder is a sample that produces a profile containing most of the common alleles for each STR marker tested. In human analysis, this ladder profile contains 208 alleles (Promega PowerPlex 16, Madison, Wisconsin, USA). Profiles generated from unknown samples are compared electronically to the ladder and assigned a corresponding allelic designation. Current, internal size-standards do not provide sufficient resolution to assign allelic designations by themselves and are affected by instrument-to-instrument variations. Most animal STR marker systems developed to date are lacking an allelic ladder. An allelic ladder is the only method for providing unequivocal allele as-

signments regardless of the detection platform used to run the analysis, and it significantly improves the reliability and repeatability of the data. The development of reliable allelic ladders is a costly and technically demanding process contributing to the lack of their development for most animal identification systems.

In the absence of an allelic ladder, exhaustive testing between analysis platforms and the development of correction factors is required to allow comparison of data profiles between different laboratories. This type of comparison is prone to errors and requires the coordination of sample exchanges and data comparisons between all the laboratories performing DNA testing on the same animal species. Therefore, conclusions based upon the data produced by 2 different laboratories could be in conflict if the interpretation of the results is not directly comparable (e.g., if the prosecutor and defense choose to have DNA evidence tested at different laboratories).

### White-tailed Deer Forensics: A Case Study

Our work in establishing and validating a panel of STR markers for white-tailed deer illustrates the many steps necessary for validation and acceptance. Our primary goal was to produce a panel of STR markers for use in legal cases involving white-tailed deer (*Odocoileus virginianus*). Beginning with a published panel of STRs, (Anderson et al. 2002), we developed a panel of STRs for commercial application (DNA Solutions, Oklahoma City, Oklahoma, USA.) and for potential use in criminal investigations. In the original population genetics investigation, 21 microsatellite markers were analyzed to determine allelic distribution, polymorphic information content, heterozygosity, and adherence to Hardy-Weinberg equilibrium (Anderson et al. 2002). From these data, we selected a subset of 13 STR markers based on the number of alleles and reproducibility of data. For the commercial and forensic panel, we developed standard protocols and established a DNA genotype database for statistical evaluation consisting of several thousand individuals.

Concurrently, we conducted several pilot studies to validate methods for evidence processing and storage. These studies tested several factors that were important for the successful and consistent extraction of DNA from white-tailed deer samples (B. Cassidy, DNA Solutions, unpublished data). For instance, samples from surfaces spotted with deer blood were used to evaluate DNA extraction techniques. Two different DNA isolation techniques were compared with each of several differ-

ent mock evidence samples. The results indicated that the surface, the method of recovery, the amount of blood, and the time after the blood was spotted on the surface were all factors affecting the ability to consistently isolate DNA and perform white-tailed deer genotyping. In one example, it appeared that a component of the dye within denim cloth interfered with the genotyping reaction, as measured by the ability to generate full genotype profiles. The DNA was successfully isolated from blood recovered from the surface of the leaves, sand, and concrete even after an extended time. However, blood on soil surfaces produced little quality DNA, most likely due to microbial degradation of the DNA. To date, we have processed over 8,000 deer samples using these protocols on a variety of sample materials.

### RECENT LEGAL CASES INVOLVING DNA TESTING IN ANIMAL FORENSICS

Despite the considerable challenges involved in using DNA evidence in animal forensic investigations, DNA evidence is being utilized in a growing number of instances. To date, relatively few criminal cases involving animal DNA evidence have actually gone to trial in U.S. courts. Typically, the crime committed does not carry a serious enough punishment to encourage the suspect to rigorously fight the charges in court. Most commonly, a suspect will choose to plead the case with the prospect of only fines, some loss of property, and possible restriction on hunting rights. Additionally, law enforcement agencies generally do not have sufficient budgets to process large volumes of animal DNA evidence in order to prosecute individuals. Cases involving human murders or federal violations, where punishments can be much more severe, are more likely to have animal evidence presented and potentially challenged in court. Several such notable cases include: the discovery of a cache of illegal drugs while executing a search warrant to obtain evidence for DNA testing associated with illegal deer hunting activity (State v. Devers, 1997), the admissibility of canine DNA testing evidence in a double homicide conviction (Washington v. Tuilefano and Lealuaialii, 1998), the admissibility of cat hair DNA testing in a murder case (Beamish v. Her Majesty the Queen, 1999), and the admissibility of swine DNA testing in a criminal conviction for making false statements to the government (US v. Boswell, 2001).

The use of DNA testing, when applied to cases involving animal theft, fraud, ownership dispute, and

poaching cases dates back to at least 1993, where prosecutors in Ontario, Canada, used DNA genotypes to link animal remains at an illegal kill site to evidence samples associated with a suspect (Guglich et al. 1993, Guglich et al. 1994). The first use of DNA analysis for the prosecution of cattle theft in the State of Louisiana was reported in June of 2000 (The Advocate, Baton Rouge). The DNA testing proved that unbranded calves in a sale transaction were in fact animals that had been reported stolen from a local rancher. The first cattle theft prosecution in California using DNA evidence was reported in the December 28, 2001 (San Francisco Chronicle). The DNA testing and parentage analysis established that a calf sold at auction was parented by a cow that had been stolen from a nearby ranch at least 16 months earlier.

Parentage analysis has been used for some time to identify the origin of suspected stolen animals. In Spain, stolen calves were identified by comparing profiles with cows on the victim's farm (Beamonte et al. 1995). Recently, our laboratory processed a case where a rancher in Texas filed suit claiming ownership of a calf nursing on his neighbor's cow. Subsequent parentage analysis revealed that the neighbor's cow was excluded as a possible parent and, furthermore, the claimant's cow could not be excluded as the possible parent. The calf was returned to the rancher with no further action.

In 2000, an Oklahoman individual was charged with illegal hunting for harvesting a deer using a firearm during bow season. Evidence was recovered from a taxidermist consisting of a set of antlers and a cape. Examination of the cape showed a clear bullet hole. The cape was subsequently sent to the National Wildlife Laboratory (Ashland, Oregon) where trace metal analysis around the hole confirmed it was made by a bullet and not an arrow point. The defense argued that although the antlers belonged to the suspect, the cape was not the one submitted to the taxidermist. Our laboratory analyzed the cape and antlers and determined DNA genotype profiles. When compared, the profiles were found to be identical. Based on this analysis, we concluded that the cape and antlers could not be excluded as originating from the same source.

In 2003, a Pennsylvania white-tailed deer farmer believed he had found a buck that had been stolen from his farm 4 years earlier. The claim was based on the fact that the antler size and conformation of this 6-year old buck was too similar to his missing animal to be coincidental. Although there was a statement that the deer was acquired

through legal means, the claimant asked law enforcement to help settle the ownership dispute. Our laboratory produced DNA genotype profiles from a court-ordered biopsy sample of the 6-year old deer and from a set of antlers that had been shed by the missing deer prior to its disappearance. They were determined to be identical and the animal was subsequently returned to the claimant. A Wall Street Journal Article (Ahmed 2003), describing the case, estimated that the buck, his offspring produced over the last 4 years, and the semen collected for future breeding were estimated to be worth over \$500,000.

One of the main goals of wildlife conservation programs around the world is to preserve and protect endangered animal species. As an example of these efforts, a project is currently underway to identify, sample, and DNA type African elephants (New Scientist 2004). Elephant DNA genotypes throughout Africa are being determined and now, through comparison with DNA profiles from confiscated ivory, law enforcement can pinpoint its likely point of origin. It is hoped that this information will help trace and possibly lead to the apprehension of the poachers.

## MANAGEMENT IMPLICATIONS

Proper handling of evidence and sound statistical treatment in population studies provides a basic starting point for the use of STRs in criminal investigation. However, these measures alone are not sufficient for forensic application. The accuracy and reproducibility of the procedures for processing evidence must be demonstrated. Compilation and analysis of data must be standardized, or the system will rely too heavily on interpretation or expert opinion and, thus, will be prone to errors and inconsistencies. If animal DNA analysis is to continue to be used effectively by law enforcement, the analysis methods must meet the same strict requirements that are currently used in human identification.

Fortunately, the aforementioned problems and challenges in animal forensics analysis are generally recognized, and several professional organizations have dedicated themselves to the improvement of animal forensics systems. Wildlife enforcement officers routinely meet to share information and discuss the most effective uses of DNA testing technology for the investigation of illegal activities involving animals. The International Society for Animal Genetics (ISAG) holds annual meetings where researchers and investigators share information and discuss requirements



for evaluating animal identification and DNA technology. This organization helps coordinate comparison testing in specific animal species to evaluate STR marker panels and makes recommendations based upon the results from laboratories all over the world. Many forensic societies (e.g. Northwest Association of Forensic Scientists, 2005) are now including numerous abstracts in their annual meeting programs detailing nonhuman DNA testing. As a whole, the forensic field is working toward the standardization of STR analysis on a multispecies level and organizing more comprehensive forensic proficiencies. DNA technology has clear implications toward wildlife management and conservation by providing the tools needed for enforcement and legal prosecution.

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## LITERATURE CITED

- ANDERSON, J. D., R. L. HONEYCUTT, R. A. GONZALES, K. L. GEE, L. C. SKOW, R. L. GALLAGHER, D. A. HONEYCUTT, AND R. W. DEYOUNG. 2002. Development of microsatellite DNA markers for the automated genetic characterization of white-tailed deer populations. *Journal of Wildlife Management* 66:67–74.
- ALTET, L., O. FRANCINO, AND A. SANCHEZ. 2001. Microsatellite polymorphism in closely related dogs. *Journal of Heredity* 92:276–279.
- AMERICAN SOCIETY OF CRIME LABORATORY DIRECTORS. 1987. Guidelines for forensic laboratory management practices. *Crime Lab Digest* 14:39–46.
- ASHWORTH, V. E. T. M., AND M. T. CLEGG. 2003. Microsatellite markers in avocado (*Persea americana* Mill.): genealogical relationships among cultivated avocado genotypes. *Journal of Heredity* 94:407–415.
- BEAMONTE, D., A. GUERRA, B. RUIZ, AND J. ALEMANY. 1995. Microsatellite DNA polymorphism analysis in a case of an illegal cattle purchase. *Journal of Forensic Science* 40:692–694.
- BLACKETT, R. S., AND P. KEIM. 1992. Big game species identification by deoxyribonucleic Acid (DNA) probes. *Journal of Forensic Science* 37:590–596.
- BLANCHETOT, A. 1991. A *Musca domestica* satellite sequence detects individual polymorphic regions in insect genome. *Nucleic Acids Research* 19:929–932.
- BONNET, A., S. THEVENON, F. MUADET, AND J. C. MAILLARD. 2002. Efficiency of semi-automated fluorescent multiplex PCRs with 11 microsatellite markers for genetic studies of deer populations. *Animal Genetics* 33:343–350.
- BUDOWLE, B., J. SMITH, T. MORETTI, AND J. DIZINNO. 2000. DNA typing protocols: molecular biology and forensic analysis. Eaton, Natick, Massachusetts, USA.
- BURKE, T., AND M. W. BRUFORD. 1987. DNA fingerprinting in birds. *Nature* 327:149–152.
- BUTLER, J. M. 2005. Forensic DNA typing: biology, technology, and genetics of STR markers second edition. Elsevier Academic Press, New York, USA.
- COLLINS, A., AND N. E. MORTON. 1994. Likelihood ratios for DNA identification. *Proceedings of the National Academy of Science* 91:6007–6011.
- COYLE, H. M., T. PALMBACH, N. JULIANO, C. LADD, AND H. C. LEE. 2003. An overview of DNA methods for the identification and individualization of marijuana. *Croatian Medical Journal* 44:315.
- DENVER DISTRICT ATTORNEY, COURT OF APPEALS DIVISION I STATE OF WASHINGTON. Opinion sheet, 2003 <<http://www.denverda.org/legalresource/Leulualii.pdf>>. Accessed 2005 August 2.
- DENISE, S., E. JOHNSTON, J. HALVERSON, K. MARSHALL, D. ROSENFELD, S. MCKENNA, T. SHARP, AND J. EDWARDS. 2004. Power of exclusion for parentage verification and probability of match for identity in American kennel club breeds using 17 canine microsatellite markers. *Animal Genetics* 35:14–17.
- DEYOUNG, R. W., S. DEMARAIS, R. L. HONEYCUTT, A. P. ROONEY, R. A. GONZALES, AND K. L. GEE. 2003. Genetic consequences of white-tailed deer (*Odocoileus virginianus*) restoration in Mississippi. *Molecular Ecology* 12:3237–3252.
- DNA ADVISORY BOARD. 2000. Quality assurance standards for forensic DNA testing laboratories. Forensic Science Communications. Volume 2(3). <<http://www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2b.htm>>. Accessed 2005 Jul 1.
- EDWARDS, A., A. CIVITELLO, H. A. HAMMOND, AND C. T. CASKEY. 1991. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *American Journal of Human Genetics* 49:746–756.
- , HAMMOND, H. A., L. JIN, C. T. CASKEY, AND R. CHAKRABORTY. 1992. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. *Genomics* 12:241–253.
- EVETT, I. W., AND B. S. WEIR. 1998. Interpreting DNA evidence, statistical genetics for forensic scientist. Sinauer, USA.
- GILMORE S., R. PEAKALL, AND J. ROBERTSON. 2003. Short tandem repeat (STR) DNA markers are hypervariable and informative in *Cannabis sativa*: implications for forensic investigations. *Forensic Science International* 131:65–74.
- GUGLICH, E. A., P. J. WILSON, AND B. N. WHITE. 1993. Application of DNA fingerprinting to enforcement of hunting regulations in Ontario. *Journal of Forensic Science* 38:84–99.
- . 1994. Forensic application of repetitive DNA markers to the species identification of animal tissues. *Journal of Forensic Science* 39:353–361.
- ICHIKAWA, Y., K. TAKAGI, S. TSUMAGARI, K. ISHIHAMA, M. MORITA, M. KANEMAKI, M. TAKEISHI, AND H. TAKAHASHI. 2001. Canine parentage testing based on microsatellite polymorphisms. *Journal of Veterinarian Medical Science* 63:1209–1213.
- JEFFREYS, A. J., V. WILSON, AND S. L. THEIN. 1985. Hypervariable 'minisatellite' regions in human DNA. *Nature* 314:67–73.
- . 1985b. Individual-specific 'fingerprints' of human DNA. *Nature* 316:76–79.
- , J. F. BROOKFIELD, AND R. SEMEONOFF. 1985c. Positive identification of an immigration test-case using human DNA fingerprints. *Nature* 317:818–819.

- , and D. B. MORTON. 1987. DNA fingerprints of dogs and cats. *Animal Genetics* 18:1–15.
- JOBLING, M. A., AND P. GILL. 2004. Encoded evidence: DNA in forensic analysis. *Nature Reviews Genetics* 5:739–751.
- KIRKNESS, E. F., V. BAFNA, A. L. HALPERN, S. LEVY, K. REMINGTON, D. B. RUSCH, A. L. DELCHER, M. POP, W. WANG, C. M. FRASER, AND J. C. VENTER. 2003. The dog genome: survey sequencing and comparative analysis. *Science* 5641:1854–1855.
- KOEHLER, J. J. 1996. On conveying the probative value of DNA evidence: frequencies, likelihood ratios and error rates. *University of Colorado Law Review* 67:859–886.
- LAGERCRANTZ, U., H. ELLEGREN, AND L. ANDERSSON. 1993. The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. *Nucleic Acids Research* 21:1111–1115.
- LANG, K. D., Y. WANG, AND Y. PLANTE. 1996. Fifteen polymorphic dinucleotide microsatellites in llamas and alpacas. *Animal Genetics* 27:293.
- MENOTTI-RAYMOND, M. A., AND S. J. O'BRIEN. 1995. Evolutionary conservation of ten microsatellite loci in four species of Felidae. *Journal of Heredity* 86:319–322.
- , DAVID, V. A., R. AGARWALA, A. A. SCHAFER, R. STEPHENS, S. J. O'BRIEN, AND W. J. MURPHY. 2003. Radiation hybrid mapping of 304 novel microsatellites in the domestic cat genome. *Cytogenetic Genome Research* 102:272–276.
- NATIONAL CRIMINAL JUSTICE REFERENCE SERVICE. 2000. Department of Justice, Office of Justice Programs, National Institute of Justice "Crime Scene Investigation: A Guide for Law Enforcement." <<http://www.ncjrs.org/pdffiles/nij/178280.pdf>>. Accessed 2005 August 2.
- NATIONAL RESEARCH COUNCIL. 1992. DNA technology in forensic science. National Academy Press, Washington, D.C., USA.
- . 1996. The evaluation of forensic DNA evidence. National Academy Press, Washington, D.C., USA.
- NEWSSCIENTIST NEWS SERVICE. 2004. DNA testing to pinpoint origin of illegal ivory. *NewScientist* 16:21.
- NORTHWEST ASSOCIATION OF FORENSIC SCIENTISTS, Fall 2004 Meeting Presentations <<http://www.nwafs.org/abstracts/Fall04.htm>>. Accessed 2005 August 5.
- PARKER, H. G., L. V. KIM, N. B. SUTTER, S. CARLSON, T. D. LORENTZEN, T. B. MALEK, G. S. JOHNSON, H. B. DEFRAANCE, E. A. OSTRANDER, AND L. KRUGLYAK. 2004. Genetic structure of the purebred domestic dog. *Science* 304:1160–1164.
- POETSCH, M., S. SEEFELDT, M. MASCHKE, AND E. LIGNITZ. 2001. Analysis of microsatellite polymorphism in red deer, roe deer, and fallow deer—possible employment in forensic applications. *Forensic Science International* 116:1–8.
- ROBERTS, M. A., T. S. SCHWARTZ, AND S. A. KARL. 2004. Global population genetic structure and male-mediated gene flow in the green sea turtle (*Chelonia mydas*): analysis of microsatellite loci. *Genetics* 166:1857–1870.
- SWITONSKI, M., I. SZCZERBAL, AND J. NOWACKA. 2004. The dog genome map and its use in mammalian comparative genomics. *Journal of Applied Genetics* 45:195–214.
- TAUTZ, D., AND M. RENZ. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Research* 12:4127–4138.
- . 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* 17:6463–6471.
- WEISING, K., F. WEIGAND, A. J. DRIESEL, G. KAHL, H. ZISCHLER, AND J. T. EPPLEN. 1989. Polymorphic simple GATA/GACA repeats in plant genomes. *Nucleic Acids Research* 17:10128.

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