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The Expanding Use of Forensics in Environmental Science

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June 1,2000/ Volume34, Issue11/ pp.262 A-266 A
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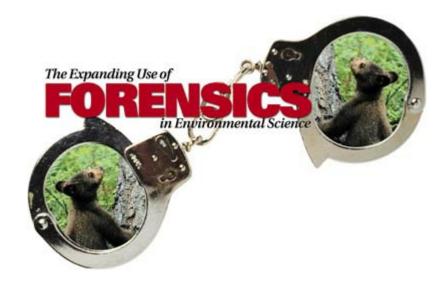
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Advances in the scientific investigation of environmental crimes are defining the emerging discipline of environmental forensics.

ANTHONY CARPI AND JEFFREY MITAL

On the morning of July 14, 1995, twin infants, barely a few weeks old, were found shot to death and disemboweled near the town dump in Winnipeg, Manitoba. In the days following the incident, a trail of forensic evidence proved crucial for identifying and convicting the perpetrators of the crime.

Taken together, the evidence was damning. Eyewitness testimony placed two men at the dump site on the evening of July 13. DNA tests revealed that blood on a knife in the suspects' possession matched that of the infants. Investigators turned to Gail Anderson, a forensic entomologist at Simon Fraser University in Burnaby, British Columbia, to corroborate the eyewitness sighting with the time of death of the victims. By tracking the life cycle of insect eggs laid in the wounds of the victims, Dr. Anderson established the time of death as the evening of July 13. The case proceeded to trial, and the judge cited the forensic DNA and entomological evidence as crucial in ordering jail time for the two offenders.

The case highlights a textbook forensic investigation that is unique from the standpoint of the victims involved—two black bear cubs. Killed for the price of their gall bladders on the black market, the young bears yielded a pair of organs too tiny to have any significant value.

Over the past decade, traditional forensic investigative techniques have increasingly been employed for environmental protection. Recently, physical science professionals have suggested that use of chemical fingerprinting and geochemical tracking to attribute liability at chemical contamination sites is helping to define the emerging discipline of environmental forensics (1, 2). The field's rapid growth is discussed in books such as *Environmental Forensics* (3)

and in the recently organized publication *International Journal of Environmental Forensics*.

To date, articles that describe the new field have focused only on chemical contamination incidents, although concurrent work by forensic scientists, wildlife geneticists, arson investigators, and parasitologists has steadily advanced the use of forensic techniques in environmental protection. Thus, a previously proposed definition of environmental forensics—the systematic investigation of a contaminated site or an event that has impacted the environment (1)—fails to reflect the true diversity of this evolving discipline.

To reflect the present range of application, an alternative definition of environmental forensics is proposed—the scientific investigation of a criminal or civil offense against the environment. This broader explanation better reflects the diversity of the field by including chemical liability inquiries, as well as investigations into wildlife and plant poaching, illegal trade of protected organisms, arson in natural areas, and liability associated with biological pollutants.

Poaching wildlife and plants

Several legal instruments prevent and restrict the taking of wildlife and plants from the environment. Hunting regulations establish limits on the types or numbers of animals that can be killed, seasons in which hunting is allowed, and methods of legally permissible hunting. Land regulations, such as laws defining wilderness areas or private property, establish boundaries on the locations from which animals and plants can be collected. Violations of the legal premises of these laws result in crimes against the environment for which forensic techniques are becoming increasingly applicable in criminal investigations.

DNA fingerprinting has proven to be a powerful tool in poaching investigations concerned with tying a victim to a specific area or circumstance. One of the technique's first applications with respect to a wildlife crime involved a deer-poaching case in Shasta County, CA, in 1992. Authorities investigating a hunter's failure to display a proper hunting license arrived at the garage of the suspect to find a large buck mule deer hanging from the rafters. Although the suspect claimed that the buck was legally taken from nearby Burney Mountain, authorities suspected otherwise; Burney Mountain only supported populations of black-tailed deer, not mule deer. Alfalfa found in the animal's mouth and gut also argued against Burney Mountain, as the grass is not found in that area.

Continued investigation uncovered a witness who recalled hearing shots fired on the night in question on a nearby private preserve. Authorities subsequently located a 'gut pile' (from field dressing an animal) on the preserve. The entrails were given to officials from the U.S. Fish and Wildlife Service (FWS), who used DNA fingerprinting to demonstrate that the chances of an arbitrary match between the viscera and the buck were one in 22 million. The suspect, confronted with the overwhelming evidence, pled guilty to the offense, was fined \$2500, and was prohibited from hunting for three years (4).

DNA fingerprinting is also being explored for tracing environmental crimes

not involving wildlife. For example, British Columbia's Ministry of Forests is developing DNA fingerprinting to identify illegally logged trees. Estimates place the annual cost of timber stolen from government and private land at C\$75-150 million in British Columbia alone. Unless caught in the act, tree thieves are difficult to identify because of problems in distinguishing legal from illegal timber harvests. Past efforts had focused on matching tree rings from a suspect tree to a stump, but tree thieves quickly responded by disposing of a thin slice of wood from the bottom of the trunk, eliminating the possibility of matching rings. DNA analysis has advantages in that poached wood can be matched with tissue from a stump at an illegal harvest at any time, even after a plank or other lumber product has been cut.

To support this latter approach, researchers are currently building databases of species-specific genetic markers that can be used to identify illegally cut trees. To

Selected forensic techniques used in environmental protection

Poaching incidents

Ballistics
DNA fingerprinting
Physical feature comparison
Serology
Time-of-death estimation:

Body temperature analysis Forensic entomology

Endangered species trade

Biochemical fingerprinting
DNA typing for species identification
Physical pattern recognition of skin or other surface
features
Protein typing

Arson in natural areas

Fiber analysis
Fingerprint comparison
Fire scene reconstruction
Tire/shoe print pattern recognition

Biological pollutant liability cases

DNA fingerprinting or population genotyping Ribotype profiling

Chemical pollutant liability cases

Chemical fingerprinting such as hydrocarbon or isotope ratio comparison
Spatial analysis of concentration profiles or migration pathways for source attribution

date, markers have been completed for yellow and western red cedar. Western red cedar, a common roofing material, is particularly vulnerable to theft because of its high value—a single cord can cost C\$1200 (US\$820), and prime cedar used in musical instruments can command as much as C\$6000 (US\$4100) per cord.

Depending on the nature of the offense, a number of other forensic techniques may be employed in criminal investigations (see box, Selected forensic techniques used in environmental protection). For example, the use of firearms during bow hunting season is forbidden in most jurisdictions. To elude detection, hunters illegally using firearms have been known to insert an arrow through a bullet wound. Two methods are under investigation to identify such violations. Gunshot primer residue detected on the hands or clothing of a hunter may be used to identify recent use of a firearm. But this analysis is confounded by the use of firearms in target shooting or other applications. A second method under investigation involves elemental analysis of the wound tissue, specifically for copper and lead residue, to confirm the transfer of particulates during bullet entry. Significantly more metal particles appear to be transferred to tissue from a bullet entry than from a metal-tipped arrow.

Physical identification is also important in forensic investigations. Parks Canada routinely takes horn casts from bighorn sheep in protected areas so that illegally poached animals can be traced back to the park. In 1993, FWS officials, aided by physical identification and other forensic techniques, tracked the killer of Charger, a large bull elk that was one of Yellowstone National Park's most photographed animals.

One evening, a poacher shot the elk, stripped the skull plate and antlers, and left the carcass by the road, to the dismay of park visitors and officials. Authorities publicized photographs of Charger featuring his distinctive, asymmetrical rack—eight points on one side and seven on the other. A local taxidermist contacted the FWS after noting similarities with an elk rack he was contracted to mount. FWS officials confirmed that the rack was Charger's through DNA fingerprinting. Although the hunter denied shooting Charger, claiming that he found the elk dead, ballistic evidence confirmed that the bullet that killed the elk came from the hunter's gun. Faced with the overwhelming evidence, the hunter pled guilty to the crime, agreed to pay more than \$30,000 in fines, and was sentenced to eight months in prison (5).

Illegal trade

The Endangered Species Act and the Convention on International Trade in Endangered Species (CITES) restrict the trade of any organism listed as endangered. In addition, the U.S. Lacey Act prohibits the interstate transport of protected organisms and the importation of species taken in violation of the laws of any sovereign country. A key component during the investigation of a violation is the association of an individual organism with a listed species. If species identification can be performed visually, enforcement is simplified, but in cases when only tissue, organs, or products from an organism are present, investigations are more complex. For this reason, DNA fingerprinting plays an important role in many cases involving endangered species.

In a November 1999 court case, a Stamford, CT, importer was found guilty of illegally transporting Russian caviar, based on forensic DNA evidence that played a key role in verifying that the roe in question came from protected sturgeon (6). In an effort to stop the precipitous decline in sturgeon populations over the past two decades, the fish are listed as protected under CITES. Although still pending appeal, the importer faces up to five years in prison for the violation.

Although DNA fingerprinting has played a major role in many endangered species cases, the method has limitations. Degradation or intentional alteration of sample tissue can hamper DNA analysis. The method also requires the compilation of large databases that represent sufficient intra- and interspecies genetic variation to accurately identify members of the protected species.

As the market for endangered species products expands, so do the techniques used for identifying the illegal trade of protected organisms. Differences in bile salt ratios have been used to distinguish gall bladders from different species of bear (7). The alignment, distribution, and appearance of skin scales have been used for species confirmation in cases involving illegal importation of reptile leather (8). Unique patterns of crossing lines in elephant ivory (see Figure 1), commonly referred to as Schreger Patterns, have been used to distinguish mammoth ivory, which can be legally traded, from trade in modern elephant ivory (9), which is restricted.

Schreger Patterns The cross section of elephant ivory illustrates Schreger patterns. The intersection of these lines form angles that can be used to infer whether the ivory has been illegally taken from an elephant or has been obtained from a legal source such as mammoth tusks.

To address the challenges that

have arisen, several institutions have opened laboratories for forensic research in environmental protection over the past decade. The FWS established the first forensic laboratory exclusively devoted to investigating wildlife crimes in 1989. The laboratory (http://www.lab.fws.gov), founded to provide "forensic support to wildlife law enforcement officers and agencies within the United States and throughout the world," has been integral to the development of research and testimony in support of wildlife protection laws. Trent University in Ontario operates a Wildlife Forensic Laboratory (http://www.trentu.ca/academic/forensic) that specializes in DNA analysis for individual and species identification in wildlife investigations.

Source: National Fish and Wildlife Forensic Laboratory

Arson in natural areas

Section 1855 of the U.S. Code on Criminal Procedures states, "Whoever, willfully and without authority, sets on fire any timber, underbrush, or grass or other inflammable material upon the public domain . . . of the United States, . . . shall be fined under this title or imprisoned not more than five years, or both" (10).

To identify suspected environmental arsons, authorities use many of the same techniques employed by their peers in arson investigations at private and commercial sites. These methods typically include analysis of burned materials (for example, trees, roots, and soil in the case of the environment) and lead the investigation toward the area of most intense burn and probable point of origin. The identification of tire tread marks and footprints left at the source area can be used to place a suspect at a specific crime scene. Incendiary devices, including cigarettes, matches, and other material recovered from a burned area can be analyzed for traces of saliva, fingerprints, and other evidence.

Sophisticated forensic techniques are increasingly being used to solve

environmental arson crimes. California is at the forefront of these investigations. In one incident, California authorities charged a man caught initiating a grassland fire with serial arson because fibers from the arsonist's matchbook matched fibers from a match found at an earlier grassland blaze.

California's penal code outlaws both the willful (intentional) and the unlawful (unintentional) destruction of any natural area by fire. According to David LeMay, deputy chief for law enforcement of the California Department of Forestry and Fire Protection, the state has been increasingly proactive in enforcing wildland fires caused by corporate negligence. One example concerns the maintenance of right-of-ways by power utilities. Improper maintenance can result in forest fires if stray tree limbs are sparked by nearby power lines. In an interesting investigative application, the department is currently exploring the use of DNA fingerprinting for attributing a downed limb with its source tree to estimate spatial proximity to nearby power lines.

Biological pollutants

Although the term environmental forensics has been commonly used in reference to chemical pollutant investigations, liability investigations involving biological pollutants also fall within this discipline. The release or transmission of infectious organisms into an environmental resource can violate a number of environmental statutes. The Clean Water Act and Clean Air Act limit the discharge of pollutants into water and air, and the Safe Drinking Water Act establishes maximum contaminant levels, required treatment technologies, notification rules, and other mandates on public drinking water. Liability investigations involving biological discharges have relied upon typical forensic techniques.

One of the best known investigations of a microbial discharge occurred in Milwaukee, WI. On April 5, 1993, the Wisconsin Division of Health was contacted after widespread employee absenteeism was attributed to gastrointestinal illness. Subsequent laboratory analysis identified high levels of the opportunistic protist *Cryptosporidium parvum* in stool samples from victims who had fallen ill. Within days after the initial reports of sickness, over 400,000 people developed gastrointestinal illness. At least 100 resulting deaths were attributed to the outbreak of cryptosporidiosis.

Wisconsin health officials suspected that the source of the pathogen was drinking water because the outbreak was geographically limited to the distribution network of only one of two city water treatment plants. Records showed that turbidity levels were exceptionally high at that plant from March 23 until the plant's closure on April 9. A team of researchers conducted a study of commercial ice cubes produced locally on March 25 and April 9, and immunofluorescent analysis confirmed the presence of *C. parvum* oocysts in city water (11).

Following the outbreak, a class-action suit was filed against the City of Milwaukee, a water-treatment chemical supplier to the city, and a meat packing company that allegedly dumped *C. parvum*-contaminated animal waste into a public storm sewer. In an effort to identify the source of the waterborne pathogens, the U.S. Centers for Disease Control and Prevention (CDC) undertook an extensive DNA-typing project on oocysts collected from the Milwaukee outbreak, other *C. parvum* outbreaks, and cattle throughout the United States.

Two distinct strains of the protist were identified, one found in both animals and humans and a second only found in humans. DNA typing of the Milwaukee organisms suggested that they were of the second strain, those only found in humans and not in cattle. The CDC study eliminated animal waste from the meat packing company as a source of the outbreak. As a result of this exoneration, an affiliate of the meat packing company proposed a minimal settlement of \$250,000 in 1998 to help fund the plaintiffs' case against the two other defendants. In October 1999, the water-treatment chemical supplier agreed to a \$1.5 million settlement of the case that accused the company of improperly advising the city on how to use a new purification chemical at its water plant. Finally, on November 30, 1999, a Milwaukee County circuit judge gave preliminary approval to a \$100,000 settlement by the City of Milwaukee, a step that incited outrage among plaintiffs because of its low value.

Significant advances have been made toward the scientific investigation of criminal or civil offenses against the environment over the past decade. In addition to chemical fingerprinting, forensic techniques such as DNA analysis, ballistics research, and fiber comparisons have made vital contributions to the field of environmental protection and help define the emerging discipline of environmental forensics. As environmental offenses continue to become more elusive, the technology to identify and investigate these acts will continue to progress.

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