Applied wildlife genetics in enforcement and management

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Abstract

The two main challenges in developing wildlife forensic serology have been 1) to apply biochemical and molecular methods to evidence samples that are often degraded or intentionally altered, and 2) to compile databases that represent sufficient intra- and interspecific variation for the application of population genetics principles to wildlife casework. For both blood and meat evidence, game species are generally differentiated from domestic animals by immunodiffusion. Species determination is done by electrophoretic methods that resolve protein and mitochondrial DNA markers. Nuclear DNA analysis is used often to ascertain the minimum number of animals involved in a poaching incident, to determine the gender of meat or blood stains, and to match the field remains with meat seized from a suspect. Tissue and blood samples from across the distribution of most mammalian game species have been obtained from wildlife professionals and cooperating zoos. The number of wildlife species for which databases have been established is expanding. These samples provide the essential standards used in both management and enforcement efforts.

Introduction

Forensic scientists have been using serological methods to assist game wardens and wildlife officers for several decades. The earliest work, performed in 1902, was simply to identify blood at a kill site (Uhlenhuth 1983). Later immunological methods were used to distinguish blood and/or tissue of wildlife, particularly deer, from that of domestic animals (Ouchterlony 1949). More recently, wildlife forensics focused on finding species specific markers at the protein level (Bunch et al. 1976, McClymont et al. 1982, Wolfe 1983). In the last five years, the research focus has shifted to finding DNA markers that can identify wildlife populations and individual animals (Carr et al. 1986, Cronin et al. 1991, Ruth and Fain 1993).

Since opening in 1989, the National Fish and Wildlife Forensics Laboratory has provided a wide range of analytical techniques for the identification of wildlife parts and products to state and federal agencies as well as foreign authorities responsible for the enforcement of wildlife laws. The establishment of a national wildlife crime laboratory has given new impetus to the development of wildlife forensic research. It has made it possible to prosecute an increasing number of cases on the basis of physical rather than circumstantial evidence. It has become clear to scientists who have recently joined this field that the commercialization of wildlife and the sale of live animals, parts of animals and products derived from animals is an international trade with substantial profits and much less risk than drug trafficking.

The Serology Section of the Laboratory uses protein and DNA analytical methods in combination for the determination of species, of individuals and of gender. Successful application of these methods generally requires that genetic variation be assessed throughout the geographical range of the target species. In its first four years, the section has performed analysis in over 450 cases encompassing over 20 different

wildlife species. This paper outlines the methods used in the examination of blood and tissue evidence in our Laboratory. We provide results for several wildlife species, focussing on North American elk and black bears to demonstrate particular developments and the problems encountered.

Methods

Serological analyses of wildlife species use the same comparative approach employed in morphological identifications. Detection of diagnostic serological characters requires blood and tissue standards. Our collection of over 15,000 specimens has been supplied primarily by officials of state wildlife agencies and zoo veterinarians. The latter have been providing samples of species not native to North America, an especially significant contribution because obtaining permits to import blood and tissue specimens can be difficult from many of the countries where these wildlife species remain.

Immunology and isoelectric focussing

The first step in identifying the source of an evidence item is immunological determination of family of origin. Forensic grade antisera are commercially available for most domestic mammals, as well as the Cervidae and Ursidae (Cappel, Organon Technika Corp.). We use a large format passive immunodiffusion system (124 x 258 mm gel frames, 1% agarose in Tris-glycine buffer). The agarose gels are poured on GelBond (FMC, Inc.) support media so that the dried stained gels can be presented in court if required. The gels are then stained with Serva Blue (Serva Feinbiochemica), destained in water, dried and scored by two analysts independently.

Species determination of blood and tissue evidence is generally done by protein electrophoresis, with two methodological refinements. Conventional electrophoresis has been replaced by isoelectric focussing due to its greater speed, repeatability, and discrimination power (Lawton and Sutton 1981). General protein stains have been replaced by histochemical and immunochemical stains to resolve only those loci that show differences in isoelectric point between species in the same family. The number of protein systems that are detectable in degraded samples, such as those often found in evidence, is substantially less than those scorable from samples collected in capture or harvest studies. We use protein loci that meet the dual criteria of being robust and variable across many mammalian species. Isoelectric focussing on the PhastGel system (Pharmacia LKB Biotechnology) and staining for glucose phosphate isomerase and superoxide dismutase (pH range 3-9), erythrocyte acid phosphatase and phosphoglucomutase (pH range 5-8), and albumin (urea gels pH range 5-6) have enabled us to identify the major mammalian game species in North America.

Individualization of wildlife with DNA profiling

A multi-locus DNA profiling method based on the work of Jeffreys (1985) has been developed at the Laboratory for the individual typing of wildlife species (Ruth and Fain 1993, Fain and Taylor 1994). While particular hybridization probes resolve more DNA fragments of different sizes in particular game species, probes found to be generally useful in characterization of wildlife are: 33.6, 33.15, MS1 and CMM101. Genetic variation is assessed by making pairwise comparisons between DNA fingerprints of individual animals. The similarity (S) of two individuals is calculated as the number of fragments common to both DNA fingerprints divided by the total number of fragments compared (Lynch 1988). The average similarity is the mean of S values from all pairwise comparisons either within or between localities. Similarity comparisons are made between all the individuals sampled from a locality.

Black bears were sampled from five localities: Humboldt County (Northern CA); San Bernardino National Forest (Southern CA); Tensas River National Wildlife Refuge (LA) Shenandoah National Park, VA; and Great Smoky Mountain National Park (TN). These are representative of the species range in the contiguous United States.

Gender determination

A new method for determining the gender of mammalian wildlife species from trace amounts of bloodstain or solid tissue has been developed at the Laboratory. The method uses the polymerase chain reaction (PCR, Saiki et al. 1988) to amplify sequences from sex-chromosome-linked genes that are conserved in mammals. The method targets both the X and Y chromosome-linked ZFX and ZFY zinc-finger protein genes (Aasen and Medrano 1990), as well as the Y-chromosome linked SRY testis-determining factor gene (Sinclair et al. 1990). The amplification products are analyzed directly; i.e., neither restriction digestion nor probe hybridization are required. Two products (446 bp: ZFX/ZXY, and 214 bp: SRY) are observed in amplifications of DNAs containing a Y-chromosome, while only the control product (446 bp ZFX/ZFX) is observed in DNAs without a Y-chromosome. The reliability of the method was tested by screening reference and blind trial samples of selected species from 6 mammalian families: Antilocapridae, Cervidae, Homidae, Odobenidae, Ovidae, Ursidae.

Results and discussion

Immunology

Using passive immunodiffusion, blood samples and tissue homogenates from 15 deer species all showed strong positive reactions with deer antisera. The species included all of those native to Europe and North America. Of over 100 deer samples tested, only a musk gland sample from a musk deer did not react positively. Deer antisera did not cross-react with any non-cervid species, including tissue standards of North American pronghorn, mountain goat, bighorn sheep, as well as African ungulates or common domestic animals. Trials on cooked meat showed that the method detected sausage samples containing 10% venison and 90% non-cervid meat sources. Bear antisera, while detecting all 7 ursid species, also cross-reacted with tissue standards from racoon, wolverine, and skunk tissue standards. Therefore, electrophoretic tests are necessary to confirm that material is ursid.

Isoelectric focussingIsoelectric focussing (pH range 3-9) and histochemical staining for glucose phosphate isomerase (GPI) and superoxide dismutase will differentiate North American black bears and grizzly bears, from racoon, skunk, and wolverine. While black bears occasionally exhibit the allele that is fixed in grizzly bears, the reverse has not been found. Superoxide dismutase (SOD) has proven a valuable species marker in both ursids and cervids as it is abundant both in blood stains and tissue. The following are estimated SOD isoelectric points for common wildlife species: white-tailed and mule deer 5.4, mountain goat 6.0, moose 6.3, big horn sheep 6.6, elk 7.6, grizzly bear 5.2 and black bear 6.3. Though mule and white-tailed deer cannot be distinguished at the SOD locus, mule deer as well as their coastal conspecifics, the black-tails, show a unique erythrocyte acid phosphatase (EAP) pattern not found in any other North American deer species. Using narrow range urea gels and immunoblotting for albumin (ALB), each of the North American cervid species show different predominant alleles. Because the predominant allele in one species can be a rare alele in a sympatric species (Smith et al. 1990), two independent protein loci should be utilized in making a species determination.

Several protein markers (GPI, SOD, and ALB) differentiate North American elk from

sympatric species. They will not distinguish elk from the red deer or from sika deer, close relatives from other continents that are now present on North American game ranches. Additional loci such as haemoglobin (Dratch 1986) must be used in these cases, and even they will not identify all hybrids between elk and other members of the genus Cervus.

DNA profiling

DNA fingerprint profiles of individual black bears from the five localities revealed that individuals from different populations shared different proportions of the total number of fragments compared. Genetic variability among black bears expressed as average similarity. The amount of band-sharing, or similarity (S), that occurred between individual DNA fingerprint profiles formed the basis of the analysis. The higher the S value between individuals, the more genetically similar they were. Average S ranged from 0.49-0.80. These values are twice as high as those determined for natural populations of wolves (S = 0.2) and elk (S = 0.4). Similarity values of this magnitude are more comparable to average S determined for a variety of domestic animal species.

The largest bear population that we studied, from San Bernardino National Forest, exhibited the least variability while individuals of the smallest population, Tensas River NWR, exhibited more variability. The proportion of the restriction fragments that were present in the DNA fingerprint profiles of every bear sampled from a location indicates the level of homozygosity extant within that population. The San Bernardino National Forest and Tensas River NWR populations exhibited the largest proportion of 'fixed' restriction fragments. Interestingly, the same San Bernardino population was the most polymorphic for the GPI protein locus, and the only one where particular black bears were homozygous for the allele that is fixed in grizzly bears. The DNA and protein data that would seem inconsistent are explained by the small number of founders (n=13), from Yosemite National Park, that reestablished this population. The few founders that bred apparently had the rare GPI allele. Differences in resolution between protein and DNA data are informative also in North American elk (Ruth and Fain 1993, Fain and Taylor 1994). While DNA fingerprint analyses show considerably more variation in Rocky Mountain elk of Yellowstone National Park origin than that revealed in the initial protein analyses (Cameron and Vyse 1978), the high band-sharing in tule elk from California show the results of a marked bottleneck. The same phenomenon is seen in the Manitoba subspecies from Elk Island National Park, Canada (Fain unpublished). The ability of DNA fingerprint profiles to reveal genetic substructure within North American black bear populations was examined by comparing the average proportions of restriction fragments shared by bears from Dickey Ridge and Timber Hollow, localities within the Shenandoah National Park separated by 38 km. The average S values between the localities was significantly lower (confidence interval = 0.99) than the average S calculated within each locality, indicating that given the habitat, terrain, and bear population density of Shenandoah National Park, a distance of 38 km is a significant barrier to gene flow (Alberte et al. 1994).

Gender determination

We have successfully applied our gender test to all of the North American cervid species as well as humans, bears, sheep, cows, and mountain goats. Interpretable amplification results were obtained for 77% of the blind trial bloodstains of these species; 23% of the samples did not produce interpretable results due to sample degradation or insufficient quantity. All blind trial samples that could be amplified were correctly identified as to gender. The gender test is currently being applied in our laboratory in cases requiring sex identification of evidence

samples from mammalian species. Conclusive results have been obtained from trace amounts of blood, as well as from meat, tooth, and hide parts.

Conclusion

Blood and tissue that are often associated with wildlife crimes constitute crucial physical evidence when characterized with analytical methods developed in biochemical genetics and molecular biology. The National Fish and Wildlife Forensic Laboratory routinely conducts species identifications, individualization and gender determination for much of North America's hunted wildlife. These include all North American deer and bear species, mountain goats, bighorn sheep, walrus, wolves, coyotes and cougars. It also includes avian wildlife, such as parrots, that are part of the pet trade.

Wildlife forensic analyses require the same population based genetic studies necessary for the long-term management of game species. Species-specific protein markers for one state, or levels of DNA similarity for a species in one province, may not be at all typical of a neighbouring locality. One reason for this is that game populations often show the genetic results of human intervention: bottlenecks, founder effects and hybridization.

Consequently, generalizations about genetic variation in game species based on few individuals or few populations are frequently inappropriate for either forensic or management purposes. There is no substitute for this baseline research as both enforcement and management decisions increasingly face the strictures of the courtroom.

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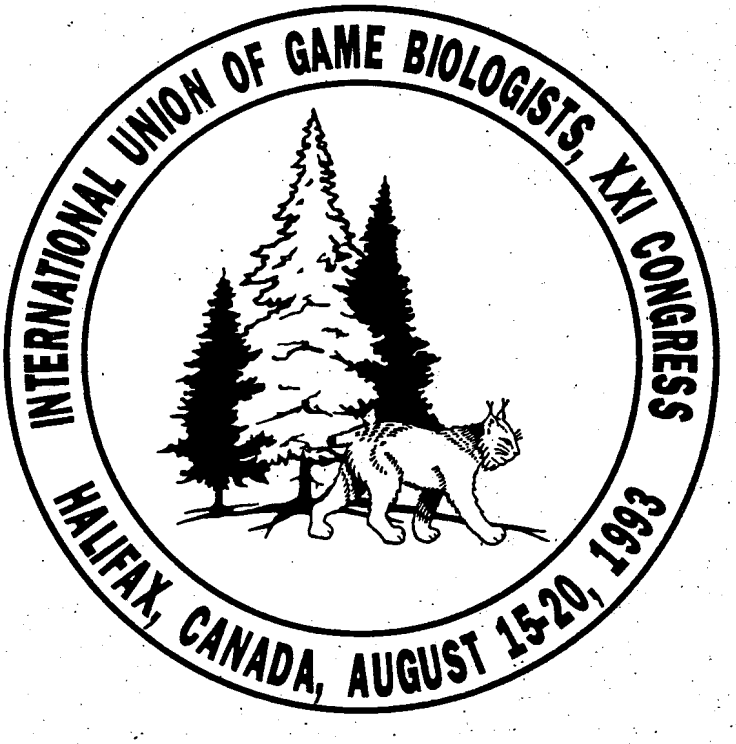

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