

Using Genetic Analyses to Identify Predators

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Introduction

Coyote and dog depredation account for much of the economic losses to livestock in the United States (National Agricultural Statistical Service, 2000, 2001). However, depredation by other species (such as members of reintroduced wolf populations) can be more socially and politically contentious. Predators are often elusive and attacks on livestock are not often witnessed but the species of predator causing stock losses can sometimes be ascertained from evidence near the carcass (such as scat or hair), the attack pattern, or size and spacing of bite wounds. However, these species assignments can be subjective and may be influenced by the experience level of personnel, the condition of the carcass, and knowledge of previous predation history at the site. Variation among conspecific predators in attack pattern, and inter-specific overlap in those patterns, may be another complication to accurate predator species identifications. There are wide ranges in accuracy of identifying species based on scat morphology (Farrell et al., 2000). Variation in individual feeding preferences (Fedriani and Kohn, 2001) may also complicate accurate species identification from scat. Sociological considerations also may influence results. For example, local or regional compensation schemes may unintentionally result in biases in predator species identification (Cozza et al., 1996). Using common field methods, the accurate identification of the gender of a predator responsible for a specific predation event is unlikely. Likewise,

although there may be assumptions about which specific individual was responsible for an attack on livestock, those assumptions may not be based on any concrete data. Clearly, an unambiguous method to determine the predator species would remove identification biases. A method to identify the specific individual responsible for kills would benefit our understanding of predation and would be useful in certain situations. Both methods, even if used strictly in research situations, might ultimately result in improved approaches to minimize livestock losses to predation.

Samples, such as hair, scat, and saliva (referred to as noninvasive samples), contain DNA, although the DNA tends to be in low quantity and degraded (Taberlet et al., 1999). Despite this difficulty, these samples can be analyzed using the polymerase chain reaction (PCR), which allows the analysis of even minute amounts of degraded DNA. Because the mitochondrial (mt) genome is small and is present in multiple copies in most cells, mtDNA lends itself well to PCR analysis. Importantly, certain regions of the mt genome are variable among species (Foran et al., 1997). By analyzing for such mtDNA differences, unknown samples (including noninvasive samples) can be identified to species. For example, Foran et al. (1997) demonstrated the ability to use mtDNA to identify scat samples from a wide range of wildlife species. Likewise, Woods et al. (1999) used mtDNA to differentiate black bear from brown bear hair collected from snares. By using highly variable nuclear DNA regions, such as microsatellite DNA loci, identification of the individual animal responsible for predation is also possible from noninvasive samples. Woods et al. (1999) were also able to differentiate

individual bears based on unique multi-locus microsatellite DNA genotypes generated from those hair samples. Additionally, the gender of an animal leaving a noninvasive sample can be determined based on analysis of regions of the sex chromosomes that vary between male and female (Woods et al., 1999; Williams et al., 2003b).

Studies that use genetic analysis of noninvasive samples to delineate regions of species overlap, census populations, or track elusive or rare species are becoming more frequent (Woods et al., 1999; Kohn et al., 1999; Palomares et al., 2002). However, the genetic analysis of noninvasive samples also has potential applications in situations involving livestock predation. Predators often leave traces of scat, hair, or saliva at a kill site, and those samples have the potential to allow the unambiguous genetic identification of the predator (Ernest et al., 2002). These noninvasive samples are not identical in utility, however. The usefulness of scat or hair found near a kill site must be carefully considered. Although found physically near a kill site, there may be some ambiguity whether the hair or scat sample was deposited at the exact time of the kill and whether it was deposited by the individual that made the kill. However, saliva left on predation wounds offers the opportunity for direct identification of the predator. Saliva has been increasingly used as a source of DNA in human criminal investigations in recent years. Despite the low quantity and quality of DNA in such samples, multi-locus genotypes have been generated from unintentionally deposited saliva samples that allowed the matching of a sample to a specific suspect (Sweet and Hildebrand, 1999). Analysis of saliva has only recently been applied to investigations of livestock predation. For example,

Williams et al. (2003b) used analysis of saliva to identify species and gender of predators killing sheep (*Ovis aries*) at a site in California. At that site the most important predator of sheep was coyotes (*Canis latrans*); (Neale et al., 1998), but other potential predators were also present (bobcat, *Lynx rufus*; black bears *Ursus americanus*; dogs, *Canis familiaris*; mountain lions, *Puma concolor*). Williams et al. (2003b) demonstrated the ability to generate microsatellite genotypes from those saliva samples. Blejwas et al. (in prep) took the identification of predators at that site further by comparing microsatellite genotypes from coyotes in the area (obtained from tissue samples; Williams et al., 2003a) to microsatellite genotypes obtained from saliva on predation wounds. Blejwas et al. (in prep) successfully identified some of the individual coyotes responsible for specific sheep kills at that study site.

Approaches for sample collection and genetic analysis

Hair samples obtained from kill sites are collected and preserved dry, in an envelope. Scat samples may be frozen or stored at room temperature in ethanol or a buffer solution (Ernest et al., 2000; Frantzen et al., 1998). To collect saliva swabs the carcass should be skinned and attack wounds distinguished from scavenging by the presence of sub-dermal hemorrhaging. Attack wounds are individually sampled using a dry, sterile swab. The swabs are air dried, then stored in an envelope or bag. Care must be taken to minimize potentially cross-contaminating samples. The cotton tip of the swabs should not be handled or touched to any surface other than the single bite it is being used to swab. Samples must be stored individually.

DNA from scat or saliva is isolated using a commercially available kit (Qiagen, Valencia, Calif.) and the manufacturer's instructions. The DNA from hair is typically isolated using a commercially available resin (Chelex 100, Bio-Rad, Hercules, Calif.). An aliquot of DNA, or an aliquot of a 1:10 dilution for scat samples, is used as a template for PCR amplifications, which are targeted to amplify specific genetic regions. For species identification, primers are used that amplify a

short fragment of the mtDNA, typically the control region (Kocher et al., 1989; Foran et al. 1997; Woods et al., 1999). This genetic region varies among species either in length (so some species result in fragments of different lengths) or in DNA sequence. For example using primers developed by Pilgrim et al. (1998), black bears produce a distinctly different fragment pattern than canids, and the felids show multiple fragments due to heteroplasmy (not shown). Other species, such as the canids, require digestion of the amplification product with restriction enzymes to resolve sequence differences. Determining gender relies on analyzing regions on the sex chromosomes which may require species-specific primers (Woods et al., 1999). Conserved primers for mammalian gender determination would be of particular use for saliva or hair samples (Woods et al., 1999), unless the fragments they amplify are large (Shaw et al., 2003). Determining an individual's genotype is accomplished by microsatellite DNA analysis. Microsatellite primers, which target these short, highly variable, genetic regions have been developed for most large and many small predators (Ostrander et al., 1993; Paetkau and Strobeck, 1995; Ernest et al., 2000).

Technical Issues

The degraded quality and low quantity of DNA from noninvasive samples makes such samples prone to contamination. Special precautions should be taken to minimize cross contamination, such as handling samples with gloves and packaging individually in the field. Laboratory precautions have been discussed by Taberlet et al. (1999) and include facilities and equipment dedicated for low-template samples, as well as additional negative controls. The nature of noninvasive samples means some samples will yield no information on species identification. However, they should not yield incorrect species identification. The degraded state of DNA from noninvasive samples also means primers targeting large DNA fragments may not result in amplification, and necessitates the use of relatively short DNA regions for all genetic analyses. For example, saliva swabs from livestock carcasses have not yielded amplification using primers that amplify a mtDNA

fragment about 600 bases long (H16498 and L15774, Foran et al., 1997), but did result in amplification of an approximately 165 base fragment using other primers (Pilgrim et al., 1998; data not shown). Although markers have been developed to differentiate even closely related species (Paxinos et al., 1997) those markers rely on relatively long genetic regions and so may not be of use with all noninvasive samples.

Although scat may contain degraded DNA from both predator and prey, saliva swab samples will likely contain degraded DNA from the predator in the presence of less degraded prey DNA (from blood), which may interfere with some identifications (Williams et al., 2003b). All types of noninvasive samples can produce erroneous microsatellite genotypes (Taberlet et al., 1999). To ensure the correct microsatellite genotype is obtained for an individual predator, additional special precautions are required. Such precautions include establishing criteria for accepting genotypes, in order to account for allelic drop out and false alleles (Taberlet et al., 1999; Fernando et al., 2003). Generating individual multi-locus microsatellite genotypes will not be practical for all samples identified to the species level, given the additional time and expense required.

Hybridization between species could also be a complicating issue for genetic species identification (Roy et al., 1994; Vila et al., 2003). Hybrids carry the mt genome of their mother, and mt analysis alone would identify a hybrid as being a member of its mother's species. Individuals that are the descendants of hybrids may also carry a misleading mt genome. For example, a dog mt haplotype was detected in coyotes in the southeastern United States, presumably as a result of a historical hybridization during range expansion into that portion of the country (Adams et al., 2003b). Similarly, wolves in certain regions in North America carry coyote mt genomes due to hybridization (Lehman et al., 1991). For accurate species identification, mt variation among individuals in a population or among species of interest may need to be established.

Discussion

Genetic methods can be successfully applied to evidence left on or near live-

stock carcasses to identify predator species, gender and individual genotype (Williams et al., 2003b; Ernest et al. 2002). Similar methods are being used to identify predators attacking humans. Genetic identification of predator species can be conclusive and may offer resolution to ambiguous or controversial cases. Clearly, genetic markers have the capacity to easily differentiate more distant species. For example, differentiating canids from felids is readily accomplished, as is differentiating either from ursids. More closely related species may require more thorough analysis and, as mentioned, differentiating among canid species can be more technically challenging (Adams et al., 2003a). One of the greatest logistical difficulties is finding carcasses of missing livestock in a suitable timeframe. On large ranches, where livestock may be most vulnerable to predation, it may not be feasible to search pastures often enough to distinguish predation wounds from scavenging. However in situations where livestock can be checked daily or more frequently, or for research purposes, success in identifying predation wounds and predator species can be high (Williams et al., 2003b; Blejwas et al., in prep). Genetic analysis can be used not only to determine the presence of a particular species at a certain location, but also to determine the identity of prey items in predator scat or stomachs (Scribner and Bowman, 1998; Fedriani and Kohn, 2001). So, for example, a scat containing both coyote and sheep DNA could indicate livestock depredation. However, we have not discussed this approach because predation could not typically be readily differentiated from scavenging using that method.

Genetic analyses offer new approaches to predator identification and can play a part in a better understanding of livestock depredation. Genetic analysis also offers a means to confirm that management programs are targeting the predators responsible for depredation. In addition to identifying predators responsible for individual cases, such data may assist investigations into prey base shifts, and the effects of multiple, overlapping predator species.

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