DNA Identification of Mountain Lions Involved in Livestock Predation and Public Safety Incidents and Investigations

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ABSTRACT

Using three case studies, we demonstrated the utility of techniques to analyze DNA from trace samples collected at sites of livestock predation and public safety incidents. Genetic analysis was used to determine species, individual identity, and relatedness between individuals. We documented the presence and individual identities of a mountain lion (*Puma concolor*) and a bobcat (*Lynx rufus*) from swab samples collected from bite wounds in domestic sheep that had been killed at the University of California Hopland Research and Extension Center, Mendocino

County, California. Four lions and two bobcats in Redwood National Park were individually identified and tested for relatedness using DNA from scats and captured animals. Another lion was genetically typed and matched at a public safety incident through blood spots left near a barn in one location in the San Joaquin Valley, and muscle sample collected from a lion captured 10 miles distant one week later. We applied statistical techniques developed for human forensic DNA analysis and a DNA database that we have compiled for California mountain lions.

INTRODUCTION

When mountain lions come into conflict with humans, livestock, pets, or endangered species, identification of species and individual identity is critical for successful management actions. However, collecting even the most basic information on these secretive and nocturnal predators can be very difficult. Recent technological advances in ecological genetic techniques allow key information to be collected even when the animal of interest is not captured. Here, we illustrate how DNA collected from trace samples such as feces, swabs from bite wounds on prey animals, and blood spots, can be used to give information about mountain lions that may come into conflict with humans.

DNA Techniques

Field-collected hair and feces can yield DNA data that provide insights into the ecology of difficult-to-study creatures such as mountain lions (Kohn and Wayne 1997; Ernest et al. 2000). Microsatellite regions can provide information on individual identity, kinship, mating systems, and population genetic structure. These regions are single locus segments of DNA randomly

distributed throughout the nuclear genome that possess rapid mutation rates and co-dominant Mendelian inheritance. Changes in the number of tandem pairs of nucleotides causes the DNA fragments to vary in size. Regions flanking each microsatellite do not vary greatly within species. These flanking regions allow production of primers for use in PCR amplification of these very specific loci. Analysis of multiple microsatellites is a sensitive way to screen genomes for variation. Microsatellites tend to be conserved within closely related taxonomic groups (families, ex. Felidae) but not across larger phylogenetic spans (Orders, ex Carnivora, which includes Felidae, Canidae and others). In a validation trial using matched pairs of scat and muscle from 15 mountain lions, we demonstrated that the microsatellite alleles from fecal DNA exactly match those from muscle DNA (Ernest et al. 2000). In contrast, primers from these same microsatellite regions did not elicit a DNA signal when tested using DNA from non-felids.

Livestock Predation

Mountain lion predation on domestic sheep at the Hopland Research and Extension Center increased from 1951 to 1997. No lion predation or evidence of lion presence on the Center's 5,300 acres was seen between 1951 and 1984, with the exception of one series of sheep kills in 1976. However, a total of 129 sheep were confirmed killed by lions from 1985 through 1996, including 28 head in 1996 (Timm, 1990; unpublished data). The actual loss of adult sheep to lions at Hopland may be twice the number of confirmed kills, and in some years may have been three to five times higher. These losses occurred despite the Center's best efforts to reduce predation through use of sheep husbandry strategies, conventional and electric fences, barn lambing, guard animals, and predator removal. While such methods are believed to have reduced losses to coyotes, they have been largely ineffective in reducing mountain lion

predation. The actual number of lions preying upon sheep at Hopland was unknown, but the California Department of Fish and Game (CDFG) has classified this area of the state (North Coast) as "high lion activity" based on the mean annual number and rate of increase of requests received by CDFG for lion control due to livestock and human safety incidents (Mansfield and Torres 1994).

Public Safety

Wildlife managers in western U.S. states and Canada reported increased requests for depredation permits for mountain lions and increased hunting harvest takes in the 1990's. While accurate lion census data is sparse, and depredation permit and hunting takes may be confounded by other factors including human population sizes and land use, these data indicate that lion numbers have increased in recent decades. Although lion attacks on humans are rare, these have also increased in recent decades (Beier 1991; Torres 1997). Through the 1990's, mountain lion sitings, unusual behavior, and attacks on humans were reported with increasing frequency in U.S. National Parks. The mean annual rate of increase in lion sitings (46%) in Yosemite National Park greatly exceeded that for visitorship to the park (5%) from 1991-1995 (L. Chow National Park Service data). In 1998, Redwood National Park initiated a research study to evaluate mountain lion use of the park in relationship to human activity.

In three case studies presented here, we tested techniques to analyze DNA from trace samples collected at sites of livestock predation and public safety incidents. Genetic analysis was used to identify species, individual identity, and relatedness between individuals.

METHODS

Case 1: Following the discovery of six domestic sheep killed by a predator at the Hopland Research and Extension Center on November 11, 1997, cotton-tipped swab samples taken from bite wounds (leg, neck and head wounds) on four sheep were collected for DNA analysis. A buccal (cheek) swab sample was collected from a lion killed on November 11, 1997 near the predation site. Case 2: Blood and buccal swabs were submitted from three lions captured for a mountain lion - human interaction study and three scats were gathered from dirt roads in the Redwood Creek region near Redwood National Park in September 1998. Case 3: A sample of vegetation with drops of blood was collected adjacent to a barn in San Joaquin County, California where a lion had startled a property owner July 1997. A muscle sample was taken at necropsy from a lion killed several days later and several miles distant in Stanislaus County, CA.

DNA was extracted from muscle, buccal swab, and fecal samples as described in Ernest et al. (2000). We analyzed DNA using polymerase chain reaction (PCR) with the following microsatellite primers: Fca 8, Fca 23, Fca 26, Fca 35, Fca 43, Fca 45, Fca 77, Fca 78, Fca 90, Fca 96, Fca 126, and Fca 132 (Menotti-Raymond et al. 1996; Menotti-Raymond et al. 1997; Menotti-Raymond et al. 1999). Products from PCR amplification were electrophoresed on polyacrylamide gels using an Applied Biosystems 373 DNA analyzer. Image analysis and fragment size determination were carried out using GeneScan 672 Analysis and Genotyper software programs (Applied Biosystems Inc.). Species of origin (mountain lion, bobcat, or non-felid) and genotype (genetic type) of scat DNA was determined based on data from Ernest et al. (2000). For samples sharing the same genotype, we computed the match probability, the likelihood that two individuals in a population could have the same microsatellite genotype

using the likelihood ratio equations (Ernest et al. 2000) and a DNA database that we have compiled for California mountain lions (Ernest, unpublished allele frequency data). Samples that displayed the same genotype and had match probabilities <1 X 10-4 were considered to be from the same lion. This value was determined by assuming that a maximum of 500 lions could have been present in each of the DNA data base regions that were used to calculate match probabilities. Mountain lion population sizes in California are unknown, but 500 is a reasonable estimate for the regions used in calculations. Match probabilities <1 X 10-4 (one in 10,000) in a maximum population of 500 limited the type I error to <5%. Type I error was the chance of misclassifying two samples with the same genotype as one lion, when in fact they were from different lions

RESULTS

Felid DNA was detected in all four of the Case 1 sheep swabs. Three of the swabs (C, D, and E) contained mountain lion DNA and one (swab B) contained bobcat DNA (Figure 1). The DNA type of the three mountain lion swab samples matched the DNA from saliva (swab A) and muscle of the suspect lion for loci that amplified. Match probabilities for the three swab genotypes C, D, and E were 6.3 X 10-3 (one chance in 160), 5.6 X 10-5 (one chance in 17,860), and 3.7 X 10-3 (one chance in 270) respectively. Swabs C and E had higher match probabilities because three (swab C) and two (swab E) loci did not amplify, therefore there was less data available and match probabilities were > 1 X 10^{-4}. The bobcat swab (B) sample amplified all loci except Fca 35. Fca 35 did not amplify in any of the known bobcat samples we tested for another study (n=20; Ernest et al. 2000).

Microsatellite DNA was amplified at all 12 loci from the buccal swab samples of the three lions captured in Redwood National Park (Case 2). Felid DNA was detected in all three of the scat samples, with two showing bobcat DNA and one showing lion DNA (Figure 2). The mountain lion scat sample showed was from a different individual than any of the three captured lions. In pair wise comparisons of the scat genotype with each of the captured lions, at least four loci displayed different alleles. Parent-cub relationships among any of the four lions were ruled out. A parent-cub relationship between two individuals requires at least one allele at each locus to be shared by descent. In each pair wise comparison, there was at least one locus at which no alleles were shared (Figure 2). Similarly, the bobcats detected by scat samples were two different individuals that were not related in a parent-kitten relationship.

In Case 3, the DNA from drops of blood collected from vegetation was typed as mountain lion DNA and matched that from a lion killed in Stanislaus county with a match probability of 7.7 X 10^{-5} (one chance in 13,000). The presence of several alleles that were uncommon in the population allowed fewer loci to be analyzed in this case (Figure 3). Uncommon alleles, by definition, have a low frequency in the population, and therefore result in a lower match probability.

DISCUSSION AND MANAGEMENT IMPLICATIONS

This study demonstrates that useful information about species, individual identity, and relatedness between individuals can be determined from the DNA extracted from trace biological material left by mountain lions. This information will be useful to assess risk to human safety

and manage human-lion interactions. For carnivores that repeatedly attack humans, pets, or livestock, DNA information may be used to determine whether "repeat offenders" are related. The finding of bobcat DNA in the wounds of domestic sheep believed to be killed by a lion at Hopland also raised new ecological and management questions. Was the bobcat scavenging a lion-killed sheep or did it kill the sheep during sheep flock disruption cause by the lion? What are the competitive interactions of lions and bobcats at kill sites? In separate studies, we have also applied trace DNA analysis for incidents involving human fatalities caused by mountain lions (Culver et al. in prep) and mortalities of endangered species (Ernest et al. in prep).

Trace samples may not always provide sufficient DNA data to differentiate individuals or confirm the same individual from two samples. DNA in fecal samples, trace amounts of blood and saliva in bite wounds is likely present in very small concentrations and is subject to degradation. Match probabilities can help determine whether there is sufficient data to be reasonably certain that two samples came from the same individual. Two of the Hopland sheep swabs (Figure 1) provided sufficient DNA to confirm the individual identities of a bobcat (swab B) and the lion (swab D) that was killed the next night (match probability 5.6×10^{-5}). Two other swabs (C and E) contained sufficient DNA to confirm species as lion. These two samples probably represent the same lion that was killed because all alleles at loci that amplified were the same as those from the lion that was killed. However, match probabilities were higher than 1×10^{-4}, therefore we cannot be certain that swabs C and E came from the lion that was killed. For ecological research to determine mountain lion numbers, movements, and activities in the vicinity of people, scat DNA can add information to telemetry and tracking studies. At Yosemite National Park in California, a minimum number of lions was determined by using data from

capture of lions and scat DNA (Ernest et al. 2000). As researchers increasingly employ DNA sampling of predators, more data will be available to assist wildlife agencies prevent and manage conflicts between humans and lions.

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FIGURE LEGEND

Figures 1, 2, and 3. DNA data from Cases 1, 2 and 3. 0/0 indicates that DNA did not amplify and NA indicates that the locus was not tested. Microsatellite loci are denoted by an Fca number.

FIGURE 1

CASE 1

	swab A lion mouth	swab B bite wound	swab C bite wound	swab D bite wound	swab E bite wound
Fca 8	**152/152**	**134/136**	**152/152**	**152/152**	**152/152**
Fca 23	**142/142**	**140/140**	**142/142**	**142/142**	**142/142**
Fca 35	**123/123**	**0/0**	**123/123**	**123/123**	**123/123**
Fca 43	**134/134**	**122/126**	**134/134**	**134/134**	**134/134**
Fca 45	**127/127**	**135/135**	**127/127**	**0/0**	**127/127**
Fca 77	**133/133**	**141/143**	**0/0**	**133/133**	**133/133**
Fca 78	**188/190**	**180/180**	**0/0**	**188/190**	**0/0**
Fca 90	**105/107**	**105/107**	**105/107**	**105/107**	**105/107**
Fca 96	**201/201**	**181/193**	**201/201**	**201/201**	**201/201**
Fca 126	**131/131**	**135/135**	**131/131**	**131/131**	**131/131**
Fca 26	**140/140**	**130/130**	**140/140**	**140/140**	**140/140**
Fca 132	**174/178**	**170/174**	**0/0**	**174/178**	**0/0**

FIGURE 2

CASE 2

	Lion 1	Lion 2	Lion 3	Scat 1	Scat 2	Scat 3
Fca 8	**152/152**	**152/152**	**152/152**	**136/140**	**136/140**	**152/152**
Fca 23	**142/142**	**142/142**	**142/142**	**132/140**	**138/140**	**142/142**
Fca 35	**123/135**	**123/123**	**123/123**	**0/0**	**0/0**	**135/135**
Fca 43	**124/134**	**134/134**	**124/136**	**124/124**	**124/124**	**136/136**
Fca 45	**127/127**	**127/127**	**127/127**	**0/0**	**157/159**	**127/127**
Fca 77	**129/133**	**133/133**	**133/133**	**139/143**	**143/145**	**133/133**
Fca 78	**186/186**	**186/188**	**188/188**	**182/182**	**178/180**	**188/188**
Fca 90	**105/105**	**105/105**	**105/105**	**107/113**	**107/113**	**105/105**
Fca 96	**191/201**	**201/201**	**201/201**	**179/181**	**179/181**	**191/201**
Fca 126	**137/139**	**131/131**	**131/139**	**135/135**	**131/139**	**131/139**
Fca 26	**140/140**	**140/144**	**140/140**	**128/128**	**128/136**	**140/140**
Fca 132	**174/188**	**162/162**	**162/188**	**172/180**	**168/180**	**162/174**

FIGURE 3

CASE 3

	Trace sample	Captured lion
Fca 8	**164/164**	**164/164**
Fca 23	**142/142**	**142/142**
Fca 35	**135/135**	**135/135**
Fca 43	**124/134**	**124/134**
Fca 45	**127/127**	**127/127**
Fca 77	**NA**	**NA**
Fca 78	**NA**	**NA**
Fca 90	**NA**	**NA**
Fca 96	**201/209**	**201/209**
Fca 126	**131/137**	**131/137**
Fca 26	**NA**	**NA**
Fca 132	**NA**	**NA**