Research Article

Salivary DNA Evidence Convicts Breeding Male Coyotes of Killing Sheep

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Abstract

*Resolving conflicts between predators and livestock producers depends on obtaining reliable information about the predators that kill livestock. We used salivary DNA obtained from attack wounds on domestic sheep carcasses to identify the species of predator responsible for the kill, as well as the sex and individual identity of coyotes (*Canis latrans*) that killed sheep. Coyotes killed 36 of 37 depredated sheep. Breeding pairs whose territories overlapped sheep grazing areas were the primary predators on domestic sheep, and only breeding pairs killed multiple sheep. Breeding males, acting alone or with their mate, were involved in 21 of 25 kills. Breeding females participated in 13 kills, but only 1 breeding female killed sheep on her own. Transient females did not kill sheep, and both kills by transient males occurred in territories with a breeding vacancy. Our results suggest that predator control should be targeted at breeding male coyotes. Salivary DNA is a potentially powerful means of both investigating predation patterns and evaluating the effectiveness of control at targeting individuals that kill livestock.* (JOURNAL OF WILDLIFE MANAGEMENT 70(4):1087–1093; 2006)

Key words

California, Canis latrans*, carnivore–livestock conflicts, coyote, microsatellite DNA, noninvasive genetic sampling, predation, saliva, wildlife damage management, wildlife forensics.*

Salivary DNA has been successfully used to indict human criminals (Sweet and Hildebrand 1999, Sweet and Shutler 1999) and shows promise for identifying which predator species and individuals are most likely to kill livestock. The advent of polymerase chain reaction (PCR)-based DNA techniques allows amplification of small quantities of degraded DNA, thereby creating new avenues for the study of elusive predators and the management problems they cause. Predator feces are one source of DNA and have been used to determine whether endangered felid species were responsible for predation on cattle in Venezuela (Farrell et al. 2000). However, individuals that merely scavenge livestock carcasses also have livestock remains in their feces. Saliva is an alternative source of DNA that, when obtained directly from attack wounds, could distinguish killers from scavengers.

Presently, most information regarding which individuals kill livestock is obtained by radiotelemetry or direct observation. Direct observation is an option only for diurnal predators in open habitats, but even then it is time-consuming, and sample sizes are likely to be small. Radiotelemetry is more informative for nocturnal or secretive carnivores, but it provides information only on collared animals. In a study of coyote (*Canis latrans*) predation on sheep in northern California, 64% of confirmed coyote kills could be assigned (most to the same male) with high confidence in one year (Sacks et al. 1999), but only 24% in subsequent years (Blejwas et al. 2002). Although most kills were assigned to breeding coyotes, radiotelemetry could not distinguish whether one or both members of the breeding pair were involved in the attack. Furthermore, it was impossible to rule out the possibility that uncollared transient coyotes were responsible for a substantial proportion of the unassigned kills. Likewise, relying on field examinations of livestock carcasses to determine which species of predator was responsible for the kill also poses problems. The accuracy of these determinations depends on the skill and experience of the observer and may be complicated when several closely related species with similar kill patterns are present in an area (Cozza et al. 1996) or when individuals kill in a non-stereotypical manner (K. M. Blejwas, University of California-Berkeley, unpublished data).

Studies using these methods have shown that predatory behavior does vary based on age, sex, or reproductive status. Older, breeding (alpha) coyotes were responsible for most domestic sheep losses in north coastal California, USA, although young nonbreeders also had access to and were located close to sheep (Sacks et al. 1999, Blejwas et al. 2002). Adult male Eurasion lynx (*Lynx lynx*) were the primary predators on domestic sheep in Norway (Odden et al. 2002), and in the French Jura, only the removal of adult male lynx reduced the number of subsequent attacks (Stahl et al. 2001).

Salivary DNA evidence has the potential to revolutionize depredation studies by producing a more accurate picture of which predator species and demographic groups are most likely to kill livestock. We used salivary DNA to assess patterns of coyote predation on domestic sheep on a range in northern California and to compare our findings with those obtained from a concurrent radiotelemetry study at the same site (Blejwas et al.

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2002). Specifically, we used salivary DNA recovered from the attack wounds of predator-killed sheep to determine the species of the predator responsible for the kill, as well as the sex and individual identity of coyotes that killed sheep. Our objectives were to determine if 1) male coyotes killed more sheep than female coyotes; 2) one or both members of the breeding pair killed sheep; and 3) transient coyotes also killed sheep.

Study Area

We conducted our study at the Hopland Research and Extension Center (HREC), a 21.7-km^2 University of California agricultural research facility located in the outer Coast Ranges of Mendocino County, in northern California, USA. The HREC maintained a year-round flock of 650–1,500 research ewes, plus lambs. Most livestock losses to predators were lambs killed during the January–April lambing season (Sacks et al. 1999, Blejwas et al. 2002). Based on field examination of sheep carcasses and kill sites, coyotes were determined to be responsible for most of these losses (Scrivner et al. 1985, Neale et al. 1998), although mountain lions (*Felis concolor*), black bears (*Ursus americanus*), domestic dogs, and golden eagles (*Aquila chrysaetos*) also killed sheep (Scrivner et al. 1985). Prior to 1996, coyotes were nonselectively removed from the entire property on a year-round basis in an effort to reduce sheep losses. Only coyotes identified by radiotelemetry as killing sheep were removed during 1996–1998 (Blejwas et al. 2002). Nonselective snaring of coyotes resumed during the 1999 lambing season in the immediate vicinity of sheep kills.

Methods

Sample Collection

A minimum of 92 sheep were killed by predators during 20 October 1997–1 May 1999. Although coyotes were implicated in most of these kills, mountain lions were suspects in 7 kills, dogs in 3 kills, golden eagles in 2 kills, and bears in at least 1 kill (K. M. Blejwas, unpublished data). We collected 25 saliva samples between October 1997 and July 1998 as part of a radiotelemetry study of coyote predation on sheep (Blejwas et al. 2002). We collected samples only from fresh kills (<24 hrs old) to maximize the possibility of recovering DNA. The HREC shepherds collected an additional 18 swabs during January–May 1999 after the radiotelemetry study ended.

We field-necropsied sheep carcasses to determine the cause of death and swabbed puncture wounds with a dry, sterile cotton swab. We used subcutaneous hemorrhaging to distinguish attack punctures from bite marks made after the sheep was already dead (i.e., from dragging or feeding on a carcass). We only swabbed hemorrhaged puncture wounds to ensure that the DNA would be from the attacking predator; we typically selected 1 or 2 wounds for swabbing. The swabs were air-dried for 24 hours before being sealed in a paper envelope, placed in a plastic bag, and stored at −20°C for 3–22 months before DNA isolation. We collected tissue samples from 117 coyotes for DNA analysis; 53 samples came from radiocollared coyotes, and we obtained the rest of the samples from uncollared coyotes that were killed on or near the study site during 1992–2000. We aged canine teeth by cementum annuli (Matson's Laboratory, Milltown, Montana) to determine whether uncollared coyotes killed after the study ended were old enough to have potentially killed sheep during the sample-collection period.

Coyote Capture and Radiotelemetry

Coyote capture and radiotelemetry procedures were described previously (Blejwas et al. 2002). Capture and handling procedures were approved by the Animal Care and Use Committees at the University of California at Berkeley (Protocol R190-1496) and the United States Department of Agriculture National Wildlife Research Center (QA-267). We classified each radiocollared coyote as a breeding resident, nonbreeding pack associate, or nonbreeding transient based on space-use patterns, reproductive condition, association with a coyote of the opposite sex, and presence at a den with pups (Andelt 1985, Sacks et al. 1999). In 1997–1998, we delineated territories for each breeding pair from the time the pair was first formed or radiocollared until the death of one (or both) members. Because home-range boundaries usually changed following the death of a breeding coyote (Blejwas 2002), we estimated home ranges for each of 3 time periods that were bounded by mortality events. We defined pair territories by the 90% adaptive kernel (ADK) isopleth in program CALHOME (U.S. Forest Service, Pacific Southwest Research Station, Fresno, California; Kie et al. 1996), using a maximum of 2 randomly selected, independent (>6 hrs apart) locations per coyote per day. We used Arcview GIS (Version 3.2, ESRI, Redlands, California) for all spatial analyses. Radiotracking was too infrequent in 1999 to delineate territory boundaries and, instead, we inferred territories from limited telemetry locations, sightings, and previous boundaries.

DNA Analysis

We extracted DNA from tissue samples using Qiagen's DNeasy tissue kit (Qiagen, Inc., Valencia, California) and the manufacturers protocol as described elsewhere (Blejwas 2002, Williams et al. 2003*a*). Isolation of DNA from saliva, species identification using restriction fragment length polymorphism, sex determination using sex determining region Y (SRY), and genotyping at 3 canid microsatellite loci (FH2010, FH2159, and FH2137) were also described previously (Williams et al. 2003*b*). All saliva samples were also amplified at 4 additional canid-specific markers (FH2001, FH2062, FH2140, and CX2235) and visualized using autoradiography. We amplified samples via PCR as follows: initial denaturation at 94°C for 3 minutes, followed by 30 cycles of 94°C for 45 seconds, 50–53°C for 45 seconds, and 72°C for 45 seconds, with a final extension at 72°C for 3 minutes. Genotypes from autoradiographs were scored independently by 2 different observers; scoring was independent of tissue genotyping and radiotelemetry information.

We performed all swab genotyping with equipment and in areas dedicated to noninvasive samples, with negative controls for all DNA isolations and amplifications. Known dog, coyote, fox, and bobcat samples were included as positive controls for species identification. We also included sheep DNA as a sample to confirm that it didn't interfere with predator identification. The canid microsatellite FH2010 served as a positive control for SRY sex determination, which was performed using a known male and female coyote for comparison (Williams et al. 2003*b*). We repeated amplifications multiple times per locus as sample volume

allowed. For all loci, we included heterozygous genotypes in the analysis if each allele amplified twice or if autoradiographs revealed clean, distinct fragments. Allelic dropout can be a problem when working with degraded DNA (Taberlet and Luikart 1999) and we observed allelic dropout; therefore, we required that a homozygous genotype be amplified 3 times to be included in our analysis.

Statistical Analysis

We used Cervus 2.0 (Marshall et al. 1998) to estimate population allele frequencies from tissue genotypes of 99 coyotes captured or killed on HREC and surrounding properties. This smaller sample excluded pups to avoid biasing population allele frequencies by overly representing a few families (Hansen et al. 1997). Although a recent study found that coyotes in central California exhibit habitat-specific genetic subdivision on a regional scale (Sacks et al. 2004), the area we sampled was both small (roughly the equivalent of 10 coyote territories) and contained within a single habitat type, and we assumed no population sub-structuring within our sample. We used Cervus 2.0 to test for the presence of null alleles at each locus. Although 3 loci showed a slight homozygote excess, there were no homozygous–homozygous mismatches between known parents and offspring, and the loci were retained in our analysis.

We analyzed all genotypes that amplified at $\geq$3 loci. We assumed that unique genotypes came from different coyotes and searched for matches with our sampled coyotes. The ability of microsatellite markers to identify individuals can be quantified as either the probability that 2 individuals drawn randomly from the population will possess identical genotypes (probability of identity) or as the probability that the saliva genotype will match a genotype drawn randomly from the population (match probability; Waits et al. 2001). We adopted the second approach, which is commonly used in forensic applications. Because close relatives share many alleles by descent, the match probability will be higher when the randomly drawn genotype belongs to a parent, offspring, or sibling. We conservatively calculated the match probability for siblings at each locus ($P_{\text{sib-locus}}$) according to the formulas in Evett and Weir (1998):

$$P_{\text{sib-locus}}(\text{homozygotes}) = (1 + p_i)^2/4$$

$$P_{\text{sib-locus}}(\text{heterozygotes}) = (1 + p_i + p_j + 2p_ip_j)/4$$

where p_i and p_j are the proportions of alleles that are A_i and A_j. The loci are unlinked according to the map of the domestic dog genome (Ostrander et al. 1993, Francisco et al. 1996); therefore, we multiplied values across loci to obtain the overall match probability (P_{sib}). For comparison, we also calculated the *observed* probability of identity (Observed P_{ID}), which is simply the proportion of all pairwise genotypes that were identical at that particular combination of loci for the full sample of 117 genotyped coyotes (Waits et al. 2001).

Without knowing the genotypes of at least one of the suspects, match probabilities cannot be calculated for samples that contain DNA from 2 coyotes (i.e., $\geq$3 alleles at $\geq$1 locus). If one genotype is known, the second can be obtained by subtraction, but this process may be complicated by allelic dropout (Taberlet and Luikart 1999). Instead, we assumed these samples represented kills by the breeding pair in that territory and determined whether the observed alleles were consistent with the combined tissue genotypes of that pair. If we did not know the genotype of one member of the pair, then we inferred it by subtracting the known genotype of the other member from the saliva genotype. When multiple kills were assigned to the same pair, we confirmed that the genotype we inferred for the unsampled coyote was the same for all kills assigned to that pair. In 1999 we compared the saliva genotypes to those of all 3 breeding pairs whose territories potentially overlapped the kill site. We assigned those kills to a specific pair only if both other pairs could be excluded (i.e., by the presence of an excluding allele).

Results

We obtained saliva samples from predation wounds on 43 sheep (25 in 1998, 18 in 1999). We attempted to determine species for 37 of the samples. Canid-specific primers amplified DNA from 36 samples (97%); 35 were identified as coyote and 1 as a possible domestic dog (this sample did not amplify well and yielded no information on sex or microsatellite genotype). Most species assignments agreed with determinations made in the field. The 2 exceptions were the possible domestic dog kill (classified as a coyote kill in the field) and a kill that had been classified in the field as a possible mountain lion kill but that genotyped as a coyote kill.

Sex-specific primers amplified for 33 of 37 samples (89%). However, sex could only be reliably determined for samples containing DNA from a single coyote because mixed male–female samples would be identified as male (Williams et al. 2003*b*). We sex-typed 12 of 15 swabs that resulted in a single coyote microsatellite genotype; all were male (Table 1). These results agreed with genders assigned by matching microsatellite genotypes amplified from saliva to known genotypes from tissue of sampled coyotes. We did not sex-type the other 3 single-genotype samples due to insufficient DNA, but the microsatellite genotype from all 3 kills matched that of the same mated female (Table 1). Considering all 15 kills attributed to single coyotes, males killed significantly more sheep than females (binomial test, % males = 80%, $n = 15$, $P = 0.014$).

We successfully genotyped 25 of 43 (58%) samples at 3 or more canid microsatellite loci (Table 1). Amplification of 3 or more alleles at some loci indicated that 10 swabs contained DNA from $\geq$2 coyotes, including 1 swab that contained DNA from $\geq$3 coyotes. Similar percentages of samples yielded multilocus genotypes for both years (60% in 1998 vs. 56% in 1999), but there was a higher percentage of single-coyote genotypes in 1998 than 1999 (73% vs. 40%). Fourteen of 15 single-coyote genotypes had a $P_{\text{sib}} \leq 0.042$ and all 4-locus genotypes had a $P_{\text{sib}} \leq 0.022$ (Table 1). We compared these single-coyote genotypes to genotypes from 117 sampled coyotes; 13 matched only one of the sampled coyotes, and 2 did not match any of the sampled coyotes. We assigned 5 swabbed sheep kills in 1998 with high confidence to coyotes based on radiotelemetry, and all 5 assignments agreed with those made by matching microsatellite genotypes. For 10 swabbed kills, radiotelemetry identified a suspect with a lesser degree of confidence (i.e., the suspect was not

Table 1. Results of DNA analysis of saliva samples obtained from attack wounds on domestic sheep at the Hopland Research and Extension Center, California, USA. Shown for all samples are the date of the kill (Date) and the number of loci at which a genotype was obtained (No. loci). The sex-typing results (Sex), sibling match probability (P_{sib}), and the proportion of identical multilocus genotypes among all possible pairs of 117 genotyped coyotes (Observed P_{ID}) are given for all single-coyote kills. Individual coyotes are identified by number (Coyote) and Status (as determined by radiotelemetry). Letters indicate the territorial affiliation (Territory) of breeders (shown in Fig. 1).

Date	Sex	No. loci	P_{sib}	Observed P_{ID}[a]	Coyote	Status[b]	Territory
20 Oct 1997	Female[c]	4	0.019	0.0000	1	B	D
22 Oct 1997	Female[c]	4	0.019	0.0000	1	B	D
14 Nov 1997	Female[c]	4	0.019	0.0000	1	B	D
17 Feb 1998		6			4,10	BP	C
21 Feb 1998	Male	4	0.017	0.0000	2	B[d]	E
4 Mar 1998		3			4,10	BP	C
5 Mar 1998		4			2,11	BP	E
13 Apr 1998	Male	3	0.070	0.0018	3	T[e]	
6 May 1998	Male	4	0.021	0.0000	4	B	F
1 Jun 1998	Male	4	0.021	0.0000	4	B	F
18 Jun 1998		7			6,12	BP	A
20 Jun 1998	Male	6	0.002	0.0000	5	T	
21 Jun 1998	Male	5	0.004	0.0000	6	B	A
12 Jul 1998	Male	4	0.015	0.0000	6	B	A
12 Jul 1998	Male	5	0.007	0.0000	4	B	F
7 Jan 1999		4			?[f]	BP	?
14 Jan 1999	Male	3	0.042	0.0005	4	B	G
29 Jan 1999		5			7,13	BP	H
18 Feb 1999	Male	6	0.004	0.0000	7	B	H
23 Feb 1999		5			4,14	BP	G
26 Feb 1999		4			?	BP	?
22 Mar 1999		7			4,14	BP	G
27 Mar 1999	Male	4	0.022	0.0000	8	U	
10 Apr 1999	Male	4	0.018	0.0000	9	U	
1 May 1999		6			15,16	BP	B

[a] We did not calculate match probabilities and Observed P_{ID} for samples that amplified genotypes from 2 coyotes, which were assumed to represent kills by breeding pairs in that territory.

[b] B = territorial breeder, BP = territorial breeding pair, T = transient, U = unknown status.

[c] We did not sex-type samples, but we determined sex by matching the microsatellite genotype to the known genotypes of sampled coyotes.

[d] We inferred the status of this uncollared male by matching his genotype to one obtained by subtracting the known genotype of the radiocollared breeding female in that territory from the genotype of a 2-coyote saliva sample.

[e] We assumed this uncollared male to be transient because the breeding males in all nearby territories were known.

[f] ? indicates the genotype was consistent with $\geq$2 breeding pairs.

located right at the kill site). For 6 of those kills, the swab genotype matched that of the suspect identified by radiotelemetry.

In 1998, 3 breeding males killed 3, 2, and 1 sheep each and 2 transient males each killed 1 sheep, and 1 breeding female made 3 kills in 1997 (Table 1). Seven out of 9 kills assigned to a breeding coyote were located within that individual's home range, and the other 2 were within 160 m of the coyote's home range boundary (Fig. 1a,b,c). Additionally, all 4 swabs that yielded multi-coyote genotypes were consistent with the genotypes of breeding pairs in the territories in which the kills were found (Fig. 1b,c).

In 1999, 9 of 10 kills were located within or immediately adjacent to the range being used by a breeding male at the termination of the radiotelemetry study in 1998. Five of those kills were consistent with the genotypes of that male or breeding pair, although 2 of 5 were also consistent with other breeding pairs (Fig. 1d). Three of the 4 single-coyote kills were located within 0.5 km of each other, but were separated in time by 4 or 5 weeks, and all were by different males (Fig. 1d).

Considering only single-coyote kills, we identified 6 different killers in 1998 and 4 in 1999; 1 breeding male killed sheep during both years (Table 1). Most coyotes (6 of 9) killed only 1 sheep each, and only breeding coyotes were assigned multiple kills. Multiple kills were typically clumped in space and time and occurred within or on the periphery of the home range (Fig. 1a,b,c). Assuming kills by $\geq$2 coyotes represented kills by breeding (alpha) pairs, breeding coyotes killed at least 21 of 25 sheep (84%).

Discussion

Several patterns emerged from our DNA analysis. First, species-specific polymorphisms indicated coyotes were responsible for most sheep kills at our study site. Given our high amplification success (97%) and the availability of mitochondrial DNA primers for a wide range of carnivores (Foran et al. 1997, Paxinos et al. 1997), this technique should prove immediately useful in areas where predator species identification is problematic. Furthermore, we successfully amplified samples that were collected in both wet and dry weather at temperatures ranging from −2–37°C, indicating this technique is practical under a range of field conditions. Second, the sex-typing results confirmed, as suggested previously (Sacks et al. 1999), that males kill more sheep than females. All females that killed sheep were members of a mated pair, and only one breeding female killed sheep on her own. In contrast, both breeding and transient males killed sheep.

The propensity for males of a species to kill more livestock than females has been viewed as a function of larger male home ranges

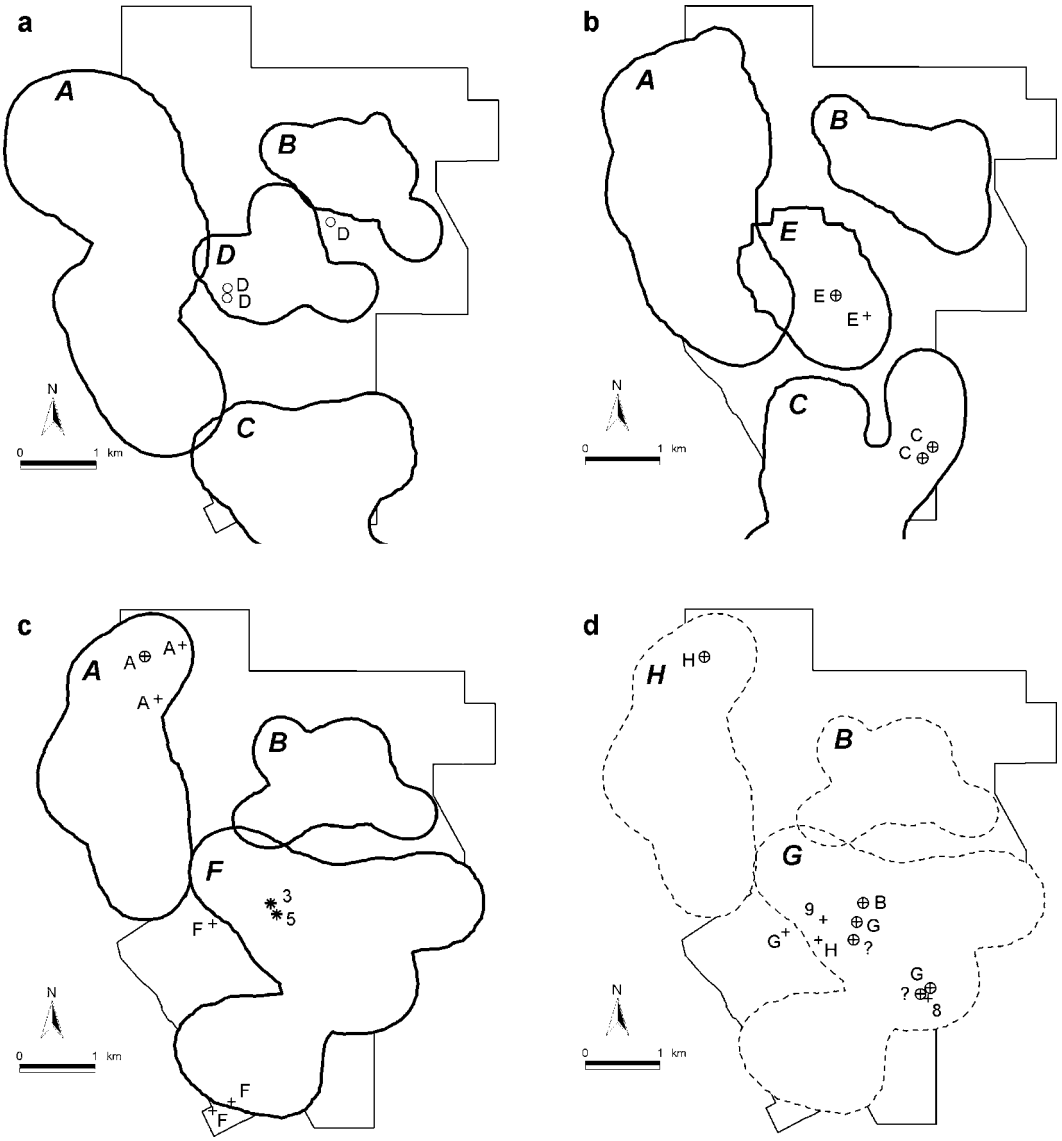

Figure 1. The location of domestic sheep carcasses in relation to breeding coyote home ranges on the Hopland Research and Extension Center, California, USA during (a) Sep–Nov 1997, (b) Jan–Mar 1998, (c) Apr–Jul 1998, and (d) Jan–May 1999. Home ranges were defined for each unique breeding pair (italic letters) during each period in 1998 (solid lines) and were inferred in 1999 (dashed lines). Symbol denotes status of coyote responsible for kill (circles = breeding females, crosses = breeding males, circled crosses = breeding pairs, asterisks = transient males or males of unknown status). Letter gives territorial affiliation, number identifies transient, ? indicates genotypes consistent with ≥2 breeding pairs.

(and therefore a higher encounter rate), larger male body size, or as intrinsic to male behavior (Linnell et al. 1999). Breeding coyotes defend a shared territory, and pairs were located together most of the time, including at kill sites (K. M. Blejwas, unpublished data), indicating that both members of the pair encountered sheep at the same rate during our study. Only one breeding female killed sheep (3 large lambs) on her own during our study; all 3 kills were atypical, with multiple punctures in the back of the head and neck (one kill also had more typical punctures on the throat), suggesting that smaller body size may put females at a disadvantage when killing larger prey. Alternatively, this may have reflected individual differences in hunting style, rather than physical limitations imposed by smaller size. Three kills by 3 different males also exhibited puncture wounds in the back of the head or neck; 2 of the kills involved smaller lambs, whereas the third was a large ewe.

It is not clear why breeding coyotes were the primary predators on sheep, but it does not appear to be a function of physical constraints on single coyotes handling larger prey. The fact that both individual coyotes and breeding pairs preyed on sheep of all sizes, including ewes, supports previous suggestions that territoriality rather than physical limitations regulates prey choice by coyotes, with breeding pairs that hunt together monopolizing larger prey within their territories (Sacks et al. 1999, Blejwas et al. 2002). Both transients that killed sheep did so following the deaths of successive breeding females in that territory (Territories E and F; Fig. 1b,c). Telemetry data from the one radiocollared transient indicated he was attempting to establish himself as the new owner of that territory at the time he made the kill (K. M. Blejwas, unpublished data).

Our study demonstrated that predator identification from salivary DNA has several advantages over radiotelemetry.

Although Sacks et al. (1999) had relatively high success in identifying the killer using radiotelemetry alone (64% of kills assigned), a single territory encompassed almost all of the lambing pastures, and 85% of those kills were assigned to the same (radiocollared) male. In subsequent years, multiple territories overlapped lambing pastures, the breeding males in those territories were not always radiocollared, and radiotelemetry identified the killer with high confidence in only 24% of kills (Blejwas et al. 2002). Salivary DNA alone exhibited a slightly higher success rate, identifying the killer in 14 of 43 kills (33%), including 2 kills assigned to uncollared individuals. This percentage would have been higher had the number of single-coyote swabs been higher.

Additionally, DNA evidence helped resolve situations where the radiotelemetry evidence was ambiguous, either because not all animals had been located the night of the kill or because multiple animals were located near a kill site. For example, transients at HREC were known to scavenge kills made by resident animals (Sacks et al. 1999, Blejwas et al. 2002) and 2 transient males were located within 300 m of a carcass the morning it was found. DNA evidence excluded both of those coyotes and instead assigned the kill to an uncollared male (the presumed breeder in that territory). Conversely, DNA evidence assigned one kill to a transient male rather than a neighboring breeding male who appeared to be expanding his territory into that area when both were located nearby.

We were unable to ascertain whether coyotes that killed sheep were resident or transient using salivary DNA alone. Theoretically, discrete and nonoverlapping distributions of kills by different individuals (or pairs) would suggest the kills were made by territorial residents. However, high breeder mortality at HREC was accompanied by changes in territory boundaries during the spring and summer of 1998 (Blejwas 2002), and the identity of coyotes that were killing sheep in the central territory also changed (Fig. 1a,b,c). The presence of multiple killers in this area in 1999 may again have reflected high breeder turnover in this area. These findings indicate that salivary DNA should be combined with radiotelemetry for a full understanding of predation patterns, particularly for heavily exploited predator populations.

Our study was clearly enhanced by having tissue genotypes from coyotes at the study site for calculating match probabilities; however, predator species identification and evaluation of control efforts could be accomplished even without those data. For example, in the absence of tissue samples, noninvasive sampling for scat or hair could be used to collect genotypes from the population at large (Woods et al. 1999, Ernest et al. 2000, Prugh et al. 2005) and similarly combined with genetic analyses of predation wounds.

We assumed samples containing DNA from $\geq$2 coyotes represented kills by breeding pairs. Although other types of pairings are also possible, particularly other combinations of pack members such as alpha–beta pairs of males (Gese and Grothe 1995), this assumption appeared reasonable for HREC. During 1996–1998, most mated pairs in territories that overlapped lambing pastures failed to produce offspring (Blejwas 2002). Furthermore, most HREC offspring dispersed from their natal territory and pack sizes during all years were small (Sacks 1996, Blejwas 2002). Transient pairs were also an unlikely alternative; although transients often frequented the same general areas, they were rarely located together and then only briefly.

Management Implications

We recommend that control efforts target breeding male coyotes. However, evaluating the ability of new or existing predator-control methods to selectively target particular demographic groups is difficult to do in the field. Salivary DNA offers a promising means of field-testing control methods by matching the DNA of individuals removed by control with the DNA from saliva swabs. Because coyotes often hunt sheep in pairs or groups, we recommend that multiple wounds from each kill be swabbed to ensure identification of all attackers. We recommend that future studies focus on determining the exact nature of these pairings or groups by using more careful procedures. Samples yielding $\geq$2 microsatellite genotypes could result either from swabbing multiple wounds with a single swab or from swabbing large wounds that represented multiple grabs by different individuals (and therefore contained DNA from >1 coyote). These problems could be avoided by swabbing only one wound with each swab and by choosing punctures that were clearly made by a single tooth.

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