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DNA persistence in predator saliva from multiple species and methods for optimal recovery from depredated carcasses

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Molecular forensics is an important component of wildlife research and management. Using DNA from noninvasive samples collected at predation sites, we can identify predator species and obtain individual genotypes, improving our understanding of predator–prey dynamics and impacts of predators on livestock and endangered species. To improve sample collection strategies, we tested two sample collection methods and estimated degradation rates of predator DNA on the carcasses of multiple prey species. We fed carcasses of calves (*Bos taurus*) and lambs (*Ovis aries*) to three captive predator species: wolves (*Canis lupus*), coyotes (*C. latrans*), and mountain lions (*Puma concolor*). We swabbed the carcass in the field, as well as removed a piece of hide from the carcasses and then swabbed it in the laboratory. We swabbed all tissue samples through time and attempted to identify the predator involved in the depredation using salivary DNA. We found the most successful approach for yielding viable salivary DNA was removing hide from the prey and swabbing it in the laboratory. As expected, genotyping error increased through time and our ability to obtain complete genotypes decreased over time, the latter falling below 50% after 24 h. We provide guidelines for sampling salivary DNA from tissues of depredated carcasses for maximum probability of detection.

Key words: depredation, DNA persistence, noninvasive DNA, predators, salivary DNA

As with other tools used for detecting wildlife species, there are trade-offs in the application of noninvasive DNA (Taberlet et al. 1999; Mills et al. 2000). Specifically, low quality and quantity of DNA can prove challenging for obtaining a robust species or individual identification and rapid degradation means there is a short time frame for collecting viable DNA. Due to these issues, when noninvasive DNA is collected from the environment, there are often errors in genotyping (Taberlet et al. 1999; Waits and Paetkau 2005). To minimize genotyping errors, a number of field, laboratory, and statistical methods have been developed (Taberlet et al. 1996; Bonin et al. 2004; Lonsinger et al. 2015). For example, field and captive experiments have

been used to develop protocols to collect the highest quality fecal DNA (e.g., Santini et al. 2007; Panasci et al. 2011; Lonsinger et al. 2015; Nakamura et al. 2017). However, the best field practices for collecting salivary DNA have not been as rigorously examined.

The use of salivary DNA as a forensic tool to identify the predator species and obtain individual identification is useful in several ways. Salivary DNA can identify the predator species and individual predators killing livestock (Williams et al. 2003; Williams and Johnston 2004; Blejwas et al. 2006; Sundqvist et al. 2008; Ernest and Boyce 2000; Caniglia et al. 2013) and improve our understanding of predator–prey

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interactions (Mumma et al. 2014; Marlow et al. 2015). Salivary DNA has also identified the predators killing endangered species (Imazato et al. 2012; Steffens et al. 2012; Marlow et al. 2015), or species of concern (Glen et al. 2010; Wengert et al. 2013; van Bleijswijk et al. 2014; Hopken et al. 2016), and game species (Kilgo et al. 2012; Mumma et al. 2014), as well as predators involved in crop depredation (Saito et al. 2008), bait uptake (Vargas et al. 2009), attacks on humans (Eichmann et al. 2004; Clarke and Vandenburg 2010; Farley et al. 2014), and other types of attacks (Ernest and Boyce 2000; Clarke and Vandenburg 2010; van Bleijswijk et al. 2014). The amplification of noninvasive DNA is also a well-established methodology for detecting species and estimating population size (Mills et al. 2000; Lukacs and Burnham 2005; Schwartz et al. 2007). The utility of this method has been demonstrated for coyotes (*Canis latrans*—Prugh et al. 2005; Morin et al. 2016), wolves (*C. lupus*—Lucchini et al. 2002; Stansbury et al. 2014; Piaggio et al. 2016), mountain lions (Ernest et al. 2000), and bears (*Ursus* spp.—Taberlet et al. 1997; Bellemain and Taberlet 2004; Wheat et al. 2016). Estimates of predator abundance from salivary DNA left on prey remains are possible as it is just as likely to sample both sexes and multiple age classes, but is best used in tandem with other noninvasive estimators (Wheat et al. 2016). Salivary DNA has also identified ungulate species and their resource partitioning when feeding on single trees (Nichols et al. 2012, 2015). Clearly, obtaining salivary DNA and identifying the depositing species has broad applications. However, studies show that amplification of noninvasive DNA (saliva, feces, hair, etc.) poses challenges and can be laboratory-intensive work due to its typical low quality and quantity (Taberlet et al. 1999; Harms et al. 2015; Lonsinger et al. 2015).

Degradation is the limiting factor for using salivary DNA to identify predators responsible for livestock depredations. Currently, little information is available on how rapidly the process of degradation occurs following depredation events. Salivary DNA from ungulate browsing on twigs was viable for 50% of samples 12 weeks after deposition (Nichols et al. 2012). In contrast, DNA from wolves and lynx (*Lynx lynx*) was shown to be viable for < 50% of samples only 48 h after deposition (Harms et al. 2015). Clearly, persistence of salivary DNA in the environment is highly variable.

Failures in obtaining individual identification from salivary DNA may stem from differences in 1) collection methods for obtaining salivary DNA, 2) persistence rates of salivary DNA over time, 3) deposition of saliva by the predator at the site of attack or feeding, and 4) saliva retention on fur of different prey species. Our study aimed to determine how these four factors affect DNA quality and identify optimal methods of field collection that increase success rates of individual identification of predators involved in livestock depredations. Our objectives were to test 1) two methods for sampling salivary DNA, including swabbing the carcass in the field, and transporting the hide from the field and swabbing it in the laboratory; 2) persistence of salivary DNA following depredation events by different predators (mountain lions, coyotes, and wolves); 3) differences

in probability of detection among different predator species; and 4) differences in probability of detection of predator salivary DNA with prey having different types of fur (calf, *Bos taurus* or lamb, *Ovis aries*). The results of this study will contribute to our understanding of the differences in probability of detection of DNA from saliva collected noninvasively from predator species, and thus aid in robust study design for the application of salivary DNA in forensic and ecological studies.

MATERIALS AND METHODS

Captive trials.—Individually housed, captive mountain lions, coyotes, and wolves were presented with prey items (calves for all predators, lambs for coyotes only) at two different facilities. Coyotes were housed at the United States Department of Agriculture, Wildlife Services, National Wildlife Research Center's (NWRC) Predator Research Facility in Logan, Utah, and mountain lions and wolves were housed at the Wildlife Science Center, Stacy, Minnesota. The trials with captive predator species were conducted as per the guidelines of the American Society of Mammalogists (Sikes et al. 2016), and reviewed and approved by the USDA-NWRC Institutional Animal Care and Use Committee (QA-1990).

We conducted experiments on captive animals in July–August 2012. We presented each study animal with a prey carcass (single predator on one carcass), allowing them to feed on it until sufficient saliva (subjective determination that enough saliva was present to collect desired samples) had been deposited on the carcass. We then removed the prey carcasses and left them outside in a sheltered area. We sampled saliva at five time intervals (0, 12, 24, 36, and 48 h) to assess the window during which DNA collection is optimal for reliable detection of predator species DNA and successful genotyping following a predation event. We tested two approaches for sampling salivary DNA: 1) swabbing the site of attack or feeding on the hide of the carcass in the field (henceforth referred to as “carcass”) as done in previous salivary DNA studies (e.g., Williams et al. 2003; Williams and Johnston 2004; Blejwas et al. 2006; Sundqvist et al. 2008; Ernest and Boyce 2000; Caniglia et al. 2013), and 2) removing and shipping saliva-saturated hides to be swabbed by personnel in the laboratory (henceforth referred to as “hide”). Using Catch-All Sample Collection Swabs (Epicentre, Madison, Wisconsin), we took two replicate samples at each time interval. We wet each foam swab with a single drop of sterile water to facilitate the removal of dried DNA molecules or buccal cells. We also collected oral swabs from the individual predators used in these trials to obtain the reference genotype for that individual. All swabs came with an individual capped sterile container; upon collection they were capped, frozen, and then shipped after the completion of trials overnight on ice packs to NWRC.

Laboratory analyses.—One technician performed all DNA extractions from swabs (two from each carcass and hide at each of five time intervals; i.e., 20 swab samples per prey carcass) using the QIAamp DNA Micro kit (Qiagen, Valencia, California) and eluting each sample in 50- μ l total volume

through two separate, 25- μ l elutions with the buffer ATE sitting on the filter for 5 min between each. Each set of extractions (10 carcass and 10 hide swabs from an individual prey) included a negative control to monitor for contamination in extraction reagents or processing. We performed extractions in a lab specifically dedicated to low quality or quantity DNA. We have extracted predator DNA previously, but always decontaminated with DNA AWAY surface decontaminant (Thermo Fisher Scientific, Waltham, Massachusetts) and used a biosafety hood with UV light for decontamination after each extraction. Further, our laboratory consists of separate rooms for extractions, pre-polymerase chain reaction (PCR) done in laminar flow hoods with UV light for decontamination after each PCR setup, PCR, and post-PCR to minimize contamination.

We genotyped saliva from coyotes and wolves using eight microsatellites for canids multiplexed into three panels for PCR amplification (Set A—Hopken et al. 2016). We followed the PCR reaction and thermocycling protocol of Hopken et al. (2016) with two exceptions: annealing was cycled for 30 s rather than 15 s, and multiplexes B and C were cycled for 45 cycles rather than 40.

We used 13 mountain lion-specific microsatellite loci (Kurushima et al. 2006). We originally tested these primers on tissue samples and found we needed to make some adaptations to amplify swabs optimally. We used a Qiagen Multiplex PCR kit (Qiagen, Valencia, California) for each of four panels (Supplementary Data SD1). We removed four loci that were uninformative or would not amplify well in these low quantity-quality DNA samples (PCOA312W, PCOA208W, PCOB309W, and PCOC108W). We further removed microsatellite panel 3A because we did not have enough DNA extract to amplify all panels for multiple replicates (see below for technical replicates) for each sample. Our microsatellite multiplex panels followed Kurushima et al. (2006) with one exception: we moved PCOB010W out of multiplex panel 2 and into panel 3B (as named by Kurushima et al. 2006) to reduce overlap in allelic distributions in multiplex panel 2. We also adapted the thermocycling regime where the initial denaturation was 95°C for 10 min for the hot start Taq polymerase in the Multiplex PCR kit. Further, we ran 40 PCR cycles and the final extension was 60°C for 30 min (Supplementary Data SD1). The rest of the cycling protocol is described in Kurushima et al. (2006). All microsatellite reverse primers used in this study were PIG-tailed to facilitate accurate genotyping (Brownstein et al. 1996). We performed three technical replicates of each PCR for each sample and across each multiplex panel for genotyping any noninvasive DNA (e.g., saliva) sample (Taberlet et al. 1999). Two technicians performed PCRs, but each ran separate panels to completion.

Statistical analyses.—We calculated the proportion of correctly amplified alleles (as determined by full genotypes of reference samples) out of all possible alleles across the three PCR replicates for each of the two carcass or two hide replicates. For example, for mountain lions, the allele proportion for each swab was 13 loci \times 2 alleles \times 3 PCR replicates = 78 (i.e., the proportion is anywhere from 0/78 to 78/78 or 0–1). We counted an allele as correct if it matched the appropriate

predator reference genotype. We used a mixed effects regression model to evaluate the proportion of alleles correctly amplified as a function of 1) sample type (hide versus carcass), 2) sample collection time, and 3) predator species. We included these three factors with interactions between predator species and sample type, and predator species and time of collection in the model. Because the two replicates collected at each time point for each of the two sample types were not independent, we treated the replicates as a repeated measure. In addition, in some cases, we offered individual predators more than one prey animal. Consequently, we included individual predators as a random effect in the model. We evaluated prey in a separate regression model because we offered only coyotes more than one prey type. That model evaluated the proportion of alleles correctly amplified for coyote samples as a function of prey type (calf or lamb), sample type, and sample collection time with the replicate swabs treated as repeated measures. We conducted all statistical analyses in R (R Core Development Team 2017).

We used known consensus genotypes from reference samples collected from predators in the study and GIMLET software to assess genotyping error rates (Valière 2001). We calculated false alleles and allelic dropout for each predator-prey combination, and for carcass swabs collected in the field over time versus hide swabs collected from samples sent to the laboratory over time. In some cases, we did not obtain reference genotypes from predator individuals; thus, we manually generated consensus genotypes as there were sufficient replicates from the carcass swabs to reliably infer the predator genotype. The resulting genotype input file for wolf and mountain lion tests on calf carcasses was too large for GIMLET, so carcass swabs taken at the captive animal facilities were analyzed separately from hide swabs taken in a laboratory setting. We also separated these collection methods for coyote-to-calf and coyote-to-lamb tests for comparison to wolf-to-calf and mountain lion-to-calf swab collections. Software including the R program Reliotype (Miller et al. 2002) could not determine the consensus genotype due to a majority of zeroes in the data set from many failed genotypes. These were maintained in the data set to accurately model the proportion of obtaining correct alleles across samples, time of collection, and type of predator or prey.

RESULTS

We successfully genotyped samples from nine wolf-to-calf, eight mountain lion-to-calf, and three coyote-to-calf tests for all five sampling time intervals as well as two additional coyote-to-calf tests having four time intervals. We also genotyped eight coyote-to-lamb tests having four time intervals (i.e., no 48-h samples). Our final data set for both coyote and wolf saliva genotypes were comprised of seven loci instead of eight because one locus (Locus 200 from Multiplex A—Hopken et al. 2016) was not amenable to scoring. All reference samples from nine wolves and two mountain lions were successfully genotyped. However, we had to infer the reference genotype by hand for a single coyote-to-calf test and two coyote-to-lamb tests. We removed data for one locus (D105) of one coyote

reference genotype because we could not infer a consensus for that locus. Consequently, for samples matched to that coyote, the proportion of correctly identified alleles was based on six loci instead of seven, but leaving the locus out should have minimal impact since we evaluated proportions of alleles correctly amplified and not raw numbers. In total, nine individual coyotes were tested, with one of the coyotes (with an inferred reference genotype) offered both a calf and a lamb.

When all predator to calf carcass data were assessed, sample type (carcass swab versus hide swab) had the strongest influence on amplification success for all three predators (Fig. 1). Time of collection had a positive correlation with amplification success, but the parameter estimate was near zero, implying a virtually neutral impact (Table 1). Exploratory data analysis showed that amplification of many coyote genotypes improved over time, which was counter to our expectation. Therefore, we plotted the wolf and mountain lion data separately from the coyote data to further explore the influence of time on obtaining the correct genotype for both swabs (Fig. 2A) and tissues (Fig. 2B). Although there is high variability, the proportion of

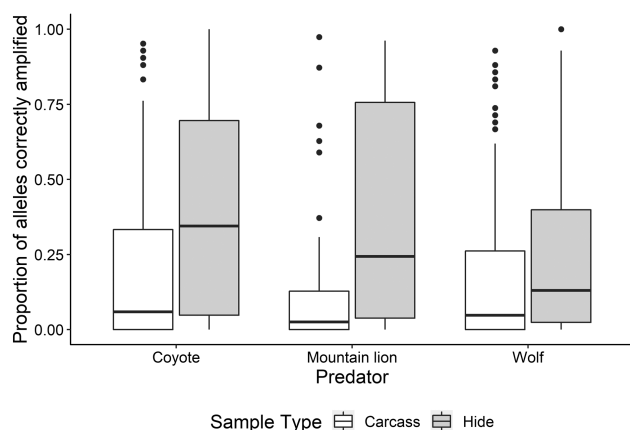


Fig. 1.—Boxplot showing the proportion of correctly amplified alleles for carcass and hide sample types by captive predator for the full data set. The bars across the columns are the medians for each category, and the length of the bars are the 25th and 75th quartiles (these show the spread of the data). Dots show data points that are outliers. “Carcass” refers to swabs collected by personnel at the captive predator facilities. “Hide” refers to swabs collected by laboratory personnel swabbing portions of hide removed from carcasses and sent to the lab from the captive facilities.

Table 1.—Regression parameters for a model evaluating the impact of sample type (carcass swab or hide swab) obtained from prey items, time of collection (0, 12, 24, 36, or 48 h), predator species (wolf, *Canis lupus*; mountain lion, *Puma concolor*; or coyote, *Canis latrans*), and their interactions on the proportion of alleles correctly amplified from saliva samples. Samples were collected from captive predators feeding on calf carcasses. Each carcass was removed and then sampled at five time intervals; some coyote-to-calf tests were not sampled at all five time intervals.

	Estimate	SE	df	t-value	Pr(> t)
Intercept	0.155	0.049	59.435	3.134	0.003
Sample type (hide)	0.169	0.034	519.213	4.983	0.000
Time of collection	0.002	0.001	523.557	2.129	0.034
Predator species (mountain lion)	-0.013	0.079	56.684	-0.168	0.867
Predator species (wolf)	0.270	0.076	56.634	3.529	0.001
Sample type (hide): Predator (mountain lion)	0.099	0.053	519.171	1.879	0.061
Sample type (hide): Predator (coyote)	-0.105	0.051	519.089	-2.081	0.038
Time:Predator (mountain lion)	-0.004	0.002	521.315	-2.289	0.022
Time:Predator (wolf)	-0.012	0.002	521.423	-7.342	0.000

alleles correctly amplified for wolves and mountain lions declined as time since sample collection increased. When only the genotypes obtained from hide swabs by personnel under laboratory conditions were assessed (Fig. 2B), the influence of time was clearer and decreased within the first 12 h and fell below 50% after 24 h. When coyote data were analyzed separately to examine differences between prey type (calf or lamb), time of collection was weakly correlated with amplification success, with a parameter estimate of 0.002 ($P = 0.051$).

Predator species had a significant influence on amplification success (Table 1) with wolves showing higher rates of correct genotypes from swabs compared to coyotes (parameter estimate = 0.270, $P = 0.001$), while mountain lions showed similar amplification success compared to coyotes (parameter estimate = -0.013, $P = 0.867$). Prey type was not a factor when obtaining a correct genotype ($P = 0.857$). Calves provided a higher proportion of accurate coyote genotypes but there was high variability in genotype success, especially for lambs as prey; therefore, the power to detect differences was low (Fig. 3). Genotyping error rates across PCR reactions as measured by allelic dropout ranged from 0.08 to 0.21 and false alleles across PCRs were 0.00 to 0.07 (Table 2). Carcass swabs had a lower genotyping error rate (allelic dropout = 0.08–0.14, false alleles = 0.00–0.05) than hide swabs (allelic dropout = 0.15–0.18, false alleles = 0.01–0.08).

DISCUSSION

We found there was a significantly better chance of obtaining correct genotypes from prey that had been fed upon by wolves compared to coyotes and mountain lions. A previous study comparing wolves and lynx also found higher genotyping success from wolf salivary DNA at kill sites (Harms et al. 2015). Differences might be due to the modes of interacting with prey by wolves, mountain lions, and coyotes. We also found that swabbing hide in the laboratory rather than the field led to more successful genotyping, which was likely due to the ability of laboratory personnel to devote time and considerable effort into carefully inspecting hide for traces of saliva (Fig. 3). Collecting saliva from depredated animals in the laboratory rather than the field is a novel sampling method for wildlife forensics that poses some challenges. For this study, we insured the hide was excised carefully to avoid prey blood as much as

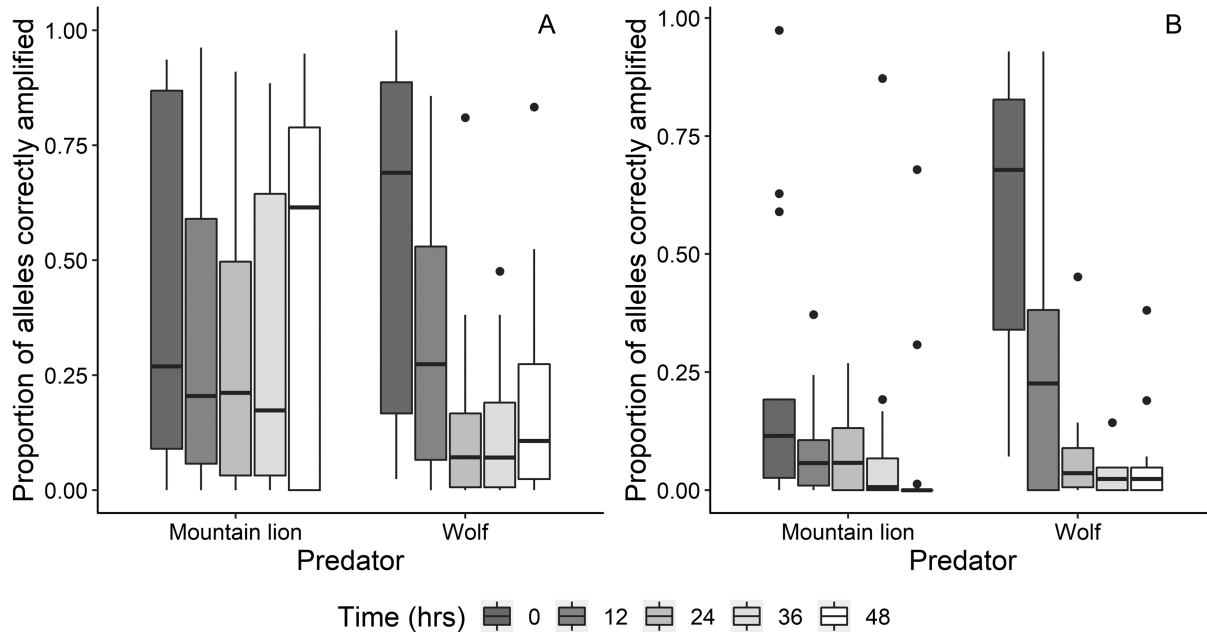


Fig. 2.—Boxplots showing the influence of time on allele amplification for wolves (*Canis lupus*) and mountain lions (*Puma concolor*) when (A) combining both sample types obtained from prey items (swabbing carcass in the field versus swabbing a portion of the hide in the lab), and (B) when only using swabs of hides obtained from prey items.

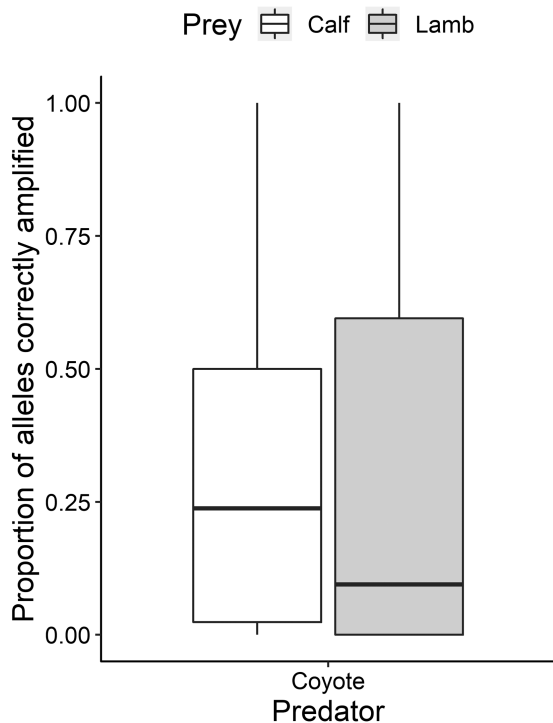


Fig. 3.—Boxplot showing the trend for higher correct genotype amplification of coyote (*Canis latrans*) DNA when calves are the prey compared to lambs.

possible, frozen expediently, carefully packaged, and shipped frozen (Appendix I) to avoid the laboratory receiving a piece of hide in a deliquescent state, which cannot then be swabbed for target buccal cells.

We had a low amplification success rate compared to most studies but we used a conservative approach for determining amplification success. Most studies accept any allele seen more

Table 2.—Genotyping error across polymerase chain reactions (PCRs) for each predator-to-prey combination, each combination with just swabs collected from the field (carcass), and for each combination with just swabs collected in the laboratory (hide). Each data set was analyzed with consensus genotype provided. AD = allelic dropout; FA = false alleles.

Predator-prey test	AD across PCR	FA across PCR
Coyote-to-calf	0.21	0.07
Coyote-to-calf carcass	0.12	0.02
Coyote-to-calf hide	0.15	0.08
Coyote-to-lamb	0.08	0.01
Coyote-to-lamb carcass	0.09	0.00
Coyote-to-lamb hide	0.18	0.01
Mountain lion-to-calf carcass	0.08	0.01
Mountain lion-to-calf hide	0.17	0.03
Wolf-to-calf carcass	0.14	0.05
Wolf-to-calf hide	0.18	0.06
Average	0.14	0.04

than one time because they do not know the real genotype of the predator individual (e.g., [Taberlet and Luikart 1999](#); [Blejwas et al. 2006](#)). We limited our positive calls to alleles that exactly matched the reference genotypes. Thus, any false alleles were considered unsuccessful amplifications (but we still accounted for false alleles in our error estimates). Our repeated sampling procedure (we collected 20 swabs for each predator-prey trial) reduced the overall genotyping success rate due to the variability in saliva distribution on the prey. While many carcass swabs from the field resulted in no amplification, the hide swabs amplified more successfully, but also included erroneous alleles. Unamplified alleles were not counted as genotyping errors but rather as zeroes in our data set, yielding lower error rate for the carcass swabs collected in the field. Even so, we were able to make strong comparisons of amplification success across these variables.

Overall, salivary DNA of wolves and mountain lions degraded through time. The data from wolf-to-calf trials were the most complete and both sample types (carcass swabs from the field or hide swabs collected in the laboratory) demonstrated rapid degradation of salivary DNA (below 50% successful genotyping of alleles within 12–24 h). This was less drastic when laboratory personnel swabbed saliva from portions of hide sent to the laboratory (Fig. 2). In a similar study, genotyping success did not drop below 50% until after 48 h (Harms et al. 2015), and another study of actual depredation events by wolves and dogs in Europe found that sampling should occur within 36 h (Caniglia et al. 2013). However, we measured success as the proportion of time a correct allele was amplified at a locus (per allele), whereas Harms et al. (2015) measured success rate as recovering a complete genotype. Furthermore, our sampling schemes were very different as our experiment was designed to compare sample collection types and sample collection times: 1) we had five time intervals and they had three; 2) we divided the saliva-covered carcass portions up for both swabbing and hide removal for all time intervals, thus reducing our chances of successful amplifications in swabbing attempts because some areas had very little saliva, whereas Harms et al. (2015) may have sampled the same spot (point with most saliva deposition) each time; and 3) we collected more swabs per time point. Thus, our results may show a more rapid decline in DNA persistence due to our study design (reduced opportunity for successful sampling), but overall it is clear from both studies that it is best to sample as quickly as possible after the depredation event. In typical forensic field investigations, we recommend using a sampling design similar to Blejwas et al. (2006) and sample all visible saliva traces at one time to maximize the opportunity for complete and accurate genotypes.

Most of our coyote genotypes and some of the mountain lion genotypes obtained from prey carcasses did not show degradation through time (Fig. 2). Instead, the genotypes were more complete and accurate in later time periods. This was likely a result of requiring multiple samples from small areas of saliva deposition, as described above. Dividing the area from which samples were obtained not only likely biased our success rate downward, but it may also be that by chance the earlier time point swabs may have been from areas with less saliva and later time intervals were from areas with a higher concentrations of intact buccal cells. Why this unexpected trend did not also occur in wolves may be related to differences in foraging strategy, or they may have simply left more saliva over a larger area relative to the other species.

Genotyping error in our study (Table 2) was low when compared to Sundqvist et al. (2008) and Caniglia et al. (2013). Similar to other noninvasive genetic studies, we found allelic dropout was more of an issue than false alleles. The data for the single predator species that interacted with two prey species (coyote-to-lamb and coyote-to-calf) did not show a significant difference in saliva being detectable between prey fur types. The nearly neutral effect we found was likely the result of some samples showing a positive trend over time, while others showed a negative trend, thus cancelling each other out.

However, the data trends demonstrated that we had proportionally higher success obtaining accurate predator genotypes from calf carcasses than from lamb carcasses. This may be due to saliva retention on fur type, or one's ability to identify saliva on one fur type over the other. While we noted the challenges of obtaining samples from sheep wool versus cow hide while collecting data, understanding if salivary DNA is more readily collected from one or the other would require further testing as our sample size lacked the power to differentiate between the two prey types. This issue should be investigated further as it could lead to design of prey-specific sample collection guidance allowing for optimal DNA capture.

In summary, wolf depredation events produced the highest probability of predator genotyping success, especially when sampled within the first 12 h. After 12 h, the genotyping success rate dropped to below 25%, suggesting this approach may not be the most effective in areas where the response to a depredation event cannot be rapid. However, if DNA can help solve depredation questions where traditional methods cannot, collecting a sample may be worth the attempt in the laboratory to get a small DNA sequence to help with species identification or a portion of the individual genotype. One could also increase the number of technical replicates performed in the laboratory (Lonsinger et al. 2015) to improve genotyping success rate if individual identification is critical. Our results provide new guidance on methods for optimal DNA recovery from depredated carcasses. Our new field collection method (removing a portion of the hide and shipping to the lab) increased genotyping success over the standard methods of swabbing depredated carcasses in the field. This method could also ease the burden of field personnel. To this end, we provide a detailed list of supplies, methods of collection, handling, and recording of relevant data, plus shipping instructions (Appendix I).

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SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Table with polymerase chain reaction (PCR) volumes and program for *Puma concolor* microsatellite loci. We amplified 17 loci in four multiplex reactions. We used the Qiagen Multiplex PCR kit (buffer 2×). The working concentration of each primer is listed after the name.

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APPENDIX I

Protocol for field collection of hide from depredated carcasses and how to ship for optimal DNA collection of predator saliva.

CARCASS HIDE SAMPLE COLLECTION PROTOCOL (To obtain predator DNA from depredated animal)

Supplies needed in the field:

- Latex or nitrile gloves
- Dissecting tools (cutting utensil: knife, scissors, razor blades, scalpel, etc.)
- Collection container (e.g., Ziploc or Glad container), bag (e.g., Whirlpak), and envelope
- Alcohol wipes
- Cooler with ice packs when possible

1. Collect samples within 24 h of the predation event.
2. Always wear **gloves** and use clean and sterile instruments.
3. First, it is important to differentiate attack wounds from scavenging. As best as you can, find areas where it appears where there are attack wounds and saliva is matted on the carcass and target this area for hide removal.
 - Once attack wounds or matted areas of saliva associated with attack have been identified on the carcass, cut a section of hide around the bite(s) or saliva-matted area using a clean, sterile knife, scissors, or razor blades. Clean the knife with alcohol wipes between hide sampling locations and prey individuals. Remove the hide leaving puncture wounds and bite patterns intact. The easiest method may be to cut around the perimeter of the target area and remove it in one piece. **Try to limit the amount prey blood and tissue as much as possible. The sample should be limited to hide rather than internal tissue (e.g., underlying muscle).**
 - Fold the excised hide in half, wool or hair side in, and place into the plastic collection container (Tupperware, Glad, or Ziploc container, Whirlpak, etc.) and put immediately on ice. If the excised skin will not fit in the container, a Ziploc plastic freezer bag may be used, but this is not ideal. Wrap a rubber band around the container to prevent the lid from popping off.
 - Label each container or bag with a unique field number.
 - **Freeze samples as soon as possible (–80°C preferred but not necessary).**
4. If you can identify areas away from the attack wounds that might have predator DNA (i.e., prey hair that appears matted with predator saliva or blood), collect samples from these areas as well. The process in step 3 can be followed or alternatively follow instructions below.
 - To collect prey hair that has predator saliva, clip hair next to skin using a razor blade or other cutting tool and place in a paper envelope labeled with the appropriate

field ID. Alternatively, sections of hide can be removed from the area where saliva is found.

- Do not lick the envelope to seal.
 - Do not get the envelope wet. The envelope may be placed in a plastic bag to protect it in the field but should be allowed to dry once back in the office. Alternatively, a few tablespoons of silica beads or multiple silica packets can be added to the plastic bag to dry out sample.
 - **Avoid areas saturated with prey blood.**
5. On the data sheet, refer to the field ID and fill in other pertinent information (date collected, name of collector, locality information, prey species, suspected predator

species, estimated time since kill, carcass condition, location on prey where hair or wound was collected).

6. Shipping instructions:

- Sample must be shipped frozen overnight to the lab. Shipping in a styrofoam cooler box is recommended. Surround the sample with as many frozen ice packs as possible.
- Ship the samples overnight as soon as possible to the processing lab
- If a sample is collected on a Friday or weekend, hold the sample in a freezer until the following Monday. UPS will not deliver on the weekend and the sample will sit in a warehouse until Monday.