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*Short communication*

# Straight from the coyote's mouth: genetic identification of prey through oral swabs of predators

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Human–carnivore conflicts often involve the depredation of domestic livestock. These depredation events are rarely observed, yet mitigation typically involves identifying the species or individual involved for removal or relocation. We tested a molecular method to identify individuals involved in depredation events using mouth swabs to determine if prey DNA could be detected, and for how long. We fed mule deer *Odocoileus hemionus* meat to captive coyotes *Canis latrans* and swabbed their mouths at five predetermined intervals between 2–72 h after consumption of the deer meat. We assessed two different molecular forensic methods to analyze the saliva swabs: qPCR for species identification and microsatellites for individual prey identification. We found that qPCR analysis was highly effective, detecting the deer DNA in the coyote saliva for up to 72 h post-deer consumption. Our results suggest that if an individual carnivore suspected of livestock depredation is captured within 72 h of a depredation incident, it is possible to confirm their potential involvement with a buccal swab and qPCR analysis. Utilizing this method could aid in more targeted and effective removal of individual problem carnivores as opposed to widespread removal of involved species.

Keywords: carnivore, environmental DNA, human–wildlife conflict, predator–prey interactions

## Introduction

Expanding human populations and encroachment into wildlife habitats in recent decades has resulted in increased conflicts between humans and carnivores (Woodroffe 2000). The most common source of human–carnivore conflict is depredation of livestock (Miller et al. 2016, Bano et al. 2021). These conflicts can be especially problematic when valuable livestock are involved or the carnivores are legally protected (Graham et al. 2005). Mitigation responses to depredations often involve lethal

removal of carnivores by state and federal agencies or retaliation by ranchers (Bano et al. 2021). Depredation events are often not evenly distributed among ranches but are instead concentrated in specific areas due to recidivism by problem individuals, causing significant economic losses for livestock owners in those areas (Muhly and Musiani 2009). Ranchers may receive financial compensation through government programs for livestock losses by some species of carnivores (Lennox et al. 2018). Even so, the economic hardships induced by depredation events can hinder local support for carnivore conservation (Miller et al. 2016).

Preventative measures to reduce depredation may involve non-lethal tools, such as livestock guard dogs or fladry (Young et al. 2018), but some attacks will still occur, and additional, more targeted actions may be needed. Attacks on livestock can be reduced by removing specific individual problem carnivores from the area (Löe and Röskaft 2004). However, depredation events are rarely witnessed, and direct observation is not a reliable method of identification (Sundqvist et al. 2008). Identifying the carnivore involved in a specific depredation event can be difficult, and relies on physical evidence at a kill site, which is often equivocal unless identification is possible from DNA detected from the bite wound (Williams et al. 2003, Piaggio et al. 2020). Environmental DNA (eDNA) from saliva remaining on prey carcasses at bite wounds has been used to identify the predator at the species level. For instance, saliva from the wounds of predator-killed sheep has been used to identify mountain lions *Puma concolor*, bobcats *Lynx rufus*, coyotes *Canis latrans*, wolves *Canis lupus* and domestic dogs *Canis familiaris* (Williams et al. 2003, Sundqvist et al. 2008, Harms et al. 2015). Species-level identification generally relies on mitochondrial sequence data from a single locus, and mitochondrial DNA occurs at a higher copy number in each mammalian cell, allowing species ID to be reliable from degraded environmental DNA.

Species identification is informative in depredation events, but individual identification would allow for more precise targeted management actions. For example, the selective removal of individuals responsible for depredation is more effective than widespread removal in reducing future conflicts (Woodroffe and Frank 2005, Lennox et al. 2018). Selective removal would also be a more ethical approach to wildlife management (Santiago-Ávila et al. 2018). Identification of specific individuals (i.e. fingerprinting) requires the use of nuclear markers (e.g. microsatellites) which have much higher polymorphism within species than mitochondrial genes. However, the copy number of nuclear DNA in cells is far lower than the copy number of mitochondrial DNA since there is generally only a single copy of the nuclear genome per cell, but many mitochondria. Thus, the identification of an individual predator involved in a depredation event can be a powerful tool in targeted management following a depredation event. However, this forensic method has an even lower probability of detection than species-level identification from carcasses (Piaggio et al. 2020).

Our study explores an alternative approach for identifying individual carnivores responsible for predation events by amplifying prey DNA from the oral cavities of carnivores suspected of predation. We tested two genetic techniques, species-specific qPCR detections and fingerprinting prey DNA using nuclear microsatellites, with the goal of determining if a suspected individual had fed on the prey species or individual carcass in question. In most cases, such as those mitigated by USDA–Wildlife Services employees in the USA, attempts to lethally remove carnivores begin within 0–48 h of a depredation event (Gehrt et al. 2011). One of the most common methods is to set live traps (e.g. foothold traps), and then euthanize any animal captured. In some cases, such as when depredation is by a threatened or endangered species (Anderson et al. 2002a) or in an urban area (Gehrt et al. 2011), there is a need to confirm the animal that caused the conflict is the one lethally removed or translocated. The confirmed presence of prey species DNA in the oral cavity of captured carnivores could identify whether the captured individual consumed the focal prey (e.g. livestock). Our goal was to determine if, and for how long, prey species DNA can be detected in the oral cavity of a predator. Our secondary goal was to determine whether prey individuals could also be identified from predator oral cavity swabs using nuclear microsatellites. We conducted this forensic experiment using captive coyotes that were fed mule deer *Odocoileus hemionus* meat, an item that is never included in their normal diet, and then collected coyote saliva at set intervals after consumption.

## Material and methods

### Study site

Experiments were conducted at the USDA National Wildlife Research Center's (NWRC) Predator Research Facility in Millville, Utah, USA. The facility maintains about 90 adult coyotes in captivity, typically housed as male–female pairs in large outdoor pens (0.001–0.01 km$^2$). Most of the coyotes are born and parent-reared on site but some are brought to the facility as wild-born pups to increase genetic diversity within the colony. Wild-born pups are hand reared until 10 weeks of age and then raised similarly to captive-born coyotes. At approximately 10 months of age, coyotes are placed into male–female pairs that are maintained throughout their lifespan to promote animal welfare and retain wild behavior (Shivik et al. 2009). The coyotes are fed 650g of commercial mink food (Fur Breeders Agricultural Cooperative Logan, Utah) at least six days each week by a caretaker entering the enclosure and scattering the food. The commercial mink food is a high protein, high-fat mixture of various meats (beef, poultry, fish and pork) that may also contain grain (corn, oats) and antibiotics. The mixture never includes mule deer or other wild game sources of meat.

### Sample collection

We used captive coyotes that were not on another concurrent study, could be housed in kennels, tolerated repeated handling

across a short period of time, and were identified by animal care staff as being willing to eat novel food items based on previous interactions with enrichment items that contained food (e.g. frozen yogurt or peanut butter in PVC tubes). These criteria resulted in 12 adult coyotes being assigned to participate in the study; two were randomly assigned to serve as controls. All 12 coyotes were housed individually in raised kennels (3.3 $m^2$) throughout the study to ensure they did not have access to any other food items (e.g. rodents, birds, reptiles, and insects) they may encounter in their outdoor enclosures.

The coyotes were fasted for 24 h at the start of the study to reduce the presence of commercial feed in the oral cavity and increase hunger so that the coyotes would be more likely to eat the novel food. After 24 h, animal care staff gave each of the treatment coyotes 650 g of mule deer meat, unprocessed and cut into chunks, while the two control coyotes received 650 g of commercial feed. Mule deer meat was donated to the study by a local hunter. Staff left the kennel area for two hours after providing the food, consistent with usual feeding procedures, because some captive coyotes will not eat when a human is present. Consumption of food was monitored at each of the sampling intervals within the first 24 h of receiving food, and the feeding bowl was removed as soon as ≥ 50% of the meat was consumed, so that the first time interval could begin, or if 24 h passed. Coyotes that refused the deer meat after 24 h were removed from the study for animal welfare purposes.

We took saliva samples from control and treatment coyotes at predetermined intervals – 2, 11, 26, 50 and 74 h after the deer or commercial feed was offered to the coyotes. Some samples were taken at different intervals because some coyotes only ate after the first two hours (Table 1). At each sampling interval, animal care staff manually restrained each coyote so the oral cavity could be thoroughly swabbed with four rayon drybud swabs (MWE (©Medical Wire and Equipment), DRYSWAB™ Fine Tip, MW113, Rayon bud), two that were sent to the USU Molecular Ecology lab that tested qPCR for analysis and two that were sent to the NWRC lab for microsatellite analysis. We obtained a sample from one control coyote before swabbing the treatment coyotes, while the other control coyote was sampled after swab samples were obtained from all treatment coyotes to detect if contamination occurred during sampling.

For each swabbing event, new nitrile gloves and disinfected coyote handling supplies were used. Immediately after sampling, the end of the sterile swab was snipped into individual sterile 1.5 ml microcentrifuge capped tubes (Olympus Plastics™, 1.7 ml clear microtube, Cat no. 24-282LR). Sample collection tubes were stored in a −70°C freezer until they were shipped to labs for extraction.

## DNA extraction

DNA was extracted from swab samples, deer meat samples, and samples of commercial food fed to the coyotes with a Qiagen DNeasy® Blood and Tissue kit following the manufacturer's protocol. Each extraction event included a negative control containing only an unused swab and reagents to monitor for contamination in the extraction process and was included in qPCR runs. Extracted DNA was stored at −70°C until further processing.

## qPCR assay design

To design an *O. hemionus* specific qPCR assay, we first compiled reference sequences from the mitochondrial gene cytochrome-b from GenBank for *O. hemionus,* and for non-target species we wished to exclude. This included 68 unique *O. hemionus* haplotype sequences from all subspecies across the species range. In addition, we included 44 sequences from *C. latrans* and five sequences each from *Bos taurus*, *Sus scrofa, Gallus gallus* and *Homo sapiens* (Supporting information). Sequence data from all species were aligned using Sequencher software ver. 5.2 (Gene Codes, Ann Arbor, MI), and species-specific primers were generated with the online tool DECIPHER (Wright 2015).

We initially chose two candidate primer pairs selected by DECIPHER for specificity testing with SYBR-Green qPCR in the laboratory. Each candidate primer set was tested on DNA extracted from ground deer, and deer sausage samples, DNA extracted from the coyotes commercial feed in a five-fold range (0.004–2.5 ng) in duplicate, seven DNA extracts of *C. latrans* tissues, and two no-template negative control samples. qPCR reaction included 7.5 µl Power SYBR-Green Mastermix (Thermo-Fisher Scientific, Waltham, Massachusettes), 900 nM of each primer, and 0.1 ng of template DNA (except feed samples) in a total reaction volume of 15 µl. qPCR cycling conditions were 95°C for 10 min, followed by 45 cycles of 95°C for 15 sec and 60°C for one minute followed by a melt curve. In-silico specificity was also evaluated by using NCBI Primer-Blast (Ye et al. 2012) to check if primer pairs were capable of amplifying DNA from non-target species not accounted for in the initial design.

One primer pair tested in SYBR-green qPCR outperformed the other in amplifying target deer DNA, as well as in excluding amplification from the commercial feed and coyote DNA samples. We then used ABI primer express software ver. 3.0.1 (Applied Biosystems, Foster City, CA) to design

Table 1. Species-specific (*O. hemionus*) primer and probe sequences and melting temperatures (Tm; °C) used in TaqmanTM qPCR assay. The amplicon size is 110 base pairs.

| Oligo | Name | Primer sequences (5′-3′) | Tm |
|---|---|---|---|
| Forward | ODHE-F2 | GTTTAATATGGGGAGGGGTATTGAG | 59 |
| Reverse | ODHE-R2 | AGGAGTATTAGCCCTAGTCTCATCTATCTT | 58.3 |
| Probe | ODHE-P | 6-FAM- TTGGAATTGATCGTAAGATTG -MGB-NFQ | 70 |

TaqMan Minor-Groove-Binder (MGB) fluorescent qPCR probe to complement our selected primer pair (Table 1). The primer set and probe from Table 1 were used in all subsequent analyses.

Assay sensitivity testing was conducted using serial dilutions of ground deer DNA extracts. Serial dilutions range from 0.1 to 0.00000128 ng per reaction and were run in triplicate with the assay to quantify the limit of detection. All samples were quantified using a Qubit™ fluorometer (ThermoFisher). Non-template controls (n = 2) and *C. latrans* (n = 7) DNA extracts were also included. qPCR reactions included 7.5 µl of Taqman™ Environmental Mastermix 2.0 (Thermo Fisher Scientific), 900 nM of each primer, 250 nM of probe, and four µl of template DNA in a total reaction volume of 15 µl. Cycling conditions were 95°C for ten min followed by 45 cycles of 95°C for 15 s and 60°C for 1 min.

### qPCR analysis

We ran all extracted swab samples through Taqman™ qPCR with the species-specific assay designed for this study. All qPCR runs included a five-step, fivefold standard curve composed of diluted *O. hemionus* genomic DNA ranging from 0.1 ng / reaction (highest step) to $1.28 \times 10^{-6}$ (lowest step), as well as no-template negative controls (n = 2) to monitor for contamination. All samples were analyzed in triplicate under the conditions described above for sensitivity testing.

### Microsatellite analysis

To determine whether DNA collected from coyote oral cavities could be used to identify individual prey animals, we used microsatellite genotyping. This lab work was conducted at the USDA National Wildlife Research Center's genetics lab. DNA was isolated from each swab following the manufacture's protocol, 'Isolation of DNA from Surface and Buccal Swabs' using a Qiagen Investigator Lyse and Spin Basket kit, QIAamp DNA Micro Kit and a QIAcube robot for automation (Qiagen, Valencia, CA). Each extraction batch included a negative control containing only an unused swab and reagents to monitor for contamination in the extraction process. Additionally, we extracted mule deer tissue and one piece of commercial food that were fed to the coyotes during the experiment. We followed the manufacturer's protocol using a Qiagen DNeasy Blood and Tissue kit and a QIAcube robot for automation (Qiagen). We stored extracted products at −20°C until further processing.

Four microsatellite loci were amplified in a single multiplex panel that amplifies cervid DNA and has been shown to amplify well with low-quality, high-quantity mule deer DNA (Table 2; Anderson et al. 2002b, Hopken et al. 2015). We performed fragment analysis for each PCR on an Applied Biosystems 3500 genetic analyzer (Life Technologies, USA), and manually binned and evaluated alleles using GENEMAPPER ver. 5.0 software (Life Technologies).

First, we performed a PCR using DNA previously isolated from the blood of five individual coyotes to determine if coyote DNA amplification can occur at any of our four microsatellite loci targeting mule deer. The PCR consisted of 5 µl Qiagen 2X buffer, 3.2 µl molecular grade water, 0.1 µl of each primer at 10 µM concentration, and 1 µl of DNA template. The PCR conditions followed Hopken et al. (2015).

Second, we performed a dilution experiment to determine if mule deer DNA would successfully amplify in the presence of varying concentrations of coyote DNA. Coyote saliva samples likely contain high concentrations of coyote DNA, and any interference between coyote and deer amplification could create ambiguous results. Using the PCR conditions described above, we ran DNA previously isolated from the tissue of two individual mule deer, in combination with coyote DNA at various ratios (Table 3). We used this format to test four different coyote samples in combination with two mule deer samples (Coyote no. 1 + Mule Deer no. 1, Coyote no. 2 + Mule Deer no. 1, Coyote no. 3 + Mule Deer no. 2, Coyote no. 4 + Mule Deer no. 2). We diluted the mule deer DNA in serial dilutions with molecular-grade water, combined with coyote DNA at full concentration, and took 1 µl of the mixed template for the PCR.

Third, we performed PCR on coyote buccal swabs from the first sampling time point of the experiment. There was a need for troubleshooting after preliminary results were unsuccessful, and non-specific amplification was abundant. Multiple rounds of PCR troubleshooting included modification to the type of polymerase, the quantity of DNA template, annealing temperature and time, extension time, total reaction volume, and demultiplexing the microsatellite primers. After determining that PCR conditions did not cause the non-specific amplification, we proceeded to amplify all swab samples using the same PCR conditions. These conditions followed Hopken et al. (2015) except for increasing

Table 2. Primer sequences for four microsatellite loci, the fluorescent dye used and the source. The original genomic resources used to develop the markers are denoted.

| Primer name | Primer sequence (5′-3′) | Dye | Reference |
|---|---|---|---|
| BM4208 | F: TCAGTACACTGGCCACCATG<br>R: CACTGCATGCTTTTCCAAAC | Ned | Bishop et al. (1994) – cattle |
| BM6506 | F: GCACGTGGTAAAGAGATGGC<br>R: AGCAACTTGAGCATGGCAC | 6Fam | Bishop et al. (1994) – cattle |
| BovPRL | F: GGAAAGTGAACATGACTGTCTAG<br>R: GCCCTCTCTTCTACAATGAACAC | Hex | Moore et al. (1994) – bovine |
| N | F: TCCAGAGAAGCAACCAATAG<br>R: GTGTGCCTTAAACAACCTGT | Hex | Jones et al. (2000) – deer |

Table 3. Sample scheme to test amplification of mule deer DNA using microsatellite analysis in the presence of various coyote DNA dilutions.

| Well | Sample |
|---|---|
| A1 | Coyote no. 1 |
| B1 | Mule deer no. 1 |
| C1 | 1:1 ratio Mule deer no. 1 to Coyote no. 1 |
| D1 | 1:10 ratio Mule deer no. 1 to Coyote no. 1 |
| E1 | 1:100 ratio Mule deer no. 1 to Coyote no. 1 |
| F1 | 1:1000 ratio Mule deer no. 1 to Coyote no. 1 |
| G1 | 1:10000 ratio Mule deer no. 1 to Coyote no. 1 |
| H1 | PCR Negative |

annealing temperature to 60°C and the number of cycles to 40. Finally, we also ran PCR with the same conditions in triplicate on the commercial food and deer meat fed to the coyotes.

### Statistical analysis

To determine the decay rate of DNA from the qPCR data across time since the food was provided, we first averaged the values of DNA quantity for each sample at each time step and used these values as the response variable for all models. We used non-linear mixed effects models with time since food was provided modeled as a negative exponential distribution and coyote identification as a fixed effect. We modeled time and amount eaten without an interaction effect, interactions between time and amount eaten, and with time alone. We ran models one at a time in this order using a stepwise approach to evaluate model significance. Only treatment coyotes were included in the models. Analysis was run in Program R (www.r-project.org), using package 'nlme' (Pinheiro et al. 2017). We were unable to run statistical analyses on the microsatellite data because results were ambiguous (Results).

## Results

Of the 12 coyotes assigned to this study, two served as controls, one was removed from the study because it did not eat ≥ 50% of the deer meat after 24 h, and nine coyotes remained as treatment animals. Of the remaining animals, during the first time interval (i.e. within two hours of receiving the food), only one female coyote ate 100% of the deer meat , one male coyote ate at least 75% of the deer meat, and three coyotes (one male, two females) ate at least 50% of the deer meat. The other treatment animals did not eat the deer meat during the first two hours and one coyote was excluded from the study because it did not eat at least 50% of the deer meat for the entire 72 h (Table 4).

### qPCR analysis

In assay specificity testing, no amplification was observed in any non-target DNA samples from *C. latrans* or from the commercial feed DNA with our *O. hemionus* qPCR assay. In sensitivity testing, our assay amplified down to 0.00000128 ng / reaction, indicating that our assay can detect very low concentrations of deer DNA.

Mule deer DNA was detected at all five time points, with DNA copy number typically declining as time progressed after feeding (Fig. 1). At the last sampling period (67.5–74 h post deer consumption) five of the nine treatment coyotes still had detectable deer DNA in their oral cavities (Table 4). Samples taken from the control coyotes remained free of contamination throughout all sampling periods, except for control coyote 1501, which showed low-level amplification in the T5 sample in one of the three replicates. It is not clear if contamination occurred during swabbing or during lab work.

While deer DNA copy number detected in saliva samples declined exponentially over time (Fig. 1), there was variation

Table 4. Oral cavity sampling data for each captive coyote given 650g of either mule deer meat (treatment) or their commercial food (control). Coyote identification codes provide the year of birth (first two digits), litter ID (third digit), and sex (final digit, odd for male and even for female). We planned to obtain saliva samples at 2, 11, 26, 50 and 74 h after coyotes received food (T1, T2, T3, T4 and T5 respectively), but some coyotes delayed consumption of the food and oral cavity sampling times were altered accordingly. At each sampling period, an estimated time since deer consumption was recorded as either these planned times or with an altered schedule of T1 had no sample taken, T2 at 6.5 h, T3 at 19.5 h, T4 at 43.5 h, and T5 at 67.5 h. Coyotes on the altered schedule are denoted with an asterisk (*). Two coyotes ate only a small amount at first but then consumed more deer meat between sampling times, so both percentages of consumption are given. Coyotes with NA at a sampling period did not have swabs taken because they did not eat enough deer meat or removed from the study early because they did not consume enough deer meat. Coyote 1600 ate < 50% of the deer meat and was therefore removed from the study to be fed her daily ration after T3 for animal welfare purposes. The quantity of DNA (*$10^{-4}$ ng) extracted using qPCR analysis are listed under T1–T5.

| Coyote ID | Status | % Consumed | Time consumed | TI | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|---|---|---|
| 1408 | Treatment | 100 | T1 | 4.539 | 0.653 | 2.284 | 1.002 | 0.258 |
| 1423 | Treatment | 75 | T1 | 832.517 | 4.408 | 6.857 | 0.201 | 0.136 |
| 1440 | Treatment | 50 | T1 | 95.839 | 0.439 | 0.091 | 0.049 | 0 |
| 1604 | Treatment | 50 | T1 | 491.532 | 1.549 | 1.928 | 0.246 | 0.053 |
| 1615 | Treatment | 50 | T1 | 221.719 | 1.284 | 0.588 | 0.928 | 0 |
| 1510* | Treatment | 100 | T2 | NA | 17.405 | 6.579 | 0.161 | 4.190 |
| 1600* | Treatment | < 5 | T2 | NA | 0.170 | 6.750 | NA | NA |
| 1705* | Treatment | 5, 95 | T2, T3 | NA | 0 | 4.511 | 1.389 | 0.350 |
| 1853* | Treatment | 25, 75 | T1, T2 | NA | 22.254 | 0.451 | 7.852 | 0.286 |
| 1802 | Control | 50 | T1 | 0 | 0 | 0 | 0 | 0 |
| 1501 | Control | < 10 | T3 | NA | NA | NA | NA | NA |

in DNA copy numbers within individual coyotes across time points, likely due to variations in oral swabbing such as coyote positioning, temperament, and amount of saliva present (Table 4). This might also be due to individual coyote mouth biomes, where such things as pH may affect detection probabilities (Davis et al. 2018). Copy number also varied among coyotes, but this was not related to the quantity of deer meat consumed. The four coyotes that did not have detectable DNA by T5 had not consumed all of the deer meat there were given; one had been removed from the study already because it did not consume sufficient deer meat to fast for 72 h of testing and the other three had only eaten ~ 50% of the deer meat. However, there was no effect for the interaction between time since feeding and quantity eaten. The main effect of amount eaten was $\beta = -0.00000811$ (SE= 0.00007, $p = 0.90$), which was reduced to $\beta = -0.0000057$ (SE= 0.00007, p= 0.94) when the interaction between amount eaten and time since feeding was included. Only time since feeding was significantly related to DNA copy number ($\beta = 0.2416$ (SE = 0.0391, $p < 0.001$).

### Microsatellites

Non-specific amplification occurred in all four microsatellite loci for the five coyote blood samples. However, this amplification did not fall within deer bins and thus we did not consider this cross-amplification as we would not mistake a coyote for a deer. Mule deer DNA amplified clearly, binned easily, and there was no non-specific amplification when combined with coyote DNA for all samples of the dilution experiment, even when deer was diluted to 1:10 000 of the amount of coyote DNA. Mule deer genotypes were consistent across all of the mixed coyote and deer samples. Thus, in the presence of coyote DNA, deer microsatellite DNA was still reliably amplified and individual alleles could be easily identified.

Amplification from coyote cheek swabs taken during the main feeding experiment resulted in multiallelic amplification in 42% of samples and clear peaks inside of deer bins 37% of the time. The commercial food sample amplified product in three of the four loci (Table 5) with peaks outside of deer bins, similar to the non-specific amplification seen in DNA from coyote cheek swabs. Amplification of the DNA extracted from the commercial food given to the coyotes during the study indicates that the ingredients in the commercial food (including bovine DNA, which was used in development in three of the four loci) amplified successfully and thus made the binning and assignment of deer alleles ambiguous.

## Discussion

We investigated if and for how long prey could be detected from DNA in the oral cavity of the predator after consumption of prey. In a controlled setting, we found qPCR to be an effective approach for prey species identification from saliva samples taken from the oral cavity of the predator. We demonstrated that prey DNA at the species level can be detected in carnivore saliva using qPCR up to 72 h after a feeding event. Our results suggest that qPCR methods could provide a useful tool in an unobserved depredation incident where identifying the individual responsible for a predation event is desirable. We demonstrate that DNA quantity is likely to be higher if oral swabbing is conducted soon after prey consumption, as most degradation occurred within eight hours. These results are consistent with studies of eDNA from predator saliva on bite wounds (Harms et al. 2015, Piaggio et al. 2020).

In field applications, a qPCR assay could implicate scavengers in addition to the primary predator, resulting in a false positive. The collection of a swab soon after a depredation event could reduce this probability but does pose a practical limitation on the utility of such an assay since the suspected

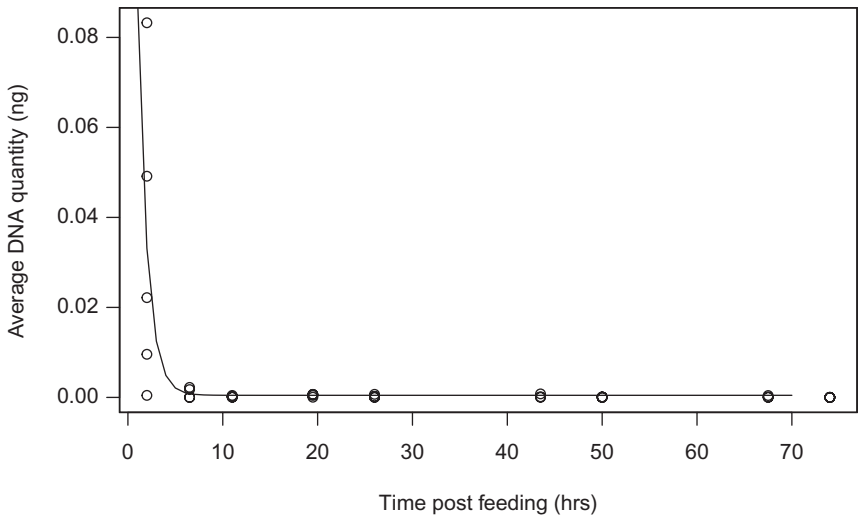


Figure 1. Exponential degradation plot showing the decline in average deer DNA quantity detected in samples from oral cavities of captive coyotes throughout sampling periods for up to 72 h after they received deer meat.

Table 5. Results from three replicates of each microsatellite PCR from the DNA extracted from commercial food and mule deer fed to the coyotes during the study. Numbers indicate scored allele sizes (bp) of the four microsatellite loci that fall within the range of established bins, question marks indicate non-specific amplification outside of established bins, and blanks represent no amplification.

| Field ID | Sample name | BM4208 | | BM6506 | | BOVPRL | | n | |
|---|---|---|---|---|---|---|---|---|---|
| Food | B-Deer-002295_1 | ? | ? | 206 | 206 | ? | ? | | |
| Food | B-Deer-002295_2 | ? | ? | 206 | 206 | ? | ? | | |
| Food | B-Deer-002295_3 | ? | ? | 206 | 206 | ? | ? | | |
| Deer tissue | B-Deer-002296_1 | 149 | 149 | 200 | 204 | 167 | 167 | 314 | 314 |
| Deer tissue | B-Deer-002296_2 | 149 | 149 | 200 | 204 | 167 | 167 | 314 | 314 |
| Deer tissue | B-Deer-002296_3 | 149 | 149 | 200 | 204 | 167 | 167 | 314 | 314 |

predator would need to be captured quickly. The animal would also need to be held while the lab results were obtained, which would take hours to days depending on sample transport time and lab availability. Future technological advances such as handheld thermocyclers will likely shorten this time. A false positive could also result if the assay amplified off-target species consumed by the predator. As with other eDNA assays, a qPCR assay for prey species should be validated with locally common prey species. Nonetheless, a well-designed qPCR assay could provide evidence of consumption in cases where forensic proof is needed, as in the case an endangered predator and/or depredation on livestock. Livestock depredation is the leading reason why carnivores are killed (Treves and Karanth 2003, Liberg et al. 2011).

A qPCR assay could also be useful in other settings, e.g., when determining whether feral or free-roaming dogs *C. familiaris* are responsible for depredations instead of wild predators. This could be important because depredation is often caused by domestic dogs (Bergman and Bender 2009, Caniglia et al. 2013, Home et al. 2017), yet depredations are most often blamed on wild predators, further eroding any willingness for coexistence. Further, it may be possible in the future to obtain dietary information by using the swab sample for metabarcoding (de Sousa et al. 2019), to determine the diet of a captured or recently deceased animal. This could provide information when scat is not collected or when more detail is needed than can be extracted from other techniques often used when animals are captured, such as obtaining whisker samples for stable isotope analysis (McLaren et al. 2015).

In our experiment, we also found that microsatellite analysis was not successful for prey species identification or individualization, most likely due to interference from ingredients in the commercial food fed to coyotes causing ambiguous amplification. However, deer microsatellites were successfully amplified in the presence of coyote DNA even when highly diluted, suggesting microsatellites could be effective in identifying deer DNA in the presence of coyote DNA. This could lead to an individual identification that could be matched to a depredated carcass if other items in the diet did not cause interference as we suspect occurred in our case. Even so, in a field setting there could also be unknown cervid/bovine species' DNA present that might interfere with microsatellite amplification. Thus, follow-up experiments should test alternate microsatellite loci designed specifically for the prey species in question, and empirically confirmed not to cross-amplify prey species that could co-occur in the oral cavity. Similarly, other DNA extraction techniques may also provide prey DNA from predator saliva. For example, an ungulate-specific assay followed by basic Sanger sequencing would allow to detect a broader range of prey species or an approach like that of Di Bernardi et al. (2021). We used qPCR instead of these other methods because qPCR is more sensitive than Sanger sequencing since it can theoretically detect down to a single DNA copy and is more cost effective than the approach described by Di Bernardi et al. (2021). Results of this study offer an important first step for additional methodology to identify prey items consumed by individual carnivores.

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### Transparent peer review

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### Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.7d7wm380z (Young et al. 2023).

### Supporting information

The Supporting information associated with this article is available with the online version.

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