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Tools and Technology

Comparing Morphological and Molecular Diet Analyses and Fecal DNA Sampling Protocols for a Terrestrial Carnivore

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ABSTRACT Analyzing predator scats for the presence of prey is a common noninvasive approach to understanding trophic interactions. Morphological analysis of prey remains has been the prevailing method of diet analysis, but molecular methods are becoming more widely used. Previous analyses suggest molecular methods detect target prey species more frequently than morphological methods. We compared these methods by analyzing coyote (*Canis latrans*) scats—collected in Tooele County, Utah, USA, in the winter of 2014—for leporids, a taxonomic group for which a molecular species identification test has been developed. We included 25 scats in which leporids were detected and 25 scats in which leporids were not detected by morphological methods. Additionally, because few studies have explored the effect of fecal sampling protocols on prey DNA detection, we analyzed subsamples taken from 5 locations on each scat to compare prey detection frequencies. We found that molecular analysis detected leporid prey in scats at a rate similar to or greater than morphological analysis, depending on the number of fecal sampling locations considered. Of the single samples, the homogenized (46%) and side (44%) samples provided the greatest rates of leporid prey DNA detection, followed by the ends (mean across both ends = 35%) and center (38%) of scats. When multiple sampling locations were considered, the homogenized-side combination (70%) had a detection rate similar to when all sampling locations were considered (76%). Our results indicate that molecular analysis detected prey more frequently than morphological analysis, but that prey detection was not equitable among fecal sampling locations and multiple sampling locations may be required. © 2017 The Wildlife Society.

KEY WORDS *Canis latrans*, fecal DNA, Leporidae, molecular diet analysis, molecular scatology, morphological diet analysis, noninvasive genetic sampling, prey identification.

Knowledge about predator–prey relationships is critical to effectively manage and conserve wildlife populations. One commonly used method of investigating these relationships is morphological diet analysis of prey remains in predator scats (Pearson 1966, Elmhagen et al. 2000, Klare et al. 2011). Scat analysis is noninvasive, making it more humane than alternative methods of diet analysis such as gastric lavage (Foster 1977, Votier et al. 2003, Barnett et al. 2010) or lethal take to obtain stomach contents (Hansel et al. 1988, Layman et al. 2005, Braley et al. 2010), particularly when target species are imperiled or difficult to capture. Morphological diet analyses have been used to evaluate how predator and prey population sizes change over time (Pearson 1966), how sympatric predators partition prey (Kozlowski et al. 2008, Spencer et al. 2014), and how predators alter their diet in response to changes in prey abundances (Dalerum and Angerbjörn 2000, Elmhagen et al. 2000). Though these methods provide insights about a population's diet, morphological analyses of scats depend on the presence of indigestible prey remains, such as bones, hair, or exoskeletons, and these parts may not always be present or identifiable. However, when indigestible prey remains are present, they can persist for extended periods of time, making the analysis of even old scats feasible (Tollit et al. 2009).

Genetic analysis of fecal samples provides an alternative method of diet analysis (Symondson 2002, Waits and Paetkau 2005). Instead of relying on the presence and identification of indigestible prey remains, molecular scatology approaches test for the presence of prey DNA in a predator's scat by using either 1) taxon-specific primers (Casper et al. 2007, Egeter et al. 2015, Mumma et al. 2016) to test for the presence of DNA from targeted prey groups (e.g., species or genus) or 2) universal primers (Deagle et al.

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2005, Lopes et al. 2015) to amplify and compare DNA sequences to reference databases to identify prey species with DNA barcoding (Valentini et al. 2009, Pompanon et al. 2012). Thus, molecular scatology may identify the presence of prey even when indigestible prey remains were not consumed, already excreted or regurgitated, or degraded too extensively for morphological identification (Casper et al. 2007, Deagle et al. 2009). Analysis of DNA may also provide higher taxonomic resolution than morphological diet analyses, particularly when indigestible prey remains can only be identified to family or order (Deagle et al. 2009, Braley et al. 2010).

Considering the quality of information that molecular scatology provides, few studies have attempted to evaluate and optimize methodological sampling approaches or compare molecular and morphological diet analyses; those that have focused primarily on marine predators (Deagle et al. 2005, 2009; Casper et al. 2007*a*; Tollit et al. 2009; Egeter et al. 2015; Mumma et al. 2016). Deagle et al. (2005) evaluated the frequency of prey detection in the scats of captive Steller sea lions (*Eumetopias jubatus*) fed a prescribed diet under different sampling strategies and found that a homogenized sample provided the highest rate of prey detection. Other studies on pinniped diets sampled from distinct fecal lobes (Deagle et al. 2009) or homogenized each scat (Casper et al. 2007*a*, *b*), but these studies did not systematically compare the effectiveness of sampling methods. Mumma et al. (2016) assessed the uniformity of prey DNA within 5 coyote (*Canis latrans*) and 5 black bear (*Ursus americanus*) scats by comparing prey species detection at 4 subsamples, but only limited inferences could be made because of the small sample size. All but one comparison between molecular and morphological diet analyses suggested that targeted prey was detected more frequently with molecular than morphological analysis (Casper et al. 2007*a*, *b*; Egeter et al. 2015; Mumma et al. 2016). However, scats of terrestrial predators are exposed to different environmental conditions, which influence DNA degradation (Piggott 2004, Brinkman et al. 2010). The physiology and behavior of predators and morphology of prey may influence contents present in the scat for morphological analysis (e.g., Symondson 2002, Tollit et al. 2006).

We investigated different fecal DNA sampling protocols for molecular scatology aimed at detecting target prey species in terrestrial carnivore scats. We evaluated prey detection rates of fecal DNA samples taken from different locations on a scat (Fig. 1), as well as a blended sample, which contained subsamples taken from multiple locations on a scat (hereafter, homogenized sample), and compared these prey detection rates to those produced from morphological diet analysis. We used the coyote as a model terrestrial carnivore and evaluated scats for the presence of 6 target leporid prey species: pygmy rabbit (*Brachylagus idahoensis*), mountain cottontail (*Sylvilagus nuttallii*), desert cottontail (*S. audubonii*), eastern cottontail (*S. floridanus*), white-tailed jackrabbit (*Lepus townsendii*), and black-tailed jackrabbit (*L. californicus*) using a mitochondrial DNA (mtDNA) species-specific primer approach (Adams et al. 2011). Based on past studies, we hypothesized molecular scatology would yield greater rates of prey detection than morphological analysis and that, among sampling protocols, the homogenized sample would provide the greatest rate of prey detection compared with subsamples collected from a single location.

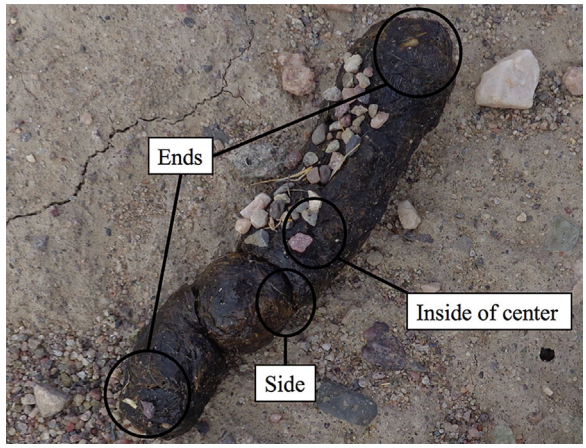

Figure 1. Diagram displaying the locations at which 5 fecal samples were collected from 50 coyote scats collected in Tooele County, Utah, USA, in the winter of 2014 for molecular scatology analysis. Approximately 500–600 μL of fecal material was collected from each of the 4 indicated locations (i.e., the side, each end, and center). A homogenized sample was collected by mixing approximately 125–150 μL from each of these 4 locations.

STUDY AREA

The study extent was approximately 3,015 km^2 and included portions of the U.S. Army's Dugway Proving Ground and neighboring federal lands in Tooele County, Utah, USA (Lonsinger et al. 2015*b*). Characterized as a Great Basin cold desert, the study site featured low-lying basins separated by abrupt range formations; elevations range from approximately 1,200 m to 2,100 m (Arjo et al. 2007). Winters at the study site are cold (coldest month is Jan, mean high = 4° C and mean low = −0° C) and summers moderate (warmest month is Jul, mean high = 36° C and mean low = 15° C, Lonsinger et al. 2015*b*). Average annual precipitation is approximately 20 cm with the greatest rainfall occurring in the spring (Arjo et al. 2007).

METHODS

We collected coyote scats in winter 2014 (13 Jan–19 Mar) along standardized transects that had been established for canid monitoring and research (Dempsey et al. 2015; Lonsinger et al. 2015*a*, *b*). Two researchers surveyed each transect. When a carnivore scat was encountered, we collected a fecal sample from the side of the scat (Stenglein et al. 2010); this sample constituted the side subsampling location (Fig. 1). Following protocols to minimize risks of contamination, we preserved a small amount of fecal material (~500–600 μL) in a tube containing 1.4 mL of DETS buffer (20% DMSO, 0.25M EDTA, 100 mM Tris, pH7.5, and NaCl to saturation; Seutin et al. 1991), bringing the total volume of buffer and scat in the tube up to 2.0 mL. Comparisons of preservation mediums have shown DETS

buffer to be superior or similar to other preservation treatments for canid fecal DNA (Panasci et al. 2011). We measured the scat (total length and diam. at widest point) and collected and froze the remaining scat in a sterile paper bag. At the time of collection, we assigned each sample a relative freshness score (1 = very fresh to 5 = very old) based on the scat condition (i.e., odor and color). Freshness scores for canid scats can provide a good indication of mtDNA amplification success for predator species identification (Smith et al. 2003), which suggests that freshness may also be important for detection of prey DNA (King et al. 2008).

We utilized the DNA extracted from the initial sample collected in the field (i.e., from the side of the scat) to confirm the identification of the carnivore species. We restricted all DNA extraction and polymerase-chain reaction (PCR) set up procedures to a clean lab, dedicated to low-quantity and -quality DNA. Fecal DNA extraction and PCR amplification followed procedures and conditions detailed by Lonsinger et al. (2015*b*). We used QIAamp DNA Stool Mini Kits (Qiagen, Inc., Valencia, CA, USA) to extract fecal DNA. To prevent contamination between samples, we handled fecal material with metal spatulas and forceps, which we sterilized with 10% bleach solution and rinsed with distilled water between samples; we used aerosol-resistant pipette tips for fluids potentially containing genetic material. To identify the carnivore species for each scat, we employed mtDNA species identification (De Barba et al. 2014). Samples that failed species identification on the first attempt were repeated once to minimize sporadic failures (e.g., due to pipetting errors). We conducted PCRs on a BioRad Tetrad thermocycler (Bio-Rad, Hercules, CA, USA). Using a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), we visualized PCR products and scored fragment sizes using Genemapper 3.7 (Applied Biosystems). We included negative controls with each extraction process and included both negative and positive controls during PCR procedures to monitor for contamination.

Following mtDNA species identification of carnivores, we thawed coyote scats to ambient temperature and collected 4 additional fecal samples of approximately 500–600 μL. One sample was collected from each end of the scat, one from within the scat, and one homogenized sample (Fig. 1). We were unable to distinguish between the leading and trailing ends of each scat and, consequently, ends of scats were arbitrarily labeled "end 1" and "end 2" during sample collection. The homogenized sample constituted approximately 125–150 μL from each of the 4 locations (i.e., side, each end, and middle) mixed together; to ensure that we collected approximately the same amount of fecal material for each sample, we added fecal material to the storage tubes until the total volume of scat and DETS buffer reached 2 mL. To minimize the potential influences of surface area (or volume) and DNA degradation, we selected scats of similar size and restricted our analysis to scats assigned a freshness score of 1 (very fresh) or 2 (fresh), respectively (Lonsinger et al. 2015*a*). All sample collection, preservation, and storage were accomplished with the same techniques as the initial sample.

Following sample collection for fecal DNA analyses, we secured each of the scats in nylon, soaked them for approximately 1 hr in hot water, and subsequently washed them in a standard washing machine with uncolored detergent for 2 cycles to remove fecal matter, leaving behind only indigestible prey remains (Kozlowski et al. 2008). Indigestible prey remains were dried in ovens at 70° C for 24 hr. We then performed traditional frequency of occurrence diet analyses, identifying and classifying prey items based on the indigestible prey remains within each scat (e.g., Kozlowski et al. 2008). We utilized reference collections and dichotomous keys from skull and dentition guides to inform classification (Glass 1973, White 1999, Martin et al. 2001). Reference collections included site-specific voucher prey specimens and hair samples provided by A. Kozlowski and utilized in Kozlowski et al. (2008), as well as hair samples provided by the Natural History Museum of Utah. We classified indigestible prey remains to prey classes aligning with previous (Kozlowski et al. 2008) and ongoing research, which included plants, insects, scorpions, reptiles, birds, kangaroo rats (*Dipodomys* spp.), small mammals (excluding kangaroo rats), ungulates, and leporids. Kozlowski et al. (2008) also included categories for anthropogenic material and miscellaneous mammals, but we did not encounter these classes. For this study, we were particularly interested in evaluating detection rates for leporids. Consequently, we further classified leporid evidence in scats to the highest taxonomic resolution possible.

To maintain a balanced sampling design and work within funding constraints, we conducted molecular analysis on samples from 50 scats: 25 scats in which leporids were detected and 25 scats in which leporids were not detected, based on morphological analyses. We extracted fecal DNA from the 4 additional samples collected from each of the 50 scats using the same procedures described above. We then used the extracted DNA from each of the 5 sampling locations (i.e., the side, each end, the middle, and a homogenized sample) to test for the presence of leporid DNA with a mtDNA species identification test that distinguishes among 6 different leporid species using 1 forward primer and 5 species-specific reverse primers (Adams et al. 2011). The previously existing primer multiplex that we employed did not distinguish between mountain and desert cottontails, but rather included a single reverse primer that amplified DNA of both species (Adams et al. 2011). Consequently, we were unable to distinguish between mountain and desert cottontails in this study. Furthermore, we were unable to determine the presence of mountain or desert cottontail in samples in which eastern cottontail had been detected ($n = 2$). Although this is a limitation of the multiplex we employed, it is not a technical limitation that could not be overcome with the development of a new primer set. For our purposes, the resolution provided by the developed primer set was sufficient (Adams et al. 2011). We conducted PCR procedures for leporid species identification following Adams et al. (2011) on a

BioRad Tetrad thermocycler and including negative and positive controls with each reaction to monitor for contamination and poor amplification performance. As before, we used a 3130xl DNA Analyzer to visualize fragment analysis products and PCR fragments were scored using Genemapper 3.7. Samples failing prey species identification (i.e., leporid species identification) on the first attempt were amplified a second time to minimize sporadic failures.

Statistical Analyses

We compared the number of scats in which leporid prey items were detected as a result of different sampling methods (i.e., molecular *vs.* morphological analysis) and sampling locations (among the genetic sampling strategies) using chi-square (χ^2) contingency tables. We used a separate 2×2 contingency table for each comparison of sampling approaches, where the dimensions represented the 2 sampling approaches being compared (e.g., morphological analysis *vs.* molecular analysis when all sampling locations were considered, morphological analysis *vs.* molecular analysis when only the side sample was considered, etc.) and the 2 possible outcomes (i.e., leporid prey detected or not detected). To account for our small sample size and reduce the probability of wrongly concluding there was not an effect, we evaluated the chi-square contingency tests at $\alpha = 0.10$. Additionally, to provide a measure of effect, we calculated odds ratios and 95% confidence intervals for each comparison (Nakagawa and Cuthill 2007). We performed all data analyses using the R statistical programming language (R Core Team 2014).

RESULTS

Morphological Analysis

The 50 scats we sampled had an average length of 15.45 cm (±0.56 SE), an average diameter of 1.98 cm (±0.04 SE), and ≤3 segments. Of these, 11 scats were classified as very fresh at the time of collection and 39 were classified as fresh. We detected leporid prey in 25 (50%) scats and failed to detect leporid prey in 25 (50%) scats. We were unable to distinguish between leporid species using morphological analysis.

Molecular Analysis and Comparison of Fecal DNA Sampling Locations

We did not detect contamination in any of our negative controls for DNA extractions or PCR procedures, and none of our positive controls for PCR failed to amplify. The mtDNA species identification tests detected leporid prey in 38 scats (76%) when all 5 sampling locations were considered (i.e., leporid prey was detected at ≥1 sampling location on the scat being considered; Fig. 2). Leporid DNA was detected in 15 scats (60%) where leporid prey remains were not detected with morphological analysis, and 23 scats (92%) where leporid prey remains had been detected. Mountain or desert cottontail, eastern cottontail, and black-tailed jackrabbit were detected in 66%, 4%, and 14% of the 50 scats, respectively. We did not detect pygmy rabbits or white-tailed jackrabbits in any of the samples analyzed.

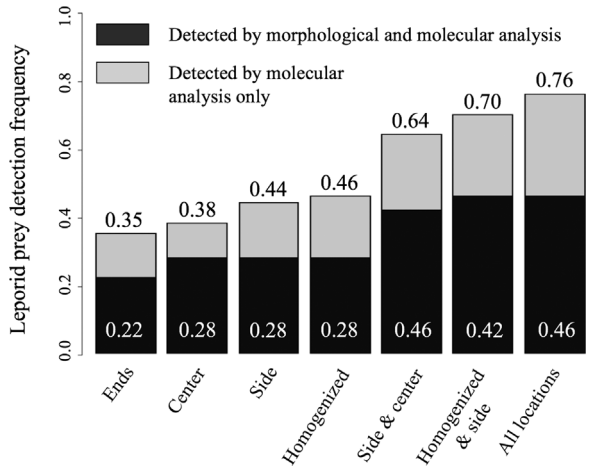

Figure 2. Comparison of leporid prey detection rates between morphological analysis and various fecal sampling strategies for molecular analysis of 50 coyote scats collected in Tooele County, Utah, USA, in the winter of 2014. Of the 50 scats we conducted genetic analyses for leporid prey on, we detected leporid hard parts in 25 (50%). The lower portion of the stacked plot indicates the proportion of scats for which both morphological and molecular analyses detected leporid prey. The upper portion of the stacked plot indicates the proportion of scats in which only molecular analysis detected leporid prey.

Among scats in which molecular analysis detected leporid prey, 8 (21%) scats contained DNA from 2 leporid prey species. Of these 8 scats, both prey species were detected at all sampling locations in 2 scats, and both prey species were detected at ≥1 location in 5 scats; in 1 scat, no >1 species was detected at a single location. For 13 (34%) of the 38 scats in which leporid DNA was detected, different prey species were detected at the 2 ends or prey was only detected at a single end.

There was no difference between the prey detection rates of the 2 end sampling locations (30% and 40%; $\chi^2_1 = 0.70$, $P = 0.40$) and we were unable to distinguish between trailing and leading ends; therefore, we pooled the prey detections from the 2 end samples. There was no difference between the rates of leporid prey DNA detection of the ends (35%), center (38%), side (44%), and homogenized (46%) samples (Table 1). Each of these sampling locations had a lower rate of prey detection than when considering all sampling locations (i.e., combining the results of all sampling locations together); the odds of detecting target prey with all sampling locations versus only the homogenized sample (the single sample location with the highest detection) was 3.67:1 (Fig. 2; Table 1). We also calculated the rates of prey detection for combinations of 2 sampling locations (i.e., when the results from 2 sampling locations were considered when determining whether a scat contained leporid prey) and found that the center-side (64%) and homogenized-side (70%) combinations provided rates of prey detection similar to (i.e., not significantly lower than) when all sampling locations were considered (Table 1). Odds ratios supported this, indicating that the odds of detecting target prey when considering all molecular sampling locations was not different from odds using the center-side or homogenized-side combinations. Although

Table 1. Comparison of leporid prey detection between morphological and molecular sampling methodologies, and among combinations of various molecular sampling strategies for 50 coyote scats collected in Tooele County, Utah, USA, in the winter of 2014. Sampling methods were compared using chi-square (χ^2) contingency tables. Morphological refers to the detection of indigestible leporid hard parts (e.g., hair, bones). Molecular refers to the detection of prey DNA sampled from the side, ends, or center of coyote scats. Homogenized samples included smaller portions from each of these locations. In some comparisons, results from 2 (e.g., center–side) or more (e.g., all locations) sampling locations were combined, where a detection of leporid DNA from ≥1 sampling location constituted a positive detection.

Sampling method 1 *vs.*	Sampling method 2	χ^2	*P*-value	Odds ratio[a]	95% CIs[a]
Molecular (all locations)	Morphological	6.18	0.01	3.13	1.2–8.2
Morphological	Molecular (ends)	2.53	0.11	1.85	0.9–3.9
Morphological	Molecular (center)	1.02	0.31	1.62	0.7–3.9
Morphological	Molecular (side)	0.16	0.69	1.27	0.5–3.0
Morphological	Molecular (homogenized)	0.04	0.84	1.17	0.5–2.8
Molecular (homogenized)	Molecular (ends)	1.27	0.26	1.58	0.7–3.3
Molecular (homogenized)	Molecular (center)	0.37	0.54	1.39	0.6–3.3
Molecular (homogenized)	Molecular (side)	0.000	1.00	1.08	0.5–2.6
Molecular (center)	Molecular (ends)	0.03	0.86	1.14	0.5–2.4
Molecular (side)	Molecular (ends)	0.80	0.37	1.46	0.7–3.1
Molecular (side)	Molecular (center)	0.17	0.68	1.28	0.5–3.1
Molecular (all locations)	Molecular (homogenized)	8.24	0.004	3.67	1.5–9.6
Molecular (all locations)	Molecular (homogenized–side)	0.20	0.65	1.35	0.5–3.7
Molecular (homogenized–side)	Molecular (homogenized)	4.97	0.03	2.71	1.1–6.8
Molecular (homogenized–side)	Morphological	3.37	0.07	2.31	1.0–5.8
Molecular (all locations)	Molecular (center–side)	1.19	0.28	1.77	0.7–4.7
Molecular (center–side)	Molecular (homogenized)	2.57	0.11	2.07	0.9–5.0
Molecular (center–side)	Morphological	1.47	0.23	1.77	0.7–4.3

[a] Odds ratios represent the odds (with 95% confidence intervals [CIs]) of detecting leporid prey with sampling method 1 *vs.* sampling method 2; an odds ratio = 1 indicates no difference.

the center-side combination did not significantly improve prey detection over single locations, the homogenized-side combination did. The odds of detecting target prey with the homogenized-side combination versus only the homogenized sample was 2.71:1 (Fig. 2; Table 1).

Comparing Molecular and Morphological Analyses

Although prey species identification may be possible from morphological analyses (e.g., when diagnostic hard parts or intact bones are present), we were unable to confidently distinguish between leporid species using morphological analysis. Thus, in our system, molecular analysis provided higher taxonomic resolution and confidence than morphological analysis. As a result, we did not distinguish between leporid species when comparing the rates of target prey detection of molecular and morphological analyses. The rate of prey detection of molecular analysis was 26% greater than morphological analysis when all sampling locations were considered ($\chi^2_1 = 6.18$, $P = 0.01$) and 20% greater when the homogenized-side sampling locations were considered ($\chi^2_1 = 3.37$, $P = 0.07$; Table 1). The odds of detecting target prey when all molecular sampling locations were considered versus morphological analysis was 3.13:1, while the homogenized-side sampling combination detected target prey 2.31 times more often than morphological analysis (Table 1). Notably, morphological analysis detected target prey more often than any individual sampling locations; however, these differences were not statistically significant and odds ratios were not different from 1:1 (Fig. 2; Table 1).

DISCUSSION

Investigations of predator–prey systems based on analysis of scats rely on accurate identification of prey remains and monitoring programs should aim to employ analysis methods that maximize prey detection. Molecular scatology methods offer wildlife practitioners and researchers an alternative analysis approach to morphological scat analysis, and may reduce the potential for false-negatives of consumed prey in the absence of indigestible hard parts in a predator's scat. Despite the capacity of molecular scatology to compliment, or even improve upon, alternative diet-analysis methods, few studies have compared multiple diet analysis approaches within a terrestrial predator–prey system or evaluated the most effective sampling methodologies for detecting prey DNA in terrestrial carnivore scats (Egeter et al. 2015, Mumma et al. 2016). The distribution of prey DNA in predator scats has the potential to greatly influence the results of molecular diet analyses, yet very little is known about how prey DNA is distributed within terrestrial carnivore scats. Consequently, our analysis more fully investigates prey DNA distribution (or uniformity) within terrestrial carnivore scats and the implications of this for fecal DNA sampling.

Comparing Fecal DNA Sampling Locations

As hypothesized, the homogenized sample had the greatest rate of prey detection, but it was not different from other sampling locations. Deagle et al. (2005) found that the homogenized sample had a greater prey DNA detection rate than subsamples taken from individual sampling locations. Homogenized samples might have greater rates of prey detection than subsamples taken from a single location if prey DNA is not evenly distributed throughout predator scats (Deagle et al. 2005). As a result, blending the scat can redistribute the prey DNA more evenly and increases the chances that it will be collected when a subsample is taken. However, homogenizing a scat entirely may not always be

practical for field-based studies. For example, preserving entire scats while in the field may not always be practical (e.g., not having freezers at remote sites) and homogenizing scats in the field may suffer from increased risk of cross-contamination when compared with conducting the homogenization in a controlled laboratory environment. We aimed to use a sampling method that would allow for a comparison between morphological and molecular diet analyses. Blending the entire scat would destroy or damage indigestible hard parts; therefore we elected to collect 4 smaller subsamples and mixed them in the DETS buffer storage tube rather than blending the entire scat (or a large portion of the scat) and taking a subsample from the blended material, as was done by Deagle et al. (2005). Our homogenized sample may have provided a slightly greater rate of prey detection than subsamples from individual locations due to an increased probability of taking a sample from an area containing leporid prey DNA, but perhaps a lower rate of prey detection than if we had blended the entire scat and distributed the prey DNA more homogeneously.

To our knowledge, this study is among the first to compare prey detection from specified subsampling locations for terrestrial carnivore scats. Studies investigating the distribution of DNA from the animal depositing the scat have found that samples taken from the outside portion of a scat (e.g., via rinsing the scat or collecting a subsample from the outside) had the greatest rates of DNA detection in carnivore and herbivore scats (Piggott and Taylor 2003, Wehausen et al. 2004, Stenglein et al. 2010). Furthermore, Stenglein et al. (2010) found that samples taken from the side of gray wolf (*Canis lupus*) scats had greater DNA detection rates than samples collected from the ends of the scats. Although we expected prey DNA may be similarly distributed within carnivore scats, it is possible that the consumption of multiple prey species, and the order of consumption, may further influence the distribution of prey DNA within scats. However, we were unable to detect a significant difference in prey DNA detection between subsampling locations based on either null hypothesis testing or odds ratios. It is possible that the relatively small sample size prevented the detection of a difference between subsampling locations and future studies should investigate the effect of fecal subsampling location on prey detection further.

Considering Multiple Sampling Locations

When results from all sampling locations were combined, the prey detection rate was maximized. However, practitioners employing molecular diet analyses may not have sufficient resources to collect and analyze up to 5 samples/scat. Alternatively, collecting only 2 samples/scat (i.e., from the side and a homogenized sample) may provide a more cost-effective alternative, as this sampling method produced prey detection rates only 6% lower and not statistically different from those based on the results of all 5 sampling locations combined. The homogenized samples had slightly greater prey detection rates than other individual sampling locations and would be the best alternative if funding constraints limited analysis to only a single sample per scat. Fully homogenized scats (i.e., blending the entire scat) may provide the greatest prey detection rates (Deagle et al. 2005) and therefore, may be the most suitable method for studies seeking to maximize prey detection. Fully homogenizing scats can provide a higher quality of samples for other, complimentary analyses such as fecal hormone analyses (Millspaugh and Washburn 2003, Beehner and Whitten 2004) or quantifying the proportion of prey DNA in scats with real-time PCR (Bowles et al. 2011). However, it can also limit subsequent morphological analyses, which may be important for calibrating historical diet analyses with contemporary analyses implementing genetic methods.

Comparing Molecular and Morphological Analyses

Overall, molecular analysis had a greater rate of prey detection than morphological analysis when the results of multiple sampling locations were combined. However, morphological analysis provided a slightly greater rate of prey detection than molecular analysis when compared with a single sampling location or the homogenized sample. This finding is in contrast to most other studies that have compared the rates of prey detection between molecular and morphological analyses, which have found that molecular analysis has a greater rate of prey detection when a homogenized sample or a sample taken from one location on a scat is analyzed (Casper et al. 2007*a*, *b*; Deagle et al. 2009; Egeter et al. 2015; Mumma et al. 2016). Tollit et al. (2009) found that molecular analysis had a lower prey detection rate, but suggested that this may have been due to a disproportionately large number of samples taken from aged scats, which have been shown to provide lower rates of prey DNA detection (Piggott 2004). We included only the freshest scats in our sample to minimize the influence of scat age on our results. Additionally, we only included samples for which we were able to successfully amplify predator DNA to confirm that the scat originated from coyotes. Thus, it is unlikely that the age of the scats significantly reduced prey DNA detection more than in other similar studies. Interestingly, our findings are inconsistent with the only other investigation of molecular scatology focusing on large, terrestrial carnivores, including coyotes. Mumma et al. (2016) conducted molecular analysis on coyote scats to detect snowshoe hare (*L. americanus*), as well as moose (*Alces alces*) and caribou (*Rangifer tarandus*), and found greater rates of prey detection from molecular analysis of a homogenized sample (like the approach used here) than morphological analysis. This difference might relate to DNA fragment lengths under investigation. Fecal DNA is often degraded, and longer fragments of DNA may have a reduced amplification success rates and detection (Murphy et al. 2000, Lonsinger et al. 2015*a*). Snowshoe hair, mountain or desert cottontail, and eastern cottontail fragment lengths are similar (173-, 172-, and 177-base pairs [bp]), but black-tailed and white-tailed jackrabbit fragment lengths are longer (293- and 348-bp), so the jackrabbit DNA might have had lower detection rates (Adams et al. 2011).

Our study demonstrates that, although molecular diet analysis can be more powerful for detecting prey than

morphological diet analysis, prey DNA detection is not always equitable across sampling locations and sampling from multiple locations on a scat can significantly improve prey DNA detection. Although evidence from past studies supports the hypothesis that molecular diet analysis improves prey detection rates over morphological analysis of predator scats (Casper et al. 2007, Tollit et al. 2009, Egeter et al. 2015, Mumma et al. 2016), our results suggest that prey detection rates may be similar at the population level if only one genetic sample (i.e., a sample collected from one discrete location on a scat) is analyzed. Thus, the value of comparing the effectiveness of diet analysis methods in additional systems is clear. This could be done by conducting a pilot study before using either type of noninvasive diet analysis approach, which would allow the comparison of sampling locations and methodological approaches to determine which approach would yield the greatest prey detection rates under particular study conditions. Furthermore, conducting a pilot study where prey DNA detection is maximized by taking genetic samples from multiple locations on scats could allow for the development of a correction factor to be applied when only one genetic sample is taken from a scat, or when morphological analyses are conducted. Additionally, it would be valuable to compare frequencies of prey detection when scats are fully homogenized to when multiple subsamples are taken from a scat and blended to determine whether fully homogenizing a scat increases prey detection significantly over taking multiple distinct subsamples. Continued work exploring sampling methodologies will further inform future noninvasive diet analysis studies and lead to practices that maximize prey detection rates.

The decision to use molecular or morphological analyses may depend on factors other than prey detection rates, such as the research question, funding availability, and tolerance for false negatives. For example, accurate estimates of diet proportions are difficult to obtain with molecular scatology (Deagle and Tollit 2007, Bowles et al. 2011, Bowen and Iverson 2013). Morphological analyses may provide sufficient information about the relative proportions of prey classes consumed, although findings can be biased by inequities in the presence and persistence of hard parts among prey. Costs will generally be greater for molecular diet analyses when compared with morphological analyses and this disparity may be exacerbated when molecular studies require the development of new species-identification tests (Mumma et al. 2016). Although molecular scatology can offer greater confidence in prey identification and may increase prey detection rates, our results support previous research indicating that prey DNA may not be uniformly distributed in predator scats (Mumma et al. 2016), and that molecular scatology can suffer from false negatives. Overall, future research across a diverse range of systems comparing the effectiveness of noninvasive methods of diet analyses will increase the quality of information that future diet analyses can provide, and will allow better-informed wildlife management and conservation decisions to be made.

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LITERATURE CITED

Adams, J. R., C. S. Goldberg, W. R. Bosworth, J. L. Rachlow, and L. P. Waits. 2011. Rapid species identification of pygmy rabbits (*Brachylagus idahoensis*) from faecal pellet DNA. Molecular Ecology Resources 11:808–812.

Arjo, W. M., E. M. Gese, T. J. Bennett, and A. J. Kozlowski. 2007. Changes in kit fox-coyote-prey relationships in the Great Basin Desert, Utah. Western North American Naturalist 67:389–401.

Barnett, A., K. S. Redd, S. D. Frusher, J. D. Stevens, and J. M. Semmens. 2010. Non-lethal method to obtain stomach samples from a large marine predator and the use of DNA analysis to improve dietary information. Journal of Experimental Marine Biology and Ecology 393:188–192.

Beehner, J. C., and P. L. Whitten. 2004. Modifications of a field method for fecal steroid analysis in baboons. Physiology & Behavior 82: 269–277.

Bowen, W. D., and S. J. Iverson. 2013. Methods of estimating marine mammal diets: a review of validation experiments and sources of bias and uncertainty. Marine Mammal Science 29:719–754.

Bowles, E., P. M. Schulte, D. J. Tollit, B. E. Deagle, and A. W. Trites. 2011. Proportion of prey consumed can be determined from faecal DNA using real-time PCR. Molecular Ecology Resources 11:530–540.

Braley, M., S. D. Goldsworthy, B. Page, M. Steer, and J. J. Austin. 2010. Assessing morphological and DNA-based diet analysis techniques in a generalist predator, the arrow squid *Nototodarus gouldi*. Molecular Ecology Resources 10:466–474.

Brinkman, T. J., M. K. Schwartz, D. K. Person, K. L. Pilgrim, and K. J. Hundertmark. 2010. Effects of time and rainfall on PCR success using DNA extracted from deer fecal pellets. Conservation Genetics 11:1547–1552.

Casper, R. M., S. N. Jarman, B. E. Deagle, N. J. Gales, and M. A. Hindell. 2007*a*. Detecting prey from DNA in predator scats: a comparison with morphological analysis, using *Arctocephalus* seals fed a known diet. Journal of Experimental Marine Biology and Ecology 347:144–154.

Casper, R. M., S. N. Jarman, N. J. Gales, and M. A. Hindell. 2007*b*. Combining DNA and morphological analyses of faecal samples improves insight into trophic interactions: a case study using a generalist predator. Marine Biology 152:815–825.

Dalerum, F., and A. Angerbjörn. 2000. Arctic fox (*Alopex lagopus*) diet in Karupelv Valley, East Greenland, during a summer with low lemming density. Arctic 53:1–8.

De Barba, M., J. R. Adams, C. S. Goldberg, C. R. Stansbury, D. Arias, R. Cisneros, and L. P. Waits. 2014. Molecular species identification for multiple carnivores. Conservation Genetics Resources 6:821–824.

Deagle, B. E., R. Kirkwood, and S. N. Jarman. 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. Molecular Ecology 18:2022–2038.

Deagle, B. E., and D. J. Tollit. 2007. Quantitative analysis of prey DNA in pinniped faeces: potential to estimate diet composition? Conservation Genetics 8:743–747.

Deagle, B. E., D. J. Tollit, S. N. Jarman, M. A. Hindell, A. W. Trites, and N. J. Gales. 2005. Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. Molecular Ecology 14:1831–1842.

Dempsey, S. J., E. M. Gese, B. M. Kluever, R. C. Lonsinger, and L. P. Waits. 2015. Evaluation of scat deposition transects versus radio telemetry for developing a species distribution model for a rare desert carnivore, the kit fox. PLoS ONE 10:e0138995.

Egeter, B., P. J. Bishop, and B. C. Robertson. 2015. Detecting frogs as prey in the diets of introduced mammals: a comparison between morphological and DNA-based diet analyses. Molecular Ecology Resources 15:306–316.

Elmhagen, B., M. Tannerfeldt, P. Verucci, and A. Angerbjörn. 2000. The arctic fox (*Alopex lagopus*): an opportunistic specialist. Journal of Zoology 251:139–149.

Foster, J. R. 1977. Pulsed gastric lavage: an efficient method of removing the stomach contents of live fish. Progressive Fish-Culturist 39:166–169.

Glass, B. P. 1973. A key to the skulls of North American mammals. Second edition. Fifth printing. Oklahoma State University, Stillwater, USA.

Hansel, H. C., S. D. Duke, P. T. Lofy, and A. Gerard. 1988. Use of diagnostic bones to identify and estimate original lengths of ingested prey fishes. Transactions of the American Fisheries Society 117:55–62.

King, R. A., D. S. Read, M. Traugott, and W. O. C. Symondson. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Molecular Ecology 17:947–963.

Klare, U., J. F. Kamler, and D. W. Macdonald. 2011. A comparison and critique of different scat-analysis methods for determining carnivore diet. Mammal Review 41:294–312.

Kozlowski A. J., E. M. Gese, and W. M. Arjo. 2008. Niche overlap and resource partitioning between sympatric kit foxes and coyotes in the Great Basin Desert of western Utah. American Midland Naturalist 160:191–208.

Layman, C. A., K. O. Winemiller, D. A. Arrington, and D. B. Jepsen. 2005. Body size and trophic position in a diverse tropical food web. Ecology 86:2530–2535.

Lonsinger, R. C., E. M. Gese, S. J. Dempsey, B. M. Kluever, T. R. Johnson, and L. P. Waits. 2015*a*. Balancing sample accumulation and DNA degradation rates to optimize noninvasive genetic sampling of sympatric carnivores. Molecular Ecology Resources 15:831–842.

Lonsinger, R. C., E. M. Gese, and L. P. Waits. 2015*b*. Evaluating the reliability of field identification and morphometric classifications for carnivore scats confirmed with genetic analysis. Wildlife Society Bulletin 39:593–602.

Lopes, C. M., M. De Barba, F. Boyer, C. Mercier, P. J. S. da Silva Filho, L. M. Heidtmann, D. Galiano, B. B. Kubiak, P. Langone, F. M. Garcias, L. Gielly, E. Coissac, T. R. O. de Freitas, and P. Taberlet. 2015. DNA metabarcoding diet analysis for species with parapatric vs sympatric distribution: a case study on subterranean rodents. Heredity 114:1–12.

Martin, R. E., R. H. Pine, and A. E. DeBlase. 2001. A manual of mammalogy with keys to families of the world. Third edition. McGraw Hill, New York, New York, USA.

Millspaugh, J. J., and B. E. Washburn. 2003. Within-sample variation of fecal glucocorticoid measurements. General and Comparative Endocrinology 132:21–26.

Mumma, M., J. R. Adams, C. Zieminski, T. K. Fuller, S. P. Mahoney, and L. P. Waits. 2016. A comparison of morphological and molecular diet analyses of predator scats. Journal of Mammalogy 97:112–120.

Murphy, M. A., L. P. Waits, and K. C. Kendall. 2000. Quantitative evaluation of fecal drying methods for brown bear DNA analysis. Wildlife Society Bulletin 28:951–957.

Nakagawa, S., and I. C. Cuthill. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biological Reviews 82:591–605.

Panasci, M., W. B. Ballard, S. Breck, D. Rodriguez, L. D. Densmore III, D. B. Wester, and R. J. Baker. 2011. Evaluation of fecal DNA preservation techniques and effects of sample age and diet on genotyping success. Journal of Wildlife Management 75:1616–1624.

Pearson, O. P. 1966. The prey of carnivores during one cycle of mouse abundance. Journal of Animal Ecology 35:217–233.

Piggott, M. P. 2004. Effect of sample age and season of collection on the reliability of microsatellite genotyping of faecal DNA. Wildlife Research 31:485–493.

Piggott, M. P., and A. C. Taylor. 2003. Extensive evaluation of faecal preservation and DNA extraction methods in Australian native and introduced species. Australian Journal of Zoology 51:341–355.

Pompanon, F., B. E. Deagle, W. O. C. Symondson, D. S. Brown, S. N. Jarman, and P. Taberlet. 2012. Who is eating what: diet assessment using next generation sequencing. Molecular Ecology 21:1931–1950.

R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82–90.

Smith, D. A., K. Ralls, A. Hurt, B. Adams, M. Parker, B. Davenport, M. C. Smith, and J. E. Maldonado. 2003. Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (*Vulpes macrotis mutica*). Animal Conservation 6:339–346.

Spencer, E., M. Crowther, and C. Dickman. 2014. Diet and prey selectivity of three species of sympatric mammalian predators in central Australia. Journal of Mammalogy 95:1278–1288.

Stenglein, J. L., M. De Barba, D. E. Ausband, and L. P. Waits. 2010. Impacts of sampling location within a faeces on DNA quality in two carnivore species. Molecular Ecology Resources 10:109–114.

Symondson, W. O. C. 2002. Molecular identification of prey in predator diets. Molecular Ecology 11:627–641.

Tollit, D., S. Haeslip, B. Deagle, S. Iverson, R. Joy, D. Rosen, and A. Trites. 2006. Estimating diet composition in sea lions: which technique to choose. Pages 293–307 *in* A. W. Trites, S. K. Atkinson, D. P. DeMaster, L. W. Fritz, T. S. Gelatt, L. D. Rea, and K. M. Wynne, editors. Sea lions of the world. Alaska Sea Grant College Program, Fairbanks, USA.

Tollit, D. J., A. D. Schulze, A. W. Trites, P. F. Olesiuk, S. J. Crockford, T. S. Gelatt, R. R. Ream, and K. M. Miller. 2009. Development and application of DNA techniques for validating and improving pinniped diet estimates. Ecological Applications 19:889–905.

Valentini, A., F. Pompanon, and P. Taberlet. 2009. DNA barcoding for ecologists. Trends in Ecology & Evolution 24:110–117.

Votier, S. C., S. Bearhop, A. MacCormick, N. Ratcliffe, and R. W. Furness. 2003. Assessing the diet of great skuas, *Catharacta skua,* using five different techniques. Polar Biology 26:20–26.

Waits, L., and D. Paetkau. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. Journal of Wildlife Management 69:1419–1433.

Wehausen, J. D., R. R. Ramey II, and C. W. Epps. 2004. Experiments in DNA extraction and PCR amplification from bighorn sheep feces: the importance of DNA extraction method. Journal of Heredity 95:503–509.

White, R. S. 1999. Animal skulls: a guide for teachers, naturalists and interpreters. International Wildlife Museum, Tucson, Arizona, USA.

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