Molecular testing of observer identification of carnivore feces in the field

Laura R. Prugh and Carol E. Ritland

Abstract Collection of carnivore feces is common in wildlife ecology studies, but misidentification of scats can compromise data quality. We tested the ability of observers to identify coyote (*Canis latrans*) feces in the Alaska Range from 2000–2002. We extracted DNA from 834 fecal samples and used an unambiguous mtDNA analysis to differentiate coyote scats from those of sympatric carnivores. We successfully amplified DNA from 78% of the extracts, and 92% of these samples were from coyotes. We rated our certainty level when collecting scats in the field, and the proportion correctly identified matched well with expected proportions. For example, 100% of scats that we rated "100% certain" were from coyotes ($n=129$), 96% of scats rated "95% certain" were from coyotes ($n=174$), and 88% of scats rated "90% certain" were from coyotes ($n=62$). Thus, we demonstrate that trained observers can identify coyote scats in the field with accuracy that should be sufficient for diet studies, even in the presence of other similar-sized carnivores. Rating observer certainty is useful for later analyses because researchers can decide what level of uncertainty is acceptable for their purposes and exclude samples accordingly.

Key words canid, *Canis latrans*, coyote, fecal DNA, mitochondrial DNA, North America, restriction enzymes, scat surveys

Biologists have been collecting carnivore feces for decades to study predator ecology (Elton 1927, Murie 1944, Litvaitis 2000). While carnivores are notoriously difficult to observe directly, their feces often are abundantly available on roads and trails. Feces have been used to study carnivore foraging ecology (Putman 1984), animal abundance, (Schauster et al. 2002, Harrison et al. 2004), parasitism (Gompper et al. 2003), hormone levels (Wasser 1996), and individual identification (Taberlet et al. 1996). However, distinguishing among the feces of sympatric, similar-sized carnivores can be difficult (Davison et al. 2002), and errors in identification could result in biased data due to the inclusion of samples from nontarget species (Bulinski and McArthur 2000, Farrell et al. 2000). Most natural areas support several species of similar-sized carnivores, which adds uncertainty to scat collection surveys.

Although proper scat identification is a fundamental assumption of studies that use fecal samples to study the ecology or distribution of carnivores, the accuracy of observers rarely has been tested. Previous tests of observer reliability have provided conflicting results. Davison et al. (2002) found that trained observers could not reliably distinguish between pine marten (*Martes martes*) and red fox (*Vulpes vulpes*) feces in Britain. However, Zuercher et al. (2003) found that indigenous and local people in Paraguay were 100% accurate when identifying carnivore feces. To our knowledge, there have been no tests of observer accuracy in North America. We

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tested the ability of observers to distinguish coyote (*Canis latrans*) feces from other sympatric carnivores in Alaska by combining molecular techniques and field data. Feces from gray wolves (*C. lupus*), red foxes, lynx (*Lynx canadensis*), or dogs (*C. familiaris*) could have been mistaken for coyote scats.

Methods

Fieldwork

We collected 1,237 feces in the central Alaska Range (63°57′N, 147°18′W) from January–March for 3 years (2000–2002) as part of a coyote foraging ecology and fecal DNA study. The area encompassed 1,000 km^2 of mountains and foothills on the northern edge of the Alaska Range, approximately 80 km south of Fairbanks. In winter we collected scats along 150 km of snowmobile trails and while snow-tracking coyotes on foot. We collected scats opportunistically in summer as well ($n = 403$). The project leader (L. Prugh) had previous experience identifying coyote feces and collected scats during all 3 years. An experienced technician assisted the project in 2000, and a technician who was initially inexperienced assisted the project in 2001 and 2002.

We randomly subsampled winter-collected scats for genetic analysis ($n = 834$). We performed no genetic tests on summer-collected scats. Snow-track surveys conducted after fresh snowfalls each year provided an index of each species' abundance. A team of 15 sled dogs regularly ran in one part of the study area, but there were no free-ranging dogs. Mustelids were present in the study area in addition to dogs, wolves, lynx, and red foxes, but their feces were uncommon and easily distinguished from coyote feces (Rendes 1999).

In 2001 and 2002 we rated our certainty that each scat originated from a coyote. We had 5 certainty categories: 100%, 95%, 90%, 70%, and 50%. We did not collect a scat if we were less than 50% certain that it was from a coyote. We used information such as the presence of animal tracks in the snow, scat morphology (diameter, volume, shape), and scat location to subjectively assess our certainty level when collecting a scat. For example, coyote and lynx scats are similar in volume and diameter, but lynx scats tend to have bulbous segments while coyote scats tend to have twisted, tapering segments (Murie 1974, Rendes 1999). Lynx often use latrine sites in treewells, whereas coyotes often defecate on trails or other conspicuous locations (authors, unpublished data). In 2000 we did not record our certainty levels, but we did record the maximum diameter of most scats.

Genetic analysis

We analyzed a segment of mitochondrial DNA (mtDNA) to test our accuracy in identifying coyote scats. We collected approximately 100 mg of frozen fecal material by scraping the surface of each sample and placing shavings into 2 mL vials with a DET storage buffer (Seutin et al. 1991), and vials were stored at -80°C. We extracted DNA from fecal samples using Qiagen Stool Mini-Kits (Qiagen, Valencia, Calif.) after centrifuging samples for 10 minutes and removing the storage buffer. We conducted DNA isolation and amplification in separate labs to minimize the risk of contaminating stock DNA with post-PCR products. We used fecal and buccal (cheek swab) samples from captive animals and pet dogs for positive controls. We obtained samples from 6 privately owned lynx, 6 red foxes at a fur farm, 2 wolves and 1 coyote at the Greater Vancouver Zoo, and 18 purebred dogs at a dog show.

We modified the method developed by Adams et al. (2003) for our genetic tests. ScatID primers (Adams et al. 2003) were used to amplify a section of the cytochrome-*b* region of mitochondrial DNA in 20-µl reactions containing: 5 µl DNA extract (directly from kit extraction), 0.5 pmol each primer, 1× reaction buffer, 1.5 mM $MgCl_2$, 1.5 units AmpliTaq polymerase (Roche, Basel, Switzerland), 0.2 mM dNTPs, and 1mg/mL BSA. We amplified DNA in PTC-100 thermocylers (MJ Research, Inc., Waltham, Mass.) using the following program: initial denaturation at 95°C for 10 min; 35 cycles of 30 s at 95°C, 30 s at 52°C, and 40 s at 72°C; final extension of 72°C for 3 min. Ten µl of the polymerase chain reaction (PCR) product was then digested with 20 units Taq^{α} I restriction enzyme (New England Biolabs Inc., Beverly, Mass.) in a 37°C water bath overnight (20 µl final volume). Digested products were visualized on 3% agarose gels after staining with ethidium bromide solution. We ran negative controls to monitor for contamination and ran positive controls of all 5 species with each batch.

Data analysis

We used a log-likelihood ratio G-test to determine how well observer certainty levels matched results from mtDNA tests. We also used a G-test to determine whether observers varied in their accuracy.

We examined the effect of season on observer certainty by comparing mean certainty levels of scats collected in summer and winter using a *t*-test with arsine square-root transformed data. We compared the mean diameters of coyote and noncoyote scats using a *t*-test, and we used a discriminant function analysis (Chatfield and Collins 1996) to determine whether scat diameter could be used to classify scats into the correct species categories. We conducted all analyses using JMP-IN 4.0 (SAS Institute Inc., Cary, N.C.).

Results

Uncut PCR products from wolf, coyote, fox, and dog DNA could not be distinguished and were approximately 200 basepairs (bp) in size. Products from lynx DNA also created a band at 200 bp, but multiple faint bands of smaller and larger sizes usually were visible as well (Figure 1). Taq$^{\alpha}$ I enzyme digested coyote DNA and created a product at 100 bp, but it did not digest wolf, lynx, dog, or fox DNA (Figure 1). Therefore, this was an unambiguous method for distinguishing coyotes from these sympatric carnivores, but we were unable to differentiate among the other canids. We identified only 1 lynx scat in our dataset and, therefore, classified results as coyote, noncoyote, or failed. We also tested DNA from 2 common prey species, snowshoe hares (*Lepus americanus*) and Arctic ground squirrels (*Spermophilus parryii*), and PCR products were not visible.

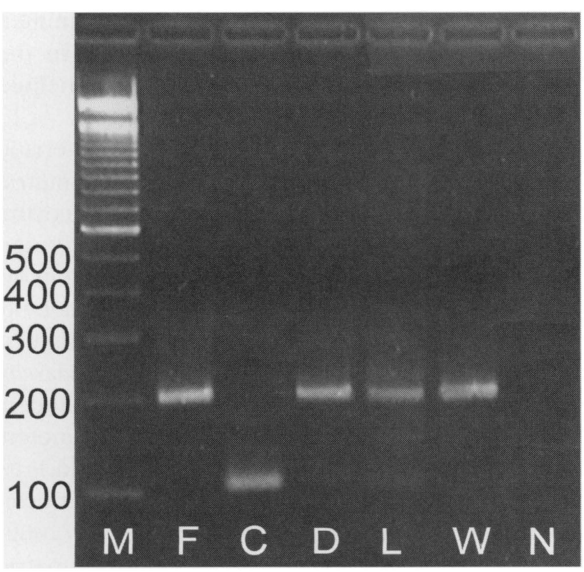

Figure 1. Photograph of 3% agarose gel showing post-electrophoresis banding patterns of cytochrome-*b* mtDNA extracted from carnivore feces collected in the Alaska Range, 1999–2002. Samples were amplified with ScatID primers (Adams et al. 2003) and digested with Taq$^{\alpha}$ I restriction enzyme. M = marker ladder (band sizes in basepairs labeled on left), F = red fox, C = coyote, D = domestic dog, L = lynx, W = wolf, and N = negative control. DNA from cheek swab and tissue samples showed the same bands for these species.

Of the 834 fecal samples tested, DNA from 647 (78%) amplified successfully. Of the successful samples, 596 (92%) were coyote. Snow track surveys did not indicate major differences in carnivore abundance. We counted a total of 259 coyote tracks, 331 red fox tracks, 126 wolf tracks, and 154 lynx tracks over 3 years. In contrast, pine marten (*Martes americana*) and wolverine (*Gulo gulo*) tracks were encountered far less frequently (15 and 20 tracks respectively), and we rarely saw scats with mustelid-like morphology.

In 2001 and 2002 we rated our certainty that scats were from coyotes, and mean certainty levels were higher in winter ($\bar{x}$=89.8 %, SE=0.45, n=872) than in summer ($\bar{x}$=78.3 %, SE=1.3, n=151; t_{1021}= -10.4, P<0.001). Based on genetic analyses of winter-collected scats, we found that observer judgment was remarkably accurate. The proportions of coyote and noncoyote scats in each certainty category were strikingly similar to expected proportions (Table 1; G_4= 3.45, P=0.49).

We hypothesized that observers may differ in their ability to identify coyote feces, and that this ability may change based on experience level. However, we found no evidence for differences in observer reliability and no indication that experience level influenced observer accuracy (Table 2). Both observers were slightly more accurate than expected in both years and did not differ in their accuracy (G_1=0.11, P=0.74).

Table 1. Observers subjectively rated their certainty that scats collected during winters 2001 and 2002 in the Alaska Range originated from coyotes. These certainty levels are compared to the actual percentages of coyote scats in the samples, which were determined by analysis of mtDNA (total n = 416). The expected number of coyote scats was calculated by multiplying the total number of scats in each certainty category by the certainty level.

Observer certainty level	No. coyote scats	No. non-coyote scats	% coyote scats	Expected no. coyote scats
100%	129	0	100.0	129
95%	167	7	95.97	165.3
90%	55	7	88.71	55.8
70%	36	7	83.72	30.1
50%	6	2	75.00	4

Table 2. Accuracy of scat identification among observers and years (i.e., experience levels) for scats collected in the Alaska Range, 2001 and 2002. Observers rated their certainty level that each scat originated from a coyote, and feces were then identified as coyote or noncoyote by analysis of mtDNA. Observer #1 had 2 years of prior scat identification experience. The expected number of coyote scats was calculated by multiplying total number of scats collected in each certainty category by the certainty level. The total number of expected and observed coyote scats is shown, with the percentage of scats originating from coyotes in parenthesis.

	Observer #1, 2001 (experienced)		Observer #1, 2002 (more experienced)		Observer #2, 2001 (inexperienced)		Observer #2, 2002 (experienced)	
Observer certainty level	expected # coyote scats	observed # coyote scats	expected # coyote scats	observed # coyote scats	expected # coyote scats	observed # coyote scats	expected # coyote scats	observed # coyote scats
100%	35	35	40	40	27	27	21	21
95%	37.05	38	57.95	60	8.55	8	40.85	42
90%	10.8	10	15.3	14	5.4	6	20.7	21
70%	11.2	13	4.2	5	5.6	8	3.5	5
50%	0.5	1	0	0	0	0	2	2
Total	94.6 (91.8)	97 (94.2)	117.5 (94.7)	119 (96.0)	46.6 (93.1)	49 (98)	88.1 (91.7)	91 (94.8)

In 2000 we did not rate observer certainty but did measure scat diameters. The diameters of coyote scats were larger ($\bar{x}$=21.7 mm, SE=0.32, n=183) than noncoyote scats ($\bar{x}$=18.4 mm, SE=0.75, n=25; t_{206}=3.66, P<0.01). This indicates that we were more likely to confuse coyote and fox scats than coyote and wolf scats because fox scats tend to be smaller than coyote scats and wolf scats tend to be larger. There was considerable overlap in the range of diameters (coyote range=13–35 mm, noncoyote range=12–26 mm). A discriminant function analysis, which determines how well a variable discriminates between naturally occurring groups, correctly classified 110 coyote scats and 17 noncoyote scats but misclassified 73 coyote scats and 8 noncoyote scats.

Discussion

We found that observers were able to identify coyote scats with >90% accuracy, despite the fact that coyote scats could be confused with those of 4 abundant species in our study area. When rating how certain we were that each scat was from a coyote, we were remarkably accurate at high certainty ratings (90–100%) and conservative at low certainty ratings (i.e., there were slightly more coyote scats than expected in the 50–70% certainty categories). Thus, our subjective certainty ratings were reliable at predicting the likelihood that a scat originated from a coyote and provided useful information about the quality of each sample. We found that observers did not vary in their ability to identify feces and that experience level did not influence accuracy. Bulinski and McArthur (2000) also found that experienced and inexperienced observers had similar accuracy when identifying macropod scats in Australia.

Our certainty levels were higher in winter than in summer, most likely due to the presence of animal tracks in the snow during winter. We only rated a scat "100% certain" if there were fresh coyote tracks associated with it and no other animal tracks present. This situation never occurred in summer. In addition, scats did not decay as rapidly in the winter since they remained frozen, and this helped to preserve useful morphological features.

Scat diameter was an important characteristic that assisted scat identification, but this metric alone was not as good at distinguishing coyote from noncoyote scats as our overall observer certainty levels. The diameters of coyote and noncoyote scats in our samples overlapped considerably and the discriminant function analysis showed that scat diameter could not be used to reliably classify scats as coyote or noncoyote. Green and Flinders (1981) found overlapping ranges in the diameters of red fox and coyote scats, and Weaver and Frits (1979) found a high degree of overlap between coyote and wolf scat diameters. Farrell et al. (2000) found a high degree of overlap among sympatric felid scat diameters in Venezuela. More importantly, their data indicate that diets constructed from scats based on diameter measurements can be highly biased when sympatric species partition resources (Farrell et al. 2000). We found that measuring scat diameter in the field was time consuming, and our results show that rating overall observ-

er certainty, though subjective, was a more efficient and effective method for reducing the number of nontarget scats in surveys.

We recommend that all scat surveyors rate their certainty when collecting scats. Although this rating is subjective, it gives relative information about sample quality that may be used to improve survey accuracy. Ideally, observers would be trained with carnivore scats of known origin, and this is possible in the presence of snow or another tracking substrate (such as mud), assuming tracks can be distinguished. We found snow-tracking to be helpful in validating and calibrating our certainty ratings because it gave us an accurate sense of the defining characteristics and the morphological variations of coyote scats. Training observers with feces from captive animals also is an option, but this is impractical for several reasons. Captive carnivores are relatively rare and it would be difficult to collect an adequate number of samples from all the sympatric species that occur in a typical study area. Captive animals also are fed different food than wild canids, which can alter scat morphology (personal observation). In addition, useful information such as the location of the scat in the field would not be available.

Some species combinations may be harder to differentiate than others, particularly when the target species are rare. Davison et al. (2002) had experienced scat collectors rate their certainty levels when collecting marten and fox scats in Britain and used an mtDNA test to verify species identity. They found that collectors often were inaccurate in their identification, even within the highest certainty level. Marten feces were absent from some parts of their study area and collectors had a tendency to misidentify fox scats as marten scats. The tendency to over-estimate the number of scats from rare species also was found in trials with known proportions of 2 different macropod species (Bulinski and McArthur 2000). This was not an issue in our study because coyotes were relatively abundant.

We recommend using molecular techniques to verify species identity in the following situations: 1) the target species is rare relative to sympatric carnivores, 2) the scat morphology of the target species is particularly similar to nontarget species, or 3) a high degree of accuracy (>90–95%) is required. Protocols have been developed to differentiate a variety of canids, felids, and mustelids using mtDNA analyses (e.g., Foran et al. 1997, Paxinos et al. 1997, Mills et al. 2000, Adams et al. 2003, Riddle et al. 2003). Molecular methods of scat identification can remove the uncertainty inherent in most scat collection surveys, but the drawback of these methods is that they can be time consuming and expensive. Materials for our project cost approximately $5.00 (U.S.) per sample. The bulk of the cost ($3.25 per sample) was spent on the Qiagen stool kits for DNA extraction. Smith et al. (2003) reported lab costs of $50 per sample for similar analyses, which presumably included labor costs. Optimization of laboratory techniques and the processing of samples can take several months, so labor costs can be high if a technician is hired.

A promising and inexpensive alternative to molecular testing is the use of trained dogs to find and accurately identify carnivore feces in the field (Wasser 1999, Smith et al. 2003). One advantage of using dogs to identify scats is their ability to find scats more efficiently than humans (Smith et al. 2003). In studies that do not use molecular techniques or trained dogs to identify feces, it is particularly important for observers to rate their certainty levels in a systematic manner. If the study area receives snow in winter, observers should use information such as animal tracks to improve their accuracy.

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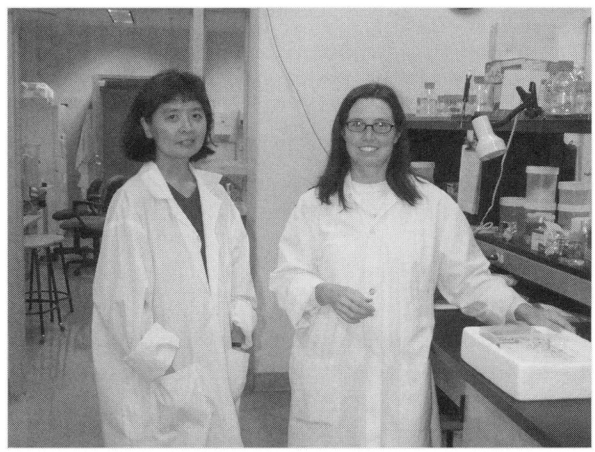

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