

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

USDA National Wildlife Research Center - Staff
Publications

U.S. Department of Agriculture: Animal and Plant
Health Inspection Service

2015

Evaluating the Reliability of Field Identification and Morphometric Classifications for Carnivore Scats Confirmed with Genetic Analysis

Robert C. Lonsinger
University of Idaho, Moscow

Eric M. Gese
Utah State University, eric.gese@usu.edu

Lisette P. Waits
University of Idaho
University of Idaho, lwaits@uidaho.edu

Follow this and additional works at: https://digitalcommons.unl.edu/icwdm_usdanwrc

 Part of the [Life Sciences Commons](#)

Lonsinger, Robert C.; Gese, Eric M.; and Waits, Lisette P., "Evaluating the Reliability of Field Identification and Morphometric Classifications for Carnivore Scats Confirmed with Genetic Analysis" (2015). *USDA National Wildlife Research Center - Staff Publications*. 1819.
https://digitalcommons.unl.edu/icwdm_usdanwrc/1819

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Animal and Plant Health Inspection Service at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in USDA National Wildlife Research Center - Staff Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.



Tools and Technology

Evaluating the Reliability of Field Identification and Morphometric Classifications for Carnivore Scats Confirmed with Genetic Analysis

ROBERT C. LONSINGER,^{1,2} *Department of Fish and Wildlife Sciences, University of Idaho, Moscow, ID 83844-1136, USA*

ERIC M. GESE, *United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, Department of Wildland Resources, Utah State University, Logan, UT 84322-5230, USA*

LISETTE P. WAITS, *Department of Fish and Wildlife Sciences, University of Idaho, Moscow, ID 83844-1136, USA*

ABSTRACT Scat surveys are commonly used to monitor carnivore populations. Scats of sympatric carnivores can be difficult to differentiate and field-based identification can be misleading. We evaluated the success of field-based species identification for scats of 2 sympatric carnivores—coyotes (*Canis latrans*) and kit foxes (*Vulpes macrotis*). We conducted scat surveys in the Great Basin desert of Utah, USA, during the winter and summer of 2013, and we detected 1,680 carnivore scats. We classified scats based on field identification, recorded morphometric measurements, and collected fecal DNA samples for molecular species identification. We subsequently evaluated the classification success of field identification and the predictive power of 2 nonparametric classification techniques—*k*-nearest neighbors and classification trees—based on scat measurements. Overall, 12.2% of scats were misclassified by field identification, but misclassifications were not equitable between species. Only 7.1% of the scats identified as coyote with field identification were misclassified, compared with 22.9% of scats identified as kit fox. Results from both *k*-nearest neighbor and classification-tree analyses suggest that morphometric measurements provided an objective alternative to field identification that improved classification of rarer species. Overall misclassification rates for *k*-nearest neighbor and classification-tree analyses were 11.7% and 7.5%, respectively. Using classification trees, misclassification was reduced for kit foxes (8.5%) and remained similar for coyotes (7.2%), relative to field identification. Although molecular techniques provide unambiguous species identification, classification approaches may offer a cost-effective alternative. We recommend that monitoring programs employing scat surveys utilize molecular species identification to develop training data sets and evaluate the accuracy of field-based and statistical classification approaches. © 2015 The Wildlife Society.

KEY WORDS *Canis latrans*, classification trees, fecal DNA, misidentification, noninvasive genetic sampling, scat deposition, scat identification, species identification, *Vulpes macrotis*.

The development of sound and effective management and conservation strategies for wildlife populations requires reliable and accurate information on species distributions and population trends. For carnivores, invasive monitoring methods requiring capture and handling of animals can be challenging and costly, often limiting their utility for large spatial extents or long-term monitoring (Gese 2001, MacKenzie 2005, Gompper et al. 2006). Noninvasive surveys (Long et al. 2008, Kelly et al. 2012) are an appealing

alternative for monitoring populations because they are simple, cost-efficient, and facilitate multispecies monitoring (Gompper et al. 2006). For many elusive or rare species, such as carnivores, scats are often the most conspicuous indication of their presence and therefore noninvasive scat surveys are a widely used means of monitoring populations (e.g., Prugh and Ritland 2005, Gompper et al. 2006, Harrington et al. 2010, Long et al. 2011). Scat surveys are frequently employed to delineate distributions (Kozłowski et al. 2012), assess relative abundances (Schauster et al. 2002, Cunningham et al. 2006, Kamler et al. 2013), develop models of resource selection (Vynne et al. 2011, Dempsey 2013), and evaluate occupancy patterns (Long et al. 2011, Schooley et al. 2012). Scat surveys can provide additional information on diet (Kozłowski et al. 2008, Marucco et al. 2008), resource partitioning (Vanak and Gompper 2009, Kamler et al. 2012),

Received: 4 November 2014; Accepted: 23 February 2015

Published: 12 June 2015

¹E-mail: Lons1663@vandals.uidaho.edu

²Present address: 875 Perimeter Drive MS1136, Moscow, ID 83844, USA.

and parasitology (Kohn and Wayne 1997). In addition, scat collections afford researchers the opportunity to obtain fecal DNA to assess measures of population genetics (Waits and Paetkau 2005), social and spatial ecology (Kitchen et al. 2005), and breeding strategies (Kitchen et al. 2006).

Correct inferences from scat surveys depend on accurate species identification, and misclassifications could bias results and potentially reduce the effectiveness of management strategies (Marucco et al. 2008, Harrington et al. 2010). Commonly, field-based species identification (hereafter, field identification) is determined through inspection of scat morphology including color, odor, overall size, and physical appearance (Vanak and Gompper 2009, Kamler et al. 2012); this is often coupled with auxiliary information, such as presence of tracks, dietary content, or distance from a den, to improve field identification confidence (Green and Flinders 1981, Prugh and Ritland 2005, Harrington et al. 2010, Kozlowski et al. 2012, Schooley et al. 2012). However, sympatric carnivores may produce scats with overlapping sizes (Green and Flinders 1981, Danner and Dodd 1982, Farrell et al. 2000, Reed et al. 2004, Gompper et al. 2006), and auxiliary information may be lacking, misleading, or uninformative. For example, counter-marking is common among conspecifics (Ferkin and Pierce 2007) and may produce confounding sign; and dietary content may not contribute to improving field identification for species with high dietary overlap (Onorato et al. 2006, Foran et al. 1997). Consequently, carnivore scats can be difficult to discriminate and confidence in field identifications can be misleading or completely erroneous (Foran et al. 1997, Paxinos et al. 1997, Farrell et al. 2000, Davison et al. 2002, Harrington et al. 2010).

Molecular species identification (hereafter, molecular identification) provides a reliable alternative to field identification (Foran et al. 1997, Kohn and Wayne 1997, Farrell et al. 2000, Dalen et al. 2004, Prugh and Ritland 2005). Comparisons of field and molecular identifications have yielded contrasting results. Coyote (*Canis latrans*) scats could be distinguished from sympatric carnivores in Alaska, USA, with high accuracy using field identification (Prugh and Ritland 2005), whereas pine marten (*Martes martes*) and red fox (*Vulpes vulpes*) scats could not be confidently discriminated in Britain (Davison et al. 2002), and experienced researchers failed to successfully identify American mink (*Neovison vison*) scats in Scotland (et al. 2010). Despite the challenges and ambiguity associated with field identification, many wildlife managers and researchers still rely on field identification, likely because of the increased costs associated with molecular identification. Conservation and management programs often suffer from limited funding and the number of imperiled species continues to rise, increasing the need to improve and evaluate cost-effective monitoring strategies (Gese 2001).

We used molecular identification to evaluate the accuracy of field identification for scats of 2 sympatric carnivores, coyotes and kit foxes (*V. macrotis*), in the Great Basin desert of western Utah, USA. Coyote populations have increased

notably over the past several decades at this site, where they are now the most abundant carnivore (Arjo et al. 2007). The kit fox is a species of conservation concern; kit foxes are significantly smaller than coyotes and have been experiencing population declines that have been contributed, in part, to increased competition with and predation by coyotes (Arjo et al. 2007). Consequently, multiple studies have been conducted in western Utah to investigate kit fox and coyote populations, some of which have employed scat surveys (Kozlowski et al. 2008, 2012; Dempsey 2013; Dempsey et al. 2014). To evaluate alternative classifications to field and molecular identification, we explored 2 common nonparametric classification approaches—*k*-nearest neighbors and classification trees—based on morphometric measurements as objective, quantitative alternatives for discriminating scats. The *k*-nearest neighbor approach is among the simplest supervised classification algorithms and assigns an unknown observation to a class based on class majority of the *k* closest training observations within the parameter space (Cover and Hart 1967, Hastie et al. 2001). Classification-tree analysis, or recursive partitioning, searches all possible binary splits of the predictor variables to identify splits that optimize classification (i.e., prediction of the species) and produces a decision tree that provides clear classification rules and information on variable importance (Breiman et al. 1984). Both *k*-nearest neighbor and classification-tree analyses require sufficiently large samples sizes, though, to develop training data sets. Consequently, using these approaches may restrict analyses to species with sufficient representation (i.e., adequate sample sizes to characterize the population), whereas field identification and molecular identification do not have this requirement. Focusing on 2 target carnivore species, we hypothesized that field identification would be more reliable for the abundant species (coyotes) than for the rarer species (kit foxes) as observed in other systems (Davison et al. 2002, Prugh and Ritland 2005). We further hypothesized that scat diameters of the 2 species would overlap (e.g., Green and Flinders 1981, Danner and Dodd 1982), but that inclusion of additional morphometric measurements (i.e., length and no. of disjoint segments) into nonparametric classification methods would provide a more accurate and objective method of identifying scats than would field identification.

STUDY AREA

The study was centered on the eastern portion of the U.S. Army's Dugway Proving Ground, Utah, and extended to surrounding federal lands managed by the U.S. Bureau of Land Management (Fig. 1). Elevations across this Great Basin Desert site ranged from 1,228 m to 2,154 m (Arjo et al. 2007). Winters were cold (Jan: \bar{x} high = 4°C, \bar{x} low = -10°C) and summers were moderate (Jul: \bar{x} high = 36°C, \bar{x} low = 15°C), with the majority of precipitation accumulating in the spring and autumn (\bar{x} annual precipitation approx. 20 cm; Arjo et al. 2007). The site was characterized at low elevations by cold desert playa (dominated by *Allenrolfea occidentalis*), cold desert chenopod

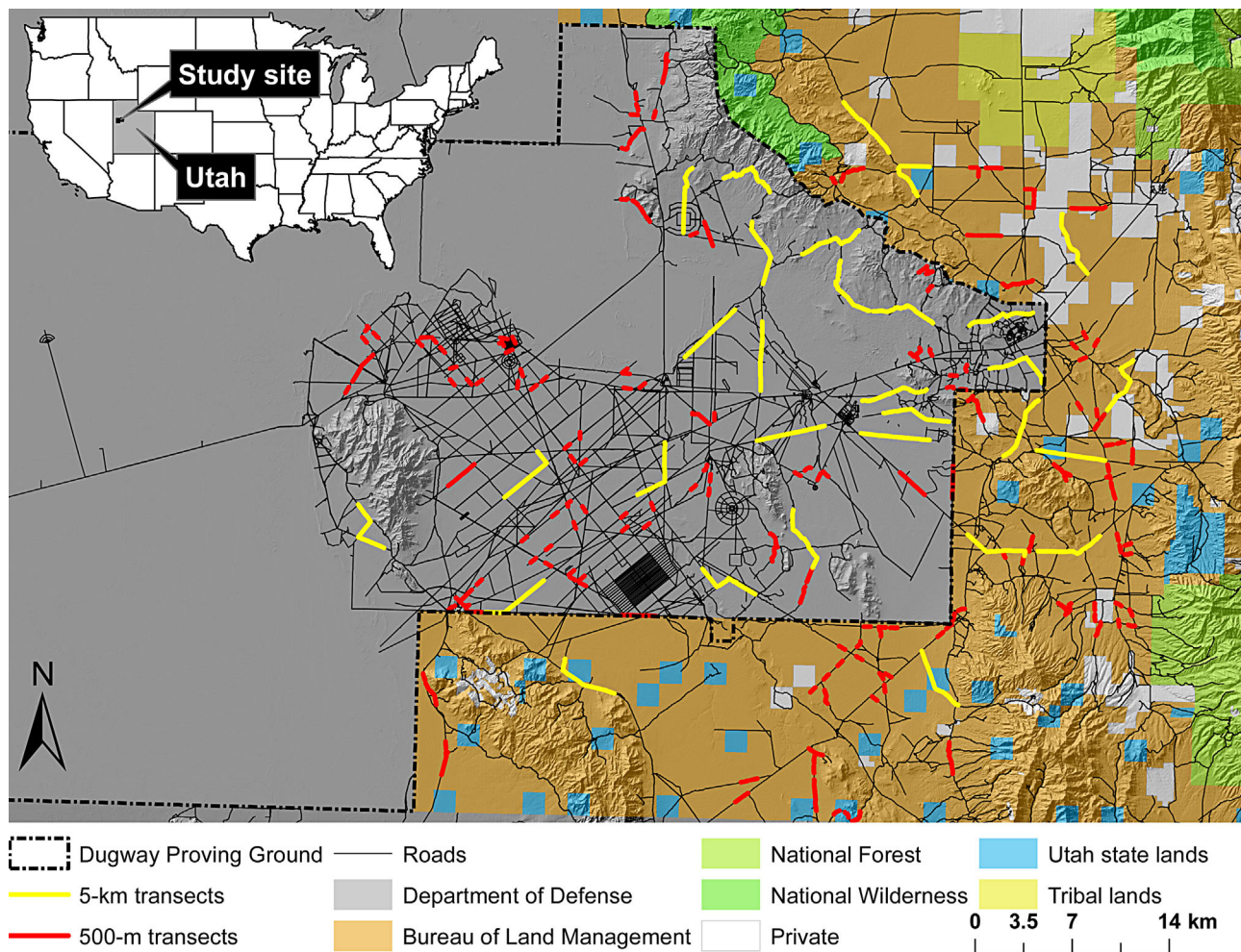


Figure 1. Location of 5 km (yellow) and 500 m (red) scat-deposition transects surveyed in Tooele County, Utah (USA) for coyote and kit fox scats in the winter and summer of 2013.

shrubland (dominated by *Atriplex confertifolia* and *Kochia americana*) and vegetated dunes, along with nonnative invasive grasslands (*Bromus tectorum*), which dominated in disturbed areas. Higher elevations supported arid shrubland (e.g., *Artemisia* spp., *Chrysothamnus viscidiflorus*) and open woodland (*Juniperus osteosperma*) complexes. *Sarcobatus vermiculatus* shrubland was distributed across the elevational gradient of the site but found more often at moderate to higher elevations.

METHODS

Field sampling and identification of carnivore scats.—We conducted surveys for carnivore scats in the winter and summer of 2013 along transects that followed 2-track or gravel roads. We surveyed 30 transects (5 km each; including 15 transects previously utilized to develop a resource selection function for kit foxes (Dempsey 2013), evaluate survey methods for kit foxes (Dempsey et al. 2014), and to evaluate scat-deposition rates for kit foxes and coyotes (Lonsinger et al. 2015), and 15 additional random transects (Fig. 1). We conducted 3–4 surveys on each 5 km transect within each season. Additionally, we selected 240 shorter random transects (500 m each) that were surveyed

once in each season (Fig. 1). Nine researchers participated in the surveys and were responsible for identifying and sampling scats. We surveyed each transect with 2 researchers, each searching half of the transect width for carnivore scats. When a carnivore scat was encountered, we determined field identification by inspecting the scat's morphology, including color, odor, overall size, and physical appearance (Kozłowski et al. 2012). We then collected a fecal DNA sample (approx. 0.7 mL) from the side of the scat (Stenglein et al. 2010), which was stored in 1.4 mL of DET buffer (20% DMSO, 0.25 M EDTA, 100 mM Tris, pH 7.5, and NaCl to saturation; Seutin et al. 1991). For a subset of scats sampled, we measured the diameter at widest point and total length with a sterilized digital caliper (resolution = 0.1 mm; Mitutoyo America Corporation, Aurora, IL) and recorded the number of disjoint segments, prior to fecal DNA sample collection. When scats consisted of multiple disjoint segments, the total length was determined by summing the lengths of the segments. Scats that lacked the typical physical structure (e.g., soft piles for which accurate measurements could not be obtained) were not measured and were excluded from subsequent analyses.

Mitochondrial DNA species confirmation.—We conducted fecal DNA extraction and mitochondrial DNA (mtDNA) polymerase-chain reaction (PCR) amplification in a laboratory dedicated to low-quality and low-quantity samples such as fecal samples. We randomized samples and extracted fecal DNA using QIAamp DNA Stool Mini Kits (Qiagen, Inc., Valencia, CA) with negative controls to monitor for contamination (Taberlet et al. 1999, Beja-Pereira et al. 2009). We performed mtDNA species identification tests by amplifying fragments of the control region using established protocols and including negative controls to monitor for contamination (De Barba et al. 2014). Qiagen Master Mix (1 × concentration), Q solution (0.5 × concentration), and 1 μL of DNA extract were combined with species identification primers into a 7 μL (total vol) multiplex. The PCR conditions for each primer were as follows: 0.29 μM SIDL, 0.20 μM H16145, 0.10 μM H3R, 0.13 μM FelidID F, 0.03 μM LRuf F, and 0.03 μM PCon R. The PCR thermal profile had an initial denaturation of 95° C for 15 min, 35 cycles of 94° C for 30 sec (denaturation), 46° C for 90 sec and 72° C for 60 sec (elongation), and a final elongation stage of 60° C for 30 min. We conducted PCR on a BioRad Tetrad thermocycler (Bio-Rad, Hercules, CA) and included negative and positive controls. We visualized results using a 3130xl DNA Analyzer (Applied Biosystems, Foster City, CA) and scored allele sizes with Genemapper 3.7 (Applied Biosystems). Species-specific PCR product lengths were 335–337 base-pairs (bp) for kit foxes and 115–120 bp and 359–363 bp for coyotes. For samples that failed to amplify for mtDNA, we repeated the species identification test once to minimize sporadic effects (e.g., pipetting errors; Murphy et al. 2007). We classified samples that contained DNA from multiple species as mixed and removed these samples and any samples that failed from subsequent analyses. We calculated the proportion of samples that were misclassified (hereafter, misclassification rate) based on field identification, including an overall misclassification rate and species-specific misclassification rates.

Statistical classification of scats.—Six species were identified with molecular identification (*see* Results) but only coyotes and kit foxes had sample sizes sufficient to evaluate species-specific classification techniques. Initially, we restricted classification of scats to samples that 1) were confirmed through molecular identification to have originated from coyotes or kit foxes, and 2) had morphometric measurements collected. For coyote and kit fox samples, we evaluated 3 predictor variables, including diameter, length, and number of segments, for normality with Shapiro–Wilk tests (Zar 1996, Razali and Wah 2011). We tested for predictor variable differences between coyote and kit fox scat using Mann–Whitney *U*-tests (Zar 1996).

To explore the ability of statistical classification algorithms to improve classification over field identification, we used 2 nonparametric classifiers, *k*-nearest neighbors and classification trees, to classify scats based on measurements. Differences in the distribution of all 3 predictors suggested that each may contribute to discriminating species, so we included all 3 predictors in classification models. The *k*-nearest

neighbor classifier can be sensitive to the *k* selected and the structure of the training data set. Consequently, we evaluated classification success for values of *k* from 1 to 20. For each *k*, we randomly selected a training data set of 100 kit fox and 100 coyote scats and then used the remaining samples for model validation. To minimize the influence of the local structure, or configuration in parameter space of a single random training data set, we repeated this procedure 500 times for each *k* and calculated the mean misclassification rate for each *k*. We then identified the *k* with the lowest mean misclassification rate for each species and overall. Classification trees may utilize predictor variables in >1 split. To account for over-fitting (i.e., branches resulting from noise or that provide limited contribution to classification), we used 10-fold cross-validation to generate an error rate for each split. We then pruned the tree back to the split corresponding to the lowest cross-validation error (Breiman et al. 1984, Therneau et al. 2014). Based on the pruned tree, we estimated variable importance, a measure of each variable's relative contribution (scaled to sum to 100) to the classification across splits (Therneau et al. 2014). We compared the performance of *k*-nearest neighbor and classification-tree models with one another and to field identification based on the misclassification rate. We used the “class” (Venables and Ripley 2002) and “rpart” (Therneau et al. 2014) packages in R (R Core Team 2014) to conduct *k*-nearest neighbor and classification-tree analyses, respectively.

We conducted additional classification-tree analyses to further assess the influence of each predictor (i.e., diam, length, and no. of segments) and explore misclassification rates by season. Using the same procedures as above, we built classification-tree models for all possible combinations of the 3 predictor variables contained in the full model and evaluated the change in misclassification rates. Seasonal variation in misclassification rates may result from differences in juvenile body size or dietary changes. Using the model with the lowest misclassification rate overall (i.e., the full model), we evaluated differences in winter and summer misclassification rates. Finally, samples from nontarget, sympatric carnivores may occur relatively infrequently in our study system, and inclusion of these species in classification approaches may increase misclassification rates of target species. To evaluate the potential influence of additional sympatric carnivores, we conducted a classification-tree analysis focusing on kit foxes. We conducted this analysis in the same fashion as reported above, but evaluated the classification success of kit fox scats versus all other scats. We limited these additional analyses to classification trees because this approach provided the lowest overall misclassification rate when comparing misclassifications of kit foxes and coyotes (*see* Results).

RESULTS

Field sampling and identification of carnivore scats.—We surveyed each 5 km transect (Fig. 1) 4 times from 8 January to 26 March 2013 (winter) and 3 times from 8 July to 28 August 2013 (summer). Sequential surveys at each site were approximately

14 days apart ($\bar{x} = 13.6 \pm 1.11$ SD, range = 9–18 days). We surveyed each 500 m transect (Fig. 1) once in each season. In total, 1,290 km of transects were surveyed across both seasons. We collected 1,680 (winter: $n = 602$; summer: $n = 1,078$) carnivore scats, and field identification and morphometric measurements were available for 1,498 scats.

Species identification.—We were able to confirm species with molecular identification for 1,203 scats. We removed those samples that failed to amplify (285) or were mixed (10) from subsequent analyses. Based on field identification, 70% (848) and 29% (345) of the scats were classified as coyote and kit fox, respectively. The remaining 1% (10) was classified as red fox (8) or bobcat (2; *Lynx rufus*). Using molecular identification, we confirmed 72% (865) and 24% (293) of the scats as coyote and kit fox, respectively, with <4% confirmed as bobcat (29), red fox (9), domestic dog (6), or cougar (1; *Puma concolor*). The overall misclassification rate, or proportion of samples that were classified as a species different from that confirmed by molecular identification, was 12.2% (Table 1). The proportion of samples misclassified by field identification was lower for coyote than for kit fox samples. Of the scats classified as coyote with field identification, 7.1% (60) were misclassified and determined to be kit fox, bobcat, domestic dog, red fox, or cougar by molecular identification (Table 1). Among scats classified as kit fox with field identification, 22.9% (79) originated from coyotes, red foxes, or bobcats based on molecular identification (Table 1). All 8 of the scats classified as red fox based on field identification were coyote. Both scats classified as bobcat by field identification were correctly classified (Table 1).

Descriptive statistics of scats.—Coyote scats were larger than kit fox scats in diameter (Mann–Whitney $U = 241,379$, $P < 0.001$) and length (Mann–Whitney $U = 228,186$, $P < 0.001$). Mean diameter and length of coyote scats were nearly 2 and 3 times larger than kit fox scats, respectively (Table 2). Coyote scats also had a greater number of disjoint segments (Mann–Whitney $U = 188,852$, $P < 0.001$) than kit fox scats (Table 2). For both coyote and kit fox scats, we found that scat diameter (coyote: $W = 0.99$, $P < 0.001$; kit fox: $W = 0.96$, $P < 0.001$) and length (coyote: $W = 0.96$, $P < 0.001$; kit fox: $W = 0.86$, $P < 0.001$) deviated from normality (Fig. 2). The number of disjoint segments

also deviated from normality for both species (coyote: $W = 0.88$, $P < 0.001$; kit fox: $W = 0.64$, $P < 0.001$). We did not find many other sympatric carnivore species (Table 2). Of those species, mean scat diameter for bobcat and domestic dog fell within the range of diameter values for coyote, but scat length was shorter for these species compared with coyote scat-length values (Table 2). Red fox scat sizes, with their high variability, overlapped the values found for kit fox scats (Table 2).

Statistical classification of scats.—The k -nearest neighbor analysis resulted in overall mean misclassification rates from 11.7% to 16.6% across k -nearest neighbors (i.e., 1–20) with $k = 3$ achieving the lowest mean misclassification rate (Fig. 3). Mean misclassification rates for coyotes ranged from 12.4% to 18.4%, with the lowest mean misclassification at $k = 3$ (Fig. 3); whereas kit fox misclassifications were lower, ranging from 8.1% to 13.2%, with the lowest value at $k = 7$ (Fig. 3). At the optimal k values, the overall mean misclassification rate was reduced, coyote misclassifications increased, and kit fox misclassifications decreased substantially, relative to field identification (Table 3).

Classification-tree analyses for kit foxes and coyotes resulted in a decision tree with 4 splits and 5 terminal nodes (Fig. 4). Cross-validation indicated the resulting classification tree did not require pruning. Diameter had the highest importance (67/100) followed by length (30/100); segments had little importance (3/100). Decision rules classified scats with diameters ≥ 15.55 mm or lengths ≥ 91.70 mm as coyotes, as were scats with diameters < 15.55 mm that were ≥ 63.75 mm in length (Fig. 4). Scats were classified as kit foxes when the diameter was < 13.75 mm and the length was < 91.7 mm, or when the diameter was < 15.55 mm with a length < 63.75 mm (Fig. 4). Misclassification rates produced by the classification-tree analysis were lower overall (7.5%) and lower for coyotes (7.2%), but were higher for kit foxes (8.5%), than those produced by the k -nearest neighbor analysis (Table 3). The classification tree produced a misclassification rate for coyotes similar to field identification (7.1%); but overall misclassification and kit fox misclassification were substantially lower than those from field identification (Table 3).

Table 1. Number of scat samples collected in western Utah, USA, during the winter and summer of 2013 that were classified to species based on field identification (determined by inspection of scat morphology including color, odor, overall size, and physical appearance) and molecular identification (determined by mitochondrial DNA). The gray diagonal represents the number of samples correctly classified based on field identification. The misclassification rate was the proportion of samples identified by field identification to a species that was in disagreement with molecular identification.

		Field identification					
		Coyote	Kit fox	Bobcat	Dog	Red fox	Cougar
Molecular identification	<i>n</i>	848	345	2	0	8	0
	Coyote	788	69	0	0	8	0
	Kit fox	27	266	0	0	0	0
	Bobcat	23	4	2	0	0	0
	Dog	6	0	0	0	0	0
	Red fox	3	6	0	0	0	0
	Cougar	1	0	0	0	0	0
Number misclassified		60	79	0	0	8	0
Misclassification rate		7.1%	22.9%	0.0%		100.0%	

Table 2. Mean (\pm SE) diameter, length, and number of disjoint segments for carnivore scat samples collected in western Utah, USA, during the winter and summer of 2013. On account of sample sizes, only coyote and kit fox scats were subsequently classified based on morphometric measurements.

Scat type	<i>n</i>	Diameter		Length		Disjoint segments	
		\bar{x}	SE	\bar{x}	SE	\bar{x}	SE
Coyote	865	20.3	0.16	127.4	2.26	2.6	0.05
Kit fox	293	11.5	0.16	45.1	1.25	1.5	0.05
Bobcat	29	18.3	0.82	73.6	9.90	1.8	0.26
Dog	6	21.2	2.77	88.6	7.69	2.3	0.33
Red fox	9	13.9	1.81	79.0	19.79	1.6	0.44
Cougar	1	15.9		65.0		1.0	

Reduced classification-tree analyses provided support for the variable importance metric. Although the full model included all 3 predictors, the number of segments did not contribute to the final decision tree (Fig. 4). Consequently, a model including only diameter and length yielded an identical decision tree and misclassification rate to the full model. When the classification tree was built with only diameter and segments, only diameter contributed to the decision tree, which contained a single split and classified scats with a diameter ≥ 15.55 mm as coyote; models built with only diameter produced identical results. Misclassification rates for coyotes (7.6%), kit foxes (16%), and overall (9.8%) increased relative to the full classification-tree model, but were still similar (coyotes) or lower (kit foxes and overall) than misclassification rates based on field identification (Table 3). Classification trees built with length and number of segments (i.e., excluding diam) resulted in a decision tree with 5 splits and 6 terminal nodes relying on both predictors. Misclassification rates increased for coyotes (10.8%), kit foxes (23.9%), and overall (14.1%) to levels exceeding field identification misclassification rates. Removing segments (i.e., including only length) resulted in a decision tree with 10 terminal nodes and produced misclassification rates for coyotes (9.2%), kit foxes (28.0%), and overall (14.0%) that exceeded field identification misclassification rates.

Among the 1,158 scats identified as coyote or kit fox (via molecular identification) with measurement collected, 435 (coyote = 309; kit fox = 126) and 723 (coyote = 556; kit

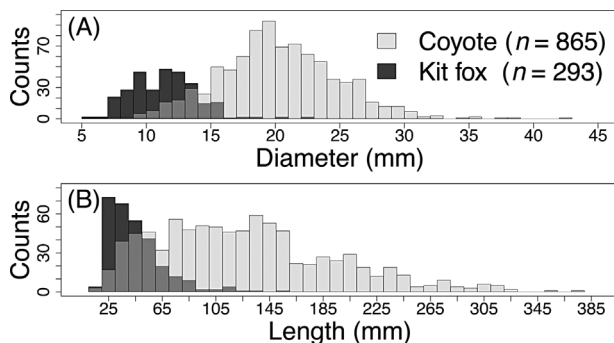


Figure 2. Distribution of (A) diameter at widest point, and (B) total length for coyote and kit fox scats collected in Tooele County, Utah (USA), in the winter and summer of 2013. The medium shade of gray indicates overlap in the distributions.

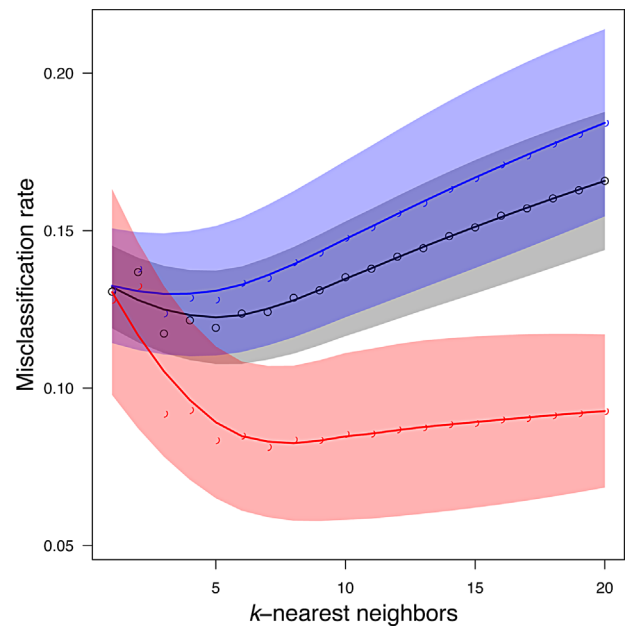


Figure 3. Mean misclassification rate (± 1 SD; bands) for scats of coyotes (blue), kit foxes (red), and overall (black) evaluated at 1–20 *k*-nearest neighbors. The minimum mean misclassification rate was achieved for coyotes (12.4%) at *k* = 3, for kit foxes (8.1%) at *k* = 7, and overall (11.7%) at *k* = 3. Scat samples were collected in Tooele County, Utah (USA) in the winter and summer of 2013.

fox = 167) were collected in winter and summer, respectively. Compared with the full classification-tree model with samples combined across seasons, the winter classification-tree model misclassification rates were lower for coyotes (4.5%) and overall (6.4%), but increased for kit foxes (11.1%). The summer classification-tree misclassification rates were higher for kit foxes (10.8%) and overall (8.0%), but were the same for coyotes (7.2%), relative to the full classification-tree model including all samples.

Less than 4% of samples were determined by molecular identification to be from carnivores other than coyotes or kit foxes. Classification-tree analysis for kit foxes versus nontarget species (i.e., all other carnivores including coyotes) resulted in a decision tree with 7 terminal nodes, which was pruned to 5 terminal nodes following cross-validation (Fig. 5). Relative to considering only kit foxes and coyotes, misclassifications of kit foxes increased to 9.6% when considering all observed carnivore species, but was still substantially lower than misclassifications from field identification. Decision rules were similar to those generated when considering only coyotes and kit foxes. Scats with diameters ≥ 15.55 mm or lengths ≥ 81.10 mm were classified as nontarget carnivores, as were scats with diameters < 15.55 mm that were ≥ 63.75 mm in length. All other scats were classified as kit foxes (Fig. 5).

DISCUSSION

Inferences drawn from scat surveys and the resulting management strategies rely on accurate species identification and therefore monitoring programs should aim to minimize misclassifications. Molecular identification of scats can

Table 3. Misclassification rates based on field identification, k -nearest neighbor classification, and classification trees for carnivore scats collected in western Utah, USA, during the winter and summer of 2013. The misclassification rate was the proportion of samples classified to a species that was in disagreement with molecular identification as determined with mitochondrial DNA. Only scats for which measurements of diameter, length, and number of disjoint segments were available were evaluated. The lowest mean misclassification rates for k -nearest neighbor classification were achieved at $k = 3$ (overall and kit foxes) and $k = 7$ (coyotes).

Scat type	Misclassification rate			
	Field identification ^a	k -nearest neighbor ^b	Classification tree ^b	Classification tree ^a
Over all	12.2%	11.7%	7.5%	8.2%
Kit fox	22.9%	8.1%	8.5%	9.6%
Coyote	7.1%	12.4%	7.2%	
Nontarget carnivores ^c				7.8%
<i>n</i>	1,203	1,158	1,158	1,203

^a Misclassification rate incorporates all carnivore scats identified to species with molecular identification.

^b Misclassification rate incorporates only scats identified as kit fox or coyote with molecular identification.

^c Includes all carnivore species (including coyote) detected except for kit fox.

provide reliable, unambiguous species identification et al. 2002, Reed et al. 2004, Prugh and Ritland 2005, Onorato et al. 2006, Harrington et al. 2010), but may be cost-prohibitive for long-term monitoring programs, particularly as the number of at-risk species increases and funding decreases. Conversely, field identification has no added cost, but may suffer from misidentification, particularly when sympatric carnivores produce scats of similar size and characteristics (Davison et al. 2002, Reed et al. 2004, Gompper et al. 2006, McCarthy et al. 2008, Harrington et al. 2010). Our statistical approach provides a method for minimizing misclassification of scats, while reducing costs compared with continued use of molecular identification. The data needed for our models can all be easily and quickly obtained in the field and provide an objective, quantitative alternative to field identification. Despite coyotes and kit foxes having overlapping morphometric measurements, we were able to substantially reduce overall misclassification rates between the 2 species using k -nearest neighbor and classification-tree methods.

Misclassification of sympatric carnivore scats from field identification is expected to be influenced by similarity in body size and resource use (e.g., prey items, habitat), which result in scats with similar characteristics (Kohn and Wayne 1997). Disparity in scat encounter rates among sympatric carnivores (with otherwise similar scats) may further

influence field identification success, with those species that are encountered less frequently being more often identified (incorrectly) as a more frequently detected species (Davison et al. 2002, Prugh and Ritland 2005). Disproportional encounter rates among sympatric carnivores may result from differences such as species abundance (e.g., relatively fewer scats of rarer species), inconspicuous size or placement (e.g., scats that are small and difficult to find, species that tend to bury scats), or removal (e.g., scat size, placement, or content may influence removal). Our use of molecular identification revealed that the number of scats that were misclassified in the field was inversely proportional to the total number of species-specific scats detected (i.e., rarer species were misclassified based on field identification more frequently). Prugh and Ritland (2005) also found that coyote scats could be discriminated in the field with high accuracy from sympatric carnivores, but suggested that field identification may be more challenging in systems with higher species richness. Researchers conducting scat surveys for pine marten and red fox could not confidently discriminate scats of the 2 species, and misclassifications increased in areas where pine martens were less abundant (Davison et al. 2002). Thus, although misclassifications may result primarily from overlap in body size and corresponding scat characteristics, misclassifications from field identification may be higher for

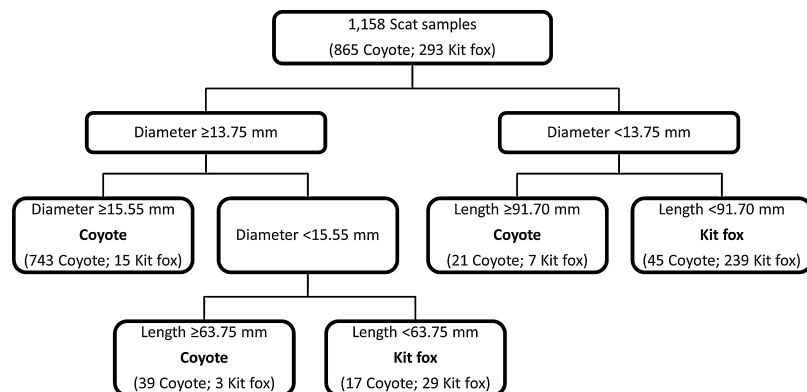


Figure 4. Classification tree for coyote and kit fox scats collected in Tooele County, Utah (USA) in the winter and summer of 2013. Terminal nodes indicate the predicted class (bold) based on the decision rules leading to the node and the number of each species that was classified to the node.

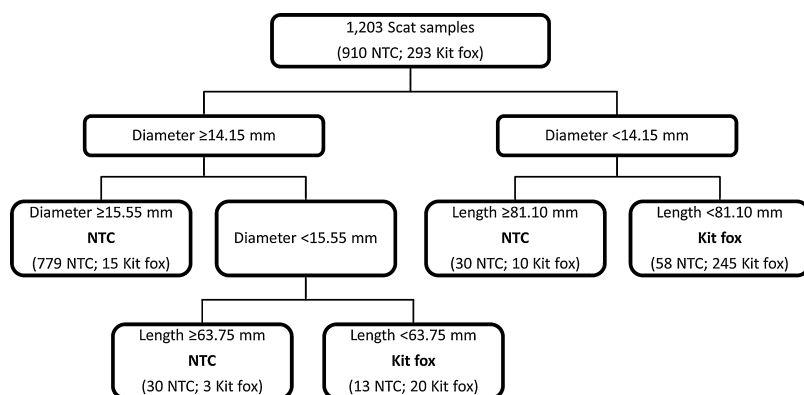


Figure 5. Classification tree for kit fox and nontarget carnivore (NTC; all other carnivore species) scats collected in Tooele County, Utah (USA) in the winter and summer of 2013. Terminal nodes indicate the predicted class (bold) based on the decision rules leading to the node and the number of each species that was classified to the node.

less frequently detected species. Consequently, scat surveys established to monitor endangered, threatened, imperiled, or otherwise rare species, may suffer from higher field-identification misclassification than those surveys being used to monitor abundant species. High levels of misclassifications may result in erroneous conclusions, such as inaccurate assessments of relative abundance or spatial distribution of species of concern (McCarthy et al. 2008).

Morphometric measurements, and primarily diameter of scats, have been used to provide quantitative thresholds for species identification (e.g., Gompper et al. 2006). Selecting a threshold based on a single measurement to discriminate common carnivore scats, such as coyotes, from sympatric carnivores may be appropriate for some objectives (Gompper et al. 2006), but ideal cut-off values likely vary by region (Weaver and Fritts 1979) and may bias results of studies investigating diets toward prey items that produce larger or smaller scats (Danner and Dodd 1982, Reed et al. 2004). In our study, we found overlap between coyote and kit fox scat sizes, but high levels of overlap in size among sympatric carnivore scats are not uncommon. Farrell et al. (2000) reported overlapping scat diameters for ocelots (*Leopardus pardalis*) and cougars, which are 2 sympatric felids with disparate body sizes. The diameters of coyote scats overlap with scats of the larger wolf (*Canis lupus*; Weaver and Fritts 1979, Reed et al. 2004) and with the smaller red fox (Green and Flinders 1981), gray fox (*Urocyon cinereoargenteus*; Danner and Dodd 1982), and swift fox (*Vulpes velox*; Harrison et al. 2001).

Previous studies have explored an alternative statistical classification method for discriminating among the scats of sympatric carnivores: parametric discriminant function analysis. A discriminant function analysis based on diameter and mass misclassified 14% of coyote scats and 35% of Mexican gray wolf (*C. l. baileyi*) scats; and misclassification increased for both species when models of only diameter, diameter and length, or diameter, mass, and length were considered (Reed et al. 2004). Although diameter and mass provided relatively high accuracy for coyote scats, classification of Mexican wolf scats was inaccurate and, in general,

measurements were deemed to be unreliable for classification (Reed et al. 2004). In another study, discriminant function analysis was evaluated as an approach to identify coyote scats from those of sympatric carnivores based on diameter, but proved unreliable and had an overall misclassification rate of 38.9% (Prugh and Ritland 2005).

Our approach differed in that we employed nonparametric classification methods, which do not require data to be normally distributed, and we incorporated information on scat diameter, length and number of segments. Unlike mass, which requires drying of scats prior to weighing, all 3 of the measures we employed can be collected quickly in the field. When comparing misclassifications for coyote and kit fox scats, we were able to improve overall classification success over field identification with both *k*-nearest neighbor and classification-tree methods but we think the best method was the classification tree because it produced the lowest overall misclassification rate.

Classification approaches remove the subjectivity commonly associated with field identification and are therefore an appealing quantitative technique that may improve classification when molecular identification is unfeasible. Classification approaches may not work effectively for all species, however, and classification success will depend in part on the variation in scat among sympatric target species, how well training data reflect true variation in the population, the proportion of nontarget species in the sample evaluated, and the selection of the appropriate predictor variables. Our results suggest that classification trees may provide a reliable method of discriminating between coyote and kit fox scats in our study system when molecular identification is unfeasible (e.g., because of funding restrictions or insufficient DNA obtained). Classification trees provided intuitive decision rules that can be easily interpreted and implemented by wildlife practitioners for future classifications. Furthermore, inspection of misclassification rates at terminal nodes can guide practitioners to those samples that are most problematic (i.e., the nodes with the highest misclassifications, either overall or for target species) and for which molecular identification might be

preferred to further reduce misclassifications. We also found that scat diameter and length were important for classifying scats. This is in contrast to studies using discriminant function analysis, which found that diameter was not a reliable metric for classifying scats (Reed et al. 2004, Prugh and Ritland 2005).

Often monitoring efforts are initiated primarily for species of conservation concern (i.e., endangered, threatened, or otherwise imperiled), such as kit foxes, and classification approaches may be restricted to species with adequate sample sizes. In our system, coyotes and kit foxes are the most abundant carnivore species. When nontarget species are detected relatively infrequently, this small proportion may not substantially change misclassification rates, as observed here. Alternatively, if samples from nontarget species are abundant and/or encountered frequently, the associated increase in sample size should allow researchers to explicitly incorporate these species into the classification models.

Our results suggest that field identification of carnivore scats can suffer from high misclassification rates, even when sympatric species have disparate body sizes. Inaccurate species identification can bias inferences drawn from scat surveys and may lead to less effective management strategies. We encourage resource managers and researchers utilizing scat surveys to employ methods to minimize or eliminate misclassifications. Although unambiguous molecular identification provides reliable classification, managers conducting long-term monitoring, surveys over large spatial extents, and/or working with limited funding may not be able to utilize molecular identification for the duration of a monitoring program or study. Alternatively, nonparametric classification based on morphometric characteristics may decrease misclassification rates over field identification. Approaches that elucidate areas of greatest misclassification, such as classification trees where misclassification rate can be identified by node, can be used to direct molecular identification analyses to those samples most likely to be misidentified, reducing overall misclassification while keeping costs low. Additionally, for studies employing molecular identification, classification techniques may provide an avenue for reliably identifying scats that fail molecular identification, because of fecal DNA degradation; this may be particularly important in environments where fecal DNA degrades more rapidly. Future projects employing scat surveys should conduct pilot studies to quantify misclassification rates and evaluate the sensitivity of downstream analyses to misclassification. By incorporating molecular identification during pilot surveys, training data sets and reliable classification schemes can be developed that may reduce future survey costs and minimize misclassifications.

ACKNOWLEDGMENTS

Funding support was provided by the U.S. Department of Defense's Environmental Security Technology Certification Program (12 EB-RC5-006) and Legacy Resource Management Program (W9132T-12-2-0050) and by the National Geographic Society's Conservation Trust (C248-13). The U.S. Army Dugway Proving Ground Natural Resource

Management Program provided logistical support. The Utah Division of Wildlife Resources provided housing. We thank B. Kluever, E. Burke, K. Cobb, J. Decotis, T. Edwards, C. Perkins, and M. Richmond for assistance with field work. J. Adams provided laboratory guidance and support. L. Gardiner, R. Irwin, T. Lasley, K. LeWallen, A. Lundgren, K. Miller, A. Reisenauer, and P. Stinebaugh assisted with DNA extractions. We thank C. Ribic, L. Berkman, L. Prugh, and an anonymous reviewer for their helpful comments on an earlier version of the manuscript.

LITERATURE CITED

- 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.
- Beja-Pereira, A., R. Oliveira, P. C. Alves, M. K. Schwartz, and G. Luikart. 2009. Advancing ecological understandings through technological transformations in noninvasive genetics. *Molecular Ecology Resources* 9:1279–1301.
- Breiman, L., J. H. Friedman, R. A. Olshen, and C. J. Stone. 1984. *Classification and regression trees*. Wadsworth, Belmont, California, USA.
- Cover, T. M., and P. E. Hart. 1967. Nearest neighbor. *Transactions on Information Theory* 13:21–27.
- Cunningham, S. C., L. Kirkendall, and W. Ballard. 2006. Gray fox and coyote abundance and diet responses. *Western North American Naturalist* 66:169–180.
- Dalen, L., A. Götherström, and A. Angerbjörn. 2004. Identifying species from pieces of faeces. *Conservation Genetics* 5:109–111.
- Danner, D. A., and N. Dodd. 1982. Comparison of coyote and gray fox scat diameters. *Journal of Wildlife Management* 46:240–241.
- Davison, A., J. D. S. Birks, R. C. Brookes, T. C. Braithwaite, and J. E. Messenger. 2002. On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. *Journal of Zoology* 257:141–143.
- 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 Genetic Resources*. doi: 10.1007/s12686-014-0257-x.
- Dempsey, S. J. 2013. Evaluation of survey methods and development of species distribution models for kit foxes in the Great Basin desert. Thesis, Utah State University, Logan, USA.
- Dempsey, S. J., E. M. Gese, and B. M. Kluever. 2014. Finding a fox: an evaluation of survey methods to estimate abundance of a small desert carnivore. *PloS one* 9:e105873.
- Farrell, L., J. Roman, and M. Sunquist. 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. *Molecular Ecology* 9:1583–1590.
- Ferkin, M. H., and A. A. Pierce. 2007. Perspectives on over-marking: is it good to be on top? *Journal of Ethology* 25:107–116.
- Foran, D. R., K. R. Crooks, and S. C. Minta. 1997. Species identification from scat: an unambiguous genetic method. *Wildlife Society Bulletin* 25:835–839.
- Gese, E. M. 2001. Monitoring of terrestrial carnivore populations. Pages 372–396 in J. L. Gittleman, S. M. Funk, D. W. Macdonald, and R. K. Wayne, editors. *Carnivore conservation*. Cambridge University Press, London, England, United Kingdom.
- Gompper, M. E., R. W. Kays, J. C. Ray, S. D. Lapoint, D. A. Bogan, and J. R. Cryan. 2006. A comparison of noninvasive techniques to survey carnivore communities in northeastern North America. *Wildlife Society Bulletin* 34:1142–1151.
- Green, J. S., and J. T. Flinders. 1981. Diameter and pH comparisons of coyote and red fox scats. *Journal of Wildlife Management* 45:765–767.
- Harrington, L. A., A. L. Harrington, J. Hughes, D. Stirling, and D. W. Macdonald. 2010. The accuracy of scat identification in distribution surveys: American mink, *Neovison vison*, in the northern highlands of Scotland. *European Journal of Wildlife Research* 56:377–384.
- Harrison, R. L., D. J. Barr, and J. W. Dragoo. 2001. A comparison of population survey techniques for swift foxes (*Vulpes velox*) in New Mexico. *American Midland Naturalist* 148:320–337.

- Hastie, T., R. Tibshirani, and J. Friedman. 2001. The elements of statistical learning: data mining, inference, and prediction. Springer, New York, New York, USA.
- Kamler, J. F., U. Stenkewitz, U. Klare, N. F. Jacobsen, and D. W. Macdonald. 2012. Resource partitioning among cape foxes, bat-eared foxes, and black-backed jackals in South Africa. *Journal of Wildlife Management* 76:1241–1253.
- Kamler, J. F., U. Stenkewitz, and D. W. Macdonald. 2013. Lethal and sublethal effects of black-backed jackals on cape foxes and bat-eared foxes. *Journal of Mammalogy* 94:295–306.
- Kelly, M. J., J. Betsch, C. Wultsch, B. Mesa, and L. S. Mills. 2012. Noninvasive sampling of carnivores. Pages 47–69 in L. Boitani, and R. A. Powell, editors. *Carnivore ecology and conservation: a handbook of techniques*. Oxford University Press, Oxford, England, United Kingdom.
- Kitchen, A. M., E. M. Gese, L. P. Waits, S. M. Karki, and E. R. Schauster. 2005. Genetic and spatial structure within a swift fox population. *Journal of Animal Ecology* 74:1173–1181.
- Kitchen, A. M., E. M. Gese, L. P. Waits, S. M. Karki, and E. R. Schauster. 2006. Multiple breeding strategies in the swift fox, *Vulpes velox*. *Animal Behaviour* 71:1029–1038.
- Kohn, M. H., and R. K. Wayne. 1997. Facts from feces revisited. *Trends in Ecology and Evolution* 12:223–227.
- Kozłowski, 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.
- Kozłowski, A. J., E. M. Gese, and W. M. Arjo. 2012. Effects of intraguild predation: evaluating resource competition between two canid species with apparent niche separation. *International Journal of Ecology* 2012:1–12. doi:10.1155/2012/629246
- Long, R. A., T. M. Donovan, P. MacKay, W. J. Zielinski, and J. S. Buzas. 2011. Predicting carnivore occurrence with noninvasive surveys and occupancy modeling. *Landscape Ecology* 26:327–340.
- Long, R. A., P. MacKay, W. J. Zielinski, and J. C. Ray, editors. 2008. *Noninvasive survey methods for carnivores*. Island Press, Washington, D. C., USA.
- Lonsinger, R. C., E. M. Gese, S. J. Dempsey, B. M. Kluever, T. R. Johnson, and L. P. Waits. 2015. Balancing sample accumulation and DNA degradation rates to optimize noninvasive genetic sampling of sympatric carnivores. *Molecular Ecology Resources* 15: DOI: 10.1111/1755-0998.12356
- MacKenzie, D. I. 2005. What are the issues with presence-absence data for wildlife managers? *Journal of Wildlife Management* 69:849–860.
- Marucco, F., D. H. Pletscher, and L. Boitani. 2008. Accuracy of scat sampling for carnivore diet analysis: wolves in the Alps as a case study. *Journal of Mammalogy* 89:665–673.
- McCarthy, K. P., T. K. Fuller, M. Ming, T. M. McCarthy, L. Waits, and K. Jumabaev. 2008. Assessing estimators of snow leopard abundance. *Journal of Wildlife Management* 72:1826–1833.
- Murphy, M. A., K. C. Kendall, A. Robinson, and L. P. Waits. 2007. The impact of time and field conditions on brown bear (*Ursus arctos*) faecal DNA amplification. *Conservation Genetics* 8:1219–1224.
- Onorato, D., C. White, P. Zager, and L. P. Waits. 2006. Detection of predator presence at elk mortality sites using mtDNA analysis of hair and scat samples. *Wildlife Society Bulletin* 34:815–820.
- Paxinos, E. E., C. McIntosh, K. Ralls, and R. C. Fleischer. 1997. A noninvasive method for distinguishing among canid species: amplification and enzyme restriction of DNA from dung. *Molecular Ecology* 6:483–486.
- Prugh, L. R., and C. E. Ritland. 2005. Molecular testing of observer identification of carnivore feces in the field. *Wildlife Society Bulletin* 33:189–194.
- R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Razali, N. M., and Y. B. Wah. 2011. Power comparisons of Shapiro–Wilk, Kolmogorov–Smirnov, Lilliefors and Anderson–Darling tests. *Journal of Statistical Modeling and Analytics* 2:21–33.
- Reed, J. E., R. J. Baker, W. B. Ballard, and B. T. Kelly. 2004. Differentiating Mexican gray wolf and coyote scats using DNA analysis. *Wildlife Society Bulletin* 32:685–692.
- Schauster, E. R., E. M. Gese, and A. M. Kitchen. 2002. An evaluation of survey methods for monitoring swift fox abundance. *Wildlife Society Bulletin* 30:464–477.
- Schooley, R. L., L. A. Cotner, A. A. Ahlers, E. J. Heske, and J. M. Levensgood. 2012. Monitoring site occupancy for American mink in its native range. *Journal of Wildlife Management* 76:824–831.
- 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.
- 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.
- Taberlet, P., L. P. Waits, and G. Luikart. 1999. Noninvasive genetic sampling: look before you leap. *Trends in Ecology and Evolution* 14:323–327.
- Therneau, T., B. Atkinson, and B. Ripley. 2014. rpart: recursive partitioning and regression trees. R package version 4. 1–8. <http://cran.r-project.org/web/packages/rpart/index.html> Accessed 8 Apr 2014.
- Vanak, A. T., and M. E. Gompper. 2009. Dietary niche separation between sympatric free-ranging domestic dogs and Indian foxes in central India. *Journal of Mammalogy* 90:1058–1065.
- Venables, W. N., and B. D. Ripley. 2002. *Modern applied statistics with S*. Fourth edition. Springer, New York, New York, USA.
- Vynne, C., J. L. Keim, R. B. Machado, J. Marinho-Filho, L. Silveira, M. J. Groom, and S. K. Wasser. 2011. Resource selection and its implications for wide-ranging mammals of the Brazilian Cerrado. *PLoS one* 6:e28939.
- Waits, L. P., 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.
- Weaver, J. L., and S. H. Fritts. 1979. Comparison of coyote and wolf scat diameters. *Journal of Wildlife Management* 43:786–788.
- Zar, J. H. 1996. *Biostatistical analysis*. Prentice Hall, Upper Saddle River, New Jersey, USA.

Associate Editor: Berkman.