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Balancing sample accumulation and DNA degradation rates to optimize noninvasive genetic sampling of sympatric carnivores

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Abstract

Noninvasive genetic sampling, or noninvasive DNA sampling (NDS), can be an effective monitoring approach for elusive, wide-ranging species at low densities. However, few studies have attempted to maximize sampling efficiency. We present a model for combining sample accumulation and DNA degradation to identify the most efficient (i.e. minimal cost per successful sample) NDS temporal design for capture–recapture analyses. We use scat accumulation and faecal DNA degradation rates for two sympatric carnivores, kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) across two seasons (summer and winter) in Utah, USA, to demonstrate implementation of this approach. We estimated scat accumulation rates by clearing and surveying transects for scats. We evaluated mitochondrial (mtDNA) and nuclear (nDNA) DNA amplification success for faecal DNA samples under natural field conditions for 20 fresh scats/species/season from <1–112 days. Mean accumulation rates were nearly three times greater for coyotes (0.076 scats/km/day) than foxes (0.029 scats/km/day) across seasons. Across species and seasons, mtDNA amplification success was ≥95% through day 21. Fox nDNA amplification success was ≥70% through day 21 across seasons. Coyote nDNA success was ≥70% through day 21 in winter, but declined to <50% by day 7 in summer. We identified a common temporal sampling frame of approximately 14 days that allowed species to be monitored simultaneously, further reducing time, survey effort and costs. Our results suggest that when conducting repeated surveys for capture–recapture analyses, overall cost-efficiency for NDS may be improved with a temporal design that balances field and laboratory costs along with deposition and degradation rates.

Keywords: *Canis latrans*, DNA degradation, genotyping error, noninvasive genetic sampling, scat deposition, *Vulpes macrotis*

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Introduction

Noninvasive genetic sampling, or noninvasive DNA sampling (NDS), is increasingly being used to monitor species that are rare, elusive or otherwise difficult to survey with traditional techniques (Waits & Paetkau 2005). Genetic material obtained from noninvasive sources (e.g. faeces, hair, feathers) can allow for species identification and individual identification, population genetic structure, genetic diversity, connectivity and sex ratios (Beja-Pereira *et al.* 2009). Combining NDS with capture–recapture and occupancy modelling approaches allows researchers to estimate population demographic parameters (Lukacs & Burnham 2005) and patterns of occurrence (Long *et al.* 2011). Many studies have opted for NDS due to logistical and animal welfare considerations, or improved cost-benefits (e.g. Prugh *et al.* 2005; Brøseth *et al.* 2010; Stenglein *et al.* 2010b).

DNA degradation and genotyping errors can influence NDS results (Taberlet *et al.* 1999; Waits & Paetkau 2005; Beja-Pereira *et al.* 2009). Accordingly, researchers have expended considerable effort to understand how factors such as sample age (Piggott 2004; Murphy *et al.* 2007; Santini *et al.* 2007), environmental conditions (Piggott 2004; Murphy *et al.* 2007; Santini *et al.* 2007; DeMay *et al.* 2013), diet (Murphy *et al.* 2003; Panasci *et al.* 2011), sample collection and storage techniques (Murphy *et al.*

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2002; Palomares *et al.* 2002; Piggott & Taylor 2003; Stenglein *et al.* 2010a; Panasci *et al.* 2011), locus length (Buchan *et al.* 2005; DeMay *et al.* 2013) and species-specific differences (Piggott & Taylor 2003; Buchan *et al.* 2005) influence the degradation of DNA. Collectively these studies indicate DNA degradation and genotyping errors vary among species and environmental conditions. General recommendations to reduce degradation and genotyping errors included sampling the freshest scats and conducting surveys during the driest and/or coldest seasons (Murphy *et al.* 2007; Santini *et al.* 2007).

While previous efforts to optimize NDS have focused on ways to minimize DNA degradation and genotyping errors, they have not explicitly incorporated sample accumulation rates. Understanding sample accumulation rates (i.e. the rate at which noninvasive genetic samples accrue and can be obtained) is critical to designing efficient sampling and may influence the optimal temporal sampling frame. Faecal DNA is a common source of noninvasive genetic samples, but sample accumulation rate is probably affected by diet, behaviour, physiology and environmental conditions. For example, seasonal variation in diet, behaviour and space use by carnivores can influence scat deposition rates and patterns (Andelt & Andelt 1984; Ralls *et al.* 2010). Additionally, heavy rain or winds can remove scats, as can conspecifics (Livingston *et al.* 2005).

The temporal sampling design of NDS can be optimized to maximize laboratory success while minimizing overall cost per successful sample. Laboratory costs are driven by the number of samples collected, polymerase chain reaction (PCR) success rates and genotyping error rates (Fig. 1). Scat accumulation rates, survey effort (spatial coverage), desired sample size (number of samples required to achieve objectives) and the number of sampling events (temporal frequency) necessary to achieve the desired sample size influence field costs (Fig. 1). Thus, to optimize the temporal design for NDS, pilot studies should consider both laboratory and field costs by incorporating DNA degradation and sample accumulation rates for each species, season and study site.

Here, we present a model for combining information on sample accumulation and DNA degradation to optimize (i.e. identify the most cost-effective) temporal sampling design for capture–recapture studies employing NDS. We use scat accumulation rates and faecal DNA degradation rates for two sympatric carnivores, kit foxes (*Vulpes macrotis*; hereafter foxes) and coyotes (*Canis latrans*), across two seasons in the Great Basin desert of Utah, USA, to demonstrate how this approach can be implemented. In regards to scat accumulation, we hypothesized that (i) scat accumulation would be greater for coyotes than foxes due to their more omnivorous diet and higher abundance and (ii) seasonal variation in diets would result in higher accumulation rates in summer than winter for both species (Andelt & Andelt 1984; Arjo *et al.* 2007; Kozlowski *et al.* 2008). Regarding DNA degradation, we hypothesized that (i) due to its higher relative abundance mitochondrial DNA (mtDNA) would have higher PCR (or amplification) success rates than nuclear DNA (nDNA), (ii) amplification success would decrease over time for both nDNA and mtDNA, (iii) amplification success would decrease more precipitously for nDNA than mtDNA and (iv) amplification success for nDNA would be higher for shorter microsatellite loci than longer loci (Buchan *et al.* 2005; DeMay *et al.* 2013).

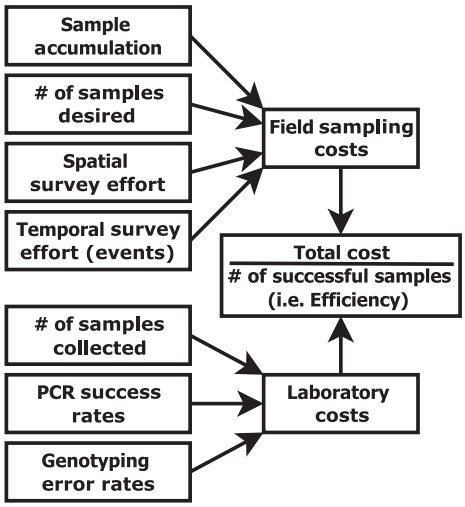

Fig. 1 Conceptual diagram showing the major components required to balance field and laboratory efficiency for optimization of noninvasive genetic sampling for capture–recapture analysis.

Materials and methods

Study area

Our investigation took place on the U.S. Army Dugway Proving Ground (DPG), in western Utah. Located within the Great Basin, DPG is characterized by basin and range formations with elevations from 1228 to 2154 m (Arjo *et al.* 2007). The site experiences cold winters and moderate summers; coldest and warmest months are January (mean high = 3.3 °C, mean low = −8.8 °C) and July (mean high = 34.7 °C, mean low = 16.3 °C), respectively. Mean annual precipitation is approximately 20 cm with the greatest rainfall occurring in spring (Arjo *et al.* 2007). Sampling seasons corresponded to periods preceding breeding (January and February) and juvenile dispersal (July and August) for target species and aligned with periods of reduced precipitation in the region (Arjo *et al.* 2007).

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Sample accumulation surveys

Scat accumulation surveys in which transects are cleared and surveyed approximately 14 days later are commonly used to estimate relative abundances of canids (Gese 2001; Schauster *et al.* 2002). Using this approach, we conducted scat accumulation surveys between September 2010 and July 2012. Scat surveys were originally initiated to evaluate relative abundance of foxes and coyotes and therefore data were available not only for our winter and summer sampling seasons, but also for spring. Fifteen 5 km transects along dirt or gravel roads were cleared and surveyed for carnivore scats approximately 14 days later (mean = 13.9 ± 0.51 SD, range = 13–16). Each 5 km transect was surveyed during two summers (2010, 2011), two springs (2011, 2012) and one winter (2011). Additionally, to expand the spatial coverage and ensure that standardized accumulation rates (scats/km/day) were similar between sampling intervals of different durations, we evaluated scat accumulation along eight shorter transects during one summer (2012), using a random starting point, direction and length (mean = 2.6 ± 0.85 SD, range = 1–3.5 km) and surveying 7 days after clearing. We determined species for each carnivore scat detected during accumulation surveys based on overall appearance, size and shape (Kozlowski *et al.* 2012).

Faecal DNA degradation

Faecal DNA degradation was assessed at DPG during two seasons, winter (initiated 8 February 2012) and summer (initiated 11 July 2012), corresponding to proposed field sampling seasons. In each season, 20 fresh scats were collected per species. Fox scats were obtained from live-captured, free-ranging individuals, and coyote scats were obtained from the USDA/NWRC/Predator Research Facility (Millville, UT, USA). Scats were frozen within four hours of collection. On average, fox and coyote scats were stored frozen for 18 months and <1 month, respectively, before being transferred to the study site, thawed and placed in the field and protected from disturbance with a frame covered with wire mesh (25 mm openings; 0.7 gauge wire). We collected faecal DNA samples from each scat at days 1, 3, 7, 14, 21, 56 and 112, or until the scat was fully utilized. Day 1 samples were collected just prior to exposure to field conditions. We added a day 5 time point during summer to provide greater resolution, as a recent study detected a significant decline in coyote faecal DNA quality as early as 5 days postdeposition (Panasci *et al.* 2011). Additionally, a severe wind event during winter buried experimental plots after day 21, so day 56 and 112 time points were only available for summer. Faecal DNA samples were collected from the side of each scat following procedures of Stenglein *et al.* (2010a), and scats were considered fully utilized when no additional samples could be collected in this manner. All samples were stored in 1.4 mL of DET buffer (20% DMSO, 0.25 M EDTA, 100 μM Tris, pH 7.5 and NaCl to saturation; Seutin *et al.* 1991). Due to natural variability in scat sizes, some smaller scats were fully utilized before completion of all time points, resulting in reduced sample sizes at later time points. To maintain more equitable sample sizes among time points during summer, we placed three additional scats for each species out at the start of the degradation study and sampled these scats in place of fully utilized scats at later time points.

DNA extraction and PCR amplification

We conducted faecal DNA extraction and PCR amplification in a facility dedicated to low-quality DNA. Faecal DNA samples were extracted using the QIAamp DNA Stool Mini Kits (Qiagen, Inc., Valencia, CA, USA) with negative controls to monitor for contamination (Taberlet & Luikart 1999; Beja-Pereira *et al.* 2009). We performed mtDNA species identification tests by amplifying fragments of the control region (Onorato *et al.* 2006; De Barba *et al.* 2014). Species-specific PCR products lengths were 336–337 base pairs (bp) for foxes and 115–120 bp and 360–364 bp for coyotes (De Barba *et al.* 2014). Samples that failed to amplify for mtDNA were repeated once to minimize sporadic effects (Murphy *et al.* 2007). For individual identification, we amplified fox and coyote samples with seven and nine nDNA microsatellite loci, respectively (Appendix S1, Supporting information). We conducted PCR on a Bio-Rad Tetrad thermocycler (Bio-Rad, Hercules, CA, USA) including negative and positive controls. PCR conditions, including primer concentrations and thermal profiles, are presented in Appendix S1 (Supporting information). We visualized results using a 3130xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and scored allele sizes with Genemapper 3.7 (Applied Biosystems). Samples were considered successful for species identification if amplification of ≥1 mtDNA fragment was achieved in either the first or second amplification attempt. We calculated mtDNA success rates as the proportion of successful samples across each time point and season. We calculated nDNA amplification success rates (number of successful amplifications/total possible) and sample success rates (proportion of samples that amplified at ≥50% of the loci) for each time point and species.

Genotyping error rates

We combined replicates for each scat (i.e. all replicates across time points with successful nDNA amplification)

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to establish consensus genotypes (Taberlet *et al.* 1999; Pompanon *et al.* 2005). To achieve a consensus genotype, we required that heterozygote and homozygote alleles be observed in two and three independent replicates, respectively. Following the methods of Broquet & Petit (2004), we classified the observation of an allele not present in the consensus genotype as a false allele (FA) and the amplification of only one allele in a heterozygous consensus genotype as allelic dropout (ADO).

Data analysis

Scat accumulation results were standardized across transects and species as daily accumulation rates (scats accumulated/days since clearing = scats/km/day). We employed a generalized linear model to test effects of season and species on scat accumulation (O'Hara & Kotze 2010). We considered a Poisson regression model with a log link function, but residuals indicated underdispersion so we based inferences on quasi-likelihood with a free dispersion parameter. We used a likelihood ratio test to compare models with and without interactions. We compared the influence of main effects and factor levels with contrast analysis (R package contrast; Kuhn *et al.* 2011; R Core Team 2014).

We evaluated PCR success, FA and ADO as binary response variables with mixed-effects logistic regression models to assess DNA degradation rates, with sample included as a random effect to resolve pseudoreplication effects due to multiple observations per sample with SAS 9.3 (SAS Institute Inc. 2011). We included time since the scat was placed in the field (log transformed), DNA type (mtDNA vs. nDNA), species (fox vs. coyote), season (winter vs. summer) and locus length as fixed effects in the model for PCR success. We excluded DNA type from models for FA and ADO as these pertain only to nDNA. We categorized nDNA locus lengths based on the midlength of alleles per locus by species (range: 90–275 bp).

Optimization of NDS temporal design

Our goal was to optimize a NDS temporal design that could be employed within a capture–recapture framework for foxes and coyotes. To this end, we derived a total cost per successful sample (i.e. sample that achieves a consensus genotype for individual identification) at sampling intervals from 1 to 56 days, where the interval represented the number of days between clearing and survey or between sequential surveys.

Both spatial survey effort and desired sample size must be selected by the researcher, but may be informed by previous research, power analyses and/or simulations (Williams *et al.* 2002). We selected a survey effort of 150 km, a length of transect which we felt provided reasonable coverage of our study site and encompasses 1350 km^2 within 2.5 km of transects, the radius of the average fox home range at DPG (Dempsey 2013). We identified desired sample sizes of 200 fox and 400 coyote samples, values approximately three times the number of individuals expected to be in our study area (Solberg *et al.* 2006).

We determined the number of samples accumulated and available for collection at each potential sampling interval (1–56 days, hereafter interval), by calculating the product of the daily accumulation rate (scats/km/day), the number of kilometres surveyed (effort) and the number of days in the interval. We combined the number of samples accumulated at each interval with our model-predicted PCR success rates to calculate the number of successful samples for each interval, considering that each interval contained scats of varying ages and levels of degradation. For example, for an interval of 3 days, we assumed that 33.3% of the scats were 1, 2 and 3 days old and that each age class was characterized by its model-predicted PCR success.

Noninvasive samples commonly suffer from genotyping errors (Pompanon *et al.* 2005), which can influence costs. For each interval, we summed the model-predicted FA and ADO rates to determine the overall predicted genotyping error rate. We then calculated the number of genotyping errors expected for samples on each day as the entrywise product of the number of successful samples and the predicted genotyping error rate for that day. The total number of samples, with a genotyping error within a given interval then, was the sum of the number of samples with a genotyping error across all days contributing to the interval. The cumulative genotyping error rate for an interval was determined as the proportion of successful samples with a genotyping error.

As genotyping errors increase, additional replicates are required to reconcile differences among genotypes (Pompanon *et al.* 2005). Within a capture–recapture framework, errors in multilocus genotypes can result in overestimates of abundance and bias survival estimates (Lukacs & Burnham 2005). Consequently, we set a goal of maintaining a probability of error $\leq 2\%$ in our data set. We assumed genotyping error rate was similar across loci, and replicates were independent. We calculated the probability of having an error in the consensus genotype at a given interval as the cumulative genotyping error rate raised to the number of replicates, then multiplied by the number of loci. We estimated our laboratory costs to be approximately \$60/sample (including labour and supplies for extraction, four independent amplifications and finalization of the consensus genotype), based on current laboratory expenses, with a 25% increase in cost for each additional pair of replicates. Thus, when the number of replicates required to maintain our goal of

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≤2% error exceeded four, we increased the number of replicates incrementally by two until the goal was achieved or eight replicates were reached. We estimated our hourly field costs to be $10/h/technician (including labour and fuel), and we could survey 150 km of transects in 160 h (e.g. two technicians working 40 h/week for 2 weeks or four technicians working 40 h/week for 1 week). For each interval, we divided the desired sample size by the total number of successful samples to determine the number of sampling events required.

We standardized cost as cost per successful sample at each interval. Thus, the total laboratory cost and field cost for each interval were each divided by the number of successful samples. We then combined these costs to obtain an overall cost per successful sample and identified the optimal intervals as those that minimized the overall cost per successful sample. Additionally, to estimate savings obtained from monitoring species concurrently, we calculated the average annual cost per successful sample by dividing the field costs in half (i.e. split between species for each given sampling event) and averaging winter and summer estimates of cost per successful sample for each species.

Results

Scat accumulation

Scat accumulation surveys were conducted along 170.5, 150 and 75 km of transects in summer, spring and winter, respectively. Rates of scat accumulation were higher for coyotes (mean = 0.076 scats/km/day ± 0.009 SE) than foxes (mean = 0.029 scats/km/day ± 0.007 SE) across seasons (Fig. 2). The likelihood ratio test comparing models with and without interactions was not significant ($P = 0.673$), and therefore, we report results for the model with main effects only. Species had a significant effect on scat accumulation when controlling for season (contrast, $z = -9.09$, $P < 0.001$; Table 1). Season contrasts controlling for species indicated that spring accumulation rates were significantly different from summer (contrast, $z = 5.99$, $P < 0.001$) and winter (contrast, $z = -3.16$, $P = 0.002$), but that summer and winter differed only marginally (contrast, $z = 1.89$, $P = 0.059$; Table 1; Fig. 2).

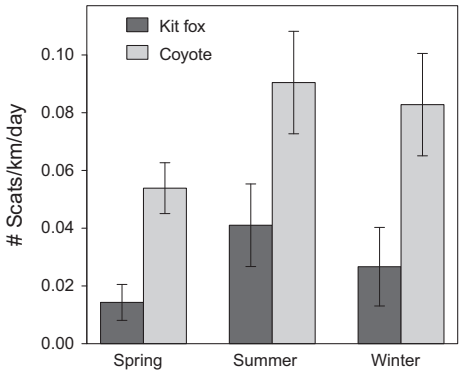

Fig. 2 Mean scat accumulation rates ±SE for kit fox (*Vulpes macrotis*; dark grey) and coyote (*Canis latrans*; light grey) at Dugway Proving Ground, Utah, collected from September 2010 to July 2012. Coyote scat accumulation rates were significantly higher than kit fox ($P < 0.001$). Spring differed significantly from summer ($P < 0.001$) and winter ($P = 0.002$). Summer and winter differed marginally ($P = 0.059$).

PCR success rates

Across time points, 95% ($n = 90$; winter) and 91% ($n = 132$; summer) of fox samples successfully amplified for mtDNA on the first PCR attempt. An additional 5% ($n = 5$) and 3% ($n = 4$) of fox samples successfully amplified for mtDNA on the second PCR attempt, giving overall fox mtDNA success rates of 100% ($n = 95$) and 94% ($n = 145$) in winter and summer, respectively. Overall coyote mtDNA success was 97% ($n = 100$) and 91% ($n = 157$) in winter and summer, respectively, with 89% ($n = 89$; winter) and 87% ($n = 136$; summer) of the samples successfully amplifying for mtDNA on the first PCR attempt and an additional 8% ($n = 8$) and 4% ($n = 7$) amplifying on the second PCR attempt. Both species exhibited high amplification success rates over time with mtDNA success rates ≥95% through 21 days in both seasons (Fig. S1, Supporting information).

Across time points, fox nDNA amplification success rates (number of successful amplifications/total possible) were 75% ($n = 665$) and 72% ($n = 1015$) in winter and summer, respectively, compared to success rates of only 68% ($n = 900$) and 45% ($n = 1413$) for coyotes. Fox nDNA sample success rates (proportion of samples successful at ≥50% of the loci) were ≥95% through day 3 (winter) and day 7 (summer), ≥70% through day 21 in both seasons and declined to <30% by day 56 (summer; Fig. S1, Supporting information). Coyote nDNA sample success rates ranged from 80% to 90% through day 5 in both seasons, remained ≥70% through day 21 in winter, but declined in summer to <50% by day 7 and <25% by day 56 (Fig. S1, Supporting information).

Models indicated that all the main effects significantly influenced PCR success (Table 2). Mitochondrial DNA had higher success than nDNA and success for both DNA types decreased over time (Fig. 3). Locus length significantly influenced nDNA PCR success, with longer loci having lower success (Fig. 3). PCR success was significantly influenced by season, with higher success in winter than summer. A significant effect of species was also detected (Fig. 3). Significant interactions among fixed effects reveal the complex nature of DNA

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Table 1 Generalized linear model and contrast analysis results with standard errors (SE) and lower (LL) and upper (UL) 95% confidence bounds for scat accumulation samples collected from September 2012 to July 2012 at Dugway Proving Ground, Utah. Species levels include coyote (*Canis latrans*) and kit fox (*Vulpes macrotis*). Season levels include spring, summer and winter

	Estimate	SE	*z*-value	*P*-value	LL	UL
Model parameters						
(Intercept)	−3.01	0.243	−12.37	<0.001*	−3.52	−2.56
Summer	0.66	0.277	2.38	0.019*	0.13	1.22
Winter	0.47	0.349	1.36	0.177	−0.23	1.16
Kit fox	−0.97	0.253	−3.83	<0.001*	−1.49	−0.49
Contrasts						
Coyote vs. Kit fox	−1.08	0.119	−9.09	<0.001*	−1.32	−0.85
Summer vs. Winter	0.26	0.137	1.89	0.059	−0.01	0.53
Summer vs. Spring	0.79	0.131	5.99	<0.001*	0.53	1.04
Spring vs. Winter	−0.53	0.167	−3.16	0.002*	−0.85	−0.19

Significant (*) *P*-values for *z* statistic evaluated at $\alpha = 0.05$.

Table 2 Mixed-effects logistic regression model results for PCR success, allelic dropout and false alleles for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) faecal DNA samples collected in 2012 during winter and summer at Dugway Proving Ground, Utah. Reported chi-square test statistics and *P*-values were generated with Type III tests of fixed effects

	PCR success		Allelic dropout		False alleles	
Fixed effect	Chi-square	*P*-value	Chi-square	*P*-value	Chi-square	*P*-value
Time	4.93	0.0263*	0.80	0.3706	0.09	0.7678
DNA type	224.06	<0.0001*	—	—	—	—
Locus length	8.73	0.0031*	0.03	0.8661	1.26	0.2614
Season	4.02	0.0449*	4.11	0.0427*	0.93	0.3337
Species	25.90	<0.0001*	0.64	0.4237	7.95	0.0048*
Time × Season	42.02	<0.0001*	0.28	0.5966	5.91	0.0150*
Time × Species	24.15	<0.0001*	4.09	0.0432*	4.94	0.0262*
Time × Locus length	13.38	0.0003*	1.03	0.3100	0.04	0.8386
Locus length × Season	1.57	0.2100	1.22	0.2699	0.15	0.7020
Locus length × Species	8.36	0.0038*	1.57	0.2098	10.16	0.0014

Significance (*) was evaluated at $\alpha = 0.05$. Time was log-transformed days since the scat was placed in the field. DNA types included mitochondrial and nuclear DNA. Locus length was based on the midpoint of each locus (range 90–275 base pairs).

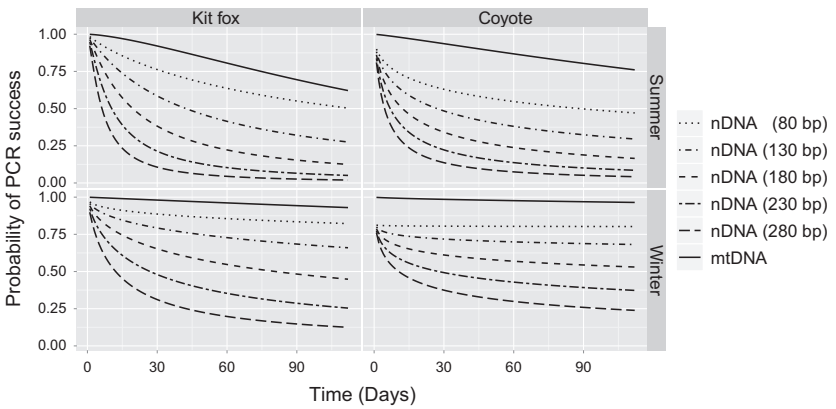

Fig. 3 Mixed-effects logistic regression model results for PCR success for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) faecal DNA samples collected in 2012 during winter and summer at Dugway Proving Ground, Utah.

degradation (Table 2). We detected significant interactions between the fixed effects of time and both season and locus length. PCR success for mtDNA and nDNA declined more slowly in winter than summer, and nDNA success declined more precipitously for longer loci than shorter loci (Fig. 3). Significant interactions

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were detected between species and both time and locus length (Table 2).

Genotyping error rates

Overall genotyping error rates varied between species (Fig. S2, Supporting information); across seasons and sampling periods, overall ADO was lower for foxes (18%) than coyotes (25%), while overall FA rate was slightly higher for foxes (5%) than coyotes (2%). Winter samples of both species had lower genotyping error rates on average than summer samples. Fox winter ADO rates ranged from 4% to 36%, whereas fox summer ADO rates ranged from 15% to 42% (Fig. S2, Supporting information). Coyote ADO rates ranged from 10% to 29% in winter and 15% to 56% in summer (Fig. S2, Supporting information). In both seasons, FA rates were low for both species (Fig. S2, Supporting information). Models for ADO and FA suggested that season and species, respectively, were the only main effects influencing each model (Table 2). Model results for ADO were influenced by a significant interaction between time and species, while model results for FA were influenced by significant interactions of time with season and species, and locus length with species (Table 2). Model-predicted cumulative genotyping error rates (combined ADO and FA rates across loci and intervals) were lower for foxes (winter mean = 20.9 ± 0.6% SE; summer mean = 25.1 ± 0.6% SE) than coyotes (winter mean = 31.5 ± 0.6% SE; summer mean = 37.4 ± 0.5% SE) and higher in summer than winter for both species.

Optimization of NDS temporal design

For fox, the predicted number of samples accumulated ranged from 4.1 (interval = 1 day) to 226.8 (interval = 56 days) in winter and 6.2 (interval = 1 day) to 345.0 (interval = 56 days) in summer. The predicted number of coyote samples accumulated ranged from 12.5 (interval = 1 day) to 697.2 (interval = 56 days) in winter and 13.5 (interval = 1 day) to 756.0 (interval = 56 days) in summer. For both species, the number of samples predicted to fail for nDNA microsatellite amplification, however, increased as interval length increased (Fig. S3, Supporting information). Across seasons and time points, a greater proportion of accumulated coyote samples were predicted to fail than fox samples (Fig. S3, Supporting information).

Based on model-predicted genotyping error rates, our goal of ≤2% probability of error in the data set could be achieved for fox with five or fewer replicates at all intervals, with four replicates being sufficient up to 34 days in winter and 16 days in summer. To achieve this goal for coyotes, up to seven replicates were required. In winter, five replicates were required for intervals of 3–16 days, six replicates for intervals of 17–49 days and seven replicates for intervals ≥50 days. For summer coyote samples, the minimum number of replicates required was five (1–3 days). Six replicates were required for intervals of 4–17 days and seven replicates for intervals of ≥18 days.

The number of sampling events necessary to obtain desired sample sizes was initially high due to the low number of samples accumulating over shorter intervals, but declined precipitously (Fig. 4). The number of sampling events was higher initially in winter than summer for both species due to seasonal differences in accumulation. The number of sampling events required was typically greater for foxes than coyotes despite the smaller desired sample size; this difference was greater in summer than winter (Fig. 4).

Overall cost per successful sample showed a similar pattern across species and seasons, but with differences in the magnitude and timing of changes. Cost per successful sample was highest for both species and seasons at the shortest intervals and was higher for foxes (Fig. 4a) than coyotes (Fig. 4b) at shorter intervals. For both species, cost per successful sample was higher in winter than summer at short intervals. Summer cost per successful sample surpassed winter costs at 7 days for coyotes and 16 days for foxes. Costs per successful sample declined as the number of required sampling events reduced field costs, until genotyping errors were sufficiently high to require additional replicates, increasing laboratory costs. The overall lower cumulative genotyping error resulted in smaller increases in overall cost for foxes (Fig. 4a) relative to coyotes (Fig. 4b). Sharp increases in cost associated with additional replicates occurred at a shorter interval for foxes (35 days) than coyotes (50 days) in winter. In summer, sharp increases in cost associated with additional replicates occurred at the same interval (17 days) for both species. When surveying species simultaneously, overall cost per successful sample was reduced (Fig. 4c) for each species, due to reduced field costs for each species individually. Average annual cost per successful sample suggested that a temporal sampling frame of approximately 14 days would reduce costs for each species and allow species to be monitored simultaneously (Fig. 4c).

Discussion

Our study is among the first to incorporate DNA degradation and sample accumulation rates to optimize NDS design; a similar approach was recently applied to ungulates (Woodruff *et al.* in press). Our

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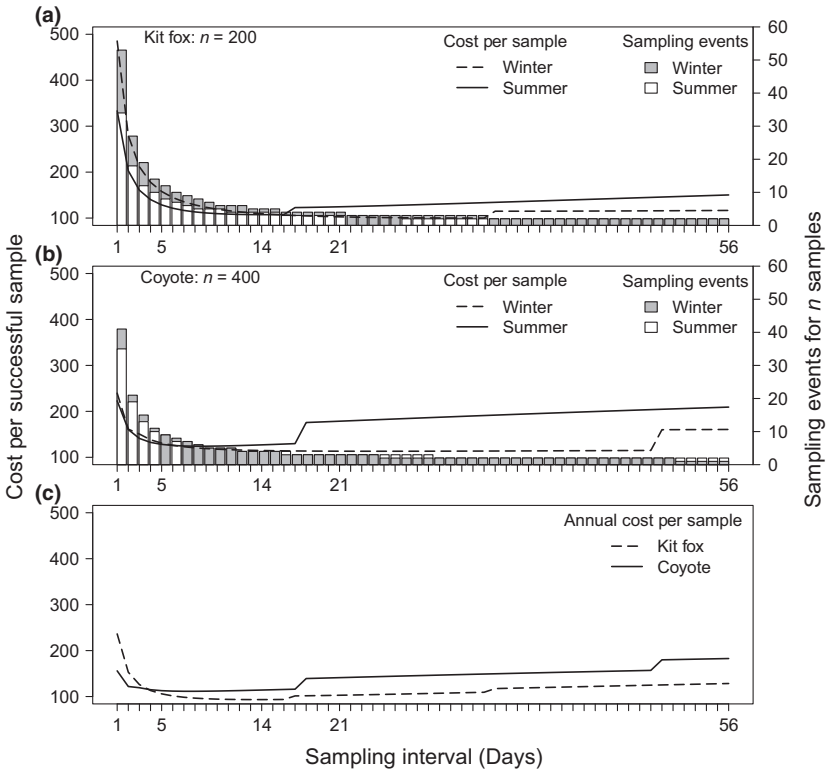

Fig. 4 Evaluation of cost ($) per successful faecal DNA sample and number of sampling events required to obtain (a) $n = 200$ kit fox (*Vulpes macrotis*) and (b) $n = 400$ coyotes (*Canis latrans*) samples from surveying 150 km of transects at Dugway Proving Ground, Utah, for a range of sampling intervals in winter and summer. Sampling intervals represent the days between an initial clear and subsequent survey or between surveys. The average annual cost for surveying each species (c) is reduced when the two sympatric species are surveyed simultaneously.

approach provides a novel method to improve efficiency of NDS and should be transferrable to systems or species where pilot studies can elucidate sample accumulation and DNA degradation rates (Fig. 1). By reducing costs, optimization approaches can make NDS an appealing monitoring strategy when funding is limited. Optimization allows NDS practitioners to increase spatial extent, temporal resolution or the number of species monitored in ongoing studies. Our study evaluated faecal DNA degradation of two carnivores under the same environmental conditions and over two seasons. Studies evaluating faecal DNA degradation rates have become customary for NDS (Murphy *et al.* 2007; Santini *et al.* 2007; DeMay *et al.* 2013), but only two have evaluated degradation for multiple species simultaneously (Nsubuga *et al.* 2004; Piggott 2004).

Factors influencing sample accumulation

The relative abundance of coyotes was higher than foxes across the study site (Arjo *et al.* 2007), and this difference probably contributed to higher observed accumulation rates for coyotes. Spring accumulation rates were significantly lower than summer and winter (Table 1; Fig. 2); winter accumulation was marginally lower than summer accumulation (Table 1). Coyotes and foxes increase their consumption of plants and insects in summer (Kozlowski *et al.* 2008), which may increase scat deposition rates (Andelt & Andelt 1984). Female foxes spend more time in or near dens during the reproduction season (Ralls *et al.* 2010), and these behavioural changes may contribute to lower accumulation rates in spring. Low spring accumulation rates suggest that from a sample accumulation perspective, summer and winter seasons are more appropriate for NDS.

Factors influencing faecal DNA degradation

Time had a significant influence on PCR success, consistent with other canid studies (Piggott 2004; Santini *et al.* 2007; Panasci *et al.* 2011). Our nDNA amplification success rates were similar to those reported by previous canid studies, including coyotes (Panasci *et al.* 2011), wolves (*Canis lupus*; Santini *et al.* 2007) and red foxes (*Vulpes vulpes*; Piggott 2004). Our fox nDNA amplification success was high relative to other canids, while coyote nDNA success was lower than previously reported (Panasci *et al.* 2011). This disparity stresses the importance of understanding interspecific and intraspecific seasonal variation in degradation rates.

Similar to other studies (Buchan *et al.* 2005; Scandura *et al.* 2006; DeMay *et al.* 2013), locus length significantly influenced nDNA PCR success, suggesting researchers may be able to improve success by selecting shorter loci. We detected a significant effect of season on degradation

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with winter samples showing less DNA degradation than summer samples. Piggott (2004) documented higher faecal DNA degradation rates in winter than summer and attributed this to increased moisture during winter. Previous studies indicate that environmental conditions such as temperature, UV exposure and humidity influence DNA degradation rates (Nsubuga *et al.* 2004; Murphy *et al.* 2007; Stenglein *et al.* 2010a). Winters and summers at DPG receive less precipitation than other seasons, but temperatures are significantly different (see Study area) and UV exposure is highest in summer. Our study design did not allow investigation of the influence of weather on degradation. We placed all samples in the field on the same day each season, and therefore, weather and time were confounded. We suspect though, that differences observed between seasons were related to broad differences in environmental conditions.

Our observed ADO and FA rates were similar to those reported in other canid studies (Piggott 2004; Santini *et al.* 2007; Stenglein *et al.* 2010b; Panasci *et al.* 2011). We were unable to detect a significant effect of time on genotyping errors, but this was likely due to small sample sizes associated with ADO and FA models. We observed a discernible, but not statistically significant increase in model-predicted ADO rates over time, but not in FA rates.

Optimization of NDS temporal design

By balancing sample accumulation and DNA degradation, an optimal NDS design can be selected that minimizes cost per successful sample. The optimal interval varies by species and season and is driven by sample collection (field) and processing (laboratory) costs. While the optimal interval is simply the interval that minimizes the cost per successful sample, additional factors should be considered such as the number of target species and interspecific differences in sample accumulation and DNA degradation. Initial costs per successful sample were calculated for sampling species independently (Fig. 4a,b). If a common interval is selected for foxes and coyotes, both species can be surveyed simultaneously on the same transects and overall field costs can be reduced (Fig. 4c). Additionally, selection of the optimal interval should consider downstream analyses. For example, demographic closure assumptions may be difficult to meet at extended intervals and small reductions in the cost per successful sample may be insufficient justification to select extended intervals.

Our results indicate a range of intervals for foxes and coyotes could be selected to improve efficiency, and these intervals are shorter in summer than winter. For example, summer cost per successful sample was minimized for foxes at day 14 and coyotes at day 9, but selection of an interval ± 2 days from these optimal intervals changed the cost per successful sample by <$1. The range of effective intervals was wider in winter. Winter cost per successful sample was minimized for foxes and coyotes at days 34 and 24, respectively, yet the cost per successful sample changed <$1 for intervals up to 8 days shorter (25–33 days) for foxes and for 24 intervals surrounding (16–40 days) the optimal interval for coyotes. We were interested in selecting a common interval that was effective for both species and consistent across seasons. Summer cost per successful sample limited the upper bound of the common interval, as cost increased sharply for both species after day 17. We thus identified an interval of 14 days as the common interval within our system (Fig. 4c). At 14 days, winter cost per successful sample was reduced and continuing to decline slowly for both species and the number of sampling events was small enough to conduct sampling over a single season.

Based on these results, we recommend NDS efforts account for sample accumulation and DNA degradation during the design phase (Fig. 1). Previous studies have recommended sampling the freshest scats possible (Murphy *et al.* 2007; Santini *et al.* 2007; DeMay *et al.* 2013). Our results show that when sampling over time within a capture–recapture framework, short intervals may be cost-prohibitive if a substantial sample size is required. Thus, we recommend sampling designs consider cost per successful sample and minimize violations of assumptions for downstream analyses.

Limitations and implications for research

Collection of fresh samples (e.g. samples known to be $\leq$1 day old) to evaluate DNA degradation is logistically prohibitive, particularly when species are rare, elusive, or difficult to capture. Consequently, many studies comparing PCR success (e.g. between species, under environmental variations, over time) have relied on samples from captive populations (Murphy *et al.* 2002, 2003, 2007; Piggott 2004; Santini *et al.* 2007; DeMay *et al.* 2013). In our study, scats used to evaluate DNA degradation varied between species in origin and length of storage. We obtained fresh scats from free-ranging foxes during live capture, but fresh scats from free-ranging coyotes were unavailable. Consequently, fresh coyote scats were obtained from a captive population. Although scats were frozen upon collection, stored for variable lengths of time and thawed prior to placement in the field, we do not feel that storage time or the freeze–thaw cycle significantly impacted PCR success. While we did not explicitly test the influence of freezing during this study, we previously evaluated PCR success of canid scats stored in a standard freezer and

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found no decline in PCR success for samples frozen for up to 1 year, when the study ended (L. P. Waits & J. R. Adams, unpublished data). Our results support this conclusion. On average, fox and coyote scats were stored frozen 18 months and <1 month, respectively. Despite the longer storage time of fox scats, observed PCR success rates were the same (mtDNA) or higher (nDNA) for foxes in both seasons and scats of both species produced high PCR success at the earliest time points (Fig. S1, Supporting information). Additionally, winter temperatures at our site fluctuate from below to above freezing (night vs. day temperatures) and scats naturally experience repeated freeze–thaw cycles, yet in this experiment, we observed higher PCR success rates for both species in winter relative to summer, suggesting that freeze–thaw cycles were not the driving cause of DNA degradation.

Variation in diets between captive and free-ranging coyotes may also influence the generalization of results to the wild population. Differences in diet could influence the rate of intestinal cell shedding or the amount of inhibitors in faecal samples. However, we do not believe that using captive coyote scats substantially influenced our results. We have data on success rates for free-ranging coyote samples collected in winter and summer 2013, and results are comparable to model-predicted results from our degradation experiment. For example, for a 14-day interval our model predicted mean nDNA success rates for coyote scats of 64.6% (winter; range 46.5–80.7%; Fig. 3) and 47.7% (summer; range 24.9–71.2%; Fig. 3), and success rates for free-ranging coyotes sampled with a 14 day interval were 78% (winter) and 55% (summer).

We analysed winter and summer degradation within the same models for PCR success, ADO and FA to increase sample size and statistical power, but winter samples were only available through day 21. Model-predicted results for winter intervals >21 days assume that trends in predicted values continue in the same way beyond 21 days (i.e. that the log odds of the outcome is linear in the log of time), and consequently, these predictions should be interpreted with caution. Missing winter data points do not affect the inferences ≤21 days, and it is during this time that the most substantial changes occurred (Fig. 3).

Monitoring and management implications

This study presents a conceptual model for optimizing NDS for capture–recapture analysis, which can be extended to any species or system where estimates of sample accumulation (e.g. hair snaring rate, scat accumulation rate) and DNA degradation rates can be quantified. We demonstrate that this novel optimization approach can effectively reduce costs of NDS monitoring programmes. By initiating a pilot study to evaluate sample accumulation and DNA degradation rates, NDS monitoring costs can be minimized, allowing monitoring to occur over larger spatial extents and at higher temporal resolutions than would be possible otherwise. Differences observed in sample accumulation and DNA degradation rates between species and across seasons, at the same study site, reiterate the importance of pilot studies for effectively implementing NDS (Taberlet *et al.* 1999; Waits & Paetkau 2005). We recommend that when possible pilot studies incorporating DNA degradation should use fresh scats collected from target populations. Additionally, practitioners optimizing NDS should compare field collected data to model-predicted results to evaluate model performance, particularly, when samples used during pilot studies have an origin other than the population being monitored.

Kit fox populations are believed to be declining, and their contemporary distribution is unclear. High mtDNA success suggests that NDS can be used to explore presence or occupancy of elusive species, such as kit fox, across large spatial areas. When employing NDS for occupancy modelling (or similar approaches), researchers should acknowledge that mtDNA amplifications may incorporate old samples violating closure assumptions and should clear transects before surveying or evaluate sample persistence (MacKenzie & Reardon 2013). Nuclear DNA success rates were sufficient to identify individuals and provide an effective capture–recapture approach to estimate population demographic parameters (Kohn *et al.* 1999; Marucco *et al.* 2011). Both mtDNA and nDNA can be used for monitoring communities or intraguild interactions and provide a cost-effective means to monitor management strategies.

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R.C.L. performed data collection, laboratory procedures, data analysis and interpretation and wrote the manuscript. E.M.G., S.J.D. and B.M.K. provided scats for DNA degradation experiments and assisted with data collection. T.R.J. assisted with statistical analyses and interpretation. L.P.W. designed the study and assisted with data interpretation. All authors assisted with the manuscript preparation.

Data accessibility

Raw data (.csv) and analysis code for scat accumulation (R script) and models of PCR success, ADO and FA (SAS scripts) are available on Dryad, doi:10.5061/dryad.23k27.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Observed per cent PCR success for mitochondrial (mtDNA) and nuclear (nDNA) DNA for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) faecal DNA samples.

Fig. S2 Observed nuclear DNA genotyping error rates (i.e. allelic dropout and false alleles) for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) faecal DNA samples.

Fig. S3 Proportion of samples accumulated for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) in winter and summer that were predicted to fail for individual identification across sampling intervals.

Appendix S1PCR conditions, including primer concentrations and thermal profiles, for mitochondrial and nuclear DNA amplification.

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