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PROJECT 12-612

Monitoring Species of Concern Using Noninvasive Genetic Sampling and Capture-Recapture Methods: Pilot Demonstration With Coyote, a Predator to Species at Risk and Game Species on DoD Lands

LISETTE WAITS

Technical Report

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ACRONYMS

ADO ..... Allelic dropout

BMGR ..... Barry M. Goldwater Range

CI ..... Confidence Interval

CPNWR ..... Cabeza Prieta National Wildlife Refuge

DNA ..... Deoxyribonucleic Acid

DoD ..... .U.S. Department of Defense

DPG ..... Dugway Proving Ground

ESA ..... Endangered Species Act

ESTCP ..... Environmental Security Technology Certification Program

FA ..... False allele

ID ..... Identification

km ..... Kilometer

LAFB ..... Luke Air Force Base

m ..... Meter

mtDNA ..... Mitochondrial DNA

nDNA ..... Nuclear DNA

NGS-CR ..... Noninvasive Genetic Sampling – Capture-Recapture

NGS-OM ..... Noninvasive Genetic Sampling – Occupancy Modeling

PCR ..... Polymerase Chain Reaction

SE ..... Standard Error

SD ..... Standard Deviation

USFWS ..... U.S. Fish and Wildlife Service

Project Synopsis

The Department of Defense (DoD) needs reliable and cost-effective methods for monitoring species of concern while minimizing constraints on military training. This project is a pilot demonstration for utilizing noninvasive genetic sampling and capture-recapture methods (NGS-CR) to provide extensive information for evaluating the status of populations and their responses to management or training actions. To facilitate this technology as an option for monitoring species on DoD installations, we initiated a monitoring program for the coyote (*Canis latrans*) on two installations where the coyote causes high mortality for species of concern and for game species. Our methods were applied on Dugway Proving Ground (DPG) in Utah where coyotes impact kit fox (*Vulpes macrotis*) populations and on the Barry M. Goldwater Range (BMGR) in Arizona where coyotes impact endangered Sonoran pronghorn (*Antilocapra americana sonoriensis*). We completed the fecal accumulation and deoxyribonucleic acid (DNA) degradation pilot study and submitted a manuscript for publication. This work identified an optimal sampling interval of 1 – 2 weeks between mark-recapture sampling sessions. We completed development of species identification (ID), sex ID, and individual ID methods for coyotes using fecal DNA.

We developed our spatio-temporal sampling design and completed multiple seasons of sampling. We obtained 379 coyote samples in season 1 and 626 in season 2 in Utah and 119 in season 1 and 180 in season 2 in Arizona. Genetic analysis and population estimation have been completed for season 1 in both study areas. Species ID success rates ranged from 79% (summer) - 90% (winter) in Utah and 84% (summer) – 100% (fall) in Arizona. Individual ID success rates were 90% in Utah during winter to 82% in Arizona during summer. Population estimates for coyotes were 120 (95% CI: 109-140) in Utah and in Arizona were broken down by region as follows: Cabeza Prieta National Wildlife Refuge: 31 (95% CI: 20-43), BMGR-East: 25 (95% CI: 16-39), and BMGR-West: 35 (95% CI: 14-77). These are the first estimates of population size for coyotes at these two DoD installations. This project will directly benefit DPG and BMGR by providing information needed to manage the coyote and to evaluate effects of military training and management actions on coyote populations. This approach will be useful to other installations that are responsible for monitoring and managing species of concern and species that directly influence species of concern.

Introduction

Lands owned by the Department of Defense (DoD) support the greatest densities of species of conservation concern (i.e., endangered, threatened or otherwise at-risk) of any federal land management agency, and under the provisions of the Endangered Species Act (ESA) and the Sikes Act, DoD is challenged to conserve these species while simultaneously adhering to the military mission. Reliable monitoring is needed to demonstrate that federally listed species have been recovered and can be considered for downlisting, and to evaluate management actions for

species-at-risk to prevent federal listing. To facilitate these efforts, DoD needs accurate and cost-effective methods for estimating population distribution, abundance, survival, reproduction, movements, and genetic diversity of species-at-risk. However, many species of vertebrates are notoriously difficult to monitor because they are wide-ranging, occur in low densities, or are otherwise not amenable to traditional methods of monitoring.

The goal of this project is to demonstrate the effectiveness of a promising new approach, based on combining noninvasive genetic sampling and capture-recapture methods (NGS-CR), that is becoming more prevalent in the scientific community (Taberlet et al. 1999, Waits and Paetkau 2005, Beja-Pereira et al. 2009), but has yet to see widespread use by DoD. Traditional capture-recapture modeling, requiring physical capture of animals, has been one of the most commonly used approaches for estimating population parameters for wild animals. NGS-CR is an attractive and innovative alternative because collection of hair, feces, or other sources of deoxyribonucleic acid (DNA) left behind by free-ranging animals can be used to identify unique individuals within the population without having to catch, handle or even observe them, which reduces stress to the species, requires less training of personnel, and can be more cost-effective than traditional methods that require trapping animals or direct observation (e.g., Prugh et al. 2005, Boulanger et al. 2008, Kendall et al. 2008, Meijer et al. 2008, Marucco et al. 2009, Brøseth et al. 2010, Stenglein et al. 2010b). Another benefit of NGS-CR monitoring is that the genetic data can also be used to estimate other important indicators of population health including genetic diversity, population connectivity, and effective population size (Schwartz et al. 2007).

We have selected coyote on Dugway Proving Ground (DPG), Utah and Barry M. Goldwater Range (BMGR), Arizona to demonstrate monitoring based on NGS-CR (Figure 1). We selected this species and these installations for multiple reasons. First, coyote occur on numerous military installations in the west (e.g., DPG, Nellis Air Force Range, Yuma Proving Grounds and BMGR, White Sands Missile Range, and Fort Bliss). They predate on listed species, species-at-risk and important game species. The San Joaquin kit fox subspecies (*Vulpes macrotis mutica*) found in California is listed as endangered under the ESA, and there is growing concern kit fox (*Vulpes macrotis*) will be petitioned for listing in other regions. In the long term, if a monitoring program can be implemented to evaluate the population size and trend of coyotes, which influence kit fox populations and effects management actions, DoD may be able to assist in proactively preventing the species from being listed under the ESA and, thus, preclude subsequent restrictions imposed by listed species on DoD lands. Thus, there is a current need to monitor coyote and determine the effects of management on species of concern. Several studies have successfully used NGS-CR for monitoring other canids (e.g., arctic fox, Meijer et al. 2008; and wolves, Marucco et al. 2009, Stenglein et al. 2010a).

The second reason we choose coyote and these installations is that we currently have funding from the DoD ESTCP program to develop NGS-CR methods for kit fox on DPG and

Sonoran pronghorn (*Antilocapra americana sonoriensis*) on the BMGR. Working at these same installations produces efficiencies and cost savings on field work and travel plus provides the opportunity to evaluate the usefulness of NGS-CR methods for studying species interactions and co-monitoring approaches. We also chose DPG as one of our focal installations to leverage current DoD funded field-based research on kit fox and coyotes (E. Gese, Utah State University and Robert Knight, DPG, Utah) that we can use to both inform our sampling design and provide alternative estimates of abundance and survival based on telemetry monitoring (Arjo et al. 2007, Kozlowski et al. 2008). We chose BMGR because coyotes are a concern and a source of mortality for the endangered Sonoran pronghorn. We have obtained support and input for our project from Daniel Garcia (Chief Environmental Science Management, Luke Air Force Base, Arizona), John Hervert (Sonoran pronghorn recovery coordinator, Arizona Game and Fish), and James Atkinson (USFWS, Cabeza Prieta National Wildlife Refuge).

Scope of Work

We have broken our demonstration into 4 main components: (1) Conduct a pilot study to evaluate scat accumulation rates, DNA degradation rates, and genotyping error rates; (2) use results from the pilot study to develop and implement a pilot spatio-temporal sampling design for collection of fecal scats; (3) implement the pilot spatio-temporal sampling design, genotype samples, obtain preliminary estimates of population parameters, perform statistical power analysis and revise spatio-temporal sampling design; and (4) implement the final spatio-temporal sampling design, genotype samples, obtain estimates of population parameters and perform cost-benefit analysis. Using funds from year 1, we have completed components 1 and 2 and one sampling period for component 3. Year 2 funds were requested to complete components 3 and 4.

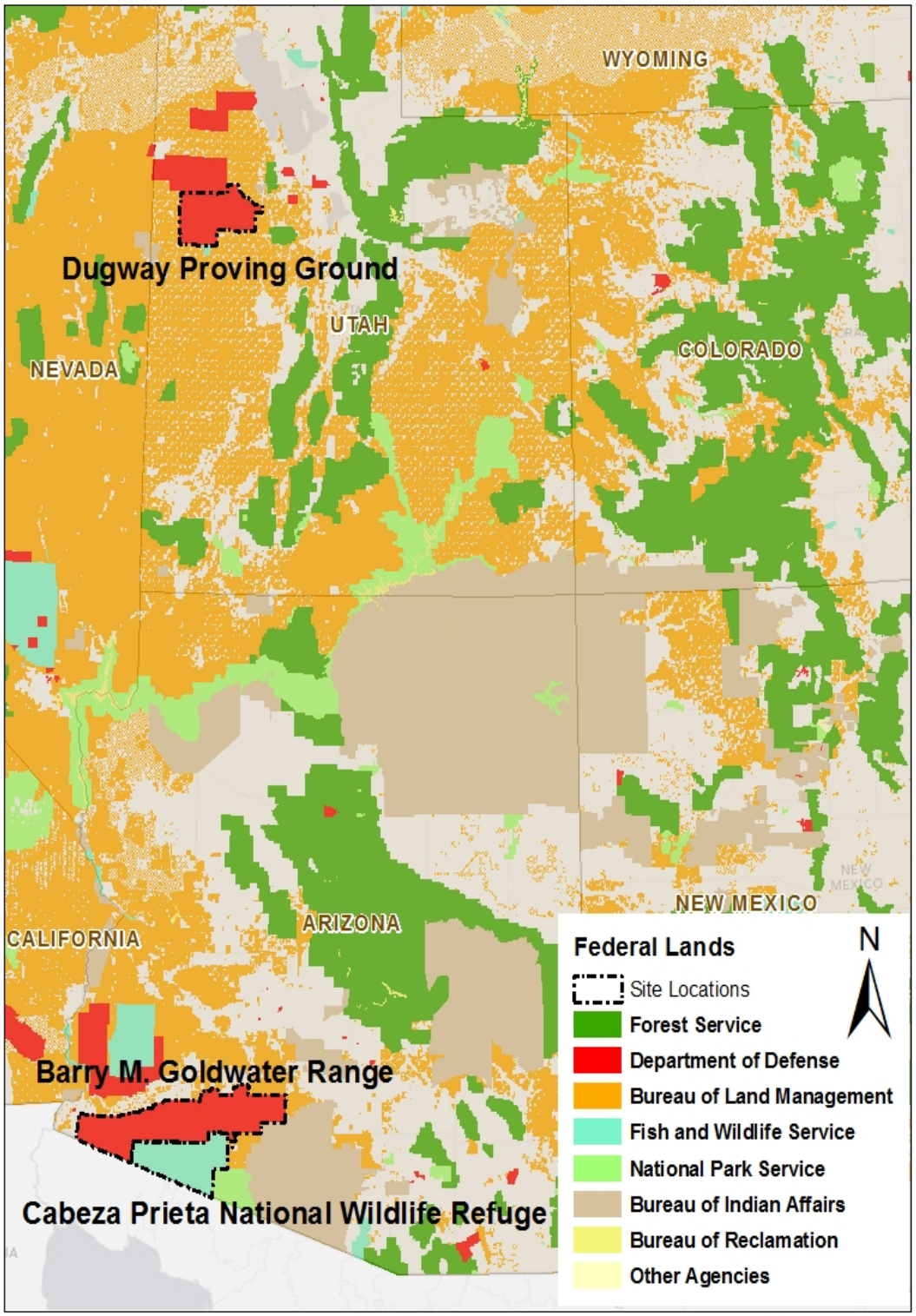

Figure 1. Location of study sites in Utah and Arizona

Study Area Descriptions

Dugway Proving Ground (DPG), Utah

The U.S. Army DGP, located at the southern end of Utah's Great Salt Lake Desert, is approximately 130 km southwest of Salt Lake City, Utah and 65 km south of Interstate 80 (DPG 2007). The western boundary of DPG lies approximately 29 km east of the Nevada border. Extending nearly 84 km east-west by 48 km north-south at its widest points, DPG encompasses 798,214 acres (DPG 2007). In response to the bombing of Pearl Harbor, DPG was founded in 1942 by presidential action as an essential facility for testing both weapons and defenses of chemical and biological weapons of mass destruction and for military training. Initially only 126,720 acres, the size of DPG has increased significantly. Currently, DPG is among the elite U.S. military installations and maintains a mission of providing premier training, testing, and evaluation for our nation's leading military forces. Testing and training related to defenses against chemical and biological weapons, remediation technology, battlefield munitions, smokes and obscurants, and survivability of military equipment in chemical or biological conditions, as well as specialized collective trainings including live-fire scenarios, all occur on DPG (DPG 2007).

Changes to the habitat on DPG and surrounding areas that may influence the distribution and abundance of coyotes and kit foxes include increases in fire frequency and severity (DPG 2007), juniper (*Juniperous osteosperma*) encroachment, and the development of anthropogenic water sources (Arjo et al. 2007). The altered fire regime has resulted in large monocultures of invasive cheatgrass (*Bromus tectorum)* as well as the spread of other invasive species, such as tumbling mustard (*Sisymbrium altissimum*) and Russian thistle (*Salsola iberica*), among others. The introduction of anthropogenic water to the landscape in the 1970s may have facilitated the increase in coyote settlement on DPG, increasing both competition and predation risk for kit foxes.

Barry M. Goldwater Range (BMGR), Arizona

The BMGR in southwestern Arizona is a 1.7 million acre training range used by U.S. and allied pilots for air-to-ground and air-to-air training missions. Established in September 1941, the range totaled 1.1 million acres divided into the western section, originally called the Yuma Aerial and Gunnery and Bombing Range, and the eastern section, known as the Gila Bend Gunnery Range and later the Ajo-Gila Bend Gunnery. During World War II, the range was expanded to 2.1 million acres and was the largest single engine advanced flying training facility in the US with more than 17,000 pilots training there during the war years (LAFB 2012a). Post-World War II, additional range expansions were necessary to accommodate jet fighters. With the closure of Luke Field from November 1946-February 1951, the range was renamed the Williams

Bombing and Gunnery Range. Escalating conflict in Korea increased demand for fighter pilots and Luke Air Force Base (LAFB) was established in February 1951. LAFB took over management of the eastern and western sections and the range was again renamed, now the Luke Air Force Range, in 1963. In 1986, Congress renamed the range again in honor of Arizona Senator Barry M. Goldwater.

Numerous expansions and reductions over the years have brought the range to its current size. Today, the eastern portion of the range is under the management of LAFB Range Management Office, while the western portion is managed by Marine Corps Air Station Yuma. Active duty, Guard, and Reserve Pilots from the Army, Navy, Marine Corps, and Air Force use the range. BMGR is the third largest tactical aviation range in the US with nine air-to-ground and two air-to-air ranges, which allow over 50 aircraft to carry out simultaneous training missions (LAFB 2012b and 2012c). While live bombs are used on a portion of the range, the majority of the range sees low flying aircraft with no munitions deployment. These large areas with little human presence are inhabited by a variety of endangered and protected species including flat-tailed horned lizard (*Phrynosoma mcallii*), lesser long-nosed bat (*Leptonycteris yerbabuenae*), cactus ferruginous pygmy owl (*Glaucidium brasilianum cactorum*), and Sonoran pronghorn (Bagne and Finch 2012). Extensive monitoring programs are in place to ensure minimal disturbance of these endangered species.

Cabeza Prieta National Wildlife Refuge (CPNWR), Arizona

Adjacent to BMGR is the Cabeza Prieta National Wildlife Refuge (CPNWR), the third largest wildlife refuge in the lower 48. The refuge is part of the largest interconnected, protected area in the lower 48 and Mexico, which includes BMGR, CPNWR, Organ Pipe Cactus National Monument, and the Pinacate Biosphere Reserve in Mexico, and is home to more than 400 plant species and over 300 wildlife species. Construction of a border fence along the international border in an attempt to reduce illegal traffic from Mexico has severed connections between the U.S. and Mexico populations of pronghorn. The border fence is expected to block gene flow between the two populations. The refuge is open and accessible most of the year upon obtaining a free permit from the CPNWR office in Ajo, Arizona; however, parts of the refuge are off-limits due to military flight training missions. Additionally, there is an annual seasonal closure from 15 March – 15 July to protect fawning pronghorn.

Methods

Pilot study on scat accumulation and DNA degradation at DPG

Scat accumulation and fecal DNA degradation (i.e., PCR success and genotyping errors) were evaluated across two seasons, which corresponded to the periods preceding reproduction

(January – March) and dispersal (July – August) for kit foxes and coyotes in DPG, Utah. While Legacy funding provided support of coyote sampling, funding from the ESTCP program supported kit fox sampling. Coyote and kit fox samples were analyzed together to increase sample size, which increases the statistical power (i.e., the probability of correctly rejecting the null hypothesis when the null hypothesis is false).

Scat accumulation surveys

Scat accumulation rates were assessed by combining data collected by our collaborators (E. Gese, B. Kluever, and S. Dempsey) at Utah State University from 2010 – 2012 and data collected during short pilot surveys in the summer of 2012. The detection of canid scats can be maximized by surveying along roadways and trails, which carnivores use for regular movements (Kohn et al. 1999). To evaluate scat accumulation rates, we cleared transects that followed small dirt or gravel roads of all carnivore scats. We subsequently surveyed these transects and assigned each carnivore scat that was detected to species based on overall appearance, size and shape. Our collaborators collected accumulation data along 15 transects (each 5 km in length; Figure 2) that were previously being used to evaluate the relative abundance of each species. These transects were cleared and subsequently surveyed ~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).

During the summer of 2012, we conducted additional scat accumulation surveys along shorter transects with starting point, direction, and length (1 - 3.5 km) being randomly selected. Additionally, to expand the spatial coverage and ensure that similar accumulation patterns were observed with shorter sampling intervals, 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 seven days after clearing. Scat accumulation surveys conducted by collaborators were not originally intended to align with our temporal sampling design (i.e., winter and summer) and included surveys in the spring. To account for seasonal differences, surveys were divided into summer, winter, or spring based on local seasonal breaks; no surveys were conducted in the fall during the dispersal period for these species. Although we did not investigate fecal DNA degradation in the spring, we elected to retain the spring accumulation data.

Scat accumulation analysis

Scat accumulation results were standardized across transects and species by evaluating accumulation by the number of scats per kilometer per day. We employed a generalized linear model to test the effects of season and species on scat accumulation (O'Hara and Kotze 2010). We considered a Poisson regression model with a log link function, but the residuals indicated

under-dispersion 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 both main effects and factor levels with contrast analysis (R package contrast; Kuhn et al. 2011; R Core Team 2014).

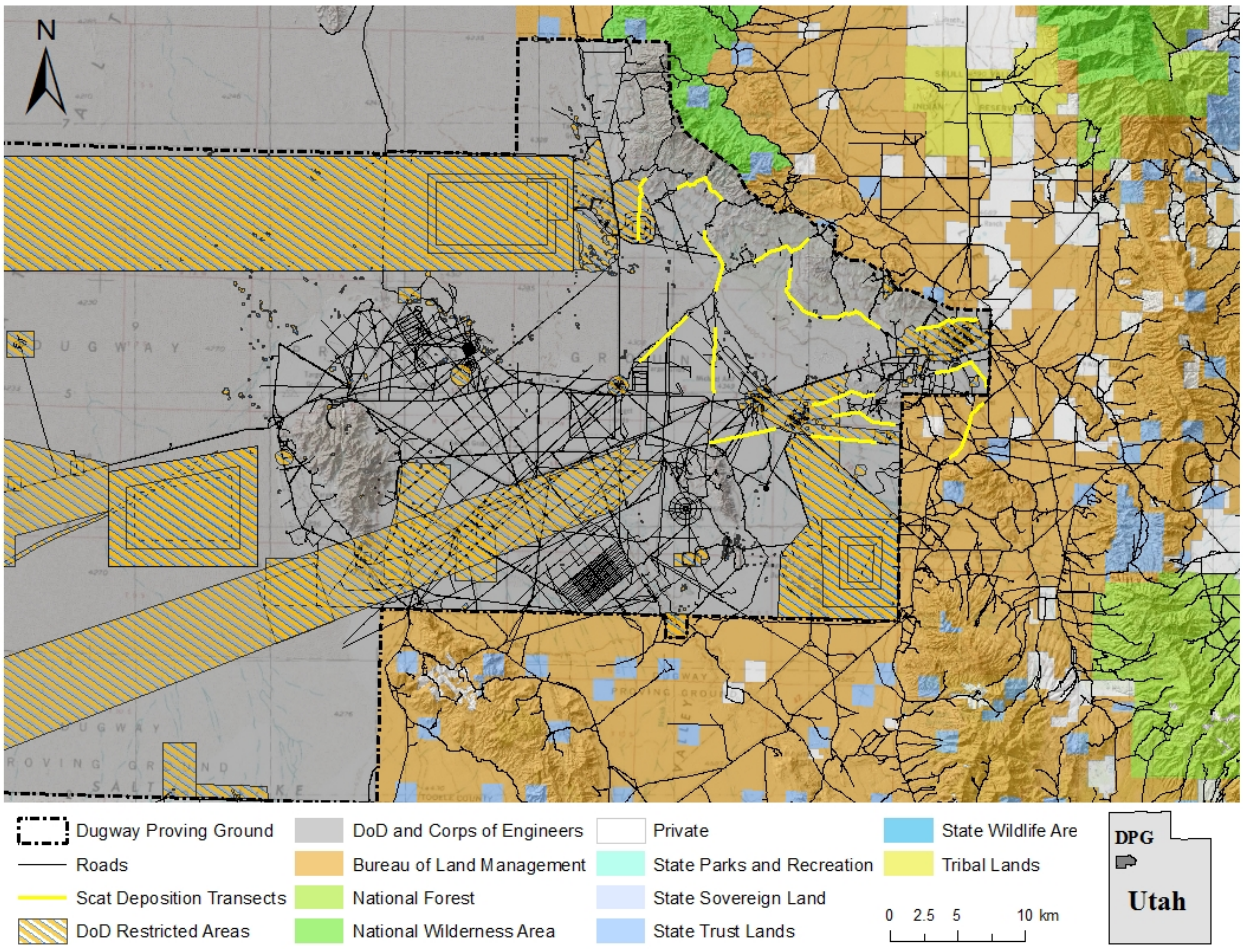

Figure 2. Location of 15 scat accumulation surveys utilized by collaborators for estimation of relative abundance of kit foxes and coyotes, Utah.

Fecal DNA degradation sampling

Fecal 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 winter, 20 fresh scats were collected for each species. Smaller scats were utilized more quickly, resulting in reduced sample sizes at later time points. In the summer, we added a day 5 time point to provide greater resolution, as a recent study of coyote fecal degradation found a significant decline in DNA quality as early as five days post-deposition (Panasci et al.

2011). Consequently, in order to maintain more equitable sample sizes among time points during the summer, we obtained three additional scats for each species and sampled these scats in place of fully utilized scats at later time points. Kit fox scats were provided by our collaborators (Eric Gese and Bryan Kluever; Utah State University) who had collected fresh scats from live-captured, free-ranging individuals. Coyote scats were obtained from the USDA/NWRC/Predator Research Facility (Millville, UT, USA). All scats were frozen within four hours of collection, transferred to the field site, and thawed to ambient temperature. We then placed scats in the field and protected them from disturbance with a frame covered with wire mesh (25mm openings with 0.7 gauge wire). We collected fecal DNA samples from each scat at 1, 3, 5 (summer only), 7, 14, 21, 56 (summer only), and 112 (summer only) days, or until the scat was fully utilized (day 1 samples were collected just prior to exposure to field conditions). Although we intended to continuing winter sampling to day 56 and 112 time points, a severe wind event buried the experimental plots after day 21, so these time points were only available for the summer. Fecal 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. Samples were stored in 1.4 ml of DETS buffer (20% DMSO, 0.25 M EDTA, 100 µM Tris, pH 7.5, and NaCl to saturation; Seutin et al. 1991).

Fecal DNA extraction and PCR amplification

We conducted fecal DNA extraction and PCR amplification at the University of Idaho in a facility dedicated to low quality DNA. Fecal DNA samples were randomized and extracted using the QIAamp DNA Stool Mini Kits (Qiagen, Inc., Valencia, CA, USA) with negative controls to monitor for contamination (Taberlet and Luikart 1999; Beja-Pereira et al. 2009). We performed mtDNA species identification (ID) tests by amplifying fragments of the control region (Onorato et al. 2006). Qiagen Master Mix (1x concentration), Q solution (0.5x concentration) and 1 µl of DNA extract were combined with species ID primers into a 7µl (total volume) multiplex with the following PCR conditions for each primer: 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 minutes, 35 cycles of 94°C for 30 seconds (denaturation), 46°C for 90 seconds and 72°C for 60 seconds (elongation), and a final elongation stage of 60°C for 30 minutes. Species-specific PCR products lengths were 335–337 base-pairs (bp) for kit foxes and 115–120 bp and 359–363 bp for coyotes. Samples that failed to amplify for mtDNA were repeated once to minimize sporadic effects (Murphy et al. 2007).

For individual ID, we amplified kit fox samples with a multiplex of seven nuclear DNA (nDNA) microsatellite loci (Ostrander et al. 1993; Fredholm and Wintero 1995; Francicso et al. 1996). The PCR conditions for the 7 µl (total volume) multiplex for each primer pair were 0.14 µM CPH3, 0.27 µM CXX403, 0.14 µM CXX250, 0.08 µM FH2054, 0.17 µM CXX20, 0.07 µM CXX173 and 0.06 µM CXX377, combined with 1x concentrated Qiagen Master Mix, 0.5x

concentrated Q solution and 1 µl of DNA extract. The PCR thermal profile had an initial denaturation of 95°C for 15 minutes, 20 touchdown cycles at 94°C for 30 seconds (denaturation), 55°C for 90 seconds (annealing; decreasing by 0.3°C per cycle) and 72°C for 60 seconds (elongation), 20 cycles at 94°C for 30 seconds (denaturation), 51°C for 90 seconds (annealing) and 72°C for 60 seconds (elongation), and a final elongation at 60°C for 30 minutes.

For coyote individual ID, we employed a multiplex with nine nDNA microsatellite loci (Ostrander et al. 1993; Holmes et al. 1994; Fransicso et al. 1996; Neff et al. 1999; Breen et al. 2001; Guyon et al. 2003) and two sex determination primers (Seddon 2005). The PCR conditions for the 7µl (total volume) multiplex for each primer pair were 0.23 µM CXX119, 0.04 µM CXX173, 0.09 µM FH2001, 0.06 µM FH2054, 0.06 µM FH2088, 0.06 µM FH2137, 0.10 µM FH2611, 0.14 µM FH2670, 0.09 µM FH3725, 0.10 µM DBX6 and 0.07 µM DBY7, combined with 1x concentrated Qiagen Master Mix, 0.5x concentrated Q solution and 2 µl of DNA extract. The PCR thermal profile has an initial denaturation of 94°C for 15 minutes, 13 touchdown cycles at 94°C for 30 seconds (denaturation), 62°C for 90 seconds (annealing; decreasing by 0.4°C per cycle) and 72°C for 60 seconds (elongation), 33 cycles at 94°C for 30 seconds (denaturation), 57°C for 90 seconds (annealing) and 72°C for 60 seconds (elongation), and a final elongation at 60°C for 30 minutes.

We conducted all PCR procedures on a BioRad Tetrad thermocycler (Bio-Rad, Hercules, CA, USA) with negative and positive controls included with each reaction. We visualized results using a 3130xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and scored allele sizes with Genemapper 3.7 (Applied Biosystems, Foster City, CA, USA). We calculated mtDNA species ID success rates as the proportion of samples identified to species across each time point and season. We calculated nDNA amplification success rates (number of successful amplifications / total number possible) and sample success rates (proportion of samples successful at ≥50% of the loci) for each time point and species.

Fecal DNA genotyping error rates

Replications necessary to establish consensus genotypes were achieved through multiple time points per scat (Taberlet et al. 1999; Pompanon et al. 2005), providing the basis for estimating genotyping error rates (Broquet and Petit 2004). We required that heterozygote and homozygote alleles be observed in two and three independent replicates, respectively, to achieve a consensus genotype. Following the methods of Broquet and 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).

Fecal DNA degradation data analysis

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 of success 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 (kit 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 mid-length of each locus by species (range: 90–275 bp). By combining coyote (Legacy funded research) and kit fox (ESTCP funded research) samples, we were able to increase our sample size and statistical power.

Optimization of NGS temporal design

Based on the scat accumulation and fecal DNA degradation data, we calculated a total cost per successful sample (i.e., a sample that achieves a consensus genotype for individual ID) at sampling intervals ranging from 1 to 56 days. The sampling interval represented the length of time between an initial clearing and a survey, or between sequential surveys when >1 survey is desired or required. We selected a survey effort of 150 km, a length of transect which we felt provided reasonable coverage of our study site. We identified a desired sample size 400 coyote fecal DNA samples, as this was approximately 3x the number of individuals we expected to be in our study area (Solberg et al. 2006). We determined the number of samples deposited and available for collection at each potential sampling interval (i.e., 1 to 56 days), by calculating the product of the daily accumulation rate, the number of kilometers surveyed (i.e., effort) and the number of days in the interval. We then combined the number of samples deposited within each interval with our model-predicted PCR success rates to calculate the number of successful samples for each interval.

Noninvasive samples commonly suffer from genotyping errors and a multiple-tube approach is typically used to establish consensus genotypes (Pompanon et al. 2005). To incorporate the influence of genotyping error on costs, we determined the overall genotyping error rate for each interval. At each potential interval, we summed the model-predicted FA and ADO rates to determine the 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 (Taberlet et al. 1999; Pompanon et al. 2005). Because errors in multilocus genotypes can result in overestimates of abundance and bias survival estimates (Lukacs and Burnham 2005), we set a goal of maintaining a probability of error ≤2% in our dataset. Assuming that genotyping error rates were similar across loci and that 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 extraction, 4 independent replicates [amplifications], finalization of the consensus genotype, and labor), with a 25% increase in cost for each additional pair of replicates. Thus, when the number of replicates required to maintain our goal of ≤2% error exceeded 4, we increased the number of replicates incrementally by 2 until the goal was achieved or we reached 8 replicates. We estimated our hourly field costs to be $10/hour/technician (including labor and fuel), and we could effectively survey 150 km of transects in 160 hours (e.g., 2 field technicians working 40 hours/week for 2 weeks). Based on each potential interval, we divided the desired samples size by the total number of successful samples to determine the required number of sampling events.

We standardized cost as the cost per successful sample at each interval, which combined field and laboratory costs. We identified the optimal interval as the interval that minimized the overall cost per successful sample while achieving desired sample sizes. The same process was used to evaluate the optimal spatio-temporal sampling design for kit foxes and was subsequently reported to ESTCP. If a common temporal sampling frame is selected for both species, sampling can be done concurrently, reducing costs.

Capture-mark recapture sampling design

Field sampling was conducted within a Pollock's robust design framework (Figure 3), which consists of two types of sampling periods (Pollock 1982, Pollock et al. 1990, Kendall et al. 1997), The first, called primary periods, are temporally separated by relatively long time intervals (e.g., several months or years) during which it is assumed that individuals in the population may reproduce, die, or move in or out of the study area. Within each primary period, there are secondary periods temporally separated by relatively short time intervals (e.g., days or weeks) during which the population is assumed to be demographically and geographically closed (i.e., very few or no individuals reproduce, die or move among subpopulations). The robust design allows for estimation of population size at each primary period, survival and temporary emigration between primary periods, and capture probability during secondary periods (Figure

3). Extensions of Pollock's robust design incorporate genotyping errors associated with NGS-CR (Lukacs et al. 2009) and partitioning of recruitment into gains from reproduction versus immigration (Sandercock 2006). Data collection under Pollock's robust design allows the most flexibility to estimate population parameters but also allows for estimation under alternative capture-recapture models such as Cormack-Jolly-Seber and several single-session estimators of abundance (Lukacs and Burnham 2005, Miller et al. 2005, Puechmaille and Petit 2007).

Employing Pollock's robust sampling design, we sampled during two primary periods in DPG in Utah that coincide with the periods preceding reproduction (January-March) and dispersal (July-August) for coyotes and kit fox. In BMGR, Arizona we sampled during the summer drought season (May-June) which is the pronghorn calving season and predispersal period of coyotes and the fall drought season (October-November) which is during dispersal for coyotes.

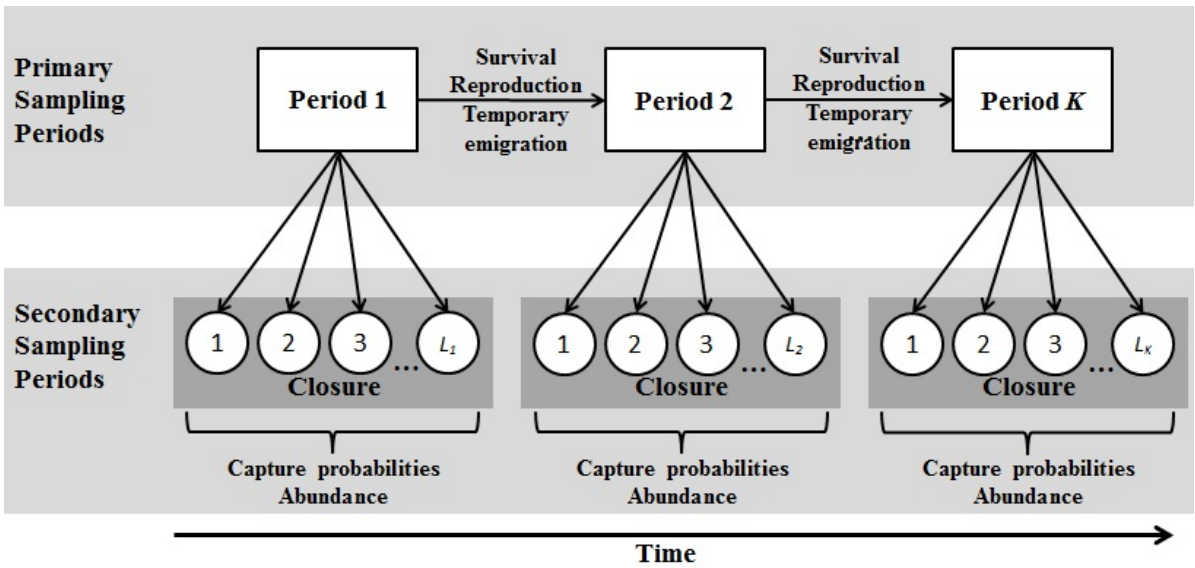

Figure 3. Pollock's robust sampling design for capture-recapture analyses.

Implementation of sampling design, Utah

Based on the optimization of NGS (*see Results*), a temporal sampling frame between secondary mark-recapture sessions of ~14 days was selected and employed in year 2 sampling. This sampling interval was cost-effective in both seasons and allowed coyotes and kit foxes to be sampled concurrently on the same transects, further reducing overall cost per successful sample. For session 1, fecal samples were collected along 150 km of transects from 5 January to 26 March 26 2013. Specifically, 30 transects (hereafter NGS-CR transects; each 5 km in length) were surveyed including the 15 transects used for scat accumulation surveys (i.e., historic transects; Figure 4) and 15 additional random transects (i.e., random transects; Figure 4). A

sampling interval of 14 days was employed and 4 sampling sessions were conducted on each transect. Furthermore, as part of an occupancy modeling portion of the research funded by ESTCP and National Geographic, surveys were conducted at 60 sites, with each site being a randomly selected 6.25 km^2 cell containing four 500 m transects (hereafter NGS-OM transects) that were each surveyed only once during a single season.

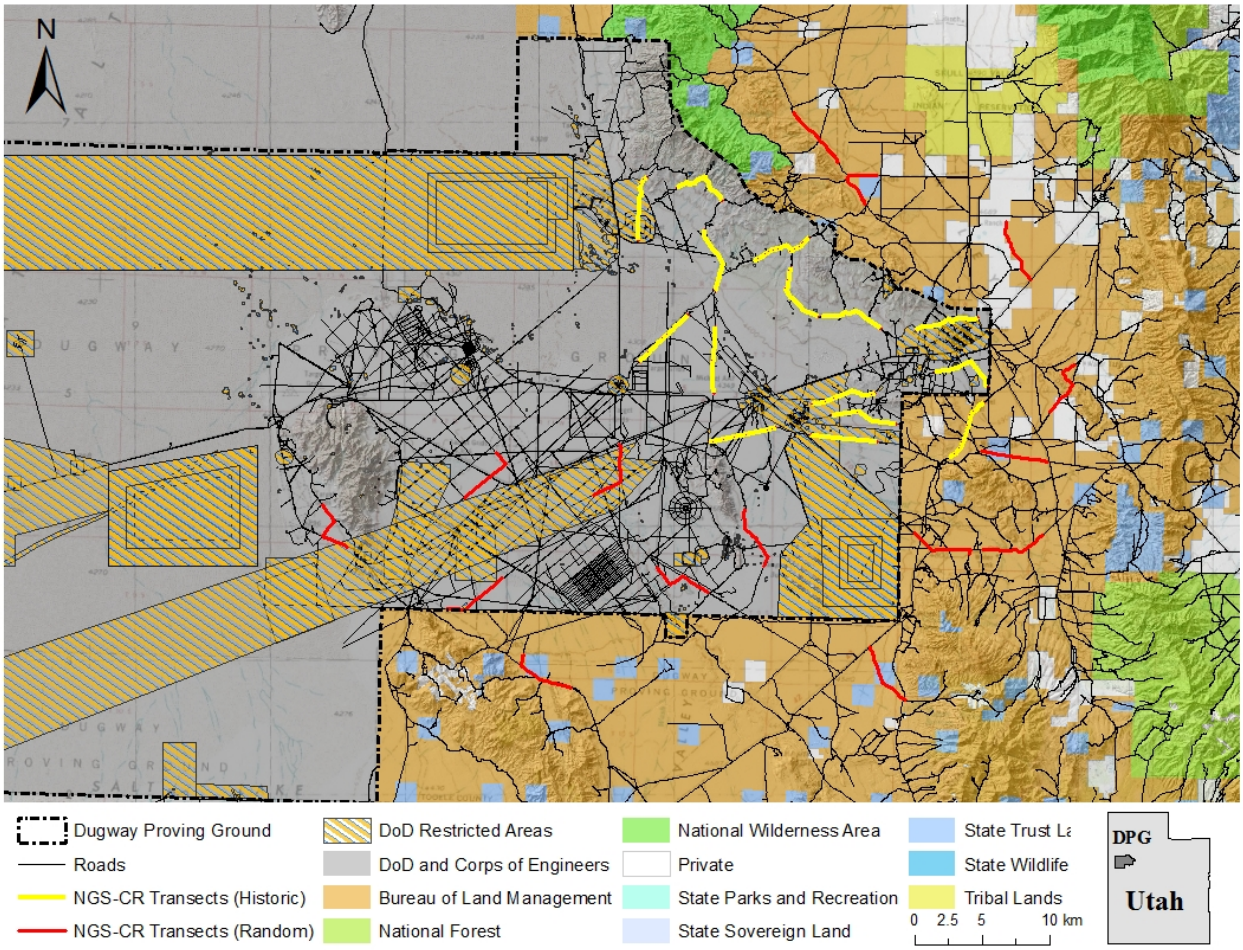

Figure 4. Location of 30 NGS-CR transects, including 15 historic transects and 15 random transects, for NGS-CR of kit fox and coyotes at Dugway Proving Ground, Utah.

All transects were surveyed by two researchers, each beginning in the middle of the transect and walking to opposite ends and back, surveying half of the transect width on each pass. All detected carnivore scats were sampled and preserved in the same manner as described for the fecal DNA degradation methods to minimize risks of contamination and further degradation. At the time of collection, scats were classified to the suspected species based on physical size and shape and assigned a relative age (very fresh – very old) based on appearance (e.g., dark vs. bleached by the sun) and odor. Using calipers, measurements of diameter at widest

point, total length, and number of disjoint segments was collected. The general habitat type, position along transect (i.e., shoulder, median, or tracks), and Global Positioning System (GPS) location were recorded for each scat. The remaining scat was collected into a labeled paper bag for diet analysis.

Session 2 (summer 2013) sampling was conducted from 8 July – 28 August 2013. As with session 1, we surveyed each of the 30 NGS-CR transects (5 km each) with temporal replication (i.e., surveyed multiple times per season), while each of the 240 NGS-OM transects (500 m each) were surveyed once in each season. In summer 2013, we cleared all of the NGS-CR transects and subsequently surveyed each transect three times.

Implementation of sampling design, Arizona

Transect sampling was conducted on the BMGR and CPNWR (Figure 5). Sampling location and interval were limited by requirements to minimize pronghorn disturbance and travel to drinkers with agency personnel, and thus, time between collection of scats varied depending on the location. Pronghorn drinkers are areas where agency personnel provide water and, in some locations, food for pronghorn. During dry periods, pronghorn and other species, such as coyotes, rely on these areas for water. Scat samples were collected at pronghorn drinkers and along trails on the approach to pronghorn water/feed sites which were accessed on foot. Drinkers were searched from one to four times each at intervals of five to seven days; intervals of scat collected along roads varied from one to seven days. Three sites were surveyed four times, three were surveyed three times, 1 site twice, and 1 site once. Approximately 75 km of roads and trails were sampled weekly including roads to and from drinkers and the road encircling the pronghorn captive pen on the CPNWR. Summer field work was conducted from 25 May 25-19 June, and we focused on the Child's Valley area of CPNWR and the BMGR.

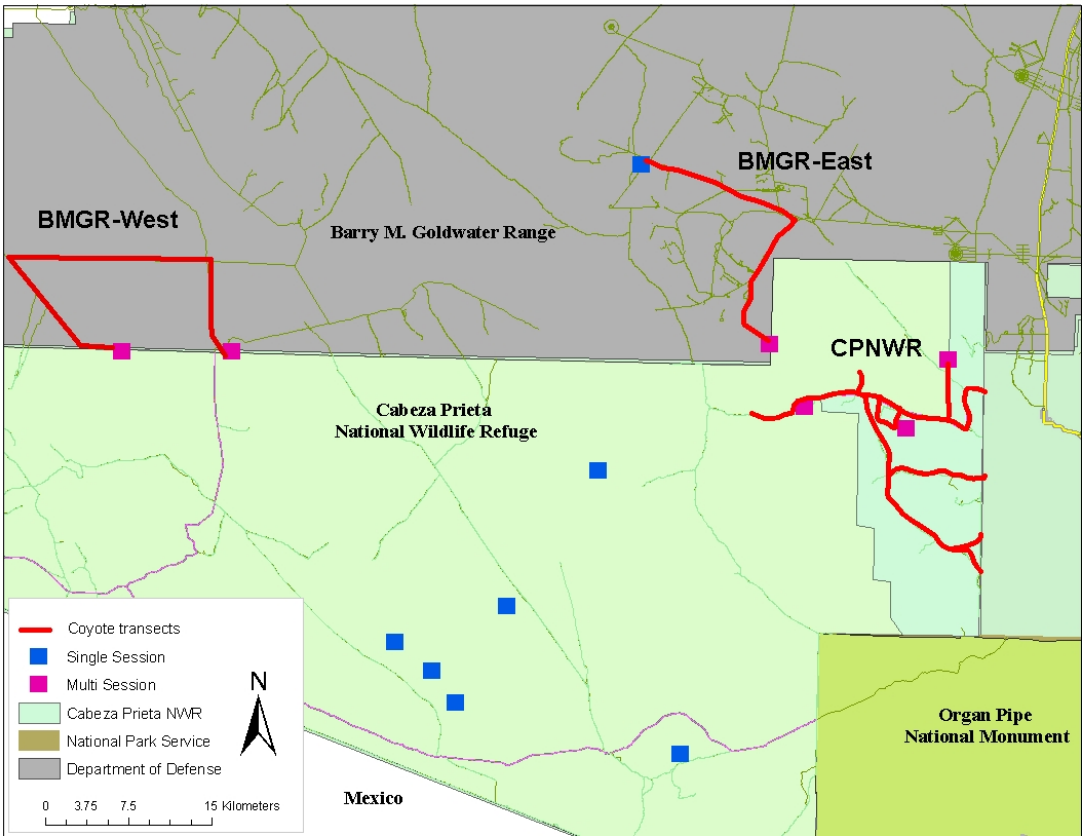

Figure 5. Location of pronghorn drinkers and roads searched for coyote scat at Barry M. Goldwater Range and Cabeza Prieta National Wildlife Refuge, Arizona.

All detected carnivore scats were sampled and preserved in the same manner as described for the fecal DNA degradation methods to minimize risks of contamination and further degradation. When a scat was found, a sample was collected from the side of the scat using sterile tweezers and placed into a 2 ml storage tube with DET buffer. The remaining scat was collected in a plastic Ziploc bag and frozen for later use in diet analysis.

NGS-CR methods

Species and individual identification

Fecal DNA extraction, amplification and scoring for coyote samples collected during year 2 were conducted following the same procedures as outlined in the fecal DNA degradation study. At our study site, coyotes are sympatric with kit foxes and red foxes (*Vulpes vulpes*) and all three species can produce scats of overlapping size (Foran et al. 1997, Green and Flinders 1981). We confirmed species ID of year 2 fecal samples collected from carnivore scats with mtDNA species ID tests (PCR conditions outlined under task 2). Samples that failed once were repeated to minimize the sporadic effects. Samples that failed twice were omitted from additional analyses.

For coyote individual ID, we employed a multiplex with nine nDNA microsatellite loci (Ostrander et al. 1993; Holmes et al. 1994; Francisco et al. 1996; Neff et al. 1999; Breen et al. 2001; Guyon et al. 2003) and two sex determination primers (Seddon 2005) as outlined above (PCR conditions outlined under task 2). To obtain a consensus genotype at each locus, we required an identical result across two replicate PCRs for heterozygotes and additional replicates were conducted when the first two PCRs did not agree. An allele was not recorded in a consensus genotype unless it was observed twice. For homozygotes, we required 3 matching genotypes. This process of testing and evaluating was repeated until a consensus genotype was obtained at ≥6 loci, the number required to meet the minimum criteria of PID sibs <0.01 (Waits et al. 2001). Across relative ages (of scats) as determined in the field, we evaluated PCR success rates as the proportion of samples with a successful amplification at ≥50% of the loci. Similarly, we evaluated genotyping error rates across relative ages following the methods of Broquet and Petit (2004). We conducted individual ID with the program GenAlEx v6.5 (Peakall and Smouse 2006), which was used to match genotypes and determine the number of unique individuals. To ensure that single-capture individuals were not the result of genotyping errors, we used RELIOTYPE (Miller et al. 2002) to determine the reliability of consensus genotypes and only retained genotypes for which we could obtain a reliability ≥95%.

Development of capture histories, Utah

For the first winter season (session 1 of year 2), we constructed two different capture histories. The first, represented the capture distribution as the number of individuals captured within each class (e.g., 51 individuals captured 1x, 37 individuals captured 2x, 13 individuals captured, and so on) and represented the input format required for population estimation with CAPWIRE (Miller et al. 2005), a single-sample population estimation approach. The second coded each individual as 1 (detected) or 0 (not detected) for each temporal sampling event and represented the input format required for population estimation with the Pollock's Robust design in program MARK (White and Burnham 1999). Thus, the latter capture histories were generated only for individuals detected on one of the 30 (5 km) transects surveyed with temporal replication.

Development of capture histories, Arizona

All mark-recapture analysis was done by region as we have no evidence of coyotes moving between sampling regions. We used three regions (CPNWR, BMGR-East, and BMGR-West) to estimate population size. CPNWR results include samples from seven drinkers and those along roads and trails to drinkers. For the first season (Summer session 1 in 2013), we constructed two different capture histories. The first, represented the capture distribution as the

number of individuals captured within each class (e.g., 29 individuals captured 1x, 10 individuals captured 2x, 5 individuals captured 3x, and so on) and represented the input format required for population estimation with CAPWIRE (Miller et al. 2005), a single-sample population estimation approach. The second coded each individual as 1 (detected) or 0 (not detected) for each temporal sampling event and represented the input format required for population estimation with the Pollock's Robust design in program MARK (White and Burnham 1999). The latter capture histories were generated only for individuals detected on the CPNWR as the sample size was insufficient in the other 2 regions.

Population estimation

We used CAPWIRE (Miller et al. 2005) in the R programming language (R Core Team 2014) and the program MARK (White and Burnham 1999) to generate estimates of population size from each of the capture histories. All unique genotypes were included in the initial CAPWIRE analysis. Model selection was based on a likelihood ratio test implemented in CAPWIRE and confidence intervals were estimated with 1000 bootstraps. Only unique genotypes that were observed on CPNWR were included in the program MARK analysis. We employed closed-capture models with a full likelihood for both the capture and recapture probabilities. We fit models with and without sex groupings. We fit models that incorporated variation in capture probability over time and compared these to null models with no time variation. Additionally, capture and recapture probabilities were constrained to be equal, as noninvasive genetic sampling does not introduce capture biases such as observed with trap-happy or trap-shy individuals. Additional demographic parameters (e.g., survival and reproduction) will be estimated within a multi-session framework when data from additional seasons are available.

Additional field sampling, Utah

We conducted summer 2013 (session 2) and winter 2014 (session 3) sampling from 8 July – 28 August 2013 and from 13 January – 20 March 2014, respectively. As with session 1, we surveyed each of the 30 NGS-CR transects (5 km each) with temporal replication, while each of the 240 NGS-OM transects (500 m each) were surveyed once in each season. In summer 2013, we cleared all of the NGS-CR transects and subsequently surveyed each transect three times. Similar to winter 2013, in winter 2014 fresh snow fall just prior to sampling eliminated the need to clear transects; thus, NGS-CR transects were not cleared and were each surveyed four times. Maintaining the optimal sampling interval across seasons, all surveys on NGS-CR transects were separated by ~14 days.

Additional field sampling, Arizona

Fall 2013 sample collection (session 2) occurred from 19 October- 3 November focusing on the same transects and regions as in summer 2013. We drove a total of 363.15 km in search of coyote scats resulting in collection of 192 samples; 3 sites were surveyed 3 times, 3 sites twice, and 4 sites once.

Results

Pilot study on scat accumulation and DNA degradation

Scat accumulation surveys

Scat accumulation surveys were conducted along 170.5 km, 150 km, and 75 km in summer, spring and winter, respectively. Rates of scat accumulation were higher for coyotes (mean = 0.076 scats/km/day ± 0.009 SE) than kit foxes (mean = 0.029 scats/km/day ± 0.007 SE) across seasons (Figure 6). The likelihood ratio test was not significant (P = 0.673) and therefore we report the 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 marginally (contrast, z = 1.89, P = 0.059; Table 1).

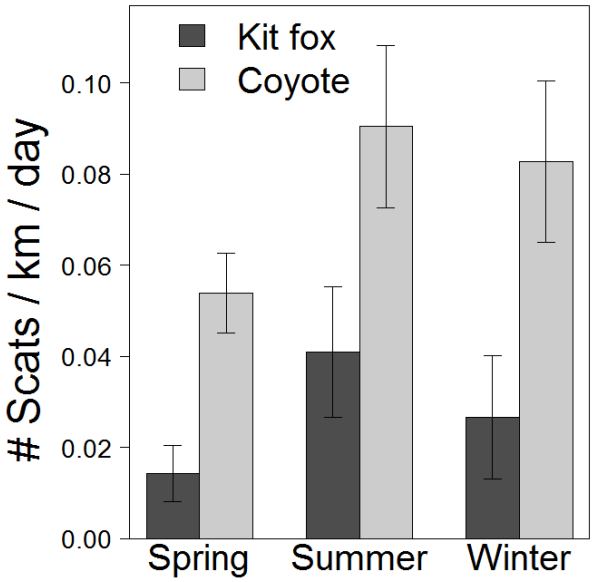

Figure 6. Mean scat accumulation rates ± SE for kit fox (*Vulpes macrotis*; dark gray) and coyote (*Canis latrans*; light gray) at Dugway Proving Ground, Utah, collected from September 2010 to July 2012.

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. Significant (*) p-values evaluated at α = 0.05.

	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	0.30	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

Fecal DNA PCR success rates

Across time points, overall kit fox mtDNA success was 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. In winter, 100% of the five kit fox and 73% of the 11 coyote samples that failed on the first amplification attempt successfully amplified on the second. In summer, however, only 31% of 13 kit fox and 33% of 21 coyote samples that failed on the first amplification attempt successfully amplified on a second attempt. Both species exhibited high amplification success rates over time with mtDNA success rates ≥95% through 21 days in both seasons (Figure 7).

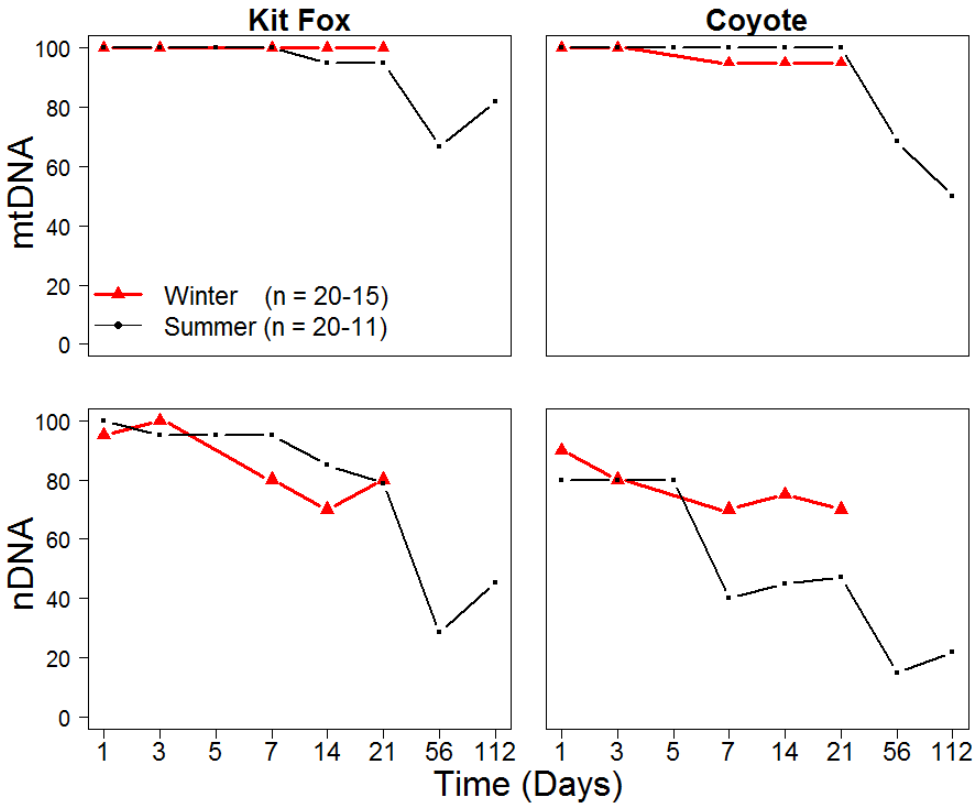

Figure 7. Observed percent PCR success for mitochondrial (mtDNA) and nuclear (nDNA) DNA for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) fecal DNA samples collected in 2012 during winter and summer at Dugway Proving Ground, Utah. Percent PCR success for mtDNA is presented as the proportion of samples identified to species across each time point and season. Percent PCR success for nDNA is presented as the proportion of samples with successful amplification at ≥50% of the loci for each time point and species.

Across time points, kit fox nDNA amplification success rates (number of successful amplifications / total number 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. Kit 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; Figure 4). Coyote nDNA sample success rates ranged from 80% to 90% through day 5. Coyote nDNA success remained ≥70% through day 21 in winter, but declined in summer to <50% by day 7 and <25% by day 56 (Figure 7).

Fecal DNA genotyping error rates

Overall genotyping error rates varied between species (Figure 5); ADO was lower for kit fox (18%) than coyote (25%) samples, while FA rate was slightly higher for kit foxes (5%) than

for coyotes (2%). Winter samples of both species had lower genotyping error rates on average than summer samples. Kit fox winter ADO rates ranged from 4% to 36%, whereas kit fox summer ADO rates ranged from 15% to 42% (Figure 5). Coyote ADO rates ranged from 10% to 29% in winter and 15% to 56% in summer (Figure 5). In both seasons, FA rates were low for both species (Figure 8). Cumulative genotyping error rates (i.e., combined ADO and FA rates) across intervals were lower for kit foxes (winter mean = 20.9% ± 0.6% SE; summer mean = 25.1% ± 0.6% SE) than for coyotes (winter mean = 31.5% ± 0.6% SE; summer mean = 37.4% ± 0.5% SE) and lower in the summer than the winter for both species.

Fecal DNA degradation and genotyping error analyses

Models indicated that all of the main effects significantly influenced PCR success rates (Table 2). Mitochondrial DNA had higher success rates than nDNA and both DNA types had decreasing success over time (Figure 9). Locus length significantly influenced nDNA PCR success, with longer loci having lower success rates (Figure 9). PCR success was significantly influenced by season, with higher success in winter than summer. A significant effect of species was also detected (Figure 9). We detected significant interactions of time with season and locus length. PCR success for both DNA types declined slower in winter than summer and nDNA success rates declined more precipitously for longer loci than shorter loci (Figure 6). Significant interactions were detected for species with both time and locus length (Table 2).

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 further 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).

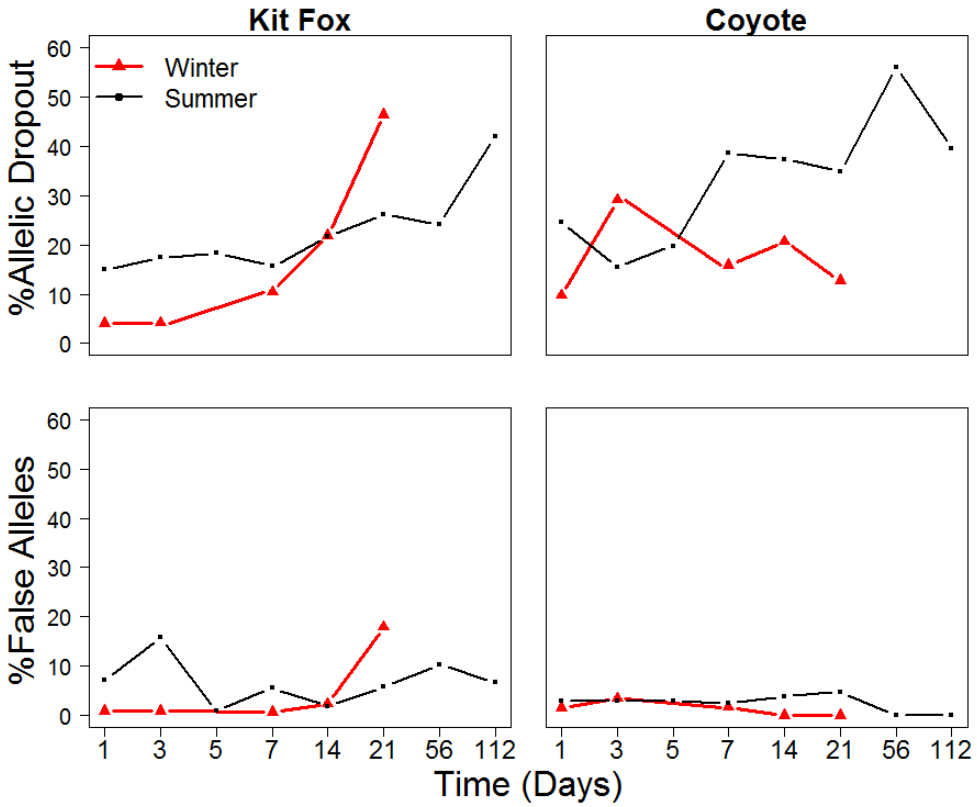

Figure 8. Observed nuclear DNA genotyping error rates (i.e., allelic dropout and false alleles) for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) fecal DNA samples collected in 2012 during winter and summer at Dugway Proving Ground, Utah.

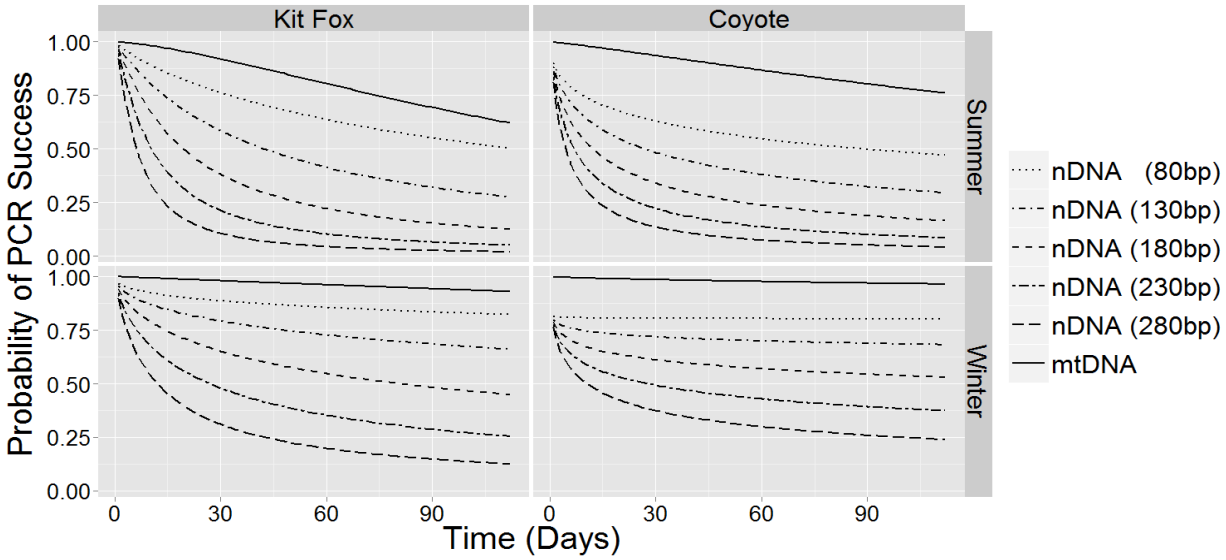

Figure 9. Mixed-effects logistic regression model results for PCR success for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) fecal DNA samples collected in 2012 during winter and summer at Dugway Proving Ground, Utah.

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*) fecal 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. Significance (*) was evaluated at α = 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).

	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

Optimization of NGS temporal design

The number of coyote samples deposited 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. Based on model-predicted genotyping error rates, we required ≤7 replicates to achieve our goal of ≤2% probability of error in the dataset for coyotes. In the winter, five replicates were required for intervals of 3 to 16 days, six replicates for intervals of 17 to 49 days and seven replicates for intervals ≥50 days. For summer coyote samples, the minimum number of replicates required was five. Six replicates were required for intervals of 4 to 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 small number of samples accumulating over shorter intervals, but declined precipitously (Figure 10). The 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 at the shortest sampling intervals. 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. Sharp

increases in cost associated with additional replicates occurred at 50 and 17 days for coyotes in winter and summer, respectively. When surveying species simultaneously, overall cost per successful sample was reduced (Figure 10) 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 ~14 days would reduce costs for each species and allow both species to be monitored together (Figure 10).

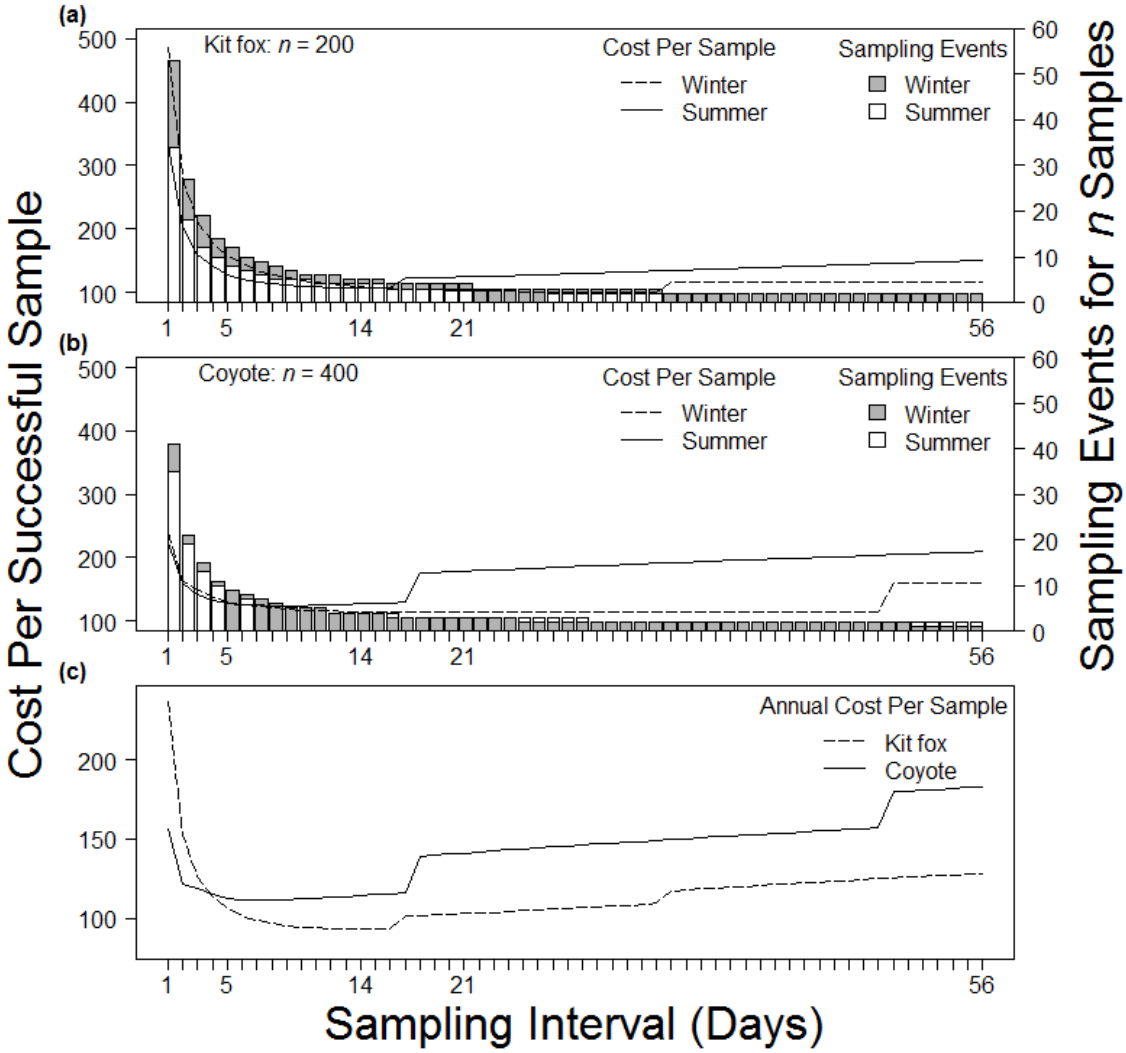

Figure 10. Evaluation of cost ($) per successful fecal DNA sample and number of sampling events required to obtain (a) n = 200 kit fox (*Vulpes macrotis*) and (b) n = 400 coyotes (*Canis latrans*) samples from surveying 150 km of transects at Dugway Proving Ground, Utah, for a range of sampling intervals 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.

NGS-CR implementation, Utah

Species and individual identification

We implemented a 14 day sampling interval between the temporally sampled transects, based on the sampling optimization scheme outlined above. We conducted transect surveys from 5 January to 26 March 26 2013. Thirty NGS-CR transects (5 km) were each surveyed four times during the winter 2013 field season, approximately 14 days apart for a total of 600km of surveys. These transects resulted in the detection of 451 carnivore scats, of which 319 were believed to have been from coyote, while 125 were identified in the field as kit fox and 7 were attributed to other carnivores (i.e., red fox, mountain lion, and bobcat). An additional 151 scats (91, coyote, 59 kit fox, and 1 other based on field ID) were detected along 120km of NGS-OM transects established for occupancy modeling, which were surveyed only once. Combined, 602 scats were sampled for fecal DNA; 410 were believed to be coyote. Genetic species identification tests (mtDNA) failed for 10% (60) of the scats. Species ID revealed that 379 samples were coyote, while 151 kit fox. An additional 12 scats were determined to be from domestic dogs (4), bobcats (3), and red foxes (2), or mixed (3). Among the scats classified as kit fox based on field ID, 23% (42) were misidentified and the majority of these were determined through mtDNA to have been from coyote (41). Only 6% (24) of the scats classified as coyote based on field ID were misidentified; these were determined to be kit fox (14), red fox (1), bobcat (2), domestic dog (4), and mixed (3). Success rates for species ID declined with relative age of the scat. The percent success by relative freshness was as follows: 1 = 97%, 2 = 96%, 3 = 89%, 4 = 64%, 5 = 47%. Thus, relative age of scat may be an effective subsampling strategy that can further reduce costs of noninvasive genetic sampling.

Among the 397 scats identified as coyote, 33 were subsequently omitted from individual ID and inclusion in population estimation due to being low quality (22; i.e., failed at >50% of the loci) or mixed (11; i.e., ≥2 alleles observed at ≥2 loci). Two additional samples were dropped due to low reliability in genotypes following 8 PCR replicates. Overall, consensus genotypes were achieved at ≥50% of the loci for >90% (344) of the coyote samples. Matching of genotypes revealed 132 unique coyote genotypes (75 males and 57 females) with 293 samples matching ≥1 other sample and comprising 81 individual coyotes, while 51 samples were identified as unique single-capture individuals.

Population estimation

Among the 132 unique coyotes captured through the fecal DNA surveys, 96 (58 males and 38 females) were captured on at least one of the 30 NGS-CR transects (5 km) that were sampled with temporal replication. The remaining 36 individuals were identified only on shorter NGS-OM transects that were sampled once for occupancy and cover a greater spatial extent,

(Figure 4). In order to incorporate all 132 individuals in capture-recapture analysis, we estimated population abundance using the single session model in CAPWIRE. The likelihood ratio test (P = <0.0001) implemented in CAPWIRE indicated that we could reject the null hypothesis that all individuals are equally likely to be captured. Thus, we estimated population size with the two-innate rates model (Miller et al. 2005). The estimated population abundance was 186 individual coyotes (95% CI: 175 – 213). Alternatively, using only the 96 individuals captured on the NGS-CR transects, we estimated abundance with multi-session models in the program MARK. We fit models with and without sex and both types of models produced similar results. In both cases, the models that incorporated variation in capture probability over time had the best fit. Abundance estimates from models incorporating sex were 71.7 (95% CI: 64.6 – 86.6) males and 46.8 (95% CI: 41.8 – 58.7) females. Overall abundance (from models excluding sex) was 119.5 (95% CI: 108.5 – 140.3).

Additional samples collected (Session 2 – 2013; Session 3 – 2014)

Summer 2013 (session 2) surveys resulted in the collection of 1078 carnivore scats, of which 797 were believed to have been from coyote, while 273 were identified in the field as kit fox and 8 were suspected to be from other carnivores (i.e., red fox [4] and bobcat [4]). Genetic species ID tests (mtDNA) failed for 21% (230) of the scats. Species ID revealed that 626 samples were coyote and 175 were kit fox. The remaining 47 scats were determined to be from bobcat (27), red fox (7), domestic dog (2), and mountain lion (1), or were mixed (10). Among the scats classified as kit fox based on field ID, 25% (53) were misidentified; the majority of these were determined through mtDNA to have been from coyote (44). Of the scats classified as coyote based on field ID, 7% (44) were misidentified and were determined to be primarily bobcat (21) or kit fox (18). Half of the scats thought to be bobcat and all of the scats believed to be red fox were identified as coyote based on mtDNA. Similar to the pattern observed with the winter (session 1) sampling, success rates for species ID declined with relative age of the scat. The percent success by relative freshness in summer was as follows: 1 = 95%, 2 = 90%, 3 = 86%, 4 = 71%, 5 = 59%. Individual ID has not been conducted since we are out of funding for processing coyote samples.

Winter 2014 (session 3) surveys resulted in the collection of 1013 carnivore scats, of which 667 and 334 were believed to have been from coyote and kit fox, respectively, based on field ID. Additionally, 12 scats were suspected to have been from bobcat (9) and red fox (3). Genetic species ID tests (mtDNA) failed for ~3% (28) of the scats and 16 scats were mixed. Species ID determined that 645 samples were coyote and 301 were kit fox. Bobcat (17) and red fox (6) were also detected. Among the scats classified as kit fox based on field ID, 11% (34) were misidentified and were determined to be from coyote (32) and red fox (2) based on mtDNA. Of the scats classified as coyote based on field ID, 5% (33) were misidentified and were determined to be bobcat (15), kit fox (14), or red fox (4). Additionally, seven of the nine scats

thought to be bobcat and all of the scats believed to be red fox were identified as coyote based on mtDNA. Individual ID has not been conducted since we are out of funding for processing coyote samples.

NGS-CR implementation, Arizona

Species and individual identification

We drove a total of 373.09 km in search of coyote scats; however, 75.7 km were heavily dragged on the BMGR, and we never found any evidence of coyote scat on these sections of road. A total of 159 carnivore fecal samples were collected with 33 scats collected along roads in CPNWR and BMGR and 125 scats at pronghorn water/feed sites. Species ID tests indicated the samples consisted of 117 coyote, 8 bobcat, 1 gray fox, 8 mixed bobcat/coyote, 3 unknown, and 11 failed. Among the 117 scats identified as coyote, 21 were subsequently omitted from individual ID and inclusion in population estimation due to being low quality (19; i.e., failed at >50% of the loci) or mixed (2; i.e., ≥2 alleles observed at ≥2 loci). Two additional samples were dropped due to low reliability in genotypes following 8 PCR replicates. Consensus genotypes were achieved at 82% (96) of the samples. Matching of genotypes revealed 49 unique coyote genotypes (29 males and 20 females) with 30 samples identified as unique single-capture individuals and 66 samples matching ≥1 other sample and comprising 19 individual coyotes.

Population estimation

To incorporate all individuals in capture-recapture analysis, we estimated population abundance using the single session model in CAPWIRE. The likelihood ratio test (P = <0.0001) implemented in CAPWIRE indicated that we could reject the null hypothesis that all individuals are equally likely to be captured. Thus, we estimated population size with the two-innate rates model (Miller et al. 2005). All mark-recapture analyses were done by region as we have no evidence of coyotes moving between sampling regions. We used three regions (CPNWR, BMGR-East, and BMGR-West) and further broke those into five regions (CPNWR, Uken, New Halliwill, Point of Pintas, Devil Hills) to estimate population size. BMGR East individuals can be further broken down into Uken (n=7) and New Halliwill (n=9) and BMGR West can be divided into Point of Pintas (n=8) and Devil Hills (n=4). While both Uken and New Halliwill are both on BMGR East, no individual coyotes were sampled at both locations. The same is true of the locations on BMGR West. CPNWR results include samples from seven drinkers and those along roads and trails to drinkers. The other sites, Uken, etc., are all individual drinkers. Results from Program CAPWIRE for three regions give the following population estimates: CPNWR: 31 (95% CI: 20-43), BMGR-East: 25 (95% CI: 16-39), and BMGR-West: 35 (95% CI: 14-77). When broken down further into five regions, estimates were the same for CPNWR, Uken: 14 (95% CI: 8-22), New Halliwill: 12 (95% CI: 8-20), Point of Pintas: 19 (95% CI: 8-44), and Devil

Hills: 13 (95% CI: 5-13). Due to insufficient sample size on BMGR-East and BMGR-West, we used a multi-session model in Program MARK only for CPNWR data and obtained a population estimate of 29 (95% CI: 23-47).

Additional samples collected (Session 2 – 2013)

Fall 2013 sampling (session 2) surveyed 363.15 km of roads. Sample collection differed markedly from summer as 91 samples were collected at drinkers, and 101 on roads and trails en route to drinkers, as opposed to summer when samples totaled 126 and 33 at drinkers and non-drinker sites, respectively. Species ID success rates were 100% and identified 180 coyote, 6 fox, 4 bobcat, 1 domestic dog, and 1 mixed carnivore. Individual ID has not been conducted since we are out of funding for processing coyote samples.

Conclusions

Our development and implementation of a protocol for conducting noninvasive genetic sampling and mark-recapture estimation of coyotes on DoD lands was very successful. By linking this work to the development of a noninvasive genetic sampling genetic protocol for kit fox and pronghorn at the same installations (Funded by ESTCP), we saved a considerable amount of money on field work and travel. Following the completion of session 1, we were able to generate population estimates with good precision for coyotes at each study site; these population estimates are the first estimates of coyote population for each installation and will assist natural resource managers in effectively managing coyote populations to reduce conflict with kit foxes and Sonoran Pronghorn. As part of our ESTCP funding we plan to complete session 4 sampling for kit fox in Utah and session 3 and 4 sampling for pronghorn in Arizona. If we receive Legacy funding for year 2, then we can add sampling of coyote and genetic analysis of samples from sessions 2 – 4. In addition to abundance estimates, session 2 – 4 will allow us to (1) estimate important demographic parameters (e.g., survival and reproduction) that can further improve management decisions, (2) conduct subsampling and power analyses to optimize sampling design, (3) complete the cost-benefit analysis of the method, and (4) provide recommendations for transference of this approach to other species and installations as a cost-effective alternative to current monitoring approaches.

Literature Cited

Arjo WM, Gese EM, Bennett TJ, Kozlowski AJ (2007) Changes in Kit Fox–Coyote–Prey Relationships in the Great Basin Desert, Utah. *Western North American Naturalist*, **67**, 389–401.

Beja-Pereira A, Oliveira R, Alves PC, Schwartz MK, Luikart G (2009) Advancing ecological understandings through technological transformations in noninvasive genetics. *Molecular Ecology Resources*, **9**, 1279–301.

Boulanger J, Kendall KC, Stetz JB *et al.* (2008) Multiple Data Sources Improve DNA-Based Mark--Recapture Population Estimates of Grizzly Bears. *Ecological Applications*, **18**, 577–589.

Breen M, Jouquand S, Renier C *et al.* (2001) Chromosome-specific single-locus FISH probes allow anchorage of an 1800-marker integrated radiation-hybrid/linkage map of the domestic dog genome to all chromosomes. *Genome research*, **11**, 1784–95.

Broquet T, Petit E (2004) Quantifying genotyping errors in noninvasive population genetics. *Molecular Ecology*, **13**, 3601–3608.

Brøseth H, Flagstad Ø, Wärdig C, Johansson M, Ellegren H (2010) Large-scale noninvasive genetic monitoring of wolverines using scats reveals density dependent adult survival. *Biological Conservation*, **143**, 113–120.

Dugway Proving Ground (DPG) (2007) *Integrated Natural Resource Plan and Environmental Assessment*. Dugway, UT.

Foran DR, Crooks KR, Minta SC (1997) Species identification from scat: An unambiguous genetic method. *Wildlife Society Bulletin*, **25**, 835–839.

Francisco L, Langston A, Mellersh C, Neal C, Ostrander E (1996) A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. *Mammalian Genome*, **7**, 359–362.

Fredholm M, Wintero A (1995) Variation of short tandem repeats within and between species belonging to the Canidae family. *Mammalian Genome*, **6**, 11–18.

Green JS, Flinders JT (1981) Diameter and pH comparisons of coyote and red fox scats. *The Journal of Wildlife Management*, **45**, 765–767.

Guyon R, Lorentzen TD, Hitte C *et al.* (2003) A 1-Mb resolution radiation hybrid map of the canine genome. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 5296–301.

Holmes NG, Strange NJ, Binns MM, Mellersh CS, Sampson J (1994) Three polymorphic canine microsatellites. *Animal Genetics*, **25**, 200.

Kendall WL, Nichols JD, Hines JE (1997) Estimating Temporary Emigration Using Capture-Recapture Data with Pollock's Robust Design. *Ecology*, **78**, 563–578.

Kendall KC, Stetz JB. Roon DA, Waits LP, Boulanger JB, Paetkau D (2008) Grizzly bear density in Glacier National Park, Montana. *Journal of Wildlife Management*, **72**,1693–1705.

Kohn MH, York EC, Kamradt DA *et al.* (1999) Estimating population size by genotyping faeces. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **266**, 657–63.

Kozlowski AJ, Gese EM, Arjo WM (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.

Kuhn M, Weston S, Wing J, Forester J (2011) contrast: A collection of contrast methods. R package version 0.17.

Lukacs PM, Burnham KP (2005) Review of capture-recapture methods applicable to noninvasive genetic sampling. *Molecular Ecology*, **14**, 3909–19.

Lukacs PM, Burnham KP, Dreher BP, Scribner KT, Winterstein SR (2009) Extending the Robust Design for DNA-Based Capture-Recapture Data Incorporating Genotyping Error and Laboratory Data. In: *Environmental and Ecological Statistics* (eds Thomson DL, Cooch EG, Conroy MJ), pp. 711–726. Springer US, Boston, MA.

Luke Air Force Base (LAFB) 2012a. BMGR military history. http://www.luke.af.mil/library/factsheets/factsheet.asp?id=14476. Last updated 07/31/12. Accessed 08/09/12.

Luke Air Force Base (LAFB) 2012b. BMGR overview. http://www.luke.af.mil/library/factsheets/factsheet.asp?id=6384. Last updated 05/01/12. Accessed 08/09/12.

Luke Air Force Base (LAFB) 2012c. BMGR range operations overview. http://www.luke.af.mil/library/factsheets/factsheet.asp?id=14477. Last updated 05/01/12. Accessed 08/09/12.

Marucco F, Pletscher DH, Boitani L *et al.* (2009) Wolf survival and population trend using non-invasive capture-recapture techniques in the Western Alps. *Journal of Applied Ecology*, **46**, 1003–1010.

Meijer T, Norén K, Hellström P, Dalén L, Angerbjörn A (2008) Estimating population parameters in a threatened arctic fox population using molecular tracking and traditional field methods. *Animal Conservation*, **11**, 330–338.

Miller CR, Joyce P, Waits LP (2002) Assessing Allelic Dropout and Genotyping Reliability Using Maximum Likelihood. *Genetics*, **160**, 357–366.

Miller CR, Joyce P, Waits LP (2005) A new method for estimating the size of small populations from genetic mark-recapture data. *Molecular ecology*, **14**, 1991–2005.

Murphy MA, Kendall KC, Robinson A, Waits LP (2007) The impact of time and field conditions on brown bear (*Ursus arctos*) faecal DNA amplification. *Conservation Genetics*, **8**, 1219–1224.

Neff MW, Broman KW, Mellersh CS, *et al.* (1999) A second- generation genetic linkage map of the domestic dog, *Canis familiaris*. *Genetics*, **151**, 803–820.

O'Hara RB, Kotze DJ (2010) Do not log-transform count data. *Methods in Ecology and Evolution*, **1**, 118–122.

Onorato D, White C, Zager P, Waits LP (2006) Detection of Predator Presence at Elk Mortality Using mtDNA Analysis of Hair and Scat Samples. *Wildlife Society Bulletin*, **34**, 815–820.

Ostrander E, Sprague G, Rine J (1993) Identification and Characterization of Dinucleotide Repeat (CA)n Markers for Genetic Mapping in Dog. *Genomics*, **16**, 207–213.

Panasci M, Ballard WB, Breck S *et al.* (2011) Evaluation of fecal DNA preservation techniques and effects of sample age and diet on genotyping success. *The Journal of Wildlife Management*, **75**, 1616–1624.

Peakall R, Smouse PE (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Pollock KH (1982) A Capture-Recapture Design Robust to Unequal Probability of Capture. *The Journal of Wildlife Management*, **46**, 752–757.

Pollock KH, Nichols JD, Brownie C, Hines JE (1990) Statistical Inference for Capture-Recapture Experiments. *Wildlife Monographs*, **107**, 3–97.

Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes, consequences and solutions. *Nature Reviews Genetics*, **6**, 847–59.

Prugh LR, Ritland CE, Arthur SM, Krebs CJ (2005) Monitoring coyote population dynamics by genotyping faeces. *Molecular Ecology*, **14**, 1585–96.

Puechmaille SJ, Petit EJ (2007) Empirical evaluation of non-invasive capture-mark-recapture estimation of population size based on a single sampling session. *Journal of Applied Ecology*, **44**, 843–852.

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

Sandercock BK (2006) Estimation of Demographic Parameters from Live-Encounter Data: A Summary Review. *The Journal of Wildlife Management*, **70**, 1504–1520.

Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. *Trends in ecology & evolution*, **22**, 25–33.

Seddon JM (2005) Canid-specific primers for molecular sexing using tissue or non-invasive samples. *Conservation Genetics*, **6**, 147–149.

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, **69**, 82–90.

Solberg KH, Bellemain E, Drageset O-M, Taberlet P, Swenson JE (2006) An evaluation of field and non-invasive genetic methods to estimate brown bear (Ursus arctos) population size. *Biological Conservation*, **128**, 158–168.

Stenglein JL, DE Barba M, Ausband DE, Waits LP (2010a) Impacts of sampling location within a faeces on DNA quality in two carnivore species. *Molecular Ecology Resources*, **10**, 109–14.

Stenglein JL, Waits LP, Ausband DE, Zager P, Mack CM (2010b) Efficient, noninvasive genetic sampling for monitoring reintroduced wolves. *Journal of Wildlife Management*, **74**, 1050–1058.

Taberlet P, Luikart G (1999) Non-invasive genetic sampling and individual identification. *Biological Journal of the Linnean Society*, **68**, 41–55.

Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: look before you leap. *Trends in Ecology and Evolution*, **14**, 323–327.

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular ecology*, **10**, 249–56.

Waits LP, Paetkau D (2005) Noninvasive genetic sampling tools for wildlife biologists: A Review of applications and recommendations for accurate data xollection. *Journal of Wildlife Management*, **69**, 1419–1433.

White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study*, **46**, S120–S139.