

A Comparison of Population Survey Techniques for Swift Foxes (*Vulpes velox*) in New Mexico

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ABSTRACT.—We compared survey techniques for estimating relative and absolute abundances of swift foxes (*Vulpes velox*) in New Mexico. For relative abundance surveys, the most efficient technique is collection of scats followed by verification of species depositing scats with DNA analysis. By collecting scats, the proportions of individual locations where swift foxes were detected were 61.9% and 67.7% during surveys in 2000 and 2001, which were greater than the proportions using scent stations (31.4%, 47.1%) or trapping (11.5%, 8.4%). By collecting scats, we detected swift foxes in 100% of the fox home ranges within the study area. If scent-station surveys are used instead, scent-station transects consisting of stations spaced at 1.6 km (1.0 mile) intervals and operated for three nights are the most practical. Searching for tracks, spotlighting and calling are much less efficient techniques. For absolute abundance surveys, trapping and resighting with cameras at bait stations was more accurate than counting unique microsatellite DNA genotypes from collected scats. Using trapping/resighting, we estimated the 95% confidence intervals for the swift fox population within the study area to be 17.8–30.0, 11.9–25.3 and 15.2–17.3 in the periods November 1999–January 2000, February 2000 and January–March 2001, respectively. We counted 63 and 27 unique genotypes in early 2000 and 2001, respectively. The numbers of unique genotypes, which were much greater than population estimates obtained from trapping and resighting, were overestimated because of the presence of transient swift foxes and poor quality DNA from scats leading to allelic drop-out and/or false alleles.

INTRODUCTION

The swift fox (*Vulpes velox*) is a small (<3.0 kg) canid that occurs in the short grass prairie from eastern New Mexico and northwestern Texas to southern Alberta and Saskatchewan (Egoscue, 1979). It was abundant historically, but the arrival of settlers led to a drastic population decline through fur trapping, habitat loss and trapping and poisoning campaigns directed against wolves (*Canis lupus*) and coyotes (*C. latrans*; Egoscue, 1979; Scott-Brown *et al.*, 1987). Populations have recovered to some extent since 1950 (Kahn *et al.*, 1997), but the swift fox was temporarily a candidate for endangered species listing by the U.S. Fish and Wildlife Service (Potter, 1982; Clark, 2001). As an alternative to a federally directed recovery program, state and national wildlife management agencies within the historical range of swift foxes, including the New Mexico Department of Game and Fish, formed the Swift Fox Conservation Team (SFCT) and developed a conservation strategy (Kahn *et al.*, 1997). Monitoring swift fox populations is an essential aspect of the conservation strategy. Measures of relative abundance rely upon an index, such as percentage of scent stations visited, to indicate population trends. Relative abundance techniques that have been used for swift foxes include trapping (Finley, 1999; Moehrenschrager and Moehrenschrager, 2001), scent-stations (Harrison and Schmitt, *in press*; Luce *et al.*, 2000), collection of scats (Sovada and Roy, 1996; Olson *et al.*, 1997), track surveys (Roy *et al.*, 1999; Hoagland, 2000) and spotlighting (Sovada and Roy, 1996; Harrison and Schmitt, *in press*). Measures of absolute abundance reflect the actual numbers of swift foxes present in

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a population. Absolute abundance techniques used for swift foxes include mark-resighting (Roell, 1999) and mark-recapture (Cotterill, 1997). Additional survey techniques that have been used for other species, but not for swift fox, include collection of scats coupled with microsatellite DNA analysis for absolute abundance (Kohn *et al.*, 1999) and calling for relative abundance (Sumner and Hill, 1980).

At present, trapping, scent station and track survey techniques are all in use for surveying relative abundance of swift foxes (Moehrensclager and Moehrensclager, 2001; Schmitt and Oakleaf, 2001). No single survey technique has been found to be suitable for all areas of swift fox range. Trapping and scent stations have been used for many carnivore surveys. They require relatively little effort to conduct, but they do rely upon a behavioral response on the part of foxes, *i.e.*, finding and entering a trap or station during the survey period. Trapping provides definitive species identification and the opportunity for collection of blood or other samples, but risks injury to the animal. Track surveys are the simplest of the relative abundance techniques and have the additional advantages that no response by foxes is required and tracks may remain visible for several weeks. However, track surveys require soils that can take and hold a clear imprint; thus, the technique is not suitable where soils do not hold tracks or where soil conditions vary over the survey area.

Observation of scats is an old technique for surveying carnivores. Until the advent of DNA analysis, it was not possible to positively identify the species producing a given scat. However, Kohn *et al.* (1999) demonstrated that scat collection combined with DNA analysis may be used to identify individuals and generate absolute abundance estimates. An advantage of scat collection is that scats may persist for several months (Kohn *et al.*, 1999); thus, the survey time period represented by scat collection is much greater than by scent stations or trapping (a few days) or by calling or spotlighting (a few minutes). Although the use of scat collection has increased recently (Taberlet *et al.*, 1999), its usefulness has not yet been widely demonstrated.

The area visible when spotlighting comprises a very small portion of a swift fox's home range; hence, this technique is limited by topography and the number of roads available. Nevertheless, in certain circumstances, such as immediately after crops are harvested, swift foxes may be attracted to specific areas where they may be efficiently spotlighted (S. Bremner, pers. comm.). Calling has not been commonly used to survey carnivores. However, individual swift foxes can be recognized by their vocalizations in response to recorded sounds (K. Weagle, pers. comm.); thus, calling potentially may be used to conduct absolute abundance surveys if swift foxes will respond adequately.

The purpose of this study was to determine the swift fox survey techniques most appropriate for New Mexico. Trapping, scent stations, collection of scats, track searches, spotlighting and calling were compared as techniques for determining relative abundance of swift foxes. We expected that scent stations would be the best technique based upon the successful application of that technique by Harrison and Schmitt (*in press*). Thus, we also examined the relationship between the percentage of scent station transects where foxes were detected and the number of nights of observation and distances between scent stations. For techniques of determining absolute abundance, trapping combined with resighting at bait stations with automatic cameras and microsatellite DNA analysis of scats were compared.

STUDY AREA

The study area was located in the Kiowa National Grasslands, northeast of Roy, New Mexico (36°15'N, 104°15'W), in Harding and Colfax counties and included private, state and federal lands. Habitat within the study area was entirely shortgrass prairie (described

as plains-mesa grassland by Dick-Peddie, 1993). Dominant plant species were blue grama (*Bouteloua gracilis*), hairy grama (*B. hirsuta*), western wheatgrass (*Pascopyrum smithii*), threeawn (*Aristida* sp.) and needle and thread (*Hesperostipa comata*). The most common shrubs were broom snakeweed (*Gutierrezia sarothrae*) and *Yucca*, spp. which occurred only in isolated stands. Topography was low rolling hills and elevation varied from approximately 1700 to 1900 m. Annual precipitation averages 390 mm and varied between 257 and 565 mm from 1975 to 2000. It was 395, 427 and 381 mm in 1998, 1999 and 2000, respectively. The entire study area is heavily grazed and cattle are present throughout the year.

METHODS

TRAPPING AND HANDLING OF SWIFT FOXES

Swift foxes were trapped during three intensive trapping sessions, January–March 1999, September–November 1999 and December 2000–March 2001. Trapping also was conducted between intensive sessions to replace collars and to relocate missing foxes. Swift foxes were captured in 25 cm × 30 cm × 81 cm single door traps (Tomahawk Traps, Tomahawk, Wisconsin) baited with beef scraps and a cod liver oil–mackerel lure (Trailing Scent, On Target A.D.C., Cortland, Illinois). Traps were placed at 0.8 or 1.6 km intervals at conspicuous locations such as road, trail and fence intersections and utility boxes. At dens, enclosure traps were used (Covell 1992) to recapture foxes for replacement of radiocollars. Captured foxes were transferred to a 30 cm × 60 cm × 76 cm restraint module (Tomahawk Traps, Tomahawk, Wisconsin) and sedated before handling. A combination of ketamine (25 mg/kg of body mass) and xylazine (2.5 mg/kg) was used initially, based upon Kreeger (1996). However, this drug combination resulted in unnecessarily long sedation and depressed breathing and heart rates. Reduction of the dosage to 10 and 1 mg/kg, respectively, did not solve these problems. Telazol (10 mg/kg; Kreeger, 1996) was more acceptable. It did not depress heart or breathing rates, but, in some instances, it caused excessive salivation and recovery with little warning. Captured foxes were dusted for fleas (to avoid transfer of fleas to the handler), inspected for sex and injury, measured, fitted with a radiocollar (telemetry system described below) and marked for individual visual identification by dyeing a unique portion of their fur with commercial hair dye (Miss Clairol black velvet). No blood was collected from radiocollared foxes. The University of New Mexico Main Campus Animal Care and Use Committee approved the animal handling procedures (protocol 9811-B). Swift foxes were trapped under New Mexico Department of Game and Fish Scientific Collecting Permit No. 2932.

Radiocollars were provided by Advanced Telemetry Systems (Isanti, Minnesota; model 16MC) and Telonics (Mesa, Arizona; model MOD-080). The receiving antenna consisted of two five-element Yagi antennas combined 180° out of phase (null) and mounted through a sunroof in the cab of the research vehicle. Tests of this configuration indicate that, under ideal conditions (both transmitter and receiver on hilltops), the signal may be detected at over 4.0 km.

RELATIVE ABUNDANCE SURVEY TECHNIQUES

We examined trapping (*see* above), scent stations, collection of scats, track searches, spotlighting and calling as techniques for determining relative abundance of swift foxes.

Field procedures.—To determine the relationship between the percentage of scent station transects where foxes were detected and the number of nights of observation and distances between scent stations, transects of scent stations were placed within the known home ranges of radiocollared foxes. Each transect consisted of five evenly spaced scent stations.

The length of the transects varied with the size of the home range. Transects were placed as much as possible within the central portions of home ranges, but availability of roads and public land resulted in placement of some transects on peripheries. Similar placement is likely when surveying areas without knowledge of existing home ranges. The separation between stations varied with the size of the home range and was approximately 0.8 km. Scent stations were operated during all seasons and consisted of 75 cm × 75 cm areas cleared of vegetation and covered with a 1:32 mixture of mineral oil and dried plaster sand. Stations were baited with approximately 4 cm³ of canned mackerel and a plaster of paris tablet (Pocatello Supply Depot, U.S.D.A., Pocatello, Idaho) soaked in a cod liver oil—mackerel mixture (Trailing Scent, On Target A.D.C., Cortland, Illinois). Automatic cameras with active infrared sensors (Trailmaster 1500 with TM 35-1 camera kit and Tm1500 Photo System, Goodson & Associates, Lenexa, Kansas) were placed at scent stations to identify visiting foxes. Cameras and receiving sensors were placed in 36 cm × 20 cm × 16 cm boat dry boxes with holes drilled to permit photographs and the infrared beam. Dry boxes and the infrared transmitting unit were strapped to wooden stakes driven into the ground. The system was set to take bursts of four photographs no less than 2 min apart when the beam was broken for 0.25 s. In some areas barbed-wire fences were built around the stations to exclude cattle. Stations were observed for six nights. It was not logistically possible to conduct scent station tests with smaller numbers of stations per transect so we subsampled the visitation data in order to simulate the results we would have obtained from smaller numbers of scent stations per home range. From each original transect of five scent stations per home range we produced one transect of five stations, five simulated transects of four stations ten simulated transects of three stations, ten simulated transects of two stations, and five simulated transects of one station. This procedure was intended to produce general guidelines for sampling. It does introduce pseudoreplication, but no statistical tests were applied to the results.

Scats were collected during two systematic surveys: September 1999–February 2000 (2000 survey) and December–March 2001 (2001 survey). Searches for scats were conducted at conspicuous locations (*see above*) along roadways that passed through known swift fox home ranges. All conspicuous locations along survey routes were searched. To minimize collection of coyote scats, we selected scats with a maximum diameter ≤20 mm (Danner and Dodd, 1982). Scats were placed in numbered paper bags during the same periods that resighting stations were operated (*see below*). We determined the species depositing scats and the number of individual swift foxes present using mitochondrial and microsatellite DNA analysis, respectively (*see below*).

We tested the hypothesis that presence of a scent lure would enhance deposition of scats by clearing scats from conspicuous locations, then depositing a lure (cod liver oil—mackerel mixture, Trailing Scent, On Target A.D.C., Cortland, Illinois) at alternate locations inside and outside of known swift fox home ranges in June 1999. Scats were collected for this purpose during July and December 1999 and January 2000.

Only preliminary studies of track searches, spotlighting and calling were conducted. Searches for tracks were conducted along unpaved roads on foot and while slowly driving a vehicle. Searches for tracks also were conducted in the vicinity of wet areas surrounding livestock water tanks, and during scat collection (*see above*). Areas both inside and outside of known swift fox home ranges were surveyed. We attempted to spotlight radiocollared foxes with one 1,000,000 candlepower spotlight while driving slowly through their home ranges. We also attempted to call foxes within visual or audible range using prerecorded tapes of rabbit distress calls and swift fox vocalizations. Tapes were played at various volumes and durations to foxes determined by telemetry to be within 0.8 km of the tape player.

Mitochondrial DNA analysis.—Before DNA analysis, the first scat sample (2000 survey) was stored in a plastic bag at room temperature and the second sample (2001 survey) was frozen at -80°C . We assumed that the scats were adequately dried before collection due to the semiarid climate of the study area. Total genomic DNA was extracted from each scat sample using the QIAamp DNA stool mini kit (Qiagen Inc., Valencia, California). The protocol for extraction established by the kit manufacturer was followed, with the exception that the incubation period for digestion was extended from 10 min to overnight and 50 μl of the supplied proteinase K was added instead of the specified 25 μl . Increased incubation period and proteinase K concentration increased final DNA concentration when compared with DNA that was isolated using the shorter incubation time. The Qiaamp mini stool kits were designed for use with fresh stool samples. The above modifications made to the protocol increased DNA yield. Each time we extracted DNA from the scat samples we also extracted a negative control on a water sample to check for contamination.

Following manufacturer's guidelines, isolated DNA was dissolved in 200 μl elution buffer supplied in the kit. The eluted DNA was separated on 0.8% agarose gels and visualized under UV light following ethidium bromide staining to determine quality and relative quantity. Eluted DNA from all scat samples was refrigerated at 4 C until later use.

For species identification, approximately 350 base pairs of the cytochrome *b* gene were amplified and sequenced. Primers L15513 and H15915 (Irwin *et al.*, 1991) were used for amplification of the cytochrome *b* gene as these primers have been shown to amplify and distinguish canid mitochondrial DNA (Mercure *et al.*, 1993; Wayne *et al.*, 1997). The PCR conditions were: denaturation at 95 C for 30 s, annealing at 45 C for 30 s and extension at 72 C for 30 s for 40 cycles. Amplification was conducted in 25 μl reactions. Reaction concentrations were 2.5 μl of 25 mM MgCl_2 , 1.2 pmol of each primer, 2.5 μl 10 \times *Taq* buffer, 2.5 μl 10 \times dNTPs, 0.125 μl *Taq* polymerase (5 Units/ μl) and 2.5 μl 1 mg/ml BSA. We performed a negative control with each PCR to check for contamination. PCR products were visualized on 0.8% agarose gels. If we did not obtain a PCR product, we tried a second time. If the second reaction did not work, we re-extracted DNA from the scat sample. Then we tried at least two more times to amplify the cytochrome *b* gene. Following visualization, amplified products were purified using QIAQuick PCR columns (Qiagen Inc., Valencia, California). The purified products were precipitated using 3 M sodium acetate and 100% ethanol, frozen for 15 min at -20°C and centrifuged for 15 min. Precipitates were washed with 70% ethanol, then dried and dissolved in 5.5 μl 10 mM Tris elution buffer pH 8.5 for sequencing. Cleaned and concentrated products were subjected to single stranded cycle sequencing amplification using ABI PRISM BigDye terminator cycle sequencing ready reaction (Applied Biosystems, Inc., Foster City, California), following manufacturers guidelines. Ethanol precipitation was performed on the sequencing reactions and products were electrophoresed on an ABI 377 DNA sequencer. Completed sequences were submitted to the Gene Bank BLAST search (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) to verify species identification of each scat sample.

Statistical analysis.—To compare the efficiency of trapping, scent stations and scat collection for detecting the presence of swift foxes, we used data from those locations where all three techniques were used during the absolute abundance surveys (*see below*). Visitation to bait/camera stations was used as a surrogate for visitation to scent stations (*see Discussion*). Due to logistic restraints, scent stations were not operated during times of absolute abundance surveys. We compared the proportion of foxes captured per trap-night, the proportion of bait/camera stations visited by foxes and the proportion of stations where scats were collected and identified as originating from swift fox, using a contingency table

(Zar, 1984:400). We used the Tukey test to compare pairs of proportions (Zar, 1984:240). For these tests we combined observations of radiocollared and uncollared foxes.

We used a *t*-test to compare the maximum diameters of swift fox and unidentified scats and a Mann-Whitney test with the normal approximation (Zar, 1984:142) to compare the number of scats found at sites with and without scent lure. We compared the proportions of sites with and without scats using a contingency table with a normal approximation (Zar, 1984:396).

ABSOLUTE ABUNDANCE SURVEY TECHNIQUES

We studied trapping combined with resighting with automatic cameras at bait stations and microsatellite DNA analysis of scats as techniques for determining absolute abundance of swift foxes. Two absolute abundance surveys were conducted: September 1999–February 2000 (2000 survey) and December–March 2001 (2001 survey).

Trapping/resighting procedures.—Following intensive trapping sessions (*see* above), radiocollared foxes were resighted and uncollared foxes were located by placing automatic cameras using active infrared sensors (*see* above) at stations baited with canned mackerel and a lure (cod liver oil—mackerel mixture, Trailing Scent, On Target A.D.C., Cortland, Illinois). Bait/camera stations were placed at 1.6 km intervals along roads and operated for four nights during November 1999–January 2000 and February–March 2001. During February 2000 cameras were operated at each station for two nights. Bait/camera stations were not at the same locations as scent stations used to determine the optimal spacing and number of nights of observation for relative abundance surveys.

To estimate the absolute swift fox population size for each sampling period we used a Lincoln-Peterson estimate for closed populations (Pollock *et al.*, 1990). The area sampled was assumed to be one average home range size diameter wide on each side of surveyed roads, based upon average home range sizes observed from June through December 1999 and September 2000 through March 2001.

Microsatellite DNA analysis.—For estimation of the number of individual swift foxes present in the study area during the 2000 and 2001 surveys, we used scats collected at conspicuous locations as described previously along the same route used for trapping and resighting foxes. We selected only scats determined by mitochondrial analysis to be from swift foxes. Seven microsatellite canid specific dimeric primer pairs were used for genotyping: CPH3, CPH7, CPH9, CPH10, CPH12 (Fredholm and Wintero, 1995), DB3 (Holmes *et al.*, 1993) and C213 (Ostrander *et al.*, 1993). Amplifications were conducted in 12.5 μ l reactions. Specific conditions of each reaction varied for each primer according to optimal conditions determined by experiments testing volume of PCR production against temperatures and amount of MgCl₂. Reaction conditions for primers were denaturation at 95 C for 20 s annealing at 60 C for 25 s and extension at 72 C for 30 s, with the exception that we adjusted annealing temperatures for primers CPH7 and CPH12 to 55 C and 57 C, respectively. We used 25 mM MgCl₂ solution in each reaction. The optimal amounts of MgCl₂ were: CPH3 and CPH12, 1.25 μ l MgCl₂; CPH7, 0.75 μ l MgCl₂; CPH9, 1.5 μ l; CPH10, DB3 and C213, 1 μ l MgCl₂. We added 0.75 pmol of each primer, 1.25 μ l 10 \times *Taq* buffer, 1.25 μ l 10 \times dNTPs, 0.125 μ l *Taq* polymerase (5 Units/ μ l) and 1.25 μ l 1 mg/ml BSA to the reactions. Negative controls were run with all reactions to check for contamination of PCR chemicals.

Reactions were separated on 2.0% agarose gels and visualized under UV light to check for amplification. Alleles were analyzed and scored by labeling one of the primers in each pair with a fluorescent dye. Labeled PCR products were loaded on a 377 ABI DNA

sequencer and visualized using Genescan Analysis Software (Applied Biosystems, Inc., Foster City, California).

We scored genotypes as unique if they did not have all elements in common with other genotypes. If only a portion of the loci amplified in two different scat samples and the samples had elements in common for some of the alleles, but incomplete data for the other loci, we did not score them as different. If a locus in two samples was homozygous for different alleles, or had two different heterozygous alleles, they were considered unique even if all the other loci matched. If only a portion of the loci amplified in one sample and a different portion amplified for a second sample, we scored the two samples as different genotypes. If two genotypes were identical at most loci, but one or two loci had the homozygous condition for one sample and the heterozygous (with one allele in common) condition for the other sample, these were scored as different genotypes, even though the homozygous condition could have been a result of incomplete amplification of target alleles (allelic drop-out). Allelic drop-out and the rules we used have potential to cause overestimation of unique genotypes.

To verify our techniques, we obtained matching blood and scat samples known to originate from the same individual, from captive swift foxes held at the Northern Prairie Wildlife Research Center, Jamestown, North Dakota. Genomic DNA was extracted from blood samples using the QIAamp blood and tissue kit (Qiagen Inc., Valencia, California). DNA extraction of blood followed the DNA extraction from blood protocol that was provided with the kit. Following manufacturers guidelines isolated DNA was dissolved in 200 μ l elution buffer supplied by the kit. To check for DNA isolation of blood and scat controls as well as unknown scat samples, the eluted DNA was separated on 0.8% agarose gels and visualized under UV light following ethidium bromide staining. Eluted DNA from all blood samples was refrigerated at 4 C until later use. We sequenced both control blood and scat and compared control microsatellite scat and blood genotypes to verify genotype matches. A sequence was obtained from all blood and scat controls and BLAST searches returned results on all control samples positive for swift fox. These results verified the technique worked and was reliable for identifying species from fecal materials.

DNA from the control blood samples was amplified multiple times with the microsatellite primers, as they often were used as positive controls for all of the scat reactions. When we achieved a genotype match with a control scat we stopped attempting to obtain microsatellite data from that particular scat. Often times the control scat had to be re-amplified because no PCR product was obtained or only a single allele at a locus amplified when we were expecting two alleles. DNA from the control scats was extracted at least 3 times.

We also had in our laboratory 13 frozen tissue (liver) samples from swift foxes collected in northeastern New Mexico during a previous study. These tissues were used to provide a control for the population genetic structure of swift foxes on our study site. We considered each scat survey and the frozen tissue samples as three different populations. We tested each "population" to determine if they were in Hardy-Weinberg equilibrium at each locus. Ideally, populations of foxes analyzed from scat sample DNA should behave the same as populations analyzed from higher quality DNA. Finally, we tested population subdivisions between scat surveys, as well as between the control blood and scats, using the method of Weir and Cockerham (1984).

We used the program Genetic Data Analysis (Lewis and Zaykin, 2001) to generate descriptive statistics for microsatellite data and to examine population genetic structure with pairwise population comparisons of Theta (θ ; Weir and Cockerham, 1984).

TABLE 1.—Capture rates for swift foxes in New Mexico

Period	Trap-nights	Without recaptures		With recaptures	
		number	%	number	%
January–March 1999	181	7	3.9	8	4.4
September–November 1999	221	13	5.9	19	8.6
January–March 2000	63	1	1.6	3	4.8
May–July 2000	71	2	2.8	4	5.6
December 2000	62	6	9.7	7	11.3
January–March 2001	107	4	3.7	10	9.3
April–June 2001	88	0	0.0	2	2.3

RESULTS

TRAPPING AND RELATIVE ABUNDANCE SURVEY TECHNIQUES

We captured 33 swift foxes plus 20 recaptures in 793 trap nights (4.2% without recaptures, 6.7% with recaptures; Table 1). Three additional foxes were captured in enclosure traps at dens. There were significant differences of capture success between periods without recaptures ($X^2 = 11.163$, $df = 6$, $P = 0.087$), with autumn and early winter producing the greatest success. There were no significant differences of capture success between periods with recaptures ($X^2 = 8.729$, $df = 6$, $P = 0.203$). A total of 36 foxes (18 males, 18 females) were radiocollared.

Scent-station tests were conducted in the home ranges of 14 radiocollared foxes (10 males, 4 females) for 420 station-nights for the purpose of determining the relationship between the percentage of scent station transects where foxes were detected and the number of nights of observation and distances between scent stations. Radiocollared and uncollared foxes made 51 and 61 visits to scent stations, respectively. Seventy-five percent of stations were visited within four nights. Percentages of transects visited leveled off after three nights for radiocollared and uncollared foxes combined (Fig. 1), but did not level off for radiocollared foxes only (Fig. 2). Sample size was inadequate for seasonal comparisons.

During the 2000 survey period we surveyed 40.5 km of roadways, examined 48 potential scat sites and found scats at 36 of those sites (75.0%). Of 194 scats collected, 141 (72.7%) were identified as swift fox with mitochondrial DNA. The median number of scats collected within a single swift fox home range was 21.5 (range, 8–63). During the 2001 survey we surveyed 37.6 km of roadways, examined 39 potential scat sites and found scats at 25 of those sites (64.1%). Of 137 scats collected, 89 (65.0%) were identified as swift fox and 4 (2.9%) were identified as coyote with mitochondrial DNA. The median number of scats collected within a single home range was 8.5 (range, 3–66). The survey route passed through eight swift fox home ranges during both surveys. We found scats that were identified as swift fox within all known swift fox home ranges and within all gaps between known home ranges where foxes had not been trapped. Home ranges were not equally surveyed, as the survey routes passed through the central portions of some ranges and peripheries of others.

At locations where traps, bait/camera stations and scat searches were located at the same site, trap success was 11.5% in 1999/2000 (95% CI 6.6–18.0%; $n = 139$ trap-nights) and 8.4% in 2000/2001 (95% CI 3.7–15.9%; $n = 95$ trap-nights). Visitation percentages to bait/camera stations were 31.4% in 2000 (95% CI 23.1–40.7%; $n = 118$ station-nights) and 47.1% in 2001 (95% CI 36.9–57.2%; $n = 102$ station-nights). At least one scat identi-

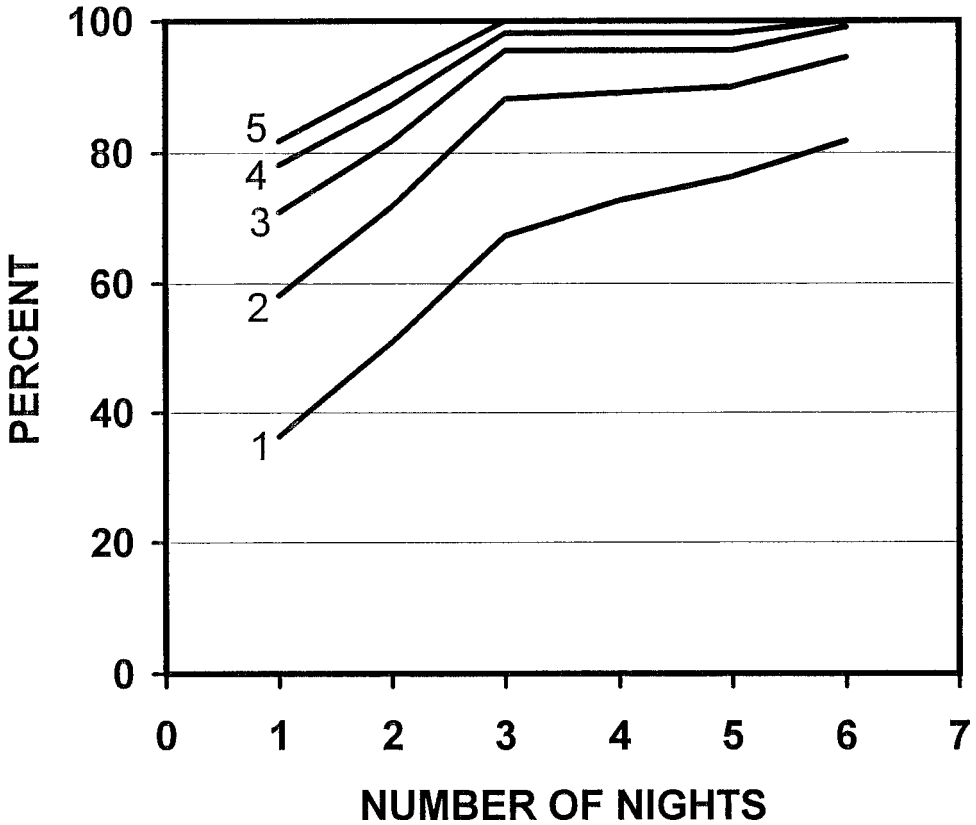


FIG. 1.—Percentages of simulated scent-station transects visited by radiocollared and uncollared swift foxes combined as a function of number of stations per home range and number of nights of observation. Numbers by lines indicate the number of scent stations per swift fox home range

fied as swift fox was found at 61.9% of locations in 2000 (95% CI 38.5–81.9%; $n = 21$), and at 66.7% of locations in 2001 (95% CI 43.0–85.4%; $n = 21$).

Detection percentages for the three techniques were not the same in 2000 ($X^2 = 32.157$, $df = 2$, $P < 0.001$), nor in 2001 ($X^2 = 64.32$, $df = 2$, $P < 0.001$). In both surveys detection by scat collection was greater than by resighting at bait/camera stations (2000: $q = 7.34$, $P < 0.001$; 2001: $q = 4.56$, $P = 0.004$), and detection at bait/camera stations was greater than detection by trapping (2000: $q = 11.122$, $P < 0.001$; 2001: $q = 18.06$, $P < 0.001$).

Scats not identified as swift fox or coyote could not be identified to species due to unclear sequences or lack of PCR products. There were no obvious visual differences of color or size between identified and unidentified scats. The average maximum diameter of scats identified as swift fox ($\bar{x} = 13.9 \text{ mm} \pm 2.8 \text{ mm SD}$, $n = 206$; Fig. 3) was not different from the average diameter of unidentified scats ($\bar{x} = 13.6 \text{ mm} \pm 2.8 \text{ mm SD}$, $n = 81$; $t = 0.924$, $df = 285$, $P = 0.356$).

Collection of scats for the test of enhancement of deposition of scats was inadequate for analysis 1 mo after deposition of lure. Scats were collected again 7 mo after the initial

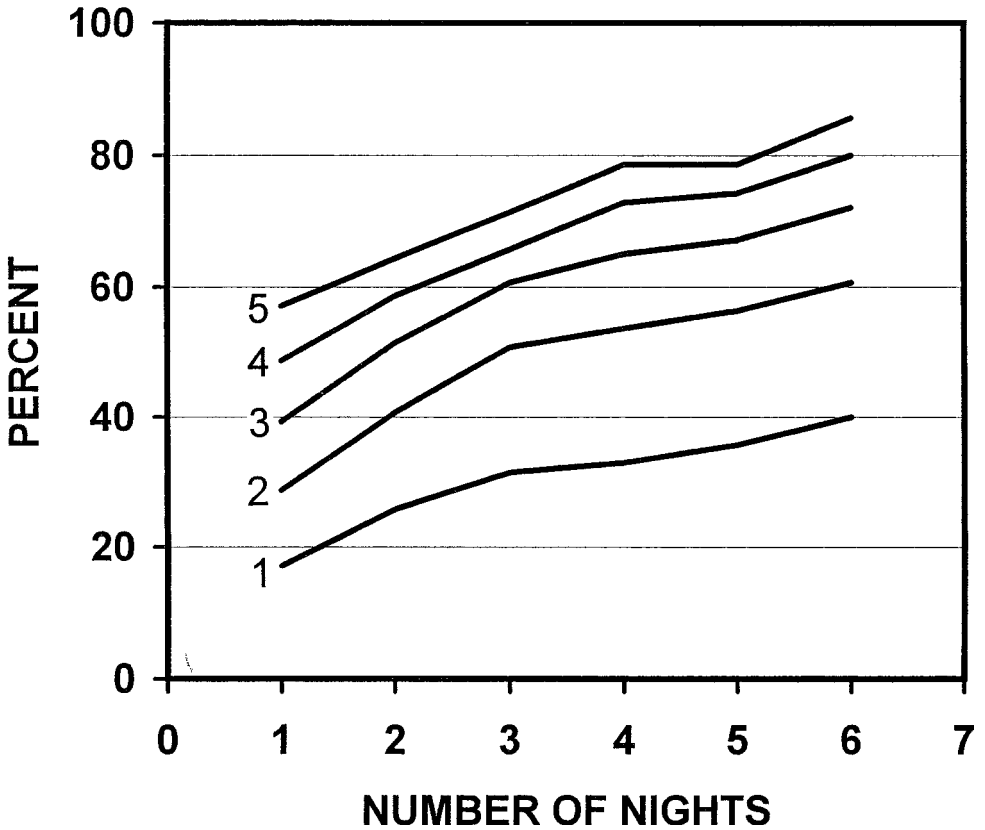


FIG. 2.—Percentages of simulated transects of scent stations visited by radiocollared swift foxes only as a function of number of stations per home range and number of nights of observation. Numbers by lines indicate the number of scent stations per swift fox home range

lure deposition. Scats were found at 63.6% of sites with lure (\bar{x} = 2.9 scats/site, range 0–16, median 1 scat/site, n = 33 sites) and at 35.5% of sites without lure (\bar{x} = 3.4 scats/site, range 0–14, median 2 scats/site, n = 31 sites). Scats were found at a higher proportion of sites with lure than at sites without lure (Z = 2.251, P = 0.026), but there was no difference between the average number of scats found at sites with and without lure (Z = 0.4223, P > 0.5).

Only one, clear, swift fox track was observed on an unprepared surface during the study. No swift fox tracks were observed at 64 locations surveyed during the test of enhancement of scat deposition by lures, during 31 km of road surveys by vehicle, nor during 12.8 km of foot surveys along roads within the home ranges of three swift foxes in July and August 1999. Spotlighting was conducted for 187 km through the home ranges of ≥ 15 foxes in May–July 1999. No foxes were seen. Eleven attempts were made to call radiocollared foxes into visual or audible range in Apr. and May 1999 and Jan. 2000. One fox responded to swift fox vocalizations by approaching the vehicle and vocalizing. No other foxes responded even though telemetry indicated they were within range of the sounds.

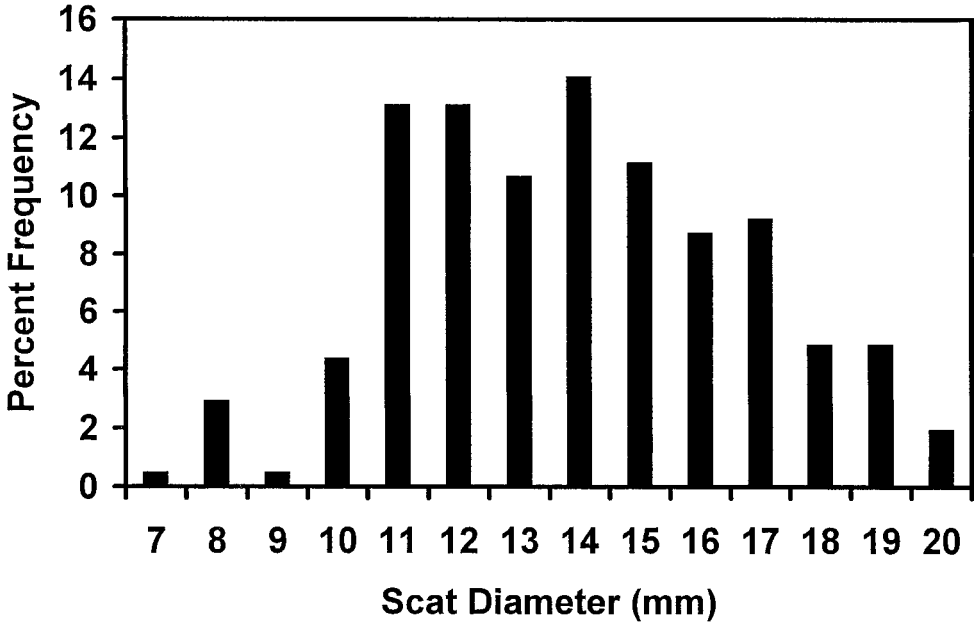


FIG. 3.—Histogram of maximum diameters of swift fox scats collected in New Mexico and identified as originating from swift foxes by mitochondrial DNA analysis. Sample size was 206

ABSOLUTE ABUNDANCE SURVEY TECHNIQUES

During the 2000 resighting period, 15 radiocollared swift foxes were available. Six radiocollared and three uncollared swift foxes were photographed during 122 station-nights. Radiocollared and uncollared foxes made 24 and 12 visits to bait/camera stations, respectively. In the period 22 November 1999–30 January 2000, the estimated population in the study area was 23.9 swift foxes (95% confidence interval: 17.8–30.0). During February 2000 the estimated population in the study area was 18.6 swift foxes (95% confidence interval: 11.9–25.3). During the 2001 resighting period 13 radiocollared foxes were available. Twelve radiocollared and three uncollared swift foxes were photographed during 107 station-nights. Radiocollared and uncollared foxes made 31 and 12 visits to bait/camera stations, respectively. During the period 14 January–21 March 2001, the estimated population in the study area was 16.2 swift foxes (95% confidence interval: 15.2–17.3). Based upon an average autumn/winter 95% minimum convex polygon home range diameter of 4.2 km during the 2000 survey and 4.5 km during the 2001 survey ($n = 4$ and 8 swift foxes, respectively), the area surveyed was 227.5 km² in 2000 and 231.3 km² in 2001. The average swift fox density was 0.105 foxes/km² in November 1999 to January 2000 (95% CI 0.078–0.132) and 0.070 foxes/km² in January–March 2001 (95% CI 0.066–0.075).

We found 63 and 27 unique genotypes from the 2000 and 2001 scat surveys, respectively. Of these, 10 genotypes appeared in both surveys. Unfortunately, scat DNA is not the best source for genetic material. As a result, we were not able to produce a PCR product for each locus for every sample. Of the seven loci examined, one (CPH12) was monomorphic for all the scats in which we obtained product. We were unable to generate enough data for two other loci (CPH10 and C213) to perform statistical analyses. Therefore, only results of the remaining four loci (CPH03, CHP07, CPH09, DB003) are discussed further.

TABLE 2.—Measures of genetic diversity at loci in swift foxes. n = individuals per population, A = number of alleles per locus, He = Hardy-Wienberg expectation of heterozygosity, Ho = observed heterozygosity, f = inbreeding coefficient, P = probability of Hardy-Weinberg equilibrium

Locus	Population	n	A	He	Ho	f	P
CPH03	Control blood	13	5	0.766	0.692	0.100	0.056
	Control scats	13	5	0.772	0.692	0.107	0.680
	2000 Survey	42	6	0.767	0.595	0.226	0.001
	2001 Survey	18	6	0.712	0.389	0.462	0.005
	Frozen tissue	13	5	0.745	0.769	-0.034	0.116
CPH07	Control blood	13	3	0.394	0.462	-0.180	1.000
	Control scats	13	3	0.342	0.385	-0.132	1.000
	2000 Survey	61	6	0.787	0.623	0.210	0.003
	2001 Survey	26	5	0.724	0.577	0.206	0.067
	Frozen tissue	14	4	0.669	0.786	-0.182	0.428
CPH09	Control blood	13	3	0.668	0.692	-0.038	0.894
	Control scats	13	3	0.680	0.615	0.099	0.667
	2000 Survey	29	3	0.670	0.448	0.335	0.070
	2001 Survey	17	4	0.713	0.471	0.347	0.010
	Frozen tissue	11	3	0.602	0.727	-0.221	0.770
DB003	Control blood	13	6	0.735	1.000	-0.381	0.327
	Control scats	13	6	0.751	0.923	-0.241	0.814
	2000 Survey	61	5	0.740	0.754	-0.019	0.203
	2001 Survey	25	5	0.770	0.800	-0.040	0.207
	Frozen tissue	14	5	0.728	0.714	0.019	0.933

All four loci were polymorphic for each population, and the number of alleles ranged from three to six. The two control samples (blood and scat) and the frozen tissue sample all were in Hardy-Weinberg equilibrium (Table 2). The 2000 survey scat sample was deficient in heterozygotes at the CPH3 and CPH7 loci, and the 2001 survey scat sample was deficient in heterozygotes at the CPH3 and CPH9 loci (Table 2).

The control blood and scat samples were essentially identical genetically and showed no population substructure based upon estimates of Theta (Table 3). Theta values for the 2000 and 2001 surveys indicated very little genetic subdivision from one year to the next (Table 3).

DISCUSSION

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The most efficient technique for determining relative abundance of swift fox in New Mexico was collection of scats followed by species verification using DNA analysis. Scent stations and trapping were the second and third most efficient techniques, respectively. In our study area scats were easily found, especially when an accumulation was present. We were able to find scats in areas where there was no evidence of swift foxes from other techniques, such as trapping or bait/camera stations. Extracting mitochondrial DNA from scats for species identification is relatively straight forward; but, depending on the quality of the sample scats, it may require multiple extractions in order to obtain DNA for PCR and sequencing. Using scats, we detected swift foxes within all known and probable home ranges in both the 2000 and 2001 surveys. We detected swift foxes at greater percentages of individual locations by collecting scats than by using scent stations or by trapping in

TABLE 3.—Values of Theta among swift fox populations. Confidence intervals (95%) for values of Theta are in parentheses

	Control blood	Control scats	2000 Survey	2001 Survey
Control scats	-0.030 (-0.020/-0.04)			
2000 Survey	0.061 (-0.005/0.140)	0.059 (-0.019/0.159)		
2001 Survey	0.028 (-0.010/0.069)	0.026 (-0.019/0.082)	0.014 (-0.108/0.045)	
Frozen tissue	0.061 (0.007/0.111)	0.059 (-0.009/0.122)		0.045 (0.000/0.106)

both 2000 and 2001. Our results were based upon visual examination of conspicuous locations along the survey route and collection of every scat sighted. We simply collected the scats available and made no effort to ensure that the samples of scats were fresh. It was not necessary in our area to use more intensive methods of locating scats (Smith *et al.*, 2001). Deposition of lure at collection sites before surveys may increase the proportion of sites where scats are found, but may not increase the number of scats collected. Deposition of lure before surveys doubles the amount of necessary field time, and in our opinion is not necessary, given the ease of collecting scats.

Sovada and Roy (1996) reported detection percentages of 30–70% when collecting scats along walking transects on roads within the home ranges of radiocollared swift foxes in Kansas. They cleared all scat from transects 2 wk before surveys. Thus, their detection percentages may have been much higher if they had used all scats available. Olson *et al.* (1997) reported a detection percentage of 66% from collecting scats by walking 1 km transects within the cores of known swift fox home ranges in Wyoming. Neither Sovada and Roy (1996) nor Olson *et al.* (1997) verified the species depositing the scats they collected. It is important to verify the species depositing scat, as the diameters of scats of several species overlap. Approximately 60% of the samples of coyote scats collected by Danner and Dodd (1982) and 32% of the coyote samples collected by Green and Flinders (1981) had diameters between 10 and 20 mm, overlapping 96% and 41%, respectively, of the scats we identified as coming from swift fox. Also, the range of diameters of red fox (*Vulpes vulpes*) scats collected by Green and Flinders (1981) is exactly the same (8–20 mm) as we found for swift foxes (Fig. 3), with the exception of one 7-mm swift fox scat.

The number of scats that must be collected to verify presence/absence in a given area depends primarily upon the success of DNA extraction. In our study collection of at least 10 scats from each site would have been adequate for confirming the presence of swift fox at 98% of sites examined.

Swift fox detection percentages on transects of scent stations within known home ranges were nearly maximized at three nights for all foxes and four nights for radiocollared foxes only, and at three stations per home range for all foxes and four stations per home range for radiocollared foxes only. Given the observed average swift fox home range size of approximately 2200 ha and assuming circular home ranges, placing five 5, 4, 3, 2 or 1 station evenly spaced in a home range requires a spacing between stations of 1.0, 1.3, 1.7, 2.6 or ≥ 5.2 km, respectively. In practice, the number of stations that may be set likely will be limited by the time available and size of the area to be surveyed. For range-wide scent-station surveys in New Mexico, scent-station transects consisting of stations spaced at 1.6 km (1.0 mile) intervals and operated for three nights are the most practical. For more intensive

examination of specific areas, operation for an additional night would produce approximately the same increase in detection percentages as decreasing the spacing to 1.3 km.

Depending upon fox density and level of effort, percentages of detection of swift foxes on transects of scent stations within known home ranges varied from 20–100% (Figs. 1, 2). Detection percentages decreased when the sample based upon all observed foxes (Fig. 1) was reduced to radiocollared foxes only (Fig. 2), indicating that visitation rates are affected by fox density. Schauster (2001) also found that scent-station detection percentages correlated consistently with swift fox density, although the significance of correlations varied between observation periods. Using transects of four stations placed 0.3 km apart and observed for 7 nights within the core areas of swift fox home ranges, Olson *et al.* (1999) observed detection percentages of 66–88%. Using transects of 16 stations placed 0.5 km apart and observed for 3 nights, Sovada and Roy (1996) observed detection percentages of 10–70%, or 100% if survey periods were combined.

We operated bait/camera stations (our surrogates for scent stations) at a separation of 1.6 km (equivalent to three stations per home range) and primarily for four nights. Thus, the detection percentages we observed at bait/camera stations were likely nearly maximized. The actual detection percentages observed at bait/camera stations (31.4%, 47.1%) were lower than observed from scent stations because scent station percentages were based upon visits to transects, which require only a single visit to any station to be considered a visit to the entire transect.

Bait/camera stations differed from scent stations that would be used in a statewide survey by the presence of bait (rather than a scented lure only) and camera units, and the absence of a prepared tracking surface. The detection percentages observed at bait/camera stations might have been greater than what would have been observed at scent stations in a statewide survey because of the presence of bait and because it was not necessary for a fox to step within the area of the prepared tracking surface. We used bait in addition to a lure in order to avoid habituating study foxes to the smell of the lure. In statewide surveys, foxes would likely be attracted to a novel lure, but foxes within the study area were tested repeatedly and might have responded less to the same lure over time. However, some foxes may have been frightened by the appearance or sounds of the camera units and may have been unwilling to approach close enough or for long enough to be photographed. At scent stations these distractions would not be present. Occasionally, foxes were photographed running away from the camera in the first photograph of a series and were not photographed in the remaining three photographs of the series. Also, on 12 station-nights during resighting surveys the infrared beam was broken, but no animal was photographed. Thus, there are compensating factors when using bait/camera stations as surrogates for scent stations. The results from observations at bait/camera stations were most likely reasonable approximations of the results that would have been obtained from scent stations during the resighting surveys.

Because we trapped before resighting and scat collection surveys, the swift fox population may have been somewhat lower during resighting and scat collection due to over-winter mortality. Hence, the trapping percentages we observed may be slightly higher relative to resighting and scat collection than would have been true if all three activities occurred simultaneously. The relatively low efficiency of trapping and potential of injury to captured animals eliminates trapping from useful survey techniques.

Track, spotlight and calling surveys are not efficient techniques in New Mexico. Precipitation is too irregular and soils in general are too hard and dry to take and hold identifiable swift fox tracks. Also, soil types vary within the range of swift foxes in New Mexico (R. Harrison, pers. obs.). Spotlighting is useful primarily as a supplement to other techni-

ques, particularly during nighttime periods when other techniques cannot be pursued. Harrison and Schmitt (in press) spotlighted one fox per 550 km of driving when surveying the entire range of swift fox in New Mexico. Sovada and Roy (1996) reported spotlighting detection percentages of 16–32% for radiocollared swift foxes in Kansas. Calling is limited by wind noise and the potential to disturb homeowners must be considered. In our study foxes appeared to be wary of vehicles and may have been reluctant to approach. Calling also requires the most extreme behavioral response by foxes: approaching an occupied vehicle.

In areas of swift fox range outside of New Mexico, the relative values of scat and track searches may be reversed from our results. DNA from scats is degraded quickly by mold, which may be a significant problem in wetter environments. However, tracks are more easily observed where soils are wetter. The efficiencies of scent stations, trapping, spotlighting, calling and trapping/resighting in other areas are likely to be similar to our results.

The sample size (number of transects) necessary to detect a change in population size may be estimated assuming a binomial distribution of transect visits (Zar, 1984:399). The results depend upon the level of Type I and Type II errors required. A Type I error (α error) results when it is believed the population changed when it really did not. A Type II error (β error) occurs when a real population change is not detected. In conservation situations Type I errors are less important than Type II errors; thus, α may be relaxed from the standard 0.05, whereas β should be relatively low, such as 0.1 or 0.05. For example, to decide whether or not a decrease of 20% in the proportion of transects visited between 2 y represented a real population decrease and, assuming $\alpha = 0.2$, $\beta = 0.1$, and first-year detection percentage = 60%, 65 independent transects each year would be required. Assuming $\alpha = 0.1$ and $\beta = 0.1$, 90 transects would be required. That many transects is practical.

The required number of independent transects determines the minimum area that can be sampled. For transects to be independent, they should be separated by at least one average home range diameter in all directions. Surveys of 65–90 transects require a minimum area approximately the size of counties in New Mexico. Lack of road access will enlarge the minimum area sampled. For comparison, Harrison and Schmitt (in press) used 80 transects to survey the entire range of swift foxes in New Mexico, which covers approximately the eastern one-quarter of the state.

Surveys for scats are the most costly of the relative abundance techniques examined here. We estimated the current cost to survey the complete range of swift foxes in New Mexico, including obtaining and analyzing a sample of 200–400 scats from 90 transects, to be \$20,000–\$30,000. Scent-station and trapping surveys require similar levels of effort. We estimated the current cost of a scent-station or trapping survey conducted over four nights to be \$15,000. The field time required for a survey for scats would be at most two months, whereas the field time required for scent-station or trapping surveys conducted over four nights could be as great as 6 to 10 mo. Because scent-station surveys are less expensive than scat surveys, the use of scent stations remains an important option.

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The absolute population size estimates obtained from microsatellite genotypes were considerably higher than those obtained from bait/camera stations. Kohn *et al.* (1999) also reported a genotype population estimate higher than a population estimate obtained from a conventional survey technique (trapping). Two factors may lead to overestimating the number of unique genotypes and the number of individual swift foxes present. First, scat samples provide nuclear DNA of low quality and quantity, resulting in allelic drop-out and incomplete amplification, leading to overestimation. Errors in assigning genotypes are

difficult to avoid (Taberlet *et al.*, 1999) and can affect population estimates dramatically (Waits and Leburg, 2000). We confirmed the prediction of Waits and Leburg (2000) that population estimates based upon genotypes may be much greater than estimates based upon conventional techniques. Second scats may remain recognizable for several months (Kohn *et al.*, 1999). The number of transient foxes included in the microsatellite population estimate potentially includes all those passing through the study area within several months, and not just those foxes present in the survey area when the survey was conducted. Furthermore, obtaining nuclear DNA for individual identification is problematic. Whereas 10–2500 copies of mitochondrial DNA can occur in one cell, only a single copy of nuclear DNA is present (Kohn and Wayne, 1997). Solutions to these problems would be to collect only fresh samples of scats (more frequent field collections) and to perform multiple DNA extractions and PCR experiments for each scat sample (Taberlet *et al.*, 1999; Waits and Leburg, 2000). Such solutions could increase costs significantly.

In general, trapping, combined with resighting at bait/camera stations, worked well. With use of this technique we did not violate the assumption that individual animals captured (trapped) during the first survey are less likely to be captured (photographed) during the second survey (Pollock *et al.*, 1990). However, infrared-triggered camera units are expensive (currently ca. \$675/station, including Trailmaster 1500 active infrared game monitor with photographic software and camera, protective boxes, mounting stakes, film and film processing). The high cost of the camera resighting technique limits its use to small areas. Trapping and retrapping is less expensive than trapping and resighting, although trapping and retrapping does violate some statistical assumptions.

To estimate the absolute abundance of swift foxes in their entire range in New Mexico, transects of bait/camera stations could be used to generate local density estimates, which could be extrapolated to fill available habitat. Assuming that 90 transects and 50 camera units were used or 200–400 scats collected, we estimated the current costs of one trapping and resighting survey to be approximately \$90,000, and one survey for scats, including microsatellite identification of individuals, to be approximately \$30,000–\$50,000.

We found that the technique of analysis of microsatellite genotypes from scats is useful for addressing population genetic structure of swift foxes. The two populations described by control blood and scat samples were essentially identical genetically, as we expected because the sources of DNA were from the same swift foxes. However, there were slight differences in the genotypic data as a result of allelic drop-out. The three populations described by the two scat surveys and the frozen tissue samples were similar (Table 3), even though DNA obtained from survey scats was of a lower quality than that obtained from control scats and blood. The frozen tissue sample population provided the genetic structuring we expected from the scat surveys. This population was in Hardy-Weinberg equilibrium for all loci, whereas the scat samples were not (Table 2). The frozen tissue sample was collected at a different period of time than the survey scat samples, which could account for some of the observed variation among samples. Because the data from the scat samples were not notably different from the other sources of swift fox populations, we believe we have properly assessed the genetic structure of the swift fox population in the survey area. Hence, our estimates of the numbers of unique genotypes in the study area were as valid as the method and DNA available from scats permitted. The survey scat samples had lower observed heterozygosities compared with the other populations, indicating that there was allelic drop-out during the PCR amplification procedure of the survey scats. Thus, it is likely that the microsatellite genotype technique overestimated the number of individuals present on our study site.

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