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Non-invasive baseline genetic monitoring of the endangered San Joaquin kit fox on a photovoltaic solar facility

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ABSTRACT: Survival of endangered San Joaquin kit foxes *Vulpes macrotis mutica* is challenged by reduced and fragmented habitat resulting from anthropogenic uses. We monitored kit foxes on the 40 km^2 proposed site for the Topaz Solar Farms (TSF) in San Luis Obispo County, California, which consisted of 76% agricultural fields and 24% grasslands. Prior to construction of the solar facility in December 2011, we used professionally trained dog-handler teams to conduct non-invasive genetic surveys annually from 2009 to 2011. We analyzed mtDNA to identify species, zinc finger genes for sex determination, and microsatellite loci to define individuals. We identified 45 individuals from 351 fresh scat samples (26 females, 18 males, and 1 individual of unknown sex), and recaptured 5 individuals between years. Kit foxes predominantly used the grasslands and rarely used agricultural fields. Samples from the TSF population had similar levels of genetic diversity to 2 areas less than 20 km away in the northern end of the Carrizo Plain National Monument. Capwire and LDNe estimates of population size using samples collected during annual November surveys indicated that ~33 individuals used the TSF over a 3 yr period. The relatively high population estimate, low recapture rates, and similar genetic diversity to 2 nearby locations suggest that individuals using the TSF site are part of a larger population using the surrounding landscape. Our study provided baseline data that, when coupled with future surveys, will help assess the effects on San Joaquin kit foxes of solar facility construction and habitat regeneration on agricultural lands removed from production.

KEY WORDS: Non-invasive surveys · Solar facility · Monitoring · Endangered species · Kit fox · *Vulpes macrotis mutica* · Detection dog · Scat

INTRODUCTION

Habitat loss and fragmentation are major causes of species extinction. Desert habitats have long been impacted by roads, railroads, fossil fuel development, urbanization, and irrigated agriculture but now face a new concern: large-scale solar power plants (Cypher et al. 2013, Katzner et al. 2013). The production of solar energy can have long-term benefits for the environment, including minimizing fossil fuel harvest and consumption and reduction of greenhouse gas emissions. However, large-scale solar facilities

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require sufficient expanses of relatively inexpensive land where solar energy potential is high. Such lands are usually located far from human population centers in areas where threatened and endangered species occur, and this is creating a conflict between solar energy development and the preservation of biodiversity throughout the arid southwestern United States (Lovich & Ennen 2011, Cameron et al. 2012, Stoms et al. 2013).

The Topaz Solar Farms (TSF) project in California is one of the world's largest solar facilities under construction. It is located in the northern end of the Carrizo Plain, which is separated from the San Joaquin Valley by the Temblor Range. The Carrizo Plain, with over 250 000 acres (ca. 101 000 ha) of arid scrub and grassland habitats protected within a National Monument, is an important area for species adapted to similar habitats of the San Joaquin Valley. The majority of these habitat types are now extremely rare within the Valley itself due to conversion of land to agricultural production and urban areas (Kelly et al. 2005, Cypher et al. 2013). The federally endangered and state threatened San Joaquin kit fox *Vulpes macrotis mutica* is one of 13 endangered species that occur in the Carrizo Plain, and requires the largest amount of habitat. By protecting the habitat required by kit foxes, it is likely that habitat required by some of the other species in the ecosystem will also be preserved (US Fish & Wildlife Service 1998).

To reduce impacts on endangered species in the area (Lovich & Ennen 2011, Cameron et al. 2012, Stoms et al. 2013), TSF is being constructed mostly on degraded habitat in the form of agricultural lands. A larger study area including potential solar project sites was initially identified to investigate variation in kit fox use across 2 predominant habitat types: cropland and grassland. Cropland was either fallowed or active, while grassland included grazing areas with high-forb content or annual grassland with many introduced and native species. The variation in habitat quality provided an opportunity to learn about patterns of habitat utilization by kit foxes on the site before, during, and after construction of the solar facility.

We monitored kit foxes in the study area prior to construction of the solar facility, annually from 2009 to 2011. We used well-established methods for conducting non-invasive genetic surveys (Schwartz et al. 2005, Cullingham et al. 2010, De Barba et al. 2010, Bozarth et al. 2011, Dutta et al. 2013, Lampa et al. 2013) that were developed specifically for San Joaquin kit foxes (Smith et al. 2003, 2006a, Ralls et al. 2010). In brief, conservation detection dog-handler teams searched transects for kit fox scats, and DNA from the fresh scats was subsequently analyzed using molecular genetic methods to identify species, sex, and unique genotypes of individuals (Ortega et al. 2004, Smith et al. 2006b, Bozarth et al. 2010).

The primary objectives of our study were to document the presence of San Joaquin kit foxes on the site and nearby properties, estimate the number of individuals present and the extent to which they used different habitats, and compare levels of genetic diversity between foxes on the TSF site and the Carrizo Plain National Monument. Our results were used to suggest placement of solar panel array fields and establish a pre-construction baseline of kit fox use. We will conduct similar surveys in future years to document combined effects of construction of the solar facility and changing habitat conditions on kit fox use of the site.

MATERIALS AND METHODS

Study area

The TSF project is located approximately 100 km west of Bakersfield, in the northern end of the Carrizo Plain, San Luis Obispo County, California (Fig. 1). It is northwest of the Carrizo Plain National Monument, which contains over 607 km^2 of scrub and grassland habitat suitable for kit fox, and is home to the largest remaining San Joaquin kit fox population (Bureau of Land Management 2010). A project Biological Resource Study Area of ~40 km^2 that included cropland and grassland habitats (see Fig. 2) was initially identified as a potential site for investigation. The northwest portions of the study area contained poor quality kit fox habitat that had been farmed for about 70 yr. Cropland was plowed yearly and grazed by cattle after harvest, leading to low numbers of small mammals (P. W. Collins 2010 unpubl. report, Results of small mammal trapping on portions of the Topaz Solar Farm project site in California Valley, Sections 4, 5, 15, 26, 28, 32-35, San Luis Obispo County, CA) and few underground kit fox dens (D. Meade pers. obs.). The southern and eastern portions had relatively better quality habitat, consisting of a mosaic of active cropland, recently fallowed cropland, and annual grassland. Cattle grazed these habitats either once a year or once every 3 yr, producing varied habitat suitability for kit foxes and their prey. The final layout of the

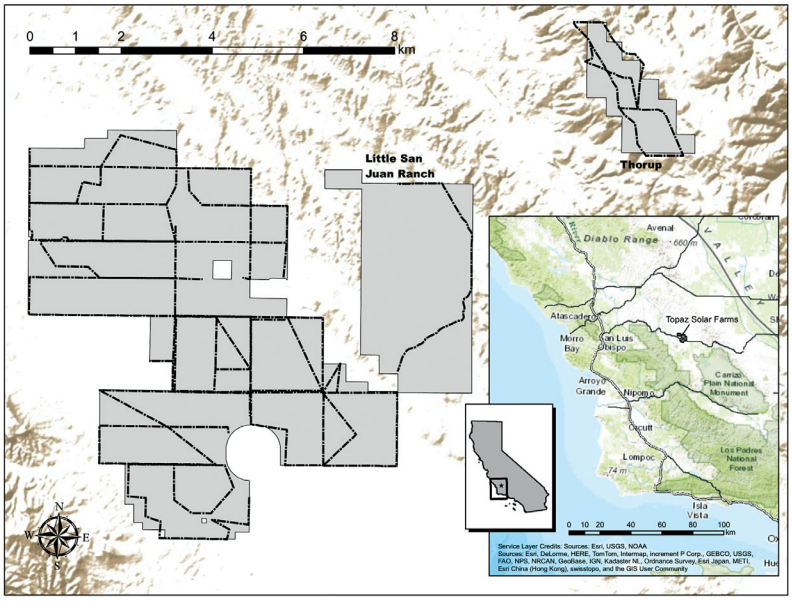

Fig. 1. Study areas with survey transects shown in dashed black lines. The main survey area (approximately 40 km^2) is located in the Carrizo Plain, San Luis Obispo County, California. The Topaz Solar Farms project is being built on 14.2 km^2. The inset maps show the location within California

TSF project is a subset of the study area with a maximum size of 14.2 km^2. Grassland vegetation grows in the aisles between and under the solar panels, and the remaining area outside of the perimeter fences will be restored to annual grassland.

Field collection of scat samples

Transects

From 18 August to 1 September 2009, and 11 November to 21 November 2009, search routes were established along 18 transects (approximately 103.31 km) running throughout the entire study area (Fig. 1). We designed transects to bisect hypothetical kit fox home ranges multiple times using previous estimates of an average home range size of approximately 4 to 11 km^2 for kit foxes in the Carrizo Plain and similar habitats (Spiegel & Bradbury 1992, White & Ralls 1993, Zoellick et al. 2002, B. Cypher pers. comm.). Transect routes utilized unpaved roads, trails, fence-lines, and vegetation in the study area. From 2 to 17 November 2010, and 1 to 16 November 2011, search routes were slightly adjusted after a final project layout was adopted. A total of 17 transects (approximately 108.04 km) were surveyed throughout the new study area boundary (Fig. 1). All surveys from 2009 to 2011 included an initial and a repeat survey session that were 1 wk apart. We also surveyed 2 private parcels east of the Topaz study area (the Little San Juan Ranch and Thorup properties; Fig. 1), in November 2010. Presence of kit foxes on these properties would confirm that they could be purchased and set aside as protected lands to partially offset the impacts of developing the solar facility. To keep track of each group and carry out appropriate comparisons, we separated the data into sampling groups: Aug 2009 TSF, Nov 2009 TSF, and 2009 All; Nov 2010 TSF, Nov 2010 surveys from the additional Little San Juan Ranch and Thorup properties ('Nov 2010 SJT'), and 2010 All; Nov 2011; all 3 yr during November on TSF ('3 yr Nov TSF'); and All Combined (all times, all locations).

Scat samples

Professional conservation detection dog-handler teams (Working Dogs for Conservation, Three Forks, MT) searched for scats using previously established methods that have been shown to increase detection and accuracy rates while also avoiding contamination of the fox scat by the dogs (Smith et al. 2003, Hurt & Smith 2009). Only fresh scats were collected for DNA analysis as determined by a freshness rating method based on their physical characteristics (Smith et al. 2003). Scats determined to be more than 8 d old were not collected, but locations for all scats detected by the dog-handler teams were geo-referenced and recorded using Global Positioning System (GPS) units (Garmin GPS III+). Fresh scats were stored in plastic bags with silica gel for desiccation (Fisher Scientific) and shipped to the Smithsonian Center for Conservation and Evolutionary Genetics laboratory in Washington, DC, for analysis.

Mapping

X–Y coordinates of each transect and the GPS location of each scat were entered into ArcGIS (ESRI Geographical Information System), and plotted over an aerial photograph with project boundary lines. Scat samples were also mapped with reference to genetic results (i.e. by species or individual identification).

Molecular methods

DNA extraction

DNA was extracted from a small piece taken from each scat sample using the QIAamp DNA stool mini kit (QIAGEN®) with modifications from the manufacturer's protocol as in Eggert et al. (2005) and an extended overnight incubation in lysis buffer and proteinase K at 56°C on a shaker. Extractions were carried out in a separate room dedicated to DNA extractions of samples from a diversity of sources including scat, hair, blood and tissue samples. This room has a positive pressure air handling system to separate the extraction laboratory air supply from sample preparation and downstream PCR applications in the main lab. Negative controls (no scat) accompanied each set of extractions and were used to check for contamination. In addition, in order to check for repeatability, DNA was extracted twice from a small subset of samples; once the methods were validated, the rest of the samples were extracted once.

Species identification

Conservation detection dogs can detect more scats and with greater accuracy in identification of species than humans (Hurt & Smith 2009). However, a handler may collect non-target scat when a dog correctly locates a latrine containing fresh scats from multiple canids (i.e. fox/coyote; Ralls & Smith 2004) and the handler unwittingly gathers scat from the non-target species, when a dog errs in scent discrimination and keys on a similar (yet incorrect) target, or when a dog selects an incorrect target when few target scats are present in order to receive a reward (Schoon 1996, Smith et al. 2003). Therefore, we used our mtDNA protocol to determine species for all scats collected (Bozarth et al. 2010). This protocol amplified a short fragment of the mtDNA control region, which is a different length in every canid species in the study area, and can be run on a sequencer as an amplified fragment length polymorphism (AFLP). Fragment length varies by species as follows: kit fox = 237 or 252–53 bp; red fox = 260–64 bp; coyote = 279–83 bp; dog = 286 bp; and gray fox = 288 bp. PCR reactions were set up as follows: a 20 µl total volume consisting of 9.3 µl of PCR water, 2.0 µl of 10× PCR buffer (No $MgCl_2$), 1.5 µl of 10 µM DNTP (2.5 µM each), 1.0 µl of primer KFSPID-F and 1.0 µl of primer KFSPID-R, 2.0 µl of $MgCl_2$ (25 mM), 0.2 µl of AmpliTaq Gold, and 3.0 µl of substrate DNA. Reactions were denatured at 95°C for 10 min, followed by 35 cycles of 95°C denaturing for 30 sec, 58°C annealing for 30 sec, and 72°C extension for 2 min, then a final extension of 72°C for 30 min, and stored at 4°C. Samples with poor amplification were diluted (1:15 up to 1:45) to minimize interference from PCR inhibitors in scat samples, and replicated as needed.

Molecular sexing

We determined the sex of the animal that deposited each scat using protocols developed in our laboratory with primers that have good specificity for canids, minimize the chances of errors due to prey item contamination, and increase amplification success (Ortega et al. 2004, Ralls et al. 2010). We amplified a small fragment (195 bp) of the zinc finger protein gene, found in both X- and Y-chromosomes, and digested the PCR product with a *Taq* I restriction enzyme yielding a clear pair of fragments for males and a single uncut fragment for females (Ortega et al. 2004, Ralls et al. 2010).

Genotyping and identification of individual kit foxes

After samples were positively identified as kit foxes, we genotyped them for individual identification using 6 microsatellite tetranucleotide repeat loci that were developed from domestic dogs (Francisco et al. 1996) and proven to reliably work for individual identification of kit foxes in our lab (*FH2137*, *FH2140*, *FH2226*, *FH2535*, *FH2561*, *Pez19*; Smith et al. 2006b). We assessed our ability to differentiate individuals by estimating the probability of a random match between unrelated individuals for all multilocus genotypes at 6 microsatellite loci (P_{ID} unbiased) and the probability of a random match between siblings (P_{ID} sibs) (Mills et al. 2000, Waits et al. 2001).

For each DNA extract, we performed at least 5 independent PCR amplifications of each locus for homozygous individuals to verify allele size and detect allelic drop out. We ran heterozygotes a minimum of 3 times to confirm both alleles. We amplified microsatellites in 10 µl volumes using 4.35 µl of PCR water, 1.0 µl of 10× PCR buffer, 1.0 µl of 10 µM DNTP (2.5 µM each), 0.25 µl of forward primer and 0.25 µl of reverse primer, 1.0 µl of $MgCl_2$ (25 mM), 0.15 µl of AmpliTaq Gold, and 1.25 µl of substrate DNA. The PCR conditions for scat extracts, as well as extract

and PCR negative controls, included an initial hot start, 35 cycles of the following profile: 94°C for 1 min, 58°C for 1 min, and 72° C for 1.5 min, and a final extension at 72°C for 30 min. We used up to 45 additional cycles to re-amplify samples with poor amplification success, usually from low starting DNA concentrations or dilutions to avoid PCR inhibitors found in scat.

We used fluorescently labeled forward primers (TET, HEX or FAM) in all of the PCR reactions (for species ID, sex ID, and microsatellite loci). We combined PCR product (1.0 to 2.5 µl) with 9.0 µl of a 5:100 mix of Gene Scan ROX-500 (Applied Biosystems) and Hi-Di Formamide (Applied Biosystems) to visualize our fragment sizes on an ABI PRISM* 3130 Genetic Analyzer (Applied Biosystems), which allowed for a plate of the 384 PCR reactions to be loaded at once. We analyzed samples using Genemapper® software to determine the size of each fragment.

We used the Excel Microsatellite Toolkit (Park 2001) to compare genotypes and defined individuals by unique genotypes and samples with matching alleles at all loci. We checked genotypes that differed at only 1 or 2 loci for accuracy of genotype and data entry. We used the program DROPOUT to look for scoring errors and allelic dropout using the chi-square homogeneity test (McKelvey & Schwartz 2004). We also compared genotypes between samples collected in 2009, 2010, and 2011 to determine if any individuals had been recaptured between survey sessions. We assigned recaptured individuals the same number previously used. In addition, we used sex to differentiate between closely related individuals that shared microsatellite genotypes.

Genetic variability

To obtain a more robust estimate of genetic diversity, we genotyped individuals at 5 additional microsatellite loci (*AHTh171*, *FH2054*, *FH2328*, *FH2848*, and *Ren162*). Since we had previously determined that we had enough power to distinguish individuals using the original 6 microsatellite loci (P_{ID} = 9.8 × 10^{-6}; see below), we selected 1 representative scat sample that amplified most reliably for each individual and genotyped these samples for additional loci using the same protocols and conditions described above. A summary of the workflow for scat sample processing is shown in Fig. S1 in the Supplement at www.int-res.com/articles/suppl/n027p031_supp.pdf. We then compared genetic variability of kit foxes in the study area to that of kit foxes in the Carrizo Plain National Monument using tissue samples collected during 2 previous studies: 32 kit foxes trapped near Soda Lake Rd between 1988 and 1991 (White & Ralls 1993) and 29 individuals trapped along Elkhorn Rd in 1998 (Bean 2002). We genotyped samples for the 11 microsatellites in the same manner as described for the TSF samples, except that, because tissue extractions yielded high quality/quantity DNA, fewer PCR replicates (2 to 3) per sample were required to obtain reliable genotypes. We tested all 3 groups (TSF, Soda Lake Rd, and Elkhorn Rd) for deviation from Hardy-Weinberg expectations and for linkage disequilibrium between loci using GenePop (Raymond & Rousset 1995). We also used GenePop to determine allelic diversity and expected and observed heterozygosity values at each locus.

Demographic parameters and Capwire estimates

To obtain estimates of the number of San Joaquin kit foxes using the study area, we analyzed samples collected during the annual November collection periods using 2 methods: LDNe as implemented in NeEstimatorV2 (Do et al. 2014) and Capwire (Miller et al. 2005). This version of LDNe estimates contemporary effective population size (Ne) from linkage disequilibrium (Waples 2006). It deals well with real-world microsatellite data sets by excluding rare alleles (Waples & Do 2008) and including a correction for missing data (Peel et al. 2013). We calculated contemporary Ne using the lowest allele frequency cutoff of 0.10 and the monogamy setting, and report results with 95% confidence intervals from the jackknife method, which has been shown to perform better than parametric confidence intervals (Waples & Do 2008). Capwire uses the number of samples per individual (as identified by genetics) as an estimate of the number of times we captured an individual and then infers probabilities of detection in the population. Capwire does urn simulations using 2 models, the equal capture probability model (ECM) and the two innate rates model (TIRM), to determine which model best fits the data. The appropriate model is chosen using a likelihood-ratio test, and TIRM is used when capture rate is heterogeneous between individuals. Capwire is similar to the Chao or jackknife estimators, or the Eggert's rarefaction estimator as calculated in the program Gimlet, which all use the resampling of individuals to estimate Ne, but Capwire performs as well if not better than the other rarefaction methods (Valière 2002).

Table 1. Species, sex, and individual identification of scat samples from San Joaquin (SJ) kit foxes *Vulpes macrotis mutica* from all surveys. Numbers of individuals recaptured are listed after the colon. The number of samples completely genotyped and assigned to an individual is listed in parentheses. Example: # detected: # recaptured (# associated scats). TSF: Topaz Solar Farms; SJT: Little San Juan Ranch and Thorup properties

Survey		Scat	San Joaquin kit foxes			Non-targets	
Date	Location	samples	Scats	Female	Male	Red fox	Coyote
Aug 2009	TSF	91	72	7 (37)	9 (35)	1 (1)	0
Nov 2009	TSF	62	48	3:1 (12)	8:7 (36)	1:1 (2)	0
Nov 2010	TSF	69	49	4:1 (22)	3:1 (17)	2:0 (16)	1:0 (3)
Nov 2010	SJT	16	16	3:0 (7)	3:1 (9)	0:0	0
Nov 2011	TSF	113	94	10:1 (49)	6:0 (33)	(8[a])	(3[a])
				+1 ind. of undetermined sex[b]			

[a]Not identified to individual; [b]Nov 2011 survey

Fig. 2. Location of the 279 San Joaquin kit fox (*Vulpes macrotis mutica*) scat samples successfully genotyped, out of 351 fresh scat samples collected from 2009 to 2011, shown with habitat types on the Topaz Solar Farm survey area and additional properties. Locations of known kit fox dens (active and natal) are also shown

RESULTS

We collected 351 fresh scat samples over the 3 yr (Table 1, Fig. 2). The dogs also located 617 older scats during the same time period. Using our mtDNA protocol, we were able to determine the species for 312 (89%) of the fresh scats. Of those 312 scats, 279 (89.4%) were San Joaquin kit fox, 27 (8.7%) were red fox *Vulpes vulpes*, and 6 (1.9%) were coyote *Canis latrans* (Table 1). Over all 3 yr, only 10.6% (n = 33) of the samples we collected were from non-target species. All but 1 kit fox sample had the 252 bp mtDNA haplotype; scat DT1014 had the shorter 236 bp kit fox haplotype first reported in Bozarth et al. (2010). We identified kit fox scats predominantly on the southern and eastern portions of the TSF (Fig. 2).

Genetic variation

Multiple PCR replicates allowed us to identify and remove samples with poor amplification (less than 3 loci), as well as confidently score alleles across loci with consistent shape profiles and repeatability (as described in Bonin et al. 2004). Because alleles were scored by 1 of 3 people and a large subset of samples was scored by more than 1 person, allele scoring was consistent across samples and years. We found a low level of spurious alleles (0.6%) and allelic dropout (3.8%) across sample genotypes. A significantly higher number of missing alleles were found at *Pez19* using DROPOUT ($p \leq 0.05$ with Bonferroni correction), but dropout at this locus did not prohibit assignment of samples to individuals. The probability of a random match between unrelated individuals for all multilocus genotypes of 6 microsatellite loci was 9.8×10^{-6} (P_{ID} unbiased), and the probability of a random match between siblings for all multilocus genotypes was 9.7×10^{-3} (P_{ID} sibs). These low P_{ID} values indicated that our 6 microsatellites were adequate to differentiate between individual foxes, including relatives. We assigned 258 of the 279 kit fox samples to an individual genotype (Table 1). The scat sample with the 236 bp mtDNA haplotype also had a unique microsatellite genotype. Sex was identified for all but 1 individual (TZ33). Our microsatellite genotyping confirmed the presence of 45 individuals over the 3 yr, including 18 males, 26 females, and 1 individual of unknown sex, for an overall 0.7:1 sex ratio of males to females. Number of scat samples per individual varied from 1 to 18, with an average of 5.3 samples for each individual detected in a given year. Scats from the same

individual were found in close proximity to each other, on the same or adjacent transects, and generally <3 km apart (see Figs. S2–S4 in the Supplement). Three individuals detected in 2009 were also found in 2010, one of which was found on the TSF property in 2009 and on the Little San Juan Ranch property in 2010. One female (TZ21) was found in the same part of the study area (south-eastern corner) in 2 yr and was the only individual identified in 2010 that was found again in 2011 (see Figs. S3 & S4 in the Supplement). Active and natal dens were also identified in the same area during both of these years.

Table 2. Genetic diversity statistics for kit fox *Vulpes macrotis mutica* detected on the Topaz Solar Farm, Soda Lake Rd, and Elkhorn Rd sites in California, based on 11 microsatellite loci. n = number of individuals; H_e = expected heterozygosity; H_o = observed heterozygosity; N_A = average number of alleles; N_{A5} = average number of alleles ≥5%; N_E = average number of effective alleles; $N_{p\ =}$ average number of private alleles; SE = 1 standard error from the mean

Location	n	H_e (SE)	H_o (SE)	N_A (SE)	N_{A5}	N_E	N_P
Soda Lake	32	0.649 (0.052)	0.647 (0.026)	6.46 (3.17)	4.091	3.510	0.636
Elkhorn	29	0.674 (0.046)	0.684 (0.027)	6.55 (3.33)	4.364	3.735	0.455
Topaz	45	0.670 (0.040)	0.627 (0.022)	6.55 (2.21)	3.909	3.364	1.091
Overall	106	0.686 (0.044)	0.649 (0.014)	8.73 (4.00)	4.088	3.537	0.780

The allelic diversity in the 11 microsatellite loci screened in individuals from the TSF ranged from 3 to 10 alleles locus^{-1}, with a mean number of 6.55 alleles locus^{-1}, which was very similar to the results for the Soda Lake and Elkhorn samples (Table 2). The most polymorphic locus was *FH2137* with 10 alleles, and the least polymorphic was *Pez19* with only 3 alleles (Table S1 in the Supplement at www.int-res.com/articles/suppl/n027p031_supp.pdf). The individuals at TSF also carried the highest number of private alleles (7 for Soda Lake, 5 for Elkhorn, and 12 for TSF). We graphed the distribution of allele frequencies from all loci using methods described in Luikart et al. (1998) (allele frequency classes from 0 to 0.1, 0.1 to 0.2, etc.) and found the expected L-shaped curve for all 3 locations (Fig. S5 in the Supplement). Analysis in GenePop revealed that all loci were under Hardy-Weinberg Equilibrium and none showed evidence of linkage disequilibrium. Allelic diversity and heterozygosity were not significantly different between individuals found on the TSF, Soda Lake Rd, or Elkhorn Rd (Table 2). The observed heterozygosities were also not significantly different than the expected values, nor did they differ significantly between populations or survey years on the study area.

Estimates of kit fox abundance

We used 170 samples that represented 32 individuals collected in November 2009 (n = 48), November 2010 (n = 39), and November 2011 (n = 83) to estimate kit fox abundance on the TSF. This total included 16 males, 15 females, and 1 individual of unknown sex. We used Capwire and LDNe to estimate the number of kit foxes for each November survey. Capwire chose the TIRM for each estimate, indicating heterogeneity in capture probability of individuals. Capwire estimated 11 ± 0 individuals for November 2009, 7 ± 0 ind. for November 2010, and 17 ± 1 ind. for November 2011, which is similar to the number of individuals identified by unique genotypes. Combining the November TSF samples for all 3 yr and all samples collected during the study produced Capwire estimates of 33 ± 1 and 46 ± 2 individuals, respectively (Fig. 3). Similarly, LDNe estimated 25.5 individuals (95% CI: 18.7–35.7) from the November surveys and 40.1 individuals (95% CI: 30.4–54.3) from all samples collected. In both cases, the Capwire estimates have a smaller 95% confidence interval that is included within the LDNe estimates. The latter 2 combined estimates are not estimates of individuals using the study site at any point in time, but represent an estimate of the total number of individuals that were exposed to sampling during the sam-

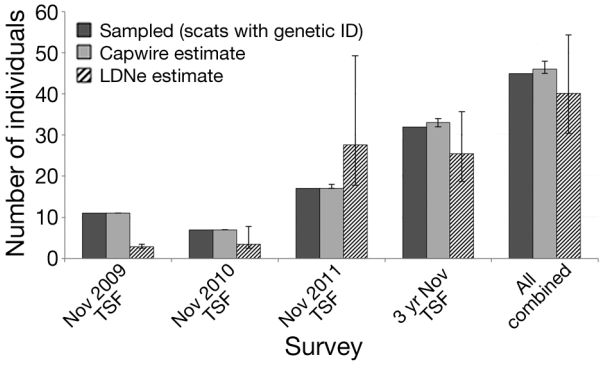

Fig. 3. Minimum number of kit fox (*Vulpes macrotis mutica*) individuals throughout the surveys. Dark grey bars: number of individual genotypes from scat surveys and genetic analysis; light grey bars: the corresponding Capwire estimates (error bars represent the Capwire min. and max. values); stippled bars: LDNe estimates (error bars represent 95% CI). 'All combined' refers to individuals detected in all of the surveys. TSF: Topaz Solar Farms

pling periods that were combined to produce the estimates, a measure that is called a super-population estimate (Schwarz & Arnason 1996). It is important to view these super-population estimates as likely referring to all individuals using the landscape rather than a population restricted to the core study area (Boulanger et al. 2004).

DISCUSSION

Our molecular analysis of scat samples was highly successful compared to other studies (Taberlet et al. 1997, Woods et al. 1999, Kohn et al. 1999); 88.9% species identification and 92.5% genotype assignment. We attributed our success in PCR amplification of problematic scat samples, particularly those that had a large proportion of insect exoskeleton remains, to stepwise dilution of samples to reduce PCR inhibitors but retain enough DNA so that amplification of the target regions was achieved. We also increased the number of PCR cycles for highly diluted samples and visualized all of our data on an automated sequencer, which allowed detection of small amounts of amplified DNA. Furthermore, we looked at sample locations for matching genotypes as described in Smith et al. (2006b) to increase our confidence in assigning samples to individuals. We found an average of 5.3 scat samples individual^{-1} yr^{-1} overall, and samples from individuals were clumped throughout the survey area (see Figs. S2–S4 in the Supplement). We collected a similar number of scat samples from males and females over all 3 yr (Table 2), indicating that both sexes were equally detectable at this time of year, in contrast to the drastic reduction in female scat samples found during reproductive denning in the spring (Ralls et al. 2010). We detected 45 individuals, 40 individuals that used the TSF study area at some time during the 3 yr, and 6 that used the Little San Juan Ranch and Thorup properties (1 individual used the TSF and Little San Juan Ranch).

The results of our analysis of genetic variation with 11 microsatellite loci showed similar patterns for the foxes in the study area and those in the Carrizo Plain National Monument to the south. The 3 localities had similar levels of heterozygosity and allelic richness. Furthermore, they did not deviate from Hardy-Weinberg equilibrium or show signatures of linkage disequilibrium. However, we found a surprisingly high number of private alleles with low allelic frequency (Table S1 in the Supplement), which could suggest low dispersal rates even though all of the groups sampled are only 15 to 30 km apart. Distributions of dispersal distances in vertebrates are typically skewed towards short distances with an extended tail of longer distances (Koenig et al. 1996). Kit foxes follow this same pattern, and dispersing individuals have been found to only move a median of 4.5 km and an average of 8 km from their natal home range (Scrivner et al. 1987). These short average dispersal distances tend to result in same-sex foxes on adjacent home ranges that are more closely related than same-sex foxes that do not live on adjacent home ranges (Ralls et al. 2001). In addition to this fine-scale clustering of related foxes, the presence of private rare alleles might result from genetic drift, past demographic events, a recent introduction of a migrant from an unsampled location, or sampling effects, as we sampled the 3 localities in different years (from 1988 to 2011). There is no evidence of long-standing barriers to gene flow between kit foxes from the study area and the locations to the south, and future research can use this information about genetic diversity to detect changes in demography and connectivity.

We detected 1 individual (male TZ15, scat DT1014) with a unique mitochondrial haplotype (236 bp) and a unique microsatellite genotype. This shorter length mtDNA haplotype was recently discovered in the Ciervo–Panoche area located in the Diablo Mountain Range and had not been previously detected in any other area (Bozarth et al. 2010). This individual provides evidence that there may be some connectivity between kit foxes on the northern end of the Carrizo Plain and those in the Diablo Mountain Range, which is located approximately 150 km to the north (Fig. 1).

Annual grassland, if appropriately grazed, provides good habitat for San Joaquin kit foxes (US Fish & Wildlife Service 1998, Germano et al. 2012). However, kit foxes have limited ability to use agricultural lands (Warrick et al. 2007). Although they may range into agricultural lands at night (Warrick et al. 2007), they typically travel on natural or man-made paths and den on nearby less-disturbed, more natural lands. Our study confirms that kit foxes avoid agricultural lands more than any of the other available habitat types. We found that kit foxes primarily used suitable grassland habitat in the southern and eastern portions of the TSF site and seldom used the agricultural lands in the northern and western portions of the site (Fig. 2). Scat density and den locations over all 3 yr paralleled these locations of kit foxes. These results are concordant with radio-tracking studies that have documented the avoidance of annual crops or almond orchards when next to natural habitats in Lokern (Nelson 2005) or the Semitropic region in northern Kern County (B. Cypher

pers. comm.). Kit foxes also avoided crops when bordered by the Bena landfill or urban areas of Bakersfield (B. Cypher pers. comm.). Agricultural lands are unattractive to kit foxes due to a lack of underground den sites (Warrick et al. 2007), low plant diversity and decreased prey availability (P. W. Collins 2010 unpubl. report; for details see 'Materials and methods'). Attacks by larger predators, particularly coyotes, are the primary source of mortality for kit foxes (Ralls & White 1995, Cypher et al. 2000, Nelson 2005). As kit foxes maintain multiple dens in their home range (Moehrenschlager et al. 2004) and attempt to escape from danger by running to the nearest den (Ralls & White 1995), they are likely more vulnerable to larger predators when traveling across or near agricultural lands with few dens.

Monitoring of endangered species is likely to be challenging for solar projects since these projects are likely to be located where desert-adapted species are abundant but hard to detect. However, even small amounts of data from non-invasive monitoring can provide very useful information. We had few recaptures across years: 3 within the TSF and one detected first on the TSF and subsequently on the Little San Juan Ranch property. One of the recaptures on TSF was a female kit fox (TZ21) detected in 2010 and recaptured in 2011 in the same part of the study area. In both years, active and natal dens were documented in the same area where she left multiple scat samples. This suggests that she may have been a resident of the area and this transect lay within her home range (Figs. 2, S3 & S4). Several factors may have contributed to our low recapture rate. First, kit foxes have high annual mortality rates in adults and dispersing juveniles (Koopman et al. 2000, Moehrenschlager et al. 2004). Second, it is possible that some of the young foxes were dispersing late in the season (Koopman et al. 2000), and mortality or movement across the landscape prevented their subsequent detection. Third, some individuals in the area may have been using or traveling across the study area on an intermittent basis, and could not be sampled during every survey. Finally, we may have failed to detect some of the foxes using the site during each survey. Despite these challenges, the Capwire and LDNe abundance estimates closely matched the number of unique genotypes found in the area, which suggests that we detected a high proportion of the individuals present during each survey.

Our finding that kit foxes were present on the Little San Juan Ranch and Thorup properties showed that these properties were suitable as 'mitigation' properties to offset any impacts of developing the TSF. We found 1 individual on both the study area and Little San Juan Ranch, demonstrating that kit foxes could move between them. Conservation of these properties to the east of the project site as well as other grasslands in the TSF conservation program prevents future development and farming of surrounding habitat that is suitable for kit fox and is connected to the Carrizo Plain National Monument. Habitat conditions for kit foxes on the TSF are expected to gradually improve, as agricultural activities (apart from carefully managed grazing) were discontinued in 2011 and cropland will be used for solar panels or be allowed to gradually revert to a more natural condition.

This study provided critical baseline data on the presence, distribution, genetic variability and habitat utilization of an endangered carnivore, and the results from our surveys were used to suggest placement of the solar panel array fields on the TSF site and establish a pre-construction baseline for kit fox use of the site. Construction of the solar facility began in December 2011 and is expected to be finished by 2015. Kit foxes have already demonstrated that they will occupy the completed array areas and that they are able to move within the project areas, which contain aboveground solar panels. The project is installing perimeter fencing that allows kit foxes and other medium and small sized mammals to enter and leave the site while deterring coyotes. We will continue to conduct non-invasive monitoring surveys on the study area each year for an additional 6 yr. Baseline monitoring studies such as this one, when coupled with future surveys, can provide invaluable information to assess and document the combined effects of construction of the solar facility, exclusion of coyotes, and changing habitat conditions on kit fox use of the area.

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LITERATURE CITED

Bean EA (2002) An evaluation of distance and mark-resight sampling to estimate abundance of San Joaquin kit fox. MSc thesis, University of Minnesota, Minneapolis, MN

Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetics studies. Mol Ecol 13:3261–3273

Boulanger J, McLellan BN, Woods JG, Proctor MF, Strobeck C (2004) Sampling design and bias in DNA-based capture–mark–recapture population and density estimates of grizzly bears. J Wildl Manag 68:457–469

Bozarth CA, Alva-Campbell YR, Ralls K, Henry TR, Smith DA, Westphal MF, Maldonado JE (2010) An efficient noninvasive method for discriminating among faeces of sympatric North American canids. Conserv Genet Resour 2:173–175

Bozarth CA, Hailer F, Rockwood LL, Edwards CW, Maldonado JE (2011) Coyote colonization of northern Virginia and admixture with Great Lakes wolves. J Mammal 92: 1070–1080

Bureau of Land Management (2010) Carrizo Plain National Monument Approved Resource Management Plan and Record of Decision. Bureau of Land Management, Bakersfield, CA

Cameron DR, Cohen BS, Morrison SA (2012) An approach to enhance the conservation-compatibility of solar energy development. PLoS ONE 7:e38437

Cullingham CI, Curteanu M, Ball MC, Manseau M (2010) Feasibility and recommendations for swift fox fecal DNA profiling. J Wildl Manag 74:849–859

Cypher BL, Warrick GD, Otten MRM, O'Farrell TP and others (2000) Population dynamics of San Joaquin kit foxes at the Naval Petroleum Reserves in California. Wildl Monogr 145:1–43

Cypher BL, Phillips SE, Kelly PA (2013) Quantity and distribution of suitable habitat for endangered San Joaquin kit foxes: conservation implications. Canid Biol Conserv 16: 25–31

De Barba M, Waits LP, Garton EO, Genovesi P, Randi E, Mustoni A, Groff C (2010) The power of genetic monitoring for studying demography, ecology and genetics of a reintroduced brown bear population. Mol Ecol 19: 3938–3951

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014) NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol Ecol Resour 14:209–214

Dutta T, Sharma S, Maldonado JE, Wood TC, Panwar HS, Seidensticker J (2013) Fine-scale population genetic structure in a wide-ranging carnivore, the leopard (*Panthera pardus fusca*) in central India. Divers Distrib 19: 760–771

Eggert LS, Maldonado JE, Fleischer RC (2005) Nucleic acid isolation from ecological samples — animal scat and other associated materials. Methods Enzymol 395:73–87

Francisco LV, Langsten AA, Mellersh CS, Neal CL, Ostrander EA (1996) A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. Mamm Genome 7:359–362

Germano DJ, Rathbun GB, Saslaw LR (2012) Effects of grazing and invasive grasses on desert vertebrates in California. J Wildl Manag 76:670–682

Hurt A, Smith DA (2009) Conservation dogs. In: Helton WS (ed) Canine ergonomics: the science of working dogs. CRC Press, Boca Raton, FL, p 175–194

Katzner T, Johnson JA, Evans DM, Garner TWJ and others (2013) Challenges and opportunities for animal conservation from renewable energy development. Anim Conserv 16:367–369

Kelly PA, Phillips SE, Williams DF (2005) Documenting ecological change in time and space: the San Joaquin Valley of California. In: Lacey EA, Myers P (eds) Mammalian diversification: from chromosomes to phylogeography (a celebration of the career of James L. Patton). University of California Press, Berkeley, CA, p 57–78

Koenig WD, Van Vuren D, Hooge PN (1996) Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends Ecol Evol 11:514–517

Kohn MH, York EC, Kamradt DA, Haught G, Sauvajot RM, Wayne RK (1999) Estimating population size by genotyping faeces. Proc R Soc Lond B 266:657–663

Koopman ME, Cypher BL, Scrivner JH (2000) Dispersal patterns of San Joaquin kit foxes (*Vulpes macrotis mutica*). J Mammal 81:213–222

Lampa S, Henle K, Klenke R, Hoehn M, Gruber B (2013) How to overcome genotyping errors in non-invasive genetic mark-recapture population size estimation: a review of available methods illustrated by a case study. J Wildl Manag 77:1490–1511

Lovich JE, Ennen JR (2011) Wildlife conservation and solar energy development in the desert southwest, United States. Bioscience 61:982–992

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. J Hered 89:238–247

McKelvey KS, Schwartz MK (2004) Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. J Wildl Manag 68:439–448

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

Mills LS, Citta JJ, Lair KP, Schwartz MK, Tallmon DA (2000) Estimating animal abundance and noninvasive DNA sampling: promise and pitfalls. Ecol Appl 10:283–294

Moehrenschlager A, Cypher BL, Ralls K, List R, Sovada MA (2004) Comparative ecology and conservation priorities of swift and kit foxes. In: MacDonald DW, Sillero-Zubiri C (eds) Biology and conservation of wild canids. Oxford University Press, Oxford, p 185–189

Nelson JL (2005) Effects of varying habitats on competition between endangered San Joaquin kit foxes (*Vulpes macrotis mutica*) and coyotes (*Canis latrans*). MSc thesis, Montana State University, Bozeman, MT

Ortega J, Franco R, Adams BA, Rails K, Maldonado JE (2004) A reliable, non-invasive method for sex determination in the endangered San Joaquin kit fox (*Vulpes macrotis mutica*) and other canids. Conserv Genet 5:715–718

Park SDE (2001) Trypanotolerance in West African cattle and the population genetic effects of selection. PhD thesis, University of Dublin

Peel D, Waples RS, Macbeth GM, Do C, Ovenden JR (2013) Accounting for missing data in the estimation of contemporary genetic effective population size (Ne). Mol Ecol Resour 13:243–253

Ralls K, Smith DA (2004) Latrine use by San Joaquin kit foxes (*Vulpes macrotis mutica*) and coyotes (*Canis latrans*). West N Am Nat 64:544–547

Ralls K, White PJ (1995) Predation on San Joaquin kit foxes by larger canids. J Mammal 76:723–729

Ralls K, Pilgrim KL, White PJ, Paxinos EE, Schwartz MK, Fleischer RC (2001) Kinship, social relationships, and den sharing in kit foxes. J Mammal 82:858–866

Ralls K, Sharma S, Smith DA, Bremner-Harrison S, Cypher BL, Maldonado JE (2010) Changes in kit fox defecation patterns during the reproductive season: implications for noninvasive surveys. J Wildl Manag 74:1457–1462

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283

Schoon GAA (1996) Scent identification lineups by dogs (*Canis familiaris*): experimental design and forensic application. Appl Anim Behav Sci 49:257–267

Schwartz MK, Ralls K, Williams DF, Cypher BL, Pilgrim KL, Fleischer RC (2005) Gene flow among San Joaquin kit fox populations in a severely changed ecosystem. Conserv Genet 6:25–37

Schwarz CJ, Arnason N (1996) A general methodology for the analysis of capture-recapture experiments in open populations. Biometrics 52:860–873

Scrivner JH, O'Farrell TP, Kato TT (1987) Diet of the San Joaquin kit fox, *Vulpes macrotis mutica*, on Naval Petroleum Reserve #1, Kern County, California, 1980–1984. US Department of Energy Report No. 10282-2168, Goleta, CA

Smith DA, Ralls K, Hurt A, Adams B and others (2003) Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (*Vulpes macrotis mutica*). Anim Conserv 6:339–346

Smith DA, Ralls K, Cypher BL, Clark HO Jr, Kelly PA, Williams DF, Maldonado JE (2006a) Relative abundance of endangered San Joaquin kit foxes (*Vulpes macrotis mutica*) based on scat-detection dog surveys. Southwest Nat 51:210–219

Smith DA, Ralls K, Hurt A, Adams B, Parker M, Maldonado JE (2006b) Assessing reliability of microsatellite genotypes from kit fox faecal samples using genetic and GIS analyses. Mol Ecol 15:387–406

Spiegel LK, Bradbury M (1992) Home range characteristics of the San Joaquin kit fox in western Kern County, California. Trans West Sect Wildl Soc 28:83–92

Stoms DM, Dashiell SL, Davis FW (2013) Siting solar energy development to minimize biological impacts. Renew Energy 57:289–298

Taberlet P, Camarra JJ, Griffin S, Uhres E and others (1997) Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Mol Ecol 6:869–876

US Fish & Wildlife Service (1998) Recovery plan for upland species of the San Joaquin Valley, California. USFWS Region 1, Portland, OR

Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. Mol Ecol Notes 2: 377–379 -i2

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Mol Ecol 10:249–256

Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv Genet 7:167–184

Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol Ecol Resour 8:753–756

Warrick GD, Clark HO Jr, Kelly PA, Williams DF, Cypher BL (2007) Use of agricultural lands by San Joaquin kit foxes. West N Am Nat 67:270–277

White PJ, Ralls K (1993) Reproduction and spacing patterns of kit foxes relative to changing prey availability. J Wildl Manag 57:861–867

Woods JG, Paetkau D, Lewis D, McLellan BN, Proctor M, Strobeck C, Woods JG (1999) Genetic tagging of free-ranging black and brown bears. Wildl Soc Bull 27: 616–627

Zoellick BW, Harris CE, Kelly BT, O'Farrell TP, Kato TT, Koopman ME (2002) Movements and home ranges of San Joaquin kit foxes (*Vulpes macrotis mutica*) relative to oil-field development. West N Am Nat 62:151–159

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