Discovery of a Remnant Population of Sierra Nevada Red Fox (Vulpes Vulpes Necator)

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Abstract

The Sierra Nevada red fox (*Vulpes vulpes necator*) occurred historically throughout the high elevations of California's Sierra Nevada and southern Cascade Mountains. Before this study, the only known remaining population in California consisted of ≤ 20 individuals restricted to the Lassen Peak region in the southern Cascades. In August 2010, we photographed a red fox in the Sonora Pass area of the Sierra Nevada, > 100 km from the Lassen Peak region. To determine if multiple individuals were present and were indigenous, we set up additional camera stations, collected genetic samples (saliva, scat, hair, and a carcass), and conducted a comparative genetic analysis between these individuals and historical and modern reference samples. Photo-detections identified at least three individuals based on pelage characteristics. Genetic analyses identified two females and one male, whose microsatellite profiles suggested they were closely related. A genetic assignment analysis indicated that all three individuals clustered most closely (> 95%) with historical samples from the Sierra Nevada, and were distinct from those in the Lassen Peak region. Additionally, mtDNA and microsatellite alleles unique to each population confirmed that the Sonora Pass individuals represent a second remnant California population of Sierra Nevada red fox. Reduced genetic diversity relative to historical levels in both remnant populations was consistent with small populations. Follow-up surveys are needed to determine the abundance and distributional extent of the Sonora Pass population, combined with research on both populations to assess demographic trajectories, determine threats, and to inform conservation efforts.

Introduction

The Sierra Nevada red fox (*Vulpes vulpes necator*) is the most endangered of three subspecies of red fox inhabiting montane areas of the western United States (U.S.). The other subspecies are the Rocky Mountain red fox (*V. v. macroura*) and the Cascade red fox (*V. v. cascadensis*). Collectively, these three subspecies, along with Sacramento Valley red fox (*V. v. patwin*), reflect a distinct evolutionary lineage restricted to the western United States since the height of the last glaciation (Aubry et al. 2009, Sacks et al. 2010). The three montane subspecies are ecologically and morphologically distinct from the Sacramento Valley and other North American subspecies, suggesting they reflect a snow-adapted lineage of montane specialists (Roest 1977, Aubry 1983, Sacks et al. 2010). The historical range of the Sierra Nevada red fox in California extends from Tulare County in the southern Sierra Nevada northward along the mountain crest to Sierra County, and in the California Cascades around Lassen Peak and Mount Shasta (Grinnell et al. 1937, Figure 1). Grinnell et al. (1937) considered the northern extent of the subspecies to be the California/Oregon border, but Sacks et al. (2010) proposed that this be extended to include red foxes in the Cascade Mountains of Oregon based on the close genetic relationship and the contiguous montane habitat

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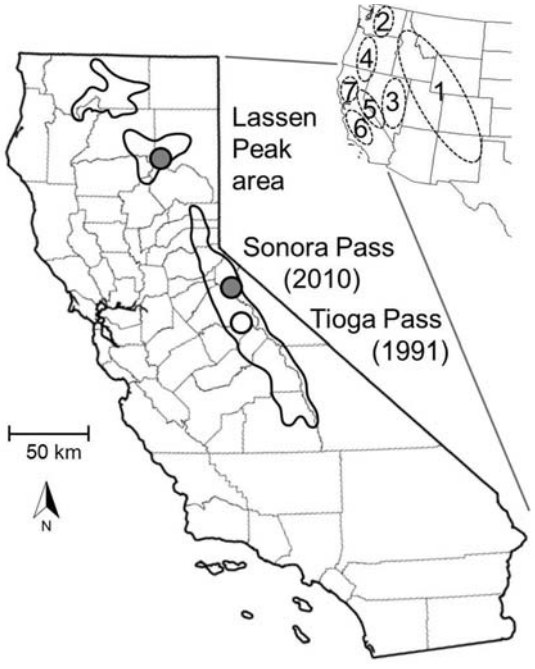

Figure 1. Historical range of Sierra Nevada red fox in California (solid black outlined polygons; Grinnell et al. 1937). White circle indicates the location of a red fox photograph taken in 1991 that was the last verifiable red fox sighting in the Sierra Nevada. Gray circles indicate the extant population in the Lassen Peak region of the southern Cascades and the site of the recent Sonora Pass detections in the Sierra Nevada. The inset map of the western USA indicates the approximate ranges of red fox reference populations analyzed in this study; 1) Rocky Mountains, 2) northern Cascades (historical), 3) Nevada State, 4) southern Cascades (historical), 5) Sierra Nevada (historical), 6) southern California (nonnative), and 7) Sacramento Valley.

between the Cascade Mountains of Oregon and California. Until recently, the only known extant population of this subspecies consisted of a small number of individuals, possibly ≤ 20, in the Lassen National Forest and Lassen Volcanic National Park (hereafter, collectively referred to as the "Lassen Peak region") of the southern Cascade Mountains of California (Perrine et al. 2010, Sacks et al. 2010, Figure 1), with occasional evidence indicating the existence of a small number of remnant individuals in the Cascades of southern Oregon (D. Clayton, J. von Kienast, K. Aubry, personal communications).

The Sierra Nevada red fox was thought to be relatively uncommon as far back as the early 1900s (Grinnell et al. 1937). In the mid-1900s the range contracted and numbers fell precipitously leading to a prohibition on trapping of red foxes in California in 1974. In 1980 the Sierra Nevada red fox was listed in California as "Threatened" (CDFG 2004). It is also currently classified as "Sensitive" by the U.S. Forest Service (U.S. Forest Service 2004). The last-known physical specimen from the Sierra Nevada-proper is a museum sample collected in 1941 from near Truckee, California (UC Berkeley Museum of Vertebrate Zoology, MVZ-95401). The last verified red fox in the Sierra Nevada was photographed in 1991 by Claudette Agard at 2940 m at the Tioga Pass near Yosemite National Park (Barrett and Golightly 1994, Figure 1). Despite extensive mesocarnivore surveys during the late 1990s and early 2000s in the Sierra Nevada and southern Cascades, red foxes were only detected in the Lassen Peak region (Zielinski et al. 2005, Perrine et al. 2010). This led to speculation that red foxes had been extirpated in the Sierra Nevada (Perrine et al. 2010).

On 11 August 2010, we photographed a red fox at a remotely triggered camera station in a survey sample unit for forest carnivores (Zielinski et al. 1995). The sample unit was located at the Sierra crest on the Humboldt-Toiyabe and Stanislaus National Forests (Figure 1). The actual detection was made using a Bushnell Trophy Cam digital camera, at 2900 m elevation, in subalpine conifer cover as defined by CWHR (California Wildlife Habitat Relationships). These initial images were overexposed, but the animal photographed had several diagnostic features including black markings on the back of the ears, black shins, and a white tail tip. Taken together, these features differentiate red foxes from the other wild canids in the region, coyote (*Canis latrans*) and gray fox (*Urocyon cinereoargenteus*).

In addition to confirming the species, it was important to assess whether the individual was indigenous, the result of a human translocation, or a natural migrant from a distinct population (e.g., Moriarty et al. 2009). Red foxes have been translocated to numerous locations in the western

U.S. from various sources (including Eastern Canada and Alaska), primarily in association with the fur-farming industry of the early to mid-1900s. As a result, escaped or released individuals have established localized nonnative populations, including in some lower-elevation portions of California (e.g., Lewis et al. 1993, Sacks et al. 2010, Statham et al. 2012). Alternatively, it was possible that this individual was a long-distance disperser from a native red fox population in the Lassen Peak region or a Great Basin mountain range from north-central Nevada (Sacks et al. 2010). To confirm the species and determine the population of origin required us to obtain DNA, and to sequence and genotype it for comparison to contemporary samples from other western native and nonnative populations and historical samples of Sierra Nevada red foxes (Sacks et al. 2010). Additionally, we wished to obtain and genotype additional samples in the area to determine whether other individuals were present.

Study Area

The focal area of data collection included a 30 km^2 area centered on Sonora Pass and overlapping the Humboldt-Toiyabe and Stanislaus National Forests (N 38.330, W 119.636). Locations of reference samples from throughout the western USA are described in detail elsewhere (Sacks et al. 2010, Statham et al. 2012).

Methods

Field Data Collection

Following the initial red fox photographic detection, we collected potential noninvasive genetic samples (hair at the camera station and saliva from a bait bag constructed from a cloth sock with chicken bait and scent lure to serve as an attractant) and initiated additional photographic and genetic survey efforts for red foxes in the area. Within one week scat searches were conducted along trails, ridges, and valleys within 1 km of the detection. We set up 11 additional remote camera stations using Cuddeback digital cameras within a 10 km radius of the original detection at elevations ranging from 1917–3041 m in subalpine conifer CWHR type habitat. The cameras were operated throughout the winter but the number deployed and number functioning at any one time was variable due to challenging weather conditions (n = 2-5). At six of the sites we included a hair snagging apparatus incorporating a series of 30-caliber rifle bore-cleaning brushes protruding from a strap affixed to the trunk below the bait (P. Figura, California Department of Fish and Game, unpublished protocol). Biweekly visits were attempted, subject to weather conditions and accessibility, to check cameras and search for and collect genetic samples. We also searched for tracks, scats, prey remains, and other sign of red foxes and sympatric mesocarnivores. All potential genetic samples were collected in individual paper envelopes to prevent cross contamination, fecal samples were later transferred to tubes with 95% ethanol.

Samples and DNA Extraction

In addition to noninvasive samples collected from bait bags, hair snares, and scats, we obtained a muscle sample from a yearling female red fox killed on state highway 395 on 12 January 2011. This animal was collected by California Highway Patrol approximately 18 km east of the initial red fox detection. The carcass was fresh, indicating that it had been killed either on the day of collection, or the previous day (4.5 months after the initial red fox detection). We extracted DNA from all noninvasive samples at a lab bench specifically designated for extraction of lower quantity/quality DNA samples. We examined two bait bags from camera stations where red fox were photographed to locate areas with residual saliva. On the bait bag recovered at the initial detection, we found multiple areas with signs of tooth punctures or tears. To recover DNA a portion of the material near the bite hole was cut out and digested in 500 µl 50 mM NaOH for 5 minutes at 96 °C. We then removed the cloth and added 75 µl 1M Tris (pH 8). The second bait bag lacked obvious signs of chewing. We attempted to extract DNA from areas that looked to have a dried residue by swabbing the area with a cotton swab soaked in phosphate buffered saline and then extracting using a DNeasy Blood and Tissue kit (Qiagen, CA). We recovered

a small number of hairs from the first bait bag and extracted the DNA by first digesting the follicles as per Pfeiffer et al. (2004), followed by purification using a modified phenol/chloroform method (Sambrook and Russell 2001). We extracted DNA from 10 scat samples using a QIAamp DNA Stool kit (Qiagen Inc.) and extracted DNA from a tissue sample from the road-killed animal using the DNeasy Blood and Tissue kit. We included an extraction blank with all extraction sets and included the extraction blanks in subsequent PCR and sequencing reactions to assess contamination. In all cases, blanks failed to amplify/sequence indicating a lack of contamination.

Genetic Analysis

Mitochondrial DNA—We used mtDNA to identify the species and, if the sample was from a red fox, the specific haplotype of all samples for comparison to our database of georeferenced sequences (described below). We amplified the 5′ region of the cytochrome *b* gene using primer pair RF14724 and RF15149 (Perrine et al. 2007) and of the D-loop using primer pair VVDL1 and VVDL6 (Aubry et al. 2009). The PCR products were purified using ExoSap-IT (Affymetrix, Inc.) and sequenced in both directions using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Inc.); we then electrophoresed products on an ABI 3730 capillary sequencer (Applied Biosystems, Inc).

We compared the resulting cytochrome *b* and D-loop haplotypes to previously published sequences to determine the geographic source of the red fox genetic samples (Perrine et al. 2007, Aubry et al. 2009, Sacks et al. 2010, Statham et al. 2012). These data included the only known population of montane red foxes in California (the Lassen Peak region, n = 23 individuals sampled during 2000-2010), along with modern and historical specimens from throughout the western mountains of the U.S. (Sierra Nevada, n = 23; southern Cascades, n = 14; northern Cascades, n = 19; Rocky Mountains, n = 51, Sacks et al. 2010), nearby native populations at lower elevations (Nevada, n = 13; Sacramento Valley, n = 42, Sacks et al. 2010, Statham et al. 2012), and a known non-native population (southern California, n = 46, Sacks et al. 2010, Statham et al. 2012).

Nuclear DNA—We PCR-amplified 14 microsatellite loci in 2 multiplex reactions as previously described to examine the nuclear DNA of samples identified as red fox (Sacks et al. 2010). To gender-type the samples, we amplified a portion of the canine amelogenin gene with homologues of differing lengths on the X and Y chromosomes (Moore et al. 2010). We genotyped all noninvasive samples $\geq$ 3 times at all 14 microsatellite loci. We used microsatellite genotypes to determine the number of distinct individuals sampled. Once individuals were identified, we genotyped the highest quantity DNA sample corresponding to each one at 21 additional loci (Moore et al. 2010) to investigate familial relationships. We quantified the genotyping error (both allelic dropout and false alleles) for each noninvasive sample based on replicate PCRs and genotypes and constructed composite (presumably error-free) genotypes for each sample. We then compared these genotypes for each sample to determine the number of unique individuals. We calculated observed (H_o) and expected (H_e) heterozygosity from the Sonora Pass genotypes for comparison to historical estimates.

We used a Bayesian model-based clustering approach implemented in the program STRUCTURE v 2.3.3 (Pritchard et al. 2000) to assess ancestry with respect to 159 samples from native red fox populations from throughout the western U.S.: northern Rocky Mountains, eastern Rocky Mountains, Nevada, northern Cascades, Sierra Nevada (historical), southern Cascades (historical and modern), and the Sacramento Valley (Sacks et al. 2010). In a previous analysis of this dataset, Sacks et al. (2010) found highest support for K = 3 and K = 4 when using the model with no prior location information; at K = 4 the historical Sierra Nevada population clustered distinctly from the remaining populations: Sacramento Valley, southern Cascades, and Rocky Mountains/Nevada-State/north Cascades. Therefore, to determine where the Sonora Pass individuals clustered, we ran the combined Sacks et al. (2010) and Sonora Pass datasets at K = 4 with no prior location in-

formation. Additionally, we ran an analysis with a geographically proximate subset of this dataset, including: modern Lassen peak region, historical southern Cascades, modern Nevada State, historical Sierra Nevada, and Sacramento Valley, along with nonnative red foxes from the San Joaquin Valley (n = 33). To determine the ancestral composition of Sonora Pass individuals relative to these six populations, we used the model with prior location-information at K = 6 (i.e., corresponding to the 6 reference populations). We ran all simulations using the admixture model with correlated allele frequencies (Falush et al. 2003) with a burn-in of 100,000, followed by 1,000,000 Markov chain Monte Carlo (MCMC) cycles.

Results

Field Data Collections

Red foxes were photographed at five of twelve camera stations, at elevations ranging from 2869 to 3041 m. The maximum distance between photographic detections was 7.4 km, while the nearest distances between photographic detections were 0.2, 0.2, 1.6, 3.3, and 5.6 km. The pelage coloration and patterns of red foxes photographed indicated at least 3 individuals; 1 red pelage and 2 distinct cross pelages (Figure 2).

Genetic Analysis

Mitochondrial DNA—Of the 13 noninvasive samples, we identified six samples originating from red fox (Table 1), as well as two from coyote, one from American marten (*Martes americana*), and one from golden-mantled ground squirrel (*Spermophilus lateralis*). These six red fox samples and the road-killed red fox all had the same cytochrome *b* and D-loop haplotype combination, C-34 (i.e., cytochrome *b* = C, D-loop = 34). This haplotype had previously been detected only in historical samples from Sierra Nevada and southern Cascades and not in any other red fox population (Table 2). Based on previous sequencing of 234 foxes throughout the western U.S. (Aubry et al. 2009, Sacks et al. 2010), haplotype C-34 had been detected in only two museum samples from the historical range of the Sierra Nevada red fox (Table 3). Haplotype J-34, which is one cytochrome *b* base-substitution from C-34, was found in 3 museum samples from the Sierra Nevada. A single museum specimen

Figure 2. Red fox photographs taken near the Sonora Pass using remote digital camera stations: A) red-pelage individual photographed at 2326 hrs on 8 October 2010, B) cross-pelage individual photographed at 0105 hrs on 7 September 2010, and C) cross-pelage individual photographed at 1315 hrs on 31 October 2010.

TABLE 1. List of samples collected from the Sonora Pass area and analyzed in this study and the mtDNA-based species type.

Sample ID	Sample Type	Species	Collection Date
S10-724	Bait bag1 (saliva)	Red fox	11 August 2010
S10-725	Bait bag1 (hair)	Red fox	11 August 2010
S10-876	Scat	Coyote	31 August 2010
S10-877	Scat	(Fail)	31 August 2010
S10-878	Scat	(Fail)	31 August 2010
S10-880	Scat	(Fail)	31 August 2010
S10-881	Scat	American marten	31 August 2010
S10-883	Scat	Red fox	4 September 2010
S10-884	Scat	Red fox	4 September 2010
S10-885	Scat	Red fox	4 September 2010
S10-887	Bait bag2 (swab)	Golden-mantled ground squirrel	23 September 2010
S10-1087	Scat	Coyote	13 December 2010
S10-1088	Scat	Red fox	13 December 2010
S11-008	Tissue	Red fox	12 January 2011

TABLE 2. Previously documented occurrences of the cytochrome *b* 'C' and D-loop '34' haplotypes in the western USA.

Population[1]	*n*	No. cytochrome *b* haplotype C (%)	No. D-loop haplotype 34 (%)	Combined haplotype C-34 (%)
Sierra Nevada (historical)	23	1 (4)	4 (17)	1 (4)
Southern Cascades (historical)	14	1 (7)	1 (7)	1 (7)
Lassen peak region (modern)	23	0 (0)	0 (0)	0 (0)
Nevada State Mountains (both)	13	1 (8)	0 (0)	0 (0)
Rocky Mountains (both)	50	0 (0)	0 (0)	0 (0)
Northern Cascades (both)	19	0 (0)	0 (0)	0 (0)
Sacramento Valley (both)	42	0 (0)	0 (0)	0 (0)
San Joaquin Valley (modern)	28	0 (0)	0 (0)	0 (0)

[1]Time periods are indicated in parentheses, where "both" indicates samples from historical and modern time periods.

TABLE 3. All known specimens identified with cytochrome *b* 'C' or D-loop '34' haplotypes.

Location	Accession number[1]	Collection year	Haplotype (cyt b-D-loop)
Sonora Pass area ($n = 7$)	(present study)	2010, 2011	C-34
East Sierra Nevada, 14 miles SW of Mono lake	MVZ33474	1923	C-34
Southern Cascades, 5 miles SE of Eagle Lake	MVZ35280	1925	C-34
East Sierra Nevada, Saddlebag Lake, near Tioga Pass	MVZ33587	1923	J-34
East Sierra Nevada, Between Ellery and Tioga Lakes	MVZ41004	1928	J-34
East Sierra Nevada, Marlette Lake near Lake Tahoe	MVZ69635	1934	J-34
Currant, Nye County, Nevada	USNM224077	1917	C-?

[1]Prefixes of the accession numbers correspond to UC Berkeley's Museum of Vertebrate Zoology (MVZ) and the National Museum of Natural History (USNM).

collected in 1917 from Currant, Nevada was identified with Cytochrome *b* haplotype C, but attempts to sequence the D-loop portion of the haplotype were unsuccessful (Sacks et al. 2010). Thus, based on the more rapidly mutating D-loop portion of the mtDNA haplotype, all known cases of haplotype 34, both historically and presently (i.e., this study), were from within the historical range of the Sierra Nevada red fox.

Nuclear DNA—We obtained complete genotypes (i.e., at all 14 microsatellite loci) for four red fox samples from the Sonora Pass area, corresponding to three distinct individuals: 1) the saliva (S10-0724) and hair (S10-0725) from the initial site, 2) a scat sample from < 1 km away (S10-1088), and 3) the road-killed animal (S11-0008; Table 4). Multiple genotyping replicates on the tissue sample were identical, while replicates of the noninvasive samples (S10-0724, S10-1088) resulted in two instances consistent with allelic dropout, resulting in an average genotyping error rate of 1.4%. Additionally two scat samples (S10-0883, S10-0885) provided low quantity DNA insufficient for genotyping at all 14 loci but provided allelic data at 13 and 8 loci, respectively, consistent with the genotype of individual 2, accounting for allelic dropout (Table 4). The remaining scat sample (S10-0884) did not provide usable microsatellite data.

Genetic gender-typing agreed with physical examination of the road killed animal, a female, and indicated the noninvasive samples were male and female (Table 4). Average observed heterozygosity (H_o) among the 3 Sonora Pass area individuals was 50.0% (SD 8%), which was higher than that of the modern Lassen population, 41.7% (SD 3%), and lower than that of the historical Sierra Nevada population, 55.2% (SD 3%), but the differences between the small Sonora Pass sample and the other two samples were not statistically significant. Additionally, H_o was underestimated for the historical population due to elevated allelic dropout associated with these museum samples (Sacks et al. 2010). Expected heterozygosity (H_e) is less sensitive to allelic dropout and therefore provided a less biased comparison between historical and modern samples. The H_e estimate for the historical Sierra Nevada population, 64.0% (SD 5%), was considerably higher than that for both the modern Sonora Pass, 50.0% (SD 6%), and Lassen, 54.0% (SD 4%), populations. Even with the small sample size in Sonora Pass, the difference in H_e between the historical Sierra Nevada (1-tailed 95% confidence limit = ≥ 0.62)

TABLE 4. Microsatellite and sex-marker genotypes of three red foxes identified from DNA in the vicinity of the Sonora Pass in California. Bolded alleles indicate errors due to allelic dropout. Italicized loci tentatively exclude parent-offspring relationships.

Sample	Individual	H_o	Sex[a]	AHT140	c01.424	FH2004	FH2010	FH2088	FH2289
S10-0724	1	0.57	F	*157/157*	181/181	216/216	224/232	*123/131*	213/213
S10-0725	1			157/157	181/181	216/216	224/232	123/131	213/213
S10-1088	2	0.64	M	155/157	181/181	216/216	224/232	*127/127*	213/213
S10-0883	2			155/157	181/181	216/216	224/232	127/127	213/213
S10-0885	2			155/**155**	181/181	216/216	-/-	127/127	-/-
S11-0008	3	0.36	F	*155/155*	181/181	212/216	224/232	*123/131*	213/213

Sample	Individual	FH2380	AHT133	FH2328	RF08.618	RF2001	RF2054	RF2457	RFCPH2
S10-0724	1	*177/177*	154/172	131/153	190/202	132/158	*194/194*	288/294	*107/107*
S10-0725	1	177/177	154/172	131/153	190/202	132/158	194/194	288/294	107/107
S10-1088	2	169/177	154/172	131/131	190/202	132/158	*194/198*	288/294	101/107
S10-0883	2	169/177	154/**154**	131/131	190/202	-/-	194/**194**	288/**288**	101/107
S10-0885	2	-/-	154/**154**	-/-	190/**190**	-/-	-/-	**294**/294	101/107
S11-0008	3	*169/169*	154/172	131/153	202/202	132/132	*178/178*	288/288	*101/101*

[a]Sex types indicate genetic sex of all 3 individuals. Individual 3 also was verified by physical examination.

TABLE 5. Q values indicating the proportional assignment of 3 Sonora Pass individuals to 6 geographically proximate red fox populations using the program Structure with prior information. Rows indicate the populations of origin and columns indicate the populations of assignment. Highlighted values on the diagonal are the proportional self-assignment of each of the reference populations.

Population[a]	*n*	SN	SV	SJ	SC	NV	LP
Sierra Nevada (historical)	22	**0.85**	0.04	0.01	0.01	0.07	0.01
Sacramento Valley	41	0.00	**0.61**	0.20	0.12	0.00	0.06
San Joaquin (nonnative)	33	0.01	0.01	**0.91**	0.01	0.06	0.00
Southern Cascades (historical)	7	0.07	0.09	0.06	**0.54**	0.22	0.01
Nevada State (modern)	11	0.01	0.01	0.01	0.01	**0.83**	0.14
Lassen Peak region (modern)	22	0.02	0.00	0.00	0.02	0.01	**0.94**
Sonora 1		**0.96**	0.01	0.01	0.00	0.01	0.02
Sonora 2		**0.96**	0.01	0.01	0.01	0.01	0.02
Sonora 3		**0.95**	0.01	0.01	0.00	0.01	0.02

[a]SN = Sierra Nevada, SV = Sacramento Valley, SJ = San Joaquin Valley, SC = Southern Cascades, NV = Nevada State, LP = Lassen Peak region.

and modern Sonora Pass (1-tailed 95% CL ≤ 0.62) estimates were of sufficient magnitude to achieve significance at alpha = 0.05.

Although we only identified a small number of red foxes at the Sonora Pass we were able to make some inferences about the sampled individuals. We tentatively excluded parent-offspring relationships for all pairs of individuals based on one or more locus-mismatches (i.e., where they did not share any alleles; Table 4). However, none of these pairs was based on double heterozygotes, indicating the possibility of allelic dropout despite our replicated genotyping. When we compared the genotypes of the three individuals typed at a further 21 loci (a total of 35 loci) we were able to exclude a parent-offspring relationship between individuals 1 and 2 at three loci, between 1 and 3 at six loci, and between 2 and 3 at six loci. The two individuals (one male, one female) sampled noninvasively at the Sonora Pass (individuals 1 and 2) shared more alleles (75 % shared alleles) than either did with the road-killed female (3). Individuals 1 and 3 shared 53.6 % of their alleles and individuals 2 and 3 shared 60.7 % of their alleles. These values were consistent both with full-sibling relationships and with unrelated individuals of a small inbred population. Therefore, a wider sampling of allele frequencies from the population is needed to assess familial relationships and reconstruct pedigrees.

Using no prior location information, the assignment analysis clustered the three Sonora Pass individuals with the historical Sierra Nevada population with q-values indicating 97.9 % ancestry on average (range: 97.6-98.2%). We obtained similar values for $K = 5$, where the Sonora Pass individuals were assigned 97.9 % on average to the historical Sierra Nevada population. Lastly, using prior location information for reference specimens, including non-native red foxes, the three Sonora Pass individuals assigned 95.6 % (SD 0.8 %) to the historical Sierra Nevada population (Table 5). This assignment value was higher than average self-assignment among historical Sierra Nevada samples, 85.2 % (SD 9 %).

Discussion

Mitochondrial and microsatellite analyses indicated that the red foxes detected in the Sonora Pass area reflected a relict population of the Sierra Nevada red fox. All seven red fox samples from this region exhibited the same haplotype, C-34. The D-loop component of this haplotype, 34, previously had been identified only in historical (pre-1941) Sierra Nevada red fox specimens where it was relatively common. Similarly, analysis of nuclear DNA assigned the modern Sonora Pass red foxes to the historical Sierra Nevada population. We observed no shared mitochondrial haplotypes between modern samples from the Lassen Peak region

and the Sonora Pass area and modern samples from these locations did not co-assign, indicating a lack of gene flow.

During this study we photographed both red- and cross-pelage red foxes. In contrast, several colleagues have shared numerous photographs of red foxes with us from the Lassen Peak region over the past 18 years, all of which have been red or red/blonde-pelaged individuals, and intensive remote camera surveys in the Lassen Peak region have failed to detect any cross or black/silver pelage animals (Perrine 2005, Perrine et al. 2010, P. Figura, California Department of Fish and Game, unpublished data). Prior to this study, the most recent physical evidence of a cross-pelage fox in the Sierra Nevada was a specimen collected near Marlette Lake, Nevada in 1934, currently housed in the UC Berkeley Museum of Vertebrate Zoology (MVZ-69635). Red-pelage animals were historically more common in northern California, while cross-pelage (and, to a lesser extent, silver/black) were more common in the Sonora Pass area. For example, cross-pelage foxes purportedly made up half of all individuals trapped between the Sonora and Mammoth passes over the course of seven years (Grinnell et al. 1937). The allele necessary for cross-pelage coloration is not present in red-pelage foxes (Våge et al. 1999). Thus the presence of cross-pelage individuals in the Sonora Pass area provides further indication of continuity with the historical population, and separation from the Lassen population.

Although montane red foxes as a whole have experienced a range wide increase in fragmentation over the past century, there is evidence to suggest that populations in the Sierra Nevada and southern Cascades may have had a degree of isolation historically (Sacks et al. 2010). Mitochondrial DNA indicates that the historical southern Cascades and Sierra Nevada populations were probably connected in the Holocene, but microsatellite analyses of historic specimens support Grinnell et al.'s (1937) range map, indicating a gap between these populations at least as far back as the early 1900s (Sacks et al. 2010). A similar gap in distribution is also seen in a sympatric mesocarnivore, the fisher (*Martes pennanti*), and recent genetic evidence indicates that northern and southern lineages have been separate for thousands of years (Knaus et al. 2011).

Explanations for such a gap are unclear. Red foxes have been recorded to disperse considerable distances. In the American Midwest, juvenile male red foxes have been reported to disperse an average of 30 km, while females disperse an average of 10 km, with 5% of the nearly 200 foxes studied dispersing over 80 km within their first year (Philips et al. 1972). The 100 km straight-line distance between the small populations of the Lassen Peak region and the Sonora Pass region consists of a substantial amount of elevational differences, numerous types of land use, and associated changes in habitat type, which may present a long term barrier to dispersal and, therefore gene flow.

Another finding of this study with important conservation implications was that red foxes were documented at the crest of the Sierra Nevada in both summer and winter (e.g., 10 August at 2900 m and 13 December at 3060 m). In general, red foxes are territorial and, therefore, breeding pairs are expected to maintain year-round territories (e.g., Aubry 1983), making this observation unsurprising in some regards. However, a telemetry study in the Lassen population found evidence that at least some individuals moved downslope in winter, suggesting the possibility of seasonal migration in Sierra Nevada red foxes (Perrine 2005). The individuals collared in that study did not breed and these movements could have reflected unsuccessful dispersal movements rather than those characteristic of the breeding segment of the population. Our documentation of foxes at high elevations in winter suggests that breeding Sierra Nevada red foxes, like red foxes elsewhere, remain seasonally resident. However, more data are clearly needed to understand space use of Sierra Nevada red foxes.

The current range of the Sierra Nevada red fox in California apparently consists of two small, geographically isolated populations that could be susceptible to extirpation by stochastic events, with little likelihood of bolstering or buffering by migration of individuals from other known populations. Ongoing survey efforts in the Sonora Pass area aim to document the extent and range of the

recently found population. The present occurrence of Sierra Nevada red foxes in the Oregon portion of the historical range is virtually unknown. Thus, we echo the calls of others (e.g., Sierra Nevada red fox conservation assessment, Perrine et al. 2010) for extensive historical range-wide surveys, including collection of DNA samples, which are needed to determine the current range of the subspecies and to provide information essential to the conservation of this subspecies.

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