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Gene flow among San Joaquin kit fox populations in a severely changed ecosystem

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Abstract

The San Joaquin kit fox (*Vulpes macrotis mutica*) was once ubiquitous throughout California's San Joaquin Valley and its surrounds. However, most of its habitat has been lost to irrigated agriculture, urban development, and oil fields. The remaining foxes are concentrated in six areas, although there are several small pockets of foxes throughout the Valley. To help conserve kit foxes, we sought an ecological understanding of the level of genetic variation remaining in these locations and the extent of gene flow among them. We collected tissue from 317 kit foxes from 8 sites and estimated genetic variability in and gene flow among sites using data from 8 polymorphic, microsatellite markers. We found no differences in both observed and expected heterozygosity between locations using Bonferonni corrected paired *t*-tests. We found differences in mean number of alleles per locus, even after we used Monte Carlo simulations to adjust for sample size differences. Population subdivision was low among sites ($F_{st} = 0.043$), yet a matrix of pairwise F_{st} values was correlated with a matrix of pairwise geographic distances. An assignment test classified only 45% of the individuals to the site where they were captured. Overall, these data suggest that kit fox dispersal between locations may still maintain genetic variation throughout most of the areas we sampled.

Introduction

The San Joaquin kit fox (*Vulpes macrotis mutica*) was once ubiquitous throughout California's San Joaquin Valley and its surrounding lands. However, most of its habitat has been converted from valley and foothill grassland, arid shrub, and oak savanna to irrigated agriculture, urban development, and oil fields. More than 50% of suitable kit fox habitat has been lost since Grinnell et al. (1937) first described the historical range (US Fish and Wildlife Service 1998). Kit foxes currently exist in suitable habitat in the San Joaquin Valley, side valleys, and surrounding foothills of the coastal ranges; the Sierra Nevada; and the Tehachapi Mountains. The highest densities of foxes exist in the southern portion of their range (US Fish and Wildlife Service 1998). In part because of this major habitat reduction, the kit fox was one of the first sub-species to be designated as "Endangered" by the US Department of Interior (US Fish and Wildlife Service 1967).

The remaining habitat for San Joaquin kit foxes consists of patches varying in size, quality,

and connectivity. Consequently, current kit fox distribution exhibits considerable heterogeneity with regard to presence and abundance. In some locations, kit foxes are relatively abundant and are continuously present. In other locations, kit foxes are less abundant and their presence may be intermittent.

Although several of the larger populations of San Joaquin kit foxes have been well studied (e.g., Ralls and White 1995; Cypher and Frost 1999; Cypher et al. 2000) little information exists concerning between-population dynamics. Koopman et al. (2000) reported that 0–79% of juvenile males (mean = 40.2%) and 0–50% of juvenile females (mean = 18.4%) dispersed from the Naval Petroleum Reserves in California (NPRC), depending on the year. Most of these dispersers died in less than 10 days. Scrivner et al. (1987) found no significant difference in the distance moved by male and female dispersers from NPRC; dispersers moved a median of 4.5 km and a mean of 8 km from their natal home ranges (Scrivner et al. 1987). However, some dispersers traveled long distances and one individual from NPRC moved greater than 120 km (Scrivner et al. 1987).

Historically, kit fox populations may have acted like a classic metapopulation, with some local extinction caused by wide annual fluctuations in food resources and hence fox reproductive success (White et al. 1996; Cypher et al. 2000) and occasional recolonization of less optimal habitat by dispersers in good years. However, it is unclear whether recolonization is still possible given the extensive habitat loss and degradation in the San Joaquin Valley, and the establishment of non-native red foxes (*Vulpes vulpes*) in some areas of historical kit fox range (US Fish and Wildlife Service 1998; Cypher et al. 2000). Therefore, we thought it is important to identify isolated and partially isolated populations, because small, isolated populations are often subject to both demographic and genetic stochasticity that increases the probability of population extinction (Berger 1990; Newman and Pilson 1997; Saccheri et al. 1998). If isolated populations are identified, the negative effects of isolation can be mitigated by managing populations to ensure low levels of connectivity (Brown and Kordrik-Brown 1977; Allendorf and Phelps 1981; Mills and Allendorf 1996; Tallmon et al. 2004).

Migration rates among wild populations can be estimated by quantifying genetic differentiation between populations, which at equilibrium under neutral models is the balance between migration and genetic drift (Wright 1969; Slatkin 1985). Unfortunately, migration rates calculated by these methods may be more reflective of historical migration than current migration (Whitlock and McCauley 1999), even when based on highly variable DNA markers such as microsatellites. Several authors have suggested ways to separate historical versus current gene flow (Slatkin 1995; O'Ryan et al. 1998). We use these approaches, plus combined field and laboratory data to make inferences about current versus historical gene flow of kit fox in the San Joaquin Valley. We also use gene flow measures to test if there is a sex bias in dispersal between female and male San Joaquin kit foxes (Stow et al. 2001; Goudet et al. 2002). We then interpret these gene flow patterns in light of the San Joaquin kit fox's natural history and ecology, and the profound habitat alteration in the San Joaquin Valley.

Methods

Samples and populations

We collected San Joaquin kit fox tissue samples (e.g., tissue or blood collected from animals trapped for radiotelemetry studies) from six areas considered to contain important kit fox populations (US Fish and Wildlife Service 1998): (1) the Carrizo Plain National Monument, San Luis Obispo Co., (35°18′ N, 119°52′ W), (2) the Naval Petroleum Reserves in California located in western Kern Co. (35°8′ N, 119°27′ W), (3) Bakersfield (35°22′ N, 119°01′ W), (4) the Panoche-Ciervo Natural Area in western Fresno-eastern San Benito counties (36°36′ N, 120°50′ W), (5) Camp Roberts (35°52′ N, 120°48′ W), and (6) Lokern Natural Area (35°24′ N, 119°33′ W). We also collected samples in two small populations occurring at Lost Hills (35°37′ N, 119°42′ W) and Los Baños (37°18′ N, 120°29′ W; Figure 1). In Bakersfield and NPRC we were able to obtain two samples temporally separated ($n_{\mathrm{NPRC}} = 46$ and 70; $n_{\mathrm{Bakersfield}} = 8$ and 87; sample sizes from other populations are reported in Table 1).

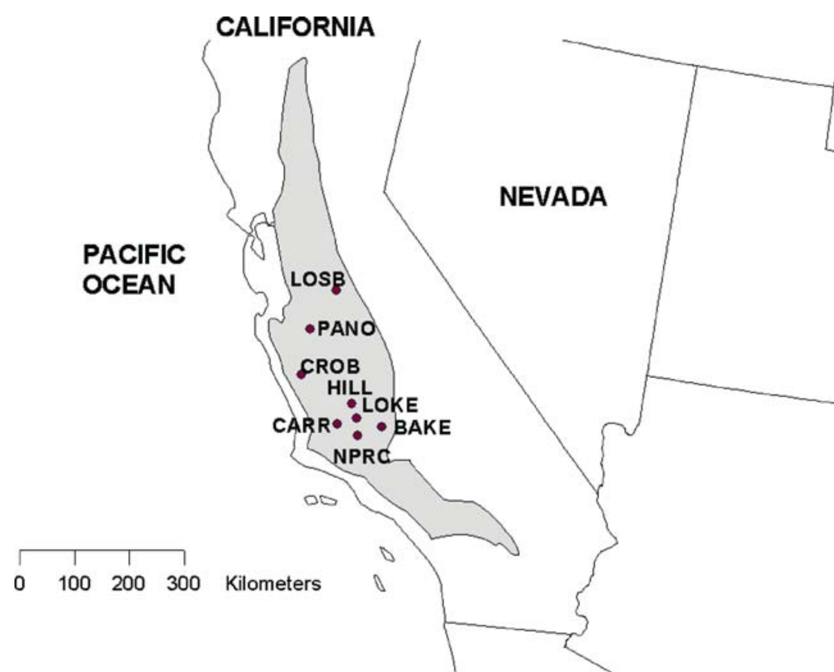

Figure 1. Map of California's Central Valley and surrounding lands. Locations are noted by four letter codes defined in Table 2.

Microsatellite DNA

We isolated DNA from kit fox tissue samples with *QIAamp*'s DNA minikit using standard protocols (*QIAGEN*, Germany). Twenty-four microsatellite primer sequences, initially developed for domestic dogs and foxes were screened to find eight unambiguous (i.e., little stutter and distinct peaks when analyzed), polymorphic DNA markers: *CXX30*, *CXX172*, *CXX173*, *CXX263*, *CXX403* (Ostrander et al. 1993), *CPH3* (Fredholm and Wintero 1995), and *F2054* and *F2140* (Francisco et al. 1996). All markers were dinucleotide repeats, except *F2054* and *F2140* that were tetranucleotide repeats. We amplified DNA using these eight primers and the polymerase chain reaction (PCR) using the published conditions. Subsequent products were electrophoresed on 8% polyacrylamide gels using an *Applied Biosystems* 373 Automated Sequencer. Microsatellite allele sizes were estimated by comparing the allele to an internal lane size standard.

Table 1. Genetic Diversity Statistics. N is the sample size for each location, A_{obs} is the mean number of alleles per locus, H_e is the mean expected heterozygosity, and H_o is observed heterozygosity. SE is one standard error from the mean. A_{stan} is the average number of alleles per locus standardized by the Monte Carlo analysis. The critical values for the lower and upper 2.5 percentile are derived from 10,000 simulations of draws corresponding to the sample size of each location (see text). $Crit_{low}$ is the critical value for the lower 2.5% of the distribution and $Crit_{high}$ is the critical value for the upper 2.5% of the distribution

Location	Code	N	A_{obs} (SE)	H_e (SE)	H_o(SE)	A_{stan}	$Crit_{low}$	$Crit_{high}$
Los Baños	LOSB	4	2.13 (0.99)	0.38 (0.21)	0.28 (0.10)	2.65	2.25	3.13
Lost Hills	HILL	6	2.00 (0.53)	0.42 (0.16)	0.50 (0.09)	2.99	2.52	3.61
Camp Roberts	CROB	15	2.88 (1.55)	0.45 (0.18)	0.40 (0.11)	3.62	3.25	4.00
Panoche	PAN	21	3.13 (1.13)	0.47 (0.17)	0.32 (0.08)	3.80	3.38	4.13
Lokern N.A.	LOKE	25	3.63 (1.69)	0.51 (0.15)	0.39 (0.07)	3.88	3.50	4.30
Carrizo Plain	CARR	35	3.88 (1.88)	0.46 (0.16)	0.46 (0.08)	4.03	3.63	4.25
Bakersfield	BAKE	95	3.25 (1.49)	0.41 (0.15)	0.36 (0.06)	4.30	4.13	4.50
Naval Petr. Res.	NPRC	116	4.63 (2.00)	0.50 (0.17)	0.40 (0.07)	4.38	4.13	4.50

Statistical analysis

The 317 kit foxes sampled in this study were collected over multiple, consecutive years and from multiple age classes. Kit foxes can live to 9-years-old, although few foxes live beyond 5 years (Spiegel 1996; White and Garrott 1999). In two of our locations, Bakersfield and NPRC, we had temporally separated samples, 9 and 8 years apart, respectively. We tested whether there was temporal stability of allele frequencies at the same collection sites by comparing allele frequencies from each time period for each location separately. If significant differences were observed between time periods then our ability to separate temporal versus spatial isolation could be confounded. However, if no differences were observed between time periods, then we have evidence for temporal stability in gene frequencies and are more confident that our results were the product of spatial separation between locations. Lugon-Moulin et al. (1999) recommended testing for differences in allele frequencies across time by using the *G*-test detailed in Goudet et al. (1996). We also calculated F_{st} between time periods in both the Bakersfield and the Naval Petroleum Reserve locations to test for temporal stability.

Mean number of alleles per locus (A_{obs}), observed (H_o) and expected (H_e) heterozygosity were computed and tests for deviations from Hardy–Weinberg (HW) proportions were conducted using program *Genepop* (Version 3.1d; Raymond and Rousset 1995). *Genepop* uses the Markov chain method of Guo and Thompson (1992) to calculate unbiased estimates of Fisher's exact test to examine the hypothesis of heterozygote excess and deficiency in the sample. Tests were also performed across all loci and locations (64 tests in total). We used a sequential Bonferroni test to reduce type I errors associated with multiple tests (Rice 1989). Subsequently, we compared allele frequency differences between locations using an unbiased estimate of the log-likelihood *G*-statistic (Goudet et al. 1996; pooled across loci using Fisher's procedure for combining probabilities; Fisher 1954).

Genetic variation was compared among locations several ways. Paired *t*-tests of arcsine-transformed H_o and H_e were conducted to detect differences in heterozygosity among the eight fox locations (Archie 1985; Paetkau et al. 1998) and the results were adjusted using sequential Bonferroni tests (Rice 1989). We also conducted one-way ANOVA with location as the factor and alleles per locus as the dependent variable to test for differences in allele frequencies between all locations. Because sample sizes differed dramatically between locations, we scaled allelic diversity by sample size using Monte Carlo simulations before comparing among locations (similar to the multiple random reductions of N approach described in Leberg 2002).

We accomplished this by writing a computer program in *Turbo Pascal* that randomly drew 2 alleles at a single locus from the NPRC allele frequency distribution; we used the NPRC gene pool as the baseline because NPRC was one of the larger San Joaquin kit fox populations (Cypher et al. 2000) and a population for which we had a large sample size. We repeated this process 10,000 times for draws of 2 alleles. Subsequently, we repeated this process for draws of size 3–232 at this same locus (232 was the total number of alleles in the NPRC population) and plotted the number of alleles drawn versus sample size. This was repeated for all loci. We used these plots to visually understand the sensitivity of our allelic diversity measures to sample size.

Next, for each of the 8 kit fox populations, independently, we determined the number of alleles actually sampled (2 times the sample size, in most cases), and drew the same number of alleles sampled from the NPRC distribution with replacement, for each locus, 10,000 times. We then computed 10,000 mean standardized number of alleles per locus (A_{stan}) and ordered them from highest to lowest. The upper and lower 2.5% (5% total) of the A_{stan} distribution were used as critical values for testing for a difference between A_{stan} and A_{obs}. For example, at the Carrizo Plain we sampled 70 alleles (35 foxes), thus we used the Monte Carlo simulation to draw 70 alleles with replacement, 10,000 times, from the NPRC population, at each locus. We calculated A_{stan} for each of the 10,000 simulations and ordered them in descending numerical order. Finally, we compared A_{obs} to this distribution and considered it to be significantly different if A_{obs} was in the 2.5% region of either tail of the distribution. In the case of the Carrizo Plain, A_{obs} was not in the tail of the A_{stan} distribution.

We used several different statistics to investigate population subdivision and migration: F_{st},

R_{st}, assignment tests, and maximum likelihood (ML) methods based on coalescent theory. We used *Fstat 2.9.1* (Goudet 1995, 2000) to calculate Weir and Cockerham's (1984) estimate of F_{st}, from which we estimated the per-generation number of migrants moving between all populations [$F_{st} \cong 1/(4\text{Nm}\alpha + 1)$], where, $\alpha = (n/n - 1)^2$ and n is the number of demes (Mills and Allendorf 1996). Subsequently, we used only our six largest populations (i.e., removing Lost Hills and Los Baños) to ensure our results were robust. We calculated R_{st} because it has been suggested that comparing R_{st} and F_{st} can provide further information about current versus historical migration (Slatkin 1995). We used the assignment test to also look at substructure (Paetkau et al. 1995; Davies et al. 1999); the assignment test calculates the likelihood of drawing an individual's particular genotype from a gene pool with a given set of allele frequencies. We used the Bayesian-based assignment test of Cornuet et al. (1999) because it performs well over a wide range of F_{st} values and is relatively insensitive to the number of loci used (Cornuet et al. 1999). We used the assignment test to assign individuals in the six major fox populations to a population of origin, and subsequently assigned the four foxes from Los Baños and six foxes from Lost Hills to one of the six major populations.

In addition, we estimated gene flow using a ML approach based on coalescent theory (Beerli and Felsenstein 2001) because this approach does not assume equal population sizes and symmetrical gene flow like F_{st} (although it still assumes an equilibrium between drift and migration). The default settings in program *Migrate* were used except we employed the "heating option" to minimize becoming trapped on a local optimum. After estimating gene flow we used a geographic information system (*ArcInfo* 7.1.2, ESRI 1997) to calculate pairwise geographic distances between the six large populations (i.e., removing Lost Hills and Los Baños) and compared geographic distance to pairwise genetic distances with a Mantel's test (Mantel 1967).

Finally, we were interested in determining if San Joaquin kit foxes followed typical mammalian patterns of sex biased dispersal; thus we compared differences in F_{st} and the variance in the "assignment index" (*vAIc*) between males and females to test for a sex bias in dispersal (Goudet et al. 2002). For testing sex biased dispersal, Goudet et al. (2002) recommend using *vAIc* when dispersal is very low (less than 10% of each population dispersing) and F_{st} if this is unknown. We present both estimates.

Results

HW Proportions

We found evidence for the temporal stability of allele frequencies between years in both NPRC and Bakersfield. The F_{st} value between the 1989 Bakersfield sample and the 1999 Bakersfield sample was 0.038 (95% CI: 0.004–0.075), and the *G*-test was only significantly different for 1 of 8 loci (*F* 2054; $P = 0.043$). The F_{st} value between the NPRC samples yielded an $F_{st} = 0.005$ (95% CI: −0.001–0.013), and again only 1 of 8 loci (*F2140*) was significantly different ($P = 0.013$).

HW proportion tests signified that not all loci were in HW proportions. Global tests revealed a heterozygote deficiency ($P = 0.001$) and showed no sign of heterozygote excess ($P = 1.0$). Testing each location separately produced a significant heterozygote deficiency in Bakersfield, Lokern, NPRC, and Panoche (Table 2). Individual tests for each locus and location revealed significant deviations from HW proportions for markers: *CPH3* (in five of eight locations; Bakersfield, Camp Roberts, NPRC, Lokern, and Panoche), *CXX403* (in three of eight locations; Bakersfield, NPRC, Panoche), *CXX30* (in one of eight locations; Lokern), and *CXX263* (in one of eight locations, Bakersfield) after the Bonferroni adjustment.

Some of the loci we used were not in HW proportions because of a heterozygote deficit, which can be produced by pooling across age classes, the presence of null alleles, selection against heterozygotes, sampling parent-offspring pairs or Wahlund effects. Each location consisted of foxes from many age classes. In many cases, the individuals sampled were known to belong to several cohorts, which has been shown to produce heterozygote deficits in other studies (e.g., Allegrucci et al. 1997). However, we found temporal stability in allele frequencies suggesting that we can pool across samples collected in different years. A more likely explanation of our heterozygote deficit is either the presence of null alleles or

Table 2. F_{is} values at 8 loci in 8 locations of kit fox. NA indicates that this locus was monomorphic. Values in bold indicate a significant ($P < 0.05$) deviation from Hardy–Weinberg proportions after Bonferroni corrections. Location codes are listed in Table 2

	LOSB	HILL	CROB	PANO	LOKE	CARR	BAKE	NPRC	Sum
CPH3	−0.286	−0.429	**0.587**	**0.748**	**0.347**	0.088	**0.033**	**0.271**	0.050
CXX30	NA	−0.250	−0.260	0.236	**0.867**	−0.065	0.087	0.282	0.039
CXX172	0.000	−0.429	0.352	0.333	0.280	0.075	−0.133	0.102	0.038
CXX173	NA	−0.111	0.000	0.077	0.085	0.114	−0.145	0.153	0.026
CXX263	NA	0.412	NA	0.000	−0.120	−0.031	**0.470**	0.254	0.042
CXX403	0.294	NA	0.431	**0.583**	0.370	0.179	**0.505**	**0.369**	−0.003
F2054	0.625	−0.333	−0.016	0.034	0.084	−0.262	0.102	0.036	0.120
F2140	0.368	−0.250	−0.343	0.172	0.141	0.019	0.042	0.114	0.089
Sum	0.250	−0.190	0.125	0.316	0.248	0.012	0.128	0.186	

the presence of closely related individuals such as parents and offspring in our samples (e.g., Ralls et al. 2001).

Genetic variation

Lokern had the highest expected heterozygosity ($H_e = 0.51$, SE $= 0.15$) and Los Baños had the lowest expected heterozygosity ($H_e = 0.38$, SE $= 0.21$; Table 1). Mean number of alleles per locus was highest in the NPRC (4.63; Table 1) and lowest in the Lost Hills and Los Baños locations (2.00 and 2.13, respectively). Bonferonni corrected paired *t*-tests showed no differences in either observed or expected heterozygosity between locations. One-way ANOVA with location as a factor and alleles per locus as the dependent variable was significant ($F_{7,56} = 2.85$, $P = 0.013$, Table 1) and the Bonferroni *post hoc* test showed significant differences between the number of alleles per locus found in the NPRC and Lost Hills, and between the NPRC and Los Baños; however, this test does not correct for sample size. Alternatively, a global *G* test for differences in allele frequencies between locations was significant, with the follow-up pairwise tests showing differences in allele frequencies between all locations except for Lokern and the NPRC ($P = 0.46$).

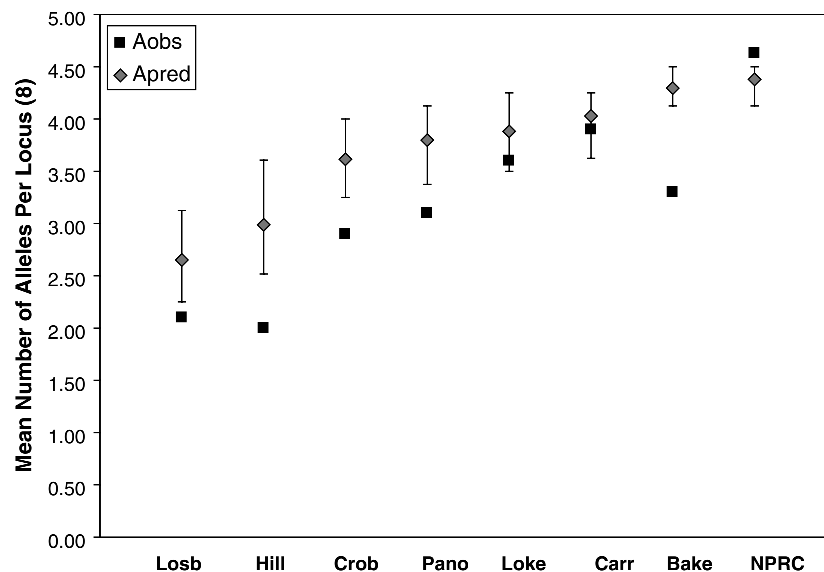

Figure 2. Observed (A_{obs}; squares) and standardized (A_{stan}; diamonds) number of alleles per locus for each kit fox location. The error bars are the 95% of the predicted distribution.

Table 3. Subdivision estimates and assignment test results. Locations codes are the same as in Table 1. Numbers below the diagonal are pairwise F_{st} estimates. Numbers above the diagonal are Nm values calculated from F_{st} estimates using equation 3 in Mills and Allendorf (1996). The numbers in parentheses are the assignment test probabilities, with the first column representing the location to which the kit fox was assigned. Each column header (in the first row) is the location from which the kit fox was captured. Each value is the proportion of times kit fox were ''assigned'' to the respective location

	BAKE	CROB	CARR	LOKE	NPRC	PANO
BAKE	– (0.59)	1.8 (0.12)	23.7 (0.12)	8.5 (0.07)	7.5 (0.03)	1.6 (0.06)
CROB	0.096 (0.20)	– (0.60)	1.8 (0.00)	2.8 (0.00)	3.8 (0.13)	3.5 (0.07)
CARR	0.008 (0.20)	0.094 (0.06)	– (0.60)	6.2 (0.03)	6.9 (0.11)	1.8 (0.00)
LOKE	0.022 (0.28)	0.064 (0.04)	0.030 (0.04)	– (0.32)	NA (0.28)	4.2 (0.04)
NPRC	0.025 (0.14)	0.047 (0.15)	0.027 (0.10)	0.000 (0.22)	– (0.28)	4.5 (0.11)
PANO	0.107 (0.05)	0.052 (0.19)	0.095 (0.00)	0.044 (0.05)	0.041 (0.14)	– (0.57)

The results of the Monte Carlo simulations demonstrated that the distribution of standardized mean number of alleles per locus (A_{stan}) values was higher than the observed mean number of alleles per locus in the Los Baños, Lost Hills, Camp Roberts, Panoche, and Bakersfield locations (Figure 2, Table 1). The variance around the observed mean number of alleles per locus (reported in Table 1) was not used because this variance is associated with the difference between loci. These results suggest that low observed mean number of alleles per locus in several locations is not only reflective of the small sample size in these populations, but may be the consequence of small population sizes leading to inbreeding effects (Figure 2). In Figure 2 we show the results of drawing 232 alleles (the number of alleles sampled in NPRC) from the NPRC location, which indicates that A_{stan} can be biased low, suggesting this is a conservative measure.

Population subdivision

F_{st} among all locations was 0.043 (95% CI = 0.02–0.064) and R_{st} was 0.041. Assuming an island model of migration, $F_{st} = 0.043$ was equivalent to approximately four migrants entering each location each generation. Using only the six locations with the largest sample sizes (removing Los Baños and Lost Hills), F_{st} was 0.036 (SE = 0.011) and R_{st} was 0.019. Pairwise estimates of migration were highest between Lokern and NPRC, and between Carrizo and Bakersfield (Table 3). The association between geographic distance and genetic distance

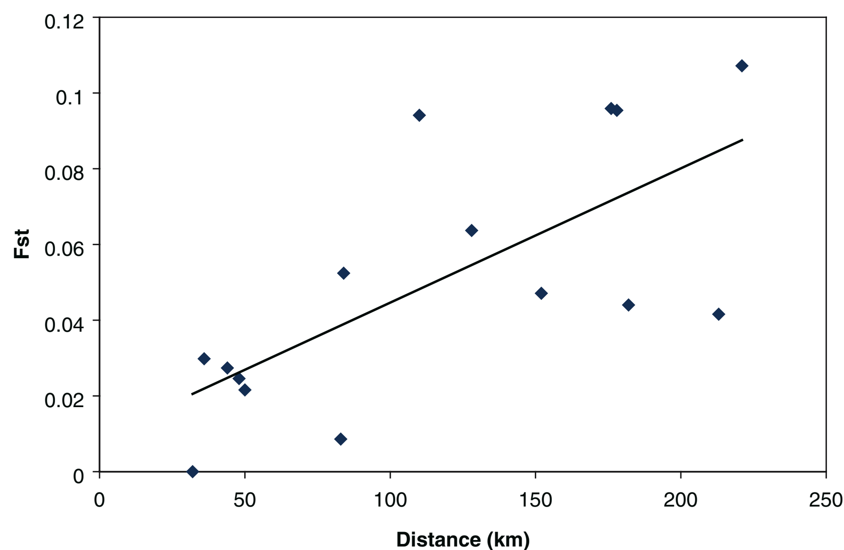

Figure 3. Geographic distance (measured in Kilometers) plotted against F_{st}. The Mantel test correlating these variables was significant.

Table 4. Migration estimates (*Nm*) from a coalescent based ML approach (using program *MIGRATE*). Locations codes are the same as in Table 1. Numbers below the diagonal are the migration from population listed in the first column to the population listed in the first row. Numbers above the diagonal are migration from the population listed in the first row to the population listed in the first column

	BAKE	CROB	CARR	LOKE	NPRC	PANO
BAKE	–	0.03	0.15	0.27	0.78	0.06
CROB	1.15	–	0.24	0.42	0.60	0.18
CARR	1.08	0.00	–	0.50	0.54	.014
LOKE	0.51	0.05	0.25	–	.049	.015
NPRC	1.46	0.23	0.41	0.20	–	.021
PANO	0.81	0.02	0.30	0.02	0.45	–

(F_{st}) was significant (Mantel's test; $z = 219.48$, $P = 0.02$; Figure 3).

Using the *GeneClass* assignment test, 45.0% of the individuals assigned to the location from which they were sampled (Table 3). Individuals had a higher likelihood of assigning to the location from which they were sampled than to any other location. However, individuals from the NPRC and Lokern only assigned to the NPRC and Lokern 28% and 32% of the time, respectively (Table 3). Because of small sample sizes we did not include the samples from The Lost Hills or the Los Baños in our initial assignment analyses. However, when we used the assignment test to classify Lost Hills' foxes to a location (other than Lost Hills) five classified with the NPRC and one classified with Panoche. We repeated this test, but allowed Lost Hills individuals to be ''self-assigned'' and found all six individuals assigning to Lost Hills. Repeating these analyses with the Los Baños samples, when self-assignment was not permitted, two of the foxes assigned to the Carrizo and two assigned to Panoche; allowing self-assignment, two foxes assigned to Los Baños, one assigned to the Carrizo, and one classified to Panoche.

The results from the ML approach based on coalescent theory suggested lower levels of migration than the F_{st} results, yet still showed a relatively high amount of movement between samples (Table 4). Only three pairs of locations produced estimates of more than 1 migrant moving between locations each generation: Bakersfield and NPRC, Bakersfield and Camp Roberts, and Bakersfield and the Carrizo Plain. In all these cases migration was strongest from Bakersfield to the other location. The matrix of exchange of migrants between locations (summing the number of migrants leaving and entering each location pair) and geographic distance was only marginally significant and negatively sloped as expected (Mantel's test, $z = 2015.28$, $P = 0.09$).

F_{st} was higher in females ($F_{st} = 0.051$; SE $= 0.025$) than in males ($F_{st} = 0.037$; SE $= 0.008$), but this result was not statistically significant ($P = 0.12$). The *vAIc* test also suggests that there is little difference between rates of female and male dispersal ($P = 0.52$).

Discussion

Genetic variation

Heterozygosity was similar across all San Joaquin kit fox locations. However, in two of the smaller sites, Lost Hills and Los Baños, there was a lower mean number of alleles per locus than in other kit fox locations. At first glance these results appear to contradict each other; however, Lost Hills and Los Baños had small samples sizes, which impacts allelic diversity more than heterozygosity. We only captured six foxes at Lost Hills despite intensive trapping efforts and only four at Los Baños; this was quite likely a census of these populations. We adjusted for sample size with Monte Carlo simulations and found lower levels of allelic diversity in Lost Hills and Los Baños, as well as lower allelic diversity in Camp Roberts, Panoche, and Bakersfield. This suggests that the NPRC (our base location from which we resampled) may have had an abnormally high level of genetic variation, was an admixture from several other unknown kit fox populations from surrounding areas, or several of

our sampled locations show evidence of inbreeding effects (Figure 3).

Kit fox populations are subject to marked population size fluctuations resulting from the high variation in annual rainfall in their desert environment and the concomitant effects on kit fox prey (White and Garrott 1999; Cypher et al. 2000). Particularly during multi-year periods of drought, kit fox abundance can decline to very low levels and local extinctions may occur. Even relatively large kit fox populations experience such episodic declines (Cypher et al. 2000). This pattern of dynamics results in kit fox populations being vulnerable to genetic bottleneck effects, as well as founder effects, in the case of local extinctions followed by colonization events.

The Lost Hills kit fox are located along an aqueduct that runs through farmland and is geographically more isolated than other kit fox populations. Seven of eight loci showed a negative F_{is} (and locus *CXX403* was fixed) at the Lost Hills. Very small populations can produce temporary heterozygote excess due to slight differences in the male and female gene pools (Robertson 1965; Luikart and Cornuet 1999) explaining our Lost Hills result. On the other hand, three loci at Los Baños were fixed (*CXX263*, *CXX173*, and *CXX30*) and four of the remaining five loci produced a positive F_{is}, suggestive of inbreeding effects. Since the time of sampling the Los Baños group of foxes has disappeared. Los Baños may have been an ephemeral fox population composed of one or two family groups with high levels of inbreeding.

The Camp Roberts and Panoche populations also are relatively small and isolated. Based on a lack of historical occurrences, kit fox abundance in the Camp Roberts region may have always been relatively low, and it is possible that the presence of foxes at Camp Roberts may represent a relatively recent colonization event (Balestreri 1981). Similarly, the Panoche population occurs in a small, relatively isolated valley. Low allelic diversity in both of these locations could reflect founder events and a low rate of genetic exchange with other locations.

The Bakersfield population differs from the other locations in that kit fox numbers may not be subject to the marked fluctuations observed in other populations (B. Cypher, unpublished data). This is a function of consistently high food availability attributable to anthropogenic influences resulting in an abundance of natural and non-natural foods (e.g., trash, pet food, handouts). However, the history of this unusual population is unclear. Although currently relatively large in size, it is plausible that this population was founded by a small number of colonizers resulting in the low observed allelic diversity relative to expected values, as found with urban red fox populations (Wandeler et al. 2003).

Gene flow

Kit fox populations have been described as metapopulations, with both large well-connected and small semi-isolated populations exchanging individuals (US Fish and Wildlife Service 1998; Cypher et al. 2000). Small kit fox populations likely face higher extinction probabilities as prey diminishes, but may be recolonized by foxes dispersing from larger populations (White et al. 1996; Cypher et al. 2000). Evidence for this metapopulation model has been poor, with only a few studies documenting interpopulation exchange of individuals, and no study showing reproduction by a migrant (Scrivner et al. 1987; Koopman et al. 2000). Our genetic results are consistent with foxes moving between locations and breeding in non-natal populations. In fact, global F_{st}, R_{st}, and our ML approach based on coalescent theory suggest minimal subdivision among San Joaquin kit fox populations. The ML estimates of gene flow were lower than F_{st} estimates, although it should be noted that ML approaches for estimating migration rates have recently been criticized (Abdo et al. 2004). Existing subdivision can be explained by geographical distance between populations (i.e., Wright's isolation by distance model; Wright 1943; Figure 3). This is consistent with mtDNA findings in kit and swift fox on a larger scale (Maldonado et al. 1997, Mercure et al. 1993).

We converted our F_{st} estimates into migration estimates. While there has been some criticism towards using this approach to produce exact estimates of migration, it still produces relative estimates of migration that can be classified as high, moderate, and low (Steinberg and Jordan 1997; Whitlock and McCauley 1999; Hedrick 1999; Mills et al. 2003). We classify migration as high if $Nm > 10$ (Vucetich and Waite 2000), low if $Nm < 1$ (Mills and Allendorf 1996), and moderate if Nm is between 1 and 10. Our results showed

no cases of low gene flow between our six largest locations (excluding Lost Hills and Los Baños because of small sample sizes); high gene flow was observed between the NPRC and Lokern, and Bakersfield and the Carrizo Plain.

These gene flow results were corroborated by assignment test results. Only 45% of kit fox were assigned to the location from which they were sampled, suggesting relatively high gene flow. Cornuet et al. (1999; Figure 6) found the number of loci used to have relatively little impact on the performance of their assignment test. Furthermore, other studies with less overall genetic variation and equal numbers of microsatellites have shown much higher assignment rates, leading us to attribute our low assignment rates to high gene flow (Manel et al. 2002).

The estimate of male gene flow was higher than that of female gene flow, although the differences were not significant. One concern is that the power of the test was diminished because we sampled both individuals that dispersed and their offspring. Dispersers that breed in their non-natal population transmit genes to both sexes in the next generation, masking any differences of sex bias in dispersal (Goudet et al. 2002). The *vAIc* test between males and females was not significant, but was likely not the most powerful test considering *vAIc* performs best when gene flow is low, but is outperformed by F_{st} when gene flow is high or unknown (Goudet et al. 2002). Overall, there is some evidence from our genetic work that kit fox dispersal may be male-biased as in many polygynous mammals, although monogamous species such as foxes often show little or no sex differences in dispersal (Greenwood 1980). This would be consistent with the demographic data where over a 14-year time horizon a higher proportion of males dispersed from the NPRC (Koopman et al. 2000).

Current versus historical gene flow

By comparing the mean values of R_{st} and F_{st} we can make some assertions to the historical level of gene flow. R_{st} is expected to be larger than F_{st} when populations have evolved independently (e.g., low historical *Nm* between populations). On the other hand, when the values between R_{st} and F_{st} are nearly equal, *Nm* has been historically large between populations and drift has been the predominant factor in creating differences between populations, not mutation (Slatkin 1995; O'Ryan et al. 1998). In this study, R_{st} was lower than F_{st} suggesting that (1) drift has been more important than mutation historically, and (2) *Nm* has been large. Thus, our data support the idea that historically there has been high gene flow among San Joaquin kit fox populations, not a series of independent populations that have only recently fused. Interestingly, distance showed greater impact on the F_{st} index of gene flow than the ML approach based on coalescence estimate. Coalescent approaches, by definition, integrate over long time spans, thus may be more reflective of historical movement. If this is the case, there is support for higher historical gene flow.

This still leaves us asking if the current, low F_{st} is solely a reflection of high historical gene flow or contemporary gene flow? This question is difficult to answer. However, while we cannot rule out that our data may be due to shared common ancestry, we can support the contention that our F_{st} values were driven by gene flow by examining field data. First, there is considerable historical documentation (e.g., Meriam 1902) of kit foxes occurring throughout the San Joaquin valley and its surrounding lands (US Fish and Wildlife Services 1998), indicating that the fox distribution in the San Joaquin Valley is not a result of recent expansion of their geographic range. Therefore, foxes have been present in the San Joaquin valley and surrounding areas for a minimum of 100 years, or 25–50 fox generations. Wright's (1969), and Nei and Chakravarti's (1977) deterministic equation [$F_{st} = 1-(1-1/(2N_e))^t$] shows that F_{st} increases over time (t) as a function of effective population size (N_e); the smaller the N_e, the more rapid the increase. Thus, for F_{st} to be maintained at 0.04 after 25 fox generations would require subpopulations with N_e greater than 300 each, or approximately 1500–3000 foxes per subpopulation (Frankham 1995). Cypher et al. (2000) estimated the abundance of kit fox in the NPRC (the largest kit fox population) to fluctuate between 46 and 363 foxes over 15 years. Therefore, unless fox populations were nearly 10-fold larger until very recently, migration must be an important factor in the partitioning of genetic variation in kit fox populations. It is highly unlikely that kit for were 10-fold larger until recently given that the impacts to the San Joaquin valley have been occurring for the majority of the last century starting with the California State Water projects occurring during the mid-1930s. However, this logic assumes a stable population

structure such that the population sizes observed by Cypher et al. (2000) were similar to historical estimates. Population stability may be a valid assumption through the 1850s, but may be unjustified after the large anthropogenic changes in the San Joaquin Valley during the middle of the 19th century. During the time of the Cypher et al. (2002) study (approximately 5 kit fox generations) in the NPRC we would expect a completely isolated population to have an F_{st} greater than 0.07 – higher than we observed. Therefore *Nm* estimates cannot be solely reflective of shared common ancestry.

This conclusion of gene flow being currently important in kit fox population dynamics is most important for understanding populations like Camp Roberts. Camp Roberts has seen a marked decline in kit fox since the time of sampling, potentially attributable to both a rabies outbreak and an increase in interference competition with coyotes (White et al. 2000). Continuing habitat conversion in the Camp Roberts region has limited opportunities for immigration into this population. Indeed, recent data suggest that this population is either extinct or on the verge of extinction (J. Eliason, personal communication).

These data suggest that at least some kit fox populations are currently in the early stages of becoming isolated. From the field data we know of several individual tagged foxes moving between population centers in recent years: for example, NPRC to Bakersfield, the Carrizo Plain and Camp Roberts; and Camp Roberts to the Carrizo. Thus, gene flow may still be occurring between some kit fox population centers. Furthermore, recent surveys using trained scent dogs to find kit fox scats (Smith et al. 2001; Smith et al. In Press) have shown that kit foxes occasionally occur in suitable habitat between known major population centers such as the NPRC and the Lokern (D. Smith and K. Ralls, unpublished data). With the continuing habitat degradation and loss in the San Joaquin Valley, isolation of some kit fox populations is a highly probable outcome. Of the populations sampled for this study, the Los Baños and Camp Robert areas are at extreme risk of becoming isolated from other kit fox populations. The Bakersfield population also is at risk of becoming isolated from the other populations included in this study.

We recommend that population and genetic monitoring programs be established that track changes in the genetic variability and gene flow of kit fox in the San Joaquin Valley. With the recent development of non-invasive genetic sampling techniques (Smith et al. 2001) these monitoring efforts can be conducted without the major expenses of trapping studies and will provide important data on changes in kit fox genetics and demography. These types of efforts will also help detect unknown populations, if they exist, or at least help quantify individual foxes that may reside, and possibly breed, in the interstitial spaces between known populations. We also advocate the continuation of demographic studies such as Cypher et al. (2000) because it is only through the synergy obtained by combining of genetics, demography, and monitoring efforts that we can fully understand the population dynamics of this species and develop successful conservation strategies.

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References

Abdo Z, Crandall KA, Joyce P (2004) Evaluating the performance of likelihood methods for detecting population structure and migration. *Mol. Ecol.*, **13**, 837–851.

Allegrucci G, Minasi MG, Sbordoni V (1997) Patterns of gene flow and genetic structure in cave-dwelling crickets of the

Tuscan endemic, *Dolichopoda schiavazii* (Orthoptera, Rhaphidophoridae). *Heredity*, **78**, 665–673.

Allendorf FW, Phelps RS (1981) Use of allelic frequencies to describe population structure. *Can. J. Fish. Aquat. Sci.*, **38**, 1507–1514.

Archie JW (1985) Statistical analysis of heterozygosity data: independent sample comparisons. *Evolution*, **39**, 623–637.

Balestreri AN (1981) S*tatus of the San Joaquin kit fox at Camp Roberts California*. Department of Army Directorate Facilities Engineering Environmental and Natural Resources Office, HQ, 7th Infantry Division, Contract No. DAKF03-81-C736, Ft. Ord.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proc. Nat. Acad. Sci.*, **98**, 4563–4568.

Berger J (1990) Persistence of different-sized populations, an empirical assessment of extinction in bighorn populations. *Conserv. Biol.*, **4**, 91–98.

Brown JH, Kodric-Brown A (1977) Turnover rates in insular biogeography, effect of immigration on extinction. *Ecology*, **58**, 445–449.

Cornuet J, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, **153**, 1989–2000.

Cypher B, et al. (2000) Population dynamics of San Joaquin kit foxes at the Naval Petroleum Reserves in California. *Wildlife Monogr.*, **145**, 1–43.

Cypher B, Frost LN (1999) Condition of San Joaquin kit foxes in urban and exurban habitats. *J. Wildlife Manage*. **63**, 930–938.

Davies N, Villablanca FX, Roderick GK (1999) Determining the source of individuals, multilocus genotyping in non-equilibrium population genetics. *Trend. Ecol. Evol.*, **14**, 17–21.

Environmental Systems Research Institute Inc. (ESRI) 1997. ARC/INFO version 7.1.2. Redlands, CA.

Fisher R (1954) *Statistical Methods for Research Workers* 12th ed. Oliver and Boyd, Edinburgh, UK.

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

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. *Genet. Res. Camb.*, **66**, 95–106.

Fredholm M, Wintero AK (1995) Variation of short tandem repeats with in and between species belonging to the Canidae family. *Mamm. Genome*, **6**, 11–18.

Goudet J (1995) FSTAT (Version 1.2), A computer program to calculate *F*-statistics. *J. Hered.*, **86**, 485–486.

Goudet J (2000) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.1) Available from http://www.unilch/izea/softwares/fstat.html Updated from Goudet (1995).

Goudet J, Perrin N, Wasser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Mole. Ecol.*, **11**, 1103–1114.

Goudet J, Raymond M, Demeeus T, Rousset F (1996) Testing genetic differentiation in diploid populations. *Genetics*, **144**, 1933–1940.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Anim. Behav.*, **28**, 1140–1162.

Grinnell J, Dixon JS, Linsdale JM (1937) *Fur-bearing Mammals of California*, Vol 2. University of California Press, Berkley.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics*, **48**, 361–362.

Hedrick PW (1999) Highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.

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

Leberg P (2002) Estimating allelic richness: effects of sample size and bottlenecks. *Mol. Ecol.*, **11**, 2445–2449.

Lugon-Moulin N, Brunner H, Balloux F, Hausser J, Goudet J (1999) Do riverine barriers, history or introgression shape the genetic structuring of a common shrew (*Sorex araneus*) population? *Heredity*, **83**, 155–161.

Luikart G, Cornuet JM (1999) Estimating the effective number of breeders from heterozygote excess in progeny. *Genetics*, **151**, 1211–1216.

Maldonado JE, Cotera R, Geffen E, Wayne RK (1997) Relationships of the endangered Mexican kit fox (*Vulpes macrotis zinseri*) to North American arid-land foxes based on mitochondrial DNA sequence data. *Southwest. Nat.*, **42**, 460–470.

Manel S, Bertier P, Luikart G (2002) Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. *Conserv. Biol.*, **16**, 650–659.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res.*, **27**, 209–220.

Mercure A, Ralls K, Koepfli KP, Wayne RK (1993) Genetic subdivision among small canids: mitochondrial DNA differentiation of swift, kit and arctic foxes. *Evolution*, **47**, 1313–1328.

Merriam, CH (1902) Three new foxes of the kit fox and desert fox groups. *Proc. Biol. Soc. Wash.*, **15**, 73–74.

Mills LS, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and management. *Conserv. Biol.*, **10**, 1509–1518.

Mills LS, Schwartz MK, Tallmon DA, Lair KP (2003) Measuring and interpreting connectivity for mammals in coniferous forests. In: *Mammal Community Dynamics: Management and Conservation in the Coniferous Forests of Western North America* (eds. Zabel CJ, Anthony RG), pp. 587–613. Cambridge University Press, Cambridge.

Nei M, Chakravarti A (1977) Drift variances of F_{st} and G_{st} statistics obtained from a finite number of isolated populations. *Theor. Popul. Biol.*, **11**, 307–325.

Newman D, Pilson D (1997) Increased probability of extinction due to decreased genetic effective population size, experimental populations of *Clarkia pulchella*. *Evolution*, **51**, 354–362.

O'Ryan C, Harley EH, Bruford MW, Beaumont M, Wayne RK, Cherry MI, (1998) Microsatellite analysis of genetic diversity in fragmented South African buffalo populations. *Anim. Conserv.*, **1**, 85–94.

Ostrander EA, Sprague GF, Rine J (1993) Identification and characterization of dinucleotide repeat (CA)n markers for genetic mapping in dog. *Genomics*, **16**, 207–213.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Mole. Ecol.*, **4**, 347–354.

Paetkau D, Waits LP, Clarkson PL, Craighead L, Vyse E, Ward R, Strobeck C (1998) Variation in genetic diversity

across the range of North American brown bears. *Conserv. Biol.*, **12**, 418–429.

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

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

Raymond M, Rousset F (1995) GENEPOP (Version 1.2), population genetics software for exact tests and ecumenicism. *J. Hered.*, **83**, 248–249.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Robertson A (1965) The interpretation of genotypic ratios in domestic animal populations. *Anim. Prod.*, **7**, 319–324.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491–494.

Scrivner JH, O'Farrell TP, Kato TT (1987) Dispersal of San Joaquin kit foxes, *Vulpes macrotic mutica,* on Naval Petroleum Reserve #1, Kern County, *California Rep No EGG* 10282-2190, EG&G Energy Measurements, Goleta, CA.

Slatkin M (1985) Gene flow and geographic structure of natural populations. *Science*, **236**, 787–792.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457–462.

Smith DA, Ralls K, Davenport KB, Adams B, Maldonado JE (2001) Canine assistants for conservationists. *Science*, **291**, 435.

Smith DA, Ralls K, Cypher BL, Maldonado JE (In Press) Assessment of Scat-detection dog surveys to determine kit fox distribution. *Wildl. Soc. Bull.*

Spiegel LK (1996) Studies of the San Joaquin kit fox in undeveloped and oil-developed areas California Energy Commission Pub No P700-96-003 California Energy Commission Publication Unit, Sacramento.

Steinberg EK, Jordan CE (1997) Using molecular genetics to learn about the ecology of threatened species: the allure and the illusion of measuring genetic structure in natural populations. In: *Conservation Biology for the Coming Decade.* (eds. Fiedler PA, Karieva PM), pp. 440–460. Chapman and Hall, New York.

Stow AJ, Sunnucks P, Briscoe DA, Gardner MG (2001) The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites. *Mole. Ecol.,* **10**, 867–878.

Tallmon DT, Luikart G, Waples RS (2004) The alluring simplicity and complex reality of genetic rescue. *Trend. Ecol. Evol.,* **19**, 489–496.

US Fish and Wildlife Service (1967) Native fish and wildlife Endangered species Federal Register 32, 4001.

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

Vucetich JA, Waite TA (2000) Is one migrant per generation sufficient for the genetic management of fluctuating populations? *Anim. Conserv.*, **3**, 261–266.

Wandeler P, Funk SM, Largiader R, Gloor S, Breitenmoser U (2003) The city-fox phenomenon: genetic consequences of a recent colonization of urban habitat. *Mole. Ecol.*, **12**, 647–656.

Weir BS, Cockerham CC (1984) Estimating F statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

White PJ, Berry WH, Eliason JJ, Hanson MT (2000). Catastrophic decrease in an isolated population of kit foxes. *Southwest. Nat.*, **45**, 204–211.

White PJ, Garrott RA (1999) Population dynamics of kit foxes. *Can. J. Zool.*, **77**, 486–492.

White PJ, Vanderbilt White CA, Ralls K (1996) Functional and numerical responses of kit foxes to a short-term decline in mammalian prey. *J. Mammal.*, **77**, 370–376.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{st} \neq 1/(4Nm+1)$. *Heredity*, **82**, 117–125.

Wright S (1943) Isolation by distance. *Genetics*, **28**, 139–156.

Wright S (1969) *Evolution and the Genetics of Populations Volume 2: The theory of gene frequencies*. University of Chicago Press, Chicago.