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Sampling Across 20 Years (1996–2017) Reveals Loss of Diversity and Genetic Connectivity in the Coachella Valley Fringe-Toed Lizard (*Uma inornata*)

Open-File Report 2019–1105

U.S. Department of the Interior
U.S. Geological Survey

Cover. A female Coachella Valley fringe-toed lizard from the NW Indio Hills population. Photograph taken by Dustin Wood, U.S. Geological Survey, June 2019.

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Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
	Mass	
nanogram (ng)	3.527×10^{-11}	ounce, avoirdupois (oz)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as

$$°F = (1.8 \times °C) + 32.$$

Abbreviations

bp	base pairs
CVFTL	Coachella Valley fringe-toed lizard
CVP	Coachella Valley Preserve
DA	discriminant axis
DAPC	discriminant analysis of principal components
ddRAD	double-digest restriction site associated DNA
DNA	deoxyribonucleic acid
MCMC	Markov chain Monte Carlo
MSATS	microsatellite loci
NGS	next-generation sequencing
NW	northwest
PC	principal component
PCR	polymerase chain reaction
RADseq	restriction site associated DNA sequencing
SNP	single nucleotide polymorphism
SPI	standardized precipitation index
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey

Sampling Across 20 Years (1996–2017) Reveals Loss of Diversity and Genetic Connectivity in the Coachella Valley Fringe-Toed Lizard (*Uma inornata*)

By Amy G. Vandergast,[1] Dustin A. Wood,[1] Mark Fisher,[2] Cameron Barrows,[3] Anna Mitelberg,[1] and Julia G. Smith[1]

Abstract

The Coachella Valley fringe-toed lizard (*Uma inornata*) is a federally threatened, aeolian sand dune obligate, endemic to the Coachella Valley, California. Historically, *U. inornata* is thought to have formed a large interconnected metapopulation across the valley, with local dune habitat and population size fluctuations linked to stochastic droughts and flooding. Since the 1950s, aeolian habitat in Coachella Valley has declined by 91–95 percent. What remains is highly fragmented by highways and development in the urban communities of the Coachella Valley, raising concerns that fringe-toed lizard movement and gene flow among remaining habitat fragments is limited or nonexistent. We examined population genetic structure across three sample periods (1996, 2008, and 2017). Over that time, this species has shifted from a panmictic condition (1996) with little or no genetic structure between sites to the current (2017) condition where there are now genetically distinct populations. Two severe droughts (2000–04 and 2012–16) may have accelerated this shift through drought-related population declines and subsequent genetic bottlenecks. Using a combination of microsatellite loci and single nucleotide polymorphisms, we found patterns of decreasing genetic connectivity and diversity over time. These patterns are consistent with reduced fringe-toed lizard movement and gene flow among isolated sand dune systems. Low effective population sizes were recovered in some sites, suggesting genetic drift in smaller and fluctuating populations is likely responsible for loss of genetic diversity. A U.S. Fish and Wildlife Service recovery objective for this species is to maintain genetic diversity; however, evidence of fragmentation suggests that genetic cohesiveness has been altered and that the diversity maintained in individual fragments is lower than in the total metapopulation. Management actions that increase genetic diversity could be implemented, including translocation. We modeled increasing gene flow between 1–10 percent, which showed that allelic richness could increase rapidly if translocated individuals can survive and reproduce. Establishing translocation protocols could help to avoid the high mortality that has occurred with other reptile translocations. Successful translocations could be a useful strategy to replenish lost genetic diversity after bottlenecks and could mitigate the loss of natural gene flow among populations.

Introduction

The Coachella Valley fringe-toed lizard (hereafter CVFTL, *Uma inornata*) is a federally threatened, aeolian sand dune obligate endemic to the Coachella Valley, California. Historically, CVFTL existed as a large interconnected metapopulation across the valley, with fluctuations in both habitat size and local population size driven by stochastic droughts and flooding. Anthropogenic habitat loss since the 1950s has resulted in a 91–95 percent decline in this lizard's aeolian habitat (Barrows and others, 2008), leaving remaining habitat patches isolated from one another, with little or no apparent connectivity between occupied patches. Previously, we examined genetic structure in CVFTL core habitat areas at two time periods, before and after a severe drought, using 11 microsatellite loci (Vandergast and others, 2016). We detected a decrease in genetic connectivity across sites, a decrease in genetic diversity at one site, and low effective population sizes (N_e) at some sites. These results suggested that drought and fragmentation had contributed to the loss of genetic connectivity and diversity.

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In the deserts of southern California, severe and prolonged droughts are predicted to accompany warming as modern climate change continues to develop (Cayan and others, 2008; Gao and others, 2012; Cook and others, 2015; Bachelet and others, 2016; Prein and others, 2016). The region is already experiencing more intense droughts: the Standardized Precipitation Index (SPI; McKee and others, 1993) calculated from 92 years of winter rain data in Palm Springs (https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/locations/CITY:US060021/detail) shows 5 years of severe drought where SPI ≤ –1.50 (fig. 1). All 5 of those years occurred after 1996, the baseline year for sampling tissues. The predictions of increased droughts portend increasing stress to desert species, especially those already challenged by habitat loss. A critical question for CVFTL recovery planning, then, is this: has genetic diversity been further eroded? If so, could management actions aimed at restoring connectivity aid in retaining more genetic diversity across the range (Frankham, 2005)?

The objectives of this study were to (1) determine if diversity and connectivity have declined over time and after recent extended droughts in the valley, (2) estimate current levels of diversity and N_e with more sensitive genomic markers, and (3) evaluate potential management scenarios aimed at increasing diversity and N_e in a simulation framework. In addition to those objectives, we also had an opportunity to conduct genetic monitoring after a translocation event. We salvaged individuals immediately prior to development of an unprotected site and moved lizards to a protected, but currently unoccupied, area of suitable habitat (Stebbins Dune). We compared individuals found post-translocation to translocated individuals to determine parentage and sibling relationships.

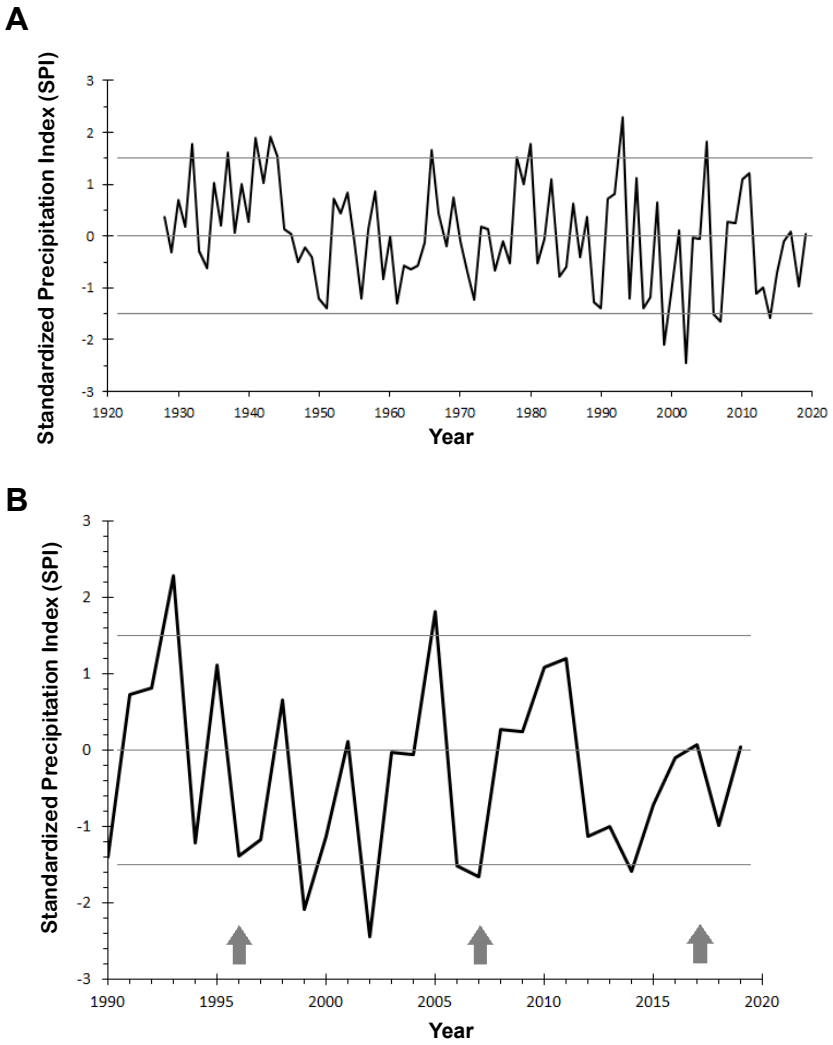

Figure 1. Standardized Precipitation Index (SPI) for winter rainfall at Palm Springs, California. Grey horizontal lines represent the mean and 1.5 standard deviations from the mean, the limit for severe wet and drought years. The SPI is calculated from the entire 92 years of rain records (plot A). Arrows (plot B) indicate years that tissues were sampled. Note that all five severe droughts occurred after the first sample, including two extreme droughts (SPI ≤ –2.0).

Methods

Field Sampling

In 2017 and 2018, we sampled CVFTL in all previously studied locations with extant populations (fig. 2; described in Vandergast and others, 2016), which are all located within conservation areas designated under the Coachella Valley Multiple Species Habitat Conservation Plan. The geographically distinct local populations that we visited between March 2017 and June 2018 included Windy Point, Train Station, Whitewater, Willow Hole, Northwest Indio Hills (previously called West Indio Hill in Vandergast and others, 2016), Central Indio Hills, and the South Coachella Valley Preserve (previously called South Thousand Palms in Vandergast and others, 2016; fig. 2). In addition, we sampled individuals that we salvaged from Section 24 and translocated to Stebbins Dune. We visually searched dune habitat and captured lizards by hand or noose. We measured mass and snout-vent length of each individual and noted sex, breeding color, and any other body conditions. We photographed all individuals with a standard color palette against a standard black background board to facilitate future morphological investigations. For genetic analysis, we clipped a small section of tail (< 1 centimeter) that we stored in 95 percent ethanol. We released all individuals at the point of capture, with the exception that we moved those salvaged in Section 24 to unoccupied suitable habitat on Stebbins Dune.

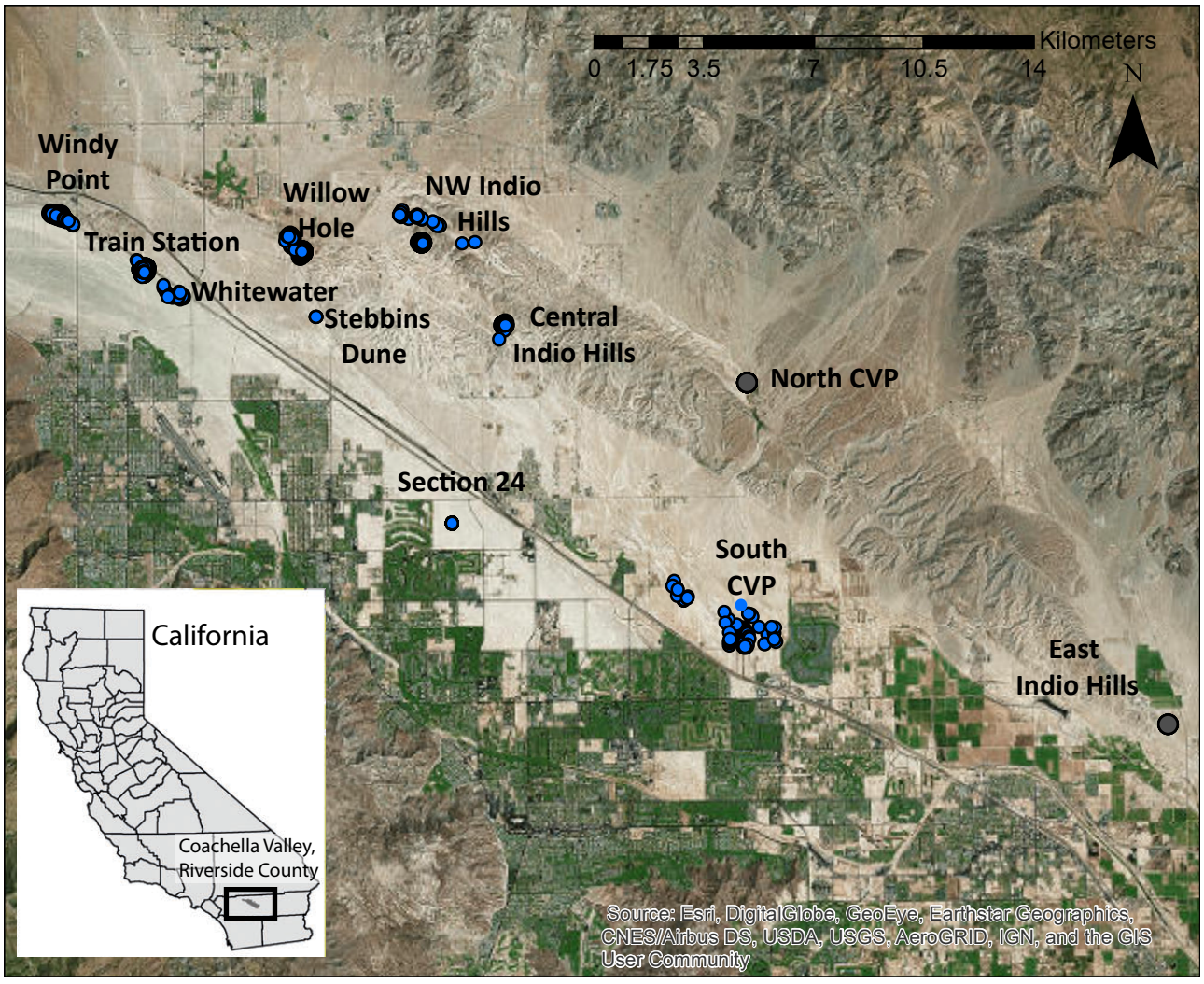

Figure 2. Study area sampled for *Uma inornata*, Coachella Valley, Riverside County, California. Blue dots represent samples collected in 2017. Two additional sites (North Coachella Valley Preserve [CVP] and East Indio Hills; grey dots) were sampled in 1996 but were no longer occupied after that. Individuals collected from Section 24 were translocated to Stebbins Dune.

DNA Extractions and Microsatellite Amplifications

Genomic deoxyribonucleic acid (DNA) was extracted from each sample following standard protocols using the Qiagen DNeasy blood and tissue kit. Eleven microsatellite loci (2L, 2M, PLKN, 2Q, 2O, 2S, 3B, TRI4H, TETQY, TETKL, and DIVQ1) were amplified in four mixed reactions (following Vandergast and others, 2016). Polymerase chain reaction (PCR) conditions were as follows: initial denaturing at 95 degrees Celsius (°C) for 15 minutes, followed by 30 cycles of 94 °C for 30 seconds, 58 °C for 90 seconds, and 72 °C for 60 seconds, followed by a 30-minute final extension at 60 °C. Fragment analysis was performed at Eton Bioscience Inc. (San Diego, California). In addition to genotyping all newly collected samples, 24 samples from the previous genetic study were rerun to check for consistency and standardize allele size calls across datasets. We edited and scored raw data in Gene-Marker v1.90 (SoftGenetics), binned and standardized allele calls using MsatAllele v1.04 (Alberto, 2009), and used Microchecker to screen for null alleles (Van Oosterhout and others, 2004).

RadSEQ Library Preparations and Illumina Sequencing

Prior to next-generation sequencing (NGS) library preparation, we quantified DNA on a Qubit fluorometer (Life Technologies), and 500 nanograms (ng) of DNA were used for library preparation. We followed the double-digest restriction-associated DNA (ddRAD) sequencing protocol developed in Peterson and others (2012) for NGS library preparation, with some modifications. We digested genomic DNAs using 20 units each of the restriction enzymes SbfI and MspI (New England Biolabs, U.S.A.) and used Agencourt AMPure beads (Beckman Coulter, Danvers, Massachusetts) to purify the digestions prior to ligating uniquely bar-coded adapters with T4 ligase (New England Biolabs). We quantified all ligation products on the Qubit fluorometer, pooled across 12 index groups in equimolar concentrations, and then size selected fragments between 415 and 515 base pairs (bp) using a Pippin Prep size fractionator (Sage Science, Beverly, Mass.). We amplified the recovered fragments from each pool using 5–10 ng of the recovered DNA, Phusion High-Fidelity Taq (New England Biolabs), and Illumina's primers. Polymerase chain reaction (PCR) products were then cleaned with Agencourt AMpure beads and quantified using the Qubit fluorometer before being pooled for sequencing (100 bp single end reads) on an Illumina HiSeq 4000 at the Genomics and Cell Characterization Core Facility at University of Oregon.

We generated single nucleotide polymorphism (SNP) datasets using the stacks version 2.2 (Catchen and others, 2013) bioinformatics pipeline. We used the *process_radtag* program to clean and filter raw reads following default settings. We then conducted initial parameter testing following the recommendations of Rochette and Catchen (2017), which involved examining a series of de novo RAD locus assemblies that used a range of values for the mismatch distance between loci within an individual (M), and the number of mismatches between loci in the catalogue (n) from 1 to 6 (fixing $n = M$ and $n = M–1$), and the minimum stack depth (m = 2–7). The final set of parameters chosen for analyses was based on the total number of loci and polymorphic loci shared by 80 percent of samples and how the distribution of SNPs per locus was affected by the range of parameter changes. Once optimal parameters were selected, we then executed the ustacks, cstacks, sstacks, and gstacks modules using the *denovo map* wrapper program.

Data Analysis

For the microsatellite and SNP datasets, we were interested in comparing genetic structure and diversity estimates among the three sample periods: 1996, 2008, and 2017–18 (hereafter 2017). Over this time period, sites have become more fragmented owing to intervening development and have undergone periodic population bottlenecks, particularly during severe droughts in the early 2000s and in the 2010s. Even though population sizes can rebound during years with better conditions, our expectations were to find increased genetic differentiation among local populations and less genetic diversity within local populations over time, if gene flow has been reduced (Slatkin, 1987; Harrison and Hastings, 1996). Because genetic drift acts more rapidly in small versus large populations, drought induced population declines could strengthen the signal of genetic differentiation, reduce genetic diversity, and decrease effective population sizes over time (Lacy, 1987; Slatkin, 1987).

Comparing Genetic Divergence

Microsatellite Dataset

We conducted several analyses to determine whether genetic differentiation increased over time. The magnitude of population differentiation (F_{ST}) was estimated using Weir and Cockerham's (1984) θ, an unbiased estimator of F_{ST} that should not depend on sample size. We also report F'_{ST}, which applies a correction to F_{ST} estimates (F_{ST}/F_{STmax}) and is appropriate for highly variable markers like microsatellites (Meirmans and Hedrick, 2011). Global calculations were performed in GENODIVE version 2.0b27, with 95 percent confidence intervals obtained by bootstrapping over loci (Meirmans and Van Tienderen, 2004). To compare global θ between sampling years, we restricted analyses to include the four local populations sampled with at least eight individuals at all three time periods (Windy Point, Whitewater, Willow Hole, and South Coachella Valley Preserve). We also calculated θ among all pairs of preserves for all three time periods, with 95 percent confidence intervals estimated with 10,000 bootstraps over loci in the program GenAlEx 6.5 (Peakall and Smouse, 2012). Pairwise estimates of θ among all local populations were plotted by geographic distance, and Mantel tests were used to assess correlation between pairwise genetic and geographic distances (Mantel, 1967). We also visually compared box plots of pairwise θ values for each time period.

We used Bayesian clustering analyses to determine the number of genetic clusters to compare among sampling periods. We inferred genetic clusters in 2017 with STRUCTURE version 2.3.4 (Pritchard and others, 2000) using the same conditions previously applied to 1996 and 2008. We used an admixture model with correlated frequencies and an additional prior incorporating location information (LOCPRIOR) because preliminary runs in STRUCTURE suggested that location improved clustering. We estimated the probability of the number of genetic clusters (K) = 1–7 using 500,000 Markov chain Monte Carlo (MCMC) iterations following 500,000 iteration burn-in, with 10 replicate runs to verify consistency across chains. Results were compiled graphically in Clumpak (http://clumpak.tau.ac.il). Optimal K was inferred by comparing the results from the mean lnP(D|K) score against maximum K (KMAX), finding where the lnP(D|K) curve plateaus, and using the Δ K criterion (Evanno and others, 2005). All sampled sites were used in each time period.

Finally, as an alternative method of visualizing genetic differences among groups (in this case, local populations), we used a discriminant analysis of principal components (DAPC) in the adegenet package (v. 2.1.0; Jombart, 2008) in R 3.5.1 (R Development Team, 2011). First, a principal components analysis (PCA) was used to determine linear combinations of alleles that describe linear variation in principal components eigenvalues (PCs). Next, DAPC was used to find the discriminant functions, which show differences among groups while minimizing variation within groups. We used the cross-validation procedure in adegenet to determine the optimal number of PCs to retain in the DAPC analysis. We split the dataset using 90 percent as a training dataset and 10 percent as a validation dataset. The DAPC was carried out with 30 replicates at each level of PC retention. We selected the number of PCs with the lowest root mean squared error (RMSE) and highest mean successful assignments for the final analysis. We included Section 24 in the DAPC analyses to examine how it may have contributed to genetic structure within the Coachella Valley.

SNP Dataset

We also conducted similar analyses using the SNP dataset to determine whether genetic differentiation had increased over time. We assessed population differentiation with two different measures. The first estimator of genetic differentiation was calculated using Weir and Cockerham's (1984) θ using GENODIVE version 2.0b27. We also used the haplotype-based Φ_{ST} statistic to estimate population differentiation incorporating both haplotype frequencies and the genetic distances among them (based on Excoffier and others [1992] and implemented in stacks version 2.2 [Catchen and others, 2013]). To compare global genetic differentiation estimates between sampling years, we restricted analyses to include the four local populations sampled at all three time periods (Windy Point, Whitewater, Willow Hole, and South Coachella Valley Preserve). We calculated θ and Φ_{ST} among all sites for all three time periods, with 95 percent confidence intervals estimated with 10,000 bootstraps over SNPs and haplotypes. Pairwise estimates of θ and Φ_{ST} among all local populations were plotted by geographic distance, and Mantel tests were used to assess correlation between pairwise genetic and geographic distances (Mantel, 1967). We also compared box plots of pairwise θ and Φ_{ST} values for each time period.

We used Bayesian clustering analyses to determine the number of genetic clusters and compare among sampling periods. We inferred genetic clusters with STRUCTURE version 2.3.4 (Pritchard and others, 2000). We used an admixture model with correlated frequencies without incorporating any location information. We specified a range for the maximum number of clusters that individuals could be assigned (K = 1–10) and performed 10 replicate runs per K using 500,000 iterations of the MCMC algorithm following a burn-in of 500,000 iterations to verify consistency across chains. Results were compiled graphically in Clumpak (http://clumpak.tau.ac.il). Because there is often a large range of uncertainty in estimating K (Pritchard and others, 2000; Meirmans, 2015), the optimal K was inferred by comparing the results from the mean lnP(D|K) score against KMAX, and the Δ K criterion (Evanno and others, 2005). All sampled sites were used in each time period except for Section 24. Given that the STRUCTURE results were very clear for SNPs (see "Results" section), we did not find it necessary to use DAPC as a secondary method to infer clusters.

Comparing Genetic Diversity

Microsatellite Dataset

Summary diversity statistics were calculated for each local population sampled in 2017. These included the number of individuals sampled (N), the number of alleles (A), observed (Ho) and expected heterozygosity (He) and the fixation index (F), calculated in GenAlEx 6.5 (Peakall and Smouse, 2012). Allelic richness was calculated using rarefaction to account for sample size differences in the program HP-Rare (Kalinowski, 2005).

We compared the allelic richness (A_r) among years at each local population. We examined allelic richness because it should be more sensitive to population size reductions than other diversity measures (Nei and others, 1975; Leberg, 2002). We also estimated effective population size (N_e) at sites for each sampling year using NeEstimator v. 2.1 (Do and others, 2014). We used the linkage disequilibrium method with a lowest allele frequency of 0.05 and report the parametric 95 percent confidence intervals around the mean.

To determine the putative relationships between individuals translocated from Section 24 and newly captured or recaptured animals at Stebbins Dune, we conducted pedigree analyses using the programs ML Relate and Colony (Jones and Wang, 2010).

To estimate potential recovery of genetic diversity through assisted gene flow, we conducted simulations of population structure over time using the simulation program EASYPOP version 2.0.1 (Balloux, 2001), which simulates neutral genetic variation using a forward time, individual-based model. We ran four simulation scenarios: (1) a single population with a large N_e = 1,000; (2) 10 small subpopulations each with N_e = 100, with maximal gene flow rates (99 percent) for 100 generations, followed by no gene flow for all generations onward; (3) for the 10 small subpopulations above, increasing gene flow to 1 percent (1 migrant per generation) after 50 generations of no gene flow; and (4) for the 10 small subpopulations, increasing gene flow to 10 percent (10 migrants per generation) after 50 generations of no gene flow. In all simulations, sex ratios were equal (overall 1:1 sex ratios have been found in CVFTL populations; A. Muth and M. Fisher, unpub. data, 2019), with identical male and female migration rates and an island migration model. We simulated 20 genetic markers with free recombination, a mutation rate of 0.0001, a stepwise mutation model, and a maximum of 100 allelic states. Initial variability was set as maximal, with randomly assigned alleles.

In each simulation, population genetic data were sampled at 1, 5, 10, 20, 50, and 100 generations after gene flow changes, and we performed 10 replicate runs of each scenario. Allelic richness at each time period was calculated using FSTAT (Goudet, 2003). The average allelic richness across 10 runs was plotted by generation to compare values over time.

SNP Dataset

We calculated summary statistics in stacks to compare genetic diversity among local populations and years. Summary statistics included the following: mean observed heterozygosity (Ho), mean expected heterozygosity (He), mean nucleotide diversity (π), and the fixation index (F) of each site. Allelic richness (A_r)was calculated at each local population across the three samples periods (1996, 2008, 2017) and across all populations for the 2017 sample using rarefaction to account for sample size differences in the program HP-Rare (Kalinowski, 2005). We also estimated effective population size (N_e) at sites for each sampling year in NeEstimator v. 2.1 (Do and others, 2014).

We used the linkage disequilibrium method with a lowest allele frequency of 0.05 and the jackknife-across-samples method (Jones and others, 2016) for 95 percent confidence intervals around the mean.

Assessing Genetic Erosion and Rescue Options

Following Frankham and others (2017), we assessed whether local populations met certain criteria that would indicate that genetic erosion has occurred and if genetic rescue could be beneficial in terms of improving heterozygosity in local populations. Frankham and others (2017) suggest calculating the mean inbreeding coefficient (*F*) as the ratio of average heterozygosity (*H*) of the receiver population (inbred) to the proposed donor population(s) (outbred).

$$F = 1 - \frac{H\text{inbred}}{H\text{outbred}} \quad (1)$$

If *F* is greater than 0.1, then it is likely that the receiver population is suffering genetic erosion in comparison to the donor population and therefore may benefit from augmentation (Frankham and others, 2017). We used our estimates of heterozygosity from the SNP dataset to calculate *F* for each local population in relation to the following donor populations: (1) South CVP (the local population with highest He and largest N_e), (2) Section 24 (to represent a site where development will occur and take is approved), and (3) a composite donor population comprised of individuals from multiple local populations. To estimate He in the composite donor population, we randomly selected 25 individuals from each of the following local populations: Windy Point, Train Station, Whitewater, Willow Hole, NW Indio Hills, and South CVP.

Results

Collections and Genotyping

We genotyped 318 lizards captured in 2017 (fig. 2). Sample sizes per sample locations ranged from 14 individuals in the Central Indio Hills to 69 in Whitewater. Four individuals were sampled post translocation at Stebbins Dune. These were hypothesized to be either recaptures or offspring of translocated animals from Section 24.

Four of the 318 samples were determined to be recaptures based on exact matching genotypes and corroborating field data. One recapture was translocated as a juvenile from Section 24 and recaptured as an adult female at Stebbins Dune. The other three individuals were likely individuals whose markings had worn off and were recaptured at original sites. The probability of two unrelated individuals having the same genotype (Probability of Identity; P_{ID}) across sites was very low and ranged from 7.5×10^{-9} at South Coachella Valley Preserve (South CVP) to 1.2×10^{-5} at Windy Point. Probability of two siblings having the same genotype (P_{sib}) ranged from 4.3×10^{-4} (South CVP) to 1.3×10^{-3} (Windy Point). Recaptures were removed from the dataset for subsequent analyses.

Microsatellite Results

We found no evidence of linkage disequilibrium among loci. However, tests for Hardy-Weinberg equilibrium indicated that one locus (locus 3B) differed significantly from Hardy Weinberg equilibrium in three sites, Whitewater, NW Indio Hills, and Section 24, with fewer observed heterozygotes than expected. This could be due to allelic drop out, purifying selection, or a Wahlund effect caused by mixed age cohorts in the sample (reviewed in Waples, 2014). This locus does not appear to be sex linked. F-statistics calculated using a correction for allelic drop out were largely identical to those without; therefore, we included all loci in our analyses.

Across the four main local populations (Windy Point, Whitewater, Willow Hole, and South CVP), global estimates of genetic differentiation increased over the three sampled time points (table 1) and were significantly different from zero in 2008 and 2017. Including all sampled sites within each sampling year, pairwise estimates of genetic differentiation and the variance among them also increased over time (fig. 3). There was no detectable pattern of increasing genetic isolation with geographic distance at any sample period (fig. 4).

Table 1. Global population differentiation estimates and 95 percent confidence intervals for microsatellite and single nucleotide polymorphism (SNP) datasets for the four preserves sampled across three time periods.

[Sites included in this analysis are Windy Point, Whitewater, Willow Hole, and South CVP]

Estimator	Sampling Period		
	1996	2008	2017
	Microsatellites		
Θ	0.012 (0.002, 0.022)	0.041 (0.015, 0.066)	0.059 (0.021, 0.097)
F'_{ST}	0.031 (0.010, 0.053)	0.097 (0.040, 0.154)	0.128 (0.052,0.204)
	SNPs		
Θ	0.021 (0.004, 0.038)	0.048 (0.025, 0.071)	0.042 (0.019, 0.068)
Φ_{ST}	0.020 (0.008, 0.033)	0.044 (0.033, 0.006)	0.030 (0.015, 0.044)

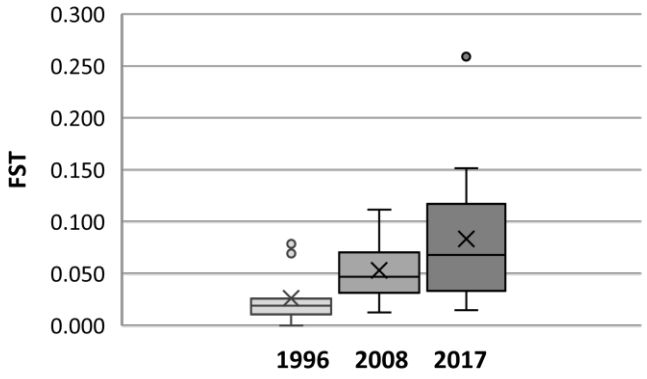

Figure 3. Pairwise genetic differentiation (F_{ST}) among populations in 1996, 2008, and 2017 estimated with microsatellite loci. Sampled sites included were the following: 1996—Windy Point, Whitewater, Willow Hole, NW Indio Hills, and South Coachella Valley Preserve (CVP); 2008—Windy Point, Train Station, Whitewater, Willow Hole, Central Indio Hills, and South CVP; 2017—Windy Point, Train Station, Whitewater, Willow Hole, NW Indio Hills, Central Indio Hills, and South CVP. Lines represent median values, boxes span the 25 percent and 75 percent quartiles around the medians. Mean values are represented by X's.

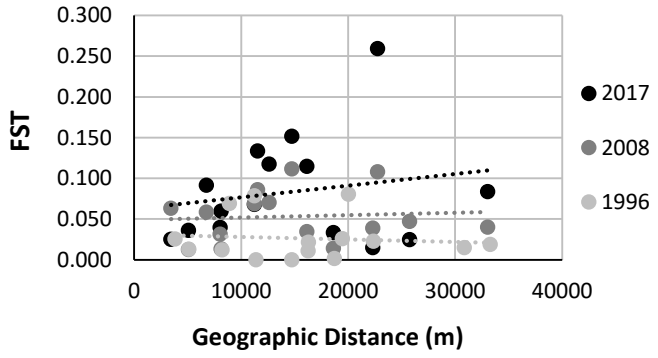

Figure 4. Pairwise genetic differentiation (F_{ST}) estimates based on microsatellite loci, plotted by geographic distance among local populations in meters. Light grey dots are estimates made in 1996, dark grey dots in 2008, and black dots in 2017. Trend lines are included as dotted lines. Values tended to increase over time.

Despite an increasing trend in genetic differentiation among sites between 2017 and the previous sample period in 2008, the structure analysis resulted in similar clustering of populations into two or three genetic clusters with a cline in assignment between Windy Point and the Central Indio Hills. In the 2017 sample at the preferred K = 2 (based on Δ K), the populations at Windy Point and Central Indio Hills appear to have unique cluster assignments (fig. 4). Train Station, Whitewater, and South CVP individuals have relatively equally mixed assignment between the two clusters. At K = 3, the two Indio Hills populations are distinguished (fig. 5). At K = 7 (which was the K with the highest probability), most individuals appear admixed (fig. 5), suggesting this level of K may be overfitting the data. These results are different from previous clustering results in both 1996 (one cluster across the entire range, Vandergast and others, 2016) and 2007 (Whitewater formed a distinct cluster, Vandergast and others, 2016).

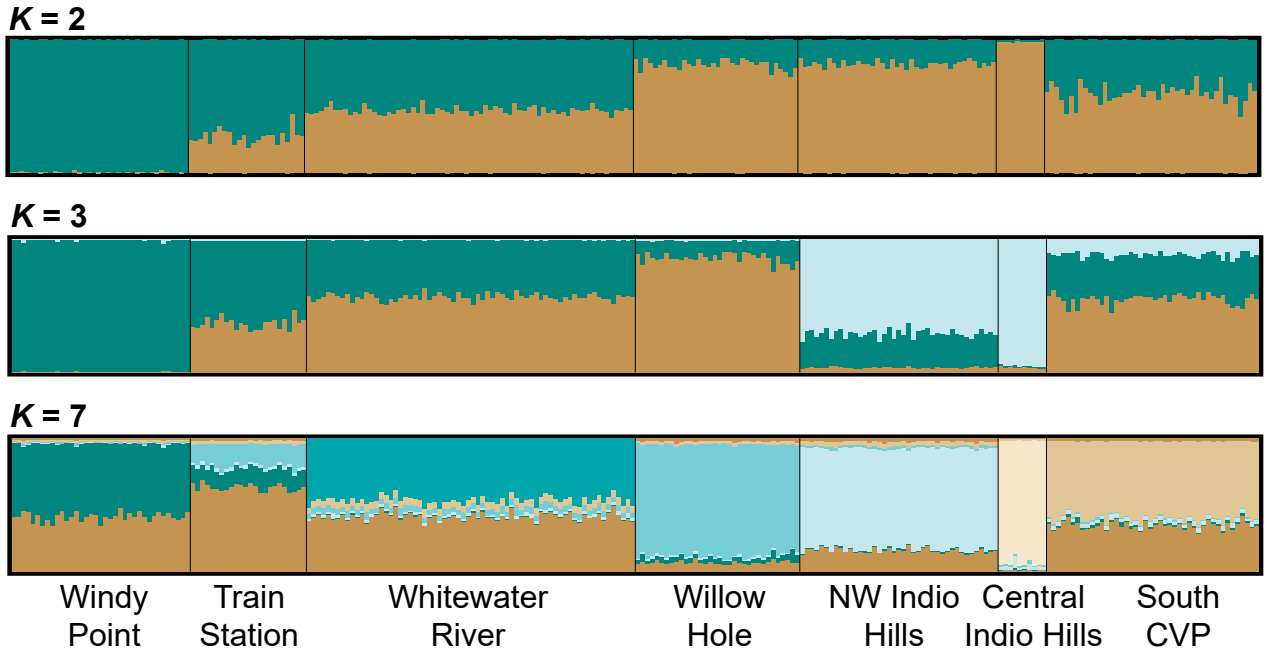

Figure 5. Structure assignment plots for number of genetic clusters (K) = 2 and K = 3 for the 2017 microsatellite dataset. K = 2 is the preferred number of clusters based on Δ K, although at K = 3 the smaller and more isolated Indio Hills sites cluster separately. K = 7 had the highest probability (Prob K = 0.995, Pritchard and others, 2000), with Willow Hole, W Indio Hills, and Central Indio Hills showing unique identity.

Cross-validation in the DAPC analysis suggested 45 PCs maximized the successful assignment rate at 0.666 and resulted in the lowest root mean square error (RMSE) of 0.341. Seven discriminant functions resulted from the DAPC analysis, with a proportion of conserved variance of 0.978 of the total variance. Resulting discriminant analysis eigenvectors (DAs) discriminated Central Indio Hills most strongly, followed by NW Indio Hills. Individuals from all other sites appeared to show more overlap, with some west-east clinal variation between Windy Point and South CVP (fig. 6). Together, clustering analyses show that there is still significant allele-sharing at microsatellite loci among most sites, even though the magnitude of differences appears to be getting larger over time based on F_{ST}.

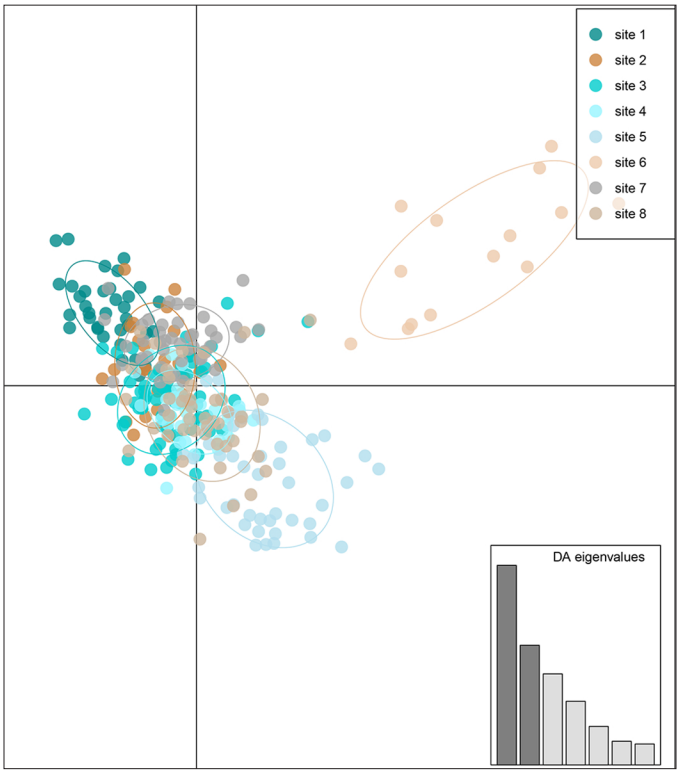

Figure 6. Sampled individuals by discriminant analysis eigenvectors (DA) 1 (x-axis) and 2 (y-axis) for the 2017 microsatellite dataset. Individuals are colored by their population assignments. Individuals from Central Indio Hills fall out most distinctly (tan, site 6), followed by NW Indio Hills (light blue, site 5). Other sites tend to cluster in a cline similar to their geographic locations from west to east, from Willow Hole (dark green, site 1) to South CVP (light brown, site 8). Site numbers correspond as follows: site 1 (dark green) = Windy Point, site 2 (brown) = Train Station, site 3 (turquoise) = Whitewater, site 4 (light turquoise) = Willow Hole, site 5 (light blue) = NW Indio Hills, site 6 (tan) = Central Indio Hills, site 7 (grey) = Section 24, site 8 (light brown) = South CVP.

Between 2008 and 2017, allelic richness decreased significantly in three sites: Windy Point (t-statistic = 3.33, 10 degrees of freedom (d.f.), $p \leq 0.0038$), Willow Hole (t-statistic = 1.884, 10 d.f., $p \leq 0.0445$), and Central Indio Hills (t-statistic = 3.161, 10 d.f., $p \leq 0.0051$). Data for these comparisons are presented in appendix 1. Overall, allelic richness was lowest in Central Indio Hills (average $A_r = 2.87$) followed by Windy Point (2.99; table 2). The site with the highest allelic richness in 2017 was South CVP (4.21; table 2). This site is the largest in terms of remaining habitat and estimated abundance during our sampling periods, although relative abundance has fluctuated dramatically in this population in the past. For example, in the mid-1980s, the census size was extremely small (A. Muth and M. Fisher, unpub. data, 2019) on a plot that had the highest density a few years earlier (Turner and others, 1984). In 1977, a flood breached the Colorado River Aqueduct (east of map area) and deposited silt, leaving just a handful of dunes.

Simulations

Simulation results showed a large (78 percent) decline in allelic richness over time in small populations with no gene flow by 100 generations (fig. 7). Loss of diversity was not linear, and declines were steepest over the first 20 generations. This may have implications for long-term monitoring of loss of diversity. Declines may appear smaller and less significant in later generations of monitoring in fragmented systems. The Coachella Valley has undergone a steady increase in urbanization over the last half century. In the translocation scenarios, restoring gene flow to 1 percent increased allelic richness threefold over the no gene flow scenario, whereas restoring to 10 percent led to a sixfold increase in allelic richness and met or exceeded the level of allelic richness in the stable population with $N_e = 1{,}000$.

Translocation Monitoring at Stebbins Dune

Forty-two individuals were captured in Section 24 and released on Stebbins Dune. The initial post-translocation sampling of this dune was opportunistic and not exhaustive; however, all captured animals showed some relationship to translocated animals from Section 24. Of the four individuals captured, one was a recapture of an individual moved from Section 24, and the other three were genetically closely related to the individuals originally moved from Section 24. This suggests that at least some translocated individuals survived one season at the new site and, given that three individuals were juveniles, reproduced. Continued monitoring of this site to detect lizards will help to determine if the translocation was successful.

Table 2. Diversity statistics based on microsatellite loci for all sites sampled in 2017

[Section 24 is included for comparison purposes, but because sampled individuals were translocated to Stebbins Dune, we did not include this site in genetic divergence estimates. Abbreviations: N, average number sampled across 11 loci; A, average number of alleles; Ar, average number of alleles rarified to 18 gene copies; Ho, observed average heterozygosity; He, expected average heterozygosity; F, fixation index = (He – Ho) / He]

Site	N	A	A_r	Ho	He	F
Windy Point	37.00	3.73	2.99	0.423	0.432	0.018
Train Station	24.27	4.46	3.64	0.552	0.537	–0.017
Whitewater	66.82	5.73	4.04	0.576	0.609	0.065
Willow Hole	34.82	5.18	4.05	0.584	0.596	0.029
NW Indio Hills	42.00	5.00	3.91	0.532	0.564	0.077
Central Indio Hills	13.91	3.00	2.87	0.583	0.505	–0.164
South CVP	44.55	6.18	4.21	0.599	0.599	0.031
Section 24	46.73	4.09	3.18	0.475	0.482	0.064

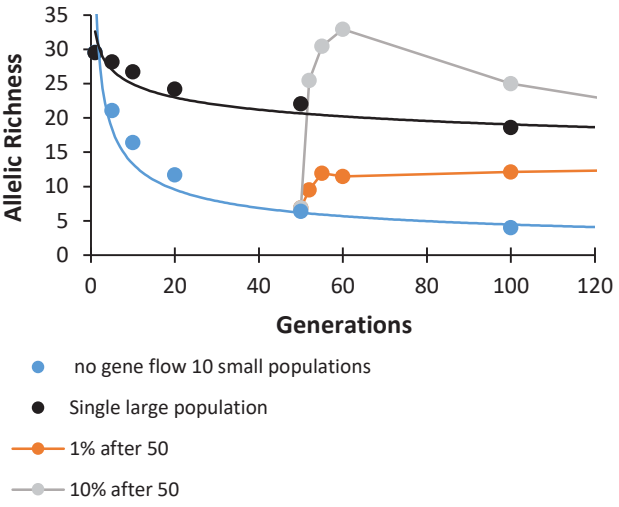

Figure 7. Simulations showing average allelic richness over time sampled at 20 loci and replicated 10 times. (1) A single large population (N_e = 1,000; in black); (2) an original large population split into 10 small populations (N_e = 100) with no gene flow (in blue); (3) at generation 50, restoring gene flow rate to 1 percent (one migrant per generation; orange); (4) at generation 50, restoring gene flow rate to 10 percent (10 migrants per generation; grey).

SNP Results

Bioinformatics

We used 55 samples that were chosen from across all sites (2–6 individuals per site) and years sampled (18–24 samples per year) to construct a catalogue of loci for our multi-year SNP dataset. The ddRAD sequencing effort yielded 49,463 loci after merging and calling final consensus sequences across the 489 samples. The mean coverage depth per individual recovered was 104.7x (min: 13.5x; max: 169.5x; standard deviation: 24.7x). All loci and variable sites produced by stacks were subjected to a final filtering approach that retained loci present across ≥ 91 percent of the sampled sites and were found in 85 percent of the individuals sequenced. We used these filtering constraints to obtain two datasets: one with all sites sampled across the sampled years (all sites) and another restricted to four local populations that were sampled at all three time periods. We used stacks to randomly select a single SNP from each of the loci once filtering constraints were applied. This process produced 659 (all sites) and 940 (four local populations) unlinked SNPs for population structure and genetic diversity analyses.

Population Structure

Across the four local populations sampled at all time periods (Windy Point, Whitewater, Willow Hole, and South CVP), global estimates of genetic differentiation increased between 1996 and 2008 and then decreased slightly in 2017, although estimates in 2017 were still higher than 1996 (table 1). This same pattern was observed in the pairwise estimates of genetic differentiation when all sampled sites were included (fig. 8). We did not detect a significant pattern of increasing genetic isolation with geographic distance among sites, but pairwise values of genetic differentiation tended to increase from 1996 to 2008 and remained similar between 2008 and 2017 (fig. 9).

Similar to microsatellite results, estimates of the number of genetic clusters across sampled years increased over time on the basis of the SNP dataset. Bayesian clustering analyses of 1996 samples suggested that all sampled sites comprised a single cluster (fig. 10). However, in 2008 and 2017, multiple clusters were detected. In the 2008 sample, two to three clusters were detected depending on the method used to choose the optimal K, with Windy Point separated from all other sites (K=2) or an additional cluster comprised of Willow Hole and Indio Hills sites (K=3). In the 2017 sample, seven genetic clusters were detected that conform to the seven sampled sites, although some individuals from each of the sites exhibit mixed assignments with the exception of Windy Point (fig. 9).

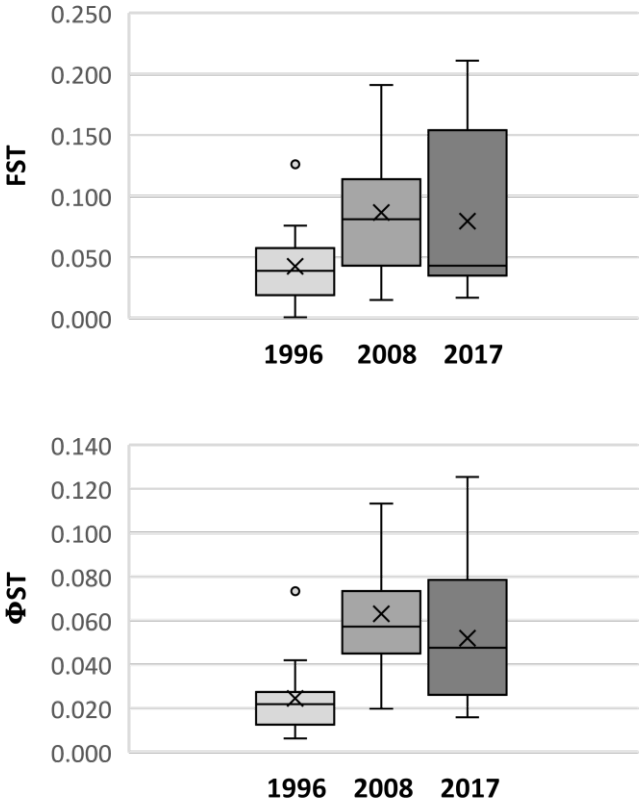

Figure 8. Single nucleotide polymorphism (SNP) pairwise genetic differentiation (F_{ST} and Φ_{ST}) among populations in 1996, 2008, and 2017. Sampled sites were the same as those included in figure 2. Lines represent median values, boxes span the 25 percent and 75 percent quartiles around the medians.

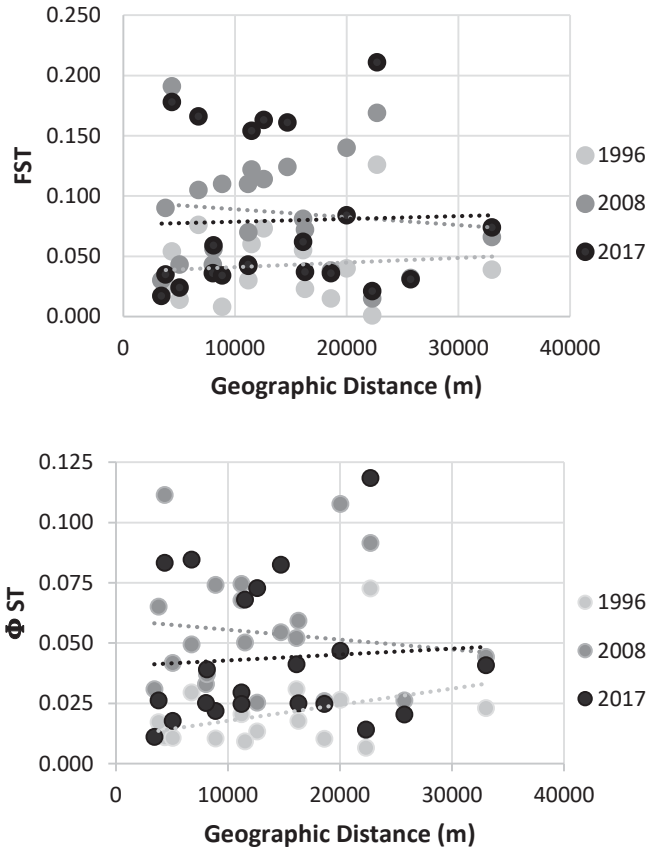

Figure 9. Pairwise genetic differentiation estimates (F_{ST} and Φ_{ST}) from single nucleotide polymorphisms (SNPs) plotted by geographic distance among sites in meters. Light grey dots are estimates between sites measured in 1996, dark grey dots in 2008, and black dots in 2017. Trend lines are included as dotted lines. Values tended to increase over time.

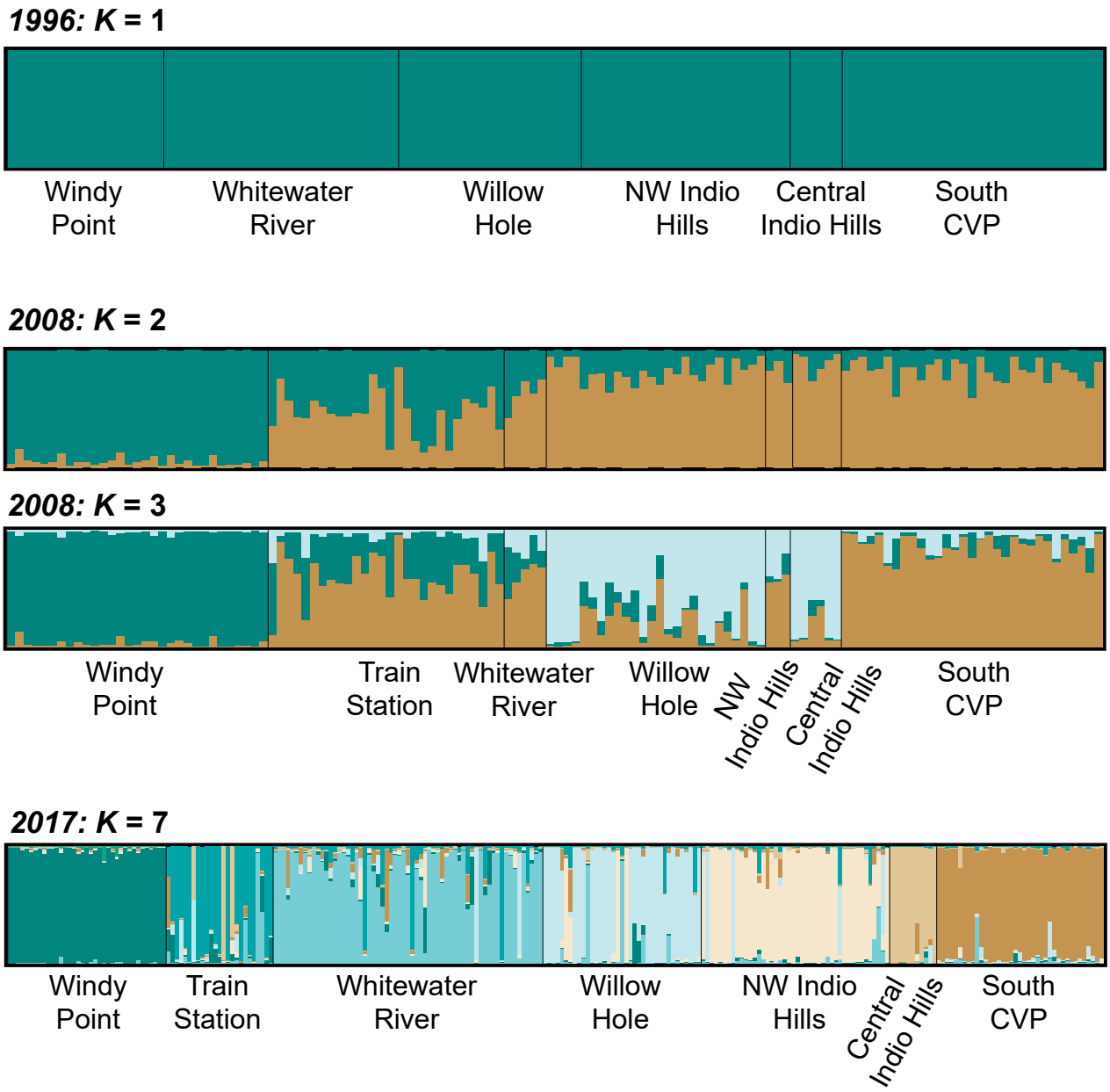

Figure 10. Structure assignment plots for 1996, 2008, and 2017 for single nucleotide polymorphism (SNP) datasets. In 2008, the number of clusters (K) = 2 is the preferred number of clusters based on Δ K plots, although at K = 3 the smaller and more isolated Indio Hills sites cluster separately. In 2017, all sites were genetically distinguishable (K = 7).

Genetic Diversity

Genetic diversity estimates for each sampled site and across years are given in table 3. For the 2017 sample, allelic richness ranged from 1.32 (Windy Point) to 1.66 (Train Station). Consistent with increasing genetic differentiation among sites, allelic richness estimates decreased among years in four of seven local populations sampled (Windy Point, Whitewater, NW Indio Hills, and Central Indio Hills). It remained relatively constant at South CVP and increased slightly at Train Station and Willow Hole. Although theory predicts that allelic richness should decrease more rapidly in a declining population than heterozygosity (Nei and others, 1975; Leberg, 2002), we found observed heterozygosity decreased from 1996 to 2017 in at least two of the sites where allelic richness had decreased (Windy Point and Central Indio Hills). In addition, we found increases in the associated fixation index (F) estimate at five of the seven sites sampled. Taken together, these downward trends in diversity indices (Ar and He) along with increases in the fixation index could indicate increased inbreeding within some sites and (or) that some populations are more susceptible to the effects of genetic drift.

Effective population size point estimates based on SNPs were generally higher than estimates obtained using the microsatellite data with some exceptions (table 4). Generally, confidence intervals around point estimates were smaller than those obtained with microsatellites, and there were fewer cases where upper confidence intervals could not be resolved (table 4). The SNP estimates of N_e at Willow Hole in 2008 and 2017 were much lower than estimates obtained with microsatellites (table 3). In 2017, three sites (Willow Hole, NW Indio Hills, and Central Indio Hills) had estimates below the short-term threshold recommendation to limit inbreeding depression (≥ 100). Owing to the limited number of samples in 2008, we were not able to compare N_e estimates across years at Whitewater and NW Indio Hills.

Genetic Erosion and Potential for Genetic Rescue

Across all three investigated rescue options, we estimated mean inbreeding coefficients (*F)* greater than 10 percent for two local populations: Windy Point and Central Indio Hills (table 5). With a composite donor population, we additionally found *F* greater than 10 percent for Train Station and that most other local populations approached the 10 percent threshold (8.9 percent or greater). The exception was South CVP, which had a lower, but still positive *F* of 4.5 percent when compared to the composite donor population. In comparison, using Section 24 as a donor site would be less beneficial generally because several recipient sites had greater heterozygosity than Section 24 (resulting in negative *F* values; table 5). Overall, these results suggest that augmentation could increase heterozygosity and genetic diversity in some sites and could be most beneficial across most local populations if a composite donor source was used.

Table 3. Diversity statistics based on single nucleotide polymorphisms (SNPs) for all sites sampled in 2017.

[Allelic richness (Ar) estimates were conducted across years at each site and were rarified by lowest number of gene copies per site (2N); only sites with six or more samples were used. Allelic richness was also estimated across sites for the 2017 sample year only (estimates in parentheses, rarified to 22 gene copies). Asterisks (*) indicate that estimates could not be obtained. **Abbreviations**:; N, mean number of individuals per locus; Ar, allelic richness; Ho, mean observed heterozygosity; He, mean expected heterozygosity; π, mean nucleotide diversity; F, fixation index = (He – Ho) / He, where π is used as the value for expected heterozygosity (Hartl and Clark 2007); NW, northwest; CVP, Coachella Valley Preserve]

Sample period	N	Ar	Ho	He	π	F
Windy Point						
1996	6	1.41	0.096	0.091	0.099	0.008
2008	31	1.30	0.091	0.094	0.095	0.018
2017	36	1.28	0.089	0.090	0.091	0.012
		(1.32)				
Train Station						
1996	*	*	*	*	*	*
2008	28	1.48	0.106	0.106	0.108	0.012
2017	24	1.60	0.112	0.109	0.111	0.005
		(1.66)				
Whitewater						
1996	10	1.51	0.107	0.109	0.115	0.008
2008	5	*	0.106	0.100	0.111	0.011
2017	61	1.44	0.111	0.114	0.115	0.029
		(1.48)				
Willow Hole						
1996	7	1.37	0.113	0.106	0.115	0.035
2008	25	1.36	0.099	0.106	0.108	0.034
2017	36	1.48	0.111	0.114	0.116	0.046
		(1.51)				
NW Indio Hills						
1996	8	1.43	0.106	0.112	0.120	0.035
2008	3	*	0.101	0.076	0.092	–0.016
2017	42	1.41	0.108	0.115	0.117	0.051
		(1.49)				
Central Indio Hills						
1996	2	*	0.094	0.111	0.109	0.022
2008	6	1.35	0.089	0.083	0.091	0.006
2017	11	1.25	0.089	0.083	0.091	0.006
		(1.38)				
South CVP						
1996	10	1.46	0.110	0.111	0.116	0.022
2008	31	1.46	0.109	0.112	0.114	0.019
2017	38	1.45	0.106	0.113	0.115	0.041
		(1.49)				
Section 24						
2017	42	(1.44)	0.103	0.110	0.111	0.031

Table 4. Effective population size (N_e) estimates across sampling years and sites estimated with microsatellite loci and single nucleotide polymorphisms (SNPs).

[All samples were combined into a single estimate for 1996 because a single genetic cluster was detected across all sites in this sampling year. INF indicates an estimated confidence interval of "infinity," suggesting there is not enough information to obtain a reliable estimate. NA indicates no point estimate could be obtained. Error in N_e estimates generally decreases with increasing sample sizes of individuals and genetic markers (Waples and Do, 2010). For site/year combinations where estimates based on one marker type did not resolve an upper confidence interval, we recommend favoring the estimate from the other marker type. In cases where multiple robust estimates are available, Waples (2016) suggests combining these using a harmonic mean, or sample-weighted harmonic mean. **Abbreviations**: N_e, effective population size; msats, microsatellite loci; snps, single nucleotide polymorphisms; CVP, Coachella Valley Preserve; NW, northwest]

Population	N_e (msats)	Sample size (msats)	N_e (snps)	Sample size (snps)
1996				
All sites	185 (89–2,009)	70	323 (163–4,554)	43
2008				
Windy Point	77 (33–INF)	30	87 (30–INF)	32
Train Station	29 (18–54)	32	22 (13–47)	29
Whitewater	39 (25–68)	40	NA	5
Willow Hole	57 (30–212)	30	42 (27–79)	26
Central Indio Hills	8 (1.7–INF)	6	3 (2–22)	6
South CVP	327 (89–INF)	45	51 (20–INF)	32
2017				
Windy Point	62 (27–1,211)	37	167 (82–2,298)	37
Train Station	52 (21–INF)	22	124 (62–1,457)	25
Whitewater	104 (60–256)	66	205 (104–1,377)	64
Willow Hole	69 (35–332)	34	44 (24–120)	37
NW Indio Hills	198 (46–INF)	41	81 (50–178)	44
Central Indio Hills	3 (2–18)	10	8 (4–17)	11
South CVP	1,211 (80–INF)	45	652 (372–2,471)	39
Section 24	NA	43	158 (81–887)	44

Table 5. Assessment of genetic erosion and the expected impact of genetic rescue from three potential donor populations.

[Criteria are described in Frankham and others (2017). These include assessing whether the recipient population is isolated (gene flow ≤ 5 effective migrants per generation), whether the population is small or has been very small for multiple generations, and whether $F > 10$ percent. Abbreviations: N_e, effective population size; He, expected heterozygosity; F, inbreeding coefficient; NW, northwest; CVP, Coachella Valley Preserve]

Recipient site	2017 N_e	Private alleles	He	Donor population			Pop. isolated	Pop. small	F> 0.1
				South CVP F	Sect. 24 F	Composite[1] F			
Windy Point	167	16	0.103	0.243	0.201	0.276	Yes	No	Yes
Train Station	124	36	0.125	0.086	0.036	0.127	Yes	Yes[2]	Yes
Whitewater	205	33	0.130	0.047	–0.006	0.089	Yes	No	No
Willow Hole	44	27	0.130	0.051	–0.001	0.094	Yes	Yes	No
NW Indio Hills	81	23	0.130	0.051	–0.002	0.093	Yes	Yes	No
Central Indio Hills	8	12	0.098	0.285	0.246	0.317	Yes	Yes	Yes
South CVP	652	51	0.137	—	–0.056	0.045	Yes	No	No
Section 24	158	32	0.129	0.053	—	0.095	Yes	No	No

[1]Composite donor population comprises equal contributions from each of the following local populations (Windy Point, Train Station, Whitewater, Willow Hole, NW Indio Hills, South CVP), with He estimated by combining 25 randomly selected individuals from each.

[2]$N_e < 100$ in 2008.

Discussion

Genetic analyses of 2017 data appear concordant with that reported after 2008; genetic differentiation has increased from the 1996 baseline. Confidence intervals in both global and pairwise F_{ST} estimates are overlapping between 2008 and 2017 estimates, suggesting more stability over this more recent time frame. Even so, clustering analyses of both marker types revealed increased distinctions among the sampled sites through time, which may indicate that differentiation will continue to climb until a new equilibrium between drift and gene flow is reached.

Differences in the patterns and magnitude of change between the different marker types (microsatellites versus SNPs) are expected and likely due to sampling error associated with each marker set. Eleven microsatellite loci represent a much smaller portion of the genome than hundreds of SNP markers. By virtue of the larger number of markers, the SNP dataset should be more robust than microsatellites to individual locus differences. However, both datasets may be sensitive to low sample sizes in some populations during some sample periods. Here, we take a holistic approach and highlight patterns that appear to be supported by both datasets. In terms of genetic differentiation (examined through Structure plots and F_{ST} estimates), both datasets robustly support that differentiation has increased from estimated levels in 1996.

In addition, a decreasing trend in allelic richness (a measure of genetic diversity) was detected in both datasets. It also appears that different sites were significantly impacted during different time periods, highlighting the stochasticity and independence of populations in this system. Between 1996 and 2008, we detected a significant loss in allelic richness in only one population, Whitewater. Between 2008 and 2017, three other populations experienced significant declines in microsatellite allelic richness and concomitant declines in SNP allelic richness (Windy Point, Willow Hole, and Central Indio Hills). Such differences among sites over sampling periods could reflect individual site differences in population stability or stochastic differences owing to the process of genetic drift and genetic sampling.

In 2017, the effective population sizes were low (upper confidence intervals < 100) in Central Indio Hills and Willow Hole (and maybe even NW Indio Hills). In contrast, Whitewater appears to have increased in N_e between 2008 and 2017. In other comparisons across years, confidence intervals generally overlapped. The SNP dataset had more instances of resolved upper confidence intervals for N_e estimates, which suggests these markers have greater information content than our microsatellite markers. In cases where multiple robust estimates are available (with resolved confidence intervals), Waples (2016) suggests it may be appropriate to estimate the harmonic mean or sample-weighted harmonic mean of these rather than favoring one estimate over another. However, in cases where confidence intervals could only be determined in one marker set, that estimate is likely more reliable and is preferred. A recent review of empirical studies suggests that population effective size should be maintained above 100 to avoid short-term inbreeding depression and fitness loss and above 1000 to retain genetic diversity over evolutionary time scales (Frankham and others, 2014). Central Indio Hills does not meet this lower threshold based on either set of markers, and Willow Hole and NW Indio Hills may be below or at this threshold. In contrast, four sites (Windy Point, Train Station, Whitewater, and South CVP) may currently exceed N_e of 1,000.

Preliminary results of the translocation experiment show that at least some of the adults captured at Section 24 produced offspring at Stebbins Dune. It is noteworthy that no adults were seen on Stebbins Dune the following year, with the exception of one that was translocated as a juvenile. Adults might not survive the translocation process, perhaps because of strong homing instincts (McCoy and others, 2014). This suggests further that very young juveniles or gravid females might be the preferred subjects for future translocations. Our evaluations of local population heterozygosity suggest that at least two local populations (Windy Point and Central Indio Hills) could be experiencing genetic erosion and that augmentation from a composite source (for example, using some individuals from all local populations) would have the potential to restore at least some genetic diversity across most local populations.

Conclusions and Future Directions

Long-term maintenance of genetic diversity is a recovery objective for CVFTL (U. S. Fish and Wildlife Service, 1984). Likewise, an objective of the Coachella Valley Multiple Species Habitat Conservation Plan, under which habitat for CVFTL is conserved, is to maintain self-sustaining CVFTL populations in four core habitat areas (South CVP, Whitewater, Windy Point, and Willow Hole). The general trends that we have detected suggest increasing genetic differentiation over the past 20 years, indicating isolation. Diversity appears to be maintained in some sites, while it has decreased in others (indicating bottlenecks or sustained small local population sizes and reduced or absent gene flow).

If, through management action, populations can retain large and stable census sizes over time, they may better meet the recovery objective of maintaining genetic diversity over the long term. However, it may prove difficult to buffer individual sites from the impacts of droughts and flooding and other stochastic events that have been linked to negative growth rates and sporadic fluctuations in population size (Barrows, 2006; Barrows and Allen, 2007), and which can act to reduce N_e. In fact, the frequency and magnitude of drought events may increase in the California deserts under some climate change predictions (Cayan and others, 2008; Bachelet and others, 2016).

Another option to slow the loss of genetic diversity over time, and replace some of what has been already been lost in some local populations through drift, could be to reconnect these populations through assisted gene flow or other management actions aimed at restoring habitat connectivity among sites. Restoring gene flow would approximate a larger linked metapopulation, such as likely existed historically prior to habitat fragmentation (Hedtke and others, 2007). Our simulations illustrate that populations connected by gene flow of between 1 percent and 10 percent will retain much more genetic diversity than if they remain isolated.

Restoring gene flow, termed "genetic rescue," is one of the primary evolutionary-based mechanisms to slow or stop the decline of dwindling populations (Whiteley and others, 2015). When migrants cross with residents, heterozygosity tends to increase, masking deleterious alleles, and increasing long-term evolutionary potential (Frankham 2015; Hedrick and Garcia-Dorado, 2016). Following criteria developed by Frankham and others (2017), we found genetic erosion has likely occurred at least in some local populations. Frankham and others (2011) and Frankham and others (2014) provide additional decision trees and tables to help determine whether it is appropriate to implement genetic rescue. An important determining factor is whether there is a significant risk of outbreeding depression. A risk of outbreeding depression could be higher in cases where the taxonomy is unresolved, there are fixed chromosomal differences, or substantial environmental differences among sites that have been separated for greater than 20 generations. A potential complicating factor to consider in translocation actions in CVFTL is whether local adaptation occurs and whether translocation efforts could reduce the effectiveness of local adaptation.

The remaining occupied habitat fragments for CVFTL span a gradient of temperatures, annual precipitation, potential competitors, sand color and grain size, and food resources; all potential features to which natural selection could respond. Lizards are capable of rapid evolution to changed habitat conditions (Losos and others, 2006; Campbell-Staton and others, 2016; Winchell and others, 2016). "Evolutionary rescue" is the process of adaptation that allows local population recovery from environmentally induced demographic effects that otherwise would have caused extinction (Carlson and others, 2014). However, the effectiveness of selection is also attenuated by small population size. For example, a simulation study suggested that when local population sizes were large ($N_e \geq 200$), isolation resulted in increased mean fitness when some loci were under heterogeneous directional selection. However, when local populations were small ($N_e < 100$), increased isolation improved local adaptation very little and overall fitness was reduced owing to increased genetic load from drift (Lopez and others, 2009). Another empirical study of the beetle *Tribolium castaneum* found that in the short term, allowing for local adaptation improved fitness and reduced extinction risk of small isolated populations. However, over the longer term (after five generations), fitness declined but was restored after admixture from other sites reduced drift load. The authors interpreted their results to suggest that relying solely on adaptation from standing genetic variation may not provide long-term benefits to small isolated populations and that active management facilitating gene flow may be necessary for longer term persistence (Stewart and others, 2017). Theoretical work also suggests that low levels of gene flow (1–10 migrants per generation and < 20 percent of recipient population size) should not swamp locally adaptive alleles (Hendrick, 1995; Mills and Allendorf, 1996). More detailed simulation studies could be conducted to critically evaluate various management options that could be implemented, such as increasing movement among specific core habitat areas, including stochastic population bottlenecks and simulating the effects of markers under different selection regimes.

While addressing questions of whether local adaptation is ongoing and how it could affect subsequent management/translocation strategies, it may be useful to continue monitoring key genetic metrics such as diversity and N_e, as well as population responses to an increasingly arid climate. Our study illustrates that genetic monitoring on a decadal time frame can detect both upward and downward trends in key genetic metrics in this species. The SNP markers generated through RADseq, when coupled with robust population sampling, generally have a greater ability to detect changes in structure and effective population size than our original, smaller microsatellite panel and so should be the preferred method for future genetic monitoring efforts.

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Appendix 1. Microsatellite Allelic Richness

Table 1–1. Microsatellite allelic richness by locus and sample period for sites that showed significant declines over time.

[Allelic richness was calculated using rarefaction based on the smallest sample size within each site]

Year	Locus 2L	2M	PLKN	2Q	2O	2S	3B	TRI4H	TETQY	TET_KL	DI_VQ1	Average over loci
Whitewater (26 gene copies)												
1996	4.00	5.85	2.00	6.78	7.00	8.71	2.93	3.86	3.86	4.99	3.86	**4.89**
2008*	4.38	5.63	2.63	5.50	6.60	7.47	2.81	3.14	4.24	3.86	3.46	**4.52**
2017	4.31	5.16	2.89	6.27	5.78	6.29	3.37	3.16	3.57	3.64	3.97	**4.40**
Windy Point (16 gene copies)												
1996	5.00	4.00	2.00	4.00	4.00	5.00	2.00	2.00	2.00	4.00	4.00	**3.45**
2008	4.64	4.17	1.64	3.84	5.21	4.72	2.21	3.36	2.96	3.57	2.87	**3.56**
2017*	2.63	3.45	1.87	3.32	4.49	4.39	2.49	2.83	1.97	2.21	2.31	**2.91**
Willow Hole (18 gene copies)												
1996	4.88	2.99	2.89	4.77	7.75	4.89	2.89	2.00	3.88	4.00	4.00	**4.09**
2008	3.83	4.82	2.27	5.20	6.78	4.85	2.45	2.83	4.47	3.74	4.68	**4.17**
2017*	3.83	4.07	2.42	4.85	7.11	4.20	2.23	3.17	3.74	2.94	4.64	**3.93**
Central Indio Hills (12 gene copies)												
2008	4.00	4.00	3.00	4.00	4.00	4.00	1.00	3.00	3.00	3.00	3.00	**3.27**
2017*	3.40	2.60	2.00	3.58	3.00	4.39	1.00	3.00	2.00	2.00	2.98	**2.72**

*Sampling period in which we detected a significant decrease in allelic richness using a paired T-test.

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