MOLECULAR ECOLOGY

Molecular Ecology (2015) **24**, 2349–2363 doi: 10.1111/mec.13176

Habitat fragmentation in coastal southern California disrupts genetic connectivity in the cactus wren (*Campylorhynchus brunneicapillus*)

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Abstract

Achieving long-term persistence of species in urbanized landscapes requires characterizing population genetic structure to understand and manage the effects of anthropogenic disturbance on connectivity. Urbanization over the past century in coastal southern California has caused both precipitous loss of coastal sage scrub habitat and declines in populations of the cactus wren (*Campylorhynchus brunneicapillus*). Using 22 microsatellite loci, we found that remnant cactus wren aggregations in coastal southern California comprised 20 populations based on strict exact tests for population differentiation, and 12 genetic clusters with hierarchical Bayesian clustering analyses. Genetic structure patterns largely mirrored underlying habitat availability, with cluster and population boundaries coinciding with fragmentation caused primarily by urbanization. Using a habitat model we developed, we detected stronger associations between habitat-based distances and genetic distances than Euclidean geographic distance. Within populations, we detected a positive association between available local habitat and allelic richness and a negative association with relatedness. Isolation-by-distance patterns varied over the study area, which we attribute to temporal differences in anthropogenic landscape development. We also found that genetic bottleneck signals were associated with wildfire frequency. These results indicate that habitat fragmentation and alterations have reduced genetic connectivity and diversity of cactus wren populations in coastal southern California. Management efforts focused on improving connectivity among remaining populations may help to ensure population persistence.

Keywords: coastal sage scrub, conservation, habitat loss, landscape genetics, songbird, wildfire

Received 15 August 2014; revision received 20 March 2015; accepted 25 March 2015

Introduction

Habitat loss is a leading cause of decline for many species worldwide, particularly in highly urbanized areas such as coastal southern California (Delaney *et al*. 2010). Indeed, this highly fragmented landscape has been frequently used as a natural laboratory for studies on post-isolation extinction processes (Soulé *et al*. 1988; Crooks & Soulé 1999; Crooks *et al*. 2001; Crooks 2002). Slowing and reversing such processes in imperilled species necessitates understanding connectivity and isolation patterns among remaining groups (Segelbacher *et al*. 2010; Luque *et al*. 2012; Manel & Holderegger 2013). Extended periods of isolation can have grave consequences for a species, potentially resulting in genetic diversity declines (Leberg & Vrijenhoek 1994), inbreeding depression (Charlesworth & Charlesworth 1987; Hemmings *et al*. 2012) and an inability to recolonize after local extinction. While increasing local population sizes is an important goal for assuaging these issues (Traill *et al*. 2010), preserving or restoring connectivity between populations may be more critical for short-term species persistence (Flather *et al*. 2011).

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Upwards of 85–90% of coastal sage scrub habitat in southern California has been lost to urbanization and agricultural development since European settlement (Westman 1981). While loss of genetic connectivity has been reported in numerous species throughout coastal southern California (McClenaghan & Truesdale 2002; Ernest *et al.* 2003; Riley *et al.* 2006; Vandergast *et al.* 2007, 2009; Delaney *et al.* 2010; Ruell *et al.* 2012; Richmond *et al.* 2013), studies on coastal sage scrub-dependent species, particularly birds, have primarily employed field observation techniques (Bailey & Mock 1998; Crooks *et al.* 2001; Preston & Kamada 2012; Kamada & Preston 2013) that do not provide information about genetic connectivity (Lowe & Allendorf 2010). Understanding the genetic structure among birds in the remaining coastal sage scrub fragments can directly inform management actions aimed at preserving species within this system.

One species of conservation concern that occupies coastal sage scrub in southern California is the cactus wren (*Campylorhynchus brunneicapillus*), a resident songbird with a relatively large range that extends from central Mexico into the American southwest. Although they are not cactus obligates throughout their range (Hamilton *et al.* 2011), cactus wrens are restricted to nesting in prickly pear (*Opuntia* sp.) and cholla (*Cylindropuntia* sp.) cacti in coastal southern California, a portion of their range that is geographically isolated from the rest. As these cacti are usually found in coastal sage scrub, cactus wren populations have declined with the loss of this habitat (Unitt 2008). Cactus wrens are a focal species in efforts to conserve the remaining coastal sage scrub habitat (Pollak 2001; Unitt 2008), and are actively monitored and managed as part of several regional habitat conservation plans; hence, analysing genetic connectivity in the species is a priority to best inform these efforts.

While urbanization is the primary driver of habitat loss and fragmentation in coastal southern California, wildfires can also quickly destroy cacti and cactus wren habitat (Bontrager *et al.* 1995; Preston & Kamada 2012). Coastal sage scrub habitat and many obligate species can recover rapidly and indeed benefit from wildfire (Westman 1981); however, burned areas may remain unsuitable for cactus wrens for years or even decades while slow-growing cacti regenerate. Over the past two decades, unusually large and intense wildfires caused significant loss or degradation of coastal sage scrub habitat in coastal southern California and reduced the abundance of cactus wrens (Mitrovich & Hamilton 2006; Hamilton 2008; Leatherman BioConsulting, Inc. 2009; Preston & Kamada 2012).

Prior observational studies suggest cactus wrens may be highly sensitive to habitat fragmentation, as they have limited dispersal capabilities (Preston & Kamada 2012; Kamada & Preston 2013) and are one of the first species to become locally extinct in recently isolated habitat patches (Crooks *et al.* 2001). With high levels of habitat loss, species with limited dispersal might be acutely at risk of experiencing reduced genetic connectivity (Barr *et al.* 2008; Lindsay *et al.* 2008; Athrey *et al.* 2012a,b); however, the effects of extensive habitat loss on cactus wren connectivity has yet to be analysed in a landscape genetic framework. Such frameworks can be useful for identifying connectivity patterns and their concordance with underlying land-use practices (Segelbacher *et al.* 2010; Luque *et al.* 2012; Manel & Holderegger 2013). Using microsatellites and a rigorous sampling scheme, we studied contemporary genetic connectivity patterns in the cactus wren in coastal southern California to assess the impacts of habitat fragmentation on the species. We characterized both genetic structure and diversity in the species using multiple, complementary analytical methods. To better understand how land-use patterns are affecting genetic connectivity and diversity in the species, we created a cactus wren dispersal habitat model to facilitate analyses. Finally, because wildfires are prevalent in the study area and represent a primary threat to cactus wren populations, we analysed the impacts of recent fire histories on genetic diversity.

Methods

Samples

We collected tissue samples in 2011–2013 from 371 cactus wrens at virtually every known aggregation throughout their coastal southern California range (Fig. 1; Table 1). Samples included growing feathers from nestlings at 6–12 days in age, blood from toenail clips of adults captured with standard mist-net and song playback techniques, and muscle or toe pads (depending on the level of decay) from deceased individuals. Only a single individual per nest was included for all analyses. Sample collection activities were authorized under a Memorandum of Understanding between the California Department of Fish and Wildlife and Barbara Kus (SCP-001504). All samples were stored in Queen's lysis buffer at −20 °C until they were processed. We conducted all extractions with the DNA tissue extraction kit (Qiagen), each with 20 μL of dithiothreitol added for a digestion step extended to 48 h. All DNA extractions were quantified on a Nanodrop spectrophotometer (Thermo Scientific) and diluted to a maximum 50 ng/μL prior to amplification.

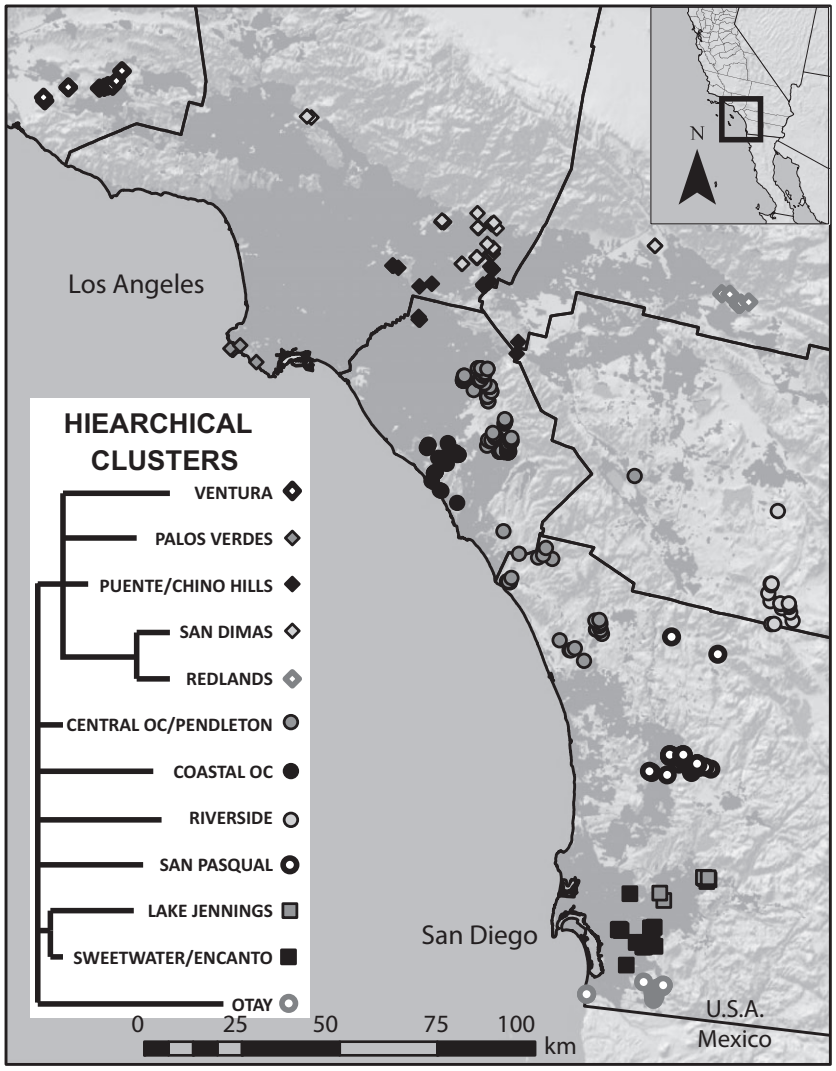

Fig. 1 Sample locations, cluster memberships from GENELAND analyses and landscape information from the study area. Common cluster memberships are designated by icon style and shading as indicated in the key. Urban area is shaded dark gray. The key represents the hierarchical clustering results in the form of a diagram, with common nodes representing clustering results at each level; note that branch lengths are arbitrary.

Genotyping and data quality assessment

We amplified 28 variable loci developed for cactus wrens in three sets using the standard conditions of the multiplex PCR kit (Qiagen) with loci combined as indicated in Table S1 (Supporting information). Details about microsatellite library development using next-generation sequencing are in Appendix S1 (Supporting information). Approximately 10% of samples were amplified and genotyped twice to assess error. Loci were checked for stepwise mutation model (SMM) consistency using MICRO-CHECKER (van Oosterhout *et al.* 2004), and Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) in GENEPOP (Raymond & Rousset 1995; Rousset 2008). We used COLONY to detect full-sib ships, the presence of which is known to confound Bayesian clustering algorithms (Anderson & Dunham 2008). For COLONY analyses, we assumed an inbreeding model and a polygamous mating system, and coded all individuals as offspring. One member of each full-sib pair detected with a probability >0.90 was then removed from the data set.

Identifying genetic structure

We employed multiple methods for characterizing genetic structure. First, we identified panmictic groups that we hereafter call 'populations' following Waples & Gaggiotti (2006). This entailed combining geographically aggregated samples with ≥5 individuals and conducting exact tests for genetic differentiation in GENEPOP. To limit the effects of individual loci on the overall test, *P*-values were set to a minimum of 0.0001 prior to combining with Fisher's method. Aggregations were assumed to be part of the same population and combined if the combined *P*-value was greater than 0.01. Combined aggregations were

Table 1 Sample sizes and genetic diversity statistics for each of the detected clusters (bold uppercase) and populations (italics), arranged from north to south: sample size (N), allelic richness (A_r), mean observed heterozygosity (H_O), mean expected heterozygosity (H_E), mean relatedness (r) with confidence interval and effective population size (N_e) with confidence interval

CLUSTER or *Population*	*N*	A_r*	H_O	H_E	*r*	N_e**
NORTH						
VENTURA	***15***	***2.82***	***0.59***	***0.57***	***0.221 (0.194–0.249)***	***23.2 (13.4–56.2)***
PALOS VERDES	***8***	***2.66***	***0.59***	***0.54***	***0.270 (0.226–0.320)***	***34.1 (12.9–∞)***
SAN DIMAS AREA	**34**	**3.03**	**0.56**	**0.60**	**0.157 (0.145–0.169)**	**72.7 (37.2–327.0)**
Glendora	*15*	*2.89*	*0.55*	*0.56*	*0.212 (0.183–0.242)*	*23.3 (13.9–51.4)*
Bonelli Park	*7*	*2.85*	*0.56*	*0.53*	*0.231 (0.187–0.284)*	*n/a*
CSPU, Pomona	*8*	*2.95*	*0.59*	*0.57*	*0.182 (0.137–0.229)*	*35.6 (18.0–223.8)*
PUENTE/CHINO HILLS	**22**	**3.10**	**0.64**	**0.63**	**0.116 (0.095–0.136)**	**21.0 (15.2–31.1)**
Puente Hills	*6*	*2.89*	*0.59*	*0.55*	*0.191 (0.133–0.250)*	*n/a*
Chino Hills	*8*	*2.87*	*0.65*	*0.56*	*0.235 (0.142–0.309)*	*10.1 (7.0–15.6)*
REDLANDS	***8***	***2.74***	***0.57***	***0.54***	***0.255 (0.190–0.327)***	***51.0 (17.5–∞)***
SOUTH						
CENTRAL OC/PENDLETON	**141**	**3.23**	**0.62**	**0.65**	**0.059 (0.057–0.062)**	**82.4 (37.6–284.1)**
El Modena	*13*	*2.99*	*0.60*	*0.59*	*0.142 (0.102–0.182)*	*17.6 (11.0–33.9)*
Central OC	*108*	*3.21*	*0.63*	*0.65*	*0.058 (0.054–0.061)*	*85.5 (37.9–407.2)*
Fallbrook	*14*	*3.00*	*0.55*	*0.58*	*0.165 (0.137–0.191)*	*50.9 (25.6–355.4)*
Southern Pendleton	*5*	*2.80*	*0.62*	*0.53*	*0.249 (0.172–0.317)*	*56.5 (11.0–∞)*
COASTAL OC	**31**	**3.06**	**0.60**	**0.62**	**0.141 (0.127–0.156)**	**17.4 (12.9–24.1)**
Bommer Canyon	*15*	*2.92*	*0.61*	*0.59*	*0.192 (0.162–0.227)*	*15.3 (10.0–26.3)*
Crystal Cove	*10*	*2.91*	*0.64*	*0.58*	*0.204 (0.155–0.257)*	*8.4 (6.1–11.7)*
RIVERSIDE	**16**	**3.00**	**0.56**	**0.60**	**0.180 (0.157–0.200)**	**59.0 (27.3–∞)**
Aguanga	*15*	*2.95*	*0.55*	*0.58*	*0.201 (0.178–0.225)*	*104.1 (31.0–∞)*
SAN PASQUAL	**40**	**3.18**	**0.65**	**0.67**	**0.095 (0.085–0.105)**	**79.4 (41.4–317.5)**
San Pasqual	*35*	*3.15*	*0.66*	*0.66*	*0.107 (0.096–0.120)*	*76.6 (37.9–442.7)*
LAKE JENNINGS	**11**	**2.99**	**0.58**	**0.61**	**0.192 (0.159–0.225)**	**19.1 (12.2–35.9)**
Lake Jennings	*9*	*2.95*	*0.59*	*0.59*	*0.207 (0.165–0.249)*	*28.0 (16.1–76.8)*
SWEETWATER/ENCANTO	**21**	**3.13**	**0.62**	**0.63**	**0.156 (0.137–0.175)**	**29.2 (19.2–52.1)**
Encanto	*5*	*2.70*	*0.57*	*0.52*	*0.235 (0.160–0.362)*	*n/a*
Sweetwater	*9*	*3.05*	*0.67*	*0.61*	*0.188 (0.141–0.243)*	*25.7 (14.7–69.8)*
OTAY	**16**	**3.15**	**0.70**	**0.66**	**0.149 (0.118–0.178)**	**20.0 (13.9–32.1)**
Otay	*14*	*3.13*	*0.69*	*0.66*	*0.156 (0.121–0.193)*	*18.1 (12.2–30.0)*

*Adjusted for minimum sample sizes of 12 alleles.

**Instances where too few data were available for the calcuation are marked "n/a".

subsequently retested, until all aggregations were grouped into significantly differentiated populations.

We implemented Bayesian clustering analyses in GENELAND 4.0 (Guillot *et al.* 2005a,b, 2008; Guillot 2008) in R 2.15.1 (R Core Team 2014), assuming the spatial model with uncorrelated alleles, running 1×10^7 Markov chain Monte Carlo (MCMC) iterations and saving every 1000th, and considering a range of clusters (K) 1–20. We assumed an uncertainty of 100 m for the geographic coordinates and assessed MCMC convergence using 10 repetitions of each analysis. We also employed BAPS 6.0 (Corander *et al.* 2008; Cheng *et al.* 2013) as an alternative clustering algorithm to provide further confidence in these results. For this analysis, we ran the 'spatial clustering of individuals' algorithm for 10 repetitions using maximum Ks of 5, 10, 15, 20 and 30 for the full data set. Clustering analyses in GENELAND and BAPS were conducted hierarchically, initially using the complete data set and then subsets of the data set successively within each detected cluster until $K = 1$ was concluded (following Coulon *et al.* 2008). For subsets, we ran BAPS for 10 repetitions considering maximum Ks of 5 and 10. Results from complete hierarchical clustering analyses are hereafter explicitly referred to as 'clusters.'

Finally, we used spatial principal component analysis (sPCA; Jombart *et al.* 2008) to further explore genetic structure. These analyses were conducted using POPGENREPORT 2.1 (Adamack & Gruber 2014) using a similar hierarchical method as previously discussed. Namely, after examining resulting biplots, further subsequent sPCA analyses were conducted on reduced data sets to better discriminate genetic structure.

Genetic diversity analyses

For both clusters and populations, we calculated observed (H_O) and expected heterozygosity (H_E) in GENALEX (Peakall & Smouse 2012), and allelic richness (A_r) using rarefaction to correct for sample size differences in HP-RARE (Kalinowski 2005). With NEESTIMATOR 2.01 (Do *et al.* 2013), we estimated effective population size (N_e) in clusters and populations via the linkage disequilibrium method (Waples & Do 2008) assuming a random mating model and using minimum allele frequencies based upon sample sizes. Confidence intervals were determined with jackknifing. We calculated a Pearson correlation coefficient (r_p) between each of these measures of genetic diversity and the geographic mid-point for each cluster to test for correlations between genetic diversity and latitude. We tested for significant heterozygote excess in each population with BOTTLENECK (Piry *et al.* 1999) with a Wilcoxon sign test. Given recent concern about the effects of varying mutational models on BOTTLENECK results (Peery *et al.* 2012), we analysed our data using the infinite alleles model (IAM), the SMM, and a range in variances and proportions of SMM in the two-phase model (TPM). Finally, we estimated average pairwise relatedness (r_{qg}, Queller & Goodnight 1989) among individuals within populations in GENALEX.

Landscape genetic analyses

We developed a cactus wren dispersal habitat suitability model to facilitate landscape analyses (methods detailed in Appendix S2, Supporting information). In short, a partitioned Mahalanobis D^2 model predicting habitat was developed from an environmental data set consisting of climatic, topographic and vegetative variables (Rotenberry *et al.* 2002, 2006). We calibrated and evaluated the performance of the model using randomly selected subsets of a data set of 353 spatially distinct locations. Using the inverse of habitat model suitability scores, we created a cost surface and measured interpopulation landscape connectivity in three ways. Least cost path distances (which represent the distance along the least cost path between locations) and weighted cost distances (which sum the cost surface values for each cell along the least cost path) were calculated using the Landscape Genetics ArcToolbox (Etherington 2011) in ARCGIS 10.2. A third connectivity measure, resistance, was calculated in CIRCUITSCAPE (McRae *et al.* 2008). While least cost paths measure a single connection route between all pairs of locations, resistances incorporate information from all possible pathways between pairs. Methods for creating the cost surface and assessing among- and within-population landscape characteristics are further detailed in Appendix S2 (Supporting information).

We tested for isolation by distance (IBD) among aggregations using Mantel tests (Mantel 1967) in IBDWS (Jensen *et al.* 2005) using both Weir & Cockerham's (1984) estimator of F_{ST} calculated in GENEPOP, and the average proportion of shared alleles, Dps, calculated in MSA 3.0 (Dieringer & Schlötterer 2003). These analyses were focused on populations identified by the Waples & Gaggiotti (2006) method, but we further divided the geographically largest population (Central Orange County) into smaller aggregations to incorporate comparisons among aggregations with large extents of suitable dispersal habitat among them in our analyses. We defined aggregations as clusters of individual sampling locations within 2 km of their nearest neighbours and within a 10 km maximum distance that were not separated by urban development. Ten kilometres represents the maximum known dispersal distance based upon field observations of cactus wrens, while most resightings were within 2 km (Atwood *et al.* 1998; Preston & Kamada 2012; Kamada & Preston 2013). This resulted in a total of 27 aggregations for pairwise comparisons. We conducted a series of Mantel tests for correlations between genetic distances and least cost path distances, weighted cost distances and resistances derived from the habitat model and Euclidean distance. We assessed the relative fit of each distance by comparing correlation coefficients. To test for a further influence of urban fragmentation, we used partial Mantel tests of a binary fragmentation index and F_{ST} or Dps while controlling for the best explanatory distance. To categorize urban areas for this analysis, we used the 2006 National Land Change Database (NLCD) Percent Developed Imperviousness layer, downloaded from the Multi-Resolution Land Characteristics Consortium (Fry *et al.* 2011). Following Fry *et al.* (2011), grid cells with more than 20% of their surface area covered by at least 20% imperviousness were classified as urban. To determine whether cluster assignments were influenced by isolation by distance, following Meirmans (2012), we tested for a correlation between cluster assignments and F_{ST} while controlling for geographic distance. This test entails creating a pairwise binomial matrix based upon whether aggregations are in the same cluster (0) or different clusters (1) and using a partial Mantel test to detect a significant association between genetic distance and cluster membership while removing the effect of geographic distance. This is one way to determine the effect of IBD on clustering results. The significance of all Mantel tests was assessed using 10 000 randomizations. Finally, based on substantial differences in the temporal scale and degree of fragmentation in the LA basin versus in Orange and San Diego Counties, we repeated all tests among northern and southern populations grouped separately.

We used multiple regression models to examine the relationships between landscape attributes and genetic diversity (A_r, H_E, and r_{qg}) within panmictic populations defined by the Waples & Gaggiotti (2006) method. Similarly, we used logistic regression to examine the relationships between landscape attributes and the presence of genetic bottlenecks. Landscape attributes include three habitat availability indices (ha suitable habitat within a 10 km radius, % urban area within a 10 km radius and nearest neighbour distance between population centroids) and fire history (time since most recent fire and number of fires between 1990 and 2010). Fire histories were determined via the CALFIRE Fire Perimeters Geodatabase version 12_1 (http://frap.cdf.ca.gov/data/frapgisdata-sw-fireperimeters_download.php), using 2 km buffers around each collection point to calculate the number of fires experienced (1990–2010) and time since most recent fire.

We compared multiple regression models using Akaike's information criteria adjusted for small sample size (AIC_c) in an information theoretic approach to find the model or collection of models that best approximated the 'truth' (Burnham & Anderson 2002). To compare models, we assessed the difference in AIC_c values for each model relative to the model with the lowest value (Δ_i), model weights (ω_i), evidence ratios (ω_i/ω_1) and adjusted coefficients of determination (adjusted R^2). The model weight represents the probability that the model is the best approximating model in the model set, and the evidence ratio is the relative likelihood that the top ranked model performs best compared to others in the set. The adjusted R^2 was used to assess model fit. We identified the best model and a subset of models comprising >90% confidence subset of best approximating models. For the >90% subset of models, we calculated 95% confidence intervals for parameter estimates to determine whether a trend was positive, negative or not present. Similarly, we compared logistic regression models to relate both fire history and suitable habitat availability to significant bottleneck signals. Finally, we used the Moran's I tool in ARCGIS to evaluate residuals from full models in order to determine whether there was spatial autocorrelation among the genetic variables analysed. Spatial patterns were not significantly different from random with *P*-values exceeding 0.75; therefore, final models were not further adjusted for spatial autocorrelation.

Results

Data quality

We genotyped all samples at 28 loci; however, we eliminated two loci because of inconsistent amplification and four that did not conform to HWE (see Table S1, Supporting information). None of the remaining loci were consistently in LD across multiple populations. The error rate in the remaining 22 loci was negligible (<0.1%). One locus, CACW4-05, had an allele that did not meet allele size expectations given the repeat type; however, this allele (142) was consistent across multiple genotyping runs and could be tracked between parents and offspring. Primers and locus information are in Table S1 (Supporting information). Eight cactus wrens, determined to be members of full-sib ships, were eliminated prior to additional analyses. In the overall data set, there were <0.01% missing data.

Genetic structure

GENELAND and BAPS results differed slightly. In the full data set analysis in GENELAND, seven clusters were detected (Figs 1, S1 and S2, Supporting information); however, in hierarchical analyses, additional clusters were detected, for a total of 12 (Figs 1 and S3–S5, Supporting information). Across repeated analyses at each hierarchical level, the MCMC converged quickly and consistently to the same results in GENELAND. BAPS, on the other hand, detected more clusters in analyses on the overall data set (9), many of which were concordant with those detected in GENELAND (Fig. S6, Supporting information). Hierarchical analyses resulted in the detection of no additional structure in BAPS. Using the Waples & Gaggiotti (2006) method, we detected 20 panmictic populations (Fig. 2) with an average pairwise F_{ST} of 0.11 (range: 0.033–0.18). Because we have greater control over the analysis procedure in GENELAND than BAPS (e.g., number of MCMC iterations) and the detected structure is very similar to that found through the Waples & Gaggiotti (2006) method (Figs 1 and 2), we only discuss the results from the GENELAND analyses here.

Our results from hierarchical sPCA analyses (Fig. S7a–d, Supporting information) were similar to those from GENELAND with more structure being revealed through additional analyses on reduced data sets. Initial results from analyses on the total data set exhibited some discrimination among the southern clusters, San Pasqual, Lake Jennings, Sweetwater/Encanto and Otay, but not among the other clusters identified in GENELAND (Fig. S7a, Supporting information). Further analyses revealed clear separation of the Riverside (Fig. S7b, Supporting information), Central OC/Pendleton and Ventura (Fig. S7c, Supporting information) clusters. A final analysis on clusters around the city of Los Angeles, including the Palos Verdes, San Dimas, Puente/Chino Hills and Redlands clusters, revealed

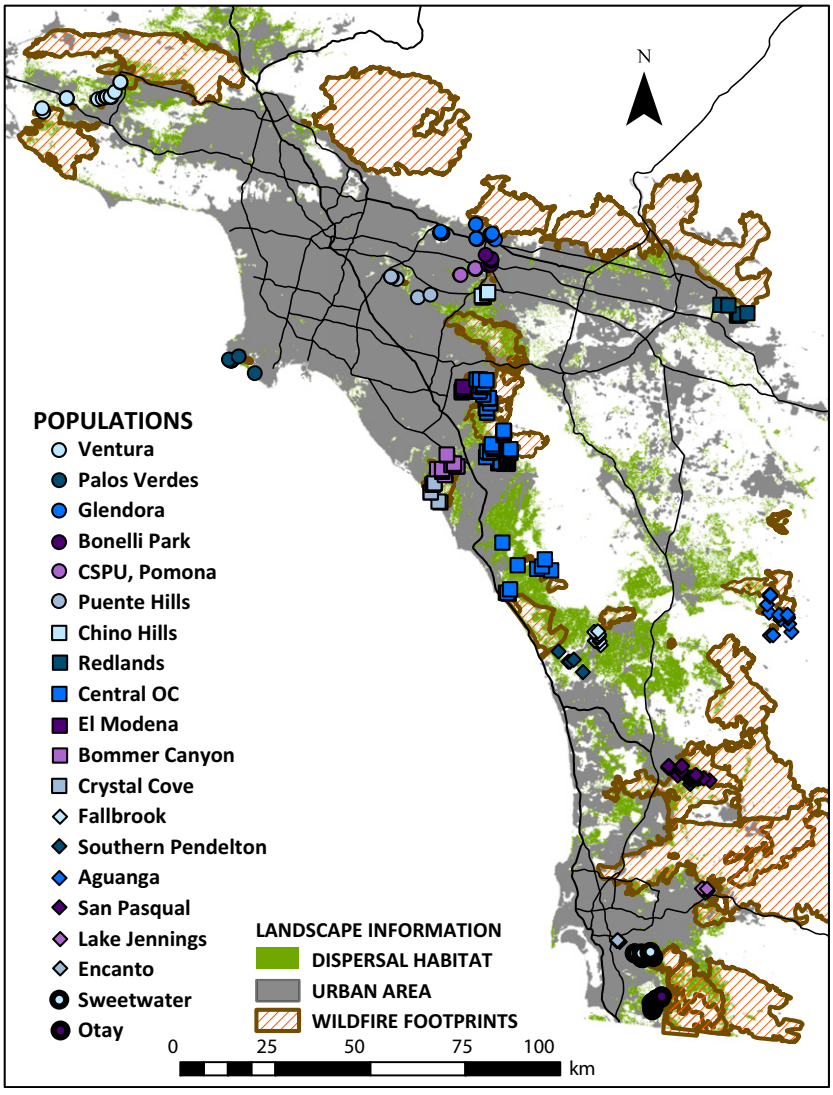

Fig. 2 Populations as determined by analyses following Waples & Gaggiotti (2006) and landscape information. Modeled dispersal habitat, urban area and wildfire footprints from 1990 to 2010 are coloured as indicated in the key. Black lines are US interstates.

some weaker separation (Fig. S7d, Supporting information).

Genetic diversity

Estimates of genetic diversity varied by relative isolation and the geographic location of individual populations (Table 1). Northern populations were generally less diverse than those in the more southern portion of the study area with significantly negative trends in A_r ($r_p = -0.50$, $P = 0.048$), H_O ($r_p = -0.54$, $P = 0.03$) and H_E ($r_p = -0.60$, $P = 0.02$). Cactus wren populations throughout coastal southern California appear to be quite small according to our N_e estimates. While N_e/census population size ratios can be very low (Frankham 1995; Leberg 2005), our results meet expectations given known low numbers of cactus wren territories in parts of the study area. We do not report the negative N_e estimates that can result from small sample size (Waples & Do 2010).

Results of tests for heterozygote excess varied across mutational models (Table S2, Supporting information), with many more being significant under the IAM, none under the SMM and fewer under the TPM as a greater percentage of SMM was considered. Altering variances in mutational size steps for the TPM affected *P*-values little. Previous studies of microsatellite mutation dynamics in birds suggest that the TPM is more appropriate than the strict SMM, with between 60 and 80% of mutations involving a single-step change (Ibarguchi *et al.* 2004; Ortego *et al.* 2008), although, Peery *et al.* (2012) reported empirical proportions of single-step mutations as low as 28% in other vertebrates. Here, we considered that populations with significant heterozygote excess test results (*P*-values <0.05) through at least 60% SMM showed support for bottleneck signals. This

Table 2 Fire histories and BOTTLENECK results by population: Fires (total 1990–2010), years since previous fire (pre-2010) and significant heterozygote excess (*$P < 0.05$) across numerous mutational models using the Wilcoxon sign test in BOTTLENECK

Population	Fires	Years since previous fire	Bottleneck
Ventura	8	3	*
Palos Verdes	6	2	*
Glendora	7	2	
Bonelli Park	4	14	
CSPU, Pomona	1	17	
Puente Hills	3	4	
Chino Hills	1	2	
Redlands	1	8	
Central OC	19	3	*
El Modena	1	18	
Bommer Canyon	4	4	
Crystal Cove	3	7	*
Fallbrook	2	2	
Southern Pendleton	1	4	
Aguanga	5	4	
San Pasqual	6	4	*
Lake Jennings	2	8	
Encanto	0	30	
Sweetwater	1	4	*
Otay	5	4	*

was the case for seven of the 20 populations (Tables 2 and S2, Supporting information).

Landscape genetic analyses

The habitat model performed well, accurately identifying known shrubland (which includes coastal sage scrub and cactus habitat) throughout the study area (Fig. 2; Appendix S2; Table S3, Supporting information). Among all aggregations, we found significant IBD in both measures of genetic differentiation (Fig. 3; Table 3; see Tables S4 and S5, Supporting information for pairwise F_{ST} values). A similarly strong signal was detected among just southern aggregations in Orange, Riverside and San Diego Counties; however, IBD was not detected among the eight northernmost aggregations (Fig. 3; Table 3). Furthermore, the least cost path distances through the habitat cost surface showed the highest correlation with genetic distance measures across the full data set, while the cost weighted distances showed the highest correlation with genetic distances in both southern and northern subsets. Resistances had lower correlation coefficients than least cost paths, and in fact were lower than Euclidean distance in both the full and southern data sets. This may indicate that the CIRCUITSCAPE resistance model, which incorporates all possible connections between sites, does not adequately reflect cactus wren gene flow at these broad spatial scales (e.g., Lee-Yaw *et al.* 2009; Miller *et al*. 2013).

While controlling for the best explanatory distance, we detected significant correlations between both measures of genetic distance and the presence of urban fragmentation in both the overall data set and the southern populations (Table 3). We also found a significant association between cluster assignment and F_{ST} after controlling for geographic distance ($r = 0.318$, $P = 0.028$).

Multiple regression models showed the strongest associations between suitable habitat and both A_r (Tables 4 and S6, Supporting information; 14× more likely than the next model) and r_{qg} (Tables 4 and S7, Supporting information; 17× more likely than the next model). The association was positive for A_r (Table 4; parameter estimate: 0.095; CI: 0.036–0.153) and negative for r_{qg} (Table 4: parameter estimate −0.033; CI: −0.016 to −0.01). We were unable to identify a best approximating model for H_E as nearly all of the models, nine of 11, were included in the 94% confidence subset, including some with poor fit. Logistic regression models assessing the occurrence of genetic bottlenecks found an association with the number of fires (Tables 4 and S8, Supporting information; 4× more likely than the next model) that was positive (Table 4; parameter estimate: 2.089; 95% CI: −0.005 to 4.184).

Discussion

Today, cactus wrens largely persist at the fringes of their historical range in coastal southern California, often wedged between urban areas and mountain ranges that are unsuitable for cactus (Unitt 2008). Our landscape analyses provide strong support that the current availability and connectivity of habitat are associated with patterns of genetic structure and diversity in cactus wren populations. Genetic differentiation was generally more strongly correlated to habitat-based distances than Euclidean distance. Additionally, we found that urban fragmentation was associated with higher levels of genetic differentiation than contiguous habitat, even when accounting for distance among sites. These associations are further reflected in cluster and population assignments, which largely mirrored underlying land-use patterns and habitat availability. The effects of available habitat were also apparent at the local population scale where suitable habitat was positively related to allelic richness and negatively to relatedness.

In contrast to the contemporary structure uncovered here, previous studies based on mtDNA data found no genetic structure among cactus wren populations in

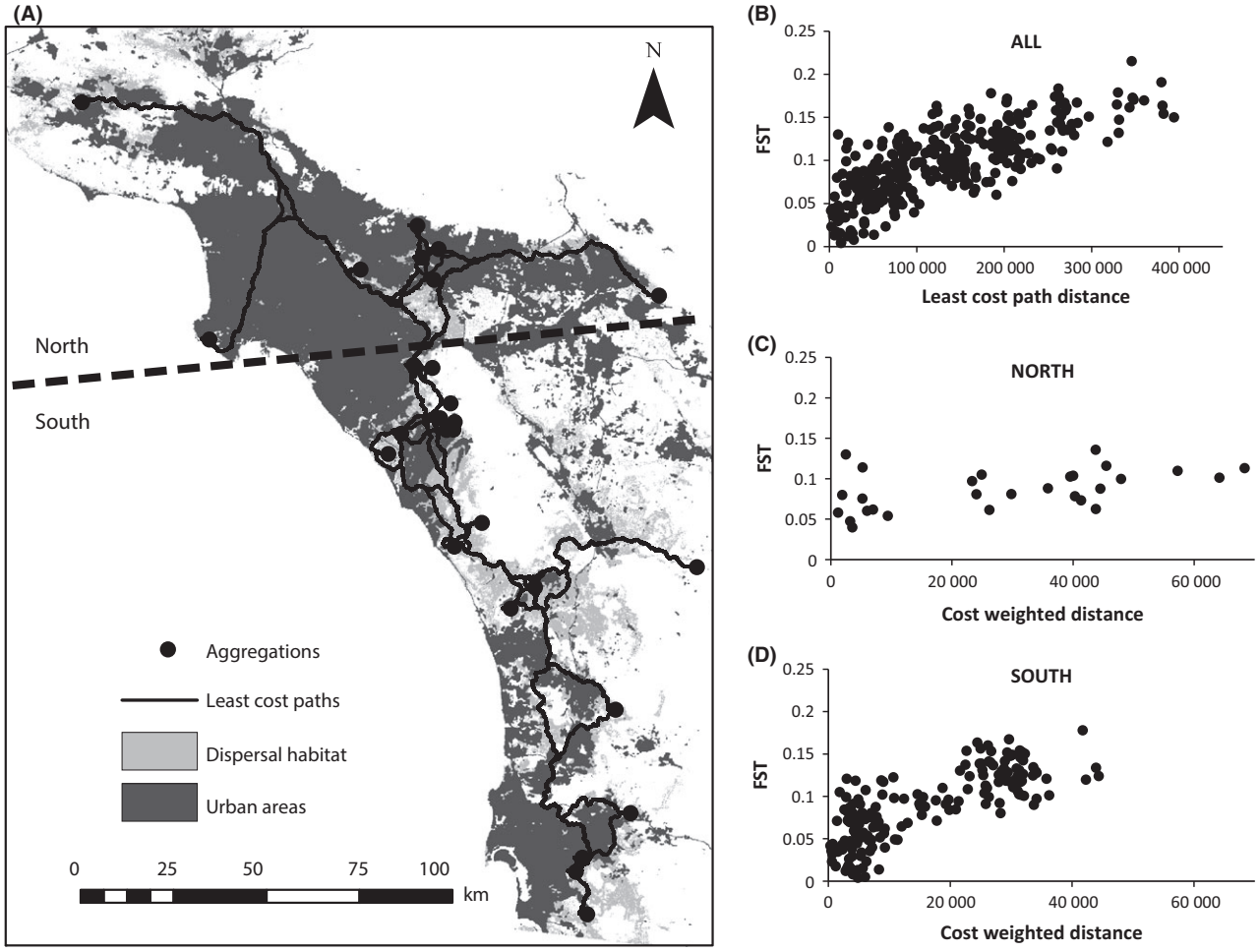

Fig. 3 Landscape Analyses. (A) Map showing 27 aggregations defined for isolation by distance analyses and least cost paths based on the habitat suitability model, and split between northern and southern subsets. (B) Plot of pairwise FST by best fit habitat distance (least cost path) for all 27 aggregations. (C) Plot of pairwise FST by cost weighted distance for eight northern aggregations. (D) Plot of pairwise FST by cost weighted distance for 19 southern aggregations.

Table 3 Correlation coefficients followed by corresponding *P*-values in italics for Mantel and partial Mantel tests for genetic isolation by Euclidean or habitat distances

	Entire study area		North		South	
Geographic distance	FST	Dps	FST	Dps	FST	Dps
Euclidean	0.7012, *<0.0001*	0.7116, *<0.0001*	0.3199, *0.1785*	0.3148, *0.1688*	0.7123, *<0.0001*	0.7548, *<0.0001*
Least cost path	**0.7541**, *<0.0001*	**0.7463**, *<0.0001*	0.3615, *0.1285*	0.3619, *0.1196*	0.7573, *<0.0001*	0.7881, *<0.0001*
Cost weighted	0.6635, *<0.0001*	0.6071, *<0.0001*	**0.4852**, *0.0827*	**0.4733**, *0.0753*	**0.7823**, *<0.0001*	**0.8050**, *<0.0001*
Resistance	0.6002, *<0.0001*	0.5795, *<0.0001*	0.3816, *0.1444*	0.3718, *0.1279*	0.6175, *<0.0001*	0.6310, *<0.0001*
Urban fragmentation	0.5192, *<0.0001*	0.4470, *<0.0001*	NC*	NC*	0.6250, *<0.0001*	0.5469, *<0.0001*
	Controlled for least cost path				Controlled for cost weighted distance	
Urban fragmentation	0.4034, *<0.0001*	0.2864, *<0.0019*	NC*	NC*	0.4766, *0.0013*	0.3319, *0.0139*

The highest correlation coefficient in each set of tests is bolded.
*Not calculated; all but one population pair separated by urban areas.

Table 4 Parameter estimates and adjusted R^2 values for best performing multiple regression and logistic regression models relating genetic diversity measures (allelic richness, pairwise relatedness and genetic bottlenecks) in each population to land use, predicted suitable dispersal habitat and fire history. Parameters from best selected models include hectares of modeled habitat predicted to be suitable for cactus wren dispersal (suitable habitat) and number of fires between 1990 and 2010 within the cluster's population polygon (number of fires). All predictor variables were ln(x + 1)-transformed

Response variable	Model parameters	Y-intercept (SE)	Parameter coefficient (SE)	Adjusted R^2
Multiple regression models				
Allelic richness	Suitable Habitat ln(x + 1)	2.10 (0.26)	0.09 (0.03)	0.33
Pairwise relatedness	Suitable Habitat ln(x + 1)	0.48 (0.10)	−0.03 (0.01)	0.28
Logistic regression model				
Genetic bottleneck	Number of Fires ln(x + 1)	−3.68 (1.71)	2.09 (1.05)	NA

coastal southern California, and generally low haplotype diversity (Eggert 1996; Zink *et al.* 2001; Teutimez 2012). This suggests both a recent range expansion into the area, likely since the last glacial maximum, 12 000 years ago (Axelrod 1978), and/or that cactus wrens were historically more genetically connected. Although cactus is naturally patchy within coastal scrublands, there is no doubt that it is even more so today, as scrub habitat has been reduced to an estimated 10–15% of its historical distribution, prior to widespread urbanization (Westman 1981).

Contemporary genetic structure

The effects of urban fragmentation on cactus wren genetic structure were most striking in areas where distinct genetic clusters or populations were detected over very small spatial scales and coincident with habitat boundaries. The Otay and Sweetwater populations in San Diego, for instance, exhibited significant genetic differentiation despite being relatively close together (Fig. 2). A habitat gap <10 km exists between these cactus wren populations that formed only in the past two decades with urban development and a major fire. Similarly, San Dimas and Puente/Chino Hills, Coastal OC and Central OC/Pendleton, and Puente/Chino Hills and Central OC/Pendleton are separated by narrow gaps (Fig. 1). Highways may also contribute to gene flow barriers. Between Coastal OC and Central OC/Pendleton, dispersing cactus wrens would have to traverse a 16-lane US interstate and extensive urban development. Intense resighting studies in these areas have never observed a bird successfully cross this gap (Preston & Kamada 2012). Numerous such large and busy highways exist throughout the study extent (Fig. 1). In contrast, genetic connectivity was relatively high among aggregations along the western slope of the Santa Ana Mountains which contains the largest amount of contiguous open space and suitable habitat and lacks large highways (Figs 1, 2 and S2, Supporting information). Both individual clustering analyses supported a single genetic cluster (Central OC) across this region. At nearly 75 km at its widest extent, this covered the largest geographic area of any cluster.

The greatest effects of isolation and genetic drift may be evident among populations in the northern extent of the study region. High F_{ST} and a lack of IBD among these populations (Fig. 3) may be indicative of a system more influenced by genetic drift rather than gene flow (Hutchison & Templeton 1999). Greater geographic isolation and generally smaller census population sizes in the north (Cooper *et al.* 2012, 2014; Preston & Kamada 2012) may contribute to this pattern. At least three of eight clusters/populations in this northern extent (Ventura, Palos Verdes, and Redlands) are likely in complete isolation given that gaps in habitat that surround these sites far exceed maximum dispersal distances. Isolation may contribute to the latitudinal trend in decreasing diversity; A_r and H_E are lowest and r_{qg} highest in Ventura, Palos Verdes and Redlands (Table 1), which represent some of the northernmost sites. Geographic isolation combined with low measured diversity suggests that these northern clusters/populations may have been small and isolated long enough to lose variation through genetic drift, and may be vulnerable to inbreeding.

Regional differences in the temporal scale of habitat loss and fragmentation may also contribute to contrasting patterns found in the northern and southern portions of the study area. The core of the northern part of the study area, Los Angeles County, was developed much earlier than the southern portion in Orange and San Diego Counties. Human populations in Los Angeles County reached 500 000 in the 1910s and 1M in the 1920s (www.dof.ca.gov/research/demographic/reports/view.php), whereas growth in San Diego County peaked later, with only 61 000 in the 1910s and 110 000 in the 1920s. By the time San Diego County reached 1M citizens in the 1960s, the population of Los Angeles County had boomed to 6M. Population growth in Orange County lagged slightly behind San Diego County. While

generation time is not known for cactus wrens, it is likely on the order of 1–3 years as is predicted for many passerines (Athrey *et al.* 2012b). Therefore, cactus wrens in Los Angeles County have experienced many more generations of habitat fragmentation than those in San Diego and Orange Counties.

Along with retaining stronger signals of isolation by distance, higher levels of genetic diversity in the southern portion of the study area may also be attributable to a lag in time since fragmentation. A_r and H_E are higher and r_{qg} lower than might be predicted in the populations near the city of San Diego (Lake Jennings, Sweetwater, Encanto, Otay) given their small N_es (Table 1); however, the effects of fragmentation on gene flow cause populations to more rapidly differentiate than genetic drift reduces genetic diversity (Leberg *et al.* 2010). Given the low estimates of N_e in these populations (Table 1) and the lack of habitat connectivity between them (Figs 1 and 2), we would expect genetic diversity in these populations to decline with time and continued isolation.

Fire and the cactus wren

An altered wildfire regime coupled with other effects of urbanization may be acting in concert to amplify loss of genetic diversity and connectivity in some sites. Wildfires are natural disturbances, but their frequency, size and intensity have been altered over the last several decades as a result of urbanization and human activities (Syphard *et al.* 2007). Recent wildfires have become a major threat to cactus wrens in coastal southern California, and can be particularly harmful for small and isolated populations. Major losses in cactus wren territories have been documented after recent fires, including Central and Coastal Orange County (Mitrovich & Hamilton 2006; Leatherman BioConsulting, Inc. 2009), San Pasqual (Hamilton 2008) and Palos Verdes (Cooper 2010). The detected association of bottleneck signatures with higher fire frequencies likely reflects these losses. Though other factors can contribute to bottleneck signals, such as reductions in population sizes related to extended drought, increased predation and restrictions to gene flow (England *et al.* 2010), our data suggest that fires are contributing to losses in genetic diversity in cactus wren populations. In addition, post-fire recovery of cactus is extremely slow. In the Coastal Orange County cluster, for instance, a 1993 fire destroyed 72% of known cactus wren territories; 20 years later, these territories largely remain unoccupied because appropriate nesting habitat is not available. Isolated aggregations affected by fire may be particularly threatened with local extirpation in the absence of habitat and connectivity restoration.

Conclusions and conservation implications

There are several findings in our study that are of particular importance for conservation. Genetic structure over relatively narrow geographic distances, for instance, suggests both that cactus wrens make short dispersing movements, which is confirmed by field observations (Preston & Kamada 2012; Kamada & Preston 2013), and that these movements are disrupted by habitat fragmentation. Populations exhibiting IBD more rapidly experience genetic diversity loss and increasing differentiation after being isolated by habitat fragmentation (Leblois *et al.* 2006; Amos *et al.* 2014). We would expect that absent restoration efforts to restore gene flow between the small and isolated populations examined in this study, genetic diversity will continue to decline and genetic differentiation will increase. Bottlenecked and low-diversity populations are more likely to suffer from inbreeding depression, accumulation of deleterious alleles and loss of adaptive genetic diversity (Shaffer 1981; Frankham 2005; Frankham *et al.* 2014). These factors can reduce individual fitness and compromise the capacity to adapt to changing environmental conditions (Reed *et al.* 2002; Reed & Frankham 2003; Markert *et al.* 2010). Further, empirical studies suggest that populations with low genetic variability may go extinct at higher rates than those with high variability (Saccheri *et al.* 1998; Higgins & Lynch 2001; Driscoll 2004).

Considering the genetic structure patterns we report here, including restoration of genetic connectivity among populations in future management plans could facilitate regional persistence of cactus wrens. Where multiple genetic clusters have formed over short habitat gaps and space is available for restoration (e.g. between Sweetwater/Encanto and Otay, Figs 1 and 2), planting cacti and other native scrub species in intervening open space may enhance gene flow. In the cases where habitat gaps are too extensive (e.g. Ventura or Palos Verdes and elsewhere) or do not offer opportunities for restoration (e.g. between Coastal OC and Central OC/Pendleton), efforts will have to focus within local populations. Increasing available habitat can increase local population sizes and make them more robust to possible extinction from drought and wildfire (Conlisk *et al.* 2014), as well as build genetic diversity over time. Translocation, which has been successful in cactus wrens (Kamada & Preston 2013), or egg-switching between populations may help to boost local genetic diversity more quickly and avoid inbreeding depression (Weeks *et al.* 2011). Continued genetic and ecological monitoring are necessary to determine whether such management actions lead to greater fitness, survivorship and, ultimately, population persistence.

In conclusion, limited dispersal ability and a tight association with patchily distributed habitat render cactus wrens vulnerable to habitat loss and fragmentation, to the extent that the persistence of many remaining populations may be threatened. These results echo a growing body of studies that also report strong area and isolation effects on genetic relatedness and diversity in other small, dispersal-limited animals throughout southern California (McClenaghan & Truesdale 2002; Vandergast *et al.* 2007, 2009; Delaney *et al.* 2010). For cactus wrens, and possibly other animals that are closely tied to coastal sage scrub, management actions aimed at restoring connectivity and increasing local population sizes are warranted, particularly given the seemingly rapid genetic erosion linked to habitat loss and fragmentation we report here. Continued analysis of connectivity in other coastal sage scrub species with different life-history traits may provide further understanding of the extent to which habitat fragmentation and other disturbance have affected coastal sage scrub inhabitants and inform ongoing conservation and restoration efforts of the habitat in coastal southern California.

Acknowledgements

We thank the Nature Reserve of Orange County (NROC), the San Diego Association of Governments (SANDAG), California Department of Fish & Wildlife and the U.S. Geological Survey, Western Ecological Research Center for both financial and logistical support. We sincerely appreciate the land managers who graciously provided property access and often logistical support as well (listed in Appendix S3, Supporting information). This study would not have been possible without much diligent and trying fieldwork by K. Allen, L. Allen, A. Houston, S. Lynn, M. Madden, R. Pottinger, T. Dixon, P. J. Falatek, A. Gallagher, M. Lipshutz, S. Nichols, J. Pietrzak, and A. Winters of USGS and D. Kamada and K. Moore of NROC. Thanks to Jonathan Q. Richmond for his review of this manuscript. Finally, we appreciate the anonymous reviewers for their input. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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B.E.K. and A.G.V. designed the study. B.E.K. and S.H. led field work. K.R.B. performed laboratory work and contributed to field work. K.R.B., A.G.V. and K.L.P. analysed the data and wrote the manuscript, with contributions from B.K., S.H. and E.P.

Data accessibility

Data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.j5h92. The first file contains microsatellite genotypes in GENALEX format and includes all samples arranged by clustering results. A second file contains both microsatellite genotypes arranged by aggregations and pairwise genetic and geographic distances used in isolation-by-distance analyses. A third file contains the output of the dispersal habitat model in img format.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Library development.

Appendix S2 Landscape variables and habitat suitability model.

Appendix S3 Acknowledgments.

Fig. S1 GENELAND results on overall dataset.

Fig. S2 GENELAND results on overall dataset and map of sample locations.

Fig. S3 GENELAND results southern San Diego part of dataset.

Fig. S4 GENELAND results within Los Angeles, San Bernardino, and Ventura Counties.

Fig. S5 GENELAND results in Glendora & Redlands.

Fig. S6 Plot of individual assignments to nine clusters with BAPS and corresponding labels of 12 clusters recovered in hierarchical GENELAND analyses.

Fig. S7 Hierarchical spatial PCA results.

Table S1 Locus information.

Table S2 Extended BOTTLENECK results.

Table S3 Habitat information within 10 km buffer zones of populations.

Table S4 Pairwise FST values among 27 aggregations (below diagonal) and associated *P*-values based on 9999 permutations (above diagonal).

Table S5 Pairwise FST values between populations identified following Waples & Gaggiotti (2006).

Table S6 Multiple regression models relating allelic richness (A) of each genetic cluster to land use, predicted suitable dispersal habitat, and fire history within each genetic cluster's population polygon.

Table S7 Multiple regression models relating average pairwise relatedness (rqg) of each genetic cluster to land use, predicted suitable dispersal habitat, and fire history within each genetic cluster's population polygon.

Table S8 Logistic regression models relating the occurrence of a genetic bottleneck at each genetic cluster to predicted suitable dispersal habitat and fire history within each genetic cluster's population polygon.