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## Introduction

The cactus wren (*Camphylorhynchus brunneicapillus*) is a habitat-restricted species in southern California, nesting strictly in prickly pear (*Opuntia* sp.) and cholla (*Cylindropuntia* sp.) cacti that exist primarily in coastal sage scrub and chaparral habitats. Long-term survival of cactus wrens in southern California relies upon the persistence of such habitat; however, urbanization, agriculture, and fire have greatly reduced cactus habitat throughout the region (Shuford & Gardali 2008). Presently, large aggregations of cactus wrens exist only in areas where urbanization and agriculture have largely been excluded, such as habitat preserves and military installations. Smaller groups are found in urban canyons, parks, and on private lands. While the exact number of extant cactus wrens is unknown, several hundred territories are thought to remain in coastal southern California. This likely represents a major reduction from historical population sizes (Unitt 2004, Shuford & Gardali 2008).

In a previous study focused on southern Orange and San Diego Counties, we detected limitations on genetic connectivity in the cactus wren that were concordant with habitat fragmentation (Barr et al. 2012). While we detected a pattern of genetic isolation by distance over the study area, we also determined that many groups of cactus wrens were much more genetically differentiated than could be attributed to geographic distances alone. Genetic structure was also detected in areas only recently fragmented by urban development, suggesting a rapid reduction in genetic connectivity among coastal cactus wren aggregations in the face of land-use alterations by urban development, agriculture, and/or wildfire. Such limitations on connectivity can have severe consequences for small populations.

Connectivity, which describes the level of movement between habitat patches by an organism during migration, dispersal, or as part of regular behavioral activity, is essential for a species' long-term persistence (Lowe & Allendorf 2010). With high connectivity between populations, genetic diversity is better preserved (Reed & Frankham 2003). Though genetic drift, small and isolated populations can naturally lose genetic diversity, potentially causing a reduction in potential for adaptation to environmental change and novel disease (Quattro & Vrijenhoek 1989, Leberg & Vrijenhoek 1994). As populations become exceptionally small, a lack of connectivity with other groups may also lead to inbreeding depression, reducing the genetic health of individuals (Charlesworth & Charlesworth 1987, Hemmings et al 2012). Demographic recovery of local populations reduced by stochastic events, such as wildfires, may also be much

slower if connectivity with source populations is low. In the case of cactus wrens, aggregations in Orange County were severely reduced by wildfires and have been slow to recover (Bontrager et al. 1995, Preston & Kamada 2012). Part of the slow recovery is attributable to the low growth rates of cacti, which need to achieve a height of one to two meters to be suitable for nesting cactus wrens. It is also likely that habitat fragmentation has disrupted connectivity with larger, nearby populations (Barr et al. 2012).

Genetic tools have long been employed for studying connectivity, and can be complementary to direct studies of movement (Bohonak 1999). Mark-recapture and re-sighting studies quantify dispersal movements, but field efforts are limited over space and time. Genetic estimates of connectivity quantify gene flow, which is the product of movement and successful breeding by individuals. These estimates typically integrate across generations, and can capture rarer long distance dispersal events that are very difficult to detect with field efforts. Patterns of gene flow and genetic drift over many generations are reflected in the genetic population structure over a species' range. By analyzing this genetic population structure, genetic connectivity patterns and the impacts of fragmentation can be inferred.

In this study, we analyze genetic population structure in the cactus wren throughout coastal southern California using microsatellite markers developed specifically for this species. Microsatellites, or short tandem repeats, are repeating regions of DNA with relatively high mutation rates. These mutation rates provide the variability to resolve the effects of recent landscape alterations on genetic population structure, such as those caused by urbanization, agriculture, and wildfire. We expand upon our previous study focused in Orange and San Diego Counties (Barr et al. 2012), adding cactus wren samples from Ventura, Los Angeles, San Bernardino, and Riverside Counties. With this full dataset, we characterize the current population genetic structure to provide information on levels of gene flow throughout the cactus wren's range in coastal southern California. We also analyze genetic diversity and recent demographic change over the study area. Understanding these patterns will aid in management of current cactus wren populations and future efforts in habitat restoration.

## Methods

### Samples

We visited known occupied and accessible (those on public lands or private lands that provided permission) cactus patches throughout the study area in 2011 and 2012. We identified potential sites using information from recent surveys by cooperators and a database of mapped cactus (data not shown; pers. comm. C. Winchell). In Orange and San Diego Counties, we monitored nests and sampled nestlings for growing feathers at 6 to 12 days of age, and captured adults where nests were inaccessible. Elsewhere in the study area, we sampled more opportunistically, either sampling nestlings of appropriate age when encountered or taking blood via toe-nail clips from adults captured using standard mist-netting techniques with song playback. We banded all individuals with a numbered metal federal band to prevent re-sampling individuals. Sample collection was authorized by a Memorandum of

Understanding between the California Department of Fish and Wildlife and B. Kus, and permit SC-001504 held by B. Kus. The Nature Reserve of Orange County (NROC) provided many of the samples from Orange County.

Samples were stored in Queen's Lysis Buffer at  $-20^{\circ}\text{C}$  until extraction. We also collected a few deceased birds discovered in or near nests, providing muscle or toepads for DNA. We extracted DNA using standard protocols provided with the DNA Tissue Extraction Kit (Qiagen), modified by adding 20  $\mu\text{L}$  of dithiothreitol to the extraction buffer and extending tissue digestion to 48 hours. We quantified all DNA extractions with a Nanodrop spectrophotometer and diluted them to  $\leq 50$  ng/ $\mu\text{L}$  to normalize PCR amplifications across samples.

### **Library Development and Genotyping**

We discovered microsatellite loci in the cactus wren genome using a modification of the techniques in Hamilton et al. (1999). Libraries were constructed by excising genomic DNA using the restriction enzyme *HincII*, and ligating these fragments to SNX linkers. Biotinylated oligonucleotide probes that included both trinucleotide and tetranucleotide repeats were then used to isolate and separate microsatellite repeat regions. These fragments were amplified via polymerase chain reactions (PCR) and sequenced on a Roche (Roche Applied Science, Penberg, Germany) 454 GL FLX DNA sequencer in the Evolutionary Genetics Core Facility (EGCF) at Cornell University, Ithaca, NY. In 3,350 captured sequences, 414 contained microsatellite repeat regions. We mapped these sequences to the Zebra Finch (*Taeniopygia guttata*) genome to identify their physical locations and facilitate library development. After eliminating loci with complex repeats, on sex chromosomes, and lacking sufficient flanking sequence for primer design, we tested the remaining 52 loci for variation using a three-primer technique (Schuelke 2000). All genotyping runs occurred on an ABI (Applied Biosystems, Foster City, CA) 3730 DNA Analyzer in the CSUPERB Microchemical Core Facility at San Diego State University or at the biotech service corporation Bio Applied Technologies Joint, Inc. in San Diego, CA.

We discovered 28 variable loci, and co-amplified these in three PCRs using a Qiagen (Venlo, The Netherlands) multiplex kit following the manufacturer's protocol. Combinations of loci are indicated in Table 1. Approximately 10% of the samples were amplified and genotyped twice to obtain an error rate. We used MICRO-CHECKER (van Oosterhout et al. 2004) to check loci for stepwise mutational model consistency, and GENEPOP ON THE WEB (Raymond & Rousset 1995, Rousset 2008) to test loci for Hardy-Weinberg equilibrium and linkage disequilibrium. These tests address assumptions made by many of the analyses used herein. Loci can exhibit departures from Hardy-Weinberg Equilibrium due to allelic dropout (i.e., missing alleles due to mutations in primer sites), selection, or sampling issues (i.e., Wahlund effect). Linkage disequilibrium occurs when loci are physically or statistically linked, and hence confound analyses due to a lack of independence.

### **Genetic Analyses**

We used multiple analyses to explore genetic population structure and patterns of diversity across the study area. First, we employed Bayesian clustering analyses to determine if individuals were arranged in distinct gene pools or clusters (Guillot et al. 2008). We also

identified groups of individuals sharing recent gene flow using a modified exact test following Waples and Gaggiotti (2006). This method is more powerful for detecting local population structure when gene flow is on-going, whereas Bayesian clustering analyses infer structure that is the product of major constraints on gene flow over many generations. Hence, the groupings of individuals suggested by the Waples and Gaggiotti (2006) method are likely in panmixia—that is, gene flow is evenly distributed within them—and we refer to them as “populations.” We refer to groups detected by the Bayesian clustering analyses as “clusters,” as these can be composed of numerous populations among which there may be some finer-scale restrictions on gene flow. We use analyses of spatial autocorrelation to examine local gene flow and connectivity patterns within clusters. Finally, we quantified patterns of genetic diversity and recent demographic change.

### *Cluster Inference*

Bayesian clustering analyses are individual-based, searching for combinations of individuals that can best be grouped together while conforming to expectations of Hardy-Weinberg Equilibrium and linkage disequilibrium. These expectations are met when a group of individuals is essentially a common gene pool in population genetics terms, without major barriers to gene flow between them for numerous generations. Since the presence of closely-related individuals can confound analyses based upon Hardy-Weinberg Equilibrium and linkage disequilibrium (Anderson & Dunham 2008), we implemented the program COLONY (Wang 2009) to identify full sibships (i.e., parent-offspring or full siblings) in the dataset. We eliminated a member of each full sibship for all analyses, except where noted.

Initially, we used the Bayesian clustering program GENELAND (Guillot et al. 2008) to identify population structure over the full dataset. This analysis takes geographic relationships into consideration along with individual genotypic data, and can identify recently developed clusters (Guillot 2008). Analyses were conducted using the uncorrelated alleles model with admixture, testing for clusters ( $K$ ) between 1 to 10 with 1 million Markov chain Monte Carlo repetitions and a 20% burn-in. Using these same parameters, we analyzed detected clusters individually in GENELAND to detect further substructure.

### *Defining Local Populations and Fine-scale Gene Flow Patterns*

To define locally panmictic populations, we grouped geographically aggregated individuals with no obvious potential barrier to movement, and conducted an exact test for genetic differentiation among them as implemented in GENEPOP ON THE WEB. Aggregations with <4 samples were excluded from this analysis. The exact test for genetic differentiation tests a null hypothesis of genetic panmixia (no genetic structure). Exact tests were conducted for each microsatellite locus and resulting  $p$ -values were combined via Fisher’s method. Automated programs like GENEPOP ON THE WEB may calculate extremely low  $p$ -values for individual loci, hence reducing the result of the overall test. Following Waples and Gaggiotti (2006), we made this test more conservative by setting  $p$ -values for individual loci to a minimum of 0.0001 prior to combining with Fisher’s method. Aggregations were determined to be in the same population if the overall  $p$ -value for the pairwise exact test between them was >0.01. To determine whether geographic distance influenced genetic structure, we calculated

pairwise  $F_{ST}$ , a measure of genetic differentiation, between these populations using GENEPOP ON THE WEB, and tested for isolation-by-distance using a Mantel test as implemented in IBDWS (Jensen et al. 2005). Finally, we visualized relationships among populations based upon  $F_{ST}$  using a principal coordinates analysis as implemented in GENALEX (Peakall & Smouse 2012), thereby allowing comparison of genetic differentiation patterns with those detected in Bayesian clustering analyses.

To estimate patterns of genetic similarity and gene flow within clusters, we calculated the spatial autocorrelation coefficient,  $r$ , in GENALEX. For this, we used 999 permutations to assess the significance of  $r$  and 999 bootstraps to obtain a confidence interval. Spatial autocorrelation quantifies the average genetic similarity between each individual and all others within binned geographic distances from that individual. These patterns can provide inferences of genetic structure within local groups, with positive spatial autocorrelation indicating distances within which gene flow occurs. Since broad-scale genetic structure can confound this analysis (Banks & Peakall 2012), analyses were conducted within three individual regions (central Orange County - northern San Diego County, southern San Diego County, and the eastern Los Angeles Basin) based upon detected patterns of population structure. We did not have enough samples with a suitable spatial arrangement to conduct this analysis in other regions. Initially, we used bins of 1000m up to the greatest distance between samples; however, to better display the results, a subset of bins is presented here.

### *Genetic Diversity*

We quantified genetic diversity within populations in the form of allelic richness in HP-RARE (Kalinowski 2005) and heterozygosity, both observed and expected, in GENALEX. Tests for heterozygote excesses were conducted in BOTTLENECK (Piry et al. 1999). This test is based upon the expectation that allelic diversity is lost more rapidly than heterozygosity during a genetic bottleneck, and thus determines whether a significant population decline has recently occurred. Finally, we implemented LDNe (Waples & Do 2008) and COLONY to calculate current effective population sizes,  $N_e$ . The former calculates effective population size based upon linkage disequilibrium, and the latter uses a sibship approach. This analysis in COLONY is the only one in which we used all genotyped individuals, full sibships included. Effective population size is an important parameter in population genetics, as it determines inbreeding rates, the strength of genetic drift, the potential for selection, and the effect of migration. It is associated with the number of successful breeding individuals in a population (Frankham 1995).

## Results

### **Data Quality**

Although 620 coastal cactus wrens were sampled in the study area, multiple nestlings from the same nest did not represent independent genetic samples; furthermore, 20 captured adults were determined to be full sibs. After eliminating redundant nestling samples and one member of each full sibship, we analyzed a dataset of 349 cactus wrens. Since closely related individuals were not used in analyses, we can infer that detected signals of population structure

are the product of gene flow and connectivity regimes rather than spurious results created by family structure (Anderson & Dunham 2008). Samples provided thorough coverage of the cactus wren range in coastal southern California (Figs. 1 - 5).

After eliminating loci that were in linkage disequilibrium, did not conform to Hardy-Weinberg equilibrium, or inconsistently amplified, 19 loci were used for all analyses (Table 1). These loci are located across the genome, falling on nine different chromosomes. Total numbers of alleles ranged from three to 18, and overall heterozygosities were generally high (mean: 0.63), as would be expected with highly polymorphic microsatellites. After re-runs, the error rate was found to be negligible (<0.1%), and there were very few missing data from failed amplifications (<0.01%).

Locus	Chr	Forward Primer	Reverse Primer	Repeat Type	Length	MP	A	$H_o$
CACW3-01	1	ACTGTTCCACCCTTGGACCTG	TGTCTGGAAACCACTGAAGAAC	Trinucleotide	250	1	7	0.85
CACW3-02	1	AATGGAAAGGAGCATCAACTG	TTCATGGTGCATACAAGATAGC	Trinucleotide	117	1	5	0.59
CACW3-03	1A	TCCTGAAATGTAATTCAGACACC	CAGAGTGTACTTAAATTGATTCTTTC	Trinucleotide	262	1	9	0.73
CACW3-04	2	CATGGATAGAGTGAGAACAATATGC	CATGAGATGGACATTATGAGCTG	Trinucleotide	125	1	4	0.31
CACW3-05	2	GATGCATATTGTCAGAGTTCAC	CTGGACTGAGCTAACAAATGATG	Trinucleotide	141	2	7	0.63
CACW3-06	3	CTCTTTGTTTGACTTAGGAGAACC	AAACCCACCAACCTCTTCC	Trinucleotide	190	2	3	0.52
CACW3-07	4	GCTCAAACCTCTGACCAAGG	TTTTGTACTTTGCTGAAGTCAATTT	Trinucleotide	199	2	5	0.51
CACW3-08	5	GCCCAGGCTCCATCACAG	ATGTCTGCTGCTCCCTCAG	Trinucleotide	98	1	4	0.36
CACW3-09	5	AGGAAGAAATAGAGGTGAGGGAAC	TGACGACTGAACAAAAGTACGAG	Trinucleotide	126	2	5	0.3
CACW3-11	22	TTCTCCTCCCTACCTCCTTT	GTGACAACAGAAAATCCCTTTA	Trinucleotide	183	1	9	0.6
CACW4-01	1	TTTTGCCTAATAAACTGGCTGAC	CACAGAACCACAACCTACATGG	Tetranucleotide	162	3	9	0.74
CACW4-03	1	CCTTACCGAAGTATGCAACAAG	TTGAGATAGAGTGTAGCCATGTG	Tetranucleotide	284	2	10	0.83
CACW4-04	1	TCTCACGTCTTACCATCCTGTG	TTGATACTTGAAACTCTCCTTCTGTC	Tetranucleotide	284	2	8	0.59
CACW4-05	2	GCTCTAAAACCTGTGGGCAAC	CGAGAACAAGATCATTAAACAGCAG	Tetranucleotide	135	2	6	0.69
CACW4-06	2	TTCTAAGCTCTCTCAATTTCTACTG	GACTGAATCAATATGTTATGGCAAC	Tetranucleotide	223	1	16	0.85
CACW4-09	3	GCTAACTGAAAGGGATTGTTGG	TTTCTGGCATGTTTCCTGTC	Tetranucleotide	180	3	18	0.81
CACW4-10	5	GGGTTGGACAAGGTGACATC	TCAATGTGCTTTGCAGGAAG	Tetranucleotide	221	3	16	0.85
CACW4-12	5	CCTGCCACCACTGTATTTCTG	AGAGGCCAAAGACTGAATGG	Tetranucleotide	300	1	4	0.55
CACW4-13	28	GCAGAACTTGGGACTTCGAC	ACTGGGCTTGTATGGATGG	Tetranucleotide	108	1	6	0.62

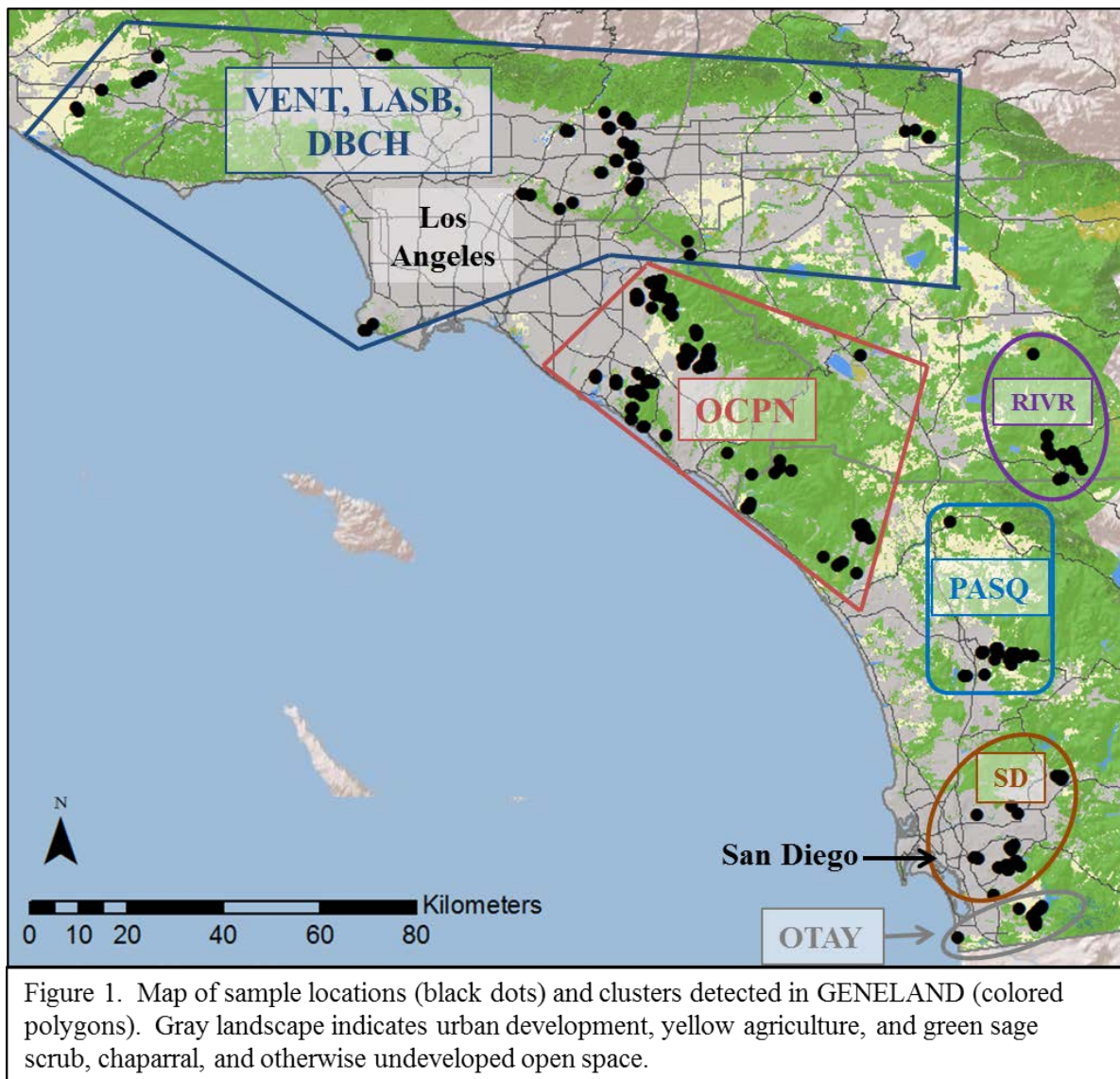
### Inference of Clusters

GENELAND identified six geographically distinct clusters over the full dataset (Fig. 1): 1) individuals from Ventura, Los Angeles, and San Bernardino Counties (VENT, LASB, DBCH); 2) Riverside County (RIVR); 3) most of Orange County and Marine Corps Base Camp Pendleton and Fallbrook Naval Weapons Station in San Diego County (OCPN); 4) San Pasqual Valley (PASQ); 5) Lake Jennings, Sweetwater Reservoir, and several urban parks and canyons in San Diego (SD); and 6) Otay River (OTAY). Notably, an individual sampled at Lake Elsinore in Riverside County was clustered into OCPN.

Substructure GENELAND analyses focused within each of three of these clusters, RIVR, PASQ, and OTAY, did not reveal any further clusters. Analyses within the clusters of cactus



wrens from Ventura, Los Angeles, and San Bernardino counties identified Ventura (VENT) as an independent cluster (Fig. 2). Removing VENT and focusing GENELAND analyses on the



remaining Los Angeles and San Bernardino County cactus wrens revealed additional clusters, including one larger cluster composed of cactus wrens widely distributed in Los Angeles and San Bernardino Counties (LASB) and a smaller cluster in the area of Diamond Bar and Chino Hills State Park (DBCH). Substructure analyses within VENT, LASB, and DBCH did not reveal any further clusters. Within OCPN, two additional clusters were apparent, one composed of wrens in the coastal reserve of NROC and another large central group occupying an extended area east of Interstate 5 through Marine Corps Base (MCB) Camp Pendleton and Fallbrook Naval Weapons Station (NWS; Fig. 3). No additional clusters were detected within the coastal OCPN cluster by GENELAND. Substructure analysis within both the central OCPN cluster (Fig. 3) and

within SD (Fig. 4) indicated two additional clusters were present within each of these areas (Data Not Shown).

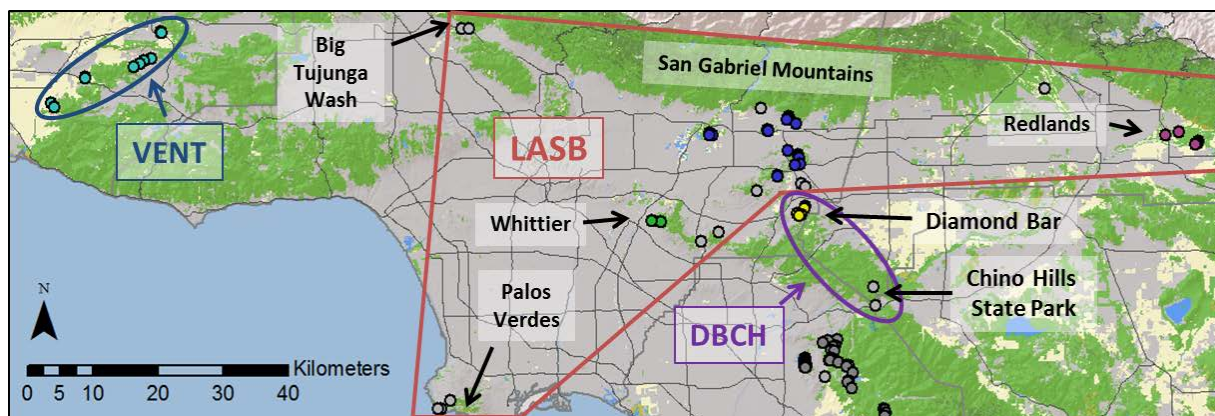


Figure 2. Map of sample locations (colored dots) and clusters detected in GENELAND (colored polygons). Gray indicates urban development, yellow agriculture, and green sage scrub, chaparral, and otherwise undeveloped open space.

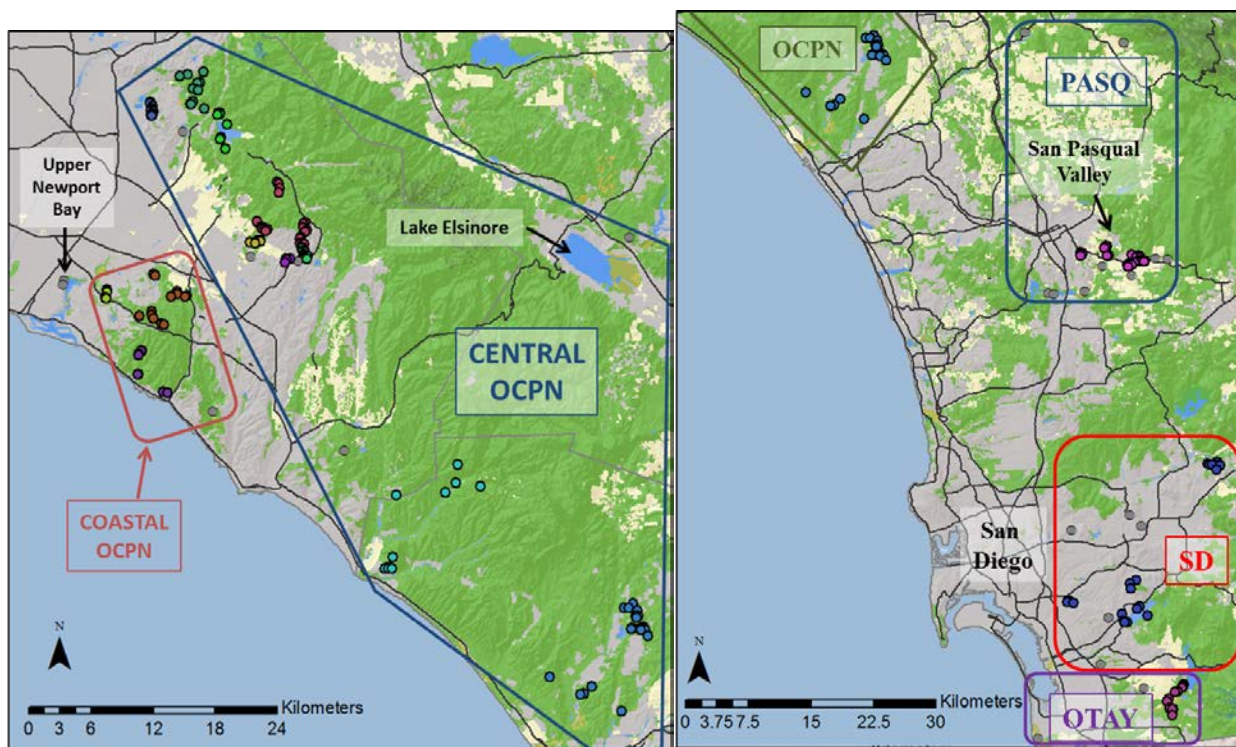


Figure 3. Map of sample locations (colored dots) and clusters detected in GENELAND (colored polygons). Gray indicates urban development, yellow agriculture, and green sage scrub, chaparral, and otherwise undeveloped open space.

Figure 4. Map of sample locations (colored dots) and clusters detected in GENELAND (colored polygons). Gray indicates urban development, yellow agriculture, and green sage scrub, chaparral, and otherwise undeveloped open space.

### Identification of Local Populations

Using the Waples and Gaggiotti (2006) method, 19 panmictic populations were detected (Fig. 5), and pairwise  $F_{ST}$  among these ranged 0.003 to 0.179 with a significant correlation with geographic distance (Fig. 6;  $r = 0.644$ ,  $p < 0.001$ ). Hence, there is an overall signal of isolation by distance in this dataset. These analyses excluded 41 individuals sampled in disparate locations and not part of aggregations of five or more. Principal coordinates analysis on these genetic distances revealed relationships between these populations that are similar to clustering results, with 51.05% of the variance explained by the two plotted coordinates (Fig. 7). For instance, most of the populations within OCPN were aggregated, as were those within LASB. Each of the other populations was dispersed throughout the coordinate space. One exception to this concordance is that cactus wrens sampled on a reserve at the University of California-Irvine were separated from the rest of OCPN despite being sorted into the coastal cluster by GENELAND.

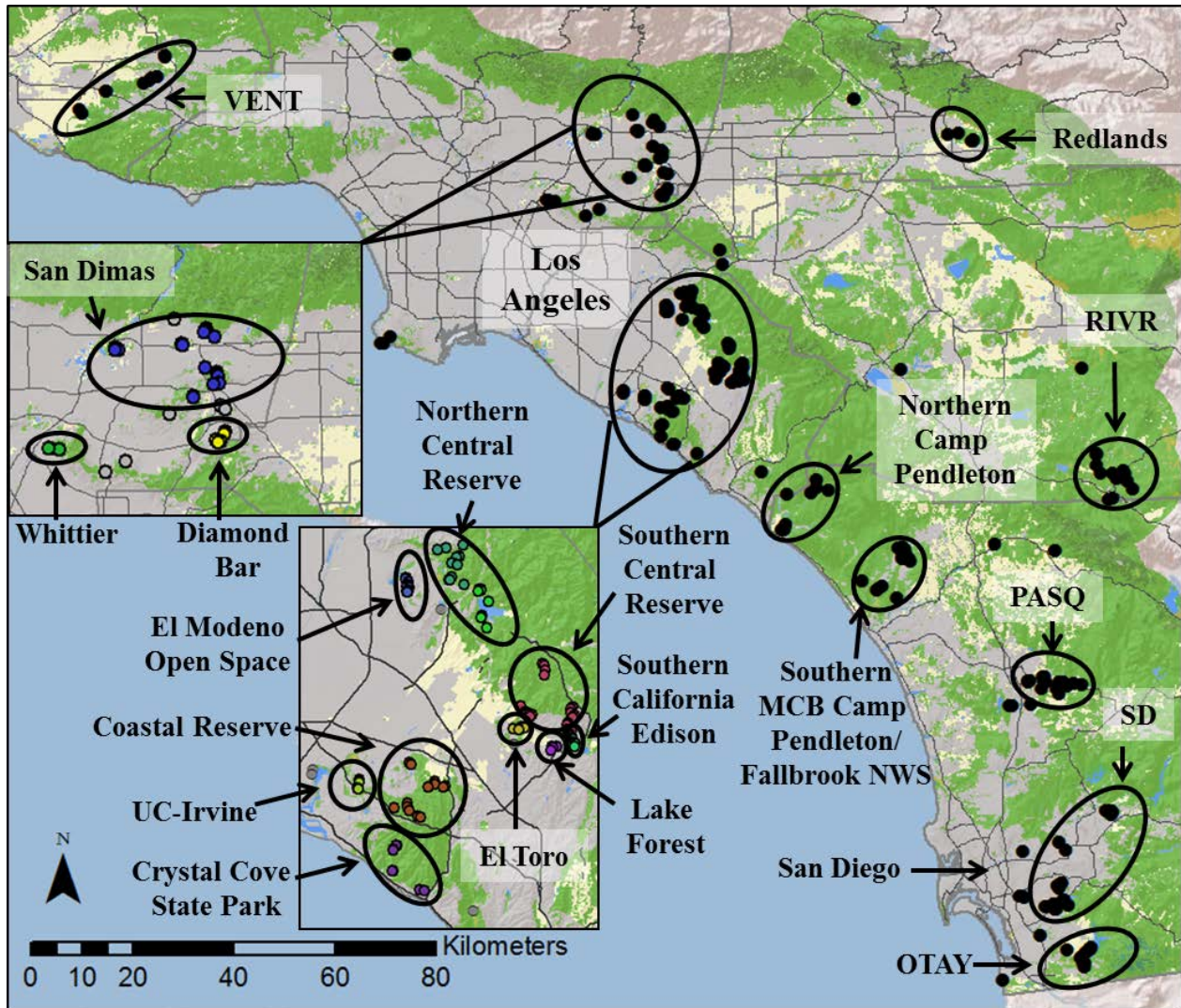
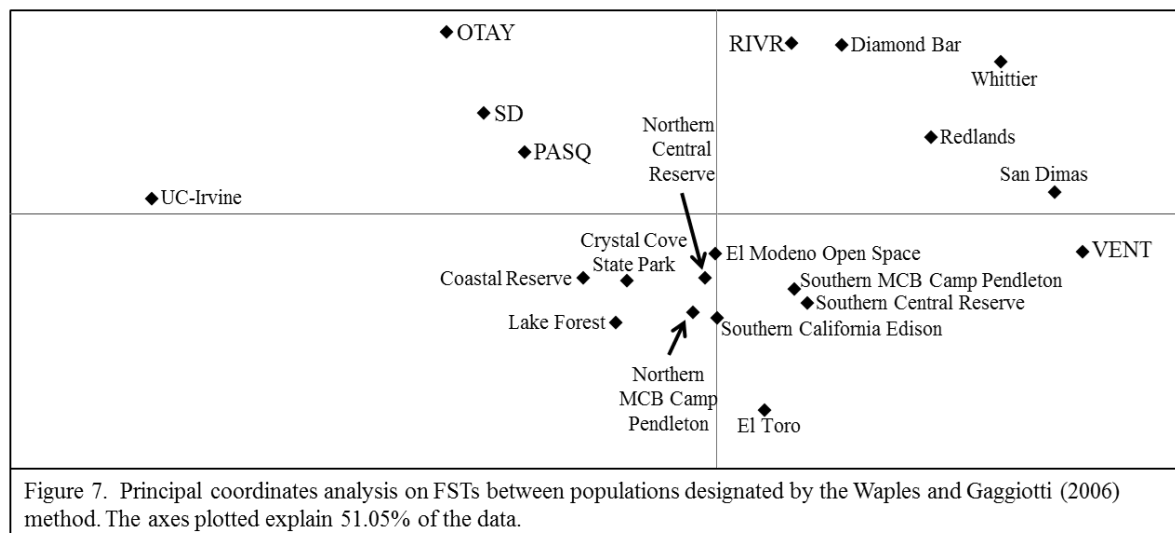
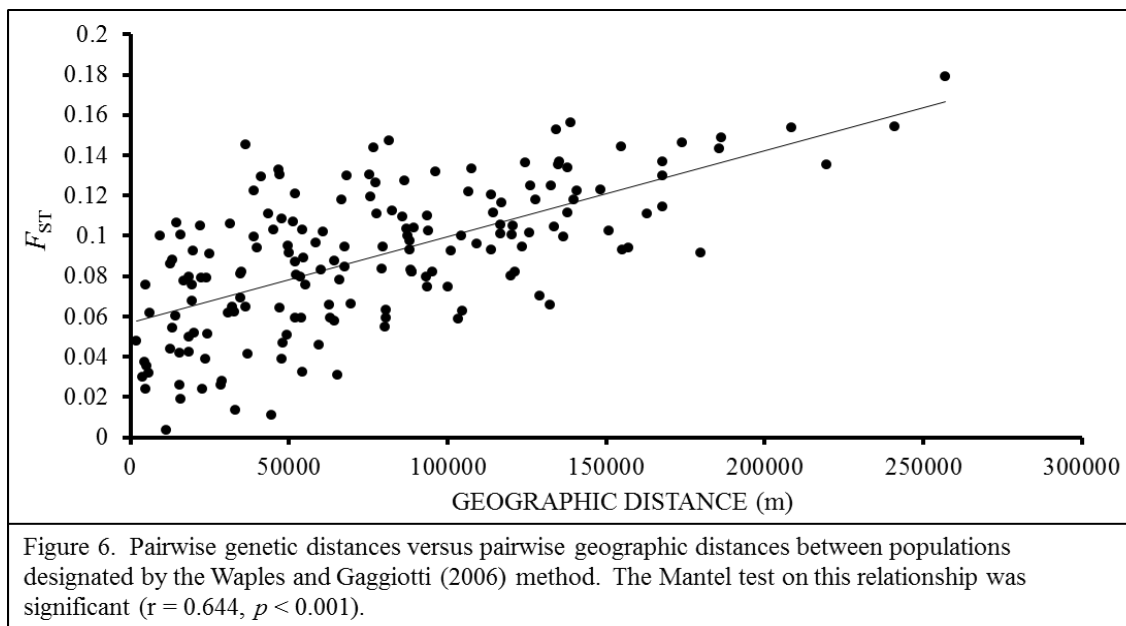
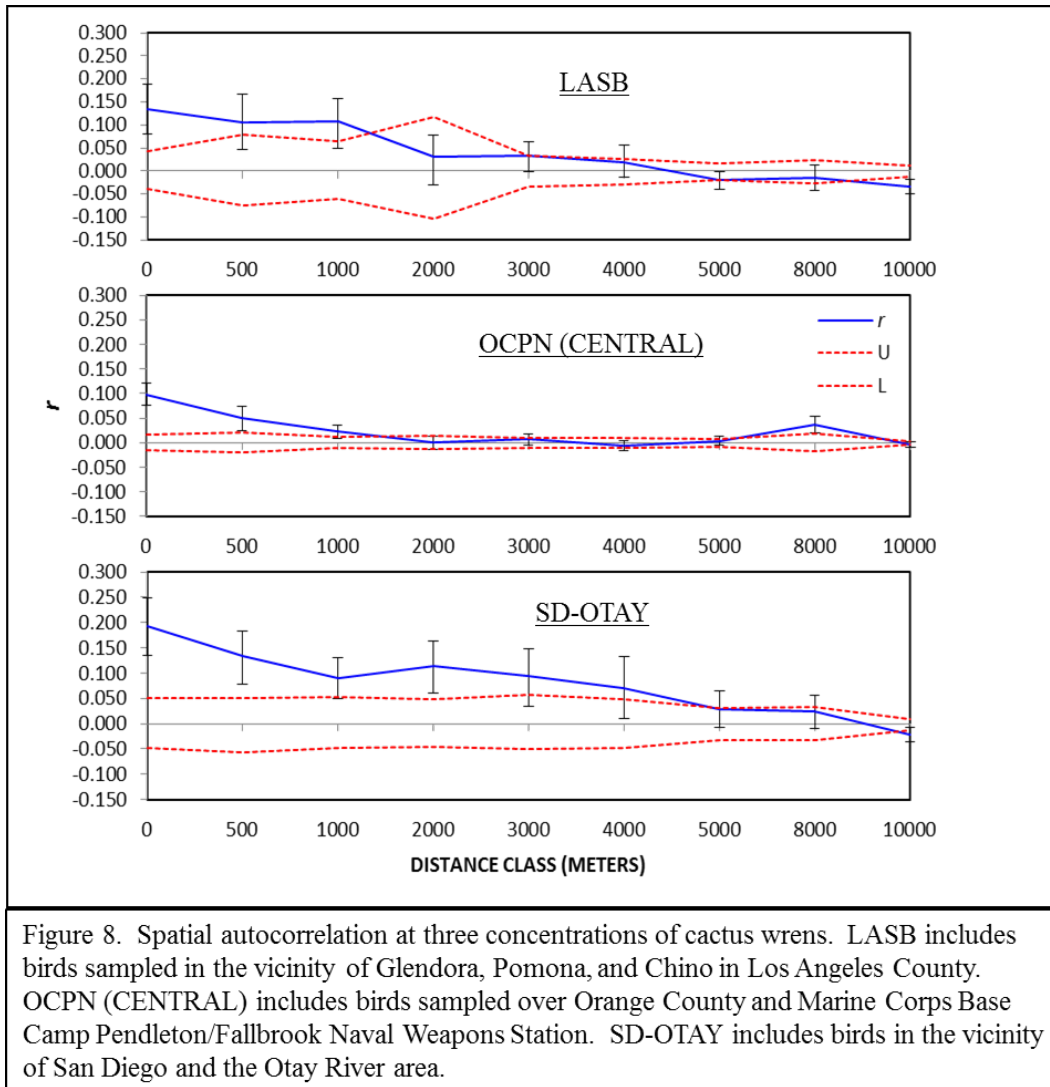


Figure 5. Map of the 349 sample locations (dots) and populations detected with the Waples and Gaggiotti (2006) method (black circles). Gray landscape indicates urban development, yellow agriculture, and green sage scrub, chaparral, and otherwise undeveloped open space.



Spatial autocorrelation analyses were focused on groups of individuals sampled across areas near San Dimas, Whittier, and Diamond Bar (noted as LASB), the central cluster in OCPN, and over San Diego and Otay (SD-OTAY). Results indicated positive relationships up to 1km in LASB (Fig. 8;  $r = 0.039$ ,  $p = 0.001$ ) and 4km in SD-OTAY ( $r = 0.129$ ,  $p = 0.001$ ). A much different spatial autocorrelation profile is evident in the central cluster in OCPN, where  $r$  is significant within 1km ( $r = 0.022$ ,  $p = 0.001$ ) and then again at 8km ( $r = 0.048$ ,  $p = 0.001$ ). None of the bins between these distances show significant spatial autocorrelation.



### Genetic Diversity

Observed and expected heterozygosity and allelic richness were similar across clusters (Table 2). One exception was in VENT, where allelic richness (VENT: 3.54; overall mean: 4.64) and expected heterozygosity (VENT: 0.512; overall mean: 0.611) were lower than observed throughout the remainder of the study area. Effective population sizes varied across the study area and between the methods we employed. Waples and Do (2010) suggested using the harmonic mean of results from multiple methods for the most reliable estimates; thus we report these as well. The largest effective population sizes were observed in the central cluster in OCPN (151.9), RIVR (112.47), and LASB (94.26). Much smaller effective population sizes were evident in DBCH (16.86) and in the coastal cluster in OCPN (35.67). We detected recent genetic bottlenecks in the form of significant heterozygote excesses in VENT, OCPN, both clusters within OCPN, PASQ, and OTAY.

Table 2. Genetic diversity indices for the populations detected via the Waples and Gaggiotti (2006) method and clusters detected in GENELAND.  $N$  = number of samples,  $A$  = allelic richness,  $H_O$  = observed heterozygosity,  $H_E$  = expected heterozygosity,  $F$  = inbreeding coefficient,  $LD-N_e$  = effective population size from LDNE,  $COL-N_e$  = effective population size from COLONY,  $Harm-N_e$  = harmonic average effective population size between LDNE and COLONY,  $H$ -excess =  $p$ -value of test for heterozygote excess. In a few locations, not enough information is available in the data to estimate either  $N_e$  (\*) or the upper limit of the confidence interval ( $\infty$ ).

CLUSTER/Population	$N$	$A$	$H_O$	$H_E$	$LD-N_e$	$COL-N_e$	$Harm-N_e$	$H$ -excess
<b>VENT</b>	<b>12</b>	<b>3.54</b>	<b>0.526</b>	<b>0.512</b>	<b>248.1 (35.5 - <math>\infty</math>)</b>	<b>26 (13 - 60)</b>	<b>47.07</b>	<b>0.04</b>
<b>LASB</b>	<b>52</b>	<b>4.7</b>	<b>0.559</b>	<b>0.584</b>	<b>133.2 (82 - 301)</b>	<b>73 (50 - 111)</b>	<b>94.26</b>	<b>0.297</b>
San Dimas	28	3.21	0.549	0.556	58.1 (39.4 - 100.2)	60 (37 - 107)	59.03	--
Whittier	4	3.16	0.592	0.508	*(23.5 - $\infty$ )	--	--	--
Redlands	6	3.1	0.561	0.536	*(68.2 - $\infty$ )	112 (25 - $\infty$ )	--	--
<b>DBCH</b>	<b>10</b>	<b>4.32</b>	<b>0.658</b>	<b>0.570</b>	<b>13.9 (9.5 - 22.6)</b>	<b>24 (10 - 221)</b>	<b>16.86</b>	<b>0.37</b>
Diamond Bar	8	3.02	0.638	0.530	8.4 (4.8 - 15.1)	28 (11 - $\infty$ )	12.92	--
<b>RIVER</b>	<b>16</b>	<b>4.46</b>	<b>0.510</b>	<b>0.568</b>	<b>325.8 (65.5 - <math>\infty</math>)</b>	<b>68 (34 - 229)</b>	<b>112.47</b>	<b>0.52</b>
<b>OCPN (CENTRAL)</b>	<b>143</b>	<b>4.94</b>	<b>0.605</b>	<b>0.623</b>	<b>166.8 (124.8 - 238.9)</b>	<b>140 (109 - 179)</b>	<b>151.9</b>	<b>0.03</b>
Northern Central Reserve	32	3.53	0.641	0.625	90.4 (59 - 174.6)	46 (29 - 77)	60.97	--
El Modeno Open Space	12	3.15	0.579	0.552	11.5 (7.1 - 20.9)	20 (10 - 50)	14.6	--
Southern Central Reserve	32	3.52	0.615	0.611	71.3 (49.7 - 117.9)	50 (32 - 85)	58.78	--
El Toro	6	2.69	0.570	0.458	4.9 (2.7 - 9.3)	12 (5 - 45)	9.96	--
Southern California Edison	16	3.36	0.651	0.610	51.9 (26.7 - 272.6)	32 (17 - 74)	39.59	--
Lake Forest	9	3.16	0.576	0.560	13.7 (9.5 - 21.3)	13 (11 - 80)	13.34	--
Northern MCB Camp Pendleton	11	3.33	0.531	0.541	18.8 (12.1 - 34.8)	44 (19 - 692)	26.34	--
Southern MCB Camp Pendleton/Fallbrook NWS	20	3.33	0.566	0.567	103.7 (49.5 - 5993.8)	54 (31 - 124)	71.02	--
<b>OCPN (COASTAL)</b>	<b>31</b>	<b>4.58</b>	<b>0.596</b>	<b>0.620</b>	<b>31.7 (24 - 44.1)</b>	<b>42 (26 - 73)</b>	<b>35.67</b>	<b>0.007</b>
Coastal Reserve	12	3.38	0.623	0.597	24.6 (14.6 - 56.7)	30 (16 - 69)	27.03	--
University of California-Irvine	5	2.96	0.526	0.522	9.9 (4.4 - 30.1)	40 (9 - $\infty$ )	15.87	--
Crystal Cove State Park	9	3.11	0.637	0.557	16 (10.3 - 29)	24 (12 - 88)	19.2	--
<b>PASQ</b>	<b>40</b>	<b>4.64</b>	<b>0.650</b>	<b>0.650</b>	<b>235.4 (109.3 - <math>\infty</math>)</b>	<b>53 (35 - 84)</b>	<b>86.49</b>	<b>0.004</b>
<b>SD</b>	<b>31</b>	<b>4.84</b>	<b>0.593</b>	<b>0.626</b>	<b>29.9 (22.9 - 41)</b>	<b>46 (30 - 77)</b>	<b>35.57</b>	<b>0.176</b>
<b>OTAY</b>	<b>14</b>	<b>4.6</b>	<b>0.673</b>	<b>0.646</b>	<b>46.4 (21.2 - 5667.4)</b>	<b>44 (24 - 137)</b>	<b>44.98</b>	<b>0.018</b>

## Discussion

The dataset analyzed here, with a large number of samples and many highly variable microsatellites, should be sensitive enough to detect fine-scale and recently developed patterns of genetic population structure in the cactus wren. Using multiple, layered analyses, we detected multiple geographically distinct genetic clusters and populations, and significant isolation by distance. These patterns correlate with observed levels of fragmentation.

### Genetic Population Structure

Detected genetic structure patterns appear to largely mirror available open space over the study area. For instance, the largest spatial extent of open space with the least urban fragmentation is encompassed by the central OCPN cluster (Fig. 3). This is separated from the coastal OCPN cluster by the Interstate 5 corridor and coincident urbanization. Extensive field surveys also confirm a lack of movement between the central and coastal clusters in OCPN (Preston & Kamada 2012). Though substructure analyses in GENELAND provide evidence for two clusters within the central OCPN cluster, these results must be interpreted in light of the significant isolation by distance also observed. Here, clustering may be influenced by sampling gaps rather than reflecting true divisions. Field observations have detected dispersal between several of the populations within the central OCPN cluster (Preston & Kamada 2012). Additionally, a second, Bayesian clustering method (STRUCTURE; Pritchard et al. 2000) employed in Barr et al. (2012) provides evidence for stepping stone gene flow in this area. For these reasons, we infer central OCPN to be a single genetic cluster. The VENT, RIVR, and PASQ clusters are also widely separated from others by fragmentation from urban development, agriculture, and fire (Fig. 1). The patterns detected at DBCH and OTAY may provide an indication of the scale at which fragmentation may disrupt genetic connectivity in the cactus wren. Both of these clusters are separated by very short distances from nearby aggregations. At OTAY, the distance is approximately 9km (Fig. 4), while DBCH occupies open space fragmented from neighboring clusters by major roadways (Fig. 2). Despite their close proximity, GENELAND results suggest significant disruptions in connectivity between these sites.

Lesser or more recent disruptions in gene flow may be indicated by the Waples and Gaggiotti (2006) method for detecting panmictic populations. For instance, aggregations of cactus wrens sampled in the El Modeno Open Space, El Toro, and the remainder of NROC's Central Reserve are differentiated from one another (Fig. 5). Notably, El Modeno and El Toro are isolated from the other sites by major roads and urbanization. Though many other aggregations within the large open space occupied by the central OCPN cluster are identified as independent populations, genetic distances between these are far lower than observed throughout the rest of the dataset (Fig. 7). For instance, genetic distance between the Northern Central Reserve and Northern Camp Pendleton 35km away is much lower ( $F_{ST} = 0.011$ ), than that between the Northern Central Reserve and El Modeno population 15km to the west ( $F_{ST} = 0.035$ ). Such patterns are prevalent throughout the study area, with higher genetic differentiation coinciding with more severe fragmentation by urban development.



Despite an overall signal consistent with habitat fragmentation and isolation, there are a few sites suggested by clustering analyses to be genetically connected despite being ostensibly geographically isolated. In particular, although it appears to be a habitat island in a huge urban expanse, Palos Verdes clusters into LASB (Fig. 2). LASB also includes cactus wrens sampled along an extended area on the southern fringe of the San Gabriel Mountains, from Big Tujunga Wash to Redlands 120 km to the east, and includes a group occupying a fragment of open space in the vicinity of Whittier. Making this cluster even more surprising is the signal of a break in genetic connectivity between it and the nearby DBCH. Both clustering analyses (Fig. 2) and the Waples and Gaggiotti (2006) method (Fig. 5) show restricted gene flow between LASB and DBCH. Small sample sizes at some collection locations and large geographic distances among collection locations may have confounded our ability to detect genetic patterns in the Los Angeles Basin (Kalinowski 2010, Meirmans 2012). For instance, while the Waples and Gaggiotti (2006) method may conclude that gene flow is not panmictic between a group of cactus wrens generally around Pomona at the heart of the LASB cluster and others near Whittier or those near Redlands, this method is not robust to the confounding effects of isolation by distance. When isolation by distance is significant, distant sites would naturally have different allele frequencies and appear genetically differentiated from one another. Overcoming this issue would require sampling intermediate sites, which, since much of the area is privately owned and the presence of cactus and cactus wrens is unknown, may not be possible. Finally, small sample sizes at Big Tujunga Wash ( $N = 2$ ) and Palos Verdes ( $N = 3$ ) restricts our ability to make conclusions about connectivity at either of these sites.

It is possible that the levels of differentiation observed among fragmented sites may result from a lack of successful breeding by dispersing individuals, rather than a lack of movement. Some of these areas have very limited available habitat, and therefore may be at carrying capacity. Field observations have detected dispersal between several of the populations detected by the Waples and Gaggiotti (2006) method (Fig. 5; Southern California Edison and the Southern Central Reserve; Preston & Kamada 2012). In this area, recent fires (Laguna Fire, 1993; Santiago Fire, 2007) have limited available habitat, and available territories may be fully occupied. If individuals disperse between sites without breeding, those individuals would neither confer gene flow between those sites nor contribute to genetic structure. These are questions that warrant further study.

While much of the extant cactus wren habitat is highly fragmented, the central cluster in OCPN may provide some insight on a dispersal regime through more contiguous open space. Spatial autocorrelation analyses indicate significant relatedness at 1km and again at 8km (Fig. 8). This pattern may be the product of many cactus wrens staying nearby or even inheriting natal territories—a pattern also reported from field observations (Preston & Kamada 2012)—but with others making regular movements up to 8km from natal areas. This is a very different pattern than detected throughout the rest of the study area, where connectivity is more limited between sites. Within the two other areas analyzed for fine-scale population structure, LASB-DBCH and SD-OTAY, patterns indicate cactus wrens are not dispersing as far. Rather, localized spatial autocorrelation was detected both in the area analyzed in LASB-DBCH (Fig. 8; 1km) and SD-OTAY (4km), indicating a limitation on dispersal distance. Notably, the coefficient of spatial

autocorrelation,  $r$ , at 1km in the central cluster in OCPN (0.022, CI: 0.009 – 0.035) is particularly lower than detected in either the LASB-DBCH analysis (0.109, CI: 0.05 – 0.157) or SD-OTAY (0.09, CI: 0.051 – 0.134). This indicates aggregations are much more genetically related within SD-OTAY and LASB-DBCH than detected in the central OCPN cluster, where more cactus wrens seem to make movements beyond their natal territories.

### Genetic Diversity

Genetic diversity is fairly similar among many of the clusters (Table 2); however, disruptions in gene flow are often evident in population structure long before genetic diversity is affected (Leberg et al. 2010). This is because genetic drift, the random survival of alleles from one generation to the next, causes populations to differentiate from one another more rapidly than it confers loss of alleles. The lower levels detected in VENT may be the product of several processes. For instance, a significant heterozygote excess indicates the cluster has experienced a genetic bottleneck, which would inherently reduce genetic diversity. Isolation combined with a relatively small effective population size may also have conferred a loss of alleles over time. Populations at the edge of a species' range often exhibit lower genetic diversity than those nearer to the core, and VENT is found at what has likely long been the margin of the cactus wren's range in southern California. Finally, it is also a possibility that this lower diversity is the product of a founder effect, with some small number of cactus wrens having initially colonized the area. Our dataset does not allow us to determine the extent to which each of these processes have contributed the lower genetic diversity detected at VENT.

Estimations of effective population sizes over the dataset can also provide some indications of connectivity levels. The discrepancies between the LD and sibship methods for estimating effective population sizes should not be discouraging in terms of their accuracy. Estimations of effective sizes are interpreted in a comparative manner, and to determine the extent to which populations have lost adaptive potential (Leberg 2005). Theory predicts minimum effective population size thresholds of 50 to avoid the negative effects of inbreeding, 500 to prevent the loss of diversity through genetic drift, and 5000 to persist in evolutionary time (Traill et al. 2010); however, it should be noted that gene flow has been shown to counter the loss of genetic diversity even when weak (Palstra & Ruzzante 2008). After estimating the harmonic mean between the methods for each site, some patterns stand out. The highest effective population sizes were detected in the central cluster in OCPN (Table 2; 151.9), RIVR (112.47), and PASQ (86.49). These are home to the largest numbers of cactus wrens in the study area (Data Not Shown). Meanwhile, the smallest effective sizes were detected within DBCH (16.86), the coastal cluster in OCPN (35.67), and in SD (35.57). These are areas we have identified as being highly isolated from other proximate aggregations. Since high levels of on-going gene flow would confer larger effective sizes to local populations, the smaller results reported here are congruent with the levels of genetic structure we report.

Importantly, populations with lower effective sizes more rapidly experience genetic drift. This may explain the striking levels of genetic differentiation between relatively proximate aggregations, such as between SD and OTAY or LASB and DBCH. With strong

isolation by distance and low effective population sizes, the removal of stepping stones between groups may have led to rapid differentiation among these sites.

Signals of genetic bottlenecks evident across the study area are not unanticipated given the known recent declines in cactus wren abundance in coastal southern California (Shufard & Gardali 2008). Notably, three of the five populations that exhibited signatures of bottlenecks were burned by recent wildfires, including PASQ (Witch Creek Fire, 2007) and both the coastal and central OCPN clusters (Table 2). The bottleneck signals in OTAY may be the result of recent limitations on connectivity with other populations, as disrupted gene flow can also cause rapid drops in effective population size (England et al. 2010). Finally, the significant signal detected in VENT could be related to any of the numerous scenarios outlined above in the discussion of the lower genetic diversity at that site.

### **Management Implications**

Perhaps the most important inference from these genetic analyses for cactus wren management is localized gene flow. Distant aggregations of cactus wrens are only genetically connected through intermediate sites. In the absence of such sites, limited dispersal capability and small effective population sizes may cause distant aggregations to rapidly differentiate, especially when faced with fragmentation by urbanization. Consequently, it appears that much of the study area is divided into numerous, small clusters. Habitat fragmentation by urbanization and agriculture is spatially coincident with many of the observed population and cluster boundaries, and may be the main cause in maintaining the observed genetic structure in the cactus wren.

Several large aggregations may warrant focused conservation effort to preserve or increase genetic connectivity. Clearly, the highest levels of connectivity in the study area exist within the central cluster in OCPN (Fig. 3). This cluster may be the most robust to stochastic processes, and efforts to limit further habitat fragmentation should help retain genetic exchange among existing aggregations. Cactus restoration in burned areas within this cluster may also be naturally recolonized by dispersers. In other more fragmented locations, small, isolated aggregations may be more susceptible to extinction by environmental perturbations, and may not be easily recolonized without additional efforts. Restoration of scrub habitat, and cactus patches sufficient for nesting may allow for increased connectivity among some of these aggregations. For example protecting and establishing additional stepping stones between SD and OTAY could help to restore connectivity in these areas (Fig. 4). Some efforts are already in place to re-establish cactus habitat lost to wildlife on the San Diego National Wildlife Refuge. In other areas where geographic distances between sites are large and the intervening landscape has been severely altered (such as between PASQ, RIVR, and VENT and other clusters), re-establishing stepping-stone connectivity may be difficult; consequently, augmentation and translocations may be necessary if local aggregations are extirpated or become too small. Cactus wrens have previously been translocated with success by NROC (Kamada & Preston 2012); however, the experiences in Orange County illustrate the necessity of understanding dispersal capabilities and natural connectivity patterns prior to performing translocations. A small group of cactus wrens was translocated to an isolated habitat patch on the Upper

Newport Bay in this area. Field observations indicated no individuals have moved into or out of this patch since the translocation (Kamada & Preston 2013). Indeed, GENELAND analyses cluster cactus wrens in this patch with those in central OCPN (Supp. Fig. 3), confirming field observations.

Notably, the central OCPN cluster extends over an area putatively occupied by two cactus wren subspecies, *C. b. anthonyi* and *sandiegensis*. Significant morphological differentiation was detected by Rea and Weaver (1990) between cactus wrens occupying coastal San Diego County and southern Orange County versus those found throughout the rest of their extensive range in the US and Mexico, leading to the designation of a unique *sandiegensis* subspecies in the region. Our data are not congruent with the suggestion by Rea and Weaver (1990) that a separation between subspecies exists along San Juan Creek in southern Orange County, but rather that gene flow is on-going through and beyond this area. Multiple genetic analyses here suggest cactus wrens from MCB Camp Pendleton to the northern extent of NROC's Central Reserve, 35km northward of San Juan Creek, are part of a common gene pool.

### **Future Study**

Several questions are apparent for future study. For instance, great geographic distances separate the cactus wrens in LASB, largely along the southern slopes of the San Gabriel Mountains despite low genetic differentiation (Fig. 2). This may indicate that cactus habitat, and cactus wrens, are present throughout this area. It is also possible that cactus wrens are capable of making long dispersing movements through this area. Furthermore, several areas exhibit surprising high levels of genetic structure between relatively proximate sites without obvious and extended impediments to gene flow. For instance, only narrow roadway corridors separate DBCH from aggregations clustered into LASB to the north and west, and the central cluster in OCPN to the south (Fig. 1). In contrast, several aggregations within LASB are divided by major roads and appear to have shared recent gene flow. Investigating the fine-scale constraints on cactus wren dispersal, such as through a focused radio telemetry study, would greatly help to understand the patterns of population structure reported here.

There is also known movement between several aggregations that have been designated as separate populations by the sensitive method we employed here (Waples & Gaggiotti 2006). Further study is warranted to determine the fate of dispersing cactus wrens in the face of limited available habitat. Preston and Kamada (2012) report that after cactus wren populations recovered in Orange County, for instance, more "floaters" were observed in the field on the margins of occupied territories. It is not known if these individuals are conferring gene flow via extrapair paternity or if they are failed dispersers. Floaters that fail to pair may do so in subsequent seasons (Preston & Kamada 2012); however, the delay decreases their likelihood of survival to breeding.

Developing a historical phylogeographic perspective would also help to better understand current genetic structure in the cactus wren. The methods utilized here are best for understanding contemporary levels of genetic structure, and it is difficult to determine how

historical distribution patterns may be influencing these results. Cactus wrens are thought to have colonized coastal southern California from the desert through the San Geronio Pass after the uplift of the Transverse and Peninsular Ranges. This is based upon mitochondrial evidence, which does not detect a deep phylogenetic divergence between cactus wrens in the desert and our study area (Eggert 1996; Teutimez 2012); however, questions about the directionality of colonization and expansion remain. Several other potential corridors between coastal and desert habitats exist, including Antelope Valley, the El Cajon Pass, passes through the San Jacinto Mountains, and through northern Baja. Certainly, multiple colonization events are possible, and the footprint of such events may exert some influence on contemporary genetic patterns. Analyses of gene sequence data may be able to provide further insight into the phylogeographic history of coastal cactus wrens. The extent to which desert and coastal populations currently exchange genes is also unknown. Many lower elevation passes are now largely developed or otherwise disturbed, and measureable gene flow may be unlikely. With additional samples from desert cactus wrens and additional genetic analyses, both historical and contemporary genetic connectivity can be quantified.

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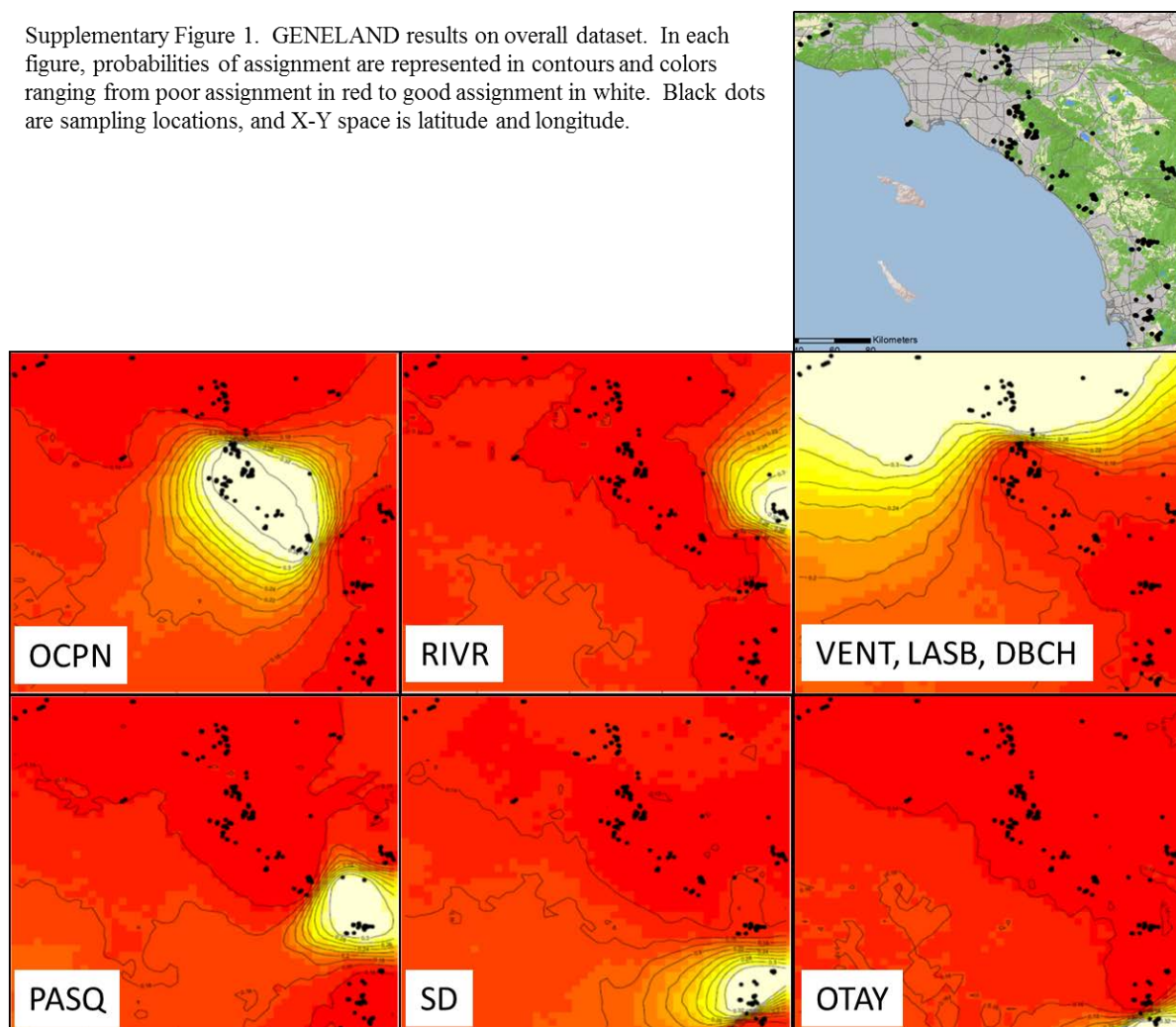
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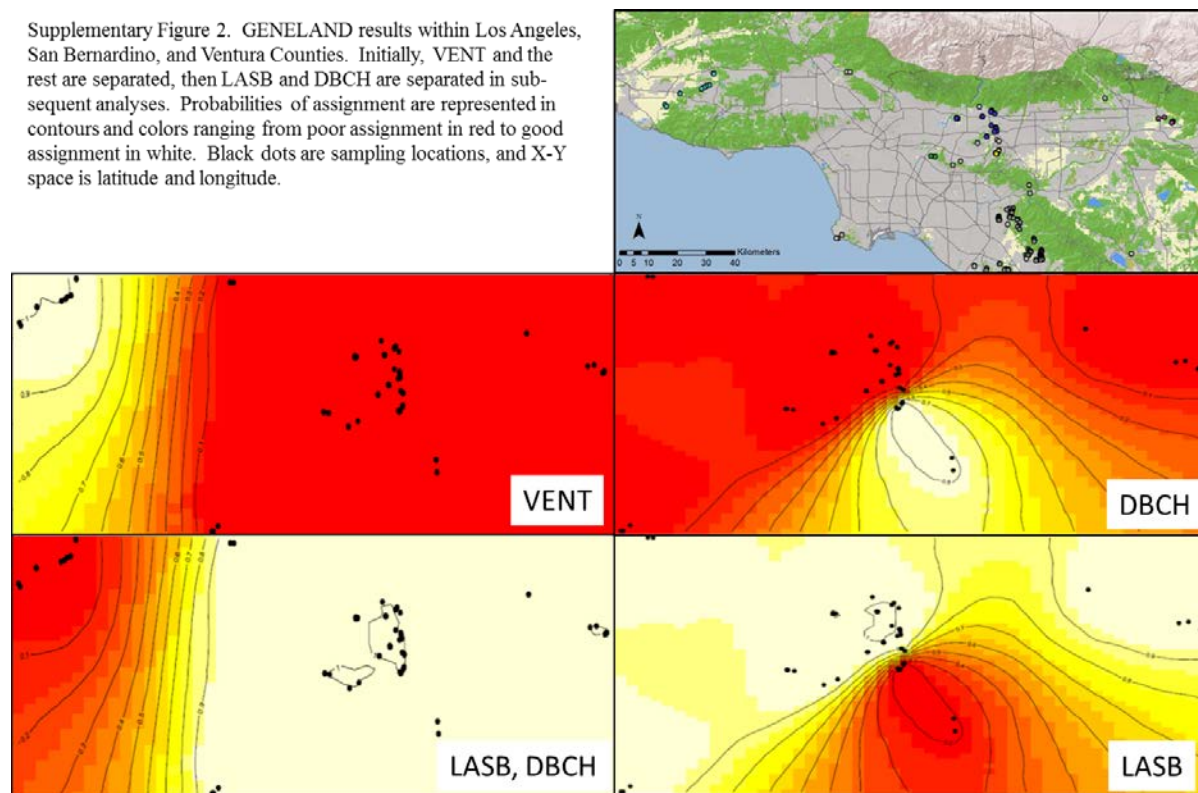
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Supplementary Figure 1. GENELAND results on overall dataset. In each figure, probabilities of assignment are represented in contours and colors ranging from poor assignment in red to good assignment in white. Black dots are sampling locations, and X-Y space is latitude and longitude.



Supplementary Figure 2. GENELAND results within Los Angeles, San Bernardino, and Ventura Counties. Initially, VENT and the rest are separated, then LASB and DBCH are separated in subsequent analyses. Probabilities of assignment are represented in contours and colors ranging from poor assignment in red to good assignment in white. Black dots are sampling locations, and X-Y space is latitude and longitude.



Supplementary Figure 3. GENELAND results from within OCPN. Probabilities of assignment are represented in contours and colors ranging from poor assignment in red to good assignment in white. Black dots are sampling locations, and X-Y space is latitude and longitude.

