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INTRODUCTION

The California gnatcatcher (*Polioptila californica*) is a resident songbird with a range extending from Ventura County, California, to the tip of the Baja peninsula, Mexico. In the U.S. and northern portion of its range in Baja California, Mexico, it nests and forages almost exclusively in native coastal sage scrub habitat (CSS), an estimated 80-90% of which has been lost since European settlement (Atwood 1993). As a consequence, the California gnatcatcher (hereafter gnatcatcher) was listed in 1993 as federally threatened under the Endangered Species Act. Since that time it has become a flagship species in southern California conservation planning, with many of the conservation related activities in this area linked directly to gnatcatchers or the CSS it inhabits (Reid and Murphy 1995). Habitat Conservation Plans, critical habitat, and other preserves have been established based on the concept that gnatcatchers required a series of large "cores" of CSS habitat for persistence and these cores can be connected via smaller CSS patches or open space linkages.

Conservation and monitoring efforts have concentrated on tracking direct loss of habitat, identifying appropriate areas for conservation, and establishing regional population trends. However, the long term persistence of gnatcatchers in a fragmented system may also depend on the extent to which aggregations function as interconnected metapopulations, linked by dispersal across the fragmented landscape. Particularly for species with small local aggregations, connectivity can be imperative for the maintenance of genetic diversity through gene flow (movement and successful reproduction), reestablishment of populations after local extinctions, and avoidance of inbreeding depression (Charlesworth and Charlesworth 1987, Frankham 2005).

We examined individual relatedness patterns and population genetic structure among gnatcatcher aggregations throughout coastal southern California from Ventura to San Diego Counties. To accomplish this goal, we developed a set of highly polymorphic microsatellite loci and sampled 268 individuals throughout the range. With genetic analyses we addressed the following questions:

1) How many genetically distinguishable populations exist across the U.S. species range?
2) Is genetic relatedness among individuals explained by the amount and distribution of suitable habitat?
3) What is the range of dispersal distances between presumptive siblings and parents/offspring?
4) What are the patterns of genetic diversity within aggregations across the U.S. range and what is the effective population size?
5) How do these results impact future management and monitoring efforts aimed at species recovery?

METHODS

Samples

We sampled gnatcatchers from throughout their range in southern California between May 2012 and September 2013. We obtained blood via toe-clipping or growing feathers from gnatcatchers captured in mistnets using song playbacks to attract birds to nets. All samples were stored in Queen's Lysis Buffer at -20^{o}C. Two muscle tissue samples were provided by the San Diego State University Museum of Biodiversity, home to the San Diego Natural History Museum's tissue collection. These muscle tissues provided sufficient quantities of genomic DNA for microsatellite library development. All extractions were performed with the DNA Tissue Extraction Kit (Qiagen), each with 20 μL of dithiothreitol added for a digestion step extended to 48 hours. DNA extractions were quantified on a Nanodrop spectrophotometer (Thermo Scientific) and diluted to a maximum of 50 ng/μL prior to amplification.

Microsatellite Library Development

A shotgun library was prepared using a Roche 454 Jr, providing 4,336 sequences with microsatellites. Of these, we screened 66 for variation using a three-primer technique (Schuelke 2000), and we found 22 of the loci were variable. We also tested loci from previously-developed libraries, finding successful cross-amplification of three loci originally developed for the cactus wren (*Campylorhynchus brunneicapillus sandiegensis*, Barr et al. 2012) and one locus for the southwest willow flycatcher (*Empidonax traillii extimus*). Genotyping runs occurred on an ABI 3730 DNA Analyzer using the GS600 size standard (Life Technologies) at Bio Applied Technologies Joint, Inc. in San Diego, CA.

Data Quality

We amplified 26 variable loci in three sets using the standard conditions of the Multiplex PCR Kit (Qiagen) with loci combined as indicated in Table 1. Approximately 10% of samples were amplified and genotyped twice to obtain an error rate. Loci were checked for stepwise mutation model (SMM) consistency using MICRO-CHECKER (Van Oosterhout et al. 2004), and exact tests for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) among loci in GENEPOP (Rousset 2008). Loci that consistently deviate from HWE may be non-neutral and loci that are physically or statistically linked do not represent independent replicate markers. Loci with inconsistent amplification or that did not consistently conform to HWE and LD expectations were eliminated from the dataset.

Identifying Populations

Multiple methods for distinguishing genetic populations were employed. First we used two Bayesian clustering algorithms to assign individuals to gene pools. Both methods use genotypic data to assign individuals to clusters that conform to theoretical expectations for randomly mating populations (maximizing Hardy-Weinberg equilibrium and linkage equilibrium).

Table 1: Information about microsatellite loci retained in analyses, including the length and motif from the original sequence, putative chromosome location (Chr), multiplex mix membership (MP), and primer sequences.

Locus	Length	Motif	Chr	MP	Forward Primer	Reverse Primer
CACW4-01	160	$(GTAT)^{6}GAATCTG(TCTA)^{11}$	1	1	TTTTGCCTAATAAACTGGCTGAC	GTTTCTTCACAGAACCACAACCTACATGG
CAGN03-13	262	$(ACT)^{7}$	3	1	AACAGTGACATACAAGAATTCAGC	GTTTCTTCAGAAACTCACAGCCAGCAC
CAGN3-15	250	$(ATC)^{15}$	5	1	TCTCCTTGGTTAGGATGCAAG	GTTTCTTCTGGATGATGATGCTTGCTG
CAGN3-35	180	(TAT)13	--	1	TTGTCTATCATTGGTCACATACCC	GTTTCTTGCACAGAGGGATTTCACAGG
CAGN3-36	291	$(ATT)^{13}$	--	1	ACAGCTCCTGGAGGAGAGAG	GTTTCTTCAAACCCTGTTTGTTAATAGTG
CAGN3-45	203	$(TTA)^{8}$	4A	1	AATCTTCTGTGGTGCCATCC	GTTTCTTAGGCCTGAGTCCGTAGCAC
CAGN4-10	129	$(AAT)^{9}$	--	1	CCGAGAGATGGACAATCCAC	GTTTCTTGGGTGCAGAGACACAAGGAG
CAGN5-03	188	$(GCACA)^{9}$	--	1	AAGAAGGAGCGGAGGACATC	GTTTCTTACGGAGGCTACACACTGCTC
CAGN5-07	263	$(ATTGG)^{11}$	--	1	GGTTGGGTTAGACTGAATTGG	GTTTCTTACCAGGTGTGAGCAGCAAC
CAGN5-09	319	$(AGAAT)^{11}$	--	1	CACCCATTCTTGTTTGATCC	GTTTCTTCAGTGATAGGAGGCATTTGG
CAGN03-12	302	$(GCT)^{6}$	3	2	GTTTGGCGAAGAGCAGGTAG	GTTTCTTCAGGCATATTGCCTTTGAGG
CAGN04-02	198	$(ACAG)^{10}$	9	2	ATCCTGCTCGAACAATCAGC	GTTTCTTACGGCCAAAGTGAGTACGG
CAGN3-39	236	$(AGG)^{10}$	--	2	ATGCCATCACTTCCCAAATC	GTTTCTTGCACTCAGCAAACAATTCAC
CAGN3-41	129	$(TGAT)^{8}$	--	2	TGAAGTCAGTGTTGAGGACCAG	GTTTCTTCATAAGCTTGACTAGATTCTCTGC
CAGN4-09	90	$(CAAT)^{9}$	--	2	CCCATCCTGCTGTGTGTG	GTTTCTTCTGGCACAAAGTTTGCACTAAAG
CAGN5-02	228	$(CAGAG)^{10}$	--	2	AACAGGTCTGTGTCCTTCCTG	GTTTCTTAGAACTTGGTGGTGCTGGAC
CAGN5-06	160	$(ATAAC)^{13}$	--	2	TTTGGGAGGTATGGGATGC	GTTTCTTACCTGCAAGCAAGAAAGCAC
CAGN5-08	205	$(AATGG)^{10}$	4	2	TGAATTTGATCCAGGGCAAG	GTTTCTTGCTATTCCCTCAGTACAGCAATG
CAGN6-02	263	$(TTATTC)^{13}$	--	2	TCCTGCAATGTCAAAGTGTTG	GTTTCTTCAATTACAATGGAATCACAAACTG

We used the Bayesian clustering program GENELAND (Guillot et al. 2005) 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 et al. 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 (MCMC) and a 20% burnin. We also used STRUCTURE (Pritchard et al. 2000) to explore genetic clusters without geographic locations included. In STRUCTURE, we used the uncorrelated alleles model with admixture, considering potential *K*s one to 10, a burnin of 100,000 MCMC steps followed by 1,000,000 additional steps, and 20 repetitions at each *K*. The top 10 highest likelihood runs at each *K* were analyzed using STRUCTURE HARVESTER (Earl 2012), which averages and graphs likelihoods across runs. We used CLUMPP (Jakobsson and Rosenberg 2007) to average results across runs, and these results were visualized in DISTRUCT (Rosenberg 2004).

As an additional means of defining genetically differentiated populations we used the method of Waples and Gaggiotti (2006). This method can be more sensitive at detecting fine scale structure than clustering algorithms. However, it can also be more sensitive to sampling gaps. Following Waples and Gaggiotti (2006), we combined geographically aggregated samples and conducted an exact test for pairwise genetic differentiation between them 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 if the overall *p*-value for the exact test was greater than 0.01. We used a minimum sample size of five to define initial aggregations for this analysis.

Individual-based Genetic Distances and Inferred Dispersal Distances

We examined the genetic distances among individuals in relation to the geographic distances and suitable habitat between them to determine how these features influence genetic structure. For these "Isolation By Distance" analyses, we calculated the genetic distance between individuals (Dps) as –ln(proportion of shared alleles) in MSA (Dieringer and Schlötterer 2003). We compared pairwise Dps to three log transformed geographic distances: 1) Euclidean distance between pairs of individuals, 2) least cost path distances through suitable habitat, and 3) weighted cost distances. Comparisons were made using Mantel tests for matrix correlations in the program IBDWS (Jensen et al. 2005). To calculate least cost paths and cost distances, a friction surface was created from a draft gnatcatcher habitat model (K. Preston, unpubl. data]. Using a threshold suitability of 0.33, we coded 150m grid cells of suitable habitat (suitability score above 0.33) with a cost value of 1, and low suitability habitat (suitability score below 0.33) with a cost value of 100 and calculated the least cost paths through this friction surface in ArcGIS 10.2.2. Because calculating least cost paths between pairs of hundreds of individuals is computationally challenging and time intensive, we restricted this analysis to San Diego County, where sampling was the most comprehensive, suitable habitat patches ranged in size and distribution, and intervening "low suitability" habitat is largely urbanized.

To estimate individual dispersal distances, we examined the spatial arrangement of genetically detected siblings or parents and offspring. We used the program COLONY (Jones and Wang 2010) to identify putative sibship pairs within our dataset based on genetic similarity. For COLONY analyses, we assumed an inbreeding model and a polygamous mating system, and

coded all individuals as offspring. We mapped the collection locations of identified sibship pairs and measured the Euclidean distance between them to estimate the scale of recent dispersal.

Genetic Diversity Patterns

We examined patterns of genetic diversity and relatedness within 16 regional aggregations of gnatcatchers, to determine how these might vary across the distribution and with local habitat availability. For regional aggregations, we calculated observed and expected heterozygosity and fixation in GENALEX, and allelic richness in HP-RARE (Kalinowski 2005). Heterozygosity is the percentage of loci that have two different alleles, and H_e is calculated to account for sampling variance. F is the fixation index. Values close to zero are expected under random mating, while substantial positive values can indicate inbreeding or undetected null alleles and negative values indicate heterozygote excess. Allelic richness (A_r) is the total number of alleles rarefied to the smallest sample size. Average pairwise relatedness among individuals within aggregations (r, Queller and Goodnight 1989) was calculated in GENALEX. We examined correlations between these diversity and relatedness indices and geographic location (latitude), sample size within aggregations (N) and percent suitable habitat within aggregations. To calculate percent suitable habitat, we created polygons surrounding collection points within each aggregation and calculated the total area and suitable habitat area within each. We used the percent suitable habitat rather than the habitat area because aggregation polygons varied in size due to sampling differences.

Finally we estimated the effective population sizes for populations and genetic clusters. N_e reflects the rate of genetic drift and inbreeding (Caballero 1994) and approximates the number of individuals that contribute equally to the next generation in an idealized population (Wright 1938). Effective population sizes were calculated in the program NeESTIMATOR 2.01 (Do et al. 2014), using the linkage disequilibrium method, assuming random mating and using a minimum allele frequency of 0.01. Confidence intervals (95%) were obtained by jackknifing over loci.

RESULTS AND DISCUSSION

Data Quality

We genotyped a total of 268 gnatcatchers sampled throughout their U.S. range (Figure 1). Seven of the 26 loci were dropped from analyses because of amplification inconsistencies or for not exhibiting Hardy-Weinberg Equilibrium (HWE). In the 19 loci we used for subsequent analyses, there was <0.1% missing data, low error rate (<0.1%), and no consistent issues with HWE or linkage disequilibrium (LD).

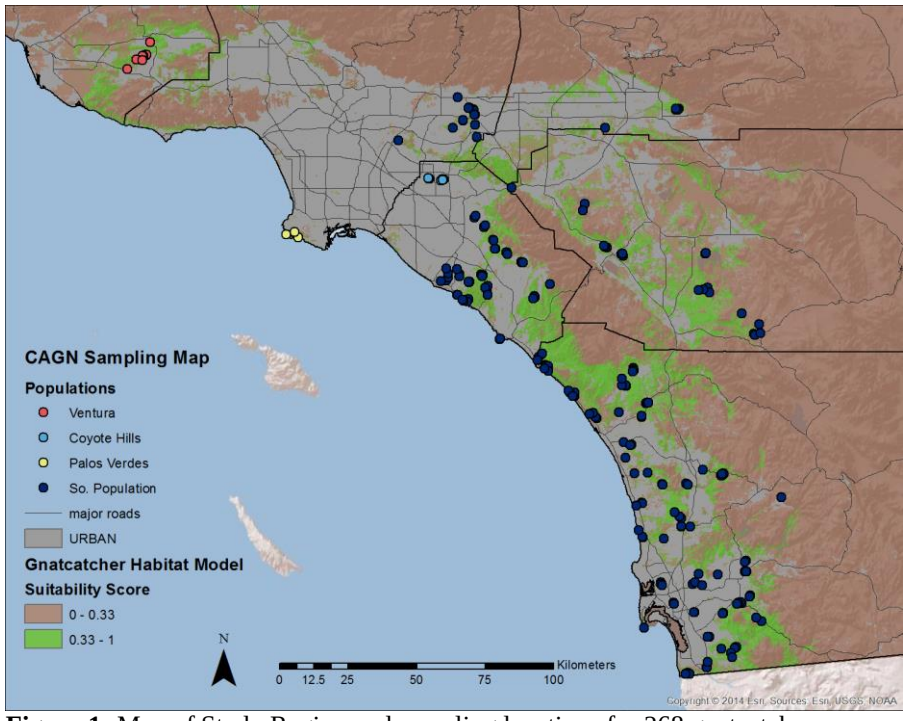

Figure 1: Map of Study Region and sampling locations for 268 gnatcatchers. Colors indicate detected population groupings. CAGN = California Gnatcatcher.

Population Structure

Both GENELAND and STRUCTURE indicated that the full range of gnatcatchers in the U.S. formed a single genetic cluster (Appendix 1). Using the Waples and Gaggiotti (2006) method, we found Palos Verdes, Ventura, and Coyote Hills comprised statistically distinguishable populations, while all other aggregations from the eastern Los Angeles Basin through southern San Diego County formed a single population (Table 2; Figure 1 colored points). Both Ventura and Palos Verdes are relatively far from other sampling locations and may be isolated by intervening urban and unsuitable habitat. The Coyote Hills population is much closer to other sampling sites, but is also surrounded by urban development. These factors may contribute to the emerging population structure detected here.

Table 2: Fisher's Exact Chi-Squared Tests for population differentiation among the four genetically distinguishable populations. These populations were defined using sequential Exact Chi-Squared Tests following Waples and Gaggiotti (2006).

Pop1	Pop2	Chi^2	df	p-value
Ventura	Palos Verdes	134.097	38	0.000
Ventura	Coyote Hills	140.011	38	0.000
Ventura	So. Population	154.122	38	0.000
Palos Verdes	Coyote Hills	66.494	38	0.003
Palos Verdes	So. Population	96.299	38	0.000
Coyote Hills	So. Population	107.449	38	0.000

Individual Genetic Distance, Habitat and Dispersal

Least cost paths generally followed suitable habitat (Figure 2A). There was no relationship between genetic differentiation among individuals and the Euclidean distance among them ($r = -0.0009$, $p = 0.508$; Figure 2B). Although correlation coefficients were still very low, genetic differentiation was significantly positively correlated with both least cost path distances and the cost weighted distances through suitable habitat (LCP $r = 0.0490$, $p = 0.008$; CD $r = 0.0431$, $p = 0.026$; Figure 2C, D). The slightly better "fit" of habitat weighted distances to genetic differentiation suggests that movement and gene flow may more often occur through suitable habitat corridors rather than across urban areas, however these relationships are weak. Previous studies have also characterized gnatcatchers as fragmentation sensitive, and that pairs are less likely to be found in very small fragments in urban areas (Crooks et al. 2004). However, a recent regional analysis of gnatcatcher occupancy found occurrence to be unrelated to patch size (Winchell and Doherty 2008).

Among the 268 individuals analyzed, sibship analysis detected 14 putative full sib pairs within our dataset (Appendix 2). Thirteen of these were detected with probabilities of 1, and one with a lower probability of 0.428. The geographic distances between these individuals averaged 28 km (median 5.2 km) and ranged from 142 m to 159 km. While the majority of intra pair distances were under 10 km, four pairs (28%) were found greater than 30 km apart, and two were over 100 km apart (Figure 3). Although the number of sibship pairs detected in our data set is relatively small, these results suggest that long distance dispersal events are possible and may even be fairly common.

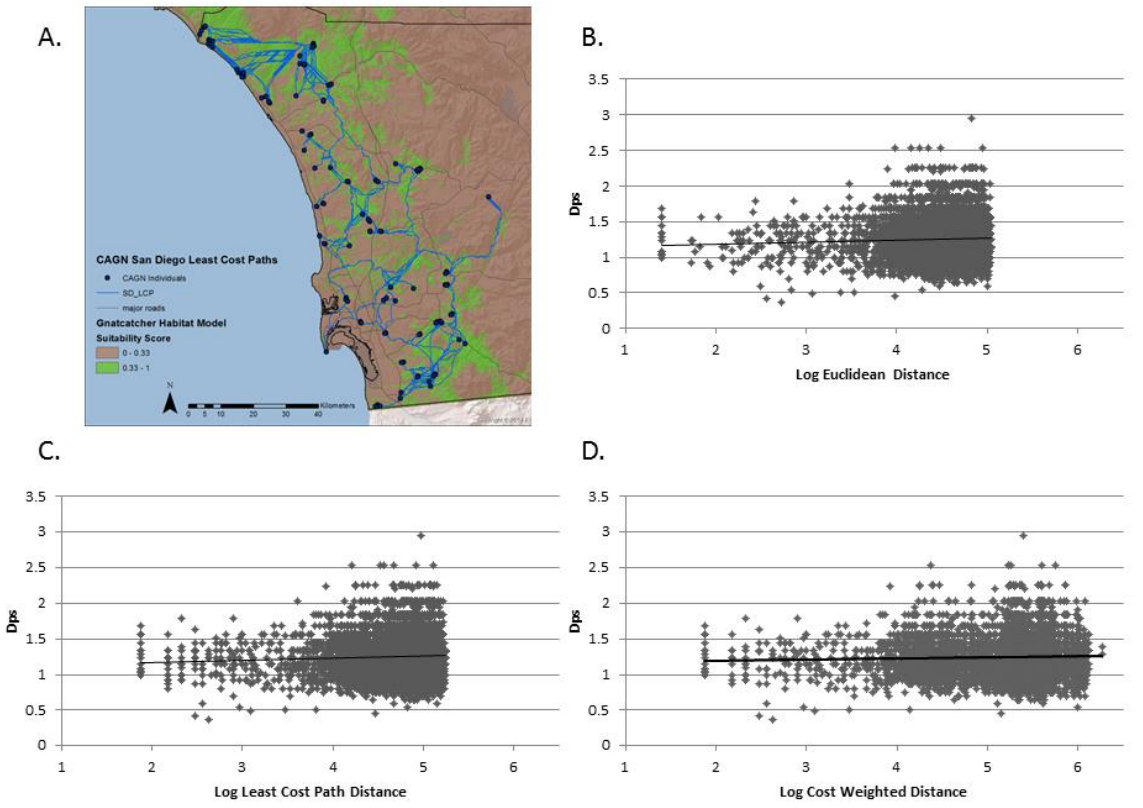

Figure 2: A) Map of least cost paths among San Diego County gnatcatchers, and isolation by distance plots between genetic distance (Dps) and B) Euclidean distances, C) least cost path distances and D) cost-weighted distances.

Genetic Diversity and Effective Population Size

We detected variable genetic diversity levels across the range, with the highest in the southernmost portion of the study area and the lowest in the north (Table 3). On average, we found gnatcatchers were most closely related in the northernmost portion of the study area and least related in the south. Significant correlations were detected between latitude H_e ($r = -0.58$, $p < 0.01$), A_r ($r = -0.66$, $p < 0.003$), and r ($r = 0.66$, $p < 0.003$) as well as between sample size and H_e ($r = 0.67$ $p < 0.005$) and A_r ($r = 0.51$, $r < 0.025$), but not sample size and r ($r = -0.37$, $p = 0.10$). Sample size and latitude were not strongly correlated. The percent of suitable habitat within aggregations was not strongly correlated to genetic diversity or relatedness.

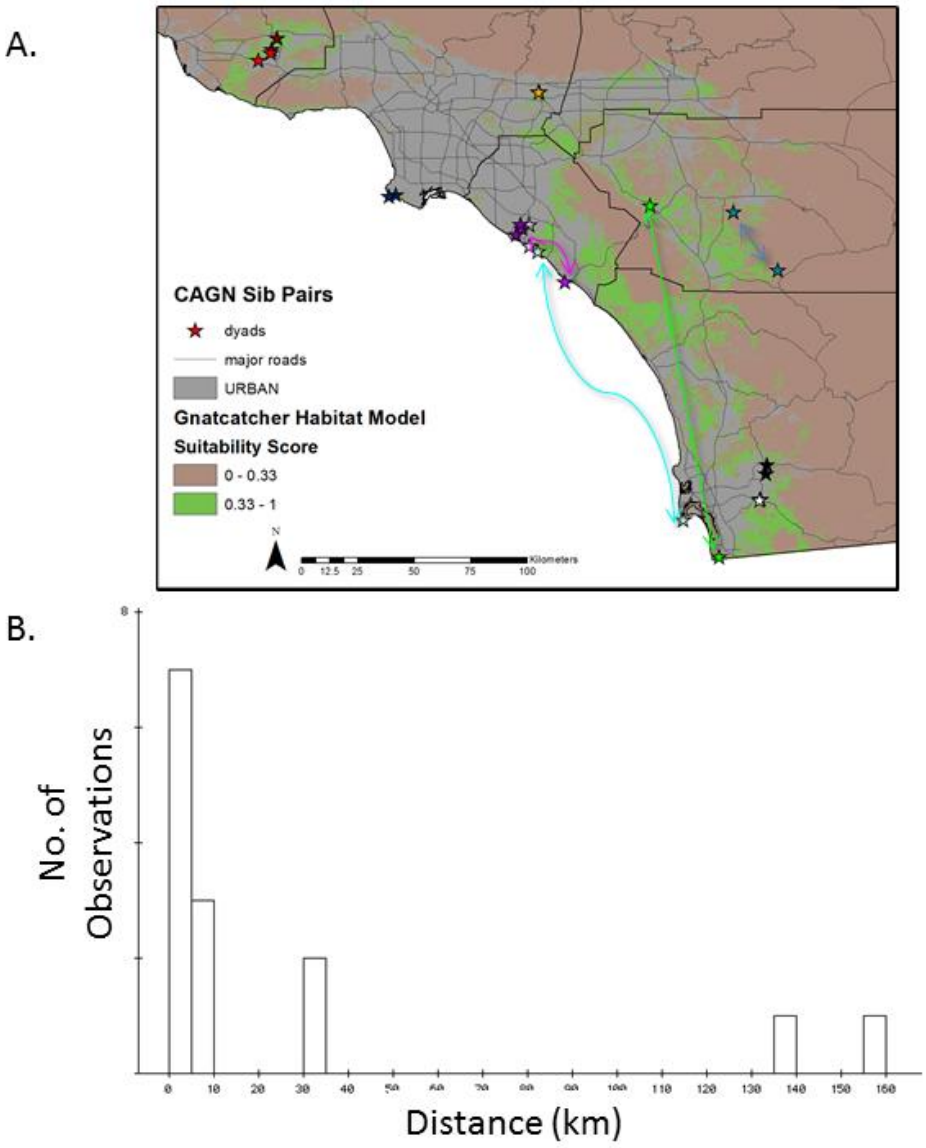

Figure 3: A) Detected sib pairs throughout the study area. Colored arrows connect long-distance pairs. B) Histogram of distances between sib pairs.

Table 3: Genetic diversity indices (H_o, H_e, A_r), inbreeding coefficients (F), relatedness (r) and effective population sizes (N_e) for the entire genetic cluster, populations, and regional aggregations.

	N	H_o	H_e	F	r	A_r	N_e
Entire Cluster	268	0.733	0.785	0.068	-0.004	4.99	1025.8 (669.8 - 2049)
Populations							
Ventura	10	0.747	0.684	-0.101	0.153	4.13	26.2 (17.1 - 49.8)
Palos Verdes	5	0.705	0.655	-0.089	0.130	4.05	24.2 (10 - inf)
Coyote Hills	10	0.768	0.713	-0.076	0.081	4.5	45.8 (25.7 - 153.9)
Southern Population	243	0.731	0.786	0.071	-0.005	4.99	1091.7 (706.8 - 2251.6)
Regional Aggregations Within Southern Population							
San Dimas - Chino Hills	22	0.686	0.722	0.043	0.078	4.6	
Redlands	5	0.653	0.654	-0.006	0.078	4.37	
Western Riverside	23	0.767	0.767	-0.003	0.013	4.92	
Santa Ana Mountains	27	0.733	0.743	0.020	0.049	4.79	
San Joaquin Hills	36	0.751	0.758	0.008	0.033	4.7	
South Camp Pendleton	35	0.746	0.779	0.047	-0.006	4.97	
Northwest San Diego	11	0.766	0.764	-0.005	-0.013	4.9	
San Pasqual	10	0.692	0.733	0.073	0.024	4.74	
Cardiff - Los Penasquitos	16	0.712	0.742	0.033	0.032	4.88	
El Cajon	12	0.665	0.738	0.118	0.035	4.61	
SD City	8	0.731	0.741	0.012	-0.013	4.87	
Sweetwater	16	0.742	0.758	0.020	0.022	4.93	
Otay - Jamul	22	0.733	0.771	0.050	0.002	5.02	

Patterns of lower diversity at range edges are common among species and expected when habitat is not contiguous (Richmond et al. 2013, Richmond et al. 2014). Lower diversity along the northern range edge in California gnatcatchers may also partially reflect smaller and more geographically isolated aggregations found at more northern latitudes (Ventura, Palos Verdes and Coyote Hills). These "leading" range edge populations may be important in allowing for future range shifts in response to climate change. There is some evidence that gnatcatcher populations have expanded northward and inland over the past decade (USFWS unpubl. data, K. Preston unpubl. data) and species distribution modeling predicts suitable habitat north of the current range boundary. Lack of relationship between genetic diversity and available habitat within local aggregations likely reflects the panmictic population structure found throughout most of the range.

The effective population size estimates for the entire cluster as well as for the large southern population (extending from eastern Los Angeles County south to San Diego County) exceeded 1000. Current recommended thresholds for effective population sizes are >100 to avoid inbreeding depression in the short term and > 1000 to retain adaptive potential in the long term (Weeks et al. 2011, Frankham et al. 2014). The southern population greatly exceeds the lower

threshold, and may meet or exceed the upper threshold. Point estimates of effective population size were much lower for the three smaller populations: Ventura (26), Palos Verdes (24) and Coyote Hills (46). While confidence intervals around N_e estimates are large for both Palos Verdes and Coyote Hills, the upper confidence interval for Ventura fell below the lower recommended threshold of $N_e > 50$. These small effective population sizes could be bolstered by increasing connectivity with the large southern population, or through increasing local abundances.

Implications for Management and Recovery

We recovered genetic signatures that are consistent with high connectivity and gene flow among most aggregations of gnatcatchers and these likely represent a single genetically connected population. These results suggest that gnatcatchers are able to move among most patches of suitable habitat given the current (or recent) spatial arrangement of CSS habitat in southern California and their intrinsic movement and dispersal behavior. As a consequence gnatcatchers form a linked metapopulation with a large effective population size over most of the southern California range. The possible exceptions to this are the outlying aggregations in Ventura and Palos Verdes and the geographically closer, but isolated aggregation in Coyote Hills. These three sites form smaller, genetically distinguishable and less diverse populations. The distribution of sib pairs throughout the region also supports this structure. Putative sibling or parent/offspring pairs were found up to 160 km apart between sites south of the Los Angeles basin. While two sib pairs were detected in Ventura and one in Palos Verdes, in all three of these cases both members of the pairs were found locally (within the same aggregation), and within 6 km of one another.

Major threats to gnatcatcher persistence in southern California include continued habitat loss associated with urban development, wildfire, climate change and drought. Given our genetic results, we anticipate that within the current southern population footprint, gnatcatchers should be able to recolonize previously occupied habitat in recently burned areas once habitat has recovered sufficiently. However, should future land use and climate change act to further reduce and fragment habitat, we would also predict concomitant reductions in genetic connectivity and local genetic diversity over time. Population monitoring and trend analysis should continue and may benefit from regional coordination across San Diego, Orange, Western Riverside, San Bernardino and Los Angeles Counties, as birds in these regions form a single genetic unit. Longer distance dispersal events and hence, regional connectivity, may also be driven by occupancy and densities in individual aggregations. Adults have been observed repeatedly chasing juveniles from occupied territories (K. Preston pers. obs.) and so we speculate that juveniles may be more apt to make longer distance flights when there are no open territories locally.

Future research efforts should also focus on increased genetic sampling and/or banding/resighting between Ventura and aggregations south and east of the Los Angeles Basin to better determine the connectivity of these regions. If limited connectivity is supported, then augmentation could be considered to boost genetic diversity at the leading range edge. Climate modeling of gnatcatcher habitat range-wide (sensu Preston et al. 2008) may help determine the importance of these more isolated northern sites to future persistence, range shifts and adaptation.

Finally, the patterns of genetic structure reported here for the California gnatcatcher differ substantially from those found in the coastal cactus wren, which was characterized by many distinct genetic clusters and populations, relatively small effective population sizes, and low genetic diversity in small populations (Barr et al. 2013, Barr et al. in review). While both of these songbirds inhabit scrub habitat in southern California, these contrasting results caution against generalizing from one species to the other even when they share the same habitat type.

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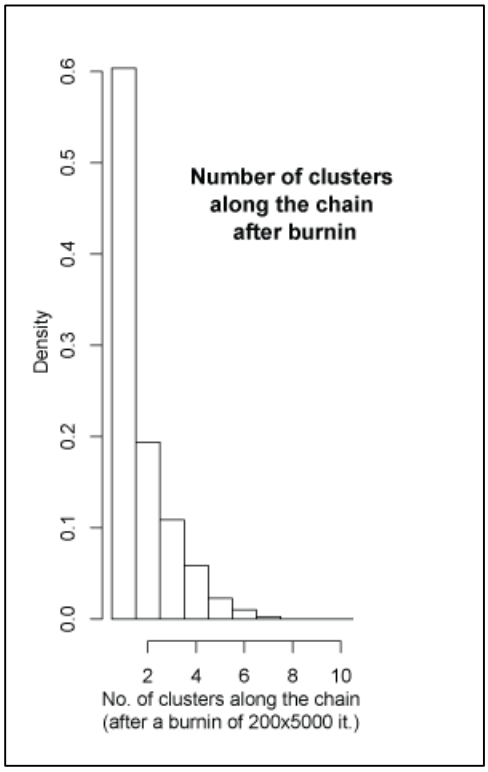
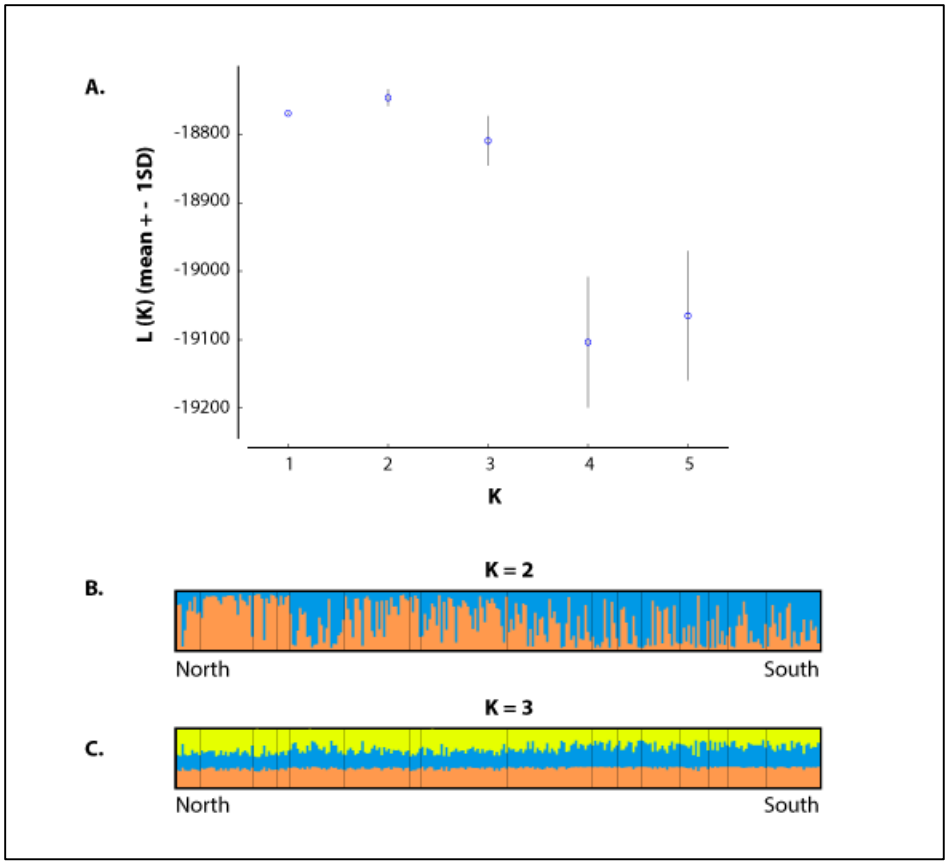
Appendix 1: Results of Bayesian clustering analyses.



Geneland Results. Number of clusters returned along the MCMC chain after burnin. A single cluster was most often recovered.



Structure Results. A) Plot of likelihood scores averaged across the top 10 runs at each K value. Highest likelihood scores were returned at K = 1, 2 or 3 clusters. B) Individual assignment plot for K of 2 arranged from North to South. Individuals of mixed assignment were found throughout the range and there was little association between geographic location and assignment. C) Individual assignment plot for K = 3 arranged from North to South. All individuals show mixed assignment with no geographic association.

Appendix 2: Full sibship dyads detected with the program COLONY, including the probability of assignment, the Euclidean geographic distance between captures, band or identification number, capture date, age, sex and geographic coordinates.

Dyad Pair	Probability	Distance (km)	Offspring ID	Capture Date	Age	Sex	Latitude	Longitude
1	1	6.555	267017521	9/6/2012	1	M	34.1962	-118.93392
			267017520	9/6/2012	1	M	34.22122	-118.8834
2	1	5.462	267017516	9/5/2012	1	M	34.23299	-118.87979
			267017518	9/5/2012	1	M	34.26959	-118.85864
3	1	0.382	233051499	8/23/2012	1	F	34.09212	-117.81593
			233051497	8/23/2012	1	F	34.09365	-117.81303
4	1	0.142	268016158	8/23/2013	1	M	33.8943	-117.90589
			268016157	8/23/2013	2	F	33.89482	-117.90477
5	1	1.022	267026824	5/8/2013	1	M	34.08891	-117.13621
			88888	5/8/2013	4	U	34.08917	-117.12703
6	1	158.835	268015427	8/19/2013	1	M	33.71634	-117.37175
			267017527	10/1/2012	1	F	32.54367	-117.09811
7	1	32.524	267026860	8/29/2013	1	M	33.69466	-117.04104
			230059917	9/26/2012	2	F	33.50161	-116.86315
9	1	2.974	267026809	7/30/2012	2	U	33.74812	-118.41255
			267017670	8/5/2012	2	M	33.75194	-118.38623
10	1	4.940	268016131	8/6/2013	1	M	33.61978	-117.90627
			268016130	8/6/2013	1	M	33.65354	-117.88822
11	1	30.600	268016129	8/6/2013	1	M	33.64187	-117.88282
			267017539	10/19/2012	2	M	33.46236	-117.71203
12	1	9.297	267017536	10/4/2012	1	M	33.65238	-117.85416
			268016144	8/13/2013	1	M	33.58283	-117.85405
13	0.428	135.154	268015425	8/13/2013	1	M	33.5641	-117.8205
			268015401	7/23/2013	1	U	32.6678	-117.23968
15	1	4.031	230059933	10/23/2012	1	M	33.57982	-117.59933
			230059931	10/23/2012	1	M	32.85316	-116.90682
16	1	0.625	267017675	8/23/2012	1	M	32.73458	-116.93666
			267017674	8/23/2012	1	F	32.73775	-116.93249

Appendix 3:

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California State University, Channel Islands
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City of Chula Vista
City of Diamond Bar
City of El Cajon
City of Escondido
City of Fullerton
City of Glendora
City of Irvine
City of Moorpark
City of Oceanside
City of San Diego
City of San Dimas
City of Thousand Oaks
City of Whittier
Conejo Open Space Conservation Authority
Conejo Recreation and Parks District
Cooper Ecological Monitoring, Inc.
County of Los Angeles, Dept. of Parks and Recreation
County of Riverside, Economic Development Agency
County of San Diego
Crystal Cove State Park
Fallbrook Naval Weapons Station
Irvine Ranch Conservancy
Many Private Landowners
Marine Corps Base Camp Pendleton
Metropolitan Water District
Nature Reserve of Orange County
North Etiwanda Preserve
Orange County Water District
Orange County Parks
Palos Verdes Peninsula Land Conservancy
Puente Hills Habitat Preservation Authority
Riverside County Habitat Conservation Authority
Riverside County Parks
San Bernardino County Flood Control District
San Bernardino County Water Conservation District
San Bernardino Valley Municipal Water District

San Diego Monitoring and Management Program
San Diego National Wildlife Refuge
San Diego Zoo Institute for Conservation Research
San Dieguito River Park
San Dieguito River Valley Conservancy
Santa Ana Watershed Association
Sweetwater Authority
UC Irvine Ecological Preserve
US Fish and Wildlife Service
Western Riverside County MSHCP
Western Riverside County Regional Conservation Authority
Western Foundation for Vertebrate Zoology