Historical Population Structure and Genetic Diversity in the Cactus Wren in Coastal Southern California

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INTRODUCTION

Museum specimens can be an invaluable source of materials for genetic studies, from molecular systematics based upon mitochondrial DNA (Johnson & Sorenson 1998, Cooper *et al*. 2001, Payne & Sorenson 2002) to population genetic surveys using suites of polymorphic markers (Athrey *et al*. 2011, Athrey *et al*. 2012). Utilizing historically collected samples affords numerous advantages, including directly detecting lost genetic diversity (Bouzat *et al*. 1998), exploring genetic structure in historical ranges by including extirpated populations (Leonard *et al*. 2005), and identifying "cryptic" invasions of non-native genotypes (Saltonstall 2002). Of particular interest for conservation is the possibility of comparing historical structure and genetic diversity patterns to those of contemporary populations. Many museum collections were created prior to major human-induced land use changes (Wandeler *et al*. 2007), particularly in the United States; hence specimens were often collected prior to the extensive habitat loss that accompanied urban development over the past century.

Coastal southern California in particular has recently experienced explosive human population growth, resulting in severe habitat fragmentation and subsequent population declines in many species. One such species, the cactus wren (*Camphylorynchus brunneicapillus*), was shown to be acutely sensitive to habitat fragmentation in recent population genetic surveys (Barr *et al*. 2012, 2013). With genetic structure essentially mirroring underlying landscape patterns and several local populations indicating both recent and long term losses in genetic diversity, the cactus wren has evidently not fared well over the previous century in coastal southern California. Little is known, however, about just how large and connected cactus wren populations were prior to the recent urbanization of much of their habitat in the area.

In this study, we mined museum collections for genetic material collected prior to widespread urban development over the second half of the 20th century in coastal southern California. We analyzed population structure and genetic diversity in these historical populations of cactus wrens using a suite of microsatellites previously developed for the species (Barr *et al*. 2012). Old and degraded sources of DNA can be difficult to amplify and present high levels of allelic dropout and null alleles, both of which can confound genetic structure analyses.

With a large number of available loci, we were able to rigorously limit analyses to only those with reliable and consistent amplification. We also utilized analyses that explicitly account for the presence of allelic dropout. With these data, we assessed historical population structure and genetic diversity in cactus wrens in coastal southern California and compared these to contemporary patterns.

METHODS

Samples

We searched museum collections for cactus wren skins via ORNIS (ornis2.ornisnet.org), and selected museums with extensive material from southern California to contact and visit. Skins were sampled following Mundy *et al*. (1997), slicing off small toepads (~1-2 mm^2) using new scalpel blades for each bird. We stored dry toepads at cool temperatures prior to DNA extraction. All DNA extractions were conducted in a fume hood in a separate laboratory from other avian DNA-based projects. We incubated chopped toepads in 500 uL of extraction solution (10 mg/mL DTT, 1% SDS, 0.1 mg/mL proteinase K, 0.02M EDTA, 0.01M Tris-HCl, and 0.01M NaCL) at 55^{o} C for 5 days and regularly agitated these by hand. After incubation, DNA was purified using phenol-chloroform extractions and Millipore Amicon Ultra 30K centrifugal filters. We further purified these products using an ethanol-sodium acetate precipitation. All disposables and pipettes used in DNA extractions were decontaminated with a CL-1000 UV Crosslinker (UVP) prior to all extractions. Blanks (negative controls) were included during each extraction session to allow testing for cross contamination downstream.

Data quality

Anticipating degraded genomic DNA, we focused on loci that were <200 basepairs and hence more likely to amplify. In our original cactus wren microsatellite library, six loci were already of an appropriate size. We also redesigned primers to amplify smaller products for an additional 11 loci (Table S1). These 17 loci were amplified using the Multiplex PCR Kit (Qiagen) with 10 µL reactions with bovine serum albumin (BSA) as an additive. We assessed data quality at several steps to focus genotyping efforts on higher quality extractions and more reliable loci. In the first step, all samples were amplified across all loci. After eliminating loci with spurious amplification patterns (e.g., slippage) and samples that did not provide any data across loci, we genotyped the remaining dataset twice more with 20 µL reactions. Individuals that genotyped as homozygous in the first run but heterozygous in the later, larger reactions were assumed to be heterozygous for analytical purposes. The larger PCRs resulted in larger amounts of product, hence we assumed these were amplifying alleles that were not detected using smaller reactions. In the few instances of discrepancies in alleles between repeated genotypes (e.g., alleles of differing sizes were amplified in different runs) genotypes were discarded and coded as missing. Negative controls were checked on electrophoresis gels.

Prior to analyses, we converted loci that were reduced in size to the number of repeats to make alleles match in the historical and contemporary datasets. We checked loci for allelic dropout in MICRO-CHECKER (van Oosterhout *et al*. 2004) and Hardy-Weinberg Equilibrium (HWE) in GENALEX (Peakall & Smouse 2006, 2012); but we did not test for linkage disequilibrium since these loci have already been shown to be unlinked (Barr *et al*. 2012, 2013).

Our contemporary dataset was reduced to locations overlapping those where historical samples were captured for comparative analyses.

To assess data quality and the potential impacts of missing data on analysis results, we plotted individual proportions of missing data and individual heterozygosity (proportion of heterozygous loci found within an individual) by collection year and tested for significant correlations by calculating Pearson's *r*. We also tested whether individual heterozygosity levels were significantly different in the total historical sample compared to the contemporary sample using a two sample t-test. We would expect to see significantly fewer heterozygous individuals in older samples if allelic dropout rates were high due to poor template DNA quality.

Identifying population structure

Geographic localities for cactus wrens in museum collections were usually too general or antiquated to assign accurate global position system (GPS) points; hence, very broad groupings were used for analyses (Figure 1). Furthermore, captures in the study area largely occurred over a 50 year period, 1885-1923. To help account for the sampling variance and genetic drift that might confound polymorphic marker datasets over numerous generations, we further divided the dataset into smaller time frames in a sliding window-type format for genetic structure analyses. For each genetic structure analysis, we analyzed samples captured 1885-1905, 1899-1910, and 1906-1923. These intervals were chosen to maximize the number of available samples (1899-1910) and to include all available data without overlapping years (1885-1905 and 1906-1923).

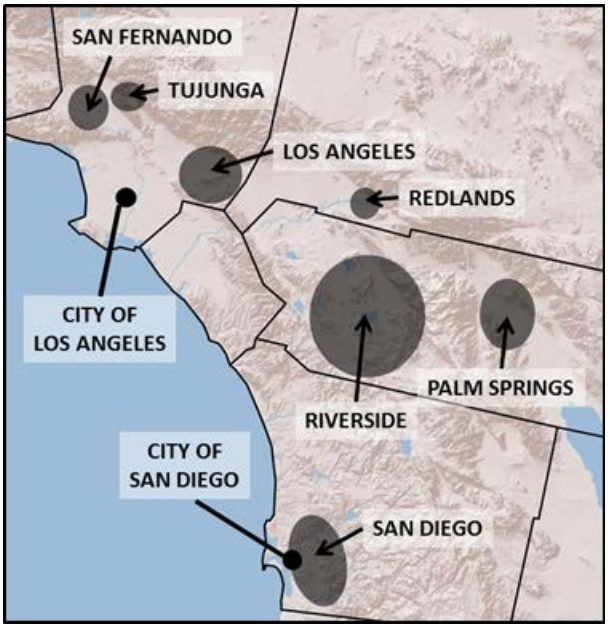


Figure 1. General locations of cactus wrens sampled in museum collections.

Pairwise Weir and Cockerham's (1984) F_{ST} and Cavalli-Sforza and Edwards (1967) distance (D_c), two measures of genetic differentiation, were calculated between groupings in FreeNA (Chapuis & Estoup 2007), both with and without a correction for null alleles. F_{ST} and D_c both scale 0 to 1, with higher numbers representing great levels of genetic differentiation. While F_{ST} accounts for allele frequency differences, D_c also takes into account the number of mutational steps between alleles. The measures are differentially affected by missing data and null alleles. Calculating both can help to interpret results. We tested for associations between geographic distance and genetic distance, or isolation by distance (IBD), using a Mantel test as implemented in IBDWS (Jensen *et al*. 2005) with 1,000 randomizations to test for significance. For these tests for IBD, we used F_{ST}s corrected for null alleles for the historical samples, but used the uncorrected calculation for the

contemporary samples. Since there is no evidence for allelic dropout in the contemporary dataset, there is no reason to use the corrected calculation. IBD can be related to dispersal patterns and levels of genetic isolation. In instances of significant IBD, it might be assumed that stepping-stone gene flow is occurring between populations, for instance. A lack of an association between genetic and geographic distances, on the other hand, might be the product of many generations of isolation, with little or no gene flow occurring. We also implemented the Bayesian clustering analyses STRUCTURE (Pritchard *et al*. 2000, Falush *et al*. 2003, Hubisz *et al*. 2009) and GENELAND (Guillot *et al*. 2005, Guillot *et al*. 2008). Analyses in STRUCTURE were conducted assuming a correlated alleles model with admixture and a location prior based upon the general capture area (Figure 1). In each analysis, we ran 1M Markov chain Monte Carlo (MCMC) steps after a 500,000 step burn-in for potential numbers of clusters (*K*) 1 – 10 with five repetitions at each *K*. These results were summarized using STRUCTURE HARVESTOR (Earl & van Holdt 2012). In GENELAND, analyses were conducted using the uncorrelated, null alleles model (a feature unique to this program) and testing for *K*s 1 - 10 with 2M MCMC steps and a 20% burn-in. Since individual GPS points were not available for historical samples, we used a very broad spatial uncertainly of 1×10^6 meters. Both of these programs use differing algorithms to detect "clusters" of individuals based upon Hardy-Weinberg Equilibrium (HWE), the product of sharing gene flow over time, while maximizing linkage equilibrium, the product of recent gene flow.

Genetic diversity

We calculated expected heterozygosity (H_E) in GENALEX, allelic richness (*A*) in HP-RARE (Kalinowski 2005), and effective population size (N_e) using the linkage disequilibrium method (Waples & Do 2010) in NeEstimator (Do *et al*. 2013). Heterozygosity is the percentage of loci that have two different alleles, and H_E is calculated to account for sampling variance. *A* is the total number of alleles scaled to account for sample size. Populations with higher H_E and *A* have higher levels of genetic diversity. N_e reflects the rate of genetic drift and inbreeding (Caballero 1994) and approximates the number of individuals that contribute equally to offspring in an idealized population (Wright 1938). Based upon genetic structure results, we pooled sites to boost sample size for an overall historical versus contemporary comparison in genetic diversities in the forms of both H_E and *A,* and tested for significance using a paired t-test. We did not make the comparisons in N_e since pooling samples over numerous generations would severely violate a basic assumption of the linkage disequilibrium method (Waples & England 2011).

RESULTS

Data quality

We genotyped a total of 225 cactus wrens over 17 loci (Table S1); however, analyses here are limited to 111 total individuals genotyped at 11 loci. There was no evidence for cross contamination as we observed neither bands from PCRs on negative controls on electrophoresis gels nor extra alleles in genotypes. Many individuals were dropped due to missing data at four or more loci after three amplification attempts. Locations with less than four individuals were also dropped since these do not provide enough information to warrant inclusion in analyses. Six loci could not be amplified consistently in the historical samples. The historical datasets exhibited much higher levels of missing data and allelic dropout than the

contemporary dataset (Table 1).

Table 1. Amplification success measures for loci across each time frame analyzed. Loci not used for genetic structure analyses because of high levels of missing data are *italicized* and shaded in dark grey. All loci were used for genetic diversity analyses. H_E = expected heterozygosity, Miss. = percentage of missing data, ADO = percentage of collection locations exhibiting significant allelic dropout as determined in MICRO-CHECKER

TIME	1885-1905			1899-1910			1906-1923			Contemporary		
LOCUS	H_E	Miss.	ADO	H_E	Miss.	ADO	H_E	Miss.	ADO	H_E	Miss.	ADO
CACW3-08	0.33	0	0	0.29	0	0	0.34	0	0	0.25	0	0
CACW3-02	0.57	0.20	0.40	0.51	0.20	0.50	0.52	0.15	0.50	0.60	0	0
CACW3-04	0.19	0.02	0	0.16	0.09	0	0.15	0.10	0	0.23	0	0
CACW3-09	0.42	0.14	0.20	0.44	0.13	0	0.45	0.10	0	0.36	0	0
CACW4-05	*0.64*	*0.56*	*0*	*0.64*	*0.54*	*0*	*0.64*	*0.46*	*0*	*0.73*	*0*	*0*
CACW3-05	0.50	0.02	0.80	0.56	0.01	0.67	0.48	0.03	0.75	0.58	0	0
CACW3-07	0.47	0	0	0.54	0	0	0.63	0.05	0	0.58	0	0
CACW4-13	0.50	0.03	0	0.48	0.03	0	0.54	0	0.25	0.59	0	0
CACW4-12	0.30	0.06	0	0.44	0.06	0	0.45	0.03	0.25	0.59	0	0
CACW4-04	*0.72*	*0.26*	*0.40*	*0.73*	*0.30*	*0.33*	*0.64*	*0.31*	*0.50*	*0.70*	*0*	*0*
CACW4-01	*0.66*	*0.44*	*0.80*	*0.58*	*0.44*	*0.83*	*0.75*	*0.33*	*0.75*	*0.80*	*0*	*0*

By collection year, we found a slight negative trend in amount of missing data per individual (Figure S1) and increasing average individual heterozygosity (Figure S2); however, the correlations were weak in both cases (missing data: r = -0.057; heterozygosity: r = 0.08). Mean individual heterozygosity, on the other hand, was found to be significantly higher in the contemporary than historical samples (Figure S3; t = -7.786, $p < 0.0001$). By removing the three loci with the most missing data (> 20%; Table 1), this difference was diminished (Figure S4; t = -0.642, p = 0.261). We interpret this result to mean that the three loci with the most missing data were also subject to the most allelic dropout resulting in lower heterozygosity than expected. Given this, we conducted all analyses with and without these three loci. Here we report genetic structure analyses without these three loci to limit the confounding effects of such excessive missing data. We report diversity statistics including all 11 loci. For diversity comparisons, missing data are of less consequence and allelic drop out in historical samples should have an opposing impact on the expected trend (higher historical diversity), and so should not confound results.

Genetic structure

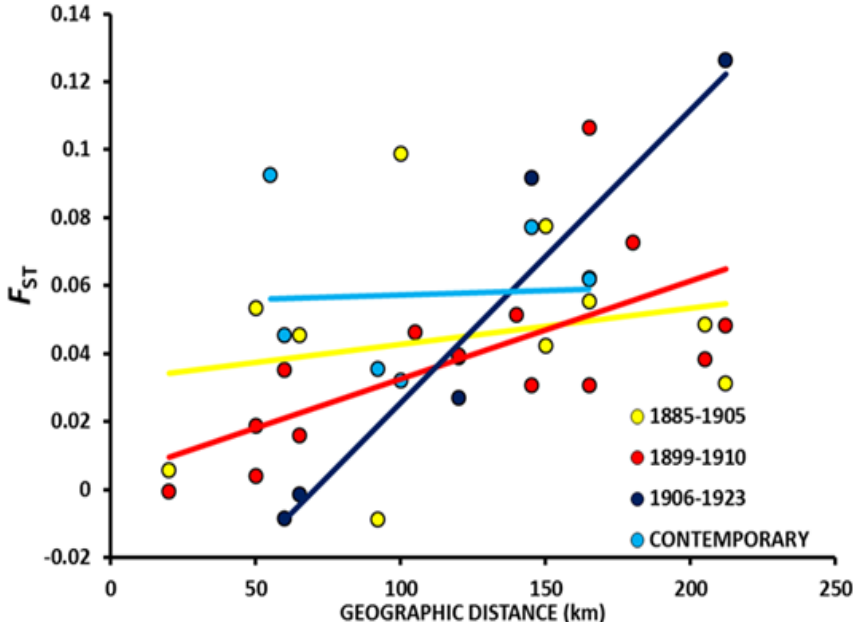
We detected similar global F_{ST}s and average D_Cs over all the datasets, historical and contemporary (Table 2). Pairwise F_{ST}s varied by time period, but were overall slightly higher in the contemporary dataset than the historical datasets (Figure 2, Tables S2-4). We detected positive associations between F_{ST} and geographic distance in each of the historical periods

Table 2. Overall diversity and differentiation by time period. N = total number of samples, $F_{ST}1$ = Weir and Cockerham's F_{ST} with confidence intervals based upon 10,000 bootstraps, $F_{ST}2$ = F_{ST} with the Chapuis & Estoup (2007) correction for null alleles and confidence intervals based upon 10,000 bootstraps, *r* = Mantel's *r* between F_{ST} and geographic distance ($F_{ST}1$ for the contemporary data and $F_{ST}2$ for the historical datasets; values significant at $p < 0.05$ are *italicized*), D_C1 = Cavalli-Sforza and Edwards distance, and D_C2 = Cavalli Sforza and Edwards distance with the Chapuis & Estoup (2007) correction for null alleles.

TIME PERIOD	N	$F_{ST}1$	$F_{ST}2$	*r*	D_C1	D_C2
1885-1905	64	0.041 (0.023 - 0.062)	0.042 (0.02 - 0.06)	0.23	0.242	0.274
1899-1910	80	0.024 (0.019 - 0.033)	0.03 (0.02 - 0.045)	*0.66*	0.219	0.264
1906-1923	39	0.04 (- 0.01 - 0.08)	0.05 (0.003 - 0.087)	*0.95*	0.264	0.298
Contemporary	128	0.05 (0.02 - 0. 08)	--	0.08	0.271	--

analyzed that was significant in the 1899-1910 and 1906-1923 datasets but not in the 1885-1905 dataset (Table 2). The trend was positive for the contemporary dataset as well, but Mantel's *r* was far lower than among historical samples, a pattern driven by higher FSTs among very close populations in the contemporary data set. This indicates gene flow may have been on-going in a stepping-stone pattern among the historical populations; conversely, a lack of a correlation among contemporary population indicates these have been in isolation from one another for an extended period of time. The 1906-1923 dataset had a particularly strong IBD signal. It should be noted that the dataset comprises fewer samples (39) and population groupings (4) than the other time frames. The strength of the IBD signal among those samples is likely an artifact of the spatial arrangement of the populations, with San Fernando, LA, and Redlands being comparably proximate to one another and San Diego much farther away.

Figure 2. Pairwise genetic distance (F_{ST}) and geographic distance by time period with Trendlines.



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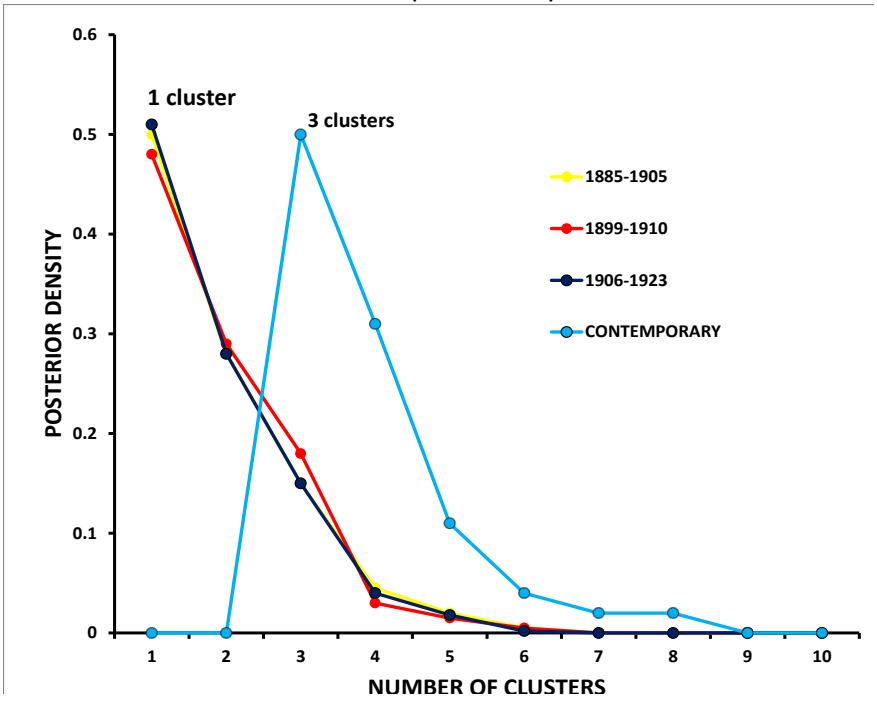
Notably, Mantel's r changes little (0.93) after log-transforming geographic distances to reduce this artifact and is still significant (p = 0.036). We did not detect population structure in any of the historical analyses using either STRUCTURE or GENELAND (Figures 3, S1); however, analyses on contemporary samples supported three clusters. These were a San Diego cluster, a Riverside cluster, and a cluster composed of Los Angeles and Redlands together. We do not display the contemporary results here since they mirror those previously reported in Barr *et al*. (2013).

Figure 3. GENELAND results for each time period analyzed here.



Genetic diversity

Based upon the genetic structure analyses, we pooled historical San Fernando and Tujunga as well as Los Angeles and Redlands for overall genetic diversity comparisons. Unfortunately, cactus wrens no longer exist in San Fernando and only two samples were available from Tujunga for the contemporary dataset; hence we could not make a comparison between historical and contemporary populations at the area. Neither A (p = 0.06) nor H_e (p = 0.30) were significantly different in contemporary samples at the three sites sampled across both time periods. However, historical populations had higher A for each of the study sites and the Los Angeles site exhibited a larger H_E than the contemporary historical versus population (Figure 4). We detected similar H_E over time for San Diego and a higher figure for contemporary samples in Riverside than the historical dataset. Estimations of Ne universally resulted in ∞ for the upper bounds of the confidence intervals and means ranged 11.9 – 50.7 among the historical datasets (Tables S2-S5).

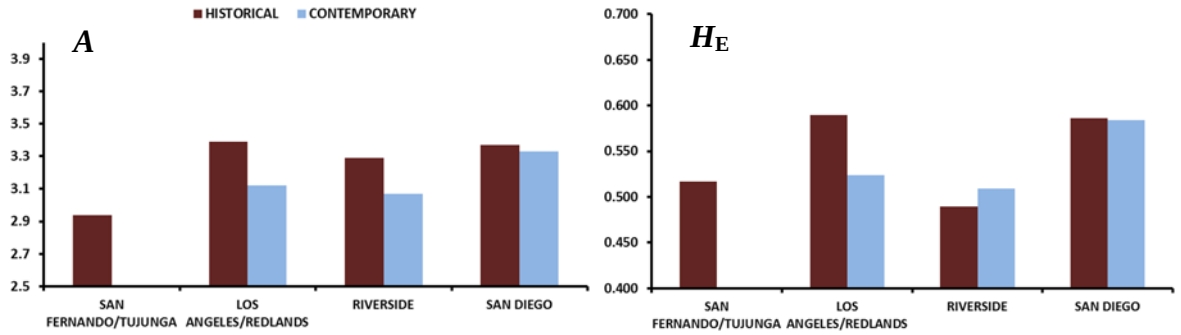


Figure 4. Genetic diversity indices allelic richness (*A*) and expected heterozygosity in historical samples versus contemporary samples. No cactus wrens currently exist in San Fernando and the two samples taken at Tujunga in Barr *et al*. 2013 were not used for this comparison.

DISCUSSION

The datasets we analyzed here generally indicate genetic structure among cactus wrens in coastal southern California is higher and genetic diversity lower today than a century ago. Previous studies on contemporary populations indicated habitat fragmentation by urbanization, agriculture, and wildfire were primary causes of these. The lack of structure among and higher genetic diversity within historical groups, prior to widespread urban and agricultural development in southern California, further supports these conclusions. The total evidence presented here and in our previous reports (Barr *et al*. 2012, 2013) supports the application of management and restoration actions aimed at improving connectivity, particularly among neighboring aggregations, to restore the potential for stepping-stone gene flow. We found no signal of historical, population structure that would indicate that aggregations have been separated over long periods of evolutionary time, and that actions taken now to increase connectivity would reverse long-standing ecological conditions.

We must interpret these results with care given the known presence of allelic dropout and high levels of missing data in some loci in the historical dataset (Table 1). Solely estimating Weir & Cockerham's (1984) F_{ST} and detecting clusters using the correlated alleles algorithm in STRUCTURE would be expected to produce unreliable results given these data collection issues. Here, we followed up on both of these *di rigor* population genetics analyses with methods that were developed specifically for datasets fraught with null alleles, the Chapuis and Estoup (2007) correction for calculating F_{ST} and a clustering algorithm for handling null alleles developed in Guillot *et al*. (2008). We also rigorously analyzed the dataset to determine where high missing data may also confound genetic structure analyses, using the contemporary dataset as a baseline since neither missing data or allelic dropout are detectable. Lower heterozygosity caused by both missing data and null alleles would inherently cause lower estimations of F_{ST} and reduce the sensitivity of clustering analyses that are inherently dependent upon the assumptions of Hardy-Weinberg Equilibrium (HWE). Using methods that account for the presence of null alleles and rigorously eliminating loci that had high levels of missing data

among historical samples, there is less genetic differentiation among historical samples (Table 2) and no evidence for genetic clusters (Figure 2, S1).

Lower information content due to amplifying a reduced set of loci and working with relatively small sample sizes collected over a broad time period may also impact our results. The central purpose of using highly polymorphic microsatellites for population genetic analyses is increasing resolution with more variable data. With a suite of diverse loci, patterns can better be detected and related to processes. Reducing the total number of loci used for analyses from 22 to 8 had little effect on the results in the contemporary datasets, with genetic structure patterns matching those in Barr *et al*. (2012) and Barr *et al*. (2013). Regardless, lower sample sizes and missing data in historical samples may simply decrease the detectability of past genetic structure despite our efforts to account for data collection issues. It should be noted, however, that genetic diversity would be predicted to be even higher in the historical samples than we detected given the levels of missing data and allelic dropout in the dataset. Intuitively, rare alleles are less likely to be detected when there are less data.

While data quality and lack of spatial resolution might limit the power of historical datasets for resolving fine-scale genetic structure, our results suggest gene flow was once on-going over coastal southern California and is more restricted now than in the past. No underlying population structure stands out in any of the historical datasets. No allelic patterns suggest that major restrictions to gene flow existed. Significant IBD in two of the historical datasets and a much higher Mantel's *r* in the third versus that of the contemporary dataset indicate gene flow was much more influential on dictating genetic structure historically (Hutchinson & Templeton 1999). Conversely, the higher levels of genetic differentiation among geographically closer population pairs resulting in small and non-significant Mantel's *r* detected in the contemporary dataset suggest the effects of genetic drift are stronger than those of gene flow for those populations. These results are not contrary to the significant IBD reported among contemporary samples in both Barr et al. 2012 and 2013. Each of those reports analyzed samples over a much finer spatial scale than we could use here for comparison with the historical samples. Indeed, IBD analyses on subsets of the data in Barr et al. 2013 also detected varying effects of genetic drift and gene flow on cactus wren population structure throughout coastal southern California (Barr *et al*. in prep.). Given these patterns, it seems that San Diego, Riverside, and Los Angeles/Redlands are presently more isolated from one another than they were a century ago.

Notably, numerous cactus wrens were present in museum collections from areas where they are now extirpated or only a few persist. For instance, the California Academy of Sciences alone has 30 cactus wren skins of individuals captured in the San Fernando where they are no longer detected today. Near Tujunga in Los Angeles County, we only detected two individuals while collecting samples for Barr *et al*. (2013), and only 2-3 territories are known to persist (D. Cooper, per. comm.); meanwhile, museums held 15 individuals captured in the area at the beginning of the 20th century. Given the sheer number of individuals collected in the early 20th century in San Fernando and Tujunga, these were likely large aggregations of cactus wrens that have declined precipitously over the past century. Though we did not detect any unique

diversity among those groups, these aggregations may have provided a connection between cactus wrens in Ventura County and coastal Los Angeles County around the Santa Monica Mountains. Today, the population in Ventura County shows evidence of isolation (Barr *et al*. 2013). San Fernando and Tujunga quite possibly was part of a connection between coastal populations and those in the desert via Antelope Valley between where Santa Clarita and Palmdale stand today. Cactus wren populations along southwestern Antelope Valley have only recently been extirpated (K. Garrett, pers. comm.). Significant IBD in the historical datasets (which is interpreted to reflect a stepping stone pattern of gene flow) and low levels of differentiation between Los Angeles, Tujunga, and San Fernando (Tables S2-4) suggests there was high connectivity among these areas. The rapid urban development of San Fernando and Tujunga in the mid-20th century was likely a major factor in the isolation of cactus wren populations in Los Angeles and Ventura.

Major extant cactus wren populations further south in the distribution were sampled very little by collectors, including the Orange County/northern San Diego County that now supports the largest and best-connected aggregations (Barr *et al*. 2012, 2013). Furthermore, limited sample availability and amplification success among samples in San Diego County provides less power for making inferences about population structure relative to other areas where cactus wrens were more frequently captured. Certainly, no evidence exists that suggest cactus wrens in San Diego were extremely different than other areas examined in the historical analyses. There is neither a preponderance of private alleles in this most southern sample site nor does it stand out as particularly more differentiated in either the historical or contemporary datasets. The lack of detail in geographic locality information also presents a hindrance for the interpretation of our results for the San Diego area. To make comparisons with historical data, we lumped together contemporary samples taken throughout the entire southwestern area of the county, from Lake Jennings, just northeast of the city of San Diego, to the Otay River area near the Mexican border. It is quite possible that collectors operated in a more reduced area, hence historical genetic diversity indices may represent much more localized signals.

A final pattern of note is the widespread allelic dropout observed among loci with NED^{TM}-labeled primers (Tables 1, S1). These loci were marked overall by very weak amplifications. It is possible that dye labels differentially affect primer annealing, an effect that would not be detected when template DNA is of high quality and concentration. Such an effect would be amplified when DNA is degraded and concentrations are low, affording fewer annealing opportunities and hence resulting in less product for genotyping. This phenomenon would be worth exploring for future researchers using old or otherwise degraded sources of DNA. They might find that NED^{TM} should be avoided.

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Table S1. Microsatellite loci and information. C = chromosome, A = number of alleles in historical samples, MP = multiplex membership.

LOCUS	A	MOTIF	RANGE	MP	DYE	FORWARD	REVERSE
CACW3-08	3	$(ACA)^{9}$	93 - 99	1	fam	GCCCAGGCTCCATCACAG	ATGTCTGCTGCTCCCTCAG
CACW3-02	4	$(ACA)^{7}(ATA)^{6}$	110 - 119	1	ned	AATGGAAAGGAGCATCAACTG	TTCATGGTGCATACAAGATAGC
CACW3-04	4	$(AAC)^{8}$	127 - 136	1	pet	CATGGATAGAGTGAGAACAATATGC	CATGAGATGGACATTATGAGCTG
CACW3-09	2	$(TTG)^{6}$	121 - 124	1	fam	AGGAAGAAATAGAGGTGAGGGAAC	TGACGACTGAACAAAAGTACGAG
CACW4-05	6	$(TTTC)^{6}$	128 - 144	1	vic	GCTCTAAAACTCTGTGGGCAAC	CGAGAACAAGATCATTAACAGCAG
CACW3-05	6	$(TGT)^{5}$	148 - 160	1	ned	GATGCATATTGTCAGAGTTCCAC	CTGGACTGAGCTAACAAATGATG
CACW3-07	5	$(AAC)^{10}$	101 113	1	pet	GCTCAAACTCCTGACCAAGG	TTGATTGAGGTAGAGAAAGTGAAA**
CACW4-13	6	$(CCAT)^{8}$	104 - 124	2	vic	GCAGAACTTGGGACTTCGAC	ACTGGGCTTGTTATGGATGG
CACW4-12	3	$(AAAC)^{8}$	112 - 120	2	fam	CCTGCCACCACTGTATTTCTG	CAGATAGCTGTGCTAACACTGAGG**
CACW4-04	6	$(TCTA)^{14}$	129 - 153	2	pet**	TGGGGAAGAAAATACTGAGGAG**	AAGCCAGGGGTGTTTTAAGG**
CACW4-01	7	$(GTAT)^{6}GAATCTG(TCTA)^{11}$	148 - 172	2	ned**	CAGTGTGACAGTGTAGCAGAGTATG**	CACAGAACCACAACCTACATGG**
CACW4-02*	--	$(TATC)^{18}$	--	--	fam**	ACGATCATCCATCTTTCTATC**	AGAAATGAATTATATAGATATAGGTGT**
CACW3-01*	--	$(ATT)^{5}G(TTA)^{4}(TTG)^{6}TTATTG\ (TTGTTA)^{3}(TCA)^{9}$	--	--	vic**	TATGACACGATTTTACTTATTATT**	GAAGAACAAACTCCATATACTACT**
CACW3-03*	--	$(CTA)^{5}CTG(CTA)^{8}(ATA)^{10}$	--	--	ned**	CATGTAATAAAATGACAACAGCAAC**	GAGGTCACCACACTAGATTGC**
CACW4-03*	--	$(AGAT)^{5}(GATA)^{14}$	--	--	vic**	ACTACACAAAAGTATATTTACTCACA**	ATCTGAAATATTTTCATCATTC**
CACW3-06*	--	$(TTG)^{6}$	--	--	ned**	TTTTTGTCTACTTTTTGTGTTTTTGG**	AAACCCACCAACCTCTTCC**

*These loci were dropped from analyses due to inconsistent amplification

**These are different primers and dyes than those reported in Barr *et al*. (2012).

Table S2. Summary of results for analyses on cactus wrens captured between 1885-1905. N = total samples analyzed, H_E = expected heterozygosity, A = allelic richness, PA = number of private alleles, N_e = effective population size from the linkage disequilibrium method, and F_{ST} = that with the Chapuis & Estoup (2007) correction for null alleles. Genetic diversity indices were calculated over all 11 loci, and F_{ST}s were limited to the 8 with the least missing data.

						Pairwise F^{ST}			
Location	N	H_E	A	PA	N_e	San Fernando	Tujunga	Los Angeles	Riverside
San Fernando	20	0.50	2.12	3	47.7 (6.4 - ∞)				
Tujunga	15	0.46	1.97	0	n/a (10.8 - ∞)	0.0059			
Los Angeles	11	0.46	1.98	3	11.9 (2.8 - ∞)	0.0456	0.0534		
Riverside	9	0.49	2.2	4	n/a (12.6 - ∞)	0.0423	0.0777	0.0989	
San Diego	9	0.49	2.13	4	n/a (10.8 - ∞)	0.0314	0.0485	0.0555	-0.0087

Table S3. Summary of results for analyses on cactus wrens captured between 1899-1910. N = total samples analyzed, H_E = expected heterozygosity, A = allelic richness, PA = number of private alleles, N_e = effective population size from the linkage disequilibrium method, and F_{ST} = that with the Chapuis & Estoup (2007) correction for null alleles. Genetic diversity indices were calculated over all 11 loci, and F_{ST}s were limited to the 8 with the least missing data.

						Pairwise F^{ST}				
Location	N	H_E	A	PA	N_e	San Fernando	Tujunga	Los Angeles	Redlands	Palm Springs
San Fernando	22	0.51	2.13	4	19.5 (5.5 - ∞)					
Tujunga	15	0.46	1.97	0	n/a (10.8 - ∞)	-0.0006				
Los Angeles	20	0.53	2.13	5	35 (9.3 - ∞)	0.0160	0.0188			
Redlands	11	0.50	2.17	3	50.7 (5.2 - ∞)	0.0391	0.0465	0.0352		
Palm Springs*	4	0.41	3.55	1	n/a	0.0727	0.1065	0.0395	0.0039	
San Diego	8	0.52	2.24	3	n/a (3.3 - ∞)	0.0483	0.0383	0.0308	0.0306	0.0514

* We attempted to assess historical structure between Palm Springs and coastal aggregations; however, too few samples were available to make strong inferences about relationships between these populations.

Table S4. Summary of results for analyses on cactus wrens captured between 1906-1923. N = total samples analyzed, H_E = expected heterozygosity, *A* = allelic richness, PA = number of private alleles, N_e = effective population size from the linkage disequilibrium method, and F_{ST} = that with the Chapuis & Estoup (2007) correction for null alleles. Genetic diversity indices were calculated over all 11 loci, and F_{ST}s were limited to the 8 with the least missing data.

						Pairwise F^{ST}		
Location	N	H_E	*A*	PA	N_e	San Fernando	Los Angeles	Redlands
San Fernando	4	0.43	2.03	1	n/a (4.6 - ∞)			
Los Angeles	13	0.57	2.35	7	n/a (19.4 - ∞)	-0.0015		
Redlands	11	0.50	2.17	2	50.7 (5.2 - ∞)	0.0270	-0.0085	
San Diego	11	0.52	2.21	3	31.6 (3.2 - ∞)	0.1263	0.0622	0.0917

Table S5. Summary of results for analyses on cactus wrens captured contemporarily, between 2011-2013. N = total samples analyzed, A = allelic richness, PA = number of private alleles, N_e = effective population size from the linkage disequilibrium method, and F_{ST} = that with the Chapuis & Estoup (2007) correction for null alleles. Genetic diversity indices were calculated over all 11 loci, and F_{ST}s were limited to the 8 with the least missing data.

						Pairwise F^{ST}		
Location	N	H_E	*A*	PA	N_e	Los Angeles	Redlands	Riverside
Los Angeles	56	0.52	2.12	1	23 (7 - 223.8)			
Redlands	8	0.51	2.14	1	51 (17.5 - ∞)	0.0455		
Riverside	16	0.51	2.14	0	59 (27.3 - ∞)	0.0320	0.0927	
San Diego	48	0.58	2.31	7	23.9 (12 - 76.8)	0.0618	0.0773	0.0355

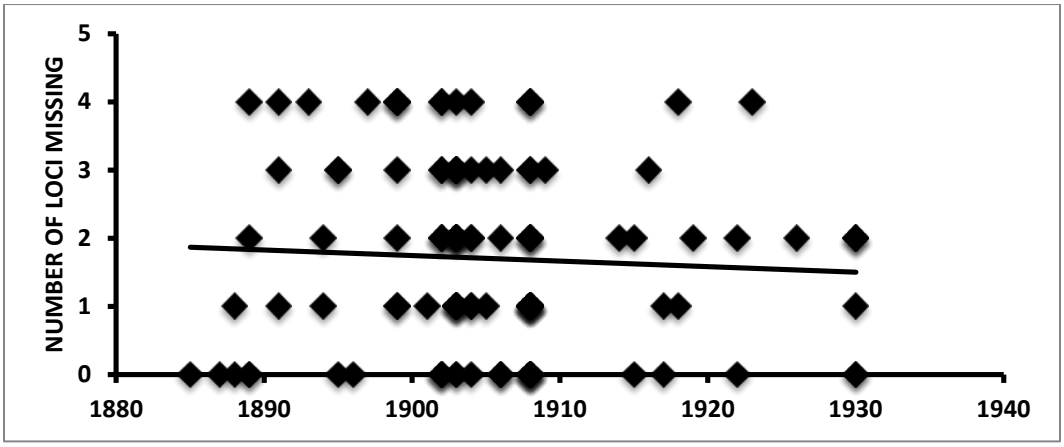

Figure S1: Missing data across individuals in the historical dataset by sampling year. The correlation between missing data and time is weakly negative, but not significant (r = -0.057).

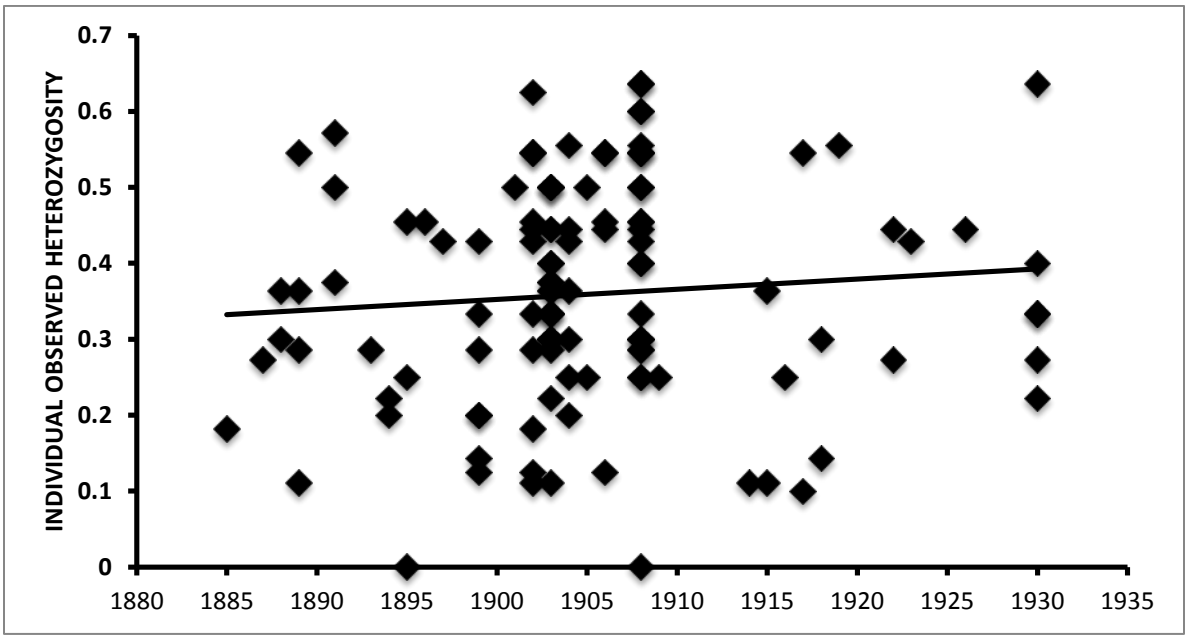

Figure S2: Proportion of heterozygous loci per individual in the historical dataset by collection year. The correlation between individual heterozygosity and time is weakly positive, but not significant (r = 0.08)

Figure S3: Proportion of heterozygous loci per individual in the historical and contemporary datasets by collection year using all 11 loci. Average individual heterozygosities are significantly different between the historical and contemporary datasets (difference between means = -0.152, t = -7.786, d.f. = 236, $p \leq 0.0001$)

Figure S4: Proportion of heterozygous loci per individual over historical and contemporary datasets with 3 loci with more than 20% missing data removed. Average individual heterozygosities are not significantly different between the historical and contemporary datasets (difference between means = -0.016, t = - 0.642, d.f. = 224, p = 0.261).

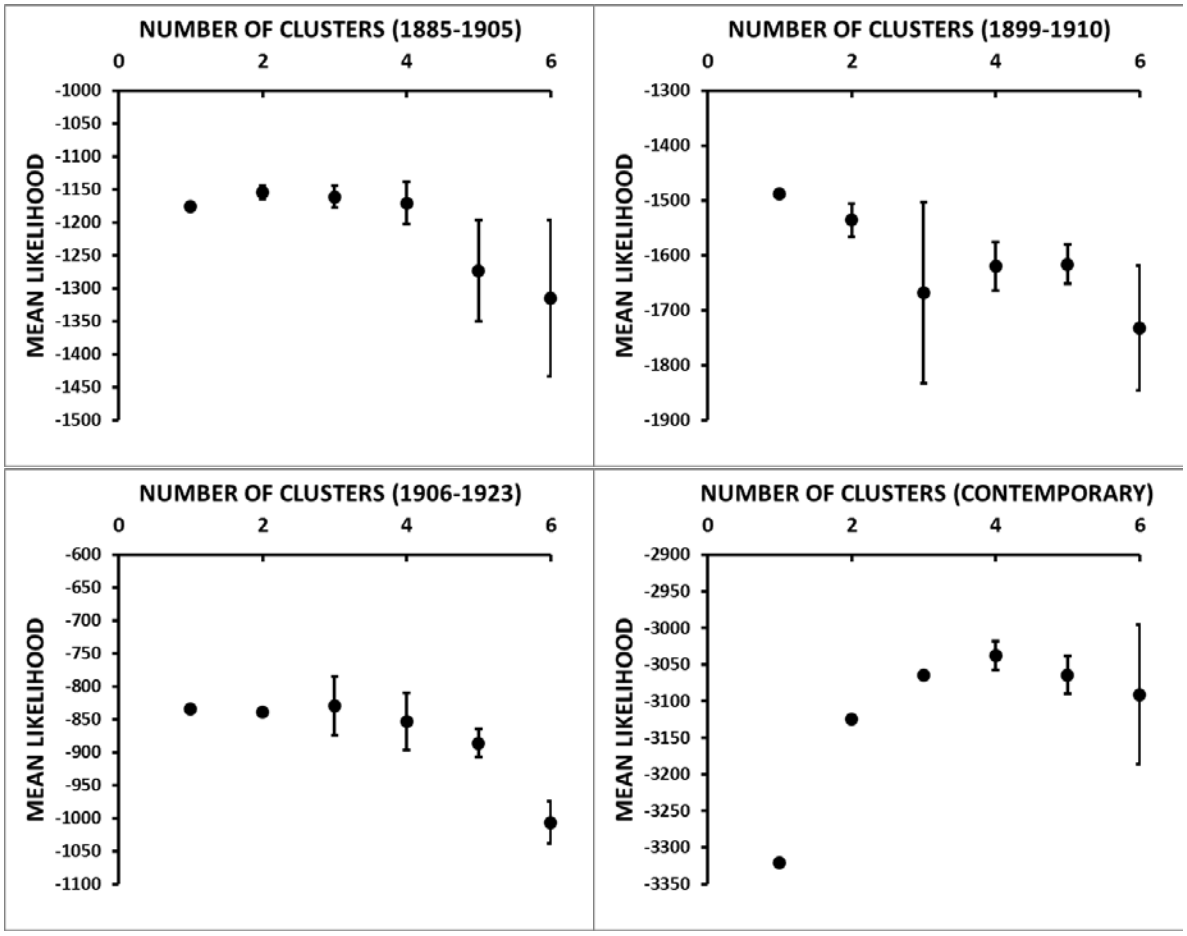

Figure S5. STRUCTURE results by year. Each of the historical datasets exhibit no evidence of structure, as evidenced by the highest likelihoods being at one and declining thereafter. Conversely, the contemporary dataset increases in likelihood up to a max at two and three clusters.