ABSTRACT

THE CACTUS WREN (*CAMPYLORHYNCHUS BRUNNEICAPILLUS*) IN SOUTHERN CALIFORNIA: HAPLOTYPE COMPARISONS AMONG COASTAL AND INLAND POPULATIONS

By

Matthew Robert Teutimez

May 2012

The Cactus Wren (*Campylorhynchus brunneicapillus:* Troglodytidae), a highly sedentary, nonmigratory bird is distributed among cactus-dominated habitats of the southwest United States and Mexico, including coastal Southern California. The coastal populations are waning and conservation efforts have been enacted to slow the decline of the coastal populations. A paucity of genetic information related to the Cactus Wren has led this study to test for genetic differentiation between coastal and inland birds. This study examined two regions of mtDNA sequences for haplotype variation in 136 individuals in 18 populations from Southern California, Arizona, Texas, and Mexico. There were seven haplotypes for CytB, seven for ND2, and nine for a coastal subset of ND2. There was a significant relationship between genetic and geographical distance within the coastal populations but no significant genetic differentiation between coastal and inland desert Cactus Wren found in this study.

THE CACTUS WREN (*CAMPYLORHYNCHUS BRUNNEICAPILLUS*) IN SOUTHERN CALIFORNIA: HAPLOTYPE COMPARISONS AMONG COASTAL AND INLAND POPULATIONS

A THESIS

Presented to the Department of Biological Sciences

California State University, Long Beach

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

Committee Members:

James W. Archie, Ph.D. (Chair)
Simon Malcomber, Ph.D.
Judith Brusslan, Ph.D.
Richard A. Erickson

College Designee:

Brian Livingston, Ph.D.

By Matthew Robert Teutimez

B.S., 2003, California State University, Long Beach

May 2012

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

ACKNOWLEDGEMENTS

This experience has given me a new understanding of frustration, struggle, joy, and anticipation. I am thankful for the many opportunities that God has allowed me through this experience. I would first like to thank my committee, especially Dr. J. Archie, my advisor, for sticking with me through the long exhaustive process of graduate work. The many days and nights of struggle and exhaustion have left me stronger and tougher for the future. I am grateful to R.A. Erickson for your vast knowledge of birds and taxonomy and your continuous extra efforts on my behalf. I want to thank Dr. S. Malcomber and Dr. J. Brusslan for your insightful comments, convenient availability, and willingness to participate in my committee. I am thankful for Dr. C. Collins, who was the impetus for my entering into grad school and who inspired my interest in birds. I am indebted to LSA Associates, Inc., my employer and family, for their continued support both financially, educationally, and emotionally and for providing me a workplace with a strong ethical foundation. I am thankful for all my lab mates who were a constant source of inspiration and help. I am grateful to USGS San Diego for their interest in this study and their willingness to share DNA, for which this study would not have come to fruition without their generous gift. As well, I am grateful to LSU Museum of Natural History for their generous and prompt loan of Cactus Wren tissues. I would like to thank P. Unitt, D. Kamada, and R. Hamilton for their willingness to teach and include me in their own Cactus Wren studies. Finally, I would like to thank my family

and friends who are a constant source of strength and support during all of the endeavors I choose to endure. This study is dedicated to my nephews, Joseph, Brennan, and Bryce, whom I love.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

FIGURE Page

LIST OF ABBREVIATIONS

µl	microliters
Φ_{st}	Phi $_{st}$ (genetic distance)
AOU	American Ornithologists' Union
bp	base pair
CR	control region
CytB	Cytochrome B
dNTP	Deoxyribonucleic triphosphate
DTT	dithiothreitol
ESA	evolutionary significant unit
FESA	Federal Endangered Species Act of 1973
GIS	Geographic Information System
HCP	Habitat Conservation Plan
h_d	haplotype diversity
IBD	Isolation By Distance
km	kilometers
LSUMNS	Louisiana State University Museum of Natural Sciences
$MgCl_2$	magnesium chloride
mi	miles
MSHCP	Multiple Species Habitat Conservation Plan
mtDNA	mitochondrial DNA
NADH2	Nicotinamide adenine dinucleotide dehydrogenase subunit 2
NCCP	Natural Communities Conservation Plan
ND2	Nicotinamide adenine dinucleotide dehydrogenase subunit 2
N_e	effective population size
NROC	Nature Reserve of Orange County
PCR	polymerase chain reactions
RMA	reduced major axis
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey

CHAPTER 1

INTRODUCTION

Cactus Wren of Southern California and Their Habitat

The Cactus Wren (*Campylorhynchus brunneicapillus*) is a non-migratory songbird and vociferous resident of various cactus-dominated habitats within the deserts of the southwest United States and Mexico, including cactus scrub habitat along the coast of Southern California (Anderson and Anderson, 1973; Garrett and Dunn, 1981; Rea and Weaver, 1990; Unitt, 2004; Hamilton et al., 2011). These coastal populations of the Cactus Wren are obligate residents of a threatened vegetation type known as coastal sage scrub (Garrett and Dunn, 1981; Rea and Weaver, 1990; O'Leary, 1990; Minnich and Dezzani, 1998; Unitt, 2008) and are further restricted within coastal sage scrub to a subset known as southern coastal cactus scrub, which comprises large localized patches of cactus-dominated stands of prickly pear (*Opuntia littoralis, O. oricola*) and occasional cholla (*Cylindropuntia prolifera*) (Holland, 1986). Historically, coastal sage scrub was a dominant feature of the coastal Southern California landscape, where it occurred in a natural mosaic with other vegetation communities such as hard chaparral, needlegrass grassland, riparian forests, and oak woodland (O'Leary, 1990). As a result of increased urbanization, cattle grazing and agricultural exploitation, regional drought, and major wildfires, 70% to 90% of the historic acreage of coastal sage scrub is estimated to have been lost within the last four decades (Klopatek et al., 1979; Westman, 1981; O'Leary,

1990; Bontrager and Gorospe, 1995; Minnich and Dezzani, 1998; Mitrovich and Hamilton, 2007; Hamilton et al., 2011). The remaining fragments of coastal sage scrub in Southern California generally occur as "islands" surrounded by ever-increasing "dead-seas" of urban development (Atwood and Noss, 1994). As a result of the rapid loss of cactus scrub, Cactus Wren populations have experienced a parallel decline that is attributable to the aforementioned factors and potentially other factors as yet not known or discovered (Simonson et al., 2005). In the early 1990s, 1,900–2,400 pairs of Cactus Wren were the baseline population estimate within Orange and San Diego Counties (Ogden Environmental and Energy Services Co., 1993). As of 2011, population estimates resulting from focused survey efforts have reported that most, if not all, known Cactus Wren populations in coastal Southern California and northwest Baja California are in a state of steep decline, with many of the populations supporting less than 20% of their original numbers (Rea and Weaver, 1990; Small, 1994; Griffith and Griffith, 1996; Garrett, 2001; Solek and Szijj, 2004; Unitt, 2004 and 2008; Garrett et al., 2006; Mitrovich and Hamilton, 2007; Clark and Dodero, 2008; Harmsworth Associates, 2008; Cooper, 2009; Leatherman Bioconsulting, 2009; Hamilton, 2009a and 2009b). Some populations disappeared entirely (Garrett, 2001; Mitrovich and Hamilton, 2007; Unitt, 2008; Cooper, 2009).

Tricky Taxonomy and Conservation Status

The December, 2011 Cactus Wren account revision in *Birds of North America* (Hamilton et al., 2011), recognizes seven subspecies (southwest U.S. and mainland Mexico: *brunneicapillus*, *seri, anthonyi, sandiegensis,* and *guttatus*; Baja California Peninsula: *bryanti* and *affinis*). The separations are based primarily on morphological

differences such as spotting in the chin/gular, chest, and abdominal areas; color differences in the flank/abdominal or back; and barring differences in the tail (Bancroft, 1923; Selander, 1964; see figures 1 through 3). Within the southwest U.S. and mainland Mexico, the nominate *C.b. brunneicapillus* (which includes the former subspecies *C.b. couesi*) extends from southeastern Arizona east to central Texas and south to Chihuahua and southern Sonora in Mexico. *C.b. anthonyi* is distributed from central Arizona west through Southern California to Los Angeles and Ventura Counties. *C.b. sandiegensis,* described by Rea (1986) and expanded upon by Rea and Weaver (1990) and Unitt (2004, 2008) is restricted to northwestern Baja California and western San Diego and Orange Counties. *C.b. seri* is found on Tiburon Island in the Gulf of California, while *C.b. bryanti* and *C.b.affinis* are found on the Baja California peninsula with *C.b. bryanti* in the north and *C.b.affinis* in the south (figure 4).

Nevertheless, the Cactus Wren in Southern California has a complex nomenclatural history that has yet to be fully resolved (Mearns, 1902; Bancroft, 1923; Grinnell and Miller, 1944; AOU, 1957; Selander, 1964; Rea, 1986; Rea and Weaver, 1990; Zink et al., 2001; Unitt 2004, 2008; Hamilton et al., 2011). Currently, the name "coastal Cactus Wren" relies more upon geographical range than a morphologically or genetically diagnosable lineage. In 1990, the United States Fish and Wildlife Service (USFWS) was petitioned to recognize *sandiegensis* as an endangered subspecies under the guidelines of the Federal Endangered Species Act of 1973 (FESA) due to low population numbers, limited distribution, and habitat loss and fragmentation (USFWS, 1994). Instead, in 1991, USFWS initiated a status review for the entire coastal California Cactus Wren population. In 1994, based on the findings of the American Ornithologists'

Union (AOU) Committee on Classification and Nomenclature, it was decided that the coastal population would be transferred from Category 2 (candidate for federal listing) to Category 3B (taxonomic problems) because it did not "constitute a distinct vertebrate population segment" under FESA (USFWS, 1994, pg. 3). This decision was made primarily due to a perceived lack of taxonomic distinctness from the widespread subspecies' of the southwest desert and Mexico (i.e., *brunneicapillus/cousei* and *anthonyi*) (USFWS, 1994). The coastal populations were considered intermediate between two recognized subspecies, *anthonyi* of the mainland and *bryanti* of Baja California, and not worthy of taxonomic recognition (USFWS, 1994). However, the debate over the subspecies distinctness of the coastal populations in relation to the inland desert populations still continues (Rea and Weaver, 1990; Unitt, 2008; Hamilton et al., 2011). According to Rea and Weaver (1990), the subspecies *anthonyi* ranges from the northern portion of coastal Southern California (i.e., southern Ventura and Los Angeles Counties) east through the deserts of southeast California, southern Nevada, and eastern Arizona, and south to northeast Baja California (south to San Felipe) and northwestern Sonora, Mexico (figure 4). Rea and Weaver (1990) described the range of *sandiegensis* as being within the coastal sage scrub of southwest California, from southern Orange County south to extreme northwest Baja California (figure 4). Its northern limit remains uncertain due to a lack of specimens from northwest San Diego County and most of Orange County (Rea and Weaver, 1990; Unitt, 2008). However, based on the seven morphological characters outlined by Rea and Weaver (1990) (i.e., speckling in the chin/gular area, chest spot shape, abdominal spotting; chest patch aggregation, color differences in the flank/abdominal and back, and barring differences in the tail), the range

anywhere between northern San Diego County and Ventura County (Hamilton et al., 2011). Regardless, the plumage of *C.b. sandiegensis* in coastal Southern California appears to be intermediate between *C.b. anthonyi* and *C.b. bryanti*, with a few *C.b. sandiegensis* individuals possessing a stronger resemblance to *C.b. anthonyi* of the inland deserts (Hamilton et al., 2011; see figures 1 through 3).

Past Research and New Investigations

Historically, there was a paucity of ecological and life history information between the California coastal and desert populations of Cactus Wren, but this has begun to change in the last two decades (Rea and Weaver, 1990; Eggert, 1996; Wheeler, 1997; Flaagan, 1999; Kristan et al., 2003; Crooks et al., 2004; Atwood and Lerman, 2007). The recent efforts of numerous investigators have mostly resulted in new information concerning the locations and sizes of local populations (Small, 1994; Atwood et al., 1994, 1998a, 1998b; Garrett, 2001; Solek and Szijj, 2004; Unitt, 2004 and 2008; Garrett et al., 2006; Mitrovich and Hamilton, 2007; Harmsworth Associates, 2008; Clark and Dodero, 2008; Cooper, 2009; Leatherman Bioconsulting, 2009; Hamilton, 2009a and 2009b) with little new ecological or life history research. The majority of documented life history and ecological records still have been derived from populations within Arizona and New Mexico (Ricklefs, 1966, 1967, 1968, 1975; Ricklefs and Hainsworth, 1966, 1968a, 1968b, 1969; Smith, 1970; Anderson and Anderson, 1973; Marr, 1981; Marr and Raitt, 1983; Simons and Martin, 1990; and Simons and Simons, 1990 and 1993). These populations live in drastically different habitats, and appropriate resource or habitat management related to desert populations is most likely different than that for coastal

populations of Cactus Wren. However, since the coastal Cactus Wren within Orange and San Diego Counties have declined so rapidly and face an uncertain future, large-acreage landowners and natural resource managers have initiated active recovery and conservation efforts. Attempts to salvage surviving portions of these waning populations have included methods such as cactus transplantation (LSA, 2005), artificial nesting structures (Sorrento, 2008), translocations of family groups (Kamada and Mitrovich, 2006; Kamada, 2008), and recognition of coastal California Cactus Wrens as federal Birds of Conservation Concern (USFWS, 2008), and designation of the subspecies *sandiegensis* as a California Species of Special Concern (Unitt, 2008). However, there is still an urgent need to obtain additional baseline biological information concerning the coastal populations of Cactus Wren. This would include genetic information relating to the distinctness of *sandiegensis* and its relationship to other subspecies/populations. The currently available genetic information on the Southern California populations of coastal Cactus Wren is minimal and limited to only one study, which utilized a short mitochondrial DNA sequence for coastal Cactus Wren (Eggert, 1996).

Genetic studies provide ecological managers new insights into the level and structure of genetic diversity within and among populations. Likewise, genetic studies can help develop genetic distribution maps to enable the most effective means of preserving a species' genetic diversity as well as determine whether the loss of a population will result in a significant loss of genetic diversity to the species (Griffiths et al., 2000). This kind of information will be crucial to ensuring that financial resources are directed to the population(s) most in need. In the absence of genetic information, efforts may end up preserving genetically indistinct populations while allowing an

inadvertent destruction of genetically distinct populations (Maehr and Lacy, 2002; Wisely et al., 2003; Zink, 2004; Pimm et al., 2006; Zink et al., 2010). At the population level, genetic information can be used to map movement patterns and assess reproductive success (Griffiths, 2000; Avise, 2000). This information can reveal the distribution of genetic variation within and among surviving portions of fragmented populations (Zink & Barrowclough, 2008) and help select the best populations, down to an individual, for a translocation/breeding program (Griffiths et al., 2000). Therefore, a continuously escalating collection of molecular data can only help natural resource managers to have a more robust understanding of wild populations and assist in formulating management decisions.

Genetic Study Focus

This study utilizes mitochondrial DNA (mtDNA) sequences from the Cactus Wren to investigate nucleotide differences in three separate mtDNA gene regions within and among *C. brunneicapillus* populations in San Diego, Orange, Los Angeles, San Bernardino, Riverside, and Imperial Counties in Southern California and *C. brunneicapillus* populations from Arizona, Texas, mainland Mexico, and Baja California, Mexico (table 1). The three mtDNA gene regions include: (1a) non-coding control region Domain I, (1b) non-coding control region Domain III, (2) the protein-coding region Cytochrome B (CytB) gene, and (3) the protein-coding region nicotinamide adenine dinucleotide dehydrogenase subunit 2 (NADH2 or ND2) gene. Many studies have utilized the molecular variation observed within populations to help understand population structure and species and subspecies delineation in other bird species (Reading and Kellert, 1993; Griffiths, 2000; Pimm et al., 2006; Funk et al., 2007; Coulon

et al., 2008; Rojas-Soto et al., 2010). Therefore, an analysis utilizing genetic sequence data will help to improve the available biological information for the Cactus Wren populations along the coast of Southern California. The highly variable mtDNA, which can evolve at the nucleotide sequence level of upwards of 1 to 10 times more rapidly than typical single-copy nuclear DNA regions (Avise et al., 1987; Hartl and Clark, 1997), has helped in the discovery of geographically significant clades in some taxa, but has revealed less genetic structuring than was originally assumed in other taxa (Wenink et al., 1996; Zink, 1997; Barrowclough et al., 2004 and 2005). MtDNA is suitable in populations that are somewhat sedentary (Haig, 1998), such as Cactus Wren, and has been used to help detect population bottlenecks and population subdivisions (Wilson et al., 1985; Brown et al., 2004).

Within the maternally inherited mtDNA, variations in nucleotide sequences compose a haplotype. In this study, a haplotype is the unique nucleotide sequence observed within individuals that is different from other individuals for the associated mtDNA region by at least one base pair. To further categorize these variable sequences, haplotypes are placed into haplogroups, which are a collection of similar haplotypes. A haplogroup is also referred to as a clade (The International HapMap Consortium, 2003). Generally, increased nucleotide differences between haplotypes imply a more distant relationship between individuals. At the population level, the total number and frequency of haplotypes present as well as their degree of differentiation is informative since a large number of haplotypes indicates a population has had a longer amount of time since shared ancestry. In contrast, a small number of haplotypes suggests a population where the individuals exhibit a shorter amount of time since shared ancestry or a small effective

population size (Avise, 2000). In comparisons between populations, if populations do not share haplotypes, then it indicates they have been isolated for a long time and are isolated from each other. If populations share a large proportion of haplotypes or belong to the same haplotype group, then gene flow between the populations is not restricted and the populations are not isolated currently or have been isolated for a relatively short period of time.

Hypotheses

This study analyzed mtDNA sequence variation of 135 different Cactus Wren individuals from 18 populations sampled from breeding territories in Orange, San Diego, Los Angeles, San Bernardino, Riverside, and Imperial Counties in California as well as from Arizona, Texas, and the mainland and Baja California peninsula of Mexico to address the following questions: (1) Is there any evidence of population-genetic structuring among Southern California populations? (2) Is there any differentiation between northern or eastern populations located in Los Angeles, Riverside, San Bernardino, and Imperial Counties from the southernmost populations located in Orange and San Diego Counties or Baja California, Mexico, that would support *C.b. sandiegensis* as an evolutionary significant unit (ESU)? (3) What do the relationships between haplotypes and frequency distribution of different haplotypes tell us about the historical population dynamics and distribution of coastal Cactus Wren?

The research hypothesis for this thesis maintains there is a significant difference between the sequences of Cactus Wren from the populations in Orange, San Diego, southern Los Angeles, western Riverside, and southwestern San Bernardino and that haplotypes found in this region are not randomly distributed among the sampled

populations. The null hypothesis for this thesis maintains there is no difference between the sequences of Cactus Wren from the populations in Orange, San Diego, southern Los Angeles, western Riverside, and southwestern San Bernardino Counties (i.e., coastal Cactus Wrens are recent colonizers from other parts of the species' range) and that haplotypes found in this region are randomly distributed among the sampled populations.

CHAPTER 2

MATERIALS AND METHODS

Population Sampling

Coastal Cactus Wren nestlings and adults were sampled for DNA in Orange County by the Nature Reserve of Orange County (NROC) and in San Diego County by the United States Geological Survey (USGS) (table 1). Nestlings were sampled in the nest by removing a few growing feathers from their breast (Horvath et al., 2005). The nestlings were banded with a unique set of colored leg bands for a corollary project (figure 5) and also banded with a USFWS aluminum band that had a unique identification number. Adult birds were lured by song playback into a polygon-shaped mist net enclosure surrounding a cactus patch that was chosen from surveys to be a highly occupied (favored) patch by the pair. The adults were sampled for blood using a toenail clip and were banded in the same manner as the nestlings. All samples were stored in Queen's lysis buffer, an AOU recommended buffer, which is a blood lysis buffer used for preserving DNA (website: http://AOU.org/committees/collections/recipes_dna_buffers.php). The DNA from feather and blood samples was extracted and prepared by USGS. Ninety-five (95) samples of extracted and purified DNA were gifted for use with this project (table 1). All samples derived from the same populations were either not from the same nest location or from the breeding pair of adults at the same nest location, thus reducing any chance of mtDNA replication due to sibling or parental

relatedness. A set of 14 organ tissue samples was provided on loan from the Louisiana State University Museum of Natural Sciences (LSUMNS) Section of Genetic Resources for use in this project (table 1). Thirty-five (35) sequences from Palos Verdes, San Bernardino, Arizona, Texas, mainland Mexico, and Baja California populations were downloaded from the online nucleotide sequence database, GenBank (http:// blast.ncbi.nlm.nih.gov; see table 1). There were 33 available ND2 sequences (Zink et al., 2001; Vasquez-Miranda, 2009) and 2 available CytB sequences (Barker, 2004).

DNA Extraction and Purification

Tissue digestion for the extraction of DNA was accomplished in two different ways. For the field-collected feather samples, DNA extractions were carried out using a modified protocol from the DNeasy blood and tissue kit (Qiagen, Valencia, California). Specifically, dithiothreitol (DTT), a cell lysis buffer, was added during the initial cell lysis step in order to aid in the digestion of keratin within the feathers. For the blood and tissue samples, DNA extraction was accomplished by using the DNeasy blood and tissue kit (Qiagen, Valencia, California) and following the standard protocol provided. The tissues were prepared in a workspace cleaned with a 20% bleach solution and a new absorbent pad placed over the workspace prior to each day of DNA extractions. During the tissue preparation, the tools were immersed in a 20% bleach solution and rinsed with distilled water after each use, then wiped and dried with a new Kim-wipe™ before subsequent use. The tissues were cut into small pieces with a new scalpel blade for each sample and placed into labeled 1.5-milliliter (mL) microtubes. The tissue samples were digested in a buffered solution of proteinase K at 55°C for 12 hours. The presence or absence of DNA for each sample was visualized on 1%–2% agarose gel containing

ethidium bromide using a DC290 camera (KODAK, Rochester, New York) and Kodak EDAS software under ultraviolet light.

DNA Amplification Using the Polymerase Chain Reaction

Four separate regions of the mtDNA genome were analyzed. A 298 base pair (bp) section of Domain I of the noncoding control region, a 298 bp section of Domain III of the noncoding control region, a 491 bp section of the ND2 protein-coding region, and a 700 bp section of CytB protein-coding region. Published primer sequences (see table 7) were used to amplify each associated fragment of the mtDNA genome. Polymerase chain reactions (PCR) were performed using a thermal cycler (Eppendorf, Hauppauge, New York) with eight-strip 0.2 mL tubes or 96-well plates, region-specific forward and reverse primers (table 7), and OneTaq DNA polymerase (New England Biolabs, Ipswich, Massachusetts) with supplied buffers. The cycling profile was the same for all different regions and was set at 1 cycle at an initial temperature of 92°C for 3 minutes, then 40 cycles of 1 minute at the denaturing temperature of 92°C, 1 minute at the primer-specific annealing temperature of 50°C, and 1 minute 15 seconds at the elongation temperature of 72°C, followed by a final extension cycle of 3 minutes at 72°C. Reactions (25 microliters [µl] or 50 µl) contained, respectively, 1 or 2 µl of genomic DNA, 5 or 10 µl 5X OneTaq Standard PCR buffer (with magnesium chloride [MgCl2]), 0.5 or 1 µl 10X deoxyribonucleic triphosphate (dNTP) mix (2mM), 1 µl forward primer, 1 µl reverse primer, 0.125 or 0.25 µl OneTaq DNA polymerase (5U/µl), and 16.375 or 34.75 µl DNA-grade water. PCR products were visualized on 1%–2% agarose gel containing ethidium bromide using a DC290 camera (KODAK, Rochester, New York) and Kodak EDAS software. PCR products were dehydrated to 10 µl and sent to the University of

Washington High Throughput Sequencing Center, Department of Genome Sciences, Seattle, Washington, for sequencing.

Data Analyses

DNA sequences were aligned using Clustal W in MEGA 5 (Tamura et al., 2011) and adjusted by eye. Phylogenetic analyses (maximum likelihood) including haplotype (η) and nucleotide diversity (π) were carried out using MEGA 5 (Tamura et al., 2011; table 2). The Hasegawa-Kishino-Yano substitution model (HKY) was the best-fit model for the data. This model is based on one rate for transitions, one rate for transversions, and variable frequencies of the nucleotides (Nei and Kumar, 2000). The evolutionary history for the CytB and ND2 regions were analyzed using a maximum likelihood tree with 500 bootstrap replicates to assess branch support. The branch lengths were measured by using the number of base pair substitutions within each mtDNA sequence (Tamura et al., 2011) (figures 10 through 12).

Measures of DNA sequence and haplotype frequency variation were calculated between populations with DnaSP version 5.10.01 (Librado and Rozas, 2009). This software was used to conduct computer simulations based on the coalescent process to produce haplotype assignments (tables 3 through 5) and measures of diversity for haplotype ($H_d = 1-\Sigma f_i^2$, where f_i is the frequency of the i^{th} haplotype) and nucleotide ($\pi = 1-\Sigma f_i f_j p_{ij}$, where p_{ij} is the sequence divergence between the i^{th} and j^{th} haplotypes) data (Avise, 2000; table 2). Haplotype diversity measurements from both MEGA and DnaSP agreed with each other.

A Geographic Information System (GIS) was used for mapping and spatial analysis of the samples. ArcGIS 9 (ESRI, Redlands, California) was used to determine

geographic distances between populations (tables 8 through 11). These geographic distances were used in Isolation By Distance (IBD) analyses. In order to reduce the inherent errors present in distance measurements taken from a two-dimensional flat surface, the digital location data were transformed into a conical equal area map projection called the Albers Equal Area Conformal Conic Projection (Snyder, 1987; ESRI, 2009). Since California is in a small scale, it is not known to have a significant latitudinal distortion (Suzanne Wechsler, Ph.D., personal communication), thus making the Albers equal area projection most reasonable. The projection utilizes a metric unit of measurement, which was desired, and is considered the most commonly used projection for maps of the conterminous United States (Snyder, 1987).

Isolation by distance was analyzed for Cactus Wren since they are considered highly sedentary, and dispersal is expected to be limited to less than 10 kilometers (km) (6.2 miles [mi]) from their natal territory (Bontrager and Gorospe, 1995; Atwood et al., 1998; Kamada, 2008; Hamilton et al., 2011). Therefore, individuals from populations that are in close proximity to one another are more likely to interact and have a higher chance to mate than individuals in more distant populations. As a result, nucleotide frequencies in populations that are closer to one another will likely be more similar than in populations that are farther apart (Slatkin, 1993; Rousset, 1997; Hutchinson and Templeton, 1999). The calculated geographic distances from GIS were used in an IBD analysis with IBDWS Version 3.21 (Jensen et al., 2005) to assess the statistical significance of the association between genetic similarity (or distance) and actual geographic distance. IBDWS uses a Mantel test, which accounts for the population as the unit of replication rather than population pairs, to check whether there is a statistically

significant relationship between the genetic distances and the associated geographic distances. In addition, IBDWS calculates the slope and intercept of the IBD relationship using reduced major axis (RMA) regression. RMA regression is considered more appropriate than standard linear regression when both the dependent and the independent variables are measured with error (Laws and Archie, 1974; Jensen et al., 2005). Estimations of the error for the slope and intercept used standard linear approximations. Both a one-delete jackknife estimation, which only uses a subset of data across population pairs, and a bootstrapping (e.g., drawing randomly from the data with replacement) of pseudoreplicates over the independent population pairs were used. IBDWS generated estimates of Phi_{st} (ϕ_{st}) between all pairs of populations using formulas provided by Weir (1990) and Excoffier et al. (1992). Phi_{st} uses pairwise comparisons of the mean genetic distance deviations between two sequences, where one sequence is chosen from each of the two populations then calculated with pairwise comparisons of the mean genetic distance between sequences within each of the two populations analyzed. These measures are used to evaluate the genetic distances distributed among populations (Nei and Kumar, 2000).

CHAPTER 3

RESULTS

I collected variable amounts of Cactus Wren DNA sequences from four discrete mtDNA genome regions in a total of 136 birds from 18 geographical locations in California, Arizona, Texas, and Mexico (table 1). I obtained 94 sequences of 298 bp of Domain I and 95 sequences of 298 bp of Domain III for the control region (CR), a noncoding region of the mtDNA genome (Randi et al., 2001). Within the protein-coding regions of the mtDNA, I obtained sequences from two regions: 78 sequences of the CytB gene including 700bp from tissue and DNA extract samples and 1073 bp from 2 GenBank sequences;, and 121 sequences of the ND2 gene including 88 sequences with 491 bp from tissue and DNA extract samples and 33 sequences from GenBank with a length of 298 bp. (table 6). Thus, two areas were located within the non-protein-coding region (i.e., Domains I and III of the CR) and two in the protein-coding region (i.e., CytB and ND2). The CR is considered the most variable region of the avian mitochondria, with suggested clock calibrations from three to five times higher than for the remainder of the mtDNA genome (Avise, 2000). Moreover, within the avian mtDNA CR, Domains I and III are considered the most variable regions because they both show an increased number of nucleotide substitutions and consistent variations in length compared to the Domain II region (Ruokonen and Kvist, 2002). Initially, the CR was intended to be the sole region of investigation for this study,; however, it was discovered that both CR

domains (i.e., Domains I and III) showed a gene duplication over a majority of the sequence, an occurrence also reported in the owl genus *Strix* (Brito, 2005; Barrowclough, 2011). This occurrence left the CR sequences non-informative for this study. Consequently, the CytB and ND2 regions were chosen for their known variability in nucleotide substitution rate, which has provided appropriate phylogenetic information (Baker and Marshall, 1997; Kidd and Friesen, 1998; Zink and Slowinski, 1995, Randi et al 2001) as well as their use in previous Cactus Wren molecular studies (Eggert, 1996; Zink et al., 2001). ND2 sequences downloaded from GenBank were considerably shorter than this study's sequences (298bp vs. 491bp). Therefore, two analyses using the ND2 samples were performed, one containing all sequences (298bp length; figure 8) and the other containing just a subset of sequences for those obtained directly from tissue and DNA extracts (491bp length; figure 9). The geographic location of the subset of 491bp ND2 sequences applies mainly to the coast with only three inland desert individuals included and more closely resembles the geographic distribution of the sequences used for the CytB analysis (figure 7 and 9).

DNA Polymorphism

CytB

The 78 sequences (excluding the outgroup sequence) from 13 populations (see table 2) had a total of 579 bp locations (sites) analyzed, with 121 sites ignored due to alignment gaps or missing data. There were 572 invariable sites and 7 polymorphic sites. One of the polymorphic sites was a singleton and 6 were parsimony informative. There were a total of 7 haplotypes (A-G) among all sequences. Haplotype diversity (h_d) is 0.675 with standard deviation of 0.037. Haplotype diversity is a measure of haplotype

variation in that it utilizes the numbers and frequencies of differences in each haplotype without regard to their sequence relationships (Avise, 2000). Nucleotide diversity (π) is 0.00250 (0.250%) with a standard deviation of 1.9E-04. The average number of nucleotide differences between sequences (k) was 1.448 (table 2). Nucleotide diversity is a measure of sequence divergence that is weighted between individuals in a population, regardless of the number of different haplotypes (Avise, 2000). According to Grant and Bowen (1998), haplotype diversity is considered high if $h_d > 0.5$ and low if $h_d < 0.5$. Nucleotide diversity is considered high if $\pi > 0.5\%$ and low if $\pi < 0.5\%$. Therefore, CytB sequences have a high h_d (0.680) and a low π (0.257%). Grant and Bowen (1998) found in marine fishes that a high h_d and a low π was reflective of a population bottleneck followed by a rapid population growth that may aid in the accumulation and retention of mutations (i.e., separations).

ND2

A total of 121 sequences from 18 populations (see table 2) were analyzed in two ways; first, with all samples available (i.e. this study's samples plus GenBank), and second, with just this study's samples (86 samples). The analysis with all samples had a total of 163 bp sites analyzed with 328 sites ignored due to alignment gaps or missing data from the shorter GenBank sequences. There were 149 invariable sites with 14 polymorphic sites. Two of the polymorphic sites were singletons and 12 were parsimony informative. Among all the sequences, there were a total of 7 haplotypes. Haplotype diversity (h_d) is 0.257 with a standard deviation of 0.051. Nucleotide diversity (π) is 0.0103 (1.03%) with a standard deviation of 2.69E-03. The average number of nucleotide differences (k) between sequences was 1.681 (table 2). ND2 sequences have a

low h_d (0.257) and a high π (1.03%). Grant and Bowen (1998) found in marine fishes that a low h_d and a high π was reflective of a divergence between geographically subdivided populations. The second analysis used 86 sequences from 13 populations (see table 2). A total of 435 bp sites analyzed with 56 sites ignored due to alignment gaps or missing data. There were 426 invariable sites with 9 polymorphic sites. Four of the polymorphic sites were singletons and 5 were parsimony informative. Among all the sequences, there were a total of 7 haplotypes. Haplotype diversity (h_d) is 0.661 with a standard deviation of 0.004. Nucleotide diversity (π) is 0.0020 (0.20%) with a standard deviation of 1.23E-03. The average number of nucleotide differences (k) is 0.888 (table 2).

Haplotype Distribution

For CytB, a total of 7 haplotypes (denoted A through G) were distributed among 78 samples (excluding the outgroup) from 13 populations (tables 3 and 5). One of the haplotypes (F) was unique to a single individual (San Bernardino 5 – Needles; figure 7). The dominant haplotype was Haplotype G with 47.5% of the individuals carrying this particular sequence. Haplotype A was the second most frequent with 30% of individuals possessing this haplotype. The remaining haplotypes (B, C, D, E) were all under 10% of the total among populations and constituted 2 to 7 individuals (table 3).

For ND2 with all samples, a total of 7 haplotypes (denoted A through G) were distributed among 121 samples from 18 populations (tables 4 and 5). Three of the haplotypes (C, E, and G) were found in single individuals. The dominant haplotype was haplotype A with 86.2% of the individuals carrying this particular sequence of DNA. Haplotype F was a distant second with a 6.5% of individuals possessing that haplotype. Haplotypes F and G represent the Baja California population, which were consistently

partitioned into a unique grouping. The remaining haplotypes (B and D) were all under 4% of the total among populations and constituted 2 to 4 individuals.

For the 491bp subset of ND2 samples, a total of 9 haplotypes (A through I) were distributed among 87 samples from 13 populations (tables 4 and 5). Three of the haplotypes (G, H, and I) were unique to single individuals. Two haplotypes were equally dominant with haplotype A including 42% of the individuals and haplotype C including 41% of the individuals. Haplotypes (B, D, E, F, and G) were unique to the Orange and San Diego county samples with less than 5% of the total and found in 2 to 4 individuals. Haplotypes H and I were found only in the inland desert population, but with only a single individual for each haplotype.

CytB

The Hasegawa-Kishino-Yano (HKY) maximum likelihood tree was rooted at the outgroup *C. megalopterus* sequence. For the ingroup sequences from Southern California, all but one were placed into a single large clade (haplogroup A). Three subclades containing 2, 5, and 7 sequences formed subclades (Haplotypes B, C, and D). The main clade groupings attained a bootstrap value of 83% (figure 10). The branches within the clades have equally strong bootstrap support. The three subclades differ by two base substitutions between the sequences and a single base substitution from the other sequences in haplogroup A. Haplotype H (singleton: San Bernardino 5-Needles) shares 2 substitutions with the outgroup (*C. megalopterus)* sequence not shared by any other sequence. *Campylorhynchus megalopterus* is known as the Grey-barred Wren and is distributed within subtropic to tropical montane forests from Jalisco to west Puebla in central Mexico (Perlo, 2006). The outgroup sequence differs by 62–67 substitutions from

the other sequences (depending on sequence length; 65 are autapomorphies). Haplotype B (7 individuals) was found in both coastal California and inland desert populations. In contrast, the other two subclade haplotypes (C and D with 2 and 5 individuals) were found only in coastal populations. The largest clade (haplogroup A exclusive of haplotypes B, C, and D) was made up of a mixture of individuals from all different populations and did not correlate with any geographical region.

ND2

With all the samples, the Hasegawa-Kishino-Yano (HKY) maximum likelihood tree grouped all sequenced individuals into two main clades with one clade strictly comprising the Baja California populations and the other clade comprising all other (US) populations. The two main clade groupings had a bootstrap value of 50% for the large clade consisting of all but one of the mainland haplotypes. The Baja clade had a bootstrap value of 99%, showing strong support (figure 11). This is also the level of support for the entire set of mainland sequences. The branches within the mainland clade have very little differentiation in the phylogram. Three sequences had two autapomorphies while two groups of sequences three and four individuals shared one substitution. The larger clade is made up of all populations from California to Texas and includes 5 mainland Mexico samples. The Baja clade consists of all populations within the Baja peninsula, all south of 30°N latitude, which is the recognized subspecies range for *C. b. bryanti* (Bancroft, 1923). The Baja sequences share 13 base changes (one of which is present in the San Bernardino Needles sequence). Of these, six are shared with the outgroup, *C. megalopterus*. The three differentiated subclades within the large mainland clade (Sweetwater Reserve-Imperial County-Otay-Fallbrook, Arizona-Imperial

County, and Bear Valley-Laguna Canyon) each share single base-pair nucleotide substitutions within each subclade. The outgroup differs from all other full sequences (non-GenBank, which were shorter) by 48–52 substitutions.

With the 491bp ND2 sequences, the Hasegawa-Kishino-Yano (HKY) maximum likelihood tree grouped all sequenced individuals into two equal clades (Haplotype A 42% and Haplotype C 41%). Haplotype A had a bootstrap value of 41%, however, the subclades (haplotypes B, D, and E) within the clades have stronger bootstrap support (figure 12). Haplotype B is made up of same El Modena samples as haplotype C in the CytB region. Haplotype E is made up of the same Camp Pendleton and Fallbrook samples as haplotype D in the CytB region. The two clades were made up of a mixture of individuals from all different populations and did not correspond to any geographical region.

Isolation by Distance

CytB

Within the CytB region, there was no significant relationship between genetic distance and log geographical distance ($r = 0.2907$; $p = 0.0800$; figure 13;table 2). The average Φ_{st} value for pairwise comparisons among groups is 0.0829 (table 8). The between groups Φ_{st} was largest for the pairwise comparisons between the Viejo-Edison (VE) population ($\Phi_{st} = 0.419$) and the remainder of populations. The large Φ_{st} values could be due to the single haplotype found within the Viejo-Edison population, although this is shared with other populations. However, none of these Φ_{st} values were significantly different from zero.

A subset of the CytB samples located in the coastal region were also tested for IBD. Within these samples there was a significant relationship between genetic and log geographical distance ($r = 0.3621$; $p = 0.0100$; table 2; figure 14). The average Φ_{st} value for pairwise comparisons among groups is 0.1340 (table 9). The between groups average Φ_{st} was largest for the pairwise comparisons between the Viejo-Edison (VE) population ($\Phi_{st} = 0.512$), which was fixed for haplotype G, and the remainder of populations. The Phi_{st} values between the Viejo-Edison population and all others were significantly different from zero.

ND2

Within the ND2 region, there was no significant relationship between genetic and geographical distance ($r = 0.3317$; $p = 0.9960$; table 2; figure 15). The average Φ_{st} value for a pairwise comparison among groups is 0.154 (table 10). The largest Φi_{st} was for the pairwise comparisons was between the Baja California (BC) populations ($\Phi_{st} = 0.554$) and the remainder of the populations. However, there were similarly large average Φ_{st} values (between 0.20 and 0.29) between population comparisons for Lake Jennings (LJ), Viejo-Edison (VE), Palos Verdes (PV), Mainland Mexico (MX), and Texas (TX). However, none of these values were significantly different from zero.

A subset of the ND2 samples located in the coastal region were also tested for IBD. Within these samples there was a significant relationship between genetic and log geographical distance ($r = 0.4669$; $p = 0.0140$; table 2). Presumably, this result is because haplotype A had its highest frequency in northern populations while haplotype C

predominated in southern populations. In addition, haplotype D was found only in the most northern (El Modena) population. The average Φi_{st} value for pairwise comparisons among groups was 0.1089 (table 2). The between groups average Φ_{st} was largest for the pairwise comparisons between the Viejo-Edison (VE) population (Φi_{st} = 0.357) and the remainder of populations and these were significantly different from zero.

CHAPTER 4

DISCUSSION

Haplotype Identification

A total of 7 haplotypes were found in the CytB sequences for 13 populations, and 7 haplotypes for 18 populations in the GenBank supplemented ND2 sequences and 9 haplotypes for 13 populations in the subset of ND2 sequences (tables 1 through 5). The disparity in the number of populations analyzed between mtDNA gene regions was due to the availability of sequences (33 sequences) from the ND2 region published on GenBank. The CytB region had only 2 sequences available in GenBank for which only one was used for the analysis since the mainland Mexico sequence gave no information for this study. The GenBank sequences were not the same length fragments that I obtained from the primers used for this study (CytB 1,073 bp and ND2 298 bp). Therefore, the analysis for the ND2 region used a smaller number of informative sites when analyzed with the GenBank sequences (i.e., 491 bp PCR sequence length vs. 298 bp GenBank sequence length, with a total of 149 bp sites analyzed with 342 sites ignored due to alignment gaps or missing data). Thus, the longer region of the ND2 gene that was not present in the GenBank sequences but was present in the other sequences did contain parsimony informative sites and was analyzed separately. The GenBank sequences were included for analysis because their localities were representative of the

species range and provided data that were more informative about subspecies distinctness (according to Rea and Weaver, 1990).

Individuals within shared geographic ranges are expected to share nucleotide substitutions and phylogenetic history if dispersal between geographic areas is limited (Avise, 2000; Jensen et al., 2005). If geographic ranges become disjunct and dispersal is prevented, long periods of time since shared ancestry should result in a reduction of shared haplotypes between disjunct regions. For my data, CytB haplotype distributions contained no geographic pattern in relation to subspecies ranges (figures 7 and 10). The maximum likelihood phylogenetic tree shows that the two haplogroups each have an almost equal number of individuals from the inland desert (45% and 55%of samples) and from the coastal populations (44%, and 56%, respectively) (table 5). The two most common haplotypes for CytB occurred in almost equal frequencies: Haplotype G 47.5% and Haplogroup A (includes haplotypes A, B, C, D, E, and F) 30% (table 5; figures 10). Three of eight haplotypes (A, B, and G) were shared among the coastal and inland desert populations. The coastal populations contained two unique haplotypes (C and D) and the inland desert contained two unique haplotypes (E and F).

Since this study concentrated on samples from San Diego and Orange Counties, the identification of haplotypes restricted to the northern and desert subspecies (*C. b. anthonyi*) is not robust (9 samples out of 79; see figure 9) and would benefit from more sequences representing *anthonyi* in the Los Angeles/Ventura County and the inland desert *anthonyi/cousei* range. However, by comparing the haplotypes found in the subset ND2 sequences, which uses the same individuals within Orange and San Diego County (75 samples out of 78) as the CytB analysis, their similarity infers that gene flow may be

limited among these coastal populations. From figure 9 it can be seen that the most common haplotype (G) is present in the coast and the inland desert; however, the second most common haplotype (A) is mainly present within the San Diego coastal populations (sans Orange County), with only a single individual from the inland desert possessing this haplotype (i.e., San Bernardino Chalk Mountains). This distribution is similar in the ND2 subset (figure 9) where the most common haplotype (A) is present in the coast but not in the inland desert. However, the absence of the most common haplotype in my samples is possibly due to the small sample size from the desert (3 of 78 samples). The second most common haplotype (C) is present in both the coast and inland desert populations, with the greatest frequency of haplotype C being in San Diego County populations. The significant isolation by distance found in the both the ND2 and CytB data suggests that there is some limitation in gene flow between northern and southern populations.

Within CytB (figure 7), Chalk Mountains of the Mojave Desert contained two haplotypes (A and B) that are both shared with coastal populations. However, other than this desert individual, this haplotype is only shared within the coastal populations. Haplotype B was found in 7 individuals: 3 San Diego populations (Bear Valley-1 of 5 individuals, Fallbrook-2 of 10 individuals, and Zoo-Safari Park-1 of 6 individuals), an Orange County population (UCI-2 of 7 individuals), and the inland desert singleton (Chalk Mountain-1 of 9 individuals). The sharing of Haplotypes A, B, and G indicates past gene flow between the coast and inland desert populations. In the ND2 subset (figure 9), only Haplotype C is found in the desert and the coast with most populations of the coast containing 1 to 5 individuals with this haplotype. Two Orange County

populations did not contain this common haplotype (i.e., El Modena and Viejo Edison). Viejo-Edison was monomorphic for common Haplotype A and for CytB Haplotype G (figure 7).

Among my sequenced samples, Haplotype C (CytB) was restricted to the El Modena population, with 4 of 7 of the samples having this unique haplotype. These same El Modena individuals (El Modena 1, 2, 5, and 6) shared the subset ND2 haplotype (D; figure 9) showing differentiation within both gene regions. The ND2 haplotype D was unique to these 4 individuals. The CytB haplotype C was also present in the downloaded GenBank sequence with the locality reported as San Diego City, California (table 6). The other three El Modena samples (El Modena 3, 4, and 7) shared the most common haplotypes for both gene regions (i.e., CytB haplotype G and ND2 haplotype A). The maximum likelihood phylogenetic tree for CytB (figure 10) showed strong bootstrap values for the separation of UCI Reserve (62; Haplotype B), and moderate bootstrap values for El Modena (42; Haplotype C), each group was supported by 3 synapomorphic substitutions. Otherwise the data did not support any further clades. Haplotypes D, E, F, and H (CytB) contained only one or two individuals and do not provide notable information on haplotype distributions. The maximum likelihood phylogenetic tree for the subset ND2 (figure 12) showed strong bootstrap values for the separation of El Modena (60; Haplotype D) and is supported by 2 synapomorphic substitutions. El Modena7 contained 2 unshared autapomorphic substitutions. The other haplotypes contained only one or two individuals and do not provide information on haplotype sharing.

The sharing of haplotypes from the coastal populations with individuals from the inland desert requires historical dispersal between populations. The likely dispersal route was through the San Gorgonio Pass between the San Bernardino Mountains to the north and the San Jacinto Mountains to the south. Currently, this portion of Riverside County contains the large cities of Beaumont, Banning, and Calimesa, with residential housing covering much of the historic habitat for the Cactus Wren. Eggert (1996) concluded that this single recognized dispersal route from the desert to the coast no longer serves as a dispersal route due to the lack of suitable habitat.

For the ND2 samples (all samples; not the subset), all United States and mainland Mexico samples contained a single haplotype group. A single haplotype was shared by a substantial majority of populations, although three small subclades characterized by one or two base pair substitutions, were also present in this haplogroup. This clade contained 112 out of 121 individuals sequenced (86% of total ND2 sequences; table 4). The remaining 9 out of 121 individuals sequenced (7.5% of total ND2 sequences) were placed in a second clade with strong bootstrap support (100; figure 11). This latter clade contains all samples from the same geographic area, the Baja California peninsula. The maximum likelihood phylogenetic tree (figure 11) reveals these two clades with a substantial level of differentiation between them (13 substitutions). The discrete Baja peninsula group and the large, nearly monotypic mainland group supports the findings of Zink et al. (2001), whose published ND2 sequences were used for this study, as well as Atwood and Lerman (2007), who found distinct differences in vocalizations of the Baja peninsula Cactus Wren compared to Southern California Cactus Wren. Zink et. al.'s study of arid land bird species placed their ND2 sequences into two distinct clades. One

clade they termed "continental" comprised samples from California, Arizona, Texas, and mainland Mexico. The other group was defined as "peninsular" and comprised samples south of 30°N in Baja California down to the southern tip of the peninsula. Their samples ranged from the southern tip of the Baja peninsula (Santa Anita 23° 10' 51.6", -109° 42' 9.828") north to the city of Camalu (30° 50' 25.494", -116° 3' 42.1776") along the western coast of the Mexican peninsula. The present study included many more samples from the Southern California coast region, particularly the San Diego and Orange County samples representing the *C.b. sandiegensis* subspecies. These samples all uniformly shared the same haplotype (Haplotype A), which was the dominant haplotype or haplogroup shared throughout the mainland. None of the samples from this study shared the Baja group haplotype and vice versa. The distinctness of the geographic patterning of this haplotype is a confirmation of the Zink et al. (2001) conclusion. Atwood and Lerman found three main groupings of vocalizations (1. Baja California Peninsula, 2. coastal Southern California, 3.Chihuahuah and Sonora Desert) with the distinctions between the groupings described as subtle. They did not find any differentiation within the coastal Southern California group. This pattern of differentiation in vocalization is consistent with the pattern of differentiation in mtDNA sequences with a notable distinction between the Baja California Peninsula and the coastal Southern California populations.

Genetic Differentiation and Isolation by Distance

Significant IBD effect were observed within the coastal populations in Southern California, however, no significant IBD effect was observed between the coastal and desert populations in Southern California. An IBD effect would be expected if there was

current or historically restricted gene flow between southern coastal and northern coastal or inland populations. There is some evidence of population differentiation within the coastal Southern California populations from northern (UCI, Viejo-Edison, El Modena) to southern (Bear Valley, Camp Pendleton, Dictionary Hill, Encanto Canyon, Fallbrook, Lake Jennings, Otay, Sweetwater Reservoir). The lack of differentiation between the eastern desert (San Bernardino) populations may be due to the small sample size and the low level of divergence in the two gene regions examined. El Modena and Viejo-Edison populations show a different haplotype distribution than all the other populations and their pairwise, interpopulation Φ_{st} values for both mtDNA regions are highest compared to other populations (tables 8 through 11). The similarity in Φ_{st} values indicates comparable differences within the CytB and ND2 sequences in coastal Southern California Cactus Wren populations, which is expected due to the maternal inheritance and single gene inheritance of the mtDNA. However, statistical difference between populations was not found, but additional samples and additional sequence data will likely result in some differences being statistically significant due to the isolation by distance patterns found in my data.

The haplotype and nucleotide diversity indices from each gene region can be compared to population demographic information from marine fishes. For both the CytB sequences and the restricted set of ND2 sequences, based on longer reads, had a high h_d and a low π, which in marine fishes indicated low effective population size preceding a period of rapid population growth where any new mutations are retained (Grant and Bowen, 1998). This is usually represented by large populations that contain one or two

common haplotypes rooted with small clusters of individuals that are only one or two mutations separated from the common haplotypes. This expectation is similar to what was found for the Cactus Wren CytB and ND2 gene regions, each of which have two main haplogroups with smaller clusters nested within them that are only a few substitution differences apart from the common haplotypes. The most common haplotypes were shared between the coastal and inland desert populations, but each area had unique alleles for one or both gene regions. During dispersal events, such as could be hypothesized for the colonization of coastal California from the inland deserts, common alleles would be expected to be shared while rare alleles would be expected to be restricted to either the source or peripheral population. Clearly, however, additional samples must be obtained, both for inland desert populations and for northern Los Angeles and Ventura County populations, and longer gene regions sequenced in order to determine the degree to which dispersal hypotheses are supported.

Conclusion

The Southern California Cactus Wren populations along the coast and in the desert share DNA haplotypes from two mtDNA gene regions and the current molecular evidence does not support historical separation of gene lineages between the coastal (*C.b. sandiegensis*) and desert (*C.b. anthonyi*) populations or within the northern (Orange County) and southern (San Diego County) populations of the coastal group. Dispersal has occurred, at least historically, between all populations in this study. However, according to the IBD analysis for both gene regions (CytB and subset ND2; figures 14 and 16), there is a significant isolation by distance within the coastal populations. This indicates a reduced amount of gene flow through dispersal within the coastal populations.

The IBD analysis for Cyt B between the inland desert and coast populations showed a p-value of 0.070, which is not considered significant, but a larger sample size from the inland desert might is necessary to substantiate the results. Atwood and Lerman (2007) found a subtle difference in the vocalizations of Cactus Wren in the desert compared to those in coastal Southern California.

There is no doubt that the Cactus Wren of Southern California are currently in peril. The federal and California state government has recognized this set of populations in Southern California as a federal Bird of Conservation Concern (USFWS, 2008) and as a California Species of Special Concern (Unitt, 2008). Multiple Southern California counties have selected the Cactus Wren as a target subspecies for their natural community conservation plans (e.g., Orange County Natural Communities Conservation Plan [NCCP]/Habitat Conservation Plan [HCP], Western Riverside County Multiple Species Habitat Conservation Plan [MSHCP], Coachella Valley MSHCP, Northwestern San Diego County MSHCP, San Diego County MCP) in Southern California (The Resource Agency, 1993). The loss of Cactus Wren habitat from removal, modification, and degradation coupled with recurring wildfire events has impacted the majority if not all of the Cactus Wren populations in Southern California. Many counties and Mexico have experienced comparable declines in habitat and Cactus Wren numbers (Garrett et al., 2006; Clark and Dodero, 2008; Unitt, 2008; Cooper, 2009). A big problem to Cactus Wren survivability after fire events is that previously burnt cactus scrub does not recover rapidly to a point able to support and sustain necessary resources (e.g., food and shelter) that a breeding Cactus Wren requires within its lifespan (oldest bird recorded is 7 years 4 months [Lutmerding and Love, 2010]). Out of 2,323 acres of cactus habitat in NROC,

1,336 (58%) remained unusable 13 years after the 1993 Laguna Beach fire (Hamilton, 2008), almost double the Cactus Wren lifespan (e.g., 2 generations).

Another hindrance to Cactus Wren survivability is their limited ability or lack of ability to disperse over large tracts of non-cactus-supported land. From this study, Baja peninsula samples exhibit substantial genetic differentiation compared to the Southern California samples. There are 2 samples located approximately 4 km west of Camalu, Baja California Norte, Mexico. These samples are within 209 km of Otay, Bear Valley, and Encanto Canyon in San Diego County. The same haplotype (F) extends south throughout Baja California for over 1,014 (table 10). There were no California haplotypes found below Camalu, Mexico, and no Camalu, Mexico haplotypes found in Southern California. This demonstrates that populations were found geographically close but exhibited a distinct gene flow barrier. The lack of gene flow has been assumed due to the differences seen in morphological characters (Rea and Weaver, 1990; Eggert, 1996) and vocalizations (Atwood and Lerman, 2007), with the disparity in dispersal potential due to lack of cactus scrub habitat. Appropriate cactus scrub habitat does not occur for over a 60 km stretch between Valle Las Palmas to the north and Ojos Negros to the east of Ensenada, Mexico (Clark and Dodero, 2008). This result of a distinct gene flow (i.e., dispersal) barrier due to a lack of suitable habitat is evidence of the lack of dispersal potential of Cactus Wren living in populations with fragmented or isolated habitat areas. It has been shown that Cactus Wren will use artificial nesting structures (Sorrentino, 2007) and even survive translocation attempts (Kamada, 2008), but it appears that the birds from the Baja peninsula will not or cannot disperse the approximately 60 km distance over non-cactus vegetation communities to other areas with suitable habitat.

The Baja California peninsula birds do occupy intermittent territories from Camalu, Mexico, over 1,014 km (630 mi) south to the tip of the Baja peninsula. On the mainland, *C. brunneicapillus* species extends over 320 km north from the US/Mexico border to Ventura County and as far east as Texas. This wide range distribution was supported and, presumably, resulted from the historic presence of a wide range of contiguous cactus dominated habitat. Within the mainland, many of the inland desert sites are over 240 km from the closest coastal population (tables 8 through 11), yet they have evidence of past gene flow (illustrated on figures 7 and 9 by the sharing of the common haplotypes between the inland desert and coastal Southern California populations). Until this past century, contiguous cactus scrub habitat existed that connected the desert to the coast in Southern California, which explains the molecular results in this study. However, the current urbanization of the Southern California landscape over the past few decades, especially in Orange, San Diego, Riverside, and San Bernardino Counties, has created large expanses between populations, many over 240 km, of non-cactus-vegetated lands, much of this area is simply non-vegetated land. Therefore, the extant Cactus Wren populations have been restricted into isolated patches that have a signature of past dispersal and gene flow, but since they possess a low dispersal capability, are currently reproductively and genetically isolated. Thus, these newly isolated populations ($\leq$ 100 years) exhibits a genetic signature of populations that led an historic population expansion from the desert to the coast. Rea and Weaver (1990) deduced that the Cactus Wren followed the formation of coastal sage scrub between 4,000 and 12,000 years. This expansion was most likely bottlenecked through the San Gorgonio pass between the San Jacinto and San Bernardino mountains and is supported by the interpretation of the

nucleotide and haplotype diversity measures reported in this study (table 2). Coastal populations show distinct morphological differences and subtle vocal differences compared to inland desert species. The results from this study found that the populations are not reciprocally monophyletic as they share common haplotypes. However, the study found unique haplotypes restricted to both areas and suggest that genetic differences have either arisen within the two areas or resulted from the pattern of population expansion from the desert to the coastal areas

The extant populations along the coast are isolated to the point where dispersal is either highly hindered, or, for some populations in Los Angeles and Orange Counties, not probable. These populations have a low probability for a natural influx of new genes into the populations. The fragmented coastal populations are highly susceptible to mechanisms of inbreeding depression due to their low effective population size (N_e), short lifespan, and low dispersal potential (Avise, 2000). Human intervention will most likely be needed for "retention and diversification" of the extant gene pool of Cactus Wren in coastal Southern California. The discovery of genetically monomorphic populations (e.g. Viejo-Edison) and populations that contain unique haplotypes (e.g. El Modena) will be beneficial for any decision making process to retain or enhance the molecular diversity of the coastal populations.

Finally, it is prudent to point out that the information contained in this study does not represent the entire genetic variation contained in the Cactus Wren genome let alone the mtDNA genome. The two regions under investigation are protein-coding regions used to carry out functions for life survival. They are likely not related to proteins that function to create the morphological features outlined in Eggert (1996) and that Rea and

Weaver (1990) used to delineate the subspecies within Southern California (figure 4). As warned by Patten (2010), the finding of no differences in the molecular information contained in DNA sequences does not negate the morphological differences found in the tangible features of the bird. If the morphological features written in the genome were able to be investigated, they may show a strong degree of separation, or, as with the two mtDNA gene regions in this study, they may not be significantly variable at all. Regardless, the main intention of this study was to provide baseline genetic information concerning coastal and inland desert populations in Southern California. It is hoped that these data will be used in evaluating existing conservation plans, guiding the preparation of new plans, contributing to the ongoing refinement of habitat and species management objectives, as well as adding new information to the general ornithological knowledge base.

APPENDICES

APPENDIX A

TABLES

TABLE 1. Cactus Wren Population Sample Sizes According to Subspecies Range

Subspecies	Population	Sample Size	Population Acronym [1,2,3]	mtDNA Region(s) Sequenced
sandiegensis	Barker – San Diego City	1	(BA)[3]	CytB
	Bear Valley	7	(BV)[1]	CytB, ND2, CR
	Camp Pendleton	5	(CP)[1]	CytB, ND2, CR
	Dictionary Hill	5	(DH)[1]	CytB, ND2, CR
	Encanto Canyon	6	(EC)[1]	CytB, ND2, CR
	Fallbrook	10	(FB)[1]	CytB, ND2, CR
	Lake Jennings	7	(LJ)[1]	CytB, ND2, CR
	Otay	10	(OT)[1]	CytB, ND2, CR
	Safari Park-Zoo	10	(SP)[1]	CytB, ND2, CR
	Sweetwater Reservoir	8	(SR)[1]	CytB, ND2, CR
	Total	**69**		
anthonyi / sandiegensis	El Modena	7	(EM)[1]	CytB, ND2, CR
	UCI Reserve	8	(UC)[1]	CytB, ND2, CR
	Viejo-Edison	7	(VE)[1]	CytB, ND2, CR
	Total	**22**		
anthonyi	California Inland Desert	15	(ID)[2,3]	CytB, ND2
	Palos Verdes	6	(PV)[3]	ND2
	Total	**21**		
Brunneicapillus/ cousei	Arizona Desert	3	(AZ)[3]	ND2
	Texas Desert	4	(TX)[3]	ND2
	Mexico Mainland	6	(MX)[3]	ND2
	Mexico - Barker	1	(MX)[3]	CytB
	Total	**14**		
bryanti / affinis	Mexico Baja Peninsula	9	(BJ)[3]	ND2
	Total	9		
	Total	**18**		
	Grand Total	**135**		

Note: Sample sizes ranged between 1 and 15 individual birds with a median of 7 and a total of 135 samples. Populations of samples whose DNA were gifted by USGS to this study and analyzed by me are indicated by the exponent number 1 above the population acronym. The number 2 indicates populations of samples whose tissues were loaned by LSUMNS to this study and analyzed by me. The number 3 indicates samples from populations attained through downloading available sequences from the GenBank online database. CytB is a 700 bp fragment of Cytochrome B, ND2 is a 400 bp fragment of the NADH dehydrogenase subunit 2 protein-coding region, and CR are Domains I and III of 298 bp fragments each of the non-protein-coding control region of the mtDNA genome.

TABLE 2. DNA Polymorphism Data for the Cactus Wren CytB and ND2 Gene Regions.

Data	CytB	ND2
Number of sequences	78	121^{A}, 86^{C}
Number of populations	13	18^{A}, 13^{C}
Total number of sites	579	163^{A}, 435^{C}
Alignment gaps or missing data	121	328^{A}, 56^{C}
Invariable sites	572	149^{A}, 426^{C}
Polymorphic sites	7	14^{A}, 9^{C}
Singleton variable sites (two variants)	1	2^{A}, 4^{C}
Parsimony informative sites (two variants)	6	12^{A}, 5^{C}
Segregating sites (S)	7	13^{A}, 9^{C}
Number of mutations (η)	7	13^{A}, 9^{C}
Synonymous changes	0	10^{A}, 6^{C}
Replacement changes	7	3^{A}, 3^{C}
Number of haplotypes	7	7^{A}, 9^{C}
Haplotype diversity (H_d)	0.67532	0.25744^{A}, 0.66129^{C}
Nucleotide diversity (π)	0.00250	0.01031^{A}, 0.00204^{C}
Average nucleotide differences (k)	1.44855	1.68072^{A}, 0.88810^{C}
θ (per sequence) from S (q-W)	1.42060	2.60763^{A}, 1.79078^{C}
Tajima's D	0.04802, $p > 0.10$	-0.94794, $p > 0.10^{A}$ -1.28709, $p > 0.10^{C}$
r (isolation by distance)	0.2907; $p = 0.0800^{CD}$ 0.3621; $p = 0.0100^{C}$*	-0.0184; $p = 0.5600^{A}$ 0.4669; $p = 0.0140^{C}$*
R^2 (reduced major axis)	0.0845^{CD} 0.1310^{C}	0.00033^{A} 0.2180^{C}
Average Φ_{st}	0.0829^{CD} 0.1304^{C}	0.1540^{A} 0.1211^{C}

Note: These results are from analyses conducted in DnaSP version 5 and IBDWS. CytB superscript C = Coastal samples, CD = Coastal and Desert samples. ND2 superscript A = All samples, C = Coastal samples. * indicates statistical significance.

TABLE 3. The Proportion of Each Population into the Seven Identified CytB Haplotypes

Location	CytB Haplotypes							Totals
	A	B	C	D	E	F	G	
Bear Valley	0	1	0	0	0	0	4	5
Percentage (%)	0	20	0	0	0	0	80	100
Dictionary Hill	2	0	0	0	0	0	1	3
Percentage (%)	66.67	0	0	0	0	0	33.33	100
El Modena	0	0	4	0	0	0	3	7
Percentage (%)	0	0	57.14	0	0	0	42.86	100
Encanto Canyon	2	0	0	0	0	0	2	4
Percentage (%)	50	0	0	0	0	0	50	100
Fallbrook	4	2	0	1	0	0	3	10
Percentage (%)	40	20	0	10	0	0	30	100
Lake Jennings	5	0	0	0	0	0	1	6
Percentage (%)	83.33	0	0	0	0	0	16.67	100
Otay	3	0	0	0	0	0	2	5
Percentage (%)	60	0	0	0	0	0	40	100
Camp Pendleton	1	0	0	1	0	0	3	5
Percentage (%)	20	0	0	20	0	0	60	100
California Inland Desert	1	1	0	0	2	1	4	9
Percentage (%)	11.11	11.11	0	0	22.22	11.11	44.44	100
Sweetwater Res.	3	0	0	0	0	0	1	4
Percentage (%)	75	0	0	0	0	0	25	100
UCI Reserve	0	2	0	0	0	0	5	7
Percentage (%)	0	28.6	0	0	0	0	71.4	100
Viejo-Edison	0	0	0	0	0	0	7	7
Percentage (%)	0	0	0	0	0	0	100	100
Safari Park-Zoo	3	1	0	0	0	0	2	6
Percentage (%)	50	16.67	0	0	0	0	33.33	100
GenBank Barker	0	0	1	0	0	0	0	2
Percentage (%)	0	0	100	0	0	0	0	100
Total Individuals	24	7	5	2	2	1	38	80
Percentage (%)	30	8.75	6.25	2.5	2.5	1.25	47.5	100

TABLE 4. The Proportion of Each Population into the Seven Identified ND2 Haplotypes

Location	ND2 Haplotypes							Totals
	A	B	C	D	E	F	G	
Fallbrook	8	1	0	0	0	0	0	9
Percentage (%)	88.9	11.1	0	0	0	0	0	100
Otay	9	1	0	0	0	0	0	10
Percentage (%)	90	10	0	0	0	0	0	100
Sweetwater Res.	7	1	0	0	0	0	0	8
Percentage (%)	87.5	12.5	0	0	0	0	0	100
Inland Desert	7	1	1	1	0	0	0	10
Percentage (%)	70	10	10	10	0	0	0	100
Bear Valley	5	0	0	0	0	0	0	5
Percentage (%)	100	0	0	0	0	0	0	100
Dictionary Hill	5	0	0	0	0	0	0	5
Percentage (%)	100	0	0	0	0	0	0	100
El Modena	7	0	0	0	0	0	0	7
Percentage (%)	100	0	0	0	0	0	0	100
Encanto Canyon	6	0	0	0	0	0	0	6
Percentage (%)	100	0	0	0	0	0	0	100
Lake Jennings	7	0	0	0	0	0	0	7
Percentage (%)	100	0	0	0	0	0	0	100
Camp Pendleton	5	0	0	0	0	0	0	5
Percentage (%)	100	0	0	0	0	0	0	100
UCI Reserve	6	0	0	0	0	0	0	6
Percentage (%)	100	0	0	0	0	0	0	100
Viejo-Edison	7	0	0	0	0	0	0	7
Percentage (%)	100	0	0	0	0	0	0	100
Safari Park-Zoo	8	0	0	0	0	0	0	8
Percentage (%)	100	0	0	0	0	0	0	100
Total Individuals	98	4	1	2	1	8	1	115
Percentage (%)	85.22	3.48	0.87	1.74	0.87	6.96	0.87	100

TABLE 5. The Proportion of Each Population into Nine subset ND2 Haplotypes.

Location	Subset ND2 Haplotypes									Totals
	A	B	C	D	E	F	G	H	I	
Fallbrook	3	0	4	0	1	1	0	0	0	9
Percentage (%)	33.33	0	44.44	0	11.11	11.12	0	0	0	100
Otay	3	2	3	0	0	1	0	0	0	9
Percentage (%)	33.33	22.22	33.33	0	0	11.12	0	0	0	100
Sweetwater Res.	3	0	4	0	0	1	0	0	0	8
Percentage (%)	37.5	0	50	0	0	12.5	0	0	0	100
San Bernardino Lanfair	0	0	1	0	0	0	0	0	0	1
Percentage (%)	0	0	100	0	0	0	0	0	0	100
San Bernardino Needles	0	0	0	0	0	0	0	0	1	1
Percentage (%)	0	0	0	0	0	0	0	0	100	100
San Bernardino Goffs	0	0	0	0	0	0	0	1	0	1
Percentage (%)	0	0	0	0	0	0	0	100	0	100
Bear Valley	3	1	1	0	0	0	0	0	0	5
Percentage (%)	60	20	20	0	0	0	0	0	0	100
Dictionary Hill	2	0	3	0	0	0	0	0	0	5
Percentage (%)	40	0	60	0	0	0	0	0	0	100
El Modena	3	0	0	4	0	0	0	0	0	7
Percentage (%)	42.86	0	0	57.14	0	0	0	0	0	100
Encanto Canyon	3	0	3	0	0	0	0	0	0	6
Percentage (%)	50	0	50	0	0	0	0	0	0	100
Lake Jennings	1	0	5	0	0	0	1	0	0	7
Percentage (%)	14.29	0	71.42	0	0	0	14.29	0	0	100
Camp Pendleton	3	0	1	0	1	0	0	0	0	5
Percentage (%)	60	0	20	0	20	0	0	0	0	100
UCI Reserve	1	0	5	0	0	0	0	0	0	6
Percentage (%)	16.67	0	83.33	0	0	0	0	0	0	100
Viejo-Edison	7	0	0	0	0	0	0	0	0	7
Percentage (%)	100	0	0	0	0	0	0	0	0	100
Safari Park-Zoo	4	0	5	0	0	0	0	0	0	9
Percentage (%)	44.44	0	55.56	0	0	0	0	0	0	100
Total Individuals	36	3	35	4	2	3	1	1	1	86
Percentage (%)	41.86	3.49	40.70	4.65	2.33	3.49	1.16	1.16	1.16	100

TABLE 6. List of Samples with Location, Subspecies, and Haplotype Identification for Both CytB and ND2 Regions

Sample ID	Subspecies (Rea and Weaver, 1990)	Location ID	County	ND2 Haplotype ID	CytB Haplotype ID
Barker-GenBank-Cytochrome B	*sandiegensis*	San Diego City	San Diego	N/A	C
Barker-GenBank-MZAH9648 México: Querétaro; Tequisquiapan, 2 km south of Estación	*cousei*	Mexico Mainland	Mexico	N/A	H
Bear Valley 1801-98439	*sandiegensis*	Bear Valley	San Diego	N/A	G
Bear Valley 871-57385	*sandiegensis*	Bear Valley	San Diego	A	N/A
Bear Valley 1801-98441	*sandiegensis*	Bear Valley	San Diego	A	N/A
Bear Valley 1801-98442	*sandiegensis*	Bear Valley	San Diego	A	G
Bear Valley 1801-98443	*sandiegensis*	Bear Valley	San Diego	A	G
Bear Valley 1801-98444	*sandiegensis*	Bear Valley	San Diego	A	B
Bear Valley 871-57385	*sandiegensis*	Bear Valley	San Diego	N/A	G
Riverside 41902	*anthonyi*	CA Inland Desert	Riverside	N/A	G
San Bernardino 5834	*anthonyi*	CA Inland Desert	San Bernardino	A	G
San Bernardino 5835	*anthonyi*	CA Inland Desert	San Bernardino	N/A	G
San Bernardino 21565	*anthonyi*	CA Inland Desert	San Bernardino	A	G
San Bernardino 21645	*anthonyi*	CA Inland Desert	San Bernardino	N/A	A
San Bernardino 21647	*anthonyi*	CA Inland Desert	San Bernardino	A	B
San Bernardino 23158	*anthonyi*	CA Inland Desert	San Bernardino	A	F
San Bernardino 23105	*anthonyi*	CA Inland Desert	San Bernardino	N/A	E
San Bernardino 23236	*anthonyi*	CA Inland Desert	San Bernardino	N/A	E
San Bernardino 31139	*anthonyi*	CA Inland Desert	San Bernardino	C	N/A
CAWR11CA USA, California Imperial Co. 3 mi SW Calipatria	*anthonyi*	CA Inland Desert	Imperial	A	N/A

TABLE 6. Continued

Sample ID	Subspecies (Rea and Weaver, 1990)	Location ID	County	ND2 Haplotype ID	CytB Haplotype ID
CAWR12CA USA, California Imperial Co. 3 mi SW Calipatria	*anthonyi*	CA Inland Desert	Imperial	A	N/A
CAWR13CA USA, California Imperial Co. 3 mi SW Calipatria	*anthonyi*	CA Inland Desert	Imperial	A	N/A
CAWR9CA USA, California Imperial Co. E side Finney Lake 5 mi. NE Brawley	*anthonyi*	CA Inland Desert	Imperial	B	N/A
CAWR10CAUSA, California Imperial Co. E side Finney Lake 5 mi. NE Brawley	*anthonyi*	CA Inland Desert	Imperial	D	N/A
Pendleton 851-60600	*sandiegensis*	Camp Pendleton	San Diego	A	G
Pendleton 1801-98411	*sandiegensis*	Camp Pendleton	San Diego	A	G
Pendleton 1801-98424	*sandiegensis*	Camp Pendleton	San Diego	A	D
Pendleton 1801-98472	*sandiegensis*	Camp Pendleton	San Diego	A	G
Pendleton 1801-98484	*sandiegensis*	Camp Pendleton	San Diego	A	A
Dictionary Hill 871-57325	*sandiegensis*	Dictionary Hill	San Diego	A	N/A
Dictionary Hill 1801-98458	*sandiegensis*	Dictionary Hill	San Diego	A	G
Dictionary Hill 1801-98460	*sandiegensis*	Dictionary Hill	San Diego	A	A
Dictionary Hill 2531-97353	*sandiegensis*	Dictionary Hill	San Diego	A	N/A
Dictionary Hill 2531-97355	*sandiegensis*	Dictionary Hill	San Diego	A	A
El Modena 168187873	*anthonyi/sandiegensis*	El Modena	Orange	A	C
El Modena 168187879	*anthonyi/sandiegensis*	El Modena	Orange	A	C
El Modena 168187882	*anthonyi/sandiegensis*	El Modena	Orange	A	G
El Modena 168187886	*anthonyi/sandiegensis*	El Modena	Orange	A	G

TABLE 6. Continued

Sample ID	Subspecies (Rea and Weaver, 1990)	Location ID	County	ND2 Haplotype ID	CytB Haplotype ID
El Modena 168187889	*anthonyi/sandiegensis*	El Modena	Orange	A	C
El Modena 168187892	*anthonyi/sandiegensis*	El Modena	Orange	A	C
El Modena 168187895	*anthonyi/sandiegensis*	El Modena	Orange	A	G
Encanto Canyon 871-57343	*sandiegensis*	Encanto Canyon	San Diego	A	A
Encanto Canyon 1801-98451	*sandiegensis*	Encanto Canyon	San Diego	A	G
Encanto Canyon 1801-98499	*sandiegensis*	Encanto Canyon	San Diego	A	N/A
Encanto Canyon 2531-97341	*sandiegensis*	Encanto Canyon	San Diego	A	G
Encanto Canyon 2531-97350	*sandiegensis*	Encanto Canyon	San Diego	A	N/A
Encanto Canyon EC4N1	*sandiegensis*	Encanto Canyon	San Diego	A	A
Fallbrook 1 NWS 851-60570	*sandiegensis*	Fallbrook	San Diego	A	G
Fallbrook 1 NWS 871-57308	*sandiegensis*	Fallbrook	San Diego	A	G
Fallbrook 1 NWS 871-57312	*sandiegensis*	Fallbrook	San Diego	N/A	B
Fallbrook 1 NWS 1801-98421	*sandiegensis*	Fallbrook	San Diego	A	D
Fallbrook 2 NWS 851-60576	*sandiegensis*	Fallbrook	San Diego	A	A
Fallbrook 2 NWS 851-60586	*sandiegensis*	Fallbrook	San Diego	A	A
Fallbrook 2 NWS 851-60587	*sandiegensis*	Fallbrook	San Diego	A	A
Fallbrook 2 NWS 851-60596	*sandiegensis*	Fallbrook	San Diego	A	B
Fallbrook 2 NWS 1801-98408	*sandiegensis*	Fallbrook	San Diego	B	A
Fallbrook 2 NWS 1801-98427	*sandiegensis*	Fallbrook	San Diego	A	G
Lake Jennings 871-57317	*sandiegensis*	Lake Jennings	San Diego	A	A
Lake Jennings 871-57365	*sandiegensis*	Lake Jennings	San Diego	A	G
Lake Jennings 871-57390	*sandiegensis*	Lake Jennings	San Diego	A	A

TABLE 6. Continued

Sample ID	Subspecies (Rea and Weaver, 1990)	Location ID	County	ND2 Haplotype ID	CytB Haplotype ID
Lake Jennings 871-57397	*sandiegensis*	Lake Jennings	San Diego	A	A
Lake Jennings 871-57398	*sandiegensis*	Lake Jennings	San Diego	A	N/A
Lake Jennings 871-57399	*sandiegensis*	Lake Jennings	San Diego	A	A
Lake Jennings 1801-98433	*sandiegensis*	Lake Jennings	San Diego	A	A
Otay 871-57336	*sandiegensis*	Otay	San Diego	A	A
Otay 1801-98446	*sandiegensis*	Otay	San Diego	A	G
Otay 1801-98448	*sandiegensis*	Otay	San Diego	A	N/A
Otay 1801-98454	*sandiegensis*	Otay	San Diego	A	A
Otay 1801-98455	*sandiegensis*	Otay	San Diego	A	N/A
Otay 1801-98456	*sandiegensis*	Otay	San Diego	A	N/A
Otay 1801-98491	*sandiegensis*	Otay	San Diego	A	N/A
Otay 1801-98495	*sandiegensis*	Otay	San Diego	A	G
Otay 1801-98497	*sandiegensis*	Otay	San Diego	B	A
Otay 1801-98498	*sandiegensis*	Otay	San Diego	A	N/A
Sweetwater Reservoir 871-57301	*sandiegensis*	Sweetwater Reservoir	San Diego	B	A
Sweetwater Reservoir 871-57321	*sandiegensis*	Sweetwater Reservoir	San Diego	A	A
Sweetwater Reservoir 871-57345	*sandiegensis*	Sweetwater Reservoir	San Diego	A	A
Sweetwater Reservoir 871-57348	*sandiegensis*	Sweetwater Reservoir	San Diego	A	N/A
Sweetwater Reservoir 1801-98452	*sandiegensis*	Sweetwater Reservoir	San Diego	A	N/A
Sweetwater Reservoir 1801-98453	*sandiegensis*	Sweetwater Reservoir	San Diego	A	G
Sweetwater Reservoir 2531-97357	*sandiegensis*	Sweetwater Reservoir	San Diego	A	N/A
Sweetwater Reservoir 2531-97358	*sandiegensis*	Sweetwater Reservoir	San Diego	A	N/A

TABLE 6. Continued

Sample ID	Subspecies (Rea and Weaver, 1990)	Location ID	County	ND2 Haplotype ID	CytB Haplotype ID
Laguna Canyon Road	*anthonyi/sandiegensis*	UCI Reserve	Orange	A	G
UCI 168187813	*anthonyi/sandiegensis*	UCI Reserve	Orange	A	G
UCI 178175815	*anthonyi/sandiegensis*	UCI Reserve	Orange	N/A	G
UCI 168187875	*anthonyi/sandiegensis*	UCI Reserve	Orange	A	B
UCI 168187877	*anthonyi/sandiegensis*	UCI Reserve	Orange	A	B
UCI 178175815	*anthonyi/sandiegensis*	UCI Reserve	Orange	A	N/A
UCI 178175823	*anthonyi/sandiegensis*	UCI Reserve	Orange	A	G
UCI 178175836	*anthonyi/sandiegensis*	UCI Reserve	Orange	A	G
Viejo 178175818	*anthonyi/sandiegensis*	Viejo-Edison	Orange	A	G
Viejo 178175827	*anthonyi/sandiegensis*	Viejo-Edison	Orange	A	G
Viejo 178175831	*anthonyi/sandiegensis*	Viejo-Edison	Orange	A	G
Viejo 178175834	*anthonyi/sandiegensis*	Viejo-Edison	Orange	A	G
Viejo 178175843	*anthonyi/sandiegensis*	Viejo-Edison	Orange	N/A	G
Viejo 178175847	*anthonyi/sandiegensis*	Viejo-Edison	Orange	A	G
Viejo 178175853	*anthonyi/sandiegensis*	Viejo-Edison	Orange	A	N/A
Viejo 178175855	*anthonyi/sandiegensis*	Viejo-Edison	Orange	A	G
Zoo 851-60595	*sandiegensis*	Safari Park	San Diego	A	A
Zoo 871-57353	*sandiegensis*	Safari Park	San Diego	A	N/A
Zoo 871-57354	*sandiegensis*	Safari Park	San Diego	A	B
Zoo 871-57357	*sandiegensis*	Safari Park	San Diego	A	N/A
Zoo 871-57368	*sandiegensis*	Safari Park	San Diego	N/A	G
Zoo 871-57383	*sandiegensis*	Safari Park	San Diego	A	N/A

TABLE 6. Continued

Sample ID	Subspecies (Rea and Weaver, 1990)	Location ID	County	ND2 Haplotype ID	CytB Haplotype ID
Zoo 871-57386	*sandiegensis*	Safari Park	San Diego	A	N/A
Zoo 871-57387	*sandiegensis*	Safari Park	San Diego	A	A
Zoo 1501-82104	*sandiegensis*	Safari Park	San Diego	A	A
Zoo WAP01tp	*sandiegensis*	Safari Park	San Diego	A	G
CAWR36PV USA, California Palos Verdes	*anthonyi*	Palos Verdes	Los Angeles	A	N/A
CAWR37PV USA, California Palos Verdes	*anthonyi*	Palos Verdes	Los Angeles	A	N/A
CAWR38PV USA, California Palos Verdes	*anthonyi*	Palos Verdes	Los Angeles	A	N/A
CAWR39PV USA, California Palos Verdes	*anthonyi*	Palos Verdes	Los Angeles	A	N/A
CAWR40PV USA, California Palos Verdes	*anthonyi*	Palos Verdes	Los Angeles	A	N/A
CAWR41PV USA, California Palos Verdes	*anthonyi*	Palos Verdes	Los Angeles	A	N/A
CAWR6AZ USA, Arizona Cochise Co. 4 mi. N Portal	*cousei*	Arizona Desert	Cochise	A	N/A
CAWR8AZ USA, Arizona Cochise Co. 4 mi. N Portal	*cousei*	Arizona Desert	Cochise	A	N/A
CAWR7AZ USA, Arizona Cochise Co. 4 mi. N Portal	*cousei*	Arizona Desert	Cochise	D	N/A
CAWR2TX USA, Texas Big Bend	*cousei*	Texas Desert	n/a	A	N/A
CAWR3TX USA, Texas Ravine	*cousei*	Texas Desert	n/a	A	N/A
CAWR4TX USA, Texas Crest III - I13	*cousei*	Texas Desert	n/a	A	N/A

TABLE 6. Continued

Sample ID	Subspecies (Rea and Weaver, 1990)	Location ID	County	ND2 Haplotype ID	CytB Haplotype ID
CAWR5TX USA, Texas Lunada	*cousei*	Texas Desert	n/a	A	N/A
CAWR14CH Mexico, Chihuahua WSW Chihuahua on Rte 16	*cousei*	Mexico Mainland	Mexico	A	N/A
CAWR74CH Mexico, Chihuahua 41 km. WSW Chihuahua on Rte.16	*cousei*	Mexico Mainland	Mexico	A	N/A
CAWR47CH Mexico, Chihuahua 41 km. WSW Chihuahua on Rte.16	*cousei*	Mexico Mainland	Mexico	A	N/A
CAWR68DU Mexico, Durango 18 km. W Durango on Rte 45	*cousei*	Mexico Mainland	Mexico	A	N/A
CAWR18SO Mexico, Sonora Lo de Campo 16.5 mi. ESE Tecaripa	*anthonyi*	Mexico Mainland	Mexico	A	N/A
CAWR15CH Mexico, Chihuahua 41km. WSW Chihuahua on Rte16	*cousei*	Mexico Mainland	Mexico	E	N/A
CAWR27SR Mexico, Baja Norte 3.5 mi. E Bahia Santa Rosalia	*bryanti*	Mexico Baja Peninsula	Mexico	F	N/A
CAWR55CL Mexico, Baja Norte 2 mi. W Camalu	*bryanti*	Mexico Baja Peninsula	Mexico	F	N/A
CAWR64MSF Mexico, Baja Norte 21.5 mi. NW Mission San Fernando on Rte. 1	*bryanti*	Mexico Baja Peninsula	Mexico	F	N/A
CAWR31LP Mexico, Baja Sur 18 km. S La Paz on Rte. 1	*bryanti*	Mexico Baja Peninsula	Mexico	F	N/A
CAWR29SI Mexico, Baja Sur 26.7 mi.by road (Rte.1) N San Ignacio	*bryanti*	Mexico Baja Peninsula	Mexico	F	N/A
CAWR78SI Mexico, Baja Sur San Ignacio	*bryanti*	Mexico Baja Peninsula	Mexico	F	N/A

TABLE 6. Continued

Sample ID	Subspecies (Rea and Weaver, 1990)	Location ID	County	ND2 Haplotype ID	CytB Haplotype ID
CAWR1442TS Mexico, Baja Sur 7.5 mi. E Todos Santos	*bryanti*	Mexico Baja Peninsula	Mexico	F	N/A
CAWR3773SA Mexico, Baja Sur 7 mi. N Santa Anita	*bryanti*	Mexico Baja Peninsula	Mexico	F	N/A
CAWR80VI Mexico, Baja Sur Villa Insurgente	*bryanti*	Mexico Baja Peninsula	Mexico	G	N/A

TABLE 7. List of mtDNA Primers Used for Sequencing

Region	Coding Region	Primers Used	Sequence	Author
Control Region Domain I	Non-coding region	L16743	5' - CTCTCCAGGAACAAC GGCCC- 3'	Tarr 1995
Control Region Domain I	Non-coding region	H417	5' - AGTAGCTCGGTTCTC GTGAG- 3'	Tarr 1995
Control Region Domain III	Non-coding region	F304	5' - CTTGACACTGATGCA CTTTG- 3'	Baker and Marshall 1997
Control Region Domain III	Non-coding region	H1261	5' - AGGTACCATCTTGGC ATCTTC- 3'	Baker and Marshall 1997
CytB	Protein-coding region	B1a-CytB	5' - CCATCCAACATCTCA GCATGATGAAA- 3'	Barker 1994
CytB	Protein-coding region	B8-CytB	5'- GGAGTCTTCAGTCTCT GGTTTACAAGAC- 3'	Barker 1994
ND2	Protein-coding region	L5215	5' - TATCGGGCCCATACC CCGAAAAT- 3'	Hackett 1996
ND2	Protein-coding region	ND2-Rev	5' - GGAGATKGAGGAGAA GGCTA- 3'	Vasquez 2009

TABLE 8. CytB Genetic Distances (Φ_{st}) and Geographic Distance (km) Among Coastal and Inland Desert Populations.

Population	BV	DH	EM	EC	FB	LJ	OT	CP	ID	SR	UC	VE	SP
BV	0.000	11	143	10	83	25	10	76	309	9	133	126	49
DH	0.000	0.000	132	9	72	17	19	64	303	3	122	114	39
EM	0.206	0.188	0.000	136	63	126	148	68	267	134	17	18	101
EC	0.000	0.000	0.213	0.000	78	27	12	69	312	8	125	119	47
FB	0.182	0.000	0.229	0.000	0.000	63	90	19	261	75	58	45	37
LJ	0.379	0.000	0.282	0.100	0.000	0.000	35	60	285	19	118	108	27
OT	0.101	0.000	0.234	0.000	0.000	0.046	0.000	80	319	16	137	131	57
CP	0.000	0.000	0.194	0.000	0.000	0.176	0.000	0.000	280	67	58	50	38
ID	0.000	0.245	0.082	0.289	0.218	0.303	0.293	0.223	0.000	304	281	264	270
SR	0.187	0.000	0.233	0.000	0.000	0.000	0.000	0.000	0.274	0.000	124	117	41
UC	0.000	0.004	0.178	0.081	0.038	0.001	0.127	0.117	0.010	0.054	0.000	18	94
VE	0.119	0.468	0.395	0.362	0.404	0.650	0.248	0.231	0.572	0.417	0.431	0.000	82
SP	0.107	0.000	0.211	0.000	0.000	0.080	0.000	0.000	0.168	0.000	0.015	0.311	0.000

Note: Analysis was conducted in IBD for F_{st}, an estimator of population differentiation. The genetic distance is on bottom and the geographic distance is on top. The greatest difference was observed within Viejo-Edison (VE) in Orange County and all the other populations (highlighted). Population names are abbreviated in the table as follows: BV = Bear Valley, DH = Dictionary Hill, EM = El Modena, EC = Encanto Canyon, FB = Fallbrook, LJ = Lake Jennings, OT = Otay, CP = Camp Pendleton, ID = California Inland Desert, SR = Sweetwater Reservoir, UC = UCI Reserve, VE = Viejo-Edison, SP = Safari Park-Zoo.

TABLE 9. CytB Genetic Distances (Φ_{st}) and Geographic Distances (km) Among Coastal Populations.

Population	BV	DH	EM	EC	FB	LJ	OT	CP	SR	UC	VE	SP
BV	0.000	11	143	10	83	25	10	76	9	133	126	49
DH	0.000	0.000	132	9	72	17	19	64	3	122	114	39
EM	0.206	0.000	0.000	136	63	126	148	68	134	17	18	101
EC	0.000	0.000	0.015	0.000	78	27	12	69	8	125	119	47
FB	0.182	0.000	0.111	0.000	0.000	63	90	19	75	58	45	37
LJ	0.379	0.000	0.179	0.033	0.000	0.000	35	60	19	118	108	27
OT	0.101	0.000	0.043	0.000	0.000	0.000	0.000	80	16	137	131	57
CP	0.000	0.000	0.074	0.000	0.000	0.138	0.000	0.000	67	58	50	38
SR	0.187	0.000	0.075	0.000	0.000	0.000	0.000	0.000	0.000	124	117	41
UC	0.000	0.000	0.150	0.000	0.049	0.271	0.000	0.000	0.125	0.000	18	94
VE	0.149	0.717	0.525	0.515	0.496	0.829	0.599	0.256	0.781	0.262	0.000	82
SP	0.107	0.000	0.087	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.586	0.000

Note: Analysis was conducted in IBD for Φ_{st}, an estimator of population differentiation. The genetic distance is on bottom and the geographic distance is on top. The greatest difference was observed within Viejo-Edison (VE) in Orange County and all the other populations (highlighted). Population names are abbreviated in the table as follows: BV = Bear Valley, DH = Dictionary Hill, EM = El Modena, EC = Encanto Canyon, FB = Fallbrook, LJ = Lake Jennings, OT = Otay, CP = Camp Pendleton, ID = California Inland Desert, SR = Sweetwater Reservoir, UC = UCI Reserve, VE = Viejo-Edison, SP = Safari Park-Zoo.

TABLE 10. ND2 Genetic Distances (Φ_{st}) and Geographic Distances (Km) Among Coastal and Inland Desert Populations

Population	BV	DH	EM	EC	FB	LJ	OT	CP	ID	SR	UC	VE	SP	AZ	PV	MX	TX	BC
BV	0.000	11	143	10	83	25	10	76	309	9	133	126	49	700	171	1071	1683	150
DH	0.000	0.000	132	9	72	17	19	64	303	3	122	114	39	705	161	1079	1689	154
EM	0.020	0.000	0.000	136	63	126	148	68	267	134	17	18	101	789	50	1188	1772	255
EC	0.000	0.000	0.081	0.000	78	27	12	69	312	8	125	119	47	710	162	1081	1693	159
FB	0.025	0.000	0.049	0.036	0.000	63	90	19	261	75	58	45	37	737	103	1128	1720	194
LJ	0.217	0.000	0.194	0.207	0.000	0.000	35	60	285	19	118	108	27	696	159	1075	1680	146
OT	0.000	0.000	0.033	0.000	0.000	0.096	0.000	80	319	16	137	131	57	706	173	1073	1688	155
CP	0.000	0.000	0.000	0.000	0.000	0.129	0.000	0.000	280	67	58	50	38	746	100	1133	1730	200
ID	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	304	281	264	270	605	312	1048	1564	242
SR	0.061	0.000	0.139	0.013	0.000	0.000	0.004	0.032	0.000	0.000	124	117	41	705	163	1077	1688	154
UC	0.081	0.000	0.118	0.067	0.000	0.000	0.017	0.028	0.000	0.000	0.000	18	94	792	46	1186	1775	252
VE	0.000	0.131	0.117	0.000	0.165	0.372	0.054	0.017	0.000	0.212	0.233	0.000	82	774	63	1171	1757	237
SP	0.000	0.000	0.014	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.052	0.000	710	137	1095	1694	162
AZ	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	837	463	984	551
PV	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.117	0.000	0.000	0.000	0.000	0.000	0.000	1231	1820	298
MX	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	723	934
TX	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.000	0.000	1534
BC	0.568	0.535	0.460	0.626	0.461	0.621	0.467	0.481	0.537	0.607	0.575	0.643	0.447	0.813	0.856	0.714	0.566	0.000

Note: Analysis was conducted in IBD for Φ_{st}, an estimator of population differentiation. The greatest distance was observed between the Baja California (BC) population of Mexico and all the other populations (highlighted). The lowest distances occurred between all mainland populations. Population names are abbreviated in the table as follows: BV = Bear Valley, DH = Dictionary Hill, EM = El Modena, EC = Encanto Canyon, FB = Fallbrook, LJ = Lake Jennings, OT = Otay, CP = Camp Pendleton, ID = California Inland Desert, SR = Sweetwater Reservoir, UC = UCI Reserve, VE = Viejo-Edison, SP = Safari Park-Zoo, AZ= Arizona, PV= Palos Verdes, MX= Mainland Mexico, TX= Texas, BC= Baja California.

TABLE 11. ND2 Genetic Distances (Φ_{st}) and Geographic Distances (Km) Among Coastal Populations.

Population	BV	DH	EM	EC	FB	LJ	OT	CP	ID	SR	UC	VE	SP
BV	0.000	11	143	10	83	25	10	76	309	9	133	126	49
DH	0.061	0.000	132	9	72	17	19	64	303	3	122	114	39
EM	0.250	0.188	0.000	136	63	126	148	68	267	134	17	18	101
EC	0.000	0.000	0.213	0.000	78	27	12	69	312	8	125	119	47
FB	0.128	0.000	0.229	0.000	0.000	63	90	19	261	75	58	45	37
LJ	0.318	0.000	0.282	0.100	0.000	0.000	35	60	285	19	118	108	27
OT	0.000	0.000	0.234	0.000	0.000	0.046	0.000	80	319	16	137	131	57
CP	0.000	0.000	0.194	0.000	0.000	0.176	0.000	0.000	280	67	58	50	38
ID	0.384	0.245	0.082	0.289	0.218	0.303	0.293	0.223	0.000	304	281	264	270
SR	0.108	0.000	0.233	0.000	0.000	0.000	0.000	0.000	0.274	0.000	124	117	41
UC	0.214	0.004	0.178	0.081	0.038	0.001	0.127	0.117	0.010	0.054	0.000	18	94
VE	0.086	0.468	0.395	0.362	0.404	0.650	0.248	0.231	0.572	0.417	0.431	0.000	82
SP	0.064	0.000	0.211	0.000	0.000	0.080	0.000	0.000	0.168	0.000	0.015	0.311	0.000

Note: Analysis was conducted in IBD for Φ_{st}, an estimator of population differentiation. The genetic distance is on bottom and the geographic distance is on top. The greatest distance was observed between the Baja California (BC) population of Mexico and all the other populations (highlighted). Population names are abbreviated in the table as follows: BV = Bear Valley, DH = Dictionary Hill, EM = El Modena, EC = Encanto Canyon, FB = Fallbrook, LJ = Lake Jennings, OT = Otay, CP = Camp Pendleton, ID = California Inland Desert, SR = Sweetwater Reservoir, UC = UCI Reserve, VE = Viejo-Edison, SP = Safari Park-Zoo.

APPENDIX B

FIGURES

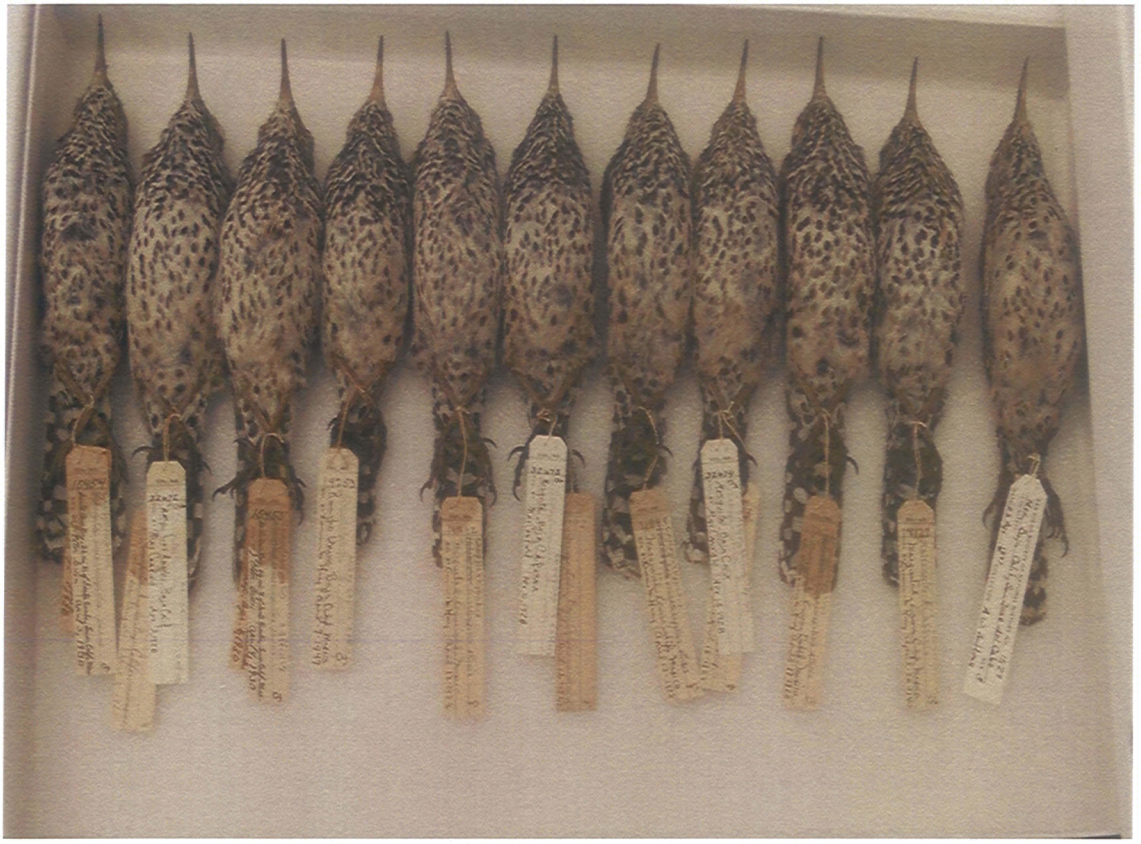

FIGURE 1. Photograph of Cactus Wren from the *C.b. bryanti* subspecies. The primary morphological features used by taxonomists to differentiate Cactus Wren are speckling in the chin/gular area, chest spot shape, abdominal spotting; chest patch aggregation, color differences in the flank/abdominal and back, and barring differences in the tail. Most notably, the *C.b. bryanti* subspecies has linearized speckling in the chin, large chest spots, and reduced color in the flank/abdominal region.

FIGURE 2. Photograph of Cactus Wren from the *C.b. anthonyi* subspecies. The primary morphological features used by taxonomists to differentiate Cactus Wren are speckling in the chin/gular area, chest spot shape, abdominal spotting; chest patch aggregation, color differences in the flank/abdominal and back, and barring differences in the tail. Most notably, the *C.b. anthonyi* subspecies has blotchy speckling in the chin, tiny chest spots, and an orange/rufous color in the flank/abdominal region.

FIGURE 3. Photograph of Cactus Wren from the *C.b. sandiegensis* subspecies. The primary morphological features used by taxonomists to differentiate Cactus Wren are speckling in the chin/gular area, chest spot shape, abdominal spotting; chest patch aggregation, color differences in the flank/abdominal and back, and barring differences in the tail. *C.b. sandiegensis* is a mixture of the *C.b bryanti* and *C.b. anthonyi* phenotypes, with a single bird having a combination of morphological characters that resemble both subspecies.

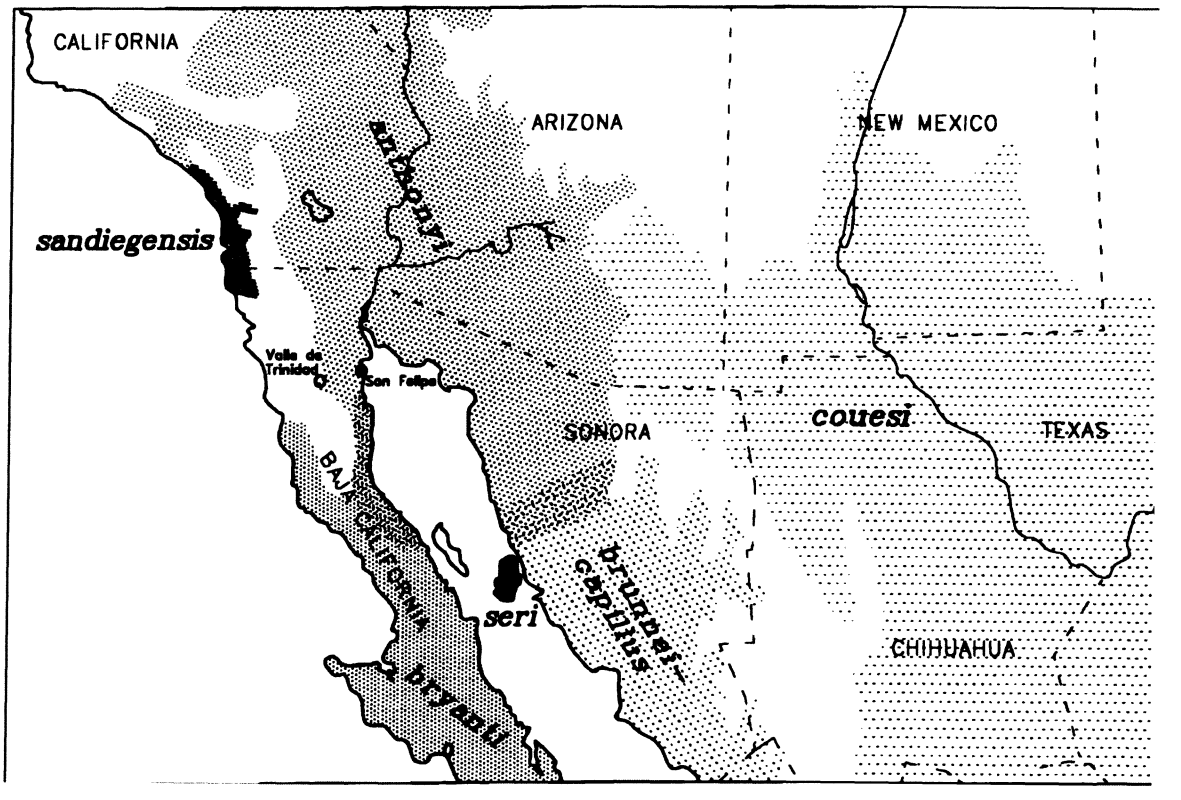

FIGURE 4: A regional map extracted from Rea and Weaver (1990) illustrating their proposed distribution of Cactus Wren subspecies in the southwest United States and Mexico.

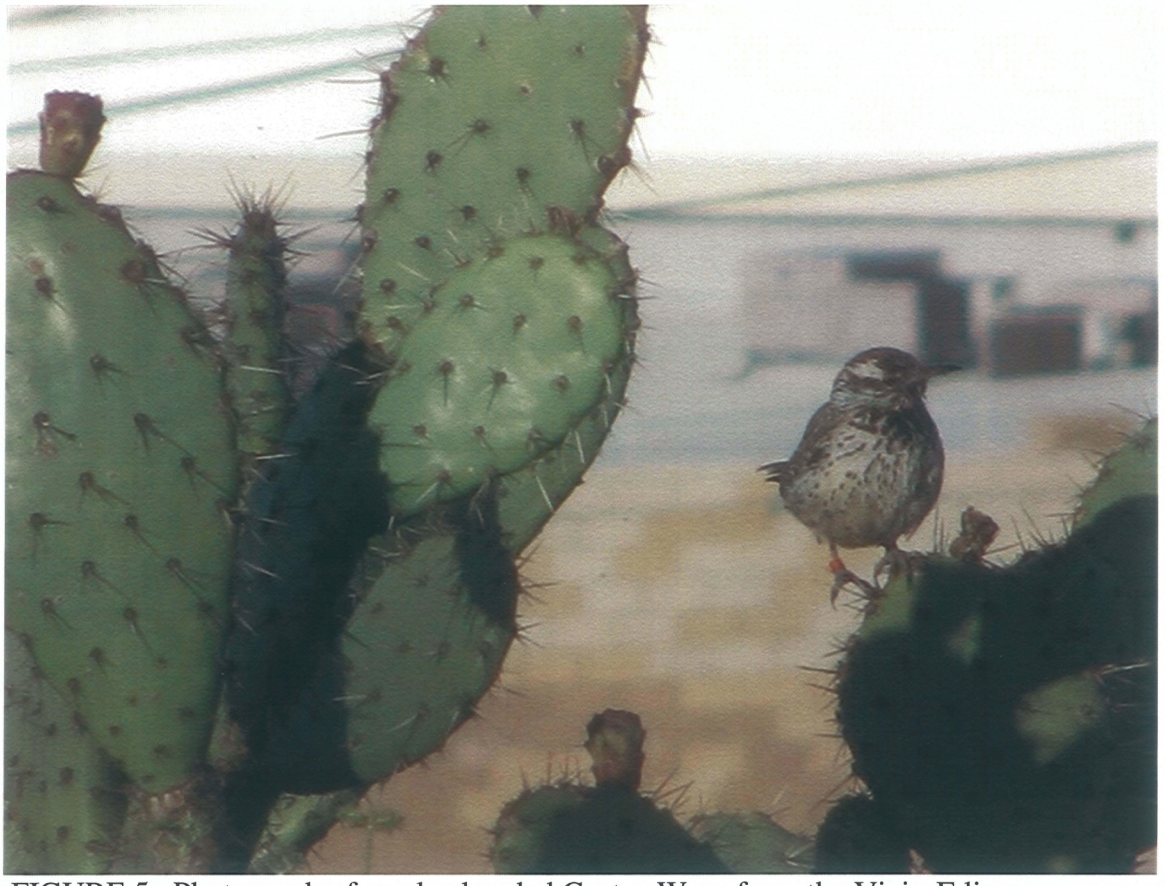

FIGURE 5. Photograph of a color-banded Cactus Wren from the Viejo-Edison population. Photograph courtesy of Eric Krieg, LSA Associates, Inc.

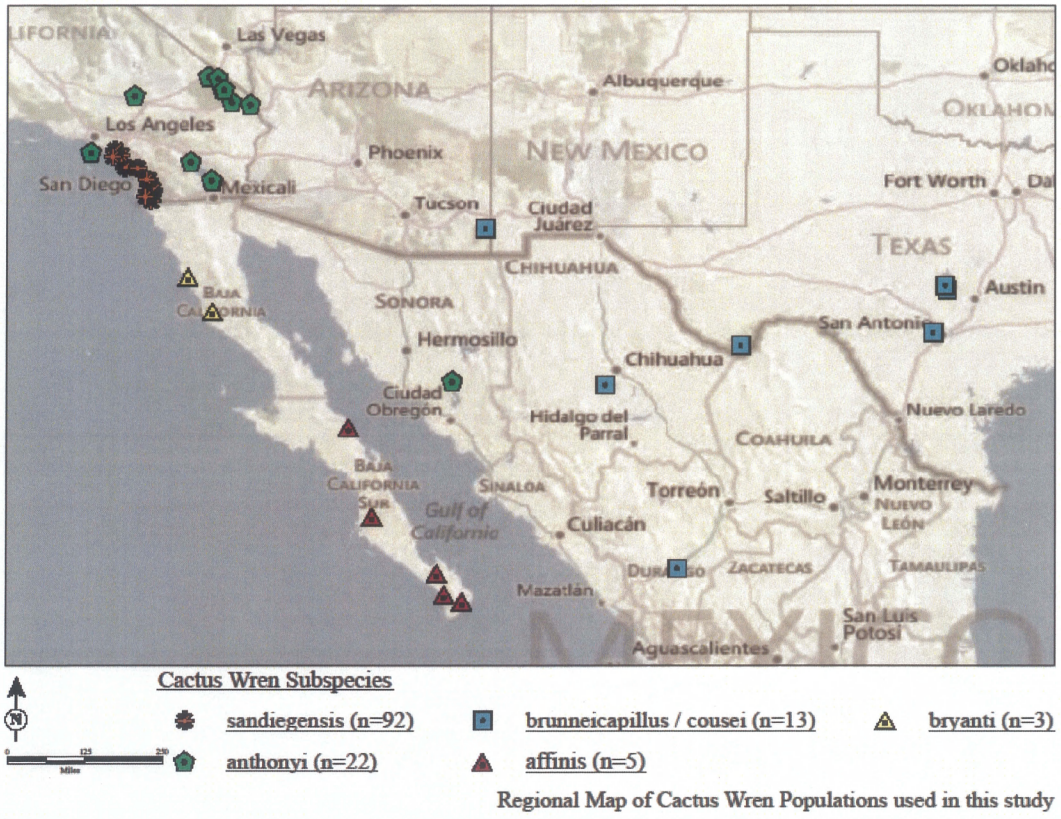

FIGURE 6. A regional map of Cactus Wren individuals analyzed in this study. The locations are derived from southern California, Arizona, Texas, and Mexico. The samples are arranged by subspecies and labeled according to the distribution set forth in Rea and Weaver (1990) (see Figure 4).

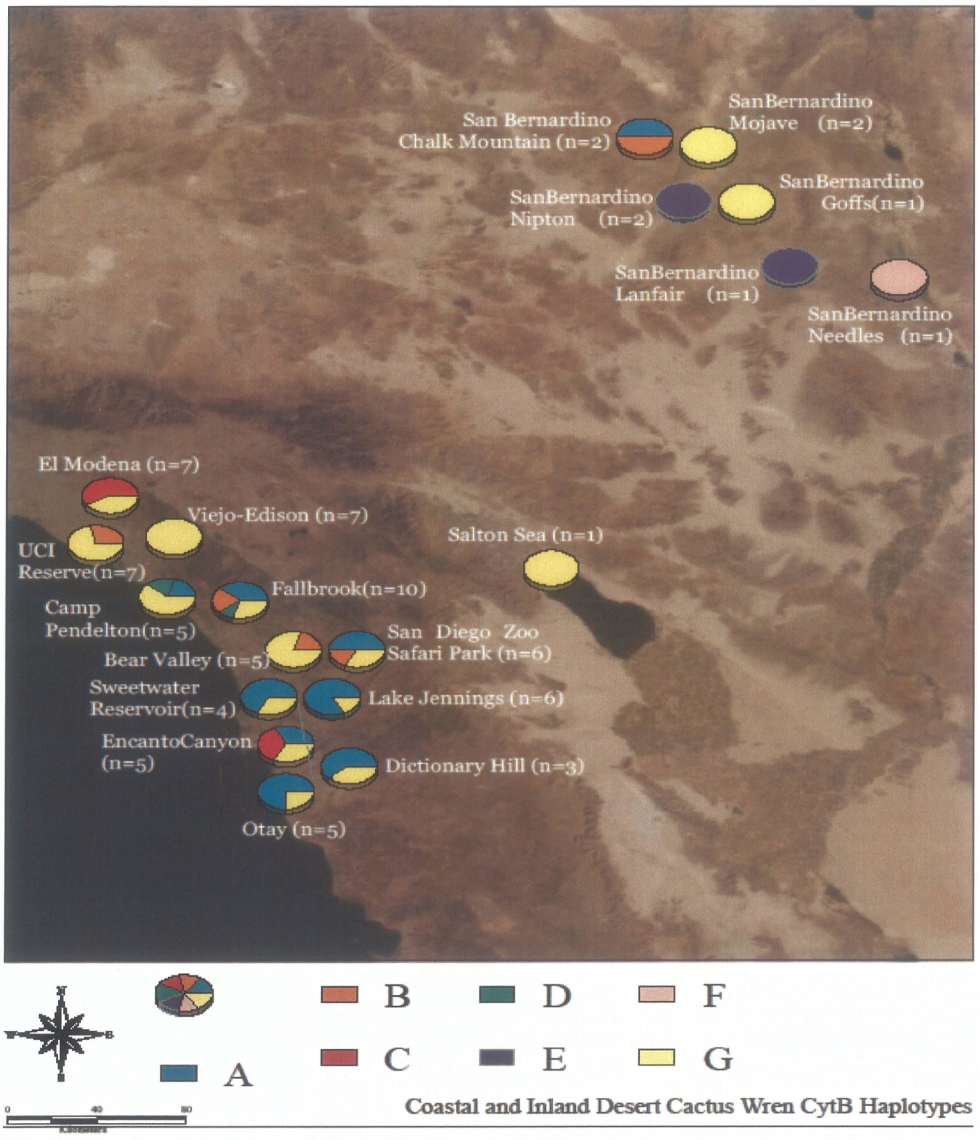

FIGURE 7. Map of Cactus Wren populations in southern California analyzed for the mtDNA CytB region. Seven haplotypes are represented by color code, and the assignment and proportion of each haplotype within the population is represented by the pie symbol.

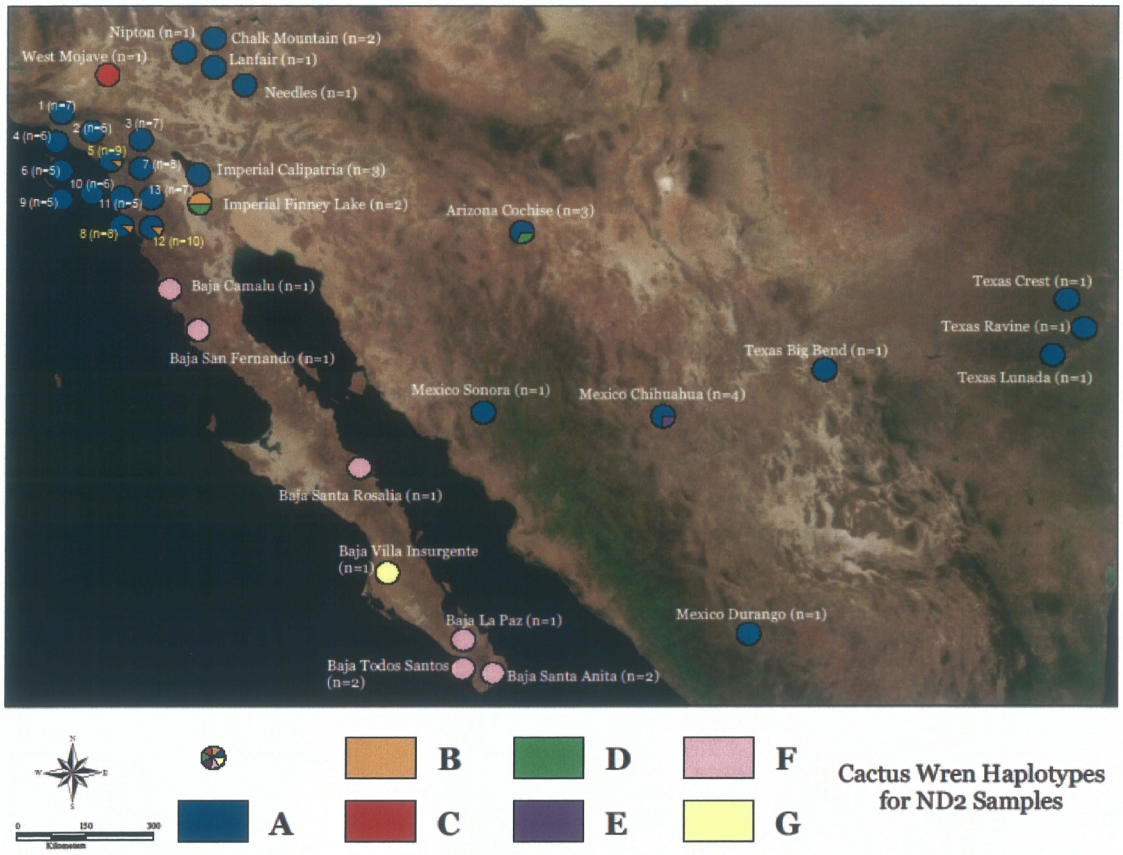

FIGURE 8. Map of 18 Cactus Wren populations in southern California analyzed for the mtDNA ND2 region. Seven haplotypes are represented by color code, and the assignment and proportion of each haplotype within the population is represented by the pie symbol. The most common haplotype on the mainland is A, and the most common haplotype on the Baja peninsula of Mexico is F with no sharing of haplotypes. 1=EM, 2=UC, 3=LJ, 4=UC, 5=FB, 6=CP, 7=SP, 8=SR, 9=BV 10=EC, 11=DH, 12=OT.

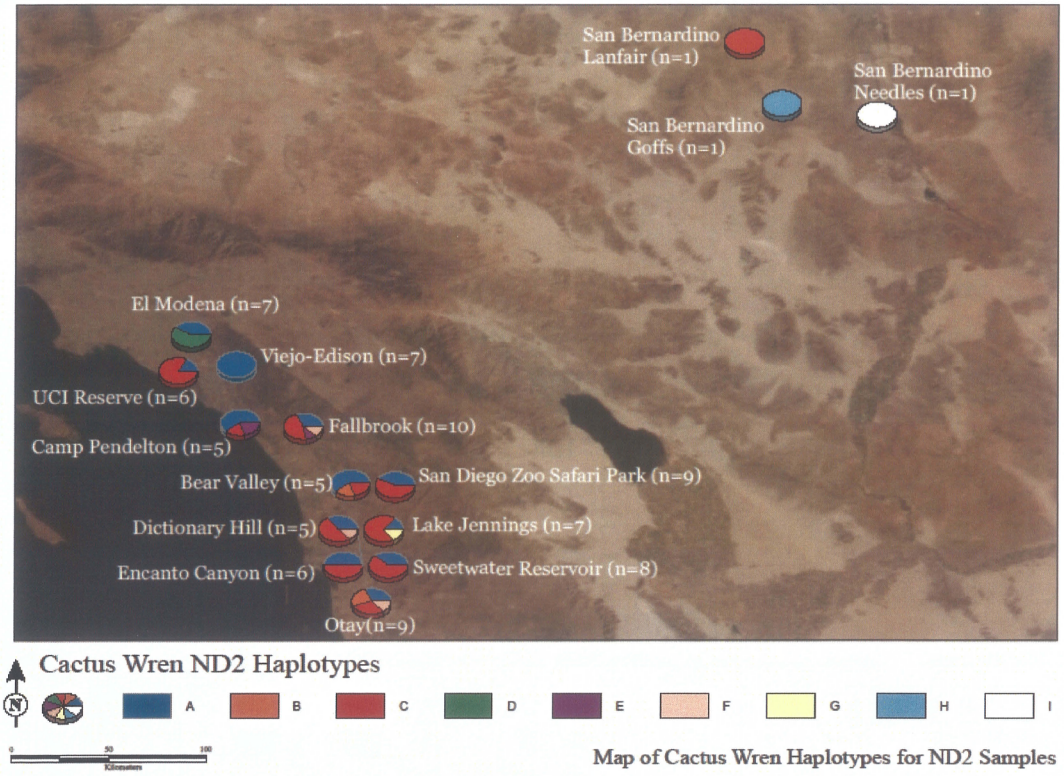

FIGURE 9. Map of 13 Cactus Wren populations in southern California analyzed for the subset ND2 region. Nine haplotypes are represented by color code, and the assignment and proportion of each haplotype within the population is represented by the pie symbol. The most common haplotype is shared between A and C. Haplotype C is found in the coast and in the desert.

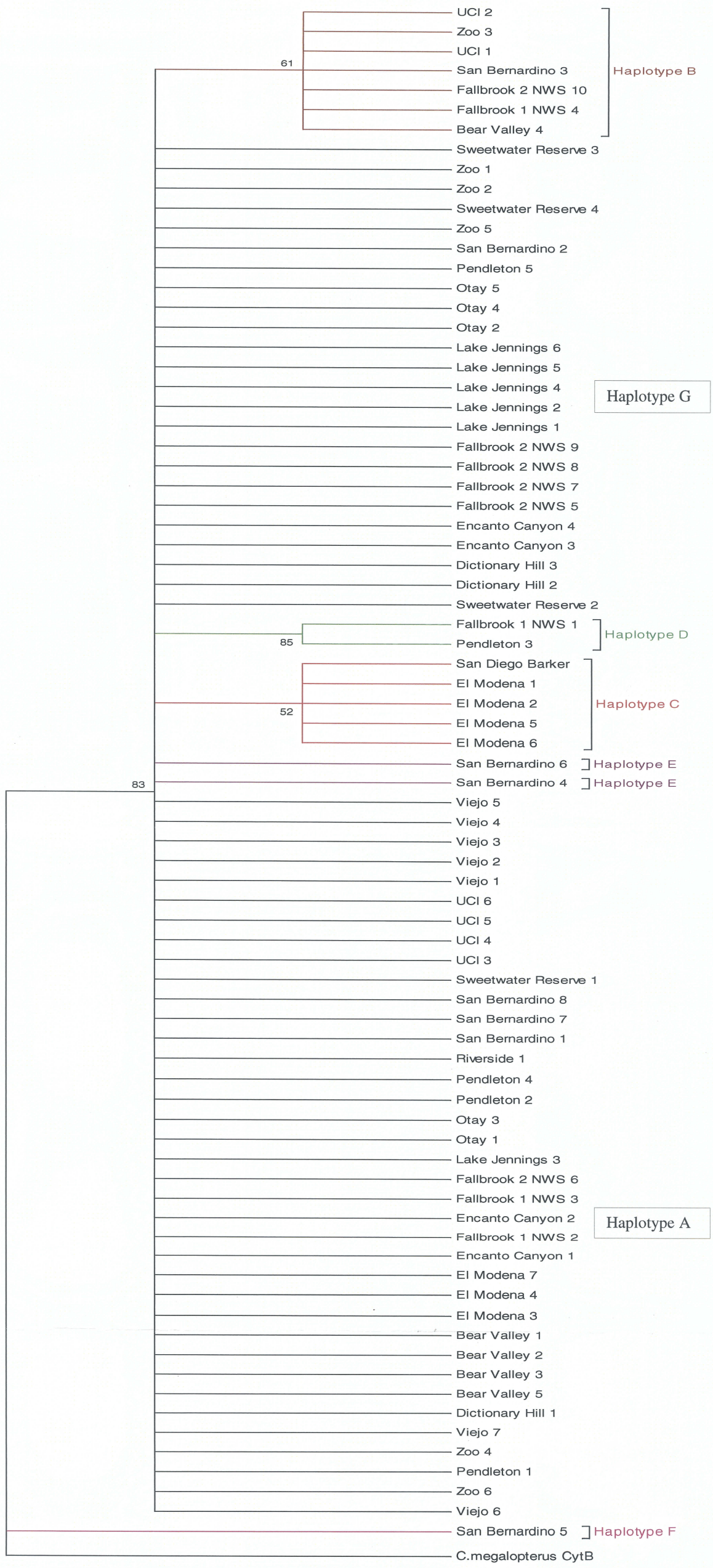

FIGURE 10. Hasegawa-Kishino-Yano maximum likelihood phylogenetic tree with 500 bootstraps that was determined from CytB mtDNA sequences. The maximum likelihood phylogenetic tree grouped all sequenced individuals into two main clades consisting of either the common Haplotypes A or G, with G grouping together a higher proportion of individuals and A grouping together a higher diversity of haplotypes. The Hasegawa-Kishino-Yano (HKY) was the best-fit model for the data. Numbers indicate bootstrap strength for the placement of sequences into the branch.

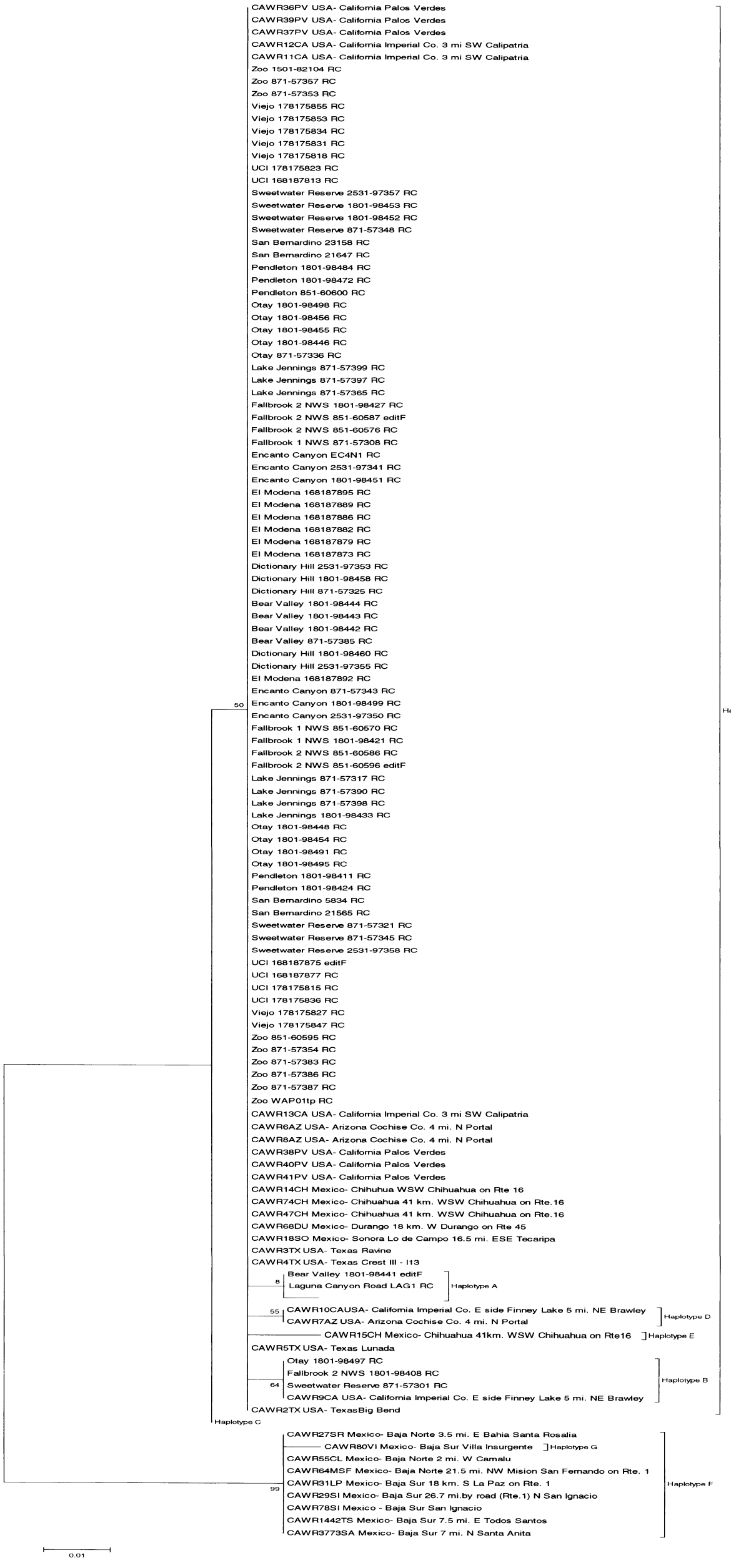

FIGURE 11. Hasegawa-Kishino-Yano maximum likelihood phylogenetic tree with 500 bootstraps that was determined from ND2 mtDNA sequences. The maximum likelihood phylogenetic tree grouped all sequenced individuals into two distinct clades, one large mainland clade with multiple haplotypes grouped within and one Baja peninsula clade with one unique haplotype. The Hasegawa-Kishino-Yano (HKY) was the best-fit model for the data. Numbers indicate bootstrap strength for the placement of sequences into the branch.

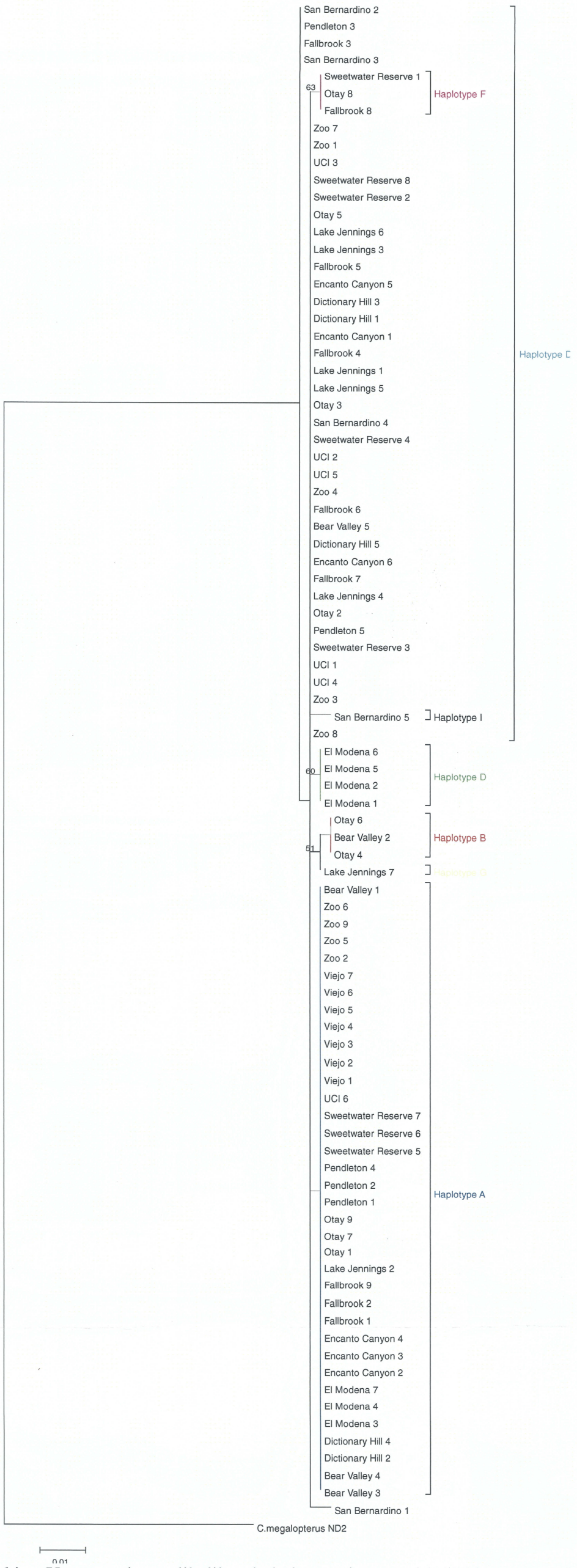

FIGURE 12. Hasegawa-Kishino-Yano maximum likelihood phylogenetic tree with 500 bootstraps that was determined from the subset of ND2 mtDNA sequences. The maximum likelihood phylogenetic tree grouped all sequenced individuals into two equal groups composed of Haplotype A and Haplotype C, one large mainland clade with multiple haplotypes grouped within and one Baja peninsula clade with one unique haplotype. The Hasegawa-Kishino-Yano (HKY) was the best-fit model for the data. Numbers indicate bootstrap strength for the placement of sequences into the branch.

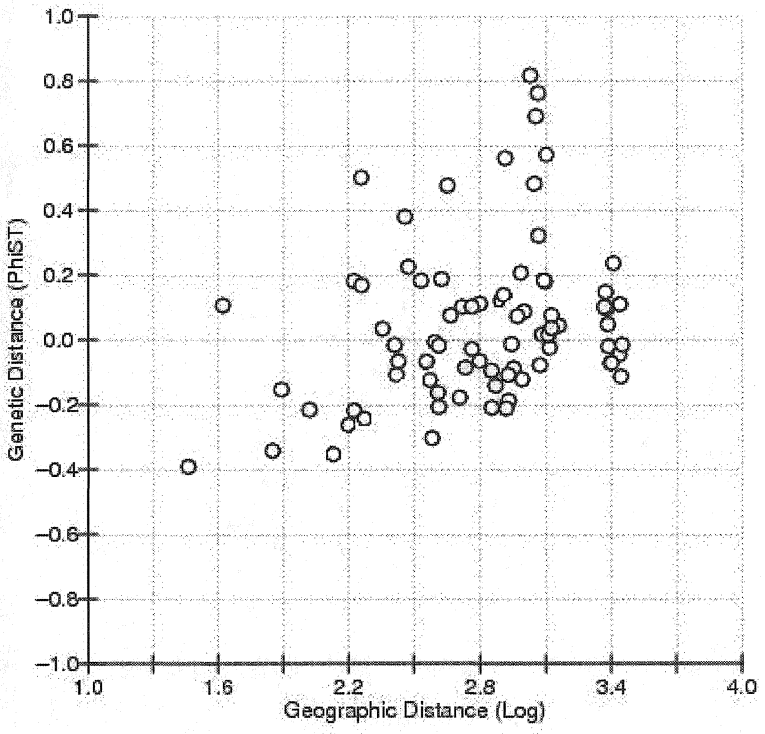

FIGURE 13. Isolation by distance estimated linear model for the CytB region. Reduced major axis regression revealed no significant relationship between genetic and geographical distance for most samples. The estimated linear model had a slope of 0.5606 D_{est}/km (SE = 0.0615) and an intercept of -1.521 (SE = 0.174) with n = 78 and R^2 value = 0.0845.

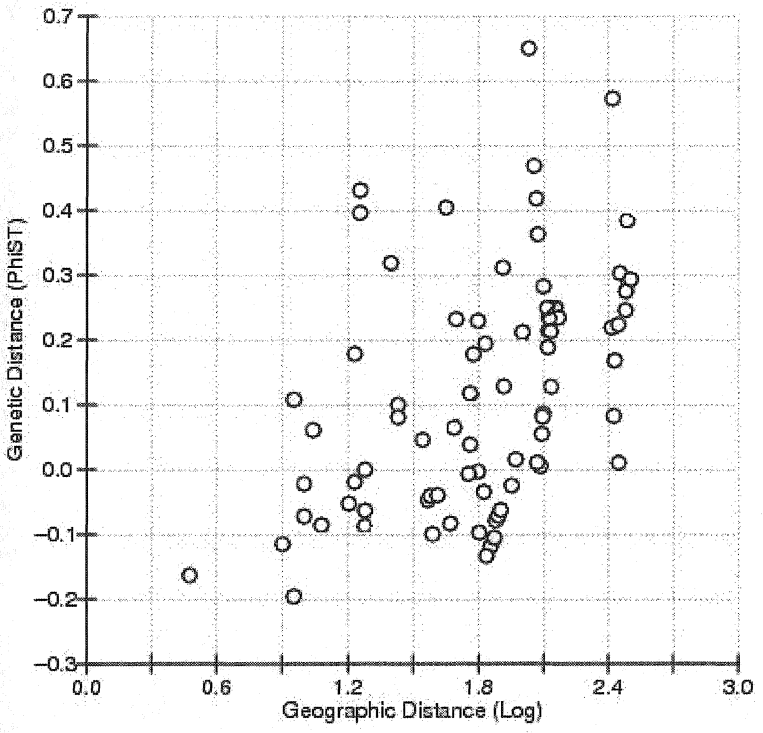

FIGURE 14. Isolation by distance estimated linear model for the CytB coastal region. Reduced major axis regression revealed a significant relationship between genetic and geographical distance for all samples. The estimated linear model had a slope of 0.7035 D_{est}/km (SE < 0.0820) and an intercept of -1.840 (SE = 0.222) with n =66 and R^2 value = 0.131.

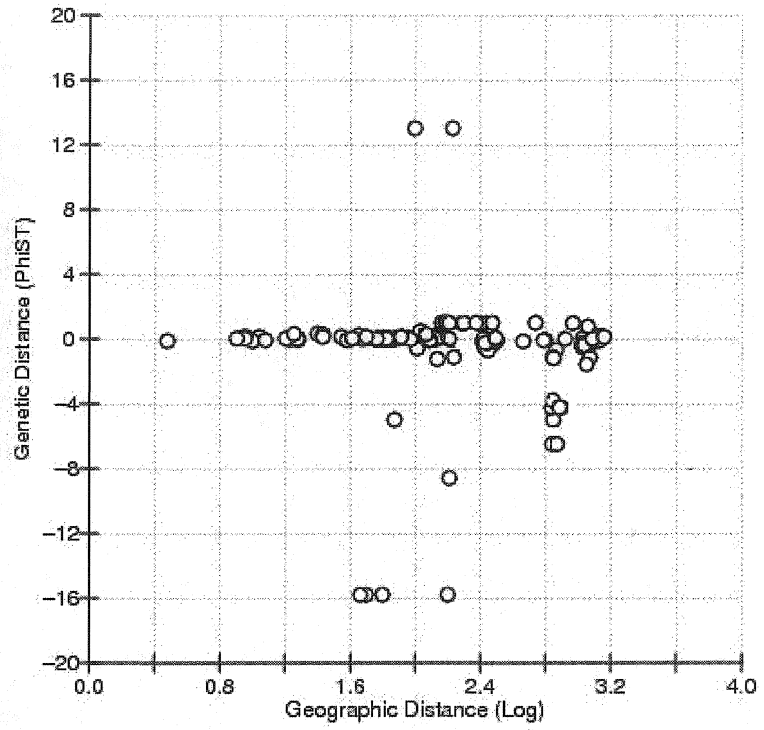

FIGURE 15. Isolation by distance estimated linear model for the ND2 region. Reduced major axis regression revealed no significant relationship between genetic and geographical distance for all samples. The estimated linear model had a slope of -5.195 D_{est}/km (SE < 0.423) and an intercept of 11.07 (SE = 0.98) with n =153 and R^2 value = 0.0003. The graph shows two distinct groups that share similar genetic composition but are separated by lengthy distances (i.e., the Baja and Mainland groups from the Discussion).

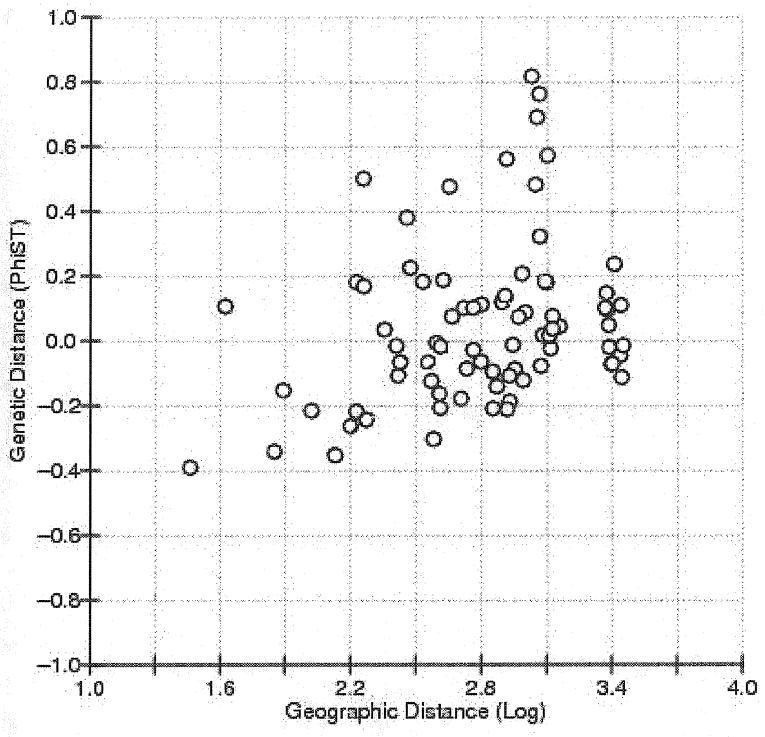

FIGURE 16. Isolation by distance estimated linear model for the subset ND2 region. Reduced major axis regression revealed a significant relationship between genetic and geographical distance for all samples. The estimated linear model had a slope of 0.3951 D_{est}/km (SE < 0.0401) and an intercept of -0.5969 (SE = 0.0747) with n =78 and R^2 value = 0.218.

BIBLIOGRAPHY

BIBLIOGRAPHY

American Ornithologists' Union. 1957. Checklist of North American birds. American Ornithologist Union 5th Edition.

American Ornithologists' Union. 1998. Checklist of North American birds. American Ornithologist Union 7th Edition.

Anderson AA, Anderson A. 1973. The Cactus Wren. Tucson: University of Arizona Press. 226 p.

Atwood JL, Noss RF. 1994. Gnatcatchers and development: A "train wreck" avoided? Illahee 10:123–132.

Atwood JL, Fugagli MR, Luttrell JC, Nicolai NC. 11 March 1994. California gnatcatchers, cactus wrens, and conservation of coastal sage scrub on the Palos Verdes peninsula. Progress Report No. 1 (1993). Manomet Observatory for Conservation Sciences, Manomet, Massachusetts 1:1–52–appendices.

Atwood JL, Bontrager DR, Fugagli MR, Hirsch K, Kamada D, Madden M, Reynolds C, Tsai S, Bowler P. 1998a. California gnatcatchers, cactus wrens, and conservation of coastal sage scrub on the Palos Verdes peninsula. Progress Report No. 5 (1997). Manomet Observatory for Conservation Sciences, Manomet, Massachusetts 5:1–41–appendices.

Atwood JL, Tsai SH, Reynolds CA, Fugagli MR. 1998b. Distribution and population size of California gnatcatchers on the Palos Verde peninsula, 1993–1997. Western Birds 29:340–350.

Atwood JL, Lerman SB. 2007. Geographic variation in Cactus Wren songs. Western Birds 38(1):29–46.

Avise JC. 1976. Genetic differentiation during speciation. Pp. 106–122 in: Molecular Evolution, F.J. Ayala (ed.). Sinauer Associates, Sunderland, MA.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC. 1987. Intraspecific Phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. Annual Review of Ecological Systematics 18:489–522.

Avise JC. 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA. 447 pp.

Baker AJ, Marshall HD. 1997. Mitochondrial control region sequences as tools for understanding the evolution of avian taxa. Avian molecular systematics and evolution. Academic Press, New York. Pp. 49–80.

Bancroft G. 1923. Some geographical notes on the cactus wren. The Condor 25:165–168.

Barker FK. 2004. Monophyly and relationships of wrens (Aves: Troglodytidae): A congruence analysis of heterogeneous mitochondrial and nuclear DNA sequence data. Molecular Phylogenetic Evolution 31 (2):486–504.

Barker FK. 2007. Avifaunal Interchange across the Panamanian Isthmus: Insights from *Campylorhynchus* Wrens. Biological Journal of the Linnaean Society 90 (4):687–702.

Barrowclough GF, Groth JG, Mertz LA, Gutierrez RJ. 2004. Phylogeographic structure, gene flow and species status in blue grouse (*Dendragapus obscurus*). Molecular Ecology 13:1911–1922.

Barrowclough GF, Groth JG, Mertz LA, Gutierrez RJ. 2005. Genetic structure, introgression, and a narrow hybrid zone between northern and California spotted owls (*Strix occidentalis*). Molecular Ecology 14:1109–1120.

Barrowclough GF, Groth JG, Odom KJ, Jonas EL. 2011. Phylogeography of the barred owl (*Strix varia*): Species limits, multiple refugia, and range expansion. The Auk 128(4):696–706.

Bontrager DR, Gorospe AL. 1995. Movement and Dispersal of California Gnatcatchers and Cactus Wrens in the San Joaquin Hills, Orange County, California. Unpublished report (Jan 1995). Ed Almanza and Associates, Laguna Beach, CA.

Brito PH. 2005. The influence of Pleistocene glacial refugia on Tawny Owl genetic diversity and phylogeography in western Europe. Molecular Ecology 14:3077–3094.

Brown LM, Ramey II RR, Tamburini B, Gavin T. 2004. Population structure and mitochondrial DNA variation in sedentary Neotropical birds isolated by forest fragmentation. Conservation Genetics 5:743–757.

Clark KB, Dodero M. 2008. Current status of the Cactus Wren in northwestern Baja California. Western Birds 39 (1):39–43.

Cooper DS. 2009. Coastal Cactus Wren summary, western Puente Hills, 2009. Unpublished Report (Sep 8, 2009). Puente Hills Landfill Native Habitat Preservation Authority, Whittier, CA.

Coulon A, Fitzpatrick JW, Bowman R, Stith BM, Makarewich CA, Stenzler LM, Lovette IJ. 2008. Congruent population structure inferred from dispersal behavior and intensive genetic surveys of the threatened Florida scrub-jay (*Aphelocoma coerulescens*). Molecular Ecology 17:1685–1701.

Crooks KR, Suarez AV, Bolger DT. 2004. Avian assemblages along a gradient of urbanization in a highly fragmented landscape. Biological Conservation 115(3):451–462.

Eggert L. 1996. A Phylogeographic Approach to Management of Coastal California Cactus Wrens (*Campylorhynchus brunneicapillus*) Masters Thesis. San Diego State University:1–88.

ESRI. 2009. Environmental Systems Research Institute (ESRI) Arcview 9 Manual. Amy Hillier Cartographic Modeling Laboratory. University of Pennsylvania; 1–72.

Flaagan KJ. 1999. Nest-site selection of the coastal cactus wren, *Campylorhynchus brunneicapillus*, in Chino Hills State Park, California. Master's thesis, California State University, Fullerton:1–appendices.

Funk CW, Mullins TD, Haig SM. 2007. Conservation genetics of snowy plovers (*Charadrius alexandrinus*) in the Western Hemisphere: population genetic structure and delineation of subspecies. Conservation Genetics 8:1287–1309.

Garrett K, Dunn J. 1981. Birds of Southern California: status and distribution. Los Angeles Audubon Society. Los Angeles, CA.

Garrett KL. 2001. Birds of the Baldwin Hills. Pages 77–126 in Biota of the Baldwin Hills, Los Angeles County, California. (Molina, K., Ed.) Community Conservancy International and Natural History Museum of Los Angeles County Foundation, Los Angeles, CA.

Garrett KL, Dunn JL, Morse B. 2006. Birds of the Los Angeles Region. R.W. Morse Company, Olympia, WA.

Grant WS, Bowen BW. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. Journal of Heredity, 89:415–426.

Grinnell J, Miller AH. 1944. The distribution of the birds of California. Pacific Coast Avifauna No. 27. 608 pp.

Griffith JT, Griffith JC. 1 March 1996. 1995 San Joaquin hills burn area California gnatcatcher and coastal cactus wren study. Prepared for California Corridor Constructors by Griffith Wildlife Biology, Calumet, MI 1995:28–appendices.

Griffiths AJF, Miller JH, Suzuki DT, Lewontin RC, Gelbart WM. 2000. Introduction to genetic analysis. 7th Edition. New York: W.H. Freeman and Company. 749 pp.

Hackett SJ. 1996. Molecular Phylogenetics and Biogeography of Tanagers in the Genus Ramphocelus (Aves). Molecular Phylogenetics and Evolution. Volume 5 No. 2, pp. 368–382.

Haig S. 1998. Molecular Contributions to Conservation. Ecology 79(2), pp. 413–425.

Hamilton RA. 2004. Target bird monitoring study, Nature Reserve of Orange County, 2004. Unpublished report (Nov 3, 2004). Nature Reserve of Orange County, Irvine, CA.

Hamilton RA. 2008. Cactus wrens in central & coastal orange county: How will a worst-case scenario play out under the NCCP? Western Tanager 75:2.

Hamilton RA. 2009a. Focused surveys for California Gnatcatchers and Cactus Wrens, Palos Verdes Nature Preserve, Palos Verdes Peninsula. Unpublished report (Nov 2009) Palos Verdes Peninsula Land Conservancy, Rancho Palos Verdes.

Hamilton RA. 2009b. 2008 surveys, Cactus Wrens and California Gnatcatchers, San Dieguito River Valley, San Diego County. Unpublished report (Jan 9, 2009). Conservation Biology Institute, Encinitas, CA.

Hamilton RA., Proudfoot GA, Sherry DA, Johnson S. 2011. Cactus Wren (*Campylorhynchus brunneicapillus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/558.

Harmsworth Associates. 2002. Coastal Cactus Wren habitat suitability assessment for the San Joaquin Hills Burn Area, 2001. Unpublished report (Mar 2002). Nature Reserve of Orange County, Irvine, CA.

Harmsworth Associates. 2008. Report on biological surveys for Upper Chiquita Canyon Conservation Easement, 2007. Unpublished report (Feb 2009). Nature Reserve of Orange County, Irvine, CA.

Hartl D, Clark A. 1997. Principles of Population Genetics. 3rd Ed. Sinauer Associates, Sunderland, MA.

Hedrick PW. 2005. A standardized genetic differentiation measure. Evolution 59:1633–1638.

Holland RF. October 1986. Preliminary descriptions of the terrestrial natural communities of California for the State of California, the Resources Agency, Department of Fish and Game, 1–154.

Horvath MB, Martínez-Cruz B, Negro JJ, Kalmár L, Godoy JA. 2005. An overlooked DNA source for non-invasive genetic analysis in birds. Journal of Avian Biology Volume 36:84—88.

Hutchinson DW, Templeton AR. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898–1914.

Jensen JL, Bohonak AJ, Kelley ST. 2005. Isolation by Distance, web service. BMC Genetics 6:13. v.3.21 http://ibdws.sdsu.edu/.

Kamada D, Mitrovich, MJ. 2006. Results of the 2006 Cactus Wren (*Campylorhynchus brunneicapillus*) translocation study in Orange County, California. Report dated December 2006 prepared for California Department of Fish and Game, San Diego.

Kamada D. 2008. Final report: Cactus Wren (*Campylorhynchus brunneicapillus*) 2007 telemetry study and the 2007 monitoring results of the 2006 Cactus Wren translocation study in Orange County, California. Report dated February 2008 prepared for California Department of Fish and Game, San Diego, and Nature Reserve of Orange County, Irvine, CA.

Kidd MG, Friesen VL. 1998. Analysis of mechanisms of microevolutionary change in *Cepphus guillemots* using patterns of control region variation. Evolution 52:1158–1168.

Klopatek JM, Olson RJ, Emerson CJ, Jones JL. 1979. Land-use conflicts with natural vegetation in the United States. Environmental Conservation 6(3):191–199.

Kristan WB, Lynam AJ, Price MV, Rotenberry JT. 2003. Alternative causes of edge-abundance relationships in birds and small mammals of California coastal sage scrub. Ecography 26(1):29–44.

Laws EA, Archie JW. 1981. Appropriate use of regression analysis in marine biology. Marine Biology 65:13–16.

Leatherman Bioconsulting, Inc. 2009. Central Reserve Cactus Wren habitat assessment and survey, 2008. Unpublished Report (Feb 2009). Nature Reserve of Orange County, Irvine, CA.

Librado P, Rozas J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

LSA Associates, Inc. 2005. Comprehensive Enhanced and Restoration Plan for Central/ Coastal Orange County Natural Communities Conservation Plan (NCCP) and Habitat Conservation Plan (HCP). Unpublished report prepared for Nature Reserve of Orange County, Irvine, CA.

Lutmerding JA, Love AS. 2010. Longevity records for North American Birds. Patuent Wildlife Reserve Center, Bird Banding Lab, Laurel, MD. [Online.] http://www.pwrc.usgs.gov/bbl/longevity/Longevity_main.cfm.

Maehr DS, Lacy RC. 2002. Avoiding the lurking pitfalls in Florida panther recovery. Wildlife Society Bulletin 30:971–978.

Marr TG. 1981. Breeding and foraging ecology of the Cactus Wren in a variable environment. Ph.D. Thesis. New Mexico State University, Las Cruces.

Marr TG, Raitt RJ. 1983. Annual variations in patterns of reproduction of the Cactus Wren (*Campylorhynchus brunneicapillus*). Southwest. Nat. 28:149–156.

Mearns EA. 1902. The Cactus Wrens of the United States. Auk 19:141–145.

Minnich RA, Dezzani RJ. 1998. Historical decline of coastal sage scrub in the Riverside-Perris Plain, California. Western Birds 29(4):366–391.

Mitrovich MJ, Hamilton RA. 2007. Status of the Cactus Wren (*Campylorhynchus brunneicapillus*) within the Coastal Subregion of Orange County, California. Unpublished report (May 2007). Nature Reserve of Orange County, Irvine, CA.

Nei M, Tajima F. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction site data. Genetics 105:207–217.

Nei M, Kumar S. 2000. Molecular Evolution and Phylogenetics. New York, New York: Oxford University Press, Inc. 333 pp.

O'Leary JF. 1990. Californian coastal sage scrub: general characteristics and considerations for biological conservation. Endangered Plant Communities of Southern California 3. Southern California Botanists Special Publication, 24–41.

Ogden Environmental and Energy Services Co. 1993. Population viability analysis for the coastal Cactus Wren within the MSCP study area. Report to Clean Water Program, City of San Diego, San Diego, CA.

Patten MA. 2010. Null expectations in subspecies diagnosis. Ornithological Monographs 67:35–41.

Perlo BV. 2006. Birds of Mexico and Central America. Princeton Illustrated Checklist. Princeton University Press. 336 pp. 98 color plates.

Pimm SL, Dollar L, Bass Jr OL. 2006. The genetic rescue of the Florida panther. Animal Conservation 9:115–122.

Proudfoot GA, Sherry D, Johnson S. 2000. Cactus Wren (*Campylorhynchus brunneicapillus*). In The Birds of North America, No. 558 (A. Poole and F. Gill, eds.). The Birds of North America, Inc., Philadelphia, PA, and The American Ornithologists' Union, Washington, D.C.

Randi E, Lucchini V, Hennache A, Kimball RT, Braun EL, Ligon JD. 2001. Evolution of the Mitochondrial DNA Control Region and Cytochrome B Genes and the Inference of Phylogenetic Relationships in the Avian Genus *Lophura* (Galliformes). Molecular Phylogenetics and Evolution 19 (2):187–201.

Rea AM. 1986. Geographic variation (of *Campylorhynchus brunneicapillus*): (1) NW peninsular and insular races, in The Known Birds of North and Middle America. Part 1 (A.R. Phillips, ed.), A.R. Phillips, Denver. p. 120.

Rea AM, Weaver KL. 1990. The taxonomy, distribution, and status of the coastal California cactus wrens. Western Birds 21:81–126.

Reading RP, Kellert SR. 1993. Attitudes toward a proposed reintroduction of black-footed ferrets (*Mustela nigripes*). Conservation Biology 7:569–580.

Ricklefs RE. 1966. Behavior of Young Cactus Wrens and Curve-billed Thrashers. Wilson Bulletin 78:47–56.

Ricklefs RE. 1967. A case of classical conditioning in nestling Cactus Wrens. Condor 69:528–529.

Ricklefs RE. 1968. The survival rate of juvenile Cactus Wrens. Condor 70:388–389.

Ricklefs RE. 1975. Patterns of growth in birds. III. Growth and development of the Cactus Wren. Condor 77:34–45.

Ricklefs RE, Hainsworth FR. 1966. The temporary establishment of dominance between two hand-raised juvenile Cactus Wrens (*Campylorhynchus brunneicapillus*). Condor 69:528.

Ricklefs RE, Hainsworth FR. 1968a. Temperature dependent behavior of Cactus Wrens. Ecology 49:227–233.

Ricklefs RE, Hainsworth FR. 1968b. Temperature regulation in nestling Cactus Wrens: development of homeothermy. Condor 70:121–127.

Ricklefs RE, Hainsworth FR. 1969. Temperature regulation in nestling Cactus Wrens: the nest environment. Condor 71:32–37.

Rojas-Soto OR, Westberg M, Navarro-Sigu AG, Zink RM. 2010. Genetic and ecological differentiation in the endemic avifauna of Tiburon Island. Journal of Avian Biology 41:398–406.

Rousset F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219–1228.

Ruokonen M, Kvist L. 2002. Structure and evolution of the avian mitochondrial control region. Molecular Phylogenetic Evolution 23:422–432.

Selander RK. 1964. Speciation in wrens of the genus *Campylorhynchus*. University of California Press, Berkeley.

Simons LS, Martin TE. 1990. Food limitation of avian reproduction: an experiment with the Cactus Wren. Ecology 71:869–876.

Simons LS, Simons LH. 1990. Experimental studies of nest-destroying behavior by Cactus Wrens. Condor 92:855–860.

Simons LS, Simons LH. 1993. Seed dispersal through removal of avian nestling fecal sacs. Southwest Naturalist 38:282–284.

Simonson SE, Stohlgren TJ, Tyler L, Gregg WP, Muir R, Garrett LJ. 2005. Preliminary assessment of the potential impacts and risks of the invasive Cactus Moth, *Cactoblastis cactorum* Berg, in the U.S. and Mexico. Unpublished report. (April 25, 2005). International Atomic Energy Agency.

Slatkin M. 1993. Isolation by Distance in equilibrium and non-equilibrium populations. Evolution 47:264–279.

Small A. 1994. California birds: their status and distribution. Ibis Publ. Co. Vista, CA.

Smith EL. 1970. Cactus Wrens attack ground squirrel. Condor 72:363–364.

Snyder JP. 1987. Map Projections – A Working Manual. U.S. Geological Survey Professional Paper 1395. United States Government Printing Office, Washington, D.C.

Solek C, Szijj L. 2004. Cactus wren (*Campylorhynchus brunneicapillus*) in the coastal scrub and chaparral bird conservation plan: A strategy for protecting and managing coastal scrub and chaparral habitats and associated birds in California. California Partners in Flight.

Sorrentino J. 2008. Manufactured homes for the birds. Accessed September 22, 2009: http://www.hcn.org/issues/40.13/manufactured-homes-for-the-birds last modified July 21, 2008.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution (submitted).

Tarr CL. 1995. Primers for amplification and determination of mitochondrial control region sequences in oscine passerines. Molecular Ecology. Vol. 4, Issue 4, pp. 527–530.

The International HapMap Consortium. 2003. The International HapMap Project. Nature 426 (6968):789–796.

The Resources Agency, Department of Fish and Game, Sacramento, CA. November 1993. Southern California coastal sage scrub: Natural community conservation planning conservation guideline 1993:1–19.

United States Fish and Wildlife Service (USFWS). September 2, 1994. Endangered and threatened wildlife and plants; 1-year finding for a petition to list the pacific coast population of the cactus wren under the endangered species act Federal Register 50 CFR Part 17.

Unitt P. 2004. San Diego County bird atlas. Proceedings of the San Diego Natural History Museum No. 39. 39th Edition. Temecula, California: Ibis Publishing. 468 pp.

Unitt P. 2008. San Diego Cactus Wren (*Campylorhynchus brunneicapillus sandiegensis*). Pages 300–305 in California Bird Species of Special Concern: A ranked assessment of species, subspecies, and distinct populations of birds of immediate conservation concern in California. (W.D. Shuford and T. Gardali, eds.) Studies

of Western Birds 1. Western Field Ornithologists and California Department of Fish and Game, Camarillo, CA, and Sacramento, CA.

Vazquez-Miranda H, Navarro-Siguenza AG, Omland KE. 2009. Phylogeography of the Rufous-naped Wren (*Campylorhynchus rufinucha*): Speciation and Hybridization in Mesoamerica. Auk 126 (4):765–778.

Website: http://AOU.org/committees/collections/recipes_dna_buffers.php, accessed February 5, 2012, at 2:24 p.m.

Wechsler S. Personal Communication. February 10, 2012. Associate Professor of Geography, California State University, Long Beach.

Weir BS. 1990. Genetic data analysis: methods for discrete population analysis. Sinauer Associates, Sunderland, MA.

Wenink P, Baker A, Rosner HU, Tilanus M. 1996. Global mitochondrial DNA phylogeography of holarctic breeding dunlins (*Calidris alpina*). Evolution 50:318–330.

Westman WE. 1981. Diversity relations and succession in Californian coastal sage scrub. Ecology 62(1):170–184.

Wheeler TR. 1997. Habitat requirements of the coastal cactus wren, *Campylorhynchus brunneicapillus*, in eastern Los Angeles County. Senior thesis in biology, Joint Sciences Department of the Claremont College.

Wilson AC, Cann RL, Carr SM, George M, Gyllensten UB, Helm-Bychowski KM, Higuchi RG, Palumbi SR, Prager EM, Sage RD, Stoneking M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biological Journal of Linnaeus Society 26:375–400.

Wisely SM, McDonald DB, Buskirk SW. 2003. Evaluation of the genetic management of the endangered black-footed ferret (*Mustela nigripes*). Zoo Biology 22:287–298.

Zink RM, Slowinski JB. 1995. Evidence from molecular systematics for decreased avian diversification in the Pleistocene epoch. Proceedings National Academy of Science USA 92:5832–5835.

Zink RM. 1997. Phylogeographic studies of North American Birds. Avian Molecular Evolution (ed. D.P. Mindell), pp 301–324. Academic Press, San Diego, CA.

Zink RM, Kessen AE, Line TV, Blackwell-Rago RC. 2001. Comparative phylogeography of some aridland bird species. Condor 103(1):1–10.

Zink RM. 2004. The role of subspecies in obscuring biological diversity and misleading conservation policy. Proceedings of the Royal Society of London, Series B 271:561–564.

Zink RM, Barrowclough GF. 2008. Mitochondrial DNA under siege in avian phylogeography. Molecular Ecology 17:2107–2121.

Zink RM, Jones AW, Farquhar CC, Westberg MC, Gonzalez-Rojas JI. 2010. Comparison of molecular markers in the endangered Black-capped Vireo (*Vireo atricapilla*) and their interpretation in conservation. Auk 127:797–806.