BEFORE THE UNITED STATES DEPARTMENT OF THE INTERIOR &
THE UNITED STATES FISH AND WILDLIFE SERVICE

In the Matter of the Petition to
Remove the California gnatcatcher
(*Polioptila polioptila californica*)
from the list of threatened species
under the Endangered Species Act

PETITION OF THE COALITION OF LABOR, AGRICULTURE, AND BUSINESS, THE PROPERTY OWNERS ASSOCIATION OF RIVERSIDE COUNTY, AND M. LOU MARSH, M.D., TO REMOVE THE CALIFORNIA GNATCATCHER FROM THE LIST OF THREATENED SPECIES UNDER THE ENDANGERED SPECIES ACT

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INTRODUCTION

Petitioners Coalition of Labor, Agriculture, and Business; Property Owners Association of Riverside County; and M. Lou Marsh, M.D., hereby petition the United States Department of the Interior and the United States Fish and Wildlife Service, pursuant to Section 4(b)(3), 16 U.S.C. § 1533(b)(3), of the Endangered Species Act (ESA), *id.* § 1531, *et seq.*, to remove the California gnatcatcher (*Polioptila polioptila californica*) from the ESA list of threatened wildlife. The gnatcatcher (*P.c. californica*) is currently listed as a threatened subspecies. Petitioners, relying upon studies published since the listing, contend that the California gnatcatcher is not a valid subspecies, and should for that reason be delisted.

PETITIONERS

Petitioner Coalition of Labor, Agriculture, and Business (COLAB) unites the independent strengths of these sectors of the economy to protect and improve the natural and business environments of San Luis Obispo and Santa Barbara Counties, California. COLAB engages in educational outreach, political action, and issue advocacy. COLAB supports the protection of private property rights, fiscal responsibility, and environmental legislation based on sound principals of science, as well as cost-effective solutions to issues associated with business and job creation. COLAB is a tax-exempt organization under Section 501(c)(6) of the Internal Revenue Code. Its

members are primarily comprised of farming and ranching families who have been stewards of the land for generations. COLAB advocates for a balanced approach to environmental regulation, especially with respect to the administration of the ESA.

Petitioner Property Owners Association of Riverside County (Association), is a tax-exempt organization under Section 501(c)(6) of the Internal Revenue Code. The Association's mission is to serve as an advocate for Riverside County property owners to ensure that the interests and private property rights of landowners are protected in the formation and implementation of public policies. The Association includes owners of real property in Riverside County whose interests are directly affected by government land use regulations, including numerous land use restrictions imposed by the ESA. In particular, the Association has two dozen members who are within the Western Riverside County Multiple Species Habitat Conservation Plan area, which includes gnatcatcher habitat.

Petitioner M. Lou Marsh, M.D., resides in unincorporated San Diego County. Dr. Marsh wishes to subdivide her lot. In order to obtain that subdivision, Dr. Marsh must obtain a coastal development permit from the California Coastal Commission. In December, 2006, the Commission denied Dr. Marsh's permit application. One of the Commission's grounds for denying the application was the presence of a pair of gnatcatchers on the property. The

Commission concluded that the development of the proposed subdivided lot would negatively affect gnatcatcher habitat, which the Commission deemed to be an environmentally sensitive habitat area under the state's Coastal Act. If the gnatcatcher were delisted, the Commission could no longer use the presence of the gnatcatcher or its habitat to deny Dr. Marsh's permit application.

ANALYSIS

In 1993, the Service listed the California gnatcatcher as a threatened subspecies. 58 Fed. Reg. 16,742 (Mar. 30, 1993). In determining that gnatcatchers represented a valid subspecies, the Service relied on a study by Atwood (1991). *See id.* at 16,742. Since 1993, several published studies have concluded that the Atwood subspecies classification is invalid and that the California gnatcatcher should be considered an undifferentiated part of one species ranging from Southern California to the southernmost tip of the Baja California peninsula in Mexico. The Service itself has initiated a formal review of the gnatcatcher's taxonomy to determine whether the subspecies classification should be withdrawn. *See* 68 Fed. Reg. 20,228, 20,230 (Apr. 24, 2003); 72 Fed. Reg. 72,009 (Dec. 19, 2007). The Service has also proposed listing the gnatcatcher as a distinct population segment (DPS). *See* 68 Fed. Reg. at 20,230-33.

Petitioners rely upon the attached paper "Subspecies status of the coastal California gnatcatcher (*Polioptila californica californica*)" (Nov. 9, 2009), as well as its appended literature, all of which are incorporated fully into this petition by reference. The paper, prepared for Petitioners' counsel by Dr. Matthew Cronin, reviews the post-listing studies to explain why the subspecies classification for the California gnatcatcher is no longer tenable. Below, Petitioners highlight the essential points of Dr. Cronin's paper.

As noted above, the subspecies classification for the California gnatcatcher is based upon the Atwood study. In that study, Atwood concluded that the California gnatcatcher subspecies classification was justified in light of observed changes in certain morphological characteristics (*i.e.*, plumage coloration and body size) in collected gnatcatcher specimens from various locales.

Studies published by Zink, et al. (2000), and Skalski, et al. (2008), have determined that Atwood (1991) was wrong on at least three points.

1. Zink, et al. (2000), determined that Atwood's observed morphological characteristics changes are *not* representative of genetic differentiation, which differentiation could support a subspecies classification. The Zink study's conclusion is all the more significant given that Atwood was a co-author. In their paper, Zink and Atwood expressly state that *P. californica* should have no subspecies.

2. Skalski, et al. (2008), determined that Atwood's statistical analyses were seriously flawed, because Atwood's supposed diagnostic characters support a geographic cline, *not* a distinct break in character distribution markers, which break could support a subspecies classification.

3. Skalski, et al. (2008), determined that Atwood's data sets were confounded: many of Atwood's specimens may not have been representative of wild gnatcatchers.

Thus, in terms of morphological, statistical, and genetic data analysis, the Atwood (1991) subspecies classification for the California gnatcatcher is not valid. Atwood, the scientist whose work is responsible for the classification of the California gnatcatcher, has published a retraction of his earlier work and has concluded that the California gnatcatcher is not a valid subspecies. (Zink, et al. 2000). New science (Zink, et al. 2000) also indicates that the California gnatcatcher does *not* qualify as an evolutionarily significant unit, and instead should be considered part of a single species of gnatcatcher whose range extends from southern Baja California, Mexico, to Southern California.

CONCLUSION

The current and best available scientific data conclusively demonstrate that the California gnatcatcher is not a valid subspecies. Petitioners therefore request that their petition to delist the California gnatcatcher from the ESA list of threatened wildlife be granted.

DATED: April 9, 2010.

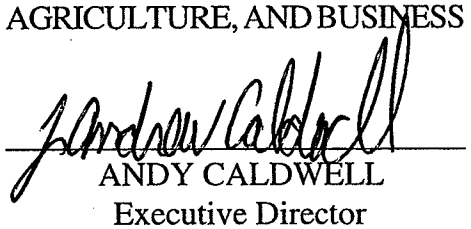
Respectfully submitted,

M. REED HOPPER
DAMIEN M. SCHIFF
Pacific Legal Foundation

By _____
DAMIEN M. SCHIFF

DATED: February 1, 2010.

COALITION OF LABOR,
AGRICULTURE, AND BUSINESS



ANDY CALDWELL
Executive Director

DATED: February 3, 2010.

THE PROPERTY OWNERS ASSOCIATION OF RIVERSIDE COUNTY

Bruce Colbert

BRUCE COLBERT
Executive Director

DATED: February 1, 2010.

PETITIONER

M. Lou Marsh, M.D.

M. LOU MARSH, M.D.

Tab 1

Subspecies status of the coastal California gnatcatcher (*Polioptila californica californica*)

Prepared for the Pacific Legal Foundation, Sacramento, California by

Matthew A. Cronin, Ph.D.
Northwest Biotechnology Company
Anchorage, Alaska

9 November 2009

Subspecies status of the coastal California gnatcatcher (*Polioptila californica californica*)

9 November 2009
Matthew A. Cronin
Northwest Biotechnology Company
Anchorage, Alaska

In this paper I review the data, designations, and interpretations of the coastal California gnatcatcher subspecies, including the following topics.

1. **Review of the scientific literature on the coastal California gnatcatcher subspecies.**

A. Morphology
B. Molecular genetics and phylogeny
C. The subjective nature of subspecies in general

2. **Review of regulatory documents**.

A. Federal Register documents regarding subspecies status of the coastal California gnatcatcher and Endangered Species Act listing
B. The coastal California gnatcatcher as a Distinct Population Segment instead of a subspecies
C. Other Endangered Species Act subspecies designations.

3. **Literature cited.**

Subspecies status of the coastal California gnatcatcher (*Polioptila californica californica*)

1. Review of the scientific literature on the coastal California gnatcatcher subspecies

Morphology

There are two species of gnatcatcher (genus *Polioptila*) whose ranges overlap in northeast Baja Peninsula, Mexico. The black-tailed gnatcatcher (*Polioptila melamura*) occurs in a range including southern Nevada and Sonora and Chihuahua, Mexico. The California gnatcatcher (*Polioptila californica*) has a range that extends from the southern end of the Baja peninsula north to southern California around Los Angeles at 31° north latitude. The California gnatcatcher was originally designated a species by Brewster (1881) but was later split into a subspecies of the black-tailed gnatcatcher (Grinnell 1926). It was then re-designated a full species based morphological, vocalization, and mitochondrial DNA (mtDNA) variation (Phillips 1980- cited by Mellink and Rea 1994, Rea 1983, Atwood 1986, 1988; American Ornithologists' Union 1989, Zink and Blackwell 1998). The California gnatcatcher has the least dense populations in the north, from El Rosario, Baja California, Mexico (latitude 30° north) north to Los Angeles. Populations in central and southern Baja California, Mexico are large and continuous (Zink et al. 2000).

There have been generally three subspecies of California gnatcatcher recognized, although five subspecies names and different range boundaries have been used by different authors. This is because there is overlap of characters and no distinct boundaries of the morphological characters used in the subspecies classifications. The morphological characters considered for gnatcatchers include body size and shape and plumage characteristics. The coastal California gnatcatcher (*Polioptila californica californica*) is the northernmost subspecies, extending to the limit of the species range. This subspecies was listed in 1993 as a threatened subspecies under the Endangered Species Act (ESA, 58 FR 16742). The ESA listing was based upon recognition of *P. c. californica* as a legitimate subspecies based on the classification of Atwood (1991).

The history of the California gnatcatcher shows inconsistent subspecies designations. Early assessments Grinnell (1926) recognized *P. c. californica* from the northern limit of the range at about 31° north latitude southward to 29° 30' north latitude, *P. c. margaritae* south of *P. c. californica* to about 24° north latitude, and *P. c. abbreviata* south of 24° in the Cape region of Baja California Sur. However, van Rossem (1931, cited by Mellink and Rea 1994) found *P. c. abbreviata* and *P. c. margaritae* to be indistinguishable and named *P. c. pontilis* (replacing yet another subspecies name *nelsoni*, Ridgway 1903, cited by Atwood 1991) as a subspecies intermediate to *P. c. californica* and *P. c. margaritae*.

Miller et al. (1957, cited by Zink et al. 2000) designated three subspecies *P. c. californica* from near Los Angeles southward to a boundary north of 30° north latitude, *P. c. pontilis* from the southern boundary of *P. c. californica* to around 27° north latitude, and *P. c. margaritae* south of 27° north latitude (Figure 1).

Phillips (1991, cited by Mellink and Rea 1994) also identified *P. c. californica* to occur from Los Angeles southward to 30° north latitude, *P. c. pontilis* in the central part of the Baja Peninsula, and *P. c. margaritae* from about 27° north latitude to the southernmost tip of Baja California. Phillips (1991, cited by Mellink and Rea 1994) evidently noted geographic variation, limited specimens, and changes in characters in storage over time (i.e., foxing) as a problem with the subspecies designations. He stated with regard to *P. c. californica*: "Geographic variation within these dark (northern) populations is indicated; need I repeat endlessly, 'There are few clean fresh-plumaged specimens'? Sorry".

Atwood (1988) described two subspecies of California gnatcatcher, *P. c. californica* and *P. c. margaritae*, with a transition of morphology at about 25° north latitude. *P. c. californica* was north of 25° and *P. c. margaritae* to the south in the Cape region of the Baja Peninsula, Mexico. Then Atwood (1990, 1991) reanalyzed his data, agreeing with criticisms (Banks 1989, Johnson 1989) that "details of intraspecific variation in *Polioptila* are difficult to assess from data provided in Atwood (1988)." Atwood (1991) described three subspecies including *P. c. californica* north of 30°, *P. c. margaritae* between 24° and 30° north latitude, and *P. c. abbreviata* south of 24° north latitude (Figure 1). Atwood (1991) noted that :

"...the naming and distributions of subspecies of *Polioptila californica* should revert to that initially proposed by Grinnell (1926): *P. c. californica* (north of 30° N), *P. c. margaritae* (from 30° N south to 24° N), and *P.c. abbreviata* (south of 24° N)."

Atwood (1991) noted that his analysis, while supporting Grinnell's (1926) designations, did not support van Rossem's (1931) classification. Unlike the other subspecies assessments, Atwood (1991) did statistical analyses of 31 morphological characters, although there are serious questions about the integrity of the data and appropriateness of the methods in these analyses (McDonald et al. 1994, Skalski et al. 2008).

Mellink and Rea (1994) subsequently identified a different subspecies scheme based on plumage coloration. This involved splitting *P. c. californica* into two subspecies: *P. c. californica* north of the U.S.-Mexican border and *P. c. atwoodi* (a new subspecies) from the U.S.-Mexican border south to about 30° north latitude (Figure 1). These authors also designated subspecies from what was designated *P. c. margaitae* in Atwood's (1991) classification. This involved recognizing *P. c. pontilis* (between 30° north latitude and 28° north latitude) and noting that *P. c. pontilis* was separable from *P. c. margaritae*. However, Mellink and Rea (1994) did not have enough specimens to assess subspecies relationships south of 26° north latitude.

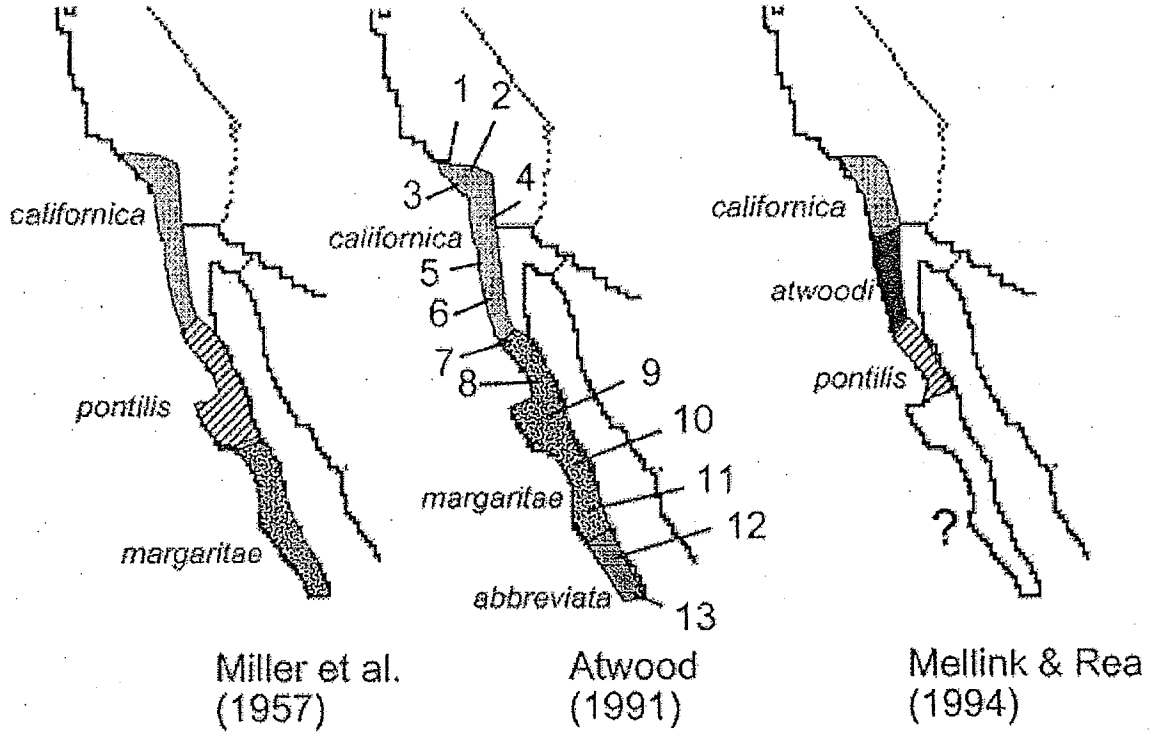


Figure 1. California gnatcatcher subspecies' ranges as designated in three different assessments (copied from Zink et al. 2000 with permission of *Conservation Biology* and R. Zink). The numbers in the Atwood (1991) scheme indicate the sampling areas for the mtDNA analysis of Zink et al. (2000).

The subspecies assessments described above were primarily based on assessments of morphology, specifically plumage coloration and body measurements. There are two aspects of this work that warrant examination. First, subspecies designations are supposed to be based on genetic relationships (technically called phylogenetic or evolutionary relationships, Cronin 1993, 2006, Zink 2004). However morphology (specifically plumage color and body measurements) is influenced by environmental factors in addition to genetics (e.g., Geist 1992). This means that body measurements and plumage color are not necessarily good characters for subspecies classification. Plumage coloration, body measurements, and other morphological characters may be influenced by environmental conditions and diet (e.g., Brush and Power 1976, James 1983, James and NeSmith 1986, Hudon and Brush 1989, Price et al. 1991, Federal Register March 30, 1993). Plumage color also changes with time since collection and storage of study skins (called "foxing"), such as those used in the analyses of Atwood (1988, 1990, 1991) and Mellink and Rea (1994). Indeed, Mellink and Rea (1994) note:

"Unfortunately, many skins from the northern end of the species' range (Los Angeles, Riverside, San Bernardino counties) are severely soiled (see also Rea and Weaver 1990:92-94). Most of these were taken early in the twentieth century and have soot-stained plumage."

It is arguably inappropriate to use morphology, particularly plumage coloration, for subspecies classification, in general, and specifically for stored study skins.

A second problem with the subspecies designations of Atwood (1991) relates to statistical analyses of morphology over the range of California gnatcatchers. Reanalysis of the gnatcatcher morphological data showed the subspecies designations to be questionable or invalid. There are no diagnostic characters for any of the subspecies. Mean values of morphological measurements may separate birds in different locations, but there is substantial overlap among areas (McDonald et al. 1994). There is also not a consensus about where there are identifiable "breaks" in character measurements, presumed to indicate genetic discontinuity and hence subspecies boundaries (Baptista and Bell 1994, Barrowclough 1992, 1994, Boyce 1995, Brush 1994, Cronin 1994, 1995, 1997, Grant 1994, Grant et al. 2004, Hurst 2004, Link and Pendelton 1994, McDonald et al.1994, Messer 1994, Roberts and Bayn 1994, Skalski 1995a, 1995b). Rigorous statistical re-evaluation of Atwood's (1991) analyses showed serious problems with the data and analyses used, calling into question the legitimacy of the subspecies designations (McDonald et al. 1994).

Recently a statistical reanalysis of the gnatcatcher morphological data of Atwood (1988, 1991) was done by Skalski et al. (2008). These authors noted:

"...the California gnatcatcher (*Polioptila californica*) which was incorrectly listed under the ESA due to misinterpretation of morphological data."

Skalski et al. (2008) showed that the types of analyses used to designate the gnatcatcher subspecies (Atwood 1988, 1991) are subject to high rates of type I statistical errors (i.e., identification of subspecies when none exist). The morphological data actually show a geographic cline (i.e., gradual change over geography), not distinct breaks in character distributions. The tests used by Atwood (1991) do not consider the possibility of a cline in morphological characters over geography. Skalski et al. (2008) tested explicit hypotheses, including a null hypothesis of a smooth cline in traits (no distinct subspecies) and an alternative hypothesis of a break in a cline (subspecies boundary), with rigorous statistical tests. Reanalysis of the morphological data with spline-regression and step-regression were robust with high resolution and did not show subspecies breaks consistent with the designations of Atwood (1991).

Skalski et al. (2008) also noted that:

"The distinction between statistical significance versus biological significance thus appears to be a matter left to the individual taxonomist. One point should be stressed, that statistical significance is not sufficient to declare different subspecies."

This is profound, because it exemplifies the contention that subspecies designations are subjective and not scientifically rigorous (Zink 2004, Cronin 2006, 2007, Haig et al. 2006).

Perhaps most importantly, the gnatcatcher data were confounded by sampling problems. A sample must be representative of the subject population for it to be legitimately used in statistical analyses. However, the gnatcatcher data used by Atwood (1988, 1991) are considered to be a "classic example of confounding" because the samples and specimens used may be non-representative of the population (Skalski et al. 2008). In other words, the birds used to designate subspecies may not represent the birds actually occurring in nature. This is because the samples were collected over a 100 year period. Recall that "foxing" (a physical change in specimens over time, especially plumage color) may occur. This can make variation of plumage color a function of time, as well as geography. This is a critical point, and Skalski et al. (2008) note (as did the earlier re-analyses of Atwood's data by McDonald et al. 1994), that both geographic location *and* year of collection, equally explain the differences in plumage brightness used by Atwood (1991) in his subspecies designations. It is important to recognize that specimens collected from northern areas were sampled in earlier years than those in southern areas, and that the subspecies were separated by boundaries along a north-south distribution. Birds from the north were apparently in storage longer than those from the south. Also recall the statement by Mellink and Rea (1994) that many skins from the northern end of the species' range are severely soiled and have soot-stained plumage. The relationships described by Atwood (1991) may reflect the dates birds were collected, not the actual distribution of plumage color traits. Skalski et al. (2008) noted that this confounding of samples used in gnatcatcher subspecies designations was not noticed by Atwood (1988, 1991).

Skalski et al. (2008) noted:

"The California gnatcatcher case study is a perfect example of the consequences of using poorly posed biological questions and off-the-shelf statistical methods. Recent genetic work by Zink et al. (2000) confirm the early papers by Atwood (1988, 1991) were wrong in identifying the occurrence of subspeciation. However, the mistake was likely more than just an α-level probability error. The north-south cline in tracts causes H_o (null hypothesis of no subspecies) to be rejected even when no subspecieation has occurred."

In summary, the subspecies designation of the coastal California gnatcatcher (Atwood 1991) was not appropriate because of the use of morphological characters subject to environmental variation, changes during long term storage, and inappropriate statistical tests.

Molecular genetics and phylogeny

Molecular genetic studies also indicate the subspecies designation of the coastal California gnatcatcher (Atwood 1991) was not valid (Zink et al. 2000, Zink 2004). Note that the author of Atwood (1991) was a co-author of the Zink et al. (2000) paper. Zink et al. (2000) analyzed 1399 nucleotides of the mitochondrial DNA (mtDNA) control region, t-RNA-Glu, and part of the ND6 gene in 64 California gnatcatchers from 13 sampling

locations from Los Angeles to the southern tip of the Baja Peninsula (Figure 1). Zink et al. (2000) reported 26 variable nucleotide positions, including 17 transitions, two transversions, and seven deletions. Fourteen of the variable positions were parsimony-informative.

Thirty-three mtDNA haplotypes were observed. All of the haplotypes were closely related, with small DNA sequence divergences (maximum divergence of 0.64%, average divergence of 0.27%). Phylogenetic analysis showed the haplotypes exhibited no geographic structure and were not consistent with the subspecies designations of Atwood (1991). In the phylogenetic tree, haplotypes did not form exclusive clusters that conformed to recognized subspecies or geographic areas. That is, the subspecies did not have distinct mtDNA sequences and did not have monophyletic mtDNA. This is also obvious from the co-occurrence of the same mtDNA haplotype in all three subspecies designated by Atwood (1991), including 11 of the 13 sample locations (Appendix in Zink et al. 2000).

Twenty-three of the 64 birds shared one haplotype that occurred in 11 of the 13 locations sampled (Appendix in Zink et al. 2000). A measure of population subdivision was low (N_{ST} = 0.074) indicating no genetic differentiation of the birds in different sampling locations. This indicates that only 7.4% of the genetic variation is among sample locations and 92.6% of the genetic variation is within locations. This pattern is consistent with populations connected with extensive gene flow (i.e. birds move and interbreed among areas). It is not consistent with genetically differentiated subspecies. Zink et al. (2000) estimated the N_{ST} value suggested an exchange of between three and four birds per generation among populations, and that one such migrant per generation was enough to result in a panmictic unit (i.e., sub-populations comprise one large interbreeding population). Zink et al. (2000) noted that:

"...the pattern of distribution of the most common haplotype, the shape of the phylogenetic tree (phylogeography), and the low N_{ST} estimate all suggest that gene flow among the gnatcatcher populations has been substantial."

There was a higher level of genetic variation in the southern sampling locations than in the northern sampling locations. Zink et al. (2000) found this pattern consistent with a recently expanded population from the southern Baja Peninsula to southern California. This analysis of the mtDNA haplotype distribution suggests that the species in each sampling area has been increasing in number and expanding its range in the recent past (Zink et al. 2000, 2001).

Regarding subspecies, Zink et al. (2000, including Atwood as co-author) noted:

"The (mtDNA) haplotype tree for California gnatcatchers *does not support recognition of ESUs or subspecies* (my italics). The N_{ST} value also reinforces the conclusion that there are no ESUs within the California gnatcatcher. Species including two or more ESUs would have an N_{ST} value an order of magnitude greater than 0.07..."

Zink et al. (2000, including Atwood as co-author) also made the following statement regarding subspecies:

"Our finding of no significant genetic divisions explains prior controversy among subspecies schemes: there probably is no general pattern of variation in morphological characters consistent with historical isolation and independent evolution of populations".

This is admission that there is no morphological basis for subspecies, as with the mtDNA data. Because Atwood is a co-author of the Zink et al. (2000) paper, this is essentially a retraction of Atwood's (1991) subspecies designations, just as Atwood (1991) retracted his earlier (Atwood 1988) subspecies designations.

To summarize, the mtDNA data do not support the coastal California gnatcatcher (*P. c. californica*) as a subspecies. Both the mtDNA phylogeny and distribution of haplotypes indicate a high level of genetic similarity and gene flow across the range of the California gnatcatcher. Zink et al. (2000 noted:

"We found that coastal sage scrub populations of California gnatcatchers are not genetically distinct from populations in Baja California".

In addition, Zink et al. (2000) acknowledge that there is no consistent basis for designating California gnatcatcher subspecies or ESUs with the previous morphological analyses.

The subjective nature of subspecies in general

It is now well established in the scientific literature that subspecies are subjectively defined and designated (Mayr 1970, Cronin 1993, 2006, 2007, 2008, In Review, Zink 2004, Haig et al. 2006). It is also clear that subspecies is a taxonomic rank, and as such designations should be based on phylogenetic (i.e. evolutionary) relationships (e.g., Avise and Ball 1990). Because subspecies are by definition members of the same species and hence can interbreed, gene flow and common ancestry may prevent distinct phylogenetic differentiation, even with molecular genetic markers (Cronin 1993). This inherent characteristic indicates that subspecies are subjective and reflect the interpretation of individual taxonomists, not unequivocal scientific data. Other categories besides subspecies, including Distinct Population Segments (DPS) and Evolutionarily Significant Units (ESU) are also scientifically subjective (Cronin 2006). It has been noted that there is a tendency of biologists to create new "species" (as with designating subspecies, DPS, and ESU as species under the ESA) to justify conservation efforts, including ESA listings. This has been called "taxonomic inflation" (The Economist 2007, Marris 2007).

2. Review of regulatory documents.

Federal Register documents regarding subspecies status of the coastal California gnatcatcher and Endangered Species Act listing

The original 1993 ESA listing determination of the coastal California gnatcatcher (58 FR 16742) noted that earlier assessments designated the coastal black-tailed gnatcatcher (*Polioptila melanura californica*) as a category 2 candidate for the list of Endangered and Threatened wildlife (47 FR 58454) and subsequently retained it as such (50 FR 37958, 54 FR 554). The U.S. Fish and Wildlife Service (FWS) in the 1993 listing determination recognized the subspecies classification of Atwood (1991), and regarding *P. c. californica* stated: "This taxon is now recognized as a subspecies of *Polioptila californica*".

The 1993 listing determination (58 FR 16742) addressed issues raised in public comments on the proposed rule. The first three issues discussed are relevant to the taxonomic status of the coastal California gnatcatcher.

Issue 1. The California gnatcatcher and its northern nominate subspecies (i.e. coastal California gnatcatcher, *P. c. californica*) are not valid taxa. Several comments questioned the change of taxonomy including changing the California gnatcatcher to a full species, *P. californica*, from a subspecies of black-tailed gnatcatcher (*P. melanura*), and whether there was a distinct subspecies of *P. californica* in southwestern California and northwestern Baja, California, Mexico. The FWS responded that they and the American Ornithologists' Union (AOU) concluded that *P. c. californica* is a valid taxon, citing Atwood (1988, 1991, and others).

Issue 2. Several comments questioned the validity of the statistical analysis used by Atwood (1991) to evaluate intraspecific morphological variation within the California gnatcatcher. FWS responded that Atwood's methods have been peer reviewed and there was no indication he used inappropriate statistical methods.

Issue 3. Several comments said that the taxonomic conclusions of Atwood (1991) were not valid because they are based on plumage color and may be environmental, and not genetic, in origin. FWS responded that it was unknown whether the traits reported by Atwood (1991) are genetically based. FWS noted that the traditional scientific approach to defining avian subspecies has been almost exclusively using morphological differences in body measurements and plumage characters, and that Atwood's conclusions are strengthened by congruent patterns in geographic variation among several species at 30° north latitude.

Assessment: The first issue of whether *P. c. californica* is a valid subspecies depends on the answers to the second and third issues. Considering the review of *P. c. californica*

taxonomy above, it is clear that the statistical analyses used by Atwood (1991) were seriously flawed (McDonald et al. 1994, Skalski et al. 2008). This makes the FWS response on issue 2 invalid. The invalidity of the statistical analyses, the discovery of changes in plumage characters over time in storage, and the mtDNA analysis (Zink et al. 2000) make the FWS response to issue 3 invalid. The genetic versus environmental origin of morphological differences is an important taxonomic consideration, but not particularly relevant in this case because there are no subspecies identifiable with the morphological data when proper statistics are applied (Skalski et al. 2008). Also, Zink et al. (2000) noted that the morphologically-based subspecies schemes are invalid, and with Atwood as a co-author, this is a retraction of the subspecies designations of Atwood (1991). The lack of genetic differentiation of the proposed subspecies also makes the subspecies designations of Atwood (1991) invalid. Therefore the FWS response to Issue 1 is also invalid and *P. c. californica* is not a valid subspecies. This means the ESA listing determination (58 FR 16742) is invalid and the subspecies was incorrectly listed (Skalski et al. 2008).

The coastal California gnatcatcher as a Distinct Population Segment instead of a subspecies

In 2003 FWS began reviewing the subspecies status of *P. c. californica* (68 FR 20228), noting:

"We originally identified the coastal California gnatcatcher as a subspecies of the California gnatcatcher. However new genetic information raises questions about the distinctiveness of the subspecies." FWS also solicited comments on consideration of the coastal California gnatcatcher as a Distinct Population Segment (DPS) instead of a subspecies, specifically asking if the recent genetic findings (i.e. Zink et al. 2000) justify a review of the taxonomy of the subspecies.

In 2004 FWS reopened the public comment period for determination of distinct vertebrate population segment (DPS) status for the California gnatcatcher (69 FR 18515). FWS notes that the mtDNA data of Zink et al. (2000) did not support a subspecies designation and that "the morphological variations previously described were not genetically based and subspecies divisions are not supported." FWS lists Atwood as a co-author in Zink et al. (2000) but does not explicitly recognize that this means the subspecies designations are retracted (i.e. the same author who designated subspecies states that the subspecies are not valid at a later date).

In 2007 FWS noted they are continuing their review of whether the listing of the coastal California gnatcatcher as a subspecies should be retained or changed (72 FR 72009).

A phone call (14 September 2009) from M. Cronin to the FWS Carlsbad, CA office was made asking if there was a decision on the gnatcatcher subspecies review. John Hazard in the FWS Carlsbad office told Cronin there is no update since 2007, and the review of gnatcatcher subspecies status is still underway.

Assessment: Zink et al. (2000) provide important information with regard to the potential designation of the coastal California gnatcatcher as a DPS instead of a subspecies. Recall that Zink et al. (2000) stated:

"The (mtDNA) haplotype tree for California gnatcatchers *does not support recognition of ESUs or subspecies* (my italics). The N_{ST} value also reinforces the conclusion that there are no ESUs within the California gnatcatcher. Species including two or more ESUs would have an N_{ST} value an order of magnitude greater than 0.07..."

Also, note that the National Marine Fisheries Service (NMFS) designates DPS of Pacific salmon (*Oncorhynchus* spp.) as Evolutionarily Significant Units (ESU, Waples 1991). That is, for the ESA, an ESU is equivalent to a DPS. Zink et al.'s (2000) conclusions mean that if the gnatcatcher does not qualify as an ESU, it should not qualify as a DPS (i.e. DPS = ESU, see Cronin 2006). However, DPS can be designated with international boundaries so the U.S.-Mexican border could possibly be used to designate a DPS regardless of the biological relationships of birds in the U.S. and Mexico.

Other Endangered Species Act subspecies designations

Recent debate over other subspecies indicates that the case of the coastal California gnatcatcher is not unique (e.g., Zink 2004, Haig et al. 2006, Cronin In Review). Other questionable subspecies listed under the ESA include the Preble's meadow jumping mouse (*Zapus hudsonius preblei*, Ramey et al. 2005, Cronin 2007) and the Perdido Key Beach mouse (*Peromyscus polionotus trissyllepsis*, Cronin 2008). It is relevant that the U.S. Fish and Wildlife Service (FWS) is doing a review of wolf subspecies taxonomy (S. Chambers, FWS, personal communication). This includes assessment of general subspecies concepts and criteria. This may be relevant to the FWS review of the gnatcatcher subspecies regarding consistency of science within the FWS.

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Tab 2

Genetics, Taxonomy, and Conservation of the Threatened California Gnatcatcher

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Abstract: *The California Gnatcatcher (*Polioptila californica*) has become a flagship species in the dispute over development of southern California's unique coastal sage scrub habitat, a fragile, geographically restricted ecosystem with high endemism. One aspect of the controversy concerns the status of the subspecies of this bird in southern California coastal sage scrub that is currently listed as threatened under the U.S. Endangered Species Act. To investigate the recent population history of this species and the genetic distinctiveness of subspecies and to inform conservation planning, we used direct sequencing of mitochondrial DNA (mtDNA) for 64 individuals from 13 samples taken throughout the species' range. We found that coastal sage scrub populations of California Gnatcatchers are not genetically distinct from populations in Baja California, which are dense and continuously distributed throughout the peninsula. Rather, mtDNA sequences from this species contain the signatures of population growth and support a hypothesis of recent expansion of populations from a southern Baja California refugium northward into the southern coastal regions of California. During this expansion, stochastic events led to a reduction in genetic variation in the newly occupied range. Thus, preservation of coastal sage scrub cannot be linked to maintaining the genetic diversity of northern gnatcatcher populations, despite previous recognition of subspecies. Our study suggests that not all currently recognized subspecies are equivalent to evolutionarily significant units and illustrates the danger of focusing conservation efforts for threatened habitats on a single species.*

Genética, Taxonomía, y Conservación de la Perlita de California Amenazado de Extinción

Resumen: *La perlita de California (*Polioptila californica*) se ha convertido en una especie insignia en la disputa sobre el desarrollo del exclusivo hábitat de chaparral de salvia costero (CSS) del sur de California, un ecosistema frágil y geográficamente restringido con un endemismo elevado. Un aspecto de la controversia tiene que ver con la situación de la subespecie de esta ave en el CSS del sur de California y que se encuentra actualmente enlistada como amenazada bajo el Acta de Especies Amenazadas de los Estados Unidos. Utilizamos un secuenciado directo de ADN mitocondrial (mtDNA) de 64 individuos de 13 muestras tomadas a lo largo del rango de distribución de la especie para investigar la historia poblacional reciente de la especie y la diferenciación de subespecies, y para documentar planes de conservación. Encontramos que las poblaciones de la perlita de California de CSS no son genéticamente distintas de las poblaciones de Baja California, las cuales son densas y tienen una distribución continua a lo largo de la península. Más bien, las secuencias de mtDNA de esta especie contienen la firma de un crecimiento poblacional y apoya una hipótesis de expansión reciente de poblaciones de un refugio sureño de Baja California hacia el norte y hacia adentro de las regiones sureñas costeras de California. Durante esta expansión, los eventos estocásticos conducen a una reducción en la variación genética en el rango recientemente ocupado. Por lo tanto, la conservación del CSS no puede ser vinculada con el mantenimiento de la diversidad genética de poblaciones norteñas de perlitas, a pesar de su previo reconocimiento como subespecie. Nuestro estudio sugiere que no todas las subespecies actualmente reconocidas son equivalentes a las unidades evolutivamente significativas e ilustra el peligro de enfocar los esfuerzos de conservación de hábitats amenazados en una sola especie.*

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Paper submitted February 8, 1999; revised manuscript accepted January 26, 2000.

Conservation Biology, Pages 1394–1405
Volume 14, No. 5, October 2000

Introduction

Since 1940, the human population of southern California has increased at a rate twice that of many developing countries (Mann & Plummer 1995). Not surprisingly this growth has had a negative effect on the native flora and fauna. One particularly hard-hit community is coastal sage scrub, where approximately 100 endemic species and subspecies of plants and animals are potentially endangered (Atwood 1993). A characteristic inhabitant of coastal sage scrub, a small, nonmigratory songbird known as the California Gnatcatcher (*Polioptila californica*), has been at the center of efforts to preserve this habitat and its unique flora and fauna. Populations of gnatcatchers in coastal sage scrub are considered threatened under the U.S. Endangered Species Act (ESA; U.S. Fish and Wildlife Service 1993, 1995) as a result of loss of 70–90% of the original habitat (Atwood 1993). The remaining highly fragmented tracts of coastal sage scrub are of high economic value because of their proximity to the Pacific Ocean and major urban, retirement, and commercial areas in Los Angeles, Riverside, Orange, and San Diego counties. Some patches of coastal sage scrub are valued at $3 million per acre (0.40 ha; Mann & Plummer 1995). Because a pair of California Gnatcatchers may occupy a year-round home range in excess of 10 acres (4 ha; Atwood 1993) the value of real estate required to support a population of, for example, 50 pairs of these birds might exceed $1 billion. Few other species better typify the conflicts and tradeoffs among legal, environmental, and economic priorities.

The abundance of gnatcatchers throughout their range reveals a potential conflict between legal and biological concerns. California Gnatcatchers occur from Los Angeles, California, to the southern tip of the Baja peninsula (Fig. 1). Northern populations are least dense, especially from El Rosario (Baja California, lat 30°N) north to Los Angeles. These threatened populations comprise many small groups of individuals, each often isolated by urban sprawl, which potentially promotes local inbreeding. In contrast, populations in central and southern Baja California and throughout Baja California Sur are large and continuous (Atwood 1993). Thus, the ESA mandates protection of populations of a species that are historically restricted (and threatened) in the United States, whereas populations elsewhere in the contiguous range are "healthy." Therefore, the species as a whole is not threatened; rather, the issue involves preservation of populations within a relatively small part of the range that transcends an international boundary (Hunter & Hutchinson 1994).

Conservation of the species has been complicated by past taxonomic studies. Before 1989, the California Gnatcatcher was classified as a subspecies of the Black-tailed Gnatcatcher (*Polioptila melanura*). Studies by Atwood (1988), however, revealed that subspecies along the coast in California and those south of 28°N latitude throughout the Baja California peninsula were distinct

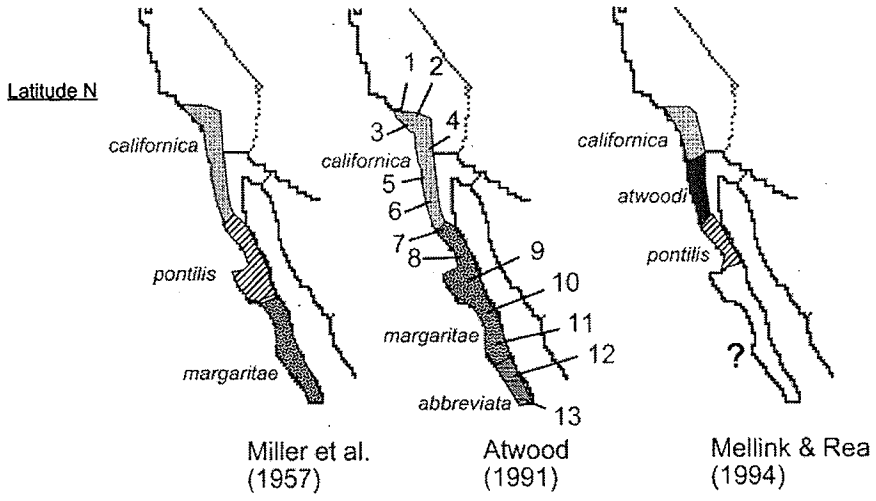

Figure 1. Three subspecies schemes proposed by Miller et al. (1957), Atwood (1991), and Mellink and Rea (1994) for the California Gnatcatcher, based on morphological characteristics of the external phenotype. Mellink and Rea (1994) did not explicitly state their recommendation for subspecific taxonomy south of 27°N latitude; they recognized P. c. margaritae, *however, for a total of at least four subspecies. Combining the treatments suggests a total of five subspecies. Sample sites for mtDNA study shown with numbers on the subspecies scheme of Atwood: 1, Los Angeles County; 2, Riverside County; 3, Orange County; 4, San Diego County; 5, Ensenada; 6, San Telmo; 7, Mision San Fernando; 8, El Rosarito; 9, San Ignacio; 10, Mulege; 11, Villa Insurgentes; 12, La Paz; and 13, Cabo San Lucas. The boundary between the states of Baja California and Baja California Sur is at 28°N latitude.*

from populations of *P. melanura* to the east. Analysis of the amount of white in the tail feathers and especially of vocalizations provided key evidence for the species-level distinctiveness of these subspecies and led to their formal recognition (American Ornithologist's Union 1989) as the California Gnatcatcher (*P. californica*). Analyses of mitochondrial DNA (mtDNA) sequences subsequently corroborated Atwood's recognition of the California Gnatcatcher (Zink & Blackwell 1998). The subspecific taxonomy of the California Gnatcatcher, however, has been controversial. Based on differing interpretations of geographic patterns of coloration, size, and shape, three recent subspecies schemes have been proposed (Fig. 1). Although these subspecies classifications differ, all suggest that the northern part of the range, including the coastal sage scrub populations, includes one or two subspecific units. The controversy over subspecies taxonomy suggests that new data are required to clarify the significance of geographic variation relative to conservation of both the species itself and of the coastal sage scrub.

From the viewpoint of conservation genetics, the issue involves the distribution of genetic diversity within the species: is the species uniform throughout its range or is it subdivided into smaller units, termed evolutionarily significant units (ESU; Ryder 1986; Barrowclough & Flesness 1996)? To qualify as an ESU (Moritz 1994; Waples 1995), phylogenetic analysis of mtDNA haplotypes must show that haplotypes from a given region are more closely related to each other than they are to haplotypes from other regions (termed reciprocal monophyly). If haplotypes from a given region do not form such an exclusive group, then either gene flow is ongoing or it has ceased recently. In either of the latter two cases, there are no geographic units that have had significant periods of isolation and independent evolution. Thus ESUs are diagnosed by the pattern of haplotype variation, not the level of sequence divergence. That is, reciprocally monophyletic groups can differ by 1% (a typical lower value for birds) to over 8% (Avise & Walker 1998). Evolutionarily significant units defined by genetic criteria constitute significant elements of biodiversity "below" the species level and are often considered units of conservation (U.S. Departments of the Interior and U.S. Department of Commerce 1996).

In cases in which direct genetic information is lacking, the taxonomic category of subspecies serves as a proxy for the ESU, as in the case of the California Gnatcatcher. The relevant conservation question here is whether putative subspecies of the California Gnatcatcher (Fig. 1) reflect ESUs or whether the genetic composition of the species is more homogeneous than previous subspecies schemes imply (Cronin 1997). Given the central role that subspecies can play under the ESA in serving as surrogates for ESUs (National Research Council 1995), testing subspecies limits is a vital component of conservation biology.

Genetic studies also provide perspective on the recent demographic history of populations. For example, Slatkin and Hudson (1991) and Rogers (1995) use recent advances in coalescence theory to show how population expansion can be distinguished from a history of long-term constant population size. Estimates of gene flow can also be derived (Wright 1931). Such inferences can complement information about patterns of genetic variation. For example, lack of ESUs might result from recent population expansion with insufficient elapsed time for differentiation.

We sequenced the rapidly evolving mtDNA control region (Taberlet 1996) and part of the ND6 gene. We sampled populations throughout the range to clarify genetic patterns of threatened and "healthy" populations. Our goals were to investigate the recent demographic history of this species, to test for the existence of ESUs and hence the validity of various subspecies schemes, and to comment on the relevance of genetic information to the conservation of this species.

Methods

We collected gnatcatchers in Mexico and plucked feathers from nestlings in the United States. Specimens are housed at the American Museum of Natural History, New York, Museo de Zoologia, Universidad Autonoma de Mexico, and the J. F. Bell Museum, University of Minnesota. Sample size at each of the 13 localities (Fig. 1) was five, except for San Diego County ($n = 3$), Riverside County ($n = 4$), and San Telmo ($n = 7$). The mtDNA was isolated from tissue or feather pulp, amplified via the polymerase chain reaction, and sequenced manually following standard protocols (Hillis et al. 1996). We used several pairs of primers (Tarr 1995; Zink et al. 1997) to obtain a sequence for the mtDNA control region, t-RNA^{Glu}, and part of ND6 (ND6E, HCR4, LCR4, HPHE-1; LMCR CCAGTACAGGAGTAATGTCG; and LCCR2M CTCTTCACAGATACAAGTGG). As a check on the control-region results, we also sequenced parts of two other mtDNA genes (318 base pairs [bp] of ND3 and 275 bp of ND2) from 12 specimens spanning the entire geographical range. We used the program PAUP* (Swofford 1999) to estimate a haplotype tree based on maximum parsimony (heuristic search, bases equally weighted); a haplotype of the Black-tailed Gnatcatcher (*P. melanura*), sister species of the California Gnatcatcher (Zink & Blackwell 1998), was used to root the tree. We bootstrapped the data set 250 times using random additions. We tested for departure from a molecular clock by performing a log-likelihood ratio test (HKY85 model with gamma correction) of the difference in likelihood for a minimum-length haplotype tree with and without a molecular clock enforced (Huelsenbeck & Rannala 1997). The significance of two times the difference in log likelihoods was assessed by a chi-square table.

We computed the amount of genetic variation within each population sample, nucleotide diversity (π), following standard equations (Nei 1987). In addition, we

computed the amount of genetic variation distributed among populations, a quantity estimated by N_{ST} (Lynch & Crease 1990). The N_{ST} analysis resembles a hierarchical ANOVA, partitioning genetic variation into among- and within-population components. The N_{ST} tends to 0 with no population subdivision, whereas a value nearing 1 indicates that populations share no recent gene-flow events or common history. We computed Tajima's (1989) D statistic to assess whether sequence evolution appeared consistent with neutral expectation.

We computed the mismatch distribution (Rogers & Harpending 1992), defined as the number of nucleotide differences between all pairs of individual California Gnatcatcher mtDNAs ($n = 64$). We calculated the mean of these differences and, following Slatkin and Hudson (1991), used the mean value to fit the observed distribution to an expected Poisson distribution; the distributions were compared with a Kolmogorov-Smirnov one-sample test. In a subsequent analysis, we divided the gnatcatcher samples into two subsets that corresponded to the 25 individuals from the five localities south of latitude 28°N and the 39 individuals from the eight localities north of latitude 28°N. We computed the mismatch distributions for these two subsets and again fit the means to Poisson distributions and performed Kolmogorov-Smirnov tests. Harpending's (1994) raggedness statistic was estimated for the distribution of pairwise differences. This statistic has been used to distinguish between stationary and growing populations of humans.

Nee et al. (1995) have shown that, for a population of approximately constant size, a plot of the logarithm of the number of lineages versus their branching times will have a characteristic concave shape, whereas an exponentially growing population exhibits a convex shape. We arbitrarily used one of the alternate minimum-length estimates of phylogenetic relationships for individual gnatcatchers and estimated the time of origin of each of the nodes on that tree. For each node, we computed the number of nucleotide substitutions to each terminal stemming from that node. The average of these substitutions over all paths from the internal node to sampled individuals was taken as the "age" of the node. This was plotted against the number of lineages segregating prior to that estimated time. For the shallowest, most terminal nodes of the tree, estimated ages are not precise because the number of substitutions along the short-terminal and near-terminal branches was frequently zero, one, or two. This sampling error can result in estimated branch lengths that are slightly negative. For such cases we collapsed the negative branches into the next most basal nodes. This circumstance arises only in the analysis of log-lineage plots for which the average age of nodes must be estimated; minimum-length parsimony trees have no negative branch lengths (for a worked example, see Barrowclough and Groth 1999).

We constructed an expected plot of log lineages versus coalescent times using the equations reviewed by Hudson (1991). The expected time between coalescent events is $T(j) = (2N_{ef})/(j(j - 1))$, where j is the number of lineages left to coalesce and N_{ef} is the effective number of females in the population. The total expected time to coalescence is $2N_{ef}(1 - 1/n)$, where n is the number of individuals sampled; for 64 individuals this is $1.97N_{ef}$. We therefore calibrated the log-lineages plot by letting the depth, in substitutions, of the gnatcatcher tree equal $1.97N_{ef}$. To compare the observed distribution of lineages versus time with the pattern expected for a population of constant size, we computed the expected time of each coalescent event $T(j)$ and set the estimated age of the earliest node on the tree equal to the expected coalescence time for a sample of 64 individuals from a stationary population.

An alternative to the graphical techniques discussed above for drawing inferences about the demographic history of populations was developed by Kuhner et al. (1998). This approach yields maximum-likelihood estimates of population size and growth rates based on a simple model of DNA evolution and a maximum-likelihood, as opposed to parsimony, estimate of the haplotype tree. Using the program FLUCTUATE (Kuhner et al. 1998), we estimated population growth rates for the entire sample of 64 gnatcatchers, as well as for the sample of 25 individuals from the five localities south of 28°N latitude and the 39 individuals from the eight localities north of 28°N latitude. In estimating the growth rate with FLUCTUATE, we used a transition-to-transversion ratio of 10.0 and a two-rate substitution model in which 90% of the sites were invariant and 10% had the same substitution rate. In this procedure, the search for the maximum-likelihood estimate over the likelihood surface was initiated with Watterson's estimate of theta(θ). To determine if the resulting estimates were stable, we iterated the search for maximum-likelihood estimates of growth and θ, but in successive iterations we used the point estimates from the previous iteration as a starting point. This procedure provides information about whether the likelihood surface is sufficiently smooth and has sufficient relief so that estimates are consistent.

Results

A total of 1399 bp, including some indels, was obtained for all 64 gnatcatchers. We analyzed data with and without deletions, and our overall conclusions are unchanged; omitting deletions results in lower resolution of the pattern of haplotype relationships. We found similar levels of variation for the 12 individuals surveyed additionally for ND2 and ND3 gene regions and no geographically segregating differences, so these data are not discussed further. Because these genes are not contiguous with the

control region, we inferred that our sequence data were mitochondrial and not derived from a nuclear homologue. Furthermore, our control-region sequences contained the "landmarks" found in other avian control regions (Baker & Marshall 1997). Also, the large number of closely related haplotypes we found argues against nuclear copies, which tend to be less variable owing to mutation repair mechanisms.

Direct sequencing of the control region revealed 26 variable positions (17 transitions, 2 transversions, 7 deletions), of which 14 were parsimony-uninformative. Of the 64 California Gnatcatchers examined, 33 exhibited unique haplotypes (Appendix). All haplotypes were closely related, with a maximum (uncorrected) interhaplotype divergence of 0.64% and an average of 0.27%. Twenty-three individuals (35.9%), representing 12 of 13 localities, shared a single haplotype, whereas the next most frequent haplotype was found in four individuals (6.3%). The N_{ST} of 0.074 suggests a lack of population subdivision; 92.6% of the genetic variation was common to populations and only 7.4% was distributed among them. The lack of structure among gnatcatcher haplotypes was confirmed by phylogenetic analysis (Fig. 2), which does not support any subspecies scheme, either previously described (Fig. 1) or unforeseen. That is, haplotypes did not form exclusive clusters that conformed to recognized subspecies or to any other geographically restricted areas. A feature common to the minimum-length trees was the basal position of several haplotypes from southern locations. A likelihood ratio test (LRT) (Kishino & Hasegawa 1989) significantly ($p = 0.01$) rejected a tree in which haplotypes were constrained to match the subspecies limits proposed by Atwood (Fig. 1). A LRT for a haplotype tree (one of the minimum-length trees) with and without a molecular clock enforced was not significant, indicating a lack of rate heterogeneity. Tajima's D statistic was significant in only 1 of 13 population samples.

The most striking genetic pattern observed (Fig. 3) was a transition in level of genetic diversity (π) between the San Ignacio and El Rosarito locales, with populations north of San Ignacio showing π values approximately 25% of those to the south of 30°N latitude.

The mismatch distribution (Fig. 4) had the overall shape associated with growing rather than constant populations (Slatkin & Hudson 1991; Rogers & Harpending 1992). Superimposed on the distribution was the Poisson distribution for a sample with the same mean, 2.33, as the observed distribution. The observed and expected distributions differed significantly (Kolmogorov-Smirnov test, $p < 0.05$). Dividing the population samples at 28°N latitude, which corresponded to the observed discontinuity in π (Fig. 3), we found that neither mismatch distribution (Fig. 5) deviated significantly ($p > 0.05$) from the Poisson expectation (mean for northern samples, 1.17 substitutions; mean for southern samples, 3.81). Harpending's (1994) raggedness value for the overall distribution of pairwise differences, 0.032, resembled those associated with growing populations. Populations with stationary sizes usually had raggedness values of 0.05–0.5, with a mode of 0.1 in their study.

Comparison of observed and expected plots of the distributions of lineages versus time requires calibration of the expected curve. The earliest (deepest) node on the tree corresponded to 6.25 substitutions, which we took as the expected coalescent time for a sample of 64 individuals, $1.97N_{ef}$. Thus, we calibrated the two curves by assuming that $1.97\mu N_{ef} = 6.25$; that is, letting $\mu N_{ef} = 3.17$, where μ is the nucleotide substitution rate. In the resultant plot (Fig. 6), we indicated the expected position of the first 10 coalescent events and used a curve to indicate the shape of the distribution for the remaining 52 closely spaced events. The observed plot (Fig. 6) was consistently to the left of the curve expected for a population of constant size (the result found by Nee et al. [1995] for a growing population).

The maximum-likelihood estimates of growth rates (+1 SD) for the entire sample of 64 California Gnatcatchers, for the five southern populations, and for the eight northern populations were 1025 + 23, 583 + 18, and 1853 + 191, respectively. (These estimates of growth rate are standardized by the mutation rate [e.g., Kuhner et al. 1998]). The three estimates were all positive and significantly different from zero, thereby rejecting a population of constant size. In three successive iterations using the program FLUCTUATE, the estimates of growth rate obtained were stable and consistent.

Discussion

Population History

The most common haplotype (36% of individuals) was found in 12 of 13 population samples. Based on the rooted haplotype tree (Fig. 2), this most common haplotype arose relatively recently. This suggests that gene flow among localities must be substantial; that is, a relatively recently arisen haplotype has spread throughout the range of the California Gnatcatcher compared to the common ancestor of all extant haplotypes. The shape of the phylogenetic tree (Fig. 2) reinforces this conclusion. Although some old, relict haplotypes were confined to the southern portions of the Baja California peninsula, there was no geographic structuring of the more recent branches. If there were substantial barriers to gene flow, one might expect that "families" of related haplotypes would be found in geographically contiguous or proximal locations. This has been found for other birds from Baja California such as LeConte's Thrasher (*Toxostoma lecontei*; Zink et al. 1997), for which mutually exclusive clades of haplotypes were found in two disjunct geographic regions. The haplotype

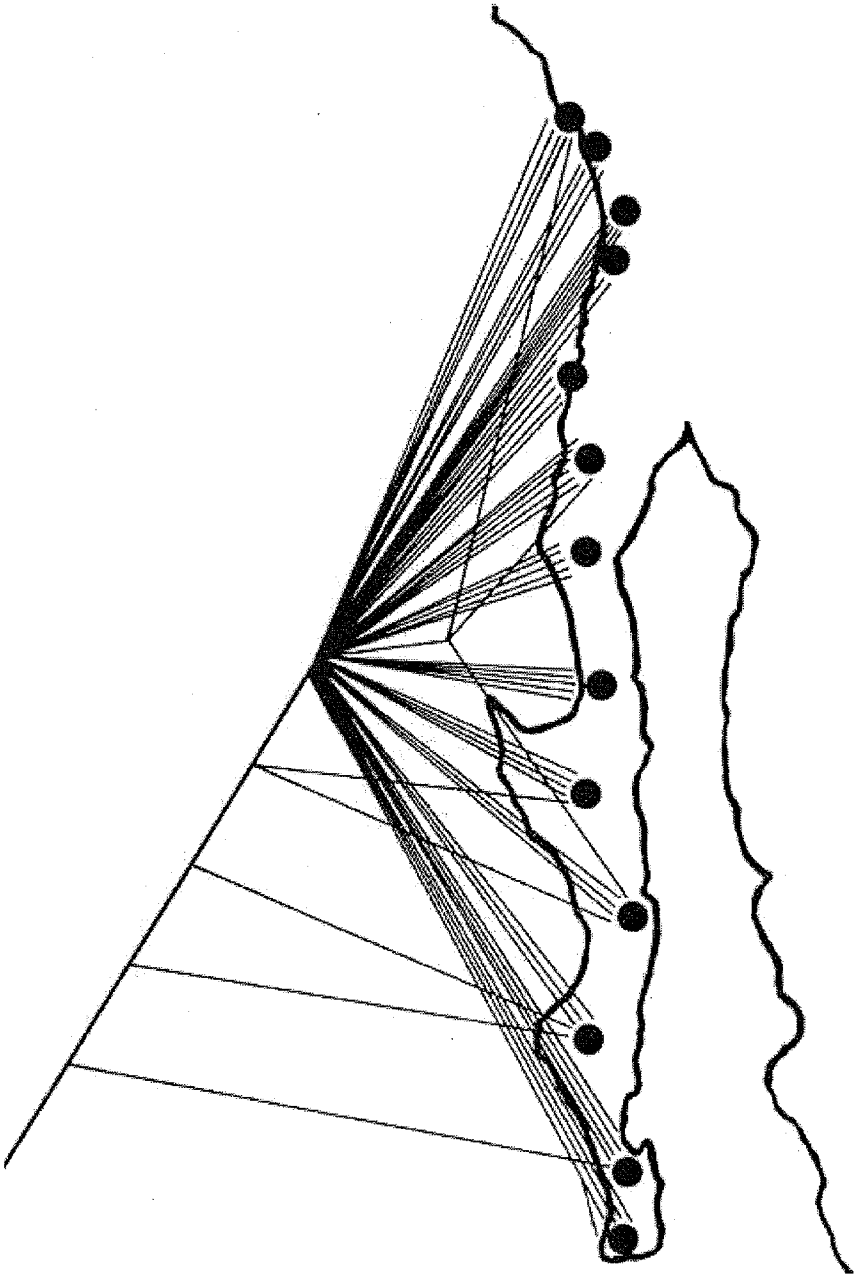

Figure 2. Strict consensus tree of haplotypes derived from 10,751 equally parsimonious trees (length 84, consistency index [ci] = 0.4, excluding uninformative characters; rescaled ci = 0.5) showing no geographic structure among 64 individuals. There is a single node at the top of this tree. No nodes were present at >65% in 250 bootstrap replicate trees. Only two nodes had bootstrap values >50%; each included one pair of haplotypes from different localities.

tree for California Gnatcatchers does not support recognition of ESUs or subspecies.

The N_{ST} value also reinforces the conclusion that there are no ESUs within the California Gnatcatcher. Species including two or more ESUs would have an N_{ST} value an order of magnitude greater than 0.07; in the case of LeConte's Thrasher, for example, N_{ST} was 0.75. Elementary but relatively robust models in population genetics (Neigel 1997) allow one to obtain an estimate of the amount of gene flow necessary to maintain an N_{ST} or F_{ST} value for an isolation-by-distance model or island model of population structure at equilibrium. The estimate of N_{ST} we obtained was equivalent to an exchange of between three and four individuals per generation among populations. Wright (1931) showed that if the amount of gene flow among populations was greater than approximately one individual per generation, the entire population could be thought of as one large panmictic unit. Thus, the pattern of distribution of the most common haplotype, the shape of the phylogenetic tree (phylogeography), and the low N_{ST} estimate all suggest that gene flow among the gnatcatcher populations has been substantial.

The sudden geographic shift in π can be attributable to two alternate phenomena, namely a range expansion

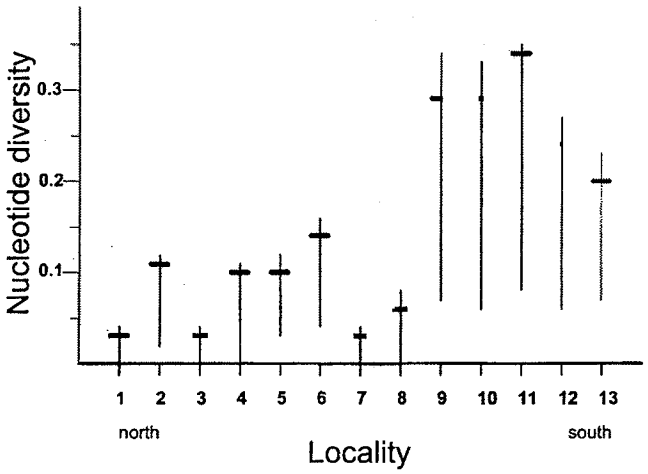

Figure 3. Pattern of geographic variation in nucleotide diversity (π; estimate and 95% bootstrap confidence intervals) showing shift between El Rosarito (locality 8) and San Ignacio (9) at 28°N latitude. The pattern is significant with a run test ($p < 0.05$)

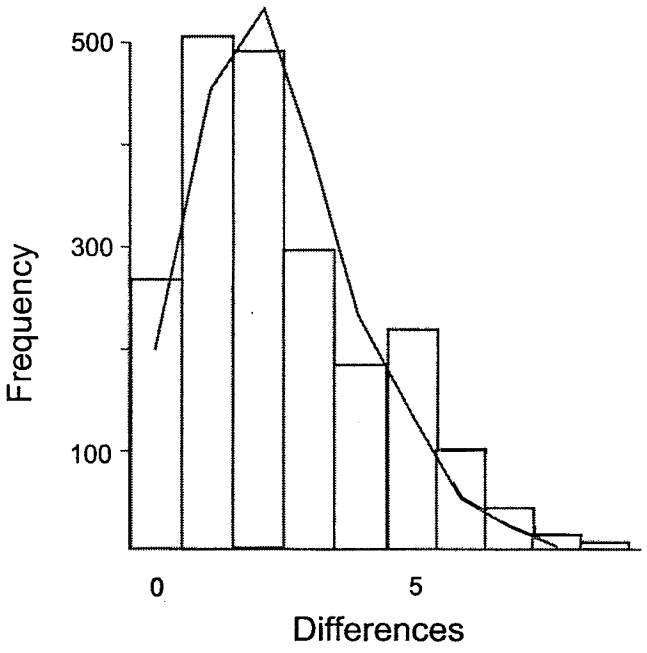

Figure 4. Mismatch distribution for 64 California Gnatcatcher sequences (histogram) and the expected Poisson distribution (line) for a growing population with the same mean.

from a southern refugium or a "selective sweep." If the northern part of the range only recently became inhabitable or otherwise available to gnatcatchers, northward emigrants dispersing from a southern refugium might represent only a part of the species' genetic diversity, leading to decreased π in the north (Hewitt 1996). Alternatively if a new advantageous mutation makes a northern haplotype selectively superior, it can spread southward rapidly and increase in frequency at the expense of older and less fit southern haplotypes. The observed pattern of variation in π is consistent with either hypothesis. Four lines of evidence support the hypothesis of recent and northward population expansion. First, phylogenetic analysis (Fig. 2) revealed several basal (i.e., oldest) haplotypes that occurred only in Baja California Sur. Older (basal) haplotypes are expected to occur disproportionately in previous refugia. Baja California south of 30°N latitude was thought to be a refugium (Magdalena Refugium) during the late Pleistocene (Hafner & Riddle 1997). Other avian (Zink et al. 1997) and nonavian (Upton & Murphy 1997) species also show genetic breaks between 28°N and 30°N latitudes. Second, the plot of the number of lineages versus the estimated age of the haplotype tree suggests an expanding population.

Third, the overall mismatch distribution (Fig. 4) was basically unimodal and had the characteristic shape associated with a growing population (Rogers & Harpending 1992). Such distributions from constant populations are often ragged (Slatkin & Hudson 1991; Harpending et al. 1993; Harpending 1994)—that is, bimodal or multimodal—unlike that for the California Gnatcatcher. In addition, the plot was quite different from those reported by Barrowclough and Groth (1999) for three populations of owls that they interpreted to be stationary in size. The results of the Kolmogorov-Smirnov test, however, indicated that our observed distribution differed from the Poisson expectation for a growing population. These tests must be interpreted with caution because the samples were not independent (Slatkin & Hudson 1991). Visual comparison of the observed and expected mismatch distributions for the 64 gnatcatchers (Fig. 4) suggests that the tail of the distribution is too long for a Poisson curve. This overall distribution, however, was for 13 population samples taken over 1000 linear km. Rogers and Harpending (1992) showed that an exponentially growing population can be characterized by a Poisson-like distribution with a mode that starts at an average pairwise difference of zero at the time of initial growth; the distribution shifts to larger values of pairwise differences as time increases while maintaining the characteristics of a Poisson distribution (for example, variance equal to mean).

Our interpretation of the topology of the sequence relationships and the geographical pattern of nucleotide diversity is that populations of California Gnatcatchers have been expanding their range northward from southern Baja California. If this were true, then the expansion in population size would be older in the southern part of the gnatcatcher range and more recent in more northern parts of the range. Therefore, we divided the gnatcatcher samples into two subsets that corresponded to the regions defined by the pattern of π (Fig. 3). Mismatch distributions (Fig. 5) fit to each subset did not differ significantly from expecta-

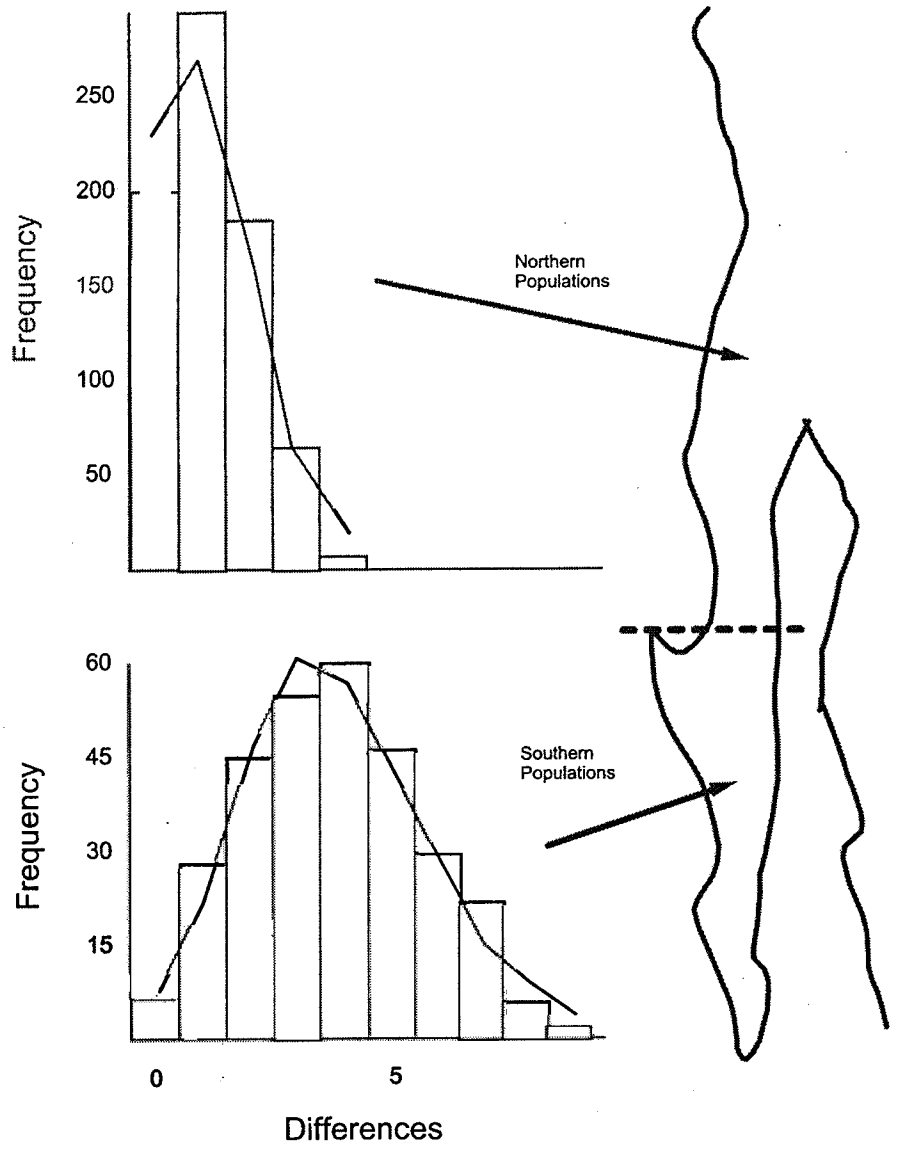

Figure 5. Mismatch distributions for northern and southern samples of California Gnatcatchers with expected Poisson distributions for growing populations with the same mean (lines).

tion. The shallow, wide distribution with the mode removed from the vertical axis in the south, and the tall, narrow distribution close to the axis in the north reflect what would be anticipated given the expansion process we suggest. Although mismatch distributions could be computed separately for each of the 13 populations samples, small sample sizes precluded this procedure.

Fourth, it might be argued that the log-lineage plot and the mismatch distributions are simply qualitative or heuristic techniques. The quantitative, maximum-likelihood method of Kuhner et al. (1998) obtains an estimate of growth rate by integrating over all possible tree topologies, rather than a single parsimony tree. In addition, the maximum-likelihood approach does not require an outgroup for rooting purposes. Consequently, it is in many ways an independent technique for addressing the question of a stable versus growing population. Using the maximum-likelihood method, we obtained estimates of growth rates for the gnatcatchers that were all positive and significantly different from zero. In addition, the growth rate for the northern eight populations was estimated to be greater by a factor of three than that for the southern five populations; this is consistent with our hypothesis that the predominant region of population expansion is the northern part of the range, emanating from a possible southern refugium.

Taken together, the tree of haplotype relationships, the geographical pattern of π, the shape of the mismatch distributions, the log-lineage plot, and the maximum-likelihood results favor a hypothesis of a relatively recent expansion of California Gnatcatcher populations from southern Baja California northward throughout the peninsula and into southwestern California. Such a recent population expansion likely explains the lack of phylogeographic pattern. Given that range expansion has recently occurred, it is unlikely that any current iso-

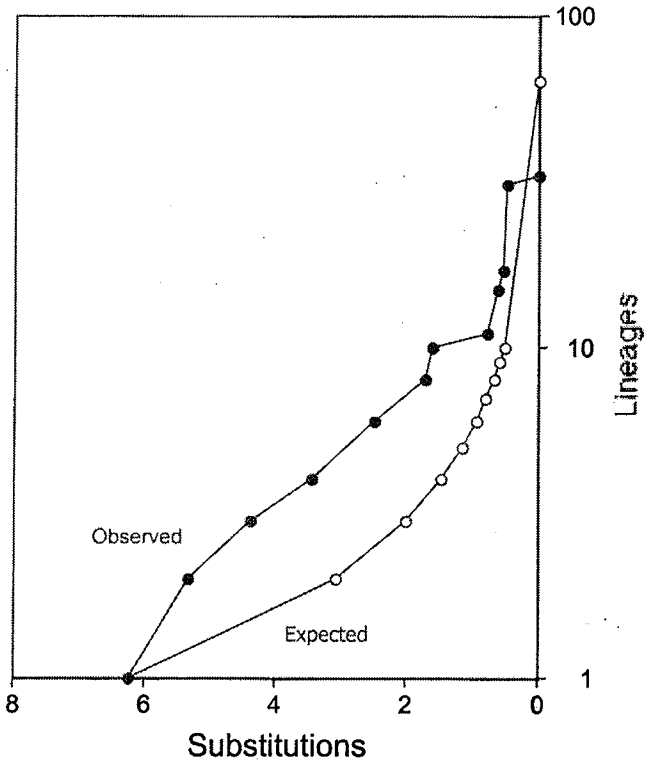

Figure 6. Number of lineages (logarithmic scale) versus estimated age (measured as number of nucleotide substitutions) of coalescent events for hypothesized relationships among California Gnatcatcher sequences (solid circles) and the expected distribution for a sample of 64 from a stationary population (open circles).

lating barriers (except distance) will result in future genetic division of the northern populations.

Conservation Implications of Population History

Our study provides a geographically thorough genetic analysis of a threatened species, encompassing both areas where populations are not threatened (providing a baseline) and where they are. Our genetically based interpretation of the recent history of the California Gnatcatcher provides perspective on current conservation questions. Put simply, based on mtDNA data, northern populations do not appear to constitute a unique component of gnatcatcher biodiversity. Nevertheless, several caveats merit consideration. One might argue that loss of northern populations could be unfortunate because populations at the margins of species' ranges might be "evolutionary laboratories" for novel genetic types (Erwin 1991). Although a few novel haplotypes exist in the coastal sage scrub populations, only an extreme view would support preservation of each unique haplotype, given their minor differences; every individual likely possesses at least one unique mutation. Second, other molecular techniques might reveal more fine-scaled geographic structuring than we have found. Third, local phenotypic adaptations might be maintained by natural selection in different parts of the range. Our data indicate, however, that no particular segment of the range of California Gnatcatchers has been evolving independently long enough (i.e., $2N_{ef}$ generations on average; Avise 1994) to have developed an exclusive set of mtDNA haplotypes (Fig. 2), and if geographic structure were found with another molecular marker (such as microsatellites), in our opinion it would be evolutionarily less "significant" than evolutionary divisions found in other species (Table 1).

Although extrapolations from our gnatcatcher study about the general significance of avian subspecies must be made with caution, our results are consistent with other studies (Ball & Avise 1992). We summarized data (Table 1) for phylogeographic surveys of 17 avian species in North America. For these species, the average number of subspecies is 6.5, and the average number of ESUs is 1.7. The data set itself is biased because the average number of subspecies per North American passerine species is 3.3 ± 3.9 (SD, n = 234, Klicka & Zink 1999); hence, species studied to date have tended to be those recognized as highly polymorphic based on classical taxonomic criteria. Nonetheless, it is likely that most biological species of birds will contain two or fewer ESUs and that subspecies on average will not be equivalent to ESUs (Avise & Walker 1998). Our findings for the California Gnatcatcher are therefore consistent with those for other bird species (Table 1).

Subspecies limits might not be predictive of ESUs in gnatcatchers and other birds (Ball & Avise 1992) because such limits are often based on single characteristics, such as plumage coloration, size, and shape, that are probably controlled by relatively few genes and influenced individually by different selective pressures. In contrast, neutral genetic characters are more likely to reflect overall demographic events and population history. Based on our mtDNA results, we predict that reanalysis of gnatcatchers will show that inconsistent patterns of variation among single morphological characters caused conflicting taxonomic opinions (Fig. 1) because different authors emphasized different characters. Our finding of no significant genetic divisions explains prior controversy among subspecies schemes: there probably is no general pattern of variation in morphological characters consistent with historical isolation and independent evolution of populations. Thus, preservation of biodiversity in California Gnatcatchers can be considered independent of subspecies designations.

Although northern populations of California Gnatcatchers do not represent discrete elements of biodiversity, our results must be interpreted in a broader context. In recent years, concern over single species has been complemented by ecosystem or community perspectives (Murphy et al. 1994). Our study reinforces this

Table 1. Molecular support for evolutionarily significant units (ESUs) in avian songbirds.[a]

Species	*Number of subspecies examined*	*Number of ESUs*
Polioptila californica	3–5	1
Passerella iliaca	15	4[b]
Melospiza melodia	19	1[c]
Spizella passerina	3	1[b]
Geothlypis trichas	6	2[d]
Molothrus ater	2	1[b]
Toxostoma curvirostre	6	2[c]
Pipilo fuscus	7	2[c]
Auriparus flaviceps	3	1[c]
Agelaius phoeniceus	10	1[b]
Campylorhynchus brunneicapillus	7	2[c]
Parus carolinensis	4	2[b]
Parus atricapillus	5	1[b]
Parus hudsonicus	4	2?[b]
Dendroica petechia	2–3	2[b]
Ammodramus maritimus	6–7	2[b]
Ammodramus caudacutus	5	2[b]

[a] *Includes North American studies (Ball & Avise 1992; Zink 1997; Avise & Walker 1998) that include two or more named subspecies for which mtDNA restriction-site or sequencing studies were performed. These studies show that the genetic structure of the California Gnatcatcher is consistent with that of other birds examined with similar molecular approaches.*
[b] *Restriction fragment studies.*
[c] *R. M. Zink, unpublished mtDNA sequence data.*
[d] *J. Klicka, personal communication*

trend because, although the gnatcatcher's widespread distribution, visibility, and legal status make it a good "flagship species" for regional conservation efforts, our genetic data show that the species poorly reflects the endemism of the coastal sage scrub community. Other species are restricted to coastal sage scrub and are relatively unstudied, and many do not share the gnatcatcher's extensive distribution to the southern tip of the Baja peninsula (Atwood 1993). Hence, further loss and fragmentation of coastal sage scrub in the United States might entail a large genetic cost, if not extinction, for other species. Programs focused at the ecosystem or community level, such as the State of California's Natural Community Conservation Planning process (O'Connell & Johnson 1997), appear most relevant to conservation of coastal sage scrub and other threatened ecosystems. That is, preservation of the California Gnatcatcher should be coupled to preservation of the coastal sage scrub ecosystem, rather than the reverse.

Acknowledgments

R. Lande, D. Tilman, J. Klicka, S. Rohwer, A. Kessen, J. Rotenberry, R. Shaw, F. Allendorf, S. Lanyon, K. Scribner, and three anonymous reviewers provided useful comments on the manuscript. C. Griffiths, J. Groth, and L. Marcus assisted with analyses. S. Hills and D. Ely provided financial support and encouragement. D. Bontrager, C. Reynolds, S. Tsai, and R. McKernan provided feather or tissue samples from the United States. O. Rojas-Soto, A. Navarro, P. Escalante-Pliego, B. Hernandez-Baños, A. Fry, J. Klicka, P. Sweet, and L. Montañez-Godoy assisted with Mexican fieldwork. Financial support was provided by the U.S. Navy, the National Fish and Wildlife Foundation, Southern California Edison, trustees of the Manomet Center for Conservation Sciences, the Building Industry Association of Southern California, the Transportation Corridor Agency, Chevron Land and Development, the University of Minnesota, and the National Science Foundation (DEB-9317945). The U.S. Fish and Wildlife Service and Mexico's Secretaria de Desarrollo Urbano y Ecologia provided collecting permits.

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BEFORE THE UNITED STATES DEPARTMENT OF THE INTERIOR &
THE UNITED STATES FISH AND WILDLIFE SERVICE

In the Matter of the Petition to
Remove the California gnatcatcher
(*Polioptila polioptila californica*)
from the list of threatened species
under the Endangered Species Act

PETITION OF THE COALITION OF LABOR, AGRICULTURE, AND BUSINESS, THE PROPERTY OWNERS ASSOCIATION OF RIVERSIDE COUNTY, AND M. LOU MARSH, M.D., TO REMOVE THE CALIFORNIA GNATCATCHER FROM THE LIST OF THREATENED SPECIES UNDER THE ENDANGERED SPECIES ACT

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INTRODUCTION

Petitioners Coalition of Labor, Agriculture, and Business; Property Owners Association of Riverside County; and M. Lou Marsh, M.D., hereby petition the United States Department of the Interior and the United States Fish and Wildlife Service, pursuant to Section 4(b)(3), 16 U.S.C. § 1533(b)(3), of the Endangered Species Act (ESA), *id.* § 1531, *et seq.*, to remove the California gnatcatcher (*Polioptila polioptila californica*) from the ESA list of threatened wildlife. The gnatcatcher (*P.c. californica*) is currently listed as a threatened subspecies. Petitioners, relying upon studies published since the listing, contend that the California gnatcatcher is not a valid subspecies, and should for that reason be delisted.

PETITIONERS

Petitioner Coalition of Labor, Agriculture, and Business (COLAB) unites the independent strengths of these sectors of the economy to protect and improve the natural and business environments of San Luis Obispo and Santa Barbara Counties, California. COLAB engages in educational outreach, political action, and issue advocacy. COLAB supports the protection of private property rights, fiscal responsibility, and environmental legislation based on sound principals of science, as well as cost-effective solutions to issues associated with business and job creation. COLAB is a tax-exempt organization under Section 501(c)(6) of the Internal Revenue Code. Its

members are primarily comprised of farming and ranching families who have been stewards of the land for generations. COLAB advocates for a balanced approach to environmental regulation, especially with respect to the administration of the ESA.

Petitioner Property Owners Association of Riverside County (Association), is a tax-exempt organization under Section 501(c)(6) of the Internal Revenue Code. The Association's mission is to serve as an advocate for Riverside County property owners to ensure that the interests and private property rights of landowners are protected in the formation and implementation of public policies. The Association includes owners of real property in Riverside County whose interests are directly affected by government land use regulations, including numerous land use restrictions imposed by the ESA. In particular, the Association has two dozen members who are within the Western Riverside County Multiple Species Habitat Conservation Plan area, which includes gnatcatcher habitat.

Petitioner M. Lou Marsh, M.D., resides in unincorporated San Diego County. Dr. Marsh wishes to subdivide her lot. In order to obtain that subdivision, Dr. Marsh must obtain a coastal development permit from the California Coastal Commission. In December, 2006, the Commission denied Dr. Marsh's permit application. One of the Commission's grounds for denying the application was the presence of a pair of gnatcatchers on the property. The

Commission concluded that the development of the proposed subdivided lot would negatively affect gnatcatcher habitat, which the Commission deemed to be an environmentally sensitive habitat area under the state's Coastal Act. If the gnatcatcher were delisted, the Commission could no longer use the presence of the gnatcatcher or its habitat to deny Dr. Marsh's permit application.

ANALYSIS

In 1993, the Service listed the California gnatcatcher as a threatened subspecies. 58 Fed. Reg. 16,742 (Mar. 30, 1993). In determining that gnatcatchers represented a valid subspecies, the Service relied on a study by Atwood (1991). *See id.* at 16,742. Since 1993, several published studies have concluded that the Atwood subspecies classification is invalid and that the California gnatcatcher should be considered an undifferentiated part of one species ranging from Southern California to the southernmost tip of the Baja California peninsula in Mexico. The Service itself has initiated a formal review of the gnatcatcher's taxonomy to determine whether the subspecies classification should be withdrawn. *See* 68 Fed. Reg. 20,228, 20,230 (Apr. 24, 2003); 72 Fed. Reg. 72,009 (Dec. 19, 2007). The Service has also proposed listing the gnatcatcher as a distinct population segment (DPS). *See* 68 Fed. Reg. at 20,230-33.

Petitioners rely upon the attached paper "Subspecies status of the coastal California gnatcatcher (*Polioptila californica californica*)" (Nov. 9, 2009), as well as its appended literature, all of which are incorporated fully into this petition by reference. The paper, prepared for Petitioners' counsel by Dr. Matthew Cronin, reviews the post-listing studies to explain why the subspecies classification for the California gnatcatcher is no longer tenable. Below, Petitioners highlight the essential points of Dr. Cronin's paper.

As noted above, the subspecies classification for the California gnatcatcher is based upon the Atwood study. In that study, Atwood concluded that the California gnatcatcher subspecies classification was justified in light of observed changes in certain morphological characteristics (*i.e.*, plumage coloration and body size) in collected gnatcatcher specimens from various locales.

Studies published by Zink, et al. (2000), and Skalski, et al. (2008), have determined that Atwood (1991) was wrong on at least three points.

1. Zink, et al. (2000), determined that Atwood's observed morphological characteristics changes are *not* representative of genetic differentiation, which differentiation could support a subspecies classification. The Zink study's conclusion is all the more significant given that Atwood was a co-author. In their paper, Zink and Atwood expressly state that *P. californica* should have no subspecies.

2. Skalski, et al. (2008), determined that Atwood's statistical analyses were seriously flawed, because Atwood's supposed diagnostic characters support a geographic cline, *not* a distinct break in character distribution markers, which break could support a subspecies classification.

3. Skalski, et al. (2008), determined that Atwood's data sets were confounded: many of Atwood's specimens may not have been representative of wild gnatcatchers.

Thus, in terms of morphological, statistical, and genetic data analysis, the Atwood (1991) subspecies classification for the California gnatcatcher is not valid. Atwood, the scientist whose work is responsible for the classification of the California gnatcatcher, has published a retraction of his earlier work and has concluded that the California gnatcatcher is not a valid subspecies. (Zink, et al. 2000). New science (Zink, et al. 2000) also indicates that the California gnatcatcher does *not* qualify as an evolutionarily significant unit, and instead should be considered part of a single species of gnatcatcher whose range extends from southern Baja California, Mexico, to Southern California.

CONCLUSION

The current and best available scientific data conclusively demonstrate that the California gnatcatcher is not a valid subspecies. Petitioners therefore request that their petition to delist the California gnatcatcher from the ESA list of threatened wildlife be granted.

DATED: April 9, 2010.

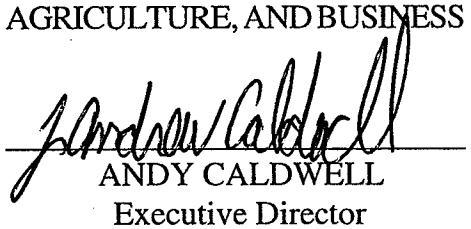
Respectfully submitted,

M. REED HOPPER
DAMIEN M. SCHIFF
Pacific Legal Foundation

By _____
DAMIEN M. SCHIFF

DATED: February 1, 2010.

COALITION OF LABOR,
AGRICULTURE, AND BUSINESS



ANDY CALDWELL
Executive Director

DATED: February 3, 2010.

THE PROPERTY OWNERS ASSOCIATION OF RIVERSIDE COUNTY

Bruce Colbert

BRUCE COLBERT
Executive Director

DATED: February 1, 2010.

PETITIONER

M. Lou Marsh, M.D.

M. LOU MARSH, M.D.

Tab 1

Subspecies status of the coastal California gnatcatcher (*Polioptila californica californica*)

Prepared for the Pacific Legal Foundation, Sacramento, California by

Matthew A. Cronin, Ph.D.
Northwest Biotechnology Company
Anchorage, Alaska

9 November 2009

Subspecies status of the coastal California gnatcatcher (*Polioptila californica californica*)

9 November 2009
Matthew A. Cronin
Northwest Biotechnology Company
Anchorage, Alaska

In this paper I review the data, designations, and interpretations of the coastal California gnatcatcher subspecies, including the following topics.

1. **Review of the scientific literature on the coastal California gnatcatcher subspecies.**

A. Morphology
B. Molecular genetics and phylogeny
C. The subjective nature of subspecies in general

2. **Review of regulatory documents**.

A. Federal Register documents regarding subspecies status of the coastal California gnatcatcher and Endangered Species Act listing
B. The coastal California gnatcatcher as a Distinct Population Segment instead of a subspecies
C. Other Endangered Species Act subspecies designations.

3. **Literature cited.**

Subspecies status of the coastal California gnatcatcher (*Polioptila californica californica*)

1. Review of the scientific literature on the coastal California gnatcatcher subspecies

Morphology

There are two species of gnatcatcher (genus *Polioptila*) whose ranges overlap in northeast Baja Peninsula, Mexico. The black-tailed gnatcatcher (*Polioptila melamura*) occurs in a range including southern Nevada and Sonora and Chihuahua, Mexico. The California gnatcatcher (*Polioptila californica*) has a range that extends from the southern end of the Baja peninsula north to southern California around Los Angeles at 31° north latitude. The California gnatcatcher was originally designated a species by Brewster (1881) but was later split into a subspecies of the black-tailed gnatcatcher (Grinnell 1926). It was then re-designated a full species based morphological, vocalization, and mitochondrial DNA (mtDNA) variation (Phillips 1980- cited by Mellink and Rea 1994, Rea 1983, Atwood 1986, 1988; American Ornithologists' Union 1989, Zink and Blackwell 1998). The California gnatcatcher has the least dense populations in the north, from El Rosario, Baja California, Mexico (latitude 30° north) north to Los Angeles. Populations in central and southern Baja California, Mexico are large and continuous (Zink et al. 2000).

There have been generally three subspecies of California gnatcatcher recognized, although five subspecies names and different range boundaries have been used by different authors. This is because there is overlap of characters and no distinct boundaries of the morphological characters used in the subspecies classifications. The morphological characters considered for gnatcatchers include body size and shape and plumage characteristics. The coastal California gnatcatcher (*Polioptila californica californica*) is the northernmost subspecies, extending to the limit of the species range. This subspecies was listed in 1993 as a threatened subspecies under the Endangered Species Act (ESA, 58 FR 16742). The ESA listing was based upon recognition of *P. c. californica* as a legitimate subspecies based on the classification of Atwood (1991).

The history of the California gnatcatcher shows inconsistent subspecies designations. Early assessments Grinnell (1926) recognized *P. c. californica* from the northern limit of the range at about 31° north latitude southward to 29° 30' north latitude, *P. c. margaritae* south of *P. c. californica* to about 24° north latitude, and *P. c. abbreviata* south of 24° in the Cape region of Baja California Sur. However, van Rossem (1931, cited by Mellink and Rea 1994) found *P. c. abbreviata* and *P. c. margaritae* to be indistinguishable and named *P. c. pontilis* (replacing yet another subspecies name *nelsoni*, Ridgway 1903, cited by Atwood 1991) as a subspecies intermediate to *P. c. californica* and *P. c. margaritae*.

Miller et al. (1957, cited by Zink et al. 2000) designated three subspecies *P. c. californica* from near Los Angeles southward to a boundary north of 30° north latitude, *P. c. pontilis* from the southern boundary of *P. c. californica* to around 27° north latitude, and *P. c. margaritae* south of 27° north latitude (Figure 1).

Phillips (1991, cited by Mellink and Rea 1994) also identified *P. c. californica* to occur from Los Angeles southward to 30° north latitude, *P. c. pontilis* in the central part of the Baja Peninsula, and *P. c. margaritae* from about 27° north latitude to the southernmost tip of Baja California. Phillips (1991, cited by Mellink and Rea 1994) evidently noted geographic variation, limited specimens, and changes in characters in storage over time (i.e., foxing) as a problem with the subspecies designations. He stated with regard to *P. c. californica*: "Geographic variation within these dark (northern) populations is indicated; need I repeat endlessly, 'There are few clean fresh-plumaged specimens'? Sorry".

Atwood (1988) described two subspecies of California gnatcatcher, *P. c. californica* and *P. c. margaritae*, with a transition of morphology at about 25° north latitude. *P. c. californica* was north of 25° and *P. c. margaritae* to the south in the Cape region of the Baja Peninsula, Mexico. Then Atwood (1990, 1991) reanalyzed his data, agreeing with criticisms (Banks 1989, Johnson 1989) that "details of intraspecific variation in *Polioptila* are difficult to assess from data provided in Atwood (1988)." Atwood (1991) described three subspecies including *P. c. californica* north of 30°, *P. c. margaritae* between 24° and 30° north latitude, and *P. c. abbreviata* south of 24° north latitude (Figure 1). Atwood (1991) noted that :

"...the naming and distributions of subspecies of *Polioptila californica* should revert to that initially proposed by Grinnell (1926): *P. c. californica* (north of 30° N), *P. c. margaritae* (from 30° N south to 24° N), and *P.c. abbreviata* (south of 24° N)."

Atwood (1991) noted that his analysis, while supporting Grinnell's (1926) designations, did not support van Rossem's (1931) classification. Unlike the other subspecies assessments, Atwood (1991) did statistical analyses of 31 morphological characters, although there are serious questions about the integrity of the data and appropriateness of the methods in these analyses (McDonald et al. 1994, Skalski et al. 2008).

Mellink and Rea (1994) subsequently identified a different subspecies scheme based on plumage coloration. This involved splitting *P. c. californica* into two subspecies: *P. c. californica* north of the U.S.-Mexican border and *P. c. atwoodi* (a new subspecies) from the U.S.-Mexican border south to about 30° north latitude (Figure 1). These authors also designated subspecies from what was designated *P. c. margaitae* in Atwood's (1991) classification. This involved recognizing *P. c. pontilis* (between 30° north latitude and 28° north latitude) and noting that *P. c. pontilis* was separable from *P. c. margaritae*. However, Mellink and Rea (1994) did not have enough specimens to assess subspecies relationships south of 26° north latitude.

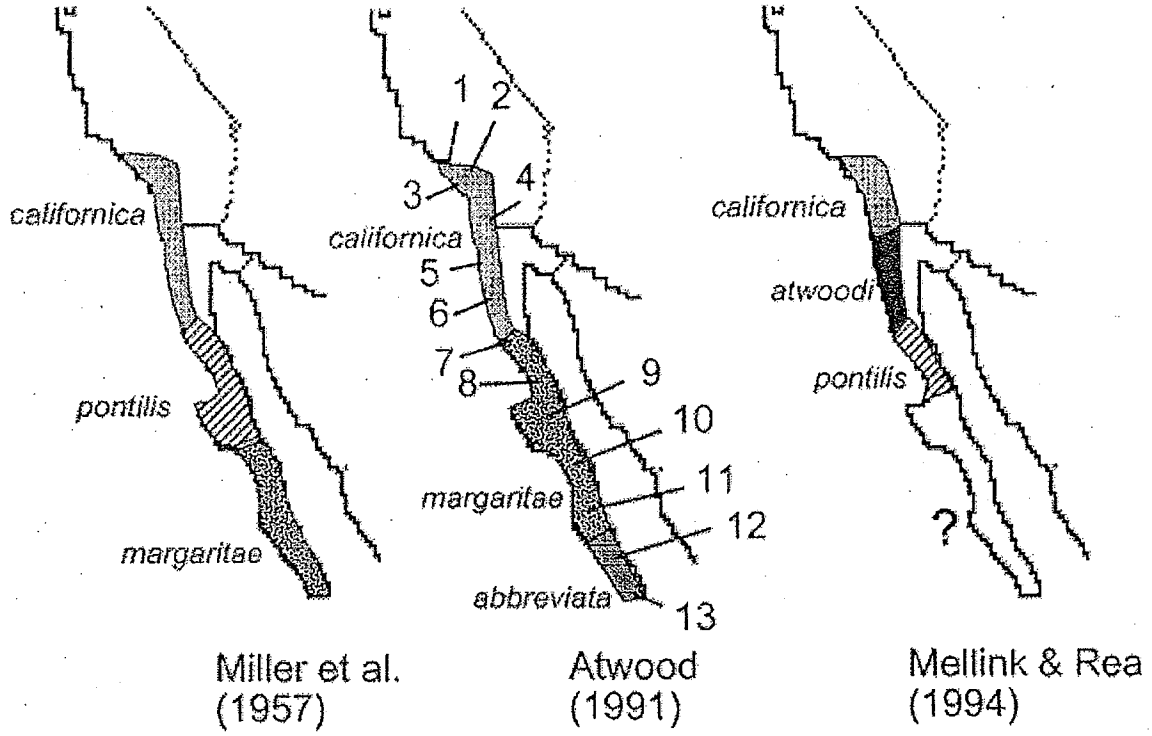


Figure 1. California gnatcatcher subspecies' ranges as designated in three different assessments (copied from Zink et al. 2000 with permission of *Conservation Biology* and R. Zink). The numbers in the Atwood (1991) scheme indicate the sampling areas for the mtDNA analysis of Zink et al. (2000).

The subspecies assessments described above were primarily based on assessments of morphology, specifically plumage coloration and body measurements. There are two aspects of this work that warrant examination. First, subspecies designations are supposed to be based on genetic relationships (technically called phylogenetic or evolutionary relationships, Cronin 1993, 2006, Zink 2004). However morphology (specifically plumage color and body measurements) is influenced by environmental factors in addition to genetics (e.g., Geist 1992). This means that body measurements and plumage color are not necessarily good characters for subspecies classification. Plumage coloration, body measurements, and other morphological characters may be influenced by environmental conditions and diet (e.g., Brush and Power 1976, James 1983, James and NeSmith 1986, Hudon and Brush 1989, Price et al. 1991, Federal Register March 30, 1993). Plumage color also changes with time since collection and storage of study skins (called "foxing"), such as those used in the analyses of Atwood (1988, 1990, 1991) and Mellink and Rea (1994). Indeed, Mellink and Rea (1994) note:

"Unfortunately, many skins from the northern end of the species' range (Los Angeles, Riverside, San Bernardino counties) are severely soiled (see also Rea and Weaver 1990:92-94). Most of these were taken early in the twentieth century and have soot-stained plumage."

It is arguably inappropriate to use morphology, particularly plumage coloration, for subspecies classification, in general, and specifically for stored study skins.

A second problem with the subspecies designations of Atwood (1991) relates to statistical analyses of morphology over the range of California gnatcatchers. Reanalysis of the gnatcatcher morphological data showed the subspecies designations to be questionable or invalid. There are no diagnostic characters for any of the subspecies. Mean values of morphological measurements may separate birds in different locations, but there is substantial overlap among areas (McDonald et al. 1994). There is also not a consensus about where there are identifiable "breaks" in character measurements, presumed to indicate genetic discontinuity and hence subspecies boundaries (Baptista and Bell 1994, Barrowclough 1992, 1994, Boyce 1995, Brush 1994, Cronin 1994, 1995, 1997, Grant 1994, Grant et al. 2004, Hurst 2004, Link and Pendelton 1994, McDonald et al.1994, Messer 1994, Roberts and Bayn 1994, Skalski 1995a, 1995b). Rigorous statistical re-evaluation of Atwood's (1991) analyses showed serious problems with the data and analyses used, calling into question the legitimacy of the subspecies designations (McDonald et al. 1994).

Recently a statistical reanalysis of the gnatcatcher morphological data of Atwood (1988, 1991) was done by Skalski et al. (2008). These authors noted:

"...the California gnatcatcher (*Polioptila californica*) which was incorrectly listed under the ESA due to misinterpretation of morphological data."

Skalski et al. (2008) showed that the types of analyses used to designate the gnatcatcher subspecies (Atwood 1988, 1991) are subject to high rates of type I statistical errors (i.e., identification of subspecies when none exist). The morphological data actually show a geographic cline (i.e., gradual change over geography), not distinct breaks in character distributions. The tests used by Atwood (1991) do not consider the possibility of a cline in morphological characters over geography. Skalski et al. (2008) tested explicit hypotheses, including a null hypothesis of a smooth cline in traits (no distinct subspecies) and an alternative hypothesis of a break in a cline (subspecies boundary), with rigorous statistical tests. Reanalysis of the morphological data with spline-regression and step-regression were robust with high resolution and did not show subspecies breaks consistent with the designations of Atwood (1991).

Skalski et al. (2008) also noted that:

"The distinction between statistical significance versus biological significance thus appears to be a matter left to the individual taxonomist. One point should be stressed, that statistical significance is not sufficient to declare different subspecies."

This is profound, because it exemplifies the contention that subspecies designations are subjective and not scientifically rigorous (Zink 2004, Cronin 2006, 2007, Haig et al. 2006).

Perhaps most importantly, the gnatcatcher data were confounded by sampling problems. A sample must be representative of the subject population for it to be legitimately used in statistical analyses. However, the gnatcatcher data used by Atwood (1988, 1991) are considered to be a "classic example of confounding" because the samples and specimens used may be non-representative of the population (Skalski et al. 2008). In other words, the birds used to designate subspecies may not represent the birds actually occurring in nature. This is because the samples were collected over a 100 year period. Recall that "foxing" (a physical change in specimens over time, especially plumage color) may occur. This can make variation of plumage color a function of time, as well as geography. This is a critical point, and Skalski et al. (2008) note (as did the earlier re-analyses of Atwood's data by McDonald et al. 1994), that both geographic location *and* year of collection, equally explain the differences in plumage brightness used by Atwood (1991) in his subspecies designations. It is important to recognize that specimens collected from northern areas were sampled in earlier years than those in southern areas, and that the subspecies were separated by boundaries along a north-south distribution. Birds from the north were apparently in storage longer than those from the south. Also recall the statement by Mellink and Rea (1994) that many skins from the northern end of the species' range are severely soiled and have soot-stained plumage. The relationships described by Atwood (1991) may reflect the dates birds were collected, not the actual distribution of plumage color traits. Skalski et al. (2008) noted that this confounding of samples used in gnatcatcher subspecies designations was not noticed by Atwood (1988, 1991).

Skalski et al. (2008) noted:

"The California gnatcatcher case study is a perfect example of the consequences of using poorly posed biological questions and off-the-shelf statistical methods. Recent genetic work by Zink et al. (2000) confirm the early papers by Atwood (1988, 1991) were wrong in identifying the occurrence of subspeciation. However, the mistake was likely more than just an α-level probability error. The north-south cline in tracts causes H_o (null hypothesis of no subspecies) to be rejected even when no subspecieation has occurred."

In summary, the subspecies designation of the coastal California gnatcatcher (Atwood 1991) was not appropriate because of the use of morphological characters subject to environmental variation, changes during long term storage, and inappropriate statistical tests.

Molecular genetics and phylogeny

Molecular genetic studies also indicate the subspecies designation of the coastal California gnatcatcher (Atwood 1991) was not valid (Zink et al. 2000, Zink 2004). Note that the author of Atwood (1991) was a co-author of the Zink et al. (2000) paper. Zink et al. (2000) analyzed 1399 nucleotides of the mitochondrial DNA (mtDNA) control region, t-RNA-Glu, and part of the ND6 gene in 64 California gnatcatchers from 13 sampling

locations from Los Angeles to the southern tip of the Baja Peninsula (Figure 1). Zink et al. (2000) reported 26 variable nucleotide positions, including 17 transitions, two transversions, and seven deletions. Fourteen of the variable positions were parsimony-informative.

Thirty-three mtDNA haplotypes were observed. All of the haplotypes were closely related, with small DNA sequence divergences (maximum divergence of 0.64%, average divergence of 0.27%). Phylogenetic analysis showed the haplotypes exhibited no geographic structure and were not consistent with the subspecies designations of Atwood (1991). In the phylogenetic tree, haplotypes did not form exclusive clusters that conformed to recognized subspecies or geographic areas. That is, the subspecies did not have distinct mtDNA sequences and did not have monophyletic mtDNA. This is also obvious from the co-occurrence of the same mtDNA haplotype in all three subspecies designated by Atwood (1991), including 11 of the 13 sample locations (Appendix in Zink et al. 2000).

Twenty-three of the 64 birds shared one haplotype that occurred in 11 of the 13 locations sampled (Appendix in Zink et al. 2000). A measure of population subdivision was low (N_{ST} = 0.074) indicating no genetic differentiation of the birds in different sampling locations. This indicates that only 7.4% of the genetic variation is among sample locations and 92.6% of the genetic variation is within locations. This pattern is consistent with populations connected with extensive gene flow (i.e. birds move and interbreed among areas). It is not consistent with genetically differentiated subspecies. Zink et al. (2000) estimated the N_{ST} value suggested an exchange of between three and four birds per generation among populations, and that one such migrant per generation was enough to result in a panmictic unit (i.e., sub-populations comprise one large interbreeding population). Zink et al. (2000) noted that:

"...the pattern of distribution of the most common haplotype, the shape of the phylogenetic tree (phylogeography), and the low N_{ST} estimate all suggest that gene flow among the gnatcatcher populations has been substantial."

There was a higher level of genetic variation in the southern sampling locations than in the northern sampling locations. Zink et al. (2000) found this pattern consistent with a recently expanded population from the southern Baja Peninsula to southern California. This analysis of the mtDNA haplotype distribution suggests that the species in each sampling area has been increasing in number and expanding its range in the recent past (Zink et al. 2000, 2001).

Regarding subspecies, Zink et al. (2000, including Atwood as co-author) noted:

"The (mtDNA) haplotype tree for California gnatcatchers *does not support recognition of ESUs or subspecies* (my italics). The N_{ST} value also reinforces the conclusion that there are no ESUs within the California gnatcatcher. Species including two or more ESUs would have an N_{ST} value an order of magnitude greater than 0.07..."

Zink et al. (2000, including Atwood as co-author) also made the following statement regarding subspecies:

"Our finding of no significant genetic divisions explains prior controversy among subspecies schemes: there probably is no general pattern of variation in morphological characters consistent with historical isolation and independent evolution of populations".

This is admission that there is no morphological basis for subspecies, as with the mtDNA data. Because Atwood is a co-author of the Zink et al. (2000) paper, this is essentially a retraction of Atwood's (1991) subspecies designations, just as Atwood (1991) retracted his earlier (Atwood 1988) subspecies designations.

To summarize, the mtDNA data do not support the coastal California gnatcatcher (*P. c. californica*) as a subspecies. Both the mtDNA phylogeny and distribution of haplotypes indicate a high level of genetic similarity and gene flow across the range of the California gnatcatcher. Zink et al. (2000 noted:

"We found that coastal sage scrub populations of California gnatcatchers are not genetically distinct from populations in Baja California".

In addition, Zink et al. (2000) acknowledge that there is no consistent basis for designating California gnatcatcher subspecies or ESUs with the previous morphological analyses.

The subjective nature of subspecies in general

It is now well established in the scientific literature that subspecies are subjectively defined and designated (Mayr 1970, Cronin 1993, 2006, 2007, 2008, In Review, Zink 2004, Haig et al. 2006). It is also clear that subspecies is a taxonomic rank, and as such designations should be based on phylogenetic (i.e. evolutionary) relationships (e.g., Avise and Ball 1990). Because subspecies are by definition members of the same species and hence can interbreed, gene flow and common ancestry may prevent distinct phylogenetic differentiation, even with molecular genetic markers (Cronin 1993). This inherent characteristic indicates that subspecies are subjective and reflect the interpretation of individual taxonomists, not unequivocal scientific data. Other categories besides subspecies, including Distinct Population Segments (DPS) and Evolutionarily Significant Units (ESU) are also scientifically subjective (Cronin 2006). It has been noted that there is a tendency of biologists to create new "species" (as with designating subspecies, DPS, and ESU as species under the ESA) to justify conservation efforts, including ESA listings. This has been called "taxonomic inflation" (The Economist 2007, Marris 2007).

2. Review of regulatory documents.

Federal Register documents regarding subspecies status of the coastal California gnatcatcher and Endangered Species Act listing

The original 1993 ESA listing determination of the coastal California gnatcatcher (58 FR 16742) noted that earlier assessments designated the coastal black-tailed gnatcatcher (*Polioptila melanura californica*) as a category 2 candidate for the list of Endangered and Threatened wildlife (47 FR 58454) and subsequently retained it as such (50 FR 37958, 54 FR 554). The U.S. Fish and Wildlife Service (FWS) in the 1993 listing determination recognized the subspecies classification of Atwood (1991), and regarding *P. c. californica* stated: "This taxon is now recognized as a subspecies of *Polioptila californica*".

The 1993 listing determination (58 FR 16742) addressed issues raised in public comments on the proposed rule. The first three issues discussed are relevant to the taxonomic status of the coastal California gnatcatcher.

Issue 1. The California gnatcatcher and its northern nominate subspecies (i.e. coastal California gnatcatcher, *P. c. californica*) are not valid taxa. Several comments questioned the change of taxonomy including changing the California gnatcatcher to a full species, *P. californica*, from a subspecies of black-tailed gnatcatcher (*P. melanura*), and whether there was a distinct subspecies of *P. californica* in southwestern California and northwestern Baja, California, Mexico. The FWS responded that they and the American Ornithologists' Union (AOU) concluded that *P. c. californica* is a valid taxon, citing Atwood (1988, 1991, and others).

Issue 2. Several comments questioned the validity of the statistical analysis used by Atwood (1991) to evaluate intraspecific morphological variation within the California gnatcatcher. FWS responded that Atwood's methods have been peer reviewed and there was no indication he used inappropriate statistical methods.

Issue 3. Several comments said that the taxonomic conclusions of Atwood (1991) were not valid because they are based on plumage color and may be environmental, and not genetic, in origin. FWS responded that it was unknown whether the traits reported by Atwood (1991) are genetically based. FWS noted that the traditional scientific approach to defining avian subspecies has been almost exclusively using morphological differences in body measurements and plumage characters, and that Atwood's conclusions are strengthened by congruent patterns in geographic variation among several species at 30° north latitude.

Assessment: The first issue of whether *P. c. californica* is a valid subspecies depends on the answers to the second and third issues. Considering the review of *P. c. californica*

taxonomy above, it is clear that the statistical analyses used by Atwood (1991) were seriously flawed (McDonald et al. 1994, Skalski et al. 2008). This makes the FWS response on issue 2 invalid. The invalidity of the statistical analyses, the discovery of changes in plumage characters over time in storage, and the mtDNA analysis (Zink et al. 2000) make the FWS response to issue 3 invalid. The genetic versus environmental origin of morphological differences is an important taxonomic consideration, but not particularly relevant in this case because there are no subspecies identifiable with the morphological data when proper statistics are applied (Skalski et al. 2008). Also, Zink et al. (2000) noted that the morphologically-based subspecies schemes are invalid, and with Atwood as a co-author, this is a retraction of the subspecies designations of Atwood (1991). The lack of genetic differentiation of the proposed subspecies also makes the subspecies designations of Atwood (1991) invalid. Therefore the FWS response to Issue 1 is also invalid and *P. c. californica* is not a valid subspecies. This means the ESA listing determination (58 FR 16742) is invalid and the subspecies was incorrectly listed (Skalski et al. 2008).

The coastal California gnatcatcher as a Distinct Population Segment instead of a subspecies

In 2003 FWS began reviewing the subspecies status of *P. c. californica* (68 FR 20228), noting:

"We originally identified the coastal California gnatcatcher as a subspecies of the California gnatcatcher. However new genetic information raises questions about the distinctiveness of the subspecies." FWS also solicited comments on consideration of the coastal California gnatcatcher as a Distinct Population Segment (DPS) instead of a subspecies, specifically asking if the recent genetic findings (i.e. Zink et al. 2000) justify a review of the taxonomy of the subspecies.

In 2004 FWS reopened the public comment period for determination of distinct vertebrate population segment (DPS) status for the California gnatcatcher (69 FR 18515). FWS notes that the mtDNA data of Zink et al. (2000) did not support a subspecies designation and that "the morphological variations previously described were not genetically based and subspecies divisions are not supported." FWS lists Atwood as a co-author in Zink et al. (2000) but does not explicitly recognize that this means the subspecies designations are retracted (i.e. the same author who designated subspecies states that the subspecies are not valid at a later date).

In 2007 FWS noted they are continuing their review of whether the listing of the coastal California gnatcatcher as a subspecies should be retained or changed (72 FR 72009).

A phone call (14 September 2009) from M. Cronin to the FWS Carlsbad, CA office was made asking if there was a decision on the gnatcatcher subspecies review. John Hazard in the FWS Carlsbad office told Cronin there is no update since 2007, and the review of gnatcatcher subspecies status is still underway.

Assessment: Zink et al. (2000) provide important information with regard to the potential designation of the coastal California gnatcatcher as a DPS instead of a subspecies. Recall that Zink et al. (2000) stated:

"The (mtDNA) haplotype tree for California gnatcatchers *does not support recognition of ESUs or subspecies* (my italics). The N_{ST} value also reinforces the conclusion that there are no ESUs within the California gnatcatcher. Species including two or more ESUs would have an N_{ST} value an order of magnitude greater than 0.07..."

Also, note that the National Marine Fisheries Service (NMFS) designates DPS of Pacific salmon (*Oncorhynchus* spp.) as Evolutionarily Significant Units (ESU, Waples 1991). That is, for the ESA, an ESU is equivalent to a DPS. Zink et al.'s (2000) conclusions mean that if the gnatcatcher does not qualify as an ESU, it should not qualify as a DPS (i.e. DPS = ESU, see Cronin 2006). However, DPS can be designated with international boundaries so the U.S.-Mexican border could possibly be used to designate a DPS regardless of the biological relationships of birds in the U.S. and Mexico.

Other Endangered Species Act subspecies designations

Recent debate over other subspecies indicates that the case of the coastal California gnatcatcher is not unique (e.g., Zink 2004, Haig et al. 2006, Cronin In Review). Other questionable subspecies listed under the ESA include the Preble's meadow jumping mouse (*Zapus hudsonius preblei*, Ramey et al. 2005, Cronin 2007) and the Perdido Key Beach mouse (*Peromyscus polionotus trissyllepsis*, Cronin 2008). It is relevant that the U.S. Fish and Wildlife Service (FWS) is doing a review of wolf subspecies taxonomy (S. Chambers, FWS, personal communication). This includes assessment of general subspecies concepts and criteria. This may be relevant to the FWS review of the gnatcatcher subspecies regarding consistency of science within the FWS.

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Tab 2

Genetics, Taxonomy, and Conservation of the Threatened California Gnatcatcher

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Abstract: *The California Gnatcatcher (*Polioptila californica*) has become a flagship species in the dispute over development of southern California's unique coastal sage scrub habitat, a fragile, geographically restricted ecosystem with high endemism. One aspect of the controversy concerns the status of the subspecies of this bird in southern California coastal sage scrub that is currently listed as threatened under the U.S. Endangered Species Act. To investigate the recent population history of this species and the genetic distinctiveness of subspecies and to inform conservation planning, we used direct sequencing of mitochondrial DNA (mtDNA) for 64 individuals from 13 samples taken throughout the species' range. We found that coastal sage scrub populations of California Gnatcatchers are not genetically distinct from populations in Baja California, which are dense and continuously distributed throughout the peninsula. Rather, mtDNA sequences from this species contain the signatures of population growth and support a hypothesis of recent expansion of populations from a southern Baja California refugium northward into the southern coastal regions of California. During this expansion, stochastic events led to a reduction in genetic variation in the newly occupied range. Thus, preservation of coastal sage scrub cannot be linked to maintaining the genetic diversity of northern gnatcatcher populations, despite previous recognition of subspecies. Our study suggests that not all currently recognized subspecies are equivalent to evolutionarily significant units and illustrates the danger of focusing conservation efforts for threatened habitats on a single species.*

Genética, Taxonomía, y Conservación de la Perlita de California Amenazado de Extinción

Resumen: *La perlita de California (*Polioptila californica*) se ha convertido en una especie insignia en la disputa sobre el desarrollo del exclusivo hábitat de chaparral de salvia costero (CSS) del sur de California, un ecosistema frágil y geográficamente restringido con un endemismo elevado. Un aspecto de la controversia tiene que ver con la situación de la subespecie de esta ave en el CSS del sur de California y que se encuentra actualmente enlistada como amenazada bajo el Acta de Especies Amenazadas de los Estados Unidos. Utilizamos un secuenciado directo de ADN mitocondrial (mtDNA) de 64 individuos de 13 muestras tomadas a lo largo del rango de distribución de la especie para investigar la historia poblacional reciente de la especie y la diferenciación de subespecies, y para documentar planes de conservación. Encontramos que las poblaciones de la perlita de California de CSS no son genéticamente distintas de las poblaciones de Baja California, las cuales son densas y tienen una distribución continua a lo largo de la península. Más bien, las secuencias de mtDNA de esta especie contienen la firma de un crecimiento poblacional y apoya una hipótesis de expansión reciente de poblaciones de un refugio sureño de Baja California hacia el norte y hacia adentro de las regiones sureñas costeras de California. Durante esta expansión, los eventos estocásticos conducen a una reducción en la variación genética en el rango recientemente ocupado. Por lo tanto, la conservación del CSS no puede ser vinculada con el mantenimiento de la diversidad genética de poblaciones norteñas de perlitas, a pesar de su previo reconocimiento como subespecie. Nuestro estudio sugiere que no todas las subespecies actualmente reconocidas son equivalentes a las unidades evolutivamente significativas e ilustra el peligro de enfocar los esfuerzos de conservación de hábitats amenazados en una sola especie.*

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Paper submitted February 8, 1999; revised manuscript accepted January 26, 2000.

Conservation Biology, Pages 1394–1405
Volume 14, No. 5, October 2000

Introduction

Since 1940, the human population of southern California has increased at a rate twice that of many developing countries (Mann & Plummer 1995). Not surprisingly this growth has had a negative effect on the native flora and fauna. One particularly hard-hit community is coastal sage scrub, where approximately 100 endemic species and subspecies of plants and animals are potentially endangered (Atwood 1993). A characteristic inhabitant of coastal sage scrub, a small, nonmigratory songbird known as the California Gnatcatcher (*Polioptila californica*), has been at the center of efforts to preserve this habitat and its unique flora and fauna. Populations of gnatcatchers in coastal sage scrub are considered threatened under the U.S. Endangered Species Act (ESA; U.S. Fish and Wildlife Service 1993, 1995) as a result of loss of 70–90% of the original habitat (Atwood 1993). The remaining highly fragmented tracts of coastal sage scrub are of high economic value because of their proximity to the Pacific Ocean and major urban, retirement, and commercial areas in Los Angeles, Riverside, Orange, and San Diego counties. Some patches of coastal sage scrub are valued at $3 million per acre (0.40 ha; Mann & Plummer 1995). Because a pair of California Gnatcatchers may occupy a year-round home range in excess of 10 acres (4 ha; Atwood 1993) the value of real estate required to support a population of, for example, 50 pairs of these birds might exceed $1 billion. Few other species better typify the conflicts and tradeoffs among legal, environmental, and economic priorities.

The abundance of gnatcatchers throughout their range reveals a potential conflict between legal and biological concerns. California Gnatcatchers occur from Los Angeles, California, to the southern tip of the Baja peninsula (Fig. 1). Northern populations are least dense, especially from El Rosario (Baja California, lat 30°N) north to Los Angeles. These threatened populations comprise many small groups of individuals, each often isolated by urban sprawl, which potentially promotes local inbreeding. In contrast, populations in central and southern Baja California and throughout Baja California Sur are large and continuous (Atwood 1993). Thus, the ESA mandates protection of populations of a species that are historically restricted (and threatened) in the United States, whereas populations elsewhere in the contiguous range are "healthy." Therefore, the species as a whole is not threatened; rather, the issue involves preservation of populations within a relatively small part of the range that transcends an international boundary (Hunter & Hutchinson 1994).

Conservation of the species has been complicated by past taxonomic studies. Before 1989, the California Gnatcatcher was classified as a subspecies of the Black-tailed Gnatcatcher (*Polioptila melanura*). Studies by Atwood (1988), however, revealed that subspecies along the coast in California and those south of 28°N latitude throughout the Baja California peninsula were distinct

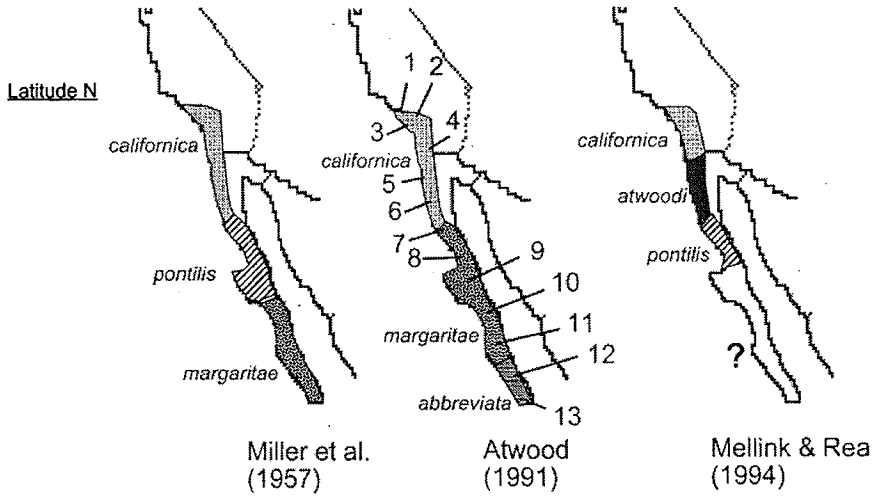

Figure 1. Three subspecies schemes proposed by Miller et al. (1957), Atwood (1991), and Mellink and Rea (1994) for the California Gnatcatcher, based on morphological characteristics of the external phenotype. Mellink and Rea (1994) did not explicitly state their recommendation for subspecific taxonomy south of 27°N latitude; they recognized P. c. margaritae, *however, for a total of at least four subspecies. Combining the treatments suggests a total of five subspecies. Sample sites for mtDNA study shown with numbers on the subspecies scheme of Atwood: 1, Los Angeles County; 2, Riverside County; 3, Orange County; 4, San Diego County; 5, Ensenada; 6, San Telmo; 7, Mision San Fernando; 8, El Rosarito; 9, San Ignacio; 10, Mulege; 11, Villa Insurgentes; 12, La Paz; and 13, Cabo San Lucas. The boundary between the states of Baja California and Baja California Sur is at 28°N latitude.*

from populations of *P. melanura* to the east. Analysis of the amount of white in the tail feathers and especially of vocalizations provided key evidence for the species-level distinctiveness of these subspecies and led to their formal recognition (American Ornithologist's Union 1989) as the California Gnatcatcher (*P. californica*). Analyses of mitochondrial DNA (mtDNA) sequences subsequently corroborated Atwood's recognition of the California Gnatcatcher (Zink & Blackwell 1998). The subspecific taxonomy of the California Gnatcatcher, however, has been controversial. Based on differing interpretations of geographic patterns of coloration, size, and shape, three recent subspecies schemes have been proposed (Fig. 1). Although these subspecies classifications differ, all suggest that the northern part of the range, including the coastal sage scrub populations, includes one or two subspecific units. The controversy over subspecies taxonomy suggests that new data are required to clarify the significance of geographic variation relative to conservation of both the species itself and of the coastal sage scrub.

From the viewpoint of conservation genetics, the issue involves the distribution of genetic diversity within the species: is the species uniform throughout its range or is it subdivided into smaller units, termed evolutionarily significant units (ESU; Ryder 1986; Barrowclough & Flesness 1996)? To qualify as an ESU (Moritz 1994; Waples 1995), phylogenetic analysis of mtDNA haplotypes must show that haplotypes from a given region are more closely related to each other than they are to haplotypes from other regions (termed reciprocal monophyly). If haplotypes from a given region do not form such an exclusive group, then either gene flow is ongoing or it has ceased recently. In either of the latter two cases, there are no geographic units that have had significant periods of isolation and independent evolution. Thus ESUs are diagnosed by the pattern of haplotype variation, not the level of sequence divergence. That is, reciprocally monophyletic groups can differ by 1% (a typical lower value for birds) to over 8% (Avise & Walker 1998). Evolutionarily significant units defined by genetic criteria constitute significant elements of biodiversity "below" the species level and are often considered units of conservation (U.S. Departments of the Interior and U.S. Department of Commerce 1996).

In cases in which direct genetic information is lacking, the taxonomic category of subspecies serves as a proxy for the ESU, as in the case of the California Gnatcatcher. The relevant conservation question here is whether putative subspecies of the California Gnatcatcher (Fig. 1) reflect ESUs or whether the genetic composition of the species is more homogeneous than previous subspecies schemes imply (Cronin 1997). Given the central role that subspecies can play under the ESA in serving as surrogates for ESUs (National Research Council 1995), testing subspecies limits is a vital component of conservation biology.

Genetic studies also provide perspective on the recent demographic history of populations. For example, Slatkin and Hudson (1991) and Rogers (1995) use recent advances in coalescence theory to show how population expansion can be distinguished from a history of long-term constant population size. Estimates of gene flow can also be derived (Wright 1931). Such inferences can complement information about patterns of genetic variation. For example, lack of ESUs might result from recent population expansion with insufficient elapsed time for differentiation.

We sequenced the rapidly evolving mtDNA control region (Taberlet 1996) and part of the ND6 gene. We sampled populations throughout the range to clarify genetic patterns of threatened and "healthy" populations. Our goals were to investigate the recent demographic history of this species, to test for the existence of ESUs and hence the validity of various subspecies schemes, and to comment on the relevance of genetic information to the conservation of this species.

Methods

We collected gnatcatchers in Mexico and plucked feathers from nestlings in the United States. Specimens are housed at the American Museum of Natural History, New York, Museo de Zoologia, Universidad Autonoma de Mexico, and the J. F. Bell Museum, University of Minnesota. Sample size at each of the 13 localities (Fig. 1) was five, except for San Diego County ($n = 3$), Riverside County ($n = 4$), and San Telmo ($n = 7$). The mtDNA was isolated from tissue or feather pulp, amplified via the polymerase chain reaction, and sequenced manually following standard protocols (Hillis et al. 1996). We used several pairs of primers (Tarr 1995; Zink et al. 1997) to obtain a sequence for the mtDNA control region, t-RNA^{Glu}, and part of ND6 (ND6E, HCR4, LCR4, HPHE-1; LMCR CCAGTACAGGAGTAATGTCG; and LCCR2M CTCTTCACAGATACAAGTGG). As a check on the control-region results, we also sequenced parts of two other mtDNA genes (318 base pairs [bp] of ND3 and 275 bp of ND2) from 12 specimens spanning the entire geographical range. We used the program PAUP* (Swofford 1999) to estimate a haplotype tree based on maximum parsimony (heuristic search, bases equally weighted); a haplotype of the Black-tailed Gnatcatcher (*P. melanura*), sister species of the California Gnatcatcher (Zink & Blackwell 1998), was used to root the tree. We bootstrapped the data set 250 times using random additions. We tested for departure from a molecular clock by performing a log-likelihood ratio test (HKY85 model with gamma correction) of the difference in likelihood for a minimum-length haplotype tree with and without a molecular clock enforced (Huelsenbeck & Rannala 1997). The significance of two times the difference in log likelihoods was assessed by a chi-square table.

We computed the amount of genetic variation within each population sample, nucleotide diversity (π), following standard equations (Nei 1987). In addition, we

computed the amount of genetic variation distributed among populations, a quantity estimated by N_{ST} (Lynch & Crease 1990). The N_{ST} analysis resembles a hierarchical ANOVA, partitioning genetic variation into among- and within-population components. The N_{ST} tends to 0 with no population subdivision, whereas a value nearing 1 indicates that populations share no recent gene-flow events or common history. We computed Tajima's (1989) D statistic to assess whether sequence evolution appeared consistent with neutral expectation.

We computed the mismatch distribution (Rogers & Harpending 1992), defined as the number of nucleotide differences between all pairs of individual California Gnatcatcher mtDNAs ($n = 64$). We calculated the mean of these differences and, following Slatkin and Hudson (1991), used the mean value to fit the observed distribution to an expected Poisson distribution; the distributions were compared with a Kolmogorov-Smirnov one-sample test. In a subsequent analysis, we divided the gnatcatcher samples into two subsets that corresponded to the 25 individuals from the five localities south of latitude 28°N and the 39 individuals from the eight localities north of latitude 28°N. We computed the mismatch distributions for these two subsets and again fit the means to Poisson distributions and performed Kolmogorov-Smirnov tests. Harpending's (1994) raggedness statistic was estimated for the distribution of pairwise differences. This statistic has been used to distinguish between stationary and growing populations of humans.

Nee et al. (1995) have shown that, for a population of approximately constant size, a plot of the logarithm of the number of lineages versus their branching times will have a characteristic concave shape, whereas an exponentially growing population exhibits a convex shape. We arbitrarily used one of the alternate minimum-length estimates of phylogenetic relationships for individual gnatcatchers and estimated the time of origin of each of the nodes on that tree. For each node, we computed the number of nucleotide substitutions to each terminal stemming from that node. The average of these substitutions over all paths from the internal node to sampled individuals was taken as the "age" of the node. This was plotted against the number of lineages segregating prior to that estimated time. For the shallowest, most terminal nodes of the tree, estimated ages are not precise because the number of substitutions along the short-terminal and near-terminal branches was frequently zero, one, or two. This sampling error can result in estimated branch lengths that are slightly negative. For such cases we collapsed the negative branches into the next most basal nodes. This circumstance arises only in the analysis of log-lineage plots for which the average age of nodes must be estimated; minimum-length parsimony trees have no negative branch lengths (for a worked example, see Barrowclough and Groth 1999).

We constructed an expected plot of log lineages versus coalescent times using the equations reviewed by Hudson (1991). The expected time between coalescent events is $T(j) = (2N_{ef})/(j(j - 1))$, where j is the number of lineages left to coalesce and N_{ef} is the effective number of females in the population. The total expected time to coalescence is $2N_{ef}(1 - 1/n)$, where n is the number of individuals sampled; for 64 individuals this is $1.97N_{ef}$. We therefore calibrated the log-lineages plot by letting the depth, in substitutions, of the gnatcatcher tree equal $1.97N_{ef}$. To compare the observed distribution of lineages versus time with the pattern expected for a population of constant size, we computed the expected time of each coalescent event $T(j)$ and set the estimated age of the earliest node on the tree equal to the expected coalescence time for a sample of 64 individuals from a stationary population.

An alternative to the graphical techniques discussed above for drawing inferences about the demographic history of populations was developed by Kuhner et al. (1998). This approach yields maximum-likelihood estimates of population size and growth rates based on a simple model of DNA evolution and a maximum-likelihood, as opposed to parsimony, estimate of the haplotype tree. Using the program FLUCTUATE (Kuhner et al. 1998), we estimated population growth rates for the entire sample of 64 gnatcatchers, as well as for the sample of 25 individuals from the five localities south of 28°N latitude and the 39 individuals from the eight localities north of 28°N latitude. In estimating the growth rate with FLUCTUATE, we used a transition-to-transversion ratio of 10.0 and a two-rate substitution model in which 90% of the sites were invariant and 10% had the same substitution rate. In this procedure, the search for the maximum-likelihood estimate over the likelihood surface was initiated with Watterson's estimate of theta(θ). To determine if the resulting estimates were stable, we iterated the search for maximum-likelihood estimates of growth and θ, but in successive iterations we used the point estimates from the previous iteration as a starting point. This procedure provides information about whether the likelihood surface is sufficiently smooth and has sufficient relief so that estimates are consistent.

Results

A total of 1399 bp, including some indels, was obtained for all 64 gnatcatchers. We analyzed data with and without deletions, and our overall conclusions are unchanged; omitting deletions results in lower resolution of the pattern of haplotype relationships. We found similar levels of variation for the 12 individuals surveyed additionally for ND2 and ND3 gene regions and no geographically segregating differences, so these data are not discussed further. Because these genes are not contiguous with the

control region, we inferred that our sequence data were mitochondrial and not derived from a nuclear homologue. Furthermore, our control-region sequences contained the "landmarks" found in other avian control regions (Baker & Marshall 1997). Also, the large number of closely related haplotypes we found argues against nuclear copies, which tend to be less variable owing to mutation repair mechanisms.

Direct sequencing of the control region revealed 26 variable positions (17 transitions, 2 transversions, 7 deletions), of which 14 were parsimony-uninformative. Of the 64 California Gnatcatchers examined, 33 exhibited unique haplotypes (Appendix). All haplotypes were closely related, with a maximum (uncorrected) interhaplotype divergence of 0.64% and an average of 0.27%. Twenty-three individuals (35.9%), representing 12 of 13 localities, shared a single haplotype, whereas the next most frequent haplotype was found in four individuals (6.3%). The N_{ST} of 0.074 suggests a lack of population subdivision; 92.6% of the genetic variation was common to populations and only 7.4% was distributed among them. The lack of structure among gnatcatcher haplotypes was confirmed by phylogenetic analysis (Fig. 2), which does not support any subspecies scheme, either previously described (Fig. 1) or unforeseen. That is, haplotypes did not form exclusive clusters that conformed to recognized subspecies or to any other geographically restricted areas. A feature common to the minimum-length trees was the basal position of several haplotypes from southern locations. A likelihood ratio test (LRT) (Kishino & Hasegawa 1989) significantly ($p = 0.01$) rejected a tree in which haplotypes were constrained to match the subspecies limits proposed by Atwood (Fig. 1). A LRT for a haplotype tree (one of the minimum-length trees) with and without a molecular clock enforced was not significant, indicating a lack of rate heterogeneity. Tajima's D statistic was significant in only 1 of 13 population samples.

The most striking genetic pattern observed (Fig. 3) was a transition in level of genetic diversity (π) between the San Ignacio and El Rosarito locales, with populations north of San Ignacio showing π values approximately 25% of those to the south of 30°N latitude.

The mismatch distribution (Fig. 4) had the overall shape associated with growing rather than constant populations (Slatkin & Hudson 1991; Rogers & Harpending 1992). Superimposed on the distribution was the Poisson distribution for a sample with the same mean, 2.33, as the observed distribution. The observed and expected distributions differed significantly (Kolmogorov-Smirnov test, $p < 0.05$). Dividing the population samples at 28°N latitude, which corresponded to the observed discontinuity in π (Fig. 3), we found that neither mismatch distribution (Fig. 5) deviated significantly ($p > 0.05$) from the Poisson expectation (mean for northern samples, 1.17 substitutions; mean for southern samples, 3.81). Harpending's (1994) raggedness value for the overall distribution of pairwise differences, 0.032, resembled those associated with growing populations. Populations with stationary sizes usually had raggedness values of 0.05–0.5, with a mode of 0.1 in their study.

Comparison of observed and expected plots of the distributions of lineages versus time requires calibration of the expected curve. The earliest (deepest) node on the tree corresponded to 6.25 substitutions, which we took as the expected coalescent time for a sample of 64 individuals, $1.97N_{ef}$. Thus, we calibrated the two curves by assuming that $1.97\mu N_{ef} = 6.25$; that is, letting $\mu N_{ef} = 3.17$, where μ is the nucleotide substitution rate. In the resultant plot (Fig. 6), we indicated the expected position of the first 10 coalescent events and used a curve to indicate the shape of the distribution for the remaining 52 closely spaced events. The observed plot (Fig. 6) was consistently to the left of the curve expected for a population of constant size (the result found by Nee et al. [1995] for a growing population).

The maximum-likelihood estimates of growth rates (+1 SD) for the entire sample of 64 California Gnatcatchers, for the five southern populations, and for the eight northern populations were 1025 + 23, 583 + 18, and 1853 + 191, respectively. (These estimates of growth rate are standardized by the mutation rate [e.g., Kuhner et al. 1998]). The three estimates were all positive and significantly different from zero, thereby rejecting a population of constant size. In three successive iterations using the program FLUCTUATE, the estimates of growth rate obtained were stable and consistent.

Discussion

Population History

The most common haplotype (36% of individuals) was found in 12 of 13 population samples. Based on the rooted haplotype tree (Fig. 2), this most common haplotype arose relatively recently. This suggests that gene flow among localities must be substantial; that is, a relatively recently arisen haplotype has spread throughout the range of the California Gnatcatcher compared to the common ancestor of all extant haplotypes. The shape of the phylogenetic tree (Fig. 2) reinforces this conclusion. Although some old, relict haplotypes were confined to the southern portions of the Baja California peninsula, there was no geographic structuring of the more recent branches. If there were substantial barriers to gene flow, one might expect that "families" of related haplotypes would be found in geographically contiguous or proximal locations. This has been found for other birds from Baja California such as LeConte's Thrasher (*Toxostoma lecontei*; Zink et al. 1997), for which mutually exclusive clades of haplotypes were found in two disjunct geographic regions. The haplotype

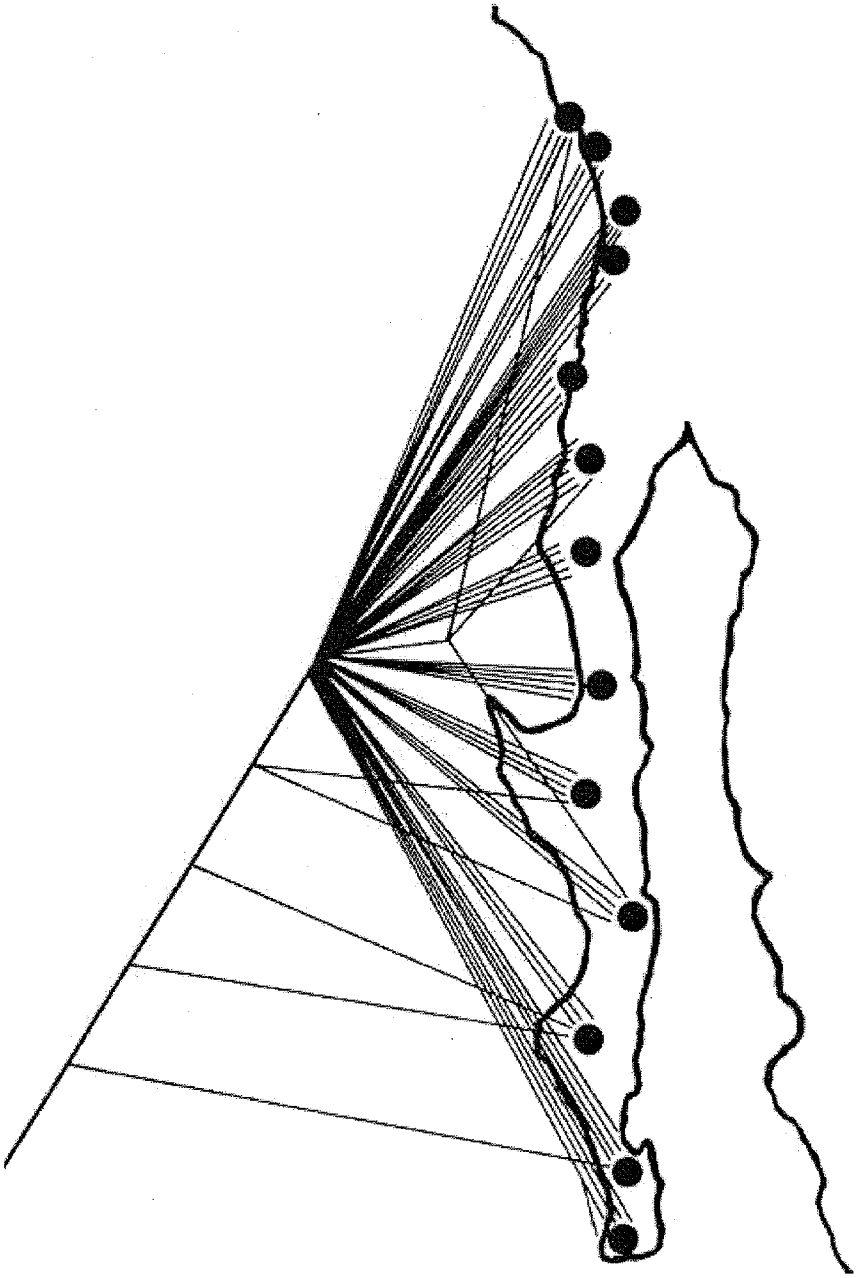

Figure 2. Strict consensus tree of haplotypes derived from 10,751 equally parsimonious trees (length 84, consistency index [ci] = 0.4, excluding uninformative characters; rescaled ci = 0.5) showing no geographic structure among 64 individuals. There is a single node at the top of this tree. No nodes were present at >65% in 250 bootstrap replicate trees. Only two nodes had bootstrap values >50%; each included one pair of haplotypes from different localities.

tree for California Gnatcatchers does not support recognition of ESUs or subspecies.

The N_{ST} value also reinforces the conclusion that there are no ESUs within the California Gnatcatcher. Species including two or more ESUs would have an N_{ST} value an order of magnitude greater than 0.07; in the case of LeConte's Thrasher, for example, N_{ST} was 0.75. Elementary but relatively robust models in population genetics (Neigel 1997) allow one to obtain an estimate of the amount of gene flow necessary to maintain an N_{ST} or F_{ST} value for an isolation-by-distance model or island model of population structure at equilibrium. The estimate of N_{ST} we obtained was equivalent to an exchange of between three and four individuals per generation among populations. Wright (1931) showed that if the amount of gene flow among populations was greater than approximately one individual per generation, the entire population could be thought of as one large panmictic unit. Thus, the pattern of distribution of the most common haplotype, the shape of the phylogenetic tree (phylogeography), and the low N_{ST} estimate all suggest that gene flow among the gnatcatcher populations has been substantial.

The sudden geographic shift in π can be attributable to two alternate phenomena, namely a range expansion

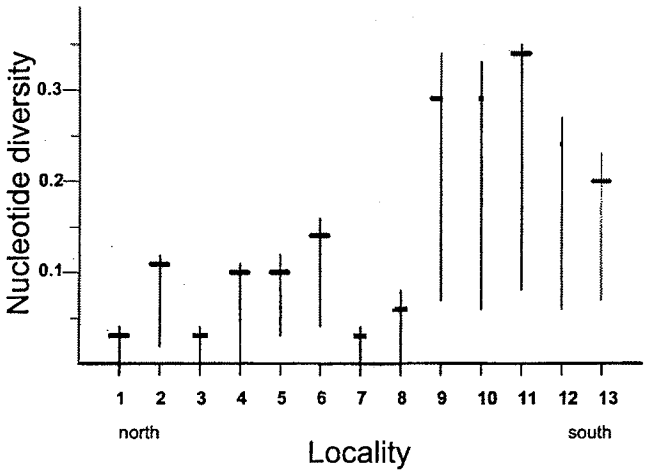

Figure 3. Pattern of geographic variation in nucleotide diversity (π; estimate and 95% bootstrap confidence intervals) showing shift between El Rosarito (locality 8) and San Ignacio (9) at 28°N latitude. The pattern is significant with a run test ($p < 0.05$)

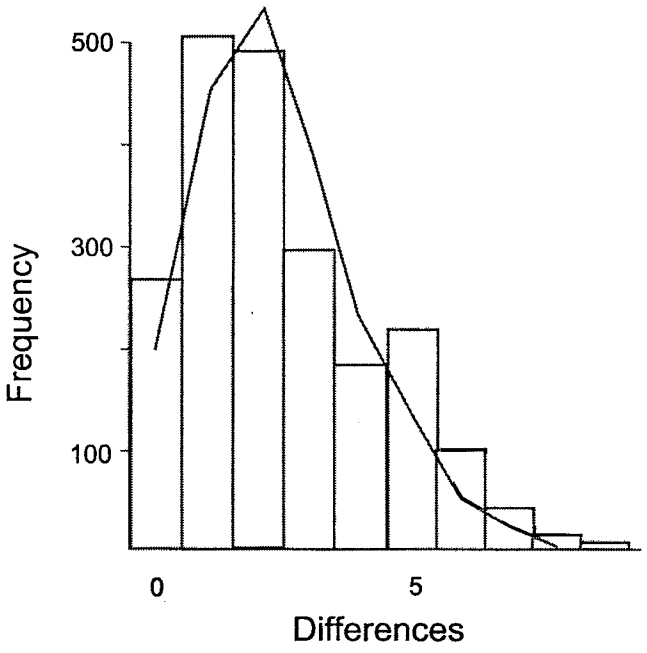

Figure 4. Mismatch distribution for 64 California Gnatcatcher sequences (histogram) and the expected Poisson distribution (line) for a growing population with the same mean.

from a southern refugium or a "selective sweep." If the northern part of the range only recently became inhabitable or otherwise available to gnatcatchers, northward emigrants dispersing from a southern refugium might represent only a part of the species' genetic diversity, leading to decreased π in the north (Hewitt 1996). Alternatively if a new advantageous mutation makes a northern haplotype selectively superior, it can spread southward rapidly and increase in frequency at the expense of older and less fit southern haplotypes. The observed pattern of variation in π is consistent with either hypothesis. Four lines of evidence support the hypothesis of recent and northward population expansion. First, phylogenetic analysis (Fig. 2) revealed several basal (i.e., oldest) haplotypes that occurred only in Baja California Sur. Older (basal) haplotypes are expected to occur disproportionately in previous refugia. Baja California south of 30°N latitude was thought to be a refugium (Magdalena Refugium) during the late Pleistocene (Hafner & Riddle 1997). Other avian (Zink et al. 1997) and nonavian (Upton & Murphy 1997) species also show genetic breaks between 28°N and 30°N latitudes. Second, the plot of the number of lineages versus the estimated age of the haplotype tree suggests an expanding population.

Third, the overall mismatch distribution (Fig. 4) was basically unimodal and had the characteristic shape associated with a growing population (Rogers & Harpending 1992). Such distributions from constant populations are often ragged (Slatkin & Hudson 1991; Harpending et al. 1993; Harpending 1994)—that is, bimodal or multimodal—unlike that for the California Gnatcatcher. In addition, the plot was quite different from those reported by Barrowclough and Groth (1999) for three populations of owls that they interpreted to be stationary in size. The results of the Kolmogorov-Smirnov test, however, indicated that our observed distribution differed from the Poisson expectation for a growing population. These tests must be interpreted with caution because the samples were not independent (Slatkin & Hudson 1991). Visual comparison of the observed and expected mismatch distributions for the 64 gnatcatchers (Fig. 4) suggests that the tail of the distribution is too long for a Poisson curve. This overall distribution, however, was for 13 population samples taken over 1000 linear km. Rogers and Harpending (1992) showed that an exponentially growing population can be characterized by a Poisson-like distribution with a mode that starts at an average pairwise difference of zero at the time of initial growth; the distribution shifts to larger values of pairwise differences as time increases while maintaining the characteristics of a Poisson distribution (for example, variance equal to mean).

Our interpretation of the topology of the sequence relationships and the geographical pattern of nucleotide diversity is that populations of California Gnatcatchers have been expanding their range northward from southern Baja California. If this were true, then the expansion in population size would be older in the southern part of the gnatcatcher range and more recent in more northern parts of the range. Therefore, we divided the gnatcatcher samples into two subsets that corresponded to the regions defined by the pattern of π (Fig. 3). Mismatch distributions (Fig. 5) fit to each subset did not differ significantly from expecta-

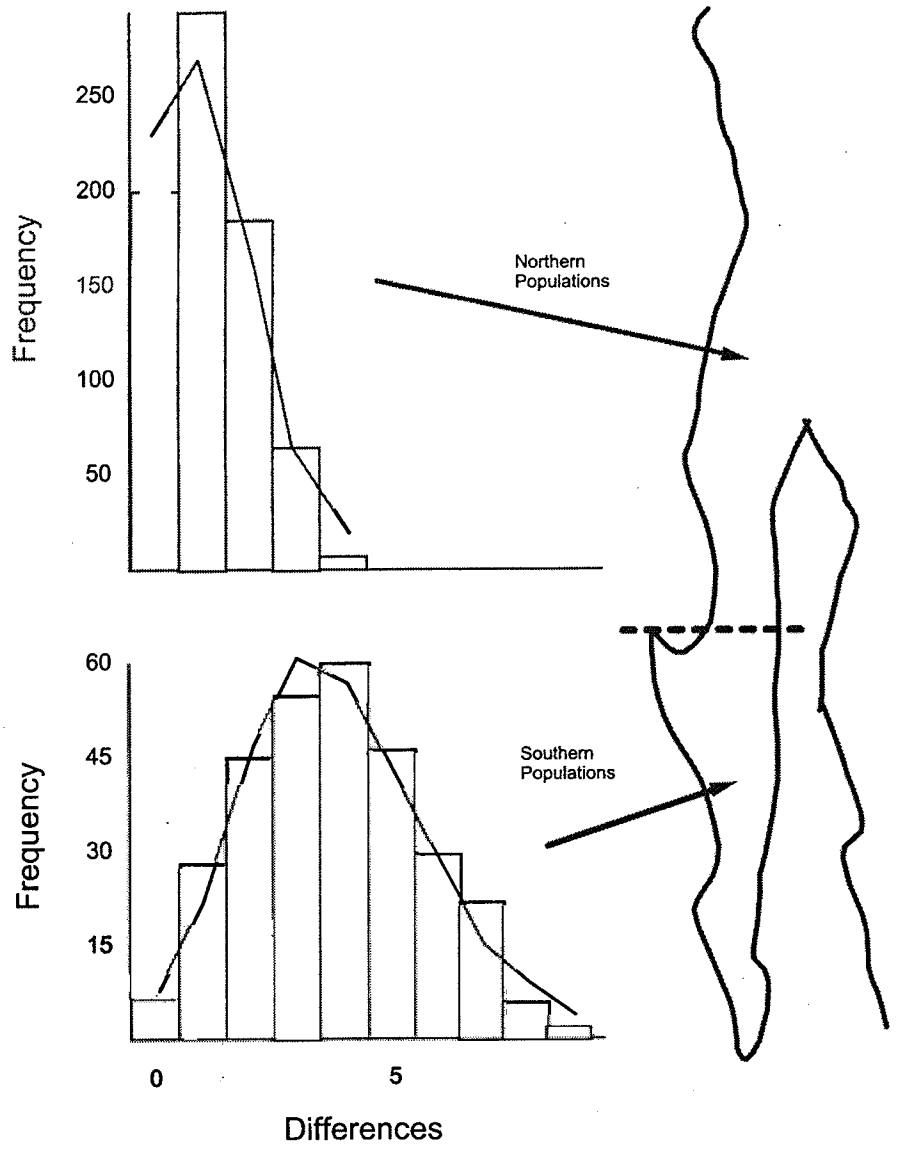

Figure 5. Mismatch distributions for northern and southern samples of California Gnatcatchers with expected Poisson distributions for growing populations with the same mean (lines).

tion. The shallow, wide distribution with the mode removed from the vertical axis in the south, and the tall, narrow distribution close to the axis in the north reflect what would be anticipated given the expansion process we suggest. Although mismatch distributions could be computed separately for each of the 13 populations samples, small sample sizes precluded this procedure.

Fourth, it might be argued that the log-lineage plot and the mismatch distributions are simply qualitative or heuristic techniques. The quantitative, maximum-likelihood method of Kuhner et al. (1998) obtains an estimate of growth rate by integrating over all possible tree topologies, rather than a single parsimony tree. In addition, the maximum-likelihood approach does not require an outgroup for rooting purposes. Consequently, it is in many ways an independent technique for addressing the question of a stable versus growing population. Using the maximum-likelihood method, we obtained estimates of growth rates for the gnatcatchers that were all positive and significantly different from zero. In addition, the growth rate for the northern eight populations was estimated to be greater by a factor of three than that for the southern five populations; this is consistent with our hypothesis that the predominant region of population expansion is the northern part of the range, emanating from a possible southern refugium.

Taken together, the tree of haplotype relationships, the geographical pattern of π, the shape of the mismatch distributions, the log-lineage plot, and the maximum-likelihood results favor a hypothesis of a relatively recent expansion of California Gnatcatcher populations from southern Baja California northward throughout the peninsula and into southwestern California. Such a recent population expansion likely explains the lack of phylogeographic pattern. Given that range expansion has recently occurred, it is unlikely that any current iso-

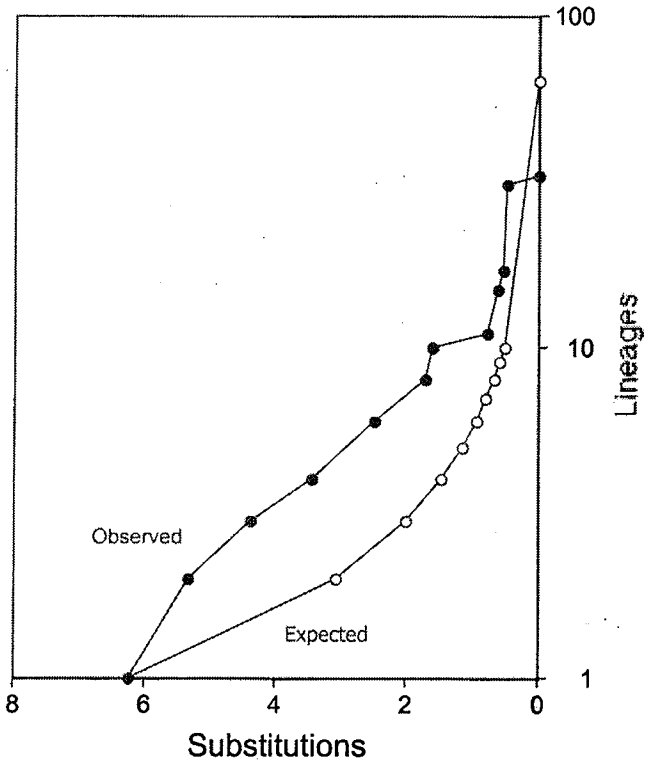

Figure 6. Number of lineages (logarithmic scale) versus estimated age (measured as number of nucleotide substitutions) of coalescent events for hypothesized relationships among California Gnatcatcher sequences (solid circles) and the expected distribution for a sample of 64 from a stationary population (open circles).

lating barriers (except distance) will result in future genetic division of the northern populations.

Conservation Implications of Population History

Our study provides a geographically thorough genetic analysis of a threatened species, encompassing both areas where populations are not threatened (providing a baseline) and where they are. Our genetically based interpretation of the recent history of the California Gnatcatcher provides perspective on current conservation questions. Put simply, based on mtDNA data, northern populations do not appear to constitute a unique component of gnatcatcher biodiversity. Nevertheless, several caveats merit consideration. One might argue that loss of northern populations could be unfortunate because populations at the margins of species' ranges might be "evolutionary laboratories" for novel genetic types (Erwin 1991). Although a few novel haplotypes exist in the coastal sage scrub populations, only an extreme view would support preservation of each unique haplotype, given their minor differences; every individual likely possesses at least one unique mutation. Second, other molecular techniques might reveal more fine-scaled geographic structuring than we have found. Third, local phenotypic adaptations might be maintained by natural selection in different parts of the range. Our data indicate, however, that no particular segment of the range of California Gnatcatchers has been evolving independently long enough (i.e., $2N_{ef}$ generations on average; Avise 1994) to have developed an exclusive set of mtDNA haplotypes (Fig. 2), and if geographic structure were found with another molecular marker (such as microsatellites), in our opinion it would be evolutionarily less "significant" than evolutionary divisions found in other species (Table 1).

Although extrapolations from our gnatcatcher study about the general significance of avian subspecies must be made with caution, our results are consistent with other studies (Ball & Avise 1992). We summarized data (Table 1) for phylogeographic surveys of 17 avian species in North America. For these species, the average number of subspecies is 6.5, and the average number of ESUs is 1.7. The data set itself is biased because the average number of subspecies per North American passerine species is 3.3 ± 3.9 (SD, n = 234, Klicka & Zink 1999); hence, species studied to date have tended to be those recognized as highly polymorphic based on classical taxonomic criteria. Nonetheless, it is likely that most biological species of birds will contain two or fewer ESUs and that subspecies on average will not be equivalent to ESUs (Avise & Walker 1998). Our findings for the California Gnatcatcher are therefore consistent with those for other bird species (Table 1).

Subspecies limits might not be predictive of ESUs in gnatcatchers and other birds (Ball & Avise 1992) because such limits are often based on single characteristics, such as plumage coloration, size, and shape, that are probably controlled by relatively few genes and influenced individually by different selective pressures. In contrast, neutral genetic characters are more likely to reflect overall demographic events and population history. Based on our mtDNA results, we predict that reanalysis of gnatcatchers will show that inconsistent patterns of variation among single morphological characters caused conflicting taxonomic opinions (Fig. 1) because different authors emphasized different characters. Our finding of no significant genetic divisions explains prior controversy among subspecies schemes: there probably is no general pattern of variation in morphological characters consistent with historical isolation and independent evolution of populations. Thus, preservation of biodiversity in California Gnatcatchers can be considered independent of subspecies designations.

Although northern populations of California Gnatcatchers do not represent discrete elements of biodiversity, our results must be interpreted in a broader context. In recent years, concern over single species has been complemented by ecosystem or community perspectives (Murphy et al. 1994). Our study reinforces this

Table 1. Molecular support for evolutionarily significant units (ESUs) in avian songbirds.[a]

Species	*Number of subspecies examined*	*Number of ESUs*
Polioptila californica	3–5	1
Passerella iliaca	15	4[b]
Melospiza melodia	19	1[c]
Spizella passerina	3	1[b]
Geothlypis trichas	6	2[d]
Molothrus ater	2	1[b]
Toxostoma curvirostre	6	2[c]
Pipilo fuscus	7	2[c]
Auriparus flaviceps	3	1[c]
Agelaius phoeniceus	10	1[b]
Campylorhynchus brunneicapillus	7	2[c]
Parus carolinensis	4	2[b]
Parus atricapillus	5	1[b]
Parus hudsonicus	4	2?[b]
Dendroica petechia	2–3	2[b]
Ammodramus maritimus	6–7	2[b]
Ammodramus caudacutus	5	2[b]

[a] *Includes North American studies (Ball & Avise 1992; Zink 1997; Avise & Walker 1998) that include two or more named subspecies for which mtDNA restriction-site or sequencing studies were performed. These studies show that the genetic structure of the California Gnatcatcher is consistent with that of other birds examined with similar molecular approaches.*
[b] *Restriction fragment studies.*
[c] *R. M. Zink, unpublished mtDNA sequence data.*
[d] *J. Klicka, personal communication*

trend because, although the gnatcatcher's widespread distribution, visibility, and legal status make it a good "flagship species" for regional conservation efforts, our genetic data show that the species poorly reflects the endemism of the coastal sage scrub community. Other species are restricted to coastal sage scrub and are relatively unstudied, and many do not share the gnatcatcher's extensive distribution to the southern tip of the Baja peninsula (Atwood 1993). Hence, further loss and fragmentation of coastal sage scrub in the United States might entail a large genetic cost, if not extinction, for other species. Programs focused at the ecosystem or community level, such as the State of California's Natural Community Conservation Planning process (O'Connell & Johnson 1997), appear most relevant to conservation of coastal sage scrub and other threatened ecosystems. That is, preservation of the California Gnatcatcher should be coupled to preservation of the coastal sage scrub ecosystem, rather than the reverse.

Acknowledgments

R. Lande, D. Tilman, J. Klicka, S. Rohwer, A. Kessen, J. Rotenberry, R. Shaw, F. Allendorf, S. Lanyon, K. Scribner, and three anonymous reviewers provided useful comments on the manuscript. C. Griffiths, J. Groth, and L. Marcus assisted with analyses. S. Hills and D. Ely provided financial support and encouragement. D. Bontrager, C. Reynolds, S. Tsai, and R. McKernan provided feather or tissue samples from the United States. O. Rojas-Soto, A. Navarro, P. Escalante-Pliego, B. Hernandez-Baños, A. Fry, J. Klicka, P. Sweet, and L. Montañez-Godoy assisted with Mexican fieldwork. Financial support was provided by the U.S. Navy, the National Fish and Wildlife Foundation, Southern California Edison, trustees of the Manomet Center for Conservation Sciences, the Building Industry Association of Southern California, the Transportation Corridor Agency, Chevron Land and Development, the University of Minnesota, and the National Science Foundation (DEB-9317945). The U.S. Fish and Wildlife Service and Mexico's Secretaria de Desarrollo Urbano y Ecologia provided collecting permits.

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