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# COMPARATIVE PHYLOGEOGRAPHY OF SOME ARIDLAND BIRD SPECIES[1]

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*Abstract.* We compared mitochondrial DNA sequences for six species distributed across the aridlands of North America to document phylogeographic patterns and assess levels of congruence. The Curve-billed Thrasher (*Toxostoma curvirostre*) and Canyon Towhee (*Pipilo fuscus*) show genetic divisions between the Sonoran and Chihuahuan Deserts, whereas the Cactus Wren (*Campylorhynchus brunneicapillus*), Black-tailed Gnatcatcher (*Polioptila melanura*), and Verdin (*Auriparus flaviceps*) do not. Most likely, species without phylogeographic structure only recently colonized their entire current range. Therefore, although these species are today part of a widespread avifauna, species' distributions were historically different from today. In Baja California, the Cactus Wren and the Verdin show phylogeographic breaks at 28°–30°N, consistent with a division previously described in the LeConte's Thrasher (*Toxostoma lecontei*) and in some members of the herpetofauna. These genetic divisions were likely caused by isolation resulting from a mid-peninsular seaway that existed one million years ago. Hence, these species appear to have been broadly sympatric for at least one million years. In contrast, the California Gnatcatcher (*Polioptila californica*) lacks such a phylogeographic division, and apparently only recently expanded into the northern part of its current range. Thus, not all species in Baja California have had similar histories, although further sampling might reveal a general pattern. Comparative phylogeography therefore provides an indirect method of evaluating the long-term stability of faunas via assessment of levels of phylogeographic congruence, and can show whether particular species are likely to have had a long period of co-association.

*Key words: Black-tailed Gnatcatcher, Cactus Wren, Canyon Towhee, Curve-billed Thrasher, mitochondrial DNA, phylogeography, Verdin.*

*Resumen.* Comparamos secuencias de ADN mitocondrial de seis especies distribuidas a través de las zonas áridas de Norteamérica para documentar los patrones filogeográficos y valorar los niveles de congruencia. El cuitlacoche piquicurvo (*Toxostoma curvirostre*) y el rascador arroyero (*Pipilo fuscus*) muestran divisiones genéticas entre los desiertos de Sonora y Chihuahua, mientras que la matraca desértica (*Campylorhynchus brunneicapillus*), la perlita colinegra (*Polioptila melanura*) y el baloncillo (*Auriparus flaviceps*) no lo hacen. Probablemente, las especies sin estructura filogeográfica han colonizado recientemente su actual área de distribución. Por lo tanto, aunque estas especies actualmente forman parte de una avifauna muy ampliamente distribuida, su distribución fue históricamente diferente a la actual. En Baja California, la matraca desértica y el baloncillo muestran interrupciones filogeográficas a los 28°–30°N, consistente con la división previa descrita en el cuitlacoche pálido (*Toxostoma lecontei*) y algunos miembros de la herpetofauna. Estas divisiones genéticas fueron aparentemente causadas por el aislamiento resultado de un brazo marino que existió hace un millón de años en la parte media de la Península. De ahí que estas especies parecen haber sido simpátricas por al menos un millón de años. En contraste, la perlita californiana (*Polioptila californica*) carece de tal división filogeográfica, y aparentemente sólo recientemente se ha expandido hacia la parte norte de su distribución actual. De esta manera, no todas las especies en Baja California han tenido historias similares, aunque futuros muestreos podrían revelar un patrón general. La filogeografía comparada, por lo tanto, provee un método indirecto de evaluación de la estabilidad prolongada de faunas a través de la valoración de los niveles de congruencia filogeográfica y puede mostrar que especies en particular pudieran haber tenido un largo periodo de co-asociación.

## INTRODUCTION

Inferences about the history of populations can be derived from phylogeographic analyses (Avise 1994). Ideally, individuals are sampled from throughout a species' range, and a molecular marker such as mitochondrial DNA (mtDNA) is assayed. Phylogenetic analysis of individuals' mtDNA haplotypes produces a tree, which can be superimposed on the species' geographic distribution to show whether the history of the pop-

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ulations has been one of isolation and divergence, high levels of gene flow, or some combination. Analyses using coalescence theory permit additional inferences about the nature of population increases and magnitude and direction of gene flow.

Extending phylogeographic analyses to sets of broadly sympatric species comprises comparative phylogeography (Bermingham and Avise 1986, Zink 1996). In theory, species that are part of a modern widespread fauna should have been affected by the same isolating barriers and ought to show similar phylogeographic patterns (Zink 1996). Hence, comparisons of species' phylogeographic patterns leads to inferences about the historical stability of the current avifauna. For example, Zink et al. (1997) showed that an isolated population of LeConte's Thrasher (*Toxostoma lecontei*) in west-central Baja California possessed a reciprocally monophyletic set of mtDNA haplotypes relative to haplotypes in the rest of the range. That is, there was a phylogeographic break at 28°–30°N corresponding to a mtDNA distance of 3.5%. In contrast, a similar mtDNA survey (Zink et al. 2000a) of the California Gnatcatcher (*Polioptila californica*) found no evidence of a phylogeographic split in Baja California or anywhere in the range. Coalescence analyses (Zink et al. 2000a) suggested that the California Gnatcatcher has recently expanded its range northward and colonized the area presently inhabited by the northernmost of the two historically isolated groups of LeConte's Thrasher. Therefore, two species in the modern avifauna that presently occupy parts of the same area have had different histories. The LeConte's Thrasher experienced a major episode of isolation and divergence, whereas the California Gnatcatcher underwent recent range expansion to achieve its current distribution. Studies of more species are needed to assess whether either of these histories is typical.

In this paper we compare species broadly distributed across the aridlands of North America (Hubbard 1973), including Baja California. We combine new mtDNA sequence information for the Cactus Wren (*Campylorhynchus brunneicapillus*), Black-tailed Gnatcatcher (*Polioptila melanura*), Canyon Towhee (*Pipilo fuscus*), and Verdin (*Auriparus flaviceps*) with that available for the LeConte's Thrasher (Zink et al. 1997), Curve-billed Thrasher (*Toxostoma curvirostre*, Zink and Blackwell-Rago 2000) and the California Gnatcatcher (Zink et al. 2000a). Because not all species occur in Baja California, our comparisons include two regions: Baja California, and the Chihuahuan plus Sonoran Deserts. Our goals were to search for major phylogeographic divisions and, if they existed, assess whether they were congruent. Additionally, where sample sizes permitted, we attempted to infer aspects of recent population history from analyses based in coalescence theory.

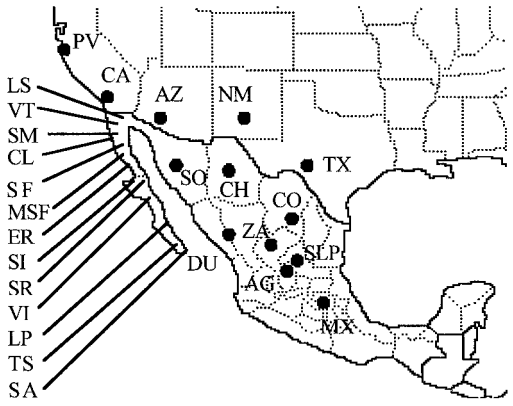


FIGURE 1. Map showing general locations of sampling sites (see GenBank accessions for precise localities). Locality codes: PV (Palos Verde peninsula), LS (Laguna Salada), VT (Valle de Trinidad), SM (Ejido San Matias), CL (Camalu), SF (San Felipe), MSF (Mision San Fernando), ER (El Rosarito), SI (San Ignacio), SR (Santa Rosalia), VI (Villa Insurgentes), LP (La Paz), TS (Todos Santos), SA (Santa Anita), CA (Salton Sea), AZ (near Tucson), NM (southern New Mexico), TX (near San Antonio), SO (Sonora near Tecoripa), CH (Chihuahua, west of city of Chihuahua), DU (Durango, north of city of Durango), ZA (Zacatecas), CO (Coahuila, north of Saltillo), SLP (San Luis Potosi, Rio Verde), AG (Aguascaliente), MX (Mexico City).

## METHODS

Sampling localities (Fig. 1) encompass most of the arid regions of North America as defined by Hubbard (1973); locality information is given in GenBank accessions (see Results). We attempted to obtain enough samples to represent large fractions of each species' range. Voucher specimens are housed at the Bell Museum of Natural History, University of Minnesota; American Museum of Natural History, New York; Museum of Natural Science, Louisiana State University; and the Universidad Autonoma Nacional de México.

Template DNA was extracted using standard

phenol chloroform (Hillis et al. 1996) and Chelex/Proteinase K protocols (Ellegren 1992, Zink and Blackwell 1996). For many specimens, we used purified mtDNA; hence, we are confident that we analyzed authentic mitochondrial sequences and not nuclear copies (Zhang and Hewitt 1996). We performed PCR using the following primers: for Control Region I (CRI), ND6E (Edwards 1993), HCR4 and LGL2 (Tarr 1995), and ND6C2 (5′-CCGCAATTAAAAACAGGCCCGC-3′); for Control Region II (CRII), LCR4, H1248, and HPHE-1 (Tarr 1995); for ND2, L5215 (5′-TATCGGGCCCATACCCCGAAAAT-3′) and H5578 (5′-CCTTGAAGCACTTCTGGGAATCAGA-3′) (Hackett 1996). We used 50-μl reaction volumes, including 0.75 μl of *Thermus flavus* polymerase, 3.0 μl of 10 mM solution of each primer, 4.0 μl of 25 mM magnesium chloride, 2.5 μl of 20X reaction buffer, and 3.0–4.0 μl of DNA template. Reactions were performed using Thermolyne Amplitron II and MJ Research PTC-100 thermocyclers with reaction conditions of 35 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C, followed by a 10-min extension at 72°C and a hold at 4°C.

We purified the PCR products using a Qiagen PCR purification kit following the manufacturer's protocols (Qiagen, Valencia, California). Sequencing reactions used the appropriate primers listed above for each gene region. Sequencing reaction products were purified using Centrisep columns with 0.05 g of Sephadex and 0.8 ml of distilled water, following manufacturer's protocols. Sequencing of these products was performed using an ABI Prism 310 automated sequencer. The resulting chromatograms were aligned and reconciled with the aid of Sequencher 3.1.1 (Gene Codes Corporation 1999).

We used Paup* (Swofford 1999) to construct haplotype trees, with heuristic random-addition searches (10 replicates) of equally weighted base pairs. Equally parsimonious trees were summarized as strict consensus trees. We bootstrapped data sets 10,000 times using the fast bootstrap option in Paup*. Congeneric species were used as outgroups except for Verdin, for which the Black-tailed Gnatcatcher was used, and Cactus Wren, for which the Green-tailed Towhee (*Pipilo chlorurus*) was used.

We used ARLEQUIN (Schneider et al. 1997) to compute nucleotide diversity ($\pi$), Tajima's (1989) D, $F_{st}$, and Nm. Nucleotide diversity includes information on both the number of haplotypes and their relative differentiation, and has been used to reconstruct the spatial history of range expansion in other birds (Merila et al. 1996). Tajima's D measures departures from selective neutrality, and $F_{st}$ is a measure of the amount of variance distributed among populations. Nm, a relative measure of gene flow, was computed from the pairwise distribution of $F_{st}$ values. Groups of samples with no apparent phylogeographic structure were combined and $\pi$, $F_{st}$, Tajima's D-value, and a mismatch distribution were computed; the latter was compared to the expected distribution based on a sudden population-expansion model (Rogers 1995) to permit inferences about population growth.

## RESULTS

### CANYON TOWHEE

Analysis of 333 base pairs (bp) of CRI resolved nine haplotypes among 28 individuals representing nine localities (GenBank accessions AF298595–298622). One haplotype was widespread, occurring in localities CH, CO, DU, SLP, TX, MX, and ZA. Phylogenetic analysis of unique haplotypes revealed three equally parsimonious trees, the consensus of which shows two groups of haplotypes (Fig. 2), representing

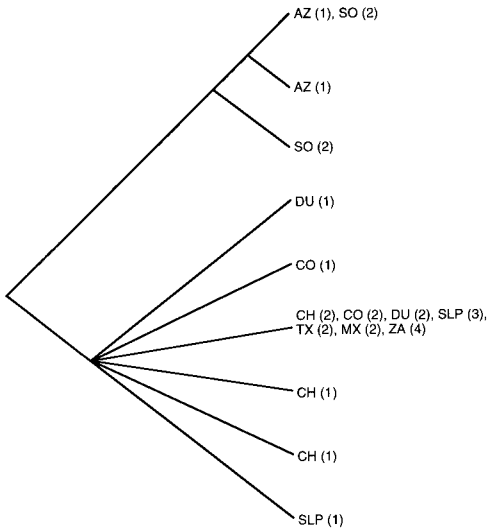


FIGURE 2. Strict consensus tree of three equally parsimonious trees of length 12 with characters equally weighted for haplotypes in Canyon Towhee. Shown are unique haplotypes and numbers of each haplotype found per locality (see Fig. 1).

TABLE 1. Samples of Canyon Towhees (*Pipilo fuscus*) used in phylogeographic analyses and estimated statistics. Localities are shown in Figure 1.

| Locality | Subspecies | *n* | Number of haplotypes | Nucleotide diversity |
|---|---|---|---|---|
| TX | *texanus* | 2 | 1 | 0.000 |
| AZ | *mesoleucus* | 2 | 2 | 0.003 |
| DU | *perpallidus* | 3 | 3 | 0.004 |
| ZA | *potosinus* (?) | 4 | 1 | 0.000 |
| SLP | *potosinus* | 4 | 3 | 0.003 |
| CO | *potosinus* (?) | 3 | 3 | 0.004 |
| SO | *intermedius* | 4 | 2 | 0.010 |
| CH | *perpallidus* | 4 | 3 | 0.003 |
| MX | *fuscus* | 2 | 1 | 0.000 |

the Sonoran Desert (locality codes SO and AZ; bootstrap support 86%), and the Chihuahuan Desert plus regions to the south on the Mexican Plateau (bootstrap support 63%). $F_{st}$ was 0.47 considering all nine samples, whereas combining samples into two geographic regions resulted in a between-region value of 0.69 (with 0.34 within populations and −0.03 among populations within regions). Nm values averaged greater than 4.0 within regions and less than 1 between regions. Nucleotide diversity ranged from 0.0 to 0.01 (Table 1), and was 0.001 for the eastern region. Tajima's D-value for the eastern region was −1.99 ($P = 0.01$), and the mismatch distribution (Fig. 3) did not differ from the sudden expansion model.

VERDIN

Analysis of 369 bp of CRII resolved 13 haplotypes among 45 individuals from 14 localities (GenBank accessions: 295205–295249). One haplotype was widespread, occurring in AZ, CA, CH, CO, DU, LS, SLP, SO, and TX. Phylogenetic analysis of unique haplotypes revealed five equally parsimonious trees, the consensus of which shows two groups of haplotypes (Fig. 4), Baja California south of 30°N (bootstrap value 65%), and the remainder of northern Baja California and the U.S. and Mexican parts of the range (bootstrap 100%). $F_{st}$ considering all 14 localities was 0.85, whereas combining samples into the two geographic regions yielded a value of 0.95 (with 0.03 within populations and 0.02 among populations within regions). Nm values averaged greater than 4.0 within regions and less than 1 between regions. Nucleotide diversity

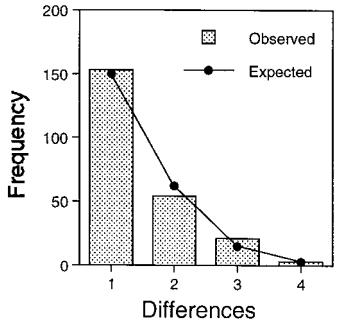


FIGURE 3. Mismatch distribution for eastern clade of Canyon Towhee.

ranged from 0.0 to 0.008 (Table 2), and for the eastern region the value was 0.003. Tajima's D-value for the eastern region was nonsignificant. A mismatch distribution (Fig. 5) for the large group of non-southern Baja samples differed significantly from the expected sudden expansion model ($P < 0.001$).

CACTUS WREN

Analysis of 298 bp of ND2 for 60 Cactus Wrens from 22 localities resolved 21 haplotypes (GenBank accessions AF291512–291571). Two haplotypes were widespread, one occurring in AZ, CA, CH, DU, PV, SF, SLP, SO, TX, and ZA, and another occurring in BS, CL, LP, MS,

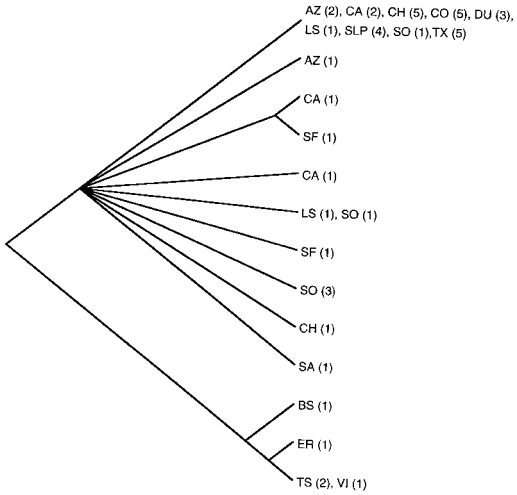


FIGURE 4. Strict consensus of five equally parsimonious trees of length 35 with characters equally weighted for haplotypes in Verdin. Shown are unique haplotypes and numbers of each haplotype found per locality (see Fig. 1).

TABLE 2. Samples of Verdins (*Auriparus flaviceps*) used in phylogeographic analyses and estimated statistics. Localities are shown in Figure 1.

| Locality | Subspecies | *n* | Number of haplotypes | Nucleotide diversity |
|---|---|---|---|---|
| TX | *ornatus* | 5 | 1 | 0.000 |
| AZ | *acaciarum* | 3 | 2 | 0.005 |
| DU | *ornatus* | 3 | 1 | 0.000 |
| CA | *acaciarum* | 4 | 3 | 0.006 |
| SLP | *ornatus* | 4 | 1 | 0.000 |
| CO | *ornatus* | 5 | 1 | 0.000 |
| SO | *ornatus* | 5 | 3 | 0.003 |
| CH | *ornatus* | 6 | 2 | 0.001 |
| LS | *acaciarum* | 3 | 3 | 0.004 |
| ER | *flaviceps* | 1 | 1 | 0.000 |
| SF | *acaciarum* | 2 | 2 | 0.008 |
| TS | *flaviceps* | 2 | 1 | 0.000 |
| VI | *flaviceps* | 1 | 1 | 0.000 |
| VT | *flaviceps* | 1 | 1 | 0.000 |

SA, SI, SR, TS, and VI. Phylogenetic analysis of unique haplotypes revealed 2,080 equally parsimonious trees, the consensus of which (Fig. 6) shows two main groupings, southern Baja California (Camalu [CL] southward; 100% bootstrap value) and the rest of the range (69% bootstrap value). One of four individuals from Camalu (31°N) possessed the eastern haplotype; only two individuals were used in phylogenetic analysis because of considerable missing data in two specimens. $F_{st}$ considering all localities was 0.79, whereas dividing the samples into the two regions (and excluding CL) resulted in a value of 0.89 (with 0.09 within populations and 0.02 among populations within regions). Nm values averaged greater than 4.0 within regions and less than 1 between regions. Nucleotide diversity values (Table 3) ranged from 0.0 to 0.007. Combined samples from the southern region possessed little nucleotide diversity ($\pi = 0.001$), whereas those from the eastern region were more variable ($\pi = 0.005$). Tajima's D-value for the southern Baja region was nonsignificant; for the eastern region it was $-2.5$ ($P < 0.001$). A mismatch distribution (Fig. 7) for the eastern region differed significantly ($P < 0.001$) from the sudden expansion model. The existence of only three haplotypes precluded testing the sudden expansion model for the southern Baja region.

BLACK-TAILED GNATCATCHER

Analysis of 980 bp of CRI, CRII, and ND2 for 34 Black-tailed Gnatcatchers from 11 localities

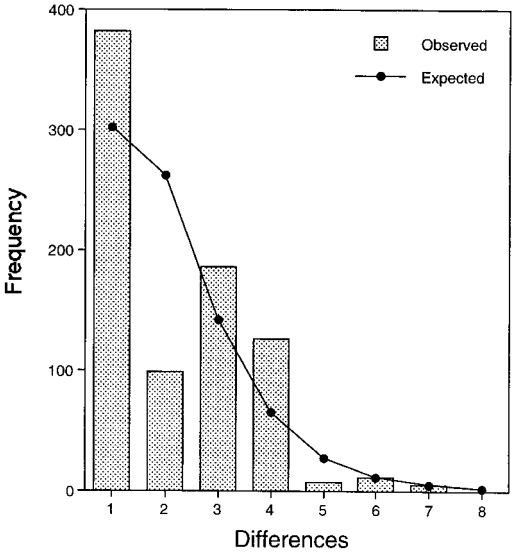


FIGURE 5. Mismatch distribution for eastern clade of Verdin.

resolved 32 haplotypes (GenBank accessions: 309037–309070). Although there appears to be a range disjunction between the Sonoran and Chihuahuan Deserts (AOU 1957), phylogenetic analysis (Fig. 8) revealed no geographic divisions; no geographic groupings of haplotypes received bootstrap support over 50%. $F_{st}$ was 0.18. Nm values averaged greater than 4.0. Nucleotide

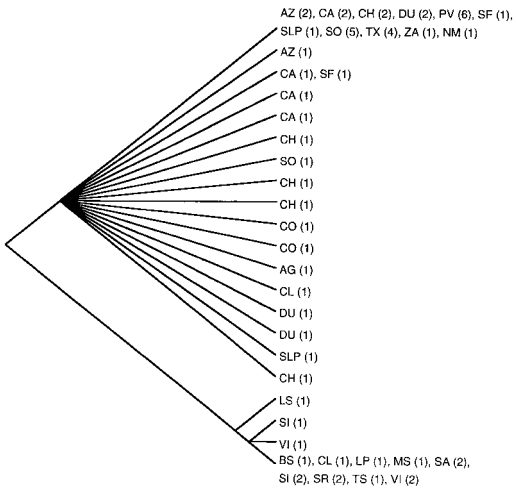


FIGURE 6. Strict consensus of 2,080 equally parsimonious trees of length 44 with characters equally weighted for haplotypes in Cactus Wren. Shown are unique haplotypes and numbers of each haplotype found per locality (see Fig. 1).

TABLE 3. Samples of Cactus Wrens (*Campylorhynchus brunneicapillus*) used in phylogeographic analyses and estimated statistics. Localities are shown in Figure 1. Nucleotide diversity is not reported for CL because there was one eastern and one southern haplotype; two other individuals proved difficult to sequence but were definitively typed as southern. The subspecies shown are based on Miller et al. (1957), which differs from the taxonomy of Rea and Weaver (1990).

| Locality | Subspecies | *n* | Number of haplotypes | Nucleotide diversity |
|---|---|---|---|---|
| AG | *guttatus* | 1 | 1 | 0.000 |
| NM | *couesi* | 1 | 1 | 0.000 |
| AZ | *couesi* | 3 | 2 | 0.004 |
| CA | *couesi* | 5 | 4 | 0.004 |
| CH | *guttatus* | 6 | 4 | 0.007 |
| CO | *guttatus* | 2 | 2 | 0.007 |
| CL | *bryanti* | 2 | 2 | NC |
| DU | *guttatus* | 4 | 3 | 0.007 |
| LP | *affinis* | 1 | 1 | 0.007 |
| LS | *couesi* | 1 | 1 | 0.000 |
| MSF | *bryanti* | 1 | 1 | 0.000 |
| PV | *couesi* | 6 | 1 | 0.000 |
| SA | *affinis* | 3 | 1 | 0.000 |
| SF | *couesi* | 2 | 2 | 0.003 |
| SI | *purus* | 3 | 2 | 0.002 |
| SLP | *guttatus* | 2 | 2 | 0.004 |
| SO | *brunneicapillus* | 6 | 2 | 0.001 |
| SR | *purus* | 2 | 1 | 0.000 |
| TS | *affinis* | 1 | 1 | 0.000 |
| TX | *couesi* | 4 | 1 | 0.000 |
| VI | *purus* | 3 | 2 | 0.002 |
| ZA | *guttatus* | 1 | 1 | 0.000 |

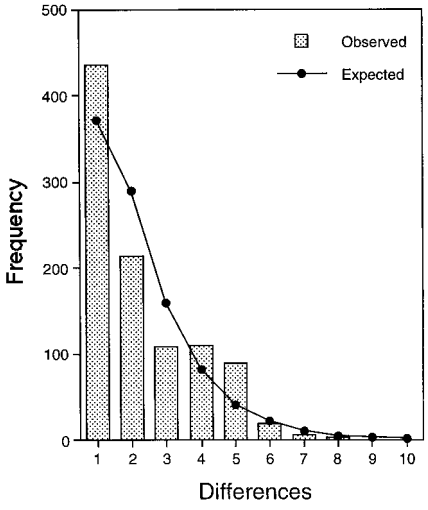


FIGURE 7. Mismatch distribution for eastern clade of Cactus Wren.

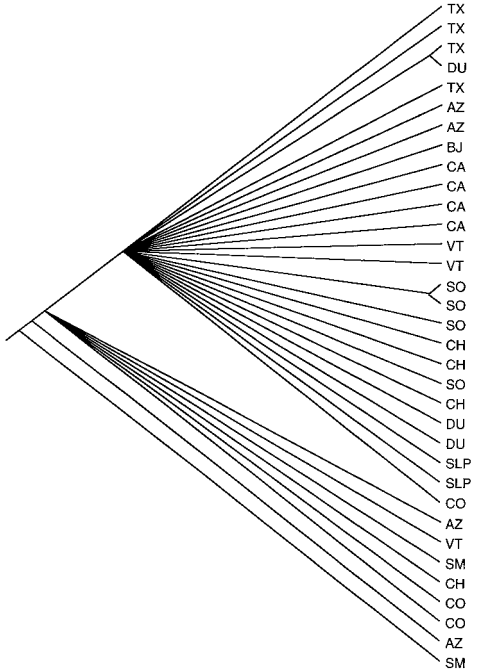


FIGURE 8. Strict consensus of 9,750 equally parsimonious trees of length 122 for haplotypes in Black-tailed Gnatcatcher. Locality codes shown in Figure 1.

diversity values (Table 4) ranged from 0.002 to 0.005, and overall, $\pi$ was 0.004. Tajima's D-value was $-1.9$ ($P = 0.02$). The mismatch distribution (Fig. 9) differed significantly from the sudden expansion model ($P = 0.03$).

TABLE 4. Samples of Black-tailed Gnatcatchers (*Polioptila melanura*) used in phylogeographic analyses and estimated statistics. Localities are shown in Figure 1.

| Locality | Subspecies | *n* | Number of haplotypes | Nucleotide diversity |
|---|---|---|---|---|
| AZ | *lucida* | 4 | 4 | 0.003 |
| CA | *lucida* | 4 | 4 | 0.003 |
| CH | *lucida* | 4 | 4 | 0.002 |
| CO | *melanura* | 3 | 3 | 0.005 |
| MS | *lucida* | 1 | 1 | 0.000 |
| DU | *melanura* (?) | 3 | 3 | 0.002 |
| SLP | *melanura* | 2 | 2 | 0.002 |
| SM | *lucida* | 2 | 2 | 0.003 |
| SO | *lucida* | 4 | 4 | 0.003 |
| TX | *melanura* (?) | 4 | 4 | 0.005 |
| VT | *lucida* | 3 | 3 | 0.004 |

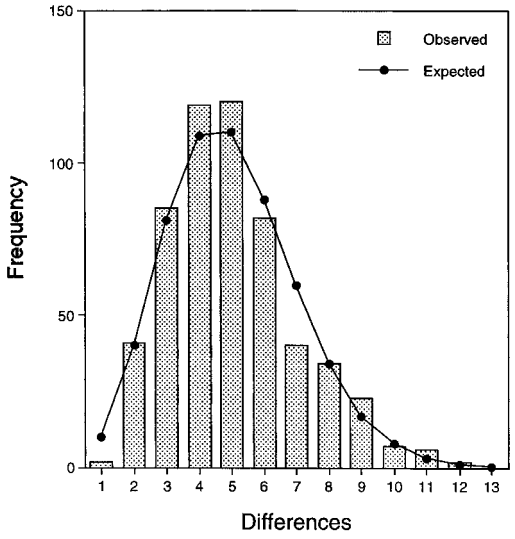


FIGURE 9. Mismatch distribution for all haplotypes in Black-tailed Gnatcatcher.

## DISCUSSION

### PHYLOGEOGRAPHIC PATTERNS AND POPULATION HISTORIES IN BAJA CALIFORNIA

The Verdin and the Cactus Wren each show a phylogeographic break at or near 30°N (our samples are not sufficiently spaced to identify the exact break), consistent with that found in the LeConte's Thrasher (Zink et al. 1997). Rea and Weaver (1990) found a similar division in the Cactus Wren based on morphological features. Upton and Murphy (1997) present data on several species in the herpetofauna that show a similar division. Upton and Murphy (1997) review evidence indicating that a seaway divided the peninsula one million years ago, which could account for isolation and divergence of populations. Because the avian species showing this division were studied for different DNA segments, it is difficult to compare levels of divergence across this common barrier. However, average uncorrected sequence divergence values between northern and southern Baja samples for the Verdin (5%), Cactus Wren (6%), and LeConte's Thrasher (3.5%) are consistent with a relatively old (e.g., one million year old) isolation event. We think it is most parsimonious to conclude that the ancestors of these species were widespread and part of the same community over the last one million years, and their ranges were fractured by the same isolating event, likely the mid-peninsular seaway.

In contrast, the California Gnatcatcher shows no evidence of a phylogeographic division throughout the Baja peninsula ($F_{st}$ = 0.07, haplotype tree unstructured; Zink et al. 2000a). Analyses based in coalescence theory suggest that the southern part of the California Gnatcatcher range corresponds to a historical refugium (based on high $\pi$-values) and that the northern part of the range has been colonized only recently (low $\pi$-values). These two parts of the gnatcatcher's range with different population histories are geographically consistent with the phylogeographic divisions found in Verdin, Cactus Wren and LeConte's Thrasher. However, given the mtDNA results, the California Gnatcatcher probably was not historically distributed north of the barrier. Hence, these four species in the modern avifauna exhibit two different histories: one including isolation and differentiation, and the other one of recent range expansion.

It is unclear when the seaway receded, allowing the terrestrial environments to reconnect. Although our samples are small, we found little introgression across this historical barrier in Verdin, Cactus Wren, and LeConte's Thrasher (Zink et al. 1997) suggesting that the habitat was only recently reconnected. In contrast, California Gnatcatcher populations have recently expanded north across the barrier to 34°N. The likely reason for the California Gnatcatcher's rapid northward spread, as opposed to limited exchange in Verdin, Cactus Wren, and LeConte's Thrasher, is that there were no conspecifics north of the barrier along the coast to impede population expansion. Because the Verdin and Cactus Wren (but not LeConte's Thrasher) are both relatively common and have Nm values similar to those in California Gnatcatchers, one might expect greater dispersal across this historical isolating barrier. It is possible that the newly evolved taxa in Verdin and Cactus Wren have not evolved sufficient ecological differences to permit range expansions and sympatry, or that the barrier has only very recently ceased to operate. If the latter were true, it would suggest the potential for very rapid range expansion in California Gnatcatcher.

More species also require study before it will be possible to estimate the percentage of the current avifauna that responded to the isolating barrier. Allozyme evidence (Zink et al. 1987) for the California Quail (*Callipepla californica*) re-

vealed no break although analyses of allozyme variation in birds typically failed to detect phylogeographic structure (Zink 1997). Because the effect of this barrier was evident in mtDNA data for the species surveyed here, its effects are likely to be pervasive when mtDNA data become available for this and other species.

It is possible to estimate population histories on either side of the Baja barrier. As noted earlier, the California Gnatcatcher appears to have been historically isolated south of the barrier. In contrast, the Cactus Wren exhibited a low $\pi$-value in the south, suggestive of a bottleneck or small population. Although small samples of Verdin prevent a third comparison, the Cactus Wren and California Gnatcatcher apparently had different population histories south and north of the barrier.

The existence of reciprocally monophyletic groups, and apparently low introgression, in the Cactus Wren and Verdin, suggest that at minimum these groups are Evolutionarily Significant Units (ESU; Moritz 1994), if not phylogenetic species (Cracraft 1989), as suggested for the LeConte's Thrasher (Zink et al. 1997). These phylogeographic splits occur at subspecies boundaries, although most subspecies are not discrete evolutionary entities (see below).

### PHYLOGEOGRAPHY OF SONORAN AND CHIHUAHUAN DESERTS

The Curve-billed Thrasher (Zink and Blackwell-Rago 2000) showed a mtDNA split at the junction of the Sonoran and Chihuahuan Deserts, with an $F_{st}$ value of 0.72 and a haplotype tree showing three reciprocally monophyletic groups: Sonoran Desert, Chihuahuan Desert south to the Mexican state of Mexico, and the southern Mexican states of Puebla and Oaxaca. The Canyon Towhee also shows a phylogeographic division (Fig. 2) between populations in the Sonoran Desert and the Chihuahuan Desert. The depths of the mtDNA divisions in the Canyon Towhee and Curve-billed Thrasher are similar, roughly 2% sequence divergence, suggesting contemporaneous geographic isolation. The reciprocally monophyletic groups apparent in each species' mtDNA haplotype trees constitute evidence for designation as ESUs (Moritz 1994) as well as phylogenetic species (Zink and Blackwell-Rago 2000). In contrast the Verdin, Black-tailed Gnatcatcher and Cactus Wren appear to have been unaffected by whatever isolating event affected Canyon Towhee and Curve-billed Thrasher. Thus, in this large part of the aridlands, there are at least two groups of species with different histories.

### REASONS FOR LACK OF CONGRUENT PHYLOGEOGRAPHIC PATTERNS

Comparative phylogeography has parallels with vicariance biogeography (Bermingham and Avise 1986, Zink 1996). As in vicariance analysis, the null hypothesis we accept is that all species had sympatric, widespread ancestors. If so, isolating events ought to have affected each species in the same fashion, leading to phylogeographic congruence, such as was generally observed in Baja California. Equally interesting, however, are species such as the Verdin, Cactus Wren and Black-tailed Gnatcatcher, which exhibit no pattern across the same region inhabited by differentiated groups of Canyon Towhee and Curve-billed Thrasher. Because comparative phylogeography deals with relatively recent evolutionary history, such situations can be investigated in an attempt to discriminate among hypotheses leading to a lack of phylogeographic structure (Zink 1996, 1997). Species that are broadly sympatric today might not have had widespread sympatric ancestors. These species should show evidence of recent population expansions, evident in the shapes of mismatch distributions. Some species might have high levels of ongoing gene flow, and might not be impeded by an environmental or geological feature that functions as a barrier to another species. Such species should exhibit low $F_{st}$ values and concomitant high Nm values, and widely distributed haplotypes on either side of a putative barrier. Alternatively, all species might have been effectively isolated by the same barrier, but differ in their ''response'' times. The time required for the evolution of reciprocal monophyly is, on average, $4N_e$ generations (Avise 1994). Hence, even if all ancestral species were fragmented in a congruent fashion, some species might not yet show evidence (reciprocal monophyly) of the isolating event if their effective population sizes were large. Evidence of geographic isolation without reciprocal monophyly would include geographic differences in haplotype frequencies, relatively high $F_{st}$ values, and the restricted geographic distribution of recently evolved haplotypes (Templeton 1998).

Some of these alternatives can be examined for species in the Sonoran and Chihuahuan Deserts. Although the mismatch distributions for Cactus Wren and Black-tailed Gnatcatcher (Fig.

7, 9) differ statistically from the expectation for sudden population expansion, they are of the same shape as that expected for an expanding population. Because we pooled samples from multiple localities, which could cause departures from expectation, we take the unimodal shape of the mismatch distribution as evidence of some degree of population expansion. Therefore, we suggest that the observed lack of phylogeographic differentiation in the Cactus Wren and Black-tailed Gnatcatcher is because they only recently occupied their modern ranges. Whether the direction of colonization was from Sonoran to Chihuahuan Desert, or vice versa, is unknown; $\pi$-values are similar in both areas. The overall mismatch distribution for the Verdin (Fig. 5) is consistent with a more stable population (Harpending 1994). Thus it is possible that a widespread Verdin ancestor was unaffected by the isolation event inferred for the other species, with persistently high levels of gene flow preventing differentiation.

To test whether Verdin, Cactus Wren, and Black-tailed Gnatcatcher might be in the incipient stages of differentiation that would eventually match that observed in Canyon Towhee and Curve-billed Thrasher, we recomputed $F_{st}$ values with the Arizona and Sonoran samples treated as an additional independent level of comparison. In each case, the $F_{st}$ value decreased, revealing no evidence that these species are ''on their way'' to becoming differentiated. Hence, we believe that the mtDNA evidence suggests that recent range expansion, if not ongoing gene flow between regions, accounts for the lack of differentiation in Verdin, Cactus Wren and Black-tailed Gnatcatcher between the Sonoran and Chihuahuan deserts (as well as the California Gnatcatcher in Baja California). Thus, the five species have not had the same history. To the extent that this small sample of species is representative, this result indicates that the modern avifauna achieved its current species composition relatively recently. Comparative phylogeography therefore can provide an indirect measure of the historical stability of faunas. Furthermore, there are implications for comparisons of particular species. For example, if one were interested in ecological interactions between, say, Verdin and California Gnatcatcher, one might make different predictions about current interactions in the southern and northern parts of their common range, reflecting a longer history of co-association in southern Baja California.

### THE TAXONOMIC SCALE OF COMPARATIVE PHYLOGEOGRAPHY

Our comparisons entailed phylogeographic divisions within ''biological species.'' Evidence also exists for phylogenetic divisions at deeper or older scales in the aridlands (Zink et al. 2000b). For example, although the Black-tailed Gnatcatcher does not occur widely in Baja California, its sister species, the California Gnatcatcher, does (Zink and Blackwell 1998). This represents a potentially older diversification event than those considered here. Eventually, by making comparisons at different historical scales, a more complete picture of the evolution of the aridlands avifauna will be obtained, showing multiple tiers of evolutionary diversification.

### SUBSPECIES AND MTDNA PHYLOGENIES

The majority of subspecies sampled in this study are not reciprocally monophyletic for mtDNA haplotypes. Possibly our data sets include too few base pairs to detect weakly differentiated yet reciprocally monophyletic groups. However, our relatively small sequence data sets for the Verdin, Cactus Wren and Canyon Towhee reveal phylogeographic structure, whereas the larger data set for the Black-tailed Gnatcatcher did not (although there were more haplotypes resolved for this species, as might be expected). Hence, we believe that our data sets are sufficient to detect reciprocally monophyletic groups, which in avian populations tend to differ by ca. 1% or greater from sister groups (Klicka and Zink 1999). This result implies that indeed most subspecies names do not denote populations or groups of populations that have been evolving independently for more than $4N_e$ generations, the time required (on average) for the evolution of reciprocally monophyletic groups of haplotypes (Avise 1994). Other studies have shown that avian subspecies names do not predict groups with independent evolutionary histories (Ball and Avise 1992), most likely because most subspecies are based on arbitrary divisions of single (usually morphological) character clines (Zink et al. 2000a). Thus, continental avian subspecies, including many of those studied here, are on average misleading indicators of patterns of evolutionary history.

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