# Temporal Analysis of mtDNA Variation Reveals Decreased Genetic Diversity in Least Terns

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# TEMPORAL ANALYSIS OF MTDNA VARIATION REVEALS DECREASED GENETIC DIVERSITY IN LEAST TERNS

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*Abstract.* The Least Tern (*Sternula antillarum*) has undergone large population declines over the last century as a result of direct and indirect anthropogenic factors. The genetic implications of these declines are unknown. We used historical museum specimens (pre-1960) and contemporary (2001–2005) samples to examine range-wide phylogeographic patterns and investigate potential loss in the species' genetic variation. We obtained sequences (522 bp) of the mitochondrial gene for NADH dehydrogenase subunit 6 (ND6) from 268 individuals from across the species' range. Phylogeographic analysis revealed no association with geography or traditional subspecies designations. However, we detected potential reductions in genetic diversity in contemporary samples from California and the Atlantic coast Least Tern from that in historical samples, suggesting that current genetic diversity in Least Tern populations is lower than in their pre-1960 counterparts. Our results offer unique insights into changes in the Least Tern's genetic diversity over the past century and highlight the importance and utility of museum specimens in studies of conservation genetics.

*Key words:* Least Tern, mitochondrial ND6 gene, genetic variation, museum specimens, temporal analysis, ancient DNA.

## El Análisis Temporal de la Variación de ADNmt Revela una Menor Diversidad Genética en *Sternula antillarum*

*Resumen.* *Sternula antillarum* ha sufrido grandes disminuciones poblacionales a lo largo del último siglo como resultado de factores antrópicos directos e indirectos. Las implicancias genéticas de estas disminuciones son desconocidas. Usamos muestras de especímenes de museo históricas (antes de 1960) y contemporáneas (2001–2005) para examinar los patrones filogenéticos en todo el rango e investigar pérdidas potenciales en la variación genética de la especie. Obtuvimos secuencias (522 pares de bases) del gen mitocondrial para la subunidad 6 de la deshidrogenasa NADH (ND6) de 268 individuos provenientes de todo el rango de la especie. Los análisis filogenéticos revelaron que no existen asociaciones con las designaciones de subespecies geográficas o tradicionales. Sin embargo, detectamos reducciones potenciales en la diversidad genética en muestras contemporáneas de *S. antillarum* de California y la costa atlántica en comparación con muestras históricas, sugiriendo que la diversidad genética actual en las poblaciones de *S. antillarum* es menor que la de sus congéneres de antes de 1960. Nuestros resultados brindan evidencias únicas de los cambios en la diversidad genética de *S. antillarum* a lo largo del último siglo y destacan la importancia y la utilidad de los especímenes de museo en los estudios de genética de la conservación.

## INTRODUCTION

Comprehensive genetic studies of wide-ranging species, such as the Least Tern (*Sternula antillarum*), are often constrained by our ability to sample on adequate spatial and temporal scales. In the case of the Least Tern, investigations can be complicated further by its status as endangered, which makes it difficult to obtain sufficient samples (USFWS 1980, 1985). The advent of enhanced molecular techniques to extract high-quality and high-yield DNA from degraded tissues, coupled with easy online access to most major museum collections worldwide (http://www.gbif.org/), permits biologists to fill gaps in sampling with museum specimens. In addition, comparing historical museum specimens with contemporary samples provides a temporal perspective on current genetic diversity. Many threatened and endangered species have experienced large reductions in population size, which can have a negative effect on genetic diversity (Frankham et al. 2002, Spielman et al. 2004). Indeed, when Spielman et al. (2004) compared threatened taxa to nonthreatened sister taxa, they found the heterozygosity of 77% of threatened taxa to be on average 35% lower than that of their nonthreatened counterparts. As loss of genetic diversity can diminish population viability and limit long-term adaptability (Lacy 1997, Reed

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and Frankham 2003, Spielman et al. 2004), assessments of genetic-diversity changes and trajectories over time provide critical information for endangered-species management.

Although actual numbers are unknown, it is thought that the Least Tern was historically abundant throughout its range (Thompson et al. 1997). However, during the 20th century it underwent large anthropogenically induced population declines (USFWS 1980, 1985, 1990, Burger 1984, Thompson et al. 1997, Kruse et al. 2001). During the late 1800s and early 1900s, Least Tern populations underwent large declines from egg collection and demand for feathers for the millinery trade (Nisbet 1973, Thompson et al. 1997). Population numbers started to rebound following passage of the Migratory Bird Treaty Act in 1918 (Thompson et al. 1997). However, populations declined again during the mid 1900s via habitat loss (USFWS 1980, 1990, Burger 1984, Kruse et al. 2001). As a result, the California subspecies (*S. a. browni*) is listed as endangered under the U.S. Endangered Species Act (USFWS 1980). The interior subspecies (*S. a. athalassos*) was not listed as a subspecies because of taxonomic uncertainty at the time of listing; however, the U.S. Fish and Wildlife Service designated "the populations of Least Terns occurring in the interior of the United States" as endangered (USFWS 1985). The east coast subspecies (*S. a. antillarum*) is state-listed as threatened or endangered in most states where it occurs (USFWS 1980, 1990).

We used museum specimens (pre-1960) and contemporary (2001–2005) samples to examine genetic diversity among Least Terns during three time periods: pre- and post 1900 to examine the influence of anthropogenically induced population declines at the turn of the century and before and after the population decline of the 1960s and 1970s (USFWS 1980, 1990, Burger 1984, Thompson et al. 1997, Kruse et al. 2001). Despite access to museum collections, our ability to obtain historical samples comparable to contemporary sampling is problematic because of the opportunistic nature of museum collecting. One way researchers might avoid this drawback is to pool samples if genetic analyses reveal genetic homogeneity among locales. Such is the case in the Least Tern. Using seven microsatellite loci and mtDNA control region sequences, Draheim et al. (2010) investigated population structure within and among traditional Least Tern subspecies and found a general lack of genetic structure. This finding suggests that older Least Tern museum specimens can be compared to contemporary samples, despite being collected at different geographical locations within a region. We confirm these results in this study via further phylogeographic analysis using mitochondrial (mtDNA) ND 6 data to justify our geographic pooling of historical samples.

## MATERIALS AND METHODS

We obtained 268 Least Tern samples (contemporary = 219, museum specimens = 49) from throughout the species' range by using several techniques: blood from live specimens, salvaged carcasses, embryos from collected eggs, and toe pads from museum specimens. We obtained dried toe-pad tissue from the American Museum of Natural History, New York; Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts; Museum of Vertebrate Zoology, University of California, Berkeley; and Florida Museum of Natural History, University of Florida, Gainesville. We collected one to 12 samples from 33 areas throughout the Least Tern's breeding range (Fig. 1, Tables 1 and 2). Along the coasts, we defined a sampling area as a group of samples collected within a breeding colony or from multiple adjacent colonies within 80 km. Inland, we defined a sampling area as a group of samples collected within 80 km along a river.

We obtained DNA from samples by using standard phenol/chloroform extractions as previously described (Haig et al. 2004). For contemporary samples, we used primers LT16130 L and LT16700 H to yield 522 bp of the mtDNA ND6 sequence (Table 3). The degraded DNA obtained from museum specimens required design of internal primers to amplify shorter overlapping sequences (150–200 bp; Table 3). To ensure our internal primers amplified the same sequence as contemporary samples we first screened internal primers with nondegraded contemporary samples. A total reaction volume of 50 μL was used with the following concentrations: 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 0.001% gelatin, 3.5 mM $MgCl_2$, 100 μM for each of the dNTPs, 0.2 μM of each primer, 100 ng of template, and 1.5 U AmpliTaq Gold Polymerase (Perkin Elmer). The following parameters were used for amplifications: 12 min denaturation at 93 °C, followed by 45 cycles of 30 sec at 93 °C and annealing at 50 °C for 45 sec. To analyze DNA sequences we used ABI Prism Big Dye DNA-sequencing chemistry on an ABI 3730 capillary DNA analyzer located in the Central Services Laboratory at Oregon State University. We used BIOEDIT (version 7.0.5) alignment software (Hall 1999) to align sequences by eye. All sequences have been archived in GenBank (accession numbers AN654131–AN654156).

### STATISTICAL ANALYSIS

Sample design was originally intended to represent the Least Tern's genetic variation throughout its breeding distribution. Museum specimens, obtained to fill gaps in this distribution, also provided us a unique opportunity to investigate the genetic implications of the population declines over the last century. However, heterogeneity in the dispersion of samples among the contemporary and historical sets led us to pool samples into the California, interior, and eastern subspecific groupings. We evaluated the appropriateness of pooling samples that encompassed broad geographic distributions with ND6 phylogeographic and population-structure analyses. We further generated a statistical haplotype tree network with 95% parsimonious connections for all contemporary and historical sequences by using the program TCS 1.13 (Clement et al. 2000). We quantified geographic differentiation within and among the three regional subspecies with Weir and

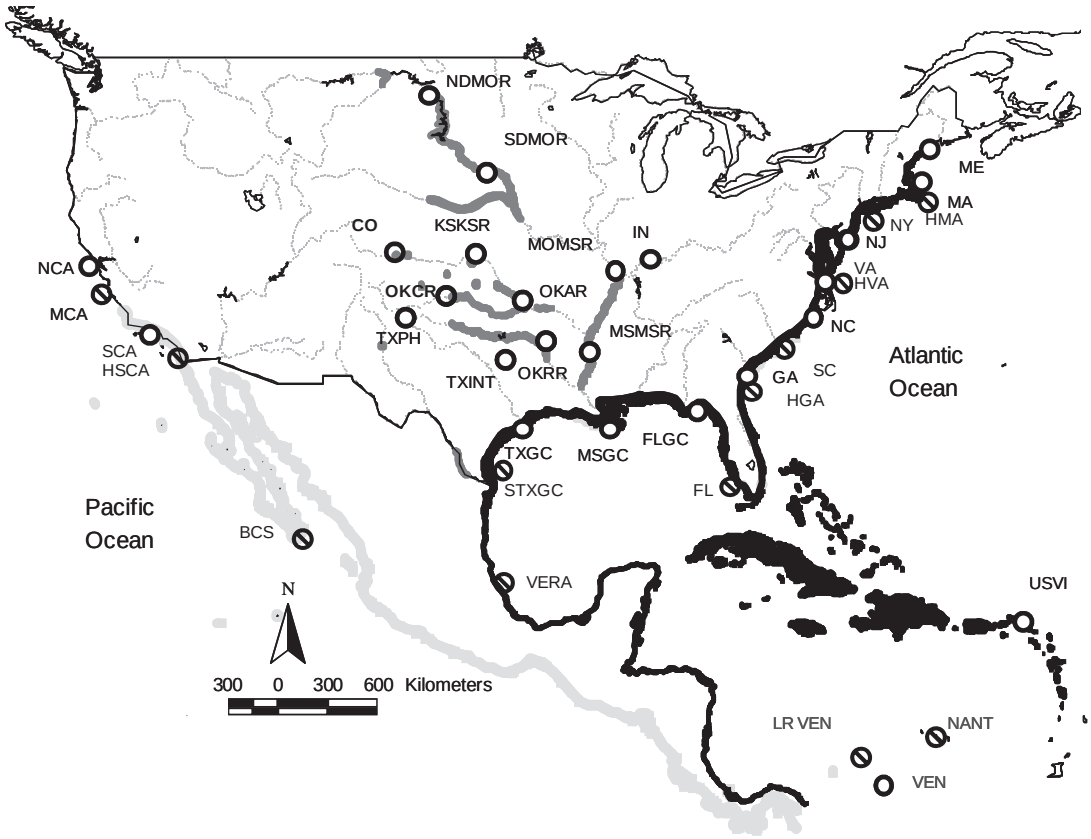


FIGURE 1. Distribution of the Least Tern included in this study and sites sampled for genetic analysis. Ranges of the California, interior, and east coast subspecies are shown in light gray, dark gray, and black, respectively. Slashed rings, museum specimens; open rings, contemporary samples. Breeding areas are identified in Table 1.

Cockerham's (1984) $\theta_{ST}$ and tested significance with 10 000 replications in program Arlequin (Schneider et al. 2000).

We evaluated genetic diversity in three ways. First, we used DNASP version 4.0 (Rozas et al. 2003) to calculate haplotype ($h$) and nucleotide ($\pi$) diversity for all areas of contemporary and historical breeding. Next, we tested for differences in genetic diversity between subspecies in contemporary samples. To eliminate bias as a result of unequal sample size, we first implemented bootstrap resampling to account for discrepancies in sample size among the subspecies. We created 100 simulated populations of 26 individuals (corresponding to the number of individuals in the contemporary California sample) for the California, interior, and east coast subspecies by random resampling with replacement as performed by POPTOOLS (Hood 2005). We calculated haplotype and nucleotide diversity for each simulated bootstrapped population in Arlequin (Schneider et al. 2000), and we compared mean haplotype and nucleotide diversity among subspecies with a parametric two sample $t$-test in the STATS package in R 2.9.2 (http://www.rb-project.org; mean ± SD).

Third, we evaluated potential loss of genetic diversity as a result of population declines with temporal comparisons of genetic diversity among historical and contemporary samples in the California and east coast subspecies. Genetic analysis of Least Tern's population structure revealed high genetic homogeneity within the California and east coast subspecies (see Results). In the absence of geographic structure, we pooled California and east coast samples into two groups for temporal analyses: (1) pre-1960 museum specimens (historical) and (2) samples taken 2001–2005 (contemporary). Lack of historical samples from the interior breeding areas precluded our including this area in the temporal analyses. The sample size for the historical temporal groups was significantly smaller, so we implemented the previously described bootstrap procedure to account for discrepancies in sample size between contemporary and historical groupings with simulated populations corresponding to the number of individuals in the historical samples (California = 13; east coast = 33). We eliminated three museum specimens collected after 1960 from temporal analyses. We calculated haplotype and nucleotide diversity for each simulated bootstrapped population in Arlequin (Schneider et al. 2000), and we compared the temporal groups' mean haplotype and nucleotide diversity by a parametric two-sample $t$-test with STATS package in R 2.9.2 (mean ± SD).

Collection over long periods could inflate diversity measures, so we performed time-series analyses and divided

TABLE 1. Breeding area information and genetic variation for mtDNA ND6 gene of Least Terns. Number of individuals sampled (*n*), number of haplotypes, haplotype diversity (*h*), and nucleotide diversity (π) for each breeding area are provided.

| Region | Sample code | County, state/region, country | *n* | No. haplotypes | *h* | π |
|---|---|---|---|---|---|---|
| Pacific coast | | | | | | |
| | NCA | Alameda, CA | 10 | 3 | 0.644 | 0.0014 |
| | MCA | Monterey, CA | 4 | 3 | 0.833 | 0.0029 |
| | SCA | San Diego, CA | 16 | 2 | 0.525 | 0.0010 |
| | HSCA | San Diego, CA (pre-1960) | 4 | 2 | 0.500 | 0.0019 |
| | BCS | Baja California Sur, Mexico | 5 | 2 | 0.400 | 0.0008 |
| Interior | | | | | | |
| | NDMOR | McLean, ND | 10 | 1 | 0.000 | 0.0000 |
| | SDMOR | Yankton, SD | 10 | 2 | 0.556 | 0.0032 |
| | KSKSR | Pottawatomie, KS | 10 | 3 | 0.378 | 0.0015 |
| | MOMOR | New Madrid, MO | 10 | 3 | 0.600 | 0.0024 |
| | OKCR | Woods, OK | 10 | 4 | 0.644 | 0.0024 |
| | OKAR | Tulsa, OK | 10 | 4 | 0.778 | 0.0033 |
| | OKRR | McCurtain, OK | 10 | 2 | 0.356 | 0.0021 |
| | TXPH | Hemphill, TX | 7 | 3 | 0.667 | 0.0031 |
| | TXINT | Dallas, TX | 10 | 1 | 0.000 | 0.0000 |
| | MSMSR | Bolivar, MS | 10 | 2 | 0.200 | 0.0004 |
| | IN | Gibson, IN | 4 | 1 | 0.000 | 0.0000 |
| | CO | Kiowa, CO | 3 | 3 | 1.000 | 0.0038 |
| East coast | | | | | | |
| | ME | Knox, ME | 10 | 4 | 0.778 | 0.0029 |
| | MA | Barnstable, MA | 12 | 4 | 0.712 | 0.0023 |
| | HMA | Barnstable, MA (pre-1960) | 4 | 3 | 0.833 | 0.0029 |
| | NY | Suffolk, NY | 2 | 1 | 0.000 | 0.0000 |
| | NJ | Cape May, NJ | 10 | 4 | 0.711 | 0.0028 |
| | VA | Accomack, VA | 10 | 3 | 0.378 | 0.0012 |
| | HVA | Virginia Beach, VA (pre-1960) | 4 | 4 | 1.000 | 0.0042 |
| | SC | Charleston, SC | 4 | 4 | 0.100 | 0.0029 |
| | GA | Glenn, GA | 8 | 4 | 0.750 | 0.0035 |
| | HGA | McIntosh, GA (pre-1960) | 8 | 6 | 0.929 | 0.0037 |
| | FL | Charlotte, Monroe, Collier, FL | 2 | 2 | 1.000 | 0.0040 |
| | USVI | St. Croix, Virgin Islands | 10 | 3 | 0.622 | 0.0021 |
| | FLGC | Bay, FL | 9 | 4 | 0.694 | 0.0020 |
| | MSGC | Harrison, MS | 10 | 4 | 0.644 | 0.0018 |
| | TXGC | Brazoria, TX | 10 | 5 | 0.756 | 0.0025 |
| | STXGC | Nueces, TX | 5 | 3 | 0.700 | 0.0015 |
| | VERA | Veracruz, Mexico | 1 | 1 | N/A | N/A |
| | NANT | Curaçao, Bonaire, Netherlands Antilles | 4 | 4 | 1.000 | 0.0035 |
| | VEN | Cumana, Venezuela | 1 | 1 | N/A | N/A |
| | LRVEN | Los Roques, Venezuela | 1 | 1 | N/A | N/A |

the museum specimens of the east coast subspecies into two groups representing comparable intervals, (1) pre-1900 ($n = 9$; 17 years), and (2) 1900–1960 ($n = 24$; 26 years), to see if timing of collection has an effect on diversity measures. By separating museum specimens into two historical temporal groups we can compare measures of genetic diversity with contemporary samples to evaluate the subsequent effect of population declines at the turn of the century and during the mid 1900s. Lack of 1900–1960 samples prevented similar analyses of the California subspecies. Sizes of samples of the historical temporal groups differed significantly different, so we implemented the previously described bootstrap procedure to account for discrepancies in sample size between contemporary and two historical groupings with simulated populations corresponding to the number of individuals in the 1900–1960 samples ($n = 9$). We calculated haplotype and nucleotide diversity for each simulated bootstrapped population in Arlequin (Schneider et al. 2000), and we compared the temporal groups' mean haplotype and nucleotide diversity with a parametric two-sample *t*-test in STATS package in R 2.9.2 (mean ± SD).

TABLE 2. Catalog numbers, location, and year of museum specimens of the Least Tern sampled for genetic variation. Prefixes represent the institutions from which the samples were taken. AMNH = American Museum of Natural History; FLMNH = Florida Museum of Natural History, University of Florida; MCZ = Museum of Comparative Zoology, Harvard University; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley.

| Location and catalog number | Year |
|---|---|
| Curaçao | |
| AMNH 747034–37 | 1892 |
| Cumana, Venezuela | |
| AMNH 188068 | 1925 |
| Los Roques, Venezuela | |
| AMNH 816441 | 1976 |
| Vera Cruz, Mexico | |
| AMNH 808740 | 1904 |
| Pacific Beach, California | |
| AMNH 753162, –66, –67, –69 | 1912 |
| Chatham, Massachusetts | |
| AMNH 26019, –20, –24, –26 | 1885 |
| Corpus Christi, Texas | |
| AMNH 79343, –45–74 | 1886 |
| AMNH 79346 | 1887 |
| AMNH 79344 | 1888 |
| AMNH 79340 | 1882 |
| Virginia | |
| AMNH 747041 | 1885 |
| Bone Island, Virginia | |
| AMNH 80194 | 1905 |
| Cobbs Island, Virginia | |
| AMNH 747030 | 1885 |
| Georgia | |
| AMNH 747031–32 | 1888 |
| Cumberland, Georgia | |
| AMNH 48523 | 1915 |
| Amelia Island, Florida | |
| AMNH 17082–83 | 1906 |
| AMNH 359013–14 | 1905 |
| New Smyrna, Florida | |
| AMNH 17560 | 1899 |
| Monroe, Florida | |
| FLMNH 15840 | 1968 |
| Collier, Florida | |
| FLMNH 2017 | 1902 |
| Cape Henry, Florida | |
| MCZ 33030 | 1884 |
| Sullivan's Island, South Carolina | |
| MCZ 210427, –428, –543, –544 | 1885 |
| San Jose del Cabo, Baja California Sur, Mexico | |
| MVZ 54739–41, –43–44 | 1929 |
| Monterey, California | |
| MVZ 91711, –13–15 | 1915 |
| Suffolk, New York | |
| MVZ 145331 | 1926 |
| MVZ 145333 | 1928 |

TABLE 3. Primers used in sequencing mitochondrial gene ND6 of the Least Tern.

| Primer | Sequence (5′–3′) | bp |
|---|---|---|
| Contemporary samples | | |
| LTND6 L | CTTAAACCTCTATCTCCAACT | 522 |
| LTND6 H | GAGTGTCATGGATGGGTA | |
| Museum specimens | | |
| LTND6 L | CTTAAACCTCTATCTCCAACT | 180 |
| LTND6-1 H | TTTTGGTAGCAGGTTGGG | |
| LTND6-2 L | CACCAACTCCAACACAACAAA | 164 |
| LTND6-2 H | TGGTTATGGGTGGAGTTG | |
| LTND6-3 L | TGTAACTACTCCCAAATCC | 150 |
| LTND6-3 H | GTGTATTCTGTGTCCTTGG | |
| LTND6-4 L | TCCTCAAGCCTCTGGAAA | 200 |
| LTND6-4 H | AATCCTTCTCCGTATTATGG | |
| LTND6-5 L | TAACAATCACCCACACCC | 165 |
| LTND6 H | GAGTGTCATGGATGGGTA | |

## RESULTS

We observed 25 unique ND6 haplotypes (522 bp) among the 268 individuals analyzed (Table 4). Sequence variants were characterized by 16 polymorphic sites (10 synonymous, five nonsynonymous base-pair substitutions): 15 sites were transitions, one site was a transversion, and there were no insertions or deletions. Haplotype 1 was identified in 53% of individuals and was observed in most breeding areas. All other haplotypes occurred in lower frequencies (0.3–8%). Thirteen of the 25 (52%) unique haplotypes were restricted to a single breeding area (Table 5). The 95% parsimony network generated by TCS revealed a starlike pattern of haplotypes on short branches radiating from a central haplotype (Fig. 2). We observed regional patterns of genetic structure comparable to those identified in previous reports (Draheim et al. 2010). While we observed some significant genetic structure among subspecies ($\theta_{ST} = 0.145$, $P < 0.001$), analyses within each traditional subspecies revealed either no structure (California: $\theta_{ST} = -0.021$, $P = 0.403$; east coast: $\theta_{ST} = –0.013$, $P = 0.619$) or extremely weak genetic structure (interior: $\theta_{ST} = 0.071$; $P = 0.045$).

Accounting for unequal sample size with the simulated bootstrap approach, we found that contemporary mean haplotype and nucleotide diversity varied by subspecies. Bootstrap mean estimates of haplotype diversity ranged from 0.457 to 0.673 (mean ± SD; California = 0.530 ± 0.046; interior = 0.457 ± 0.100; east coast = 0.673 ± 0.080), and mean nucleotide diversity ranged from 0.0011 from 0.0015 (California = 0.0011 ± 0.0001, interior = 0.0013 ± 0.0005, east coast = 0.0015 ± 0.0004; Fig. 3). In all comparisons differences were statistically significant ($P < 0.001$).

Comparisons of the haplotype composition of historical and contemporary samples suggest higher haplotype diversity in the historical sample, despite a much smaller sample size

TABLE 4. Sites of variation of 25 haplotypes of mtDNA ND6 of the Least Tern.

| | Nucleotide site | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Haplotype | 68 | 93 | 177 | 199 | 218 | 234 | 246 | 354 | 381 | 409 | 435 | 436 | 438 | 447 | 507 | 515 |
| H1 | A | T | C | G | C | T | C | A | C | G | C | A | C | C | T | A |
| H2 | | | T | | | | | | | | | | | | | |
| H3 | | | | | | | | | | | | | | T | | |
| H4 | G | | | | | | | G | | | | | | | C | |
| H5 | | | | | | | T | | | | | | | | | |
| H6 | G | | | | | | | G | | | | G | | | C | |
| H7 | G | | | | | | | | | | | | | | C | |
| H8 | | | | | | C | | | | | | | | | | |
| H9 | | | | | | | | | | | | | T | | | |
| H10 | | | | | | | | | | | | | | | | T |
| H11 | G | | | | | | | G | | | T | | | | C | |
| H12 | | | | | T | | | | | | | | | | | |
| H13 | | | T | | | | | | | A | | | T | | | |
| H14 | | | T | | | | | | | | | | | | C | |
| H15 | | | | | | | | | | | | | | | C | |
| H16 | G | | | | | | | | | | | | | | | |
| H17 | | | | | | | | | T | | | | | | C | |
| H18 | | | T | | | | | G | | | | | | | | |
| H19 | | | T | C | | | | | | | | | | | | |
| H20 | | | T | C | | | T | | | | | | | | | |
| H21 | G | | T | | | | | | | | | | | | C | |
| H22 | G | | T | C | | | | | | | | | | | | |
| H23 | | | T | | | | | | T | | | | | | | |
| H24 | G | C | | | | | | | | | | | | | C | |
| H25 | G | C | | | | | | | | | | | | | | |

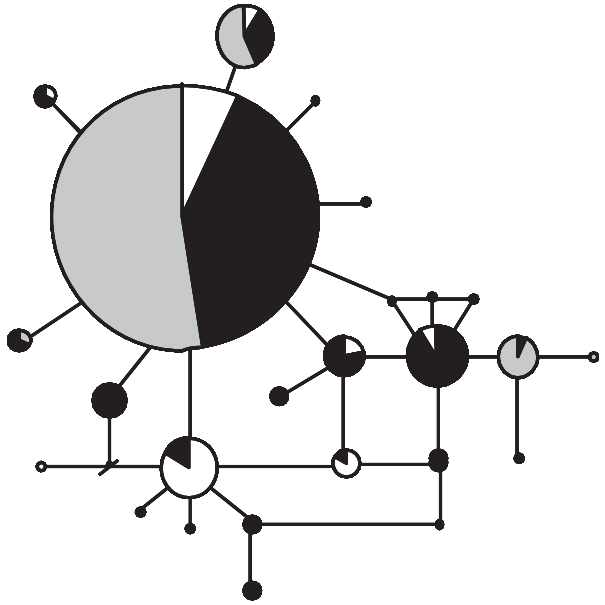

FIGURE 2. The 95% parsimony network generated by the program TCS from mtDNA ND6 haplotypes of the Least Tern. Circle sizes are proportional to the number of individuals sharing the haplotype. Shades refer to the proportion of samples that came from a geographic region. California Least Terns are shown in white, interior Least Terns in gray, and east coast Least Terns in black. Black dashes are inferred haplotypes.

(Table 5). Three of five historical California haplotypes and nine of 13 historical east coast haplotypes were not found in the contemporary samples. Eight contemporary haplotypes were absent from the historical samples, suggesting potential bias due to the smaller size of the historical sample or to nonoverlapping sampling. However, on closer inspection, we found that most of these contemporary haplotypes occur at low frequency; indeed, 84% of contemporary samples shared a haplotype with historical samples. Temporal analysis using bootstrap resampling to control for unequal sample size revealed haplotype diversity was significantly higher in the California and east coast historical samples (mean = 0.748 ± 0.088; mean = 0.871 ± 0.033, respectively) than in the contemporary samples (mean = 0.539 ± 0.083; mean = 0.666 ± 0.073; $t' = -17.1$, $P < 0.001$; $t' = -25.5$, $P < 0.001$, respectively; Fig. 4). Similarly, nucleotide diversity was significantly higher in California and east coast historical samples (mean = 0.0024 ± 0.0005; mean = 0.0037 ± 0.0004, respectively) than in the contemporary samples (mean = 0.0011 ± 0.0002; mean = 0.0023 ± 0.0004; $t' = -20.6$, $P < 0.001$; $t' = -26.5$, $P < 0.001$, respectively; Fig. 4).

In samples from the east coast, the number of haplotypes in pre-1900, 1900–1960, and contemporary samples was similar (Table 1). In the eastern subspecies, however, time-series analyses using bootstrap resampling to account for unequal sample size revealed significantly higher genetic diversity in museum specimens irrespective of temporal group (pre-1900: $h = 0.872 \pm 0.057$, $\pi = 0.0038 \pm 0.0007$; 1900–1960:

TABLE 5. Distribution of 25 mtDNA ND6 haplotypes among all areas of Least Tern breeding samples. Individuals are grouped according to geographic location (west to east) and temporal group. See Table 1 for codes for areas.

| | Contemporary samples | | | | | | | | | | | | | | | | | | | | | | | | | Museum specimens | | | | | | | | | | | | | | | California | | East coast | | | | Interior |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Haplotype | NCA | SCA | NDMOR | SDMOR | KSKSR | MOMOR | OKCR | OKAR | OKRR | TXPH | TXINT | MSMSR | CO | IL | ME | MA | NJ | VA | GA | USVI | FLGC | MSGC | TXGC | | HSCA | BCS | MCA | HMA | NY | HVA | SC | HGA | FL | STXGC | NANT | VERACRUZ | VEN | LRVEN | | Contemporary | Historical | Contemporary | Historical | pre-1900 | 1900–1960 | Contemporary |
| H1 | 5 | 7 | 10 | 5 | 8 | 6 | 6 | 4 | 8 | 4 | 10 | 9 | 1 | 4 | 4 | 6 | 3 | 8 | 4 | 6 | 5 | 6 | 5 | | | | | | 2 | 1 | | 2 | | 3 | 1 | | | | | 12 | | 47 | 9 | 5 | 4 | 75 |
| H2 | 4 | 9 | | | | | | | | | | | | | | | | | | | | | | | 3 | | | | | 1 | | 2 | | | | | | | | 13 | 3 | | 3 | 2 | 1 | |
| H3 | 1 | | | | | | | | | | | | | | | | | | | | 1 | 1 | | | | | | | | | | | | | | | | | | 1 | | 2 | | | | |
| H4 | | | | 5 | 1 | | 1 | 2 | 2 | 2 | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | 13 |
| H5 | | | | | 1 | 3 | 2 | 3 | | 1 | | 1 | 1 | | 2 | 1 | | | | | | 2 | 1 | | | | 2 | | | | | | 1 | | | | | | | | 2 | 4 | 1 | | 1 | 12 |
| H6 | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| H7 | | | | | | | 1 | | | | | | 1 | | 3 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | | | | | | | | 1 | | | | 1 | 1 | | | | | 12 | 3 | 3 | | 2 |
| H8 | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| H9 | | | | | | | | | | | | | | | | 3 | 5 | 1 | 1 | 2 | 2 | | 1 | | | | | | | | | | | | | | | | | | 15 | | | | |
| H10 | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| H11 | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| H12 | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | 1 | | | | |
| H13 | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | 1 | | | | | |
| H14 | | | | | | | | | | | | | | | | | | | | | | | | | | 4 | 1 | | | | | | | | | | | | | | 5 | | | | | |
| H15 | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | 1 | | | 1 | 2 | 1 | 1 | 1 | | | | | | 2 | | 5 | 5 | | |
| H16 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | 1 | 1 | | |
| H17 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | 1 | | | | | | | 2 | 2 | | |
| H18 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | 1 | 1 | | |
| H19 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | | | | | 1 | | | | | | | | 3 | 3 | | |
| H20 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | 1 | | | | | | | | | | | 2 | 1 | 1 | |
| H21 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | 1 | 1 | | |
| H22 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | 1 | | 1 | |
| H23 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | 1 | | 1 | |
| H24 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | |
| H25 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | |
| Total | 10 | 16 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 7 | 10 | 10 | 3 | 4 | 10 | 12 | 10 | 10 | 8 | 10 | 9 | 10 | 10 | 219 | 4 | 5 | 4 | 4 | 2 | 4 | 4 | 8 | 2 | 5 | 4 | 1 | 1 | 1 | 49 | 26 | 13 | 83 | 33 | 24 | 9 | 104 |

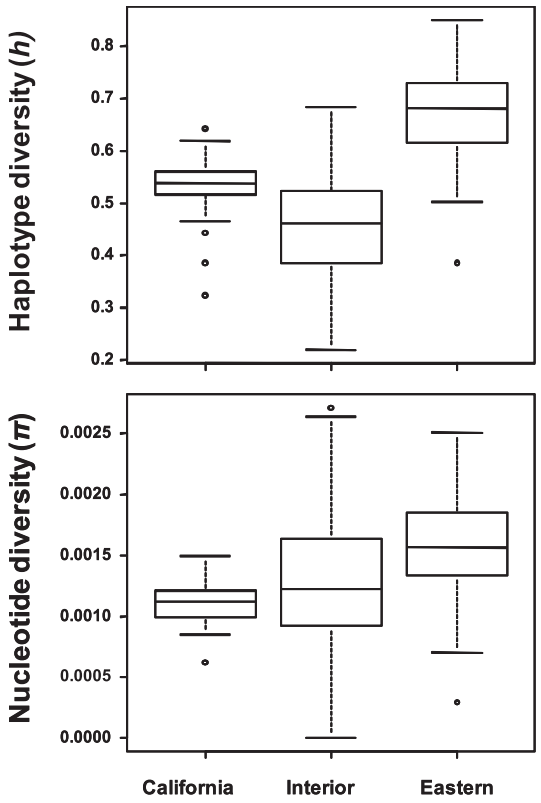


FIGURE 3. Comparisons of haplotype and nucleotide diversity of traditional Least Tern subspecies in which bootstrap resampling was used to control for differences in sample size (line within the box = median, box = 25–75% interquartile range, lines = ±1.5 times the interquartile range, dots outside the box = outliers outside 1.5 times the interquartile range above the upper quartile and below the lower quartile).

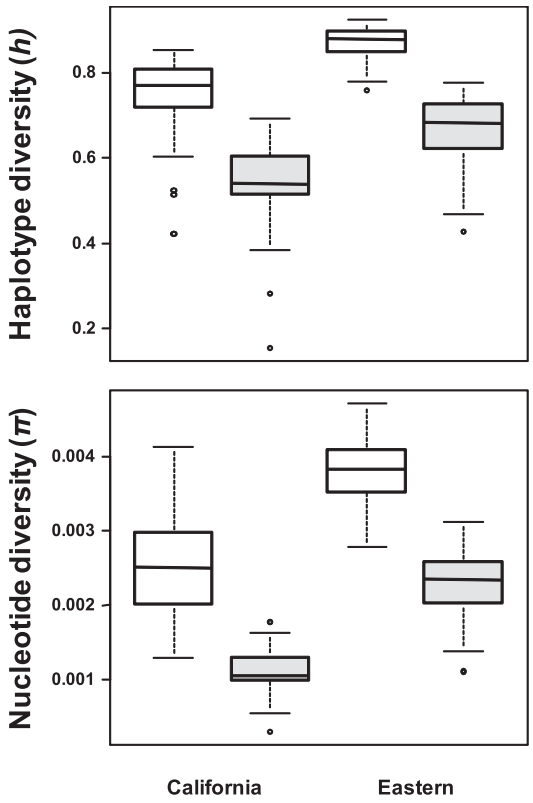


FIGURE 4. Comparisons of haplotype and nucleotide diversity in historical (pre-1960) and contemporary (2000–2005) samples of the Least Tern in which bootstrap resampling was used to control for sample size differences (line within the box = median, box = 25–75% interquartile range, lines = ±1.5 times the interquartile range, dots outside the box = outliers outside 1.5 times the interquartile range above the upper quartile and below the lower quartile).

$h = 0.769 \pm 0.116$, $\pi = 0.0031 \pm 0.0005$; contemporary: $h = 0.654 \pm 0.121$, $\pi = 0.0016 \pm 0.0006$; Fig. 5). Thus sampling over large time periods did not overly bias historical genetic diversities. We observed a trend of genetic diversity decreasing with time when all temporal groups were compared. For example, haplotype diversity was significantly higher in the pre-1900 samples than in 1900–1960 and contemporary samples ($h$: $t' = -7.9$, $P < 0.001$; $t' = -16.3$, $P < 0.001$, respectively) and was significantly higher in 1900–1960 samples than in contemporary samples ($t' = -6.8$, $P < 0.001$; Fig. 5). Likewise, nucleotide diversity was significantly higher in pre-1900 samples than in 1900–1960 and contemporary samples ($h$: $t' = -7.5$, $P < 0.001$; $t' = -24.1$, $P < 0.001$, respectively) and significantly higher in the 1900–1960 samples than in the contemporary samples ($t' = -20.0$, $P < 0.001$; Fig. 5)

## DISCUSSION

Traditional population-genetic analyses that report estimates of genetic diversity capture only a snapshot of the population in its current state. However, studies that compare historical and contemporary samples permit evaluations of population changes and highlight trends over time. In this study, we demonstrated that contemporary California and east coast Least Tern populations have lower genetic diversity than pre-1960 populations. Despite much smaller sample sizes, historical samples contained more haplotypes than did samples of contemporary populations, and analyses of historical and contemporary genetic diversity identified significantly lower haplotype and nucleotide diversity in contemporary samples.

Time-series analyses within the east coast subspecies revealed genetic diversity in historical samples was not overly inflated by sampling over long periods. While in direct comparisons of haplotype number the sample groups were similar, bootstrap analyses accounting for unequal sample size revealed museum specimens have higher genetic diversity irrespective of temporal group. Furthermore, we observed a trend of decreasing genetic diversity over time. Thus loss of genetic diversity in east coast Least Terns correlates with bottlenecks caused by anthropogenically induced population declines around 1900 and during the mid 1900s (USFWS 1980, 1990, Burger 1984, Thompson et al. 1997, Kruse et al. 2001).

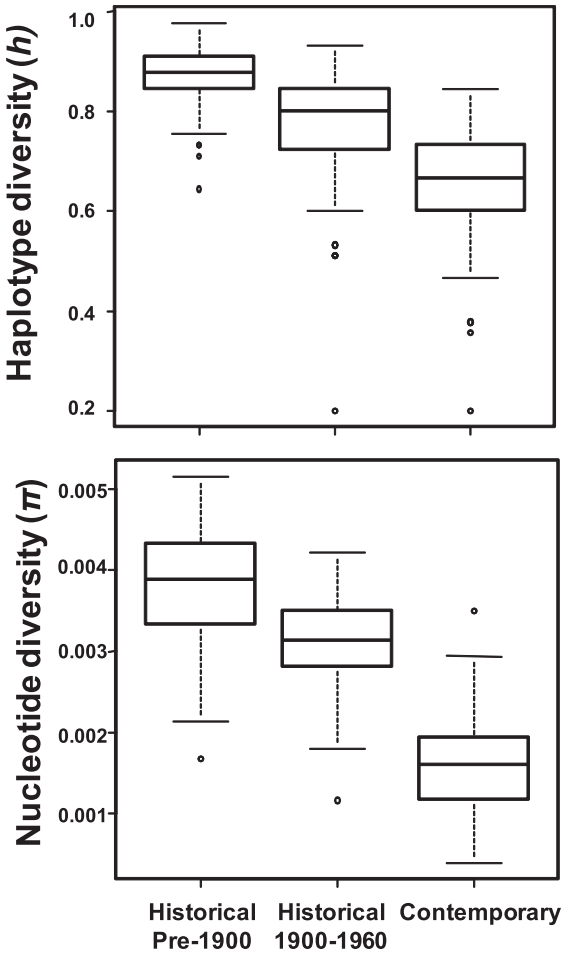


FIGURE 5. Comparisons of haplotype and nucleotide diversity between pre-1900 historical, 1900–1960 historical, and contemporary (2000–2005) samples of the Least Tern from the east coast in which bootstrap resampling was used to control for differences in sample size (line within the box = median, box = 25–75% interquartile range, lines = ±1.5 times the interquartile range, dots outside the box = outliers outside 1.5 times the interquartile range above the upper quartile and below the lower quartile).

Least Terns have experienced large-scale habitat destruction, disturbance from human recreation, harvest, and pollution, resulting in small effective population sizes, which reinforce the negative genetic effects. Reduced mtDNA genetic diversity suggests increased homozygosity at nuclear loci, which can disfavor endangered species' recovery (diminishing population viability and limiting long-term adaptability (Frankham et al. 2002, Lacy 1997, Spielman et al. 2004, Allendorf and Luikart 2007, Tollefsrud et al. 2009).

Previous microsatellite analyses of contemporary (2001–2005) populations indicated minimal evidence of past population bottlenecks in the form of heterozygote excess (Draheim et al. 2010). This discrepancy is expected given the disparate sensitivities of mtDNA and microsatellite analyses to demographic factors. For mtDNA the effective population size is one quarter that for nuclear loci, so mtDNA is more sensitive to factors such as genetic drift (Shaw et al. 2004). In addition, in microsatellites the excess of heterozygotes after population bottlenecks is transient and detectable only over a few generations until the mutation–drift equilibrium is reestablished (Luikart and Cornuet 1998). Unfortunately, we were unable to amplify nuclear microsatellite loci from many of the museum specimens, which prevented direct temporal comparisons of historical and contemporary allelic diversity.

Our temporal analyses may have been influenced by limitations of the museum collections we used. For example, several areas of historical breeding were not represented in the contemporary sample and vice versa. We recognize this is a potential source of sample bias. However, in view of high genetic homogeneity within subspecies (California $\theta_{ST} = -0.021$, $P = 0.403$; east coast $\theta_{ST} = -0.013$, $P = 0.619$) we believe bias due to disparate sampling within subspecies is negligible. However, because museum specimens were not available from the interior region for comparison, further study is needed to investigate potential loss of genetic diversity in that area.

Museum collections are being used increasingly to facilitate studies of avian molecular systematics and phylogeography (Armenta et al. 2005, Brito 2005, Burg et al. 2005, Ruokonen et al. 2005, Ohlson et al. 2007, and others). However, relatively few studies have incorporated older museum specimens to assess changes in genetic diversity over time, despite their potential utility for informing conservation and management of threatened and endangered species (but see Godoy et al. 2004, Muñoz-Fuentes et al. 2005, Brown et al. 2007, Taylor et al. 2007, Haig et al. 2011). Our results highlight the importance and utility of incorporating museum specimens in conservation-genetic studies.

Museum collections as a source for augmenting samples and/or as historical samples for temporal comparisons are invaluable to our understanding of current patterns of genetic diversity (Haig et al. 2011). However, existing collections can function only as sources of past materials. Future anthropogenic effects such as changes in climate and land use will continue to affect wildlife populations as alteration and fragmentation of natural habitats cause patterns of gene flow and dispersal to be disrupted. In expectation of predicted trends, we encourage collection curators, in cooperation with researchers, to grow their collections of current specimens (with exemption of endangered species of which collection is restricted). Today's samples will serve as pivotal temporal resources in future conservation-genetic studies.

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