AN ABSTRACT OF THE THESIS

Hope M. Draheim for the degree of Master of Science in Wildlife Science presented on June 1, 2006.

Title: Phylogeography and Population Genetic Structure of Least Terns (*Sterna antillarum*).

Abstract approved_____

Susan M. Haig

Historically, least terns (*Sterna antillarum*) were one of the most common tern species in North America. However, population declines have resulted from direct and indirect anthropogenic pressures on their breeding and foraging habitat. Three subspecies of least terns have been described within the United States: California least tern (*S. a. browni*), Interior least tern (*S. a. athalassos*), and East Coast least tern (*S. a. antillarum*). California and Interior subspecies are listed as endangered under the U.S. Endangered Species Act. However, the taxonomic status of least terns is a highly contentious issue which has implications for setting conservation priorities at erroneous levels of taxonomic distinctness. Thus, understanding population structure and taxonomy is critical for successful conservation of least terns. To clarify the phylogeographic patterns and population structure and evaluate the traditional subspecific designations, we examined variation in two mitochondrial DNA (mtDNA) genes and 10 microsatellite loci among least terns in North America.

MtDNA control region sequences and 10 polymorphic microsatellite loci were used to evaluate traditional subspecific designations and genetic structure in least terns. While highly variable, results from mtDNA control region sequences and microsatellite loci did not support the three traditional subspecies that occur in the United States. However, mtDNA pairwise θ_{ST} comparisons and AMOVA analyses indicated some genetic structure between the California and the remaining Interior/East Coast breeding areas indicating restriction to female-mediated gene flow.

We evaluated phylogeographic patterns and demographic history of least terns using the mtDNA NADH dehydrogenase subunit 6 (ND6) sequences. Phylogeographic analysis revealed no association with geography or traditional subspecies designations. Population genetic analysis did reveal slight genetic differentiation between the California breeding areas and all other Interior/East Coast breeding areas. ND6 data indicate least terns have undergone a recent population expansion. Temporal comparisons between four contemporary breeding areas and their historical counterparts found significant difference in nucleotide diversity and seven historical haplotypes were absent from contemporary breeding areas suggesting loss of genetic diversity.

This study is the most comprehensive evaluation of the genetic status of least terns, or any tern species, to date. It points to the need for better information on breeding site fidelity and natal philopatry across the species range was well as population-specific movements throughout the annual cycle. These finding should provide a helpful perspective to those planning conservation efforts throughout the species range.

Phylogeography and Population Genetic Structure of Least Terns (*Sterna antillarum*)

by
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APPROVED:

Major Professor, representing Wildlife Science

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Hope M. Draheim, Author

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CONTRUBUTION OF AUTHORS

Dr Susan Haig was involved in the design, analysis, and writing of each manuscript.

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Phylogeography and Population Genetic Structure of Least Terns (*Sterna antillarum*)

Chapter 1. Introduction

Conservation genetics is a relatively new discipline that combines evolutionary and population genetic theories and applies them to relevant conservation issues (Frankham et al. 2002). The underlying assumption in conservation genetics is that an accurate understanding of species taxonomy, evolutionary relationships, and population genetic structure are key components in evaluating population viability (Haig 1998). Genetic concerns in endangered species conservation include inbreeding depression, reduction in gene flow, loss of genetic variation as a result of small population size, and appropriate taxonomic designations for management strategies (Frankham et al. 2002). Determining how genetic diversity is partitioned over the landscape and how populations interact assists managers in identifying units of particular conservation relevance for endangered species recovery (Moritz 1994).

Widespread availability of molecular markers makes possible multi-scale estimates of gene flow, genetic diversity, and inbreeding. The field of phylogeography defined by Avise et al. (1987), uses geographic distributions and phylogenetic relationships to interpret demographic history and biogeography of natural populations, subspecies, and other grouping units. Consequently, it has become possible to more rigorously test theories in population genetics and phylogeography. Mitochondrial DNA (mtDNA) and microsatellite DNA are genetic markers that have been used to look at phylogeny, phylogeography, and population structure in avian species (Wenink et al. 1994; Gorman 2000; Zink et al. 2000; Abbot & Double 2003a; Abbot & Double 2003b;

Buehler & Baker 2003; Randi et al. 2003; Sgariglia & Burns 2003; Jones et al. 2005; McCoy et al. 2005; Nicholls & Austin 2005; and others).

The large scale distribution of least terns (*Sterna antillarum*) makes this species optimal for investigating phylogeographic patterns. Least terns are small colonial nesting terns of the subfamily Sternidae (Laridae, Charadriiformes) that have a cosmopolitan geographic distribution containing multiple breeding ranges (Thompson et al. 1997). They breed along Pacific and Atlantic coastlines, the interior rivers of mid to low-latitudinal North American as well as within the Caribbean.

Although historically abundant throughout their range (Thompson et al. 1997), least terns have experienced population declines due to direct and indirect anthropogenic pressures on their breeding and foraging habitats (U.S. Fish and Wildlife Service [USFWS] 1980, Burger 1984, USFWS 1990, Kirsch & Sidle 1999, Kruse et al. 2001). As a result, California (*S. a. browni*) and Interior (*S. a. athalassos*) subspecies in the United States are listed as endangered under the U.S. Endangered Species Act (USFWS 1985). The Eastern subspecies (*S. a. antillarum*) is state-listed as threatened or endangered in most states where it occurs (USFWS 1980 & 1990).

The taxonomic status of least terns is a highly contentious issue that has implications for setting conservation priorities at erroneous levels of taxonomic distinctness. At least five subspecies of least tern have been described based on morphological characteristics: *S. a. antillarum* (Lesson 1847), *S. a. athalassos* (Burleigh & Lowery 1942), *S. a. browni* (Mearns 1916), *S. a. mexicana* (Van Rossem & Hachisuka 1937), and *S. a. staebleri* (Brodkorb 1940). Three subspecies in the United States (*S. a. antillarum*, *S. a. athalassos*, and *S. a. browni*) are recognized by the American

Ornithologists Union (AOU 1957). The taxonomic status of the two subspecies described from Mexico (*S. a. mexicana* and *S. a. staebleri*) is uncertain (Garcia & Ceballos 1995, Patten & Erickson 1996). In addition, least terns have a wide ranging, highly mobile, colonial nesting life history that can affect taxonomic groupings and population structure. Implementing molecular methodologies can resolve taxonomic confusion and identify patterns of population genetic structure in least terns.

Research presented in this thesis was designed to apply phylogenetic and population genetic methodologies to investigate genetic variation and patterns of genetic differentiation within least terns. Individual chapters were prepared as manuscripts for submission to peer-reviewed journals and each addresses a different aspect of our study. Chapter 2 evaluates least tern subspecies designations that occur within the United States and evaluates the degree of exchange between and within such groupings. Chapter 3 investigates range wide phylogeographic patterns and demographic history of least terns. These data will be the most comprehensive molecular work that has been done to date on this species or any species of tern.

Chapter 2. Subspecific status and population genetic structure of least terns (*Sterna antillarum*) inferred by mitochondrial DNA control region sequences and microsatellite DNA.

ABSTRACT

The taxonomic status of least terns (*Sterna antillarum*) is a highly contentious issue. Thus, it has implications for setting conservation priorities at erroneous levels of taxonomic distinctness. Three described least tern subspecies occur within the United States: California least tern (*S. a. browni*), Interior least tern (*S. a. athalassos*), and East Coast least tern (*S. a. antillarum*). The California and Interior subspecies are listed as endangered under the U.S. Endangered Species Act due to precipitous recent population declines. Understanding population structure and taxonomy is critical for successful conservation of least terns. We sampled 417 individuals from 20 least tern breeding areas that represent all three subspecies. We used 840 bp of the mitochondrial DNA (mtDNA) control region and 10 variable microsatellite loci to examine the genetic structure within and among the three subspecies. Results from mtDNA control region sequences and microsatellite DNA loci did not support traditional subspecies designations. However, mtDNA pairwise θ_{ST} comparisons and AMOVA analyses detected some genetic structure between the California and the remaining Interior and East Coast breeding areas suggesting some restriction to female-mediated gene flow.

INTRODUCTION

The subspecies concept has been extensively applied within avian taxa since Linnaeus first introduced intraspecific classifications in 1753 (AOU 1957). Indeed, ornithologists have spent considerable time and effort refining the theory and debating its utility (Mayr 1942; Wilson & Brown 1953; Amadon 1949; Smith & White 1956; Barrowclough 1982; Johnson 1982; Mayr 1982; Avise & Nelson 1989; Ball & Avise 1992; Mallet 2001; Patten & Unitt 2002; Zink 2004; Philmore & Owen 2006 and others). Definitions have varied from "any geographically distinct natural population that was not sufficiently different to be a separate species" (Mayr 1942) to more quantitative definitions such as the "75 % rule" that states a population may be described as a separate subspecies only if 75% its individuals differ from a previously described subspecies (Amadon 1949).

Today, the debate over taxonomic definitions has widened with passage of conservation legislation that mandate or allow birds to be protected below the species level (e.g., subspecies, evolutionary significant units, distinct population segments, and more; Haig et al. in press). Thus, there can be legal ramifications depending on how these units are defined. These issues come to the forefront with endangered species, such as the least tern (*Sterna antillarum*), where various populations have been and are being considered for listing under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service [USFWS] & National Marine Fisheries Service [NMFS] 1996). Thus, resolving least tern taxonomy is critical lest conservation priorities be set at erroneous levels of taxonomic distinctness. In addition, understanding how populations interact with each other provides insight into demographic and evolutionary patterns affecting their current structure and status (Dearborn et al. 2003).

Least terns are small terns that nest in colonies of up to 500 pairs on open beaches and islands near fresh or salt water (e.g., exposed sparsely vegetated sand or dried mudflats or river sandbars). Least terns occur along both Pacific and Atlantic coastlines, the Interior Rivers of mid to low-latitudinal North American as well as within the Caribbean (Thompson et al. 1997). Population counts during the 1980's, 1990's, and 2000's estimate the total population of least terns breeding in the United States to be around 59,000 birds (California 4000, Interior 17,600, and East Coast 37,000, respectively; Thompson et al. 1997; Keane 1998; Lott 2006) Although actual numbers are unknown, it is thought that least terns historically were abundant throughout their range. However, during the 1800's least terns were almost extirpated due to demand for feathers for the millenary trade. Population numbers started to rebound after the passing of the Migratory Bird Treaty Act in 1918 (Thompson et al. 1997) but populations began declining again during the 1960's and 1970's due to habitat loss via river channel augmentation, irrigation diversions, dam construction, housing development and subsequent human recreation. As a result, the California (*S. a. browni*) and Interior (*S. a. athalassos*) subspecies are listed as endangered under the U.S. Endangered Species Act (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).

At least five subspecies of least tern have been described based on morphological characteristics (*S. a. antillarum* [Lesson 1847], *S. a. athalassos* [Burleigh & Lowery 1942], *S. a. browni* [Mearns 1916], *S. a. mexicana* Van Rossem & Hachisuka 1937], and *S. a. staebleri* [Brodkorb 1940]). Three subspecies in the United States (*S. a. antillarum*, *S. a. athalassos*, and *S. a. browni*) are recognized by the American Ornithologists Union

(AOU 1957). The taxonomic status of the two subspecies described from Mexico (*S. a. mexicana* and *S. a. staebleri*) is uncertain (Garcia and Ceballos 1995, Patten and Erickson 1996).

The need to clarify appropriate conservation units for least terns has led to two genetic studies that revealed little genetic differentiation among traditional least tern subspecies. Using 12 polymorphic allozyme loci Thompson et al. (1992), found no genetic differentiation between the Interior and East Coast subspecies. Whittier (2001) sequenced a portion of the mitochondrial cytochrome-b region and two nuclear intron genes among the U.S. subspecies (East Coast: n = 17, Interior: n = 22, California: n = 14) and found no genetic differentiation between the three subspecies. Although, one intron gene indicated genetic differentiation between the California and Interior breeding areas. Draheim (Chapter 3) found no genetic break between the Interior and East Coast breeding areas using mtDNA ND6 sequence data. However, there was significant genetic structure between California breeding areas and the Interior/East Coast breeding areas (Chapter 3).

Implementing multiple molecular methodologies can resolve taxonomic confusion and identify patterns of population genetic structure in least terns. Mitochondrial DNA (mtDNA) and microsatellite DNA are genetic markers that have been used to examine phylogeny, phylogeography, and population structure in avian species (Wenink et al. 1994; Gorman 2000; Zink et al. 2000; Abbot & Double 2003a; Abbot & Double 2003b; Buehler & Baker 2003; Randi et al. 2003; Sgariglia & Burns 2003; Jones et al. 2005; McCoy et al. 2005; Nicholls & Austin 2005; and others). Mitochondrial DNA has a matrilineal mode of inheritance whereby exact copies of the

maternal mitochondrial genome are passed to offspring. This mode of inheritance results in an effective population size one fourth that of nuclear alleles and subsequent high rates of haplotype extinction (Avise 2004). This clonal feature coupled with high rates of mutation (bias of transition over transversion changes) make mtDNA sequence data useful for intraspecific phylogeography (Avise et al. 1987; Zink 1997). Microsatellites are hypervariable, bi-parentally inherited genetic markers and are capable of detecting fine-scale genetic variation within species (Goldstein & Schotterer 1999). To date, no molecular work has been done on least terns using the mitochondrial control region or multiple nuclear microsatellite loci. Thus, we have two primary objectives: 1) evaluate least tern subspecies designations that occur within the United States and 2) evaluate the degree of exchange between and within such groupings.

MATERIAL AND METHODS

Criteria to delineate subspecies is intensively debated and remains a contentious issue. Heritable morphological, behavioral, genetic, and preferably multiple, characters are equally valid for delimiting subspecies (Haig et al., in press). However, for the purposes of our analysis we are defining subspecies in a genetic context as a group of breeding areas occupying a geographic region that demonstrate consistent genetic differences from another group of breeding areas occupying a different geographic region. Genetic differences consist of significant variation in mtDNA haplotype and microsatellite allele frequencies with the presence of high frequency unique haplotypes or alleles, and strong association between haplotype lineages and geography.

Sampling

We obtained 417 least tern samples from several tissue sources: blood samples from live specimens, salvaged carcasses, and embryos from collected eggs. Samples were preserved in 1 ml of tissue storage buffer (100 mM Tris-HCl pH 8, 100 mM EDTA, 10 mM NaCl, and 0.5% SDS) and stored at -80˚C until DNA extraction. Eight to 61 samples were collected from 20 breeding areas throughout least terns breeding range (Figure 2.1, Table 2.1). Sampling areas along the coastal breeding areas were defined as a group of individual samples collected within a breeding colony or collected from multiple adjacent colonies. Sampling areas along the interior river breeding distribution were defined as a group of individual samples collected within 50 river miles. Additionally, breeding areas that occurred within the described geographic ranges of the traditional subspecies were grouped accordingly (Table 2.1).

Figure 2.1. Map of least tern breeding areas included in current study. Breeding area names are listed in Table 2.1. Distributions of currently recognized subspecies are shown in light gray (California least tern), dark gray (Interior least tern) and black (East Coast least tern).

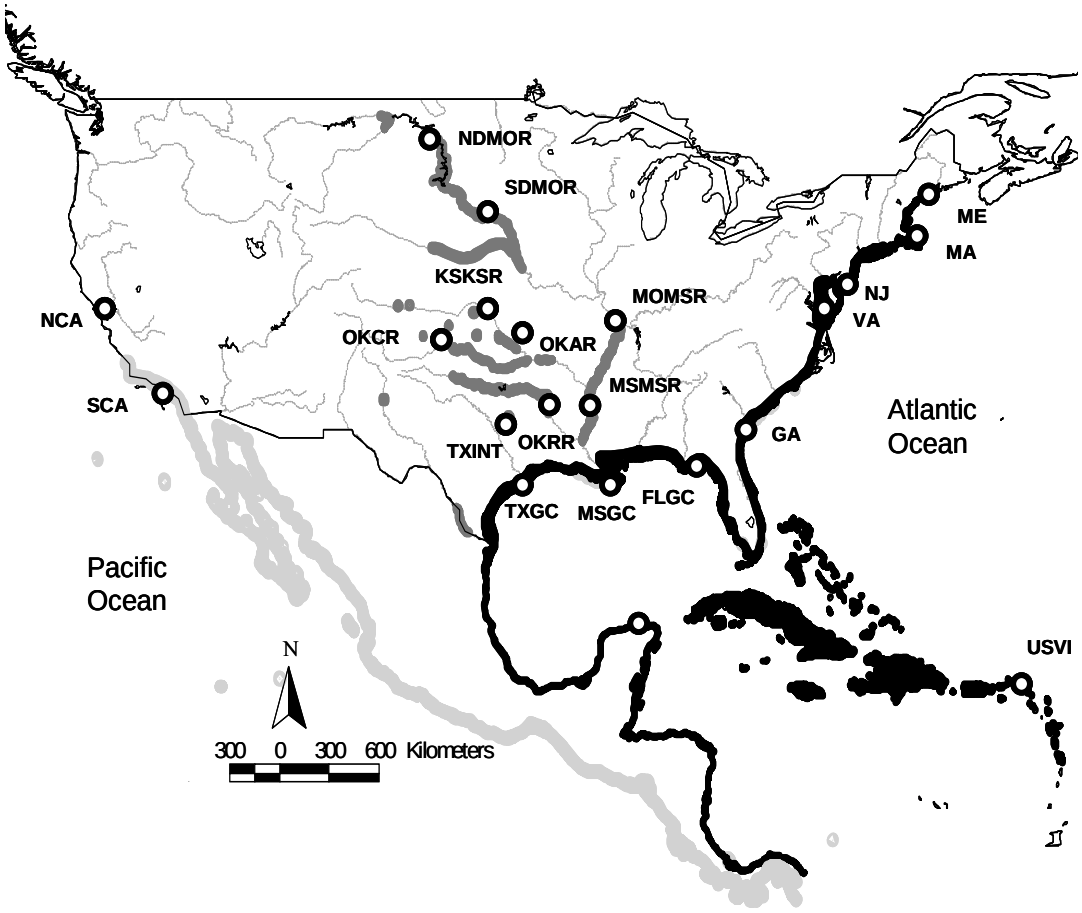


Table 2.1. Within breeding area genetic variability for mtDNA control region (840 bp) and nine microsatellite loci for least terns in the United States.

			MtDNA						Microsatellites			
Subspecies	Sample Code	County, State	*N*	No. Hap	(*h*)	(π)	*D*	F_S	*N*	a	H_E	H_O
California (*S. a. browni*)												
	NCA	Alameda, CA	10	4	0.89	0.0028	-0.127	-9.515*	26	5.2	0.563	0.531
	SCA	San Diego, CA	10	4	0.53	0.0017	-1.667*	-16.715*	24	5	0.572	0.504
Interior (*S. a. athalassos*)												
	NDMOR	McLean, ND	10	6	0.89	0.0020	-0.279	-11.771*	20	5	0.582	0.490
	SDMOR	Yankton, SD	10	7	0.91	0.0055	0.820	-6.271*	30	5.4	0.582	0.563
	KSKSR	Pottawatomie, KS	10	7	0.91	0.0048	-1.073	-6.820*	18	5	0.595	0.594
	MOMSR	New Madrid, MO	10	9	0.98	0.0044	-0.612	-7.306*	14	4.7	0.595	0.536
	OKCR	Woods, OK	10	9	0.98	0.0058	0.287	-6.356*	14	5.1	0.582	0.521
	OKAR	Tulsa, OK	10	7	0.93	0.0054	-0.398	-6.049*	35	6.2	0.599	0.537
	OKRR	McCurtain, OK	10	9	0.98	0.0067	-0.046	-5.456*	18	5.1	0.558	0.539
	TXINT	Dallas, TX	10	6	0.89	0.0028	-0.280	-9.728*	16	4.9	0.613	0.625
	MSMSR	Bolivar, MS	10	7	0.87	0.0028	-0.788	-9.728*	15	5.5	0.757	0.720
East Coast (*S. a. antillarum*)												
	ME	Knox, ME	10	5	0.80	0.0040	-1.516*	-7.806*	21	6.2	0.555	0.548
	MA	Barnstable, MA	12	8	0.92	0.0051	-0.061	-9.119*	61	8	0.589	0.554
	NJ	Cape May, NJ	10	8	0.96	0.0065	0.123	-5.568*	12	5.9	0.572	0.515
	VA	Accomack, VA	10	10	1.00	0.0047	-1.208	-7.001*	10	4.8	0.583	0.620
	GA	Glenn, GA	8	8	1.00	0.0069	-0.321	-3.497*	8	5.2	0.663	0.613
	USVI	St. Croix, VI	10	7	0.91	0.0060	-0.224	-5.879*	24	6.1	0.617	0.558
	FLGC	Bay, FL	9	9	1.00	0.0060	-1.197	-4.882*	17	5.7	0.514	0.459
	MSGC	Harrison, MS	10	8	0.93	0.0052	-1.473	0.002	19	6.3	0.519	0.463
	TXGC	Brazoria, TX	10	8	0.96	0.0058	-1.098	-6.030*	15	6.1	0.604	0.540

Number of individuals sampled (*N*), haplotype diversity, number of haplotypes, (*h*), nucleotide diversity (π), Tajima's *D,* Fu's F_S, mean number of alleles per locus (*A*), expected heterozygosity (H_E), observed heterozygosity (H_O) for breeding areas of least terns. Significant values ($P < 0.05$) are followed by asterisks.

DNA Extraction, Marker Isolation, and Amplification

DNA extraction --Ten microliters of blood or 1 mm^3 of tissue was digested in 400 µl of extraction buffer A (50 mM Tris pH 8.0, 10 mM EDTA pH 8.0, 200 mM NaCl, 2% SDS) and 20 mg/ml of Proteinase K. Samples were vortexed and incubated overnight at 50°C. DNA was extracted from samples using equal volumes (~ 400 ml) of phenol (saturated with 10 mM Tris pH 8.0) and chloroform/isoamyl alcohol (25:1). DNA was cleaned and concentrated by centrifugation dialysis using Microcon 30,000 MW cutoff columns (Amicon Bioseparations). Stock DNA was then diluted to a concentration of 25-100 ng/µl.

PCR amplification of mitochondrial DNA— A ~1800 bp segment containing the ND6 and control region of the mtDNA genome was amplified by long PCR using conserved mtDNA primers L16087 (5'- TGGTCTTGTAARCCAAARANYGAAG-3, Desjardins & Morais 1990) and H1248 (5′-CATCTTCAGTGCCATGCTTT-3′, Tarr et al. 1995). Each 50 µl reaction contained 1 mm $Mg(OAc)_2$, 800 µm dNTPs, 0.64 µm of each primer, 1.2 U r*Tth* XL polymerase (GeneAmp XL PCR kit, Roche Molecular Systems, Branchburg, NJ, USA), 1× XL Buffer II and 1 µL template DNA (≈25–100 ng). The PCR profile was 93°C for 1 min; 15 cycles of 93°C for 50 s, 62°C for 10 min; 25 cycles of 93°C for 50 s, 62°C for 10 min + 15 s autoextend per cycle; 72°C for 7 min. Sequences were aligned with known ND6 and control region sequences of a variety of tern and gull (i.e. Charadriiformes) species from Genbank to confirm the sequence was mitochondrial and not a nuclear homolog. The least tern control region sequence indicated the presence of a string of C repeats at the 5' end and a repetitive AC at the 3' end. Internal primers LETE70 L (5'-ATACGCTCACATGCACCT-3') and LETE 1000 H (5'-

ACTGTCGTTGACGTATAACAA-3') were designed to anneal 90 bp down stream from the 5' end and 50 bp upstream of the AC repeat at the 3' end to yield a 840 bp sequence of the least tern mtDNA control region. Amplifications for 199 individuals were performed using a PTC 100 thermal cycler (MJ Research). 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 um of each primer; 50-100 ng of template; and 1.5 U *AmpliTaq* Gold Polymerase (Perkin Elmer). The following parameters were used for amplifications: 12 min. denaturation at 93^{o}C, followed by 35 cycles of 30 seconds at 93^{o}C, annealing at 50^{o}C for 30 seconds, and elongation at 72^{o}C for 1 min. A final 10 min. period of elongation at 72^{o}C followed the last cycle. PCR amplification quality was assessed by visualizing 10 µl of the product with ethidium bromide on 1% agarose gels. Successful PCR reactions were cleaned and concentrated by centrifugation dialysis using Microcon 30,000 MW cutoff filters (Amicon Bioseparations). Sequences were generated using ABI Prism Big Dye Terminator Cycle Sequencing chemistry on an ABI 3730 capillary DNA analyzer located in the Central Services Laboratory at Oregon State University. Ambiguities were resolved by comparing light and heavy-strand sequences or from overlap of different fragments. Sequences were aligned by eye using BIOEDIT (version 7.0.5) alignment software (Hall 1999). All sequences were archived in GenBank (Accession nos ##-##).

PCR amplification of microsatellite DNA—PCR was used to screen for amplification and variability in least terns at 20 microsatellite loci with primer sequences developed for the red-billed gull (*Larus novaehollandiae*), black-legged kittiwake (*Rissa tridactyla*), roseate tern (*S. dougallii*), and black oystercatcher (*Haematopus bachmani*)

(Given et al. 2002; Tirard et al. 2002; Szczys et al. 2005; Gust et al. in prep). Ten microsatellite loci did not amplify or were monomorphic in least terns and were thus excluded from further analysis. Variable microsatellite loci for analysis included: Hbau4 (Gust et al. in prep); *K6*, *K16*, *K32* (Tirard et al. 2002); *RBG13, RBG18, RBG27, RBG28* (Given et al. 2002); and *SDAAT 20, SDAAT 27* (Szczys et al. 2005). Amplifications for 417 individuals were performed using a PTC 100 thermal cycler (MJ Research). DNA was amplified using a PCR profile with the following steps: initial denaturation for 5 min at 94°C, followed by 29 cycles of: 30 s at 94°C, 30 s at an annealing temperature (Table 2.2), 60 s at 72°C, then an additional 10 min extension step at 72°C. Ten microliter reactions were prepared using 50-100 ng of DNA in 10 mM Tris-HCl; 50 mM KCl; 2.0 mM $MgCl_2$; 0.25 mM of each dATP, dCTP, dGTP, dTTP; 15 mM of each primer; and 0.5 units of *Taq* polymerase (Promega). Amplified products were sized on an ABI 3100 Genetic Analyzer at Oregon State University's Center for Gene Research. Genotype analysis was performed using software applications Genescan Analysis 3.2 and Genotyper 2.5 (Applied Biosystems).

Statistical Analysis

Mitochondrial DNA variation--We used DNASP version 4.0 (Rozas et al. 2003) to calculate haplotype diversity (*h*), the probability that two randomly chosen individuals have different haplotypes; and nucleotide diversity (π), the average pairwise nucleotide differences for control region haplotypes. Relationships between haplotypes were inferred by estimating a statistical haplotype tree network with 95 percent parsimonious connection from the program TCS 1.13 (Clement et al. 2000). Tajima's *D* (Tajima 1989)

Table 2.2. Least tern microsatellite primer sequences, annealing temperatures, product size, total number of alleles at each locus, and references of primer sequences used in this study.

Locus	Primer sequence (5'-3')	Anealing Temp (C°)	Size	No. Alleles	Reference
RBG13	F: CAGGAGGGAAAGCCCATATG R: GACAGGCAGGAAAGAATCTC	58	217-233	9	Given et al. 2002
RBG18	F: AAAGGGCTGCTCATAGTACG R: GTAGCATCATGTCTTCCCGC	50	139-184	10	Given et al. 2002
RBG27	F: GGAATTTTCGTTGGCAGGAT R: GAAATCACAGTGAAAACGCC	50	186-200	7	Given et al. 2002
RBG28	F: ACAAACTTCTGGTGCCCC R: TACACACCCCATTGCATTTC	50	158-194	22	Given et al. 2002
K6	F: AAAAAGAAAGCACCCTCTTC R: AAGTGGGATATGAAAGATGC	52	115-123	2	Tirard et al. 2002
K16	F: TGCAATTTGTACAACCAGATTT R: GGGTTCCTGTTTGCAATGAA	53	128-142	10	Tirard et al. 2002
K32	F: CATTGCACGAGTGTTAAGCTG R: AAGGGTGCCTGTCCTTGTC	53	126-164	28	Tirard et al. 2002
SDAAT20	F: CTGGCTATGCTGCAGACTGA R: GCATCAAGTGCTCGATACCA	58	198-257	29	Szczyz et al. 2005
SDAAT27	F: TGAAACAGATGAATCAAACCA R: ATCTGGTCTCCCTCCAGCTT	60	234-237	4	Szczyz et al. 2005
HBaμ4	F: GTCCTGCTGGTTTATATC R: TTCTGCTGAGGTCCTACG	55	136-148	6	Gust et al. (in prep)

and Fu's F_S (Fu 1997) were estimated to infer demographic factors and test assumptions of neutrality. A large and significant value of *D* (positive or negative) can indicate deviation from neutrality and can be used to infer demographic processes (i.e. population expansion or historical bottlenecks). Similarly, a large and significant negative value of Fu's F_S may be indicative of population expansion. All tests were performed using ARLEQUIN version 2.0. (Schneider et al. 2000).

Microsatellite variation— Program GENEPOP (version 3.4) (Raymond & Rousset 1995) was used to quantify genetic variation in each breeding area using mean number of alleles (*A*), observed (H_O), and expected (H_E) heterozygosity for each locus and over all loci. Deviations from Hardy-Weinberg (HW) proportions and tests for linkage disequilibrium were evaluated using Fisher's exact tests as performed in program GENEPOP (Raymond & Rousset 1995). Tests were performed over all loci and breeding areas. We used sequential Bonferroni corrections for multiple tests (Rice 1989). Heterozygote excess was used to detect recent small population bottlenecks using program BOTTLENECK (Cornuet & Luikart 1997). The program BOTTLENECK was run under the two phase model as suggested by the authors.

Genetic Structure-- Molecular variance was assessed using separate analyses of molecular variance (AMOVA; Excoffier et al. 1992) at different hierarchical levels (within breeding areas, among breeding areas, among traditional subspecies). Although using F_{ST} to is prone to pitfalls for evaluating gene flow it remains a useful index for comparative purposes (Neigel 2002). Thus, to determine the degree of genetic differentiation we used Weir and Cockerham's (1984) estimate of F_{ST} (θ_{ST}) to estimate global $\theta_{ST,}$ pairwise θ_{ST} between traditional subspecies and pairwise θ_{ST} between breeding

areas implemented in ARLEQUIN version 2.0. (Schneider et al. 2000). Microsatellite pairwise θ_{ST} was calculated only for breeding areas with greater than or equal to 14 individuals to limit sampling error caused by small sample size. We used sequential Bonferroni corrections for multiple tests (Rice 1989). Mantel tests (Mantel 1967) were calculated using program ISOLDE in GENEPOP to access isolation by distance.

Microsatellite population genetic structure was further evaluated using the Bayesian-clustering method of Pritchard et al. (2000) as performed by the program STRUCTURE. This program uses genotypic data to determine a number of genetic clusters (K) based on Hardy-Weinberg expectations using no prior breeding area information. To estimate the number of genetic clusters we performed five independent runs of K = 1-17 using simulations of 1×10^6 iterations after a burn-in period of 5×10^5 iterations. The most likely number of populations was determined by the log likelihood of K and the posterior probability of K (P(K|X)) as determined by the method described in Pritchard et al. (2000).

Principle component analysis (PCA) was also used to project the relationship among breeding areas using microsatellite allelic frequencies. We computed PC scores based on the covariance among allele frequencies using PC-ORD version 4.33 (McCune & Mefford 1999). The largest allele at each locus was omitted to account for non independence of allele frequencies within each locus.

RESULTS

Genetic Variation

Mitochondrial DNA--Control region sequences (840 bp) were characterized by 44 polymorphic sites: 38 polymorphic sites were transitions, 6 sites were transversions, and no insertions or deletions were present. Sixty-eight unique haplotypes were observed among 199 individuals (Table 2.3). Mean nucleotide composition in the least tern control region (A 23.69%; C 28.20%: T 30.82%, G 17.29%) was similar to other Charadriiform species [Wenink et al. 1994; Buehler & Baker 2003; Funk et al (in review)]. The 95 % parsimony network generated by TCS revealed a number of loops which are indicative of homoplasies and the presence of reverse/parallel mutations (Figure 2.2; Posada & Crandall 2001).

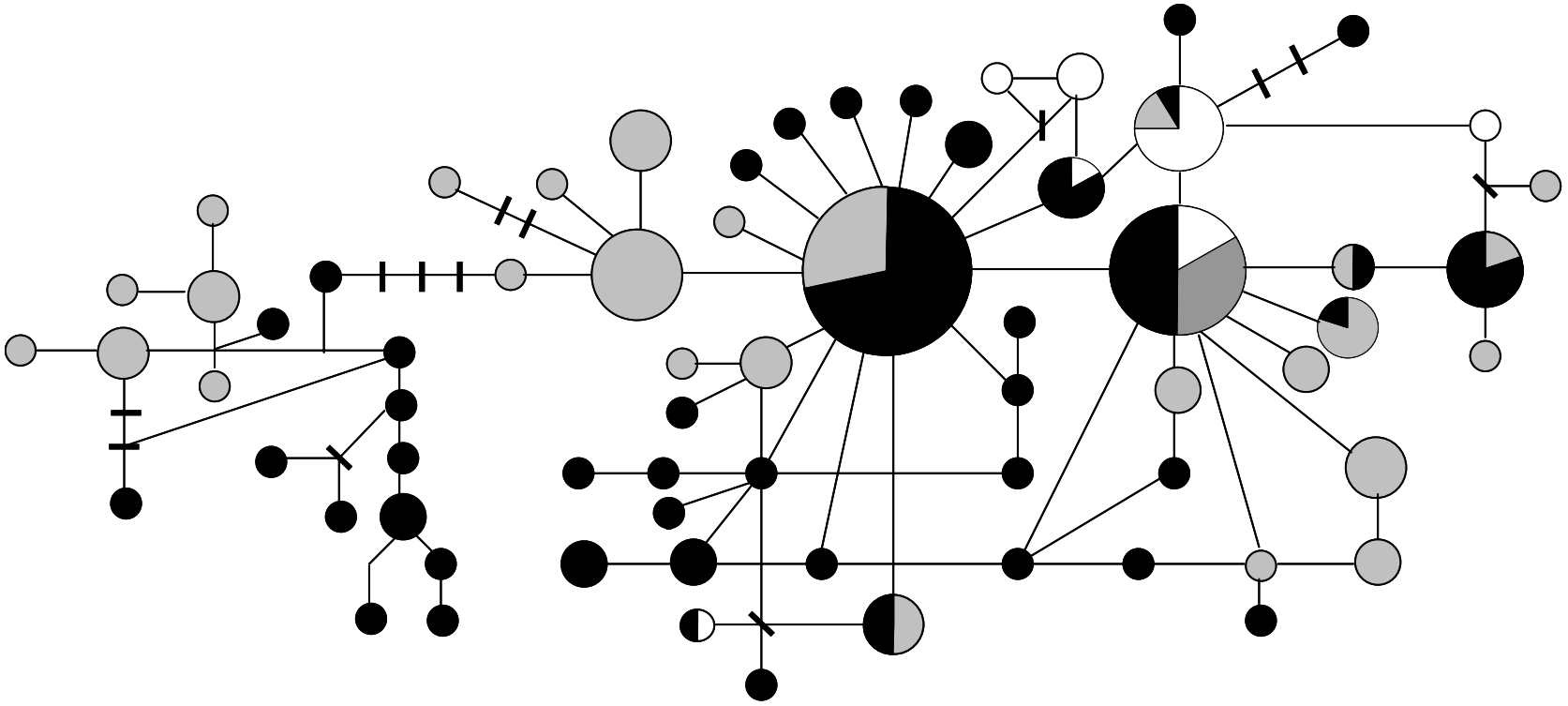
The mtDNA haplotype network showed a general lack of association between haplotype lineages and geography (Figure 2.2). The most common haplotype (H14) was shared by 10.01% of all individuals. Two haplotypes (H4 and H8), each one step removed from H14, comprised the basal haplotypes for two possible haplotype lineages suggesting slight haplotype structure. Most adjacent haplotypes differed by only one or two mutational steps with exception of H33 lineage which differed by three mutational steps from H8.

Control region sequences indicated 58 haplotypes were restricted to a single subspecies designation whereas only 41 haplotypes were restricted to a single breeding area (Table 2.3). Subsequently, within breeding area haplotype diversity was high ranging from 0.553 to 1 and averaging 0.912 ± 0.104. The highest genetic variation was

Table 2.3. Distribution of 68 mtDNA control region haplotypes among all least tern breeding areas.

	brownii		*athalassos*										*antillarum*								
Haplotype	SCA	NCA	NDMR	SDMR	KSKR	MOMS	OKAR	OKCR	OKRR	TXINT	MSMR	ME	MA	NJ	VA	GA	USVI	FLGC	MSGC	TXGC
H1	1																			
H2	7	2		1				1										1		
H3	1	3																		
H4	1	2	3			1		1	1			3	2					1	1	2
H5		1																		
H6		1													1		3	1		
H7		1																	1	
H8			2	1	1		2			3	4									
H9			2		2						1									
H10			1	1		1			2		1									
H11			1		1					1	0									
H12			1		3															
H13				3		1		1												
H14				2		1	1		1	1		4	2		1	1	1		3	2
H15				1																
H16				1		1	1							1		1			1	
H17					1															
H18					1															
H19					1	2	2	1			1	1							1	
H20						1			1											
H21						1	1			2										1
H22						1														
H23							1					1								
H24							2	1	1											
H25								1	1											
H26								1												
H27								2												
H28								1		1	1									
H29									1											
H30									1	2										1
H31									1											
H32											1									
H33											1									
H34												1								
H35													3	1						
H36													1	2						
H37													1							
H38													1		1					
H39													1							
H40													1				2	1		
H41														1	1					
H42														1						
H43														1						
H44														2						
H45														1	1	1		1		
H46															1		1			
H47															1					
H48															1					
H49															1					
H50															1					
H51																1				
H52																1				
H53																1				
H54																1				
H55																1				
H56																	1			
H57																	1			
H58																	1			
H59																		1		
H60																		1	1	
H61																		1		
H62																		1		
H63																			1	
H64																			1	
H65																				1
H66																				1
H67																				1
H68																				1
Total	10	10	10	10	10	10	10	10	10	10	10	10	12	10	10	8	10	9	10	10

Figure 2.2. The statistical 95 % parsimony network generated by TCS based on mtDNA control region haplotypes for least terns. Circle sizes are proportional to the number of individuals sharing the haplotype. Shades refer to the proportion of samples that came from a traditional subspecies designation. California least tern haplotypes are shown in white, Interior least tern haplotypes shown in gray, and East Coast least tern haplotypes shown in black. Dashes represent inferred haplotypes.



found in Cape May County, NJ; Accomack County, VA; St. Croix County, VI; and Bay County, FL while the lowest genetic variation was found in San Diego County, CA (Table 2.1). Tests for population bottlenecks or expansions all reveal negative and significant Fu's *F* values with exception of the Harrison County, MS breeding area. Tajima's *D* was negative for all breeding areas but only significant for the San Diego County, CA; and Knox County, ME breeding areas (Table 2.1). Thus, suggesting possible population expansion for these breeding areas.

Microsatellite variation—Among 10 variable loci, the total number of alleles per locus ranged from two at locus K6 to 11 at locus K32. Mean number of alleles per locus and breeding area varied from 4.7 in New Madrid County, MO to 8.0 in Barnstable County, MA. Average observed heterozygosity within breeding areas ranged from 0.469 in Bay County, FL to 0.72 in Bolivar County, MS, and expected hetrozygosity ranged from 0.514 in Bay County, FL to 0.757 in Bolivar County, MS (Table 2.1).

Microsatellites revealed no significant linkages ($P < 0.05$) between loci after corrections for multiple tests. However, Hardy-Weinberg probability tests across breeding areas revealed significant deviations from Hardy-Weinberg expectations ($P < 0.001$) in the form of heterozygote deficiencies. Individual tests for all loci and all breeding areas revealed a significant heterozygote deficiency after sequential Bonferroni adjustments for multiple tests at locus Sdaat20 in 12 breeding areas (Alameda County, CA; San Diego County, CA; McLean County, ND; Yankton County, SD; New Madrid County, MO; Tulsa County, OK; McCurtain County, OK; Bolivar County, MS; Barnstable County, MA; Cape May County, NJ; St. Croix County, VI; and Harrison County, MS) most likely due to presence of null alleles (Shaw et a1. 1999). Thus, locus

SDAAT20 was eliminated from further analyses. Significant heterozygote deficiency was also detected at locus K32. Since this deficiency was found only in Tulsa County, OK it is most likely explained by population history rather than genotyping error. The program BOTTLENECK detected no significant excess of hetrozygosity in any breeding areas, indicating they have not experienced recent reductions in effective population size or genetic bottlenecks.

Population genetic structure

Mitochondrial DNA-- AMOVA revealed that the majority of the variation in haplotype variation was explained by variation within breeding areas (Table 2.4). Hierarchical analysis making assumptions of traditional subspecific groupings indicated 89% of the total variance (θ_{ST} = 0.109, P < 0. 0.001) was explained by variation within breeding areas, 4% was explained by variation among breeding areas within traditional subspecies (θ_{SC} = 0.038, P = 0.026), and only 7% was explained by variation among groups (θ_{CT} = 0.074, P < 0. 0.001). The greatest variation among groups was found when breeding areas were grouped into the California vs. Interior/East Coast breeding areas (θ_{CT} = 0.110, P < 0. 0.001) (Table 2.4). When only breeding areas within traditional subspecies were considered, hierarchical analysis showed some structure for the California and Interior subspecies (θ_{ST} = 0.167, P < 0. 0.019; θ_{ST} = 0.061, P < 0.013; respectively), however there was no significant differentiation among breeding areas in the East Coast subspecies (θ_{ST} = 0.005, P < 0. 0.364).

Global θ_{ST} among all breeding areas was 0.083 (P < 0.05; Table 2.4). Genetic

Table 2.4. Analysis of molecular variance (AMOVA) at mtDNA control region and 9 microsatellite loci of least tern breeding areas.

	MtDNA				Microsatellites			
Groups	df	% var	θ	*P*	df	% var	θ	*P*
All breeding areas								
Among breeding areas	19	8.29	$\theta_{ST} = 0.083$	$P < 0.001$	19	2.45	$\theta_{ST} = 0.025$	$P = 0.05$
Within breeding areas	179	91.71			814	97.55		
California breeding areas								
Among breeding areas	1	16.67	$\theta_{ST} = 0.167$	$P = 0.019$	1	0.11	$\theta_{ST} = 0.001$	$P = 0.375$
Within breeding areas	18	83.33			98	99.89		
Interior breeding areas								
Among breeding areas	9	6.01	$\theta_{ST} = 0.061$	$P = 0.013$	8	0.22	$\theta_{ST} = 0.002$	$P = 0.252$
Within breeding areas	81	93.99			361	99.78		
East Coast breeding areas								
Among breeding areas	8	0.49	$\theta_{ST} = 0.005$	$P = 0.364$	5	1.22	$\theta_{ST} = 0.012$	$P = 0.001$
Within breeding areas	80	99.51			298	98.78		
Subspecies								
Among subspecies	2	7.38	$\theta_{CT} = 0.074$	$P < 0.001$	2	2.74	$\theta_{CT} = 0.027$	$P < 0.001$
Among breeding areas within subspecies	17	3.51	$\theta_{SC} = 0.038$	$P = 0.026$	14	0.59	$\theta_{SC} = 0.006$	$P < 0.001$
Within subspecies	179	89.11	$\theta_{ST} = 0.109$	$P < 0.001$	757	96.76	$\theta_{ST} = 0.033$	$P < 0.001$
California vs. Interior/East Coast breeding areas								
Among groups	1	11.02	$\theta_{CT} = 0.110$	$P < 0.001$	1	1.44	$\theta_{CT} = 0.014$	$P = 0.016$
Among breeding areas within groups	18	5.46	$\theta_{SC} = 0.061$	$P < 0.001$	15	2.01	$\theta_{SC} = 0.020$	$P < 0.001$
Within breeding area	179	83.52	$\theta_{ST} = 0.165$	$P < 0.001$	757	96.55	$\theta_{ST} = 0.035$	$P < 0.001$

differentiation between traditional subspecific groupings using pairwise θ_{ST} were significant and ranged from 0.04 to 0.15 (Table 2.5). The California subspecies was the most differentiated from the other two subspecies. Pairwise θ_{ST} values among breeding areas ranged from 0.0 to 0.59 (Table 2.6). The greatest levels of differentiation were observed between San Diego County, CA and all other breeding areas. Similarly, birds from Alameda County, CA were significantly differentiated from all breeding areas with the exception of McCurtain County, OK; Bay County, FL; and Harrison County, MS. Comparisons between Interior breeding areas and East Coast breeding areas indicated a random pattern of population differentiation. Twenty-two (21%) of the pairwise comparisons between Interior/East Coast breeding areas were significant but showed no obvious geographic pattern. A Mantel test revealed that a moderate but significant proportion of the variation in genetic distance among breeding areas was explained by geographic distance ($r = 0.524$, $P = 0.001$; Figure 2.3).

Microsatellite DNA— Similar to the mitochondrial data, microsatellite DNA AMOVA analyses revealed that the majority of variation in allele frequency was explained by variation within breeding areas (Table 2.4). Hierarchical analysis making assumptions of traditional subspecific groupings indicated 96.7% of the total variance ($\theta_{ST} = 0.033$, $P < 0.\ 0.001$) was explained by variation within breeding areas, 0.5% was explained by variation among breeding areas within traditional subspecies ($\theta_{SC} = 0.006$, $P = 0.026$), and only 2.7% was explained by variation among groups ($\theta_{CT} = 0.027$, $P < 0.\ 0.001$). AMOVA analysis testing the assumption of a California vs. an Interior/East Coast group revealed only 1.44% of the variation was explained by these groupings. Within traditional subspecies, there was little genetic structure within the East Coast

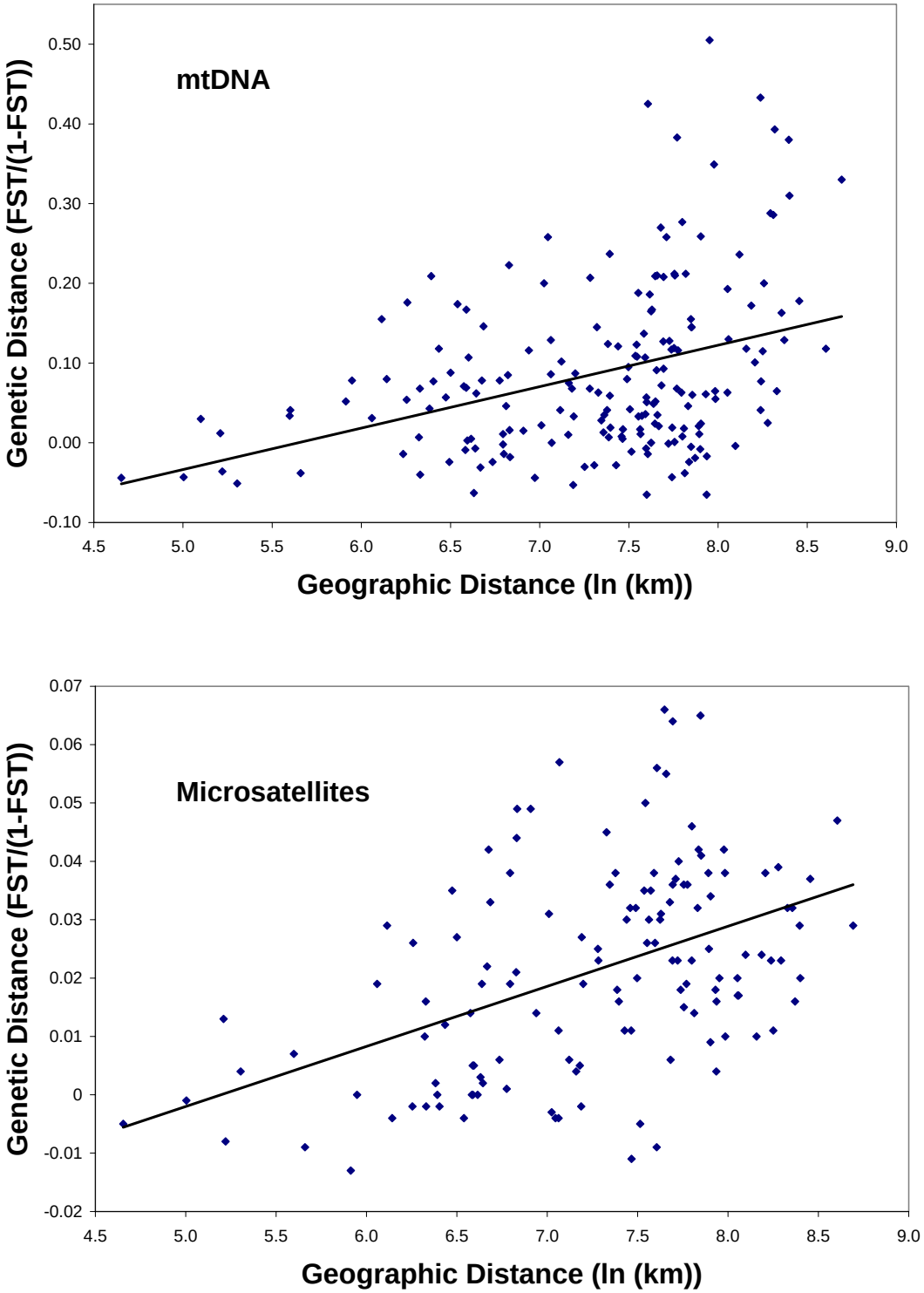
Table 2.5. Pairwise θ_{ST} values among traditional least tern subspecies of mtDNA control region sequences (left side of slash) and microsatellite loci (right side of slash) Statistical significance above the diagonal ($P < 0.05$).

	California	Interior	Eastern Coastal
California		*/*	*/*
Interior	0.119/0.033		*/*
Eastern Coastal	0.149/0.021	0.038/0.030	

Table 2.6. Pairwise θ_{ST} values among least tern breeding areas of mtDNA control region sequences (below diagonal) and statistical significance ($P < 0.05$; above the diagonal).

Site	NCA	SCA	NDMOR	SDMOR	MOMOR	KSKSR	OKAR	OKCR	OKRR	TXINT	MSMSR	ME	MA	NJ	VA	GA	USVI	MSGC	TXGC	FLGC
NCA		*		*	*	*	*	*	NS	*	*	*	*	*	NS	*	*	NS	NS	*
SCA	0.167		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
NDMOR	0.210	0.425		*	NS	*	NS	*	NS	NS	NS	NS	*	*	NS	*	*	*	NS	NS
SDMOR	0.167	0.270	0.209		NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
KSKSR	0.145	0.349	0.007	0.116		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
MOMOR	0.123	0.208	0.086	0.077	0.071		NS	NS	NS	NS	*	NS	*	*	NS	*	NS	NS	NS	NS
OKAR	0.109	0.258	0.068	0.005	0.003	-0.043		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
OKCR	0.121	0.165	0.102	-0.009	0.078	-0.051	-0.044		NS	*	NS	NS	*	*	NS	NS	NS	NS	NS	NS
OKRR	0.107	0.212	0.087	-0.063	0.043	0.012	-0.036	-0.038		NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
TXINT	0.188	0.383	-0.028	0.200	0.062	0.054	0.052	0.078	0.080		NS	NS	*	*	NS	*	*	*	NS	NS
MSMSR	0.277	0.505	0.005	0.258	0.034	0.174	0.088	0.176	0.146	0.068		*	*	*	*	*	*	*	*	*
ME	0.129	0.310	0.046	0.117	0.034	-0.038	-0.024	0.008	0.021	0.024	0.127		NS	NS	NS	NS	NS	NS	NS	NS
MA	0.163	0.380	0.155	0.128	0.080	0.116	0.063	0.119	0.060	0.145	0.186	0.041		NS	NS	NS	NS	NS	NS	NS
NJ	0.200	0.393	0.210	0.049	0.075	0.137	0.057	0.108	0.036	0.209	0.237	0.107	-0.014		NS	NS	NS	NS	NS	NS
VA	0.077	0.286	0.068	0.091	0.041	0.033	0.011	0.042	0.024	0.051	0.145	-0.014	-0.024	0.030		NS	NS	NS	NS	NS
GA	0.236	0.433	0.212	0.019	0.085	0.124	0.028	0.068	0.008	0.207	0.223	0.095	0.013	-0.044	0.046		NS	NS	NS	NS
USVI	0.118	0.330	0.178	0.065	0.063	0.115	0.041	0.101	0.025	0.172	0.193	0.055	-0.008	-0.005	0.018	-0.001		NS	NS	NS
FLGC	0.011	0.130	0.093	0.041	0.057	0.022	0.015	0.016	0.000	0.078	0.155	0.001	0.021	0.059	-0.028	0.069	-0.017		NS	NS
MSGC	0.072	0.259	0.052	0.063	0.011	-0.018	-0.031	-0.007	-0.002	0.031	0.118	-0.065	-0.019	0.035	-0.065	0.010	-0.004	-0.040		NS
TXGC	0.118	0.288	0.065	0.061	0.033	0.017	-0.011	0.017	-0.014	0.019	0.129	-0.043	0.000	0.035	-0.030	0.007	-0.007	-0.024	-0.053	

Figure 2.3. Scatter plot of genetic distance among least tern breeding areas versus geographic distance for mtDNA control region haplotypes (Mantel test; $r = 0.524$, $P = 0.001$) and microsatellite loci (Mantel test; $r = 0.690$, $P < 0.001$).



subspecies (θ_{ST} = 0.012, P = 0. 0.001) and no significant genetic structure within the California or Interior subspecies (θ_{ST} = 0.001, P < 0. 0.375; θ_{ST} = 0.002, P < 0. 0.252) (Table 2.4).

Microsatellite analysis revealed small but significant global genetic differentiation (θ_{ST} = 0.025 (P < 0.05; Table 2.4). Genetic differentiation between traditional subspecies using pairwise θ_{ST} ranged from 0.021 to 0.033 (Table 2.6) and pairwise θ_{ST} values for population comparisons ranged from 0.0 to 0.065 (Table 2.7). Unlike the mtDNA data no traditional subspecific grouping indicated higher pairwise θ_{ST} values. However, most population comparisons involving either of the California breeding area were significant. Alternatively, comparisons between Interior and East Coast populations that showed a random pattern of population differentiation; 61% of population comparisons between subspecies designations were significant (Table 2.7). Comparisons within subspecies were generally not significant with exception of St. Croix County, VI and the two most northern populations of the East Coast subspecies (Knox County, ME; Barnstable County, MA). A Mantel test revealed that a significant proportion of the variation in genetic distance among populations was explained by geographic distance (r = 0.69, P = 0.001; Figure 2.3).

Using Bayesian clustering inference, the highest log likelihood value and posterior probability was observed for K = 2 suggesting least terns are subdivided into two genetic clusters: an Interior cluster and California/East Coast cluster (Table 2.8). The first cluster proportionally favored individuals originating from the Interior subspecies in which 75% of the Interior genotypes were assigned. The second cluster chiefly consisted of individuals originating from both California and East Coast

Table 2.7. Pairwise θ_{ST} values among least tern breeding areas with 14 or more individuals of nine microsatellite loci (below diagonal) and statistical significance ($P < 0.05$; above the diagonal).

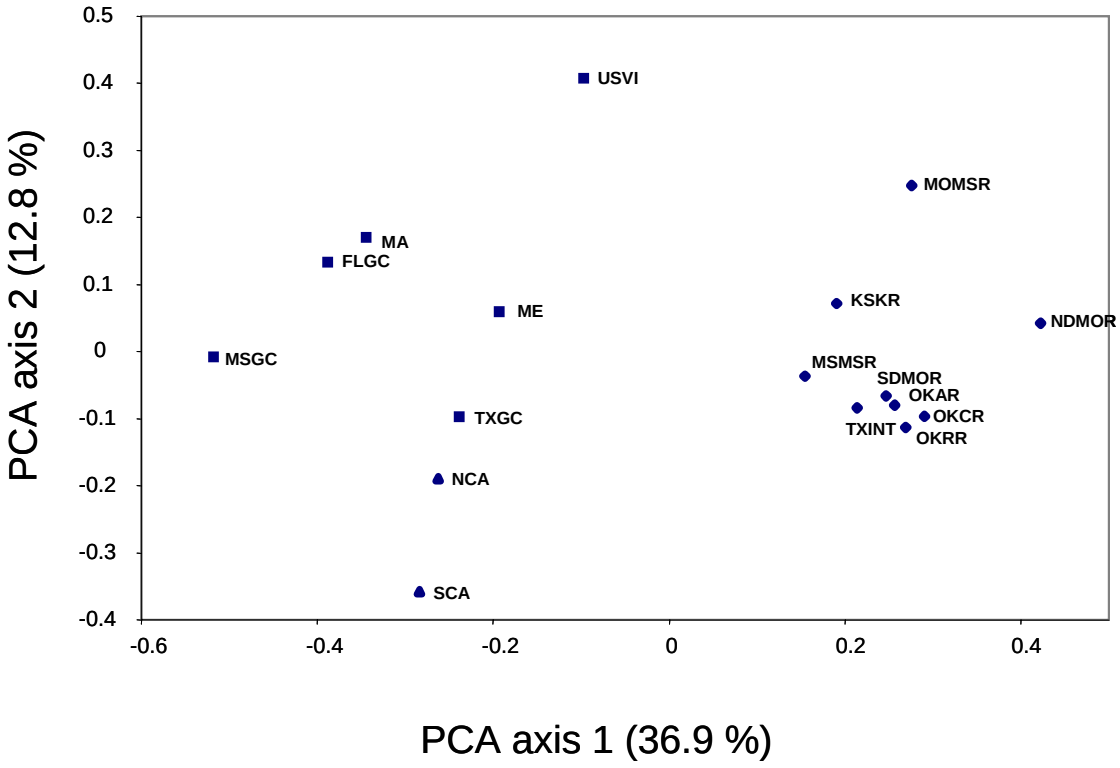
Site	NCA	SCA	NDMOR	SDMOR	KSKSR	MOMOR	OKAR	OKCR	OKRR	TXINT	MSMSR	ME	MA	USVI	MSGC	TXGC	FLGC
NCA		NS	*	*	*	*	*	*	*	NS	*	NS	*	*	NS	NS	*
SCA	0		*	*	*	*	*	*	*	*	*	*	*	*	*	NS	*
NDMOR	0.055	0.056		NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	*	*	*
SDMOR	0.031	0.033	0		NS	NS	NS	NS	NS	NS	NS	*	*	*	*	*	*
KSKSR	0.041	0.042	0.018	0.014		NS	NS	NS	NS	NS	NS	NS	*	NS	*	NS	*
MOMOR	0.050	0.036	-0.004	-0.002	0.014		NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
OKAR	0.035	0.037	0.005	0.000	0.005	-0.001		NS	NS	NS	NS	NS	*	*	NS	NS	NS
OKCR	0.030	0.030	0.006	0.000	0.001	0.004	0.000		NS	NS	NS	*	*	*	*	*	*
OKRR	0.038	0.036	0.019	0.003	0.002	0.013	-0.005	-0.005		NS	NS	NS	*	*	*	*	NS
TXINT	0.026	0.019	0.011	-0.003	0.002	-0.002	-0.008	-0.009	-0.011		NS	NS	*	*	NS	NS	NS
MSMSR	0.023	0.020	0.011	-0.004	0.007	-0.004	-0.013	0.000	-0.004	-0.009		NS	*	*	*	NS	NS
ME	0.016	0.020	0.032	0.018	0.035	0.014	0.027	0.026	0.033	0.016	0.016		NS	*	NS	NS	NS
MA	0.032	0.029	0.065	0.040	0.032	0.036	0.042	0.046	0.038	0.034	0.023	0.011		*	NS	NS	NS
USVI	0.047	0.029	0.037	0.032	0.02	0.011	0.036	0.025	0.032	0.023	0.021	0.020	0.023		NS	NS	NS
FLGC	0.025	0.017	0.064	0.038	0.035	0.031	0.023	0.038	0.039	0.024	0.017	0.010	0.005	0.016		NS	NS
MSGC	0.006	0.009	0.066	0.045	0.038	0.049	0.049	0.044	0.057	0.042	0.029	0.015	0.004	0.024	-0.002		NS
TXGC	0.010	0.023	0.038	0.018	0.027	0.030	0.022	0.019	0.019	0.019	0.012	0.004	0.010	0.026	0.006	-0.002	

Table 2.8. Inferred number of genetic clusters (*K*) for least terns using microsatellite DNA.

K	Ln likelihood	P (*K*|*X*)
1	-9704.4	~0
2	-9607.3	~1
3	-9690.1	~0
4	-9768.4	~0
5	-10204.1	~0

subspecies (California = 63%; East Coast = 73%, respectively). Similarly, principle component analysis revealed a strong grouping of the Interior breeding areas and a widely distributed grouping of California and East Coast breeding areas along both the PC1 axes. The U.S. Virgin Islands breeding area was separated from all other sites primarily along second principle component axis (PC2) (Figure 2.4). Although PC axes 3 and 4 accounted for 10.1% and 8.2% of the variation, respectively, breeding areas did not separate out along these axes.

Figure 2.4. Microsatellite principle component analysis (PCA) showing genetic relationships among least tern breeding areas with 14 or more individuals.



DISCUSSION

Subspecies

Results from the present study of 840 bp of the mtDNA control region and nine microsatellite DNA loci did not support the three traditional subspecies based on our subspecies criteria. Lack of support for traditional subspecies designations is emphasized by the star-like phylogeny of haplotypes that shows no correlation with geography. Although the number of haplotypes restricted to a traditional subspecies was high (58), they occurred at low frequencies (0.01 – 0.19) and 42-71% of individuals within each traditional subspecies shared haplotypes with individuals originating from another subspecies. Values were not large enough to be considered valid under the 75% rule (Amadon 1949). Low levels of differentiation are further reflected in mtDNA pairwise θ_{ST} comparisons between least tern breeding areas (mean $\theta_{ST} = 0.087$), particularly comparisons between Interior and East Coast breeding areas (mean: $\theta_{ST} = 0.073$). Lack of genetic differentiation was also observed using AMOVA analyses where only 7% of the variance in haplotype frequencies was explained by traditional subspecific groupings, a low value relative to those values observed among recognized avian subspecies (0-81%; Fry & Zink 1998; Liebers et al. 2001; Moum & Árnason 2001; Randi et al. 2003; Nicholls & Austin 2005)

One noteworthy finding is population pairwise θ_{ST} values increased when comparisons included a California breeding area (mean: $\theta_{ST} = 0.225$). Slight population subdivision was also observed in the AMOVA analyses where 11% of the variance in haplotype frequencies was explained by a California vs. Interior/East Coast grouping, an increase from that explained by prior subspecific groupings (Table 2.4). As expected, the

moderate level of population subdivision detected for the California breeding areas is not limited to the mtDNA control region, Draheim (Chapter 3) also suggested California subspecific population subdivision using the mtDNA ND6 gene. Likewise, a comparison of ND6 gene AMOVA analysis revealed that more of the variance in haplotype frequencies was explained when breeding areas were grouped into California vs. Interior/East Coast comparisons. Indeed, pairwise comparisons of either California breeding area was larger using ND6 sequences (Chapter 3, mean; $\theta_{ST} = 0.299$) than those observed in the control region for the same breeding areas. The discrepancy between levels of population subdivision between the mitochondrial genes might be explained by the number of parallel/reverse substitutions in the control region that could obscure the initial phylogenetic signal (Posada and Crandall 2001). Alternatively, since ND6 is a coding gene, higher θ_{ST} values might be indicative of adaptive genetic variation (Quinn 1997). It should be noted that although the mtDNA data showed increased subdivision when breeding areas from California subspecies were considered the magnitude of the observed differentiation was in the lower to middle range of values observed among recognized avian subspecies (0.036–0.950; Fry and Zink 1998; Valliantoes et al. 2002; Benedict et al. 2003; Eggert et al. 2004; Idaghdour et al. 2004; Pitra et al. 2004; Solorzano et al. 2004).

Microsatellite data also indicated little population subdivision among the three traditional subspecific designations. As with the mitochondrial DNA data, we found a number of unique alleles (34) but none that occurred at a frequency higher than 3% within a traditional subspecies. Furthermore, observed pairwise θ_{ST} values between subspecies were significant but extremely low (mean: $\theta_{ST} = 0.032$) when compared to

values found in other avian subspecific groupings from microsatellite loci (0.023–0.571; Chan & Arcese 2002; Eggert et al. 2004; Pitra et al. 2004; Jones et al. 2005). The AMOVA analyses also reflected an absence of genetic structure; only 2.57% of the variance in microsatellite allele frequency was explained by traditional subspecific designations.

In contrast to the mitochondrial DNA, microsatellite population pairwise θ_{ST} values did not increase (mean: $\theta_{ST} = 0.025$) when comparisons included a California breeding area. Also, the AMOVA analyses did not improve by dividing breeding areas into a California vs. an Interior/East Coast group, the amount of variance that was explained by a California break was lower than the traditional subspecific groupings (1.44%).

Lack of support for the three traditional subspecific designations is not limited to results in this study, two separate analyses using a variety of genetic makers indicated similar patterns. Using allozymes, Thompson et al. (1992) found no genetic differentiation between the Interior and Eastern subspecies. However, these results should be taken with caution due to the small sample size of the Interior subspecies (n = 4) that all samples originated from Texas, a possible subspecies hybrid zone. To further address the subspecies issue, Whittier (2001) sequenced a portion of the mitochondrial cytochrome-b region and two nuclear intron genes for individuals from the U.S. subspecies (Eastern: n = 17, Interior: n = 22, California: n = 14). MtDNA analyses revealed no genetic differentiation between the three subspecies but one intron gene indicated differences between the California and Interior breeding populations. It should

be noted however, that both genetic markers revealed low amounts of genetic variation (mtDNA haplotypes = 3, nuclear intron = 3 alleles).

In addition to molecular data morphological, behavioral and geographic ranges can also be used to determine if a subspecies is "diagnosable distinct" (Mayr & Ashlock 1991). However, previous least tern studies that examined factors such as vocalizations, behavior, and morphological characteristics found little to no support for differences between traditional subspecies based on such characteristics and argue that distinctions were arbitrary or clinal (Burleigh & Lowery 1942; Massey 1976; Thompson et al. 1992). One morphological study using refined colorimetry concluded validation for the three traditional subspecies using lightness of feathers on the dorsum and hue of feathers on the hind neck (Johnson et al. 1998). However, these results could also be clinal in nature.

Least Tern Gene Flow

Many field studies have investigated dispersal patterns in least terns using banding and resight efforts (Atwood & Massey 1988; Massey & Fancher 1989; Boyd 1993; Renken & Smith 1995). The degree and spatial scale of natal and breeding site fidelity appears to vary among breeding areas. Estimates of natal philopatry ranged from 5-82% where breeding site fidelity ranged from 28-97% (Thompson et al. 1997, and references therein). It has been suggested that variation in site fidelity may depend on behavioral differences due to landscape type (i.e. coastal vs. interior rivers; Renken & Smith 1995) but our genetic data do not support this.

While molecular data suggest frequent gene flow among traditional subspecies, it has not been confirmed in studies using conventional methods (banding/resight records,

satellite transmitters, or radio telemetry) (Boyd 1993; Lingle 1993; Johnson & Castrale 1993). The exception involves a single record of one individual banded on the Gulf Coast of Texas and later found nesting in Kansas (Boyd & Thompson 1985). Most natal dispersal has been found between colonies within traditional subspecies (Boyd 1993; Lingle 1993; Johnson & Castrale 1993). However, this conclusion could be biased as large scale monitoring efforts have not been carried out.

Microsatellite Bayesian clustering and principle component analyses revealed a tight clustering of the Interior breeding areas and a weaker clustering among the California and East Coast breeding areas. Loose genetic clustering of the two coastal subspecies is unexpected given the strong association between genetic and geographic distance ($r = 0.690$, $P = 0.001$; Figure 2.3). However, mixing of the California and East Coast breeders could occur on migration or in winter as has been observed in other tern species (e.g. Royal Terns, *S. maxima*; Buckley & Buckley 2002). Least terns breeding on the Pacific Coast are thought to winter on the west coast of southern Mexico and as far south as Peru. Breeding least terns on the Atlantic Coast winter from eastern Mexico and Central America as far south as northern Brazil (Patten & Erickson 1996; Thompson et al. 1997). California and Gulf Coast least terns migrate along the coast and birds could meet in Central America (possibly in Isthmus of Panama). However, the exact geographic distribution of the least tern wintering range is incomplete and limited band recoveries make resolution difficult.

Conservation Implications

Mitochondrial control region and microsatellite data do not provide support for the existence of traditional subspecies designations within least terns. California, Interior, and East Coast least terns exhibit high genetic connectivity between these groups, however genetic connectivity and demographic connectivity are not synonymous. Only a few migrants each generation are needed to genetically homogenize disparate breeding populations, whereas the same level of movement is not an adequate amount for maintenance of viable populations or for recolonization of an extinct population (Wright 1931, 1940; Mills & Allendorf 1996). In addition, neutral mtDNA control region and microsatellite loci are not likely to reflect adaptive variation expected for different environments or life histories which could impact the potential for evolutionary change.

Although molecular tools have demonstrated an ability to identify evolutionary divergent lineages, decisions to protect species and groupings below the species level should reflect life history, ecology, population dynamics, as well as genetics. It is difficult to put these results in context without appropriate life history data. Our findings emphasize the need for range wide breeding site fidelity and natal philopatry information as well as population-specific movements throughout the annual cycle.

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Chapter 3. Phylogeography, population history, and temporal analysis of least terns (*Sterna antillarum*)

ABSTRACT

We used mitochondrial (mtDNA) sequence data to investigated range wide phylogeographic patterns and demographic history in least terns (*Sterna antillarum*). Sequences (522 bp) of the NADH dehydrogenase subunit 6 gene were obtained from 271 individuals. Phylogeographic analysis revealed no association with geography or correspondence with traditional subspecies designations. However, we found moderate support for population subdivision between California subspecies and all other least tern breeding areas. Mantel tests for "isolation by distance" revealed a moderately significant correlation between genetic distance and geographic distance ($r = 0.460$, $P = 0.001$). A star-like haplotype network, unimodal mismatch distribution, small Harpending's raggedness index (HRI = 0.03, P = 0.93) and a highly significant Fu's F_S (-17.28, P < 0.001) indicate that least terns have undergone a recent population expansion. In addition, we investigated potential changes in genetic diversity between four contemporary (2001-2005) least tern breeding areas and their historical (pre-1912) counterparts. Temporal comparisons revealed significantly lower overall nucleotide diversity in the contemporary breeding areas.

INTRODUCTION

Phylogeography uses geographic distributions and phylogenetic relationships to interpret demographic history and biogeography of natural populations, subspecies, and other grouping units (Avise et al. 1987). Mitochondrial DNA (mtDNA) has proven to be useful for examining phylogeny, phylogeography, and population structure among avian species (Wenink et al. 1994; Gorman 2000; Zink et al. 2000; Abbot & Double 2003a; Abbot & Double 2003b; Buehler & Baker 2003; Randi et al. 2003; Sgariglia & Burns 2003; Haig et al. 2004; Funk et al. in review; and others). The matrilineal mode of inheritance in mtDNA results in an effective population size one fourth that of nuclear alleles that results in high rates of haplotype extinction (Avise 2004). This clonal feature, coupled with high rates of mutation (bias of transition over transversion changes), has made the mtDNA control region particularly useful for examining intraspecific avian phylogeography. However, rapid evolution of the control region can lead to multiple substitutions (i.e., homoplasy), masking phylogenetic signals, which can be problematic in intraspecific analyses (Tameria & Nei 1993; Posada & Crandall 2001). Thus, fast evolving mitochondrial protein coding genes can provide a useful alternative for avian phylogeographic studies. The mitochondrial NADH dehydrogenase subunit 6 (ND6) in particular has a high substitution rate when compared to other coding genes (Desjardis & Morais 1990; Jones & Gibbs 1997).

Least terns (*Sterna antillarum*) are small North American terns (Order Charadriiformes) that nest in colonies of up to 500 pairs on open freshwater or ocean beaches and islands (e.g., exposed sparsely vegetated sand or dried mudflats or river sandbars; Thompson et al. 1997). Historically, least terns were one of the most common

tern species in North America (Thompson et al. 1997). However, direct and indirect anthropogenic pressures on least tern breeding and foraging habitat have caused population declines (United States Fish and Wildlife Service [USFWS] 1980; Burger 1984; USFWS 1990; Kirsch & Sidle 1999; Kruse et al. 2001). As a result, the California (*S. a. browni*) and Interior (*S. a. athalassos*) subspecies are listed as endangered under the U.S. Endangered Species Act (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).

Recent population surveys have identified three disparate breeding regions (Thompson et al. 1997) (Figure 3.1). Pacific Coast breeding colonies range from Central California south to the Mexican border, along both coasts of Baja, and from Sonora south to Oaxaca in Mexico (Patten & Erickson 1996; Thompson et al. 1997). Interior breeding areas includes major inland rivers systems of the United States as well as alkali wetlands of the Great Plains (USFWS 1990; Patten & Erickson 1996; Thompson et al. 1997). East coast breeding colonies range from southern Maine south to Florida, along the Gulf of Mexico from Florida west to Texas, and in Central America from Tamaulipas, Mexico south to Belize, and include Caribbean breeding colonies (i.e. Bahamas, Cuba, Jamaica, Hispaniola, Puerto Rico, and the Lesser Antilles; Patten & Erickson 1996; Thompson et al. 1997).

The taxonomic status of least terns is not well resolved. At least five subspecies of least terns have been described based on morphological characteristics (*S. a. antillarum* [Lesson 1847], *S. a. athalassos* [Burleigh & Lowery 1942], *S. a. browni* [Mearns 1916], *S. a. mexicana* Van Rossem & Hachisuka 1937], and *S. a. staebleri*

[Brodkorb 1940]). Three subspecies in the United States (*S. a. antillarum*, *S. a. athalassos*, and *S. a. browni*) are recognized by the American Ornithologists Union (AOU 1957). However, the taxonomic status of the two Mexican subspecies (*S. a. mexicana* and *S. a. staebleri*) is uncertain (Garcia & Ceballos 1995; Patten & Erickson 1996). Furthermore, with the exception of one morphological study based on colorimetry (Johnson et al. 1998), previous studies using morphology and/or molecular analyses found no support for the three United States subspecies, although most studies had limited sampling (Burleigh & Lowery 1942; Massey 1976; Thompson et al. 1992; Whittier 2001).

Comprehensive genetic studies of wide ranging species, such as least terns, are often constrained by the ability to sufficiently sample throughout the species distribution. Least tern sampling is further hampered by its endangered status. Subsequently, integrating museum specimens permits biologist to fill in any potential sampling gaps as well as evaluate potential temporal changes in genetic diversity and improve our understanding of current patterns of diversity.

In this study, we used sequence data from the mtDNA ND6 gene to obtain range wide phylogeographical patterns in least terns. We applied statistical parsimony analysis to discover patterns of genetic differentiation, specifically to determine if distinct lineages exist and if they correlate with current taxonomy. We also applied methods to reconstruct past population history. In addition, a number of pre-1912 museum specimens from four of the contemporary breeding areas were compared with contemporary samples to evaluate potential temporal changes in genetic diversity.

MATERIALS AND METHODS

Sampling

We obtained 271 least tern samples from throughout their range representing all currently recognized subspecies using several sampling techniques: blood samples from live specimens, salvaged carcasses, embryos from collected eggs, and toe pads from museum specimens. Blood and tissue samples were preserved in 1 ml of tissue storage buffer (100 mM Tris-HCl pH 8, 100 mM EDTA, 10 mM NaCl, and 0.5% SDS) and stored at -80˚C until DNA extraction. Dried toe pad tissue was obtained from the American Museum of Natural History; Museum of Comparative Zoology, Harvard University; Museum of Vertebrate Zoology, University of California Berkeley; and Florida Museum of Natural History, University of Florida. One to 12 samples were collected from 36 breeding areas throughout the least tern breeding range (Figure 3.1, Table 3.1, Appendix 3.1). Sampling areas along coastal breeding areas were defined as a group of individual samples collected within a breeding colony or collected from multiple adjacent colonies. Breeding areas along the interior river breeding distribution were defined as a group of individual samples collected along 50 river miles. Additionally, breeding areas that occurred within the described geographic range of a traditional subspecies were grouped accordingly (Table 3.1). For temporal comparisons historical (pre-1912) museum specimen samples that were originally collected from within 60 kilometers of contemporary (2001-2005) breeding areas were compared with there contemporary counterparts: Barnstable County, Massachusetts; Accomack County, Virginia; Glenn County, Georgia, and San Diego County, California.

Figure 3.1. Map of least tern breeding area included in current study. Breeding areas are listed in Table 3.1. Distributions of currently recognized subspecies are shown in light gray (California least tern), dark gray (Interior least tern) and black (East Coast least tern).

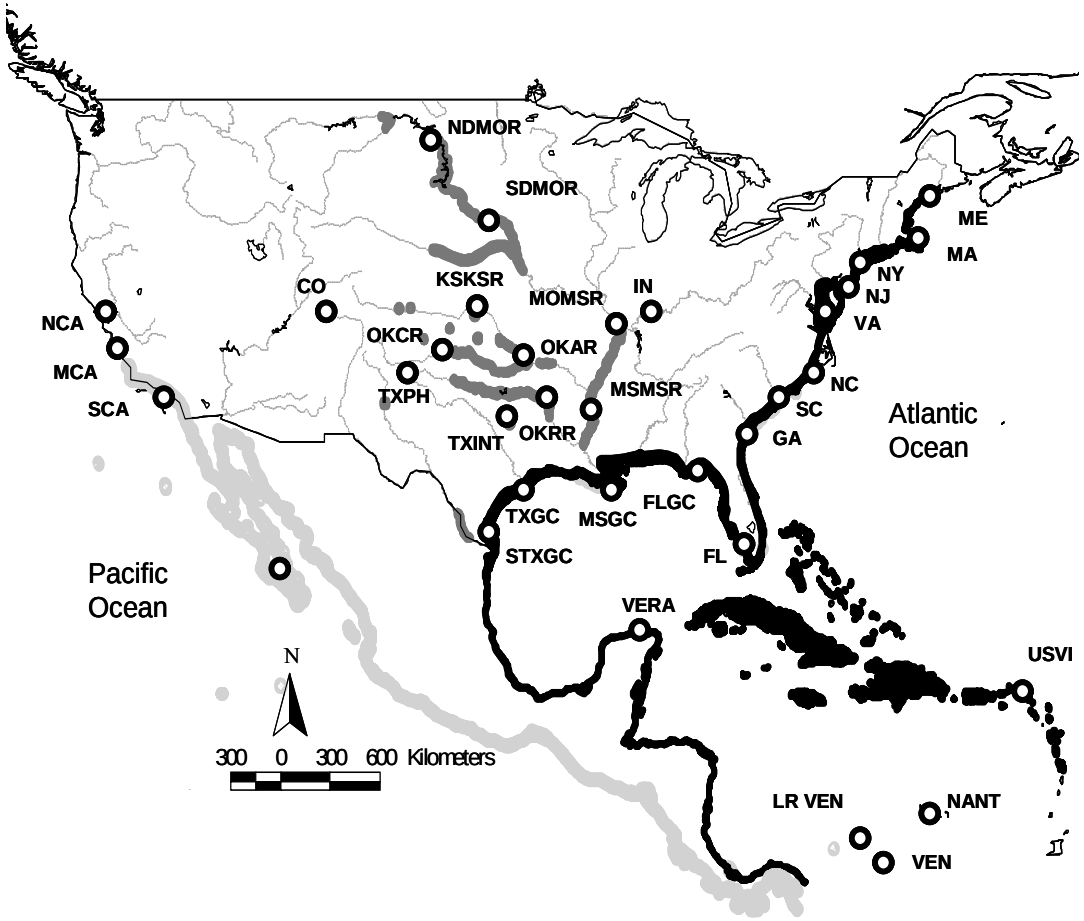


Table 3.1. Breeding area information and within population genetic variation for mtDNA ND6 gene of least terns organized by traditional subspecific designations. Number of individuals sampled (*N*), number of haplotypes, haplotype diversity (*h*), and nucleotide diversity (π) for each breeding area.

Subspecies	Sample Code	County, State/Region, Country	*N*	No. Hap	(*h*)	(π)
California (*S. a. browni*)						
	NCA	Alameda, CA	10	3	0.644	0.0014
	MCA	Monterrey, CA	4	3	0.833	0.0032
	SCA	San Diego, CA	10	2	0.318	0.0010
	HSCA	San Diego, CA (pre-1912)	5	2	0.400	0.0015
	BCS	Baja California Sur, Mexico	2	4	0.533	0.0009
Interior (*S. a. athalassos*)						
	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	0.356	0.0021
	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	0.0033
	TXINT	Dallas, TX	10	1	0.000	0.0000
	MSMSR	Bolivar, MS	10	2	0.200	0.0003
	IN	Gibson, IN	4	1	0.000	0.0000
	CO	Kiowa, CO	3	3	1.000	0.0038
East Coast (*S. a. antillarum*)						
	ME	Knox, ME	10	4	0.778	0.0028
	MA	Barnstable, MA	12	4	0.318	0.0006
	HMA	Barnstable, MA (pre-1912)	4	2	0.500	0.0019
	NY	Suffolk, NY	2	1	0.000	0.000
	NJ	Cape May, NJ	10	4	0.711	0.0028
	VA	Accomack, VA	10	3	0.378	0.0012
	HVA	Virginia Beach, VA (pre-1912)	6	2	0.333	0.0014
	SC	Charleston, SC	4	3	0.833	0.0020
	GA	Glenn, GA	8	4	0.750	0.0004
	HGA	McIntosh, GA (pre-1912)	8	5	0.875	0.0028
	FL	Charlolette, Monroe, Collier, FL	4	3	0.833	0.0023
	USVI	St. Croix, VI	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	Curacao, Bonaire, Netherland Antilles	4	4	1.000	0.0035

Table 3.1 continued

Subspecies	Sample Code	County, State	*N*	No. Hap	(*h*)	(*π*)
	VEN	Cumana, Venezuela	1	1	N/A	N/A
	LRVEN	Los Roques, Venezuela	1	1	N/A	N/A

DNA Extraction, Marker Isolation, and Amplification

DNA extraction --Ten microliters of blood or 1 mm^3 of tissue was digested in 400 µl of extraction buffer A (50 mM Tris pH 8.0, 10 mM EDTA pH 8.0, 200 mM NaCl, 2% SDS) and 20 mg/ml of Proteinase K. Samples were vortexed and incubated overnight at 50°C. DNA was isolated from samples using equal volumes (~ 400 ml) of phenol (saturated with 10 mM Tris pH 8.0) and chloroform/isoamyl alcohol (25:1) followed by an additional cleaning and concentration step using centrifugation dialysis using Microcon 30,000 MW cutoff columns (*Amicon Bioseparation*). DNA was extracted from museum specimens using DNAeasyTM tissue kits (Qiagen). Stock DNA was then rediluted to a concentration of 25-100 ng/µl.

PCR amplification of blood and tissue samples — Initial screening of variation in the mtDNA genome included the control region, ND2, and ND6 gene. Sequencing of a subset of samples representing a gradient of breeding areas throughout the least terns range revealed the control region to be the most variable (see Chapter 2) followed by the ND6 and ND2 gene, respectively. However, initial phylogenetic analysis revealed high homoplasy levels (Homoplasy Index = 0.74; see Figure 2.2) in the control region and lack of variation in the ND2 genes. Therefore, the ND6 gene represented the most promising mitochondrial genetic marker to evaluate phylogeographic structure in least terns.

An ~1800 bp segment of the mtDNA genome, including the ND6 gene and the control region, was amplified by long PCR using conserved mtDNA primers L16087 (5'-TGGTCTTGTAARCCAAARANYGAAG-3'; Desjardins & Morais 1990) and H1248 (5′-CATCTTCAGTGCCATGCTTT-3′; Tarr 1995). Each 50 µL reaction contained

1 mm $Mg(OAc)_2$, 800 µm dNTPs, 0.64 µm of each primer, 1.2 U r*Tth* XL polymerase (GeneAmp XL PCR kit, Roche Molecular Systems, Branchburg, NJ, USA), 1× XL Buffer II and 1 µL template DNA (≈25–100 ng). The PCR profile was 93°C for 1 min; 15 cycles of 93°C for 50 s, 62°C for 10 min; 25 cycles of 93°C for 50 s, 62°C for 10 min + 15 s autoextend per cycle; 72°C for 7 min. Primers LT16130 L and LT16700 H were designed to yield 522 bp of the ND6 least tern sequence (Table 3.2). Amplifications for all tissue and blood extractions were performed using a PTC 100 thermal cycler (MJ Research). A total reaction volume of 50 µl was used with the following concentrations: 10mM Tris-HCl at pH 8.3; 50mM KCl; 0.001% gelatin; 3.5mM $MgCl_2$; 100µM for each of the dNTPs; 0.2µm 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 35 cycles of 30 seconds at 93°C, annealing at 50°C for 30 seconds, and elongation at 72°C for 1 min. A final 10 min. period of elongation at 72°C followed the last cycle.

PCR amplification of ancient DNA—The degraded DNA obtained from museum specimens called for design of internal primers to amplify shorter overlapping sequences (150-200 bp; Table 3.2). A total reaction volume of 50 µl was used with the following concentrations: 10mM Tris-HCl at pH 8.3; 50mM KCl; 0.001% gelatin; 3.5mM $MgCl_2$; 100µM for each dNTP; 0.4um of each primer; 100 ng of template; and 3.0 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 seconds at 93°C, annealing at 50°C for 45 seconds, and elongation at 72°C for 1 min. A final 10 min. period of elongation at 72°C followed the last cycle.

Table 3.2. Least tern ND6 sequencing primers used in the present study.

Primer Name	Primer sequence (5'-3')
LT16130 L	CTTAAACCTCTATCTCCAACT
LT16280 L	CACCAACTCCAACACAACAAA
LT16310 H	TTTTGGTAGCAGGTTGGG
LT16450 L	TGTAACTACTCCCAAATCC
LT16480 H	TGGTTATGGGTGGAGTTG
LT16550 L	TCCTCAAGCCTCTGGAAA
LT16580 H	GTGTATTCTGTGTCCTTGG
LT16660 L	TAACAATCACCCACACCC
LT16775 H	AATCCTTCTCCGTATTATGG
LT16700 H	GAGTGTCATGGATGGGTA

PCR amplification quality was assessed by visualizing 10 µl of the product with ethidium bromide on 1% agarose gels. Successful PCR reactions were cleaned and concentrated by centrifugation dialysis using Microcon 30,000 MW cutoff filters (Amicon Bioseparations). Sequences were generated using ABI Prism Big Dye Terminator Cycle Sequencing chemistry on an ABI 3730 capillary DNA analyzer located in the Central Services Laboratory at Oregon State University. Ambiguities were resolved by comparing light and heavy-strand sequences or from overlap of different fragments. Sequences were aligned by eye using BIOEDIT (version 7.0.5) alignment software (Hall 1999). All sequences were archived in GenBank (Accession nos ##-##).

Statistical Analysis

We used DNASP version 4.0 (Rozas et al. 2003) to calculate haplotype diversity (*h*), the probability that two randomly chosen individuals have different haplotypes; and nucleotide diversity (π), the average pairwise nucleotide differences for ND6 haplotypes. Relationships between haplotypes were inferred by estimating a statistical haplotype tree network with 95 percent parsimonious connection using the program TCS 1.13 (Clement et al. 2000).

Demographic factors were inferred by comparing mismatch distributions of pairwise nucleotide differences among ND6 haplotypes and calculating Rogers's (1995) model of sudden population expansion. Deviations from the sudden population expansion model were further tested using Harpending's Raggedness index (HRI; Harpending 1994). Tests of assumptions of neutrality were also performed by Tajima's *D* (Tajima 1989) and Fu's F_S (Fu 1997). A large significant value of D (positive or

negative) can indicate deviation from neutrality which can potentially be used to make inferences about demographic processes (i.e. population expansion or historical bottlenecks). Similarly, a large significant negative value of Fu's F_S may be indicative of a population expansion. All tests were performed using ARLEQUIN version 2.0. (Schneider et al. 2000). Sampling museum tissue provided an opportunity to evaluate potential temporal changes in genetic diversity among some breeding areas. Diversity indices (h, π) for the historical and contemporary Barnstable County, Massachusetts; Accomack County, Virginia; Glenn County, Georgia; and San Diego County, California breeding areas were compared using Welch's approximate t-test, which corrects for unequal sample size and variance (Welch 1938; Sokal & Rohlf 1995)

We assessed molecular variance among samples obtained from 2000-2005 using separate analyses of molecular variance (AMOVA; Excoffier et al. 1992) at different hierarchical levels among samples (within, among breeding areas, among subspecies). Although using F_{ST} is prone to pitfalls for evaluating gene flow it remains a useful index for comparative purposes (Neigel 2002). Therefore, to examine genetic differentiation we used Weir and Cockerham's (1984) estimate of F_{ST} (θ_{ST}), to estimate global $\theta_{ST,}$ pairwise θ_{ST} between traditional subspecies, and pairwise θ_{ST} between breeding areas implemented in ARLEQUIN version 2.0. (Schneider et al. 2000). We used sequential Bonferroni corrections for multiple tests (Rice 1989). A Mantel test (Mantel 1967) was calculated using program ISOLDE in GENEPOP (version 3.4) to evaluate isolation by distance (Raymond & Rousset 1995).

RESULTS

Phylogenetic Analysis

ND6 sequences (522 bp) were characterized by 17 polymorphic sites: 16 sites were transitions, 1 site was a transversion, and no insertions or deletions were present. Twenty-six unique haplotypes were observed among 271 individuals. Haplotype 1 dominated most breeding areas occurring at a 52% frequency, all other haplotypes occurred in lower frequencies (0.3 – 8%). Fifteen of the 25 (60%) unique haplotypes were only observed within a specific traditional subspecies (California = 1, Interior =2, East Coast = 12, respectively). Eight of the 25 (32 %) unique haplotypes were restricted to a single breeding area (Table 3.3). The 95 % parsimony network generated by TCS revealed a star like pattern with haplotypes occurring on short branches radiating from a central haplotype (Figure 3.2). Most haplotypes were separated by only one or two base substitutions suggesting a recent population expansion.

Mismatch distribution and test for neutrality--Distributions of pairwise differences between haplotypes for all recent samples indicates a smooth curve as predicted for sudden expansion, thus it did not reject Rogers (1995) model of sudden expansion ($P > 0.93$) (Figure 3.3). Furthermore, Harpending's Raggedness index was low indicating significant fit of the observed and expected distributions (HRI = 0.03, P = 0.93). Fu's F_S test of all recent samples supported population expansion (-17.28, $P < 0.001$), whereas Tajima's D was negative but not significant (-1.33, $P < 0.06$).

Population Subdivision--Genetic differentiation among contemporary breeding areas with 8 or more individuals estimated by θ_{ST} was 0.11 ($P < 0.05$; Table 3.4).

Table 3.3. Distribution of 25 mtDNA ND6 haplotypes among all least tern breeding areas. Asterisks denote breeding areas with museum specimen sampling. Shaded breeding areas indicate temporal comparisons for San Diego County, California; Barnstable County, Massachusetts; Accomack County, Virginia; and Glenn County, Georgia.

Haplotype	29	68	93	177	199	218	234	246	354	381	409	435	436	438	447	507	515
H1	A	A	T	C	G	C	T	C	A	C	G	C	A	C	C	T	A
H2	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.
H3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
H4	.	.	.	T	.	.	.	.	.	.	A	.	.	T	.	.	.
H5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
H6	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
H7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
H8	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
H9	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
H10	.	G	.	.	.	.	.	.	G	.	.	.	.	.	.	C	.
H11	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
H12	.	G	.	.	.	.	.	.	G	.	.	.	G	.	.	C	.
H13	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
H14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
H15	.	G	.	.	.	.	.	.	G	.	.	T	.	.	.	C	.
H16	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H17	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.
H18	.	.	.	T	.	.	.	.	G	.	.	.	.	.	.	.	.
H19	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.
H20	.	.	.	T	C	.	.	T	.	.	.	.	.	.	.	.	.
H21	.	G	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.
H22	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.
H23	G	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.
H24	.	G	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.
H25		G	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.

	S. a. browni					*S. a. athalassos*												*S. a. antillarum*																			
Haplotype	NCA	MCA*	SCA	HSCA*	BCS*	NDMOR	SDMOR	KSKSR	MOMOR	OKCR	OKAR	OKRR	TXPH	TXINT	MSMSR	IL	CO	ME	MA	HMA*	NY*	NJ	VA	HVA*	SC*	GA	HGA*	FL*	USVI	NANT*	FLGC	MSGC	TXGC	STXGC*	VERACRUZ	VEN*	LRVEN*
H1	5		**4**		1	10	5	8	6	6	4	8	4	10	9	4	1	4	**7**		2	3	**8**	**1**		**4**	**2**	1	6		5	6	5	4			
H2	4		**7**	**4**																				**1**			**2**			1							
H3	1																														1	1					
H4				**1**																																	
H5		1			4																						**1**										
H6		2						1	3	2	3		1		1		1	2	**1**													2	1	1			
H7		1			1																			**1**	**2**		**1**	2						1			
H8																																	1				
H9										1							1	3	**2**			1	**1**	**2**	**1**	**2**			2	1	1	1	2		1		
H10							5	1		1	2	2	2									1															
H11											1																										
H12									1																												
H13																			**2**			5	**1**			**1**			2		2		1				
H14																		1																			
H15																										**1**											
H16																														1							
H17																									**1**					1							
H18																				**1**																	
H19																				**2**								1									
H20																				**1**				**1**													
H21																											**1**										
H22																											**1**										
H23																																		1			
H24																																					1
H25																																				1	
Total	10	4	11	5	6	10	10	10	10	10	10	10	7	10	10	4	3	10	12	4	2	10	10	6	4	8	8	4	10	4	9	10	10	7	1	1	1

Figure 3.2. The statistical 95 % parsimony network generated by TCS based on ND6 least tern haplotypes. Circle sizes are proportional to the number in individuals sharing the haplotype. Shades refer to the proportion samples that came from a traditional subspecies designation. California least tern shown in white, Interior least tern shown in gray, and East Coast least tern shown in black. Black dashes are inferred haplotypes.

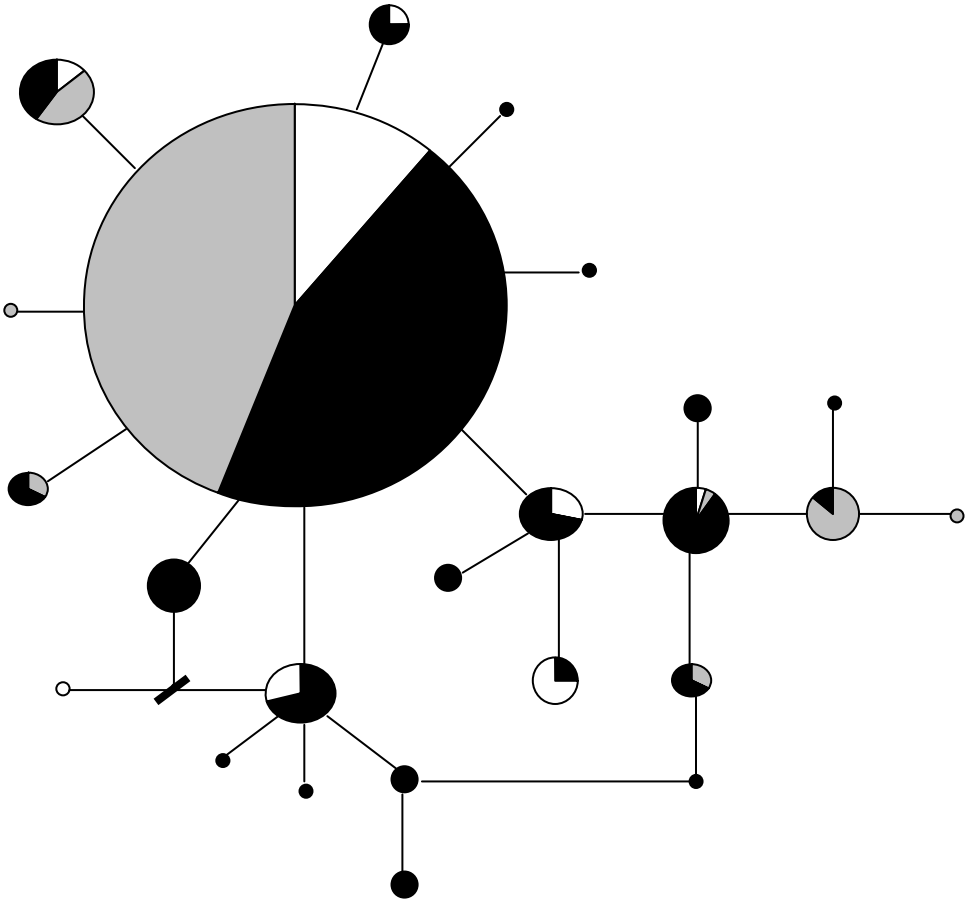
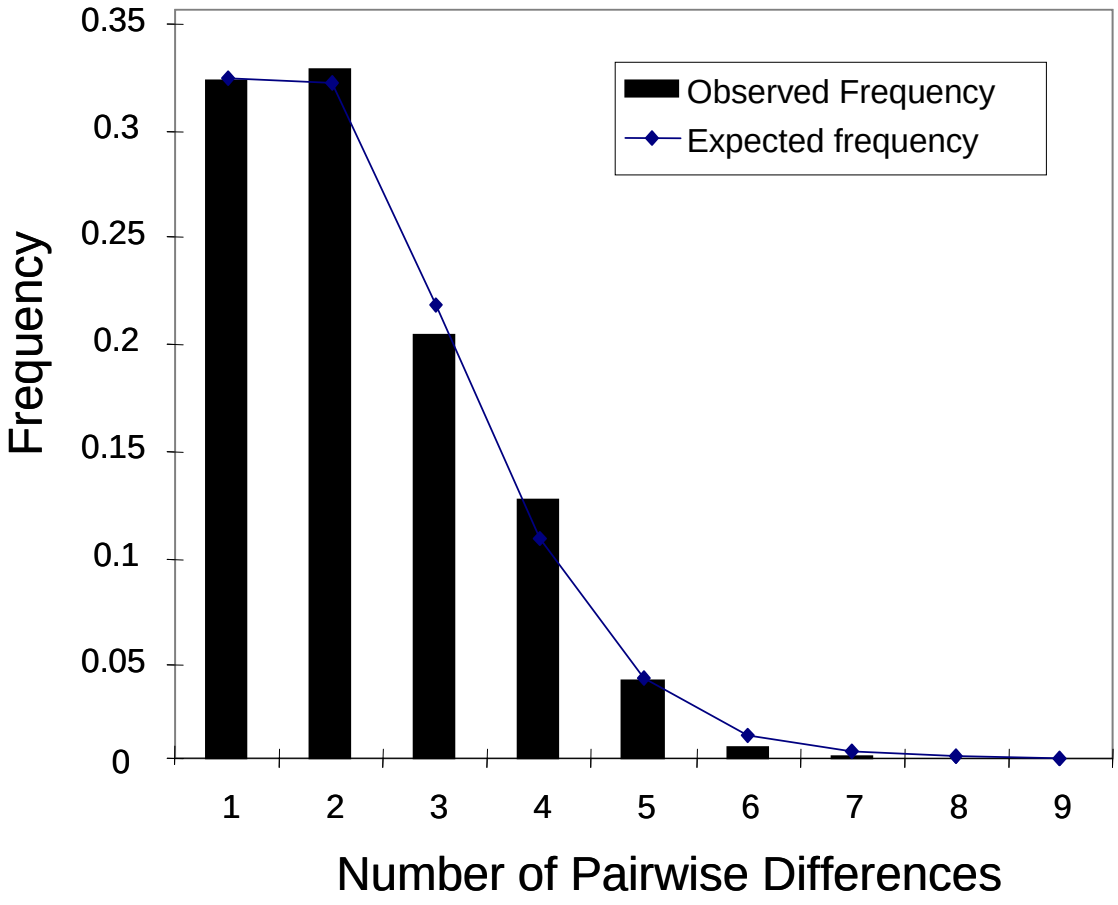


Figure 3.3. Pairwise mismatch distribution for current least tern ND6 haplotypes. Frequency distribution of pairwise nucleotide differences among least terns (bars) and distribution expected under a sudden expansion model (filled diamonds).



Pairwise θ_{ST} values for subspecies comparisons ranged from 0.025 to 0.154 (Table 3.5) and breeding area comparisons ranged from 0.0 to 0.59 (Table 3.6). Significant θ_{ST} values ($P < 0.05$) were found for most pairwise comparisons including either California subspecies breeding areas. A Mantel test revealed a moderate but significant proportion of the variation in genetic distance among breeding areas was explained by geographic distance ($r = 0.460$, $P = 0.001$; Figure 3.4).

AMOVA indicated that the majority of the variation in haplotype variation was explained within breeding areas (Table 3.4). When breeding areas within subspecies were considered, hierarchical analysis showed no structure for the California and East Coast subspecies ($\theta_{ST} = 0.011$, $P < 0.$ 0.187; $\theta_{ST} = 0.014$, $P < 0.$ 0.267; respectively). However, there was shallow differentiation between breeding areas in the Interior subspecies ($\theta_{ST} = 0.082$, $P = 0.038$). Hierarchical analysis using traditional subspecific groupings indicated 85% of the total variance ($\theta_{ST} = 0.146$, $P < 0.$ 0.001) was explained by variation within breeding areas, 5% was explained by variation among breeding areas within subspecies ($\theta_{SC} = 0.049$, $P < 0.05$), and only 10% was explained by variation among groups ($\theta_{CT} = 0.102$, $P < 0.$ 0.001). The highest variation among groups was found when breeding areas were grouped into California vs. Interior/East Coast breeding areas ($\theta_{CT} = 0.226$, $P < 0.$ 0.001; Table 3.4).

Temporal Analysis— Nucleotide diversity was significantly higher in the historical samples ($t' = -2.994$, $P = 0.03$ Figure 3.5). Haplotype diversity was generally higher but not significant in the historical samples ($t' = -0.539$, $P = 0.60$; Figure 3.5). If least terns have experienced a loss of haplotype diversity over the last hundred years, we would expect a pattern in which: 1) the historical samples consists of an array of common

contemporary and unique historical haplotypes and 2) the contemporary samples would consist of fewer haplotypes with a bias towards the common haplotypes. Seven historical haplotypes (H4, H7, H18, H19, H20, H21, and H22) were not present in the contemporary samples. However, temporal comparison revealed haplotype composition from both time periods deviated from expectations suggesting sampling bias. For example, Barnstable County, Massachusetts, comparison revealed no overlap in haplotype composition. Also, three contemporary haplotypes including H13, which occurred at a high frequency, are absent from the historic breeding areas.

Table 3.4. Analysis of molecular variance (AMOVA) at the mtDNA ND6 gene of least tern samples from 2000-2005.

Groups	df	% var	θ	*P*
All breeding areas				
Among breeding areas	20	11.13	$\theta_{ST} = 0.111$	$P < 0.001$
Within breeding areas	186	88.87		
California breeding areas				
Among breeding areas	1	1.15	$\theta_{ST} = 0.012$	$P = 0.187$
Within breeding areas	19	98.85		
Interior breeding areas				
Among breeding areas	9	8.18	$\theta_{ST} = 0.082$	$P = 0.038$
Within breeding areas	97	91.62		
Eastern breeding areas				
Among breeding areas	8	1.39	$\theta_{ST} = 0.014$	$P = 0.267$
Within breeding areas	80	98.61		
Traditional Subspecies				
Among subspecies	2	10.21	$\theta_{CT} = 0.102$	$P < 0.001$
Among breeding areas within subspecies	18	4.42	$\theta_{SC} = 0.049$	$P = 0.047$
Within breeding areas	186	85.37	$\theta_{ST} = 0.147$	$P < 0.001$
California vs. Interior/East Coast groups				
Among groups	1	22.63	$\theta_{CT} = 0.226$	$P < 0.001$
Among breeding areas within groups	19	4.76	$\theta_{SC} = 0.062$	$P = 0.046$
Within breeding areas	186	72.61	$\theta_{ST} = 0.274$	$P < 0.001$

Table 3.5. Pairwise θ_{ST} values among traditional least tern subspecies (2000-2005 samples) of mtDNA ND6 sequences (below diagonal) and statistical significance ($P < 0.05$, above the diagonal).

	California	Interior	East Coast
California		*	*
Interior	0.158		*
East Coast	0.144	0.025	

Table 3.6. Pairwise θ_{ST} values among sites of for mtDNA ND6 sequences 2000-2005 least tern breeding areas (below diagonal) and statistical significance ($P < 0.05$, above the diagonal).

Site	NCA	SCA	NDMOR	SDMOR	MOMOR	KSKSR	OKAR	OKCR	OKRR	TXINT	MSMSR	ME	MA	NJ	VA	GA	USVI	MSGC	TXGC	FLGC
NCA		*	NS	*	*	*	*	*	*	NS	NS	*	*	*	NS	*	*	*	NS	*
SCA	0.110		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
NDMOR	0.267	0.586		NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	*	NS	*	NS	NS
SDMOR	0.400	0.499	0.444		NS	NS	NS	NS	NS	*	*	NS	*	*	*	NS	NS	*	*	NS
MOMOR	0.167	0.342	0.095	0.228		NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
KSKSR	0.148	0.372	0.000	0.229	-0.054		NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
ORAR	0.178	0.324	0.133	0.105	-0.071	-0.038		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
OKCR	0.167	0.342	0.095	0.137	-0.073	-0.076	-0.087		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
OKRR	0.182	0.368	0.111	0.089	0.011	-0.061	-0.029	-0.061		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TXINT	0.267	0.586	0.000	0.444	0.095	0.000	0.133	0.095	0.111		NS	NS	NS	*	NS	*	NS	*	NS	NS
MSMSR	0.222	0.512	0.000	0.417	0.009	-0.042	0.071	0.035	0.095	0.000		NS	NS	*	NS	*	NS	NS	NS	NS
ME	0.206	0.358	0.173	0.123	-0.013	-0.004	-0.046	-0.076	-0.014	0.173	0.120		*	NS	NS	NS	NS	NS	NS	NS
MA	0.204	0.448	0.041	0.407	0.045	0.002	0.095	0.063	0.105	0.041	-0.011	0.136		NS	NS	*	NS	NS	NS	NS
NJ	0.267	0.404	0.267	0.217	0.146	0.128	0.111	0.101	0.095	0.267	0.242	0.110	0.119		NS	NS	NS	NS	NS	NS
VA	0.167	0.409	0.000	0.292	0.028	-0.061	0.044	-0.014	-0.016	0.000	0.000	0.033	-0.033	0.077		NS	NS	NS	NS	NS
GA	0.230	0.371	0.230	0.005	0.070	0.041	-0.001	-0.025	-0.044	0.230	0.206	-0.049	0.179	0.022	0.047		NS	NS	NS	NS
USVI	0.182	0.368	0.111	0.196	0.044	-0.014	0.028	-0.023	-0.026	0.111	0.095	-0.014	0.027	-0.022	-0.068	-0.044		NS	NS	NS
MSGC	0.137	0.356	0.064	0.254	0.040	-0.017	0.046	0.004	0.005	0.064	0.052	0.036	-0.033	-0.015	-0.077	0.018	-0.085		NS	NS
TXGC	0.138	0.360	0.044	0.272	-0.068	-0.070	-0.034	-0.068	0.009	0.044	-0.032	-0.019	0.012	0.147	-0.023	0.068	0.009	-0.016		NS
FLGC	0.148	0.326	0.063	0.173	-0.023	-0.056	-0.029	-0.074	-0.043	0.063	0.031	-0.061	0.020	0.035	-0.062	-0.040	-0.081	-0.056	-0.0493	

Figure 3.4. Scatter plot of mtDNA ND6 haplotype genetic distance among 2000-2005 least tern breeding areas versus the natural logarithm of geographic distance (Mantel test; $r = 0.460$, $P = 0.001$).

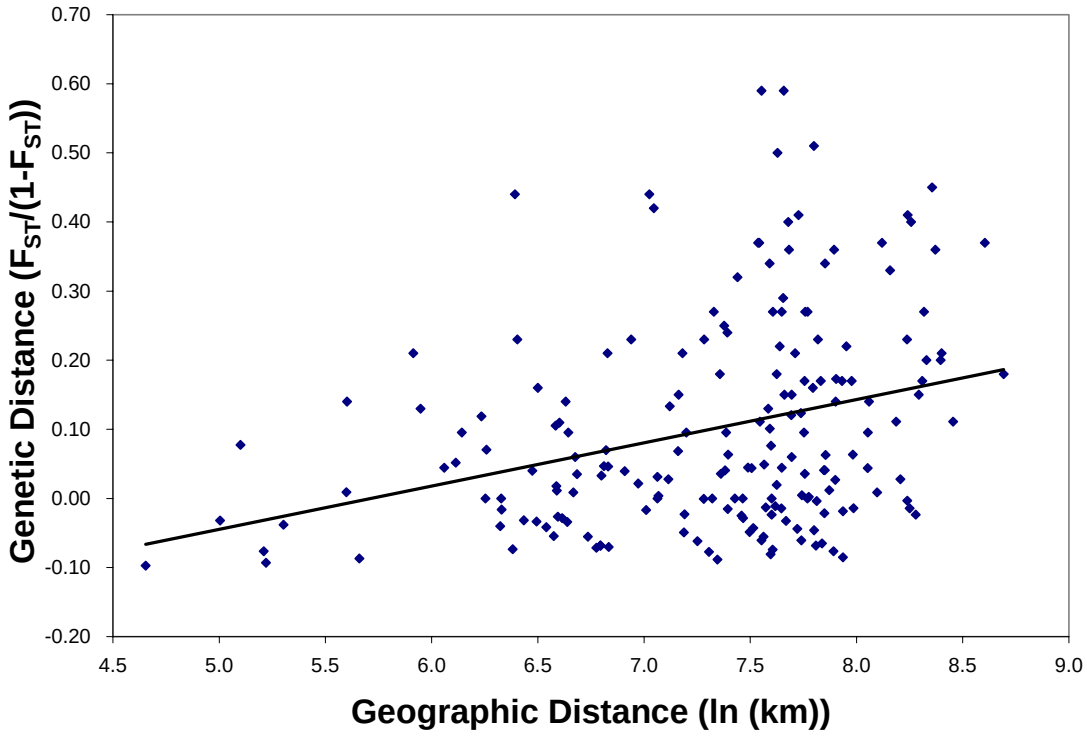
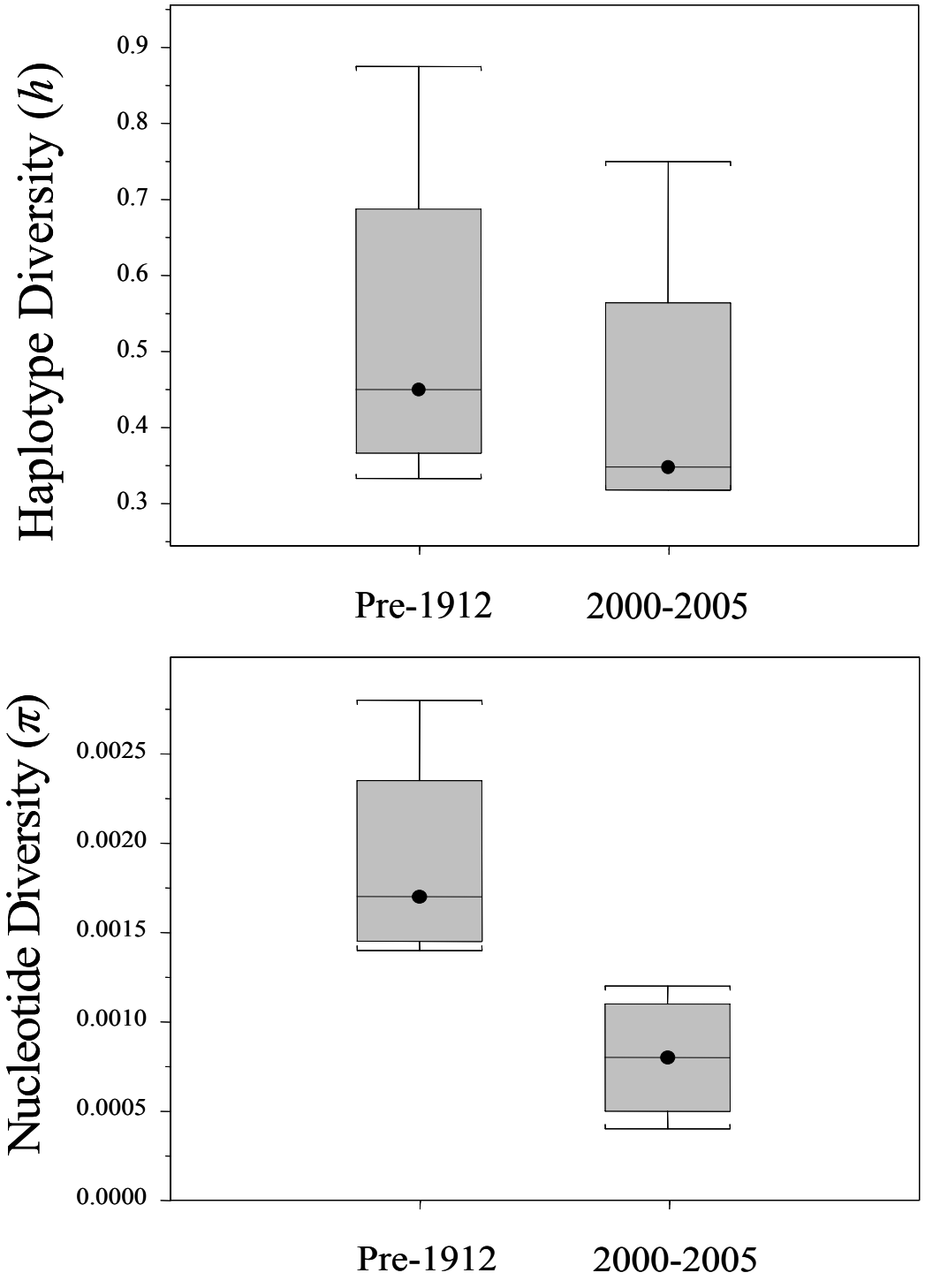


Figure 3.5. Haplotype and nucleotide diversity for temporal comparisons of historic (pre-1912) and contemporary (2000-2005) least tern breeding areas: Barnstable County, Massachusetts; Accomack County, Virginia; Glenn County, Georgia; and San Diego County, California (Box plots: dots = median, box = interquartile range, lines = minimum and maximum values).



DISCUSSION

Population history

Phylogeographic analysis of least tern mitochondrial ND6 sequences across their breeding range revealed no association with geography or correspondence to traditional subspecies designations. However, a Mantel test for "isolation by distance" revealed a moderately significant correlation between genetic distance and geographic distance. The ND6 haplotype network exhibited a star like pattern with haplotypes occurring on short branches radiating from a common central haplotype, a pattern consistent with a population expansion. Population expansion was confirmed by a unimodal mismatch distribution, a small non-significant Harpending's Raggedness index (HRI = 0.03, P = 0.93), and by a large negative Fu's F_S (-17.28, P < 0.001). This pattern has been observed in a number of North American avian species undergoing population expansions (Swainson's thrush *Catharus ustulatus*, Ruegg & Smith 2002; MacGillivray's warbler *Oporornis tolmiei*, Mila et al. 2000; Wood duck *Aix sponsa* Peters et al. 2005; and others).

Intraspecific geographic variation among Nearctic avian taxa has been linked to the Late Pleistocene (Avise & Walker 1998; Avise et al. 1998; Johnson & Cicero 2004; but see Klicka & Zink 1997, 1999; Zink et al. 2004). Avian species that have undergone such glacial induced vicariant events have shown patterns of distinct phylogroups and/or shallow genealogies exhibiting mutational patterns consistent with a population expansion. At the glacial maximum of the Wisconsin glaciation (~18,000 years before present) the most northern Interior and East Coast breeding areas were covered by the Laurentide ice sheet (Dawson 1992). A retreat of least terns to their southern breeding

distributions during the glacial periods and a northward range expansion during postglacial warming could account for the low genetic diversity and evidence for a population expansion revealed by the ND6 data. However, these patterns can be obscured by the amount of gene flow or dispersal capability in least terns.

Genetic variation and historical samples

Least terns have undergone two recent bottlenecks. During the late 1800's and early 1900's, least terns where almost extirpated due to demand for feathers for the millenary trade. Population numbers started to rebound, however, after the passing of the Migratory Bird Treaty Act in 1918 (Thompson et al. 1997). Populations declined again during the 1960's and 1970's due to habitat loss via river channel augmentation, irrigation diversions, dam construction, housing development and subsequent recreation (USFWS 1980; Burger 1984; USFWS 1990; Kirsch & Sidle 1999; Kruse et al. 2001).

Genetic diversity comparisons of historical and contemporary breeding areas revealed significantly lower nucleotide diversity in the contemporary samples for Barnstable County, Massachusetts; Accomack County, Virginia; Glenn County, Georgia; and San Diego County, California. In addition, seven historical haplotypes (H4, H7, H18, H19, H20, H21, and H22) were not present in the contemporary samples suggesting loss of genetic diversity. While this comparison suggests that least terns have lost genetic diversity over the past century, this conclusion is problematic. Haplotype composition from both time periods deviated from expectations suggesting sampling bias. For example, the Barnstable County, Massachusetts comparison, revealed no overlap in haplotype composition. Also, three contemporary haplotypes are absent from the historic

breeding areas and it is unlikely that these haplotypes have evolved over the past 100 years (mtDNA substitution rate of ~2% per million years; Klicka & Zink 1997 and references therein). Most historical sample sizes are half that of present day samples and are prone to sample error caused by small sample size.

Current Population structure

Similar to phylogenetic patterns, population genetic structure analysis indicated a lack of concordance between traditional subspecies designations and genetic differentiation. Hierarchical analysis, making assumptions about subspecific groupings indicated that only 10% of the variation was explained by traditional subspecific designations. Additional AMOVA analyses revealed a greater amount of the variation in haplotype frequency was explained when breeding areas were grouped into California vs. Interior/East Coast breeding areas. The California vs. Interior/East Coast spilt is further supported by a majority of pairwise θ_{ST} values between the two California breeding areas and the remaining Interior and East Coast breeding areas. These values were higher and statistically significant suggesting some restriction to female-mediated gene flow. Unfortunately, small sample sizes prevented analysis of genetic differentiation for breeding areas outside the continental U.S., with exception of the U.S. Virgin Islands.

Lack of mtDNA phylogeographic structure over large distances is not uncommon in many colonial nesting species (e.g., shy albatrosses *Thalassarche cauta*, Abbot & Double 2003; Audouin's gull *Larus audouinii* Genovart et al. 2003; snowy plovers *Charadrius alexandrinus*, Funk et al. in review), including species with high natal philopatry (e.g., black-legged dittiwake *Rissa tridactyla*, McCoy et al. 2005; common

guillemot *Uria aalge*, Moum & Árnason 2001; and Buller's albatross *Thalassarche bulleri* Van Bekkum et al. 2005).

On the surface, there appears to be lack of concordance between demographic and genetic studies in least terns. Field studies investigating dispersal patterns in least terns using banding and resight efforts reported that the degree and spatial scale of fidelity to natal sites ranges greatly (5-82%; Atwood & Massey 1988; Massey & Fancher 1989; Boyd 1993; Renken & Smith 1995; Thompson et al. 1997). For example, Boyd and Thompson (1985) found a chick banded on the Texas Coast nesting in Kansas. However Boyd (1993) found high natal philopatry for banded chicks in Kansas and Northwestern Oklahoma (82%).

The ND6 sequence data is consistent with previous studies using morphological and/or molecular analyses which found no support for separate subspecific status of Interior and East Coast least terns (Burleigh & Lowery 1942; Massey 1976; Thompson et al. 1992; Whittier 2001; Draheim (Chapter 2). However, we did find mild support of population subdivision between California subspecies and all other least tern breeding areas. These ND6 results should be interpreted with caution. If geographic structure exists, a recent population expansion could cloud the signal making it difficult to separate population structure from recent and historical gene flow. Given the shared history of the three subspecies it is clear that the mtDNA ND6 gene is not informative enough to reflect potential genetic structure.

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Appendix 1.1. Voucher numbers, sampling location, and collection date for least tern museum specimens used in the present study. Prefixes represent the institutions from which the samples were collected. AM = American Museum of Natural History; MCZ = Museum of Comparative Zoology, Harvard University; FLMNH = Florida Museum of Natural History, University of Florida; and MVZ = Museum of Vertebrate Zoology, University of California Berkeley.

Voucher Number	Breeding Area	Country	Date
AMNH747034	Curacao	Aruba	1892
AMNH747036	Curacao	Aruba	1892
AMNH747035	Curacao	Aruba	1892
AMNH747037	Curacao	Bonaire	1992
AMNH188068	Cumana	Venezuela	1925
AMNH816441	Los Roques	Venezuela	1976
AMNH808740	Vera Cruz	USA	1904
AMNH753162	Pacific Beach, CA	USA	1912
AMNH753166	Pacific Beach, CA	USA	1912
AMNH753169	Pacific Beach, CA	USA	1912
AMNH139885	L.A. Coast, CA	USA	1908
AMNH753167	Pacific Beach, CA	USA	1912
AMNH26026	Chatham, MA	USA	1885
AMNH26024	Chatham, MA	USA	1885
AMNH26019	Chatham, MA	USA	1885
AMNH26020	Chatham, MA	USA	1885
AMNH79345-374	Corpus Christi, TX	USA	1886
AMNH79346	Corpus Christi, TX	USA	1887
AMNH79344	Corpus Christi, TX	USA	1888
AMNH79343	Corpus Christi, TX	USA	1886
AMNH79340	Corpus Christi, TX	USA	1882
AMNH747041	VA	USA	1885
AMNH80194	Bone Island, VA	USA	1905
AMNH747030	Cobbs Island, VA	USA	1885
AMNH747031	GA	USA	1888
AMNH48523	Cumberland, GA	USA	1915
AMNH747032	GA	USA	1888
AMNH17082	Amelia Island, FL	USA	1906

Appendix 1.1 (Continued)

Voucher Number	Breeding Area	Country	Date
AMNH17083	Amelia Island, FL	USA	1906
AMNH17560	New Smyrna, FL	USA	1899
AMNH359014	Amelia Island, FL	USA	1905
AMNH359013	Amelia Island, FL	USA	1905
FLMNH3629	Charlotte, FL	USA	1958
FLMNH2017	Collier, FL	USA	1902
FLMNH15848	Monroe, FL	USA	1968
FLMNH41910	Monroe, FL	USA	2000
MCZ33031	Cape Henry, VA	USA	1884
MCZ33032	Cape Henry, VA	USA	1884
MCZ33033	Cape Henry, VA	USA	1884
MCZ210427	Sullivan's Island, SC	USA	1885
MCZ210428	Sullivan's Island, SC	USA	1885
MCZ210543	Sullivan's Island, SC	USA	1885
MCZ210544	Sullivan's Island, SC	USA	1885
MVZ54739	Baja California Sur	Mexico	1929
MVZ54740	Baja California Sur	Mexico	1929
MVZ54741	Baja California Sur	Mexico	1929
MVZ54742	Baja California Sur	Mexico	1929
MVZ54743	Baja California Sur	Mexico	1929
MVZ54744	Baja California Sur	Mexico	1929
MVZ91711	Monterey, CA	USA	1915
MVZ91713	Monterey, CA	USA	1936
MVZ91714	Monterey, CA	USA	1936
MVZ91715	Monterey, CA	USA	1936
MVZ101466	McIntosh, GA	USA	1881
MVZ145331	Suffolk, NY	USA	1926
MVZ145333	Suffolk, NY	USA	1928

Chapter 4. Conclusions

4.1 Summary

This thesis provides the most comprehensive examination of phylogeographic patterns and gene flow of least terns to date. The results of this study indicate little evidence to support the uniqueness of the three traditional least tern subspecies designations based on mitochondrial and microsatellite DNA. Collectively, the results from this study should provide impetus for least tern taxonomy to be revisited by the American Ornithologist Union.

4.2 *Taxonomic evaluation and gene flow*

- MtDNA control region sequences and microsatellite DNA data provided no support for the three traditional U.S. least tern subspecies. Furthermore, gene flow between the three traditional subspecies is high. Genetic patterns observed for both molecular markers are characterized by isolation by distance.
- Levels of gene flow between breeding areas are sufficient to homogenize disparate breeding areas. However, the control region sequences did reveal moderate restriction of female mediated gene flow between California least terns and the Interior/East Coast least terns.
- Subspecies taxonomy needs to be revisited by the AOU Committee and Taxonomy and Nomenclature.

4.3 *Phylogeography, demographic history and temporal analysis*

- Phylogeographic analysis using ND6 sequence data revealed lack of correlation with geography and traditional subspecies designations. However, AMOVA

analyses and pairwise θ_{ST} revealed slight population subdivision between California least terns and the Interior/East Coast breeding areas. Investigations into the demographic history of least terns revealed patterns similar to many North American avian taxa that have undergone a recent population expansion.

- Comparison of museum specimens collected around the turn of the century (pre-1912) and contemporary samples (2001-2005) from the same geographic areas revealed significantly lower nucleotide diversity in the contemporary samples. Also, seven pre-1912 haplotypes were missing from the present day samples suggesting loss of genetic diversity. Small sample sizes for historic breeding areas are problematic and should be increased in future analyses.

4.4 *Conservation Implications*

- California, Interior, and East Coast least terns exhibit high genetic connectivity between the geographically distributed groups. However, the same level of movement is not an adequate amount for maintenance of viable populations or recolonization of an extinct population (Wright 1931, 1940; Mills & Allendorf 1996). Although molecular tools have demonstrated an ability to identify evolutionary divergent lineages, decisions to protect species and groupings below the species level should reflect life history, ecology, population dynamics, as well as genetics.
- Least tern conservation would benefit from large scale dispersal studies within and between breeding ranges and accurate wintering/migratory distributions and movements.

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