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Mitochondrial Evidence for Panmixia despite Perceived Barriers to Gene Flow in a Widely Distributed Waterbird

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Abstract

We examined the mitochondrial genetic structure of American white pelicans (*Pelecanus erythrorhynchos*) to: 1) verify or refute whether American white pelicans are panmictic and 2) understand if any lack of genetic structure is the result of contemporary processes or historical phenomena. Sequence analysis of mitochondrial DNA control region haplotypes of 367 individuals from 19 colonies located across their North American range revealed a lack of population genetic or phylogeographic structure. This lack of structure was unexpected because: 1) Major geographic barriers such as the North American Continental Divide are thought to limit dispersal; 2) Differences in migratory behavior are expected to promote population differentiation; and 3) Many widespread North American migratory bird species show historic patterns of differentiation resulting from having inhabited multiple glacial refugia. Further, high haplotype diversity and many rare haplotypes are maintained across the species' distribution, despite frequent local extinctions and recolonizations that are expected to decrease diversity. Our findings suggest that American white pelicans have a high effective population size and low natal philopatry. We suggest that the rangewide panmixia we observed in American white pelicans is due to high historical and contemporary gene flow, enabled by high mobility and a lack of effective physical or behavioral barriers.

Key words: *American white pelican, colonial waterbird, gene flow, mitochondrial DNA, panmixia, Pelecanus erythrorhynchos*

Past events of global climate change, as observed during the Pleistocene glaciations, produced barriers that drastically altered the genetic structure of many taxa through population bottlenecks, local adaptation, and genetic drift (Avise 2009). Many avian species occupied 2 major glacial refugia in North America during the Pleistocene: 1 east and 1 west of the North American Continental Divide (NACD), where the Rocky Mountains and Great Plains acted as historical barriers to gene flow. As a result, east–west genetic differentiation is common among widespread migratory bird species, including Swainson's thrush (*Catharus ustulatus*; Ruegg and Smith 2002), wood duck (*Aix sponsa*; Peters et al. 2005), and numerous warblers (reviewed in Kelly and Hutto 2005). In addition to historical barriers, genetic structuring is influenced by behavioral and ecological life-history characteristics, such as migration and dispersal, which affect gene flow. To understand genetic patterns resolved by molecular markers, it is important to take both contemporary and historical processes into account (Bossart and Prowell 1998).

The evolution of ecologically important and complex life-history traits, such as migratory behavior, is better understood with the use of genetic markers (Webster et al. 2002; Davis et al. 2006). In some migratory bird species, some populations migrate whereas others do not, and in many species, populations differ in the migratory routes used (Davis et al. 2006). The effect of different migration patterns on genetic variation is not well understood. Nonmigration in some populations may promote differentiation through limiting dispersal and gene flow and can be a cause of speciation. For example, southern nonmigratory populations of yellow warblers are a different subspecies (*Dendroica petechia bryanti*) than northern migratory populations (*D. p. aestiva*; Salgado-Ortiz et al. 2009). Further, population differentiation resulting from different migratory routes has been observed in some species (*Sylvia atricapilla*, Perez-Tris

et al. 2004) and not in others (*Phylloscopus trochilus*, Bensch et al. 1999; *Dendroica caerulescens*, Davis et al. 2006). Many widespread migratory songbirds in North America show migratory and genetic differentiation between eastern and western populations (reviewed in Kelly and Hutto 2005), with few exceptions (Colbeck et al. 2008). Determining the timing of sequence divergence of mitochondrial DNA between populations with different migratory routes may suggest the amount of population segregation required for complex and ecologically important traits to diverge.

Studies of genetic variation commonly use different markers with varying rates of evolution to help ensure that inferences made from contemporary patterns of variation are placed in the appropriate historical context (Lukoschek et al. 2008; Avise 2009). Nuclear (e.g., microsatellites) and mitochondrial (mtDNA) markers often reveal contrasting patterns of genetic variation, largely due to differing modes of inheritance, effective population sizes (Birky et al. 1983), and rates of evolution (e.g., Brunner et al. 1998; Hurles et al. 1998; Lu et al. 2001; Yang and Kenagy 2009). Moreover, greater genetic structure has been detected using mtDNA compared with nuclear markers in some species exhibiting the uncommon phenomenon of rangewide panmixia (e.g., European plaice [*Pleuronectes platessa*], Hoarau et al. 2004). Therefore, employing mitochondrial markers when nuclear markers such as microsatellites provide little or no resolution is a more comprehensive approach to examining patterns of genetic variation and the processes by which they are influenced.

The American white pelican (*Pelecanus erythrorhynchos*) is a migratory waterbird with a breeding range consisting of colonies scattered over much of the continental United States and southern Canada: from the Pacific coast to the Great Lakes (Evans and Knopf 1993). These colonies experience drastic fluctuations in numbers that lead to local extinctions and recolonizations (Anderson and King 2005). The breeding range of American white pelicans is currently expanding eastward (Pekarik et al. 2009) and has traditionally been divided into eastern and western metapopulations (as in Hanski and Gilpin 1991) separated by the NACD (Anderson and King 2005). Band-recovery data, which suggest largely separate migratory pathways for breeding individuals from colonies east and west of the NACD (Vermeer 1977; Anderson JGT and Anderson KB 2005), have been used to substantiate the designation of 2 discrete metapopulations because they suggest low levels of gene flow between them (Anderson and King 2005). Though this small amount of gene flow appears sufficient to prevent differentiation in nuclear microsatellite markers (Reudink et al. 2011), it is unclear if it is enough to obscure historic patterns of differentiation. In addition, a few nonmigratory colonies exist in Mexico and coastal Texas (Chapman 1988). The extent to which individuals from these colonies may be differentiated genetically from individuals from migratory colonies is not certain, although microsatellites suggest they are not genetically different (Reudink et al. 2011).

The apparent lack of genetic differentiation in American white pelicans has not been explored using mitochondrial markers, which can reveal historically rooted genetic differences. In fact, some pelagic seabirds with life-history characteristics similar to pelicans exhibit no broad-scale structure with microsatellites but show differences in mitochondrial DNA within ocean basins (e.g., Morris-Pocock et al. 2008). Mitochondrial DNA may reveal genetic differentiation of the southern nonmigratory populations stemming from a historic divergence in migratory behavior or enhanced genetic drift in the isolated southern colonies. In light of the recent range expansion and large distances between new colonies (>500 km; Pekarik et al. 2009), we expect to see differentiation in the newly established and peripheral colonies as a result of founder effects and enhanced genetic drift (Vucetich and Waite 2003). Mitochondrial markers may also reveal a deep phylogenetic break across the NACD that stemmed from separate eastern and western glacial refugia during the Pleistocene. Alternatively, significant differences in haplotype composition between populations, but very few nucleotide differences between haplotypes, would suggest recent expansion from a single glacial refugium, which is rare for widespread bird species (Colbeck et al. 2008).

Using DNA samples obtained from colonies across the breeding range of American white pelicans, we sequenced an 812–base pair (bp) fragment of the mitochondrial control region to construct a map of the observed haplotypes and their geographic distributions. Herein, we test for the following: 1) large-scale genetic structure between the proposed eastern and western metapopulations and the southern nonmigratory colonies; 2) fine-scale genetic structure between pelican colonies; and 3) distinct ancestral lineages indicative of more than 1 historic glacial refugium.

Methods

Field Methods

We collected tissue, blood, and feather samples from 19 colonies across the breeding range of American white pelicans (see Reudink et al. 2011 for additional details). The location of study colonies is presented in Figure 1.

Laboratory Methods

We extracted DNA using a QIAGEN DNAeasy Tissue Extraction kit (QIAGEN) according to the manufacturer's instructions. For feathers, the final elution was performed using 100 μl of elution buffer heated to 65 °C. We amplified an 812-bp section of the mitochondrial control region in 367 individuals with PCR primers Av438FDloopB (5′TCACGTGAAATSAGCAACCC) and Av16137tPro (5′ARAATRCCAGCTTTGGGAGTTGG) (Gibb et al. 2007). Amplification was done in 15-μl volumes with final reaction concentrations of 1.5× PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.4 mg/ml bovine serum albumin, 0.4 μM of each primer, 0.05 U/μl of Taq polymerase, and 10 ng of template DNA. The PCR conditions were as follows: 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 57 °C for 60 s, and 72 °C for 60 s

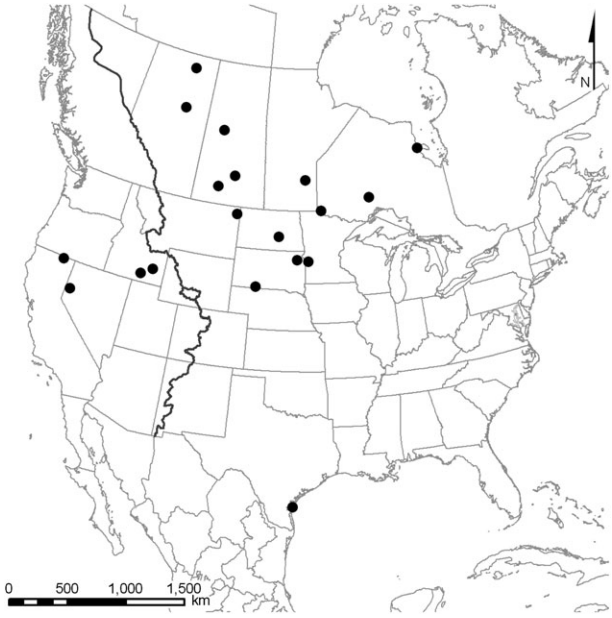

Figure 1. Sampling locations across the American white pelican (*Pelecanus erythrorhynchos*) breeding range. Each circle represents 1 of the 19 colonies sampled and the bold black line represents the North American Continental Divide.

followed by a final extension of 72 °C for 10 min. We quantified the amount of amplified DNA by electrophoresis in a 1% agarose gel stained with ethidium bromide and comparison with a low mass ladder (Invitrogen).

We diluted the amplified samples to 2.86 ng/μl (~2.5 ng/100 bp in 7 μl) with distilled deionized water and cleaned them using an exonuclease I/shrimp alkaline phosphatase (ExoSAP) protocol (New England Biolabs, Beverly, MA) to remove excess reactants prior to sequencing. PCR products were sequenced in both the forward and reverse directions using BigDye Terminator Cycle Sequencing reagents according to the manufacturer's instructions (Applied Biosystems, Foster City, CA) and were left at 4 °C overnight. We tested the quality of the PCR reaction relative to a positive control of a known sequence. We purified the sequencing reaction products by ethanol precipitation and resuspended the DNA in HiDi formamide before visualizing the DNA fragments using an ABI Prism 3730 genetic analyzer (Applied Biosystems).

To ensure that mitochondrial DNA sequences were generated from this process, as opposed to nuclear homologs, which are common in avian species (Sorenson and Quinn 1998), we cloned PCR products from 2 individuals using Promega pGemTEasy Vector system and Invitrogen Max efficiency DH5α competent cells. Thirteen clones were sequenced from each individual to screen for the presence of multiple alleles, which would suggest the simultaneous amplification of nuclear homologs. Of the 8 unique clonal sequences that differed from the consensus sequence, nucleotide differences were not observed more than once per individual, indicating that these differences are most likely a result of PCR error and not the amplification of multiple alleles from nuclear pseudogenes. In addition, a single band of expected size was observed for all amplified samples on the agarose gel, supporting the conclusion that only 1 region was amplified. Sequence identity was confirmed through a nucleotide–nucleotide basic alignment search tool search of the National Center for Biotechnology Information database (Altschul et al. 1990; http://blast.ncbi.nlm.nih.gov/Blast.cgi), which identified the mitochondrial control region of the Australian pelican (*Pelecanus conspicillatus*; Gibb et al. 2007) as the most similar sequence with 94% homology (no American white pelicans were in the database previously). The sequence homology between clones also confirms that only 1 control region was amplified, which is important because the presence of a duplicate control region was suspected in the pelican family (Pelecanidae; Gibb et al. 2007).

Sequence Data Analysis

We edited and aligned sequences manually using MEGA version 4.1 (Kumar et al. 2008). We confirmed forward and reverse sequences by alignment with each other, and haplotypes sampled only once were confirmed by reamplification and sequencing of these types. We estimated genetic diversity using ARLEQUIN version 3.11 (Excoffier et al. 2005) to calculate haplotype diversity (h, the probability that 2 haplotypes drawn randomly from a population are different; Halldorsson et al. 2004) and nucleotide diversity (π, the mean number of pairwise differences per site between 2 sequences; Nei 1987). Haplotype richness and the number of private haplotypes in a sampling location were calculated manually, and a rarefaction test was performed using ADZE version 1.0 (Szpiech et al. 2008) to assess differences in these diversity

indices when adjusted to the lowest sample size ($N = 6$) and to $N = 15$ (excluding those locations where $N < 15$). The rarefaction test estimates the number of distinct and private haplotypes expected in a random subsample of specified size drawn from the population (Hurlbert 1971; Petit et al. 1998). Both Tajima's D and Fu's F_s tests were applied to test for in situ population growth and neutrality. D is calculated by comparing the number of segregating sites in relation to the average number of nucleotide differences between DNA sequences (Tajima 1989), and F_s is the probability of exhibiting an excess of rare alleles compared with a neutral population (F_s should be considered significant if $P < 0.02$; Fu 1997). All calculations were permuted 10 000 times.

To identify genetic subdivisions between the eastern, western, and southern regions, we performed an analysis of molecular variance (AMOVA) using ARLEQUIN (Excoffier et al. 2005), which compares haplotype variation within groups with variation between groups. Pairwise F_{ST} statistics based on haplotype frequencies were also calculated using ARLEQUIN ($\alpha = 0.05$) to estimate genetic distances between regions and between colonies. Additionally, we performed a spatial analysis of molecular variance (SAMOVA) using SAMOVA version 1.0 (Dupanloup et al. 2002), which aims to define groups of samples that are maximally differentiated from each other yet geographically homogenous. We performed a SAMOVA for a range of K (2–10) and 100 simulated annealing processes on: 1) all sampling locations and 2) only locations with a sample size >15 to derive the most probable model of genetic clustering. We compared AMOVA and SAMOVA results for the a priori hypothesis of $K = 3$ (east, west, and south) to see if they produced similar clustering of genetic variation.

We used FaBox (Villesen 2007) to calculate haplotype frequencies and Network version 4.5 (http://www.fluxus-engineering.com/sharenet.htm) to construct a phylogenetic network using the median-joining option (Bandelt et al. 1999) with maximum parsimony construction (Polzin and Daneschmand 2003) to illustrate the relationships between haplotypes. The best model of nucleotide substitution was determined by evaluating Akaike's Information Criterion (Akaike 1974) in jModelTest v. 0.1.1 (Posada 2008). For Bayesian inference of phylogeny, we used the Markov chain Monte Carlo analysis in MrBayes v. 3.1 (Ronquist and Huelsenbeck 2003) using 40 000 000 generations and a sample frequency of 5000. After discarding 25% as burn-in, 6000 trees were produced. We used TreeView (Page 1996) to visualize the resulting phylogenetic tree, which was rooted with a mitochondrial control region sequence from the Australian pelican (GenBank accession number: DQ780883; Gibb et al. 2007).

Results

Haplotype Diversity

In addition to the 3′ end of the control region, the 812-bp sequence amplified contained a short variable fragment (116 bp) resembling part of the 3′ end of the cytochrome b gene and 57 bp of transfer RNA threonine. This gene arrangement is consistent with that observed for the Australian pelican (*Pelecanus conspicillatus*; Gibb et al. 2007). The region contained 27 variable sites producing 32 distinct haplotypes in 367 individuals (Supplementary Table S1). Sequences have been deposited in GenBank (accession numbers: HQ315688–HQ315719). Haplotypes 1 and 2 were extremely common, accounting for 72% of individuals, whereas rare haplotypes (<1% of haplotypes) comprised a large portion of the remaining individuals (7%; Table 1). Haplotype diversity (h) was high, whereas nucleotide diversity (π) was very low (Table 2). Given the high haplotype diversity observed, sampling error at some colonies due to insufficient sample size is likely and was taken into account in subsequent analyses. Allelic richness and the number of private alleles were similar among colonies after adjusting for sample size (Table 2). All but one of Tajima's D and of Fu's F_s tests for neutrality produced negative values (single exceptions being Pelican Lake, Alberta, and Pipestone Rocks, Manitoba), although only Pipestone Rocks had a significant D value whereas 11 colonies had significant F_s values (Table 2). However, these tests cannot differentiate between a recent demographic expansion and weak selection acting on mildly deleterious mutations located elsewhere in the mitochondrial genome (e.g., Fry 1999), to explain these deviations from neutrality.

Genetic Structure

An AMOVA performed with sampling locations partitioned according to eastern, western, and southern regions showed that 100% of the total variation was explained by variation within groups ($\varphi_{ST} = 0.000$, $P = 0.341$; Table 3). Negative pairwise F_{ST} values calculated between regions were interpreted as zero and are due to an imprecision in the algorithm used by the ARLEQUIN software when very low deviations in frequencies across regions are observed (Excoffier et al. 2005; Table 4).

Pairwise F_{ST} values between colonies were generally extremely low (Supplementary Table S2). Small yet significant F_{ST} values were obtained between Pipestone Rocks and 5 other colonies, Lake Nipigon and 7 other colonies, and Portage Lake and 3 other colonies. However, after a Bonferroni correction for multiple comparisons (which reduces α to 0.002), there were no significant F_{ST} values.

When dividing the breeding range into 3 groups, a SAMOVA conducted on all colonies found Pipestone Rocks and Lake Nipigon, independently, to be maximally differentiated from the remaining colonies, with 6.89% of the variation explained by variation between groups ($\varphi_{ST} = -0.059$, $P = 0.006$; Table 3). However, when only colonies with sample sizes >15 were included, a SAMOVA found Lake Nipigon, Portage Lake, and the remainder of the sampled colonies to be the most different groups, with variation between these groups explaining 4.33% of the

Table 1 Frequencies of control region haplotypes among sampling locations

Sampled region	*N*	1	2	3	4	5	7	8	9	10	11	14	19	24	27	Rare (<1%)
Akimiski Island, Nunavut	9	4	2												1	2
Lake of the Woods, Ontario	6	3	2									1				
Lake Nipigon, Ontario	25	10 (40%)	10 (40%)		1					2					1	1 (4%)
Pipestone Rocks, Manitoba	8	6														2
Last Mountain Lake, Saskatchewan	24	13 (54%)	6 (25%)	1			1	3								
Reed Lake, Saskatchewan	23	10 (43%)	6 (26%)				1		2	1			1			2 (9%)
Dore Lake, Saskatchewan	19	11 (58%)	3 (16%)							1	1	2				1 (5%)
Portage Lake, Alberta	18	12 (67%)	2 (11%)	2								1				1 (6%)
Pelican Lake, Alberta	11	5	5	1												
Marsh Lake, MN	24	12 (50%)	4 (17%)	1						2	2			1		2 (8%)
Chase Lake, ND	15	7 (47%)	4 (27%)	1	1								1			1 (7%)
Lacreek National Wildlife Refuge, SD	35	16 (46%)	5 (14%)	3	1	1						3				6 (17%)
Bitter Lake, SD	10	6	2			1				1						
Medicine Lake, MT	21	10 (48%)	3 (14%)	1	1	2	1			1						2 (10%)
Blackfoot Reservation, ID	10	5	2				1			1	1					
Minidoka National Wildlife Refuge, ID	6	3	1	1								1				
Anaho Island, NV	33	17 (52%)	5 (15%)	2				2	1				2			4 (12%)
Clear Lake, CA	46	23 (50%)	8 (17%)			2	3		3	1				2	2	2 (4%)
Padre Island, TX	24	13 (54%)	7 (29%)	1					1					1		1 (4%)
Pooled region																
East	248	125 (50%)	54 (22%)	10	4	4	3	3	2	8	3	7	2	1	2	20 (8%)
West	95	48 (51%)	16 (17%)	3		2	4	2	4	2	1	1	2	2	2	6 (6%)
South	24	13 (54%)	7 (29%)	1					1					1		1 (4%)

The first row contains the haplotype label. Percentages indicate the proportion of samples with a particular haplotype from a sampling location and are only given for haplotypes with relatively high frequencies. The frequencies of rare haplotypes (those present in <1% of individuals) are combined for each region.

variation observed ($\varphi_{ST} = -0.039$, $P = 0.011$; Table 3). Significant support was not obtained for any value of K, and F_{CT} increased as K approached 1 (data not shown).

Phylogenetic Analysis

The haplotype-spanning network revealed a star-like topology with many rare haplotypes (diverged by only 1 nucleotide) radiating out from 2 common haplotypes (1 and 2; Figure 2). There were no observed differences in haplotypes originating from each of the 3 regions and low incidences of homoplasy. jModelTest selected TrN+G (Tamura and Nei 1993) as the most appropriate model of nucleotide substitution. The Bayesian phylogeny created using MrBayes was unable to resolve haplotype groupings, with the majority of bootstrap values below 50 (data not shown).

Discussion

Our analysis of mitochondrial control region haplotypes from 367 individuals revealed a lack of genetic structure across the entire breeding range of American white pelicans. This lack of structure was unexpected because: 1) The NACD, a presumed major geographic barrier to dispersal, should limit gene flow; 2) Differences in migratory behavior should promote population differentiation; and 3) Many widespread North American migratory bird species show historic patterns of differentiation resulting from inhabiting multiple glacial refugia (Ruegg and Smith 2002; Kelly and Hutto 2005; Peters et al. 2005). Many species with life-history characteristics similar to pelicans (e.g., coloniality, high mobility, broad ranges, low annual fecundity, long life span), such as pelagic seabirds, show fine-scale mtDNA structure (Friesen et al. 2007). For example, marbled murrelets (*Brachyramphus marmoratus*; Friesen et al. 2005), red-legged kittiwakes (*Rissa brevirostris*; Patirana et al. 2002), razorbills (*Alca torda*; Moum and Árnason 2001), and Cory's shearwater (*Calonectris diomedea* spp.; Gomez-Diaz and Gonzalez-Solis 2007) show differences in mtDNA within ocean basins. The lack of genetic structure detected using microsatellite markers (Reudink et al. 2011) did not negate our expectation of significant genetic structure in mtDNA because a lack of concordance between mitochondrial and nuclear DNA is common (e.g., Brunner et al. 1998; Hurles et al. 1998; Lu et al. 2001; Morris-Pocock et al. 2008; Yang and Kenagy 2009), particularly in species with high dispersal capabilities and recent colonization histories (Hoarau et al. 2004). Additionally, mitochondrial evidence for rangewide panmixia is rare (Coltman et al. 2007; Neethling et al. 2008).

The lack of mtDNA structure in this study corroborates results on American white pelican population genetic structure obtained using microsatellite markers (Reudink et al. 2011), suggesting that gene flow in American white pelicans precludes genetic differentiation of both genomes at broad and fine scales. We suggest that the lack of genetic differentiation between regions on either side of the NACD refutes the current classification of American white pelicans into eastern and western metapopulations (Anderson and King 2005). Despite banding data indicating largely separate eastern and western migratory pathways (Anderson JGT and Anderson KB 2005), the lack of broad-scale structure detected in this study suggests that gene flow is high enough

Table 2 Haplotype diversity (±standard deviation [SD]), nucleotide diversity (±SD), allelic richness (A_r), and private allele (A_p) estimates for the raw haplotype frequencies (actual N) and frequencies adjusted to $N = 6$ and $N = 15$ by rarefaction analysis

				Actual N		Adjusted $N = 6$		Adjusted $N = 15$			
Sampled region	N	h	π	A_r	A_p	A_r	A_p	A_r	A_p	Tajima's D	Fu's F_s
Akimiski Island, Nunavut	9	0.806 ± 0.120	0.00172 ± 0.00131	5	2	3.917	1.715			−1.04	−1.69
Lake of the Woods, Ontario	6	0.806 ± 0.120	0.00107 ± 0.00100	3	0	3.000	0.000			−0.05	−0.43
Lake Nipigon, Ontario	25	0.697 ± 0.060	0.00112 ± 0.00090	6	1	3.093	0.397	4.650	0.871	−0.59	−2.21
Pipestone Rocks, Manitoba	8	0.464 ± 0.200	0.00124 ± 0.00105	3	1	2.500	1.290			**−1.53**	0.20
Last Mountain Lake, Saskatchewan	24	0.652 ± 0.081	0.00097 ± 0.00081	5	0	2.955	0.431	4.208	0.296	−0.77	−1.51
Reed Lake, Saskatchewan	23	0.759 ± 0.071	0.00144 ± 0.00108	8	2	3.627	0.852	6.150	1.379	−1.21	**−3.93**
Dore Lake, Saskatchewan	19	0.655 ± 0.112	0.00114 ± 0.00092	6	0	3.195	0.339	5.329	0.583	−1.10	**−2.60**
Portage Lake, Alberta	18	0.556 ± 0.130	0.00079 ± 0.00072	5	0	2.804	0.276	4.627	0.481	−1.35	**−2.44**
Pelican Lake, Alberta	11	0.636 ± 0.089	0.00090 ± 0.00081	3	0	2.541	0.000			0.20	−0.02
Marsh Lake, MN	24	0.732 ± 0.086	0.00158 ± 0.00114	8	1	3.596	0.714	6.227	1.150	−1.14	**−3.45**
Chase Lake, ND	15	0.743 ± 0.094	0.00132 ± 0.00103	6	0	3.502	0.613	6.000	0.329	−0.77	**−2.61**
Lacreek National Wildlife Refuge, SD	35	0.771 ± 0.067	0.00143 ± 0.00105	12	2	3.872	0.767	7.032	1.326	−1.35	**−8.61**
Bitter Lake, SD	10	0.644 ± 0.152	0.00107 ± 0.00093	4	0	3.067	0.211			−0.66	−1.18
Medicine Lake, MT	21	0.767 ± 0.090	0.00151 ± 0.00111	9	1	3.864	0.708	7.199	0.981	−1.37	**−5.47**
Blackfoot Reservation, ID	10	0.756 ± 0.130	0.00132 ± 0.00107	5	0	3.667	0.406			−0.94	**−2.10**
Minidoka National Wildlife Refuge, ID	6	0.800 ± 0.172	0.00124 ± 0.00110	4	0	4.000	0.013			−1.23	**−1.81**
Anaho Island, NV	33	0.716 ± 0.077	0.00137 ± 0.00102	9	2	3.539	1.007	6.168	1.506	−0.76	**−4.57**
Clear Lake, CA	46	0.720 ± 0.063	0.00140 ± 0.00103	10	0	3.523	0.548	6.008	0.727	−1.33	**−4.90**
Padre Island, TX	24	0.641 ± 0.081	0.00121 ± 0.00095	6	0	2.905	0.400	4.500	0.351	−0.95	−2.02
Pooled region											
East	248	0.696 ± 0.027	0.00127 ± 0.00094							**−2.00**	**−29.62**
West	95	0.715 ± 0.046	0.00136 ± 0.00100							**−1.64**	**−13.11**
South	24	0.641 ± 0.081	0.00121 ± 0.00095							−0.95	−2.02

Significance (indicated in bold) assigned to $P < 0.05$ for Tajima's D and $P < 0.02$ for Fu's F_s tests for neutrality.

across the NACD to prevent population differentiation. In contrast, many other widespread North American bird species exhibit structuring on either side of the divide because the Rocky Mountains and Great Plains act as a barrier to gene flow (Ruegg and Smith 2002; Lovette et al. 2004; Kelly and Hutto 2005; Peters et al. 2005). Alternatively, if pelicans have an extremely high effective population size, gene flow across the NACD may be low and insufficient time may have passed for east–west differences to evolve. However, given the additional evidence from microsatellite markers (Reudink et al. 2011) and banding data (Anderson JGT and Anderson KB 2005) suggesting some degree of movement between eastern and western regions, it is reasonable to conclude that gene flow may exceed the 1-to-10-migrant-per-generation rule leading to genetic homogeneity (Mills and Allendorf 1996).

Contrary to our expectations, neither differences in haplotype diversity nor significant genetic differentiation was observed in recently established or peripheral colonies. In addition, high haplotype diversity and many rare

Table 3 Comparison of explained variance calculated using AMOVA for pooled regions (east, west, south) and the optimal group selection using SAMOVA (all sampling locations and only those with $N \geq 15$)

Source of variation	% of variation	P value
AMOVA (all colonies), groups: 1) east, 2) west, and 3) south		
Among groups	−0.61	0.81
Among groups within colonies	0.59	0.24
Within colonies	100.02	0.34
φ_{sr}		0.00
SAMOVA (all colonies), groups: 1) Pipestone Rocks, 2) Lake Nipigon, and 3) remaining colonies		
Among groups	6.89	0.006
Among groups within colonies	−0.97	0.32
Within colonies	94.07	0.30
φ_{sr}		0.06
SAMOVA (only colonies where $N \geq 15$), groups: 1) Lake Nipigon, 2) Portage Lake, and 3) remaining colonies		
Among groups	4.33	0.01
Among groups within colonies	−0.44	0.28
Within colonies	96.11	0.16
φ_{sr}		0.04

Table 4 Pairwise differentiation between pooled sampling regions

	East	West	South
East		−0.001	−0.015
West	0.51		−0.008
South	0.94	0.63	

F_{ST} values are shown above the diagonal and P values are shown below the diagonal.

haplotypes are maintained across the species distribution, suggesting a high effective population size. A high effective population size may be maintained despite local extinctions and recolonizations if these processes redistribute individuals among breeding sites and thereby negate founder effects and genetic drift. Further, the lack of genetic structure across the species' range counters the existence of behavioral barriers to gene flow, such as natal philopatry and sex-biased dispersal, which are common in species with similar life-history characteristics, such as pelagic seabirds (Friesen et al. 2007). Pelicans may exhibit lower levels of philopatry than other colonial waterbirds due to fluctuating colony numbers driven by variable water conditions, which may force pelicans to disperse and breed elsewhere in any given year (Reudink et al. 2011). These dispersal events may happen in large groups over long distances, frequently enough to prevent the loss of genetic variation at newly established and peripheral colonies, and may facilitate a gradual range shift in response to environmental changes at breeding sites.

Nonmigration can promote genetic differentiation (Buerkle 1999) and speciation (Salgado-Ortiz et al. 2009) by limiting dispersal and gene flow, but we observed no genetic differences between the isolated nonmigratory pelican colony in coastal Texas and the northern migratory colonies. Perez-Tris et al. (2004) also found little differentiation between migratory and nonmigratory blackcap (*Sylvia atricapilla*) populations, which they attributed to either the remnant of an ancestral nonmigratory population from which all others diverged or secondary colonization of sedentary populations since the initial demographic expansion that followed the last glacial cycle. However, in our study, haplotype diversity in Texas did not differ from other colonies, as one would expect in an ancestral population. Moreover, breeding colonies in Mexico and coastal Texas have likely been there for centuries (Oberholser and Kincaid 1974; Chapman 1988), and microsatellite markers intimate high contemporary gene flow (Reudink et al. 2011). We suggest that gene flow was likely maintained between the migratory and nonmigratory colonies historically as well as contemporarily, resulting in a lack of differentiation in mtDNA. One possible explanation for high gene flow in the nonmigratory colony is that some migratory individuals remain in the south to breed with local adult nonmigrants. However, our data do not allow us to rule out a secondary colonization event.

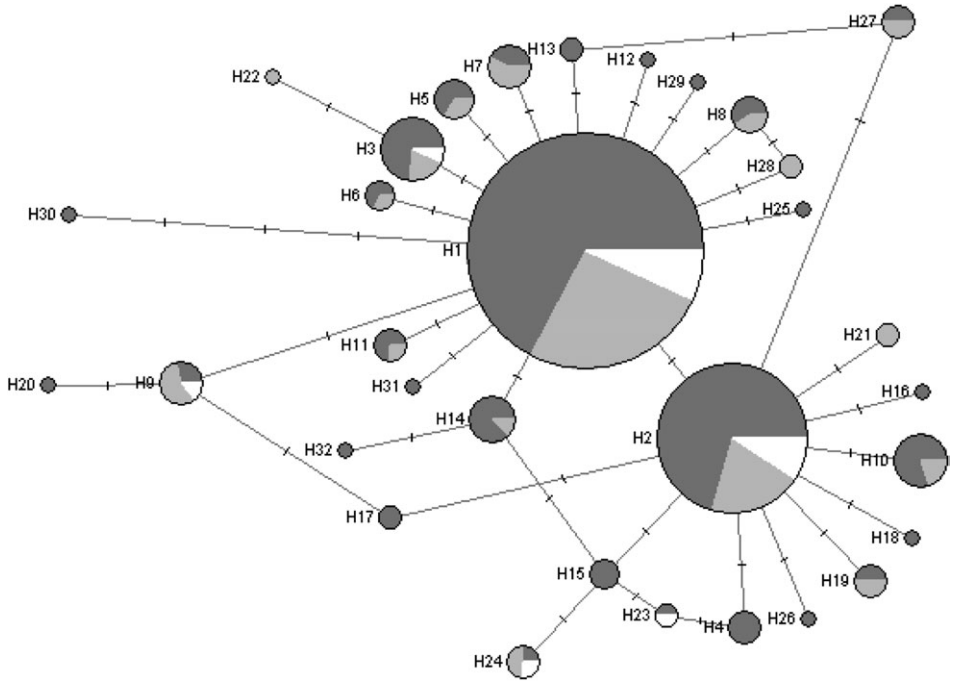

Figure 2. Maximum parsimony network of 32 mitochondrial control region haplotypes in American white pelicans (*Pelecanus erythrorhynchos*). The size of the circle is proportional to the frequency of the haplotype in the population; shading indicates the region in which the haplotype occurs (east, dark gray; west, light gray; south, white).

Unlike nearly all other widespread North American migratory bird species studied (e.g., Swainson's thrush, Ruegg and Smith 2002; wood duck, Peters et al. 2005; American redstart [*Setophaga ruticilla*], Colbeck et al. 2008; and all warblers with continental distributions, Kelly and Hutto 2005), we found no evidence to suggest contemporary populations of pelicans may have originated from more than 1 glacial refugium during the Pleistocene. The star-shaped haplotype network and high haplotype diversity coupled with low nucleotide diversity at all colonies suggest a recent expansion from a single population following a bottleneck (Grant and Bowen 1998). However, because of the high mobility and dispersal capabilities of American white pelicans (Evans and Knopf 1993; Pekarik et al. 2009), we cannot discount the possibility that they may have occupied multiple refugia during the Pleistocene and experienced continuous gene flow between them or that all but 1 lineage went extinct.

The lack of phylogeographic structure in the American white pelican underscores the idea that species that share similar life-history characteristics and occupy similar contemporary distributions may have very different evolutionary histories (Zink 1996; Colbeck et al. 2008). Pelicans have maintained high genetic diversity and exhibit no genetic differentiation despite putative barriers to dispersal (such as the NACD) that promote differentiation in other species. How genetic homogeneity is maintained in pelicans warrants further study into the interplay between demographic (e.g., frequent local extinctions and recolonizations) and behavioral processes (e.g., natal philopatry). Understanding these interactions may allow us to predict how highly mobile species such as American white pelicans will fare in the face of large-scale environmental change.

Supplementary Material

Supplementary Tables S1 and S2 can be found at http://www.jhered.oxfordjournals.org/.

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