Genetic Diversity and Population Structure of Reddish Egrets along the Texas Coast

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Abstract.—Little is known about the ecology and population structure of the Reddish Egret (*Egretta rufescens*). Furthermore, the effects of the near extirpation on genetic variation of Reddish Egrets are unknown. A 223-base-pair region of the mitochondrial DNA (mtDNA) control region was sequenced in 149 Reddish Egret samples from sixteen breeding colonies along the Texas Coast to investigate genetic diversity and population structure. Despite experiencing a severe reduction in population size, Reddish Egrets retained a moderate amount of haplotype (0.705) and nucleotide diversity (0.005). A mismatch distribution among haplotypes and non-equilibrium relationship between the number and similarity of haplotypes (Fu's Fs) are consistent with historical population size changes. No evidence of genetic structuring was found among either colonies or color morphs. Reddish Egrets along the Texas Coast appear to form a single panmictic population. The results of this first conservation genetic study of Reddish Egrets serve as a foundation for management and additional research. *Received 2 September 2008, accepted 16 February 2009.*

Key words.—conservation genetics, genetic diversity, genetic structuring, mtDNA, Reddish Egret.

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The Reddish Egret (*Egretta rufescens*) is the rarest species of heron in North America. Reddish Egrets are coastal wetland specialists, found along the Gulf of Mexico, in the Caribbean and Bahamas, the Atlantic Coast of Florida and the Pacific Coast of Mexico (Lowther and Paul 2002). Historically considered common, Reddish Egrets were nearly extirpated in the United States in the early 1900s by plume hunters (Paul 1991). The population in the United States made a modest recovery after the passage of the Migratory Bird Treaty Act in 1918. Currently, there are about 2,000 breeding pairs in the United States. Populations in Mexico and the Caribbean have not been surveyed (Lowther and Paul 2002). In North America, the majority of the breeding population is located along the Texas Gulf Coast (Paul 1991). Because the Reddish Egret is rare compared to other heron species and relies completely on coastal wetlands, it is listed as a species of concern by the U.S. Fish and Wildlife Service and has been designated as threatened by the State of Texas (U.S. Fish and Wildlife Service 1995; Texas Parks and Wildlife 2007).

The population in Texas has fluctuated in abundance over the past eight decades. The population initially experienced a rapid recovery after 1918, numbering about 3,206 pairs in 1939, two-thirds of which nested on Green Island in the lower Laguna Madre of Texas (Paul 1991). In the early 1960s, the population experienced a precipitous decline and only 552 pairs were found in Texas by 1965 (Paul 1991). The cause of the decline is unknown, but human disturbance, predation and pesticides have been implicated (Paul 1991). The population in Texas made a second modest recovery to 1,500 pairs by 1990, comprising about 75% of the United States population (Paul 1991). The greatest breeding concentrations occur in the Laguna Madre along the lower Texas Coast, which extends from the southern tip of Corpus Christi Bay to Port Isabel, averaging 64% of the breeding population in Texas between 1996 and 2005, and in some years as high as 94% (Texas Colonial Waterbird Society unpublished data) (Fig. 1).

Reddish Egrets are restricted to a narrow band of coastal habitat and the limited amount of nesting habitat along the coast is unevenly distributed (Lowther and Paul 2002). Coastal development, loss of nesting and foraging habitat, and disturbance by humans are potential factors affecting the current United States population. Managing Reddish Egrets is difficult because of the lim-

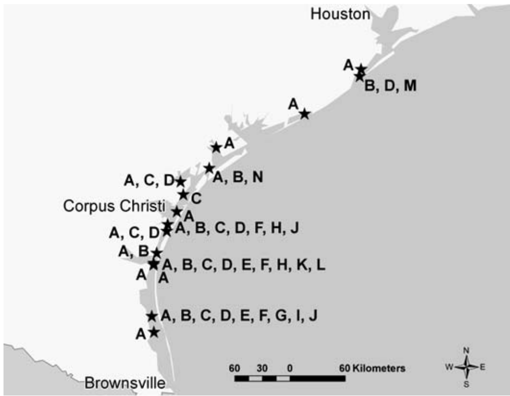

Figure 1. Reddish Egret colonies along the Texas Coast from which feather samples were obtained for genetic analysis during spring 2005, 2006 and 2007. The numbers represent colony identifications.

ited knowledge regarding the species ecology and population structure. Knowledge of population structuring would be useful for focusing management efforts and predicting the effects of disturbance on Reddish Egrets. For instance, if Reddish Egrets display nest site fidelity, genetic variation may be structured among breeding colonies, in which case genetic variation found within individual colonies may be vulnerable to local disturbance, pollution and loss of nesting habitat or foraging grounds. Since most Reddish Egrets in the United States breed in Texas, an understanding of the species' conservation status and population structuring in this region is critical for conservation to be effective.

The near extirpation of Reddish Egrets suggests that the population may have experienced a genetic bottleneck and concomitant loss of genetic diversity (Nei *et al.* 1975), similar to other avian species (Glenn *et al.* 1999; Bellinger *et al.* 2003). Of further concern is the series of population size fluctuations that Reddish Egrets have experienced during the past century. Repeated bottlenecks and genetic drift may have substantially eroded genetic variation. Finally, Reddish Egrets have white and dark color morphs. There appear to be differences in foraging behavior and foraging habitats between the color morphs (Green 2006). The behavioral differences may lead to assortative mating, which may result in genetic structuring between the color morphs. Thus, management units for Reddish Egrets may plausibly involve spatial or behavioral components. The goals of this study were to: (1) determine genetic variability and the effects of demographic history on the Reddish Egret population in Texas and (2) assess the genetic structure within this breeding population, facilitating the delineation of management units.

METHODS

Study Site

Most (~64%) of the Texas breeding population of Reddish Egrets nest in the Laguna Madre, a shallow (average depth 1 m), hypersaline lagoon 185 km long and 7 km wide located along the lower Texas Coast (Fig. 1) (Tunnel 2002). The Laguna Madre consists mostly of two habitat types: sea grass meadows and wind tidal flats. In the upper Laguna Madre, seagrasses cover about 243 km^2, with shoalgrass (*Halodule wrightii*) being the dominant species (Quammen and Onuf 1993). Other species include manatee-grass (*Cymodocea syringodium*), wigeon grass (*Ruppia maritima*), clover grass (*Halophila engelmannii*) and turtle grass (*Thalassia testudinum*). In the lower Laguna Madre, seagrasses cover about 114 km^2 with manatee-grass being the dominant seagrass (Quammen and Onuf 1993). Wind tidal flats cover about 114 km^2 in the upper Laguna Madre and over 820 km^2 in the lower Laguna Madre (Quammen and Onuf 1993). Reddish Egrets nest colonially on islands with other wading bird species. The species typically nests in low vegetation such as prickly pear cactus (*Opuntia* sp.) and sea oxeye (*Borrichia* sp.). However, nesting substrate can range from bare ground to tall trees.

DNA Collection, Extraction, and Amplification

We collected Reddish Egret samples during spring 2005, 2006 and 2007 from 16 colonies distributed along the Texas Coast (Fig. 1). We captured pre-fledgling individuals prior to dispersal from their natal colonies. We plucked 1-3 feathers from the tail and back of each individual as a source of DNA. Plucked feathers are minimally invasive and have proven to be a good source of DNA in several avian studies (Taberlet and Bouvet 1991; Leeton *et al.* 1993; Fowler *et al.* 2004). Feathers from each individual were placed in sterile bags, stored on ice, and frozen at -20°C.

We extracted DNA from feather samples using the DNEasy Tissue Kit and Tissue Protocol (Qiagen, Valencia, CA) and attempted to sequence several mitochondrial DNA (mtDNA) loci using degenerate avian primers from Sorenson (2003) to determine if sufficient genetic variability was present for analysis. The control region (domain I), ATPase8, and ND_2 were chosen, as these regions have proven useful in fine-scale population genetic studies of birds (Burg and Croxall 2001; Sorenson 2003; Oyler-McCance *et al.* 2005). We successfully amplified portions of the control region

and ATPase8, but were unable to amplify ND_2. Preliminary analysis of sequence data from ATPase8 revealed no variable sites in five individuals, but several nucleotide substitutions were observed in the control region. We then designed primers 5'-CCTGTACTAGAACCATACTCTAGGTGGG-3' and 5'TGTGAAGAGCGAGTGCTAAGT-3' to amplify a 223 base-pair region that contained the variable sites. We amplified DNA via the polymerase chain reaction (PCR) in 25 µl reaction volumes containing 1.5 µl DNA, 10.8 µl water, 0.1 µl (10 pmol) of each primer, and 12.5 µl PCR master mix, consisting of a buffer, dNTPs, $MgCl_2$, and a thermal stable polymerase (Amplitaq Gold, Applied Biosystems, Foster City, California). Reactions were performed under the following conditions: initial denaturation at 94°C for twelve minutes, followed by 32 cycles of 94°C for 50 sec, 67.5°C for one minute, and 72°C for four minutes, and a final extension of 72°C for 30 minutes. The PCR products were then electrophoresed on a 1% agarose gel containing ethidium bromide and visualized under UV light. The PCR products from successful reactions were purified by an enzymatic method (ExoSAP-IT USB Corporation, Cleveland, OH) and prepared for sequencing using the BigDye Terminator Cycle Sequencing Kit v.1.1 (Applied Biosystems). Unincorporated dye terminators were removed from the sequencing reaction using the DyeEx 2.0 spin kit (Qiagen). The sequencing reactions were then electrophoresed on an ABI Prism 3130 automated sequencer (Applied Biosystems). We sequenced in both directions and sequences were aligned and assembled using SEQUENCHER 4.6 (GeneCodes, Ann Arbor, Michigan). The sequences were deposited in GenBank under accession numbers EU552506–EU552519.

Data Analysis

We estimated genetic diversity at the nucleotide (π) and haplotype (H) level using the program DnaSP (Rozas *et al.* 2003). Nucleotide diversity is the average number of nucleotide differences per site and H is the probability that any two randomly sampled haplotypes are different (Nei 1987).

We determined the degree of genetic structuring by examining an unrooted, parsimony-based minimum spanning network using the program TCS (Clement *et al.* 2000). We quantified genetic differentiation among the sampling sites (colonies) and between color morphs by computing θ, an estimator analogous to Weir and Cockerham's (1984) re-formulation of Wright's Fst (Wright 1951) using the computer program Arlequin (Michalakis and Excoffier 1996; Excoffier *et al.* 1992; Schneider *et al.* 2000). We then conducted an Analysis of Molecular Variance (AMOVA) to partition variance within and among colonies. Also, we estimated the genetic differentiation, D, among colonies using the diversity index proposed by Jost (2008, eq. 13) because Fst performs poorly as an indicator of differentiation when heterozygosity is high. The D statistic provides an unbiased index of genetic structure, where 0 indicates no differentiation and 1 indicates complete differentiation (Jost 2008). We used DnaSP (Rozas *et al.* 2003) to calculate Fu's Fs (Fu 1997), a statistical test for departures from neutrality, which can indicate historical population size changes and allowed us to assess the effect of these changes on genetic diversity. We created a mismatch distribution of pairwise differences using DnaSP to compare the expected distribution for a population at equilibrium with the observed distribution (Slatkin and Hudson 1991; Rozas *et al.* 2003).

We assessed the recovery of the Reddish Egret population by comparing the current population size with an estimate of historical population size. We first calculated the uncorrected p-distance, the proportion of nucleotide sites where sequences differ among haplotypes (Nei and Kumar 2000) among haplotypes using the computer program MEGA 3.1 (Kumar *et al.* 2004). We estimated an effective population size (N_e) based on the equation $N_e = 10^8 \times 0.5 \times p/g$ (Avise *et al.* 1988) where N_e is the long-term effective population size of females, p is the uncorrected mean p-distance within samples, and g is the generation time. Since Reddish Egrets do not breed until four years of age (Lowther and Paul 2002), we used a conservative estimate of eight years for the generation time.

RESULTS

Genetic Diversity and Population Structuring

We sampled 149 Reddish Egrets from 16 colonies along the Texas Coast. Sample sizes for each colony ranged from one to 44 individuals, a function of the size of the colony and proportion of Reddish Egrets present (Table 1). One hundred forty-eight were hatch-year birds; the remaining individual was a salvaged adult. A 223 base-pair portion of the control region was sequenced for all 149 individuals. We found 14 haplotypes and eleven variable sites; all haplotypes were within six mutational steps (Fig. 2). Haplotype A was the most common and widely distributed haplotype, found in 74 of the individuals and in 14 of the 16 colonies sampled (Table 1). Estimates of nucleotide and haplotype diversity in the sample were relatively high, with $\pi = 0.005$ ($s^2 = 0.00045$) and $H = 0.705$ ($s^2 = 0.033$). Seven colonies were represented by a single sample and were not included in analyses of genetic structure (Table 1). We found little evidence of genetic structuring among colonies ($\theta = -0.004$, D = 0.135) and between the two color morphs ($\theta = -0.012$). The majority of the variation came from within colonies (Table 2).

Demographic History and Effective Population Size

The mismatch distribution of observed haplotype variation revealed a departure from expected variation under a constant

Table 1. Reddish Egret DNA sample sizes from 16 colonies along the Texas Coast during 2005, 2006 and 2007.

Colony ID	n	dark morphs	white morph	Haplotypes (*n*)
1	4	4	0	A(*4*)
2	5	3	2	B(*2*), D(*2*), M(*1*)
3	1	1	0	A(*1*)
4	4	4	0	A(*2*), B(*1*), N(*1*)
5	5	5	0	A(*3*), C(*1*), D(*1*)
6	1	1	0	A(1)
7	1	1	0	C(*1*)
8	1	1	0	A(*1*)
9	25	17	8	A(*13*), B(*4*), C(*4*), D(*1*), F(*1*), H(*1*), J(*1*)
10	6	0	6	A(*3*), C(*2*), D(*1*)
11	6	4	2	A(*4*), B(*2*)
12	43	29	14	A(*22*), B(*7*), C(*6*), D(*3*), E(*1*), F(*1*), H(*1*), K(*1*), L(*1*)
13	1	1	0	A(*1*)
14	1	1	0	A(*1*)
15	44	33	11	A(*17*), B(*10*), C(*3*), D(*4*), E(*3*), F(*2*), G(2), I(*2*), J(*1*)
16	1	0	1	A(*1*)

population size (Fig. 3). We estimated the Fu's Fs statistical test of neutrality to be -5.2, which was significantly different from zero and is further evidence that the population has undergone size fluctuations in the past. The uncorrected p-distance among all haplotypes was 0.005 and we assumed an eight-year generation time based on a four-year sexual maturation period for Reddish Egrets (Lowther and Paul 2002), which resulted in a long-term N_e of 31,250 females.

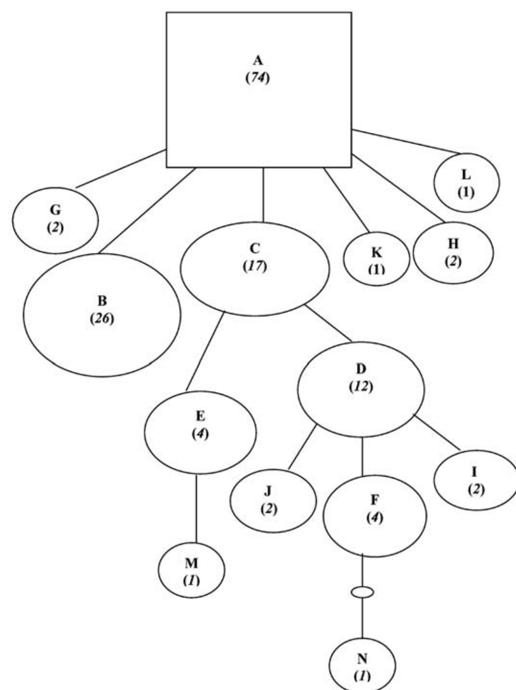

Figure 2. Minimum spanning network depicting parsimony-based relationships among 14 haplotypes found in 149 Reddish Egret mtDNA samples in Texas during 2005, 2006 and 2007. Letters represent unique haplotypes (*n*) and the area is proportional to frequency in the overall population. The small oval represents a hypothetical, intermediate haplotype that was not sampled. Haploype A is the most common haplotype.

DISCUSSION

Notwithstanding a history of significant population fluctuations, Reddish Egrets along the Texas coast appear to have maintained much greater genetic diversity than other species that have undergone severe population declines, such as the Whooping Crane (*Grus americana*). The Whooping Crane experienced a severe decline in population size in the early 1900s, dropping to a low of 14 individuals in 1938, and as a result lost two-thirds of their haplotype diversity. Only three haplotypes have been identified

Table 2. Analysis of Molecular Variance (AMOVA) for hierarchical population groups based on 142 Reddish Egrets sampled from the Texas Coast during 2005, 2006 and 2007.

Source of Variation	d.f.	Sum of Squares	Percentage of Variation
Among colonies	8	4.767	-0.41
Within colonies	133	83.895	100.41

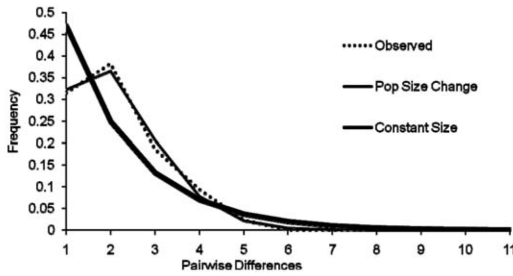

Figure 3. Mismatch distribution of the observed haplotype variation from 149 Reddish Egret mtDNA samples compared to the expected under stable population conditions and expected under a population expansion.

in the current population (Glenn *et al.* 1999). As shown in Whooping Cranes, a severe reduction in population size can cause a genetic bottleneck, resulting in loss of genetic diversity (Nei *et al.* 1975). However, the impact of a bottleneck on population genetic diversity depends on how rapidly the population declines, the size of the bottleneck population and the duration of the bottleneck. When a population suffers a genetic bottleneck, rare alleles initially are lost, but the population can maintain diversity if the population recovery is rapid (Allendorf 1986). If population abundance remains low, diversity can continue to be lost due to genetic drift (Allendorf 1986). There are several possible explanations for the relatively high genetic diversity observed in Reddish Egrets along the Texas Coast. First, the Reddish Egret decline may have been less severe than that of the Whooping Crane. The population status in the early 1900s is unknown, but even the 1965 low of 552 breeding pairs is a reasonably large number, especially considering that juvenile birds (< four years of age) were not included in population estimates. Secondly, population recovery may have been sufficiently rapid so as not to have lost diversity due to genetic drift. Although Reddish Egrets in Texas have recently experienced several episodes of population decline, each time population recovery appears to have been rapid. Finally, recolonization by individuals that immigrated from areas where genetic diversity remained high, such as Mexico or the Caribbean, could have contributed to the high genetic diversity. The observed genetic similarity within the region is consistent with an open population, as gene flow is expected to homogenize populations (Charlesworth 2003).

We detected no pattern in the distribution of haplotypes to suggest that genetic structuring exists in Reddish Egrets along the Texas Coast. Both estimates of differentiation show little evidence of genetic structuring among colonies, suggesting that females demonstrate weak fidelity to colonies, or that they disperse at some point in their lives. Thus, our results support managing Reddish Egrets along the Texas Coast as a single breeding population. It is possible that the Reddish Egrets may form one large panmictic population along the Gulf Coast and possibly beyond. The existence of one large population could explain the high level of genetic diversity found in the Texas population by allowing the effective population size to remain high during periods of population fluctuation and serve as a source of immigrants.

Although the Reddish Egret's demographic history does not show drastic reductions in genetic diversity, the population size fluctuations appear to have had detectable effects on the differentiation and frequency of haplotypes as evidenced by the mismatch distribution and Fu's Fs. The minimum spanning network (Fig. 2) shows that the 14 haplotypes are closely related (within six mutational steps). A star-shaped phylogeny, as shown in Fig. 2, is characteristic of a population that has recently expanded in size from a small number of founders (Avise 2000).

Although the Reddish Egret population in Texas has recovered from previous lows, it still appears smaller than historical accounts suggest. Estimates of N_e, although subject to large variance, provide a means of comparing current and historic population sizes (Roman and Palumbi 2003). Historical accounts described Reddish Egrets as common in Texas in the 1800s (Paul 1991). Based on the number of haplotypes and the similarity among these haplotypes, we estimated the historical population size to be 10-15 times larger than the current breeding population in the United States. Thus, although the

population may have recovered slightly from severe declines in the last century, it still seems to be well below historical numbers.

The present study assists in understanding the conservation needs for Reddish Egrets by providing information on the genetic diversity and population structuring along the Texas Coast. The study focuses on an important portion of the Reddish Egrets' range as the Texas Coast contains the majority of breeding individuals in the United States. Although we found little structuring within our study area, further study is needed, examining the genetic diversity and population structuring throughout the species' range, as it seems probable that structuring may exist between the Gulf Coast and Pacific Coast populations.

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