AN EXAMINATION OF GENE FLOW AMONG DISTINCT MANAGEMENT UNITS OF THE REDDISH EGRET (*Egretta rufescens*)

by

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

The Reddish Egret (*Egretta rufescens*) is one of the least studied herons in North America. It ranges from Baja California to the Bahamas, north to Texas and Louisiana and southwards to Central America and the northern part of South America. I examined gene flow and genetic diversity among populations across the range of the species. I specifically tested hypothesized distinct management units (Western, Central, and Eastern) based on geographic distributions and the findings of Hill et al. (2012). We collected blood and feather samples from nestlings (n = 145) of eight sample sites (Baja California, Chiapas, Yucatan, Tamaulipas, Texas, Louisiana, Florida and the Bahamas). We extracted DNA from collected samples and used ten microsatellite markers and the mitochondrial control region to estimate deviations from Hardy-Weinberg equilibrium, genetic differentiation, population structure, and gene flow. In all analyses, I detected more differentiations among groups and regions (Fst = 0.21) than among populations within groups (Fst = 0.09). Our results revealed three primary breeding concentration centers, one in each of the management units (Baja California in the Western region, Chiapas for the Central region, and Bahamas for the Eastern region) providing further support for the previously established management units. We found greater differentiation among populations in our mtDNA analysis suggesting less movement across populations and management units and greater philopatry by females than by males. Recent banding and telemetry data also support differences in movement patterns between males and females. Lastly, gene flow between the Baja California population and the other populations is weak; whereas, we detected weak to moderate gene flow between sampling sites in Central and Eastern management units.

I. INTRODUCTION

Genetic tools and estimating levels of genetic variation are useful and important for conservation and population management of animals (Ellstrand & Elam, 1993; González et al., 2014). Conservation genetics can help reveal genetic patterns, population differentiation, resolve taxonomic problems, and reveal evolutionary directions (González et al., 2014). The International Union for Conservation of Nature (IUCN) has stated the conservation of genetic diversity is one of the most important components of biodiversity conservation (McNeely et al., 1990). Genetic diversity is critical for species persistence for several reasons including the ability to respond to environmental changes as there is a direct link between heterozygosity and population fitness (Reed & Frankham, 2003). Losing genetic variation reduces the survival potential that a population will have in facing environmental changes (Simberloff, 1988). Additionally, genetic diversity helps to decrease the probability of extinction for the population under severe conditions and selection pressures (Miño & Del Lama, 2014).

Populations experiencing a bottleneck are subject to greater impacts of genetic drift (Nei et al., 1975) whereby alleles can become randomly fixed or lost from the population; this decrease in allelic diversity increases the probability of extinction, especially under severe situations. Additionally, after a bottleneck event genetic variability is reduced (Nei et al., 1975) and inbreeding depression may happen unless there is a quick recovery (Bouzat et al., 1998; Brown, Harrisson, Clarke, Bennett, & Sunnucks, 2013; B. Charlesworth & Charlesworth, 1999; Coates, 1992; N C Ellstrand & Elam, 1993). Inbreeding depression can lead to mating between related individuals and may result in

reduced fitness owing to decreased genetic diversity and increased deleterious alleles (Reed & Frankham, 2003).

Genetic drift, random changes in frequencies of alleles in populations from one generation to another (Ellstrand & Elam, 1993), is one of the reasons reduced genetic variation and heterozygosity in small populations may lead to an inbred population (Reed & Frankham, 2003). Genetic drift changes the distribution of genetic variation in two ways: (1) by decreasing variation within populations and (2) by increasing differentiation between populations (Ellstrand & Elam, 1993). In contrast to large populations where the chances of genetic drift are small, in small populations allelic frequencies may fluctuate unpredictability (Ellstrand & Elam, 1993). In species with small populations where variation within a species may be less and potentially high differentiation between subpopulations, genetic drift may play an important role in determining genetic structure (Ellstrand & Elam, 1993).

Inbreeding, a likely consequence of finite population size, can also result in the loss of genetic variation and reduced heterozygosity and increased homozygosity (Charlesworth & Charlesworth, 1987; Ellstrand & Elam, 1993; Frankham, 1996; Keller & Waller, 2002). Even though inbreeding may decrease the frequency of deleterious alleles (Barrett & Charlesworth, 1991), it is possible that in small populations, deleterious alleles become fixed and cause more harm compared to larger populations (Ellstrand & Elam, 1993). Inbreeding depression, through reduced heterozygosity and or fixation of deleterious alleles, will lead to decreased fitness and increased probability of extinction (Charlesworth & Charlesworth, 1987; Newman & Pilson, 1997; Reed & Frankham, 2003).

Inbreeding and drift reduce genetic diversity, whereas gene flow, the transfer of alleles between populations, has an important role in increasing genetic variations in small populations (Ellstrand & Elam, 1993; Ellstrand, 1992). The best consequences of gene flow is homogenizing population structure and decreasing differentiation among populations of same species, which reduces the impact of genetic drift and diversifying selection (Lande, 1992; Wright, 1930). Based on Slatkin et al. (1989), one immigrant every second generation or one inter-population mating per generation will be sufficient to maintain differentiation, illustrating the importance of gene flow for keeping genetic variation in populations (especially small populations). However, the level of changes in local diversity will depend on the level of variation in source and sink populations (Ellstrand & Elam, 1993). Although gene flow is considered beneficial in conservation genetics, under some circumstances it could be harmful for small populations by decreasing local adaptation which may lead to decreased fitness through outbreeding depression (Ellstrand & Elam, 1993).

The Reddish Egret (*Egretta rufescens*) is one of the least studied herons in North America (Lowther & Paul, 2002). This plumage-dimorphic species has experienced numerous threats, most significant during the late 19th and early 20th centuries from plumage hunting, habitat loss, coastal development and contaminants (Paul, 1978); the global population is currently estimated at 5,000 to 7,000 breeding pairs (Paul, 1991; Green, 2006; Wilson et al., 2014). Although this species was considered weakly migratory based on adults remaining in and around the breeding area throughout the year, recent banding and telemetry works suggest some individuals exhibit long distance dispersal (e.g.

post-breeding) and migration (Hill et al., 2012; Geary et al. 2015; Koczur and Ballard, unpubl. data).

The Reddish Egret can be found along the Gulf coast and Pacific coast in North America (Fig. 1, Lowther & Paul, 2002). Their range extends southward and eastward to Central America and the Caribbean islands, respectively (Paul, 1978). Considered a habitat specialist, this species uses habitat comprised of broad, saline coastal flats for foraging and associated barrier islands (natural and dredge-spoil) for nesting (Paul, 1991). These habitat types have decreased during the past 100 years presumably affecting Reddish Egret populations (Lowther & Paul, 2002; Paul, 1978; Paul, 1991).

Previous research on Reddish Egrets showed no differences between color morphs in initiation date of nesting, clutch size, nest success, and incubation time (Holderby et al., 2012). A previous genetic study on colonies in Texas, which has the greatest concentration of breeding pairs in the whole range (Green et al., 2011; Green, 2006), found no differences among colonies and high levels of genetic diversity, all of which are evidence for high gene flow among colonies (Bates et al., 2009). The other previous genetic study compared genetic diversity across three different locations within the whole range (Baja California, Texas – Tamaulipas, and Bahamas) and found different structures among them but not among sample sites within a location (Hill et al., 2012). Neither of these studies have detected any recent bottlenecks in these populations (Bates et al., 2009; Hill et al., 2012).

Hill et al. (2012), in addition to limited banding data, provided the basis for the Reddish Egret Working Group (REWG) to establish three management units (Western, Central, and Eastern) for the Reddish Egret Conservation Plan (Wilson et al., 2014, for more info see www.reddishegret.org). However, recent banding data (Green & Palacios,

unpubl. data) has revealed movement between Western and Central populations (i.e. Baja California birds to Chiapas, Mexico) and between the Central and Eastern populations (i.e. Yucatan, Mexico birds to Florida).

In this study, I will investigate genetic differentiation across the range of the species (Western, Central, Eastern Management Units) and expand on Hill et al.'s (2012) study, by including breeding populations that potentially bridge the gap between the disjunct Baja, Texas and Bahamas populations. I will examine gene flow across the species' range using regions of mtDNA and microsatellites (Hill & Green, 2011) and expand on previous study of mitochondrial variation of Reddish Egrets in Texas (Bates et al., 2009).

Research Objectives

For this study, my primary objective was to examine genetic structure and gene flow of Reddish Egrets across their range, expanding on the Hill et al. (2012) study, and specifically testing the validity of the three established management units (Wilson et al. 2014). We also examined potential bottlenecks, expanding the findings of Bates et al. (2009) from Texas, across the species' range since the recent history of the Reddish Egret shows declines, some dramatic, in the global population, which may result in loss of genetic variation from bottleneck, genetic drift, and/or inbreeding depression.

II. METHODS AND MATERIALS

Collecting Samples

Blood and feather samples were collected from 153 nestling Reddish Egrets at breeding sample sites, hereafter 'sample sites' (Chiapas=30, Yucatan=27, Florida=10, Louisiana=9, Baja=20, Texas=25, Bahamas=20, Tamaulipas=12); I collected blood/feather samples from only one nestling per nest and all nestlings were returned to their nests after sample collections (Table 1). Blood samples (about 4 µl) were collected from brachial vein using a 25 gauge needle to puncture the vein and then transferred to 1.5 ml centrifuge tubes containing 600 µl cellular lysis buffer from Puregene (Qiagen) kit. Feather samples were taken from nestling flight feather or retrice and transferred to a sterilized zip lock bag and kept at 4°C until processed. From 153 total samples, 91 samples (all samples from Yucatan, Louisiana, Florida, Chiapas, and 23 samples from Texas) had not been previously analyzed. The remainder of the samples (Baja, Bahamas, Tamaulipas, and two samples from Texas) were chosen randomly from previous a previous study for use in this study (Hill et al., 2012). After DNA extractions, nine samples (two from Chiapas, two from Texas, four from Yucatan, and one from the Bahamas) were excluded from further analyses because of low quality DNA; therefore, my total sample size was 144 individuals from eight sample sites.

Laboratory Methods

DNA from whole blood samples were extracted using a Puregene (Qiagen) kit following the manufacturer's protocol. I used 12 microsatellite primers, designed by Hill & Green (2011), modified by adding a sequence-specific forward primer with CAG tag at

the 5′ end (Miles et al., 2009). Samples were amplified through a nested method for the first PCR, they were amplified in 6µl volumes (4.84 µl water, 0.625 µl 10X taq buffer $MgCl^2$ included, 0.0625 µl 10 µM of each of two primers, 0.0625 µl 10 mM each of dNTPs, 0.0625 µl 400 ng/µl BSA, 0.03125 µl 5 U/µl taq polymerase, and 0.25 µl tDNA) using a touchdown protocol (94°C for 3 minutes, 20 cycles at 94° C for 40 second, 60° C (-0.5° C) for 40 seconds, and 72° C for 30 seconds, 12 cycles at 94° C for 40 seconds, 50° C for 40 seconds, 72° C for 30 seconds, and a final extension at 72° C for 5 minutes). For the second PCR, the products from the first PCR were used as tempate and instead of a forward primer, universal fluorescent-labeled dyes (FAM, VIC, PET, and NED) (Miles et al., 2009) were used with the same total volume and touchdown protocol. For the last part before genotyping, 0.5 µl of the final product was incubated with 8 µl formamide and 0.5 µl size standard at 95° C for 3 minutes.

For mtDNA control region, we used primers designed by Bates et al. (2009). All the samples were amplified in 12 µl reactions (10.06 µl water, 1.25 µl 10X taq buffer $MgCl^2$ included, 0.125 µl 10 µM of each of two primers, 0.125 µl 10 mM each of dNTPs, 0.125 µl 400 ng/µl BSA, 0.0625 µl 5 U/µl taq polymerase, and 0.125 µl tDNA) using the thermal profile suggested by Bates et al. (2009). Then, 8 µl of the PCR amplicons were cleaned with 2 µl Exosap-It and incubation at 37 °C for 15 minutes and 80° C for 15 minutes. We used 1 µl of PCR-cleanup products for cycle sequence reactions with 5 µl total volume (0.5 µl 5X BigDye buffer, 1 µl BigDye v3.1, 0.12 µl 10 µM of one of the primers, and 2.38 µl water) under cycle sequence reaction protocol of 96° C for 2 minutes, 30 cycles of 96° C for 10 seconds, 50° C for 5 seconds, 60° C for 4 minutes. For cycle sequence clean ups, 15 µl water was added to all samples and a total of 20 µl products filtered through a Sephedex

G-50 column. As the last step before sequencing, 0.5 µl of final products were cooked with 8µl formamide at 95˚C for 3 minutes.

To determine sex of individuals sampled, we used primers developed by Huang et al. (2012) and Wang et al. (2011). All samples were amplified through a nested method: for the first PCR in 12 µl total volume (10.06 µl water, 1.25 µl 10X taq buffer $MgCl^2$ included, 0.125 µl 10 µM of each of two primers, 0.125 µl 10 mM of each of dNTPs, 0.125 µl 400 ng/µl BSA, 0.0625 µl 5 U/µl taq polymerase, and 0.125 µl tDNA), and amplified under a touchdown protocol 94˚C for 3 minutes, 6 cycles at 94˚C for 30 seconds, 58˚C (-0.5˚C) for 40 seconds, and 72˚C for 40 seconds, 17 cycles at 94˚C for 30 seconds, 54.5˚C for 40 seconds, and 72˚C for 40 seconds, and for the final extension 72˚C for 5 minutes. For the second PCR, products of the first PCR were used in 12 µl reaction volumes (9.93 µl water, 1.25 µl 10X taq buffer $MgCl^2$ included, 0.125 µl 10 µM of each of 3 primers, 0.125 µl 10 mM of each of dNTPs, 0.125 µl 400 ng/µl BSA, 0.0625 µl 5 U/µl taq polymerase, and 0.125 µl of first PCR products) under the same touchdown protocol.

Collecting Data

Microsatellite samples were genotyped with Applied Biosystems 3500xL Genetic Analyzer and peaks read using Genemapper software version 4.1 for each sample and each locus. After initial analysis, two primers (Er42 and Er46) were excluded because of low functionality, resulting in ten primers used for downstream analyses.

All mtDNA reactions were sequenced with an Applied Biosystems 3500xL Genetic Analyzer. Sequenced data were transferred to Geneious Pro version 5.6 (Kearse et

al., 2012) for assembling and aligning with reference samples selected from NCBI ("National Center for Biotechnology Information," n.d.).

For genetic sex determination, I ran 8 µl of final sex marker products on 2% agarose gel for 45 minutes under 90 volts. The 100 bp and 250 bp represent the W and Z bands, respectively. Females were determined by presence of W and Z bands while males just showed the Z band.

Statistical Analysis

Analysis of Microsatellite Data

Arlequin version 3.5 (Excoffier et al., 2005) was used to estimate Hardy-Weinberg equilibrium, F_{ST}, R_{ST}, and to conduct an analysis of molecular variance (AMOVA). Hardy-Weinberg equilibrium estimations were run with 1,000,000 steps in the Markov-chain and 100,000 dememorization steps and were tested at the loci level to examine consistency within a population from generation to generation (Guo et al., 1992). Fst and Rst are measures of interpopulations differentiations; Fst uses allelic frequency (Höglund, 2009) without regard to a mutation model, and Rst uses the sum of squared number of repeat differences from following the mutation model (Slatkin, 1995). I ran 100 permutations of significance and 1,000 permutations for Mantel test for both Fst and Rst. For the Fst analysis, I interpreted the range of values as follows: < 0.05 as no to low differentiation, 0.05 - 0.20 as moderate differentiation, 0.21 - 0.30 as high differentiation, and > 0.30 as substantial differentiation. I conducted an AMOVA for 1,000 permutations to estimate genetic structure and differentiation among management units and sample sites. For the analysis, we *a priori* grouped sample sites based on Hill et al. (2012) and the geographical

distribution within management units: Region 1 (Eastern) including Florida and Bahamas, Region 2 (Central) including Texas, Louisiana, Yucatan, Chiapas and Tamaulipas, and Region 3 (Western) including Baja California (Wilson et al, 2014).

The Program Structure version 2.3, which uses a Bayesian clustering method to assign individuals to population clusters, was used to determine the best number of clusters (i.e., populations) given the data without bias from geographical distribution for microsatellite data (Pritchard et al., 2000). I tested *K* values from 1 to 9, each ran 10 times with a burn-in period of 100,000 followed by 700,000 Markov Chain Monte Carlo iterations to estimate the mean likelihood of each k. You can find the parameter setting in appendix 1. I determined the best k based on the highest log likelihood values (Ciofi et al., 2002; Hampton et al., 2004) defined as ln Pr(X/k) (Pritchard et al., 2000) and Δ k (Evanno et al., 2005) with Structure Harvester (Earl & vonHoldt, 2012).

To investigate the existence of a bottleneck event in any of the populations, we used Bottleneck software version 1.2 (Cornuet & Luikart, 1996) and ran Infinite alleles model (Kimura & Crow, 1964), Stepwise mutation model (Luikart et al., 1998), and Two-phase model mutations (Gordon Luikart & Cornuet, 1998) with 30 units of variance for Two-phase Model, 70% of proportion of Stepwise mutation model in Two-phase model and 10,000 iterations (Cornuet & Luikart, 1996).

Analysis of mtDNA Data

Popart version 1.7 ("Popart," n.d.) was used to develop a median-joining network (Bandelt et al., 1999). The program Mega version 6 (Tamura et al., 2013) was used to find the best model and G (gamma) value for Arlequin analyses. Arlequin version 3.5 (Excoffier

et al., 2005) was used to examine Fst, AMOVA, and haplotype inference. Fst estimations were run with 1,000 permutation to test for significance, 1,000 permutations for the Mantel test, and 0.05 level of significance both for Slatkin's and Reynolds distances (Reynolds et al., 1983; Slatkin et al., 1989). For Fst analysis, I interpreted the range of values as follows: < 0.1 as no differentiation, 0.1 - 0.30 as low differentiation, 0.31 – 0.50 as moderate differentiation, and 0.51 – 0.80 as high, and > 0.81 as substantial differentiation. I ran the AMOVA with 10,000 permutations for three regions with eight sample sites using the same protocol reported previously for the microsatellite data. I ran haplotype inferences to examine shared haplotypes among populations, and frequencies and relative frequencies of haplotypes in populations.

III. RESULTS

Sex Ratios

The number of males, females and sex ratio for each sample site, each region and in total are provided in Table 1. In five out of eight sample sites, the number of males was higher than the number of females (Florida, Tamaulipas, Texas, Yucatan, and Louisiana). While the ratio for Eastern and Central regions was roughly 1:1, there were more females than males in the Western region; overall the ratio was ~ 1:1 (71 females: 73 males) across the range and all sample sites.

Microsatellite Data

Fourteen of 80 loci-sample site combinations significantly deviated from Hardy–Weinberg equilibrium (Table 2). Seven loci-sample site combinations were monomorphic and therefore no Hardy – Weinberg equilibrium was calculated. The number of alleles per locus ranged from one to ten alleles. Locus Er41 had the most significant difference between observed and expected heterozygosity among sample sites (four populations out of eight) and locus Er31 has the most monomorphic structure among all sample sites (4 populations out of eight). Chiapas with five loci was the most out of equilibrium sample site among all Reddish Egret sites.

We found ten and eight high to substantial pairwise differences for Fst and Rst, respectively, between Reddish Egret sample sites (Table 3). For Fst values, the high to substantial (> 0.2) differences were between Florida and Louisiana, Tamaulipas and Louisiana, Tamaulipas and Bahamas, Tamaulipas and Baja, Chiapas and Baja, Texas and

Baja, Yucatan and Baja, Louisiana and Bahamas, Louisiana and Baja, and Bahamas and Baja. For Rst values, the high to substantial differences were between Tamaulipas and Louisiana, Yucatan and Tamaulipas, Florida and Baja, Baja and Tamaulipas, Chiapas and Baja, Texas and Baja, Louisiana and Baja and Bahamas and Baja.

The analysis of molecular variance revealed there is more differentiation between regions than among sample sites within a region (Table 4). For the analyses of composition of genomes among populations (Program Structure), I found differing results for log likelihood and Δ k. The number of clusters based on Δ k suggested two clusters, followed by four for the all Reddish Egret sample sites (Fig. 2a). Based on log likelihood, five and four clusters are more likely (Fig. 2b). The $K = 2$ barplot (Fig. 3a) suggests that the localities Baja, Bahamas and Chiapas potentially represent two demes and possibly two breeding centers (i.e. there is less genetic differentiation within each populations), while the other sampling sites are a mix of the two demes. My results also show that the population structure of Reddish Egrets in Chiapas is different from the structure in Bahamas and Baja, whereas, Bahamas and Baja have similar structure (Fig. 3a). The $K = 4$ barplot (Fig. 3b) still supports that Baja, Bahamas, and Chiapas are breeding centers, indicating there is less mixing with other sites at these three centers. Based on four clusters, samples from Baja, Chiapas and the Bahamas correspond to three unique genetic demes each, while Texas, Louisiana, Yucatan, Florida and Tamaulipas exhibit a mixture of the three demes, with the fourth genetic deme highly represented in Texas, Yucatan, Louisiana, and Tamaulipas.

For bottleneck, I found potential evidence in Reddish Egrets from Tamaulipas and Chiapas (Table 5).

mtDNA Data

I detected 10 haplotypes across all sample sites (Fig. 4, Table 6). Yucatan had the most shared haplotypes (Hap. 1, Hap. 3, Hap. 5, Hap. 6, Hap. 8, Hap. 9 and Hap. 10). Texas, Yucatan, Tamaulipas, and Louisiana have shared haplotype with all other populations. There were no shared haplotypes between Bahamas and Chiapas, Baja and Chiapas, Florida and Tamaulipas, and Texas and Florida. Hap. 3 is the most shared haplotype occurring in six populations. Chiapas and Bahamas each had a single representative haplotype. Five more other haplotypes were unique to either Baja (2), Florida (1), or Yucatan (2).

The Kimura 2P mutation model was selected as the best model and used for all further analyses. I found 14 pairwise loci-sample site comparisons with high to substantial levels of differences (Table 7). Estimates of migrants per generation revealed > 1 individuals migrate per generation for 7 pairwise comparisons. The remaining comparisons had values < 1 suggesting one individual migrates between two populations every 2-3 generation (Table 7).

The AMOVA analysis showed higher variance components and percentage of variation among regions than among sites within regions (Table 8).

IV. DISCUSSION

The analyses of observed versus expected heterozygosity showed most loci were under Hardy – Weinberg equilibrium. Locus Er41 was the most out of equilibrium locus (four out of eight sample sites, Chiapas, Bahamas, Baja and Florida). In all of these four sample sites, observed heterozygosity was less than expected. For Florida, the result could be the result of small sample size. For the three other populations, possible explanations include natural selection and migration. Natural selection could lead this locus to be more homozygote than heterozygote. It is also possible that new members from other populations move to these populations and skewed the result. In my study, natural selection does make more sense since based on Structure results, each of these locations (Chiapas, Baja and Bahamas) have their own genetic demes indicating less mixing and accepting less emigrants. Locus Er31 is monomorphic in four sample sites (Florida, Tamaulipas, Chiapas, and Bahamas). For Florida and Tamaulipas, this could be due to low sample size whereas with Chiapas and the Bahamas, reduced gene flow (less immigrants) and genetic adaption is a likely explanation.

In my research, I found more variation among regions than among sites within a region for both molecular markers (mtDNA and microsatellites). I grouped, *a priori*, all 8 Reddish Egrets sample sites into three management units as Hill et al. (2012) suggested; my results support these findings. Although I support the delineation of three management units: Western region (Baja), Central region (Texas, Tamaulipas, Louisiana, Chiapas and Yucatan) and Eastern (Florida and Bahamas), there are some high to substantial differences between sites within a same region (Tamaulipas and Yucatan, Tamaulipas and Louisiana) based on microsatellite data. Low sample size from Tamaulipas and Louisiana might be a

reason as banding and telemetry data has shown considerable movement between Texas, Tamaulipas and Louisiana (Geary et al., 2015). Based on microsatellite data, individuals from Baja have high to substantial differences with all other Reddish Egret populations; breeding individuals in Baja have the greatest geographic separation from all other sample sites. However, banding data revealed some movements from Baja to Chiapas (Green & Palacios, unpubl. data). Compared with the mtDNA results, there are only three high to substantial differences for Baja (with Louisiana, Chiapas and Bahamas). While Louisiana may again be because of low sample size from that sample site, differences between Baja and Chiapas and Baja and Bahamas may have other explanations. Samples sites in Chiapas and Bahamas have only one haplotype each. The haplotype present in Chiapas is only found in other central sample sites. The haplotype in the Bahamas (hap. 3) was found in Baja population as well but there are three more haplotypes in Baja that are not in Bahamas.

There are two demes in two clusters structure: a) Chiapas, b) Bahamas and Baja. The first three sample sites represent a single cluster, whereas, other sample sites are mixture of the first two. Similar structure between Reddish Egrets of Baja and Bahamas can be explained as these two sample sites potentially have same ancestor but have diverged over time as our four cluster results indicate separated structuring in Baja and Bahamas.

There are four demes in four clusters structure: a) Chiapas, b) Bahamas, c) Baja, and d) Texas, Yucatan, Louisiana, Tamaulipas. Three first demes are restricted to individual sample sites. This limitation and single structure for any of these locations is likely because of less migration to these populations. This does not mean there is no migration to any of these locations but there is lack of evidence for much if any gene flow

between these demes. Banding data shows there is a movement from Baja to Chiapas, but there is no evidence that individual breed in Chiapas. Also, no movement from or to Bahamas and no movement to Baja has been recorded from banding or telemetry data. Each of these locations belong to one of the three management units (Baja for Western, Chiapas for Central, and Bahamas for Eastern) and appear to be important breeding centers for each of the units. Previous research indicated Texas as important breeding center across the range of the species. However, Hill et al. (2012) did not sample from any other sites geographically close to Texas (i.e. Louisiana, Yucatan, Chiapas). Our results indicate that Texas is part of larger geographic deme that includes Texas, Louisiana, Tamaulipas and Yucatan; telemetry data supports movement between these sample sites (Geary et al., 2015; Koczur & Ballard, unpubl. data). While Texas may not represent a unique breeding center like Chiapas, Texas still contains the largest number of breeding individuals across the range and is still important for the long term conservation of the species (Green, 2006; Wilson et al., 2014).

While Florida shows some similarities in structure compared to neighboring demes, the overall structure appears different. Also, the haplotype result is different for Florida. There are two haplotypes in Florida, one of which is represented in the Bahamas and the other belongs to Florida and cannot be found in any of the other sample sites. The small sample size from Florida might limit our inferences, but we suggest some differences observed in Florida for both microsatellite and mtDNA variation suggest that genetic variation in Florida is unique. Limited banding and telemetry data has not revealed any movement or migration to or from Florida to other sample sites (Green, unpubl. data;

Meyer & Kent, unpubl. data). Increased sample size for Florida may strengthen our inferences about this site.

The interesting finding of shared haplotypes between the Bahamas and Baja warrants some explanation. The only haplotype found in the Bahamas was also found in Baja and possible scenarios include: 1) females are long distance migrants; however, this is not supported by any of our analyses or banding/telemetry studies, 2) since Baja and Bahamas have the same ancestral structure, it is possible the haplotype in these two populations had the same mutations under selection, but this is highly unlikely, and 3) historically, before the recent declines of Reddish Egret populations, there were more breeding colonies (e.g. populations) between the Western and Central regions with shorter distances apart which enabled females more opportunities to move between populations with their short distance migrations and movements.

I found no evidence of recent bottleneck event in any of the sites with the exception of possible bottleneck in Tamaulipas. While I cannot rule out a bottleneck event, it is more likely a result of small sample size from the Tamaulipas population. Hill et al. (2012) and Bates et al. (2009) did not detect any recent bottleneck event and we infer that our results do not strongly support evidence for any recent bottleneck.

In general there was more differentiation among sites based on mtDNA than microsatellites. Sine mtDNA is a maternally–inherited gene and haploid, it shows less movement for females compare to microsatellite markers. Also, banding and telemetry data support this conclusion as well. Differential movement between sexes has been shown in long-term banding data (Green and Palacios, unpubl.data) and satellite telemetry in juveniles (Geary et al. 2015), with males dispersing greater distances than females.

Presumably, male juveniles may be the dispersing sex and migrate to find new colonies with better environmental and foraging habitats. My results demonstrate movement within and between management units and suggest likely differences in gene flow between female and males. Future studies using telemetry and banding across the range of the Reddish Egret would yield considerable insight into potential differences in movement between males and females; important information necessary for the conservation of North America's rarest heron.

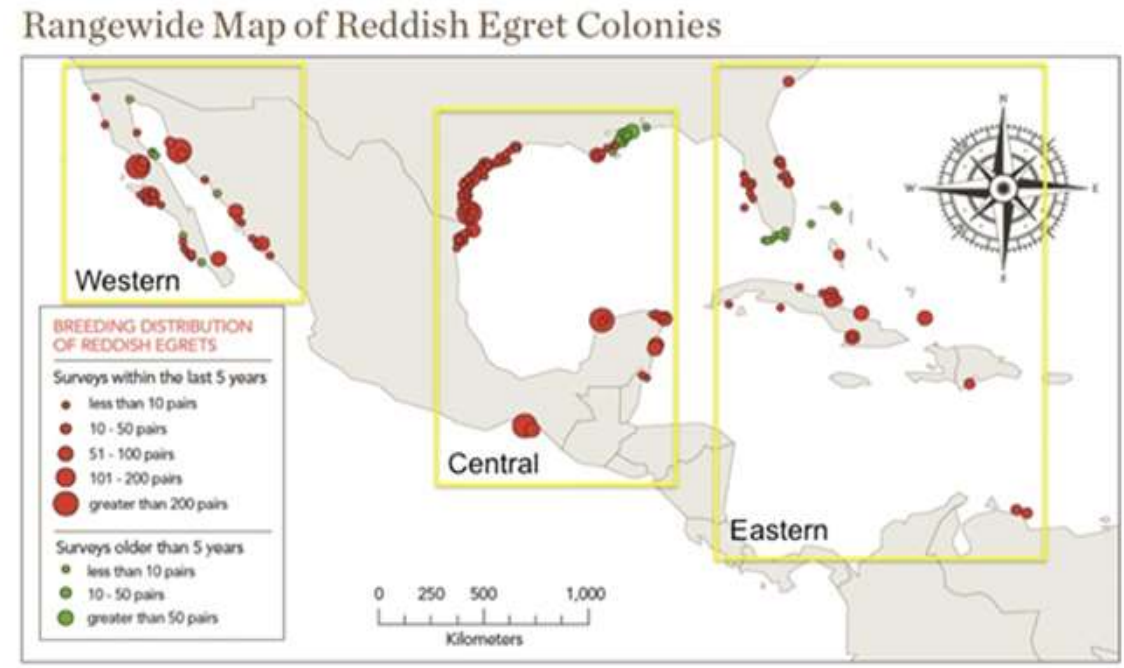
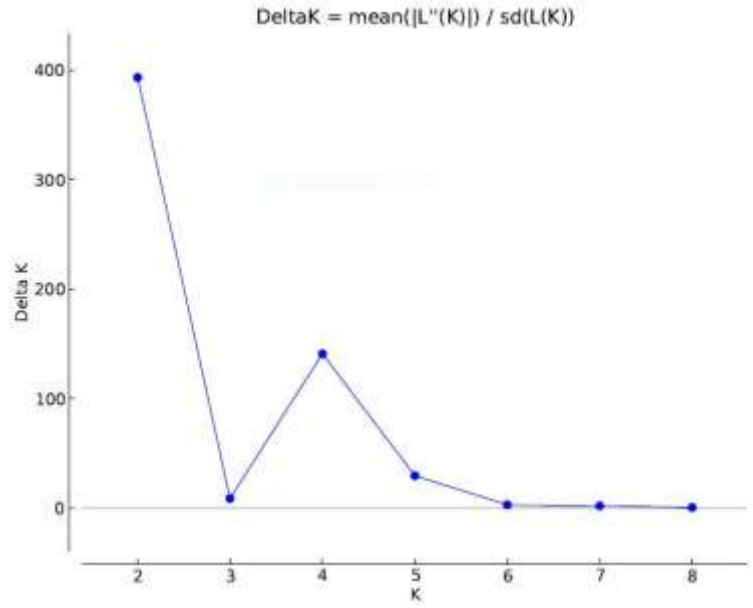


Figure 1. Spatial distribution of Reddish Egret (*Egretta rufescens*) breeding colonies across the species range. Yellow boxes indicate Management Units established by the Reddish Egret Working Group (Wilson at el., 2014).

a)



b)

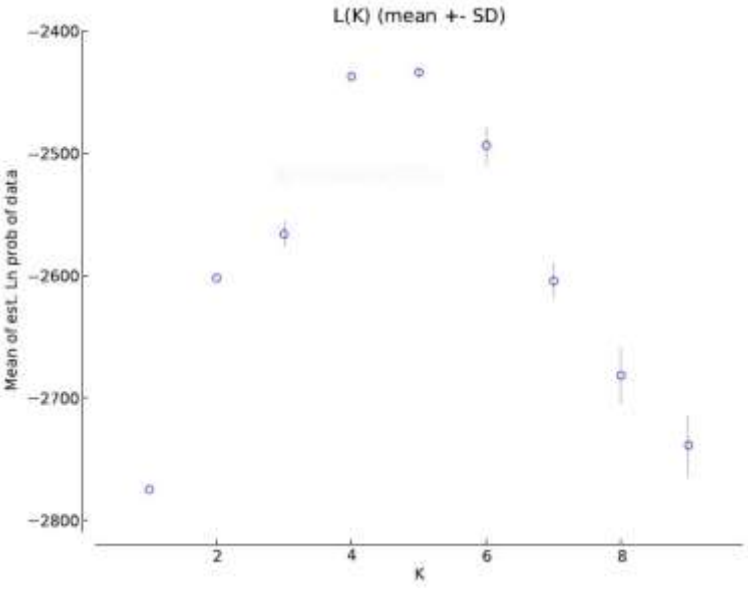
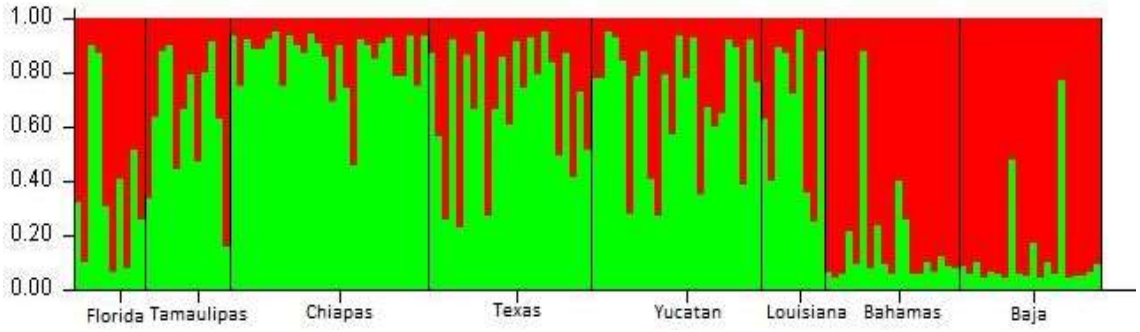


Fig 2. Summarized results from StructureHarvester of the Structure analysis indicating the most likely number of genetic demes represented among our Reddish Egret (*Egretta rufescens*) sample sites. a) The delta k graph which shows two clusters which followed by four would fit best with our data. b) The log likelihood graph shows four or five clusters as most likely number of genetic demes.

a)



b)

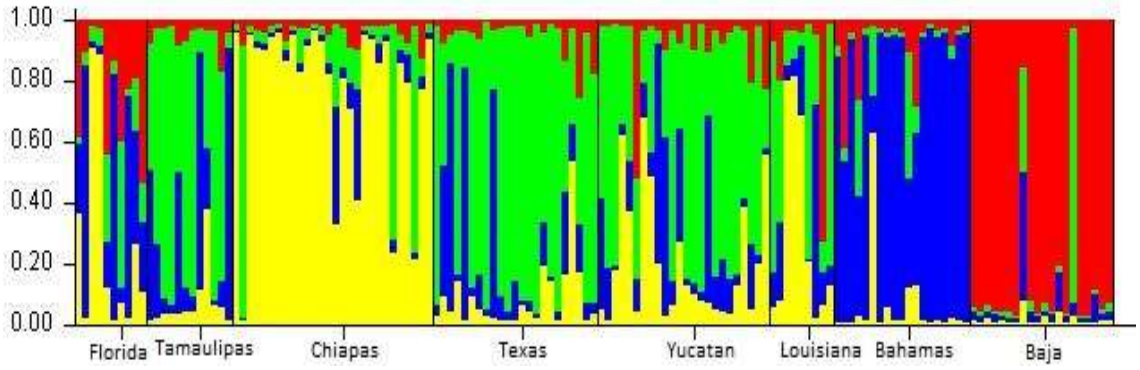


Fig 3. Barplots of individual assigment probablilities from the Structure analysis for K = 2 and K = 4 in Reddish Egret (*Egretta rufescens*) populations. a) Plot of two clusters which reveal two demes: 1) Chiapas, 2) Bahamas and Baja. First two demes are made from one structures. Other sample sites made of mixture of two demes b) Plot of four clusters which reveal four demes: 1) Chiapas, 2)Bahamas, 3)Baja, 4) Texas, Yucatan, Louisiana, Tamaulipas and Florida. First three demes have their own structure while fourth deme is mixture of the first three and one more structures.

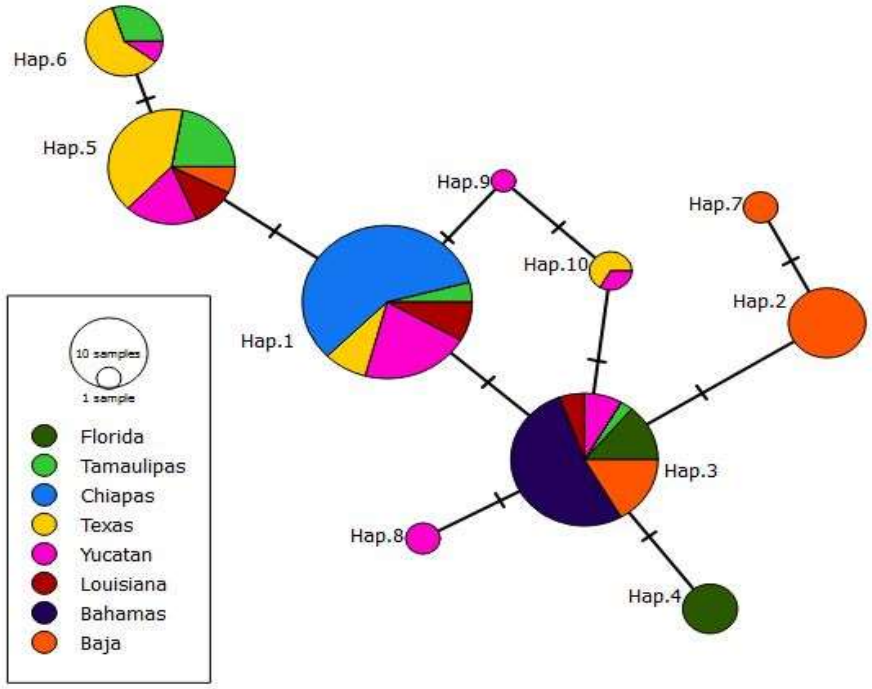


Fig 4. Network-joining graph between haplotypes among sample sites of Reddish Egret (*Egretta rufescens*). There are 10 haplotypes in total. Each haplotype shown as a circle and size of circles depends to the number of individuals carry that haplotype. Each location has its own color to show what percentage of each haplotype belongs to which population. Haplotypes seperated from each other with one mutation unit (small bars on networking lines between two circles).

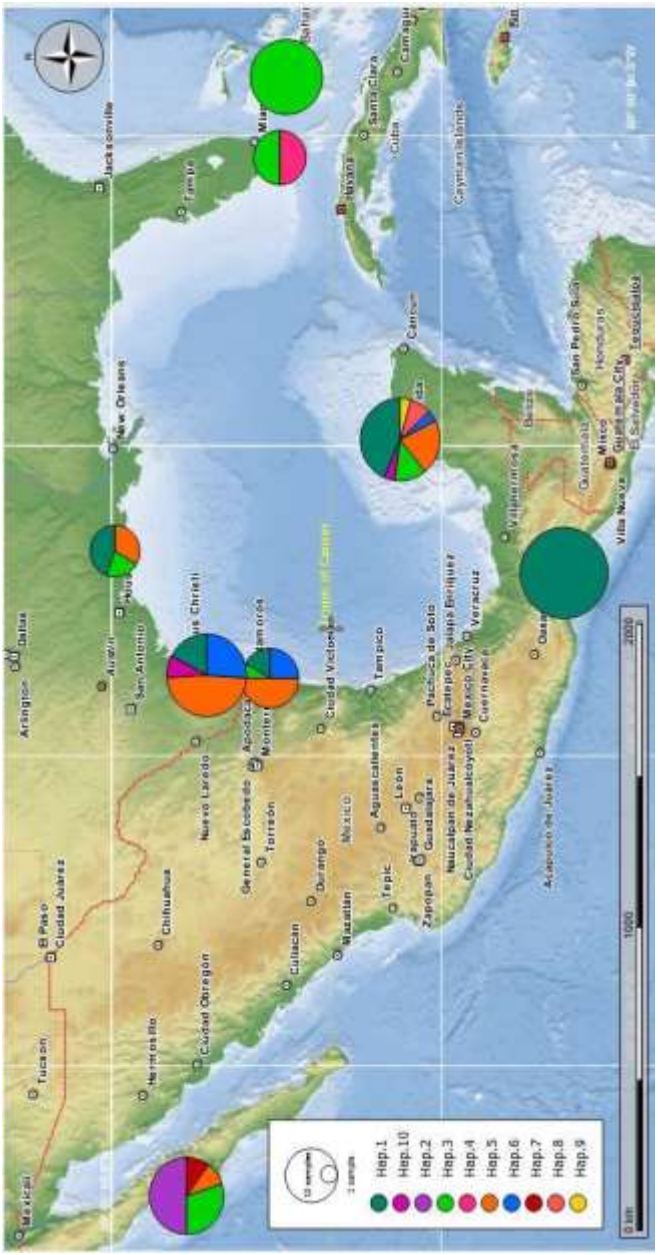


Fig 5. Percentages of different haplotypes in each location of Reddish Egert (*Egretta rufescens*) sample sites. Size of circles depend to sample size of each location (numbers in circles). Each haplotypes represented with specific color to show precentage of each haplotype in each population.

Table 1. Sampling sites for Reddish Egrets (*Egretta rufescens*) including sample size, location, and number of males, females and sex ratios.

Regions	Sample sites	Sample size	Longitude	Latitude	Male	Female	Sex ratio (M:F)
Central	Tamaulipas	12	-97.5	25.3	7	5	1.4 : 1
	Chiapas	28	-94.1	16.1	8	20	1 : 2.5
	Texas	23	-97.4	27.2	16	7	2.1 : 1
	Yucatan	24	-89.8	21.3	14	10	1.4 : 1
	Louisiana	9	-93.4	29.9	7	2	3.5 : 1
Eastern	Bahamas	19	-78.1	24.8	8	11	1 : 1.4
	Florida	10	-80.7	25	7	3	2.3 : 1
Western	Baja	20	-114.2	27.8	7	13	1 : 1.8
	Total	145			73	71	1.03:1

Table 2. Heterozygosity and Hardy – Weinberg equilibrium for all loci and all sample sites of Reddish Egrets (*Egretta rufescens*). Observed and expected heterozygosity, p-value and number of alleles for each sample site for each locus.

Locus	Heterozygosity	FL	Tam	Chp	Tx	Yuc	Loui	Bah	Baja
Er21	Obs. Het.	X	0	0.04	0.27	0.12	0.1	0.05	0.15
	Exp. Het.	X	0.16	0.11	0.38	0.19	0.1	0.05	0.15
	P - value	X	0.4	0.06	0.29	0.21	1	1.0	1.0
	# alleles	1	2	2	4	2	2	2	3
Er22	Obs. Het.	0.4	0.63	0.63	0.43	0.42	0.67	0.58	**0.65**
	Exp. Het.	0.34	0.68	0.70	0.60	0.66	0.68	0.68	**0.54**
	P – value	1.0	0.21	0.17	0.06	0.053	1.0	0.34	**0.00**
	# alleles	2	3	4	4	3	3	3	**3**
Er41	Obs. Het.	**0.1**	0.27	**0.04**	0.43	0.33	0.22	**0.05**	**0.00**
	Exp. Het.	**0.48**	0.52	**0.17**	0.48	0.45	0.52	**0.23**	**0.10**
	P – value	**0.02**	0.22	**0.00**	0.67	0.15	0.17	**0.01**	**0.02**
	# alleles	**2**	2	**3**	2	3	2	**2**	**2**
Er43	Obs. Het.	**0.3**	**0.33**	0.35	0.61	0.42	0.78	0.65	0.55
	Exp. Het.	**0.5**	**0.63**	0.30	0.58	0.53	0.53	0.63	0.58
	P – value	**0.03**	**0.02**	1.0	0.35	0.29	0.23	0.10	0.81
	# alleles	**4**	**4**	3	3	3	2	3	4
Er51	Obs. Het.	0.4	0.82	**0.65**	0.71	0.87	1.0	0.88	0.5
	Exp. Het.	0.82	0.87	**0.87**	0.86	0.81	0.82	0.74	0.60
	P – value	0.09	0.26	**0.00**	0.41	0.81	0.42	0.63	0.36
	# alleles	5	8	**10**	9	9	7	6	4
Er23	Obs. Het.	0.5	0.64	**0.18**	0.54	0.74	**0.11**	0.37	**0.35**
	Exp. Het.	0.8	0.51	**0.46**	0.58	0.70	**0.54**	0.33	**0.53**
	P – value	0.09	0.17	**0.00**	0.88	0.51	**0.00**	1.0	**0.02**
	# alleles	5	3	**4**	5	5	**3**	4	**4**
Er44	Obs. Het.	0.62	0.2	**0.61**	0.35	0.58	0.77	0.42	0.35
	Exp. Het.	0.77	0.33	**0.71**	0.46	0.45	0.80	0.4	0.30
	P – value	0.15	0.30	**0.04**	0.33	0.19	0.16	1.0	1.0
	# alleles	5	2	**4**	3	2	5	2	2
Er31	Obs. Het.	X	X	X	0.17	0.04	0.11	X	0.25
	Exp. Het.	X	X	X	0.16	0.04	0.11	X	0.30
	P – value	X	X	X	1.0	1.0	1.0	X	0.47
	# alleles	1	1	1	2	2	2	1	2
Er45	Obs. Het.	X	0.58	0.07	0.30	0.58	0.66	X	0.05
	Exp. Het.	X	0.43	0.07	0.51	0.48	0.52	X	0.14
	P – value	X	0.49	1.0	0.09	0.16	0.54	X	0.08
	# alleles	1	2	2	2	2	2	1	2
Er24	Obs. Het.	0.67	0.54	**0.33**	0.73	0.75	0.71	**0.42**	0.35
	Exp. Het.	0.79	0.79	**0.51**	0.78	0.73	0.79	**0.65**	0.40
	P – value	0.29	0.05	**0.00**	0.50	0.89	0.46	**0.00**	0.26
	# alleles	5	6	**5**	5	7	6	**4**	6

Loci in bold are statistically significant ($\alpha \leq 0.05$). Calculation for monomorphic loci shown with X.

Table 3. Genetic differentiation (Fst and Rst values) for microsatellite data of Reddish Egret (*Egretta rufescens*) samples. Rst values and Fst values are presented above and below the diagonal. Values above 0.2 considered as high to substantial difference.

	Florida	Tamaulipas	Chiapas	Texas	Yucatan	Louisiana	Bahamas	Baja
Florida	-	0.194	0.042	-0.03	-0.012	0.045	0.061	0.245
Tamaulipas	0.136	-	0.131	0.168	0.227	0.292	0.109	0.453
Chiapas	0.103	0.177	-	0.033	0.038	0.127	-0.015	0.327
Texas	0.063	0.178	0.030	-	0.043	0.127	0.035	0.334
Yucatan	0.096	0.188	0.030	0.024	-	0.054	0.057	0.225
Louisiana	0.229	0.319	0.167	0.097	0.082	-	0.158	0.329
Bahamas	0.182	0.203	0.023	0.040	0.039	0.214	-	0.342
Baja	0.183	0.238	0.226	0.212	0.212	0.309	0.240	-

Underlined values are statistically significant ($\alpha \leq 0.05$)

Table 4. Analysis of Molecular Variation (AMOVA) using microsatellite markers to examine variation among regions and among sample sites within regions of Reddish Egrets (*Egretta rufescens*)

Source of variation	d.f.	Sum of squares	Variance Components	Percentage of Variance	P-value
Among regions	2	64.012	0.306	12.29	0.000
Among sample sites within regions	5	45.932	0.207	8.32	0.005

Higher variance components and percentage of variance in among regions compare to among sample sites within regions show more differentiations.

Table 5. Bottleneck results for all sample sites using three different mutation models for Reddish Egrets (*Egretta rufescens*): Infinite Alleles Model (IAM), Two-Phase Model (TPM) and Stepwise Mutation Model (SMM)

Population	p-value IAM	p-value TPM	p-value SMM
Florida	0.084	0.119	0.367
Tamaulipas	0.024	0.042	0.381
Chiapas	0.187	0.153	0.040
Texas	0.078	0.256	0.467
Yucatan	0.065	0.475	0.504
Louisiana	0.239	0.415	0.516
Bahamas	0.170	0.459	0.501
Baja	0.385	0.521	0.099

The significant p – values underlined.

Table 6. Frequencies and relative frequencies of each haplotype of mtDNA control region in all sample sites for Reddish Egrets (*Egretta rufescens*).

	Florida	Tamaulipas	Chiapas	Texas	Yucatan	Louisiana	Bahamas	Baja
Number of samples	10	12	28	23	23	9	19	20
Haplotype Frequencies								
Hap. 1	0	2	28	4	10	4	0	0
Hap. 2	0	0	0	0	0	0	0	10
Hap. 3	5	1	0	0	3	2	19	6
Hap. 4	5	0	0	0	0	0	0	0
Hap. 5	0	6	0	11	5	3	0	2
Hap. 6	0	3	0	6	1	0	0	0
Hap. 7	0	0	0	0	0	0	0	2
Hap. 8	0	0	0	0	2	0	0	0
Hap. 9	0	0	0	0	1	0	0	0
Hap.10	0	0	0	2	1	0	0	0
Haplotype Relative Frequencies								
Hap. 1	0	0.167	1.000	0.174	0.435	0.440	0	0
Hap. 2	0	0	0	0	0	0	0	0.500
Hap. 3	0.500	0.083	0	0	0.130	0.220	1.000	0.300
Hap. 4	0.500	0	0	0	0	0	0	0
Hap. 5	0	0.500	0	0.478	0.217	0.330	0	0.100
Hap. 6	0	0.250	0	0.261	0.043	0	0	0
Hap. 7	0	0	0	0	0	0	0	0.100
Hap. 8	0	0	0	0	0.087	0	0	0
Hap. 9	0	0	0	0	0.043	0	0	0
Hap. 10	0	0	0	0.087	0.043	0	0	0

Table 7. Genetic differentiation (Fst) and migration rates (M) for Reddish Egrets (*Egretta rufescens*) based on mtDNA data. Fst values (lower diagonal) greater than 0.5 considered as high to substantial differences. M-values (upper diagonal) indicate average number of individuals moving between two sample sites per generation.

	Florida	Tamaulipas	Chiapas	Texas	Yucatan	Louisiana	Bahamas	Baja
Florida	-	0.392	0.294	0.397	0.635	0.056	0.238	0.792
Tamaulipas	0.560	-	0.219	0.200	0.564	0.00	0.129	0.768
Chiapas	0.629	0.695	-	**3.051**	**2.242**	0.422	0	0.376
Texas	0.557	0.717	0.141	-	**Inf**	0.703	**2.865**	0.632
Yucatan	0.440	0.470	0.182	0.060	-	**2.122**	**2.219**	0.749
Louisiana	0.900	1.00	0.542	0.416	0.191	-	0.232	0.200
Bahamas	0.677	0.795	1.00	0.149	0.184	0.683	-	0.355
Baja	0.387	0.394	0.571	0.441	0.400	0.725	0.585	-

For Fst values, the underlined are statistically significant and the M-values in bold represent movements with more than one individual per generation (Inf: Infinite).

Table 8. Analysis of Molecular Variation (AMOVA) using mtDNA marker to examine variation among regions and among sample sites within regions of Reddish Egrets (*Egretta rufescens*).

Source of variation	d.f.	Sum of Squares	Variance components	Percentage of variation	P-value
Among regions	2	34.635	0.398	44.93	0.000
Among sample sites within regions	5	13.326	0.134	15.11	0.005

Higher value of Variance components and Percentage of variation in among regions compare to among sample sites within regions reveal more differentiation among regions than among sample sites within regions.

APPENDIX SECTION

Running Length

Length of Burnin Period: 100000
Number of MCMC Reps after Burnin: 700000

Ancestry Model Info

Use Admixture Model
* Infer Alpha
* Initial Value of ALPHA (Dirichlet Parameter for Degree of Admixture): 1.0
* Use Same Alpha for all Populations
* Use a Uniform Prior for Alpha
** Maximum Value for Alpha: 10.0
** SD of Proposal for Updating Alpha: 0.025

Frequency Model Info

Allele Frequencies are correlated among Pops
* Assume Different Values of Fst for Different Subpopulations
* Prior Mean of Fst for Pops: 0.01
* Prior SD of Fst for Pops: 0.05
* Use Constant Lambda (Allele Frequencies Parameter)
* Value of Lambda: 1.0

Advanced Options

Estimate the Probability of the Data Under the Model
Frequency of Metropolis update for Q: 10

LITERATURE CITED

Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, *16*(1), 37–48. doi:10.1093/oxfordjournals.molbev.a026036

Barrett, S. C., & Charlesworth, D. (1991). Effects of a change in the level of inbreeding on the genetic load. *Nature*, *352*(6335), 522–524. doi:10.1038/352522a0

Bates, E. M., Deyoung, R. W., & Ballard, B. M. (2009). Genetic Diversity and Population Structure of Reddish Egrets Along the Texas Coast Genetic Diversity and Population Structure of Reddish Egrets along, *32*(3), 430–436.

Bouzat, J. L., Cheng, H. H., Lewin, H. a., Westemeier, R. L., Brawn, J. D., & Paige, K. N. (1998). Genetic Evaluation of a Demographic Bottleneck in the Greater Prairie Chicken. *Conservation Biology*, *12*(4), 836–843. doi:10.1046/j.1523-1739.1998.97164.x

Brown, S. M., Harrisson, K. a, Clarke, R. H., Bennett, A. F., & Sunnucks, P. (2013). Limited population structure, genetic drift and bottlenecks characterise an endangered bird species in a dynamic, fire-prone ecosystem. *PloS One*, *8*(4), e59732. doi:10.1371/journal.pone.0059732

Charlesworth, B., & Charlesworth, D. (1999). The genetic basis of inbreeding depression. *Genetical Research*, *74*(3), 329–340. doi:10.1017/S0016672399004152

Charlesworth, D., & Charlesworth, B. (1987). Inbreeding Depression and its Evolutionary Consequences. *Annual Review of Ecology and Systematics*. doi:10.1146/annurev.es.18.110187.001321

Ciofi, C., Milinkovitch, M. C., Gibbs, J. P., Caccone, a, & Powell, J. R. (2002). Microsatellite analysis of genetic divergence among populations of giant Galapagos tortoises. *Molecular Ecology*, *11*(11), 2265–2283. doi:10.1046/j.1365-294X.2002.01617.x

Coates, D. J. (1992). Genetic consequences of a bottleneck and spatial genetic structure in the triggerplant Stylidium coroniforme (Stylidiaceae). *Heredity*.

Cornuet, J. M., & Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, *144*(4), 2001–2014. doi:Article

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. doi:10.1007/s12686-011-9548-7

Ellstrand, N. C. (1992). Gene flow by pollen: implications for plant conservation genetics. *Oikos*, *63*(1), 77–86. doi:10.2307/3545517

Ellstrand, N. C., & Elam, D. R. (1993). Population Genetic Consequences of Small Population Size: Implications for Plant Conservation. *Annual Review of Ecology and Systematics*. doi:10.1146/annurev.es.24.110193.001245

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, *14*(8), 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x

Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, *1*, 47–50. doi:10.1111/j.1755-0998.2010.02847.x

Frankham, R. (1996). Relationship of genetic variation to population size in wildlife. *Conservation Biology*, *10*(6), 1500–1508. doi:10.1046/j.1523-1739.1996.10061500.x

Geary, B., Green, M., & Ballard, B. (2015). Movements and survival of juvenile reddish egrets Egretta rufescens on the Gulf of Mexico coast. *Endangered Species Research*, *28*(2), 123–134. doi:10.3354/esr00682

González, B. a, Orozco-Terwengel, P., von Borries, R., Johnson, W. E., Franklin, W. L., & Marín, J. C. (2014). Maintenance of genetic diversity in an introduced island population of guanacos after seven decades and two severe demographic bottlenecks: implications for camelid conservation. *PloS One*, *9*(3), e91714. doi:10.1371/journal.pone.0091714

Green, M. C. (2006). Status Report and survy recommendations on the Reddish Egret (Egretta rufescens). *U.S. Fish and Wildlife Service, Atlanta, GA.*, 37pp.

Green, M. C., Hill, A., Troy, J. R., Holderby, Z., & Geary, B. (2011). Status of Breeding Reddish Egrets on Great Inagua , Bahamas with Comments on Breeding Territoriality and the Effects of Hurricanes Status of Breeding Reddish Egrets on Great Inagua , Bahamas with Comments on Breeding Territoriality and the Effects of Hurr. *Waterbirds*, *34*(2), 213–217.

Guo, S. W., Guo, S. W., Thompson, E. A., & Thompson, E. A. (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*, *48*(2), 361–372. doi:10.2307/2532296

Hampton, J. O., Spencer, P. B. S., Alpers, D. L., Twigg, L. E., Woolnough, A. P., Doust, J., … Pluske, J. (2004). Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: A case study with feral pigs. *Journal of Applied Ecology*, *41*(4), 735–743. doi:10.1111/j.0021-8901.2004.00936.x

Hill, A., Green, C., & Palacios, E. (2012). Genetic diversity and population structure of North America's rarest heron, the reddish egret (Egretta rufescens). *Conservation Genetics*, *13*(2), 535–543. doi:10.1007/s10592-011-0305-y

Hill, A., & Green, M. C. (2011). Characterization of 12 polymorphic microsatellites for the Reddish Egret, Egretta rufescens. *Conservation Genetics Resources*, *3*(1), 13–15. doi:10.1007/s12686-010-9267-5

Höglund, J. (2009). *Evolutionary conservation genetics*. Oxford University Press.

Holderby, Z., Simper, W., Geary, B., & Clay, M. (2012). Potential Factors Affecting Nest Initiation Date , Clutch Size and Nest Success in the Plumage Dimorphic Reddish Egret Potential Factors Affecting Nest Initiation Date , Clutch Size and Nest Success in the Plumage Dimorphic Reddish Egret, *35*(3), 437–442.

Huang, X., Zhou, X., Lin, Q., Fang, W., & Chen, X. (2012). An efficient molecular sexing of the vulnerable Chinese egret (Egretta eulophotes) from faeces samples. *Conservation Genetics Resources*, *4*(2012), 391–393. doi:10.1007/s12686-011-9557-6

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., … Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647–1649. doi:10.1093/bioinformatics/bts199

Keller, L., & Waller, D. (2002). Interbreeding effects in wild populations. *Trends in Ecology and Evolution*, *17*(May), 230–241.

Kimura, M., & Crow, J. F. (1964). The number of alleles that can be maintained in a finite population. *Genetics*, *49*, 725–738.

Lande, R. (1992). Neutral Theory of Quantitative Genetic Variance in an Island Model with Local Extinction and Colonization. *Evolution*, *46*(2), 381–389. doi:Doi 10.2307/2409859

Lowther, P. E. and Paul, R. T. (2002). *The Birds of North America*.

Luikart, G., & Cornuet, J. M. (1998). Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology*, *12*(1), 228–237. doi:10.1046/j.1523-1739.1998.96388.x

Luikart, G., Sherwin, W. B., Steele, B. M., & Allendorf, F. W. (1998). Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. *Molecular Ecology*, *7*(8), 963–974. doi:10.1046/j.1365-294x.1998.00414.x

McNeely, J. A., Miller, K. R., Reid, W. V., Mittermeier, R. A., & Werner, T. B. (1990). *Conserving the world's biiological diversity*. World Conservation Union, World Resources Institute, Conservation Inernational, World Wildlife Fund - US, and the World Bank, Washington, D. C.

Miles, L. G., Isberg, Æ. S. R., Moran, Æ. C., Hagen, C., Glenn, Æ. T. C., Á, P. Á. T. Á. T., & Glenn, T. C. (2009). 253 Novel polymorphic microsatellites for the saltwater crocodile (Crocodylus porosus). *Conservation Genetics*, 963–980. doi:10.1007/s10592-008-9600-7

Miño, C. I., & Del Lama, S. N. (2014). Genetic differentiation among populations of the Roseate Spoonbill (Platalea ajaja; Aves: Pelecaniformes) in three Brazilian Wetlands. *Biochemical Genetics*, *52*(7-8), 321–37. doi:10.1007/s10528-014-9650-0

National Center for Biotechnology Information. (n.d.). Retrieved September 3, 2015, from http://www.ncbi.nlm.nih.gov/

Nei, M., Maruyama, T., & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution*, *29*(1), 1–10. Retrieved from http://www.jstor.org/stable/2407137

Newman, D., & Pilson, D. (1997). Increased Probability of Extinction Due to Decreased Genetic Effective Population Size : Experimental Populations of Clarkia pulchella. *Evolution*, *51*(2), 354–362. doi:10.2307/2411107

Paul, R. (1978). History and current status of reddish egrets in the United States. *Proceedings of the Colonial Waterbird Group*, *1*, 179–184. Retrieved from http://www.jstor.org/stable/1520918

Paul, R. (1991). Status Report - egretta rufescens (GMELIN) Reddish Egret. *U.S. Fish and Wildlife Service, Houston, Texas.*, 73pp.

Popart. (n.d.). Retrieved August 27, 2015, from http://www.popart.otago.ac.nz/index.shtml

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959. doi:10.1111/j.1471-8286.2007.01758.x

Reed, D. H., & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conservation Biology*, *17*(1), 230–237.

Reynolds, J., Weir, B. S., & Cockerham, C. C. (1983). Estimation of the coancestry coefficient: Basis for a short-term genetic distance. *Genetics*, *105*(3), 767–779.

Simberloff, D. (1988). The Contribution of Population and Community Biology to Conservation Science. *Annual Review of Ecology and Systematics*. doi:10.1146/annurev.es.19.110188.002353

Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, *139*(1), 457–462. doi:Article

Slatkin, M., Slatkin, M., Barton, N. H., & Barton, N. H. (1989). A Comparison of Three Indirect Methods for Estimating Average Levels of Gene Flow. *Evolution*, *43*(7), 1349–1368. Retrieved from internal-pdf://slatkin_barton_evolution_1989-3716207877/Slatkin_Barton_Evolution_1989.pdf

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–2729. doi:10.1093/molbev/mst197

Wang, Z., Zhou, X., Lin, Q., Fang, W., & Chen, X. (2011). New primers for sex identification in the Chinese egret and other ardeid species. *Molecular Ecology Resources*, *11*(1), 176–9. doi:10.1111/j.1755-0998.2010.02879.x

Wilson TE, Wheeler J, Green MC, P. E. (2014). *Reddish egret conservation action plan. Reddish Egret Conservation Planning Workshop*. Corpus Christi, TX.

Wright, S. (1930). Evolution in Mendelian populations. *Genetics*, *16*, 97 – 159. doi:10.1016/S0092-8240(05)80011-4