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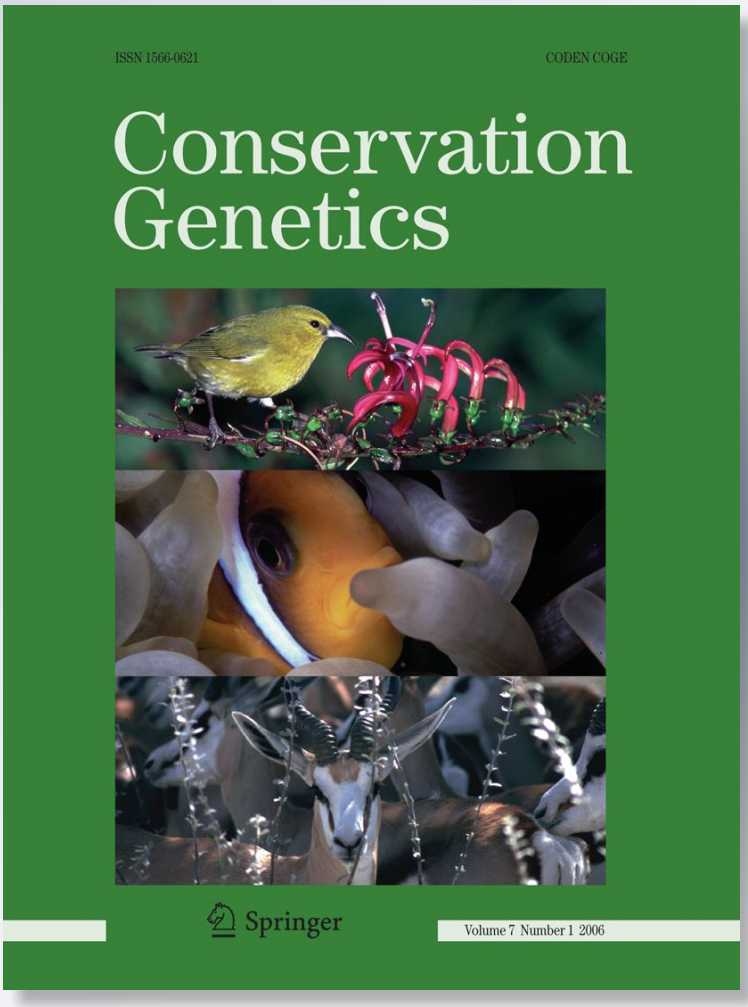

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RESEARCH ARTICLE

Genetic diversity and population structure of North America's rarest heron, the reddish egret (*Egretta rufescens*)

Austin Hill · Clay Green · Eduardo Palacios

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Abstract The global distribution of the reddish egret is characterized by disjunct colonies occurring from the Pacific side of Northwest Mexico to the Caribbean. We examined distantly isolated colonies of reddish egret to determine global population genetic structure. We used seven polymorphic microsatellites to accomplish five goals: (1) to assess range wide population differentiation among reddish egret (*Egretta rufescens*) populations, (2) identify extent of gene flow, (3) determine any historical occurrence of bottlenecks, (4) assess genetic differentiation between color morphs, (5) clarify subspecies status of *E. r. dickeyi,* a completely dark morph population located in and around the Baja California peninsula, Mexico. Genetic differentiation was dramatic (global Fst = 0.161) throughout the reddish egrets range extending from Baja California, Mexico to Great Inagua, Bahamas. Differentiation occurred among three distinct regions (Fst = 0.238) but not among colonies/islands within regions suggesting regional philopatry. Genetic diversity (alleles per locus, and heterozygosity) in Baja California Sur, Mexico and Great Inagua, Bahamas populations is lower than in the Texas/Mexico population due to minimal dispersal between regions and smaller population sizes. Dark and white color morphs when present within the same region showed no differentiation. Patterns of recent population bottlenecks are not evident in each of the three regional populations. With evidence of limited gene flow in addition to low genetic diversity and prospects of habitat loss we recommend that reddish egrets be managed as three distinct or evolutionary significant units.

Keywords Reddish egret · Microsatellite · Plumage dimorphism · Genetic diversity · *Egretta rufescens* · Philopatry

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Introduction

The reddish egret (*Egretta rufescens*), with its narrow habitat requirements and limited distribution, has an estimated global population of 5,000–7,000 adults (Paul 1991; Green 2006). Reddish egret is a plumage dimorphic coastal wading bird that is a year-round resident predominantly in the Southeastern United States (mainly Texas and Florida) as well as on the Pacific and Gulf Coasts of Mexico and the Bahamas (Fig. 1, Cooke 1913; Paul 1991). Three subspecies are currently recognized: *E. r. rufescens* is the nominate race representing populations in Laguna Madre de Tamaulipas, Mexico, Bahamas and the Gulf Coast states of the U.S while *E. r. dickeyi* and *E. r. colorata* are suggested to represent populations in the Baja California peninsula region and the Yucatan peninsula, respectively; these subspecies have not been evaluated using molecular tools and appear to be weakly differentiated morphologically

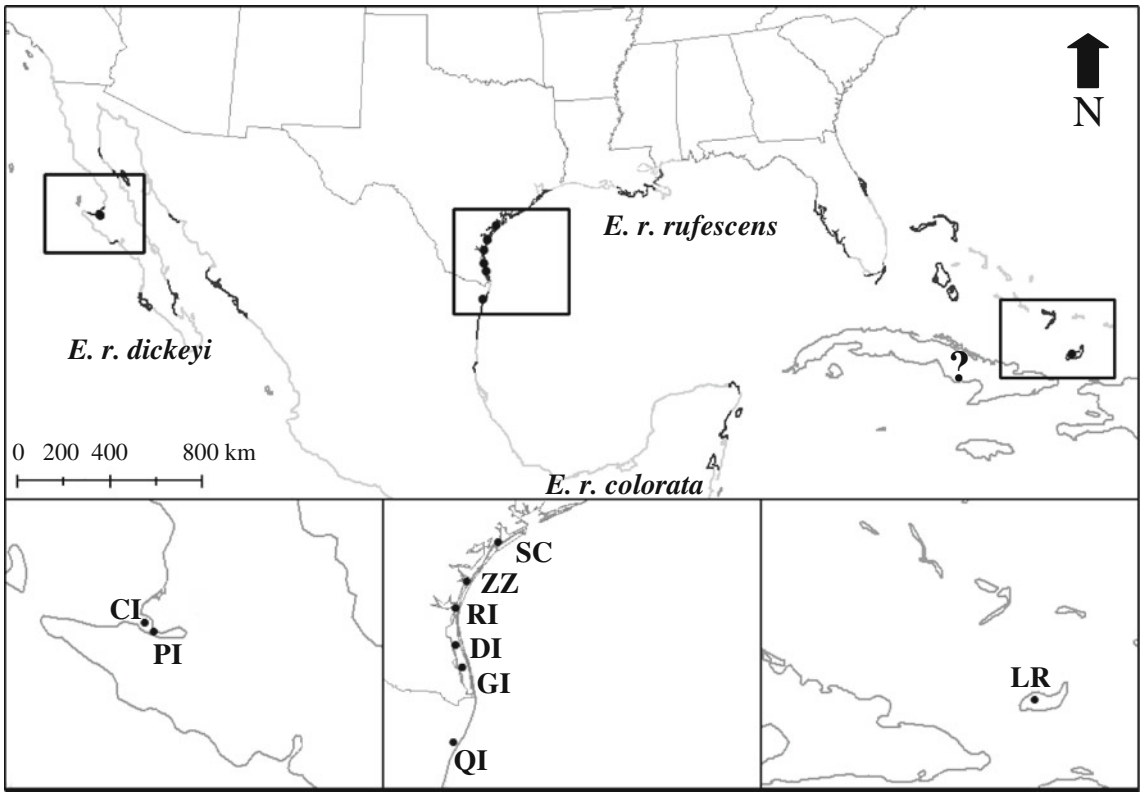

Fig. 1 Location of sampling sites for reddish egrets (*Egretta rufescens*): Concha Island (*CI*), and Piedra Island (*PI*), Baja California Sur, Mexico; Queso Island (*QI*), Tamaulipas, Mexico; Green Island (*GI*), Dubbs Island (*DI*), Rabbit Island (*RI*), Zig Zag Island (*ZZ*), and Second Chain Islands (*SC*), Texas, USA; and Lake Rosa (*LR*), Great Inagua, Bahamas. Dark gray shading indicates known breeding distribution of reddish egrets. "?" indicates unconfirmed breeding status of reddish egrets in Cuba. See Table 1 for more sampling site information

(Lowther and Paul 2002). The largest concentration of *E. r. rufescens* is in Texas having an estimated 900–950 breeding pairs: reddish egrets in this region consist of both color morphs (Green 2006). *E. r. dickeyi*, subspecies found in northwest Mexico, is comprised completely of dark morph individuals while the population in Great Inagua, Bahamas is unique from the others in that it is comprised of primarily (~90%) the white morph (Allen 1955; Scott and Carbonell 1986; Green et al. 2011). Blake (1977) suggests that *E. r. colorata* exhibits slightly paler neck and ornamental plumes and a slightly larger body size though it is more likely that these differences represent seasonal changes rather than geographical variation (Paul 1991).

Current populations in Texas are considered "threatened" due to near extirpation during the plume trade at the turn of the twentieth century and current threats such as habitat loss (Lowther and Paul 2002). The population in Texas has made an apparent recovery from hunting exploits but is currently below historic highs due to recent anthropogenic impacts (Pemberton 1922; Paul 1991; Green 2006). In Mexico, reddish egrets are officially listed, most recently to 'species of special concern', although very little is known about its population status, population trend, and habitat threats. In the Bahamas, little historical information is available for population estimates of reddish egrets, however recent surveys indicate declining population trends especially on Great Inagua (Scott and Carbonell 1986; Green et al. 2011).

The unique dimorphic plumage of reddish egret also warrants an examination of the genetic differentiation between the two color morphs. Color morphs occur in both sexes and consist of dark, slate colored individuals and all white individuals. Kondrashov and Shpak (1998) suggests that assortative mating may divide a population even in the absence of natural selection. Assortative mating occurs in reddish egrets although the courting of the opposite color morph has been observed (Audubon 1843; Pemberton 1922) and mixed-morph pairing at nests has been reported (Lowther and Paul 2002; Green et al. 2011). Each color morph of the reddish egret is attracted differentially to their own plumage coloration during flock formation with all individuals of both morphs landing at like-plumaged decoys (Green and Leberg 2005). In reddish egrets, it has also been shown that color morphs may differ in foraging behaviors based on their degree of crypsis to prey (Green 2005). The hypothesis that color polymorphisms are linked

to alternative strategies is supported by the finding that a majority of known cases of species polymorphisms are associated with reproductive parameters and behavioral, life-history, and physiological traits, which can all have a genetic basis (Roulin 2004). The adaptive significance of two color morphs in reddish egrets may be linked to alternative strategies, suggesting that a genetic assessment of the differentiation between morphs is warranted.

Studies of population structure evaluate how genetic variation is partitioned across a species' range and the ecological factors that contribute to genetic structure. Often a species does not occur as a single panmictic population, but rather as several subdivided populations with each experiencing the heterogeneities of the various geographic locations. Population genetics can inform management decisions and conservation planning through the ability to distinguish populations within a species that have unique evolutionary trajectories. Geographic separation of populations when accompanied by a lack of immigration by individuals or gene flow to other populations can lead to population differentiation, the first stage of allopatric speciation (Coyne and Orr 2004). The vagility of organisms like birds allows them to overcome many of the physical barriers compared to other vertebrates (Avise 1996; Crochet 2000) although behavioral barriers to dispersal such as philopatry can be sufficient to prevent gene flow and promote genetic differentiation in birds (Avise 1996). The objectives of our study were to assess the genetic diversity and the genetic structure of reddish egret populations throughout most of its range and to assess genetic differentiation between color morphs using polymorphic microsatellite markers. Using mtDNA, Bates et al. (2009) found no evidence of genetic structure for reddish egrets within the state of Texas. Disjunct populations, subspecies designations, varying color morph frequencies and the residential nature of reddish egrets suggests possible differentiation across the range. The results of this analysis will yield insights into population connectivity, population structure, and the maintenance of color polymorphism in this species. Knowledge of the genetic structure of the species has considerable importance for the management and conservation of North America's rarest species of heron.

Materials and methods

Field sampling

We collected 8–37 genetic samples from each of nine breeding colonies in April–July 2006, March–July 2007, March–July 2008, January 2009, and March 2009 for a total of 223 individuals (Fig. 1; Table 1). Sampling locations spanned from the western end of the species range, Baja California Sur, Mexico, to the Texas/Mexico Gulf coast, and to the easternmost portion of its range, Great Inagua of the Bahamas. Blood was obtained from the brachial vein of nestlings or fledglings during the breeding season using a 25 gauge needle. We drew approximately 4–6 μl of blood from the bird into a capillary tube and then

Table 1 Sampling locations and sample sizes

Location	Sample site	Abbreviation	Lat/long	Sample size
Lower Laguna Madre, Texas	Dubbs Island	DI	26°43′20.49 N 97°25′38.80 W	31
Lower Laguna Madre, Texas	Green Island	GI	26°23′31.07 N 97°19′27.03 W	13
Upper Laguna Madre, Texas	Rabbit Island	RI	27°14′47.73 N 97°24′51.24 W	32
Ayers Bay, Texas	Second Chain	SC	28°11′34.48 N 96°48′52.10 W	20
Upper Laguna Madre, Texas	Zig Zag Island	ZZ	27°37′52.47 N 97°15′47.02 W	32
Laguna Madre de Tamaulipas, Mexico	Queso Island	QI	25°19′05.10 N 97°27′00.90 W	15
Laguna Ojo de Liebre, Baja California Sur, Mexico	Isla Concha	CI	27°49′32.67 N 114°14′02.25 W	8
Laguna Ojo de Liebre, Baja California Sur, Mexico	Isla Piedra	PI	27°42′10.51 N 114°09′29.61 W	35
Great Inagua, Bahamas	Lake Rosa	LR	21°03′12.44 N 73°24′46.00 W	37

deposited the blood into a vial containing 600 µl of Puregene cell lysis solution (Qiagen, Valencia, CA). Blood was typically collected from only one individual per nest to reduce the possibility of similar genotypes from siblings being incorporated in the analysis. If blood was collected from more than one individual from a nest, then an individual from that nest was chosen randomly for inclusion in the analysis.

Laboratory methods

We performed DNA extraction using (Qiagen Valencia, CA) Puregene DNA isolation protocol for avian whole blood. We screened 13 microsatellite loci primers and found 12 that could be successfully amplified in all 223 samples: Er21, Er22, Er41, Er42, Er43, Er51, Er23, Er44, Er31, Er45, Er24, and Er46 using methods as follows (Hill and Green 2011). We carried out PCR reactions using a BIO-RAD PTC-100 thermocycler in a volume of 20 µl under a standard protocol (Hill and Green 2011). WellRed dye (Sigma Proligo) labeled PCR products for all loci ran through a Beckman Coulter CEQ 8800 DNA sequencer for microsatellite detection and scoring using a CEQ DNA 600 size standard.

Statistical analysis

We assessed the suitability of 12 loci for further use in structure and differentiation analysis by exploring Hardy–Weinberg Equilibrium and linkage disequilibrium. In populations where a deficiency in heterozygosity was present, we used the program MICROCHECKER to determine whether deviations from Hardy–Weinberg expectations (HWE) were due to the presence of null alleles (Oosterhout et al. 2004). We assessed standard measures of genetic variation for nine colonies at the remaining loci including gene diversity (Nei 1973, 1987), number of alleles, allelic richness, and observed heterozygosity. Nei's gene diversity (H_e), allelic richness, and number of alleles were calculated using FSTAT (Goudet 1995; 2001). ARLEQUIN version 3.1 (Excoffier and Schneider 2005) was used to test for deviations from HWE (Guo and Thompson 1992) and linkage disequilibrium. We measured population differentiation using the Raymond and Rousset (1995a) exact test performed in GENEPOP (Raymond and Rousset 1995b).

We assessed the genetic structure across the entire range of the reddish egret by calculating pairwise Fst and Rst for each pair of sampled colonies using ARLEQUIN (Excoffier and Schneider 2005). We used 16,000 permutations to test the significance of covariance components and fixation indices. We performed a hierarchical analysis of genetic structure in ARLEQUIN (Excoffier and Schneider 2005) using analysis of molecular variance (AMOVA) to test the hypothesis that variation is partitioned according to subspecies designations. For the AMOVAs, examined colonies were placed into two groups to fit sampled subspecies divisions and three groups based on distance between regions.

Program STRUCTURE 2.3.1 was used to assess the most probable number of groups or populations without bias from existing subspecies designations or geographical distribution (Pritchard et al. 2000). We tested K values (1–10) 20 times each, using a burn-in time of 10,000 followed by 50,000 iterations to attain a mean value of the likelihood of each K; the most likely number of populations, K, was assessed by observing ΔK (Evanno et al. 2005). The program was executed using the admixture model and the correlated allele frequencies option, which is the most conservative and appropriate approach for populations with individuals that may share ancestry from another population (Pritchard et al. 2009). To test the hypothesis of no differentiation between color morphs, we calculated pairwise Fst values between dark morphs across geographic regions (Baja California, Texas/Mexico), white morphs across geographic regions (Texas/Mexico, Great Inagua, Bahamas), and between white and dark morphs within the Texas/Mexico Gulf region. Program STRUCTURE was also used to identify true populations and how color morph corresponds to predicted values of K.

To test the hypothesis of recent population bottlenecks due to the potential impacts of the plume trade early in the 20th century (Paul 1991), we used program BOTTLENECK 1.2 to determine whether populations had experienced a recent population reduction (Cornuet and Luikart 1996; Piry et al. 1999). We used both the stepwise mutation model (SMM) and the two phase model (TPM) to perform the Wilcoxon 1-tailed test which is most powerful when using less than 20 loci (Piry et al. 1999). The SMM is recommended for testing microsatellite data but the TPM may be more appropriate (Luikart and Cornuet, 1998; Di-Rienzo et al. 1994). As recommended by Piry et al. (1999) when using the TPM, we incorporated 95% single step mutations into the TPM with a variance of 12 and performed 16,000 iterations.

Results

Hardy–Weinberg, linkage disequilibrium and genetic diversity

Twenty-one of 108 loci-colony combinations showed significant departures from HWE before Benjamini and Yekutieli (2001) corrections for multiple comparisons. After correction, 13 of 108 loci-colony combinations

exhibited a departure from HWE ($P < 0.009$, adjusted critical value). No single locus deviated from HWE in all nine populations. Fourteen of 594 loci-pair-colony combinations and one of 66 loci-pair combinations across all populations showed linkage disequilibrium before correction for multiple comparisons. Upon Benjamini and Yekutieli correction, only one significant linkage between loci occurred within colonies and none across all colonies ($P < 0.007$, adjusted critical value). Using MICRO-CHECKER, 14 of 108 loci-colony pairings analyzed exhibited positive results for the presence of null alleles with 12 of the possible null alleles within three loci. To be conservative in our analyses we removed loci Er41, Er42, and Er24 which contained a majority of the possible null alleles in addition to Er22 which exhibited evidence of excess heterozygosity in three of the nine sampled colonies. The remaining eight loci were examined again for departures from HWE across the hypothesized three populations. Er23 exhibited a departure from HWE in each of the three groups and was subsequently removed. All further analyses used seven loci: Er21, Er43, Er51, Er44, Er31, Er45, and Er46.

The number of alleles per locus ranged from two to 12, while allelic richness and gene diversity were significantly different across all colonies with colonies on the Texas/Mexico Gulf having greater allelic richness and gene diversity than colonies in Baja California and the Bahamas ($\chi^2 = 27.42$, $P < 0.001$, Online Resource 1; $\chi^2 = 21.42$, $P < 0.01$, Online Resource 2). Observed ($\chi^2 = 20.78$, $P < 0.01$) and expected heterozygosity ($\chi^2 = 22.03$, $P < 0.005$) differed significantly across all colonies with the mean ranging from 0.35 to 0.70 and 0.41 to 0.59, respectively (Table 2).

Population structure

Exact tests for population differentiation found significant differences in allele frequencies for 20 of 36 pairwise comparisons. Estimates of Fst and Rst revealed significant differentiation in 20 of 36 pairwise population comparisons. Fst and Rst values ranged from 0.00 to 0.38 and 0.00 to 0.53, respectively (Table 3). Global Fst value estimated over all populations and loci was 0.161 ($P < 0.001$) while our global Rst estimates were 0.15 ($P < 0.001$). Comparisons of sampling colonies within Texas/Mexico Gulf populations showed no signs of significant differentiation. Baja California and Great Inagua, Bahamas populations exhibited large significant differentiation from Texas/Mexico Gulf populations as well as between each other. Results of the AMOVA suggest that differentiation is greatest among the three geographic regions (22.88%, df = 2,445, $P = 0.004$) and not between populations within regions (0.34%, df = 6,445, $P = 0.280$). Using program BOTTLENECK, we detected no evidence for a recent population reduction in any of the populations ($P > 0.05$).

Bayesian clustering analysis, as performed by program STRUCTURE (Pritchard et al. 2000), gave the strongest support to a structure that recognized three distinct genetic units though several individuals exhibit assignment probabilities for populations other than their respective sampling region (Fig. 2). STRUCTURE found no evidence of substructure within any of the three genetic units when analyzed independently. Approximation of ΔK (Evanno et al. 2005), identified a peak value at $K = 3$ (Online Resource 3). Populations on Isla Piedra and Isla Concha in Baja California, Mexico formed a single cluster and all Texas/Mexico Gulf populations formed a second cluster, while the population in Great Inagua, Bahamas formed the last unique cluster.

No structure was identified between dark morphs and white morphs within Texas as little to no differentiation was observed (Fst = 0.002, Table 4). Across the species range, differentiation between color morphs aligned with geographic populations. This is also supported by previous results in structure which did not support clusters $K = 4$–9.

Discussion

The results of this study suggest that genetic structure of the reddish egret is most pronounced at the regional level and not between colonies within regions. Fst and Rst values from the Baja California peninsula, Mexico and Great Inagua, Bahamas were significantly large when compared with each other as well as between the Texas/Mexico Gulf regions. Results from program STRUCTURE also suggest the existence of three major regions of distinct genetic units. A previous study using mtDNA to look at population structure of reddish egrets along the Texas coast found no structuring among 16 colonies; our results in Texas using microsatellites support these earlier findings (Bates et al. 2009).

Despite the vagility of the reddish egret there seems to be some regional philopatry. Due to their unique foraging habitat, specifically wind-tidal flats, there does not appear to be a continuous line of suitable coastal habitat, which may limit movement. This leads to many disjunct colonies that are most often associated with coastal lagoons or otherwise open, calm, shallow saltwater habitats and may play a large role in the resulting population structure. The reddish egret is considered a "weakly" migratory species based on adult birds remaining in and around breeding areas throughout the year; some minimal northward movement occurs after breeding in both Texas and Baja California (Cooke 1913; Lowther and Paul 2002; Green

Table 2 Number of alleles, allele size range, observed heterozygosity (H_o), expected heterozygosity (H_e) for seven microsatellite loci in nine reddish egret colonies

Primer	Texas/Mexico Gulf[a]						Baja, Mexico[b]		Bahamas[c]	Overall
	DI	GI	RI	SC	ZZ	QI	CI	PI	LR	
Er21										
# Alleles	2	2	2	2	2	1	1	1	1	2
Range	141–147	141–147	141–147	141–147	141–147	147	147	147	147	141–147
H_o	0.06	0.15	0.06	0.05	0.09	0.0	0.0	0.0	0.0	0.05
H_e	0.06	0.15	0.06	0.05	0.09	0.0	0.0	0.0	0.0	
Er43										
# Alleles	3	3	3	3	4	3	2	2	2	4
Range	144–152	144–152	144–152	144–152	140–152	144–152	144–148	144–148	144–148	140–152
H_o	0.48	0.46	0.44	0.50	0.44*	0.73	0.38	0.40	0.38	0.47
H_e	0.58	0.62	0.51	0.60	0.56	0.55	0.46	0.46	0.39	
Er51										
# Alleles	10	8	11	10	9	8	3	3	6	12
Range	278–333	293–328	278–333	278–343	278–328	278–328	298–313	298–308	278–333	278–343
H_o	0.90	0.92	0.94	0.90	0.84	0.93	1.0	0.49	0.76	0.85
H_e	0.88	0.87	0.88	0.88	0.86	0.84	0.59	0.59	0.73	
Er44										
# Alleles	4	3	4	3	2	3	2	2	2	5
Range	190–206	190–206	190–206	190–206	198–206	190–206	198–206	198–206	198–206	190–206
H_o	0.42	0.62	0.53	0.55	0.47	0.60	0.38	0.20	0.35	0.46
H_e	0.51	0.46	0.50	0.50	0.47	0.53	0.53	0.23	0.41	
Er31										
# Alleles	2	2	2	2	3	1	2	2	1	3
Range	292–298	292–298	292–298	292–298	292–304	292	292–298	292–298	292	292–304
H_o	0.03	0.15	0.09	0.05	0.19	0.0	0.38	0.29	0.0	0.13
H_e	0.03	0.15	0.15	0.05	0.20	0.0	0.33	0.41	0.0	
Er45										
# Alleles	2	2	2	2	2	2	1	1	1	2
Range	180–188	180–188	180–188	180–188	180–188	180–188	188	188	188	180–188
H_o	0.48	0.23	0.59	0.40	0.50	0.60	0.0	0.0	0.0	0.31
H_e	0.51	0.47	0.51	0.51	0.51	0.48	0.0	0.0	0.0	
Er46										
# Alleles	6	5	5	5	5	5	2	4	4	6
Range	130–166	130–166	142–166	142–166	142–166	142–166	154–158	154–166	154–166	130–166
H_o	0.61	0.46	0.63	0.60	0.72	0.67	0.13	0.40	0.38	0.51
H_e	0.69	0.41	0.65	0.68	0.69	0.57	0.13	0.37	0.43	
Over all loci										
Avg. alleles/locus	4.14	3.57	4.14	3.86	3.86	3.29	1.86	2.14	2.43	
H_o	0.43	0.43	0.47	0.44	0.46	0.70	0.45	0.35	0.47	
H_e	0.47	0.45	0.47	0.47	0.48	0.59	0.41	0.41	0.49	

* Significant departure from Hardy–Weinberg expectations after Benjamini & Yekutieli correction

[a,b,c] Represents the three different hypothesized populations. See Table 1 for more locality information

and Ballard, unpubl. banding and telemetry data). Long distance dispersal appears to be rare and present mainly in juveniles (Bent 1926; Telfair and Swepston 1987; Paul 1991). The genetic evidence from pairwise Fst and AMOVA suggest that a regional philopatric behavior exists and little migration takes place among regions.

A large debate has played out over the last 20 years over the criteria used to define an evolutionary significant unit

Table 3 Estimates of population differentiation. Shading represents the three different hypothesized populations

	DI	GI	RI	SC	ZZ	QI	CI	PI	LR
DI	–	0.01	0.00	0.00	0.00	0.00	*0.21*	*0.23*	*0.23*
GI	0.01	–	0.03	0.00	0.02	0.01	*0.32*	*0.34*	*0.36*
RI	0.00	0.01	–	0.00	0.01	–0.01	*0.18*	*0.21*	*0.26*
SC	0.01	0.01	0.00	–	–0.01	0.00	*0.20*	*0.22*	*0.25*
ZZ	0.00	0.01	0.01	0.01	–	0.00	*0.19*	*0.21*	*0.26*
QI	0.04	0.00	0.00	0.01	0.00	–	*0.27*	*0.29*	*0.33*
CI	*0.24*	*0.30*	*0.14*	*0.15*	*0.15*	*0.11*	–	0.03	*0.34*
PI	*0.37*	*0.47*	*0.25*	*0.29*	*0.27*	*0.24*	0.05	–	*0.38*
LR	*0.17*	*0.26*	*0.15*	*0.23*	*0.17*	*0.25*	*0.40*	*0.53*	–

Fst is shown above diagonal, Rst is shown below diagonal. Italicized values are statistically significant at $\alpha = 0.01$

Table 4 Fst between reddish egret color morphs and between geographic regions

	Baja red	TX/MEX red	Bahamas white	TX/MEX white
Baja red	–			
TX/MEX red	*0.23*	–		
Bahamas white	*0.35*	*0.23*	–	
TX/MEX white	*0.22*	0.00	*0.23*	–

Italicized values are statistically significant at $\alpha = 0.01$

(ESU; Ryder 1986; Waples 1991; Dizon et al. 1992; Moritz 1994; Vogler and DeSalle 1994; Pennock and Dimmick 1997; Dimmick et al. 1999; Crandall et al. 2000; Fraser and Bernatchez 2001), though the goal to preserve biodiversity at an organizational level below that of the species has remained consistent with each definition. Though some definitions are stricter than others, several authors recommend the use of genetics as a basis for recognizing an ESU and moreover recognize that in support of genetic data should be ecological data (life history, morphology, and behavior) and that ESU recognition may vary from case to case (Crandall et al. 2000; Moritz 2002). Our results indicated that these distinct populations should be treated as three ESU's due to the high degree of genetic differentiation between them and the current geographical isolation that prevents gene flow among them. Future molecular research of populations in Florida and southern Mexico (e.g. Chiapas, Yucatán) may reveal stepping stones between distinct populations of Bahamas and Texas and Texas and Baja California, respectively. Yucatán (then Cuba) might also be stepping stone between Texas and Bahamas based on recent satellite telemetry studies (B. Ballard, pers. comm.). While other regions may serve as stepping stones between the three studied populations, our results nevertheless reveal that populations in Baja California, Texas and Great Inagua, Bahamas are genetically differentiated and warrant management and conservation as distinct populations.

A population that experiences a dramatic decline in size can undergo a loss of genetic variation as has occurred in the greater prairie chicken (*Tympanuchus cupido*; Bouzat et al. 1998; Bellinger et al. 2003). The history of plume hunting with regards to the reddish egret is best known to have occurred in Florida and Texas with both populations being affected significantly. The Florida population was briefly extirpated and the population in Texas has since recovered, but still remains below the highest estimates in the historical records (Paul 1991). Little to nothing is known of historical populations in places other than Texas and Florida including Baja California, Mexico and Great Inagua, Bahamas. Program BOTTLENECK failed to detect evidence of any recent genetic bottleneck in any of the populations in this study. Bates et al. (2009) did not detect reduced genetic diversity in reddish egrets from Texas but did find signals of a population expansion indicating a possible recovery from a previous low. Initial impacts of a genetic bottleneck include the loss of unique alleles, but a population can preserve genetic diversity if recovery occurs quickly (Allendorf 1986; Coates 1992). If a population fails to rebound and remains small after a decline, it becomes more vulnerable to the forces of genetic drift and inbreeding (Allendorf 1986; Ellstrand and Elam 1993; Frankham 1995). These results suggest the population in Texas recovered quickly enough to mitigate the impacts of a severe population reduction though current anthropogenic

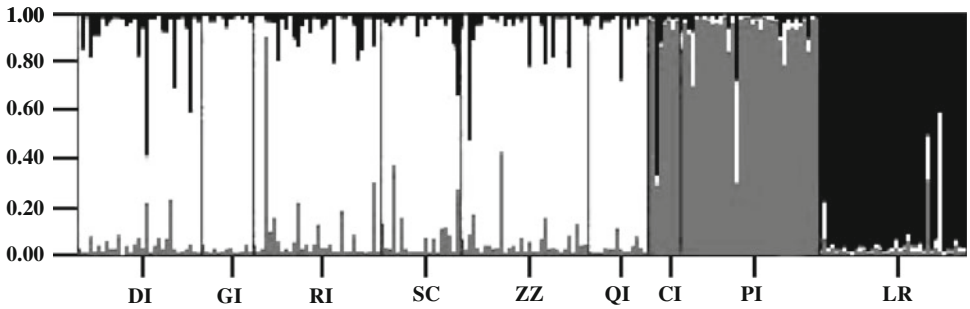

Fig. 2 $K = 3$ clusters as performed by program STRUCTURE. See Table 1 for locality information

impacts may be limiting them from reaching historic population levels.

Differentiation between the two color morphs was non existent within the Texas/Mexico Gulf region. Dark and white individuals within Texas/Mexico are more similar to each other than they are to like-colored individuals of the other regions. Although many of the mating events between reddish egrets appear to be assortative based on color morph, courtship between opposite color morphs has been observed and extra pair copulations are not uncommon for other herons and egrets (Gladstone 1979; Ramo 1993). Gene flow between color morphs through mixed morph mating events though not common can occur with enough frequency to prevent differentiation between the morphs (Mills and Allendorf 1996). It is very likely that within reddish egret populations, enough mixed morph mating events take place whether as typical pair bond or as an extra pair copulation event to prevent any differentiation from occurring between color morphs within the same population (Green et al. 2011; Hill and Green, unpublished data). As it is the case that a pair of dark morphs may have white offspring (Green and Hill, unpublished data), the potential for imprinting upon the offspring and attraction to like individuals may also be a factor promoting mating between different morphs (Immelman 1972; Slagsvold et al. 2002).

This is the first known range-wide examination of genetic variation in the family Ardeidae (herons, egrets). Our results indicate three evolutionarily distinct population units that support the subspecies *E.r. dickeyi* in Baja California and propose a new unique population within *E. r. rufescens* occurring in Great Inagua, Bahamas. The limited availability of suitable habitat and philopatric tendencies has created geographic isolation between populations of a plumage dimorphic waterbird that is a species of concern. Small populations sizes in each of the regions puts the reddish egret at risk to stochastic demographic and environmental events in the short term and loss of genetic variation in the long term. We advocate the preservation of all known reddish egret breeding colonies and foraging locations; as the rarest and least studied heron in North America, this research reveals the importance of focusing conservation efforts on regional populations and the protection of any and all breeding sites for reddish egret.

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