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CONSERVATION GENETICS OF ROATÁN SPINY-TAILED IGUANAS, *CTENOSAURA OEDIRHINA*

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***Abstract.*—Roatán Spiny-tailed Iguanas, *Ctenosaura oedirhina*, are assessed as Endangered by the IUCN Red List of Threatened Species and listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Occurring in less than 1% of the available habitat on Roatán, due primarily to hunting pressure, this species faces severe fragmentation. Herein we used a combination of mitochondrial and nuclear DNA to elucidate contemporary levels of genetic diversity and genetic structure across the range of this species. Our results reveal generally low levels of genetic diversity within groups at each site sampled, coupled with moderate to high levels of genetic differentiation among these sites. Although contemporary differentiation among sites is substantial, alleles and haplotypes shared among those sites suggest historical connectivity across Roatán and Barbareta. However, despite past connectivity, our data indicate contemporary disruption of movement among isolated sites, resulting in the high level of observed genetic differentiation. Our data further suggest increased inbreeding within sites, which, coupled with small population size, makes each group more vulnerable to stochastic events and disturbances. In order to manage for the long-term persistence of this species, a captive breeding program may be essential; however, data regarding relatedness within sites and basic reproductive information must be gathered prior to beginning such a program.**

***Resumen.*—*Ctenosaura oedirhina,* o la Iguana de cola espinosa de Roatán, se encuentra listada En Peligro según la lista Roja de la IUCN y bajo el Apéndice II de la Convención Internacional de Especies Amenazadas de Fauna y Flora Silvestres (CITES). Esta especie ocupa menos de 1% del hábitat disponible de la isla de Roatán, debido principalmente a la cacería ilegal, enfrentando así fragmentación severa. A partir de esto, fueron empleados una combinación de marcadores de ADN mitocondrial y nuclear para elucidar los niveles contemporáneos de diversidad y estructura genética a lo largo de su rango de ocupación. Nuestros resultados revelaron bajos niveles de diversidad genética dentro de cada grupo analizado para cada localidad muestreada junto a niveles de diferenciación genética que iban de moderados a elevados entre las localidades muestreadas. Aunque la diferenciación actual entre localidades es sustancial, los alelos y haplotipos compartidos entre localidades sugiere que existió una conectividad histórica entre estas, extendiéndose esta evidencia hasta la isla Barbareta. Sin embargo, independientemente de la conectividad histórica, existe interferencia en el movimiento de las iguanas entre localidades, resultando esto en los niveles elevados de diferenciación genética observada en cada localidad. Ocasionando esto, a la vez, la presencia de varias poblaciones genéticamente aisladas. Adicional a esto, nuestros resultados demuestran una alta señal de endogamia dentro de las localidades muestreadas, la cual, junto a los pequeños números poblacionales, ocasiona que las poblaciones en Roatán sean más vulnerables a disturbios en el hábitat y eventos estocásticos. Por consiguiente, el manejo de esta población a largo plazo para su conservación pudiera requerir de la implementación de un programa de reproducción en cautiverio. Sin embargo, información sobre los niveles de parentesco para cada localidad e información básica sobre reproducción debe ser colectada antes de iniciar un programa de esa categoría.**

Key Words.—endangered; fragmentation; Honduras; *palearis* clade; population genetics

INTRODUCTION

Fragmentation of natural habitats is one of the greatest threats to biodiversity, as it often results in a decrease in overall habitat availability, and changes the quality and configuration of the habitat (Ehrlich and Ehrlich 1970; Soulé 1983). Species living within fragmented habitats often suffer from reduced population sizes and decreased migration potential. From a genetic perspective, fragmentation can result in lower diversity within each fragment, increased differentiation among fragments, increased levels of inbreeding, lower evolutionary potential, and an overall higher risk of extinction. The degree to which fragmentation affects a species is dependent upon initial migration patterns and genetic subdivision, and the cumulative diversification that may occur through genetic drift and inbreeding following further population subdivision (Crnokrak and Roff 1999; Frankham et al. 2010; Allendorf and Luikart 2013). Understanding the effect that habitat fragmentation has on a given species is thus of immense conservation concern. When dealing with endangered species, often already having small populations, the risk of extinction is all the more elevated in fragmented landscapes.

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In theory, recolonization events can counter the effects of fragmentation and prevent extinction. However, when anthropogenic causes are at play, recolonization rarely exceeds population decline and extirpation continues (e.g., Bolger et al. 1997). Corridors are often suggested as a means of increasing migration and recolonization events, however, understanding the effectiveness of corridors is complex. Studies have shown that various taxa respond differently to corridors (Wiens 1997). Reptiles in particular have demonstrated difficulty adapting to corridor use, depending on habitat quality (e.g., Boudejemandi et al. 1999). In addition to habitat quality, other factors may play a role in preventing migration in general or while using corridors. When dealing with species that are subject to harvesting, the amount of protection afforded across a landscape may play a larger role in determining the degree of isolation than the habitat itself. In other words, if harvesting cannot be prevented in areas between fragments, the quality of the habitat becomes less important (e.g., Goode et al. this volume).

Species that have inherently small populations due to range restrictions, such as those occurring on small islands, will be increasingly affected by fragmentation, as they do not have the ability to expand or shift their ranges (Frankham 1998). Roatán Spiny-tailed Iguanas, *Ctenosaura oedirhina* (de Queiroz 1987), exemplify a narrow-range insular endemic whose population may be suffering the effects of human-mediated fragmentation. These iguanas are endemic to Roatán, Barbareta, and a few satellite cays located within the Bay Islands, Honduras (McCranie et al. 2005; Pasachnik 2013). This species has been recognized as the second most vulnerable reptile species in Honduras (Wilson and McCranie 2003), is Endangered by the International Union for Conservation of Nature (IUCN; Pasachnik et al. 2010), and listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES; species in which trade must be controlled in order to avoid utilization incompatible with their survival; Pasachnik and Ariano 2010). Although the Honduran government designated *C. oedirhina* as in need of protection in 1994 (Pasachnik et al. 2010), virtually no protection is actually afforded to this species by the government. Instead, the protection that is given comes largely from grassroots efforts within the local community, by prohibiting habitat destruction and harvesting on private property.

Goode et al. (this volume) showed that this species is found in almost all habitat types on the island, but that their distribution is largely influenced by the amount of protection afforded to them. Of the approximate 160 km^2 expanse of Roatán, *C. oedirhina* is found only in small pockets across the island (less than 1% by area), where hunting is prevented by grassroots efforts (Goode et al. this volume). The objective of our research was to understand the genetic structure of this species, within and among its remaining populations. We used mtDNA and microsatellite data to evaluate contemporary levels of genetic diversity within and among sample sites across the distribution of *C. oedirhina*. We also evaluated patterns of spatial genetic structure to understand the level to which habitat fragmentation and harvesting may be associated with disrupted connectivity among populations of *C. oedirhina*. Any patterns revealed by our analyses will shed light on the condition of this species and can be used to develop informed strategies directed at best management practices for its long-term survival.

MATERIALS AND METHODS

Study site.—Roatán is the largest and middle island of the Bay Islands and is located approximately 48 km north of mainland Honduras. A series of hills run along the spine of the island, reaching 235 m at the highest point (McCranie et al. 2005). The Bay Islands, and Roatán in particular, are becoming an increasingly popular tourist destination. From 1985 to 2013, the urban areas of the island increased from 0.95 km^2 to 14.50 km^2, and the sandy beach areas decreased from 3.28 km^2 to 0.38 km^2 (Aiello 2007; Goode et al. this volume). A consequence of this increased development has been an influx of people from the mainland, who bring with them the custom of consuming iguana meat. Thus, harvesting seems to be increasing on Roatán as the population from the mainland grows (Pasachnik et al. 2012). Hence, though habitat destruction is increasing, *C. oedirhina* is most affected by the local level of protection afforded to them more than habitat type availability (Goode et al. this volume). The exact study locations are not recorded herein due to the status of this species. If desired, additional information concerning these locations may be requested from the authors.

Field collection.—We collected DNA samples from 108 individuals across the geographic range of *C. oedirhina* on the islands of Roatán and Barbareta, Honduras, during 2010 and 2011 (Fig. 1, Table 1). We took a digital photograph upon capture and snout-vent length, tail length, sex, and mass were recorded. In addition, we gave each individual a unique mark, with PIT tags, bead tags (Rodda et al. 1998), and paint, to avoid re-sampling. We drew a 0.3 ml sample of blood from the caudal vein of each individual and stored it in an EDTA buffer (Longmire et al. 1992) for molecular analysis. In order to prevent infection, we disinfected the puncture site with ethanol before the blood was drawn and sealed it with a topical adhesive afterward.

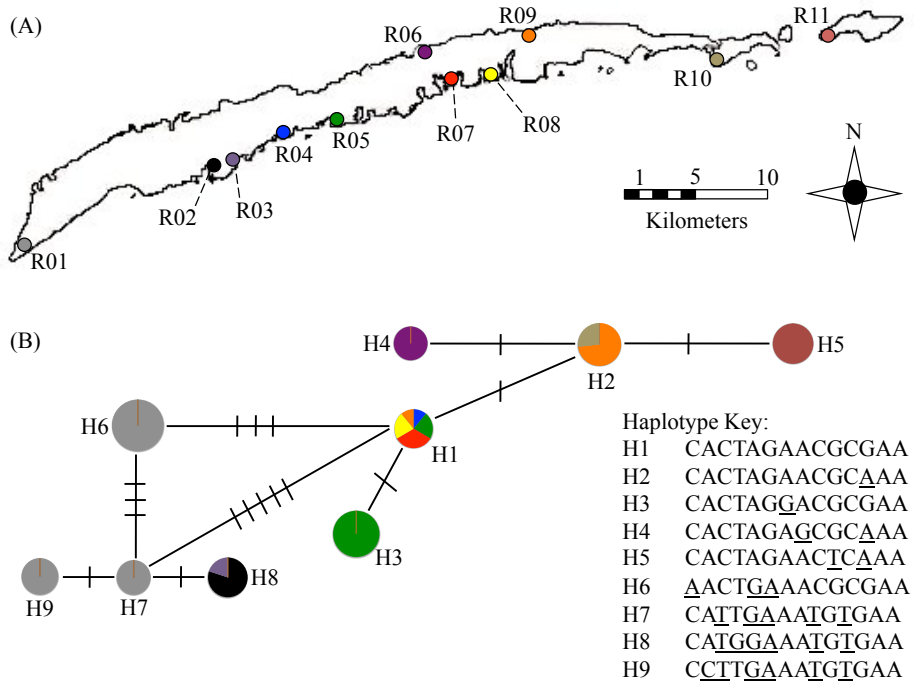

FIGURE 1. (A) Sampling sites for *Ctenosaura oedirhina* distributed across Roatán (R01–R10) and Barbareta (R11), Honduras. Site numbers correspond to those presented in Table 1 and each site is color-coded. (B) A mtDNA haplotype network constructed from a 674 bp region of the ND4 gene. Pie charts represent the nine individual haplotypes (H1–H9) and color codes represent proportional contribution of individuals from each sample site to the total number of observations of a haplotype (e.g., H1 was observed at R04, R05, R07, R08, and R09). The size of each pie chart is scaled to indicate the proportional contribution of each haplotype to the total sample. Haplotype identities are based on 12 single nucleotide polymorphisms included in the key.

DNA sequencing.—We extracted total genomic DNA (gDNA) by tissue digestion in cell lysis buffer (10 mM Tris, 100 mM EDTA, 2% SDS, pH = 8.0) with proteinase K (Invitrogen, Inc., Grand Island, New York, USA), treatment with RNase A (Qiagen, Inc., Valencia, California, USA), ammonium acetate precipitation of proteins, and alcohol precipitation of DNA before suspension in TLE buffer (10 mM Tris, 0.1 mM EDTA, pH = 8.0). We confirmed gDNA quality by agarose gel electrophoresis and quantified gDNA concentration using an ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, Delaware, USA). We diluted each sample to a concentration of ~10 ng/µl for use as template in polymerase chain reaction (PCR).

Mitochondrial DNA analysis.—We assessed mitochondrial DNA variation by amplifying 675 bp of NADH dehydrogenase subunit 4 using primers ND4 (5'–

TABLE 1. Sample sites, number of samples collected in 2010 and 2011 (N), number of sequences used to estimate haplotype diversity (n_S), number of samples used to estimate microsatellite allele frequencies (n_M), and the average number of individuals successfully genotyped (n_G) per sample location for *Ctenosaura oedirhina* across its range in Honduras. Descriptive statistics are provided for the full microsatellite data set (12 Loci; P = proportion polymorphic loci, H_O = Observed Heterozygosity, H_E = Expected Heterozygosity, k = average number of alleles per locus, and k_E = the effective number of alleles per locus), and rarefied allelic richness (Â), gene diversity (G = H_E), and inbreeding coefficients (F_{IS}) are provided for two reduced data sets (10 loci and 8 loci).

	Sample Sizes				12 Loci					10 Loci					8 Loci				
Sample Site	N	n_S	n_M	n_G	P	H_O	H_E	k	k_E	k	Â	H_O	G	F_{IS}	k	Â	H_O	G	F_{IS}
R01	43	35	41	39.8	0.75	0.33	0.42	4.0	2.5	4.6	2.6	0.40	0.42	0.24	5.3	3.0	0.50	0.53	0.05
R02	9	8	7	6.9	0.75	0.35	0.40	2.8	2.2	3.2	2.8	0.42	0.52	0.15	3.5	3.0	0.52	0.53	0.02
R03	2	2	2	2.0	0.50	0.42	0.25	1.7	1.5										
R04	1	1																	
R05	22	22	15	14.5	0.83	0.37	0.42	3.3	2.3	3.8	2.8	0.45	0.51	0.22	4.3	3.1	0.59	0.56	0.05
R06	1	1	1	1.0	0.25	0.25	0.13	1.3	1.3										
R07	2	2	2	2.0	0.58	0.33	0.30	1.9	1.8										
R08	2	2																	
R09	12	10	10	9.8	0.83	0.44	0.45	3.8	2.6	4.3	3.1	0.53	0.50	0.21	4.9	3.6	0.62	0.66	0.07
R10	6	4	5	4.8	0.58	0.33	0.36	2.8	2.3	3.2	2.8	0.39	0.52	0.20	3.8	3.6	0.62	0.49	0.21
R11	10	8	9	8.8	0.58	0.26	0.32	2.9	2.4	3.3	3.2	0.31	0.57	0.07	3.9	3.0	0.52	0.39	0.24
Grand Mean	10.0	8.6	10.2	9.95	0.63	0.34	0.34	2.7	2.1	3.7	2.9	0.42	0.51	0.18	4.3	3.2	0.52	0.57	0.09

CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC –3'; Sites et al. 1996) and ND4R1 (5'– CGA AAC ACC TCT CGG TTT GC –3'; Pasachnik et al. 2009). We conducted amplifications in a total volume of 15 µl using 3.0 µl 5X PCR buffer, 1.2 µl 8 mM dNTPs, 0.75 µl of 10 mM forward primer, 0.75 µl of 10 mM reverse primer, 0.15 µl Taq polymerase, 5.15 µl ddH_2O, and 4.0 µl gDNA template. PCR cycling was performed by denaturing at 94° C for 3 min., followed by 30 cycles of 94° C for 30 s, 50° C for 30 s, 72° C for 90 s, and a final extension at 72° C for 5 min. We verified PCR success by gel electrophoresis, and purified successful reactions using ExoSap (exonuclease I/shrimp alkaline phosphatase; New England BioLabs, Ipswich, Massachusetts, USA). We performed sequencing reactions using the original PCR primers. We assembled forward and reverse sequences for each template using GENEIOUS R6 (v6.1.8; BioMatters, Inc., San Francisco, California, USA). We corrected incongruent base calls manually by examining the electropherograms for the forward and reverse reads. We verified alignment for the 90 sequences representing nine haplotypes identified herein (Accession Numbers: KM883205-KM883213) using GENEIOUS R6 with the aid of 12 published sequences representing five haplotypes (Accession Numbers: GU331999-GU332001 and GU906221-GU906222), which were also used to augment our estimates of haplotype diversity. We differentiated haplotypes and characterized molecular diversity (number of haplotypes, haplotype diversity, and nucleotide diversity) within and among sample sites using DnaSP v5 (Librado and Rozas 2009).

Nuclear DNA analysis.—We assessed nuclear DNA variation using 12 microsatellite loci from the genome of *Ctenosaura melanosterna* known to successfully amplify in the genome of *C. oedirhina* (Stewart et. al 2012). We amplified all loci using the touchdown PCR conditions given in Stewart et al. (2012) and labeled PCR products for individual loci with one of four fluorescent dyes (6-FAM, NED, PET, or VIC). We subsequently combined markers into three multiplexes, which we separated by electrophoresis using an ABI 3130XL Genetic Analyzer (Applied Biosystems, Grand Island, New York, USA). We calibrated fragment sizing with the LIZ 500 (-250) or GeneScan600 size standard and implemented them using the microsatellite plugin for GENEIOUS R6 (v6.1.8; BioMatters).

Of the 12 microsatellite markers targeted for analysis, two loci (*Ctme217*, *Ctme220*) were monomorphic across all sample sites, and each of the other loci had a fixed allele in at least one sample site. For markers that were polymorphic among sample sites, we found no consistent deviations from Hardy-Weinberg equilibrium (MCMC permutation test using 1,000 batches of 10,000 dememorization steps followed by 10,000 iterations in GenePop v 4.2; Raymond and Rousset 1995) when using the Dunn-Sidak stepwise Bonferroni correction (Sokal and Rohlf 1995). Notably, however, markers *Ctme212* and *Ctme216* showed significant heterozygote deficiency at two sites (R01 and R05), and marker *Ctme427* showed significant heterozygote deficiency at two sites (R01 and R11). Although not significant after Dunn-Sidak correction, markers *Ctme212* and *Ctme216* were fixed (five of nine sites for each) or demonstrated heterozygote deficiency ($P < 0.05$ at four of nine sites for each) across all sample sites. Further, genetic diversity at three sites is characterized using fewer than five individuals (R03, R06, and R07). We therefore made descriptions of genetic diversity and analyses of population differentiation and spatial genetic structure using a data set based on eight polymorphic loci and including six sample sites.

For those loci in Hardy-Weinberg and linkage equilibrium, we estimated rarefied allelic richness ($\hat{A}$; Petit and Mousadik 1998; Leberg 2002), gene diversity ($G = H_E$; Nei 1987) and the inbreeding coefficient (F_{IS}) using FSTAT v2.9.3 (Goudet 1995). We characterized each site sampled by taking the average over loci for each estimate of genetic diversity (Table 1).

We estimated genetic differentiation among sample sites in three ways. First, we used GENEPOP v3.4 (Raymond and Rousset 1995; Rousset 2008) to test for genotypic differentiation between each pair of sites (MCMC permutation test using 1,000 batches of 10,000 dememorization steps followed by 10,000 iterations in GenePop v4.2; Raymond and Rousset 1995) followed by the stepwise Bonferronni procedure (Sokal and Rohlf 1995). Second, we examined spatial genetic structure among sample sites using the clustering algorithm STRUCTURE (v2.3; Pritchard et al. 2000). We modeled the genetic structure of *C. oedirhina* on Roatán using an empirically determined allele frequencies parameter ($\lambda = 0.727$), under an admixture model with correlated allele frequencies. We allowed k to vary from two to six, and our strategy resulted in four potentially informative groups based on the method of Evanno et al. (2005). Third, we calculated F_{ST} (Weir and Cockerham 1984) and examined this statistic as a function of geographic distance to summarize spatial patterns of pairwise population differentiation over the entire study area. We used the statistical package R (R Development Core Team, Vienna, Austria) to implement 10,000 iterations of Mantel's permutation test (Mantel 1967) to determine the pattern of genetic isolation with respect to geographic distance (isolation-by-distance).

Finally, we used a Bayesian clustering algorithm implemented in BayesAss v3.0 (Wilson and Rannala 2003) to detect the signature of recent movement among sampled sites on Roatán. We used five replicate runs (each with a different seed) with a burn-in of 10^6 iterations, sampling for 10^7 iterations, and data collection every 10^3 steps during sampling. We empirically determined values for the migration (m), allele

TABLE 2. Observed haplotype distribution and haplotype frequencies for a 675 base region of subunit four of NADH dehydrogenase at 11 *Ctenosaura oedirhina* sample sites on Roatán and Barbareta in the Bay Islands of Honduras.

Sample Site	Haplotype								
	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
R01						28	2		5
R02								8	
R03								2	
R04	1								
R05	2		20						
R06				1					
R07	3								
R08	2								
R09	1	11							
R10		4							
R11					11				
Frequency	0.09	0.15	0.20	0.01	0.11	0.28	0.02	0.10	0.05

frequencies (a), and inbreeding (f) switching proposals such that acceptance rates were between 20% and 40%, as suggested by Rannala (2007). We estimated the mean signature of movement between sample sites by taking the average of the off-diagonal values, which represents the proportion of the individuals sampled at each site thought to be of migrant ancestry.

RESULTS

Mitochondrial DNA analysis.—We observed nine haplotypes characterized by 12 single nucleotide polymorphisms among the 11 sites sampled. Genetic variation within sample sites was characterized by k = 0–1.24 nucleotide differences (median = 0), resulting in haplotype diversities (H_d) from 0–0.346 (median = 0) and nucleotide diversities (π) from 0–0.0019 (median = 0). In contrast to the relatively low within-site measures, global measures of genetic diversity revealed differentiation among sites: k_T = 3.061, H_{dT} = 0.838, and π_T = 0.0047. Each sample site was characterized by one to three haplotypes (Fig. 1, Table 2) with variable signal of site differentiation (range = 0–1; Table 3), but generally high pairwise differentiation (mean F_{ST} = 0.951; Hudson et al. 1992).

Nuclear data analysis.—Genetic diversity was generally low across sample sites. The full data set (i.e., the 12 locus data set) revealed 1.3–2.6 effective alleles per locus (mean = 2.09; Table 1) and a proportion of polymorphic loci ranging from 0.25 to 0.83 (mean = 0.63; Table 1). Accordingly, expected heterozygosity was generally low across the study site (range = 0.13–0.45, mean = 0.34; Table 1). The reduced microsatellite data set (i.e., the eight locus set) revealed substantial subdivision among populations. Most population pairs demonstrated significantly different allele frequencies, with the notable exceptions of the R09–R10 pair ($P > 0.10$, Fisher's Combined Probability across loci) and the R02–R09 pair ($P > 0.05$, Fisher's Combined Probability across loci).

Analysis of genetic structure revealed that the six sample sites included in this analysis formed four genetic clusters (Fig. 2). Site R01, at the west end of the study area, formed a single cluster. Sites R02 and R05 formed a second cluster, and Sites R09 and R10 formed a third cluster. Finally, Site R11, at the east end of the study area formed a distinct cluster. Notably, all individuals demonstrated some level of admixture among the four genetic groups, but the signal of differentiation is nevertheless substantial (Fig. 2).

Global genetic differentiation, based on the 8-locus data set, was moderate (F_{ST} = 0.128) with pairwise estimates of F_{ST} ranging from 0.036 (Sites R09 and R10; Table 4) to 0.176 (Sites R01 and R11; Table 4). The level of genetic differentiation, however, is independent of geographic distance (Mantel Test; $r = 0.10$, $P > 0.05$; Fig. 3), suggesting a broad-scale lack of connectivity between the sites sampled.

TABLE 3. Estimates of F_{ST} for *Ctenosaura oedirhina* across its range in Honduras based on haplotype data analyzed using DnaSP; sites R04 and R06 are excluded because they are represented by only one sequence.

	R01	**R02**	**R03**	**R05**	**R07**	**R08**	**R09**	**R10**
R01	—							
R02	0.83	—						
R03	0.83	0.00	—					
R05	0.81	0.99	0.99	—				
R07	0.77	1.00	1.00	0.91	—			
R08	0.77	1.00	1.00	0.91	0.00	—		
R09	0.81	0.99	0.99	0.91	0.91	0.91	—	
R10	0.83	1.00	1.00	0.96	1.00	1.00	0.00	—
R11	0.87	1.00	1.00	0.97	1.00	1.00	0.92	1.00

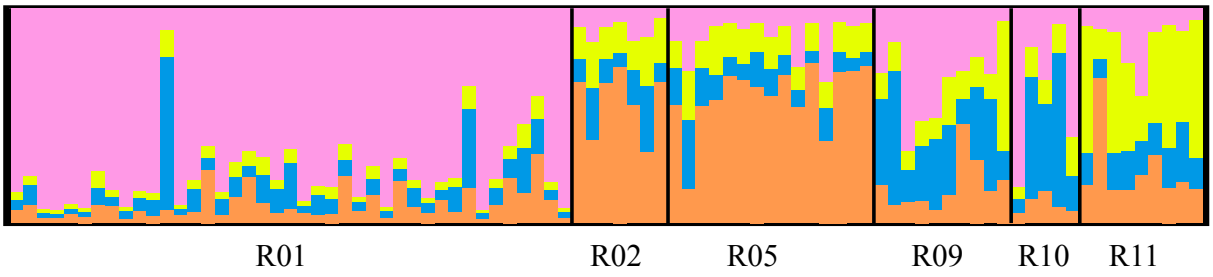

FIGURE 2. STRUCTURE results showing genetic clusters for *Ctenosaura oedirhina* by color (K= 4). Each vertical bar represents an individual, with colors corresponding to the proportional assignment of its multi-locus genotype. Sample sites share some portion of genetic information across the range of the species (Roatán and Barbareta, Honduras) as indicated by the shared colors between sites, but are distinct by the predominant color (pink, orange, blue, or yellow) that identifies each group.

Bayesian estimates of gene flow among sample sites were generally positive (range = 0.009–0.130) but not discernable from zero based on 95% credible limits (Table 5). Mean estimates of gene flow were qualitatively higher between sample sites on Roatán proper (m = 0.049) than from Roatán to Barbareta or Barbareta to Roatán (m = 0.037 and m = 0.027, respectively).

DISCUSSION

We used mtDNA (ND4) and microsatellite data to assess genetic structure for *Ctenosaura oedirhina* occupying 11 sample sites distributed across the islands of Roatán and Barbareta located within the Bay Islands, Honduras. Our analyses revealed generally low levels of genetic variation within populations for both the mtDNA and microsatellite data sets. Both data sets suggest historical connectivity among sample sites on Roatán and Barbareta, as revealed by the sharing of some ND4 haplotypes among sites coupled with the signal of historical admixture among sites in the microsatellite data. Nevertheless, the signature of past connectivity is overwhelmed by that of contemporary erosion of genetic diversity and disruption of movement among sample sites. In other words, though each sample site is characterized by low estimates of genetic diversity (i.e., few alleles per locus and low gene diversity, low nucleotide and haplotype diversity), the moderately high level of differentiation indicates that the genetic constitution of each group is different, thus global genetic diversity is reasonably high. Such a pattern has likely resulted from small population sizes and increased rates of genetic drift as a consequence of fragmentation (Allendorf and Luikart 2013). Our data, therefore, elucidate a high degree of spatial structuring that is consistent with strong barriers to movement as suggested by a moderately high global F_{ST} and lack of correlation between genetic and geographic distances. This suggests that the sample sites comprise a set of isolated genetic units that are subdivided into groups, largely defined by impassable intervening areas. Given that this species is already an endangered narrow-range insular endemic, further subdivision and isolation is increasingly threatening.

The level of isolation observed between groups of *C. oedirhina* across Roatán proper is consistent with that observed between populations separated by significant barriers to dispersal. For example, measures of genetic differentiation among islands (based on microsatellite data) of *Conolophus* spp. (Tzika et al. 2008), *Varanus komodoensis* (Ciofi and Bruford 1999), and *Cyclura cychlura cychlura* (Colosimo et al. 2014) were significant and qualitatively similar to those observed among samples sites for *C. oedirhina* across Roatán. In each of those cases, however, there was little signature of within-island genetic structuring, as can be observed

TABLE 4. Multilocus estimates of F_{ST} based on the 8-locus microsatellite data set for *Ctenosaura oedirhina* across its range in Honduras.

	R01	R02	R05	R09	R10
R01	—				
R02	0.17	—			
R05	0.17	0.04	—		
R09	0.08	0.07	0.12	—	
R10	0.05	0.14	0.15	0.04	—
R11	0.18	0.11	0.13	0.09	0.14

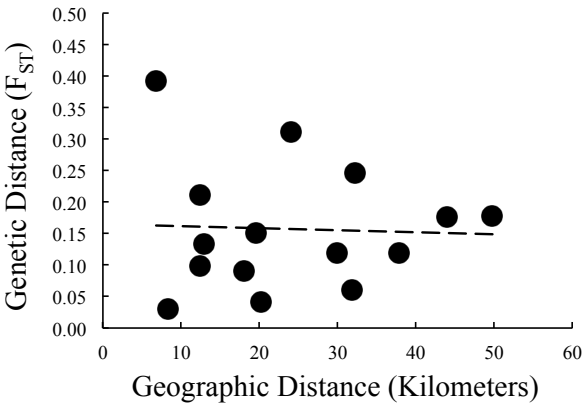

FIGURE 3. The relationship between genetic distance (F_{ST}) and linear geographic distance (km) for *Ctenosaura oedirhina* suggests that differentiation among groups of spiny-tailed iguanas on Roatán is not a function of distance-limited dispersal (Mantel Test, $r = 0.10$, $P > 0.05$).

TABLE 5. Estimates of migration (m ± 1 SD) for *Ctenosaura oedirhina* from the sample site indicated in the row title to the sample site indicated in the column title. The diagonal (in bold) represents the proportion of microsatellite variation of non-migrant origin; estimates of m are based on the 8-locus data set.

	R01	R02	R05	R09	R10	R11
R01	**0.95 ± 0.02**	0.01± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
R02	0.03 ± 0.03	**0.71 ± 0.03**	0.13 ± 0.06	0.08 ± 0.05	0.03 ± 0.03	0.03 ± 0.03
R05	0.03 ± 0.02	0.11 ± 0.05	**0.79 ± 0.05**	0.04 ± 0.03	0.02 ± 0.02	0.02 ± 0.02
R09	0.11 ± 0.06	0.05 ± 0.05	0.06 ± 0.05	**0.71 ± 0.04**	0.03 ± 0.02	0.04 ± 0.03
R10	0.09 ± 0.05	0.04 ± 0.04	0.04 ± 0.04	0.07 ± 0.05	**0.72 ± 0.04**	0.04 ± 0.03
R11	0.03 ± 0.03	0.04 ± 0.04	0.04 ± 0.03	0.05 ± 0.04	0.03 ± 0.02	**0.81 ± 0.06**

on Roatán. Such genetic differentiation among sample sites might be explained by strong social interactions, philopatry, or limited dispersal capabilities (Allendorf and Luikart 2013). However, given the extremely small size of Roatán, and direct estimates of habitat utilization, it is clear that connectivity among sample sites is disrupted by disturbance (i.e., increased poaching pressure) rather than being an artifact of the biology of *C. oedirhina* or natural vicariance. In an extensive analysis of habitat utilization, Goode et al. (this volume) have shown that stable densities of iguanas only occur in areas afforded protection from poaching, though additional suitable areas are available. Further, the lack of correlation between genetic and geographic distance coupled with a moderately high global F_{ST} (= 0.128) strongly suggest that there are limited corridors between populations, and that genetic diversity is locally and independently governed within sample sites.

The consequences of disrupted connectivity between elements of a putative metapopulation can be severe. Decreased local population size, genetic drift, and the resulting potential inbreeding depression contribute to the erosion of genetic diversity (Crnokrak and Roff 1999). Our data reveal evidence of small population sizes, consistent with ecological work by Goode et al. (this volume), and locally decreased genetic diversity within sample sites on Roatán. Indeed, estimates of gene diversity (H_E; Nei 1987) on Roatán are consistently lower than those reported for sample sites on the same island. For example, estimates for groups of *C. cychlura cychlura* on Andros Island ranged from 0.44 to 0.70 (Colosimo et al. 2014) and estimates for *Sphenodon punctatus* (Moore et al. 2008) ranged from 0.73 to 0.78, which are 10–44% higher than the estimates observed for *C. oedirhina*. The estimates observed for *C. cychlura cychlura* and *Sphenodon punctatus* are consistent with estimates taken from Marine Iguanas (*Amblyrhynchus cristatus*) occurring on a subset of the Galápagos Islands (Fernandina, San Cristóbal, and Santiago; Steinfartz et al. 2009) comprising larger samples, and taken from larger islands with lower human population densities. Although, the samples sizes reported here are smaller than those reported in Steinfartz et al. (2009), they are consistent with those reported by Colosimo et al. (2014). Hence, it is unlikely that the erosion of genetic diversity on Roatán is solely explained by sampling error and underestimates of these metrics.

Our data, coupled with the ecological work by Goode et al. (this volume) suggest that groups of Roatán Spiny-tailed Iguanas are negatively impacted by fragmentation resulting from anthropogenic pressures. Groups characterized at individual locations appear to have been historically connected given that haplotypes and alleles are shared among sampling locations across the island. Although these data suggest that the population of *C. oedirhina* on Roatán may have once been large and panmictic, individuals can now only be found in a few locations and in relatively low numbers (Goode et al. this volume). This has resulted in the current subdivisions, which appear to be evolving independently. The result is an increase in signal of local inbreeding (see F_{IS} in Table 1) and an apparent erosion of local genetic diversity. Given that this subdivision is likely relatively recent for *C. oedirhina*, the signal of inbreeding is relatively low at this moment; however, the reduced genetic variation and reduced gene flow among groups will quickly elevate the degree of inbreeding and likely make each population more vulnerable to environmental changes (increased temperature, altered precipitation, infectious diseases), demographic stochasticity (random changes in life expectancy or reproductive output), and continued human-related disturbances (Frankham et al. 2010).

As global measures of genetic diversity (i.e., genetic differentiation) are relatively high, augmenting exchange between groups might be a useful conservation strategy for maintaining population viability. That is, though each sample group holds limited genetic diversity, the groups combined hold higher diversity, as each group has different genetic variants. Therefore, exploring options for moving individuals among sites or maintaining a captive breeding program that facilitates exchange may be worthwhile. In many instances captive programs have prevented extinction, such as with the Jamaican Rock Iguana, *Cyclura collei* (see Wilson et al. this volume). That being said, such management strategies should not be entered into lightly and careful organization and monitoring must be in place before and during the process (see Alberts 2004).

Baseline data concerning the level of within-group relatedness and overall reproductive output would

provide insight into the potential for local adaptation, and overall potential for a successful breeding program on Roatán. In general, captive breeding programs should consider economic constraints, biological suitability (i.e., which species can be raised and bred), and potential for success during the planning process (Allendorf and Luikart 2013). Considering these potential limitations, managers should look closely at sites R01, R05, R09, and R11 as potential sources for breeding stock or locations for captive breeding programs. Taken together, these sites represent a cross-section of the highest population densities (Goode et al. this volume) and extant genetic variation (based on the 12 locus data set) on Roatán and the eastern island (site R11; Barbareta). Indeed, Barbareta may be an ideal location for a captive breeding effort as it is privately owned and protected, thus limiting the required economic input for successful program development. Many iguana species breed successfully in captivity, particularly when within their native range. For example, *C. bakeri*, the sister species to *C. oedirhina,* had a successful breeding facility in place for many years, in its native range (Stesha Pasachnik, pers. obs.). Thus, it seems likely that *C. oedirhina* would respond similarly to such a program. Nevertheless, experimental evidence regarding mate-choice dynamics and inbreeding and outbreeding factors should be considered prior to establishing such a program. If deemed reasonable, a captive breeding program could have benefits beyond rescuing within-population genetic diversity. Headstarting individuals in a captive breeding program may also have a substantial positive impact on population growth.

Goode et al. (this volume) showed that this species is a generalist. Thus, reintroduction should result in increased local recruitment and decreased negative impacts of inbreeding. Captive breeding may also provide the added benefit of increased effective fecundity because eggs would be protected from harvesting for human consumption, a common practice in Central America (Pasachnik et al. 2012, 2014). A successful captive breeding program could also facilitate education and outreach initiatives, involving local inhabitants of Roatán in the protection of their endemic species. Although captive breeding has a high potential for success, such a program must be viewed as a temporary means of management and not the sustainable solution. Rather, if captive breeding is implemented, efforts must be directed concurrently toward formal, legislated, habitat protection as well as the establishment of protected, high-quality dispersal corridors. Establishing protected habitat corridors has a high potential to facilitate connectivity between isolated groups, thus ensuring gene flow between populations and establishing a self-sustaining metapopulation with the capacity to respond to short- and long-term ecological dynamics, as was likely the case historically.

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