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RESEARCH ARTICLE

Genetic structure, diversity, and interisland dispersal in the endangered Mariana Common Moorhen (*Gallinula chloropus guami*)

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ABSTRACT

The Mariana Common Moorhen (*Gallinula chloropus guami*) is a highly endangered taxon, with fewer than 300 individuals estimated to occur in the wild. The subspecies is believed to have undergone population declines attributable to loss of wetland habitats on its native islands in the Mariana Islands. We analyzed mitochondrial DNA (mtDNA) sequences (control region and ND2 genes) and nuclear microsatellite loci in Mariana Common Moorhens from Guam and Saipan, the two most distal islands inhabited by the subspecies. Our analyses revealed similar nuclear genetic diversity and effective population size estimates on Saipan and Guam. Birds from Guam and Saipan were genetically differentiated (microsatellites: $F_{ST} = 0.152$; control region: $F_{ST} = 0.736$; ND2: $F_{ST} = 0.390$); however, assignment tests revealed the presence of first-generation dispersers from Guam onto Saipan (1 of 27 sampled birds) and from Saipan onto Guam (2 of 28 sampled birds), suggesting the capability for long-distance interpopulation movements within the subspecies. The observed dispersal rate was consistent with long-term estimates of effective numbers of migrants per generation between islands, indicating that movement between islands has been an ongoing process in this system. Despite known population declines, bottleneck tests revealed no signature of historical bottleneck events, suggesting that the magnitude of past population declines may have been comparatively small relative to the severity of declines that can be detected using genetic data.

Keywords: Mariana Common Moorhen, *Gallinula chloropus guami*, Guam, Saipan, mitochondrial DNA, microsatellites, migration, genetic structure

Estructura genética, diversidad y dispersión entre islas en *Gallinula chloropus guami,* un taxón federalmente amenazado

RESUMEN

Gallinula chloropus guami es un taxón últamente amenazado, para el que se estima que existen menos de 300 individuos en vida silvestre. Se cree que esta subespecie ha experimentado disminuciones poblacionales debidas a la pérdida de los humedales en sus islas nativas. Analizamos secuencias de ADN mitocondrial (región control y ND2) y loci de microsatélites en individuos de *G. c. guami* de Guam y Saipán, las islas más distantes habitadas por la subespecie. Nuestros análisis revelaron similitud en la diversidad genética nuclear y en los estimados del tamaño efectivo de las poblaciones en Saipán y Guam. Las aves de Guam y Saipán estuvieron diferenciadas genéticamente (microsatélites: $F_{ST} = 0.152$; región control: $F_{ST} = 0.736$; ND2: $F_{ST} = 0.390$), pero las pruebas de asignación revelaron la presencia de individuos dispersantes de primera generación desde Guam hacia Saipán (1 de 27 aves muestreadas) y desde Saipán hacia Guam (2 de 28 aves muestreadas), lo que sugiere una capacidad de movimientos inter poblacionales de larga distancia en la subespecie. La tasa de dispersión observada fue consistente con los estimados de largo plazo del número efectivo de migrantes por generación entre islas, lo que indica que el movimiento entre islas ha sido un proceso continuo en este sistema. A pesar de los declives poblacionales documentados, las pruebas de cuellos de botella no revelaron una señal histórica de eventos de reducción poblacional, lo que sugiere que la magnitud de los declives poblacionales pasados podría haber sido relativamente pequeña comparada con la severidad de los declives que pueden ser detectados con datos genéticos.

Palabras clave: ADN mitocondrial, estructura genética, *Gallinula chloropus guami*, Guam, microsatélites, migración, Saipán

INTRODUCTION

The Mariana Common Moorhen (*Gallinula chloropus guami*) is a subspecies of the Common Moorhen (*Gallinula chloropus*) that inhabits islands with suitable wetland habitat in the Mariana Archipelago, including the islands of Guam, Rota, Tinian, and Saipan (Ripley 1977, Taylor 1998). Mariana Common Moorhens were extirpated from the island of Pagan due to large quantities of ash and cinder deposited from a volcanic eruption in May 1981 and the destruction of vegetation by feral ungulates (Stinson et al. 1991, U.S. Fish and Wildlife Service 1991). On Guam, the moorhen is the only native freshwater bird species left after the extirpation of the Mariana Mallard (*Anas platyrhynchos oustaleti*), White-browed Crake (*Amaurornis cinerea*), and Nightingale Reed-Warbler (*Acrocephalus luscinius*; Reichel et al. 1992, Reichel and Lemke 1994). In 1984, the Mariana Common Moorhen was listed as Endangered due to its small population size and loss of wetland habitat on the islands that it inhabits due to changing agricultural practices, encroachment by undesirable vegetation, and clearing and filling of wetlands for development (U.S. Fish and Wildlife Service 1984). The introduced brown treesnake (*Boiga irregularis*) is also a threat on Guam through egg and chick predation (Takano and Haig 2004a). In 2001, the total adult moorhen population was estimated to be 287, with 154, 41, 2, and 90 adult moorhens on Saipan, Tinian, Rota, and Guam, respectively (Takano and Haig 2004a). However, Takano and Haig (2004a) considered these estimates to be conservative based on the lower response rate of female moorhens compared with males when using vocalization playbacks during surveys of larger wetlands.

Given the limited wetland habitat remaining in the Mariana Islands, protection and restoration of wetlands has become a conservation priority within the Marianas (U.S. Fish and Wildlife Service 1991, 2009). Moorhens are opportunistic and will colonize new or restored wetland habitats (Worthington 1998), especially if they occur within the normal dispersal range of the species, thereby facilitating connectivity of populations and minimizing population isolation. In this study, we investigate the population genetic structure of the endangered Mariana Common Moorhen in an effort to provide information to better conserve this subspecies. Specifically, we address the following questions: (1) How much genetic diversity occurs in the remaining Mariana Common Moorhen populations, and to what extent are these populations genetically differentiated? (2) Do the genetic data provide evidence of bottlenecks that reflect the declines in population size that have occurred in Mariana Common Moorhen populations? (3) Is there evidence for contemporary dispersal between Mariana Common Moorhen populations? We address these questions using a suite of 8 nuclear microsatellite markers, DNA sequence data from the mitochondrial control region and ND2 genes, and samples of Mariana Common Moorhen DNA obtained from Guam and Saipan, the 2 most distal islands of the Marianas currently inhabited by the taxon.

METHODS

Genetic Samples

Blood samples from Mariana Common Moorhens were collected in 2000 and 2001 from Saipan ($n = 27$) and Guam ($n = 28$). Birds were captured at night using spotlights and 1.5 m pole nets (Takano 2003). Moorhens that were sampled on Guam were captured on Fena Reservoir (81 ha), the largest permanent wetland area on Guam. The moorhens from Saipan occurred in an abandoned World War II concrete tank (0.5 ha) with concrete platforms and aquatic vegetation, providing habitat for 20–30 adults and 15–21 juveniles (Takano 2003). Following guidelines approved by the American Ornithologists' Union (Gaunt and Oring 1997), blood samples (0.5 ml) were collected from the brachial vein with a 26-gauge needle. Blood samples were stored in cryogenic tubes containing a buffer solution (100 mM Tris HCl pH 8.0, 100 mM EDTA pH 8.0, 10 mM NaCl, 0.5% SDS) and frozen at –80°C until analysis.

Molecular Methods

DNA was extracted by a standard phenol–chloroform extraction as previously reported by Haig et al. (2004) or by following the standard protocol for DNeasy Blood and Tissue Kits (QIAGEN, Valencia, California, USA). A 1.5 kb portion of the mitochondrial genome containing both the ND6 gene and the control region was amplified from 6 Mariana Common Moorhen samples with a GeneAmp XL PCR kit (Applied Biosystems, Foster City, California, USA) using primers L16087 and TS778H (Wenink et al. 1994, Sorenson 2003). Using the sequence generated from these amplifications, a moorhen-specific primer MH96L (5′-GCATAATGCGCATTCGATCC-3′) was developed to obtain a control region sequence. Using primer MH96L in combination with TS778H, a 705 bp portion of domain I and II of the control region was amplified and sequenced. A large 1.1 kb portion of the ND2 gene was amplified using primers L5216 and H6313 (Sorenson 2003), and bidirectional sequencing of this gene was completed using these primers in concert with the primer H5766 (Sorenson et al. 1999). PCR reactions were performed in a total reaction volume of either 50 µl or 20 µl with the following reaction concentrations: 10 mM Tris-HCl at a pH of 8.3; 50 mM KCl; 0.001% gelatin;

TABLE 2. Genetic diversity measures and sample sizes (*n*) for nuclear microsatellite loci and mitochondrial control region and ND2 genes of Mariana Common Moorhens on Guam and Saipan, Mariana Islands, between 2000 and 2001. Diversity measures for the microsatellites include observed heterozygosity (H_o), expected heterozygosity (H_e), and average number of alleles per locus (A_{nuc}). Nucleotide diversity (π), haplotype diversity (*H*), and haplotype richness (A_{mt}) are summarized for the 2 mitochondrial genes.

		Microsatellites			mtDNA (control region, ND2)		
Population	*n*	H_o	H_e	A_{nuc}	π	*H*	A_{mt}
Guam	27	0.485	0.577	5.18	0.002, 0.001	0.613, 0.501	3, 2
Saipan	28	0.497	0.599	5.18	0, 0	0, 0	1, 1

3.5 mM or 2.5 mM $MgCl_2$; 100 μm for each of the dNTPs; 0.2 μm or 0.4 mM of each primer; 100 ng of template; and 1.5 U AmpliTaq Gold Polymerase (Applied Biosystems). Thermal cycling parameters were as follows: 12 min denaturation at 93°C, followed by 35 or 40 cycles of 30 s at 93°C, annealing at 50°C for 30 s, and elongation at 72°C for 3 min. A final 10 min elongation step at 72°C completed each reaction. Polymerase chain reaction (PCR) amplification quality was visualized via agarose gel electrophoresis.

Bidirectional sequences for all samples were generated using ABI Prism Big Dye Terminator Cycle Sequencing chemistry on an automated capillary sequencer (ABI Prism 3730 Genetic Analyzer, ABI Prism 3730 Data Collection Software 2.0, ABI Prism DNA Sequencing Analysis Software 5.1.1; Applied Biosystems [ABI], Foster City, California, USA). Sequences for each sample were aligned and edited to obtain a composite sequence using program BioEdit 5.0.9 (Hall 1999) or SeqMan 8.0.2 (DNAStar, Madison, Wisconsin, USA).

We obtained nuclear microsatellite genotypes for each individual at 8 loci. Primer sequences and annealing temperatures for all loci are provided in Appendix Table 1. Microsatellite sequences were identified using high-throughput DNA sequencing data of Mariana Common Moorhens (sensu Jennings et al. 2011), processed using program *SSR_pipeline* (Miller et al. 2013). PCR primers were labeled with 5′6-FAM or HEX. PCR took place in 10 μl reactions containing 1× PCR buffer (Promega, Madison, Wisconsin, USA), 0.5 μm of each primer, 2.0 mM $MgCl_2$, 100 μm of each dNTP, and 1 U Taq DNA polymerase (Promega). Thermal cycling included an initial 2 min denaturation at 93°C, followed by 35 cycles of 30 s at 93°C, 30 s at the annealing temperature specified in Appendix Table 1, and elongation at 72°C for 45 s. A final 10 min elongation completed each reaction. Amplification products were analyzed on an ABI 3100 capillary DNA automated sequencer. We used ABI Genescan analysis software to size fragments with reference to the ABI internal lane standard GeneScan 500 ROX and scored allele sizes with ABI GeneMapper 4.1 analysis software.

Analyses of Genetic Diversity and Population Differentiation

Quantitative estimates of genetic diversity and differentiation were obtained using Arlequin 3.5.1.2 (Excoffier and Lischer 2010). Genetic diversity measures calculated for each mitochondrial DNA (mtDNA) gene included nucleotide diversity (π), haplotype diversity (*H*), and the number of unique haplotypes identified in each population (A_{mt}). For the microsatellites, we calculated observed and expected heterozygosity values (H_o and H_e, respectively) and the average number of alleles per locus (allelic richness; A_{nuc}) in each population. Prior to all microsatellite analyses, we used program GDA 1.1 (Lewis and Zaykin 2002) to identify deviations from Hardy-Weinberg genotypic proportions and to test for linkage disequilibrium between pairs of loci. The significance of each of these tests was evaluated using sequential Bonferonni corrections. We likewise used the microsatellite data to estimate contemporary effective population sizes (N_e) for each Mariana Common Moorhen population using NeEstimator 2.01 (Do et al. 2013). This analysis applied the linkage-disequilibrium estimator (LDNe) approach (Hill 1981), using an allele frequency cutoff value of 0.02 as recommended by Waples and Do (2010) and the assumption of monogamous breeding (Loyau and Schmeller 2012). Alternative contemporary N_e estimates were obtained using the approximate Bayesian computational approach implemented in ONeSAMP (Tallmon et al. 2008), assuming uniform priors with minimum and maximum N_e values of 2 and 100, respectively.

When population sizes are small, sampling of related individuals is likely to occur. Furthermore, our samples from Saipan were collected at a much smaller spatial scale than those obtained from Guam (see above). We therefore characterized the patterns of relationships among our samples from each population to determine whether birds from Saipan were potentially more related to one another. We used program ML-RELATE (Kalinowski et al. 2006), which employed a maximum-likelihood approach to classify pairs of individuals as being either unrelated, half siblings, full siblings, or parent and offspring. We tested for homogeneity of frequencies of the categories between

populations using a simple chi-square test for independence (Preacher 2001).

Patterns in genetic structure were quantified for each dataset using an Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) implemented in Arlequin. Estimates of F_{ST} were calculated between Guam and Saipan, with *P*-values for each estimate obtained by using 10,000 randomization replicates. The microsatellite data were also analyzed using STRUCTURE 2.2.3 (Pritchard et al. 2000), to identify the number of genetic clusters and to assign each individual to one of the identified clusters. Analyses assumed numbers of clusters (*K*) ranging from 1 through 7, and were performed using an initial 10^6 burn-in steps, followed by 10^7 analysis replicates based on the uncorrelated allele frequency model and the no admixture model. Ten replicate analyses were performed for each value of *K*. We evaluated analysis outcomes using the ΔK procedure of Evanno et al. (2005), and summarized across analysis replicates using program CLUMPP (Jakobsson and Rosenberg 2007). A haplotype network was constructed for each mtDNA gene using the Median Joining network construction approach (Bandelt et al. 1999) implemented in NETWORK 4.6.1.2 (www.fluxus-engineering.com), which allowed us to visualize the relationships and degree of divergence of haplotypes for each gene.

Tests for Genetic Bottlenecks

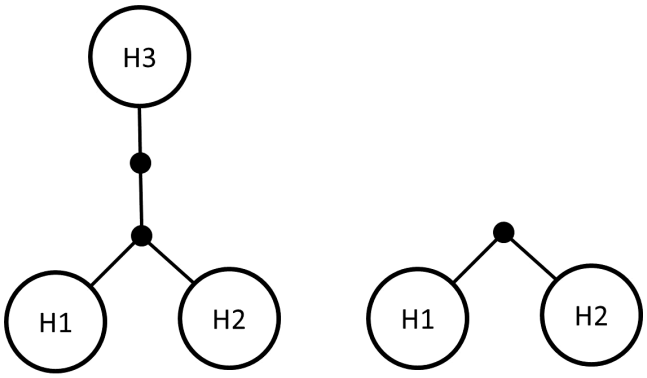
We used the microsatellite data to test for evidence of recent genetic bottlenecks within the Saipan and Guam populations of the Mariana Common Moorhen. Analyses were performed using the heterozygosity excess approach implemented in BOTTLENECK 1.2.02 (Piry et al. 1999). Analyses were run with the strict stepwise mutational model (SMM), the infinite alleles model (IAM), and the two-phase model (TPM). The latter is considered to most closely reflect the mutational processes in microsatellites (Di Rienzo et al. 1994). We therefore performed our TPM analyses, using a range of parameter values derived from observed values seen in empirical analyses of avian pedigrees (Miller et al. 2012). Specifically, we ran our TPM analyses using values of either 60% or 80% pure stepwise mutations, and specified variances of multistate mutational jumps to be either 4, 9, 16, or 25. All bottleneck analyses were performed using 10,000 analysis replicates, with results across loci derived from the Wilcoxon signed-rank test (Cornuet and Luikart 1996).

To complement the explicit tests for population bottlenecks, we also used the maximum-likelihood framework of Leblois et al. (2014) to determine the parameters of a historical population size contraction on each island. Analyses were conducted using MIGRAINE 0.4 (http://kimura.univ-montp2.fr/~rousset/Migraine.htm) via the OnePopVarSize model, which is designed to estimate mutation rate (μ)–scaled ancestral and contemporary effective population size parameters ($\theta_{anc} = 2N_{anc}\mu$; $\theta = 2N\mu$) and their confidence intervals using microsatellite data from a population. Analyses were performed assuming the Generalized Stepwise Mutation (GSM) model, using 15,000 estimated points and 2,000 runs per point in each of 3 iterations of the parameter inference process.

Historical Gene Flow and Contemporary Interisland Dispersal

We used program MIGRATE 3.6.6 (Beerli 2006, 2009) to quantify long-term interisland gene flow rates. MIGRATE calculates a mutation rate–scaled long-term effective population size parameter for each population ($\theta = 4N_e\mu$), but, more importantly for our purposes, can also estimate the asymmetrical effective migration rate parameter between populations (i.e. $M = 4N_em$, where m is the immigration rate into each population, N_e is the long-term effective population size, and N_em reflects the long-term effective number of migrants per generation). Analyses were conducted using the Brownian motion mutational model with variable mutation rates among loci and evaluated using Bayesian inference. Uniform priors bounded by 0 and 10 (with a delta of 1) were applied to the effective population size parameter estimates, whereas uniform priors bounded by 0 and 40 were used for the migration rate parameter (delta set to 4). Markov chain settings for a single analysis run included 100 replicates of 750,000 steps each, with each replicate adopting a static heating scheme using 4 chains (mathematical temperatures of 1, 1.5, 3, and 10^6) and a swapping interval of 1. The first 250,000 steps of each replicate were discarded as burn-in, and parameters were recorded every 250 steps during the remaining 500,000 steps. The entire analysis was repeated twice to ensure convergence of parameter estimates.

As a counterpoint to the MIGRATE analysis, we used the microsatellite data to identify evidence for contemporary dispersal between Guam and Saipan. This analysis was performed using program GENECLASS2 (Piry et al. 2004), which we used to independently implement the Bayesian migrant detection analysis approaches of Rannala and Mountain (1997) and Baudouin and Lebrun (2001). Probabilities associated with disperser classifications were derived using the simulation procedure of Paetkau et al. (2004), based on 10,000 simulations. We also considered the outcomes of the STRUCTURE analyses (described above) to provide heuristic evidence for the presence of dispersers. If an individual was sampled in one population but was primarily assigned to a different population, then we took this as reasonable evidence indicating that the sampled individual had dispersed into the population where it was detected.



	Haplotype frequencies				
	Control region			ND2	
Population	H1	H2	H3	H1	H2
Guam	11	13	3	11	16
Saipan	-	-	28	-	28

FIGURE 1. Haplotype network illustrating the genealogy of control region and ND2 mitochondrial DNA haplotypes detected in this study of Mariana Common Moorhens on Guam and Saipan in the Mariana Islands between 2000 and 2001. Frequencies of each haplotype on Guam and Saipan are also provided.

We compared contemporary interisland dispersal rates with long-term effective migration rates (N_em) estimated by MIGRATE as follows: An estimate of the contemporary interisland dispersal rate (d) was calculated as the fraction of all sampled individuals ($n = 55$) determined to be first-generation dispersers by GENECLASS2. Incorporating the contemporary effective population size estimates derived from programs ONeSAMP and NeEstimator, we obtained estimates of the effective numbers of contemporary dispersers between islands as N_ed. Contemporary N_e estimates obtained from ONeSAMP and NeESTIMATOR did not differ between islands or estimation approaches (see Results). Thus, the average of all individual contemporary effective population size estimates was used to calculate N_ed, an estimate of contemporary interisland dispersal that could be compared with the long-term effective migration rate (N_em) obtained using MIGRATE.

RESULTS

Genetic Diversity and Differentiation

The mtDNA analyses revealed 3 control region haplotypes and 2 ND2 haplotypes in birds on Guam (Figure 1, Table 2; Genbank Accession #KT384197–KT384201), with only 1 of the haplotypes from each gene detected in birds on Saipan, thereby producing diversity estimates (π and H) of 0 (Figure 1). Nuclear genetic diversity was relatively similar between Guam and Saipan populations (Table 2), as were the N_e estimates obtained from ONeSAMP (Table 2; Guam: $N_e = 24.3$, Saipain: $N_e = 22.2$). Linkage disequilibrium–based estimates of N_e were over twice as high for Guam ($N_e = 38.3$) as for Saipan ($N_e = 16.6$); however, 95% confidence intervals for all estimates overlapped, indicating that no significant differences existed between islands or between estimation procedures (Table 3). Tests for Hardy-Weinberg equilibrium were significant for locus MCMH-7 among samples from Saipan, and loci MCMH-8 and MCMH-10 were identified as deviating from expectations among Guam samples after sequential Bonferonni corrections. Tests for linkage disequilibrium identified one pair of loci in each population that differed from expectations (Guam: MCMH-5 and MCMH-12, $P < 0.001$; Saipan: MCMH-6 and MCMH20, $P < 0.001$), a result that could have been observed by chance alone. Analyses of the relationships among individuals from each sample indicated that there was a slight, but significant, difference in the frequencies of related vs. unrelated individuals in each sample ($\chi^2 = 12.092$, df = 3, $P = 0.007$). Overall, there were ~4% more unrelated pairs of individuals sampled on Guam than on Saipan (~75% vs. ~71%, respectively; Table 4).

TABLE 3. Effective population size estimates (N_e) and 95% confidence limits for Mariana Common Moorhens from Guam and Saipan, Mariana Islands, obtained using linkage disequilibrium–based estimates (LDNe) and approximate Bayesian computation approaches (ONeSAMP).

	LDNe		ONeSAMP	
Population	N_e	95% CL	N_e	95% CL
Guam	38.3	21.7–85.8	24.3	18.6–38.1
Saipan	16.6	11.2–24.9	22.2	17.3–31.8

Genetic differentiation between island populations was revealed in our analyses (microsatellites: $F_{ST} = 0.152$, $P < 0.001$; control region: $F_{ST} = 0.736$, $P < 0.001$; ND2: $F_{ST} = 0.390$, $P < 0.001$). Results of our STRUCTURE analyses were consistent with the F_{ST} values (Figure 2). The analysis suggested that the $K = 2$ solution was the most likely of all the evaluated scenarios, and, in general, individuals from each island were assigned to the island from which they were originally sampled.

Evidence for Genetic Bottlenecks

We identified no signature evidence of a genetic bottleneck in the Guam or Saipan population. Tests based on the stepwise mutational model and two-phase model were not significant, with P-values in excess of 0.65 across all combinations of model parameters examined (results not

TABLE 4. Frequencies (with percentages in parentheses) of pairwise relationships of Mariana Common Moorhens inferred for samples of birds obtained from Guam ($n = 27$ individuals; 351 pairwise relationships) and Saipain ($n = 28$ individuals; 378 pairwise relationships).

Relationship	Guam	Saipan
Unrelated	262 (75%)	268 (71%)
Parent and offspring	16 (4%)	40 (10%)
Full siblings	17 (5%)	25 (7%)
Half siblings	56 (16%)	45 (12%)

shown). The lowest *P*-values were observed in analyses based on the infinite alleles model, where *P*-values nonetheless were well above conventional significance thresholds (Guam: $P = 0.139$; Saipan: $P = 0.120$). Estimates of historical ($2N_{anc}\mu$) and contemporary ($2N\mu$) effective population size parameters obtained from MIGRAINE also provided no clear evidence for population size changes, having broad confidence intervals for which lower confidence limit values could in some cases not be estimated. On Guam, MIGRAINE calculated a historical population estimate of 1.5 (95% CI: not estimated to 103.5) compared with a contemporary value of 1.2×10^{-2} (95% CI: 9.6×10^{-4} up to 673.6). On Saipan, MIGRAINE estimated a historical value of 1.3 (95% CI: not estimated to 726.0) relative to a contemporary parameter estimate of 5.0 (95% CI: not estimated to 758.4).

Historical Gene Flow and Contemporary Interisland Migration

Parameter estimates from both replicates of the MIGRATE analysis were very similar (Table 5). Point estimates of θ were greater for Saipan than for Guam, but 95% credibility intervals overlapped, indicating no evidence for differences in the effective population size parameters obtained by each procedure. Likewise, estimates and credibility intervals for $M = 4N_em$ were very similar between runs and for both populations (Table 5), leading to average point estimates of long-term effective numbers of migrants per generation (N_em) of 0.785 for Guam and 0.873 for Saipan.

When using the Rannala and Mountain (1997) approach for migrant detection, GENECLASS2 identified 3 individuals as first-generation migrants: one from Saipan into Guam, and 2 from Guam into Saipan. The approach of Baudouin and Lebrun (2001) provided identical results. All migrant assignments had associated *P*-values < 0.001. Our qualitative evaluations of the STRUCTURE analysis results were largely concordant with these findings. One individual sampled from Guam was primarily assigned to the Saipan cluster, whereas 2 Saipan birds were more closely allied with the Guam cluster (Figure 2). Based on the outcome of the GENECLASS2 and STRUCTURE analyses, we estimate the contemporary dispersal rate between islands to be $3/55 = 0.055$. Assuming an average contemporary effective population size of 25.35 (average across islands and estimation procedures; Table 3), our analyses collectively suggest a contemporary effective number of dispersers per generation (N_ed) of 1.38.

TABLE 5. Parameter estimates from 2 replicate analyses of long-term effective population size and effective migration rates of Mariana Common Moorhens on Guam and Saipan, Mariana Islands, 2000–2001, obtained from program MIGRATE. Modes of the posterior distribution of each parameter are provided, as are the lower (LCL) and upper (UCL) 95% credibility limits for each estimate. θ represents a mutation-scaled long-term effective population size for each population ($4N_e\mu$). MIGRATE calculates the parameter *M*, which reflects the quantity $4N_em$, where N_em quantifies the long-term number of effective migrants per generation into a population; estimates of N_em are the primary result of interest from these analyses. Estimates of θ are presented primarily to illustrate the similar outcomes of both runs.

		Mode	LCL	UCL
Run 1	θ (Guam)	1.49	0.81	2.30
	θ (Saipan)	2.99	1.91	4.31
	$4N_em$ (Saipan to Guam)	3.03	1.23	5.52
	$4N_em$ (Guam to Saipan)	3.37	1.44	7.44
Run 2	θ (Guam)	1.35	0.68	2.25
	θ (Saipan)	3.01	1.97	4.32
	M (Saipan to Guam)	3.24	1.52	5.68
	M (Guam to Saipan)	3.61	1.47	6.80
Average	θ (Guam)	1.42	0.75	2.28
	θ (Saipan)	3.00	1.94	4.32
	M (Saipan to Guam)	3.14	1.38	5.60
	M (Guam to Saipan)	3.49	1.46	7.12

DISCUSSION

With a total population of just a few hundred individuals (Takano and Haig 2004a), the small size of the Mariana Common Moorhen population poses numerous challenges for recovery efforts. At this time, the key limiting factor is the availability of suitable wetland habitats. An interim recovery objective for the taxon involves achieving a stable population size of at least 975 adult birds (600 on Guam, 300 on Saipan, and 75 on Tinian; U.S. Fish and Wildlife Service 1991). This recovery goal can only be achieved if primary habitats are preserved and historical habitats are restored or compensated for if already altered. In this investigation, we used genetic analyses to obtain new insights into the genetic diversity, population structure, population history, and interisland movements of Mariana Common Moorhens that are relevant to recovery objectives for the subspecies.

Nuclear genetic diversity, as reflected by the microsatellite loci, was similar between Guam and Saipan (Table 2). Mitochondrial diversity, however, was higher on Guam,

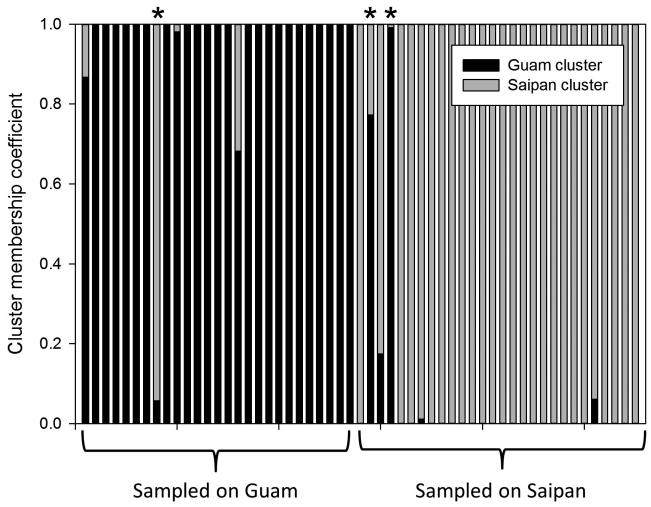


FIGURE 2. Results from program STRUCTURE, identifying the number of genetic clusters and assigning each tested individual of Mariana Common Moorhen on Guam and Saipan, Mariana Islands, to an identified cluster. Individuals indicated by asterisks were identified as being first-generation migrants in the program GENECLASS2 using 2 different Bayesian approaches.

given the detection of just a single mtDNA haplotype on Saipan for each of the 2 genes that were investigated. The single control region and ND2 haplotypes detected on Saipan were identical to 1 of the 3 control region and 2 ND2 haplotypes identified on Guam (Figure 1), suggesting that there is greater variation in the number of maternal family groups on Guam relative to Saipan. This finding may be partially due to the fact that Saipan samples were collected from a single, small nesting area, and may not necessarily reflect the situation across the whole island. We note, however, that while analyses of the interindividual relationships suggested that the sample from Saipan was comprised of more related individuals, the difference in the relative proportions of related vs. unrelated individuals between islands was very small (<4%; Table 4) and likely not sufficient to account for this difference in diversity. In contrast to the mtDNA results, nuclear microsatellite genetic diversity estimates were very similar between islands (Table 2), and estimates of contemporary effective population sizes (N_e) derived from the microsatellite data did not significantly differ between islands or among estimation procedures (Table 3). Effective population sizes are influenced by factors such as variation in reproductive output, fluctuating census population sizes, reproductive strategies, and the demographic or social structure of populations (Caballero 1994). Our results suggest that these types of life history parameters and demographic processes are likely similar between moorhen populations on Guam and Saipan.

Although population sizes of Mariana Common Moorhens are known to have declined, our analyses did not reveal a signature of prior genetic bottlenecks. The power of bottleneck detection tests depends primarily on sample sizes, the number of loci examined, and the magnitude of any population size reduction that a population may have experienced (Cornuet and Luikart 1996, Luikart et al. 1998, Peery et al. 2012). We consider our sample sizes to be unproblematic, especially given that they comprised a substantial percentage of the total population on each island (30% [27/90] on Guam and 18% [28/154] on Saipan). We likewise suggest that an acceptable number of loci were used in analyses, and that additional loci are unlikely to change the analysis outcomes. This inference comes from the *P*-values associated with the bottleneck tests. With a sample of 8 loci, we could expect to observe *P*-values that at least approached significance thresholds if a true bottleneck had occurred. Under this scenario, additional data may help to increase the power of analyses to the point where credible inference of a historical bottleneck may be made. However, in our analyses, *P*-values from most tests were in excess of 0.65, well above any "approximate significance" threshold that might encourage us to incorporate more data into the analysis. Given the exclusion of these 2 factors, our analyses suggest that the magnitude of the historical population decline was insufficient to generate a detectable genetic bottleneck. The MIGRAINE analyses, which estimated historical and contemporary effective population size parameters, yielded similar conclusions. In particular, parameter estimates resulting in very broad confidence intervals or in confidence intervals that cannot be estimated usually occur when there has been small (or no) past population size change (R. Leblois personal communication). Thus, the outcome of these analyses supports the position of Stinson et al. (1991), who suggested that Mariana Common Moorhen populations were reduced by only 36–52% during the 20th century. Population size reductions of this magnitude may be difficult to detect based on genetic data (Cornuet and Luikart 1996).

Our analyses identified evidence for direct movement of moorhens between Guam and Saipan (Figure 2). Previous radio-telemetry studies did not find any evidence for such long-distance movements. Instead, interisland movements were detected between Saipan and Tinian (Takano and Haig 2004b), 2 islands separated by only ~7 km of open ocean. The only recorded long-distance interisland movement of Mariana Common Moorhens involved the colonization of a 0.6-ha golf course stabilization pond on Rota by 2 adults and 3 juveniles in 1995 (Worthington 1998). In general, avian populations on Rota are declining (Amar et al. 2008), and Mariana Common Moorhens were previously extirpated from Rota, likely due to loss of wetland habitat and the introduction of mammalian predators (Steadman 1992). This suggests that the colonists on Rota originated from Guam, Tinian, or Saipan

(~75 km, ~100 km, and ~120 km away, respectively). Our data, however, suggest that even longer distance movements between Guam and Saipan can occur. One of the 27 sampled Guam birds (~4%) and 2 of the 28 sampled Saipan birds (~7%) were identified as first-generation migrants (Figure 2), leading to an estimated overall dispersal rate (d) of 0.055 (3/55). Based on the average contemporary effective population sizes of Mariana Common Moorhens (Table 3), our estimate of the contemporary effective number of dispersers per generation ($N_e d = 1.38$) is only slightly higher than the estimated long-term effective numbers of migrants per generation onto Guam and Saipan ($N_e m = 0.785$ and 0.873, respectively; Table 5). Thus, the dispersal events detected in this study appear to be generally compatible with the long-term estimates of migration rates between islands, suggesting that interisland migration has been relatively constant over time.

We suggest that our detection of long-distance movement has positive repercussions for the management of Mariana Common Moorhens. Ultimately, these results suggest that it may be possible to consider targeting additional islands (i.e. the island of Pagan) for population establishment and habitat restoration or construction. If moorhens fly long distances, then the prospects for creating demographically and genetically isolated populations is reduced, thereby minimizing the chance of local extinctions occurring in smaller populations within the Mariana Islands. We note, however, that Pagan is ~320 km from Saipan, a distance that exceeds the ~120 km movements between Guam and Saipan suggested by our data. If appropriate wetland habitats are available on Pagan, it remains possible that the best opportunity to establish a new population may come from human-assisted translocations of birds to the island, and by ensuring the availability of other smaller wetlands that could serve as stepping-stone habitats between islands. Once established, the potential for demographic connectivity between Pagan, Saipan, and other islands may be better evaluated using resightings of banded birds or through new genetic investigations.

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APPENDIX TABLE 1. Primer sequences, repeat motifs, and annealing temperatures for 8 microsatellite loci used to investigate the population genetic structure of the endangered Mariana Common Moorhen on Guam and Saipan, Mariana Islands, between 2000 and 2001. The range of fragment sizes (base pairs), number of alleles, and observed and expected heterozygosities for each locus (H_o and H_e, respectively) are also provided.

Locus	Primer sequence	Repeat motif	Annealing temperature	Fragment size range	Number of alleles	H_e	H_o
MCMH-5	5′-CAGAAAAGTGAGAAATCAGAA-3′ 3′-TTTTTCCCTTAATAGCTTGA-5′	GT	45°C	110–154 bp	11	0.847	0.656
MCMH-6	5′-TTCTTTAACAGAATAGGGACA-3′ 3′-CAAGGAAAATACGTTGTAACT-5′	TG	47°C	132–162 bp	10	0.592	0.483
MCMH-7	5′-CCAATCTAGAGTTTTGAAGTG-3′ 3′-GCAAAGAGCTTACAATCTAAT-5′	TG	47°C	149–173 bp	6	0.472	0.180
MCMH-8	5′-ACATGGTAATTTCTTTTGCT-3′ 3′-CTCTATGTTGCATGTCTCTCT-5′	GT	56°C	124–146 bp	8	0.747	0.525
MCMH-10	5′-GGCATATGTCTTCACCTG-3′ 3′-AACAAATGCTTTTATGTTCC-5′	TG	45°C	101–117 bp	6	0.708	0.459
MCMH-12	5′-CTTGAAGTGTCTTGTTCCTT-3′ 3′-TAATCTCACTGGAAAGAATGA-5′	AC	54°C	130–164 bp	12	0.844	0.672
MCMH-14	5′-TCTCATTTGACTATCACCAAT-3′ 3′-CATAGAATCATGGAGAGGTTA-5′	GT	47°C	118–148 bp	7	0.703	0.492
MCMH-20	5′-GGTAAGTCCTTCATATCCTCT-3′ 3′-AGTCACCTCCTCCATTTT-5′	TG	56°C	113–141 bp	7	0.516	0.361