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RESEARCH ARTICLE

Interisland genetic structure of two endangered Hawaiian waterbirds: The Hawaiian Coot and Hawaiian Gallinule

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ABSTRACT

Most of Hawaii's endemic avifauna are species of conservation concern. Some of Hawaii's endangered waterbirds, however, have increased in number as a result of intensive management of wetlands. To inform these conservation efforts, we examined interisland genetic structure and gene flow within 2 Hawaiian endemic waterbirds, the Hawaiian Coot (*Fulica alai*) and the Hawaiian subspecies of the Common Gallinule (*Gallinula galeata sandvicensis*), using microsatellite and mitochondrial loci. Hawaiian Coots and Hawaiian Gallinules occupy coastal wetlands and exhibit similar life history characteristics and generation times, although they may differ in dispersal propensity. Mark–resight data for Hawaiian Coot indicate interisland movements, whereas Hawaiian Gallinules are sedentary. Genetic diversity is partitioned across the landscape differently for Hawaiian Coots and Hawaiian Gallinules; patterns of variation are likely influenced by behavioral and ecological mechanisms. Hawaiian Coots exhibit low levels of structure at microsatellite loci ($F_{ST} = 0.029$) and high levels of gene flow among islands. Conversely, Hawaiian Gallinules are highly structured across marker types (microsatellite $F_{ST} = 0.205$, mtDNA control region $F_{ST} = 0.370$, mtDNA ND2 $F_{ST} = 0.087$), with restricted recent gene flow. Patterns of gene flow have changed after the population declines in the early to mid-1900s. Gene flow estimates indicate historical dispersal from Kauai to Oahu in both species, while recent estimates show individual Hawaiian Coots dispersing from Oahu and restricted gene flow between islands for the Hawaiian Gallinule. Changes in gene flow through time suggest that patterns of dispersal may be an artifact of the availability of habitat, which may be indirectly associated with the synergistic influences of population density and wetland quality. Despite recent population size increases for both species, continued threats to Hawaiian waterbirds (i.e. nonnative mammalian predators and invasive plants, avian disease, altered hydrology, and saltwater inundation of freshwater wetlands) will likely require continued active management to maintain viable populations.

Keywords: Fulica alai, *Gallinula galeata sandvicensis*, gene flow, Hawaiian Coot, Hawaiian Gallinule, population genetic structure

Estructura genética inter-isla de dos aves acuáticas en peligro de Hawái: *Fulica alai* y *Gallinula galeata sandvicensis*

RESUMEN

La mayoría de la avifauna endémica de Hawái son especies de preocupación para la conservación. Sin embargo, algunas aves acuáticas en peligro de Hawái han aumentado en número como resultado del manejo intensivo de los humedales. Para evaluar estos esfuerzos de conservación, examinamos la estructura genética inter-isla y el flujo génico adentro de dos aves acuáticas endémicas de Hawái, *Fulica alai* y *Gallinula galeata sandvicensis*, usando loci microsatelital y mitocondrial. Ambas especies ocupan la costa de los humedales y exhiben características de sus historias de vida y tiempos generacionales similares, aunque pueden diferir en la propensión a dispersarse. Los datos de marcado y re-avistaje de *F. alai* indican movimientos inter-isla, mientras que *G. g. sandvicensis* es sedentaria. La diversidad genética se divide de modo diferente a través del paisaje para *F. alai* y *G. g. sandvicensis*; los patrones de variación están probablemente influenciados por mecanismos comportamentales y ecológicos. *F. alai* muestra bajos niveles de estructura en los loci microsatelitales ($F_{ST} = 0.029$) con altos niveles de flujo génico entre islas. Contrariamente, *G. g. sandvicensis* muestra una alta estructuración a través de los tipos de marcadores (microsatélite $F_{ST} = 0.205$, región de control del ADNmt $F_{ST} = 0.370$, ND2 ADNmt $F_{ST} = 0.087$) con un flujo génico reciente restringido. Los patrones de flujo génico han cambiado luego de la disminución poblacional desde principios hasta mediados de 1900. Las estimaciones de flujo génico indican una dispersión histórica desde Kauai hacia Oahu en ambas especies, mientras que las estimaciones recientes muestran a los individuos de *F. alai* dispersándose desde Oahu y un flujo génico restringido entre islas para *G. g. sandvicensis*. Los cambios en flujo génico a través del tiempo sugieren que los

patrones de dispersión pueden ser un artefacto de la disponibilidad de hábitat, lo que puede estar asociado indirectamente con las influencias sinérgicas de la densidad poblacional y la calidad de los humedales. A pesar de la mejora reciente en el tamaño poblacional de ambas especies, la continuidad de las amenazas para las aves acuáticas de Hawái (mamíferos depredadores y plantas invasoras no nativas, enfermedades aviares, alteración de la hidrología e inundación de agua salada de los humedales de agua dulce) requerirá probablemente un manejo activo continuo para mantener poblaciones viables.

Palabras clave: estructura genética poblacional, flujo génico, *Fulica alai, Gallinula galeata sandvicensis*

INTRODUCTION

Hawaii is the most remote island archipelago in the world. Post-colonization isolation likely promoted the diversification, and endemism, of many of Hawaii's fauna and flora. As with other island ecosystems in Oceania, however, most of Hawaii's endemic fauna and flora are endangered or extinct. Indeed, Hawaii is home to 66 endemic avian species, of which 37.9% are extinct; 53.0% are listed as vulnerable, endangered, or critically endangered; 4.5% are near threatened; and only the remaining 4.5% are listed as species of least concern (under IUCN Red List criteria; IUCN 2018). Hawaiian species endangerment has been attributed to a range of anthropogenic influences, including the loss or modification of habitat, introduced predators and plants, disease, altered hydrology, and hybridization with nonnative species (U.S. Fish and Wildlife Service [USFWS] 2011, Paxton et al. 2018). Among Hawaii's endangered waterbirds, however, positive species-level population trends have been observed over the past several decades, based on count data from the period 1986–2015 (State of Hawaii Division of Forestry and Wildlife [DOFAW] 1980–2015, Reed et al. 2011, Underwood et al. 2013). Protection of wetland areas—including the establishment of several national wildlife refuges, increased aquaculture, and active management (e.g., control of nonnative predators and invasive plants and manipulation of water levels to mimic historical hydrological regimes)—likely played a large role in the reversal of population trajectories for these species (Reed et al. 2011, Underwood et al. 2013).

Two of Hawaii's endemic waterbirds, the Hawaiian Coot (*Fulica alai*) and the Hawaiian subspecies of the Common Gallinule (*Gallinula galeata sandvicensis*), were considered "abundant" and widely distributed throughout the main Hawaiian islands prior to the 1900s (USFWS 2011), though no estimates (pre-1900s) of population size are available. Statewide counts conducted in the mid-1900s, however, detected <1,000 Hawaiian Coots and just 60 Hawaiian Gallinules (USFWS 2011). Hawaiian Gallinules were last observed on the islands of Hawaii in 1898, Maui in 1900, and Molokai in 1973, whereas Hawaiian Coots were likely not extirpated from any of the main Hawaiian islands. Hawaiian Coot and Hawaiian Gallinule populations increased starting in the mid-1970s, coincident with the protection of wetland areas and increased aquaculture (Reed et al. 2011). Population declines that started in the late 1800s altered genetic diversity for both species (Sonsthagen et al. 2017). Large reductions (between −38.4 and −51.4%) in mitochondrial diversity were observed between Hawaiian Coot samples collected before and after the decline; minimal differences were observed in the distribution of allelic and haplotypic frequencies between sampled time periods. Conversely, for the Hawaiian Gallinule, allelic frequencies were strongly differentiated between time periods, signatures of a genetic bottleneck were detected, and biases in mean effective population size were observed at microsatellite loci. Kauai was considered a population stronghold for both species during the decline because ~70% of the Hawaiian Coot population and ~66% of the Hawaiian Gallinule population occurred there (Shallenberger 1977). As more wetlands have been restored, the distribution of populations across the islands appears to have changed. Surveys from 1986 to 2015 show that ~28% of the Hawaiian Coot population and ~58% of the Hawaiian Gallinule population are estimated to be on Kauai (DOFAW 1980–2015, J. G. Underwood personal observation). The current census population size for the Hawaiian Coot is 1,777 ± 310 individuals (Underwood et al. 2013), and it occupies its historical range (Figure 1A). The Hawaiian Gallinule's current distribution is restricted to the islands of Oahu and Kauai, with most of the birds occurring on Kauai (~1,000 individuals range-wide; Figure 1B; DOFAW 1980–2015, J. G. Underwood personal observation).

Hawaiian Coots and Hawaiian Gallinules occupy coastal wetlands, with Hawaiian Gallinules preferring more densely vegetated areas. Both species exhibit similar life history characteristics and generation times (7 yr for Hawaiian Coot and 6 yr for Hawaiian Gallinule; BirdLife International 2016a, 2016b), though they may differ in dispersal propensity (Bannor and Kiviat 2002, Pratt and Brisbin 2002). Mark–resight data ($n = 4$; Riggs 2016, J. G. Underwood personal observation) and large shifts in bird numbers among islands during statewide surveys (Shallenberger 1977, Engilis and Pratt 1993, DOFAW 1980–2015) indicate interisland movements by Hawaiian Coots. Conversely, Hawaiian Gallinules are thought to be highly sedentary and to maintain territories year-round (Bannor

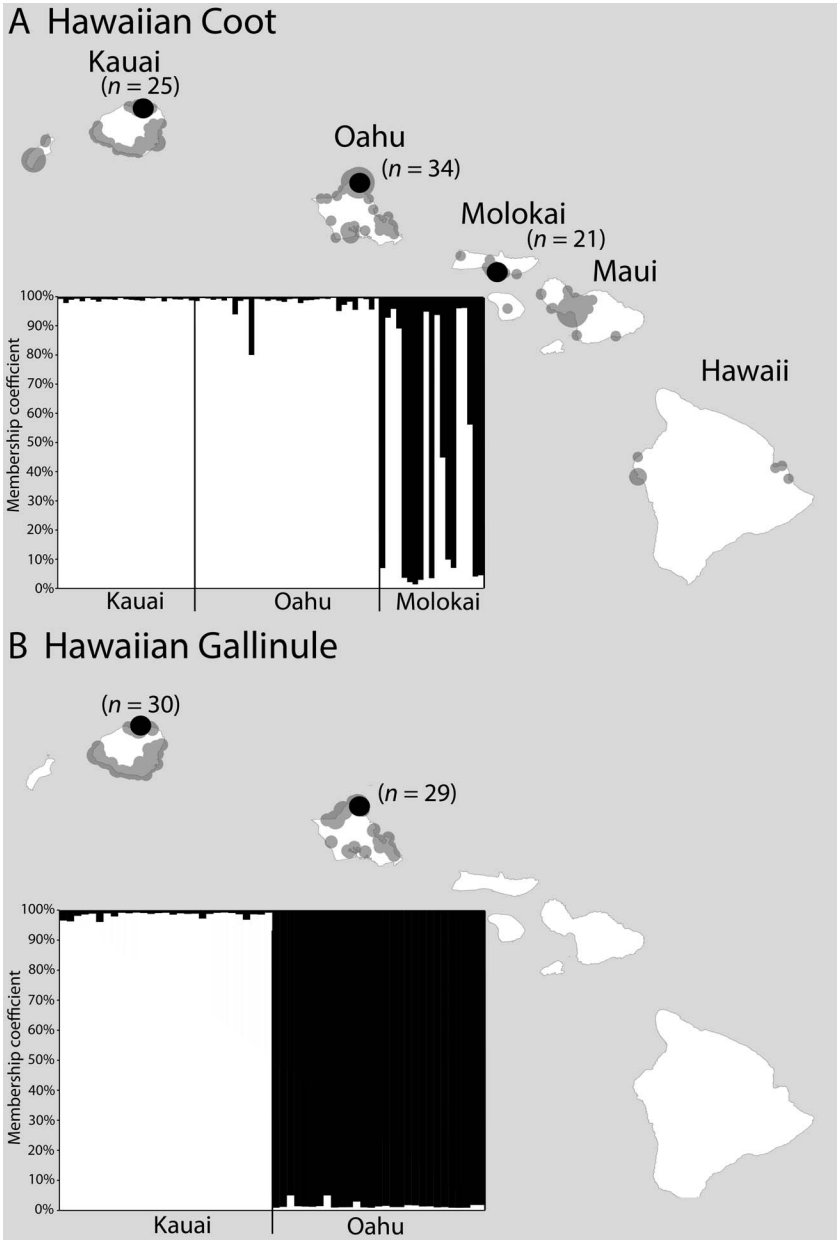

Figure 1. Current distribution (shaded in gray), sample size, and average membership coefficient of individual Hawaiian Coots (**A**) and Hawaiian Gallinules (**B**) from sampled Hawaiian islands into the 2 clusters inferred from 16 and 13 microsatellite loci, respectively, in Structure (Pritchard et al. 2000).

and Kiviat 2002). Despite the sedentary nature of Hawaiian Gallinules, they have colonized wetlands (either unoccupied or created through restoration and management) as numbers increased post-decline (based on genetic data; van Rees et al. 2018b), and intraisland dispersal events have been observed (van Rees et al. 2018a). Differences in dispersal propensity are postulated to have played a role in how the population decline influenced genetic diversity (Sonsthagen et al. 2017). Here, we examine the population genetic structure of Hawaiian Coot and Hawaiian Gallinule to fill knowledge gaps identified in the Recovery Plan for Hawaiian Waterbirds (USFWS 2011) and inform conservation efforts, such as reestablishment on the main Hawaiian islands. Specifically, we assessed the extent of genetic structure and gene flow among sampled islands based on 16 (Hawaiian Coot) and 13 (Hawaiian Gallinule) autosomal microsatellite loci along with mitochondrial (mtDNA) control region and NADH dehydrogenase (ND) 2 loci.

METHODS

Sample Collection

Hawaiian Coots and Hawaiian Gallinules were trapped on James Campbell National Wildlife Refuge, Oahu, Hawaii, from 2012 to 2013, and on Hanalei National Wildlife Refuge, Kauai, Hawaii, in 2015 (Figure 1). Hawaiian Coots were also trapped at Kaunakakai Wastewater Reclamation Facility, Molokai, Hawaii, from 2008 to 2013 (Figure 1A). Tissue samples were opportunistically collected during 2011–2015 from Hawaiian Coots and Hawaiian Gallinules on Kauai that were found dead, either on the refuge or in other wetlands, and stored in tissue preservation buffer (4.0 M urea, 0.2 M NaCl, 10 mM EDTA, 0.5% N-lauroylsarcosine, and 100 mM tris-HCl). Blood samples were collected from the brachial vein for individuals trapped on Kauai and Oahu and stored in blood preservation buffer (Longmire et al. 1988). Feather samples were collected from Molokai birds and stored in coin envelopes at room temperature. Distances between islands are as follows: 100 km (Oahu and Molokai), 175 km (Oahu and Kauai), and 280 km (Molokai and Kauai).

Laboratory Techniques

Genomic DNA was extracted using a "salting out" procedure described by Medrano et al. (1990), with modifications described in Sonsthagen et al. (2004). Genomic DNA concentrations were quantified using fluorometry and diluted to 50 ng mL^{-1} working solutions. Genotype data were collected at 16 loci for Hawaiian Coot (Fal02, Fal04, Fal07, Fal08, Fal10, Fal12, Fal14, Fal16, Fal17, Fal19, Gch03, Gch07, Gch12, Gch14 [Sonsthagen et al. 2014]; KiRa10 [Brackett et al. 2013]; and Pho110 [Grueber et al. 2008]) and 13 loci for Hawaiian Gallinule (Fal08, Fal10, Fal12, Fal14, Fal17, Fal19, Gch06, Gch12, Gch13, Gch17, Gch19 [Sonsthagen et al. 2014]; KiRa9 [Brackett et al. 2013]; and Pho110 [Grueber et al. 2008]). The forward primer for locus KiRa9 was modified for this study (KiRa9.1F: 5′–GCGAGACTTGAAGTAGTGG–3′). Polymerase chain reaction (PCR) amplifications and thermocycler conditions followed Talbot et al. (2011). In addition, 10% of the samples were extracted, amplified, and genotyped in duplicate for quality control. No inconsistencies in genotype scores were observed between replicates.

Individual Hawaiian Coots and Hawaiian Gallinules were sequenced at 2 mtDNA loci: we amplified an 824 base pair (bp) and 826 bp fragment, respectively, of control region using primer pairs CR200L and CR1029H as well as a 752 bp and 753 bp fragment, respectively, of ND2 with primer pairs ND2_224L and ND2_1003H (Sonsthagen et al. 2017). PCR amplifications, cycle-sequencing protocols, and post-sequencing processing followed Sonsthagen et al. (2007). For quality control purposes, we extracted, amplified, and sequenced 10% of the samples in duplicate. No inconsistencies in mtDNA sequences were observed between replicates.

Analysis of Genetic Diversity

We calculated allelic richness, observed and expected heterozygosities, Hardy-Weinberg equilibrium, and linkage disequilibrium in FSTAT 2.9.3 (Goudet 1995). Tests for null alleles and allelic dropout were implemented in MicroChecker (van Oosterhout et al. 2004). Haplotype (h) and nucleotide (π) diversity were calculated at mtDNA loci in Arlequin 2.0 (Schneider et al. 2000). Fu's F_S (Fu 1997) and Tajima's D (Tajima 1989) were calculated to test the hypothesis of selective neutrality for mtDNA loci and implemented in Arlequin. We applied critical significance values of 5%, which requires $P < 0.02$ for Fu's F_S (Fu 1997). An unrooted haplotype network for mtDNA loci was constructed in Network 5.0.0.0 (Fluxus Technology 2015) using the reduced median method (Bandelt et al. 1995), to illustrate possible reticulations in the gene tree because of homoplasy or recombination.

Analysis of Genetic Structure

The degree of population genetic structure within Hawaiian Coot and Hawaiian Gallinule was assessed by calculating overall and pairwise F_{ST} and R_{ST} for microsatellite and F_{ST} and Φ_{ST} for sequence data in Arlequin, adjusting for multiple comparisons using Bonferroni correction ($\alpha = 0.05$). Pairwise Φ_{ST} was calculated using a Tamura-Nei nucleotide substitution model (Tamura and Nei 1993).

We used the Bayesian clustering program Structure 2.3.2 (Pritchard et al. 2000, Hubisz et al. 2009) to assign individuals to clusters based on their microsatellite allelic frequencies and to infer the occurrence of genetic structure without a priori knowledge of putative populations. Data were analyzed using an admixture model assuming correlated frequencies and sample location information as a prior with a 50,000 burn-in period, 500,000 Markov chain Monte Carlo iterations, and number of possible populations (K) ranging from 1 to 5; the analysis was repeated 10 times to ensure consistency across runs. We followed the method of Evanno et al. (2005) to determine the most likely number of clusters given the data.

Analysis of Gene Flow

Estimates of gene flow among islands were calculated in BayesAss 3.0.4 (Wilson and Rannala 2003) and Migrate 3.6.8 (Beerli and Felsenstein 1999, 2001). These programs differ in the temporal scale at which they estimate gene flow rates. BayesAss estimates gene flow (m) over the last several generations (short term) and does not assume that populations are in migration–drift or Hardy-Weinberg

equilibrium. Migrate, by contrast, estimates gene flow rates (effective number of migrants per generation based on nuclear loci, N_{em}, and effective number of female migrants per generation based on mitochondrial loci, N_{fm}) and effective population sizes (θ) based on the coalescent and assumes that populations are in migration–drift equilibrium. Gene flow estimates generated in Migrate, therefore, reflect a more historical signature than those generated in BayesAss.

BayesAss was run with the default delta values for allelic frequency (a), gene flow rate (m), and inbreeding (f). Subsequent runs incorporated different delta values to ensure that proposed changes between chains at the end of the run were between 20% and 40% of the total chain length (Wilson and Rannala 2003). Once delta values (Hawaiian Coot: $\Delta a = 0.70$, $\Delta m = 0.60$, $\Delta f = 0.60$; Hawaiian Gallinule: $\Delta a = 0.70$, $\Delta m = 0.25$, $\Delta f = 0.60$) were within the accepted proportion of proposed changes (Hawaiian Coot: $a = 38\%$, $m = 23\%$, $f = 34\%$; Hawaiian Gallinule: $a = 25\%$, $m = 26\%$, $f = 32\%$), data were run 3 additional times (30 million iterations, 3 million burn-in, and sampling frequency of 2,000) with different random seeds to ensure convergence across runs.

Migrate was run with a full gene flow model, θ (composite measure of effective population size, $4N_e\mu$ for microsatellite loci and $N_f\mu$ for mtDNA, and mutation rate), and all pairwise gene flow parameters were estimated individually from the data and were compared to a restricted island model for which θ was averaged and pairwise gene flow parameters were symmetrical between populations. All loci and individuals were included in a single input file (mtDNA and microsatellite data were analyzed separately). Any individuals for which data were missing for a particular locus were still included in the analysis, and information for that locus was denoted as missing. Gene flow was estimated using maximum likelihood search parameters: 10 short chains (2,500 trees used out of 500,000 sampled), 5 long chains (10,000 trees used out of 2 million sampled), and 5 static chains (start temperatures: 1, 1.5, 3, 6, 12; swapping interval = 1) with 5 million burn-in per chain. Models were run 3 times to ensure the convergence of parameter estimates. The alternative model was evaluated for goodness-of-fit given the data using a log-likelihood ratio test. The resulting statistic from the log-likelihood ratio test is equivalent to a chi-square distribution with degrees of freedom equal to the difference in the number of parameters estimated in the 2 models (Beerli and Felsenstein 2001).

RESULTS

Genetic Diversity

Null alleles and allelic dropout were not detected for any loci assayed for Hawaiian Coot or Hawaiian Gallinule. Microsatellite loci did not deviate from Hardy-Weinberg equilibrium and were in linkage equilibrium. Indices of genetic diversity based on microsatellite loci were similar across sampled sites for both species (95% confidence intervals overlapped), although more private alleles were observed within Hawaiian Coots from Molokai (Table 1).

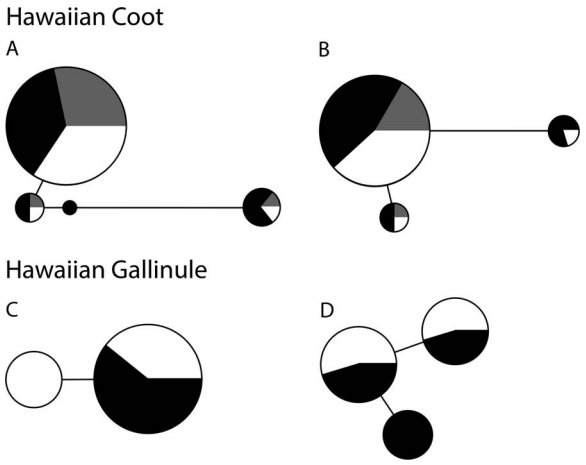

Figure 2. Parsimony networks illustrating relationships of mtDNA control region (**A**, **C**) and ND2 (**B**, **D**) haplotypes in Hawaiian Coots and Hawaiian Gallinules sampled on Kauai (white), Oahu (black), and Molokai (gray). The size of the circle node corresponds to the frequency of each haplotype.

Within the mtDNA control region sequences, 4 haplotypes characterized by 11 variable sites were observed for Hawaiian Coot and 2 haplotypes characterized by a single variable site were observed for Hawaiian Gallinule (Figure 2). ND2 had greater variation for Hawaiian Gallinule than the mtDNA control region; 3 haplotypes were characterized by 2 variable sites. Three ND2 haplotypes were observed within Hawaiian Coot characterized by 5 variable sites (Figure 2). Similar to the microsatellite data, indices of genetic diversity were similar across sampled sites for each species based on the mtDNA sequence data, with a few exceptions (Table 1). Within Hawaiian Coot, the Kauai population had lower levels of haplotype diversity than the Oahu and Molokai populations, albeit not significantly (based on overlapping 95% confidence intervals; Table 1). Within Hawaiian Gallinule, the Kauai population had greater diversity at mtDNA control region than the Oahu population. Tajima's D was significant and negative for Hawaiian Coot Kauai and Molokai populations, based on control region and ND2 data (Table 1) suggestive of fluctuations in population size (Tajima 1989).

Genetic Structure

Patterns of spatial variation at allelic and haplotypic frequencies differed between species. Signatures of genetic structure were detected in Hawaiian Coot, based on

Table 1. Indices of genetic diversity, including mean (± SD) numbers of alleles and haplotypes, allelic richness, number of private alleles and haplotypes, observed and expected heterozygosity (H_o/H_e), haplotype (h) and nucleotide diversity (π), and sample size (n), based on 16 and 13 microsatellite loci, 824 and 826 bp of mtDNA control region, and 752 and 753 bp of mtDNA ND2, respectively, for 3 Hawaiian Coot populations and 2 Hawaiian Gallinule populations. Significant values are in bold.

	Hawaiian Coot			Hawaiian Gallinule	
	Kauai	Oahu	Molokai	Kauai	Oahu
Microsatellites					
Number of alleles	4.6 ± 1.8	4.9 ± 1.6	4.6 ± 1.0	2.2 ± 0.7	2.3 ± 0.5
Allelic richness	4.3 ± 1.5	4.6 ± 1.2	4.5 ± 0.9	–	–
Private alleles	6	8	10	3	3
H_o (%)	62.4 ± 2.5	60.4 ± 2.1	55.4 ± 2.9	35.8 ± 2.5	43.2 ± 2.6
H_e (%)	60.2 ± 4.1	62.1 ± 2.6	62.3 ± 3.6	37.8 ± 5.6	42.5 ± 3.4
n	25	34	19	29	29
mtDNA control region					
Number of haplotypes	3	4	3	2	1
Private haplotypes	0	1	0	1	0
h	0.157 ± 0.096	0.411 ± 0.097	0.186 ± 0.110	0.520 ± 0.497	–
π	0.001 ± 0.001	0.004 ± 0.002	0.001 ± 0.001	0.001 ± 0.001	–
Fu's F_s	1.3	4.5	1.6	1.6	–
Tajima's D	**−2.3**	0.4	**−2.2**	1.5	–
n	25	33	21	30	28
mtDNA ND2					
Number of haplotypes	3	3	3	2	3
Private haplotypes	0	0	0	0	1
h	0.157 ± 0.096	0.322 ± 0.097	0.318 ± 0.164	0.514 ± 0.046	0.690 ± 0.028
π	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001
Fu's F_s	−0.5	1.7	0.3	1.5	1.2
Tajima's D	**−2.0**	−0.5	**−1.8**	1.5	1.5
n	25	33	12	21	27

microsatellite data (F_{ST}; Table 2). Molokai was differentiated from Kauai ($F_{ST} = 0.049$, $P < 0.001$) and Oahu ($F_{ST} = 0.045$, $P < 0.001$); however, no structure was detected between Kauai and Oahu. The spatial pattern of genetic partitioning was also uncovered in Structure ($K = 1$, Ln$P|K = -3{,}067$; $K = 2$, $\Delta K = 106$, Ln$P|K = -2{,}983$; $K = 3$, $\Delta K = 23$, Ln$P|K = -2{,}980$; Figure 1A). Kauai and Oahu birds clustered into one group, with Molokai containing birds clustering into Kauai-Oahu cluster ($n = 7/19$) and a second group ($n = 10/19$). The sample location prior was informative ($r < 1$). Analyses based on R_{ST} were not significant (overall and pairwise; Table 2). Differentiation was not detected (overall and pairwise) at mtDNA control region or ND2 haplotypic data for Hawaiian Coot (Table 2). Conversely, a strong signature of genetic structure was observed within Hawaiian Gallinule across all marker types (F_{ST}, R_{ST}, and Φ_{ST}; Table 2). Structure uncovered 2 groups with individuals forming island-specific clusters with high membership coefficients ($K = 1$, Ln$P|K = -1{,}072$; $K = 2$, $\Delta K = 358$, Ln$P|K = -948$; $K = 3$, $\Delta K = 2$, Ln$P|K = -952$; Figure 1B), and the sample location prior was informative ($r < 1$).

Gene Flow

Asymmetric gene flow was uncovered across marker types and analyses for both Hawaiian Coot and Hawaiian Gallinule (Figure 3). Gene flow estimates for Hawaiian Coot varied across temporal scales, though patterns were similar for estimates based on the coalescent. Frequency-based estimates (BayesAss) showed more individuals dispersing from Oahu into Kauai and Molokai, with restricted gene flow between Kauai and Molokai within the past several generations. Conversely, more Hawaiian

Table 2. Estimates of genetic differentiation (F_{ST}, R_{ST}, and Φ_{ST}) calculated from 16 Hawaiian Coot and 13 Hawaiian Gallinule microsatellite loci, mtDNA control region, and mtDNA ND2. Significant values ($\alpha = 0.05$) are in bold.

	Microsatellites		mtDNA control region		mtDNA ND2	
	F_{ST}	R_{ST}	F_{ST}	Φ_{ST}	F_{ST}	Φ_{ST}
Hawaiian Coot	**0.029**	0.025	0.012	0.019	–0.023	–0.023
Hawaiian Gallinule	**0.205**	**0.092**	**0.370**	**0.370**	**0.087**	**0.118**

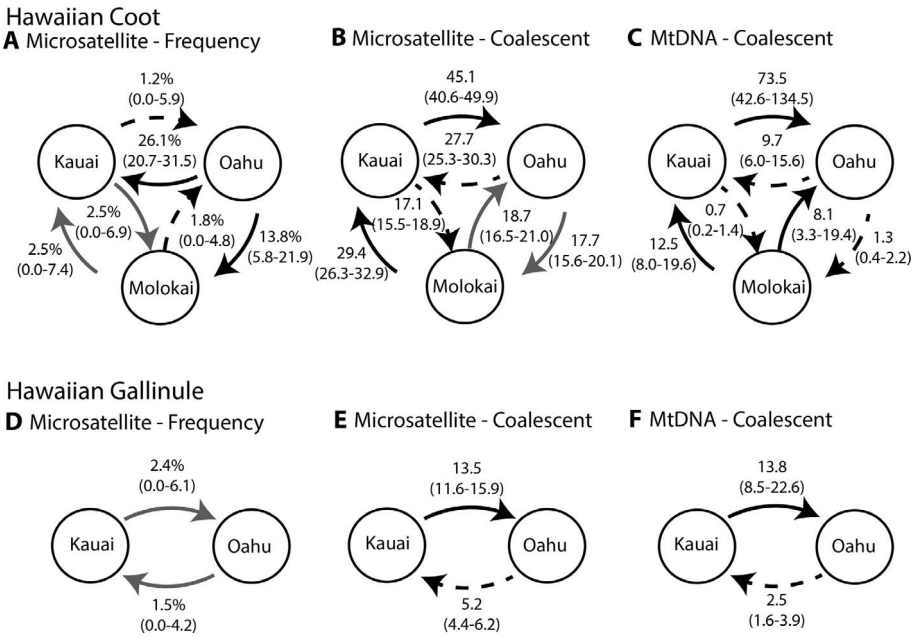

Figure 3. Gene flow estimated among 3 Hawaiian Coot populations and between 2 Hawaiian Gallinule populations calculated from 16 and 13 microsatellite loci, respectively, and from mtDNA control region and ND2 in BayesAss (frequency-based) and Migrate (coalescent-based). Black arrow lines indicate main biases in the directionality of gene flow between population pairs; in instances with bidirectional gene flow, dashed arrow lines indicate the lower rate between population pairs. Gene flow estimates with overlapping 95% confidence intervals between population pairs are indicated by gray arrow lines. Parameter estimates are reported as m (proportion of migrants) for frequency-based and as N_{em} or N_{fm} (number of migrants or female migrants per generation) for coalescent-based gene flow values.

Coots were dispersing from Molokai into Kauai and Oahu (95% CI overlap for mtDNA) and from Kauai into Oahu, based on the coalescent (Migrate). Within Hawaiian Gallinule, the directionality of gene flow was similar across temporal scales with dispersal from Kauai into Oahu, though gene flow was restricted for frequency-based estimates.

The full model (all parameters allowed to vary independently) had significantly higher likelihoods than the restricted model (symmetric interpopulation gene flow rates and θ) across marker types, indicating asymmetric gene flow within Hawaiian Coot (microsatellites LnL(full) = –6,219, LnL(test) = –6,545, $P < 0.001$; mtDNA LnL(full) = –71, LnL(test) = –250, $P < 0.001$), and Hawaiian Gallinule (microsatellites LnL(full) = –1,275, LnL(test) = –1,328, $P < 0.001$; mtDNA LnL(full) = –152, LnL(test) = –214, $P < 0.001$).

DISCUSSION

Dispersal propensity appears to be a strong determinant in the patterns of genetic structure observed within both study species. The Hawaiian Coot, for which interisland movements have been detected from banding data (Riggs 2016, J. G. Underwood personal observation), exhibits low levels of structure at microsatellite loci and high levels of gene flow among islands. The presence of 2 genetic groups suggests that Hawaiian Coots may have been isolated in at least 2 areas during the decline, presumably a Kauai-Oahu group and a Maui Nui (Maui, Molokai, Lanai)-Hawaii group. As Hawaiian Coots increase in numbers and reestablish previously unoccupied wetlands, individuals representing the 2 genetic clusters are coming into contact, as evidenced by the presence of individuals with membership coefficients predominantly assigned to one group or the other in Molokai. Genetic drift has greater influence over small populations, and the population declines influenced genetic diversity within both species (Sonsthagen et al. 2017). Therefore, genetic drift may have played a role in the formation of 2 genetic clusters within the Hawaiian Coot. Genetic data from Maui and Hawaii are needed to confirm this hypothesis.

Unlike the Hawaiian Coot, the Hawaiian Gallinule is highly structured across marker types, with restricted recent gene flow; genetic drift likely had a larger influence on how genetic diversity is partitioned within this species. The limited evidence of interisland dispersal by Hawaiian Gallinules is in contrast to observations of direct movement exhibited by a closely related island endemic, the Mariana Common Moorhen (*G. chloropus guami*), for which 3 dispersal events between Guam and Saipan (~120 km) were detected (Miller et al. 2015). The extent of

genetic structure observed within the Hawaiian Gallinule is similar to (albeit higher than) levels observed at microsatellite loci ($F_{ST} = 0.152$) in the Mariana Common Moorhen. While interisland movements of Mariana Common Moorhens may be associated with dry periods (Takano and Haig 2004), there is contradictory evidence regarding the influence of dry or wet seasons on dispersal propensity within the Hawaiian Coot and Hawaiian Gallinule (e.g., Engilis and Pratt 1993, Reed et al. 2011, Riggs 2016). Thus, other factors, either ecological or evolutionary, are likely driving patterns of dispersal within Hawaiian waterbirds.

Patterns of gene flow may be an artifact of the availability of habitat, which may be indirectly associated with the synergistic influences of several factors, such as population density and wetland quality, on individual dispersal propensity (Clobert et al. 2009). During the population decline, Kauai maintained a majority of the Hawaiian Coot and Hawaiian Gallinule populations (Shallenberger 1977). Coalescent-based estimates of gene flow, which reflect a more historical signature than frequency-based estimates, indicate an outward dispersal of individuals from Kauai to Oahu. The directionality of gene flow supports the inference that Kauai was a stronghold for Hawaiian Coots and Hawaiian Gallinules and suggests that density was sufficiently high to promote interisland movement, at least before the decline. Post-decline patterns of dispersal have changed. Gene flow is restricted between islands for the Hawaiian Gallinule; conversely, individual Hawaiian Coots appear to be dispersing from Oahu. Recent movement of Hawaiian Coots from Oahu to adjacent islands is consistent with mark–resight data. Birds banded in unmanaged (presumably marginal) wetlands were resighted at other wetlands within Oahu, and 2 birds were observed on Kauai, 1 on Molokai, and 1 on Maui, whereas Hawaiian Coots banded on managed habitats remained on (or returned to) the managed areas (Riggs 2016, J. G. Underwood personal observation). Among the main Hawaiian islands, Oahu has experienced the second-highest coastal wetland loss since the post-Polynesian settlement of the islands (–71%) and has approximately half of the coastal wetland area of Kauai (similar wetland area as Molokai; see Table 2; van Rees and Reed 2014). The limited availability of coastal wetlands on Oahu, coupled with nearly half of the world's population of Hawaiian Coots being present on Oahu (USFWS 2011), has likely promoted recent dispersal of individuals from Oahu to adjacent islands as the population has recovered from the decline and as wetlands have presumably neared their carrying capacity. The apparent lack of recent Hawaiian Gallinule gene flow between Kauai and Oahu, despite genetic evidence that birds dispersed between the islands historically, suggests that Hawaiian Gallinule populations have not reached densities high enough to promote interisland movement or that the behavior of Hawaiian Gallinules has changed post-decline.

Expanses of ocean between islands are often a strong barrier to dispersal (Grant 2001), and within the Hawaii archipelago they have likely played a large role in the evolution of (sub)species endemic to individual islands (e.g., VanderWerf et al. 2010, Lerner et al. 2011, Shaw and Gillespie 2016). Even in introduced species, the varying selection pressure on individual islands has promoted morphological and behavioral divergence from the mainland counterparts (Hamao 2015) and among islands (Foster et al. 2018) within relatively short periods (<100 yr). Processes driving population divergence within the Hawaiian waterbirds studied here, however, appear to be different from those influencing Hawaiian avifauna that occupy forest ecosystems, in that (sub)species unique to individual islands have not evolved in waterbirds. Ocean crossings do not appear to be a deterrent of dispersal for Hawaiian Coots or (historically) for Hawaiian Gallinules. Rather, the evolutionary trajectories of the Hawaiian Coot and Hawaiian Gallinule were altered by microevolutionary processes acting on genetic diversity during the population decline (Sonsthagen et al. 2017); therefore, the genetic structure observed here likely reflects recent processes rather than historical conditions. We hypothesize that recent population declines and subsequent recovery likely played a larger role in how genetic diversity is partitioned across the landscape than differences in selection regimes among islands, given that genetic drift is a strong evolutionary force when acting on small populations and that islands (at least until recently in the case of the Hawaiian Gallinule) are connected via gene flow.

Conservation Implications

Conservation of Hawaii's endemic waterbirds requires data on demographic and behavioral processes to inform species' recovery. Population resilience to stochastic processes is dependent, at least in part, on the ability of individuals to interact across the landscape, which can be inferred by patterns of genetic variation. Genetic diversity is partitioned across the landscape differently for the Hawaiian Coot and Hawaiian Gallinule; patterns of variation are likely influenced by behavioral and ecological mechanisms. Minimum-population-size targets listed in the species' recovery plan criteria for both the Hawaiian Coot and the Hawaiian Gallinule are 2,000 individuals, with further evaluation needed using population viability analyses (USFWS 2011). While the population target sizes are based on estimates of census numbers prior to population declines, high levels of genetic structure within the Hawaiian Gallinule at both the interisland and intraisland spatial scales (see van Rees et al. 2018b), coupled with evidence from the present study of restricted gene flow between islands, warrant further evaluation of

this target. High genetic structuring coupled with restricted gene flow within the Hawaiian Gallinule indicates that populations occupying Kauai and Oahu are acting as independent demographic units, requiring larger census sizes for long-term viability. Limited genetic structure observed within the Hawaiian Coot, however, may make this species more resilient to perturbations and stochastic processes, given that genetic diversity is panmictic throughout a large segment of its range and that islands are connected through a network of dispersal.

Continued threats to Hawaiian waterbirds (i.e. nonnative mammalian predators and invasive plants, avian disease, altered hydrology, and saltwater inundation of freshwater wetlands) will likely require active management in perpetuity to maintain viable populations (Underwood et al. 2013). In light of current predictions of sea-level rise (28–43 cm by 2100), island ecosystems will face increased challenges such as reduced land mass, inundated atolls, and saltwater intrusion into freshwater areas (Legra et al. 2008). For species such as the Hawaiian Coot and Hawaiian Gallinule that reside in coastal wetland habitats and prefer freshwater wetlands, saltwater intrusion may further alter the population dynamics of these wetland specialists (e.g., van Rees and Reed 2018). Potential loss of habitat from sea-level rise, threats they currently face, and their differential dispersion tendencies highlight the reality that despite recent improvement in the population size of these species, they remain imperiled.

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