Conserv Genet (2018) 19:129–142
DOI 10.1007/s10592-017-1020-0

RESEARCH ARTICLE

Small-scale genetic structure in an endangered wetland specialist: possible effects of landscape change and population recovery

Charles B. van Rees[1] · J. Michael Reed[1] · Robert E. Wilson[2] · Jared G. Underwood[3,4] · Sarah A. Sonsthagen[2]

Received: 7 June 2017 / Accepted: 6 October 2017 / Published online: 24 October 2017

Abstract The effects of anthropogenic landscape change on genetic population structure are well studied, but the temporal and spatial scales at which genetic structure can develop, especially in taxa with high dispersal capabilities like birds, are less well understood. We investigated population structure in the Hawaiian gallinule (*Gallinula galeata sandvicensis*), an endangered wetland specialist bird on the island of O`ahu (Hawai`i, USA). Hawaiian gallinules have experienced a gradual population recovery from near extinction in the 1950s, and have recolonized wetlands on O`ahu in the context of a rapidly urbanizing landscape. We genotyped 152 Hawaiian gallinules at 12 microsatellite loci and sequenced a 520 base-pair fragment of the ND2 region of mitochondrial DNA (mtDNA) from individuals captured at 13 wetland locations on O`ahu in 2014–2016. We observed moderate to high genetic structuring (overall microsatellite $F_{ST} = 0.098$, mtDNA $F_{ST} = 0.248$) among populations of Hawaiian gallinules occupying wetlands at very close geographic proximity (e.g., 1.5–55 km). Asymmetry in gene flow estimates suggests that Hawaiian gallinules may have persisted in 2–3 strongholds which served as source populations that recolonized more recently restored habitats currently supporting large numbers of birds. Our results highlight that genetic structure can develop in taxa that are expanding their range after severe population decline, and that biologically significant structuring can occur over small geographic distances, even in avian taxa.

Keywords Habitat fragmentation · Metapopulation · Connectivity · Microsatellites · Urbanization · Moorhen

Introduction

Research on anthropogenic impacts on population connectivity (Ricketts 2001; Baguette and Van Dyck 2007), and growing attention to connectivity's effects on population persistence (Fahrig and Merriam 1985; Hanski and Gaggiotti 2004), have made the study of fragmented populations highly germane to biodiversity conservation (Opdam 1991; Hawkes 2009; Goldberg and Waits 2010; Murphy et al. 2010). Fragmented populations suffer increased extirpation risk because these populations are often relatively isolated, and smaller, making them more strongly influenced by stochastic processes, all of which may result in increased likelihood of inbreeding depression, and low evolutionary potential due to decreased genetic diversity (Keller and Weller 2002; Mills 2007; Frankham et al. 2009). As connectivity among habitat patches is reduced, genetic drift can promote divergence in allelic and haplotypic frequencies among populations, resulting in genetic structuring when there is little or no emigration or immigration to counteract it (Selkoe and Toonen 2006; Broquet and Petit 2009). Conversely, habitat connectivity (Taylor et al. 1993) can ameliorate many risk factors and allow physically disjunct populations to persist as part of a network (Macdonald and Johnson 2001; Crooks and Sanjayan 2006) or, under particular circumstances, as a metapopulation of interconnected habitats (Hanski 1999;

✉ Charles B. van Rees
Charles.van_Rees@tufts.edu

[1] Department of Biology, Tufts University, 200 College Avenue, Medford, MA 02155, USA

[2] Alaska Science Center, U. S. Geological Survey, Anchorage, AK 99508-4626, USA

[3] Pacific Reefs National Wildlife Refuge Complex, U.S. Fish and Wildlife Service, Honolulu, HI 96850, USA

[4] Present Address: Don Edwards San Francisco Bay National Wildlife Refuge, Fremont, CA 94555, USA

Smith and Green 2005; Doleman 2012). Thus, genetic structure can be a useful indicator of the interactions between spatially isolated populations, that provides potentially valuable information for their conservation (Gibbs and Reed 2007; Mills 2007).

Though many studies have investigated population connectivity in stable populations, the development of genetic structure in growing populations (e.g., recovering or following colonization) is less well-understood. Specifically, it is unclear at which temporal and spatial scales fragmentation (and consequently population differentiation) will occur in dynamic, human-altered landscapes. Research on the population structure of fragmented, recovering populations at small spatial scales may yield valuable insights into the demographic and genetic effects of recovery and landscape change of populations at larger scales (Greenbaum and Fefferman 2017; Moreno-Mateos et al. 2017).

Islands are particularly vulnerable to landscape changes because anthropogenic pressures can more readily affect a significant portion of the available habitat (Fordham and Brook 2010). Here we investigate the population structure of an endangered bird, the Hawaiian gallinule (`Alae `ula, *Gallinula galeata sandvicensis*), whose populations have been recovering from near-extinction in a highly modified landscape. The island of O`ahu, a moderately sized (1500 km^2) volcanic island in the central Pacific Ocean once supported a diversity of terrestrial habitats, including extensive lowland forests and coastal freshwater wetlands (Ziegler 2002). Throughout the twentieth century, urban land cover has increased markedly with growing tourist visitation and resident population size while agricultural land use on the island decreased (Giambelluca 1986; Klasner and Mikami 2003; NOAA Coastal Services Center 2014). O`ahu is now heavily urbanized, supporting > 60% of the population of the state of Hawai`i (953,000 of 1.4 million people) in ~10% of its land area. More than 65% of O`ahu's freshwater lowland wetlands have been lost to land conversion, primarily because they occupied ideal locations for agricultural residential areas (van Rees and Reed 2014). Due to the island's topography, wetland habitats have always been spatially separated on the landscape, but habitat loss and anthropogenic landscape change may have introduced new barriers to dispersal among animal populations persisting in remaining wetland patches on the island. Accordingly, information on the population structure of wetland-specialist taxa on the island would be valuable for their conservation and management.

The Hawaiian gallinule is an endangered waterbird subspecies endemic to the Hawaiian Islands that lives only in shallow, coastal, freshwater wetlands. Hawaiian gallinules are hypothesized to be recent colonizers of the Hawaiian Islands (Fleischer and McIntosh 2001), with archaeological evidence of gallinules only found in deposits post-dating human colonization of the islands (~1500 years before present, James 1987). Hawaiian gallinules were formerly distributed among most of the main Hawaiian Islands (Hawai`i, Maui, Moloka`i, O`ahu, and Kaua`i), but were extirpated from all islands except Kaua`i and O`ahu during a period of severe population decline throughout the 19th and early twentieth centuries (Schwartz and Schwartz 1949; Munro 1960; Banko 1987). This decline was driven by the introduction of invasive mammalian predators (esp. small Indian mongoose *Herpestes javanicus*, domestic dogs *Canis lupis familiaris*, and cats *Felis catus*), habitat loss and degradation by agriculture and later urbanization, and habitat modification by exotic invasive plants (e.g., *Urochloa mutica*) (Griffin et al. 1989; Reed et al. 2012). The population reached a minimum estimated total population size of 57 individuals in the 1950s (Engilis and Pratt 1993). The establishment of National Wildlife Refuges on O`ahu and Kaua`i, and associated predator control and habitat restoration have helped Hawaiian gallinules make a steady recovery over the last 50 years, with current statewide population estimates from 400 to 1000 total individuals (Reed et al. 2011; Underwood et al. 2013; USFWS pers. comm.). Currently, between 200 and 300 Hawaiian gallinules persist on O`ahu (Reed et al. 2011; USFWS 2011) in a number of spatially isolated, relict or artificially maintained wetland habitats. These habitat patches occur within state, county, and federal protected areas, private farms, golf courses, and waterways. Wetland habitats are separated by a highly heterogeneous landscape of highways, agriculture, dense urban and suburban areas, and active military bases. To date, no formal studies have been undertaken to understand the genetic structure of gallinule populations among these physically isolated patches.

Hawaiian gallinules are a member of the family Rallidae (rails), which are known for their remarkable propensity to colonize islands and disperse long distances over open water despite many species being reluctant fliers (Ripley 1977). Rails are often freshwater wetland specialists, and can exhibit limited dispersal and subsequent genetic structuring under conditions of habitat loss and landscape change (e.g. California Ridgway's rail, *Rallus obsoletus obsoletus*, Wood et al. 2017). Several rail species in North America are migratory (e.g. yellow rail *Coturnicops noveboracensis*, Sora *Porzana carolina*, common gallinule) with vagrant sightings well outside of their breeding range, indicating substantial dispersal abilities (Ripley 1977; Taylor and Van; Perlo 1998). In contrast to the migratory behaviors of other *G. galeata* subspecies, Hawaiian gallinules do not migrate, and are thought to be highly sedentary. For example, they have not colonized restored or unoccupied habitats on other islands in their historical range despite gradual population recovery (USFWS 2011). Hawaiian gallinules are weakly seasonal breeders, and can have multiple broods per year (average clutch size is three to six eggs; Byrd and Zeillemaker 1981) with an estimated generation time of 5.9 years

(BirdLife 2016), though individuals are observed breeding at age 2 (van Rees, pers. obs.). For species with known limited dispersal propensities and relatively rapid generation times, understanding the level of connectivity (effective dispersal) among local wetlands is important when assessing the spatial ecology and likely persistence of small endemic populations (Van Strien et al. 2014). O`ahu's small size, rapid landscape change, and the discrete distribution of wetland habitats provide an excellent study system for examining the spatial scales at which population structure may be influenced by changing landscapes, including habitat loss and matrix alteration, as well as by population recovery.

The structure and connectivity of O`ahu's Hawaiian gallinule population is also pertinent to the long-term conservation prognosis of the subspecies. The majority of habitat patches on O`ahu support fewer than 30 individuals (CVR pers. obs.), and therefore each population may have a high extinction risk without genetic and demographic rescue. As has been demonstrated in other habitat specialist birds (e.g., Florida scrub-jay, *Aphelocoma coerulescens*, Chen et al. 2016), habitat fragmentation and reduced dispersal lead to increased inbreeding coefficients, affecting vital rates important to population persistence (e.g., increased hatch failure). Consequently, we investigated the spatial patterns of genetic diversity within Hawaiian gallinules on O`ahu to evaluate the effects of population decline and recovery in an urbanized, fragmented landscape.

Methods

Sample Collection

We captured Hawaiian gallinules using walk-in Tomahawk live-traps baited with cracked corn, cat food, or other attractive food items (e.g. mango fruit *Mangifera sp*.). We captured Hawaiian gallinules at 13 coastal wetlands on O`ahu in summer (May–July) from 2014 to 2016 (Fig. 1). Wetland names and their corresponding four-letter codes are given by region in Table 1: North Shore (includes TURT, JCNR, SHRI, WAIM, LOTU), Windward (KLIP, HAMA, ENCH, KAWA, OLOM), Maunalua (KEAW), and Pearl Harbor (POUH, HONO). Our sampled wetland sites represent all known major habitats for the species on O`ahu. James Campbell National Wildlife Refuge was sampled only in 2014. Marine Corps Base Hawai`i, Kahuku, Kawainui marsh, and Pouhala marsh were sampled only in 2016. All other wetlands were sampled in 2015 and 2016. Inter-site Euclidean distances ranged from 1.5 to 54 km, with a mean of around 30 km. Notably, sample sizes for some wetlands (POUH, HONO, KEAW, KAWA, and SHRI) are small; however, sample sizes represent between 20 and 100% of birds occupying individual wetlands. Due to small sample size and close geographic proximity, samples from Pouhala and Hono`uli`uli wetlands were pooled into a regional population, Pearl Harbor (PEHA). We collected 3–6 body feathers from post-fledging Hawaiian gallinules and stored feather samples in plastic bags at room temperature. We extracted DNA using DNeasy Blood and Tissue Kits (QIAGEN; Valencia, CA).

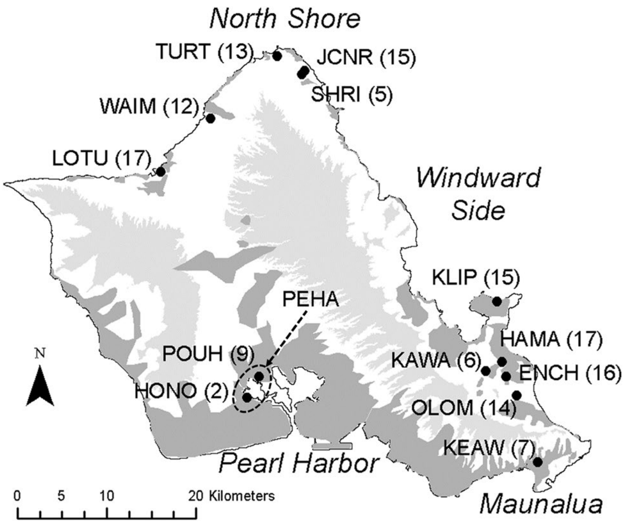

Fig. 1 Locations of sampled wetlands on O`ahu; four-letter codes are defined in Table 1. Note that PEHA (Pearl Harbor) represents the combination of two adjacent wetlands with smaller sample size, POUH (Pouhala Marsh) and HONO (Pearl Harbor National Wildlife Refuge, Honouliuli Unit). Light gray regions indicate mountain ranges (elevation > 300 m) and darker gray represents developed and urban areas. Parenthetic values indicate the number of birds sampled at each site

Laboratory techniques

We genotyped individuals at 12 autosomal microsatellite loci: Fal08, Fal10, Fal12, Fal14, Fal 17, Fal19, Gch06, Gch12, Gch13, Gch17, Gch19 (Sonsthagen et al. 2014), and KiRa9 (Brackett et al. 2013). The forward primer for locus KiRa9 was modified for this study (KiRa9.1F: 5′-GCGAGACTTGAAGTAGTGG-3′). We amplified microsatellites using polymerase chain reaction (PCR), and electrophoresed with fluorescent-tailed primers following the protocols described in Sonsthagen et al. (2004). We also sequenced 520 base pairs (bp) of the NADH dehydrogenase 2 (ND2) region of mitochondrial DNA (mtDNA) using primer pair ND2_224L (5′-CTMCTACTATTCTCCAGCAC-3′) and ND2_720H (5′-GCCTGCTAGGGAKAG-3′), following Sonsthagen et al. (2007) for PCR amplifications, cycle-sequencing protocols, and post-sequence processing. Lastly, 10% of samples were randomly selected, reamplified, and genotyped in duplicate for quality control. No inconsistencies in genotype scores were observed

Table 1 Code names and regions of sampled wetlands on O`ahu that are depicted in Fig. 1, listed in clockwise order starting at the western side of the island

Code	Region	Wetland
LOTU	North Shore	Private lotus farm, Hale`iwa
WAIM	North Shore	Waimea Valley Park
TURT	North Shore	Arnold Palmer Golf Course at Turtle Bay Resorts
SHRI	North Shore	Shrimp farms leased from USFWS, Kahuku
JCNR	North Shore	James Campbell National Wildlife Refuge-Ki`i unit
JCSF	North Shore	Combination of SHRI and JCNR
KLIP	Windward	Klipper Golf Course at Marine Corps Base Hawaii
KAWA	Windward	Kawainui Marsh State Wildlife sanctuary
HAMA	Windward	Hamakua Marsh State Wildlife sanctuary
ENCH	Windward	Ka`elepulu wetland and Kuilima Estates at Enchanted Lakes
OLOM	Windward	Olomana Golf Links
KEAW	Maunalua	Keawawa Wetland and Hawea Heiau, Hawaii Kai
POUH	Pearl Harbor	Pouhala Marsh State Wildlife sanctuary
HONO	Pearl Harbor	Pearl Harbor National Wildlife Refuge-Hono`uli`uli unit
PEHA	Pearl Harbor	Pearl Harbor region (combination of HONO and POUH)

between replicates. Sequences are accessioned in GenBank (MF673902–MF673904).

Analysis of genetic diversity

We calculated allelic richness (AR), inbreeding coefficient (F_{IS}), observed and expected heterozygosity (H_O and H_E, respectively) and tested for Hardy–Weinberg equilibrium and linkage disequilibrium among microsatellite loci in FSTAT version 2.9.3.2 (Goudet 1995). We estimated haplotype (h) and nucleotide (π) diversity of the ND2 region of mtDNA using Arlequin 3.1 (Schneider et al. 2000). We tested for selective neutrality for sequence data from the mtDNA ND2 region using Fu's F_S (Fu 1997) and Tajima's D (Tajima 1989) in Arlequin. We constructed an unrooted haplotype network for mtDNA ND2 using the reduced median method (Bandelt et al. 1995) in NETWORK 5.0.0.0 (Fluxus Technology Ltd.).

Because we collected samples from both adult and hatch-year individuals, we used Queller and Goodnight's (1989) index of relatedness (r_{xy}) to calculate levels of familial relationship between pairs of individuals within wetlands using IDENTIX 1.1 (Belkhir et al. 2002). Relatedness values of <0.5 represent first order (i.e. full-sibling, parent-offspring) relationships, 0.25 for second order (i.e. half-sibling) relationships, and 0 for unrelated individuals.

Estimation of genetic structure

We calculated estimates of inter-population variance in allelic and haplotypic frequencies (F_{ST}) in Arlequin; P-values were adjusted for multiple comparisons using the B-Y method (Benjamini and Yekutieli 2001; Narum 2006) with a starting α of 0.05. We used RecodeData version 1.0 (Meirmans 2006) to calculate the maximum possible F_{ST} values for the sampled suite of microsatellite loci. Hierarchical analysis of molecular variance (AMOVA) was performed using Arlequin to determine the magnitude of spatial variance in haplotypic and allelic frequencies among wetland regions on the island. Because the Pearl Harbor and Maunalua regions were both represented by only a single wetland (Pearl Harbor made up of the combined wetland PEHA and Maunalua by KEAW), and groups must be represented by more than one population to avoid violating assumptions of the AMOVA analysis, we made comparisons only between the Windward side and North Shore regions. The Pearl Harbor region could not be considered a group based on the original populations HONO and POUH because of the small sample sizes of these sites. We tested for isolation by distance by comparing a matrix of Euclidean geographic distances and genetic distances (F_{ST}) between all wetland pairs using a Mantel test implemented in the isolation By distance web service (Jensen et al. 2005).

Finally we used STRUCTURE 2.3.2.1 (Pritchard et al. 2000; Hubisz et al. 2009) to infer the occurrence of population structure among sampled individuals without using prior knowledge of sample collection locations based on our genotypic microsatellite data. STRUCTURE was conducted using an admixture model assuming correlated frequencies and with sample group information as a prior, with the possible number of populations (K) ranging from 1 to 11 (search strategy; burn-in of 50,000 iterations followed by 500,000 Markov chain Monte Carlo iterations); the analysis was repeated ten times. We followed the method of Evanno et al. (2005) and maximum likelihood (Pritchard et al. 2000) to determine the most likely number of clusters (K) given the data. The results were summarized in STRUCTURE HARVESTER Web v0.6.94 (Earl and von Holdt 2012).

Estimation of gene flow

We estimated gene flow between wetlands in MIGRATE v3.6.11 (Beerli and Felsenstein 1999, 2001) based on the 12 microsatellite loci. We ran MIGRATE with a full gene flow model, θ (4 Neμ or Nfμ, composite measure of effective population size and mutation rate), and all pairwise gene flow parameters (M) 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. We estimated gene flow using a maximum likelihood search parameter; ten short chains (5000 trees used out of 1,000,000 sampled), five long chains (15,000 trees used out 3,000,000 sampled), five static chains (start temperatures: 1, 1.5, 3, 6, and 12; swapping interval = 1) with a 5M burn-in per chain. Models were run three 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 χ^2 distribution with the degrees of freedom equal to the difference in the number of parameters estimated in the two models (Beerli and Felsenstein 2001).

We aggregated several wetlands that were adjacent and shared low genetic structure to reduce the number of estimated model parameters. JCNR and SHRI were pooled into JCSF (James Campbell-Shrimp Farms), and HAMA, OLOM, KAWA, and ENCH were combined into WIND (Windward side), resulting in a total of eight unique wetland patches in our gene flow analysis.

Results

Genetic diversity

We obtained multi-locus genotypes for 152 Hawaiian gallinules at 13 wetland sites on O`ahu (sample distribution shown in Fig. 1). Each individual had a unique multi-locus genotype. Four hatch-year birds had pairwise r_{xy} values > 0.5 with adults sampled from the same wetland (HAMA n = 2, KEAW n = 1, WAIM n = 1), suggestive of a parent-offspring relationship. The four hatch-year birds were omitted from subsequent analysis; our final data set comprised 148 individuals (Table 2). Number of alleles per microsatellite locus ranged from 1 to 4, with a mean of 2.2 and standard deviation of 0.5 alleles per locus. The average number of alleles per locus was similar across sampled wetlands with values ranging from 2.1 to 2.3 (Table 2). Within wetlands, observed heterozygosity ranged from 33.9 to 52.0% (Table 2). All loci and populations conformed to the assumptions of Hardy–Weinberg equilibrium, and all loci were in linkage equilibrium.

We observed three unique haplotypes at the ND2 region of the mtDNA (n = 148) characterized by two variable sites (Fig. 2). Number of ND2 haplotypes observed per wetland ranged from 1 to 3. Individuals from KLIP and KEAW were represented by a single haplotype (1), while all other wetlands were represented by at least two haplotypes. In wetlands with more than one ND2 haplotype observed, moderate levels of haplotype (h = 0.516–0.726) and nucleotide (π = 0.0026–0.0043) diversity were detected (Table 2).

Genetic structure

Moderate to high genetic structure was detected based on the 12 microsatellite loci (F_{ST} = 0.098, p < 0.001, Table 3) and mtDNA (F_{ST} = 0.248, p < 0.001, Table 3). The upper limit of F_{ST} for our microsatellite data set is 0.584; therefore the F_{ST} of 0.098 accounts for 16.8% of the maximum possible level of genetic structure. KLIP and KEAW were differiented from most wetlands with the highest number of significant comparisons, followed by LOTU, HAMA, and TURT based on microsatellite data and WAIM and LOTU based on mtDNA variance. The regional analysis uncovered within-population structure (microsatellites F_{ST} = 0.096, p < 0.001; mtDNA F_{ST} = 0.248, p < 0.001); however, region (North Shore vs. Windward side) did not explain a significant portion the variance at either marker type (microsatellites F_{CT} = 0.007, p = 0.301; mtDNA F_{CT} = 0.112, p = 0.086). We observed no evidence of isolation by distance among wetlands based on microsatellite data or mtDNA (Mantel test, microsatellites: r = 0.107, p = 0.131; mtDNA: r = − 0.134, p = 0.926).

STRUCTURE uncovered genetic partitioning of Hawaiian gallinules on O`ahu; two clusters were identified based on Evanno et al. (2005) method (ΔK = 53.3; LnPr|K = − 2417.6, Fig. 3a) and four clusters were identified based on the maximum likelihood given the data (ΔK = 2.0, LnPr|K = − 2374.3, Fig. 3b). Based on Evanno's method, individuals from KLIP had high membership coefficient to group 1 (white), and individuals from WAIM had moderate coefficients (Fig. 3a). The individuals representing the remaining wetlands clustered into group 2 (black). Based on Pritchard et al. (2000) method, individuals from KEAW (group 2; light gray) and KLIP (group 1; white) formed wetland specific clusters (Fig. 3b). Individuals from KAWA, HAMA, ENCH, OLOM, and PEHA clustered together into group 3 (dark gray). Individuals from TURT and half of the LOTU individuals clustered into group 4 (black). Individuals from WAIM, SHRI, JCNR, and KAWA, had intermediate membership coefficients. The four-population model detected one known dispersal event (ENCH87856);

Table 2 Estimates of genetic diversity of Hawaiian gallinule on O`ahu, Hawai`i, including; average number of alleles, allelic richness (AR), observed and expected heterozygosity (H_o, H_e, respectively), inbreeding coefficient (F_{IS}), and sample size calculated from 12 microsatellite loci, as well as, number of haplotypes (no. hapl.), haplotype diversity (*h*), and nucleotide diversity (π), Fu's Fs, and Tajima's D calculated from 295 bp of mtDNA ND2

	LOTU	WAIM	TURT	SHRI	JCNR	KLIP	KAWA	HAMA	ENCH	OLOM	KEAW	PEHA
Microsatellites												
No. Alleles	2.3 (0.5)	2.3 (0.5)	2.3 (0.5)	2.2 (0.7)	2.3 (0.5)	2.0 (0.4)	2.3 (0.5)	2.1 (0.3)	2.3 (0.5)	2.3 (0.7)	2.2 (0.4)	2.3 (0.5)
AR	2.1 (0.4)	2.1 (0.3)	2.2 (0.5)	2.2 0.7)	2.2 0.4)	1.8 (0.3)	2.2 (0.4)	2.0 (0.3)	2.1 (0.4)	2.1 (0.4)	2.1 (0.4)	2.1 (0.3)
H_e (%)	43.3 (4.1)	45.2 (3.4)	42.9 (5.5)	42.6 (6.5)	45.3 (3.3)	33.5 (4.8)	45.1 (4.0)	39.0 (4.9)	41.5 (4.7)	43.9	38.1	40.7
H_o (%)	42.6 (3.5)	52.1 (4.2)	34.0 (3.8)	50.0 (6.5)	43.9 (3.7)	40.0 (3.7)	42.8 (5.9)	35.3 (3.4)	44.1 (3.5)	41.1 (3.8)	39.7 (5.8)	45.9 (4.4)
F_{IS}	0.015	−0.160	0.215	−0.200	0.032	−0.204	0.057	0.098	−0.066	0.067	−0.048	−0.137
n	17	12	13	5	15	15	6	17	16	14	7	11
mtDNA												
No. Hapl	2	2	2	2	3	1	2	3	3	3	1	2
h	0.458 (0.090)	0.167 (0.130)	0.539 (0.060)	0.600 (0.180)	0.676 (0.070)	0.000 (0.000)	0.600 (0.180)	0.662 (0.070)	0.575 (0.080)	0.582 (0.090)	0.000 (0.000)	0.533
π	0.001 (0.001)	0.000 (0.000)	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.000 (0.000)	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.000 (0.000)	0.001 (0.001)
Fu's Fs	2.5	−0.5	1.5	0.6	0.6	–	0.6	0.5	0.1	0.0	–	1.0
Tajima's D	1.3	−1.1	1.2	1.2	1.1	0.0	1.2	0.9	0.2	0.0	0.0	1.3
n	17	12	13	5	15	15	6	17	16	14	7	11

Standard deviations are in parentheses

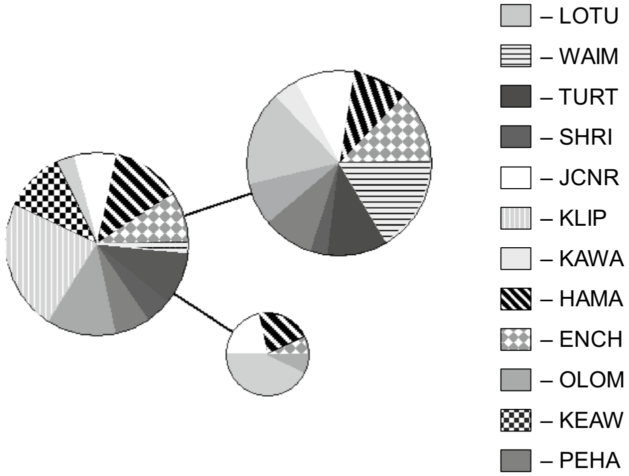

Fig. 2 Network diagram illustrating relationships of mtDNA haplotypes among sampled wetlands of Hawaiian gallinules on O`ahu. The size of the circle corresponds to the frequency of each haplotype, and connecting lines represent a change in a single nucleotide. Each sampled wetland has a unique shading pattern

that individual hatched in KEAW and dispersed to ENCH; this individual's assignment profile more closely resembles those of birds from KEAW than ENCH. The sample group information was informative (r < 1.0) for both models. The average proportion of cluster membership across all individuals within each wetland are shown over their respective sampling location in Fig. 4.

Gene flow

Asymmetrical gene flow was detected based on the microsatellite data; the full model (all parameters allowed to vary independently) had significantly higher likelihoods than did the restricted model (symmetric interpopulation M and θ) indicating asymmetric gene flow among wetlands [LnL(full) = − 4436, LnL(test) = − 4789, p < 0.001]. Gene flow (Nem) maximum-likelihood estimates and 95% confidence intervals are provided in Table 4, and gene flow between populations is displayed schematically in Fig. 5. We observed asymmetrical gene flow (as indicated by non-overlapping 95% confidence intervals) between 17 of 66 pairwise wetland combinations. Overall, most gene flow occurred from other wetlands into JCSF and WIND, with JCSF receiving the highest magnitude of asymmetrical gene flow from the greatest number of wetlands. Highly asymmetrical gene flow was detected from KLIP outward to almost every other wetland, and limited dispersal into KLIP. PEHA and TURT both had moderate levels of dispersal outward, with gene flow into both wetlands only from KLIP. It is important to note that gene flow estimates for KEAW are based on few individuals (n < 10); though sampling represents all birds occupying the wetland.

Table 3 Pairwise F_{ST} values for all sampled wetlands, separated by region

	North Shore					Windward side					Maunalua	Pearl H.
	LOTU (17)	WAIM (12)	TURT (13)	SHRI (5)	JCNR (15)	KLIP (15)	KAWA (6)	HAMA (17)	ENCH (16)	OLOM (14)	KEAW (7)	PEHA (11)
LOTU	–	**0.099**	**0.066**	0.090	**0.116**	**0.205**	0.017	**0.101**	**0.068**	0.049	**0.236**	**0.060**
WAIM	0.139	–	**0.077**	0.071	0.035	**0.090**	0.005	**0.066**	0.037	0.052	**0.139**	0.075
TURT	**0.211**	0.240	–	**0.080**	**0.113**	**0.233**	0.037	**0.169**	**0.110**	**0.092**	**0.252**	**0.105**
SHRI	**0.297**	0.446	−0.116	–	0.012	**0.261**	0.044	**0.102**	0.033	0.068	0.142	0.022
JCNR	0.081	**0.208**	−0.022	−0.048	–	**0.209**	0.039	0.040	0.024	0.036	**0.101**	**0.051**
KLIP	**0.765**	**0.919**	**0.523**	0.516	**0.493**	–	**0.135**	**0.232**	**0.180**	**0.143**	**0.266**	**0.213**
KAWA	0.123	0.176	−0.152	−0.154	−0.094	**0.720**	–	0.032	0.004	−0.003	**0.119**	0.032
HAMA	**0.201**	**0.320**	−0.014	−0.100	−0.040	**0.357**	−0.058	–	0.035	0.044	**0.159**	**0.070**
ENCH	0.130	0.162	−0.063	−0.066	−0.042	**0.531**	−0.144	−0.007	–	−0.004	**0.142**	−0.008
OLOM	**0.292**	**0.387**	−0.030	−0.148	−0.002	0.331	0.059	−0.051	0.003	–	**0.153**	0.006
KEAW	**0.697**	**0.885**	0.411	0.331	**0.389**	0.000	0.571	**0.260**	**0.433**	0.225	–	**0.215**
PEHA	0.161	0.174	−0.089	−0.084	−0.034	**0.621**	−0.176	0.000	−0.082	−0.003	**0.501**	–

Microsatellite data are above the diagonal, and mitochondrial ND2 region data below. Values in bold text are statistically significant (P values for comparisons based on microsatellite data were corrected for multiple comparisons using the B-Y method; α = 0.05, P = 0.0009). Sample sizes for each wetland are shown in parentheses

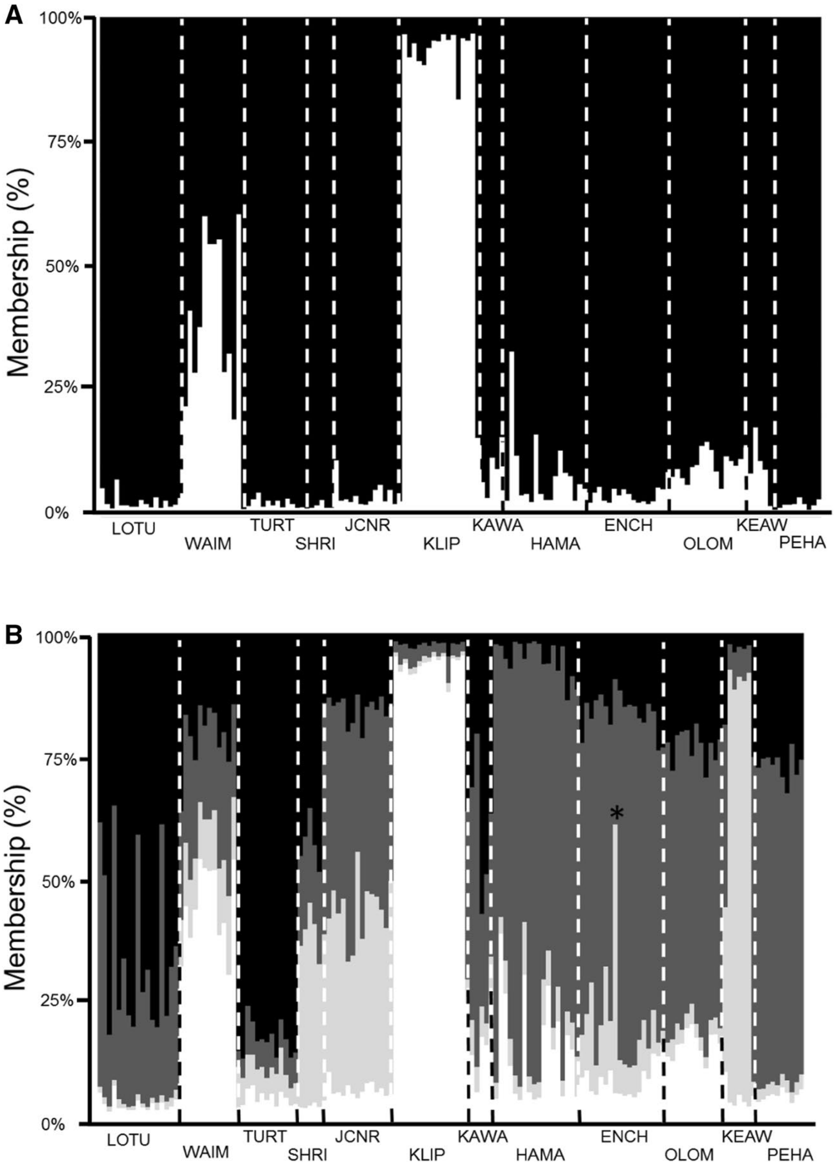

Fig. 3 Average membership coefficient of genotyped Hawaiian gallinules that were sampled at 12 wetlands on O`ahu, Hawaii; **a** shows assigned membership among two clusters (the most likely number according to the Evanno method), and **b** shows membership among four clusters (the most likely number according to the maximum-likelihood method) inferred with data from 12 microsatellite loci in STRUCTURE. Dashed lines separate individuals from different wetlands. Asterisk denotes an individual banded at KEAW (Keawawa wetland, Maunalua region) that was later resighted and captured at ENCH (Enchanted Lakes, Windward side)

Discussion

Hawaiian gallinules on O`ahu exhibit moderate to high genetic structure (sensu Wright 1978) among small and geographically close wetlands surrounded by a diverse and increasingly urbanized landscape matrix. These results are particularly noteworthy considering the small distances by which wetland habitats on the island are separated (1.5–55 km) coupled with moderate to high levels of genetic structure observed at two marker types (pairwise msat $F_{ST} \leq 0.261$; pairwise mtDNA $F_{ST} \leq 0.919$; Table 3). Genetic drift had limited variation upon which to act in this population, ($AR \leq 2.2$; Table 2), so dispersal among remnant patches was likely markedly reduced in order to promote the levels of genetic partitioning observed. Hawaiian gallinules experienced a severe and recent population bottleneck (Engilis and Pratt 1993) and are relatively recent colonizers of Hawai`i (James 1987; Fleischer and McIntosh 2001). We thus hypothesize that the population bottleneck and subsequent isolation of some habitat patches throughout population recovery promoted the rapid development of genetic structure among wetlands through the process of genetic drift.

The magnitude of genetic differentiation observed among wetlands in this study is higher than that observed for highly vagile bird species (e.g., Swainson's warbler *Limnothlypis swainsonii*, microsatellite $F_{ST} = 0.023–0.049$, Winker et al. 2000), and more closely resembles values for other wetland specialist birds in habitat fragmented by urbanization (e.g. white-fronted chats *Epthianura albifrons*, microsatellite $F_{ST} = 0.035–0.183$, Major et al. 2014), highly sedentary tropical birds (e.g. bicolored antbirds *Gymnopythis bicolor*,

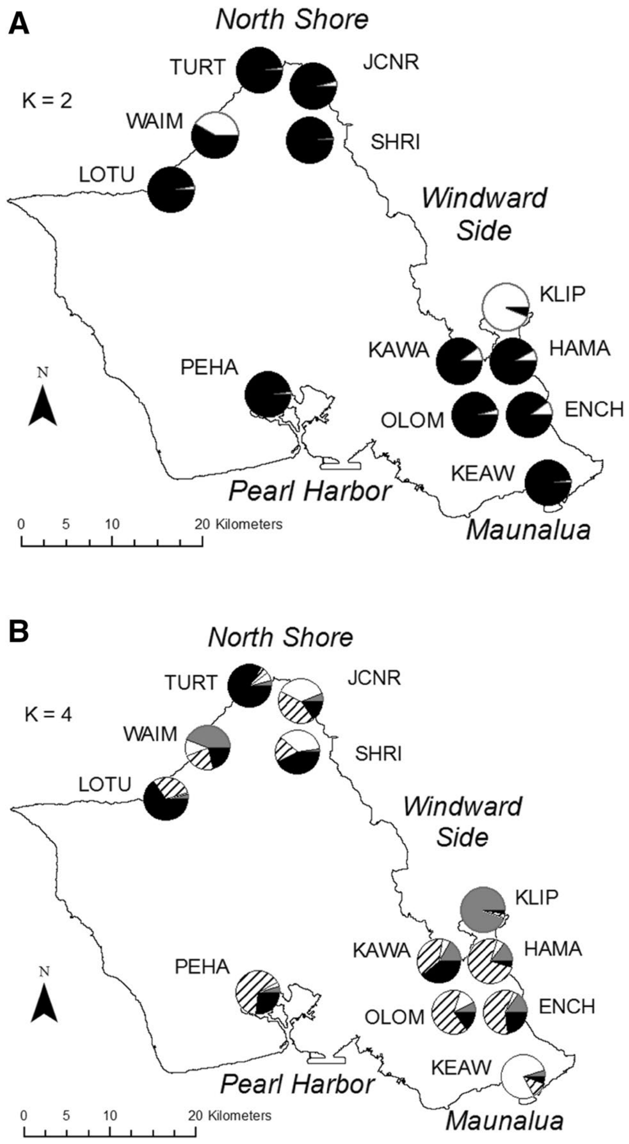

Fig. 4 Within-wetland average membership coefficients of genotyped Hawaiian gallinules sampled at 12 sites on O`ahu, Hawai`i, USA. Image **a** shows assigned membership among two clusters (the most likely number according to the Evanno method), and **b** shows membership among four clusters (the most likely number according to the maximum-likelihood method) based on data from 12 microsatellite loci analyzed in STRUCTURE

microsatellite $F_{ST}=0.012–0.278$, Brown et al. 2004), and other rail species (Girard et al. 2010; Miller et al. 2012, 2015). Yellow rails, a migratory species, exhibit moderate genetic structure (microsatellite $F_{ST}=0.083–0.113$) between an isolated population in Oregon, with the rest of the breeding population distributed over much of Eastern North America; no genetic structure was detected within the contiguous distribution (Miller et al. 2012). High genetic structuring (microsatellite $F_{ST}=0.19–0.63$; distance 100–850 km) was found among nonmigratory Californian populations of black rail (*Laterallus jamaicensis coturniculus;* Girard et al. 2010). Similarly, the closely related Marianas common moorhen (*Gallinula chloropus guami*) is also highly structured, with interisland estimates (F_{ST}) of 0.152 for microsatellites and 0.390 for the ND2 region of mitochondrial DNA (distance ~200 km), between Saipan and Guam (Miller et al. 2015). Unfortunately no studies of within-island structure for Marianas moorhen or between-island structure for Hawaiian gallinules have been conducted for comparison.

Although inter-population estimates of genetic structure observed here are similar across a range of avian taxa, assessments described above were conducted at a much larger geographic scale relative to our study (distances 100–10,000 km vs 1.5–55 km, respectively). Indeed, the spatial scale of our study is smaller than most reports of notable avian population differentiation (e.g., Nicholls et al. 2006; Roberts et al. 2011; Nogueira et al. 2014; Vidal et al. 2015; Greenberg et al. 2016; Levy et al. 2016), even for sedentary tropical species (Brown et al. 2004; Abalaka et al. 2015). Genetic structure observed in our study is similar in magnitude and spatial scale to that observed by Delaney et al. (2010) in what was described as the highest amount of genetic structure observed over small distances in avian taxa. The small spatial scale at which genetic structure is observed within Hawaiian gallinule of O`ahu highlights two important drivers of genetic structure: low dispersal propensity and the influence of genetic drift as a result of a severe bottleneck event. We hypothesize that the alteration of O`ahu's landscape and the reduction of habitat for waterbirds (Griffin et al. 1989; van Rees and Reed 2014) has likely played a role in the creation of a mosaic of spatially isolated wetlands enabling genetic drift to quickly act and promote genetic structure among populations in close geographic proximity.

Landscape and behavioral ecologists have challenged the notion that inter-patch Euclidean distances alone are an effective measure of potential population isolation or connectivity, suggesting instead that the type of landscape cover making up that distance (i.e., the landscape matrix) plays a larger role in many terrestrial systems (Ricketts 2001; Rodewald 2003; Revilla and Wiegand 2008; Aben et al. 2012). We observed a small number of populations (in particular KLIP, KEAW, WAIM, and TURT) that show strong and consistent structure that is not correlated with physical distance. The patterns of genetic structure we observed may be explained, at least in part, by the influence of the physical structure of the landscape matrix on Hawaiian gallinule dispersal among wetlands (i.e., functional connectivity; Taylor et al. 1993; Baguette and Van Dyck 2007), the history of O`ahu's Hawaiian gallinule population, and source-sink dynamics among persisting populations (Pulliam 1988).

The severity of the population decline experienced by Hawaiian gallinule populations in the late 1800s to

Table 4 Gene flow estimated among Hawaiian gallinules sampled on eight wetlands in O`ahu, based on 12 microsatelite loci

Destination	Origin								
	LOTU	WAIM	TURT	JCSF	KLIP	WIND	KEAW	PEHA	Total immigration
LOTU	–	2.9 (2.0–3.8)	**5.2 (3.8–6.5)**	1.7 (1.1–2.4)	3.1 (2.1-4.0)	1.8 (1.2–2.5)	2.6 (1.7–3.4)	1.3 (0.8–1.9)	18.7
WAIM	1.6 (1.1–2.2)	–	1.0 (0.7–1.5)	1.6 (1.2–2.2)	**5.1 (4.0–6.4)**	1.0 (0.4–1.5)	1.8 (1.3–2.5)	**1.8 (1.3–2.5)**	12.3
TURT	2.0 (1.5–2.6)	1.2 (0.8–1.7)	–	1.2 (0.8–1.6)	**2.9 (2.3–3.8)**	0.6 (0.4–1.0)	3.0 (2.3–3.8)	4.3 (3.5–5.4)	13.2
JCSF	**4.2 (2.9–6.0)**	**5.5 (4.0–7.6)**	**10.3 (7.8–13.6)**	–	**5.9 (4.3–8.2)**	2.7 (1.9–3.7)	**9.6 (7.2–12.8)**	**4.2 (2.9–6.0)**	38.2
KLIP	2.2 (1.8–2.8)	2.5 (2.0-3.1)	1.6 (1.2-2.0)	1.3 (1.0–1.7)	–	0.4 (0.3–0.6)	0.6 (0.4–0.9)	1.3 (1.0– 1.7)	7.8
WIND	**4.4 (2.7–5.8)**	2.0 (1.4–3.7)	**2.1 (1.4–3.0)**	2.3 (1.7–3.2)	**2.6 (1.9–3.6)**	–	2.0 (1.4–2.8)	**3.5 (2.5–4.7)**	14.5
KEAW	1.9 (1.0–2.8)	2.1 (1.4–2.9)	2.0 (1.4–2.9)	2.2 (1.5–3.1)	**4.8 (3.1–6.3)**	2.2 (1.6–3.2)	–	**2.9 (2.1–4.0)**	16.1
PEHA	2.1 (1.6–3.0)	0.7 (0.5–1.2)	2.6 (2.0-3.7)	1.2 (0.8–1.8)	**2.4 (1.8–3.4)**	0.9 (0.6–1.4)	1.1 (0.8–1.6)	–	8.8
Total emigration	18.4	16.9	24.8	11.4	26.7	9.6	20.7	19.4	–

Effective number of migrants per generation (N_em) and 95% confidence intervals are listed for each population pair, where the columns are the wetland of origin and the rows are the wetland destination. Comparisons in bold text indicate the dominant direction of asymmetrical gene flow between wetland pairs and values in gray text represent comparisons with overlapping 95% confidence intervals. Total immigration for each wetland is shown in the right-most column, and emigration in the bottom row. Total immigration and emigration rates were calculated by totaling mean gene flow values to and from each individual wetland

mid-1900s (gallinules reduced to ~60 individuals across the state of Hawai`i) and subsequent recovery (Shallenberger 1977; Reed et al. 2011), likely erased signatures of genetic structure prior to the decline. Genetic drift acting on remnant populations during the bottleneck and subsequent recolonization likely played a large role in current patterns of genetic structure observed. Hawaiian gallinules on O`ahu likely survived the population bottleneck in a few (2–3) isolated strongholds (as indicated by high pairwise F_{ST} estimates) and subsequently colonized unoccupied or newly available habitats (created through restoration or active management) as numbers increased. Wetland size and quality differ widely between contemporary habitats, and may have influenced dispersal rates and population connectivity via habitat effects and source-sink dynamics (Pulliam 1988; Pfluger and Balkenhol 2014). JCNR and HAMA, two wetlands that currently support larger populations of Hawaiian gallinules, are weakly differentiated from most other populations on the island, and are known to have supported few if any birds prior to recovery (Shallenberger 1977). Limited genetic structure coupled with larger census sizes suggests that these now presumably high-quality habitats received a high number of immigrants from other wetland populations during the recovery period (Fig. 5). Spatially isolated wetlands (e.g. KEAW, a wetland located in an urban development surrounded by mountains, and KLIP, located on a fenced military base) exhibited asymmetrical gene flow to most other wetlands on the island, notably those in the WIND and JCSF regions. Prior to the decline, these wetlands (KEAW, KLIP, and TURT) may have been strongholds for Hawaiian gallinules as they are located on restricted military and private properties, and potentially experienced reduced persecution from hunting, which was legal until

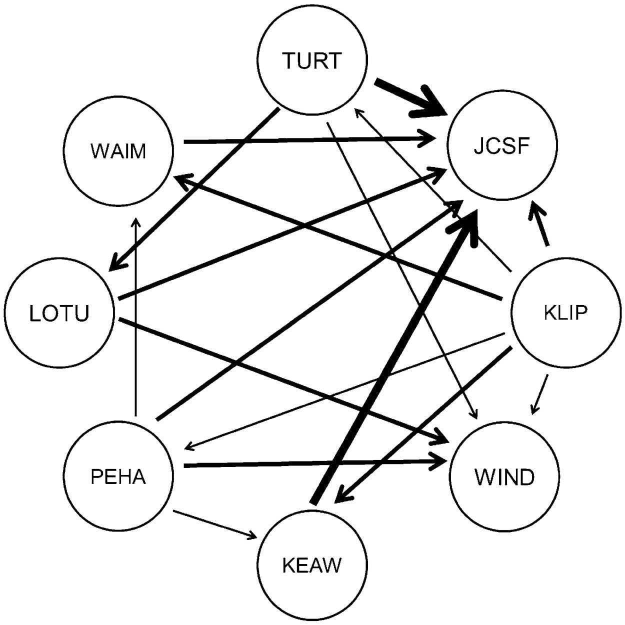

Fig. 5 Diagram showing direction of prevailing gene flow between wetland sites where Hawaiian gallinules were sampled on O`ahu, Hawai`i, USA. Gene flow estimates with non-overlapping 95% confidence intervals between wetland pairs are shown. Arrow thickness represents the magnitude of gene flow (N_em): small arrows 1.0–2.4, medium arrows, 2.5–5.0, and large arrows > 5.0

1939 (Shallenberger 1977). Wetland habitats in WIND and JCSF supported few Hawaiian gallinules prior to the 1970s and 1980s (Shallenberger 1977; Banko 1987; Silbernagle, USFWS, pers. comm.), after which significant habitat management and predator control efforts began. Once management was enacted and protected habitat became available, WIND and JCSF wetlands apparently received immigrants from throughout the island (see Fig. 5), as large populations developed quickly where birds had not been observed for some time (Shallenberger 1977; Banko 1987).

Hawaiian gallinules suffer mortality near roads (K. Doyle, Hawai`i State Department of Fisheries and Wildlife, pers. comm.), are typically shy of humans, and are rarely seen flying > 2 m off the ground. These factors suggest mechanisms by which anthropogenic landscape change could limit dispersal success through psychological, mortality-based, and physical barriers (Zeller et al. 2012). Population structure in other wetland birds (e.g. white-fronted chat, Major et al. 2014) is driven in part by urbanization around remaining habitats, regardless of the level of protection within these habitats; it may be that observed structure in Hawaiian gallinules follows similar patterns. Though the low connectivity of urban wetlands like KEAW and KLIP to nearby wetlands supports this hypothesis, observed structure between TURT and two adjacent wetlands (WAIM and JCNR) might be a notable exception, given that O`ahu's North shore is among the island's least-developed regions and that these wetlands are embedded in a largely rural matrix. However, there are other possible barriers to dispersal that might apply within O`ahu's North shore, such as fence lines and dense scrub-forest. Linear features like roads can increase mortality and act as barriers to movement in many taxa (Andrews 1990; Vanak et al. 2010), including cursorial birds occupying open habitats (e.g. *Tympanuchus pallidicinctus*, Wolfe et al. 2007). In contrast, connectivity between spatially distant populations can be increased by corridors, or other landscape features that encourage or facilitate movement (Baum et al. 2004; Tewksbury et al. 2002). In the case of Hawaiian gallinules, birds use streams as breeding and foraging habitat (Banko 1987), and it is suspected that they may disperse more readily along stream margins and drainage canals (M. Silbernagle, USFWS, pers. comm). Finally, steep topography and mountain ranges may physically or psychologically (e.g. behavioral inhibition; sensu Harris and Reed 2002) impede dispersal. The Ko`olau mountain range, for example, separates the windward wetlands from PEHA, KEAW, and wetlands on the leeward side of the North shore (Fig. 1). These additional possible matrix effects on dispersal rates may also play a role in the observed structure among wetlands that are not located in urbanized areas. Though perhaps not of direct conservation concern, the influence of natural barriers on gene flow may be exaggerated by more recent modifications to the landscape. Given the diversity of potential landscape-relevant drivers of genetic structure in Hawaiian gallinules, formal analysis of patterns observed in this study using a landscape genetic framework (Manel et al. 2003; Storfer et al. 2010) should be considered an important next step in research on this threatened population.

Conservation implications

Our results illustrate the development of genetic structure in a taxon that is expanding its range after severe population decline, showing that biologically significant structuring can occur over small geographic distances. This raises an important, management relevant possibility—that populations recovering in altered landscapes may experience isolation or poor connectivity after recruitment. Given the relationship between fragmentation and extinction risk (Saunders et al. 1991; Fahrig 2003), this genetic structuring could be of conservation concern. For example, the recovery plan for the Great Lakes population of the Piping plover *Charadrius melodus* in the U.S. has as one of its delisting criteria the maintenance of genetic diversity sufficient for population persistence (USFWS 2003). If genetic structuring of this kind can be attributed to landscape characteristics, methods that increase connectivity in the context of altered landscapes (e.g. translocations, Wright et al. 2014; corridors, Tewksbury et al. 2002; stepping stone habitats; Saura et al. 2014) may be useful in ameliorating the potential demographic and genetic effects of fragmentation. The influx of gene flow we observed for James Campbell National Wildlife Refuge and Hamakua Marsh wildlife sanctuary suggests that restored and managed wetlands can be rapidly recolonized by Hawaiian gallinules. This suggests that wetland restoration and management may be effective tools for the management of this subspecies.

Acknowledgements Funding for this project was provided by the Tufts Institute of the Environment, Tufts Graduate School of Arts and Sciences, Nuttall Ornithological Club, U.S. Geological Survey (USGS) Alaska Science Center, U.S. Fish and Wildlife Service (USFWS), Wilson Ornithological Society, Sigma Xi Grants-in-Aid of Research, and Tufts Water Diplomacy IGERT (NSF 0966093). We are grateful to Kevin Sage, Meg Fowler, Sandra Talbot, Christy Haughey, and Lisa Pajot for lab management, logistics, and mentoring C. van Rees in laboratory techniques. We thank Marty Kawasaki, Annie Miller, Amanda Sandor, Alejandra Muñoz, and Matthew Pedrotti (Team Gallinule) and Aaron Nadig and Joy Browning (USFWS) for their invaluable work in the field. We also thank the Livable Hawai`i Kai Hui, Katie Doyle, Ati Jeffers-Fabro and Hawai`i DOFAW, Turtle Bay Resorts, Olomana Golf Links, the anonymous owners of a Lotus farm and of a Shrimp farm on Hawai`i's North Shore, Hugo Devries and Cindy Turner for their help with capture, banding, and survey logistics. We are grateful to the U.S. Marine Corps Base Hawai`i, especially Lance Bookless and Todd Russell for their permission and assistance sampling birds at the Klipper golf course. This research used resources of the Core Science Analytics and Synthesis Advanced Research Computing program at the USGS. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the USFWS.

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. We also thank John M. Pearce of the USGS Alaska Science Center and two anonymous reviewers for their insights and helpful suggestions on earlier drafts of this paper

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Abalaka J, Hudin NS, Ottosson U, Bloomer P, Hansson B (2015) Genetic diversification and population structure of the range restricted rock firefinch *Lagonosticta sanguinodorsalis*. Conserv Genet 16:411–418

Aben J, Adriaensen F, Thijs KW, Pellikka P, Siljander M, Lens L, Matthysen E (2012) Effects of matrix composition and configuration on forest bird movements in a fragmented Afromontane biodiversity hotspot. Anim Conserv 15:658–668

Andrews A (1990) Fragmentation of habitat by roads and utility corridors: a review. Austr Zool 26:130–141

Baguette M, Van Dyck H (2007) Landscape connectivity and animal behavior: functional grain as a key determinant for dispersal. Landsc Ecol 22:1117–1129

Bandelt HJ, Forster P, Sykes BC, Richards MB (1995) Mitochondrial portraits of human populations. Genetics 141:743–753

Banko WE (1987) CPSU/UH avian history report 12A: history of endemic Hawaiian birds. Part I. Population histories—species accounts, freshwater birds: Hawaiian gallinule alae-ula. Department of Botany, University of Hawai`i at Manoa, Honolulu, HI

Baum KA, Haynes KJ, Dillemuth FP, Cronin JT (2004) The matrix enhances the effectiveness of corridors and stepping stones. Ecology 85:2671–2676

Beerli P, Felsenstein J (1999) Maximum likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152:763–773

Beerli P, Felsenstein J (2001) Maximum Likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc Natl Acad Sci 98:4563–4568

Belkhir K, Castric V, Bonhomme F (2002) IDENTIX, a software to test for relatedness in a population using permutation methods. Mol Ecol Resour 2:611–614

Benjamini Y, Yekutieli D (2001) The Control of false discovery rate under dependency. Ann Stat 29:1165–1188

BirdLife International (2016) *Gallinula galeata*. The IUCN Red List of Threatened Species 2016: e.T62120280A95189182. doi:10.2305/IUCN.UK.2016-3.RLTS.T62120280A95189182.en

Brackett CL, Maley JM, Brumfield RT, McRae SB (2013) Characterization of microsatellite loci for a threatened species, the King Rail, *Rallus elegans*, using a next-generation sequencing protocol. Conserv Genet Resour 5:1189–1191

Broquet T, Petit EJ (2009) Molecular estimation of dispersal for ecology and population genetics. Ann Rev Ecol Evol Syst 40:193–216

Brown LM, Ramey II RR, Tamburini B, Gavin TA (2004) Population structure and mitochondrial DNA variation in sedentary Neotropical birds isolated by forest fragmentation. Conserv Genet 5:743–757

Byrd GV, Zeillemaker CF (1981) Ecology of nesting Hawaii common gallinules at Hanalei, Hawaii. Western Birds 12:105–116

Chen N, Cosgrove EJ, Bowman R, Fitzpatrick JW, Clark AG (2016) Genomic consequences of population decline in the endangered Florida scrub jay. Curr Biol 26:2974–2979

Crooks KR, Sanjayan M (2006) Connectivity Conservation. Conservation Biology Book series. Cambridge University Press, Cambridge

Delaney KS, Riley SPD, Fisher RN (2010) A rapid, strong, and convergent genetic response to urban habitat fragmentation in four divergent and widespread vertebrates. PLoS ONE 5(9):e12767

Doleman PM (2012) Mechanisms and processes underlying landscape structure effects on bird populations. In: Fuller RJ (ed) Birds and habitat relationships in changing landscapes. Cambridge University Press, Cambridge, pp 93–124

Earl DA, von Holdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Engilis A Jr, Pratt TK (1993) Status and population trends of Hawaii's native waterbirds 1977–1987. Wils Bull 105:142–158

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. Ann Rev Ecol Evol Syst 34:487–515

Fahrig L, Merriam G (1985) Habitat patch connectivity and population survival. Ecology 66:1762–1768

Fleischer RC, McIntosh CE (2001) Molecular systematics and biogeography of the Hawaiian avifauna. Stud Avian Biol 22:51–60

Fordham DA, Brook BW (2010) Why tropical island endemics are acutely susceptible to global change. Biodivers Conserv 19:329–342

Frankham R, Ballou JD, Briscoe DA (2009) Introduction to conservation Genetics, 2nd edn., Cambridge University Press, Cambridge

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selections. Genetics 147:915–925

Giambelluca TW (1986) Land-use effects on the water balance of a tropical island. Nat Geog Res 2:125–151

Gibbs JP, Reed JM (2007) Population and genetic linkages of vernal-pool associated amphibians. In: Calhoun AJK, deMaynadier PG (eds) Science and conservation of vernal pools in Northeastern North America, CRC Press, Boca Raton, pp 149–167

Girard P, Takekawa JY, Beissinger SR (2010) Uncloaking a cryptic, threatened rail with molecular markers: origins, connectivity and demography of a recently-discovered population. Conserv Genet 11:2409–2418

Goldberg CS, Waits LP (2010) Comparative landscape genetics of two pond-breeding amphibian species in a highly modified agricultural landscape. Mol Ecol 19:3650–3663

Goudet J (1995) Fstat (vers. 1.2): a computer program to calculate *F*-statistics. J Hered 86:485–486

Greenbaum G, Fefferman NH (2017) Application of network methods for understanding evolutionary dynamics in discrete habitats. Mol Ecol 26:2850–2863

Greenberg R, Cammen KM, Wilson AG, Olsen BJ, Ballentine B, McInerny NC, Fleischer RC (2016) Geographic population structure and subspecific boundaries in a tidal marsh sparrow. Conserv Genet 17:603–613

Griffin CR, Shallenberger RJ, Fefer SI (1989) Hawaii's endangered waterbirds: a resource management challenge. In: Sharitz RR, Gibbons JW (eds) Freshwater wetlands and wildlife. U.S. Department of Energy, Oak Ridge, pp 1165–1175

Hanski I (1999) Metapopulation ecology. Oxford University Press, Oxford

Hanski I, Gaggiotti OE (2004) Metapopulation biology: past, present, and future. In: Hanski I, Gaggiotti OE (eds) Ecology, genetics, and evolution of metapopulations. Elsevier, Amsterdam, pp 3–22

Harris RJ, Reed JM (2002) Behavioral barriers to non-migratory movements of birds. Ann Zool Fennici 39:275–290

Hawkes C (2009) Linking movement behavior, dispersal and population processes: is individual variation a key? J Anim Ecol 78:894–906

Hubisz MA, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Res 9:322–1332

James HF (1987) A late Pleistocene avifauna from the island of Oahu, Hawaiian Islands. Doc des Lab de Geol de Lyon 99:221–230

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. BMC Genet 6:13. http://ibdws.sdsu.edu/ Accessed 11 Mar 2005

Keller LF, Weller DM (2002) Inbreeding effects in wild populations. Tree 17:230–231

Klasner FL, Mikami CD (2003) Land use on the island of Oahu, Hawaii 1998. USGS water-resources investigations Report 02-4301. U.S. Geological Survey, Honolulu

Levy H, Clucas GV, Rogers AD, Leache AD, Ciborowski K, Polito MJ, Lynch HJ, Dunn MJ, Hart T (2016) Population structure and phylogeography of the Gentoo Penguin (*Pygoscelis papua*) across the Scotia Arc. Ecol Evol 6:1834–1853

Macdonald DW, Johnson DDP (2001) Dispersal in theory and practice: consequences for conservation biology. In: Clobert J, Danchin E, Dhondt AA, Nichols JD (eds) Dispersal Oxford University Press, New York, pp 358–372

Major RE, Johnson RN, King AG, Cooke GM, Sladek JLT (2014) Genetic isolation of endangered bird populations inhabiting salt marsh remnants surrounded by intensive urbanization. Anim Conserv 17:419–429

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trend Ecol Evol 18:189–197

Meirmans PG (2006) Using the AMOVA framework to estimate a standardized genetic differentiation measure. Evol Int J org Evol 60:2399–2402

Miller MP, Haig SM, Mullins TD, Popper KJ, Green M (2012) Evidence for population bottlenecks and subtle genetic structure in the Yellow Rail. Condor 114:100–112

Miller MP, Mullins TD, Haig SM, Takano L, Garcia K (2015) Genetic structure, diversity, and interisland dispersal in the endangered Mariana common Moorhen (*Gallinula chloropus guami*). Condor 117:660–669

Mills SL (2007) Conservation of wildlife populations: demography, genetics, and management. Blackwell, Malden, MA

Moreno-Mateos D, Barbier EB, Jones PC, Jones HP, Aronson J, Lopez-Lopez JA, McCrackin ML, Meli P, Montoya D, Rey Benayas JM (2017) Anthropogenic ecosystem disturbance and the recovery debt. Nat Commun 8:14163. doi:10.1038/ncomms14163

Munro GC (1960) Birds of Hawaii. Tuttle Company, Rutland

Murphy MA, Evans JS, Storfer A (2010) Quantifying *Bufo boreas* connectivity in Yellowstone national park with landscape genetics. Ecology 91:252–261

Narum SR (2006) Beyond Bonferroni: less conservative analyses for conservation genetics. Conserv Genet 7:783–787

Nicholls JA, Austin JJ, Moritz C, Goldizen AW (2006) Genetic population structure and call variation in a passerine bird, the satin bowerbird, *Ptilonorhynchus violaceus*. Evol Int J org Evol 60:1279–1290

NOAA Coastal Services Center (2014) 2011 Hawaii Oahu high Research Land Cover. Coastal Change Analysis Program. Charleston, South Carolina. http://coast.noaa.gov/digitalcoast/dataregistry/#/ Accessed 16 Jun 2014.

Nogueira DM, Pope LC, Burke T, Alves MAS (2014) Genetic differentiation over a short water barrier in the Brazilian tanager, *Ramphocelus bresilius* (Passeriformes: Thraupidae) an endemic species of the Atlantic forest, Brazil. Conserv Genet 5:1151–1162

Opdam P (1991) Metapopulation theory and habitat fragmentation: a review of holarctic breeding bird studies. Landsc Ecol 5:93–106

Pfluger FJ, Balkenhol N (2014) A plea for simultaneously considering matrix quality and local environmental conditions when analyzing landscape impacts on effective dispersal. Mol Ecol 23:2146–2156

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure from multilocus genotype data. Genetics 15:945–959

Pulliam HR (1988) Sources, sinks, and population regulation. Am Nat 132:652–661

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evol Int J org Evol 43:258–275

Reed JM, Elphick CS, Zuur AF, Ieno EN (2011) Long-term population trends of endangered Hawaiian waterbirds. Pop Ecol 53:473–481

Reed JM, DesRochers DW, Vanderwerf EA, Scott JM (2012) Conservation reliance and long-term persistence of Hawaii's endangered avifauna. Biosci 62:881–892

Revilla E, Wiegand T (2008) Individual movement behavior, matrix heterogeneity, and the dynamics of spatially structured populations. Proc Natl Acad Sci 105:19120–19125

Ricketts (2001) The matrix matters: effective isolation in fragmented landscapes. Am Nat 158:87–99

Ripley SD, Lansdowne JF, Olson SL (1977) Rails of the world: a monograph of the family rallidae. D.R. Godline, Boston

Roberts DG, Baker J, Perrin C (2011) Population genetic structure of the endangered Eastern Bristlebird, *Dasyornis brachypterus*; implications for conservation. Conserv Genet 12:1075–1085

Rodewald AD (2003) The importance of land uses within the landscape matrix. Wildl Soc Bull 31:586–592

Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. Conserv Biol 5:18–32

Saura S, Bodin O, Fortin M-J (2014) Stepping stones are crucial for species' long-distance dispersal and range expansion through habitat networks. J Appl Ecol 51:171–182

Schneider S, Roessli D, Excoffier K (2000) Arlequin version 2.0: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva

Schwartz CW, Schwartz ER (1949) The game birds in Hawaii. Hawaii Division of Fish and Game and Board of Commissioners of Agriculture and Forestry, Honolulu

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Lett 9:615–629

Shallenberger RJ (1977) An ornithological survey of Hawaiian wetlands. U.S. Army, Engineer District, Honolulu

Smith MA, Green DM (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography 28:110–128

Sonsthagen SA, Talbot SL, White CM (2004) Gene flow and genetic characterization of Northern Goshawks breeding in Utah. Condor 106:826–836

Sonsthagen SA, Talbot SL, McCracken KG (2007) Genetic characterization of common Eiders (*Somateria mollissima*) breeding on the Yukon–Kuskowim delta, Alaska. Condor 109:879–894

Sonsthagen SA, Wilson RE, Underwood JG (2014) Development and characterization of microsatellite markers for the Hawaiian coot, *Fulica alai* and Hawaiian gallinule, *gallinula chloropus sandvicensis*, through next-generation sequencing. Conserv Genet Resour 6:765–767

Storfer A, Murphy MA, Spear SF, Holderegger R, Waits LP (2010) Landscape genetics: where are we now? Mol Ecol 17:3496–3514

Tajima F (1989) The effect of change in population size on DNA polymorphism. Genetics 123:597–601

Taylor B, van Perlo B (1998) Rails: a guide to the rails, crakes, gallinules and coots of the world. Yale University press, New Haven

Taylor PD, Fahrig L, Henein K, Merriam G (1993) Connectivity is a vital element of landscape structure. Oikos 68:571–572

Tewksbury JJ, Levey DI, Haddad NM, Sargent S, Orrock JL, Weldon A, Danielson BJ, Brinkerhoff J, Damschen EI, Townsend P (2002) Corridors affect plants, animals, and their interactions in fragmented landscapes. Proc Natl Acad Sci 99:12923–12926

U.S. Fish and Wildlife Service (2003) Recovery plan for the great lakes piping plover (*Charadrius melodus*). Region 3, fort snelling. U.S. Fish and Wildlife Service, Minnesota

U.S. Fish and Wildlife Service (2011) Recovery plan for Hawaiian waterbirds, second revision. U.S. Fish and Wildlife Service, Portland, OR

Underwood JG, Silbernagle M, Nishimoto M, Uyehara K (2013) Managing conservation reliant species: Hawai`i's endangered endemic waterbirds. PLoS ONE 8:e67872. doi:10.1371/journal.pone.0067872

van Rees CB, Reed JM (2014) Wetland loss in Hawai`i since human settlement. Wetlands 34:335–350

Van Strien MJ, Keller D, Holderegger R, Ghazoul J, Kienast F, Bolliger J (2014) Landscape genetics as a tool for conservation planning: predicting the effects of landscape change on gene flow. Ecol Appl 24:327–339

Vanak AT, Thaker M, Slotow R (2010) Do fences create an edge-effect on the movement patterns of a highly mobile mega-herbivore? Biol Conserv 143:2631–2637

Vidal M, Hernández MA, Luis A, Dominguez J (2015) Lack of genetic structure in Kentish Plover *Charadrius alexandrinus* from the Iberian coast. What's wrong with the endangered north-western population? Bird Conserv Int 25:153–165

Winker K, Graves GR, Brau MJ (2000) Genetic differentiation among populations of a migratory songbird: *Limnothlypis swainsonii*. J Avian Biol 31:319–328

Wolfe DH, Patten MA, Shochat E, Pruett CL, Sherrod SK (2007) Causes and patterns of mortality in Lesser Prairie-chickens *Tympanuchus pallidicinctus* and implications for management. Wildl Biol 13(sp1):95–104

Wood DA, Bui T-VD, Overton CT, Vandergast AG, Casazza ML, Hull JM, Takekawa JY (2017) A century of landscape disturbance and urbanization of the San Francisco Bay region affects the present-day genetic diversity of the California Ridgway's rail (*Rallus obsoletus obsoletus*). Conserv Genet 18:131–146

Wright S (1978) Variability within and among natural populations. Evolution and the Genetics of populations, vol 4., University of Chicago Press, Chicago

Wright DJ, Spurgin LG, Collar NJ, Komdeur J, Burke T, Richardson DS (2014) The impact of translocations on neutral and functional genetic diversity within and among populations of the Seychelles warbler. Mol Ecol 23:2165–2177

Zeller KA, McGarigal K, Whitely AR (2012) Estimating landscape resistance to movement: a review. Landsc Ecol 27:777–797

Ziegler AC (2002) Hawaiian natural history, ecology, and evolution. University of Hawaii Press, Honolulu