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RESEARCH ARTICLE

Island life and isolation: The population genetics of Pacific Wrens on the North Pacific Rim

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ABSTRACT

Conservation of intraspecific variation is a growing focus of conservation biology. Island populations can make up a large portion of the variation of widespread species, as they are often isolated and exhibit differences in phenotype and genetic structure compared with mainland populations. We genotyped 169 Pacific Wrens (*Troglodytes pacificus*) from 9 locations and 6 subspecies in Alaska, USA, and British Columbia, Canada, to examine the population structure, genetic diversity, and likelihood of genetic rescue of island populations of conservation concern. We found that 25% of genetic variation was partitioned among conservation units delineated by subspecies, suggesting that the present framework of managing subspecies as separate units is warranted. Populations found farthest from possible mainland sources had the lowest genetic diversity. The Attu Island population, subspecies *T. p. meligerus*, had the lowest genetic diversity and highest genetic divergence of all sampled locations, a signal also found in Attu Island populations of Song Sparrow (*Melospiza melodia*) and Rock Ptarmigan (*Lagopus muta*). Currently, populations in western Alaska are unlikely to be connected to populations in mainland locations through dispersal, and thus are unlikely candidates for natural genetic or demographic rescue.

Keywords: *Troglodytes pacificus*, Aleutian Islands, conservation genetics, endemism

Vida y aislamiento en islas: Genética de poblaciones de *Troglodytes pacificus* en el borde del Pacífico Norte

RESUMEN

La conservación de la variación intraespecífica es un tema creciente en biología de la conservación. Las poblaciones insulares pueden representar una gran proporción de la variación de especies ampliamente extendidas, ya que usualmente están aisladas y muestran diferencias en fenotipo y estructura genética en comparación con las poblaciones continentales. Determinamos el genotipo de 169 individuos de *Troglodytes pacificus* provenientes de 9 localidades y 6 subspecies en Alaska y Columbia Británica para examinar la estructura poblacional, la diversidad genética y la probabilidad de rescate genético de poblaciones insulares de interés para la conservación. Encontramos que el 25% de la variación genética está particionada entre unidades de conservación delineadas por subespecie, sugiriendo que el marco actual de manejo de las subespecies como unidades separadas está garantizado. Las poblaciones que se encuentran más lejos de posibles fuentes continentales tienen la menor diversidad genética. La población de la Isla Attu, de la subespecies *T. p. meligerus*, tiene la menor diversidad genética y la mayor divergencia genética de todas las localidades muestreadas, una señal que también se encuentra en las poblaciones de *Melospiza melodia* y *Lagopus muta* de la Isla Attu. Actualmente, es poco probable que las poblaciones del oeste de Alaska estén conectadas a las localidades continentales por medio de dispersión y, por ende, son candidatos poco probables para realizar un rescate genético o demográfico natural.

Palabras clave: endemismo, genética de la conservación, Islas Aleutianas, *Troglodytes pacificus*

INTRODUCTION

The evolution of endemic populations is often associated with isolation and adaptation to island life (Grant 1998, Whittaker and Fernández-Palacios 2007). Islands harbor almost 10 times as many endemic plant and vertebrate

populations as mainland areas (Kier et al. 2009), and might contain a large portion of the genetic variation of widespread species (Wilson et al. 2009). However, humans have had negative impacts on island populations, primarily through the introduction of invasive species (Reaser et al. 2007, Gibson et al. 2013). Thus, there is a growing focus on

the value of conserving island populations (Van Dyke 2008, Kier et al. 2009). The use of conservation genetics approaches can enable researchers to identify unique populations, assess the genetic diversity of these populations, and estimate the likelihood of declining populations being rescued through gene flow (Frankham et al. 2010).

Much of the research on island endemism in birds has focused on species radiations that have occurred in tropical systems such as the Galapagos Islands (Grant 1998); however, some high-latitude systems also have high population-level biodiversity, such as the Aleutian Islands in Alaska, USA (Gibson and Byrd 2007). Many phenotypically based subspecies of birds have been described from these islands (Gibson and Withrow 2015), and population genetics research has often supported this taxonomy, although disagreements between genetic and phenotypic datasets at these shallow levels of divergence are expected to be frequent (Shields and Wilson 1987, Holder et al. 2004, Pruett and Winker 2005a, 2005b, Pruett et al. 2010, Winker 2010).

Historically, the majority of the Aleutian Islands lacked mammalian predators, but humans introduced a variety of exotic species (Ebbert and Byrd 2002), including the Norway rat (*Rattus norvegicus*) and Arctic fox (*Alopex lagopus*), both known predators of nesting birds in the Aleutian Islands (Bailey 1993, Major and Jones 2005). Population declines and extinctions of many seabirds were linked to Arctic fox predation; foxes were therefore removed from many of these islands by the U.S. Fish and Wildlife Service (USFWS; Ebbert and Byrd 2002). Although predation of nesting seabirds by rats has been identified as a major management problem by the USFWS (Buckelew et al. 2011), rats are much more difficult to eradicate. An experimental rat removal effort on a small island in the central Aleutian Islands led to population increases of some bird species (Croll et al. 2016), suggesting that rats continue to have negative impacts on many Aleutian bird populations. Populations of several unique subspecies of landbirds might also be targets of rat predation, including one of the most phenotypically variable species in Alaska, the Pacific Wren (*Troglodytes pacificus*).

Pacific Wrens have between 7 and 10 subspecies in Alaska, with the majority being from the Aleutian Islands and neighboring areas (AOU 1957, Gibson and Withrow 2015). The Pacific Wren was recently split from the Winter Wren (*Troglodytes hiemalis*) based on song, genetic structure, and reproductive isolation in areas of sympatry (Toews and Irwin 2008); the species was also split from the Eurasian Wren (*Troglodytes troglodytes*) in Europe and Asia, primarily due to differences in genetic structure (Drovetski et al. 2004, Chesson et al. 2010). However, a detailed within-species assessment of the population genetics of Pacific Wrens in Alaska is currently insufficient

to assess the conservation status of the populations and subspecies occurring there, and the need for additional information has been identified as a priority for future research (Toews and Irwin 2012). Pruett and Winker's (2008) 4-population examination of Pacific Wrens in Alaska based on mitochondrial (mt) DNA sequences showed that 2 populations from the Aleutian Islands (Attu Island and Adak Island) had the same 3 haplotypes, and that these 3 haplotypes were not found in other areas of Alaska. However, these haplotypes differed from Alaskan mainland haplotypes by only 1 to 4 mutations. A pattern of few haplotypes that are separated by few mutations is commonly found in Aleutian Island populations of birds (Holder et al. 2004, Pruett and Winker 2005b, 2008, Winker et al. 2013), suggesting that mtDNA is not evolving rapidly enough to reveal population splits since the last glacial cycle.

Studies that have used multilocus, microsatellite-based approaches have been more successful at identifying genetically distinct, isolated, and genetically depauperate populations in the Aleutian Islands, probably due to recent population divergences (Pruett and Winker 2005a, Pruett et al. 2010). For 2 species with distributions on the Aleutian Islands and mainland Alaska, the Song Sparrow (*Melospiza melodia*) and Rock Ptarmigan (*Lagopus muta*), populations on the most remote islands have the lowest genetic diversity and the most genetic divergence from locations on or near the Alaskan mainland (Pruett and Winker 2005a, Pruett et al. 2010). Remote populations with limited diversity are those likely to be at highest risk of extinction, as the probability of genetic rescue from neighboring populations is low (Frankham et al. 2010).

Pacific Wrens have several subspecies that have been identified as being of conservation concern in Alaska (<http://accs.uaa.alaska.edu/education-and-outreach/species-lists/>): *Troglodytes pacificus alascensis* of the Pribilof Islands, *T. p. helleri* of the Kodiak Archipelago, *T. p. kiskensis* of the central Aleutian Islands, *T. p. meligerus* of the western Aleutian Islands, and *T. p. semidiensis* of the Semidi and adjacent islands (Table 1, Figure 1). The reasons for their listing status include a lack of knowledge about population sizes, their restricted distributions, and the possible threat of rat predation on islands (http://aknhp.uaa.alaska.edu/species_summary_reports/pdfs/332.pdf). We used population genetics approaches to further examine the conservation status of Pacific Wrens throughout their distribution in Alaska and in western British Columbia, Canada, focusing on these taxonomic conservation units and island populations. Using 8 microsatellite loci, we genotyped birds from 10 locations in Alaska and British Columbia, including samples from each of the subspecies of conservation concern, to answer the following questions: (1) What is the population structure of Pacific Wrens in Alaska? Do conservation

TABLE 1. Sampling locations, subspecies based on Gibson and Withrow (2015), sample sizes (*n*), and genetic diversity values for Pacific Wrens (*Troglodytes pacificus*) in Alaska, USA, and western British Columbia (BC), Canada. *H_O* = average observed heterozygosity, *H_E* = unbiased average expected heterozygosity, *A_R* = allelic richness, *P_A* = private alleles, and SD = standard deviation.

| Location | Subspecies | <i>n</i> | <i>H_O</i> (SD) | <i>H_E</i> (SD) | <i>A_R</i> (SD) | <i>P_A</i> |
|--------------------------------|---------------------------------|----------|---------------------------|---------------------------|---------------------------|----------------------|
| Attu Island | <i>meligerus</i> ^a | 20 | 0.21 (0.26) | 0.21 (0.26) | 1.88 (1.10) | 0 |
| Rat Islands ^b | <i>kiskensis</i> ^a | 19 | 0.45 (0.22) | 0.53 (0.24) | 3.53 (2.17) | 7 |
| Andreanof Islands ^c | <i>kiskensis</i> ^a | 20 | 0.35 (0.24) | 0.41 (0.27) | 2.76 (2.06) | 1 |
| St. George Island | <i>alascensis</i> ^a | 10 | 0.53 (0.34) | 0.37 (0.25) | 2.45 (1.04) | 2 |
| Chirikof Island | <i>semidiensis</i> ^a | 16 | 0.38 (0.28) | 0.46 (0.29) | 3.16 (1.53) | 2 |
| Kodiak Island ^d | <i>helleri</i> | 20 | 0.61 (0.23) | 0.69 (0.25) | 4.91 (2.59) | 4 |
| Copper River Delta | <i>pacificus</i> ^e | 7 | 0.66 (0.29) | 0.70 (0.24) | 4.89 (3.11) | 4 |
| Middleton Island | <i>helleri</i> | 17 | 0.61 (0.18) | 0.67 (0.24) | 4.56 (2.18) | 7 |
| Southeast Alaska ^f | <i>pacificus</i> ^e | 20 | 0.66 (0.13) | 0.70 (0.20) | 4.74 (2.24) | 1 |
| Graham Island, BC | <i>pacificus</i> | 20 | 0.72 (0.24) | 0.71 (0.22) | 4.94 (2.52) | 7 |

^a Subspecies considered to be of greatest conservation need by the Alaska Natural Heritage Program (<http://accs.uaa.alaska.edu/alaska-natural-heritage-program/>).
^b Includes Kiska Island (*n* = 18) and Semisopochnoi Island (*n* = 1).
^c Includes Adak Island (*n* = 19) and Kanaga Island (*n* = 1).
^d Includes Kodiak Island (*n* = 19) and Ushugat Island (*n* = 1).
^e Might include an undescribed subspecies (Phillips 1986).
^f Includes Juneau (*n* = 3), Prince of Wales Island (*n* = 9), and Ketchikan (*n* = 8).

units identified using genetic tools correspond with the conservation units presently identified based on subspecies status? (2) What is the genetic diversity of Pacific Wren populations? Do populations found farther from the mainland of Alaska have lower diversity than other populations, as has been found for other Aleutian birds? (3) How much contemporary gene flow is occurring among populations of Pacific Wrens, and what is the

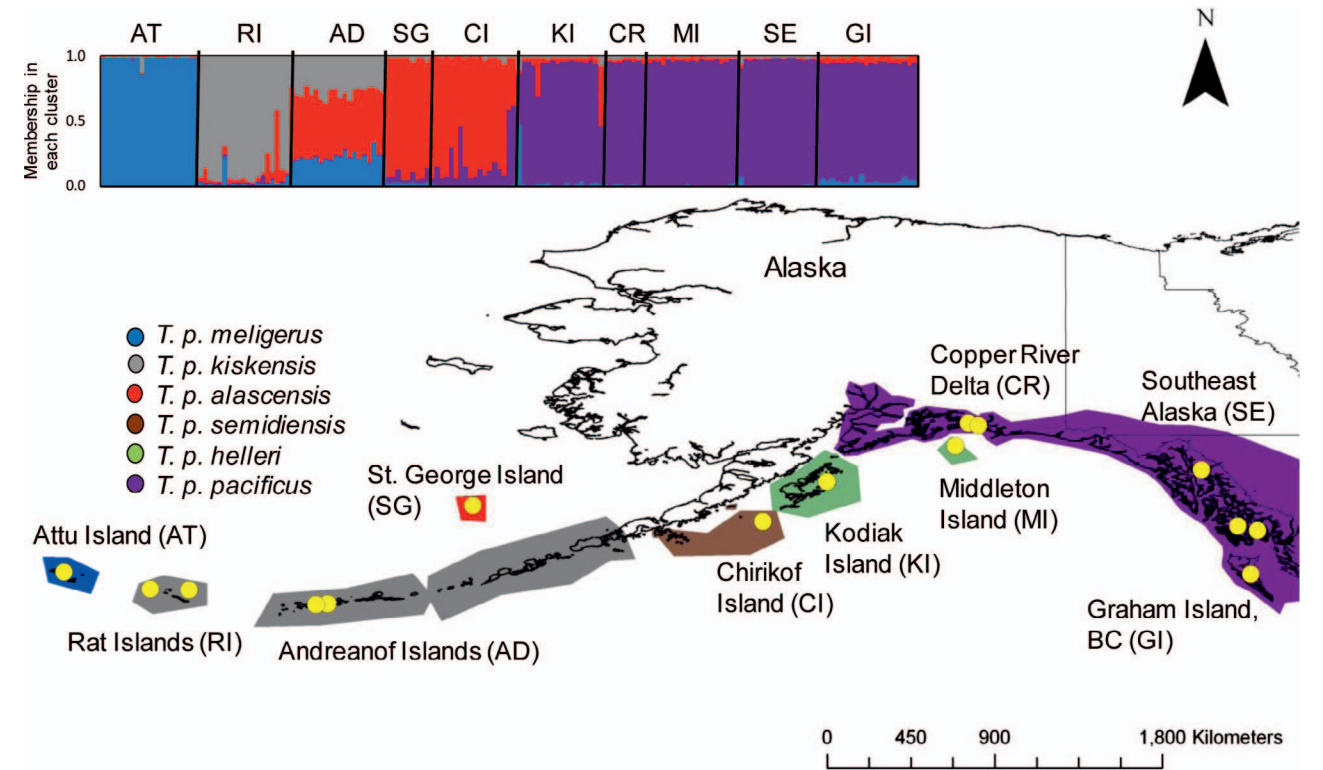


FIGURE 1. Distributions of Pacific Wren (*Troglodytes pacificus*) subspecies in Alaska, USA, and western British Columbia (BC), Canada, including sampling locations for genetic testing (yellow circles) and the proportions of individual membership of Pacific Wren populations in each of 4 genetic clusters identified using STRUCTURE (Pritchard et al. 2000, Falush et al. 2003).

likelihood of natural demographic or genetic rescue of island populations?

METHODS

Using a QIAamp DNA Mini Kit (QIAGEN, Valencia, California, USA), we extracted whole genomic DNA from the tissue of 169 Pacific Wrens sampled from 9 areas in Alaska and 1 in British Columbia (Figure 1, Table 1). Individuals from migratory populations were sampled during the breeding season. All specimens were deposited in the University of Alaska Museum of the North (Fairbanks, Alaska, USA; Appendix Table 4). We amplified 8 microsatellite loci using fluorescent dye-labeled primers (Ase19, Ase34, Ase43, and Ase56 from Richardson et al. 2000; Trpa12, Trpa21, Trpa22, and Trpa27 from Pruett et al. 2014). Amplifications were genotyped using an ABI 3130 automated sequencer (Applied Biosystems, Foster City, California, USA) and manually scored using GeneMapper (Applied Biosystems). Ten percent of extractions were reamplified and genotyped to ensure data quality.

We tested for deviations from Hardy-Weinberg equilibrium using 1,000,000 steps in a Markov chain with 100,000 dememorization steps. We also evaluated each locus for linkage disequilibrium using 10,000 permutations of the data. Tests were performed using Arlequin 3.5.2.2 (Excoffier and Lischer 2010).

We assessed the genetic diversity of populations in each location through observation of average observed heterozygosity (H_O) and average unbiased expected heterozygosity (H_E), also using Arlequin. Due to unequal sample sizes, we used FSTAT (Goudet 2002) to rarefy the data to the smallest sample size ($n = 7$; Table 1) to determine allelic richness (A_R). We determined the number of private alleles in each population and the frequency of those alleles using CONVERT (Glaubitz 2004). We tested whether or not the genetic diversity (H_E and A_R) of populations on islands was correlated with straight-line distance from the nearest mainland population (km) or with island size (km²) using a Pearson product-moment correlation analysis.

We examined population structure using several methods. First, we used Arlequin to compute pairwise genetic differences between populations (F_{ST}). To determine whether F_{ST} values were significantly greater than 0, the data were permuted 10,000 times. Bonferroni corrections were used when multiple tests were performed (Sokal and Rohlf 1995). Second, we used a simple Mantel test to determine whether there was a signal of isolation by distance in our dataset. Using zt (Bonnet and Van de Peer 2002), we compared a matrix of pairwise $[(1 - F_{ST})/F_{ST}]$ values with log-transformed straight-line geographic distance. We used 10,000 iterations of the dependent matrix to perform the test. Third, we used a principle component analysis (PCA) to determine genetic relation-

ships among individuals. We used the *adeigenet* package (Jombart 2008) in R (R Core Team 2016) to perform PCA analyses. Lastly, we used the Bayesian Markov chain Monte Carlo (MCMC) approach in STRUCTURE 2.3.4 to place individual genotypes into genetic clusters (K) such that they were in Hardy-Weinberg and linkage equilibrium (Pritchard et al. 2000, Falush et al. 2003). Initially, each Markov chain had a burn-in of 50,000, followed by 50,000 further iterations of the data examining K values ranging from 1 to 15 clusters, and we performed each analysis 8 times using the admixture model and the correlations model. We performed initial runs to refine our search for the optimal K value. We used the Evanno method (Evanno et al. 2005) to estimate the most likely number of genetic clusters using STRUCTURE HARVESTER (Earl and vonHoldt 2012). We then performed 3 runs for each K value ($K = 1-8$) with burn-ins of 500,000 and with 1,000,000 subsequent iterations to ensure that the Markov chain had converged on the optimal likelihood. We used CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) to combine STRUCTURE runs based on the 3 runs with $K = 1-8$ to aid with the visual display of output.

We also sought to determine how genetic variation was partitioned among subspecies, among individuals within subspecies, and within individuals. We did not perform analyses among populations within subspecies because we only sampled 1 location within some subspecies' ranges (Figure 1). Using Arlequin, we conducted an analysis of molecular variance (AMOVA) with 10,000 permutations of the data.

We inferred recent gene flow among locations using an assignment test in GENECLASS2 (Piry et al. 2004) using the methods of Rannala and Mountain (1997) and Paetkau et al. (2004). Assignment tests have been shown to be effective for identifying recent instances of dispersal among populations (Berry et al. 2004). We used the method of Underwood et al. (2007) to exclude or assign individuals as immigrants; this method is a very conservative method for identifying immigrants. Individuals were considered to be excluded from their location of origin when the probability of exclusion was $>99\%$ ($\alpha = 0.01$). If individuals were excluded from their population of origin, they were assigned to another location when $P > 0.32$. This value was chosen based on the lowest mean Bayesian probability for a population with a sample size larger than 10; in this instance, Middleton Island. When individuals could not be assigned with certainty to any population they were considered to be from an unsampled location, as all areas where Pacific Wrens are found were not sampled.

RESULTS

Hardy-Weinberg tests showed disequilibrium for locus Ase34 from Graham Island and Trpa22 from Chirikof

TABLE 2. Pairwise F_{ST} estimates among Pacific Wren (*Troglodytes pacificus*) sampling locations in Alaska, USA, and western British Columbia (BC), Canada. Values in bold are significantly different from zero after Bonferroni correction (adjusted $P < 0.001$). Subspecies names are given with each location in parentheses.

| | Attu Island | Rat Islands | Andreanof Islands | St. George Island | Chirikof Island | Kodiak Island | Copper River Delta | Middleton Island | Southeast Alaska | Graham Island, BC |
|--|-------------|-------------|-------------------|-------------------|-----------------|---------------|--------------------|------------------|------------------|-------------------|
| Attu Island (<i>T. p. meligerus</i>) | — | | | | | | | | | |
| Rat Islands (<i>T. p. kiskensis</i>) | 0.47 | — | | | | | | | | |
| Andreanof Islands (<i>T. p. kiskensis</i>) | 0.50 | 0.22 | — | | | | | | | |
| St. George Island (<i>T. p. alascensis</i>) | 0.76 | 0.50 | 0.47 | — | | | | | | |
| Chirikof Island (<i>T. p. semidiensis</i>) | 0.66 | 0.39 | 0.17 | 0.33 | — | | | | | |
| Kodiak Island (<i>T. p. helleri</i>) | 0.41 | 0.19 | 0.16 | 0.29 | 0.13 | — | | | | |
| Copper River Delta (<i>T. p. pacificus</i>) | 0.45 | 0.18 | 0.24 | 0.31 | 0.28 | 0.03 | — | | | |
| Middleton Island (<i>T. p. helleri</i>) | 0.39 | 0.20 | 0.17 | 0.30 | 0.18 | 0.01 | 0.01 | — | | |
| Southeast Alaska (<i>T. p. pacificus</i>) | 0.42 | 0.20 | 0.27 | 0.33 | 0.28 | 0.06 | 0.00 | 0.04 | — | |
| Graham Island, BC (<i>T. p. pacificus</i>) | 0.36 | 0.18 | 0.22 | 0.27 | 0.24 | 0.03 | 0.00 | 0.02 | 0.00 | — |

Island; all other loci were in equilibrium. Because there did not appear to be a pattern of disequilibrium across locations, all loci were included in subsequent analyses. All loci were in linkage equilibrium.

The populations with the lowest genetic diversity were in locations west of Kodiak Island (Figure 1), with Attu Island having the lowest values (Table 1). Genetic diversity in island populations declined with geographic distance from the mainland of Alaska or Canada (H_E : $r = -0.76$, $P = 0.02$; A_R : $r = -0.74$, $P = 0.02$). Genetic diversity was not significantly correlated with island size (H_E : $r = 0.54$, $P = 0.13$; A_R : $r = 0.55$, $P = 0.12$). The highest number of private alleles ($n = 7$; Table 1) was found in 3 populations, those on the Rat Islands, Middleton Island, and Graham Island. All of these, except 1 private allele, were found at low frequencies (<0.07); Copper River Delta had an allele with a frequency of 0.14.

All comparisons between Kodiak Island, Copper River Delta, Middleton Island, Southeast Alaska, and Graham Island had F_{ST} values that were not significantly different from 0 (Table 2). In contrast, populations in locations west of Kodiak Island had pairwise F_{ST} values that differed significantly from 0 in comparisons with populations from all other locations (Table 2). On average, populations in the 2 most geographically remote locations, Attu Island ($F_{ST} = 0.49$, $SD = 0.13$) and St. George Island ($F_{ST} = 0.40$, $SD = 0.16$), had the highest F_{ST} values in comparisons with other populations. However, geographic distance was not correlated with genetic distance ($r = 0.14$; $P = 0.16$).

Based on STRUCTURE output, the Evanno et al. (2005) method identified the most likely number of genetic clusters (K) as 4. Based on the proportion of membership of each individual in each cluster, Kodiak Island and locations to the east corresponded to 1 genetic cluster, Chirikof Island and St. George Island to a second cluster, Rat Islands to a third cluster, Attu Island to a fourth cluster, and the Andreanof Islands showed admixture between the 3 western clusters (Figure 2). STRUCTURE outputs with K values different from $K = 4$ showed divergences between eastern and western populations. When K was set to 6 clusters, St. George Island was separated from all other locations (Appendix Figure 3). The results of the PCA analysis were similar to the STRUCTURE results ($K = 4$), with Attu Island individuals clustering together, Rat Islands and Andreanof Islands forming a cluster, Chirikof Island and St. George Island clustering together, and the remaining populations forming a single group (Figure 2).

Genetic variation in the data was partitioned primarily within individuals ($\sim 70\%$; $P < 0.001$) and among subspecies ($\sim 25\%$; $P < 0.001$). The remaining variation, $\sim 5\%$ ($P = 0.004$), was partitioned among individuals within subspecies (Table 3).

Based on assignment tests, only 5 birds (3%) were excluded from their location of origin. One wren sampled from Attu Island was assigned to the Rat Islands, but with a low probability ($Pr = 0.17$), well below the cutoff outlined by Underwood et al. (2007). One bird from Adak Island and one bird from Graham Island could not be assigned

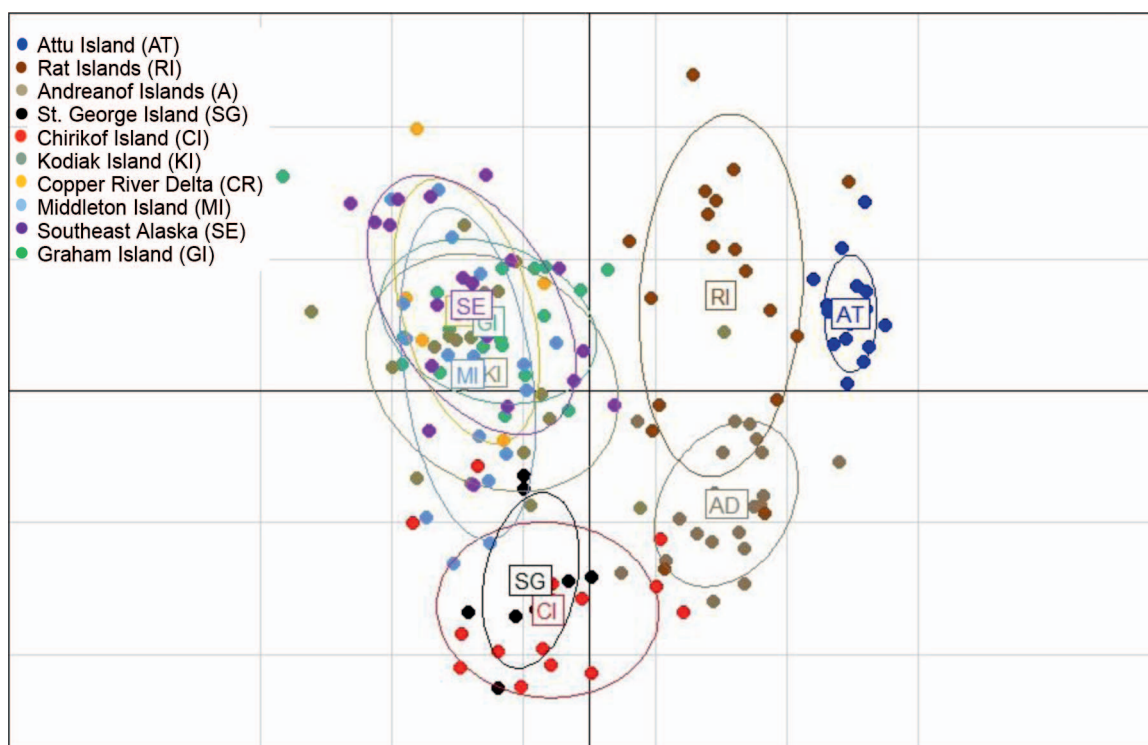


FIGURE 2. Results of principal component analysis (PCA) based on microsatellite allele frequencies for Pacific Wrens (*Troglodytes pacificus*) in Alaska, USA, and western British Columbia, Canada. Ellipses encompass 75% of points.

with high probability to an alternative location ($Pr < 0.06$ for all locations), suggesting that these 2 individuals were from unsampled locations. One individual from St. George Island was slightly more likely to have come from Kodiak Island ($Pr = 0.32$) than from Graham Island ($Pr = 0.28$), and a bird from Chirikof Island was assigned to neighboring Kodiak Island ($Pr = 0.47$). Conversely, 164 individuals (97%) could not be excluded from their population of origin, indicating that there was little dispersal overall among these populations.

DISCUSSION

There are effectively 2 datasets in our study, the grouping of populations based on phenotype (the recognized subspecies) and our nuclear DNA dataset. Variation in the first is likely driven mostly by selection, whereas

variation in the latter is mostly effected by neutral processes and gene flow (or its absence). At shallow levels of evolutionary divergence, these 2 signals commonly disagree (Winker 2010), yet integrating these 2 different perspectives on divergence is critical for conservation, lest we inadvertently lose the very diversity necessary to see a lineage through a period of climatic change (Winker 2009, in press). Here, we consider our results in the context of the current North Pacific conservation framework, which focuses on subspecies, then extend that framework to the finer level of resolution that our population genetics results provide.

Genetic Structure and Subspecies

Subspecies, based on phenotypic differences, have been used to describe units for conservation of Alaskan populations of Pacific Wrens. Designating conservation

TABLE 3. Partitioning of genetic variance among subspecies (groups), among individuals within subspecies, and within individuals based on analysis of molecular variance (AMOVA) of Pacific Wrens in Alaska, USA, and western British Columbia, Canada. Asterisks indicate significance of at least $P < 0.003$.

| Source of variation | df | Sum of squares | Variance components | Percentage of variation |
|-------------------------------------|-----|----------------|---------------------|-------------------------|
| Among subspecies | 5 | 127.19 | 0.45 * | 25 |
| Among individuals within subspecies | 162 | 240.02 | 0.10 * | 5 |
| Within individuals | 168 | 216.00 | 1.29 * | 70 |
| Total | 335 | 583.21 | 1.84 | |

units based on this criterion of subspecies suggests that subspecies reflect some degree of genetic identity (and may even be on independent evolutionary trajectories) and that populations within subspecies are likely to be connected via gene flow. Genetic studies provide insights into these phenomena. Initial studies, using mtDNA sequences, did not show genetic differences among most subspecies of Pacific Wren (Pruett and Winker 2008). However, our assessment using relatively rapidly evolving microsatellite loci showed that a large proportion of the genetic variance within the dataset was partitioned among subspecies. Four of these subspecies are considered to be of conservation concern (Table 1).

A close association between genetic differences and phenotypic differences (subspecies) was found for wrens on Attu Island (*T. p. meligerus*; Figure 1). Pairwise F_{ST} , PCA, and STRUCTURE results all showed that this population was different from populations in all other sampled locations (Table 2, Figure 2), suggesting that it is on its own evolutionary path. However, there was a single individual from Attu Island that was not assigned with high probability to its location of origin and was assigned only marginally to the Rat Islands. It is possible that this bird came from an unsampled population west of Attu Island. The complete distribution of Pacific Wrens is currently unknown, but is likely to extend to the Commander Islands of Russia (contra Dickinson and Christidis 2014, Clements et al. 2016). Pruett and Winker (2008) used mtDNA sequences to examine 2 birds from the Commander Islands and found that they had the same haplotype that was found on Attu Island and in the Andreanof Islands. Wrens from the Commander Islands are considered by some to be a separate species and subspecies (*T. troglodytes pallescens*) from those on Attu Island (Johansen 1961), but their taxonomy has not been critically revised since Pacific Wrens were split from Eurasian Wrens (Dickinson and Christidis 2014, Clements et al. 2016). Shared mtDNA haplotypes can occur across species boundaries (Funk and Omland 2003) and, given that microsatellite data have provided a more refined view of Pacific Wren population genetics, sharing of haplotypes does not necessarily indicate that populations are currently connected through gene flow. For example, populations on Attu Island and the Andreanof Islands share several mtDNA haplotypes (Pruett and Winker 2008), but are clearly different using microsatellite markers (Figure 1, Table 2).

Some subspecies appear to harbor cryptic genetic differences that are not evident using phenotype alone. Based on the most recent evaluation of subspecies of Alaskan birds (Gibson and Withrow 2015), the subspecies *T. p. kiskensis* includes populations on the Rat Islands and Andreanof Islands (Figure 1). However, based on our F_{ST} results, these 2 populations are genetically different from

one another and from populations in all other locations (Table 2). In contrast, our STRUCTURE and PCA results suggested the possibility of connectivity (Figure 2). Older assessments of subspecies from these locations described 2 different subspecies (AOU 1957), *T. t. kiskensis* (Rat Islands) and *T. t. tanagensis* (Andreanof Islands). In this study, all individuals from both locations assigned to their population of origin, suggesting that they are currently isolated from one another and from other locations. Given the current isolation of these populations and the larger number of private alleles in the Rat Islands population than in other Aleutian Island populations (Table 1), the signal of admixture might have been caused by historical effects such as shared colonization histories rather than by recent gene flow. A similar signal is found in Song Sparrows on the Aleutian Islands, in which populations show gradual genetic differences, suggesting a stepping-stone colonization pattern (Pruett and Winker 2005a).

In one case, the F_{ST} and STRUCTURE output differed from one another with respect to subspecies. Wrens from St. George Island in the Pribilof Islands are described as being a distinctly different subspecies (*T. p. alascensis*) from those on Chirikof Island (*T. p. semidiensis*; Gibson and Withrow 2015, Withrow 2015). Our F_{ST} results showed that St. George and Chirikof Island wrens differed substantially (Table 2); however, our PCA and STRUCTURE results showed that wrens in these locations were very similar. Assignment tests showed little or no contemporary gene flow into or out of these locations, suggesting historical connectivity, possibly due to similar colonization sources. To test this hypothesis, sampling of birds from the Alaska Peninsula is needed. However, birds in this area appear to be patchily distributed and rare. We attempted to collect samples from 2 locations on the Alaska Peninsula and a nearby location in the Shumagin Islands, but failed to detect a single individual.

Wrens from Kodiak Island and areas east were not genetically differentiated from one another based on F_{ST} , PCA, and STRUCTURE results (Table 2, Figure 2). However, they are considered to be 2 different subspecies, *T. p. helleri* and *T. p. pacificus* (Table 1). Assignment tests did not show substantial current gene flow among these locations, but gene flow would appear to be enough to limit the effects of genetic drift.

Population Genetic Divergence and Diversity

A general pattern in the data was that populations farther from the mainland of Alaska or Canada were genetically differentiated from populations in other locations. This suggests that geographic distance might be associated with genetic distance. However, a test of isolation-by-distance did not show a correlation. The difficulty of island colonization might have led to small population sizes and pronounced effects of genetic drift in each individual

island population, a pattern also shared with Rock Ptarmigan in Alaska (Pruett et al. 2010).

Both Rock Ptarmigan and Song Sparrows show a pattern of lower genetic diversity in island populations that are distant from mainland locations (Pruett and Winker 2005a, Pruett et al. 2010), a pattern also found in Eurasian Wrens in the north Atlantic (Amouret et al. 2016). Eurasian Wrens in Iceland have lower diversity and are farther from possible source populations than those found on the Faroe Islands of Scotland. We found that Pacific Wrens also show this pattern, with the lowest diversity values found on the Aleutian Islands and on St. George Island in the Pribilof Islands (Figure 1, Table 1). Distance from the nearest mainland location was negatively correlated with genetic diversity but not with island size, suggesting that this pattern was caused by the difficulty of colonization rather than by habitat availability. However, Pacific Wrens on islands west of Kodiak Island have very specific nesting requirements along rocky shorelines (Gibson and Byrd 2007), possibly limiting nesting opportunities. The presence of introduced Arctic foxes might also have restricted the nesting habitat of wrens and thus population size. In at least one instance, wrens on islands where foxes were never introduced were found in upland areas away from the shore (Gibson and Byrd 2007). If foxes were constraining habitat use, now that foxes have been removed from most of the Aleutian Islands, birds might begin to use more diverse habitats for nesting, thereby increasing population sizes, as has been found for several species of seabird, waterfowl, shorebird, and passerine (Lavers et al. 2010, Mini et al. 2011).

Pacific Wrens nesting on Kodiak Island and locations eastward are found in forested habitats, whereas birds west of Kodiak Island are found in treeless areas. This change corresponds to the break in genetic structure evident in the STRUCTURE (Figure 1), PCA (Figure 2), and F_{ST} results (Table 2); wrens found on Kodiak Island and areas eastward form a single genetic cluster that is different from the 3 clusters found among wrens in the treeless areas. In addition, populations of wrens in the forested areas have higher genetic diversity than those in the treeless areas (Table 1). Based on these findings, one explanation could be that large, connected forested habitats cause an increase in dispersal distances, facilitating gene flow and retention of diversity, especially if population sizes in forested areas are large. However, the change in habitat type coincides with colonization into areas distant from the mainland and thus into areas that might be more difficult to colonize and disperse among. These hypotheses are not mutually exclusive, and both might have played a role in the lower genetic diversity and greater divergences found in areas west of Kodiak Island.

Another transition that occurs across the Alaskan distribution of Pacific Wrens is that from partially or fully

migratory populations in eastern Alaska to completely sedentary populations in western Alaska (Phillips 1986). However, changes in migratory movement do not match the genetic break as closely as the change in habitat. Wrens from Kodiak Island and locations westward are considered to be nonmigratory. However, there has been very limited research into the migratory behavior of Pacific Wrens, and a more gradual transition from migratory to partially migratory to fully sedentary, as seen in Song Sparrows (Patten and Pruett 2009), is possible. A combination of habitat transition, change in population size, and movement might provide an explanation for the genetic signals in our dataset.

Genetic Rescue and Conservation Implications

Island birds are often at greater risk of extinction due to human-caused habitat changes, including the introduction of invasive predators. Island populations that have experienced population declines and that are found long distances from mainland source populations likely have the lowest probability of natural genetic or demographic rescue due to a low probability of immigration (Frankham et al. 2010). By examining the genetic diversity of island populations and gene flow among these populations, we found 2 patterns in the Pacific Wren data. First, Pacific Wrens found on islands near possible source areas in Canada or Alaska had higher genetic diversity than those found in distant areas (Table 1). Wrens on islands such as Graham Island, Middleton Island, Kodiak Island, and islands in southeast Alaska had very low F_{ST} values in comparisons between these locations (Table 2), suggesting that there are high levels of connectivity among these locations and that these areas have a high probability of both genetic and demographic rescue. Second, wrens found on islands west of Kodiak Island likely have a lower probability of genetic rescue through natural dispersal. All westerly locations, assessed using assignment tests, showed limited current gene flow. Thus, long-distance movements among these locations is unlikely.

Based on our genetic results, we support the continued use of subspecies as conservation units, but with the understanding that some subspecies contain more than one genetic unit (e.g., the Rat Islands and Andreanof Islands populations in subspecies *T. p. kiskensis*). Populations from subspecies identified as being of conservation concern (Tables 1, 2) appear to be genetically differentiated, have lower genetic diversity than other locations, and are unlikely candidates for natural genetic rescue through immigration if populations decline. Although Pacific Wrens probably occur on the Commander Islands of Russia, their distribution on the mainland of Russia is unknown. Wrens that were sampled from areas in central Russia had mtDNA haplotypes that differed by >20 mutations from the haplotypes of wrens in Alaska (Pruett

and Winker 2008). Thus, natural genetic rescue from Russia into the western Aleutian Islands seems unlikely.

Arctic foxes and Norway rats have been on the Aleutian Islands and other islands in the Bering Sea region for at least 200 yr, i.e. for 50–100 generations of Pacific Wrens. The initial and cumulative effects on population size and genetic diversity are unknown, but Pacific Wren population size did not change significantly after the experimental eradication of rats from an island in the Aleutians (Croll et al. 2016). This suggests that rat predation may have a negligible impact on Pacific Wrens. However, the experiment only examined a single, very small island that had a very limited number of wrens. Predation of wrens by foxes has not been observed, and a study of Rock Ptarmigan found that losses in genetic diversity in Aleutian populations did not coincide with the timing of fox introductions; rather, changes in diversity were more likely to have occurred during colonization (Pruett et al. 2010). However, research on Common Eiders (*Somateria mollissima*) inhabiting the western Aleutian Islands suggests that fox predation could have caused a population bottleneck in this species (Sonsthagen et al. 2013).

Song Sparrows, Rock Ptarmigan, and Pacific Wrens all show similar patterns of population divergence and loss of diversity in Aleutian Island populations. Research on other landbird species with similar distributions might show similar patterns. The Aleutian Islands and other islands off the coast of Alaska harbor many unique avian subspecies and should be conserved as an important region for high-latitude biodiversity.

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Author contributions: C.L.P. and K.W. conceived the idea and designed the study. A.R., C.S., and C.L.P. performed genetic analyses and analyzed data. C.L.P. wrote the manuscript, and all authors edited the manuscript.

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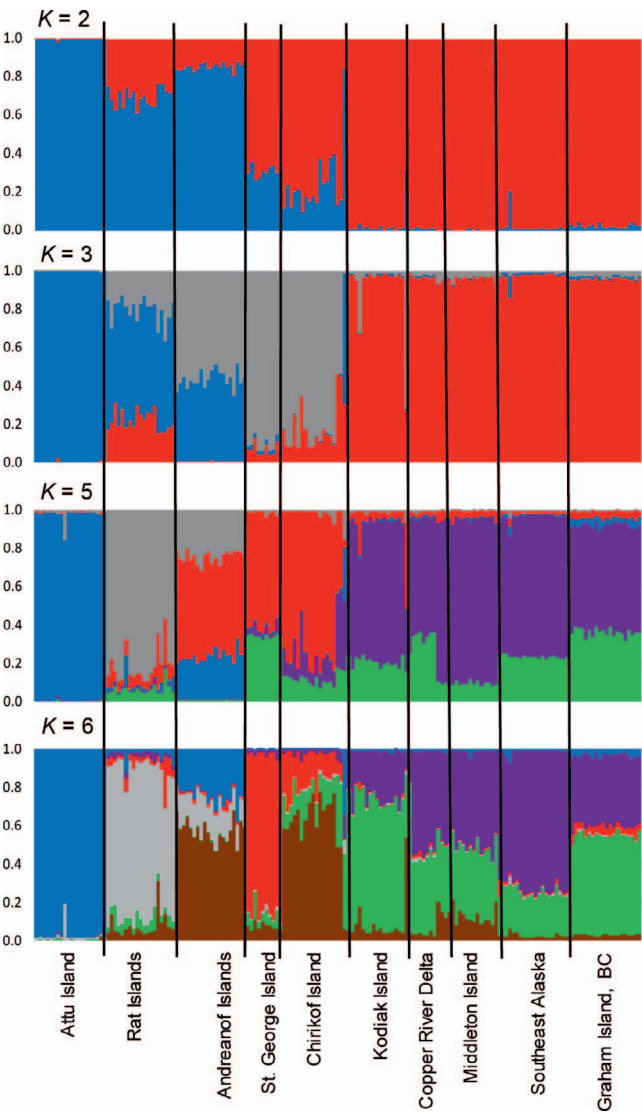
APPENDIX TABLE 4. Voucher numbers for specimens of Pacific Wren (*Troglodytes pacificus*) used in this study.

| Subspecies | Museum ^a | Catalog numbers | Locality | Geographic coordinates |
|--------------------------|---------------------|---|---|------------------------|
| <i>T. p. meligerus</i> | UAM | 20511, 24612, 24613, 27167, 27168, 27172, 27175–27183, 27186, 27190–27192, 27683 | Alaska: Aleutian Is., Attu Is. | 52.8436°N, 173.2003°E |
| <i>T. p. kiskensis</i> | UAM | 8792 | Alaska: Aleutian Is., Semisopochnoi Is. | 51.9546°N, 179.4907°E |
| | UAM | 27140, 27141, 27143–27147, 27150, 27151, 27154–2157, 27159–27163 | Alaska: Aleutian Is., Kiska Is. | 51.9767°N, 177.5360°E |
| | UAM | 8353, 9187, 9188, 10058, 11189, 12078, 13240, 14678, 17129, 27128, 27130–27138 | Alaska: Aleutian Is., Adak Is. | 51.8314°N, 176.6320°W |
| <i>T. p. alascensis</i> | UAM | 8354 | Alaska: Aleutian Is., Kanaga Is. | 51.8099°N, 177.2090°W |
| | UAM | 13979–13981, 14115, 18523, 19863, 27124–27127 | Alaska: Pribilof Is., St. George Is. | 56.5888°N, 169.6150°W |
| <i>T. p. semidiensis</i> | UAM | 27106–27121 | Alaska: Chirikof Is. | 55.8147°N, 155.6160°W |
| <i>T. p. helleri</i> | UAM | 7526–7529, 8352, 8355, 8392, 8393, 9861, 11163, 11164, 11314, 11523–11525, 11534, 11535, 14741, 22058 | Alaska: Kodiak Archipelago, Kodiak Is. | 57.7369°N, 152.5150°W |
| | UAM | 28583 | Alaska: Kodiak Archipelago, Ushagat Is. | 58.9242°N, 152.3061°W |
| | UAM | 29137–29148, 30709, 30711, 30715, 30716, 30718 | Alaska: Middleton Is. | 59.4511°N, 146.3170°W |

APPENDIX TABLE 4. Continued.

| Subspecies | Museum ^a | Catalog numbers | Locality | Geographic coordinates |
|------------------------|---------------------|--|---|------------------------|
| <i>T. p. pacificus</i> | UAM | 12099, 22578, 23499, 27065, 27066, 27132, 29247 | Alaska: Copper River Delta | 60.5642°N, 145.6840°W |
| | UAM | 11313, 12075, 27063, 27681 | Alaska: Juneau | 58.3020°N, 134.4200°W |
| | UAM | 7633, 7635, 7747, 8395, 8916, 8917, 8919 | Alaska: Prince of Wales Is. | 55.5890°N, 132.6730°W |
| | UAM | 7629, 7632, 7694, 8918, 11536, 14594 | Alaska: Ketchikan | 55.3675°N, 131.7040°W |
| | UAM | 8353, 11526, 11527, 11529, 11531, 11532, 13897, 13898, 13901, 13966, 13971, 14093, 14354, 14593, 15200, 18532, 27054–27057 | British Columbia: Haida Gwaii, Graham Is. | 53.2736°N, 132.0440°W |
| | | | | |

^a UAM = University of Alaska Museum.



APPENDIX FIGURE 3. Proportion of membership in genetic clusters (*K*) for Pacific Wren (*Troglodytes pacificus*) subspecies in Alaska, USA, and western British Columbia (BC), Canada, based on STRUCTURE output (Pritchard et al. 2000, Falush et al. 2003). See Figure 1 for the colors used to denote subspecies.