

High levels of gene flow among song dialect populations of the Puget Sound white-crowned sparrow

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Abstract

Populations within a species can show geographic variation in behavioral traits that affect mating decisions or limit dispersal. This may lead to restricted gene flow, resulting in a correlation between behavioral variation and genetic differentiation. Populations of a songbird that differ in a learned behavioral trait, their song dialects, may also differ genetically. If song dialects function as mating barriers, evolutionary processes such as genetic drift should lead to divergence in allele frequencies among dialect populations. The Puget Sound white-crowned sparrow (*Zonotrichia leucophrys pugetensis*) is an excellent study system with a well-defined series of song dialects along the Pacific Northwest coast. A previous study found low genetic differentiation based on four microsatellite loci; however, available loci and analyses techniques have since dramatically improved and allow us to reassess gene flow in this species. We also add extra samples to fill in gaps and add a new level of analysis of geographic variation. Based on acoustic similarities, we group six song dialects into two geographically larger “northern” and “southern” song themes. One southern dialect is acoustically more similar to dialects in the north, which makes the genetic profile of birds singing this dialect particularly interesting. Traditional *F*-statistics, analysis of molecular variance as well as Bayesian techniques confirmed the earlier result that geographic variation in song does not correlate with the neutral genetic structure of the sampled dialect populations. The song themes also did not differ genetically, and the origin of the extralimital northern-theme dialect cannot be determined. We compare this result to findings in several other species and discuss how the timing of learning and dispersal allow vocalizations to vary independently of patterns of genetic divergence.

KEYWORDS

gene flow, genetic variation, geographic variation, song dialect, *Zonotrichia leucophrys pugetensis*

1 | INTRODUCTION

Dispersal promoting gene flow is crucial for maintaining the genetic connectivity of populations. Behaviors that occur in sexual interactions, such as male–male competition over access to mates or mating decisions, may influence the reproductive success of individuals

after dispersal. These behaviors can limit the success of interacting and mating individuals and act as barriers to gene flow (Dieckmann & Doebeli, 1999; Smith, 1966). Thus, when we observe differences in these behaviors among groups of individuals, we can ask whether these traits correlate with genetic differentiation and may indicate early stages of speciation (Grant & Grant, 1997). Birdsong, which

typically varies within a species, is one such behavioral trait (Catchpole & Slater, 2003; Slabbekoorn & Smith, 2002) and may reduce gene flow between dialect populations (Baker, 1975).

Songbirds produce species-specific songs that allow recognition and discrimination of potential mates and social competitors. Young songbirds learn their songs mainly through imitation of songs of adult conspecifics (Beecher & Brenowitz, 2005). As with the genetic divergence of isolated groups, both stochastic processes (such as cultural drift) and selective pressures can lead to geographic variation in song among populations within a species (Podos & Warren, 2007). Due to the function of song in mate attraction, variation in song may potentially separate groups of individuals and result in genetic differentiation among populations. In multiple species, it has been found that singing non-local or non-shared songs have lower pairing success, indicating that dispersal may not be genetically effective (Hegelbach, 1986; MacDougall-Shackleton, Derryberry, & Hahn, 2002; Payne, Payne, & Doehler, 1988; Poesel, Nelson, & Gibbs, 2012). Thus, it is essential to use genetic techniques to estimate dispersal.

An analysis of trace elements in feathers in *Zonotrichia leucophrys pugetensis* provides evidence for some dispersal among dialects (Poesel, Nelson, Gibbs, & Olesik, 2008). However, we do not know whether successful breeding resulting in gene flow follows dispersal. Overall, males tend to breed within the area where they learned song(s) the previous year (Nelson & Poesel, 2014). As a result, this subspecies shows distinct song dialects along the Pacific Northwest coast from California into British Columbia, as defined by the structure of the terminal trill (Figure 1; Baptista, 1977; Nelson, Hallberg, & Soha, 2004). These dialect differences are salient to the birds: Males respond more strongly to playback of local than foreign dialects indicating that local songs are a more effective territorial signal (Nelson & Soha, 2004a). Moreover, males respond differently to songs of two different themes, a “northern” and a “southern” cluster of song dialects (Nelson & Soha, 2004a). Baptista (1977) defined themes by stable differences in the ordering and identity of phrases within the song. Northern themes have a buzz, a pair of note complexes or complex syllables, and a second buzz preceding the terminal trill, whereas southern themes have only one note complex and one buzz before the trill (Figure 1). The song themes and dialects have been stable for over 45 years, with only small changes in the locations of boundaries (Chilton & Lein, 1996; Nelson et al., 2004). Baptista (1977) identified the Columbia River between Oregon and Washington as the border between the northern and southern themes. Five or six northern-theme dialects occur north of this border, and four southern-theme dialects occur south of it (Nelson et al., 2004). In addition, we found one northern-theme dialect (dialect 12) on the southern Oregon coast (Figure 1; Baker, 1987). How this extralimital northern-theme dialect population became established and whether it differs genetically from the surrounding southern-theme dialects are questions we sought to answer here.

To understand the relationship between song variation and genetic structuring of populations, it is crucial to know not only the extent to which song plays a role in mate choice and male–male interactions, but also whether males learn their song before or after dispersal. We know

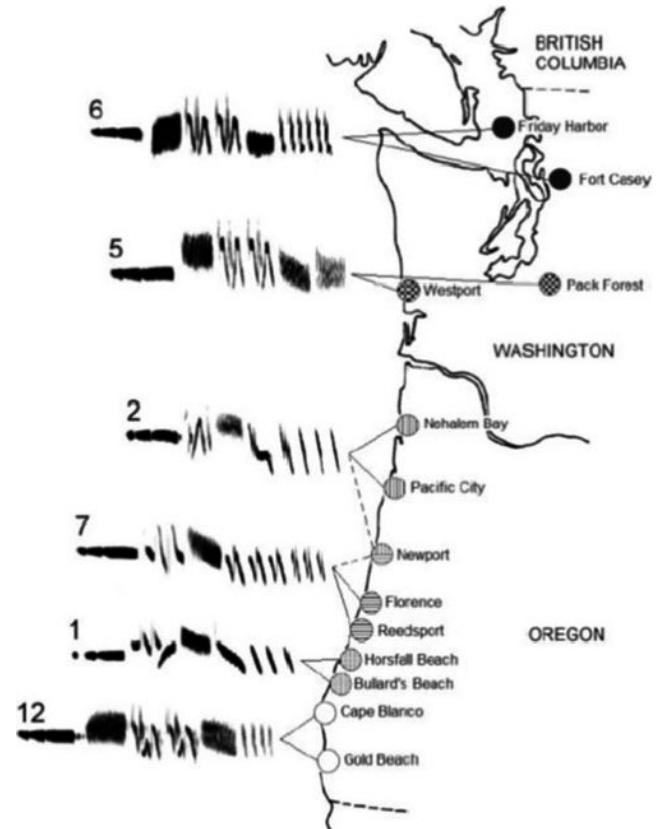


FIGURE 1 Map of the Pacific Northwest showing 13 sample sites of Puget Sound white-crowned sparrows in six song dialect areas. Spectrograms of one representative song from each sampled dialect are shown along with dialect identification numbers. Dialects 6, 5, and 12 represent the northern song theme with a second buzz between the whistle and the note complex. Dialects 2, 7, and 1 represent the southern song theme. Adapted from Soha et al. (2004) with two additional sample sites (Reedsport, Fort Casey). The Columbia River marks the border between Washington and Oregon

much of this information for the Puget Sound white-crowned sparrow. Males of this subspecies memorize song in the first few months of life, and as in many songbird species, males commonly learn multiple song types from different tutors (Nelson, 1999; Nelson & Poesel, 2014). These song types are defined by differences in the note complex part of the song, and when they end with a similar trill, they are within the same dialect. Close to dialect boundaries, young males are especially likely to encounter tutors singing different dialects and themes. In addition, at other locations, young birds may hear foreign dialects from one of the few resident adult males that sing non-local dialects. As a result, yearling males often sing more than one song type, sometimes of different dialects and themes, when they first establish a breeding territory. Through the process of selective attrition, they usually retain a single type as their adult song that matches a song type in the social neighborhood where they establish territory (Marler & Nelson, 1992; Nelson, 2000). Once song is crystallized, males retain it throughout their life without major changes or additions (Nelson & Poesel, 2009).

In the closely related but sedentary subspecies, *Zonotrichia leucophrys nuttalli* Baker, Thompson, Sherman, Cunningham, and Tomback

(1982) found a correlation between genetic variation and geographic distance. In the migratory subspecies, *Zonotrichia leucophrys oriantha* MacDougall-Shackleton and MacDougall-Shackleton (2001) documented some genetic divergence between dialect populations (independent of geographic distance) and so did Soha, Nelson, and Parker (2004) in *Z. l. pugetensis*. However, in both cases, a single population that appeared especially divergent might primarily explain the results. This limited evidence and generally inconsistent results across species (Table S2) warrant further investigation with an increased sample size and new techniques.

Tools for the analysis of minor genetic differences have dramatically improved over the last decade. Here, we use Bayesian clustering techniques (STRUCTURE and DAPC in adegenet) and a three times larger set of 14 nuclear microsatellite loci specifically identified in *Z. l. pugetensis* (Poesel, Gibbs, & Nelson, 2009) than was previously used by Soha et al. (2004) to analyze population genetic structure. We test for differentiation not only among song dialects as in Soha et al. (2004), but also between the two song themes, with the goal of determining the genetic association of the extralimital northern-themed dialect in the south. We also sampled at two additional sites. One of these new sites (Reedsport) enabled us to include Dialect 7, which was omitted from the final analysis in Soha et al. (2004). If song dialects restrict gene flow, we expect genetic differences among dialects. If the two song themes reflect relatively early founder events in geographically distinct areas (Baker & Thompson, 1985), and if dispersal between themes is restricted, we expect genetic differences between the two song themes.

2 | MATERIAL AND METHODS

2.1 | Study system and sample collection

As part of a long-term study of *Z. l. pugetensis*, 285 males across a latitudinal gradient of ~700 km along the Pacific Northwest coast of Oregon and Washington (Figure 1) were sampled in April through June of 1999–2001. This includes the sample set of 252 males reported in Soha et al. (2004); in particular, we almost doubled the sample sizes for dialects 6 and 7. Briefly, at each of 13 sample sites, 16–26 males were caught in mist-nests or seed-baited traps and banded with a USGS metal band on one leg and a unique pair of colored plastic bands on the other. A maximum of 100 μ l of blood was taken from the brachial wing vein and stored in Longmire's lysis buffer (Longmire, Maltbie, & Baker, 1997).

2.2 | Song recording and dialect identification

The songs of most of the 285 males were recorded and used to assign each sample site to a particular song dialect (see details in Soha et al., 2004). The dialects of *Z. l. pugetensis* along the Pacific Northwest coast have been described in previous studies (Baptista, 1977: dialects 1 through 6; DeWolfe & Baptista, 1995: dialect 7; Chilton & Lein, 1996: dialects 8 and 9; Soha et al., 2004: dialects 10–13). Each location was assigned to the dialect sung by the majority of birds, on average 86%

(see table 2 in Soha et al., 2004; additionally, Reedsport is dialect 7; Fort Casey is dialect 6).

2.3 | DNA extraction and microsatellite analysis

We extracted genomic DNA using a phenol–chloroform protocol and quantified DNA concentration with a Smart Spec 3000 spectrophotometer (Bio-Rad Laboratories, Hercules, CA). We then genotyped all 285 individuals by amplifying 14 microsatellite loci using primers specifically developed for *Z. l. pugetensis* and following methods described in detail in Poesel et al. (2009). To minimize observer bias, the dialect identity of these individuals was unknown when genotyping. Briefly, we size-separated the amplified PCR products on an ABI PRISM[®] 3100 DNA Genetic Analyzer (Applied Biosystems, Foster City, CA) and scored allele sizes using GENEMAPPER 3.7 software (Applied Biosystems). We used the program MICRO-CHECKER (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004) to check the dataset for scoring errors and null alleles.

We summarized genetic diversity among the six song dialect populations as the total number of alleles, observed heterozygosity (H_O) and expected heterozygosity (H_E) as reported by ARLEQUIN 3.5 (Excoffier, Laval, & Schneider, 2005). We calculated the inbreeding coefficient F_{IS} following the formula $(\text{mean } H_E - \text{mean } H_O) / \text{mean } H_E$ and allelic richness (AR) using HPR are with rarefaction to 80 gene copies and the lowest sample size ($n = 40$, dialect 12; Kalinowski, 2005). For each dialect, we also counted the number of alleles detected only in that dialect (private alleles, PA).

2.4 | Genetic structure

To investigate differences in allele frequencies among dialect populations, we used four methods that complement one another: To compare allelic differentiation between designated populations, we calculated (i) traditional F -statistics and (ii) analysis of molecular variance (AMOVA), and to define clusters of genetically similar samples, we used Bayesian methodology, specifically (iii) Bayesian clustering analysis and (iv) a multivariate analysis using discriminant analysis of principal components (PCs). We also used two techniques that assess geographic patterns of differentiation: isolation by distance and spatial analysis of shared alleles (SASHA). Finally, we estimated dispersal rates among groups under three hypothetical scenarios of genetic structure.

We calculated pairwise F_{ST} values across the 13 study sites in ARLEQUIN (Excoffier et al., 2005). We conducted standard AMOVA computations using ARLEQUIN to partition genetic variation within and among dialects and themes and test for structure. We tested significance using 10,000 permutations of randomized group arrangement.

For the Bayesian clustering analysis, we used the program STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) to assign individuals to one or more cluster while maintaining Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium at each locus to identify the optimal number of clusters (K ; Pritchard et al., 2000). We used an admixture model and assumed correlated allele frequencies

(Falush, Stephens, & Pritchard, 2003). We used the LocPrior clustering algorithm implemented in STRUCTURE to take information on sample location into account. We ran two separate models. In the first model, we used the six dialect groups as prior population designation and conducted five independent iterations for each value of $K = 1-13$ sampling locations with a burn-in of 50,000 followed by 200,000 Markov chain Monte Carlo (MCMC) repetitions. In the second model, we used birds singing northern and southern song themes as prior population designation and conducted five replicate runs of $K = 1-2$ with a burn-in of 50,000 followed by 200,000 MCMC repetitions. For both models, we determined the most likely number of genetic clusters (K) based on $\ln(\Pr(X|K))$ values to identify the k for which $\Pr(K = k)$ is highest (Pritchard et al., 2000). We summarized results with the "BestK" option in the online version of CLUMPAK (<http://clumpak.tau.ac.il/>, Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015).

For the fourth method, we conducted a discriminant analysis of principal components (DAPC; Jombart, Devillard, & Balloux, 2010) as implemented in the R package adegenet 1.4-1 (ade4; Jombart, 2008). Here, we assessed the degree of differentiation of individuals among a priori defined dialect or theme groupings using discriminant analysis of PC axes summarizing the allele frequency data from dialect populations. In addition, we independently assessed the number of clusters using the function find.clusters, which runs successive K -means clustering algorithms with increasing numbers of clusters based on variation in the PCs observed in the dataset (Jombart et al., 2010). We chose the optimal number of clusters based on the lowest associated Bayesian information criterion (BIC value). We performed cross-validation of DAPC using varying numbers of PCs (with the xavDapc function in adegenet) and found that 110 PCs best described the dataset consisting of dialects and 122 PCs the dataset of themes. However, Jombart, Devillard, Dufour, and Pontier (2008) suggest that DAPC is unstable when the number of PCs exceeds $N/3$. We therefore ran all subsequent analyses using the $N/3$ criteria as a conservative measure, balancing the chances of overfitting our data against the likelihood of eliminating the signal in our dataset (95 and 81 PCs, respectively, for the analyses of song dialects and themes). For the DAPC on themes, we excluded the 42 individuals singing dialect 12, because we were interested in assigning this dialect, a northern-theme dialect occurring in the southern end of the sampling range, to the genetically most similar group. To determine how accurate our assignments of known-origin individuals were to their sampled dialect or theme, we conducted leave-one-out cross-validation analyses using 10,000 iterations on both theme and dialect groupings.

2.5 | Analysis of isolation by distance

To test whether population genetic differences followed an isolation-by-distance model, as would occur if gene flow between populations follows a stepping stone model (Hutchison & Templeton, 1999), we performed Mantel tests on site-pairwise F_{ST} values and geographic distance. We also tested for isolation by distance between populations within themes using a third matrix in which "1" indicated that two populations shared the same (northern or southern) song theme

and "0" indicated that they did not. Isolation by distance within themes might occur if the northern and southern themes originated from different founder populations and subsequently expanded with little gene flow between themes. We also performed partial Mantel tests to correlate between-site F_{ST} values with binary, same/different matrices of either song dialect or song themes, while controlling for geographic map distance. We used the program NTSYS-pc 2.1 (Rohlf, 2000) to calculate Mantel correlations, using 10,000 random permutations of the matrices to estimate p -values.

2.6 | Spatial analysis of shared alleles

We used SASHa (Kelly, Oliver, Sivasundar, & Palumbi, 2010) to further investigate potentially subtle genetic subdivision across geographic space. This analysis uses spatial and allele information to detect non-random allele distribution against an expectation of panmixia and is particularly useful in systems with high gene flow among populations. SASHa calculates the difference between the observed mean (OM) of all geographic distances between every pair of identical alleles and the expected mean (EM) under panmixia. Where OM is less than EM, alleles are considered to be aggregated (restricted allelic dispersal). Where OM is larger than EM, alleles are considered overdispersed (panmictic). We used 1,000 permutations to estimate p -values for each of the 14 loci. We then ran the analysis for all loci combined and present this result. We used the jackknifing procedure to run the overall analysis on the dataset after removing each allele, which allows the identification of those alleles that contribute to the distribution.

2.7 | Analysis of dispersal rate

We used the program MIGRATE-N 3.6 to test various hypotheses regarding connectivity of Puget Sound white-crowned sparrow populations based on dialects and themes (Beerli & Palczewski, 2010). MIGRATE-N jointly estimates effective population size θ ($4 N_e \mu$) and migration M (m/μ) between populations in a coalescent framework assuming an n -island model of population connectivity. Here, M represents the relative importance of migration vs. mutation in generating variation within and among populations. We constructed three hierarchically nested models and tested model fit using Bayes factors comparisons. The three models included the following: (i) a panmictic model in which all of the sampled populations are part of the same population, (ii) a theme model in which populations within the same (northern or southern) theme are grouped and we estimate symmetrical dispersal between these populations, and (iii) a dialect model in which populations within the same dialect are grouped and we estimate a stepping stone dispersal/migration model with symmetrical rates between adjacent dialects. If the panmictic model is rejected, then there is sufficient evidence to detect structuring of populations based on song characteristics. We used Bayesian inference with a Brownian motion microsatellite model allowing mutation rates to vary among loci. We ran preliminary trials to establish uniform priors for both θ (Min = 0, Max = 200, Delta = 20) and M (Min = 0, Max = 1,000, Delta = 100). We ran three replicates of each model with static

heating using four chains (default settings), discarded 200,000 trees as burn-in, and recorded 40,000 steps with increments of 50 (resulting in six million samples for each model). We used effective sample size values greater than 1,000 and the distribution of the posteriors to assess convergence.

To take multiple testing in the analyses of deviations from HWE, F_{ST} , and SASHA into account, we controlled for the false discovery rate (FDR; Benjamini & Hochberg, 1995).

3 | RESULTS

3.1 | Genetic diversity

All 14 microsatellite loci were highly polymorphic (9–22 alleles each). We detected some cases of null alleles using MICRO-CHECKER, but overall null allele frequencies were low, with the highest values of 5–8% for five (F09, C06, C07, C12, E02) of the 14 loci. These values have a limited effect on population genetics analyses (Chapuis & Estoup, 2007), and thus, we included all loci in subsequent analyses. Of 209 total alleles sampled, 15 were PA that occurred at low frequencies. Dialects 6 and 7 had the highest numbers of PA (Table 1). \bar{x} ($\pm SD$) of expected heterozygosity (H_E) and AR for all sampled dialects were 0.83 ± 0.08 and 11.52 ± 0.31 , respectively. Comparison to observed heterozygosity (H_O) values did not indicate consistent deviations from HWE as 77 of 84 (92%) locus-by-location tests for deviations were not significant after controlling the FDR. Inbreeding coefficients (F_{IS}) were small (\bar{x} ($\pm SD$) overall value: 0.03 ± 0.02) among all dialect populations (Table 1).

3.2 | Genetic structure

\bar{x} ($\pm SD$) overall F_{ST} was 0.004 ± 0.004 indicating little genetic differentiation among song dialects. Correspondingly, genetic differentiation was not significant in any of the 15 pairwise comparisons of dialect populations after controlling the FDR (Table S1). The highest pairwise F_{ST} value among dialects equaled 0.009. Between themes, F_{ST} equaled 0.001 ($p = .23$).

Using AMOVA, we partitioned the total genetic variation within individuals, among individuals within dialects, among dialects within

themes, and among themes. The great majority (>95%) of the total variation in allele frequencies occurred within individuals regardless of hierarchical grouping, as we would expect in panmictic populations. Still some occurred among individuals within dialects and among dialects within themes (Table 2).

Bayesian cluster analysis for song dialects using values of $\ln(\text{Pr Data})$ as described by Pritchard et al. (2000), returned the highest probability for $K = 1$. Cluster analysis using the `find.clusters` algorithm in the `adegenet` package using no a priori groupings of populations marginally identified $K = 3$ (2–4) as a potentially suitable number of clusters. However, discriminant analysis on those clusters did not distinguish group membership any better than random, as individuals were assigned with similar probabilities to each cluster.

We retained 91.7–94.2% of the total variation in the original data using the standard $N/3$ number of components to describe genetic variation in the dataset. With a priori groupings based on song dialects, a DAPC analysis on song dialects showed no signature of clustering among groups, suggesting significant overlap of dialects in genetic variance space (Fig. S1). Cross-validation on dialect DAPC analyses suggested no better than random assignment of individuals to their dialect, with 24.9% reassignment accuracy as compared to 16.7% expected by chance for each of the six dialects ($n = 285$, $Z = 2.41$, $p = .99$).

A priori groupings based on song themes (excluding individuals of dialect 12) visually indicated some separation along the first (and only) discriminant function (Figure 2a). However, in this DAPC analysis, only 57.6% of cases were correctly classified to theme, not significantly different from the 50% expected by chance for each of the two song themes ($n = 245$, $Z = 1.69$, $p = .95$; Figure 2b). Individuals in dialect 12, the northern-theme dialect geographically located in the south, were equally likely to be classified to either the northern or the southern theme (Figure 2a).

3.3 | Isolation by distance

There was no significant correlation between geographic distance and pairwise F_{ST} values between sites (Mantel $r = .07$, $p = .47$). The partial Mantel test for the correlation between sites while controlling theme identity gave an identical result (Mantel partial $r = .07$, $p = .47$;

TABLE 1 Standard diversity indices obtained from 14 microsatellite loci analyzed for six song dialect populations of Puget Sound white-crowned sparrows using ARLEQUIN

Dialect/theme	<i>n</i>	\bar{x} Na ($\pm SD$)	PA	AR ($\pm SD$)	H_O ($\pm SD$)	H_E ($\pm SD$)	F_{IS}
D6/N	51	11.57 \pm 4.05	4	11.29	0.81 \pm 0.11	0.83 \pm 0.08	0.02
D5/N	44	11.14 \pm 3.30	2	11.12	0.74 \pm 0.10	0.83 \pm 0.08	0.00
D2/S	67	12.43 \pm 3.65	2	11.43	0.81 \pm 0.10	0.84 \pm 0.06	0.04
D7/S	43	12.36 \pm 3.34	5	11.91	0.81 \pm 0.11	0.83 \pm 0.09	0.05
D1/S	40	11.71 \pm 3.45	1	11.86	0.79 \pm 0.12	0.83 \pm 0.10	0.03
D12/N	40	10.71 \pm 3.29	1	11.50	0.79 \pm 0.10	0.83 \pm 0.07	0.05

Dialects are classified into northern (N) and southern (S) themes and listed here from north to south (see also Figure 1).

Shown are number of sampled individuals (*n*), mean number of alleles per locus (mean Na), private alleles (PA), allelic richness (AR) calculated for 80 genes in HP-Rare (Kalinowski, 2005), mean observed (H_O) and expected (H_E) heterozygosities, and inbreeding coefficient (F_{IS}).

TABLE 2 Results from an AMOVA of allele frequencies in six song dialects within two song themes as calculated in ARLEQUIN

Source of variation	df	Sum of squares	Variance components	Percentage of variation	p-value
Among themes	1	6.36	-0.004	0	.90
Among dialects within themes	4	29.59	0.01	0.26	<.01
Among individuals within dialects	279	1673.09	0.27	4.62	<.001
Within individuals	285	1558.00	5.47	95.19	
Total	569	3267.04	5.74		

Figure 3). Controlling for geographic distance with partial Mantel tests, the correlations between genetic distance and either shared song dialects or shared song themes were not significant (Mantel partial $r = -.09$, $p = .16$ and $r = .01$, $p = .54$, respectively).

3.4 | Spatial analysis of shared alleles

Spatial analysis of shared alleles analysis confirmed population panmixia: The mean observed geographic dispersal of alleles was similar to the expected dispersal (OM = 260.0 km, EM = 261.7 km; $p = .54$; Fig. S2).

3.5 | Dispersal rates

The panmictic model was the most strongly supported (>99%) of the three models tested in MIGRATE. Models representing dialects or themes received little support (<1%). The posterior distribution of theta values across all iterations of each locus as well as across all loci varied between 51 and 70. Based on a microsatellite locus mutation rate of 10^{-3} or 10^{-4} (Ellegren, 2004), we calculated an effective population size of between 70,125 and 96,250 individuals.

4 | DISCUSSION

Dialect populations of *Z. l. pugetensis* along the Pacific Northwest coast show clear geographic variation in song at two levels: local dialects and larger song themes (Figure 1). Our analyses using multiple methods indicate that acoustic variation among these song dialects and themes does not correlate with genetic differentiation. Our results on song dialects confirm those of a previous study by Soha et al. (2004), which also found evidence for gene flow across song dialect boundaries; here, we also show that gene flow occurs across themes. Our findings, along with previous song analyses in this subspecies, support the conclusion that the persistence of geographic variation in song in *Z. l. pugetensis* is best explained by dispersal of individuals across dialect boundaries followed by post-dispersal song dialect sharing through selective song attrition, with little influence of genetic processes (Nelson, Marler, & Morton, 1996; Nelson et al., 2004). Vocal dialect boundaries do not appear to act as barriers to gene flow in this subspecies; rather,

the sampled dialect populations are part of one large, genetically uniform population.

4.1 | Factors promoting gene flow

Genetic differentiation and structuring among populations of a species are influenced by the factors that affect gene flow, including dispersal, assortative mating, and population size (Hartl & Clark, 1997). The relative importance of each factor depends in part on the distribution of a species, which mirrors the distribution of suitable habitat. We discuss the relevance of each of these factors for song dialect populations of *Z. l. pugetensis*.

4.2 | Dispersal

Our analysis of dispersal rates across dialect populations indicates that all of the sampled dialect populations should be treated as one large panmictic population, implying that individual *Z. l. pugetensis* frequently cross-dialect boundaries. Behavioral evidence supports this conclusion. We have observed males singing non-local dialects, particularly at sites close to dialect borders (D. A. Nelson, personal observation, 1999-2008). Some of these are first-year birds rehearsing more than one dialect but will eventually retain only the local, sometimes non-natal, dialect. Others are adults that have crystallized a non-local dialect. In addition, females will also disperse, in many songbirds even farther than males (Greenwood, 1980). Thus, females may be exposed to different song dialects and may not show a clear preference for one particular dialect as needed for genetic differentiation to occur. Tracking female dispersal requires following color-banded birds, radio telemetry or isotope or trace element analyses of biological tissues. In a recent study of these same *Z. l. pugetensis* dialect populations, we found dispersal in both males and females across dialect boundaries based on trace element profiles in feathers (Nelson et al., in review). Even a few migrants per generation will prevent genetic differentiation between populations (Grant, Grant, & Petren, 2002; Hansson et al., 2000; Keller et al., 2001).

In any case, high dispersal rates are facilitated by the lack of physical barriers, such as mountain ridges or areas of inhospitable habitat, which have been shown to result in genetic differentiation in other species (Manthey, Klicka, & Spellman, 2011). The Pacific Northwest coast from California into Washington, where we sampled songs and DNA,

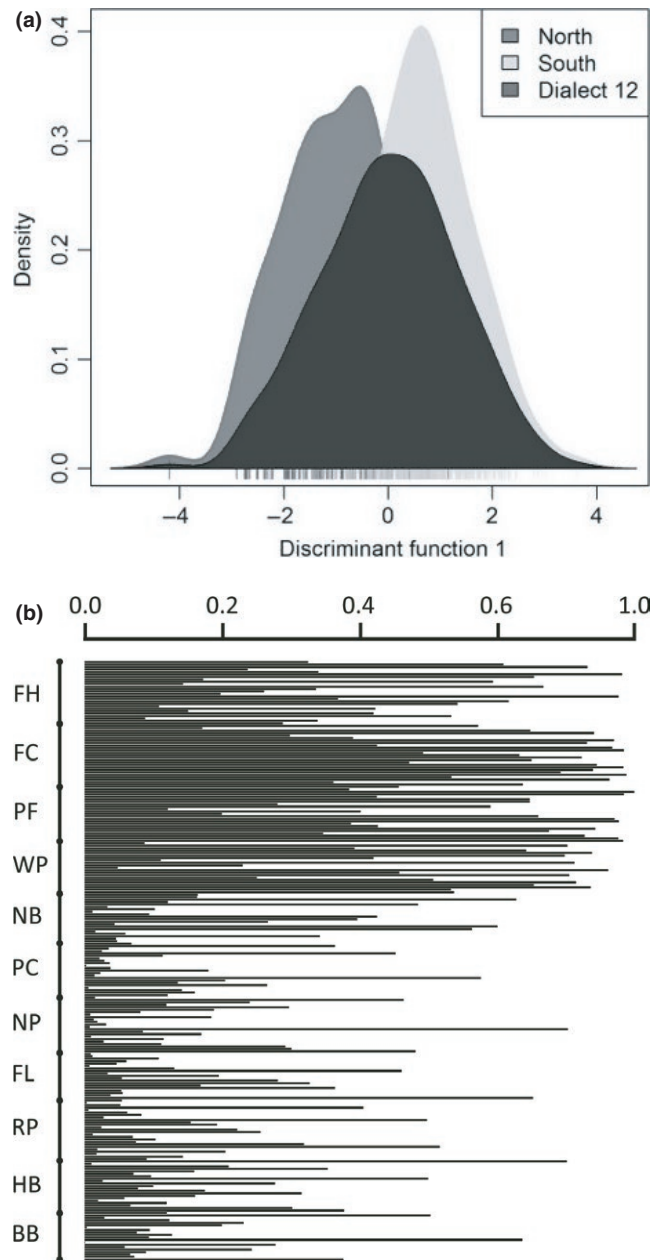


FIGURE 2 Results of discriminant function analysis in the R package *adegenet*. Panel (a) shows densities of individuals on the discriminant function between northern and southern song themes. Northern theme is shown in dark gray, southern theme in light gray. The location of individual densities of the disjunct dialect 12, a northern theme occurring in the south, is added in black. Panel (b) shows the posterior probability of assignment of individuals to either northern (black) or southern (white) themes (excluding dialect 12) as calculated in *adegenet*. Populations on the y-axis are from top to bottom (north to south): Friday Harbor (FH), Fort Canby (FC), Pack Forest (PF), Westport (WP), Nehalem Bay (NB), Pacific City (PC), Newport (NP), Florence (FL), Reedsport (RP), Horsfall Beach (HB), and Bullard's Beach (BB)

shows no major ecological discontinuities that would suggest differences in environmental selective pressures. Furthermore, *Z. l. pugetensis* readily accepts human-influenced habitats such as gardens, parks,

and pastures, which promotes a relatively continuous distribution of this subspecies (Baptista, 1977; Chilton & Lein, 1996). Some birds do inhabit more isolated habitat patches such as regenerating forest clearings or forest patches following fire, and it would be interesting to sample these birds to test for founder effects in both song and genetic make-up. The congeneric rufous-collared sparrow *Zonotrichia capensis* breeding in patchy mountainous habitat is genetically heterogeneous among sites while birds sampled at more continuous lowland sites are not (Lougheed & Handford, 1992). However, MacDougall-Shackleton and MacDougall-Shackleton (2001) argue that a possible correlation between song and genetic variation in *Z. l. oriantha* occupying patchy mountainous habitat is better explained by fitness consequences of dispersal to non-natal dialect singers than habitat patchiness itself.

4.3 | Assortative mating

In many songbird species, including the white-crowned sparrow, males are restricted to learning song types (and thus dialects) in the first few months of life. These songs therefore reflect the neighborhood of song learning. When females preferentially mate with males singing local songs of their own natal dialect, genetic structure may correlate with song variation. Even though song sharing and dialects seem to affect some mating decisions in *Z. l. pugetensis* (Poesel et al., 2012), strong assortative mating with respect to song sharing or song dialects seems unlikely in this subspecies. Yearling males that shared song with at least one territorial neighbor had higher social mating success than non-sharers in one dialect population of this subspecies (Poesel et al., 2012). However, in the same population, birds engage in extra-pair copulations and extra-pair mate choice does not correlate with song sharing. Furthermore, females whose mates had been removed gave the same responses to the local dialect as they did to songs containing phrases from a foreign dialect in a playback experiment (Nelson & Soha, 2004b). Like males, females are probably exposed to multiple song dialects during dispersal, and therefore, they do not develop strong song preferences; as a result, mating may occur randomly with respect to song dialects.

4.4 | Population size

In small populations, genetic drift can affect genetic diversity such that some alleles may accumulate while others are lost due to chance. The number of individuals sharing each dialect is unknown for *Z. l. pugetensis*, but based on the genetic data, we estimated an effective population size over all dialects of at least 70,000 breeding individuals. This suggests an average of over 10,000 breeding birds for each of the six dialects. This number is larger than for the non-migratory *Z. l. nuttalli*, for which Baker (1982) estimated 400–800 breeding birds in each of six dialects. This is not surprising, given that the song dialects of *Z. l. pugetensis* stretch over longer areas than in *Z. l. nuttalli*. Overall, our estimate of population size suggests that drift would be weak within populations of *Z. l. pugetensis*. Combined with the evidence for dispersal, this further explains the observed lack of genetic differentiation between dialect-defined populations.

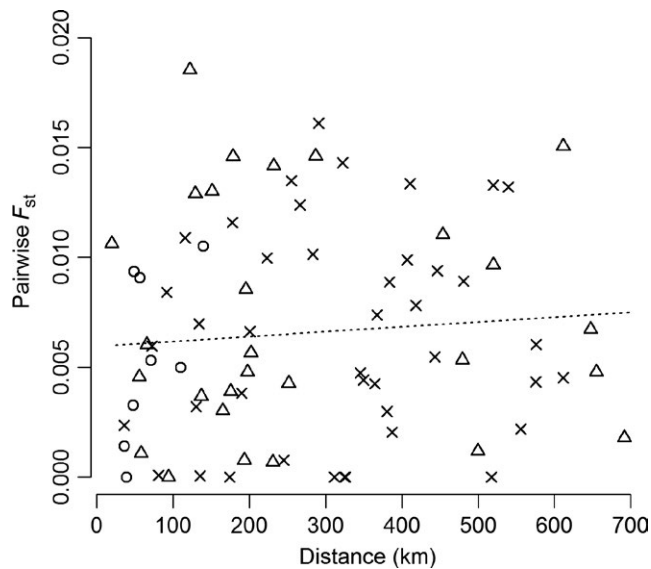


FIGURE 3 Pairwise genetic (microsatellite) distance vs. geographic distance between populations. For illustrative purposes, we included the dotted line indicating a relationship with a simple linear regression ($R^2 < .01$, $p = .54$). Circles represent populations with the same dialect, triangles with different dialects but the same theme, and crosses with different themes

4.5 | Persistence of song dialects despite dispersal between dialects

The relationship between vocal variation and genetic structure strongly depends on the timing of song learning relative to juvenile dispersal and the geographic scale of song variation relative to dispersal distance (Wright & Wilkinson, 2001). In *Z. l. pugetensis*, social learning facilitates the sharing of local song types. Young males often overproduce song types, that is, memorize and practice more song types than they eventually retain. After dispersal, they discard song types that do not match those of other males in a local neighborhood (Nelson, 2000). As discussed by Soha et al. (2004), this mechanism can explain the persistence of song dialects despite gene flow between dialect areas. From a functional perspective, males may experience social benefits from sharing local songs. Previously we found that male *Z. l. pugetensis* that share song with at least one neighbor are more likely to attract a social mate in their first year than males that do not share a neighbor's song type (Poesel et al., 2012). This effect extends beyond the first year, over the male's lifetime (Nelson & Poesel, 2013). Mating preferences based on song seem weak in this subspecies (see above) but may occur in other species or subspecies (MacDougall-Shackleton et al., 2002; O'Loghlin & Rothstein, 1995; Payne et al., 1988).

4.6 | Song themes

The two song themes in *Z. l. pugetensis* differ clearly in acoustic structure, with northern-theme songs containing two buzzes and two note complexes and southern-theme songs containing only a single buzz and note complex. Such large-scale groupings of songs have been

observed in other species. For example, northern and southern populations of swamp sparrows *Melospiza georgiana* show a correlation between song features and genetic variation across groups (Balaban, 1988). In *Z. l. pugetensis*, the two themes suggest two centers of origin for the dialects, as discussed by Soha et al. (2004), and we thus expected the founder populations to differ genetically. However, we found no evidence for genetic differentiation between these two groups in our sample. This might indicate that over time, any initial differentiation has eroded due to dispersal and population expansions. Consequently, we cannot establish the genetic origin of the founders of dialect 12: Whether local birds were tutored by northern-theme singers, as has been shown to occur in *Z. l. nuttalli* (Baptista, 1974), or whether vacant habitat in southern Oregon was founded by northern-theme singers stopping short on migration.

4.7 | Comparison to earlier work and other *Zonotrichia* (sub)species

Building on the study by Soha et al. (2004), we included two additional sample sites, used three times as many highly variable microsatellite loci, and analyzed the data with multiple statistical approaches that have recently become available. Our results confirmed that clearly defined song dialect populations show little genetic differentiation. Soha et al. (2004) reported that dialect 7, sampled at one site, Florence only, differed genetically from other sites, but this result was not replicated in the current study with a second sample site (Reedsport) for this dialect and an overall larger sample size ($n = 43$ vs. $n = 23$).

Two other subspecies, *Z. l. nuttalli* and *Z. l. oriantha*, have also been studied with respect to the correlation between song variation and genetic structure. These subspecies differ in migratory behavior and size of dialect populations, but overall, based on small F_{ST} values (Table 3), neither subspecies shows a strong connection between song and genetic structure. Studies of the congeneric rufous-collared sparrow *Z. capensis* found some clinal variation in gene frequencies, but this genetic variation did not correlate with song variation (Handford & Nottebohm, 1976; Loughheed & Handford, 1992).

4.8 | Weak correspondence between neutral genes and song variation in songbirds

We studied a system where the only obvious difference among populations is in a learned vocal character: song. Our intention was to discover whether such song differences could represent the early stages of the speciation process, as hypothesized by Baker (1982). A very small amount of genetic variation can be explained by dialects as revealed by the AMOVA, but we do not think that this is biologically significant. A disconnect between vocal variation and genetic population structure similar to that found here has been observed in at least six other bird species that learn their vocalizations and that lack apparent morphological variation (Table S2). These studies have all yielded similarly small estimates of genetic distances (Table 3). This agreement is remarkable given natural variation among study systems, the use of a wide range of analytical methods (traditional and Bayesian

TABLE 3 Genetic distance measures among population subdivisions based on distinct vocal features in one hummingbird, one parrot, and seven songbird species (including three subspecies of *Zonotrichia leucophrys*)

Reference	Species	Genetic marker (#)	Genetic distance	\bar{x} value (\pm SD)
4	<i>Campylopterus curvipennis</i>	Microsatellite (10)	F_{ST}	0.021 \pm 0.002
17	<i>Amazona auropalliata</i>	mtDNA	Tamura-Nei	0.027
18	<i>Amazona auropalliata</i>	Microsatellite (8)	F_{ST}	0.006
12	<i>Ptilonorhynchus violaceus</i>	Microsatellite (11)	F_{ST}^a	0.051 \pm 0.043
13	<i>Colluricincla harmonica</i>	Microsatellite (11)	F_{ST}^b	0.032 \pm 0.011
8	<i>Sylvia atricapilla</i>	Microsatellite (17)	F_{ST}^c	0.105 \pm 0.009
7	<i>Nectarinia osea</i>	mtDNA, Microsatellite (5)	F_{ST}^d	0
19	<i>Nectarinia osea</i>	Microsatellite (5)	F_{ST}^e	0.046 \pm 0.058
10	<i>Zonotrichia capensis</i>	Allozymes	F_{DT}^f	0.06
6	<i>Z. capensis</i>	Allozymes	$D_{(G)}^g$	0.06 \pm 0.02
11	<i>Z. leucophrys oriantha</i>	Microsatellite (8)	F_{ST}^h	0.016
1, 2	<i>Z. l. nuttalli</i>	Allozymes	F_{ST}	0.042
20	<i>Z. l. nuttalli</i>	Allozymes	F_{DT} ; Nei's D^i	0.033; 0.014 \pm 0.031
5	<i>Z. l. nuttalli</i>	Allozymes	Nei's D	0.029 \pm 0.019
9	<i>Z. l. nuttalli</i>	SNP	F_{ST}^j	0 \pm 0
	<i>Z. l. pugetensis</i>	SNP	F_{ST}	0 \pm 0
16	<i>Z. l. pugetensis</i>	Microsatellite (4)	F_{ST}^k	0.014 \pm 0.009
15	<i>Z. l. pugetensis</i>	Microsatellite (14)	F_{ST}	0.004
3	<i>Melospiza georgiana</i>	Allozymes	Nei's D^l	0.007 \pm 0.007
14	<i>Passerina cyanea</i>	Allozymes	F_{ST}	0.004

Species are presented in taxonomic order. A summary of further statistical analyses of each study can be found in Appendix Table S2.

References: 1 Baker (1982); 2 Baker et al. (1982); 3 Balaban (1988); 4 González and Ornelas (2014); 5 Hafner and Petersen (1985); 6 Handford and Nottebohm (1976); 7 Leader, Geffen, Mokady, and Yom-Tov (2008); 8 Linossier, Zsebök, Baudry, Aubin, and Courvoisier (2016); 9 Lipshutz et al. (2017); 10 Loughheed and Handford (1992); 11 MacDougall-Shackleton and MacDougall-Shackleton (2001); 12 Nicholls, Austin, Moritz, and Goldizen (2006); 13 Pavlova et al. (2012); 14 Payne and Westneat (1988); 15 this study; 16 Soha et al. (2004); 17 Wright and Wilkinson (2001); 18 Wright, Rodriguez, and Fleischer (2005); 19 Yoktan et al. (2011); 20 Zink and Barrowclough (1984).

^a F_{ST} among vocally distinct populations calculated from values in Table 3.

^b F_{ST} calculated as $\bar{x} \pm SD$ of six of 60 pairwise F_{ST} values that were significantly different from zero.

^c F_{ST} value among populations that shared song, personal communication with J. Linossier.

^dNegative F_{ST} value was set to zero.

^e F_{ST} calculated as $\bar{x} \pm SD$ of F_{ST} values among all populations, personal communication with E. Geffen.

^fCorrected F -statistics for dialect-total (F_{DT}) as reported in Table S2.

^g $\bar{x} \pm SD$ of $D_{(G)}$ calculated from values from Table 6 in Handford and Nottebohm 1976.

^hAmong dialect regions within the Sierra Nevada, personal communication with E. MacDougall-Shackleton.

ⁱ $\bar{x} \pm SD$ genetic distance (Nei's D) calculated from values in Table 1 while excluding six values among sample localities with the same song dialect (RCA-LR, DP-PL-CC, LIM-MH, VIS-CGR).

^j $\bar{x} \pm SD$ of F_{ST} values among localities within *Z. l. nuttalli* and within *Z. l. pugetensis* calculated from values in Table S2. All F_{ST} values within *Z. l. nuttalli* and all but one within *Z. l. pugetensis* were negative. Negative F_{ST} values do not have a biological meaning and can be considered equal to zero; thus, we set all negative F_{ST} values to zero.

^kOverall F_{ST} value (Table 1) based on 55 site-wise comparisons which include four comparisons of sites that share a song dialect with a mean $F_{ST} = 0.003$ (outlier site Florence included).

^lThe values of Nei's D reported in Balaban (1988) are relative measures of genetic distance and should be treated with care for comparisons with other studies and species.

analysis), differences in the number and type of genetic markers used (allozymes, microsatellites, mitochondrial DNA, single nucleotide polymorphisms), the variability of these loci (number of alleles), the number of sample sites, the number of individuals sampled at each site, and the description of song and its variability. Furthermore, these populations within species differ widely in their life history (sedentary to migratory) and the occupied ecological niches (grasslands to woodlands, continuous to patchy habitat).

In contrast, several studies comparing morphologically distinct subspecies, which presumably represent longer divergence times, have found genetic differences between (some) subspecies that correlate with song differences (Dingle, Halfwerk, & Slabbekoorn, 2008; Irwin, Thimgan, & Irwin, 2008; Lipshutz, Overcast, Hickerson, Brumfield, & Derryberry, 2017; Ortiz-Ramírez, Andersen, Zaldívar-Riverón, Ornelas, & Navarro-Sigüenza, 2016; Toews & Irwin, 2008). We cannot rule out that the use of neutral genetic markers is not sufficient to detect the

fine scale genetic structure of populations (Reed & Frankham, 2001). Furthermore, in *Z. l. pugetensis* and in at least some of the other species studied, any genetic divergence would have to be recent because of common geological history.

We conclude that our findings for *Z. l. pugetensis* in which males learn song early in life, are consistent with most studies to date of song and population structure within species or subspecies of songbirds. The processes of learning and dispersal mean that vocalizations are free to vary independently of patterns of divergence in neutral genetic markers.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL NOTE

Procedures were approved by The Ohio State University IACUC Protocol 2000A005.

REFERENCES

- Baker, M. C. (1975). Song dialects and genetic differences in white-crowned sparrows (*Zonotrichia leucophrys*). *Evolution*, *29*, 116–241.
- Baker, M. C. (1982). Vocal dialect recognition and population genetic consequences. *American Zoologist*, *22*, 561–569.
- Baker, M. C. (1987). Intergradation of song between two subspecies of white-crowned sparrows on the west coast of North America. *Ornis Scandinavica*, *18*, 265–268.
- Baker, M. C., & Thompson, D. B. (1985). Song dialects of white-crowned sparrows: Historical processes inferred from patterns of geographic variation. *Condor*, *87*, 127–141.
- Baker, M. C., Thompson, D. B., Sherman, G. L., Cunningham, M. A., & Tomback, D. F. (1982). Allozyme frequencies in a linear series of song dialect populations. *Evolution*, *36*, 1020–1029.
- Balaban, E. (1988). Cultural and genetic variation in swamp sparrows (*Melospiza georgiana*). *Behaviour*, *105*, 250–290.
- Baptista, L. F. (1974). The effects of songs of wintering white-crowned sparrows on song development in sedentary populations of the species. *Ethology*, *34*, 147–171.
- Baptista, L. F. (1977). Geographic variation in song and dialects of the Puget Sound white-crowned sparrow. *Condor*, *79*, 356–370.
- Beecher, M. D., & Brenowitz, E. A. (2005). Functional aspects of song learning in songbirds. *Trends in Ecology & Evolution*, *20*, 143–149.
- Beerli, P., & Palczewski, M. (2010). Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics*, *185*, 313–326.
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Statistical Methodology)*, *57*, 289–300.
- Catchpole, C. K., & Slater, P. J. (2003). *Bird song: Biological themes and variations*. Cambridge, UK: Cambridge University Press.
- Chapuis, M. P., & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, *24*, 621–631.
- Chilton, G., & Lein, M. R. (1996). Long-term changes in songs and song dialect boundaries of Puget Sound White-crowned Sparrows. *Condor*, *98*, 567–580.
- DeWolfe, B. B., & Baptista, L. F. (1995). Singing behavior, song types on their wintering grounds and the question of leap-frog migration in Puget Sound White-crowned Sparrows. *Condor*, *97*, 376–389.
- Dieckmann, U., & Doebeli, M. (1999). On the origin of species by sympatric speciation. *Nature*, *400*, 354–357.
- Dingle, C., Halfwerk, W., & Slabbekoorn, H. (2008). Habitat-dependent song divergence at subspecies level in the grey-breasted wood-wren. *Journal of Evolutionary Biology*, *21*, 1079–1089.
- Ellegren, H. (2004). Microsatellites: Simple sequences with complex evolution. *Nature Reviews Genetics*, *5*, 435–445.
- Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics*, *1*, 47–50.
- Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, *164*, 1567–1587.
- González, C., & Ornelas, J. F. (2014). Acoustic divergence with gene flow in a lekking hummingbird with complex songs. *PLoS One*, *9*, e109241. <https://doi.org/10.1371/journal.pone.0109241>
- Grant, P. R., & Grant, B. R. (1997). Genetics and the origin of bird species. *Proceedings of the National Academy of Sciences*, *94*, 7768–7775.
- Grant, P. R., Grant, B. R., & Petren, K. (2002). A population founded by a single pair of individuals: Establishment, expansion, and evolution. In A. P. Hendry, & M. T. Kinnison (Eds.), *Microevolution rate, pattern, process* (pp. 359–382). The Netherlands: Springer Science & Business Media.
- Greenwood, P. J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, *28*, 1140–1162.
- Hafner, D. J., & Petersen, K. E. (1985). Song dialects and gene flow in the white-crowned sparrow, *Zonotrichia leucophrys nuttalli*. *Evolution*, *39*, 687–694.
- Handford, P., & Nottebohm, F. (1976). Allozymic and morphological variation in population samples of rufous-collared sparrow, *Zonotrichia capensis*, in relation to vocal dialects. *Evolution*, *30*, 802–817.
- Hansson, B., Bensch, S., Hasselquist, D., Lillandt, B. G., Wennerberg, L., & Von Schantz, T. (2000). Increase of genetic variation over time in a recently founded population of great reed warblers (*Acrocephalus arundinaceus*) revealed by microsatellites and DNA fingerprinting. *Molecular Ecology*, *9*, 1529–1538.
- Hartl, D. L., & Clark, A. G. (1997). *Principles of population genetics* (4th ed.). Sunderland, MA: Sinauer associates.
- Hegelbach, J. (1986). Song dialects and mating success in corn bunting males *Emberiza calandra*. *Ornithologischer Beobachter*, *83*, 253–256.
- Hutchison, D. W., & Templeton, A. R. (1999). Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, *53*, 1898–1914.
- Irwin, D. E., Thimman, M. P., & Irwin, J. H. (2008). Call divergence is correlated with geographic and genetic distance in greenish warblers (*Phylloscopus trochiloides*): A strong role for stochasticity in signal evolution? *Journal of Evolutionary Biology*, *21*, 435–448.
- Jombart, T. (2008). ADEGENET: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 1403–1405.

- Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*, 94.
- Jombart, T., Devillard, S., Dufour, A. B., & Pontier, D. (2008). Revealing cryptic spatial patterns in genetic variability by a new multivariate method. *Heredity*, *101*, 92–103.
- Kalinowski, S. T. (2005). hp-rare 1.0: A computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Resources*, *5*, 187–189.
- Keller, L. F., Jeffery, K. J., Arcese, P., Beaumont, M. A., Hochachka, W. M., Smith, J. N., & Bruford, M. W. (2001). Immigration and the ephemerality of a natural population bottleneck: Evidence from molecular markers. *Proceedings of the Royal Society B: Biological Sciences*, *268*, 1387–1394.
- Kelly, R. P., Oliver, T. A., Sivasundar, A., & Palumbi, S. R. (2010). A method for detecting population genetic structure in diverse, high gene-flow species. *Journal of Heredity*, *101*, 423–436.
- Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*, 1179–1191.
- Leader, N., Geffen, E., Mokady, O., & Yom-Tov, Y. (2008). Song dialects do not restrict gene flow in an urban population of the orange-tufted sunbird, *Nectarinia osea*. *Behavioral Ecology and Sociobiology*, *62*, 1299–1305.
- Linossier, J., Zsebök, S., Baudry, E., Aubin, T., & Courvoisier, H. (2016). Acoustic but no genetic divergence in migratory and sedentary populations of blackcaps, *Sylvia atricapilla*. *Biological Journal of the Linnean Society*, *119*, 68–79.
- Lipshutz, S. E., Overcast, I. A., Hickerson, M. J., Brumfield, R. T., & Derryberry, E. P. (2017). Behavioural response to song and genetic divergence in two subspecies of white-crowned sparrows (*Zonotrichia leucophrys*). *Molecular Ecology*, *26*, 3011–3027.
- Longmire, J. L., Maltbie, M., & Baker, R. J. (1997). *Use of "lysis buffer" in DNA isolation and its implication for museum collections*. Texas, US: Museum of Texas Tech University.
- Loughheed, S. C., & Handford, P. (1992). Vocal dialects and the structure of geographic variation in morphological and allozymic characters in the rufous-collared sparrow, *Zonotrichia capensis*. *Evolution*, *46*, 1443–1456.
- MacDougall-Shackleton, E. A., Derryberry, E. P., & Hahn, T. P. (2002). Nonlocal male mountain white-crowned sparrows have lower paternity and higher parasite loads than males singing local dialect. *Behavioral Ecology*, *13*, 682–689.
- MacDougall-Shackleton, E. A., & MacDougall-Shackleton, S. A. (2001). Cultural and genetic evolution in mountain white-crowned sparrows: Song dialects are associated with population structure. *Evolution*, *55*, 2568–2575.
- Manthey, J. D., Klicka, J., & Spellman, G. M. (2011). Isolation-driven divergence: Speciation in a widespread North American songbird (Aves: certhiidae). *Molecular Ecology*, *20*, 4371–4384.
- Marler, P., & Nelson, D. A. (1992). Action-based learning: A new form of developmental plasticity in bird song. *Netherlands Journal of Zoology*, *43*, 91–103.
- Nelson, D. A. (1999). Ecological influences on vocal development in the white-crowned sparrow. *Animal Behaviour*, *58*, 21–36.
- Nelson, D. A. (2000). Song overproduction, selective attrition and song dialects in the white-crowned sparrow. *Animal Behaviour*, *60*, 887–898.
- Nelson, D. A., Hallberg, K. I., & Soha, J. A. (2004). Cultural evolution of Puget sound white-crowned sparrow song dialects. *Ethology*, *110*, 879–908.
- Nelson, D. A., Marler, P., & Morton, M. L. (1996). Overproduction in song development: An evolutionary correlate with migration. *Animal Behaviour*, *51*, 1127–1140.
- Nelson, D. A., & Poessel, A. (2009). Does learning produce song conformity or novelty in white-crowned sparrows, *Zonotrichia leucophrys*? *Animal Behaviour*, *78*, 433–440.
- Nelson, D. A., & Poessel, A. (2013). Song sharing correlates with lifetime social pairing success but not territory tenure in the Puget Sound white-crowned sparrow. *Behavioral Ecology and Sociobiology*, *67*, 993–1000.
- Nelson, D. A., & Poessel, A. (2014). Tutor choice and imitation accuracy during song learning in a wild population of the Puget Sound white-crowned sparrow. *Behavioral Ecology and Sociobiology*, *68*, 1741–1752.
- Nelson, D. A., & Soha, J. A. (2004a). Perception of geographical variation in song by male Puget Sound white-crowned sparrows, *Zonotrichia leucophrys pugetensis*. *Animal Behaviour*, *68*, 395–405.
- Nelson, D. A., & Soha, J. A. (2004b). Male and female white-crowned sparrows respond differently to geographic variation in song. *Behaviour*, *141*, 53–69.
- Nicholls, J. A., Austin, J. J., Moritz, C., & Goldizen, A. W. (2006). Genetic population structure and call variation in a passerine bird, the satin bowerbird, *Ptilonorhynchus violaceus*. *Evolution*, *60*, 1279–1290.
- O'Loughlen, A. L., & Rothstein, S. I. (1995). Culturally correct song dialects are correlated with male age and female song preferences in wild populations of brown-headed cowbirds. *Behavioral Ecology and Sociobiology*, *36*, 251–259.
- Ortiz-Ramírez, M. F., Andersen, M. J., Zaldívar-Riverón, A., Ornelas, J. F., & Navarro-Sigüenza, A. G. (2016). Geographic isolation drives divergence of uncorrelated genetic and song variation in the Ruddy-capped Nightingale-Thrush (*Catharus frantzii*; Aves: Turdidae). *Molecular Phylogenetics and Evolution*, *94*, 74–86.
- Pavlova, A., Amos, J. N., Goretskaia, M. I., Beme, I. R., Buchanan, K. L., Takeuchi, N., & Sunnucks, P. (2012). Genes and song: Genetic and social connections in fragmented habitat in a woodland bird with limited dispersal. *Ecology*, *93*, 1717–1727.
- Payne, R. B., Payne, L. L., & Doehlert, S. M. (1988). Biological and cultural success of song memes in indigo buntings. *Ecology*, *69*, 104–117.
- Payne, R. B., & Westneat, D. F. (1988). A genetic and behavioral analysis of mate choice and song neighborhoods in indigo buntings. *Evolution*, *42*, 935–947.
- Podos, J., & Warren, P. S. (2007). The evolution of geographic variation in birdsong. *Advances in the Study of Behavior*, *37*, 403–458.
- Poesel, A., Gibbs, H. L., & Nelson, D. A. (2009). Twenty-one novel microsatellite DNA loci isolated from the Puget Sound white-crowned sparrow, *Zonotrichia leucophrys pugetensis*. *Molecular Ecology Resources*, *9*, 795–798.
- Poesel, A., Nelson, D. A., & Gibbs, H. L. (2012). Song sharing correlates with social but not extrapair mating success in the white-crowned sparrow. *Behavioral Ecology*, *23*, 627–634.
- Poesel, A., Nelson, D. A., Gibbs, H. L., & Olesik, J. W. (2008). Use of trace element analysis of feathers as a tool to track fine-scale dispersal in birds. *Behavioral Ecology and Sociobiology*, *63*, 153–158.
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.
- Reed, D. H., & Frankham, R. (2001). How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution*, *55*, 1095–1103.
- Rohlf, F. J. (2000). *NTSYS-PC numerical taxonomy and multivariate system, version 2.1*. Setauket, NY: Exeter Publishing.
- Slabbekoorn, H., & Smith, T. B. (2002). Bird song, ecology and speciation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *357*, 493–503.
- Smith, J. M. (1966). Sympatric speciation. *American Naturalist*, *100*, 637–650.
- Soha, J. A., Nelson, D. A., & Parker, P. G. (2004). Genetic analysis of song dialect populations in Puget Sound white-crowned sparrows. *Behavioral Ecology*, *15*, 636–646.
- Toews, D. P., & Irwin, D. E. (2008). Cryptic speciation in a Holarctic passerine revealed by genetic and bioacoustic analyses. *Molecular Ecology*, *17*, 2691–2705.
- Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Resources*, *4*, 535–538.



- Wright, T. F., Rodriguez, A. M., & Fleischer, R. C. (2005). Vocal dialects, sex-biased dispersal, and microsatellite population structure in the parrot *Amazona auropalliata*. *Molecular Ecology*, *14*, 1197–1205.
- Wright, T. F. & Wilkinson, G. S. 2001: Population genetic structure and vocal dialects in an amazon parrot. *Proceedings of the Royal Society of London. Series B: Biological Sciences* *268*, 609–616.
- Yoktan, K., Geffen, E., Ilany, A., Yom-Tov, Y., Naor, A., & Leader, N. (2011). Vocal dialect and genetic subdivisions along a geographic gradient in the orange-tufted sunbird. *Behavioral Ecology and Sociobiology*, *65*, 1389–1402.
- Zink, R. M., & Barrowclough, G. F. (1984). Allozymes and song dialects: A reassessment. *Evolution*, *38*, 444–448.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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