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Genetic Analysis of Song Dialect Populations in Puget Sound White-Crowned Sparrows

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ABSTRACT: The relationship between cultural variation and biological variation among natural populations has been the subject of both theoretical and empirical study. *Zonotrichia leucophrys pugetensis* is one of three subspecies of white-crowned sparrow known to form geographical song dialects. We investigated whether these dialects correspond to genetic differences among *Z. l. pugetensis* populations. We compared allele frequencies at four microsatellite loci in males from 11 sites spanning six dialects over the subspecies' range in Oregon and Washington. Cluster analysis and genotype assignment tests indicated no tendency for sample sites within dialect areas to be genetically more similar than are sites from different dialect areas. AMOVA tests revealed high within-site variation and low but significant cross-site and cross-dialect-area variation. Finally, genetic distance between sites was not correlated with dialect differences when the effect of geographic distance was controlled statistically. We compare our finding of low genetic differentiation among *Z. l. pugetensis* dialect populations to results of previous studies on *Z. l. nuttalli* and *Z. l. oriantha*. Because genetic structuring appears weaker than cultural (song dialect) structure in this species, we discuss the behavioral mechanisms underlying dialect maintenance in the presence of apparent gene flow.

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The coevolution of culturally and genetically transmitted systems of information received attention from theoreticians beginning in the 1980s (Boyd and Richerson, 1985; Cavalli-Sforza and Feldman, 1981; Lumsden and Wilson, 1981; Pulliam and Dunford, 1980). The first models, which treated culture as a system of dual inheritance in which genes and learning interact, were developed largely with human cultural traditions in mind, in part because there are relatively few examples of culture in animals. More recent studies have addressed how early mate preference learning (sexual imprinting), a trait common in birds, might influence speciation (Irwin and Price, 1999; Kondrashov and Shpak, 1998; Laland, 1994; ten Cate and Vos, 1999). These studies suggest that sexual imprinting, modeled as a culturally transmitted trait, may lead to genetic subdivision of populations.

Bird song provides a well-documented example of a culturally inherited trait in nonhuman animals (Mundinger, 1980). Within certain genetic limitations, or song learning “predispositions,” most songbirds acquire particular songs through imitation of models produced by conspecifics. When birds acquire their songs in the same geographic area in which they themselves establish breeding territories, local similarities in the details of song structure can result (Krebs and Kroodsma, 1980). Regional differences in song structure, in turn, can arise when inaccuracies in song imitation, or improvisations, spread through subpopulations located in different areas of the breeding range. The resulting geographic distributions of shared song structure are called song dialect areas. We will use the term “dialect” to refer to the cultural trait itself.

In species such as the white-crowned sparrow (*Zonotrichia leucophrys*), in which song is memorized early in life (Marler, 1970; Nelson, 1998) and males are the primary singers, there are two general ways in which song dialect areas might be maintained over time. First, young males might wander and memorize songs of multiple dialects during their first summer, and then retain only the song that matches the dialect of the area in which they end up settling to breed the following spring (Nelson, 2000). The gene flow occurring in this case would lead to the decoupling of cultural (song) and genetic evolution. Patterns of cultural and genetic variation would also be decoupled if males routinely memorized new songs on the breeding grounds after dispersal, but this has little empirical support in migratory populations of this species (Nelson, 1998; Nelson et al., 2001).

Second, young males might memorize the song of their natal dialect and not disperse outside of the dialect area boundaries. In this case, by displaying philopatry to their natal dialect area, males would limit the rate of gene flow between dialect areas. Gene flow between dialect areas would be further restricted if females were also to breed preferentially in the area of their natal dialect by mating assortatively based on song. Under these conditions, genetic drift (Marler and Tamura, 1962) or local selection (Nottebohm, 1969) might then cause dialect populations to diverge over time into genetically distinct populations. Boyd and Richerson (1987) provide a formal model of how cultural “markers” can evolve in the context of locally adapted populations, but even in the absence of local adaptation, gene flow between dialect areas could be reduced simply as a consequence of when birds learn and how they recognize their songs (Laland, 1994). Models suggest that speciation could occur via assortative mating (Kondrashov and Shpak, 1998). Passerines are highly speciose, and one theory proposes that song learning and the resulting song dialects have facilitated speciation in

this taxon (see Baker, 1982; cf. Baptista and Trail, 1992). The possibility that genetic divergence of song dialect populations represents a first step in the process of speciation has inspired a number of investigations into whether such divergence has, in fact, occurred.

Studies of the rufous-collared sparrow *Zonotrichia capensis* in South America have found no congruence between song dialect areas and genetic variation—in particular, differences in allozyme frequencies (Handford and Nottebohm, 1976; Loughheed and Handford, 1992; Nottebohm and Selander, 1972) or in mtDNA sequences (Loughheed et al., 1993). In the brown-headed cowbird, *Molothrus ater*, body size and beak flange color distributions indicate substantial gene flow between subspecies with different learned flight whistle dialects, and between dialect areas within one subspecies (Fleischer and Rothstein, 1988). In contrast, Balaban (1988) reported parallel variation in allozyme frequencies and population-wide song repertoires in the swamp sparrow, *Melospiza georgiana*. The most promising evidence for genetic differences between song dialect populations came from a study done by Baker and colleagues (1982) on a sedentary subspecies of white-crowned sparrow, *Z. l. nuttalli*. Two criticisms of this work (Hafner and Petersen, 1985; Zink and Barrowclough, 1984) suggested that the genetic variation reported among *Z. l. nuttalli* dialect populations might (1) primarily reflect variation owing to geographic distance and (2) not be concordant with actual song dialect boundaries. Nonetheless, the original investigators (Baker and Cunningham, 1985; Baker et al., 1984) maintain that allozyme differences do correspond to dialect differences in *Z. l. nuttalli* populations at Point Reyes, California. We include a reanalysis of the *Z. l. nuttalli* data by using analytical methods similar to ours both to reexamine the previously reported results and to allow more direct comparison with those presented here for *Z. l. pugetensis*.

More recently, MacDougall-Shackleton and MacDougall-Shackleton (2001) examined genetic variation among dialect populations in a migratory subspecies of white-crowned sparrow, *Z. l. oriantha*. They measured variation in microsatellite loci among males from eight dialect areas. Microsatellite loci have gained favor as a marker in studies of population structure because of their presumed selective neutrality and their relatively rapid mutation rate. By using this more sensitive marker, they found that a significant but small amount of genetic variation occurred among dialect areas rather than among sites within dialect areas, suggesting that dialect and genetic variation are to some extent correlated in this subspecies.

In the study presented here, we investigated genetic variation among song dialect populations in the third of the three subspecies of white-crowned sparrow known to form vocal dialects (Baptista, 1977; Chilton and Lein, 1996). This subspecies, *Z. l. pugetensis*, breeds in coastal areas of the northwestern United States, where it forms relatively larger dialect areas than do *Z. l. nuttalli* and *Z. l. oriantha*. The trill at the end of this subspecies' song was described as a dialect marker by Baptista (1977) because trill variants have a more limited geographical distribution than do syllables in the song's introduction. These easily distinguished trill-based dialects persist today (Figure 1), and a series of song playback tests has shown that the territorial response of *Z. l. pugetensis* males is influenced more by variation in the trill than by variation in the other phrases (Nelson and Soha, in press). We therefore used the terminal trill as our dialect marker. To test the hypothesis that genetic distance is correlated with differences in

song structure, we analyzed variation at four microsatellite loci in males from 11 sites spanning six dialect areas over the range of the subspecies in Oregon and Washington.

METHODS

Samples

We collected blood samples from 252 male white-crowned sparrows in 11 populations distributed across six dialect areas along the coast of Oregon and Washington states from 1999–2001 (Figure 1). Site names and abbreviations are listed in Table 2. We sought to sample two sites from each dialect except the northernmost (dialect 6), such that distances between neighboring sites were similar both within and across dialect boundaries. Realization of this goal was limited by the presence of urban areas (where birds existed but could not readily be studied), and by the varying size of dialect areas. Birds were mist-netted or caught in seed-baited traps and sexed by examination of the cloacal protuberance. We placed a metal band on one leg of each bird and a unique pair of colored bands on the other. Up to two capillary tubes of blood (100 μ l or less) were taken from the brachial wing vein and stored in Longmire's lysis buffer (0.1 M Tris, 0.1 M EDTA, 0.01 M NaCl, 0.5% SDS at pH 8.0; Longmire et al., 1986).

Song analysis

We recorded songs from most males just before capture or within a few days after release, using a Sennheiser MKH70 shotgun microphone and Sony TCD10 digital tape recorder. To map the distribution of song dialect areas, we also recorded songs from 321 males at 33 other sites from 1997–2001. Sound spectrograms were generated by using Signal (Engineering Design, 1999) and inspected visually by D.A.N. and J.A.S. to assign songs to dialects. Our recordings are archived in the collection of the Borror Laboratory of Bioacoustics at The Ohio State University (<http://blb.biosci.ohio-state.edu>).

Based on the terminal trill, we classified the dialect sung by each recorded male as local, foreign, or hybrid (containing a combination of trill notes from local and foreign dialects). Figure 2 depicts variation within and between two dialects, including a hybrid song. Partial songs with no terminal trill were recorded from some males. We classified these songs as local if the last phrase in the recorded song was typical of the local dialect, and as inconclusive otherwise. Figure 1 depicts the locations of the dialects. Known contact zones between dialects are 20–30 km wide and contain males singing both of the dialects to the north and south and/or hybrid songs. With the exception of Newport, we avoided genetic sampling from dialect hybrid zones.

To compare geographic variation in song to that in genes, we used two measures of song similarity: a binary measure of dialect identity (same/different trill), and a continuous measure based on acoustic measurements of the songs occurring at each site. The binary measure was used to test the hypothesis that a categorical distinction based on the trill alone is correlated with genetic differentiation. The continuous

measure addressed the possibility that all parts of the song correspond in a graded manner to genetic differences among populations. Following methods described elsewhere (Nelson et al., 2001), we measured 31 frequency and temporal features of song phrases other than the introductory whistle in 12–16 songs, each from a different male, at each of our 11 study sites. We excluded the whistle because it does not vary geographically (Nelson DA, unpublished data). We next used principal components analysis to derive 11 principal components that summarized 78% of the variation in the original 31 variables. To represent the “average” song at each site, we then calculated the site mean for each of the 11 principal components. We used the Euclidean distance between the average song at each site in this 11-dimensional space as our continuous measure. We compared our subjective visual assessment of song similarity to a UPGMA cluster phenogram of this acoustic distance between songs at the 11 sites (Figure 3).

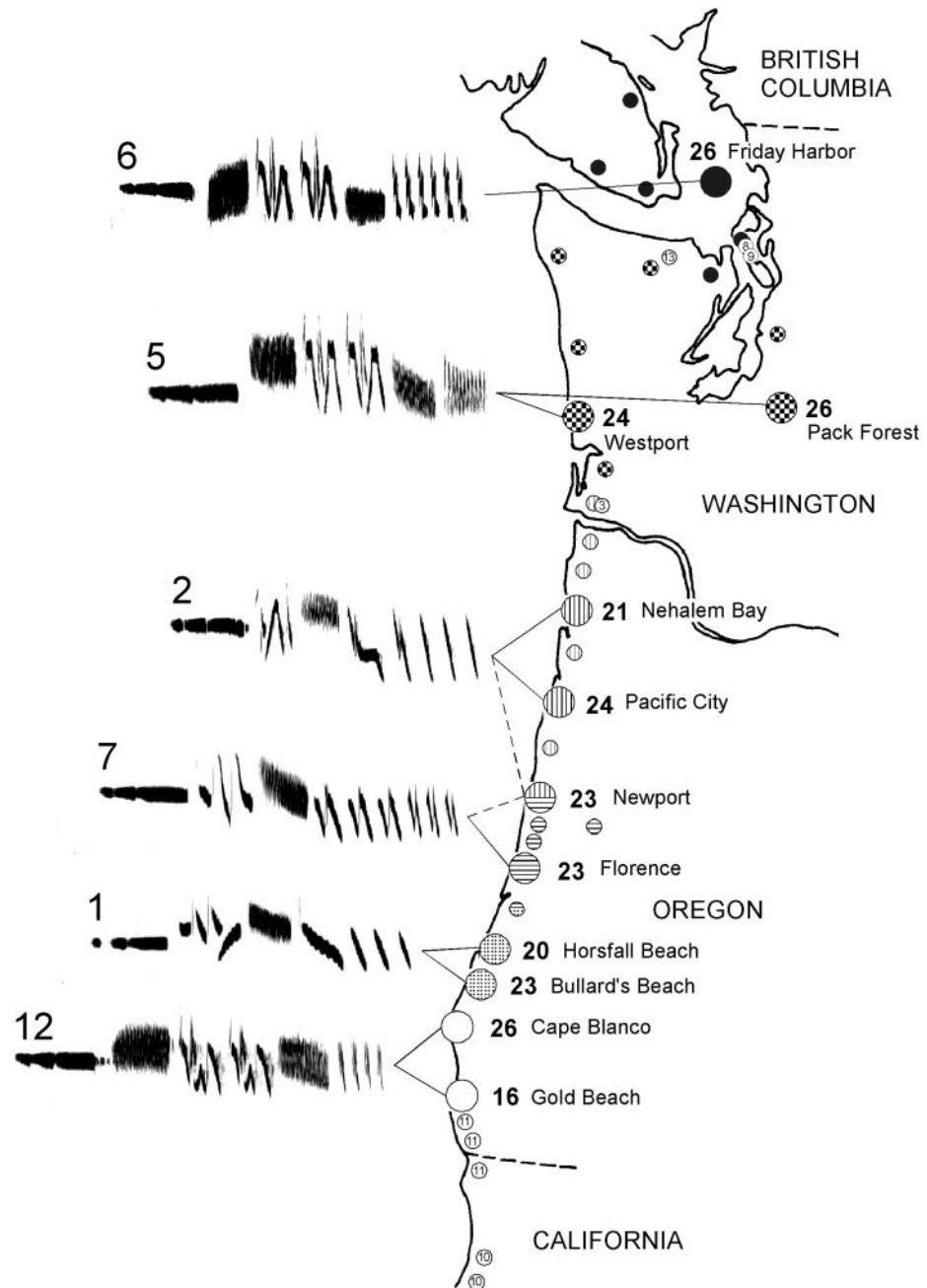
Genetic assay

Variation was assessed at four microsatellite loci previously described in other species (Table 1). DNA was extracted from blood samples and purified by using phenol and chloroform, and the concentration of purified DNA was calculated from spectrophotometer readings. We ran 5- μ l PCR reactions including 40 ng DNA, 20 mM Tris-HCL (pH 8.4), 50 mM KCl, 1 mM each dNTP, 1 μ M each primer, and 0.25 units Taq polymerase. MgCl₂ was included at concentrations of 3 mM for locus GF01, 2 mM for loci YW16 and GF12, and 1.5 mM for locus YW01. Bovine serum albumin was included at 0.2 μ g/ml for YW01 and GF12, 0.18 μ g/ml for YW16, and 0.16 μ g/ml for GF01. All loci were amplified under the following thermal conditions: two cycles of 45 s at 94C, 30 s at 55C, and 30 s at 72C; then 10 cycles of 30 s at 94C, 30 s at 54C–50C in a “touchdown” series (two cycles at each annealing temperature), and 30 s at 72C; and finally 36 cycles of 30 s at each of 94C, 49C, and 72C.

PCR products were electrophoresed in 7.5% polyacrylamide gels, stained with ethidium bromide, and photographed under ultraviolet fluorescence. Premanufactured DNA ladder segments (123-bp and 1-kb mixtures, GIBCO BRL) were used initially as size reference markers. Once all individuals had been genotyped, relative allele sizes were verified in cross-sample-site comparison gels.

Figure 1

Map of *Z. l. pugetensis* sample sites (large dots) in the northwestern United States. Numbers of males sampled at each site and site names are indicated. Site markers are shaded based on dialect. Spectrograms of one representative song from each sampled dialect are shown along with dialect identification numbers. Small dots indicating additional recording sites are included to illustrate dialect ranges. Dialect numbers are from Baptista (1977; dialects 1 through 6), DeWolfe and Baptista (1995; dialect 7), Chilton and Lein (1996; dialects 8 and 9), and the present study (dialects 10–13). Of the six dialects illustrated here, dialects 1, 2, and 7 represent Baptista's (1977) southern theme and dialects 5, 6, and 12 represent the northern theme.



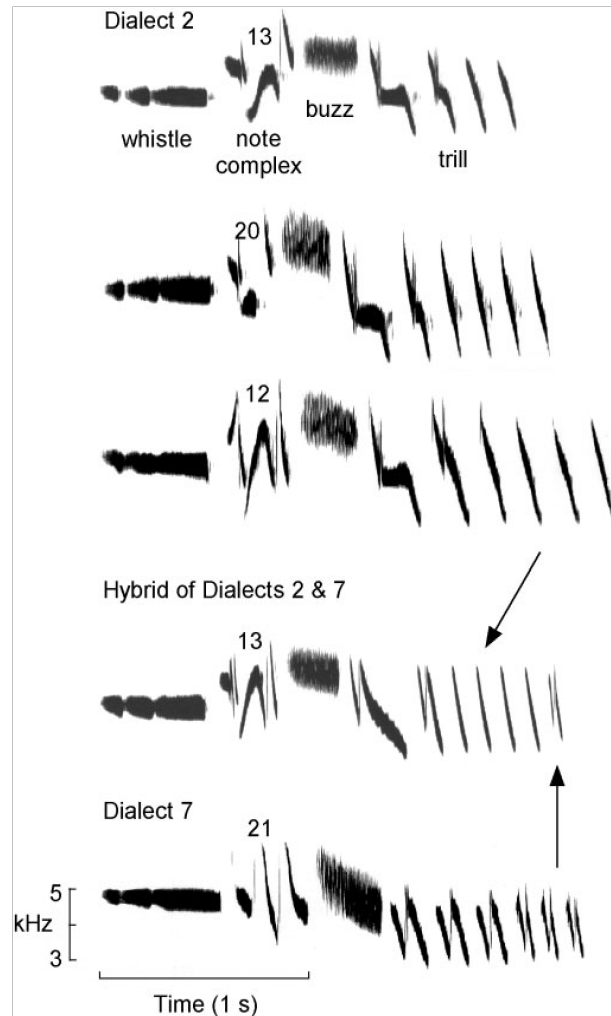
Data analysis

Within each sample site, each locus was tested for HardyWeinberg equilibrium, and all pairs of loci were tested for linkage disequilibrium by using Arlequin software (version 2.0; Schneider et al., 1998). Site-pairwise F_{ST} comparisons, AMOVAs (16,000 permutations), and genotype assignment tests were also performed by using Arlequin. The sequential Bonferroni procedure was applied to ascertain significance in the site-pairwise F_{ST} comparisons. Allele frequency data were entered into PHYLIP (Phylogeny Inference Package, version 3.5; Felsenstein, 1993) and used to generate 1000-

bootstrap consensus trees, using the UPGMA algorithm on Nei's standard genetic distances (Nei's D) between sites (Nei, 1972)

Figure 2

Song variation within and between dialects, including hybrid song. Four distinct phrase types are labeled on the top song. The top three songs are from males at Nehalem Bay (dialect 2). Each of these songs has a distinct note complex type (numbered 13, 20, and 12) in the introduction, yet the terminal trill is similar in all songs. Note complex types 12 and 13 were common at Nehalem Bay (67% and 30% of 27 males), whereas type 20 was rare. At the bottom is a dialect 7 song from Florence with note complex and trill distinct from dialect 2. In the middle is a "cultural hybrid" song from Newport: the note complex and first trill are similar to dialect 2, whereas the last trill syllable resembles dialect 7.



The genotype assignment test calculates the likelihood of finding each individual's genotype in a potential source population, assuming Hardy-Weinberg equilibrium in the population based on allele frequencies observed in the sample (Paetkau et al., 1995). We addressed two questions by using assignment tests. First, of all sites sampled, are individual birds most likely assigned to their own sites? Second, for birds at sites that share a dialect with a second site, is each individual's assignment to the other site in its dialect area more likely than to all sampled sites outside the dialect area? For these analyses, we calculated per-site percentages and averaged these across sites.

Mantel tests (10,000 permutations) of correlations between geographic distance, Nei's standard genetic distance, and song similarity of birds at each site (expressed either by binary

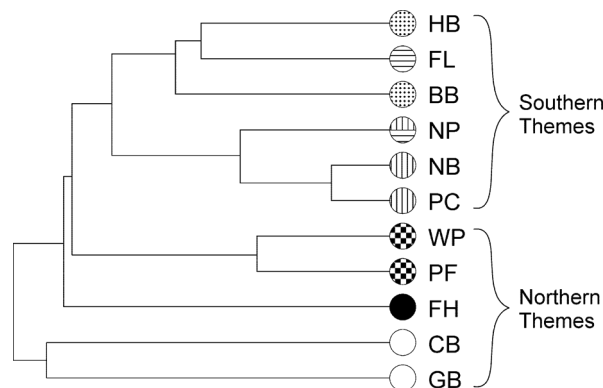


Figure 3

UPGMA phenogram of acoustic distances between the average song at each site. With one exception (Florence: see text) sites from the same dialect area cluster together based on acoustic structure. Distances were calculated from principal components analysis of 31 acoustic measurements of songs recorded from most of the birds sampled for the genetic analysis as well as additional birds at each site. Relative distance is represented in branch lengths.

or graded measures), as well as partial correlation of genetic distance and song similarity controlling for geographic distance, were done by using FSTAT (version 2.9.1; Goudet, 2000). It is appropriate to use binary data in one matrix (Manly, 1991; Schnell et al., 1985); in this application, the test becomes a two-sample comparison (same group versus different group) for nonindependent observations. The data matrices used in the Mantel tests are presented in Appendix 1. To compare our results to those of a previous study on *Z. l. oriantha* (MacDougall-Shackleton and MacDougall-Shackleton, 2001), we also ran the Mantel test using log F_{ST} instead of Nei's D.

Table 1
Microsatellite loci at which allele frequencies were compared

Locus	Source species	Reference	Alleles	F_{ST} (0)
YW01	<i>Dendroica petechia</i>	Dawson et al., 1997	20	0.002
YW16	(yellow warbler)	Dawson et al., 1997	8	0.025
GF01	<i>Geospiza fortis</i>	Petren, 1998	10	0.018
GF12	(medium ground finch)	Petren, 1998	18 ^a	0.011

Source species are those for which primers were originally developed. Also listed are total numbers of different alleles seen across all *Z. l. pugetensis* populations in the present study, and the overall F_{ST} calculated from allele frequencies at each locus.

^a One or more nonamplifying alleles were probably also present at this locus (see Results).

Z. l. nuttalli: a reanalysis

Baker and colleagues presented an extensive series of studies on the relationship between song dialects and population structure in the sedentary subspecies *Z. l. nuttalli*. These studies were completed before the Mantel test became widely known and preferred for analyzing data on geographic variation. These studies instead used simple correlation techniques, partitioned into within- and between-dialect comparisons,

and ANCOVA to control for geographic distance effects (Baker et al., 1984). The concern with these approaches is that the observations are not statistically independent; hence, the degrees of freedom may be inflated (Manly, 1991). The Mantel test also treats observations from each site separately, instead of averaging sites within dialects as was done originally. Because these investigators published their data, we were able to reanalyze this data set by using the Mantel test (10,000 permutations) on matrices of genetic distance (Nei's D), geographic distance, and song distance between nine sites in four dialect areas. For song distance we again used two matrices, one containing binary values (indicating same or different dialect, using Baker and colleagues' dialects as defined by complex syllables), and the other containing a continuous measure of song dissimilarity. All matrices used in the *Z. I. nuttalli* reanalysis are presented in Appendix 2.

RESULTS

Song dialects

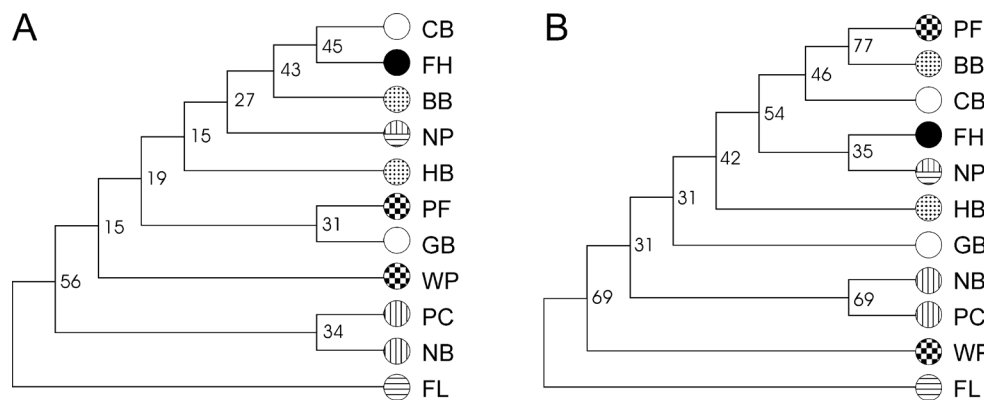


Figure 4
Consensus phenograms constructed from 1000 bootstrap calculations of Nei's genetic distance, based on allele frequencies, by using the UPGMA method. Song dialects do not cluster together based on genetic distances. Bootstrap values are shown at each node. (A) The tree on the left includes data from locus GF12; the tree on the right (B) excludes it. Total widths of trees are $D = 0.073$ and $D = 0.085$. Distance is not represented in branch lengths.

The two judges agreed in their subjective classifications of the recorded songs into dialects, based on the terminal trill, except that one judge initially grouped dialects 1 and 2 together. After referring to the criteria Baptista (1977) used to define these two particular dialects, both judges were in perfect agreement on the classification of recorded songs into these two dialects as well. On average, 85.6% of males recorded in each population sang the local dialect. At Newport, 52.4% of the males sang a hybrid trill (Figure 2). These results are summarized in Table 2. The phenogram resulting from the UPGMA cluster analysis based on measurements of 31 acoustic variables is shown in Figure 3. A distinction between geographically northern and geographically southern themes or “superdialects” is apparent in the phenogram. This distinction, which is based on the sequence of phrase types within each song (as can be seen in the spectrograms in Figure 1) was first noted by Baptista (1977) in his description of geographic variation in *Z. l. pugetensis* song. The sole disagreement between the acoustic phenogram in Figure 3 and our subjective binary classification based on the trill is the grouping of Florence (a dialect 7 site) with Horsfall Beach (a dialect 1 site). This might have resulted from the presence of several males at each site that sang the other site’s dialect (trill).

Microsatellite properties

Table 2
Songs recorded from male white-crowned sparrows at each study site

Site	Dialect number	No. of males recorded	2-Dialect males	Local songs	Foreign songs	Hybrid songs	Partial songs
Friday Harbor (FH)	d6	24/26		87.5%			12.5%
Pack Forest (PF)	d5	26/26		96.2%			3.8%
Westport (WP)	d5	20/24	1	77.5%	7.5%		15.0%
Nehalem Bay (NB)	d2	18/21		100%			
Pacific City (PC)	d2	23/24		91.3%			8.7%
Newport (NP)	d2	21/23		47.6% ^a		52.4%	
Florence (FL)	d7	20/23	1	67.5%	10.0%	22.5%	
Horsfall Beach (HB)	d1	19/20	1	86.9%	2.6%	10.5%	
Bullard’s Beach (BB)	d1	14/23	1	89.3%	10.7%		
Cape Blanco (CB)	d12 ^b	22/26	1	97.7%	2.3%		
Gold Beach (GB)	d12 ^b	15/16		100%			
Total/Average		222/252	5	85.6%			

The number of males whose songs were recorded is given as fraction of males included in genetic analysis. Also shown are the number of males at each site that sang two dialects, and proportions of recorded birds singing local, foreign, and hybrid songs. Partial songs too short to classify were recorded from some birds.

^a This number is an upper bound because dialect 2/dialect 7 hybrid trills predominated; “local” songs at this site might actually be incomplete hybrids.

^b Very few songs at Cape Blanco or Gold Beach contained terminal trills, so we classified these songs as “local” if all other song components were typical of dialect 12.

A total of 56 alleles were detected at the four microsatellite loci across all 11 sample sites. The number of alleles per locus ranged from eight to 20 (Table 1). Linkage disequilibrium tests showed that all pairs of loci segregated independently in each population, with one exception for each of five locus pairs and two exceptions for the sixth pair. These seven exceptions were spread across six populations. This low number of exceptions (seven of 66), and their spread across the populations, suggests that the four loci do segregate independently.

Hardy-Weinberg expectations of heterozygosity were met in all samples at loci YW16 and YW01, and in most (nine of 11) populations at locus GF01. At locus GF12, significant deviation from Hardy-Weinberg expectations in all but one population (FH) indicated a heterozygote deficiency at this locus. This suggests the presence of one or more null (nonamplifying) alleles at GF12. The overall F_{ST} value given by the observed allele frequencies at GF12 alone falls within the range of low F_{ST} values given by each of the other loci (Table 1). However, to ensure that inclusion of GF12 did not bias the results, the four interpopulation tests described in the next section were carried out both with and without data from this locus.

Site-level comparisons

Site-pairwise F_{ST} s indicate whether a significant proportion of the variation seen among the individuals from those sites is attributable to variation between, rather than within, the two sites. Four pairs of sites share dialects (PF and WP, NB and PC, HB and BB, CB and GB). None of these pairs yielded significant F_{ST} values (mean F_{ST} = 0.003 with data from all loci). Of the 51 remaining cross-dialect comparisons, only one (without GF12) or three (with GF12) were significant, all of which involved FL (versus PF, WP, and FH). Without sequential Bonferonni adjustment, 13 (without GF12) or 16 (with GF12) pairwise F_{ST} values were significant, including nine of the 11 FL comparisons in both cases. This pattern of site-pairwise F_{ST} values suggests that the FL sample is distinct but that otherwise there is no tendency for sites from different dialects to differ in allele frequencies.

Low but significant site differentiation was demonstrated by an AMOVA that compared variation within and among the 11 sites together. Of the total variation, variation within sites accounted for 98.7% when GF12 was included, and for 98.9% when GF12 was excluded. The remaining 1.3% and 1.1%, respectively, were accounted for by among-site variation. The corresponding F_{ST} values were 0.013 and 0.011 ($p < .01$ for each). The following analyses address the extent to which this site differentiation corresponds with dialects rather than resulting merely from geographic isolation.

UPGMA consensus phenograms based on 1000 bootstrap calculations of Nei's genetic distance are shown in Figure 4. These phenograms do not show clustering of sites from the same song dialect, either with or without the data from locus GF12, and low bootstrap values indicate weak structuring overall.

By using the genotype assignment test, either 45.0% or 42.0% of individuals (with or without data from GF12) were assigned to their own site over all other sites sampled. This percentage is significantly higher than the chance value of 9.1% ($p < .001$, binomial test). For individuals from one of two sites sharing the same dialect, only 10.5% or 13.1% were assigned to the other site in their dialect over all sites outside the dialect. These percentages are close to the 10% expected by chance ($p = .09$ and $p = .05$, respectively). Together, these results suggest that although single sites are genetically distinct enough to enable correct assignment of many genotypes, sites within a dialect area are genetically no more similar to each other than are two sites from different dialect areas.

Dialect-level comparisons

Inclusion of the data from locus GF12 did not affect the results of the site-level comparisons above, so GF12 data were included in the two dialect-level tests described below. However, because the predominant song at Newport could not be classified as either dialect 2 or dialect 7 (Figure 2), we excluded this site from dialect-level tests. This left Florence in a dialect category by itself. We had hoped to obtain another sample site in dialect 7, but the next public land to the south (Reedsport) was in a contact zone between dialects 7 and 1. Fifteen of 16 birds recorded on private land between Florence and Reedsport sang pure dialect 7. Given the uniquely high genetic divergence of Florence from all other sites (as revealed by site-pairwise F_{ST} values and UPGMA phenograms), the following analyses were done with and without Florence in order to evaluate the influence of this outlier population on the results. If the results do not stand up without Florence, then we cannot conclude that there is a general correlation between dialects and genetic structure.

An AMOVA comparing within-site, among-site-within-dialect-area, and among-dialect-area components of total variance in allele frequencies revealed that when Florence was included, the variation was partitioned among these three levels as follows: 98.54% within sites, 0.42% among sites within dialect areas, and 1.04% among dialect areas. For this among dialect-area value, $p = .021$ (60.001 SE), indicating significant variation among dialect areas. Excluding Florence, however, changed the partitioning of variance to 99.03%, 0.47%, and 0.50% respectively, yielding $p = .076$ (60.002 SE) for the level of variation among dialect areas.

Mantel tests with 10,000 permutations indicated that as expected, because dialects are regional variants of song, dialect identity (a binary measure based on shared trill types) and geographic distance were significantly correlated, whether or not Florence was included ($r = .38$ or $-.45$, $p = .05$). In contrast, intersite Nei's D and geographic distances were not correlated, either including Florence ($r = .09$, $p = .57$) or excluding it ($r = .01$, $p = .94$). Finally, partial correlation of Nei's D and dialect identity, which removes the effect of geographic distance, was not significant either with Florence ($r_{part} = .20$, $p = .09$) or without ($r_{part} = .15$, $p = .30$).

Additional Mantel tests using a matrix of continuous acoustic distances based on acoustic properties of entire songs, which also takes into account intra- and intersite variation, yielded similar patterns of correlation: acoustic and geographic distance were significantly correlated ($r = .50$ with Florence and $r = .51$ without, $p = .05$ for both), and partial correlation of Nei's D and acoustic distance, controlling for geographic distance, was not significant ($r_{part} = .03$, $p = .93$ with Florence and $r_{part} = .03$, $p = .81$ without). This final analysis included Newport because dialect identity was not of concern.

Because we analyzed data from only four microsatellite loci, concern might arise that our negative Mantel test results are a consequence of insufficient power to detect genetic differences among dialects. To address this concern, we ran separate Mantel tests using Nei's D calculated from each locus separately and each combination of two and three loci to examine whether the partial correlation between Nei's D and acoustic distance increases with the number of loci analyzed (Figure 5). We found no such increase (Spearman's $r_s = .197$, $N = 15$, $p = .48$ for the Mantel r_{part} values, and $r_s = .188$,

$p = .50$ for the Mantel p values), so we believe it is unlikely that adding more loci would increase the partial correlation between Nei's D and acoustic distance to the point of significance.

To compare our results to those on *Z. l. oriantha*, we also ran Mantel tests using $\log F_{ST}$ instead of Nei's D as the measure of genetic distance. Using our graded acoustic distance measure, which is most similar to the subjectively defined acoustic measure used for *Z. l. oriantha*, partial correlation of $\log F_{ST}$ and acoustic distance controlling for geographic distance was not significant ($r_{part} = .06$, $p = .60$ with Florence and $r_{part} = .11$, $p = .52$ without).

Z. l. nuttalli: reanalysis

In contrast to the lack of correlation in migratory *Z. l. pugetensis* and *Z. l. oriantha* (MacDougall-Shackleton and MacDougall-Shackleton, 2001), geographic distance and genetic distance were significantly correlated in sedentary *Z. l. nuttalli* (Mantel $r = .67$, $p = .01$). When geographic distance between sample sites was controlled, there was no significant correlation between genetic distance (Nei's D) and song distance. This conclusion held for both the binary representation of song distance (same or different dialect based on the complex syllables; $r_{part} = .35$, $p = .60$), and the continuous measure based on four song phrases ($r_{part} = .59$, $p = .65$). The latter hypothesis was tested because geographical dialect boundaries vary in some cases depending upon which phrases are used to define the dialects (Kroodsma et al., 1985). Finally, we note that mean F_{ST} among dialects in *Z. l. nuttalli* was 0.042, about four times higher than in either of the migratory subspecies.

DISCUSSION

We found little genetic differentiation among Puget Sound white-crowned sparrows from 11 sites spanning six song dialect areas. Although site-level AMOVAs indicated significant genetic variation among sites regardless of dialect, pairwise F_{ST} tests revealed that most of this intersite variation was contributed by one genetically distinct site, Florence. The genetic divergence of Florence from almost all of the other sites is puzzling because Florence is centrally located in the subspecies' range. One possibility is that the habitat at Florence was disturbed in the recent past, such that the site experienced a bottleneck or recolonization event, and that current gene flow has not yet masked this effect. In any case, inclusion of Florence accounted for the statistically significant genetic variation between dialect areas. No significant effect of dialect on the genetic structure of *Z. l. pugetensis* populations exists that is general to all song dialects.

Cluster analysis (consensus phenograms) and genotype assignment tests indicated no tendency for sites from the same dialect area to be genetically more similar than sites from different dialect areas, and Mantel tests revealed no partial correlation between dialect identity and genetic distance (Nei's D). Overall, these results suggest a

lack of genetic divergence between song dialect populations of Puget Sound white-crowned sparrows.

We found no correlation between genetic distance and geographic distance among sample sites. Although this might mean that the subspecies is panmictic, it might also mean that the historical pattern of colonization or isolation of dialects has not been linear. Support for nonlinear colonization comes from the cultural history apparent in the distribution of song types: the northern and southern themes suggest two centers of origin for the dialects, and the presence of an isolated northern theme dialect in southern Oregon (Figure 1) might have resulted from dispersal of northern birds into the range of the southern theme. Finally, the four microsatellite loci we examined might not vary enough to differ geographically. We consider this last possibility unlikely, because the four loci had a total of 56 alleles and an average experiment-wide heterozygosity of 0.66. In addition, a small number of population-pairwise F_{ST} values were significant, so some variation does exist across sites.

Subspecies differences in genetic structure?

Three subspecies of white-crowned sparrow have now been examined for genetic structure corresponding to song dialects. The low genetic differentiation among dialect areas of *Z. l.*

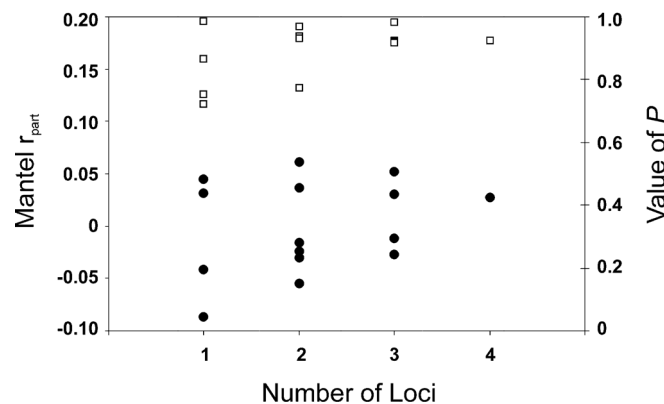


Figure 5

Partial correlation (Mantel r_{part}) of Nei's D with acoustic distance (filled circles), and the associated P values (open squares), versus the number of loci analyzed. All combinations of one through four loci were tested. Within the four loci we used, adding more loci did not significantly increase r_{part} or cause p to approach significance, suggesting that adding more loci would not change the results of the Mantel test.

pugetensis reported here differs from the significant structuring among dialect areas of *Z. l. nuttalli* described by Baker and colleagues (1982), although the reanalysis of those data presented here does not support the original authors' conclusion. Our results more closely match those of a recent, methodologically more similar study of *Z. l. oriantha* (MacDougall-Shackleton and MacDougall-Shackleton, 2001), and *Z. l. pugetensis* more closely resembles migratory *Z. l. oriantha* in life history and behavior than it does the

sedentary *Z. l. nuttalli* (Nelson et al., 1996). The *Z. l. oriantha* study concluded that song dialects are associated with genetic population structure in this subspecies. In *Z. l. pugetensis*, given the small amount of genetic variance associated with dialect structure (1%) and the lack of significance of the Mantel test results, we conclude that song dialects do not reflect population genetic structure across all dialects. The actual results of the two studies, when directly comparable, are strikingly similar.

Three types of analyses were used in both our study and the *Z. l. oriantha* study. First, cluster analysis using UPGMA phenograms gave similar results in both cases, despite the use of different genetic distance measures (Nei's D versus F_{ST}). In both phenograms, bootstrap values were low, and sites from the same dialect did not cluster together.

Second, hierarchical AMOVA analyses in both studies revealed that although nearly 99% of the variation in allele frequency exists within sites, a significant portion of the remaining variation is between dialect areas rather than between sites within dialects. As stated above, in our study this result depends on the inclusion of our Florence sample. The *Z. l. oriantha* study did not report whether any one site might have similarly contributed unusually high among-dialect-area variation. From the phenogram in the *Z. l. oriantha* study, it appears possible that the Mammoth Lakes site, which is the only representative of its dialect (and which lies within the geographically largest known Sierra Nevada dialect area; Harbison et al., 1999), might have contributed enough variation to affect the results of the hierarchical AMOVA (Figure 4) (MacDougall-Shackleton and MacDougall-Shackleton, 2001).

Third, neither study found a bivariate correlation between geographic distance and Nei's standard genetic distance, although different correlation techniques were used in each case. Both studies used Mantel tests to assess the partial correlation between dialect similarity and genetic distances, controlling for effects of geographic distance. We found no significant partial correlation in *Z. l. pugetensis* using Nei's D or $\log F_{ST}$, the latter measure having been chosen in the *Z. l. oriantha* study.

The studies differed in one possibly critical methodological respect. In our study, we used both a binary dialect similarity matrix and a similarity matrix based on multiple acoustic measurements, whereas the *Z. l. oriantha* study used a single subjective, graded measure of song similarity. The *Z. l. oriantha* study provided few details on how the similarity of song elements was decided and whether the subjective assessment was repeatable with different judges (see Jones et al., 2001). Repeatability is a concern because, by using a published catalog of song elements (song phrases in the terminology of Harbison et al., 1999), we cannot reconstruct the phenogram based on subjective song similarities reported in that study (MacDougall-Shackleton and MacDougall-Shackleton, 2001, Figure 2). The acoustic distance metric used is just as critical as the estimate of genetic distance, as the former represents the hypothesis

Table 3

Summary of studies of genetic and song variation in three subspecies

	<i>Z. l. nuttalli</i>	<i>Z. l. oriantha</i>	<i>Z. l. pugetensis</i>
Results	Genetic differentiation between dialects ^a	Slight but significant genetic differentiation	No genetic difference between dialects
Dialects sampled	4	8	56 ^b
Linear range of study area	27 km	220 km	689 km
Approx. % of range sampled	,5%	,10%	7580%
Sites sampled	9	14 ^c	911 ^b
Mean males per site sampled	29	14 ^c	23
Molecular marker	Allozymes	Microsatellite DNA	Microsatellite DNA
No. loci	8	8	4
No. alleles	20	73	56
Migratory	No	Yes	Yes
Dialect areas	Small	Small	Large
Dialect contact zones	2 km wide	None known	2030 km wide
Habitat	Continuous	Patchy	Continuous

Shown here are results and methods of studies on song and genetic variation in white-crowned sparrows, along with aspects of each subspecies' natural history. References include *Z. l. nuttalli*: Baker et al. (1982) and *Z. l. oriantha*: MacDougall-Shackleton and MacDougall-Shackleton (2001).

^aAs reported in the original study. Our Mantel reanalysis revealed no genetic differentiation.

^bDifferent analyses included different numbers of dialects and sites.

^cBased on those sites included in analysis.

concerning the aspect of song variation to which the birds respond. By using two metrics to represent acoustic similarity, we tested two hypotheses concerning the possible relationship between song structure and genetic differentiation. Our conclusion is that neither of the levels of song variation examined, the terminal trill alone or the entire song, appear to reflect the partitioning of genetic variation.

Comparison to *Z. l. nuttalli*: a reanalysis

The results for *Z. l. nuttalli* have already been thoroughly discussed elsewhere (Baker and Cunningham, 1985; Hafner and Petersen, 1985; Kroodsma et al., 1985; Zink and Barrowclough, 1984). Our reanalysis using the Mantel test yields results rather similar to our own: when the effect of geographic distance is controlled statistically, no correlation is found between genetic distance (measured by Nei's *D*) and dialect differences, using either of two acoustic measures. As previous investigators have suggested (Baker et al., 1982; Zink and Barrowclough, 1984), there is a strong effect of geographic distance on genetic differentiation, possibly as a result of this subspecies' sedentary nature and relatively short dispersal distances (Nelson et al., 1995: Table 2).

Ecological influences on genetic structure

To assess whether our results are in fact divergent from those found in *Z. l. oriantha* and *Z. l. nuttalli*, it is important to consider whether biological or ecological differences between the subspecies might influence genetic divergence of their dialect populations. The rate of genetic drift—and thus population divergence—depends on population size (Kimura and Ohta, 1969). Most populations of *Z. l. pugetensis* in Oregon and Washington are considerably larger than those of *Z. l. oriantha* in the Sierra Nevada. Local populations of *Z. l. pugetensis* on the central Oregon coast number in the hundreds and are probably effectively continuous for tens of kilometers. Most populations of *Z. l. oriantha* in the Sierra Nevada contain fewer than 20 pairs (DeWolfe and DeWolfe, 1962; Orejuela and Morton, 1975). In the absence of gene flow, *Z. l. oriantha* dialect populations would be expected to diverge more rapidly than those of *Z. l. pugetensis*. Some gene flow is likely, however (see below), and habitat characteristics might affect the rate of gene flow within each subspecies. Based on our observations in both areas, the breeding habitat of *Z. l. oriantha* in the Sierra Nevada is patchier than is the coastal habitat of *Z. l. pugetensis*, and patchier populations might be expected to experience more restricted gene flow. Both subspecies are migratory, however, so the question is whether habitat patchiness influences loyalty to the natal dialect in migrants.

We conclude that although ecological differences might explain some difference between *Z. l. pugetensis* and *Z. l. oriantha* in partitioning of genetic variation among dialect populations, we cannot rule out the influence of methodological differences between our study and the *Z. l. oriantha* study. In addition to the analytical differences described above, the number of loci (four versus eight, respectively), total alleles (56 versus 73), sites (11 versus 14), dialects (6 versus 8), and males sampled per site (23 versus 14) differed between the two studies. Ecological and methodological differences between these two studies, as well as the *Z. l. nuttalli* study, are listed in Table 3.

Dialect maintenance in the absence of genetic differentiation

Low genetic differentiation between song dialect populations in *Z. l. pugetensis* might reflect recent population divergence. Because migratory songbirds are highly mobile and cultural evolution of song can occur rapidly (white-crowned sparrow: Harbison et

al., 1999; corn bunting, *Miliaria calandra*: Holland et al., 1996; village indigobird, *Vidua chalybeata*: Payne, 1985), a population of birds singing a variant of the common song type could disperse and establish itself in suitable neighboring habitat over a relatively short time span. Although one dialect known today was recorded in 1952 (dialect 6: archived at the Macaulay Library of Natural Sounds) and six were described spectrographically in 1970 (Baptista, 1977), *Z. l. pugetensis* dialects could be of recent enough origin that insufficient time has passed for genetic differences to accumulate, even if assortative mating is currently occurring. We cannot distinguish between recent population divergence and ongoing gene flow as causes of the low genetic divergence we observed. Just as our negative result is difficult to interpret, a significant correlation between genetic distance and song dialects would not prove that song differences maintain genetic divergence. Genetic differences could be produced by founder effects in isolated populations; such differences may appear if populations are sampled soon enough after founding that gene flow has not had time to homogenize gene frequencies. Genetic surveys provide useful data on the possibility of gene-culture coevolution, but need to be supported with direct evidence that song differences influence mating decisions by birds.

In any case, gene flow across dialects probably counteracts differentiation owing to genetic drift among *Z. l. pugetensis* populations today. Evidence for this includes multiple-dialect or foreign-dialect singers in the wild (Table 2). The percentage of foreign-dialect singers in *Z. l. pugetensis* is no greater than that in *Z. l. oriantha* (11.2% based on data in Orejuela and Morton, 1975) or in *Z. l. nuttalli* (9.7% based on data in Baker and Thompson, 1985). In each subspecies, at least some of these foreign-dialect singers are probably individuals that hatch in one dialect area and breed in another. Additional behavioral evidence for ongoing gene flow comes from the observation that young white-crowned sparrows commonly acquire (memorize and rehearse) multiple song dialects during their early sensitive phase (Nelson et al., 1996) and, in *Z. l. pugetensis* at least, subsequently retain the dialect that matches their neighbors' songs when they settle on a breeding territory the following spring (Nelson, 2000). Anecdotal descriptions of song overproduction and attrition in the wild exist for *Z. l. nuttalli* (DeWolfe et al., 1989) and *Z. l. oriantha* (Baptista and Morton, 1988), so this behavior may be common in white-crowned sparrows. This flexibility in dialect choice could reflect selection for mobility in first-year birds, in which case it seems possible that gene flow has long occurred among dialect populations in this species.

Acquisition of multiple dialects by young white-crowned sparrows, followed by selective attrition guided by the neighbors' dialect in the spring, provides a mechanism for dialect maintenance in the absence of natal dialect philopatry and reduced gene flow between dialect populations. Under this scheme, dialects are "formed" not at the song acquisition stage, but at the song retention stage. This suggests that breeding within the natal dialect area itself is less crucial to reproductive success than is the ability to claim and defend a territory, with a song that is shared by territorial neighbors, within the range of dialect areas that a bird experiences while young. This scenario of cultural differentiation in the absence of genetic differentiation does not support the idea that dialect populations in this species differ genetically. It does, however, support the idea that sharing song with neighbors confers social benefits that may increase reproductive success.

APPENDIX 1

Matrices of distances between 11 sampling sites studied in *Z. l. pugetensis*

Site	FH	PF	WP	NB	PC	NP	FL	HB	BB	CB	GB
FH		198	198	322	379	444	510	572	607	644	689
PF	0.065		140	179	225	284	346	406	442	479	520
WP	0.075	0.093		135	192	256	322	383	417	453	500
NB	0.086	0.124	0.123		57	122	189	250	285	322	367
PC	0.053	0.072	0.115	0.080		65	132	193	228	265	310
NP	0.048	0.098	0.101	0.100	0.086		67	128	163	200	245
FL	0.178	0.183	0.238	0.180	0.200	0.143		62	97	134	179
HB	0.068	0.083	0.096	0.147	0.101	0.064	0.137		36	73	117
BB	0.045	0.088	0.095	0.075	0.075	0.076	0.167	0.074		37	83
CB	0.043	0.066	0.124	0.090	0.065	0.052	0.128	0.069	0.045		48
GB	0.102	0.081	0.170	0.174	0.105	0.089	0.142	0.105	0.124	0.060	

Site	FH	PF	WP	NB	PC	NP	FL	HB	BB	CB	GB
PF	<i>3.33</i>										
WP	<i>3.88</i>	<i>1.43</i>									
NB	3.38	3.29	3.37								
PC	3.38	3.18	3.30	<i>0.65</i>							
NP	3.70	3.04	3.11	<i>1.71</i>	<i>1.52</i>						
FL	3.28	3.66	3.66	<i>2.90</i>	<i>2.97</i>	<i>2.68</i>					
HB	3.13	3.44	3.53	<i>2.87</i>	<i>2.94</i>	<i>3.17</i>	<i>2.02</i>				
BB	3.71	3.54	3.45	<i>3.14</i>	<i>2.89</i>	<i>3.11</i>	<i>2.42</i>	<i>2.17</i>			
CB	<i>4.58</i>	<i>3.63</i>	<i>3.87</i>	4.14	4.07	3.80	4.25	3.91	3.67		
GB	<i>3.97</i>	<i>4.26</i>	<i>4.36</i>	4.16	3.89	3.90	4.25	3.65	3.64	<i>3.65</i>	

In the upper matrix, Nei's *D* values are below the diagonal, and geographic distances (km) are above the diagonal. The lower matrix contains continuous-scale acoustic distances between sites. Italicized values are the same theme, and bold values share the same trill (dialect).

APPENDIX 2

Matrices used in reanalysis of *Z. l. nuttalli* data (Baker, 1982)

Site	RCA	LR	DP	PL	CC	LIM	MH	VIS	CGR
RCA		4	5	6	15	18	20	24	27
LR	0.008		1	2	12	14	16	20	24
DP	0.011	0.017		1	11	14	16	19	23
PL	0.016	0.026	0.022		10	12	15	18	22
CC	0.026	0.038	0.020	0.007		3	5	9	12
LIM	0.024	0.035	0.034	0.006	0.010		3	7	9
MH	0.047	0.061	0.046	0.016	0.017	0.016		4	7
VIS	0.065	0.084	0.046	0.040	0.031	0.043	0.017		4
CGR	0.049	0.063	0.034	0.020	0.010	0.023	0.008	0.013	

Site	RCA	LR	DP	PL	CC	LIM	MH	VIS	CGR
LR	0								
DP	2	2							
PL	2	2	0						
CC	4	4	3	3					
LIM	6	6	6	6	5				
MH	6	6	6	6	5	0			
VIS	8	8	8	8	7	5	5		
CGR	8	8	8	8	4	6	6	2	

The upper matrix contains Nei's *D* values below the diagonal (from Table 1 in Hafner and Petersen, 1985) and geographic distances (km) above (measured in Baker et al., 1982: Figure 1). The lower matrix contains continuous-scale acoustic distances (range = 0–8). A value of 8 indicates that songs at the two sites differ at all four song parts described in Baker and Thompson (1985). At each of these four parts, zero was assigned if the two sites shared the same syllable type(s); one, if multiple syllable types occurred at one or both sites and some but not all types were shared; and two, if the songs at each site contained only distinct syllable types. Bold values represent shared complex syllables (dialects as defined by Baker and Thompson, 1985).

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