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The role of history and ecology as drivers of song divergence in Bell's and Sagebrush sparrows (*Artemisiospiza*, Aves: Passerellidae)

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Bell's and Sagebrush sparrows (*Artemisiospiza belli* and *A. nevadensis*) are phenotypically and genetically distinct, but data have yielded contradictory findings for *A. belli* subspecies. Disjunct populations of *A. b. canescens* from the San Joaquin Valley and Mojave Desert are phenotypically indistinguishable but diagnosable from Coast Range *A. b. belli*. However, San Joaquin Valley *A. b. canescens* shares allozymes and mitochondrial DNA (mtDNA) with *A. b. belli* whereas Mojave Desert *A. b. canescens* is genetically distinct. Furthermore, Great Basin *A. nevadensis* is closer in mtDNA to non-desert *A. belli* subspecies than to Mojave Desert *A. b. canescens*, with which it contacts across an aridland transition. We assessed concordance of song with genetics and phenotype for these taxa, and also analysed songs and mtDNA for *A. b. clementeae* which is endemic to arid San Clemente Island off the coast of southern California. Songs of open, arid habitat populations (*A. nevadensis*, *A. b. canescens*, *A. b. clementeae*) are consistently more similar to each other than they are to songs of coastal *A. b. belli.* We examined bioclimatic and land cover conditions to understand the basis for these patterns across ecoregions, and discuss the effect of the acoustic environment on song.

ADDITIONAL KEYWORDS: acoustic adaptation – biogeography – convergence – cultural evolution – song variation – subspecies.

INTRODUCTION

When phylogenetic history disagrees with patterns of phenotypic variation within or between closely related species, ecological similarity often explains the contradiction as it can drive phenotypic convergence in independent lineages. Individuals from allopatric populations that are subject to similar ecological pressures may evolve phenotypic similarities that are locally adaptive but incongruent with genetic patterns. Strong selection (Greenberg *et al.*, 1998; Brehm *et al.*, 2001; Hoekstra *et al.*, 2005) and/or phenotypic plasticity (Price, 2006; Charmantier *et al.*, 2008; Vedder *et al.*, 2013) in response to ecological pressures are often invoked as mechanisms for environmental adaptation, the latter being especially critical in the successful colonization of new habitats (Thibert-Plante & Hendry 2011). Such local adaptation can occur rapidly in the absence or presence of gene flow (Hoekstra *et al.*, 2005; Nosil, 2012).

In oscine birds, songs represent a culturally inherited trait that is tied to both the ecology and the morphological phenotype of individuals (Christensen, 2006; Derryberry, 2009; Giradeau *et al.*, 2014). More specifically, the 'acoustic adaptation hypothesis' predicts that certain properties of song will vary with habitat because of differential patterns of sound degradation (Boncoraglio & Saino, 2007; Ey & Fischer, 2009). Evolutionary studies that incorporate song shed valuable insight into the role of cultural evolution in allopatric divergence and speciation at both shallow and deeper taxonomic levels (e.g. Toews & Irwin, 2008; Aleixandrei *et al.*, 2013; Mason *et al.*,

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2014a, 2017; Derryberry 2018). Because song is a sexual signal used for mating and territorial interactions, vocal performance has been shown to affect reproductive success (Ballentine *et al.*, 2004; Christensen, 2006). Furthermore, divergence in song between lineages can impact the potential for reproductive isolation through ecological speciation (Ballentine *et al.*, 2013) or upon secondary contact (Bambrilla *et al.*, 2008). Song divergence often shows patterns that are congruent with ecological, phenotypic and genetic differences between populations (e.g. Cicero, 1996; Caro *et al.*, 2013; Lipshultz *et al.*, 2017). However, because song traits vary in geographically structured patterns that may arise through different mechanisms (Podos & Warren, 2007), songs are especially useful to study in genetically differentiated populations where ecology and phenotype do not match phylogeny. In such cases of discordance, three hypotheses are possible: (1) song relationships will follow genetic patterns and evolutionary relatedness, thus being passed down along genealogical lineages; (2) songs will evolve due to selective pressures and may match phenotype and/or ecology, independent of genetic relationships; and (3) patterns of song variation will differ from those of phylogeny and ecology, or not vary at all. Songs may evolve variation through natural selection from a particular acoustic environment (Boncoraglio & Saino, 2007), through sexual selection on particular song traits (Byers & Kroodsma, 2009) or through cultural drift (Koetz *et al.*, 2007; Benedict & Bowie, 2009). Convergent patterns of phenotype and song variation that do not match phylogenetic relationships would probably be caused by natural selection pressures from similar adaptive environments.

BELL'S AND SAGEBRUSH SPARROWS

We studied song variation in Bell's and Sagebrush sparrows (*Artemisiospiza belli* and *A. nevadensis*, which were conspecific until recently; Chesser *et al.*, 2013; Fig. 1) to address three fundamental questions: (1) How do the songs of mainland taxa vary in the context of contradictory genetic, phenotypic and ecological differences among populations? (2) How do the songs differ on San Clemente Island, which has an endemic and federally threatened subspecies of *A. belli*. (3) Does phylogenetic history or ecology appear to be more important in driving song divergence in this group? On mainland California, *A. b. belli* is a resident of chamise chaparral in the central and southern Coast Ranges, with a small disjunct population in the central Sierra Nevada foothills. Inland, *A. b. canescens* is a short-distance migrant that nests in more arid scrubland of the southern San Joaquin Valley and the Mojave Desert. This subspecies occurs in limited secondary contact with *A. nevadensis* in eastern California and western Nevada, where the Mojave and Great Basin deserts meet (Cicero & Johnson, 2007; Cicero & Koo, 2012); the extent to which these two taxa hybridize is under investigation. The recently elevated species *A. nevadensis*, a longer-distance migrant, nests primarily in Great Basin sagebrush. On San Clemente Island, *A. b. clementeae* is non-migratory and breeds restrictedly in small patches of maritime sage scrub dominated by boxthorn (*Lycium californicum*) mixed with cactus. Finally, *A. b. cinerea* (not studied herein) is restricted to Baja California where it is resident in arid and semi-arid scrub.

Taxa of *A. belli* and *A. nevadensis* were originally described on the basis of size and colour differences (Table 1). Previous quantitative studies (Johnson & Marten, 1992; Cicero & Johnson, 2006; Cicero, 2010; Cicero & Koo, 2012) provide evidence that the three mainland US taxa are morphologically distinct. Specifically, *A. nevadensis* is diagnosably larger than both *A. b. belli* and *A. b. canescens*, and *A. b. belli* is smaller than *A. b. canescens*, although the latter two taxa overlap somewhat in size (Johnson & Marten,

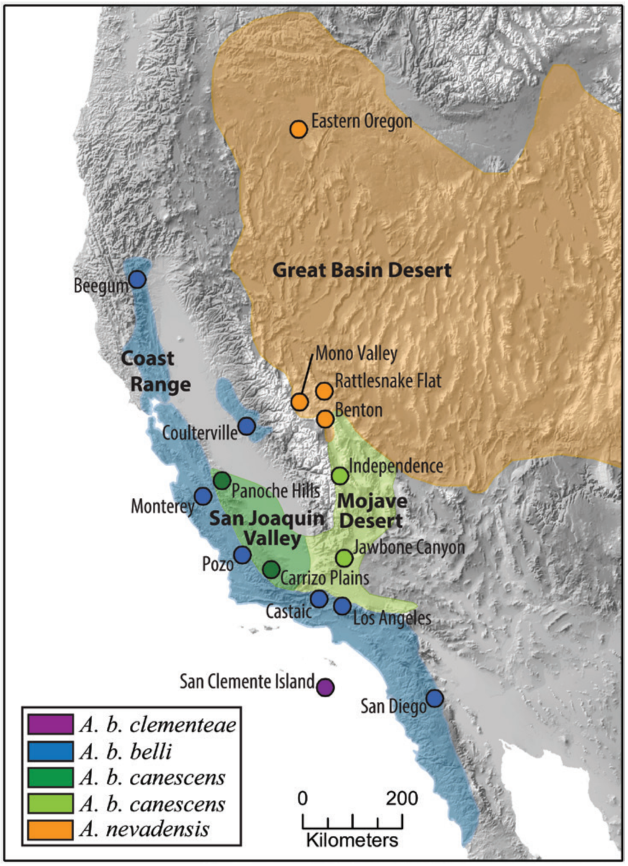

Figure 1. Recording sites (dots) with range distributions of taxa (see key). Site names correspond to those of Cicero & Koo (2012).

Table 1. Summary of phenotypic, ecological, behavioural, and mtDNA differences among the five taxa of *Artemisiospiza*; populations of *A. b. canescens* are divided into San Joaquin Valley (SJV) and Mojave Desert (MD) regions

Subspecies	Size*	Colour	mtDNA†	Primary habitat	Migration
A. b. belli	Small	Dark	AC	Chaparral and coastal sage scrub	Non-migratory
A. b. cinereus	Small	Pale	No data	Arid and semi-arid scrub	Non-migratory
A. b. clementeae	Small	Dark	This study	Maritime arid scrub	Non-migratory
A. b. canescens -SJV	Medium	Pale	AC	Alkali desert scrub	Short-distance migrant
A. b. canescens -MD	Medium	Pale	AD	Alkali desert scrub	Short-distance migrant
A. nevadensis	Large	Pale	BC	Great Basin sagebrush	Long-distance migrant

*Morphological data have been analysed extensively for *A. b. belli*, *A. b. canescens* and *A. nevadensis* (Cicero & Johnson, 2006, 2007; Cicero, 2010; Cicero & Koo, 2012). Morphological variation also has been studied for *A. b. clementeae* (Cicero et al., unpublished).
†mtDNA haplotype groupings AC, AD and BC are from Cicero & Koo (2012).

1992; Cicero & Johnson, 2006). Within *A. b. canescens*, individuals from the San Joaquin Valley and Mojave Desert are not morphologically distinct (Cicero & Koo, 2012). Although *A. b. clementeae* is most similar in size to *A. b. belli*, it is morphological distinct (van Rossem, 1932; Johnson, 1972). Differences in colour are also characteristic, with arid-adapted forms (*A. nevadensis*, *A. b. canescens* and *A. b. clementeae*) having paler plumage than coastal *A. b. belli.*

Genetic data from allozymes (Johnson & Marten, 1992) and mitochondrial DNA (mtDNA; Cicero & Johnson, 2007; Cicero & Koo, 2012) are congruent with phenotype in supporting *A. nevadensis* as a distinct species. However, the genetic data contradict phenotype and ecology when comparing *A. b. canescens* and *A. b. belli*. Although these two subspecies are distinct in size, colour and habitat (Table 1), populations of *A. b. canescens* from the San Joaquin Valley are more closely related in both allozymes (Johnson & Marten, 1992) and mtDNA (Cicero & Koo, 2012; Table 1) to neighbouring *A. b. belli* from the Coast Range than they are to other *A. b. canescens* from the Mojave Desert. The relationship of *A. b. clementeae* has not been tested with molecular data prior to this study.

As oscines that learn their songs during a critical period in early development (e.g. days 10–50 in White-crowned Sparrows, *Zonotrichia leucophrys*; Marler, 1970), Sagebrush and Bell's sparrows acquire their songs through acoustic input from their father or neighbouring males, and maintain that song repertoire (defined by unique song types) for the rest of their lives. In one study in south-eastern Idaho (Rich, 1981), individuals surveyed across 3–4 years sang the same song each year in the same territory. Because *A. b. canescens* from the San Joaquin Valley move upslope after breeding into the range of nesting *A. b. belli* (Grinnell & Miller, 1944; Johnson & Marten, 1992), where they are potentially exposed to *A. b. belli* songs, it is possible that juvenile *A. b. canescens* could acquire song traits of *A. b. belli* if they were still in their critical learning period. Accordingly, we might predict that song relationships would match genetic patterns whereby *A. b. canescens* songs from the San Joaquin Valley are more similar to songs of *A. b. belli* than to those of *A. b. canescens* from the Mojave Desert. Alternatively, because song traits are known to reflect acoustic adaptation to habitat (Wiley, 1991; Dingle *et al.*, 2008; Derryberry, 2009), we might expect that songs of *A. b. canescens* from the San Joaquin Valley and Mojave Desert, areas that are characterized by open arid scrub, would be more similar to each other than to songs of *A. b. belli* from dense chamise chaparral in the Coast Range. Although we do not have any specific predictions about song relationships for insular *A. b. clementeae*, this subspecies occupies arid maritime habitat that appears more similar to habitat of inland *A. b. canescens* than to that of *A. b. belli* on the adjacent mainland (B. Karin and C. Cicero, pers. observ.). Given the distinctiveness of *A. nevadensis* as a species, we expect songs of that taxon to be distinct from those of *A. belli* despite limited secondary contact and sympatry with *A. b. canescens* from the Mojave Desert.

By studying the songs of these taxa, we gain a better understanding of the role of cultural evolution in population divergence. Is song evolution inextricably linked to genealogical history, or can evolution at the lineage and cultural level be independent and respond to different mechanisms? Specifically in this system we assess whether patterns of song variation are concordant with phylogenetic relationships (songs are most similar between San Joaquin Valley *A. b. canescens* and Coast Range *A. b. belli*) or with phenotype and ecology (songs are most similar between San Joaquin Valley and Mojave Desert *A. b. canescens*). We include songs of *A. nevadensis* and *A. b. clementeae* to provide a comprehensive dataset for song analysis and to make more detailed comparisons of the song data to phenotypic, ecological and genetic patterns. Using these data, we explore mechanisms of song divergence in relation to both evolutionary history and ecology.

MATERIAL AND METHODS

Recordings

We recorded songs at the same sites used in earlier genetic and morphological studies (Johnson & Marten, 1992; Cicero & Johnson, 2006; Cicero & Koo, 2012) to facilitate comparisons with previous findings. To supplement this data set, we added recordings for two sites that we obtained from Xeno-Canto (https://www.xeno-canto.org, last accessed March 2015) and the Macaulay Library (https://www.macaulaylibrary.org, last accessed March 2015). In total, the recordings we studied included 4456 songs from 247 individual Sagebrush and Bell's sparrows. After discarding recordings that were too quiet or had interference, we selected a single song from 228 birds which we analysed in detail for this study (Table 2; Supporting Information, Table S1). Of the 16 sites sampled, 12 sites were recorded in 2011–2012 and four sites were recorded between 1991 and 2004. The sites include seven populations of *A. b. belli*, two populations of San Joaquin Valley *A. b. canescens*, two populations of Mojave Desert *A. b. canescens*, four populations of *A. nevadensis* and the single island population of *A. b. clementeae* (Fig. 1). We were unable to obtain songs of one additional subspecies of *A. belli*, *A. b. cinerea* from Baja California, Mexico.

We recorded singing males that we encountered while we walked along a transect at each site. At desert sites occupied by *A. b. canescens* and *A. nevadensis*, the terrain was open and level enough to walk anywhere. We recorded birds at these sites while we walked in a relatively straight line, straying only to get closer to singing individuals. At coastal sites inhabited by *A. b. belli*, dense chaparral prevented walking in transects. There, we recorded individuals heard while we walked on a trail or road. We began recording birds at dawn and continued until they ceased singing, which generally occurred at approximately 10:00 or 11:00 h. From 1991 to 2004, we recorded songs using a Sony TCM-5000EV cassette recorder, Sennheiser ME88 directional microphone and SME-BA3 amplifier. In 2011 and 2012, we used a Marantz PMD661 digital recorder with an ME67 Sennheiser directional microphone to record the birds. All recordings are deposited at the Museum of Vertebrate Zoology, University of California, Berkeley, and are accessible through the museum's online database (https://arctos.database.museum).

Sonographic analysis

We visualized the songs as sonograms (Fig. 2) using RAVEN Pro 1.4 Interactive Sound Analysis Software (Charif *et al.*, 2010) with standardized settings (brightness: 92, contrast: 65, resolution: 256). We removed excess background noise (e.g. wind, other birds, planes, swarms of bees) by utilizing a bandpass filter and occasionally erasing the noise manually. In cases of quiet recordings, we adjusted the brightness and contrast to better visualize the notes in the songs.

Individuals that we recorded produced nearly identical songs in one singing bout, although they might add or subtract a few notes from the beginning or end of the song on occasion. Other studies have found a similar repertoire pattern (e.g. Rich, 1981; Wiens, 1982). The added notes were nearly always an identical sequence, usually a replication of another section of the song and therefore would not dramatically affect our analyses (because most measures were based on sequence similarity). Because we could easily determine an individual's repertoire by visual inspection of the sonograms, we chose the most typical song from each individual for analysis.

We developed a lexicon of 72 distinct note types as seen in the sonograms, and classified these into categories of notes that included downstrokes, upstrokes, trills, broken-whistles and chevrons, among others types (for descriptions see Dryad data repository doi:10.5061/dryad.jc181bs). For example, we defined T1 (trill number one) as a short-duration, large-bandwidth trill and T2 as a longer-duration, lower-frequency trill. Our definitions reflect unique note attributes but do not include specific durations or frequency ranges due to variability between taxa. For each of the 228 songs analysed in this study (Table 2), we systematically logged the sequence of notes from this lexicon and used the selection feature in RAVEN to measure total song duration, minimum song frequency, maximum song frequency and frequency range (maximum minus minimum frequency). We also recorded the number of notes in each song and divided that number by the song duration to determine the number of notes per second (note rate).

From the total number of notes in each song, we determined the number of unique note types as well as the proportion of each of five note categories that were most consistent among songs (upsweeps, downsweeps, trills, chevrons and broken-whistles; see Fig. 2 for examples). For example, the complete lexicon consisted of 11 different types of trills produced in the 228 analysed samples. The number of trill notes in a particular song divided by the total number of notes in the song equals the proportion of notes that are trills, or the frequency of trills. By automating this process using the statistical software R (R Core Team, 2015), we determined the frequency of trills, upsweeps, downsweeps, chevrons and broken-whistles in each song (song data and all R scripts available on Dryad doi:10.5061/dryad.jc181bs). Although we defined other note categories in addition to these five, the others were much less common and therefore not included as proportions because many individuals would have had a value of zero. This gave a total of

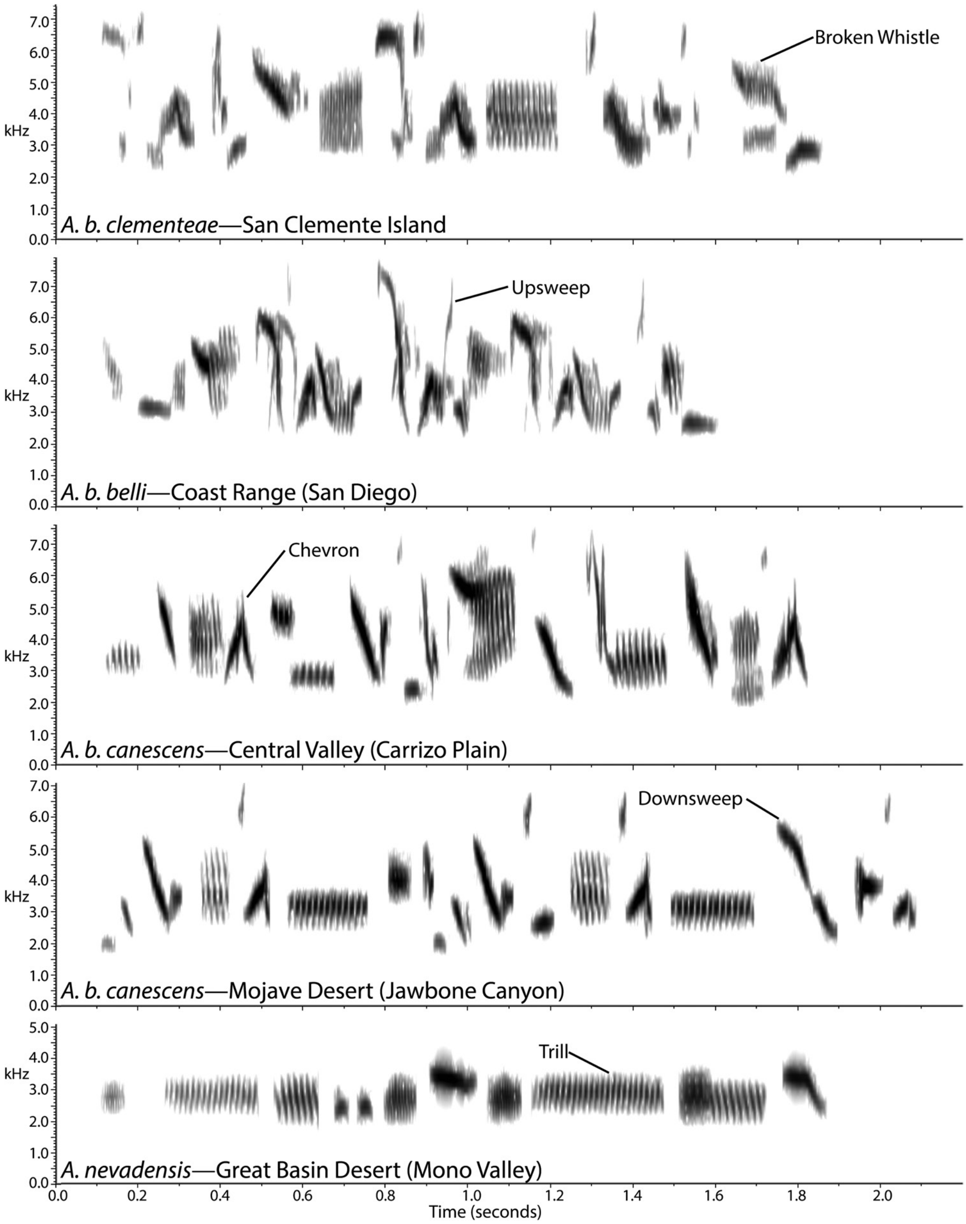

Figure 2. Sample sonograms from five sites representing each of the taxa and geographical regions analysed in this study. Examples of the five most common note types are labelled.

12 continuous, quantitative song variables from each individual for statistical analysis: duration, minimum frequency, maximum frequency, frequency range, number of notes, number of note types, number of notes per second, proportion of trills, proportion of chevrons, proportion of upsweeps, proportion of downsweeps and proportion of broken-whistles. Further analyses of the song data were based on grouping the sampled recordings into five geographical ecoregions according to taxonomy and prior phenotypic and genetic patterns of variation: San Clemente Island (*A. b. clementeae*), Coast Range (*A. b. belli*), San Joaquin Valley (*A. b. canescens*), Mojave Desert (*A. b. canescens*) and Great Basin (*A. nevadensis*).

MOLECULAR GENETICS

We sequenced two mitochondrial genes, NADH dehydrogenase subunit 2 (*ND2*; 1041 bp) and cytochrome B (*CytB*; 1143 bp), and combined those with samples of *CytB* on GenBank (Cicero & Koo, 2012), for 32 ingroup samples and two outgroups (*Amphispiza bilineata*) using standard Sanger sequencing methods for fresh tissues (see Table S2 for sample localities and GenBank

Table 2. Recordings of *Artemisiospiza belli* and *A. nevadensis* used in this study

Site	Recordist	Year	Number of birds recorded	Number of songs recorded	Number of birds analysed
Beegum	C. Cicero	2012	16	384	16
Coulterville	B. Karin	2011	19	260	12
Monterey	B. Karin	2012	9	106	7
Pozo	B. Karin	2011	18	235	20
Castaic	B. Karin	2011	11	120	11
Los Angeles	C. Cicero	2004	9	264	8
San Diego	C. Cicero	2012	14	533	14
San Clemente Island	J. McAntee, J. Bradley[*]	2011	10	125	10
Panoche Hills	B. Karin	2011	20	253	20
Carrizo Plains	B. Karin	2011	19	194	15
Jawbone Canyon	B. Karin	2011	38	460	30
Independence	N. K. Johnson	1991,1993	20	188	20
Benton	N. K. Johnson, C. Cicero	2002	15	700	19
Mono Valley	C. Cicero	2012	15	494	13
Rattlesnake Flat	B. Karin	2012	8	59	6
Eastern Oregon	D. Herr[†], R. Little[†]	1994	7	81	7
Total:			**247**	**4456**	**228**

Sites are labelled as in Figure 1; see figure for distributions of taxa associated with sites. Except where noted, all recordings are archived in the Museum of Vertebrate Zoology, University of California, Berkeley.

[*] Recordings from Xeno-Canto (XC83986).

[†] Recordings from Macaulay Library, Cornell Laboratory of Ornithology (Catalogue Numbers: 106570, 106571, 106572, 106573, 106579, 106531, 106532).

accession numbers). To assess the genetic relationship of *A. b. clementeae*, we extracted DNA from three 1908 specimens and one modern specimen using a Qiagen kit, with the historical samples being extracted in a DNA clean room. To capture mtDNA from the historical samples, we prepared genomic libraries for each individual following Meyer & Kircher (2010) with the Sequence Capture using PCR-generated Probes method (SCPP; Peñalba *et al.*, 2014), and sequenced the samples on a single lane of the Illumina Hiseq 2500 device. The single modern sample of *A. b. clementeae* was included with fresh tissues of other subspecies during Sanger sequencing. To recover homologous sequences to our PCR-generated probes for the 1908 samples, which we derived from PCR-amplification of fresh tissues as described above, we made use of the data processing pipeline that accompanied the publication of the SCPP method (https://www.github.com/MVZSEQ/SCPP). Sequences generated for this study add to existing molecular data sets (allozymes: Johnson & Marten, 1992; mtDNA: Cicero & Koo, 2012) for *A. belli* and *A. nevadensis*.

For further analyses we aligned sequences by eye, concatenated the two mitochondrial genes, and conducted maximum likelihood (ML) phylogenetic analyses in RAxML v8.1.15 (Stamatakis, 2014) and Bayesian inference (BI) in MrBayes v.3.2 (Ronquist & Huelsenbeck, 2003). The optimal partitioning scheme and evolutionary model were assessed with PartitionFinder v1.1.1 (Lanfear *et al.*, 2012) using the Bayesian information criterion (BIC) and the greedy algorithm, specifying an HKY model for MrBayes and GTRGAMMA for RAxML on three separate partitions for combined *CytB* and *ND2* first, second and third codon positions. We ran the ML analysis for 100 rapid bootstraps and the BI analysis for 10 million generations with four chains sampling every 1000 generations. Adequate convergence of the Markov chains was assessed by eye in Tracer v1.6 (Rambaut & Drummond, 2013) with all effective sample size (ESS) values well over 5000, and the first 25% of sampled trees were discarded as burnin to generate the consensus tree. Haplotype networks were generated in R (R Core Team, 2016) using the pegas package (Paradis, 2010) and colour coded by population.

ENVIRONMENTAL DATA

To compare the environmental space occupied by populations in the five different geographical ecoregions, we used the same set of locality points as Cicero & Koo (2012) and added localities from San Clemente Island.

Because high-accuracy localities from San Clemente Island were unavailable, those points were randomly generated with ArcGIS (ArcGIS Desktop: Release 10; Environmental Systems Research Institute) within a polygon composed of the medium- and high-density habitats for *A. b. clementeae* (Turner, 2009). For all populations, we used localities within the known breeding ranges of the taxa and excluded points in the contact zone between *A. b. canescens* and *A. nevadensis* (Cicero & Johnson, 2007). The final dataset included 886 coordinates, which we assigned to taxa manually based on known distributions (see Supplementary Information).

For the environmental data, we downloaded bioclimatic variables at 30-second resolution from WorldClim (http://worldclim.org/version1, last accessed October 2015), eliminated highly correlated variables and used the same eight environmental predictor variables as Cicero & Koo (2012): Isothermality (Bio3); Temperature Seasonality (Bio4); Maximum Temperature of the Warmest Month (Bio5); Minimum Temperature of the Coldest Month (Bio6); Mean Temperature of the Driest Quarter (Bio9); Precipitation of the Driest Month (Bio14); Precipitation Seasonality (Bio15); and Precipitation of the Wettest Quarter (Bio16). We used ArcGIS Spatial Analyst to extract the values of each of the eight variables at each locality, and performed a principal component analysis (PCA) on the resulting environmental data in R using the methods previously stated. This analysis differs from Cicero & Koo (2012) in the inclusion of locations from San Clemente Island, which required the use of WorldClim instead of PRISM climate data used in the earlier study. We also downloaded Global Ecological Land Units data (ELUs, Sayre *et al.* 2014, available from https://catalog.data.gov/dataset/global-ecological-land-units-elus, last accessed October 2015) and generated maps to visually compare bioclimate vs. land cover among ecoregions using open access categorical data. In addition, we extracted the bioclimatic and landcover categorical data for each point locality to compare habitat occupancy among populations in our five ecoregions.

STATISTICAL ANALYSES

As a first step in assessing song variation, we used R to perform an ANOVA on each of the 12 song characters, generated boxplots for each song variable by ecoregion, and assessed pairwise statistical differences between populations using Tukey's Honestly Significant Difference (HSD) test in the 'agricolae' package (de Mendiburu, 2014) with the default settings and alpha = 0.05. We also performed a PCA on the set of 12 continuous song characters using the prcomp command in the 'stats' package in R (R Core Team, 2015), scaling the variables before analysis for unit variance. We assessed if these song characters could distinguish the five populations by running a linear discriminant function analysis (DFA) in the 'MASS' package in R (Ripley *et al.*, 2017) using a random half of samples as training data ($N = 114$) and testing the predictions on the other half of samples ($N = 114$).

We generated a matrix of presence–absence data for each of the 72 distinct note types in the lexicon for each individual (1 = present, 0 = absent), and used these data to generate a maximum parsimony tree with PAUP* (Swofford, 2003) and for BI using the discrete character model in the program MrBayes 3.2 (Ronquist & Huelsenbeck, 2003). We ran the Markov chain for 50 million generations with sampling every 1000, and checked for convergence by eye using Tracer v1.6. We discarded the first 25% of trees to produce the Bayesian consensus tree, and visualized the results as a network in the program Haploviewer (http://www.cibiv.at/~greg/haploviewer, Max F. Perutz Laboratoratories, Center for Integrative Bioinformatics, Vienna, last accessed May 2015). Similar to a genetic haplotype network, the resulting network displays a unique 'song haplotype' for a particular set of note types found in the song. Two individuals with different songs may share the same 'song haplotype' if they use exactly the same repertoire of notes. When applied to song data, this novel method allows for visualization of song relationships and comparison of song similarity both within (size and number of haplotypes) and between (network distance) populations.

Using R, we computed the simple matching coefficient for each pair of songs (228 × 228 comparisons = 51 984 pairs) according to the formula described by Cicero & Benowitz-Fredericks (2000). The simple matching coefficient for each pair is equal to the sum of the number of matching notes plus the number of non-matching notes (i.e. the remainder of the lexicon of notes), divided by the total number of notes in the lexicon. The resulting pairwise coefficients comprised a symmetrical 228 × 228 matrix of similarity values. We then computed a dissimilarity matrix by subtracting each simple matching coefficient from 1, and used those data to perform a cluster analysis and build a neighbour-joining tree using the 'phangorn' package for R (Schliep, 2011). We plotted the results as dendrograms using the 'ape' package for R (Paradis *et al.*, 2004). For both analyses, the clustered tips fell into well-distinguished, geographically structured groups for nearly all samples (see Supporting Information). We therefore averaged the simple matching coefficients for individuals within each site to obtain a compressed matrix of mean dissimilarity values, and performed a second UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and neighbour-joining analysis for these averaged distance values to generate new site-averaged dendrograms.

We assessed the correlation between song and phenotype or genetics in distance matrices using a Mantel test in the *ape* package in R. We compared the site-averaged song distance matrix as described above with a matrix of site-averaged raw pairwise genetic (mtDNA) distances and another distance matrix of site-averaged morphological Euclidean distances scaled by the maximum. Morphological data for *A. b. belli*, *A. b. canescens* and *A. nevadensis* are from Cicero & Koo (2012); measurements for *A. b. clementeae* are unpublished (C. Cicero *et al.*, in prep.). The data included seven morphological variables: wing length, tail length, bill length, bill depth, bill width, tarsus plus toe length and body mass. We averaged the data by site because each dataset is composed of unique sets of individuals from a site (except for a few sites where recorded birds were vouchered) and different numbers of samples were analysed from each site. For cases where sites were non-overlapping across datasets, we used the most closely located site from the same subspecies or population while excluding the contact zone near Bishop (e.g. the East Oregon song site was compared with Plush, OR, and Denio, NV). Because we do not have morphological data for the Los Angeles site, we excluded this site from the song and genetic distance matrices in the Mantel test comparisons. We did not conduct a Mantel comparison with the environmental data because those data were extracted from point localities across the ranges of Sagebrush and Bell's sparrows rather than from individual sites with recordings, and it would be difficult and contrived to create compatible site-averaged data.

RESULTS

Phylogenetic analysis

We obtained nearly identical mtDNA results from the ML and BI analyses, and consistently recovered three major clades (Fig. 3): (1) *A. b. belli*, *A. b. clementeae* and San Joaquin Valley *A. b. canescens*; (2) *A. b. nevadensis*; and (3) Mojave Desert *A. b. canescens*. The analyses differed only in that the ML tree did not support the monophyly of Mojave Desert *A. b. canescens*, but instead placed them as closely related genetically in a polytomy near the root of the tree. Haplotype networks for individual genes showed identical population groupings (Fig. 3 inset). We successfully obtained data from three 1908 specimens and one modern tissue sample of *A. b. clementeae*, all of which produced nearly identical sequences (differing by two single nucleotide polymorphisms in *CytB*) and shared haplotypes with specimens of *A. b. belli*. As far as we are aware, these are the only genetic data available for this federally threatened subspecies endemic to San Clemente Island.

Acoustic parameters

The ANOVA was significant ($P < 0.05$) for all 12 acoustic characters among the five geographical ecoregions. Box and whisker plots of the data and results of Tukey's post-hoc test are visualized in Figure 4, with different letters corresponding to significantly different groups. Populations of *A. b. canescens* from the San Joaquin Valley and Mojave Desert showed similar song characteristics in most variables, whereas some song characters from *A. b. belli* from the Coast Range had a markedly different distribution. However, San Joaquin Valley *A. b. canescens* was more similar to Coast Range *A. b. belli* than to Mojave Desert *A. b. canescens* in the use of broken whistles (Fig. 4L). Interestingly, *A. b. belli* was more similar to *A. b. canescens* from the Mojave Desert than from the San Joaquin Valley in the use of trills (Fig. 4H). Songs of *A. nevadensis* from the Great Basin were distinctive from all *A. belli* in having a much lower maximum frequency (Fig. 4B) and frequency range (Fig. 4C), and in using fewer downsweeps in their songs (Fig. 4K). We also found that *A. nevadensis* had particularly small note repertoires (number of note types), as did *A. b. clementeae* compared to other taxa (Fig. 4F). Songs of *A. b. clementeae* were allied with *A. b. canescens* for most of the song characters, and differed significantly from the two *A. b. canescens* groups in only one song trait (proportion of broken whistles; Fig. 4L). Our data showed that *A. b. belli* had the most variable songs (highest number of note types; Fig. 4F), with more total notes delivered at the fastest rate (highest number of notes per second; Fig. 4G), whereas *A. nevadensis* had the longest, least variable songs that were given at the slowest rate (Fig. 4D–G).

PCA on the 12 song characters showed 100% separation of the songs of Coast Range *A. b. belli* and Great Basin *A. nevadensis* along PC1 (Fig. 5). Songs with intermediate characteristics defined *A. b. canescens* from both the Mojave Desert and the San Joaquin Valley along the same axis. However, those from the San Joaquin Valley formed a tight (i.e. less variable) group within the space occupied by *A. b. canescens* as a whole. Furthermore, unlike songs from the Mojave Desert, they showed no overlap with *A. nevadensis* songs. Songs of San Clemente Island *A. b. clementeae* occupied the same PCA space as *A. b. canescens*, overlapping with songs from both the San Joaquin Valley and the Mojave Desert. PC1 accounted for 44% of the variance in song characters (Table S3), which reflected differences among regions in maximum frequency, frequency range, number of

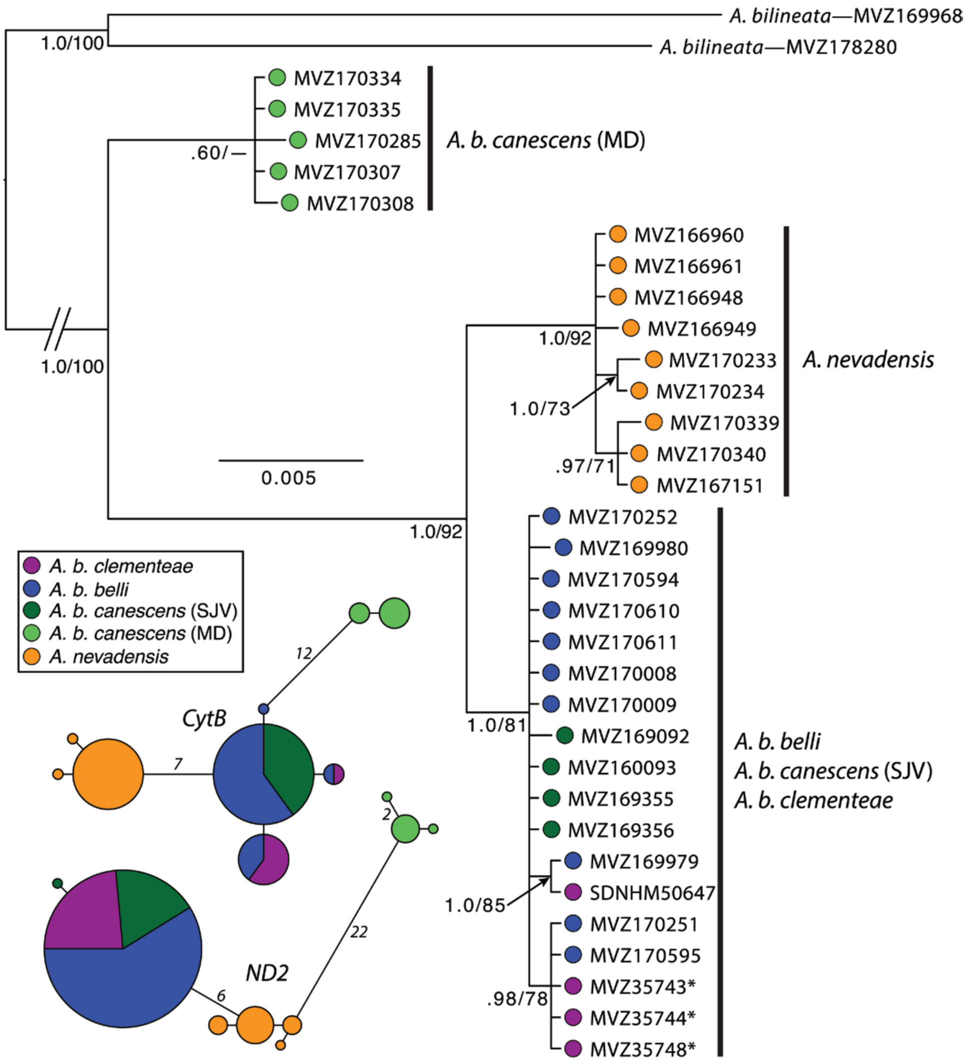

Figure 3. Phylogenetic tree for concatenated *ND2* and *CytB* from Bayesian inference (BI) analysis, with support shown as BI posterior probabilities (left) and maximum likelihood bootstrap replicates (right). Inset: haplotype networks for *ND2* and *CytB*, with number of changes between haplotypes written on connections with more than one change, and the size of haplotype circles corresponding to the number of individuals with that haplotype. Tree tips and haplotypes are colour coded according to population as in the key. Historical 1908 specimens used for sequencing *A. b. clementeae* are indicated by an asterisk. Scale bar represents changes per site.

notes, number of note types and number of notes per second. Variance in the proportion of chevrons, trills, upsweeps and broken whistles also contributed to PC1. PC2 explained 14% of the total variance and mostly reflected differences within regions in duration, minimum frequency, proportion of downsweeps and number of notes. The first nine axes explained 99% of the variance.

Using the same set of acoustic song characters, DFA correctly classified 102 of 114 songs used to test the model. Of the 12 songs that were incorrectly classified, eight were Mojave Desert *A. b. canescens* (seven classified as San Joaquin Valley *A. b. canescens* and one classified as *A. nevadensis*), three were *A. b. belli* (two classified as San Joaquin Valley *A. b. canescens* and one classified as Mojave Desert *A. b. canescens*), and one was *A. b. clementeae* classified as a San Joaquin Valley *A. b. canescens*. Stepwise analysis suggested that the number of notes per second was the most important variable in distinguishing songs among groups.

Measures of note similarity

UPGMA and neighbour-joining trees based on pairwise simple matching coefficients between sites, as

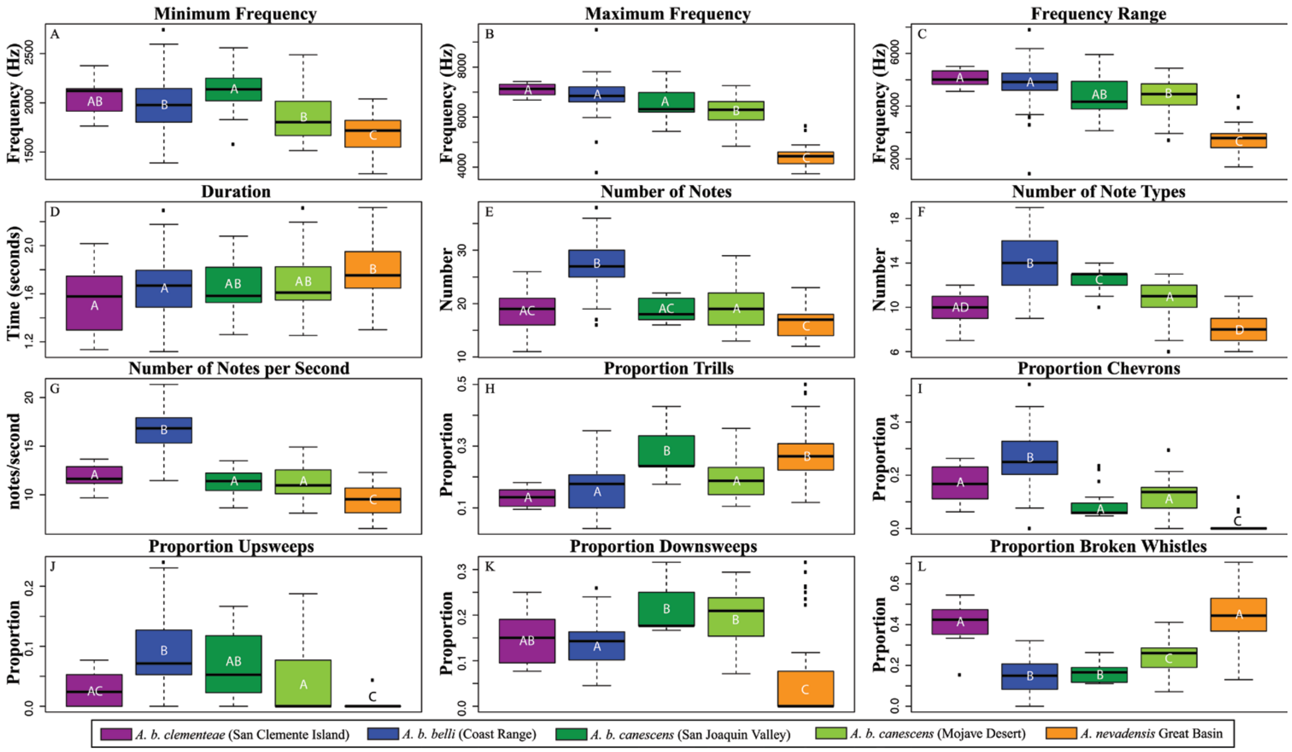

Figure 4. Box and whisker plots of 12 measured song characters between study regions. ANOVA was significant for all 12 variables against populations. Groups that are significantly different by Tukey's HSD test are labelled with different letters.

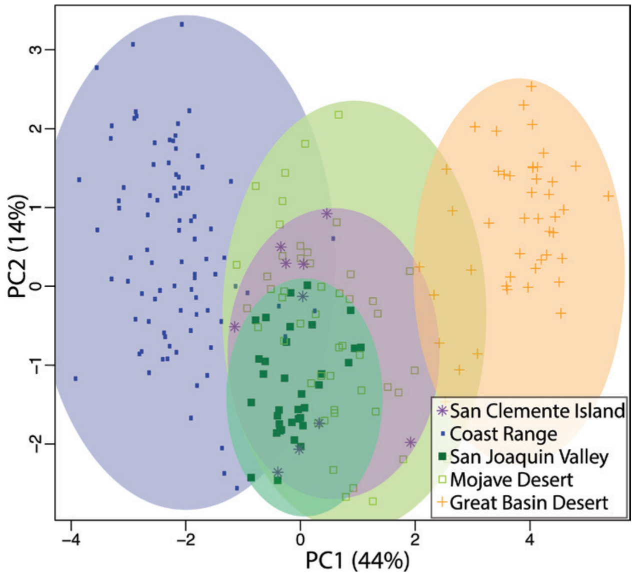

Figure 5. Principal component analysis of 12 song characters on the first two axes. See Table S3 and Fig. S4 for loadings and proportion of variance for all principal components.

well as Bayesian and maximum parsimony analyses of the presence–absence of the 72 note types defined in our lexicon, all reflect some measure of the number of note types shared between individual songs. These different analyses showed general agreement in some respects, and notable differences in others. Here we present UPGMA (Fig. 6A) and neighbour-joining (Fig. 6B) dendrograms and the Bayesian consensus tree visualized as a network (Fig. 7). Trees with all 228 individual tips are provided in the Supporting Information (Figs S1–3).

All analyses showed geographically distinct clusters of song type. In the UPGMA tree of site-averaged simple matching coefficients (Fig. 6A), songs formed two groups that clearly separated Coast Range *A. b. belli* from all other populations. Within the second group, *A. nevadensis* also formed a distinctive cluster. While the two sampled populations of *A. b. canescens* from the Mojave Desert (Jawbone Canyon and Independence) likewise grouped together, songs of *A. b. canescens* from the two sites in the San Joaquin Valley (Carrizo Plains and Panoche Hills) were dissimilar. Rather, songs of Carrizo Plains individuals shared more note types with songs of Mojave Desert *A. b. canescens* and San Clemente Island *A. b. clementeae* than they did with those of Panoche individuals. Songs of *A. b. clementeae* were nested within the interior group of populations and shared the most note types with Mojave Desert *A. b. canescens.*

The neighbour-joining tree (Fig. 6B) showed the same two groupings as the UPGMA, with *A. b. belli*

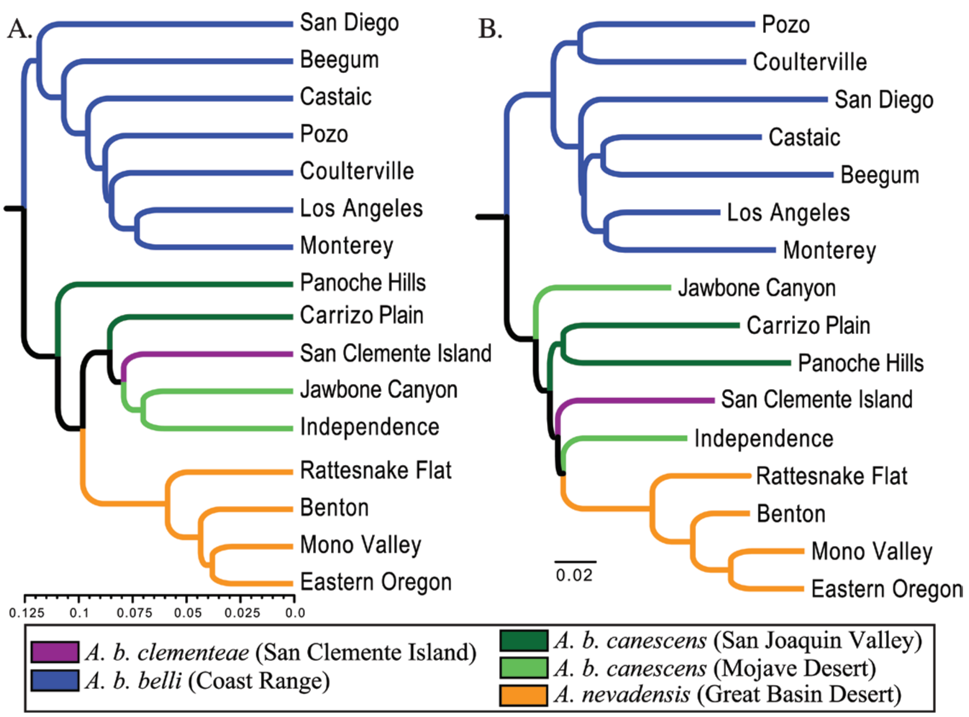

Figure 6. Midpoint-rooted UPGMA dendrogram (A) and midpoint-rooted neighbour-joining tree (B) of site-averaged pairwise simple matching coefficients for note repertoires. Colours correspond to population assignment (see key). Dendrograms of un-averaged data are provided in the Supporting Information. Scale bars in both trees represent distance between clusters based on the Simple Matching Coefficient.

and *A. nevadensis* separated from other taxa. However, unlike the UPGMA analysis, songs of *A. b. canescens* from the San Joaquin Valley (Carrizo Plains and Panoche Hills) clustered together, whereas those from the Mojave Desert did not form a group. As in the UPGMA tree, songs of *A. b. clementeae* were most similar to those of *A. b. canescens*, and shared the most note types with songs from Independence in the Mojave Desert.

The Bayesian consensus tree network (Fig. 7) showed a pattern similar to both the PCA and the UPGMA analyses, with songs from mainland populations forming distinct, geographically structured groups. Songs of *A. b. belli* and *A. nevadensis* were most dissimilar in their note types, with intermediate songs characterizing *A. b. canescens* from both the Mojave Desert and the San Joaquin Valley. As in the UPGMA dendrogram, island songs of *A. b. clementeae* shared the most note similarities with *A. b. canescens* from the Mojave Desert. The Bayesian network revealed that individuals of *A. b. canescens* from the San Joaquin Valley (Carrizo Plains and Panoche Hills) had smaller note repertoires (shared more note types) compared to Mojave Desert *A. b. canescens*. Carrizo Plains individuals, in particular, had almost no variation in repertoire as seen by the largest circle in Figure 7. Both the Carrizo Plains and Panoche Hills populations showed note similarity to Mojave Desert *A. b. canescens*. However, the network does not provide evidence that the two San Joaquin populations share unique song characters, as the two major branches for San Joaquin Valley populations in the network correspond to Carrizo Plains and Panoche Hills, separately. The largest note repertoire was seen in populations of *A. b. belli*, which had numerous unique song repertoires and very few shared repertoires (circles larger than a single individual) among individuals within the subspecies.

The Mantel test comparison of song distance vs. genetic distance showed no significant correlation ($P = 0.487$), whereas song distance and morphological distance were significantly correlated ($P = 0.003$). Genetic distance and morphological distance were not significantly correlated ($P = 0.313$).

ENVIRONMENTAL DATA

Bioclimatic variables showed a pattern of site occupancy (Fig. 8) that is consistent with Cicero & Koo (2012) despite the use of a different climate dataset (WorldClim vs. PRISM) to allow for inclusion of

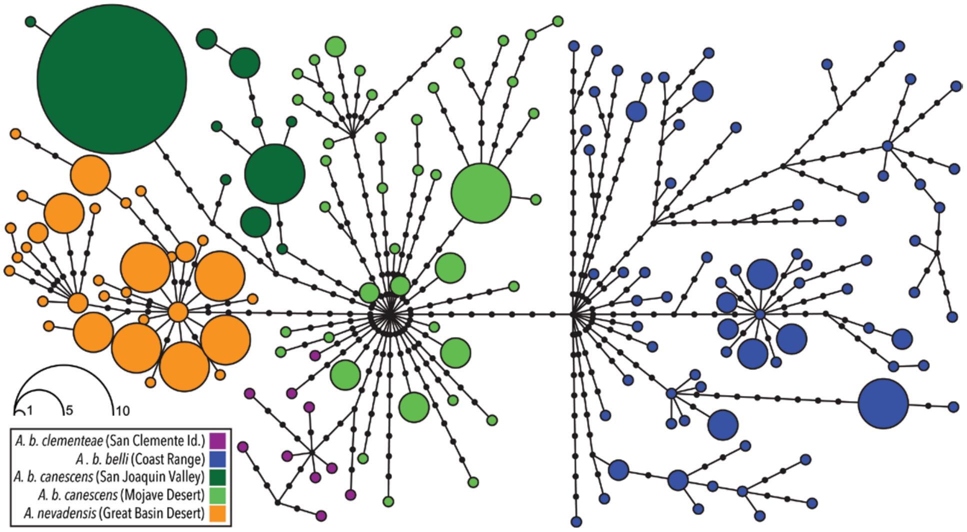

Figure 7. Network of Bayesian tree based on presence–absence data for notes used in song repertoires. Each black dot indicates a single difference in note type used in the song, thus separating unique song repertoires. Colours correspond to geographical population assignment (see key), and sizes of the circles correspond to the number of individuals that share the exact same set of note types in their song.

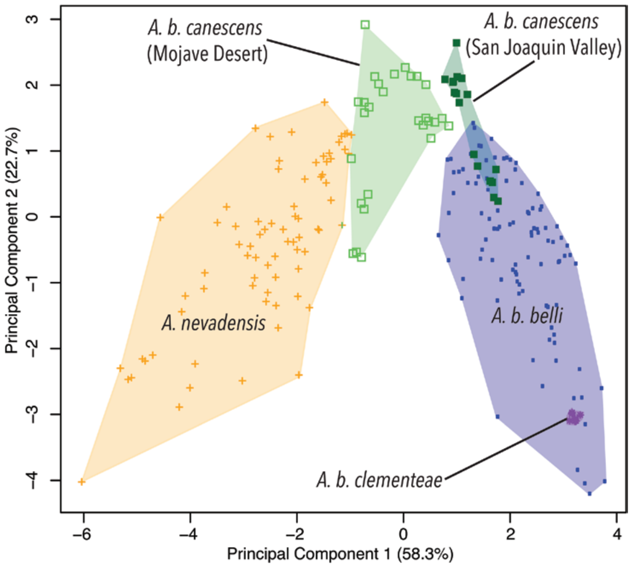

Figure 8. Principal component analysis of bioclimatic variables showing the environmental space inhabited by each population, colour-coded identically to Figure 5.

A. b. clementeae localities. Specifically, *A. b. belli* and *A. nevadensis* show no overlap in their bioclimatic environment in the PCA, whereas Mojave Desert *A. b. canescens* occupies sites that are bioclimatically intermediate. The environmental spaces for different taxa varied primarily along PC1, which accounted for 58.3% of the variation in the data and was strongly associated with all bioclimatic variables except for Bio5 (Maximum Temperature of the Warmest Month). PC2 accounted for 22.7% of the total variance and distinguished the environmental space (primarily Bio5 and Bio9, Mean Temperature of the Driest Quarter) occupied by *A. b. canescens* vs. the other taxa. Table S4 provides a matrix of PCA loadings.

Analysis of categorical data for bioclimate and land cover (Fig. 9) showed different patterns of similarity depending on the data source. The categorical results for bioclimate indicate that the environments of *A. b. canescens* in the San Joaquin Valley, *A. b. belli* and *A. b. clementeae* all share warm semi-dry characteristics (proportions of localities in that category are 68.6, 67.3 and 100%, respectively). However, San Joaquin Valley and Mojave Desert *A. b. canescens* also shared the highest values for hot dry or warm dry climates (31.4 and 72.0% of localities, respectively). Coastal *A. b. belli* was the only subspecies with a warm–wet environment (21.2% of localities), whereas Great Basin *A. nevadensis* was unique in being dominated by a cool semi-dry climate (84.8% of localities). In contrast, land cover data (Fig. 9) showed the greatest similarity in vegetation among San Clemente Island (94.0% shrubland), Mojave Desert (71.2% shrubland) and Great Basin (73.9% shrubland) localities. San Joaquin Valley localities had the next highest percentage (42.9%) of shrubland, with Coast Range localities being distinguished as having the lowest percentage (22.4% shrubland). Overall, the land cover results are more concordant with the song similarity analyses than the bioclimatic data.

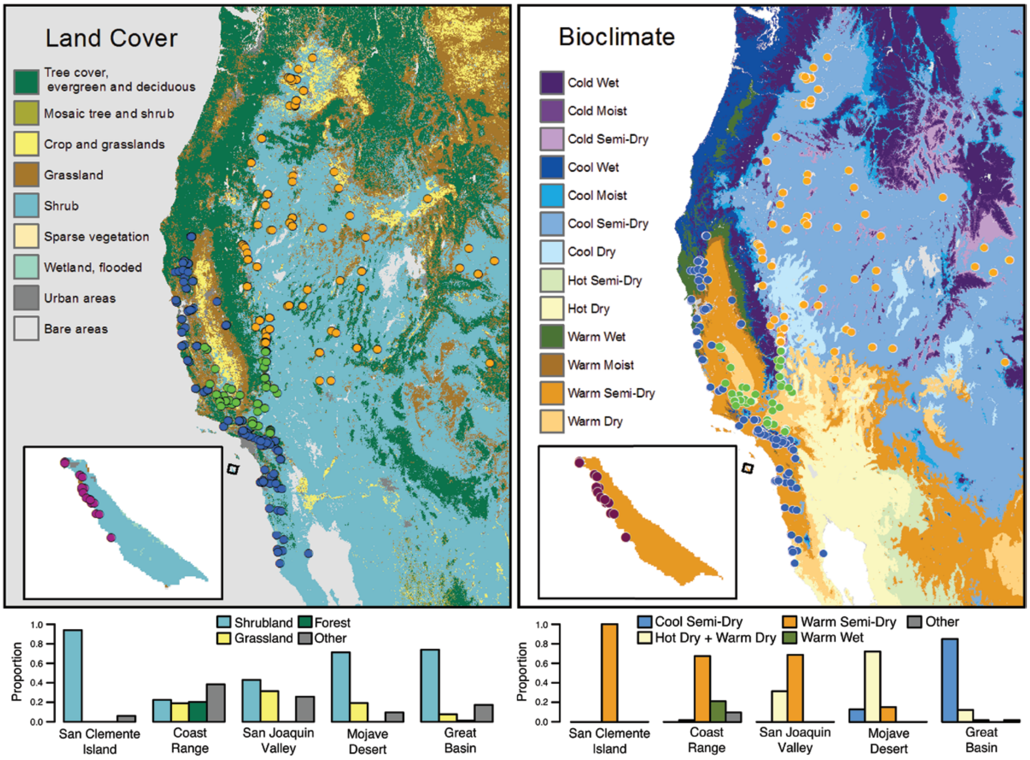

Figure 9. Maps of land cover (left) and bioclimate (right) in the western United States. Dots correspond to environmental niche model analysis from Cicero & Koo (2012) and are colour coded by subspecies: *A. b. clementeae* (purple), *A. b. belli* (blue), *A. b. canescens* (green) and *A. nevadensis* (orange). The bar graphs below each map correspond to the proportion of extracted point values within each land cover or bioclimatic category; warm dry and hot dry bioclimates were combined for simplicity. San Clemente Island shows similar land cover to desert sites (i.e. *A. b. canescens* and *A. nevadensis*) in concordance with song affinities, but is similar in bioclimate to coastal sites (i.e. *A. b. belli*) and the San Joaquin Valley in concordance with molecular results. Base map data available from ESRI (Sayre *et al.* 2014).

DISCUSSION

PHYLOGENETIC RELATIONSHIPS

In this study, we built upon the *CytB* dataset of Cicero & Koo (2012) with the addition of another mitochondrial gene, *ND2*, and sequences of *A. b. clementeae* that include historical 1908 museum specimens. We uncovered with higher support the same general patterns as Cicero & Koo (2012), placing San Joaquin Valley *A. b. canescens* with *A. b. belli* (also supported by allozymes, Johnson & Marten, 1992), and showing both taxa to be more closely related to *A. nevadensis* than to Mojave Desert *A. b. canescens* (Fig. 3). This relationship renders *A. belli* paraphyletic with respect to *A. nevadensis*, which was recently elevated from a subspecies of *A. belli* to full species status (Chesser *et al.*, 2013), although this result should be interpreted with caution as we have not specifically tested placement of the root of the tree.

The mtDNA data place *A. b. clementeae* within the *A. b. belli* and San Joaquin Valley *A. b. canescens* clade. All four specimens of *A. b. clementeae* sequenced for this study share identical mitochondrial haplotypes (of two haplotypes) with *A. b. belli* (Fig. 3). The lack of mitochondrial divergence from *A. b. belli* suggests that *A. b. clementeae* dispersed to San Clemente Island relatively recently and quickly underwent morphological change (bill length and body size; Ridgway, 1898; Johnson, 1972) in this novel environment. Niche divergence accompanied by phenotypic plasticity has been invoked to explain lineage diversification among mainland taxa of *A. belli* and *A. nevadensis*, including the contradictory molecular and phenotypic patterns exhibited by *A. b. canescens* in the San Joaquin Valley vs. Mojave Desert (Cicero & Koo, 2012). On San Clemente Island, adaptation through phenotypic plasticity is more likely than phenotypic change acting on existing genetic variation because founder effects of island dispersal would probably restrict genetic diversity in insular populations. We argue below that acoustic adaptation to local environments may have led to the observed patterns of song divergence in the group. This is appealing as songs represent a phenotype that, similar to morphology and coloration, are adapted to the environment (Boncoraglio & Saino, 2007), but are less constrained by genetics in oscine songbirds.

MAINLAND SONG EVOLUTION

In comparison to genetic evolution, songs and dialects of oscine passerines can evolve much more rapidly between generations due to selective influences from environmental and behavioural factors (e.g. Soha *et al.*, 2004; Xing *et al.*, 2013). A primary goal of this study was to compare patterns of song variation with phenotypic, genetic and ecological variation among mainland populations of Bell's and Sagebrush sparrows. Specifically, we asked whether observed song differences are concordant with phylogenetic relationships (prediction: songs of San Joaquin Valley *A. b. canescens* are most similar to those of *A. b. belli*) or with phenotype and ecology (prediction: songs of San Joaquin Valley *A. b. canescens* are most similar to those of Mojave Desert *A. b. canescens*). We included songs of *A. nevadensis* to provide a broader geographical perspective on song evolution among mainland sparrow populations, and because *A. nevadensis* and *A. b. canescens* occur in limited secondary contact or sympatry in eastern California and western Nevada (Cicero & Johnson, 2007; Cicero & Koo, 2012).

Our analyses of overall song characteristics (Figs 4, 5) and note repertoire (Figs 6, 7) all agree with the second prediction that songs of *A. b. canescens* from the San Joaquin Valley and Mojave Desert are most similar to each other, and are clearly distinctive from those of Coast Range *A. b. belli*. The DFA further confirms these patterns, indicating that songs can be accurately assigned to subspecies, but songs of San Joaquin Valley vs. Mojave Desert *A. b. canescens* are difficult to separate. The patterns of song variation are concordant with morphological variation (significant correlation with Mantel test) and ecology of the subspecies (Fig. 9), but contradict patterns of genetic variation (no significant correlation with Mantel test) in which San Joaquin Valley *A. b. canescens* and *A. b. belli* share allozymes and the same mitochondrial haplotype (Johnson & Marten, 1992; Cicero & Koo, 2012; Fig. 3). Songs of *A. nevadensis*, on the other hand, form their own distinctive grouping that supports the species-level distinction of this taxon based on genetic, morphological and ecological traits (Cicero & Koo, 2012; Chesser *et al.*, 2013; Figs 6, 7).

The contradiction between phenotypic and genetic results when comparing *A. b. canescens* and *A. b. belli* could be the product of ecological pressures and strong selection at different sites. In subspecies of song sparrows (*Melospiza melodia*), for example, pronounced differences in body size, bill morphology and plumage colour (e.g. Chan & Arcese, 2002; Patten & Pruett, 2009; Pruett & Winker, 2010) have occurred without accompanying geographical structure in mtDNA (Zink, 1991; Fry & Zink, 1998) and reflect local adaptation to diverse environments. Likewise, morphological adaptation in tidal swamp sparrows (*M. georgiana*, Greenberg *et al.*, 1998) and selection for camouflage in beach mice (*Peromyscus polionotus*, Mullen *et al.*, 2009) are incongruent with molecular data. Although genetic markers examined to date in *A. belli* (allozymes: Johnson & Marten, 1992; mtDNA: Cicero & Koo, 2012; Fig. 3) do not distinguish between San Joaquin Valley *A. b. canescens* and Coast Range *A. b. belli*, birds in the San Joaquin Valley phenotypically resemble other *A. b. canescens* from the Mojave Desert (Cicero, 2010). Furthermore, although San Joaquin Valley sites are bioclimatically distinct from other regions, they share characteristics of the Mojave Desert including higher summer temperatures and reduced winter precipitation (Cicero & Koo, 2012; Fig. 9, Table S4). San Joaquin Valley sites also show similarities to Mojave Desert sites in satellite-based land cover data, with Coast Range sites containing much denser leaf cover than both arid interior regions (Fig. 9; B. Karin and C. Cicero, pers. observ.). Therefore, the phenotypic differences between San Joaquin Valley and Coast Range sparrows could be driven by phenotypic plasticity (Cicero & Koo, 2012). However, additional markers from the nuclear genome need to be sequenced to test this prediction.

Song divergence between taxa could be a product of the same environmental pressures that led to phenotypic differences. In the Great Basin, environmental conditions and large territories are thought to place selective pressures on song structure in *A. nevadensis* to favour long-distance transmission and locatability of singing males (Rich, 1985). The evolution of song in this species has occurred concordantly with phenotype (large body size, pale plumage), which likewise reflects adaptation to that environment (Cicero & Koo, 2012). At a landscape scale, studies of *A. nevadensis* song have shown that variation is greater between rather than within populations, but whether such differences are due to habitat structure, chance events ('epiphenomena'), or other factors is unclear (Rich, 1981; Wiens, 1982). Within the range of *A. belli*, the Coast Range can be classified as a more complex acoustic environment with more varied terrain and thicker scrub than either the San Joaquin Valley or the Mojave Desert (B. Karin and C. Cicero, pers. observ.). In addition to its topographic and ecological complexity, the Coast Range also experiences greater precipitation seasonality (Cicero & Koo, 2012; Table S4), which has been shown to affect acoustic variability in songbirds (Medina & Francis, 2012). These differences are probably reflected in the more complex and variable songs typical of Coast Range *A. b. belli* (Figs 2, 4; song complexity here is defined by the number of notes and note types characteristic of the song). Selective pressures on song in the San Joaquin Valley and Mojave Desert are probably comparable because of the relatively flat topography and less-dense vegetation that characterizes both of those inland regions. Thus, our data support the acoustic adaptation hypothesis because songs of mainland *A. belli* have evolved independently of genetic relationships but similarly to morphology and ecology, and therefore are likely to be adapted to the respective breeding environments of each subspecies.

This view is reinforced by our evidence that *A. b. belli* and neighbouring *A. b. canescens* from the San Joaquin Valley do not seem to be influenced by each other's songs where they co-occur. Breeding occurs earlier in the San Joaquin Valley than in the Coast Range because of the higher summer temperatures (Cicero & Koo, 2012), and individuals of *A. b. canescens* disperse upslope from the valley into Coast Range sites after they have finished nesting but while resident *A. b. belli* are still actively singing and breeding (Grinnell & Miller, 1944; Johnson & Marten, 1992). If such close contact occurs during the potentially critical period of song learning, it seems plausible that some subspecific song characters would be transmitted to heterosubspecific populations. However, no interbreeding has ever been recorded between these two subspecies, and no transmission of song characters between the subspecies is apparent. Dispersing *A. b. canescens* individuals are not in breeding condition (i.e. they have small gonads, are moulting and are not singing; Johnson & Marten, 1992; C. Cicero pers. observ.), and the lack of singing at this time reduces the likelihood of introducing interior song characters into coastal populations. Conversely, the lack of transmission of coastal song characters into interior populations suggests that juvenile *A. b. canescens* may be past their critical learning period when they disperse into areas with singing *A. b. belli*, or that there is selection against incorporating aspects of *A. b. belli* songs when they return downhill into a different acoustic environment. Furthermore, our data show that the songs of *A. b. belli* are fairly consistent across its range, and do not show greater similarity to *A. b. canescens* songs near the point of range contact (Figs 5–7), although they do show similarity in maximum frequency and the proportion of broken whistles when comparing all populations of each subspecies (Fig. 4). We therefore hypothesize that the songs of *A. b. belli* and *A. b. canescens* have adapted to the acoustic environment of the Coast Range and San Joaquin Valley, respectively, and it may be disadvantageous to incorporate vocal features from the other subspecies.

SAN CLEMENTE ISLAND SONGS

Divergence and adaptation of island populations has interested evolutionary biologists for decades (e.g. MacArthur & Wilson, 1967; Grant & Grant, 2007). Off the coast of Mexico, dark-eyed Juncos (*Junco hyemalis*) on Guadalupe Island have diverged in mtDNA, morphology and song variables from mainland populations, indicating a cryptic lineage (Aleixandrei *et al.*, 2013). The lack of opportunity to learn mainland songs, and different sexual selection pressures, have been invoked to explain song divergence in dark-eyed juncos as well as Anna's hummingbirds (*Calypte anna*) on that island (Mirsky, 1976). The California Channel Islands are less isolated than Guadalupe Island, but nonetheless have intrigued biologists because of their natural and geological history, many endemic plants and animals, and conservation issues (e.g. Diamond, 1969; Johnson, 1972; Lynch & Johnson, 1974; Schoenheer *et al.*, 1999). Recent studies have shed light on genetic and morphological divergence in bird species on the Channel Islands (e.g. Delaney *et al.*, 2008; Caballero & Ashley, 2011; Greenberg & Danner, 2012, 2013; Mason *et al.*, 2014b; Wilson *et al.*, 2015). However, little is known about song divergence on these islands.

Our study shows clearly that *A. b. clementeae* songs on San Clemente Island are more similar to those of inland *A. b. canescens* than neighbouring mainland *A. b. belli* (Figs 5–7), in contrast to mtDNA which shows that *A. b. clementeae* is most closely related to *A. b. belli* (Fig. 3). However, different analyses conflict as to whether island songs should be grouped with those from the San Joaquin Valley or Mojave Desert. *Artemisiospiza b. clementeae* does not occur on any of the other California Channel Islands (Johnson, 1972), and San Clemente Island has never been connected to the mainland (Sullivan & Kershner, 2005). Therefore, colonization probably occurred through a single event from the mainland (Johnson, 1972). Although the source of this colonization is unknown, early reports suggested that the birds were common on the island (Grinnell, 1897), and the mtDNA affinities suggest that colonization probably occurred from coastal southern California. This deserves further research, however, because *A. b. belli* is sedentary (especially in the southern portion of its range; Martin & Carlson, 1998) while inland taxa are more migratory and likely to disperse to the island (as documented by a specimen identified as *A. nevadensis* from San Clemente Island collected in 1939, Los Angeles County Museum #19703).

Our molecular results support *A. b. clementeae* as closely allied with *A. b. belli* in the Coast Range and *A. b. canescens* in the San Joaquin Valley, which presents yet another case within Bell's sparrow of song relationships inconsistent with genetic relationships. Morphological differences (longer bill: Johnson, 1972) and environmental data (Greenberg & Danner, 2012; this study) allow us to make inferences about potential selection pressures on song evolution in *A. b. clementeae* independent of phylogeny. Our data clearly placed sites occupied by *A. b. clementeae* within the bioclimatic niche space of mainland *A. b. belli*, which is most probably explained by the maritime influence of coastal California. However, the vegetative land cover on San Clemente Island more closely resembles that of the Mojave Desert and southern San Joaquin Valley (Fig. 9). Although San Clemente Island has a maritime climate with cool summers and mild winters (Jorgensen & Ferguson, 1984), it is considered semi-arid (Sullivan & Kershner, 2005) and experiences a higher maximum mean summer temperature than the other California Channel Islands and Coronados Island in Mexico (Greenberg & Danner, 2012). Higher temperatures correlate positively with larger bill surface area in Song Sparrows on the islands, presumably to dissipate heat as an adaptation to summer heat stress (Greenberg & Danner, 2012, 2013). Thus, the larger bill morphology of *A. b. clementeae* (van Rossem, 1932; Johnson, 1972) compared to mainland *A. b. belli* may similarly reflect an adaption to warmer summers on San Clemente Island.

Bill morphology (gape, size, shape) plays a functional role in avian sound production and in the structure and evolution of vocal signals (Palacios & Tubaro, 2000; Podos & Nowick, 2004; Riede *et al.*, 2006; Derryberry *et al.*, 2018). However, this effect may vary depending on the ecology of the species. For example, bill length was negatively correlated with acoustic frequency in woodcreepers (Dendrocolaptinae) inhabiting humid forest but not xeric, open-country habitats (Palacios & Tubaro, 2000). In house finches (*Carpodacus mexicanus*), birds inhabiting more disturbed urban areas showed an increase in bill length that coincided with changes in frequency range (higher minimum song frequency, lower upper frequency limit) compared to those in rural areas (Giraudeau *et al.*, 2014). Morphological evolution has also shaped acoustic signal evolution in Darwin's finches, with larger-billed and larger-bodied species having lower rates of syllable repetition and narrower frequency bandwidths (Podos, 2001). In *A. b. clementeae*, some frequency characteristics of the song are more similar to Coast Range *A. b. belli* than they are to inland *A. b. canescens.* However, song rate, note complexity, repertoire and syllable composition are clearly more similar to those of *A. b. canescens.* Although bill morphology in conjunction with ecology may have shaped the acoustic structure of songs of island Bell's sparrow, further study is needed to tease apart the contributions of phylogeny, morphology and ecology in *A. b. clementeae* song evolution. Sampling of nuclear markers may further resolve phylogenetic relationships and allow for an estimation of the time since isolation on the island, which may influence acoustic adaptation and cultural drift following colonization (Potvin & Clegg, 2015).

VARIABILITY IN SONG TRAITS AND ACOUSTIC ENVIRONMENT

PCA revealed that the songs of *A. nevadensis* and *A. belli* subspecies can be distinguished by a number of factors including the number of note types, number of notes per second, maximum frequency and frequency range (Fig. S4). Variation in these song characteristics are probably important for acoustic transmission in the respective breeding environments (e.g. Rich, 1985; Derryberry, 2009). On the other hand, variation within a taxon was strongly influenced by the number of notes in a song and its duration. The number of note types or notes per second did not increase when song length increased, indicating reuse of longer duration notes which was consistent with our observations. By lengthening their song with additional notes, birds are able to transfer additional information without sacrificing song features that may be adapted to acoustic transmission in a specific environment.

Research has shown that frequency (especially maximum frequency), syllable structure and temporal properties of bird songs are associated with habitat type and acoustic attenuation in the environment (e.g. Waas, 1988; Wiley, 1991; Derryberry, 2009). Furthermore, temporal characteristics are less affected by wind and temperature gradients than frequency variables, and thus may be more important for sound transmission in open-country species (Morton, 1975). More complex and high-pitched songs can travel further in dense and complex acoustic environments, whereas simpler and lower-pitched songs can travel further in open country habitats where there is less interference. In the windy, sagebrush steppe habitat of the Great Basin, songs of *A. nevadensis* are characterized by low frequencies and broad-band signals with rapid frequency modulation that are well-suited for transmission and locatability in that environment (Rich, 1985; this study). Likewise, the fast tempo, pure tones, and high number of notes and note types of *A. b. belli* songs may transmit better in the acoustically complex Coast Range environment (hilly, dense chaparral) when compared to the flat, more open-country habitat of the other taxa. By increasing the number of notes and note types in a song, there is a greater chance that encoded information will be transmitted. Furthermore, if songs are limited to a maximum duration (possibly around 2–2.5 s) due to physiological constraints, *A. b. belli* could have evolved songs with a faster tempo in order to accommodate a greater number of notes and note types in its song.

Acoustic environment may also be driving differences in the proportion of certain note types found between the taxa. Chevrons and upsweeps, for example, were more typical of *A. b. belli* songs and thus may be especially well suited to the Coast Range. On the other hand, *A. b. belli* produced relatively few trills compared to *A. b. canescens* (especially from the San Joaquin Valley) and *A. nevadensis*, and acoustic properties of a trill may allow it to transmit better in flatter, more open desert habitats.

We did not collect ecological data to quantify factors affecting the acoustic properties of different sites in this study. Nonetheless, our use of remote sensing and weather station data support the acoustic adaptation hypothesis that local habitat differences have played a significant role in song evolution between populations of Bell's and Sagebrush sparrows. More detailed studies that measure the acoustic environment and other ecological pressures would shed further light on the role of ecology in shaping song traits in these taxa. Furthermore, song playback experiments would provide information on the extent to which song may function in reproductive isolation and lineage divergence.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. UPGMA tree.
Figure S2. Neighbour-joining tree.
Figure S3. Bayesian tree.
Figure S4. PCA loadings for 12 continuous song characters.
Table S1. Sound recordings and accession numbers.
Table S2. Genetic specimens and associated GenBank accession numbers.
Table S3. Loadings matrix of song characteristics on the first nine principal components, and proportion of total variance explained by each principal component.
Table S4. Loadings matrix of bioclimatic variables on the first six principal components.