

High inter-population connectivity and occasional gene flow between subspecies improves recovery potential for the endangered Least Bell's Vireo

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ABSTRACT

Increasingly, genomic data are being used to supplement field-based ecological studies to help evaluate recovery status and trends in endangered species. We collected genomic data to address 2 related questions regarding the Least Bell's Vireo (*Vireo bellii*), an endangered migratory songbird restricted to southern California riparian habitat for breeding. First, we sought to delineate the range limits and potential overlap between Least Bell's Vireo and its sister subspecies, the Arizona Bell's Vireo, by analyzing samples from the deserts of eastern California, southwestern Nevada, Utah, and Arizona. Second, we evaluated genetic structure among Least Bell's Vireo populations in coastal California and estimated effective population size. Clustering analyses based on 10,571 single-nucleotide polymorphisms (SNPs) from 317 samples supported 2 major groups that aligned closely to the previously defined subspecies ranges. The first cluster included birds in the Central Valley, all coastal drainages, and westernmost deserts of California, with no further sub-structuring among coastal drainages. Almost all birds from the Amargosa River in eastern California and eastward assigned to the second cluster; however, low levels of gene flow were detected across the subspecies groups, with greater rates of gene flow from Arizona Bell's Vireo to Least Bell's Vireo than the reverse. Admixed individuals occurred in the California deserts; and although smaller than coastal populations, desert populations may be important for maintaining and replenishing genetic diversity and facilitating the movement of potentially adaptive genes between subspecies. Within Least Bell's Vireo, local populations in coastal drainages comprised a single genetic population, with some evidence of close relatives distributed across drainages, suggesting that these could function as a well-connected metapopulation. These results are consistent with previous Least Bell's Vireo banding studies that reported high rates of dispersal among drainages. Effective population size for both subspecies was high, suggesting that adaptive potential has been maintained despite previous declines.

Keywords: contact zone, effective population size, Least Bell's Vireo, population genomic structure, range limits, *Vireo bellii*

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LAY SUMMARY

- Maintenance of genetic diversity is essential for species to be able to persist and adapt to changing environmental conditions.
- We examined dispersal and genetic diversity in the endangered Least Bell's Vireo, a migratory songbird dependent upon riparian habitat for breeding, and in its closest subspecies, the Arizona Bell's Vireo.
- Analysis of blood samples collected from vireos throughout southern California, Nevada, Utah, and Arizona revealed that in general birds from the Mojave Desert west are Least Bell's Vireos, while those to the east are Arizona Bell's Vireos. However, we found low levels of gene exchange across subspecies and that 9% of birds in the desert region were of mixed ancestry.
- Occasional dispersal and successful breeding across the California desert could be a source of novel adaptive genetic variation that could increase the capacity of Least Bell's Vireo to adapt to climate change across its range.

La alta conectividad inter-poblacional y el flujo génico ocasional entre subspecies mejoran el potencial de recuperación para la subespecie en peligro *Vireo bellii pusillus*

RESUMEN

Cada vez más, los datos genómicos se utilizan para complementar estudios ecológicos basados en el campo y ayudar a evaluar el estado de recuperación y las tendencias en especies en peligro. Recolectamos datos genómicos para abordar dos preguntas relacionadas a *Vireo bellii pusillus*, un ave canora migratoria en peligro de extinción restringida para su reproducción al hábitat ribereño del sur de California. En primer lugar, buscamos delinear los límites de los rangos de distribución y la posible superposición entre *V. b. pusillus* y su subespecie hermana, *V. b. arizonae*, mediante el análisis de muestras de los desiertos del este de California, suroeste de Nevada, Utah y Arizona. En segundo lugar, evaluamos la estructura genética entre las poblaciones de *V. b. pusillus* en la costa de California y estimamos el tamaño efectivo de la población. Los análisis de agrupamiento basados en 10.571 polimorfismos de nucleótido único provenientes de 317 muestras respaldaron dos grupos principales que se alinearon estrechamente con los rangos previamente definidos de las subespecies. El primer grupo incluyó las aves en el Valle Central, todos los drenajes costeros y los desiertos más occidentales de California, sin una subdivisión adicional entre los drenajes costeros. Casi todas las aves del Río Amargosa en el este de California y hacia el este se asignaron al segundo grupo; sin embargo, se detectaron bajos niveles de flujo génico entre los grupos de subespecies, con tasas de flujo génico más altas desde *V. b. arizonae* hacia *V. b. pusillus* que al revés. Se encontraron individuos mestizos en los desiertos de California; y aunque más pequeñas que las poblaciones costeras, las poblaciones del desierto pueden ser importantes para mantener y reponer la diversidad genética y facilitar el movimiento de genes potencialmente adaptativos entre las subespecies. Dentro de *V. b. pusillus*, las poblaciones locales en los drenajes costeros constituyeron una sola población genética, con algunas evidencias de parientes cercanos distribuidos a través de los drenajes, lo que sugiere que estos podrían funcionar como una metapoblación bien conectada. Estos resultados son consistentes con estudios anteriores de anillado de *V. b. pusillus* que informaron altas tasas de dispersión entre los drenajes. El tamaño efectivo de la población para ambas subespecies fue alto, lo que sugiere que el potencial adaptativo se ha mantenido a pesar de las disminuciones previas.

Palabras clave: estructura genómica de la población, límites de distribución, tamaño efectivo de la población, *Vireo bellii*, *Vireo bellii pusillus*, zona de contacto

INTRODUCTION

Increasingly, genomic data are being used to supplement field-based ecological studies within the context of endangered species recovery (Smith *et al.* 2018). Genomic data make it possible to assess the genetic diversity of populations, which is the basis for adaptive potential, or the capacity to adapt to changing conditions. Adaptive potential is critical to population viability and long-term persistence, and as such is fundamental to species recovery. In addition to selection, genetic diversity of populations is influenced by 2 main factors: population size, and inter-population connectivity (Hoffmann *et al.* 2017), which for endangered species are both typically low. When populations are small, inbreeding depression and genetic drift can act to reduce genetic diversity. Fragmentation can leave populations with little or no gene flow (dispersal and successful breeding) among them, which can increase genetic divergence among sites (Schlaepfer *et al.* 2018). These genetic changes can occur rapidly in species with small, isolated populations (Vandergast *et al.* 2022a), and have been shown to contribute to extinction risks in a range of organisms (Saccheri *et al.* 1998, Spielman *et al.* 2004, Bozzuto *et al.* 2019).

Another way that genomic data can inform endangered species management is through an assessment of genetic structure and gene flow among geographically adjacent subspecies and populations within their zone of contact (Klicka *et al.* 2016, Oyler-McCance *et al.* 2015, Ruegg *et al.* 2018, Vandergast *et al.* 2022b). This can be used to identify range boundaries of listed taxa, which has implications for their legal protection under the Endangered Species Act (Forester and Lama 2022). In addition, it can identify the extent and consequences of gene flow that might be occurring between subspecies (Turbek *et al.* 2023).

We examined both of these aspects of genetic diversity in the U.S. federally endangered Least Bell's Vireo (*Vireo bellii pusillus*; LBVI), a migratory songbird restricted to riparian habitat for breeding, and an ecologically well-studied species (Kus *et al.* 2022). Historically, the range of LBVI extended from coastal northern Baja California, Mexico, through the Central Valley of California, and included sites in the deserts of eastern California (USFWS 1998). By the mid-1980s the species had been extirpated from most of its range in response

to habitat loss (Goldwasser *et al.* 1980, Kus *et al.* 2022) and brood parasitism by the Brown-headed Cowbird (*Molothrus ater*) (Laymon 1987, Rothstein 1994, Kus 1999, Kus and Whitfield 2005), with remaining populations concentrated in coastal southern California and northern Baja California. Habitat in this region is naturally fragmented in that it occurs along rivers in discrete watersheds. Moreover, it also exists within a matrix of urban development that could affect movement of birds among drainages.

In the more than 3 decades since LBVI was federally listed as endangered, loss of riparian habitat has generally slowed and in some cases has been reversed by restoration efforts (Kus 1998, Kus *et al.* 2022). The total number of breeding pairs has grown from estimates in the low hundreds to low thousands (USFWS 2006), and LBVI has expanded back into some of its previously occupied coastal range and has been regularly sighted in the Central Valley. In addition, desert sites in eastern California historically occupied by vireos have recently been recolonized. This recovery has been largely attributed to protection and restoration of remaining riparian habitat and cowbird control efforts (USFWS 2006).

Recolonization of California desert sites provides a new opportunity to increase our understanding of the eastern range limits of LBVI and identification of areas of potential genetic exchange with its sister subspecies, the Arizona Bell's Vireo (*Vireo bellii arizonae*; AZBV). The range of the LBVI, as described in the available literature, includes the Mojave and Colorado Deserts within California (hereafter California desert), while the western edge of the range of the AZBV has been limited to the greater Colorado River Valley (Ridgway 1904, Klicka *et al.* 2016; Figure 1). Nuclear single nucleotide polymorphism (SNP) data were used to assess relationships among all described subspecies of the Bell's Vireo and showed that LBVI and AZBV formed 2 distinct genetic clusters, with a contact zone somewhere in the eastern California desert (Klicka *et al.* 2016). However, given sparse sampling (one sample in the California desert), Klicka *et al.* (2016) recommended further sampling to better delineate the spatial distributions of LBVI and AZBV subspecies, and to resolve the taxonomic status of populations in eastern California and neighboring states.

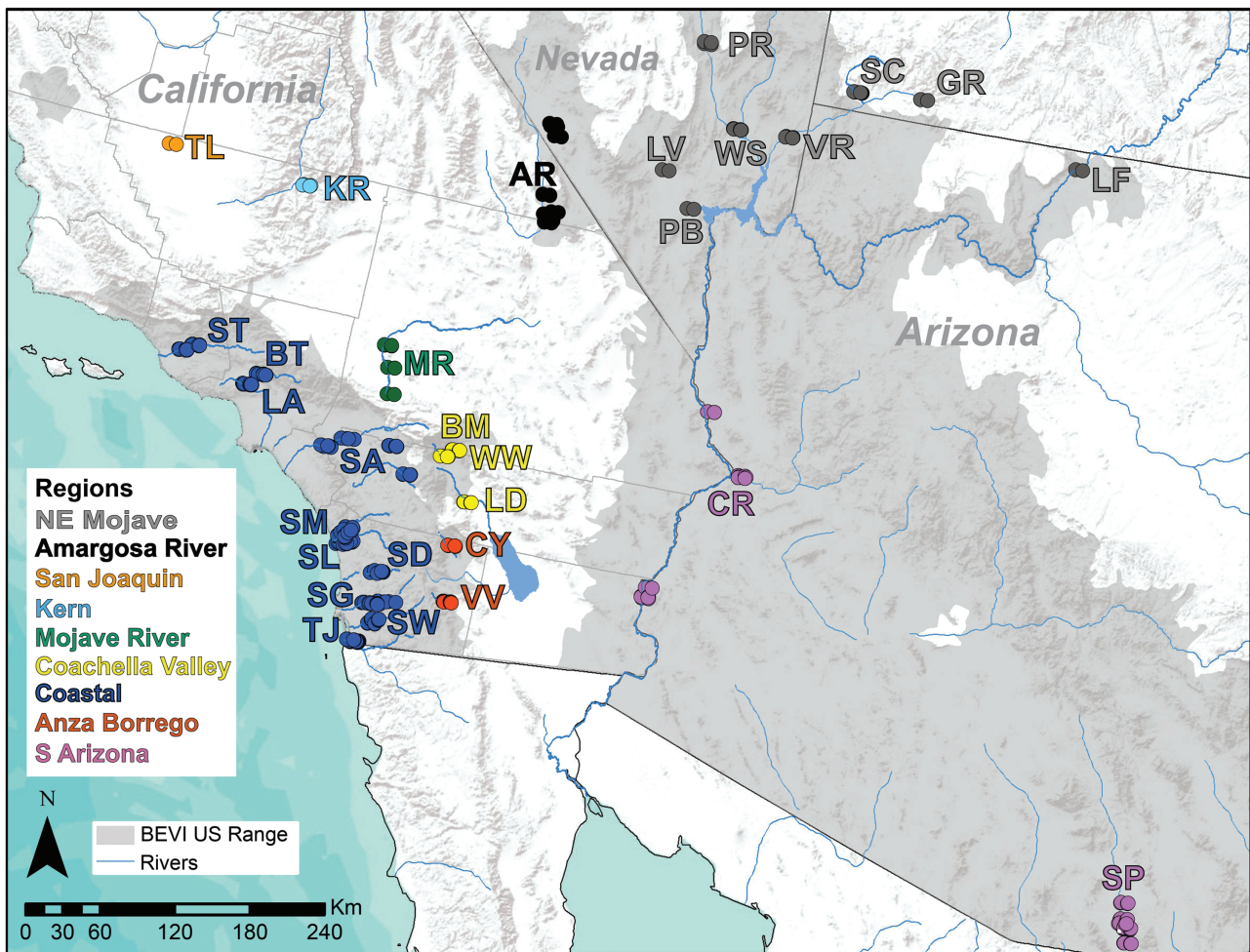


FIGURE 1. Map of collection locations of *Vireo bellii* in California, Nevada, Arizona and Utah. Location codes are colored by region to correspond to Table 1. Approximate U.S. range of Bell's Vireo is shaded grey.

Although not federally listed, AZBV has been similarly impacted by habitat degradation and brood parasitism as LBVI. It is listed as endangered by the State of California and is on the Audubon Society's Priority Bird list. The Colorado River Basin population was nearly extirpated during the mid-1900s (Rosenberg *et al.* 1991) and subsequently rebounded in response to habitat protection and cowbird management. Delineating locations where contact and gene flow have occurred between the 2 subspecies could be important in identifying areas where lineage mixing, adaptation and evolutionary change are likely to occur; these areas could act as adaptive linkages that continue to allow for the exchange of genetic variation between the desert and coastal subspecies. Adaptation to drier and warmer conditions may be particularly important, as southern California is predicted to become warmer with more variable and extreme precipitation and drought events (Cayan *et al.* 2010, Berg and Hall 2015, Kam and Sheffield 2016, Swain *et al.* 2018).

We collected and analyzed population-level genomic data to assess the population structure and diversity among aggregations of LBVI with focused sampling of major drainages in coastal and interior California. We also examined range limits and potential contact or overlap with AZBV in California, Arizona, Nevada and Utah. These data can help estimate genetic essential biodiversity variables (Hoban *et*

al. 2022) including effective population size, the distribution of genomic diversity, and levels of genetic exchange among drainages, providing a baseline to help managers evaluate status, recovery and effectiveness of actions across the study area.

METHODS

Field Sampling

We collected blood and pin feather samples between 2011 and 2021 at all the major coastal drainages supporting LBVI populations, many of which have been monitored long-term (Figure 1, Table 1). These included the Tijuana River, San Diego River, Sweetwater River, San Dieguito River, San Luis Rey River, Santa Margarita River, Santa Ana River, Los Angeles River, Hansen Dam Recreational Area/Tujunga Wash, and the Santa Clara River. In addition, we collected samples in 2021 at 2 sites recently colonized in central California; the Kern National Wildlife Refuge (NWR) in Tulare Lake and the south fork of the Kern River near Lake Isabella. Desert sampling began in 2021, guided by eBird reports and expert knowledge, and included collection at Anza Borrego, the Mojave River and Amargosa River in California, Ash Meadows NWR (Amargosa River), the Virgin River and Las Vegas Wash in Nevada, and the San

TABLE 1. Sample codes corresponding to Figure 1. Drainage, region and subspecies assignments are given, along with the number of individuals sampled (N) and number adjusted for missing data ($\text{adj}N$). Observed heterozygosity (H_o), unbiased expected heterozygosity (uH_e) and inbreeding coefficients (F_{is}) were calculated. Population diversity statistics not reported for sites with fewer than 3 individuals sampled. The last row of the table contains a summary across all drainages, including the sum of samples (N and $\text{adj}N$) and ranges of other values ($n\text{Loc}$, H_o , uH_e , F_{is}).

Code	Drainage	Region	Subspecies	Years sampled	N	$\text{adj}N$	$n\text{Loc}$	H_o	uH_e	F_{is}
TL	Tulare Lake, Kern NWR	San Joaquin	<i>pusillus</i>	2021	1	1.00	10,552	NA	NA	NA
KR	Kern River	Kern	<i>pusillus</i>	2021	2	2.00	10,571	NA	NA	NA
ST	Santa Clara River	Coastal	<i>pusillus</i>	2020	27	24.28	10,571	0.2201	0.2188	-0.0059
LA	Los Angeles River, Sepulveda Basin	Coastal	<i>pusillus</i>	2020	5	4.96	10,571	0.2243	0.2265	0.0098
BT	Hansen Dam, Big Tujunga Wash	Coastal	<i>pusillus</i>	2020	12	11.85	10,571	0.2222	0.2250	0.0123
SA	Santa Ana River	Coastal	<i>pusillus</i>	2020	27	25.14	10,571	0.2236	0.2207	-0.0132
SM	Santa Margarita River	Coastal	<i>pusillus</i>	2011–2019	29	27.83	10,571	0.2177	0.2226	0.0222
SL	San Luis Rey River	Coastal	<i>pusillus</i>	2011–2013	25	23.52	10,571	0.2221	0.2233	0.0053
SD	San Dieguito River	Coastal	<i>pusillus</i>	2020	9	8.89	10,571	0.2247	0.2282	0.0152
SG	San Diego River	Coastal	<i>pusillus</i>	2020	29	28.64	10,571	0.2270	0.2303	0.0145
SW	Sweetwater River	Coastal	<i>pusillus</i>	2020	8	7.85	10,571	0.2252	0.2259	0.0032
TJ	Tijuana River	Coastal	<i>pusillus</i>	2020	29	28.23	10,571	0.2260	0.2257	-0.0015
VV	Vallecito Valley	Anza Borrego	<i>pusillus</i>	2021	8	7.96	10,571	0.2226	0.2226	-0.0002
CY	Coyote Creek	Anza Borrego	<i>pusillus</i>	2021	6	5.97	10,571	0.2180	0.2238	0.0258
LD	Deep Canyon, Living Desert Zoo and Gardens	Coachella Valley	<i>pusillus</i> *	2022	3	2.99	10,571	0.2259	0.2328	0.0293
BM	Big Morongo Canyon	Coachella Valley	<i>pusillus</i> *	2022	3	2.95	10,571	0.2297	0.2145	-0.0710
WW	Whitewater River	Coachella Valley	<i>pusillus</i>	2022	8	7.93	10,571	0.2243	0.2291	0.0209
MR	Mojave River	Mojave River	<i>pusillus</i> *	2021–2022	8	7.96	10,571	0.2293	0.2333	0.0173
AR	Amargosa River	Amargosa River	<i>arizonae</i> *	2021	21	20.70	10,571	0.2225	0.2234	0.0044
LV	Las Vegas Wash	NE Mojave	<i>arizonae</i>	2021	1	1.00	10,521	NA	NA	NA
PB	Pabco Wash	NE Mojave	<i>arizonae</i>	2022	1	1.00	10,521	NA	NA	NA
PH	Pahrnagat NWR	NE Mojave	<i>arizonae</i>	2022	2	1.97	10,568	0.2101	0.2181	0.0368
WS	Muddy River, Warm Springs NRA	NE Mojave	<i>arizonae</i>	2022	5	4.95	10,571	0.2243	0.2269	0.0115
VR	Virgin River	NE Mojave	<i>arizonae</i>	2022	6	5.96	10,571	0.2233	0.2261	0.0126
SC	Santa Clara River, UT	NE Mojave	<i>arizonae</i>	2022	5	4.98	10,571	0.2217	0.2170	-0.0216
GR	Grafton	NE Mojave	<i>arizonae</i>	2022	2	1.99	10,569	NA	NA	NA
LF	Lee's Ferry	NE Mojave	<i>arizonae</i>	2022	1	1.00	10,469	NA	NA	NA
CR	Colorado River	S Arizona	<i>arizonae</i>	2021	17	16.51	10,571	0.2230	0.2219	-0.0050
SP	San Pedro River	S Arizona	<i>arizonae</i>	2021	17	16.90	10,571	0.2214	0.2253	0.0176
Summary Across All Sites					317	307	10469–10571	0.2101–0.2297	0.2145–0.2333	-0.071–0.0368

*Drainages contained some individuals with mixed assignment.

Pedro River and lower Colorado River in Arizona. In 2022, we expanded our study area to encompass the entire north-west portion of the range of AZBV, and collected samples at Pabco Wash, the Muddy River, and Pahrnagat NWR in Nevada, the Santa Clara River and several locations on the Virgin River in Utah, Lee's Ferry in the Grand Canyon of Arizona, and in the Coachella Valley and Mojave River in California (Figure 1, Table 1).

Adult vireos were captured in mist nets placed in their territories, using song playbacks to attract birds (usually males) to nets. Small quantities of blood were collected by venal puncture and transferred onto GenSaver 2.0 DNA cards (GenTegra). Alternatively, 1–3 growing feathers were collected in cell lysis buffer. Blood cards were stored in envelopes at room temperature with silica beads while growing feathers in buffer were kept frozen until extraction at the USGS

Western Ecological Research Center (WERC), San Diego Field Station genetics laboratory.

Genomic Data Extraction and Library Preparations

We extracted all samples using the Gentra Puregene Kit (Qiagen) according to manufacturer's protocol with minor modifications, including cell lysis in the presence of Proteinase K and dithiothreitol (DTT), an overnight DNA precipitation step, and 5–30 min centrifugation steps at 21,194 g and 4°C. For samples with low yields, the same protocol was performed on the feather storage buffer. We quantified DNA with the Qubit fluorometer (Life Technologies), and included samples with 125–400 ng of DNA for next-generation sequencing (NGS) library preparation at the USGS WERC San Diego Field Station genetics laboratory.

In total, 344 samples were prepared for sequencing. We followed the double-digest restriction-associated DNA (ddRAD) sequencing protocol developed in Peterson *et al.* (2012) for NGS library preparation modified to use separate indexing reads. We digested genomic DNAs using 20 units each of the restriction enzymes *EcoRI* and *MseI* (New England Biolabs) and used Solid Phase Reversible Immobilization (SPRI) beads (Cytiva) to purify the digestions prior to ligating uniquely bar-coded adapters with T4 ligase (New England Biolabs). We quantified ligation products with a Qubit fluorometer, pooled across index groups in equimolar concentrations, and selected fragments between 250 and 400 base pairs (bp) using a Pippin Prep size fractionator (Sage Science). We amplified the recovered fragments from each pool using 5–10 ng of the recovered DNA, Phusion High-Fidelity *Taq* (New England Biolabs), and Illumina's primers (Illumina, Inc.). Polymerase chain reaction (PCR) products were then cleaned with SPRI beads and quantified using the Qubit fluorometer (Life Technologies) before being pooled for sequencing (150 bp paired-end reads) on Illumina HiSeqX and NovaSeq platforms at MedGenome, Inc. (Foster City, CA). Samples from different sampling locations and regions were mixed across libraries and sequencing lanes to avoid confounding batch effects.

SNP Calling and Quality Filtering

Raw sequence demultiplexing, quality filtering, and genotyping were performed using Stacks v2.60 (Rochette and Catchen 2017) on the USGS Yeti High Performance Computing platform. We demultiplexed our raw sequence files, removed reads with uncalled or low-quality bases (phred score < 10) and discarded reads with ambiguous barcodes using the *process_radtag* program. We performed a reference-based assembly using the genome scaffolds of the most closely related species available, *Vireo altiloquus* (Genbank Genome Assembly GCA_013396875.1, Bioproject PRJNA545868). We used Stacks 2.60 to process our RAD loci for the reference-based assembly, because of the ability to easily integrate a reference genome within the SNP calling pipeline. We used BWA 0.7.17 to align demultiplexed forward reads to the *Vireo altiloquus* reference genome using the “mem” command with default settings (Li *et al.* 2009). These reads were then exported and sorted as .bam files using SAMtools 1.10 (Li *et al.* 2009) and used as input for the *gstacks* program within Stacks to build a catalog of individual RAD loci (Rochette *et al.* 2019). For comparison, we also performed a *de novo* assembly; however,

this resulted in fewer loci and no discernable differences in population clustering (see [Supplementary Material](#) for full *de novo* methods and results). We retained the reference-assembled dataset for all downstream analyses.

We used the *populations* module within Stacks to generate a Variant Call Format (vcf) file that was used for downstream filtering. We used the functions within SNPfiltR (DeRaad 2022) and vcfR (Knaus and Grünwald 2017) to explore and visualize differing filtering thresholds based on genotype quality, minimum and maximum read depth, allele balance, number of alleles present, missing data per sample and SNP, minor allele count, and genomic distance between SNPs. Final filtering steps removed called genotypes with low confidence by requiring a minimum depth of 5 and a minimum genotype quality of 30, which resulted in the removal of 43.9% and 5.5% of called genotypes, respectively (see Puritz *et al.* 2014 and O'Leary *et al.* 2018 for discussion of these thresholds). We then filtered heterozygous genotypes for which an allele balance fell outside the range of 0.25–0.75 (Puritz *et al.* 2014), which removed 8.14% of all heterozygous genotypes (0.96% of all genotypes) from our reference aligned SNP dataset. We set a maximum mean depth cutoff of 50 to remove genotypes with outlier depth values, which removed 0.22% of all SNPs. To identify failed samples, we initially chose to filter out samples missing calls at more than 80% of their genotypes, which removed 27 samples. We then removed invariant sites generated during the filtering process, which resulted in 0.2% remaining SNPs being dropped. We explored how missing data might influence patterns of sample clustering using the visualization tools of SNPfiltR using a suite of possible missing data thresholds (25%, 15%, and 5% missing data). Based on these explorations, we removed SNPs with more than 5% missing data. We filtered for a single randomly selected SNP per locus to reduce linkage within rad loci (O'Leary *et al.* 2018) and imposed a minor allele count filter of 32 (equivalent to a minor allele frequency of ~0.05). Finally, we looked at the effects of removing SNPs that were out of Hardy-Weinberg equilibrium (HWE). We tested for significant departures from HWE in SNPs grouping by drainage (with > 15 samples), regions and subspecies (following Pearman *et al.* 2022). We used exact tests (Wigginton *et al.* 2005) implemented in the R package *dartR* v.2.9.7 (Gruber *et al.* 2018, Mijangos *et al.* 2022). Significant departures were assessed at $\alpha \leq 0.05$ and corrected for multiple tests using the BY method (Benjamini and Yekutieli 2001). A small number of loci (22 total) deviated significantly from HWE when samples were grouped by subspecies. Deviations from HWE are not unexpected when multiple populations are combined (Waples 2015), and further, removing these had no effect on patterns of population structure ([Supplementary Material Figure 2](#)). Therefore, we did not remove these SNPs from the final dataset.

Population Genetic Analyses

Genetic diversity indices including observed heterozygosity (H_o), unbiased expected heterozygosity (uH_e) and inbreeding coefficients (F_{IS}) were estimated with *dartR* v.2.0.4 (Mijangos *et al.* 2022) at each sampling location with greater than 5 samples and grouped by region. Three genetic clustering analyses were used to explore the pattern of genetic structure among samples in the full dataset. First, a principal component analysis (PCA) was implemented in adegenet 2.1.3 (Jombart and Ahmed 2011), using R 4.2.2 (R Development Team 2018),

to visualize the genotypes in multidimensional space. The `find.clusters` command was used to assess the best supported number of clusters in the dataset. This procedure runs successive *K*-means with an increasing number of clusters (*K*), after transforming data using a principal component analysis. We ran the analysis using the first 100 PCs as an arbitrary cut off to reduce analysis time. For each model, goodness of fit was measured by the Bayesian Information Criterion (BIC), with the lowest BIC indicating the best-supported *K*-value. We also applied the maximum-likelihood approach of ADMIXTURE (Alexander *et al.* 2009) to define the best-supported number of genetic clusters (*K*) in the data when fit to a population genetic model that assumes Hardy-Weinberg equilibrium. We performed 10 replicate analyses to evaluate up to 9 clusters. To assess the best value of *K*, we performed 5-fold cross-validation and determined the *K*-values with the lowest cross validation error and examined the individual assignment plots. Third, population structure was also analyzed using PopCluster (Wang 2022), which provides estimates of recent gene flow rates (last 3 generations) from the final model. We first evaluated up to 9 clusters with 20 replicate runs. After selecting the optimal *K*, we ran the PopCluster model with migration for 20 replicate runs to estimate gene flow rates among clusters from the individual admixture estimates.

We examined the subset of coastal LBVI aggregations independently to determine whether additional substructure was supported among coastal drainages. We used the `find.clusters` command in *adegenet* 2.1.3 in R to test for genetic clusters as described above. Pairwise F_{ST} values (Weir and Cockerham 1984) were estimated between all coastal drainages and significance assessed with 10,000 bootstraps in *StAMPP* 1.6.3 in R, and applying the B-Y correction for multiple tests (Benjamini and Yekutieli 2001). We further tested for isolation by distance by comparing linearized F_{ST} by log transformed geographic distance using a Mantel test for matrix correlation. Sites with fewer than 5 individuals were excluded from this analysis. We looked for evidence of recent dispersal among coastal breeding populations by examining the spatial distribution of first order relatives (i.e., parent-offspring or siblings). When genetic structure is very weak or absent, admixture-based migration models cannot be reliably applied (Faubet and Gaggiotti Oscar 2008, Meirmans 2014). However, pedigree analysis has been applied to estimate dispersal among close relatives in a wide array of birds and other species (Peery *et al.* 2008, Norman and Spong 2015, Vandergast *et al.* 2019; reviewed in Millon *et al.* 2019). In Bell's Vireo, dispersal typically occurs when first-year adults return from the wintering grounds and establish breeding territories. We used the program *Colony* 2.0.6.8 (Jones and Wang 2010) to perform a maximum likelihood pedigree analysis and identify first-order relatives. Analyses were run assuming an initial error rate of 0.001 for all markers and with the following settings: all individuals coded as offspring, monogamous breeding, low sibship prior, FLPL combined likelihood scoring method, very long run length.

We estimated contemporary effective population size (N_e) for each genetically defined cluster and for coastal LBVI aggregations with > 10 samples using the linkage disequilibrium method (Waples and Do 2008) within the program *NeEstimator* 2.1 (Do *et al.* 2014). We assumed random mating for each group, calculated 95% confidence inter-

vals for point estimates by jackknifing across samples (Jones *et al.* 2016). We applied a standard correction for physical linkage based on the number of chromosomes as suggested by Waples *et al.* (2016) for genomic datasets. We used the number of chromosomes reported for species in the genus *Vireo*, $2N = 80$; $1N = 40$ (Degrandi *et al.* 2020).

RESULTS

A total of 744.7 million of 956.1 million reads (79.8%) were aligned to the reference genome with the remainder discarded due to insufficient mapping quality (5%), excessive soft-clipping (12.8%) or unmapped (2.4%). The average number of aligned reads per sample was 2.5 million. From these reads, a total of 2,033,408 loci were built from the reference assembly and effective per-sample coverage ranged from 1.1x to 35.5x, mean 11.6x (stdev, 5.4x). The final filtered dataset contained 10,571 SNPs with 3.0% overall missing data per locus sampled from 317 vireos (Table 1). Mean missing data per individual was 2.9% (median: 0.09%, range: 0–31%).

Genetic Structure, Gene Flow, and Diversity Within the Sampled Range of *Vireo bellii*

All 3 clustering analyses supported 2 major groups that closely corresponded to the previously defined subspecies ranges of LBVI and AZBV (Figure 1). In the PCA, PC 1 described 3.55% of the variation and separated most AZBV from most LBVI with a small number of individuals positioned intermediately (Figure 2). PC 2 further separated a subset of Coachella Valley, Anza Borrego, Mojave and some coastal samples, but encompassed < 1% of the variation. Admixture analysis also supported 2 clusters ($K = 2$, Figure 3). The first cluster consisted of birds sampled in the Central Valley (Kern River and Tulare Lake), all coastal drainages from Ventura County to San Diego County, and western desert sites including Anza Borrego, Coachella Valley, and the Mojave River, although western desert sites showed higher proportions of admixed ancestry than coastal drainages. Almost all individuals from the Amargosa River in California and eastward assigned to the second cluster. Five birds in the California desert were substantially admixed (25% or more): 3 sampled in Coachella Valley (2 BM, 1 LD), 1 in the Mojave River (MR) and 1 in the Amargosa River (AR, Figure 3). PopCluster analyses also supported $K = 2$ and individual assignment proportions were close to identical and highly correlated to those estimated with ADMIXTURE (correlation coefficient = 0.999). Recent gene flow rates (estimated over the last 3 generations) from AZBV into LBVI were higher (0.011) than from LBVI into AZBV (0.001). Genetic diversity metrics did not vary substantially among sites (Table 1) nor region (Table 2). At the regional level, Coachella Valley and Mojave River had the highest heterozygosity (Table 2).

LBVI Population Structure and Diversity

There was no evidence of further genetic structure among LBVI breeding populations in coastal drainages, and the lowest BIC score supported $K = 1$ (Figure 4). Pairwise F_{ST} among populations within the LBVI coastal drainages averaged 0.0053 (range: 0.0011–0.0097), with a significant pattern of isolation by distance (Mantel $R = 0.288$, $P \leq 0.029$). All but 3 pairwise F_{ST} comparisons were statistically

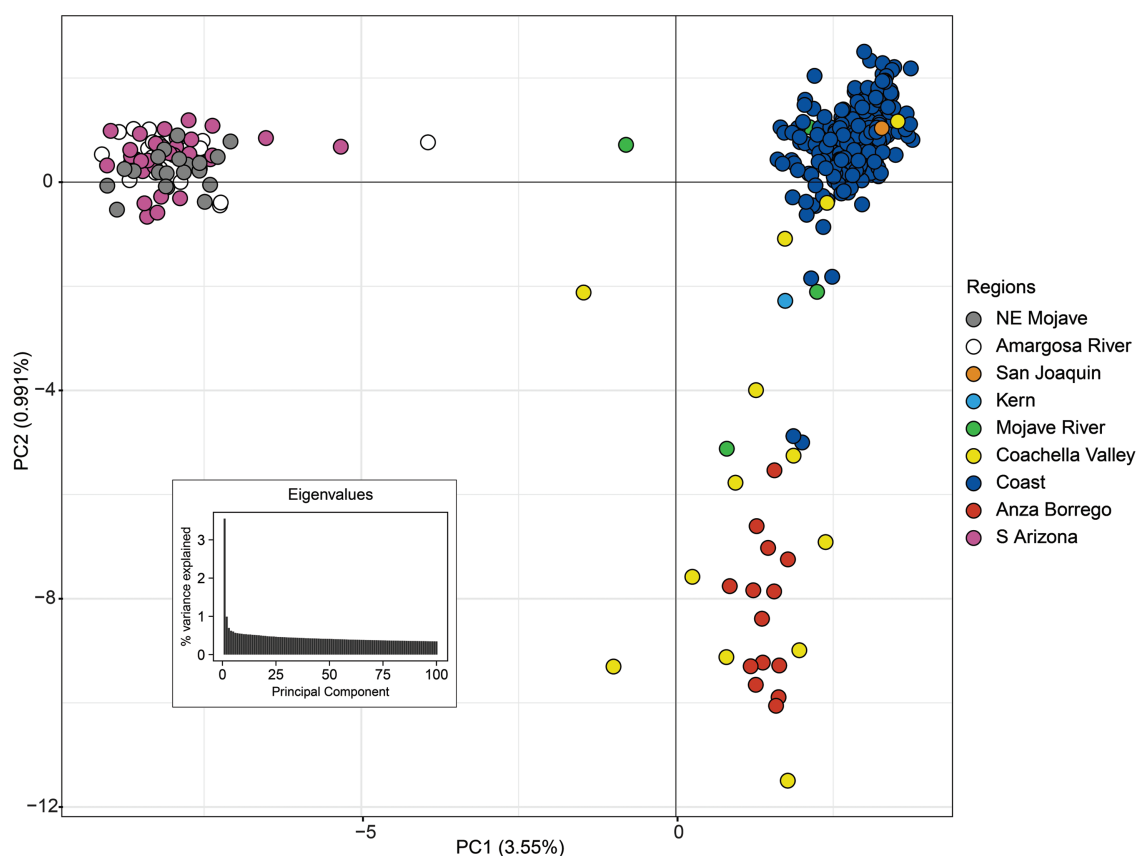


FIGURE 2. PCA Axis 1 and Axis 2. Two major groups were separated along axis 1 which encompassed 3.55% of variation. Individuals are colored by region.

significant after B-Y correction (Los Angeles River (LA)–San Dieguito River (SD), LA–San Diego River (SG), SD–SG). The pedigree analysis assigned 29 individuals (15% of the total individuals genotyped in coastal drainages) into 13 groups of first-order relatives with high probability ($P = 1.0$; Table 3). Most family groups were sampled ≤ 2 km apart and within the same river drainage (Table 3). However, 2 family groups had members distributed across separate drainages (San Diego River–Santa Ana River (SA): 129 km apart; Santa Margarita River (SM)–Tijuana River (TJ): 85 km apart), indicating some long-distance dispersal among drainages. These long-distance dispersers represent 16.7% of the identified family groups and 1% of the genotyped individuals in the coastal drainages. Among the coastal drainages, the Santa Clara River had the lowest unbiased expected heterozygosity while the San Diego River had the highest (Table 1). The N_e point estimate of LBVI and AZBV was both large and similar to one another, with overlapping confidence intervals (Table 4). Upper confidence intervals could not be estimated for localized N_e estimates for any of the LBVI coastal drainage breeding populations, even with sample sizes of up to 29 individuals (Table 4).

DISCUSSION

Contact Zones with Arizona Bell's Vireo

Our genomic data supported 2 major genetic clusters largely concordant in range with the described Bell's vireo subspecies *V. b. pusillus* and *V. b. arizonae*, with a shift in assignment

from mostly LBVI in the western desert to mostly AZBV in the Amargosa River and further east. Gene flow estimates were low ($\sim 1\%$ or less) and uneven, with more gene flow from AZBV into LBVI. Mapping of admixed individuals suggests that gene flow between these groups occurs mostly in the California desert populations. We found evidence of low levels of admixture in the California desert, and a few individuals with highly mixed ancestry ($>25\%$) in the Mojave River, Coachella Valley and Amargosa River. While our sampling is sparse in the California desert, so is the Bell's Vireo distribution in this region, particularly in the eastern Mojave Desert, with few recently recorded sightings (Kus *et al.* 2022, eBird 2023), and low proportions of modeled suitable habitat which occurs in small, isolated patches (Preston *et al.* 2021). An exception to this is the Amargosa River in California, which supports a growing vireo population in the wake of habitat restoration and cowbird control (McCreedy 2009, 2016, 2022). Our analysis places this breeding population as AZBV.

Steady and low levels of gene flow across these subspecies ($\sim 1\%$ or less) are unlikely to swamp adaptive alleles (Hedrick 1995, Lopez *et al.* 2009) or lead to outbreeding depression (Frankham *et al.* 2011). The maintenance of occasional gene flow between AZBV and LBVI could in fact increase heterozygosity, allow for new gene combinations and potentially enhance adaptive potential by allowing for the movement of adaptive genetic variation between these groups. For example, recent simulations of a co-distributed riparian passerine, the Southwestern Willow Flycatcher

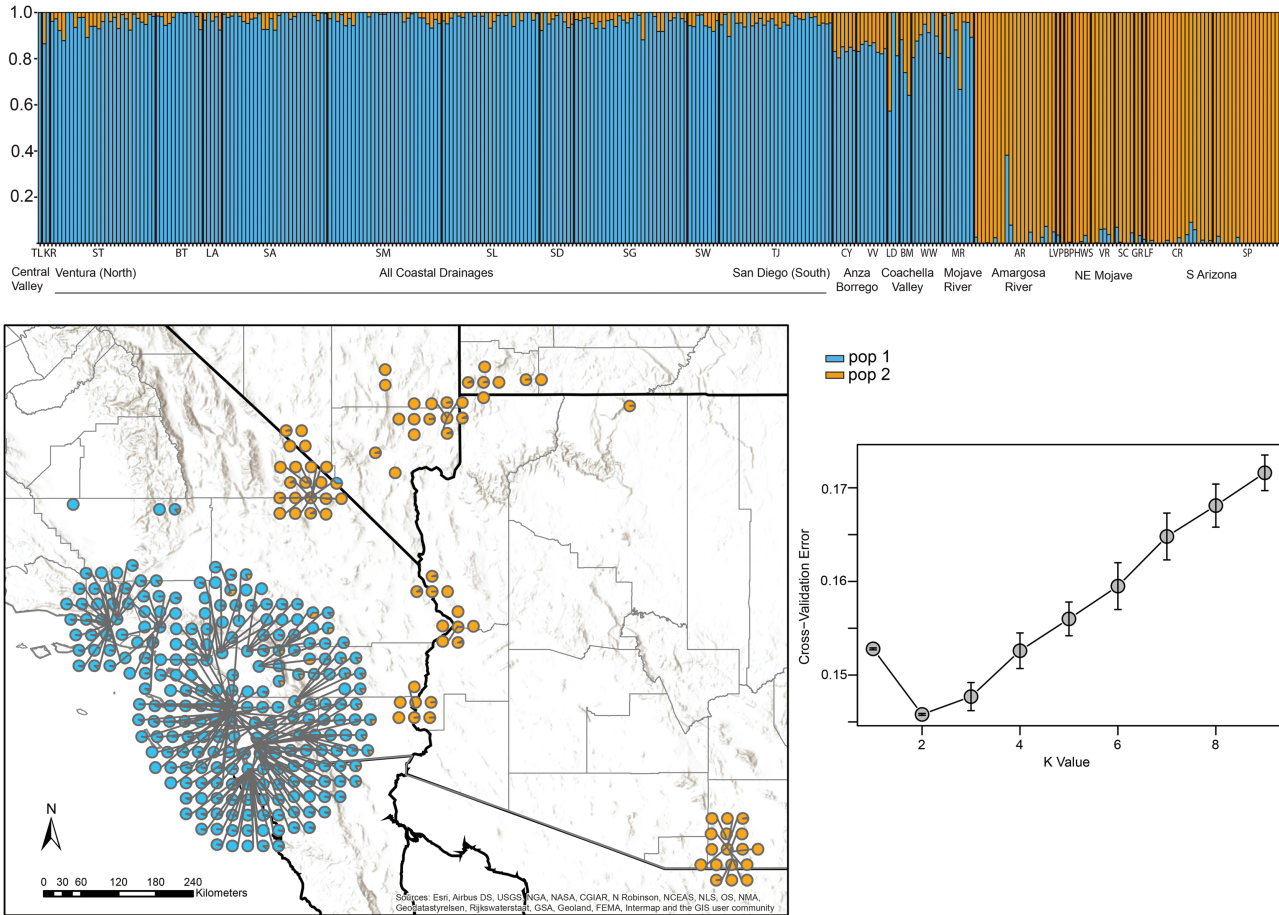


FIGURE 3. Admixture assignment of the reference-based assembly dataset, map of individual assignments at the best-supported $K = 2$, and boxplots of cross validation error (CV) from 10 replicates of each K -value between 1 and 9. Pop 1 included coastal drainages from Ventura to San Diego counties, Kern River and Tulare Lake of the Central Valley and western-most desert sites in Anza Borrego, Coachella Valley, and the Mojave River. Pop 2 included individuals from Arizona, Utah, Nevada and most individuals sampled at the Amargosa River in California. Individuals of mixed assignment were found at the Mojave River, Amargosa River, and in the Coachella Valley.

TABLE 2. Number of individuals adjusted for missing data (adjN), number of loci (nLoc), observed heterozygosity (H_o), unbiased expected heterozygosity (uH_e) and inbreeding coefficients (F_{IS}) by region.

Region	adjN	nLoc	H_o	uH_e	F_{IS}
Amargosa River	20.70	10,571	0.2225	0.2234	0.0044
Anza Borrego	13.93	10,571	0.2206	0.2250	0.0193
Coachella Valley	13.86	10,571	0.2257	0.2283	0.0115
Coastal	191.18	10,571	0.2230	0.2253	0.0102
Mojave River	7.96	10,571	0.2293	0.2333	0.0173
Northeast Mojave	22.84	10,571	0.2213	0.2256	0.0191
South Arizona	33.41	10,571	0.2221	0.2242	0.0092

(*Empidonax traillii extimus*), found that dispersal and movement of adaptive genes among breeding grounds greatly improved the viability of the population under climate change (Forester et al. 2023), a result corroborated by empirical analysis of historical samples which suggested population growth occurred in San Diego after gene flow from desert sites (Turbek et al. 2023). This highlights the importance of maintaining aggregations in the desert regions of eastern California to potentially facilitate gene exchange, even though they are smaller than coastal aggregations. Indeed,

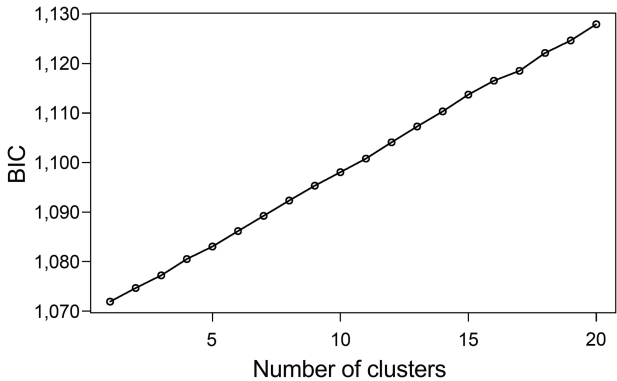


FIGURE 4. K -means clustering analysis of the subset of LBVI coastal drainages evaluated by Bayesian Information Criterion (BIC) scores of successive K -values. One cluster had the lowest BIC score and was selected as the best-supported model.

the Coachella Valley and Mojave River regions had among the highest expected heterozygosity estimates despite lower local abundance. Whether individuals of mixed ancestry have higher fitness could be further investigated in these areas, and habitat management for vireos in these sites may help to maintain or increase local breeding populations.

TABLE 3. First-order relative dyads estimated in Colony with high probability ($P = 1$). Site codes follow Table 1. Dyad members are denoted by age (AHY = adult, after hatch year, HY = fledgling hatch year, nestling = sampled in the nest), sex (Male, Female or U = unknown) and date of capture (month, day, year). The distances between capture locations are given in kilometers (km).

Sites	Member 1	Member 2	Distance between capture locations (km)
BT-BT	AHY Male, 6/16/2020	AHY Male, 6/16/2020	1.90
SG-SA	AHY Male, 5/22/2020	AHY Female, 6/17/2020	129.33
SG-SG	AHY Male, 5/19/2020	AHY, U, 5/29/2020	1.67
SG-SG	AHY Male, 5/19/2020	AHY Male, 5/29/2020	2.02
SG-SG	AHY Male, 5/28/2020	AHY Male, 5/29/2020	0.56
SL-SL	Nestling, U, 7/5/2011	Nestling, U, 6/1/2012	0.04
SM-SM	AHY Male, 7/7/2011	Nestling, Female, 6/29/2012	0.07
SM-TJ	Nestling, Male, 6/30/2011	AHY Male, 6/2/2020	85.24
SM-SM	Nestling, U, 6/14/2012	Nestling, U, 5/2/2013	0.03
ST-ST	AHY Male, 5/27/2020	AHY Male, 5/28/2020	0.53
TJ-TJ	AHY Male, 4/27/2020	AHY Male, 4/27/2020	0.44
TJ-TJ	AHY Male, 5/26/2020	AHY Male, 6/2/2020	0.93
TJ-TJ	AHY Female, 5/26/2020	AHY Male, 6/2/2020	1.96

TABLE 4. Estimates of effective population size (N_e) for subspecies units and for coastal drainages with sample sizes > 10 .

Subspecies	Drainage	Sample size	LD N_e (95% CIs)
<i>pusillus</i>	All	239	912 (725 – 1,216)
	Santa Clara River	27	379 (103 – ∞)
	Hansen Dam	12	65 (22 – ∞)
	Santa Ana River	27	1,261 (114 – ∞)
	Santa Margarita River	29	152 (70 – ∞)
	San Luis Rey River	25	386 (92 – ∞)
	San Diego River	29	214 (95 – ∞)
	Tijuana River	28	161 (79 – ∞)
<i>arizonae</i>	All	78	879 (595 – 1,328)

LBVI Genetic Connectivity and Diversity

Previous monitoring studies have found that Least Bell's Vireo local breeding populations in coastal southern California have grown in abundance and have expanded in range northward over the past few decades (USFWS 2006, Kus *et al.* 2022). High genetic similarity among these drainages with isolation by distance, relatively even diversity, and evidence of recent movement of close relatives among some drainages suggest coastal breeding populations may make up a metapopulation connected by high gene flow. Further, individuals in the Central Valley and western desert assigned to the LBVI cluster, suggesting LBVI has persisted or has been re-established in these areas of the historically occupied range.

The findings of a single genetic cluster and the distribution of close relatives across coastal breeding populations are consistent with observations of banded bird dispersal spanning several decades of monitoring. Although adult LBVI show high inter-annual fidelity to breeding territories, ~20% of first-year birds disperse away from their natal drainages (Kus *et al.* 2022), and in some years and sites up to 49% do so (Houston *et al.* 2021). Distance moved from the natal drainage varies depending on proximity to other habitat, but in coastal populations can be as high as 200 km (Kus *et al.* 2022). Indeed, movement has been

documented among all of the major drainages within San Diego County, as well as dispersal out of San Diego County into the Los Angeles basin and as far north as the Santa Clara and Ventura rivers, some 200 km away (Kus *et al.* 2022). In addition, movement of a banded adult from the San Antonio Dam in the Los Angeles basin to the Mojave River has been documented (M. Madden personal observation), confirming the role of coastal LBVI populations in recolonization of desert sites.

Overall, LBVI has a large genetic effective size that likely exceeds 500. Generally, the literature suggests that N_e greater than 50–100 is sufficient to avoid inbreeding depression and N_e greater than 500–1,000 is sufficient to retain allelic richness and longer-term evolutionary potential (Frankham *et al.* 2014). Although not a main objective of this study, we found that AZBV has a large N_e of similar magnitude to LBVI. This may suggest that ongoing habitat management has helped both subspecies retain N_e sufficient to preserve evolutionary potential (Frankham *et al.* 2014). These estimates also provide a baseline for any future genetic monitoring efforts (Hoban *et al.* 2022). N_e estimates for local breeding populations of LBVI were not well resolved (undefined or large upper confidence intervals), although point estimates were in the same order of magnitude as the number of territories censused at the time of genetic sampling (Houston and Kus 2013, Ferree *et al.* 2014, Pottinger and Kus 2019, Griffith Wildlife Biology 2020a, 2020b, Hall and Plesetz 2020, Zembal *et al.* 2020, Kus 2021). However, in simulations of local populations connected with high gene flow, local N_e estimates become upwardly biased and more reflective of the larger metapopulation than the number of local breeders, as the pool of contributing parents encompasses a greater area than the sampled site (Waples and Do 2010, Waples and England 2011). Therefore, for breeding populations of LBVI along the coast, N_e is not a good proxy for local abundance or the number of breeding birds in a single drainage. Given that breeding populations in coastal drainages appear to function as a metapopulation, synchronization and standardization of range wide abundance and occupancy monitoring could help estimate the total number of breeding birds and temporal trends of the entire population.

Conclusions

Local LBVI breeding populations appear to be well connected by dispersal and gene flow, with a large effective size. These characteristics are consistent with management as a single genetic unit. Habitat restoration and cowbird control efforts throughout the previously occupied range likely enhance recovery and expansion by relaxing novel ecological and evolutionary pressures on LBVI. Movement among drainages coupled with isolation by distance in LBVI are encouraging indications that the benefits of these management activities extend well beyond the geographic limits of individual projects and contribute to overall stability of the range wide population. Occasional dispersal and gene flow across the desert regions of California likely contribute to higher diversity in this area and could be a source of novel adaptive genetic variation which could facilitate further expansion into the drier desert region and increase adaptive capacity to climate change across the subspecies range.

Supplementary material

Supplementary material is available at *Ornithological Applications* online.

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Ethics statement

This research was conducted in compliance with U.S. Geological Survey Institutional Animal Care and Use Committee (IACUC) protocols and authorized under federal 10(a)(1)(A) permits TE-829554 and MBPER0004080, federal migratory bird scientific collecting permit MBPER0040600, and state collecting permits for California, Nevada, Utah and Arizona.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

A.G.V. and B.E.K. conceived the study, B.E.K. designed and led the field data collection, D.A.W., A.G.V., A.M., J.G.S., and E.R.M. analyzed the data, A.G.V., B.E.K., D.A.W., A.M., and J.G.S. wrote the manuscript.

Data Availability

Analyses reported in this article can be reproduced using the data provided by Wood *et al.* (2023). Raw sequence data are accessible as an NCBI Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra/PRJNA999641>).

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