Report on the differences between eastern and western populations of Bell's Vireo (*Vireo bellii)* in New Mexico.

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Introduction

Riparian habitats across the Southwestern United States are at risk due to habitat destruction, climate change, and invasive species (Knopf et al. 1988, Sogge et al. 2008). Gaining better understanding of species that live in these habitats is of utmost priority for biologists. Some important lines of inquiry include: assessing species status of divergent subspecies, determining the role that riparian corridors play in genetic connectivity, and inferring effective population sizes of isolated populations. New Mexico in particular provides an interesting and important study system, as its isolated riparian corridors can be seen as stepping stones between the eastern and western ranges of many species. It is not yet known how these disjunct riparian corridors affect genetic connectivity in widespread species of the Southwest. Southwestern New Mexico, namely the continental divide, roughly corresponds to the Cochise Filter Barrier, which has been shown to serve as an important ecological barrier for many taxa (e.g., Curve-billed Thrasher, Northern Cardinal, Verdin; Provost et al. 2018, Provost et al. 2021).

Bell's Vireo (*Vireo bellii*) is a characteristic songbird of shrubby habitats in the central to southwestern United States and northern and northwestern Mexico. In the southwestern United States, it is confined primarily to riparian zones. It has been declining across much of its range over the past century and is considered a threatened species in New Mexico (Sauer et al. 2015). There are four subspecies that are distributed across the longitudinal extent of the species' range, from east to west: *V. b. bellii*, *V. b. medius*, *V. b. arizonae*, and *V. b. pusillus* (Kus et al. 2010). The subspecies differ subtly in plumage, body size, and vocalizations. *Vireo b. medius* occurs in much of Texas and is thought to be the subspecies that occurs in eastern and central New Mexico, within the Pecos River and Rio Grande drainages (Hubbard 1970). *Vireo b. arizonae* is common across southern Arizona and is the subspecies thought to occur in southwestern New Mexico in the Gila River drainage (Hubbard 1971).

A previous genetic study (Klicka et al. 2016) found that the eastern and western pairs of subspecies formed distinct genetic groups with species-level divergence in mitochondrial DNA.

They also found significant genome-wide divergence in nuclear DNA. The fact that *V. bellii* contains some deep genetic divergence makes the species ideal for understanding how the fragmented riparian habitat may affect population structure. Importantly, Klicka et al. (2016) were unable to sample the New Mexico populations, which prevented determination of whether the eastern and western groups represented two distinct species (Chesser et al. 2017). Additionally, the study left uncertainty as to which subspecies occurred in the major drainages of New Mexico and whether any regions contained admixed populations. Here, we aim to resolve the question of species status and taxonomic identity of New Mexico populations, while also leveraging the dataset to understand the genetics of this species for the purpose of its effective management and conservation.

Methods

Sampling

We sampled 43 individuals of *V. bellii* using muscle tissue (Table S1). Of those, 20 were from New Mexico, ten were from Arizona (putative *V. b. arizonae*), and 12 were from Texas (putative *V. b. medius*). Within New Mexico, 11 samples were from the Rio Grande Valley and three each from the Mimbres, Pecos, and Gila rivers (Figure 1). All samples were from vouchered museum specimens archived in four institutions (Table S1).

Genomic DNA was extracted using QIAgenDNeasy extraction kits (Qiagen Inc., Valencia, CA, USA) following manufacturer's protocols. We quantified the amount of DNA in the extractions using a Qubit 3.0 Fluorometer. One sample for use in 10X chromium sequencing was extracted at Discovery Life Sciences using a high molecular weight extraction for optimal reference quality. We selected this individual (MSB:Bird:60504) from the Gila River drainage because individuals from nearby Arizona sites were thought not to be admixed based on a previous genetic study (Klicka et al. 2016).

Sequencing

Two genomic sequencing methods were used for this study, one for the reference genome and one for the rest of the samples. The reference genome was sequenced using the 10X Chromium platform at Discovery Life Sciences. This sequencing facility prepared the library and sequenced it on 18% of an Illumina NovaSeq S4 lane. The other 42 samples were used for whole-genome resequencing, where short, unlinked reads from the whole genome are sequenced and aligned to a reference for future analyses. Whole-genome resequencing libraries were prepared at facilities at the University of New Mexico according to the KAPA library preparation kit protocol and sequenced on one lane of a NovaSeq S4 at the Oklahoma Medical Research Facility. We selected three libraries representing individuals from the Rio Grande, Eastern New Mexico, and Missouri for higher coverage for use in Pairwise Sequential Markovian Coalescent (PSMC) analyses of effective population size over time. These were chosen to assess how connected effective population sizes are across New Mexico and to compare to the core of the species' range.

Reference Genome

Our reference genome was assembled using the Supernova v2.1.1 assembler provided by 10X Genomics for the purpose of assembling *de novo* genomes sequenced with their Chromium platform. The maximum number of reads was set to achieve 56x coverage based on a preliminary run, and all other parameters were standard. We then used ARKS v1.2.2 (Assembly Roundup by linked-read Kmer mapping Scaffolder; Coombe et al. 2018) and LINKS v1.8.7 (Long Interval Nucleotide Kmer Scaffolder; Warren et al. 2015) to correct the assembly for downstream analyses. This resulted in a reference series of scaffolds, or individual segments of the genome sequence with unknown position relative to other segments. Scaffolds represent either full chromosomes or segments of chromosomes. We used QUAST v 5.0.2 (i.e., quality assessment tool for genomic assemblies; Gurevich et al. 2013) to calculate summary statistics about the reference and BUSCO v5.2.1 (Benchmarking Universal Single-Copy Orthologs; Simão et al. 2015) to assess genome completeness as measured by number of expected genes detected. This and all future analyses were performed on computational clusters maintained by the University of New Mexico's Center for Advanced Research Computing.

Single Nucleotide Variant Calling of Nuclear and Mitochondrial DNA

All datasets were aligned to the reference using the BWA-MEM v0.7.17 (Burrow-Wheeler Alignment tool's Maximum Exact Matches) algorithm (Li & Durbin 2009). We performed two different methods of variant calling from whole genomic data, one for PSMC analysis and one for all the whole-genome resequenced libraries used for population genomic analyses. For PSMC analysis, we used a pipeline of SAMtools v1.12 (Sequence Alignment Map tools; Li et al. 2009), Picard (Broad Institute), and BCFtools v1.12 (Binary variant Call Format tools; Li et al. 2009) to call variants and generated a diploid consensus sequence as input for the analysis. This pipeline was run for the three individuals sequenced at high coverage and for the reference genome. For all 42 resequenced genomes, we followed a pipeline based on the Genome Analysis Toolkit's (GATK; McKenna et al. 2010) Best Practices to call SNVs (Single Nucleotide Variants) for downstream population genomic analyses. This approach entailed marking duplicates and sorting the output of BWA-MEM using the GATK v4.1.9 tool MarkDuplicatesSpark, then calling and genotyping variants separately for each scaffold (individual genome segment) of the reference genome assembly. We did this using GNU (GNU's Not Unix) Parallel (Tange 2018) and the GATK tools HaplotypeCaller, GenomicsDBImport, and GenotypeGVCFs. The resulting per-scaffold VCF (Variant Call Format) files were combined using GATK's GatherVcfs, then filtered with SelectVariants and VariantFiltration, keeping only single nucleotide polymorphisms with a depth of coverage greater than 4, quality score greater than 30.0, and quality-by-depth greater than 2. We further pruned these VCFs using VCFtools v0.1.15 (Danecek et al. 2011) to account for linkage disequilibrium.

We implemented a similar pipeline to that used for PSMC (SAMtools, Picard, and BCFtools) to generate mitochondrial DNA sequences for birds not sampled by Klicka et al. (2016). Unlike the pipeline used for PSMC, we did not permit heterozygous sites. We used a sequence of the ND2 gene from Klicka et al. (2016) as a reference. We aligned reads from all

whole-genome resequencing libraries to this single-gene reference and used those reads to determine if that mitochondrial haplotype was from the eastern or western mitochondrial haplotype group.

Population Structure

We used two approaches to determine the population structure of *V. bellii*. First, we used the genlight Principal component analysis (glPca) method within adegenet (Jombart 2008) to conduct a principal component analysis on the dataset to obtain a non-model-based estimate of population structure. Second, we estimated ancestry coefficients based on a sparse Nonnegative Matrix Factorization (sNMF; Frichot et al. 2014), implemented in the R package LEA 2.2.0 (Landscape and Ecological Associations studies; Frichot & François 2015), to infer the best-fit number of populations (*k*) and construct assignment plots with admixture coefficients. Admixture coefficients represent what proportion of an individual's genome was derived from a given population. We performed 100 replicates for each value of *k* tested. We also calculated F_{ST}, an index that assesses population divergence, using VCFtools v0.1.15 (Danecek et al. 2011). F_{ST} provides a fine scale metric for population divergence.

Conservation Genetics

We used three metrics to assess the conservation genetic health of *V. bellii* populations. First, we assessed per-site heterozygosity(what percentage of sites had two different alleles) of the individual used for the reference genome, as calculated during assembly with Supernova v2.1.1 (Shengbin et al. 2014). Second, we used an implementation of the PSMC (Li & Durbin 2011) to leverage our reference genome and genomes sequenced to a higher coverage to produce an estimate of effective population size throughout time (Allendorf et al. 2010, Oh et al. 2019). This analysis allows for detection of major events, such as bottlenecks; an assessment of the impact of range contractions at the Last Glacial Maximum (Klicka et al. 2016); and determination of what the effective population size was prior to human-mediated declines. We also used pixy v1.2.3 (Korunes & Samuk 2021) to calculate population-level nucleotide diversity, using input that included invariant sites identified by the following tools: GATK v4.1.9 (McKenna et al. 2010), VCFtools v0.1.15 (Danecek et al. 2011), and SAMtools v1.12 (Li et al. 2009). Input with invariant sites included was used to avoid biased estimates of nucleotide diversity (Korunes & Samuk 2021). We calculated nucleotide diversity at both a subspecies level and for individual populations as defined by clusters of sampling sites.

Morphological measurements

We measured standard morphological characters of wing chord, tail length, tarsus length (intertarsal joint to most distal, undivided tarsal scute), and bill length (anterior of nares to bill tip) from 33 adult specimens, including the 20 individuals sequenced in this project from New Mexico (with specimens housed at the Museum of Southwestern Biology) and additional specimens from the Louisiana State University Museum of Zoology, University of Kansas Biodiversity Institute, and University of Arizona. Not all of these specimens were sequenced for this project. All measurements were taken by ABJ. One specimen (MSB:Bird:60560) had a broken tarsus that precluded reliable measurements.

Plumage color comparisons

We selected New Mexico specimens representing the five brightest and dullest of the series and ordered them with respect to brightness. Prior to arranging the series, specimen labels were obscured to avoid biases associated with locality. Most variation in plumage was the extent of yellow-green in the flanks, breast, and belly of each of the specimens. These were then compared to known *V. b. medius* and *V. b. arizonae* from Texas and Arizona, respectively.

Results

Sequencing

The whole genome resequencing was successful for all individuals, although with some variance in individual coverage. Samples targeted for lower coverage (n=39) ranged from 3.2–16.4x mean depth, and higher coverage samples (n=3) ranged from 12.4–34.9x mean depth. Sequencing for the reference genome resulted in higher depth than expected (approximately 70x) and had to be down-sampled to achieve the 56x recommended coverage.

Reference Genome

Our reference genome represents the highest quality reference genome in *Vireonidae*, particularly with respect to the only other available reference genome in the genus *Vireo*. Relative to the *V. altiloquus* genome sequenced by the Birds 10K Project (Zhang 2015), our ARKS and LINKS corrected reference had a contig N50 that was 2.36x higher and scaffold N50 that was 11.08x higher, meaning much more of the genome was confidently assembled. The exact values of our contig and scaffold N50s were 134.24 kilobase pairs (kb) and 7.31 megabase pair (Mb), respectively, which is exceptional given the low cost of assembly.

Mitochondrial DNA

Mitochondrial data from individuals not sequenced in a prior project (Klicka et al. 2016) revealed three key findings. The first two are that populations from far eastern and western New Mexico do indeed have the mitochondrial DNA of Texas and Arizona *V. bellii* respectively. The third key finding that was not expected based on prior research was that individuals in the Rio Grande Valley had a mix of *V. bellii* mitochondrial DNA haplotypes. Within the Rio Grande Valley, the Sevilleta NWR individuals had two eastern and one western haplotype(s), and birds north of Elephant Butte Lake had one eastern and seven western haplotypes (Figure 1). Although there was a striking difference in frequency, the difference between the two sampling areas was not statistically significant (Fisher's Exact Test $p>0.10$). Although only two individuals were sampled, the population along the Rio Grande in west Texas had one eastern and one western mitochondrial haplotype, despite no other Texas birds having a western haplotype (n=15, Klicka et al. 2016).

Population structure

The population structure revealed a complex pattern of population divergences inconsistent with past taxonomic hypotheses, as illustrated by Figures 2 and 3. First, we found that populations from Arizona and the Mimbres and Gila drainages in New Mexico clustered

together. Second, birds from Texas, Kansas, and the Pecos drainage of New Mexico clustered together. The birds from West Texas were variably inferred to have ancestry from *V. b. arizonae*, although the amount of ancestry did not align with geography, which may suggest recent admixture (Figure 3). Finally, birds from the Rio Grande Valley in New Mexico were also genetically intermediate between eastern and western populations but were much more closely aligned to western populations (Figure 2). Although all New Mexico Rio Grande samples were closer to western populations, the birds sampled from farther north along the river (i.e., Sevilleta National Wildlife Refuge) were genetically more similar to eastern populations than were those collected farther south (i.e., Elephant Butte; Figure 2, Table 1). This pattern of divergence is inconsistent with previously hypothesized subspecies boundaries based on phenotype (Hubbard 1970).

Conservation Genetics

The per-site heterozygosity, which, when calculated across a whole genome, is a good estimate of population-wide nucleotide diversity (Dutoit et al. 2016), was 0.0045. This value is intermediate when compared to genome-wide estimates from other passerines (Dutoit et al. 2016, Brüniche-Olsen et al. 2019). Results of our PSMC analysis of effective population size (N_e) through time suggested a relatively stable (i.e., no more than five-fold change in size) population for the past million years for all sampled populations. However, the exact demographic pattern differed between eastern and western populations, with patterns diverging approximately 150,000 years ago. For western New Mexico populations, the population remained stable after that divergence, followed by a steeper decline in the past 30,000 years to the most recently inferred N_e of approximately 130,000 (totaling a two-fold decline; Figure 4a). The central New Mexico population was more similar to the western population but ended with a higher N_e and appeared to increase for a period after the mid Pleistocene transition (gray line, Figure 4b). Note that this sample had lower coverage than the other two and may be less accurate. For eastern New Mexico populations, the population size increased over two-fold before declining rapidly starting 70,000 years ago (Figure 4c). This decline was over three-fold from the peak but still ended higher than western New Mexico populations with an N_e of approximately 220,000.

Population-level estimates of nucleotide diversity (π) performed with pixy v1.2.3 (Korunes & Samuk 2021) were consistent with estimates from whole genome data and PSMC analyses (Table 2). When geographic sampling regions were considered independently, the nucleotide diversity of those comprising *V. b. medius* (combined π=0.0049, mean region-level π =0.0048) were consistently higher than *V. b. arizonae* (combined π=0.0040, mean region-level π=0.0038). The reduction of this estimate relative to the reference genome may be due to an increased false negative rate of variant calls on samples with lower coverage. Populations along the Rio Grande had intermediate nucleotide diversity (combined π=0.0042, mean region-level π=0.0040).

Morphological measurements

Tail length had the highest degree of variation in measurements (43.2 – 54.5 mm). Tail length has previously been shown to be a useful character to separate subspecies groups (Phillips

1991), with the western *V. b. pusillus* and *V. b. arizonae* having longer tails than eastern *V. b. medius* and *V. b. bellii*. The series of specimens from across the drainages of New Mexico shows this pattern; the Gila specimens having the longest tails (mean = 51.2) and those from the Pecos having the shortest tails (mean = 48.4; Tables 3 and S2). However, there was notable overlap between populations, and this trait did not appear to be diagnostic. Wing chord had the second highest amount of variation (51.6 – 59.0 mm) with longer winged birds on average in the Rio Grande and Pecos drainages. These two most variable characters were plotted against each other and show a high degree of overlap among populations on both axes (Figure 5).

Plumage color comparisons

Our series of specimens displayed some geographic variation in plumage (Figure 6). Variation was continuous from bright to dull, and in many cases the order of adjacent specimens could have been swapped without a noticeable difference. When focal specimens were arranged in series from bright to dull, individuals from the Pecos drainage of New Mexico were typically brighter and those from the Mimbres and Gila drainages were duller, corresponding to their presumed source populations in adjacent Trans-Pecos Texas and Arizona, respectively. Individuals from the Rio Grande showed notable variation, and birds from the Sevilleta appeared brighter on average than those from further south along the river (Figure 6). Dorsally, several of these specimens seemed to have more intense feather wear on the head and neck such that it affects the feather structure in the form of fewer barbules. A hypothesis for this extra wear is that these were individuals hatched the previous year, which might tend to have duller, weaker plumage that is more prone to wear than older individuals hatched at least two years prior. Thus, some of the variation in plumage we observed may be due to the age of the individuals when they were collected. Populations from Texas and Arizona were the brightest and dullest of the series, respectively.

Discussion

We demonstrated a unique and unexpected pattern of divergence in New Mexico's *V. bellii* populations. Most importantly, the birds along the Rio Grande were closely allied to individuals from the western subspecies but showed a variable amount of eastern ancestry across their latitudinal range. However, despite the variation, the farthest east (Pecos drainage) and west (Gila drainage) populations in New Mexico clearly form two distinct lineages from each other and are comparable to adjacent populations in Texas and Arizona, respectively. In addition to the genetic divergence of these populations, we determined that plumage coloration and tail length vary greatly across southern New Mexico. On average, individuals from Arizona and the Mimbres and Gila drainages were duller while those from Texas and the Pecos were brighter. Rio Grande birds varied substantially but those from farther north tended to be brighter yellow-green, consistent with their proportionally larger genetic component derived from eastern populations. We also determined preliminary subspecies assignments of *V. bellii* in New Mexico. Birds from the Gila River are genetically aligned with *V. b. arizonae* and Pecos River birds with *V. b. medius*. These results are consistent with past studies and were expected given the continuity of these drainages with populations in Arizona and Texas down river of our samples. Rio Grande samples were admixed, with mitochondrial and nuclear DNA from both subspecies,

but were most closely aligned to *V. b. arizonae* on a whole genome level. This suggests that the Rio Grande constitutes a hybrid population. Curiously, individuals sampled at Sevilleta NWR had a higher proportion of *V. b. medius* ancestry than the population farther south, although this is the Rio Grande population farthest from west Texas populations of *V. b. medius*. The isolated location and North-South orientation of the Rio Grande habitat corridor represents an unusual biogeographic scenario, which may facilitate the formation of admixed populations in other taxa. Finally, we determined that *V. bellii* populations had high effective population sizes and intermediate modern genetic diversity.

Conservation Genetic Implications

The effective population sizes of different populations were overall high (i.e., always greater than 100,000, Figure 4a-c), and the genetic diversity was intermediate when compared to genome-wide estimates from other passerines (Table 2; Dutoit et al. 2016, Brüniche-Olsen et al. 2019). Although the high effective population size and moderate genetic diversity demonstrate that *V. bellii* is not at risk of immediate depletion of genetic diversity, the threat to its habitat in the Southwest (e.g., habitat destruction, drought, invasive species) and range-wide population declines suggest the need for continued caution. Indeed, this genetic variation may be maintained in part by habitat connectivity between the two subspecies facilitated by the Rio Grande and intermediate drainages. Empirical examples (Robinson et al. 2019) and simulation studies (Kyriazis et al. 2021) suggest that a transition from a historically large to a small population size can lead to an excess of deleterious, recessive alleles. This could mean that habitat loss, particularly within the isolated riparian populations of central New Mexico, could have a larger than expected negative impact on the species due to the loss of gene flow between populations. Such gene flow between disparate populations can both facilitate adaptation to changing climatic conditions and genetic rescue from deleterious alleles (Brown & Kodric-Brown 1977, Oziolor et al. 2019).

Evolutionary Implications

Our analyses tie into past niche modeling work (Klicka et al. 2016) to understand how *V. bellii* evolved over the past million years, which could help illuminate the population genetic dynamics of other riparian-associated species in the southwestern United States. First, eastern and western lineages had notably different population trajectories associated with their modern population structure; eastern populations increased in the mid Pleistocene, while western ones remained stable. Both populations decreased in the late Pleistocene, but the eastern ones maintained higher effective population sizes. The eastern population's increase began just before the start of the last interglacial (130,000 years ago; black dashed line, Figure 4) and lasted until the end of the mid-Pleistocene transition (70,000 years ago; gray dashed line, Figure 4). The eastern population then declined from the mid-Pleistocene transition to the present, a period for part of which glaciation increased greatly and likely caused significant range contractions in *V. bellii* (Klicka et al. 2016). Previous work by Klicka et al. (2016) indicates that after the last glacial period, their ranges expanded to meet along the Rio Grande in New Mexico.

The two sampled regions of the Rio Grande in New Mexico showed a curious pattern where the one farther north from the Texas Rio Grande populations showed higher *V. b. medius* ancestry. This result could suggest that the river was originally colonized by *V. b. medius,* or an admixed population with higher *V. b. medius* ancestry, then individuals closer to current *V. b. arizonae* populations (e.g., those in the area north of Elephant Butte Reservoir) experienced higher gene flow from *V. b. arizonae* that occupy drainages between the Rio Grande and the Black Range. Additionally, populations in western Texas appeared to be genetically intermediate between "pure" eastern and western birds (Figure 3). Although this may represent an artifact of ancestry assignment, it is consistent with results based on F_{ST} that find that western Texas individuals are more closely related to *V. b. arizonae* (West Texas-Arizona F_{ST}=0.039) than are populations in south Texas (South Texas-Arizona F_{ST}=0.083; Table 1). Further sampling of individuals along the Rio Grande will provide additional insight into this pattern.

Continental hybrid populations are rare in birds, so the Rio Grande populations of *V. bellii* allow for a unique opportunity to understand their evolutionary history. We have yet to explore the heterogeneity of parental ancestry across the genome of these hybrid birds, but such investigations can potentially reveal signatures of selection against hybrid ancestry (Martin et al. 2019). Additionally, demographic modeling (Gutenkunst et al. 2009) and genomic scans of selection (Irwin et al. 2016) might reveal the degree to which eastern and western populations are connected by this hybrid population and whether gene flow may have resulted in introgression of universally beneficial loci between eastern and western taxa (Hendrick 2013, Oziolor et al. 2019).

Taxonomic Implications

From a taxonomic standpoint, there are two clear lineages of *Vireo bellii* that come into contact along the Rio Grande with some degree of gene flow. But do these lineages represent one species or two? An argument for one species can be made because the admixture along the Rio Grande, and possible admixture in west Texas, suggests that these lineages are not fully reproductively isolated from those in other drainages. This scenario, and adherence to strict applications of the Biological Species Concept (Mayr 1942), would support the recognition of one species. Alternatively, modern extensions of the Biological Species Concept include an increased acceptance that gene flow between sister species is common (Grant & Grant 1992, Coyne & Orr 2004, Ottenburghs 2019). This would suggest that treatment as two species may be warranted. The two species treatment is supported by the apparent sharp turnover between the largely *V. b. arizonae* populations north of Elephant Butte Lake and the *V. b. medius* along the Rio Grande in west Texas, despite these lineages occupying similar habitats within a shared habitat corridor and without clear barriers to dispersal. However, without finer-scale sampling, the nature of this transition cannot be fully known. As for the subspecies status of New Mexico populations, Pecos birds appear to represent *V. b. medius* and birds of western drainages (Gila and Mimbres) represent *V. b. arizonae*. Delimiting hybrid populations is a taxonomic challenge, and although *V. bellii* along the Rio Grande in New Mexico are genetically most similar to *V. b. arizonae*, a transition to *V. b. medius* may exist within the state. Furthermore, it is apparent that coloration and morphometric measurements are not sufficient for the identification of individuals

along the Rio Grande in New Mexico as being affiliated with either subspecies, so assessment of the taxonomic affinities of unsampled populations will likely require further genetic sampling (Figures 5 and 6). We suggest that it is best to consider Rio Grande populations admixed until further genetic assessment is completed. However, the geographic position of the Rio Grande population makes it a stepping stone for range-wide genetic connectivity between the most diverged forms of the *V. bellii* complex, suggesting that it will have high value for conservation as riparian habitat contracts in the future. Future exploration of these genomic data will undoubtedly lead to additional insights about the role of this population in genetic connectivity. However, based on present results, it seems that the populations of this riparian specialist should be considered as genetically linked among riparian corridors at a regional scale.

Figures and Tables

Figure 1: A) Map of all samples in this study (to the exclusion of the one sample from Missouri; see Table S1), color-coded by mitochondrial DNA (mtDNA). Orange samples have eastern mtDNA and blue have western mtDNA haplotypes, with assignment based on ND2 data from Klicka et al. (2016) and newly sampled individuals from out study. To show our sampling of the Rio Grande more clearly, we included inset maps of B) the northern Rio Grande Valley samples and C) the southern Rio Grande Valley samples.

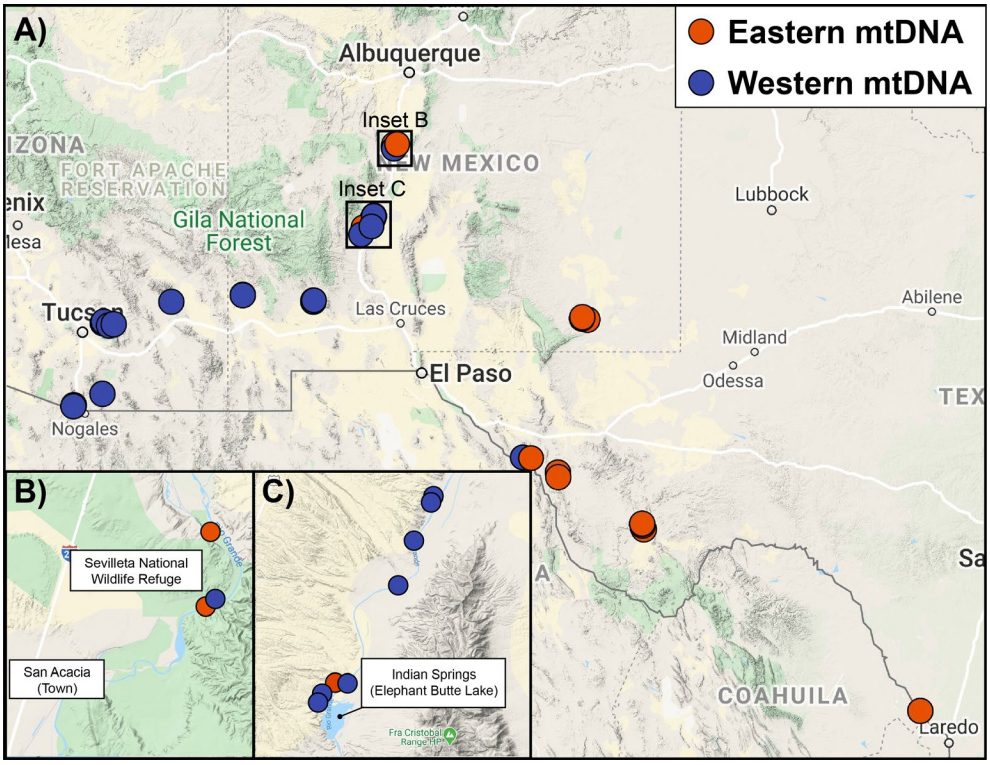

Figure 2: Analyses of population structure in Bell's Vireo (*Vireo bellii*) based on whole-genome data. Primary graph shows results of a non-model-based clustering approach, specifically a principal component analysis (PCA) of single nucleotide polymorphism data. Each cluster is labeled with its respective population and is surrounded by a 95% confidence ellipse. Axes are labeled with the weight of their respective principal components (PC). The second PC onward did not add much more explanation of variance, as illustrated by the inset bar plot. Note one west Texas bird from the Rio Grande consistently clustered with south Texas individuals and is not included in the confidence ellipse.

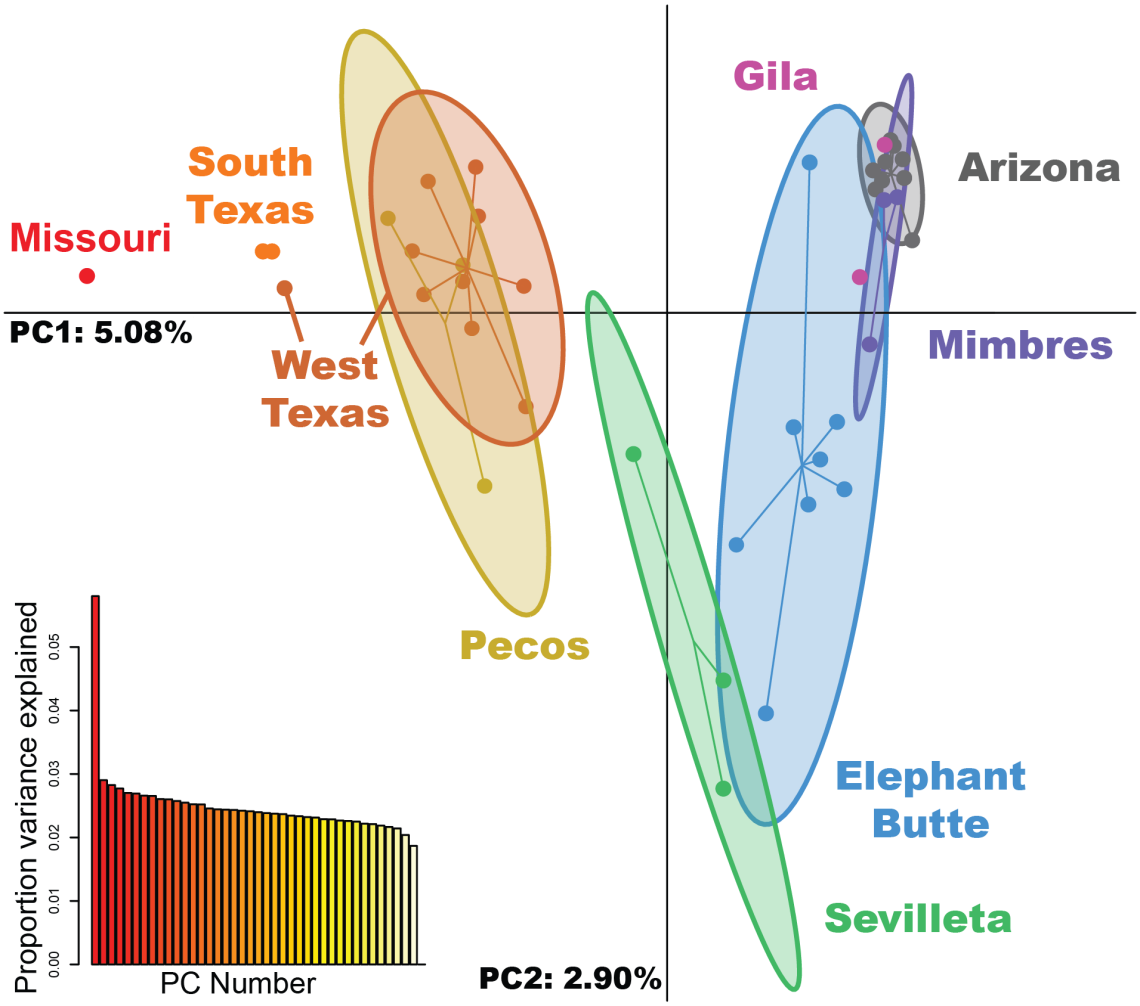

Figure 3: Ancestry proportions for two historic populations (represented by different colors) estimated with a model-based clustering approach using sparse Nonnegative Matrix Factorization (sNMF). Labels at the top of the figure represent sampling regions. The individual marked with an asterisk is the individual from west Texas that clustered with south Texas birds.

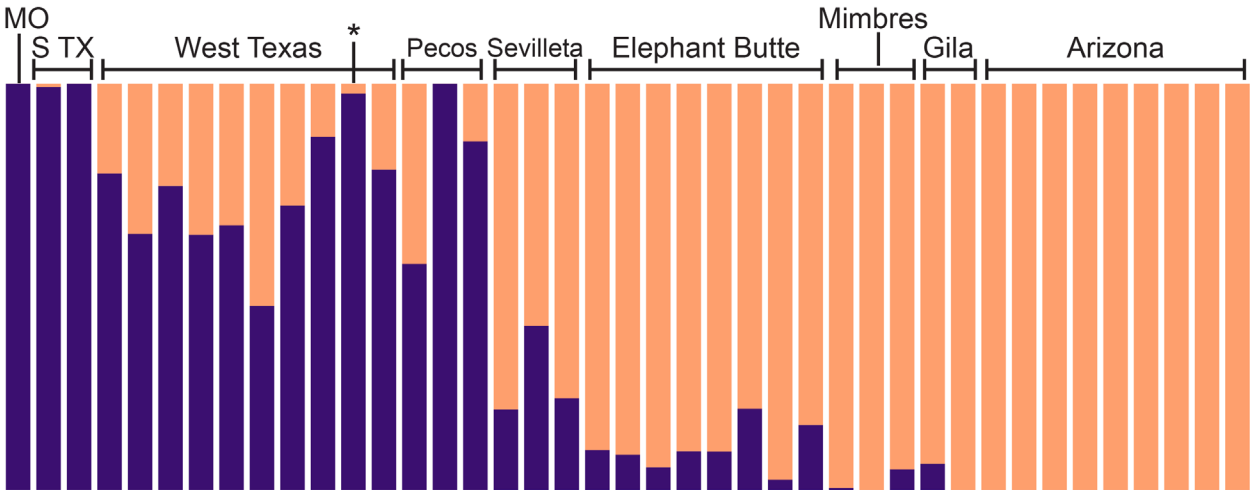

Figure 4: Plot of effective population size through time produced by Pairwise Sequential Markovian Coalescent (PSMC) analysis for the three high coverage New Mexico samples from the A) Gila, B) Rio Grande, and C) Pecos drainages. Note that the Rio Grande sample had notably lower depth of coverage than the other two, likely decreasing accuracy. The red lines represent estimated effective population size at a given point in time, with lighter red lines representing bootstrap replicates. The black vertical dashed line corresponds to the approximate time of the last interglacial (i.e., the last period of warmth before the last glacial period; 120,000 years ago). The gray vertical line represents the end of the mid-Pleistocene transition (70,000 years ago), after which ice sheet extent increased dramatically (Willeit et al. 2019). The period of time after the mid-Pleistocene transition shows some degree of decline for all populations, particularly toward the present.

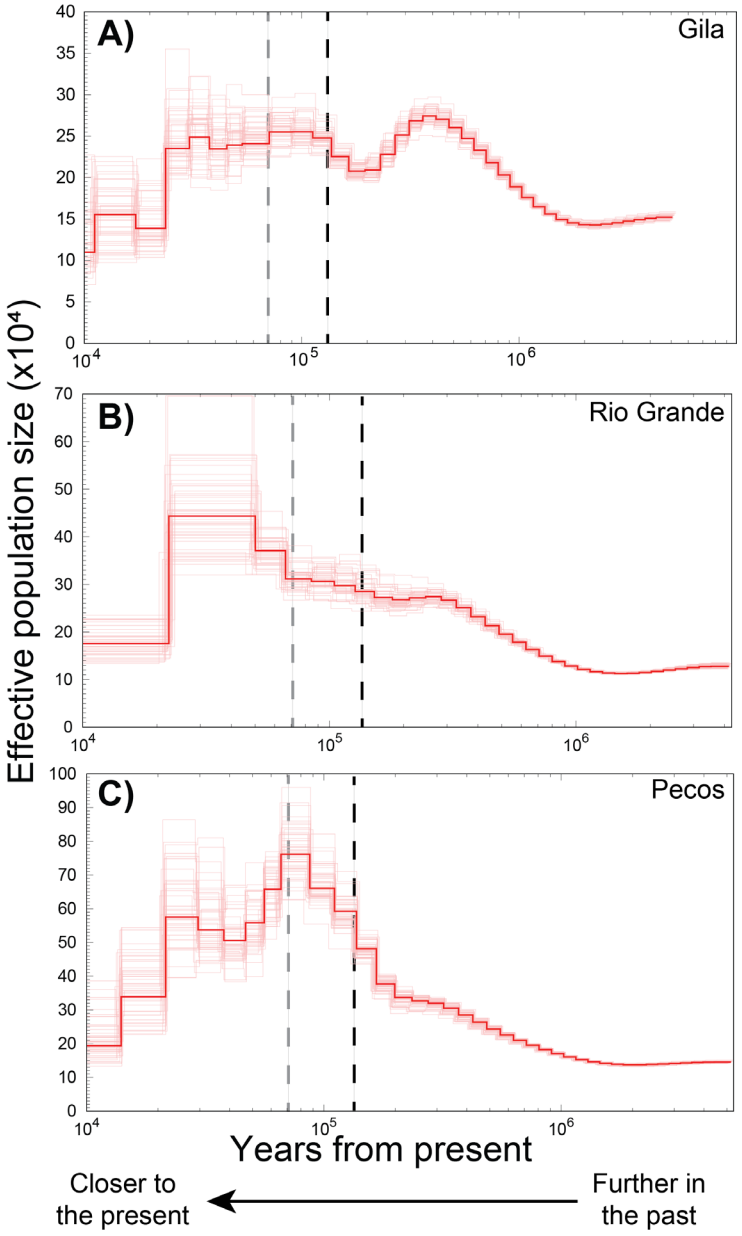

Figure 5: Figure depicting key morphometric data (tail length and wing chord) of male Bell's Vireo (*Vireo bellii*) specimens from Arizona, New Mexico, and Trans-Pecos Texas. Color corresponds to location; small points represent individual samples, and large points represent population averages.

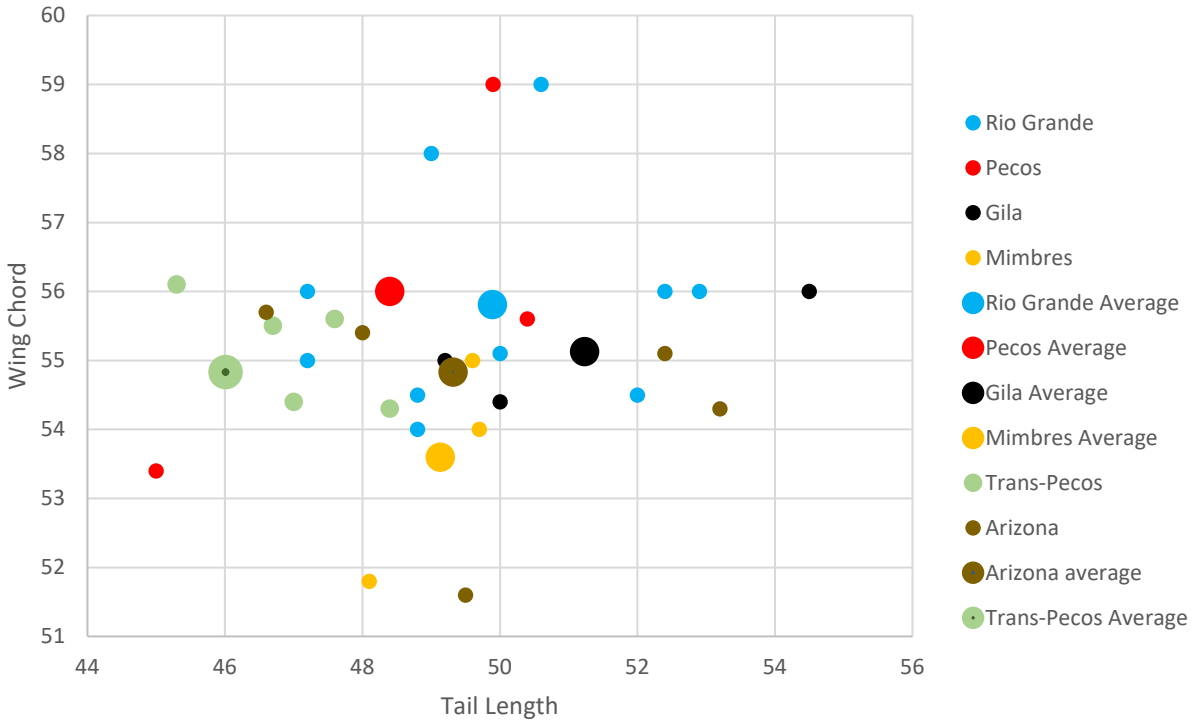

Figure 6. Comparison of the brightest and dullest New Mexico Bell's Vireo (*Vireo bellii*) specimens to specimens from adjacent Trans-Pecos Texas and southern Arizona representing *V. b. medius* and *V. b. arizonae* respectively. Top three specimens are from Arizona, bottom three are from Trans-Pecos Texas. Middle ten specimens are from New Mexico, with the red line indicating the split between the dullest specimens above and the brightest specimens below.

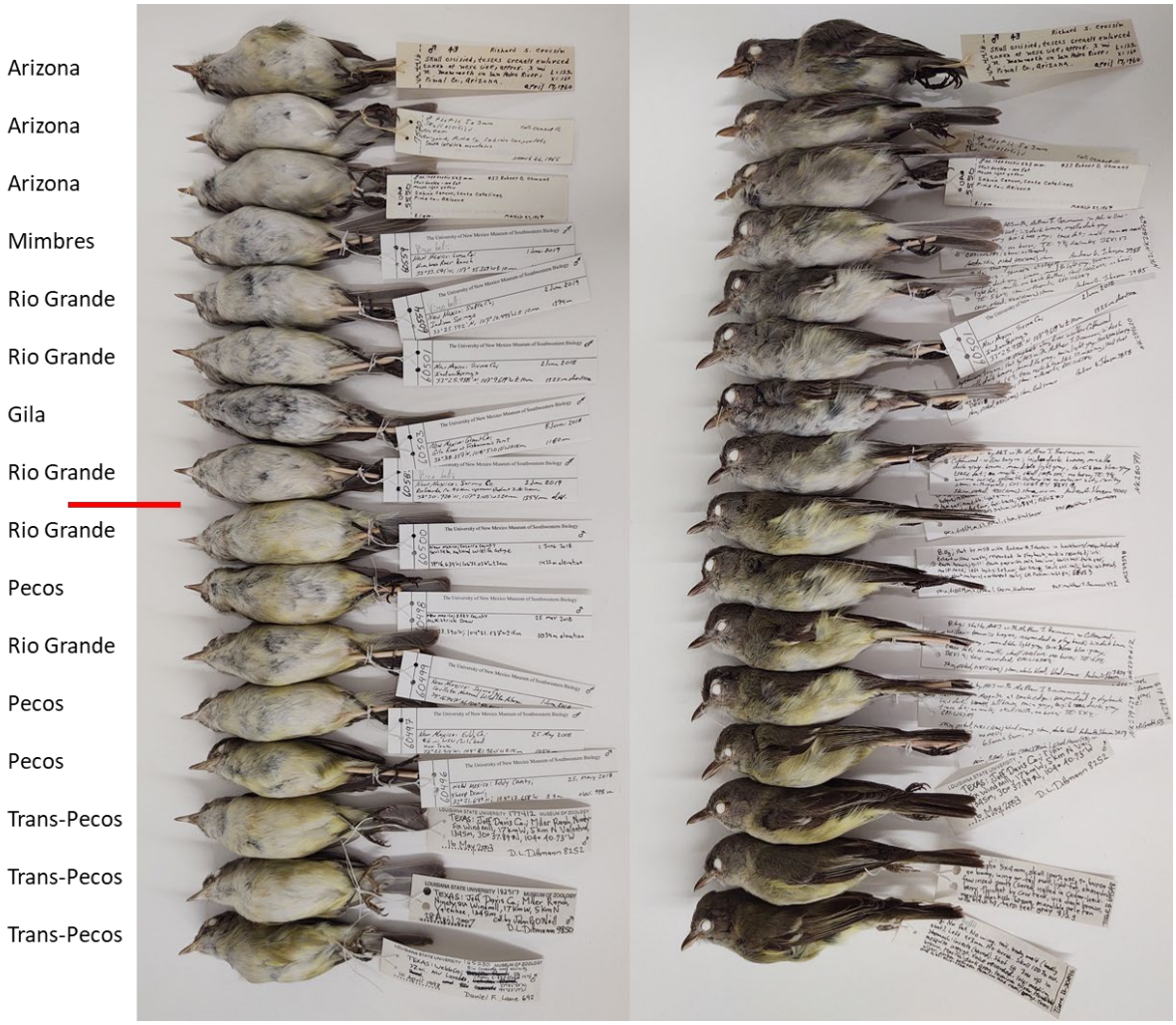

Table 1: Table of pairwise F_{ST} values between populations, where higher values correspond with greater divergence between respective populations. Subspecies and the New Mexico Rio Grande birds are delimited by thicker lines.

Populations	Missouri	South Texas	West Texas	Pecos	Sevilleta	Butte	Mimbres	Gila	Arizona
Missouri	-	-0.015	0.008	0.015	0.053	0.092	0.114	0.107	0.126
South TX	-0.015	-	0.005	0.009	0.043	0.062	0.077	0.070	0.083
West TX	0.008	0.005	-	-0.003	0.021	0.028	0.034	0.027	0.039
Pecos	0.015	0.009	-0.003	-	0.022	0.031	0.041	0.036	0.047
Sevilleta	0.053	0.043	0.021	0.022	-	0.019	0.021	0.014	0.034
Butte	0.092	0.062	0.028	0.031	0.019	-	0.009	0.008	0.011
Mimbres	0.114	0.077	0.034	0.041	0.021	0.009	-	-0.004	0.006
Gila	0.107	0.070	0.027	0.036	0.014	0.008	-0.004	-	0.012
Arizona	0.126	0.083	0.039	0.047	0.034	0.011	0.006	0.012	-

Table 2: Table of nucleotide diversity values of each population of Bell's vireo (*Vireo bellii*). Values for single sampling regions are given in the first row, and values from subspecies are given in the bottom row (with the broader cells corresponding to their constituent populations). RGV = Rio Grande Valley.

Missouri	South Texas	West Texas (RGV)	Trans-Pecos	Pecos	Sevilleta	Butte	Mimbres	Gila	Arizona
0.0052	0.0050	0.0048	0.0047	0.0048	0.0039	0.0042	0.0037	0.0036	0.0042
V. b. bellii	***V. b. medius***				**New Mexico RGV**		***V. b. arizonae***		
0.0052	0.0049				0.0042		0.0040		

Table 3: Means and standard deviations of male Bell's Vireos (*Vireo bellii*) morphological characters across southern Arizona, four river drainages in New Mexico (NM), and Trans-Pecos Texas.

Drainage	Wing chord	Tail length	Tarsus length	Bill length	Body mass
Arizona(n=6)	54.9 ± 1.9	49.3 ± 2.9	18.8 ± 0.9	6.7 ± 0.3	8.1[#]
NM Gila (n=3)	55.1 ± 0.8	51.2 ± 2.9	18.8 ± 0.7	6.9 ± 0.1	8.4 ± 0.4
NM Mimbres (n=3)	53.6 ± 1.6	49.1 ± 0.9	18.0 ± 0.4	6.7 ± 0.3	8.2 ± 0.3
NM Rio Grande (n=10)	55.8 ±1.6	49.9 ±2.1	18.2 ± 0.7*	6.8 ± 0.4	8.4 ± 0.3
NM Pecos (n=3)	56.0 ±2.8	48.4 ± 3.0	18.8 ± 0.3	6.8 ± 0.3	8.3 ± 0.9
Trans-Pecos (n=7)	54.8 ± 1.0	46.0 ± 1.9	18.3 ± 0.6	7.0 ± 0.3	8.2 ± 0.8

[#]n=1

*n=9

Table S1: Specimen table. Columns 1-3 denote the scientific name of the sample. Columns 4-5 denote the museum and associated specimen number. Columns 6-8 denote the location of the sample's collection. Column 9 denotes the mitochondrial DNA group of the sample (if known) and whether the sample was only measured for this study (i.e., no genetic analyses performed). Institution abbreviations are as follows: University of Kansas Biodiversity Institute (KU), Louisiana State University Museum of Zoology (LSUMZ), Museum of Southwestern Biology (MSB), San Diego State University (SDSU), University of Arizona Museum of Natural History (UAZ), and University of Texas El Paso Biodiversity Collections (UTEP). RGV = Rio Grande Valley.

Genus	Species	Subspecies	Institution	ID	Country	State	Location	mtDNA
Vireo	*bellii*	*medius*	MSB	MSB:Bird:60496	United States	New Mexico	Pecos River	Eastern
Vireo	*bellii*	*medius*	MSB	MSB:Bird:60497	United States	New Mexico	Pecos River	Eastern
Vireo	*bellii*	*medius*	MSB	MSB:Bird:60498	United States	New Mexico	Pecos River	Eastern
Vireo	*bellii*	*arizonae*	MSB	MSB:Bird:60503	United States	New Mexico	Gila River	Western
Vireo	*bellii*	*arizonae*	MSB	MSB:Bird:60504	United States	New Mexico	Gila River	Western
Vireo	*bellii*	*arizonae*	MSB	MSB:Bird:60505	United States	New Mexico	Gila River	Western
Vireo	*bellii*	*arizonae*	MSB	MSB:Bird:60557	United States	New Mexico	Mimbres River	Western
Vireo	*bellii*	*arizonae*	MSB	MSB:Bird:60559	United States	New Mexico	Mimbres River	Western
Vireo	*bellii*	*arizonae*	MSB	MSB:Bird:60564	United States	New Mexico	Mimbres River	Western
Vireo	*bellii*	*?*	MSB	MSB:Bird:60501	United States	New Mexico	Rio Grande	Western
Vireo	*bellii*	*?*	MSB	MSB:Bird:60547	United States	New Mexico	Rio Grande	Western
Vireo	*bellii*	*?*	MSB	MSB:Bird:60554	United States	New Mexico	Rio Grande	Western
Vireo	*bellii*	*?*	MSB	MSB:Bird:60556	United States	New Mexico	Rio Grande	Eastern
Vireo	*bellii*	*?*	MSB	MSB:Bird:60560	United States	New Mexico	Rio Grande	Western
Vireo	*bellii*	*?*	MSB	MSB:Bird:60499	United States	New Mexico	Rio Grande	Western
Vireo	*bellii*	*?*	MSB	MSB:Bird:60500	United States	New Mexico	Rio Grande	Eastern
Vireo	*bellii*	*?*	MSB	MSB:Bird:60502	United States	New Mexico	Rio Grande	Eastern
Vireo	*bellii*	*?*	MSB	MSB:Bird:60543	United States	New Mexico	Rio Grande	Western
Vireo	*bellii*	*?*	MSB	MSB:Bird:60548	United States	New Mexico	Rio Grande	Western
Vireo	*bellii*	*?*	MSB	MSB:Bird:60583	United States	New Mexico	Rio Grande	Western
Vireo	*bellii*	*arizonae*	SDSU	2903	United States	Arizona	Southeast Arizona	Western
Vireo	*bellii*	*arizonae*	SDSU	2904	United States	Arizona	Southeast Arizona	Western
Vireo	*bellii*	*arizonae*	SDSU	2905	United States	Arizona	Southeast Arizona	Western
Vireo	*bellii*	*arizonae*	SDSU	2906	United States	Arizona	Southeast Arizona	Western
Vireo	*bellii*	*arizonae*	SDSU	2907	United States	Arizona	Southeast Arizona	Western
Vireo	*bellii*	*arizonae*	SDSU	2908	United States	Arizona	Southeast Arizona	Western
Vireo	*bellii*	*arizonae*	SDSU	2909	United States	Arizona	Southeast Arizona	Western

Vireo	*bellii*	*arizonae*	SDSU	2910	United States	Arizona	Southeast Arizona	Western
Vireo	*bellii*	*arizonae*	SDSU	2911	United States	Arizona	Southeast Arizona	Western
Vireo	*bellii*	*arizonae*	SDSU	2917	United States	Arizona	Southeast Arizona	Western
Vireo	*bellii*	*medius*	SDSU	2912	United States	Texas	West Texas	Eastern
Vireo	*bellii*	*medius*	SDSU	2913	United States	Texas	West Texas	Eastern
Vireo	*bellii*	*medius*	SDSU	2914	United States	Texas	West Texas	Eastern
Vireo	*bellii*	*medius*	SDSU	2915	United States	Texas	West Texas	Eastern
Vireo	*bellii*	*medius*	SDSU	2916	United States	Texas	West Texas	Eastern
Vireo	*bellii*	*medius*	LSUMZ	LSUMZ:Bird:177411	United States	Texas	West Texas	Eastern
Vireo	*bellii*	*medius*	LSUMZ	LSUMZ:Bird:177412	United States	Texas	West Texas	Eastern
Vireo	*bellii*	*medius*	LSUMZ	LSUMZ:Bird:177413	United States	Texas	West Texas	Eastern
Vireo	*bellii*	*medius*	UTEP	UTEP:Bird:3117	United States	Texas	West Texas	Western
Vireo	*bellii*	*medius*	UTEP	UTEP:Bird3130	United States	Texas	West Texas	Eastern
Vireo	*bellii*	*medius*	LSUMZ	LSUMZ:Bird:165230	United States	Texas	Lower RGV	Eastern
Vireo	*bellii*	*medius*	LSUMZ	LSUMZ:Bird:174440	United States	Texas	Lower RGV	Eastern
Vireo	*bellii*	*bellii*	KU	KU:Bird:89925	United States	Missouri	Northwest Missouri	Eastern
Vireo	*bellii*	*medius*	LSUMZ	LSUMZ:Bird:165230	United States	Texas	Lower RGV	measured only
Vireo	*bellii*	*medius*	LSUMZ	LSUMZ:Bird:182517	United States	Texas	West Texas	measured only
Vireo	*bellii*	*medius*	LSUMZ	LSUMZ:Bird:181902	United States	Texas	West Texas	measured only
Vireo	*bellii*	*medius*	LSUMZ	LSUMZ:Bird:197431	United States	Texas	West Texas	measured only
Vireo	*bellii*	*medius*	LSUMZ	LSUMZ:Bird:197432	United States	Texas	West Texas	measured only
Vireo	*bellii*	*arizonae*	UAZ	UAZ:Bird:7520	United States	Arizona	Southeast Arizona	measured only
Vireo	*bellii*	*arizonae*	UAZ	UAZ:Bird:5550	United States	Arizona	Southeast Arizona	measured only
Vireo	*bellii*	*arizonae*	UAZ	UAZ:Bird:4416	United States	Arizona	Southeast Arizona	measured only
Vireo	*bellii*	*arizonae*	KU	KU:Bird:23417	United States	Arizona	Southeast Arizona	measured only
Vireo	*bellii*	*arizonae*	KU	KU:Bird:18893	United States	Arizona	Southeast Arizona	measured only
Vireo	*bellii*	*arizonae*	KU	KU:Bird:18897	United States	Arizona	Southeast Arizona	measured only

Table S2: Morphological measurements* of male Bell's Vireo (*Vireo bellii*) specimens from southern Arizona, across four river drainages in New Mexico (NM), and Trans-Pecos Texas. Institution abbreviations are as follows: University of Kansas Biodiversity Institute (KU), Louisiana State University Museum of Zoology (LSUMZ), Museum of Southwestern Biology (MSB), and University of Arizona Museum of Natural History (UAZ).

Catalog No.	Locality	Wing chord	Tail length	Tarsus length	Bill length	Body mass
UAZ:Bird:7520	Arizona	57.5	52.4	20.4	6.7	-
UAZ:Bird:5550	Arizona	55.1	53.2	18.5	6.3	8.1
UAZ:Bird:4416	Arizona	54.3	48	17.9	7	-
KU:Bird:23417	Arizona	51.6	46.6	18.7	6.8	-
KU:Bird:18893	Arizona	55.7	46.2	18.2	6.3	-
KU:Bird:18897	Arizona	55.4	49.5	19	6.8	-
MSB:Bird:60503	NM: Gila River	54.4	50.0	18.9	7.0	8.4
MSB:Bird:60504	NM: Gila River	55.0	49.2	19.4	6.9	8.8
MSB:Bird:60505	NM: Gila River	56.0	54.5	18.0	6.8	8.0
MSB:Bird:60557	NM: Mimbres River	51.8	48.1	17.6	6.5	7.8
MSB:Bird:60564	NM: Mimbres River	54.0	49.7	18.3	6.7	8.4
MSB:Bird:60559	NM: Mimbres River	55.0	49.6	18.2	7.0	8.4
MSB:Bird:60560	NM: Rio Grande	54.0	48.8	-	6.9	8.1
MSB:Bird:60548	NM: Rio Grande	54.5	48.8	17.3	6.9	7.7
MSB:Bird:60501	NM: Rio Grande	54.5	52.0	18.1	7.0	8.3
MSB:Bird:60499	NM: Rio Grande	55.0	47.2	18.3	6.4	8.6
MSB:Bird:60556	NM: Rio Grande	55.1	50.0	18.1	6.5	8.7
MSB:Bird:60554	NM: Rio Grande	56.0	47.2	17.5	7.0	8.4
MSB:Bird:60583	NM: Rio Grande	56.0	52.4	18.2	6.4	8.5
MSB:Bird:60547	NM: Rio Grande	56.0	52.9	18.0	6.7	8.7
MSB:Bird:60502	NM: Rio Grande	58.0	49.0	19.8	6.8	8.4
MSB:Bird:60500	NM: Rio Grande	59.0	50.6	18.7	7.7	8.7
MSB:Bird:60497	NM: Pecos River	53.4	45.0	19.1	7.0	7.7
MSB:Bird:60496	NM: Pecos River	55.6	50.4	18.6	6.9	9.3
MSB:Bird:60498	NM: Pecos River	59.0	49.9	18.7	6.4	8.0
LSUMZ:Bird:174440	Trans-Pecos Texas	54.9	43.9	18.1	6.5	8.8
LSUMZ:Bird:165230	Trans-Pecos Texas	56.1	45.3	18.3	7.3	7.9
LSUMZ:Bird:182517	Trans-Pecos Texas	54.4	47	18	6.8	8.5
LSUMZ:Bird:181902	Trans-Pecos Texas	54.3	48.4	17.8	6.7	7.4
LSUMZ:Bird:97431	Trans-Pecos Texas	55.6	47.6	18.1	6.8	9.4
LSUMZ:Bird:197432	Trans-Pecos Texas	55.5	46.7	19.6	7.4	7.7
LSUMZ:Bird:177412	Trans-Pecos Texas	53.0	43.2	18.0	7.2	7.4

*Measurements in millimeters; mass in grams.

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