

Geographic variation and divergence of songs in the Olive Sparrow species complex

Ronald A. Fernández Gómez,¹  Jorge E. Morales-Mávil,¹  and J. Roberto Sosa-López^{2,3} 

¹Instituto de Neuroetología, Universidad Veracruzana, Xalapa, Veracruz, Mexico

²Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca (CIIDIR), Instituto Politécnico Nacional, Oaxaca, Mexico

³Dirección de Catedras, Consejo Nacional de Ciencia y Tecnología (CONACYT), Ciudad de México, Mexico

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ABSTRACT. Phenotypic traits such as songs are important in species recognition. Variation in acoustic traits can form barriers to gene flow and promote speciation. Therefore, understanding song divergence is crucial in groups with controversial taxonomy such as Olive Sparrows (*Arremonops rufivirgatus*), a widespread Neotropical species of songbird with multiple allopatric populations. Taxonomic authorities disagree on the number of Olive Sparrow subspecies, placing them into either two or three main groups. These groups may represent separate species based on morphological traits, but trait divergence within the complex has not been examined. We studied geographic variation in the characteristics of the songs of Olive Sparrows at two geographical levels: among three proposed groups and among five allopatric populations. In a second analysis, we evaluated the strength of acoustic divergence within the complex by comparing acoustic distances among groups and allopatric populations of Olive Sparrows with the acoustic distance among three recognized species in the genus *Arremonops*. We analyzed 802 songs from 174 individuals across 81 locations and measured 12 variables to describe the fine structural characteristics of the songs of Olive Sparrows, Green-backed Sparrows (*A. chloronotus*), Black-striped Sparrows (*A. conirostris*), and Tocuyo Sparrows (*A. tocuyensis*). We found significant acoustic variation in the Olive Sparrow complex at both geographical levels. Our divergence analysis also revealed that vocal divergence within the complex is similar to or greater than that found between recognized species in the genus. Together, these results suggest that acoustic diversity within the Olive Sparrow complex probably originated by isolation in tandem with selective and/or non-selective factors.

RESUMEN. Variación geográfica y divergencia del canto del Rascador Oliváceo (*Arremonops rufivirgatus*)

Los caracteres fenotípicos, como los cantos, son importantes para el reconocimiento de especies. La variación en caracteres acústicos puede formar barreras que previenen el flujo genético y promueven especiación. Por lo tanto, es crucial entender la divergencia en el canto en grupos en los cuales la taxonomía es controversial como en *Arremonops rufivirgatus*, una especie de ave canora Neotropical, ampliamente distribuida con múltiples poblaciones alopatricas. Las autoridades taxonómicas están en desacuerdo con respecto al número de subespecies de *Arremonops rufivirgatus*, ubicándolas en dos o tres grupos principales. Estos grupos pueden representar especies diferentes basado en caracteres morfológicos, pero la divergencia dentro del complejo no ha sido evaluada. Nosotros estudiamos la variación geográfica en las características del canto de *Arremonops rufivirgatus* en dos niveles geográficos: a través de los tres grupos propuestos y a través de cinco poblaciones alopatricas. En un segundo análisis, evaluamos la magnitud de la divergencia acústica dentro del complejo, comparando distancias acústicas entre los grupos y las poblaciones alopatricas de *Arremonops rufivirgatus* con la distancia acústica entre tres especies reconocidas en el género *Arremonops*. Analizamos 802 cantos de 174 individuos a través de 81 localidades y medimos 12 variables para describir las características estructurales finas de los cantos de *Arremonops rufivirgatus*, *A. chloronotus*, *A. conirostris* y *A. tocuyensis*. Nosotros encontramos variación acústica significativa en el complejo de *Arremonops rufivirgatus* en los dos niveles geográficos. Nuestros análisis revelaron que la divergencia local dentro del complejo es similar o mayor que la encontrada entre especies reconocidas en el género. Estos resultados sugieren que la diversidad acústica dentro del complejo de *Arremonops rufivirgatus* se originó probablemente por aislamiento geográfico en tandem con factores selectivos y/o no-selectivos.

Key words: acoustic variation, allopatry, birdsong, divergence, isolation, song evolution, species limits, vocalization

The acoustic signals of birds are used for during inter- and intra-species competition, species recognition, to advertise territories, and to attract mates (Catchpole and Slater

Corresponding authors. Email: jrobertososa@gmail.com (J.R.S.L.); jormorales@uv.mx (J.E.M.M)

2008). Because vocalizations encode information about a signaler's identity, acoustic variation provides a mechanism to mediate discrimination between members belonging to different populations (Bradbury and Vehrencamp 2011, Wilkins et al. 2013). In allopatry, birds could accrue variation in their vocal behavior, resulting in divergent signals that later, in sympatry, could result in behavioral discrimination, assortative mating, and reproductive isolation (Matens 1996, Wilkins et al. 2013).

Divergence in learned songs could have evolved due to selective (e.g., cultural selection, natural selection, or through selection on song as a social and sexual signal) and/or non-selective factors (e.g., cultural drift and genetic drift; Wilkins et al. 2013). Variation in learned songs is also influenced by the evolutionary history of a species, with a number of studies providing evidence of corresponding variation between song and genetic divergence, e.g., Pacific Wren (*Troglodytes pacificus*) vs. Winter Wren (*T. hiemalis*; Toews and Irwin 2008), Rufous-naped Wren (*Campylorhynchus rufinucha*; Vázquez-Miranda et al. 2009, Sosa-López et al. 2013), White-browed Brush-finch (*Arremon torquatus*; Cadena and Cuervo 2010), Wedge-tailed Sabrewing (*Campylopterus curvipennis*; González et al. 2011), White-crowned Sparrow (*Zonotrichia leucophrys*; Lipshutz et al. 2017), and Timberline Wren (*Thryorhynchus browni*; Camacho-Alpizar et al. 2018), and concordance between vocal traits and phylogenetic relationships (e.g., McCracken and Sheldon 2002, Price and Lanyon 2002).

Generally, studies showing variation in vocal traits congruent with genetic and/or morphological variation across geography involve species with isolated populations (Cadena and Cuervo 2010, Sosa-López et al. 2013, Sosa-López and Mennill 2014, Sandoval et al. 2017). For example, Sandoval et al. (2017) evaluated phenotypic variation between isolated populations of White-eared Ground-sparrows (*Melospiza leucotis*) in Central America and found differences in voice, plumage, and morphological traits. Cadena and Cuervo (2010) found morphological, genetic, and vocal differences among eight isolated populations of Stripe-headed Brush-finches (*Arremon torquatus*) in Central and South America, and Buainain et al. (2017)

found morphological and acoustical differences among two isolated groups of Pectoral Sparrows (*Arremon taciturnus*) in South America. These studies suggest that allopatry plays an important role in the origin of acoustic diversity within a species complex.

We examined the geographic variation in the songs of the Olive Sparrow (*Arremonops rufivirgatus*), a Neotropical songbird with five allopatric populations ranging from the southern United States to northern Costa Rica (Howell and Webb 1995, Rising 2011, Fig. 1). This species inhabits deciduous and semideciduous forests with thorny shrubs (Howell and Webb 1995). Because of its long-debated taxonomic history, this species complex represents an ideal group for exploring vocal geographic variation and its taxonomic implications. Based on plumage coloration and distribution, some authorities have described either eight (Dickinson and Christidis 2014, Gill and Donsker 2019) or nine (del Hoyo et al. 2016, Clements et al. 2019) subspecies within the complex (Fig. 1). Del Hoyo et al. (2016) recognize nine subspecies divided into three groups: the *rufivirgatus* group (subspecies: *rufivirgatus*, *ridgwayi*, and *crassirostris*) distributed from southern Texas to southern Veracruz, the *superciliosus* group (subspecies: *sinaloae*, *sumichrasti*, *chiapensis*, and *superciliosus*) distributed from Sinaloa, Mexico, to the Pacific coast of Costa Rica, and the *verticalis* group (subspecies: *verticalis* and *rhyptothorax*) restricted to the Yucatan Peninsula. Dickinson and Christidis (2014) recognize eight subspecies within the same three groups as del Hoyo et al. (2016), with the *rufivirgatus* group including the subspecies *rufivirgatus* and *crassirostris*. Clements et al. (2019) recognize nine subspecies divided into the *rufivirgatus* group (subspecies: *rufivirgatus*, *ridgwayi*, *crassirostris*, *verticalis*, and *rhyptothorax* subspecies) and the *superciliosus* group. However, other authorities lump all subspecies into two or three taxonomic groups (Dickinson and Christidis 2014, del Hoyo et al. 2016, Clements et al. 2019). These different taxonomic classifications are likely a direct result of variation in phenotypic traits within the complex. Despite the taxonomic controversy around this species, phenotypic trait divergence and its relationship to the geographic distribution of Olive Sparrows have not been examined.

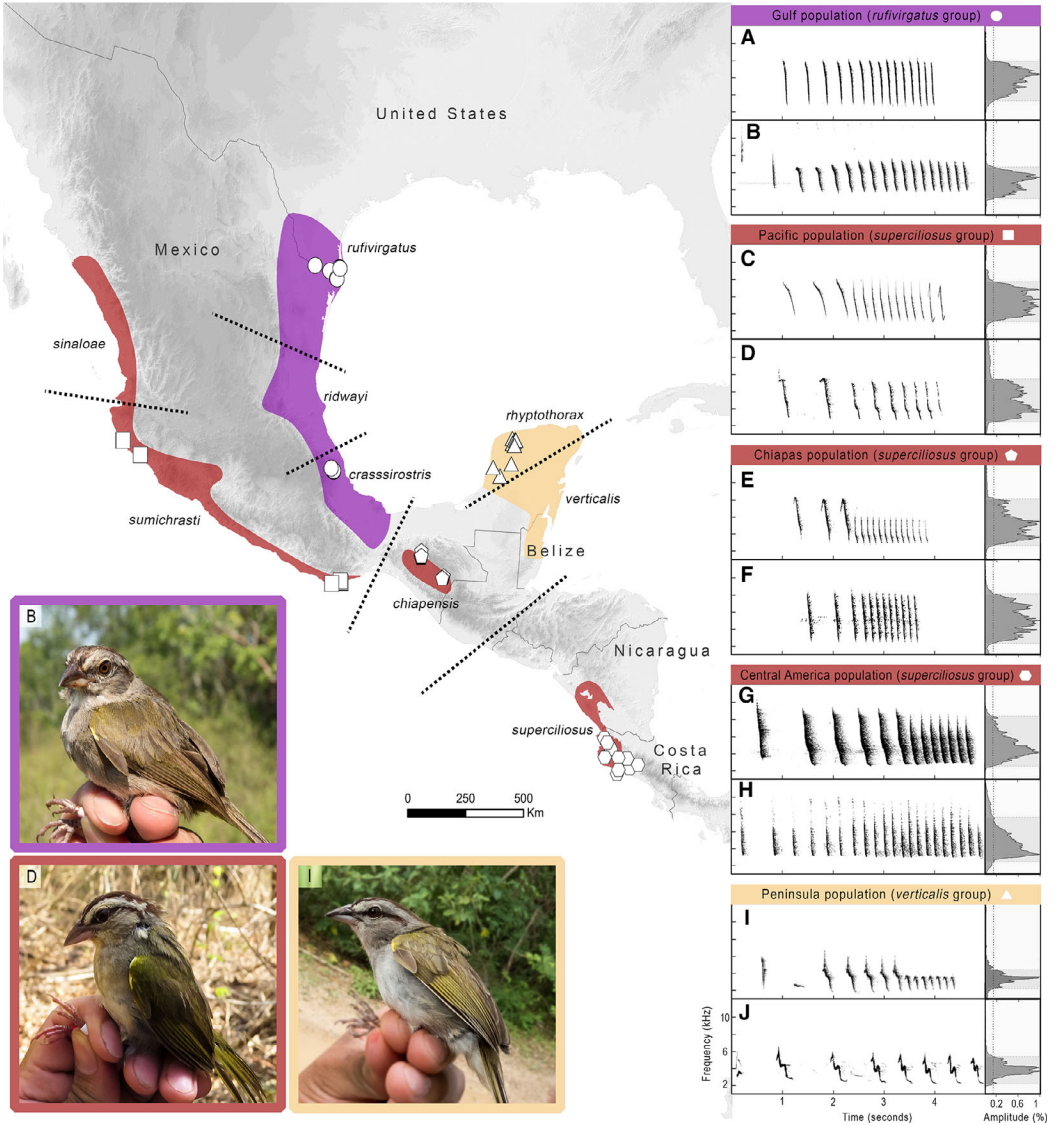


Fig. 1. Map of North and Central America showing the distribution ranges, photographs, and recording localities of Olive Sparrows (*Arremonops rufivirgatus*). Colors represent the three taxonomic groups (del Hoyo et al. 2016), where purple = *rufivirgatus* group, red = *superciliosus* group, and yellow = *verticalis* group. Dotted lines show the approximate geographic boundaries of each subspecies (Ridgely et al. 2012). Symbols indicate allopatric populations, where circles = Gulf, squares = Pacific, pentagons = Chiapas, hexagons = Central America, and triangles = Peninsula. Spectrograms and power spectra depict song exemplars of the five allopatric populations. Letters represent a sampled locality, including (A): Atascosa N.W.R., Texas, U.S.A.; (B): Chavarrillo, Emiliano Zapata, Veracruz, México; (C): Estación de Biología Chamela, San Patricio, Jalisco, México; (D): Cacaluta, Huatulco, Oaxaca, México; (E): Cañón del Sumidero P.N., Tuxtla-Gutiérrez, Chiapas, México; (F): Los Girasoles, Comitán, Chiapas, México; (G): Lomas de Barbudal, R.B., Guanacaste, Costa Rica; (H): Garabito, Puntarenas, Costa Rica; (I): Área reforestación MAPSA, Mérida, Yucatán, México; and (J): Hopelchen, Campeche, México. The 15% threshold amplitude reference is indicated with a dashed line for all power spectra. [Colour figure can be viewed at wileyonlinelibrary.com]

Our objective was to describe the pattern of geographic variation in acoustic traits throughout the geographic distribution of the Olive Sparrow complex. First, we describe geographic variation in the songs of Olive Sparrows and test whether song divergence corresponds to either allopatric populations or the three taxonomic groups proposed by del Hoyo et al. (2016). Second, we evaluate the strength of song divergence within the Olive Sparrow complex by comparing acoustic distances among taxa within the complex to acoustic distances among recognized species in the *Arremonops* genus. Our motivation for this study was to better understand the role that song divergence may play in resolving taxonomic uncertainty over species limits within the Olive Sparrow complex.

METHODS

Locations and acoustic sampling. Although there are no published studies of vocal behavior of Olive Sparrows, our observations in both the field and laboratory suggest that Olive Sparrows do not have repertoires of different song types, but sing the same song type with some variation among renditions (e.g., number of elements; Fig. S1). To sample the widest possible diversity and variation in the songs of Olive Sparrows, we included songs from multiple geographically distant locations whenever possible. To do this, we obtained recordings in the field as well as recordings from libraries of natural sounds. We recorded Olive Sparrows in the field at 10 locations in México from 2016 to 2018, including (1) Chavarrillo, Emiliano Zapata, Veracruz (19.4362°N, 96.8026°W), (2) Estación de Biología Chamele, San Patricio, Jalisco (19.4991°N, 105.0422°W), (3) Cacaluta, Huatulco, Oaxaca (15.7344°N, 96.1602°W), (4) Cañón del Sumidero National Park, Tuxtla Gutiérrez, Chiapas (16.8070°N, 93.0921°W), (5) Rancho Los Girasoles, Comitán, Chiapas (16.1966°N, 92.1490°W), (6) La Fortuna, Tzimol, Chiapas (16.1342°N, 92.1982°W), (7) Fraccionamiento Los Héroes, Mérida, Yucatán (20.9903°N, 89.5394°W), (8) Parque Kai luum, Mérida, Yucatán (21.0437°N, 89.6578°W), (9) Área reforestación MAPSA, Mérida, Yucatán (20.8928°N, 89.6318°W), and (10) Tekik de Regil, Timucuy, Yucatán

(20.8036°N, 89.5616°W). We were not able to obtain recordings of the subspecies *ridgwayi*, *sinaloae*, and *verticalis* because the songs of these subspecies are not represented in sound libraries. However, we do not believe that the absence of these subspecies in our analysis affected our results because our objective was to test for vocal differences among allopatric populations and taxonomic groups.

At each location, we used mist-nets to capture birds and gave them unique combinations of colored leg bands to facilitate identification of individuals. We identified unbanded birds as unique individuals based on observations of territorial behavior and considered the low dispersion of the species (Brush 2005). We assumed that birds in isolated patches of scrub > 100 m apart were different individuals. We recorded from 07:00 to 11:00 and from 16:00 to 18:00 in different territories each day to further minimize the possibility of recording the same individuals. We recorded songs using digital recorders (Tascam DR40 and Marantz PMD661) and shotgun microphones (Audio-technica AT897 and Sennheiser ME67/K6). All songs were recorded in 16-bit WAV format at a sampling rate of 44.1 kHz.

We obtained recordings from four natural sound libraries and private libraries, including the Macaulay Library (Cornell Lab of Ornithology, Ithaca, NY), xeno-canto (<https://www.xeno-canto.org/>), Biblioteca de Sonidos Aves de México (<http://www1.inecol.edu.mx/sonidos/menu.htm>), and a private collection (Sounds of Nature) (information about recordings and libraries can be found in Tables S1 and S2). For analysis, we selected recordings with the highest signal-to-noise ratio. For multiple recordings made at a single location, we chose recordings made on different dates, reducing the possibility of analyzing songs from the same individual as coming from two different birds. In our final recording selection, we included two recordings made at the same location, but on different dates, and two recordings made at the same location on the same date. However, if a recordist's comment regarding the last recordings indicated that there were two different birds, we included both recordings in our analysis.

For the first analysis, we included only Olive Sparrows songs, randomly selecting 2–6

high-quality songs per individual to account for within-individual singing variation (because Olive Sparrows may sing the same song type with some variation among renditions). We obtained 614 songs of 117 individuals ($\bar{x} = 5.3 \pm 0.1$ [SD] songs per bird) from 34 locations (*rufivirgatus* group, $N = 24$ individuals; *superciliosus* group, $N = 75$ individuals; and *verticalis* group, $N = 18$ individuals; see Table S1 for detailed information about the number of songs per individual).

For the second analysis, we included an additional 188 songs belonging to 57 individuals of three additional species in the analysis, encompassing all species in the genus *Arremonops* (Fig. S1). As with Olive Sparrows, the vocal repertoires of congeneric species have not been examined; thus, we used multiple songs per individual. In this analysis, we included the songs of 117 Olive Sparrows used in the first analysis plus the songs of 19 Green-backed Sparrows (*A. chloronotus*; $\bar{x} = 3.1 \pm 1.8$ songs per bird), 24 Black-striped Sparrows (*A. conirostris*; $\bar{x} = 3.6 \pm 1.7$ songs per bird), and 14 Tocuyo Sparrows (*A. tocuyensis*; $\bar{x} = 3.1 \pm 1.2$ songs per bird). We obtained recordings in the field and from two natural sound libraries (Macaulay Library and xeno-canto; Tables S1 and S2). Prior to analysis, all recordings made in mp3 format or at 24 bits were uncompressed (WAV format) and downsampled to 16 bits at 44.1 kHz sample rate, respectively. All recordings were high-pass filtered at 1 kHz and low-pass filtered at 12 kHz to eliminate background noise and normalized to -6 dB using Audacity 2.3.0 (Audacity Team 2018).

Song measures and acoustic analysis. We quantified 12 fine structural characteristics of the songs of Olive Sparrows that described the frequency and temporal traits of songs. We included four measurements based on the spectrum (Araya-Salas and Smith-Vidaurre 2017, Sueur 2018, Araya-Salas et al. 2019), including (1) mean frequency (the average of the frequencies in the signal weighted by their amplitude), (2) standard deviation of the mean frequency, (3) first quartile frequency (based on the power spectrum, the smallest discrete frequency in kHz where the summed energy under the frequency value has to exceed 25% of the total energy), and (4) third quartile frequency (based on the power spectrum, the smallest

discrete frequency in kHz where the summed energy under the frequency value has to exceed 75% of the total energy).

We included an additional eight measurements from spectrograms, including (5) song duration from the beginning of the first element to the end of the last element in a song, (6) number of elements (i.e., with an element defined as a continuous trace on a spectrogram, Catchpole and Slater 2008), (7) element rate (number of elements per second), (8) minimum frequency (lowest frequency in kHz defined by the 15% amplitude threshold in a selection), (9) maximum frequency (highest frequency in kHz defined by the 15% amplitude threshold in a selection), (10) bandwidth (difference between the maximum and minimum frequencies in a song), (11) peak frequency (frequency of maximum energy in a song in kilohertz), and (12) entropy (energy distribution of the frequency across the signal, where broadband sounds with energy randomly distributed across frequencies would tend to have values ~ 1 , whereas tonal sounds with most of the energy concentrated in few frequencies will tend to have values ~ 0).

Most variables (except song duration, number of elements, and element rate) were calculated using the threshold method. The threshold method for signal detection is used to standardize measurements by establishing a threshold that corresponds to a percent value of the amplitude relative to the maximum value of amplitude detected in a signal (Podos 1997, Ríos-Chelén et al. 2017). A threshold value is arbitrary and depends on the quality of a recording (i.e., recordings with a high noise-to-signal ratio will have larger threshold than recordings with poor quality). Thus, for our analysis, a threshold of 15% of the absolute amplitude for signal detection allowed us to include most of the recordings in our study (Sueur 2018; Fig. S2). Measurement extraction was performed using the functions “specan” and “frange” to create spectrograms (1024 samples, with 98% overlap, and Hanning window) in R package “warbleR” (R Core Team 2013, Araya-Salas and Smith-Vidaurre 2017) that uses codes and functions from the packages “seewave” (Sueur et al. 2008), “tuneR” (Ligges et al. 2018), and “monitoR” (Katz et al. 2016).

Statistical analyses. For the first analysis, we reduced the number of variables for

the analysis by conducting a principal component analysis (PCA). We conducted the analysis using the correlation matrix with varimax rotation and extracted component factors with eigenvalues > 1. We eliminated superfluous variables from the analysis by removing variables with $KMO \leq 0.6$, suggested as a minimum requirement for sampling adequacy (Kaiser 1974). We eliminated one variable at a time until we achieved a general $KMO \geq 0.7$ (i.e., calculated for all variables combined). Using this technique, we eliminated two of the 12 variables. The PCA assumptions were met, with a positive correlation determinant ($9.5E-7$), acceptable sampling adequacy (KMO test = 0.74), and sphericity indicating strong correlations among variables (Bartlett's test $\chi^2_{45} = 8443.9$, $P < 0.001$). The principal component analysis of the acoustic variables produced three principal components that together explained 88.06% of total variation in the 10 acoustic variables (Table 1). The first component explained 57.27% of the total variation and was strongly associated with the standard deviation of the mean frequency, third quartile frequency, highest frequency, bandwidth, and entropy. The second component explained 15.72% of the total variation and was strongly associated with mean frequency, first quartile frequency, and peak frequency. The third component explained 15.07% of the total variation and was strongly associated with song duration and number of elements.

We conducted our analysis at two levels: (1) whether song variation differed among the three taxonomic groups and (2) among allopatric populations. We conducted a linear mixed model (LMM) analysis per component (for a total of three analyses). Each principal component was included as a dependent variable, the three taxonomic groups (i.e., *rufivirgatus*, *superciliosus*, and *verticalis*) or the five allopatric populations (i.e., Gulf, Pacific, Chirapas, Central America, and Peninsula) as fixed factors, and subjects (individual identity) as random effects, allowing us to include multiple songs per individual in the analysis (Garamszegi 2016, Gratton and Mundry 2019). Because we included songs recorded in different years from natural sound libraries, we included recording year as a covariate in our analyses to rule out possible effects of recording year. We found no significant effect

Table 1. Loadings of the first three principal components summarizing 10 acoustic variables measured in songs of Olive Sparrows. Eigenvalues and the percentage of variation explained are presented for each component.

	Components		
	First	Second	Third
Eigenvalues	5.73	1.57	1.51
Variance explained (%)	57.27	15.72	15.07
Mean frequency (kHz) ^a	0.62	0.75	−0.11
SD mean frequency ^b	0.91	−0.10	−0.18
First quartile frequency (kHz) ^b	0.23	0.92	−0.11
Third quartile frequency (kHz) ^a	0.73	0.61	−0.09
Song length (s) ^b	−0.07	−0.12	0.92
Number of elements ^c	−0.17	−0.10	0.89
Maximum frequency (kHz) ^a	0.76	0.50	−0.25
Bandwidth (kHz) ^c	0.81	0.37	−0.28
Peak frequency (kHz) ^b	−0.07	0.87	−0.11
Entropy ^b	0.95	0.15	0.08

Variables with the strongest loading are shown in bold. Principal component analysis was based on the correlation matrix and varimax rotation. Components with eigenvalues > 1 were extracted.

^aThese variables had a normal distribution and were not transformed.

^bThese variables were log-transformed.

^cThese variables were square root-transformed.

of year of recording for any of the three principal components (PC1: $F_{1,111.6} = 0.1$, $P = 0.72$; PC2: $F_{1,110.3} = 3.2$, $P = 0.07$; PC3: $F_{1,109.5} = 0.8$, $P = 0.36$). Thus, we report all LMM results without recording year as a covariant. We computed a Bonferroni post hoc test to test pairwise differences within each level (i.e., taxonomic groups and allopatric populations).

We then tested for isolation-by-distance with a Mantel test. First, we generated both an acoustic distance matrix and a geographic distance matrix. The acoustic distance matrix was created by averaging the principal component scores by individual. We then computed the distances among individuals by calculating the Euclidian distances at each of the three principal components. The geographic distance matrix was calculated using the geographic distances among individual territories in kilometers using the software

Geographic Distance Matrix Generator (ver 1.2.3; Ersts 2012).

In the second analysis, we explored the extent of vocal divergence among the three taxonomic groups and among the five allopatric populations. To do this, we assessed divergence between groups and between allopatric populations. We then compared them to the divergence among recognized species in the genus *Arremonops*. First, we computed a new principal component analysis using the 12 acoustic parameters extracted from songs of individuals in the Olive Sparrow complex and the three additional *Arremonops* species, averaging all measurements per individual ($N = 174$). The principal component analysis was conducted using the correlation matrix and varimax rotation. We extracted components with eigenvalues > 1 . We followed the same procedure to eliminate superfluous variables from the analysis explained above, and we eliminated four of the 12 variables. The PCA assumptions were met, with a positive correlation determinant ($5.8E-8$), acceptable sampling adequacy (KMO test = 0.77), and sphericity indicated strong correlations among variables (Bartlett's test $\chi^2_{28} = 2823.8$, $P < 0.001$). The analysis resulted in two principal components that together explained 93.23% of total variation in the eight acoustic variables (Table 2). The first component explained 79.06% of total variation and was strongly associated with mean frequency, first and third quartile frequency, high frequency, bandwidth, peak frequency, and entropy. The second component explained 14.17% of total variation and was strongly associated with the standard deviation of the mean frequency.

We estimated the acoustic divergence scores as the pairwise distance between different taxa of *Arremonops* sparrows for each principal component. We used Hedges' g to calculate the pairwise distance between taxon means, accounting for within-taxon variance and small sample sizes (Hedges and Olkin 1985). Hedges' g is interpreted in a way similar to Cohens' d values where 0.2 represents a small effect size, a Hedges' $g = 0.5$ represents a medium effect size, a Hedges' $g = 0.8$ represents a large effect size, and Hedges' $g = 1.2$ or 2 represent very large and huge effects, respectively. In other words, if the means of two groups do not differ by 0.2 standard

Table 2. Loadings of the first two principal components summarizing eight acoustic variables measured for the songs of three groups in the Olive Sparrow complex and three additional species of *Arremonops* sparrows, including Green-backed Sparrows, Black-striped Sparrows, and Tocuyo Sparrows. Eigenvalues and the percentage of variation explained are presented for each component.

	Components	
	First	Second
Eigenvalues	6.32	1.13
Variance explained (%)	79.07	14.17
Mean frequency (kHz) ^a	0.98	-0.09
SD mean frequency ^b	0.63	0.73
First quartile frequency (kHz) ^b	0.89	-0.39
Third quartile frequency (kHz) ^a	0.98	0.00
Maximum frequency (kHz) ^a	0.97	-0.03
Bandwidth (kHz) ^c	0.94	0.07
Peak frequency (kHz) ^a	0.79	-0.52
Entropy ^b	0.87	0.41

Variables with the strongest loadings are shown in bold. Principal component analysis was based on the correlation matrix and varimax rotation. Components with eigenvalues > 1 were extracted.

^aThese variables had a normal distribution and were not transformed.

^bThese variables were log-transformed.

^cThese variables were square root-transformed.

deviations or more, the difference is trivial, even if statistically significant (Hedges and Olkin 1985, Cohen 1992). Similar approaches have allowed estimates of the divergence among taxa using effect size estimators like Cohen's d (Toews and Irwin 2008, Sosa-López and Mennill 2014). We estimated the pairwise divergence scores between: (1) the three groups in the Olive Sparrow complex (e.g., *rufivirgatus* vs. *superciliosus*), (2) the five allopatric populations (e.g., Gulf vs. Pacific), and (3) the three additional recognized *Arremonops* species (e.g., Green-backed Sparrows vs. Black-striped Sparrows). Prior to analysis, we normalized non-normally distributed variables using log and square root transformations to reduce the effect of outliers (Whitlock and Schluter 2009). Statistical analyses were conducted in Statistica 10.0 (StatSoft 2011) and R (R Core Team 2013) using the packages lme4 and ade4 (Dray and Dufour 2007, Bates et al. 2015).

RESULTS

Geographic variation. Songs of Olive Sparrows varied considerably throughout their range. Results of the LMM showed significant differences in the songs for all three principal components among taxonomic groups ($F = 13.3\text{--}36.2$, $P < 0.001$; Table 3, Fig. 2A, B, C), and among allopatric populations ($F = 11.2\text{--}26.3$, $P < 0.001$; Table 3, Fig. 2D, E, F).

At the taxonomic group level, the post hoc test showed that songs differed significantly among the three taxonomic groups in all three components (Table S3). For the first component, the *superciliosus* group had the highest value scores, the *rufivirgatus* group had intermediate values, and the *verticalis* group had the lowest values (Fig. 2A). This indicates that the *superciliosus* group had the songs with the greatest variation in mean frequency (i.e., standard deviation of mean frequency), highest third quartile and maximum frequencies, the broadest bandwidth, and more randomly distributed frequencies (i.e., entropy), whereas the *verticalis* group had songs with the lowest frequencies, narrowest bandwidth, and less randomly distributed frequencies. For the second component, the *rufivirgatus* group had the highest value scores, the *superciliosus* group had intermediate values, and the *verticalis* group had the lowest values (Fig. 2B). This indicates that the *rufivirgatus* group had songs with energy concentrated at higher frequencies (i.e., mean and peak frequency) and the lowest minimum frequencies (i.e., first quartile frequencies), whereas the *verticalis* group had songs with energy concentrated in lower frequencies. For the third component, the *rufivirgatus* group

had the highest value scores, and the *superciliosus* group had the lowest values (Fig. 2C). This indicates that the *rufivirgatus* group had the longest and fastest songs, and the *superciliosus* group had the shortest songs with the slowest element rate.

At the allopatric population level, the LMM showed that songs differed significantly in all three components among populations in the *superciliosus* group (Table 3). A post hoc test revealed that the Peninsula allopatric populations were significantly different from the rest of the populations in the first component (Fig. 2D), whereas, for the third component, the Chiapas population was significantly different from all the others (Fig. 2F). All allopatric populations in the *superciliosus* group only differed significantly in the third component (Fig. 2F). For the first component, the Central America population had the highest value scores with the greatest variation in mean frequency (i.e., standard deviation of mean frequency), highest maximum frequencies, the broadest bandwidth, and more randomly distributed frequencies (i.e., entropy), whereas the Peninsula population had the lowest value scores with the lowest frequencies, narrowest bandwidth, and less randomly distributed frequencies. For the third component, the Central America population had the highest value scores with the longest and fastest songs, whereas the Chiapas population had the lowest values (Fig. 2F).

The Mantel test revealed a significant relationship for the third component (PC3: $r = 0.09$, $P = 0.016$), a tendency for the first component (PC1: $r = 0.08$, $P = 0.05$), and no relationship for the second component (PC2: $r = 0.03$, $P = 0.17$). These analyses suggest that the third component (associated with song

Table 3. Linear mixed models results of the first three principal components summarizing 10 acoustic variables measured for the songs among taxonomic groups and allopatric populations within the Olive Sparrow complex.

Components	Model	SS	MS	<i>F</i> (df)	<i>P</i>
First	Taxonomic groups	19.27	9.64	36.2 (2, 113.58)	< 0.001
	Allopatric populations	25.23	6.31	23.7 (4, 112.90)	< 0.001
Second	Taxonomic groups	12.09	6.05	19.9 (2, 112.38)	< 0.001
	Allopatric populations	13.61	3.40	11.2 (4, 111.78)	< 0.001
Third	Taxonomic groups	7.91	3.95	13.3 (2, 111.97)	< 0.001
	Allopatric populations	31.19	7.79	26.3 (4, 110.77)	< 0.001

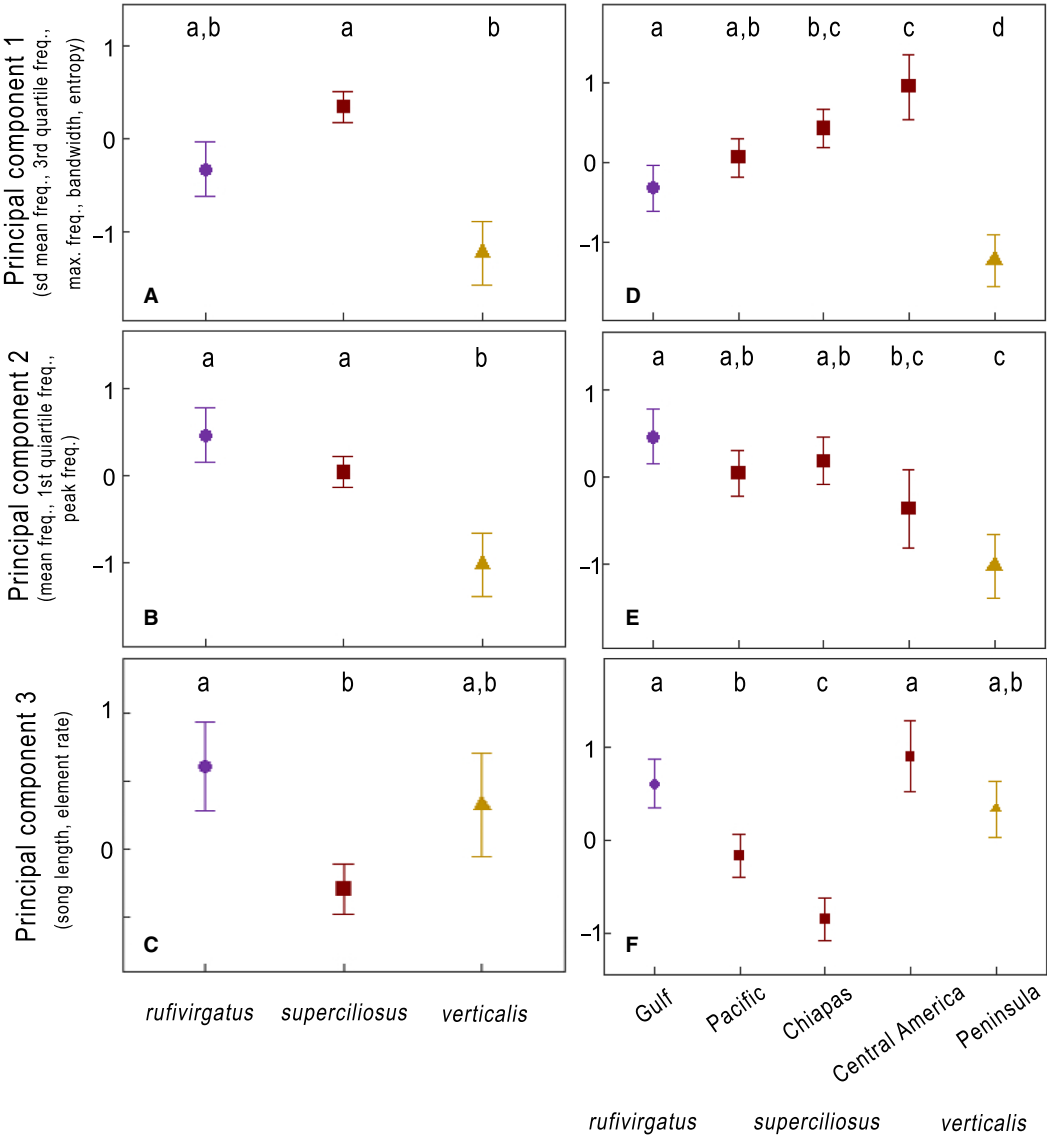


Fig. 2. Plots depicting the mean values of the three principal component scores summarizing spectral and temporal variables in the songs of Olive Sparrows among groups (A–C) and among allopatric populations (D–F). Error bars show 95% confidence intervals. Letters above error bars show results of Bonferroni post hoc tests, with different letters indicating significant differences between groups. [Colour figure can be viewed at wileyonlinelibrary.com]

duration and number of elements in songs) tended to increase with distance, with a similar tendency for the first component (associated with standard deviation of mean frequency, third quartile frequency, maximum frequency, bandwidth, and entropy).

Acoustic divergence. We estimated acoustic divergence scores as the pairwise

distance between different taxa of *Arremonops* sparrows for each principal component. For all two components, most pairwise comparisons had Hedges' *g* values > 0.2 (only one allopatric population pairwise comparison had a value < 0.2; Table 4, Fig. 3A). For Olive Sparrows at the taxonomic group level, the average Hedges' *g* value was 1.30 for PC1

Table 4. Acoustic divergence pairwise scores for taxa in the Olive Sparrow complex and recognized *Arremonops* species.

Pairwise comparisons	Components	
	First	Second
Other recognized species of <i>Arremonops</i>		
GBSP vs. BSSP	2.11	0.81
GBSP vs. TOSP	1.97	1.29
BSSP vs. TOSP	3.48	0.22
Taxonomic groups		
<i>rufivirgatus</i> vs. <i>superciliosus</i>	0.38	1.27
<i>rufivirgatus</i> vs. <i>verticalis</i>	2.05	0.50
<i>superciliosus</i> vs. <i>verticalis</i>	1.48	1.61
Allopatric populations		
Gulf vs. Pacific	0.46	1.05
Gulf vs. Chiapas	0.10	1.49
Gulf vs. Central America	1.04	2.10
Gulf vs. Peninsula	2.05	1.49
Pacific vs. Chiapas	0.30	0.59
Pacific vs. Central America	0.72	1.34
Pacific vs. Peninsula	1.85	1.30
Chiapas vs. Central America	0.81	0.69
Chiapas vs. Peninsula	1.66	1.62
Central America vs. Peninsula	1.05	1.88

Pairwise comparisons are provided for three recognized species of *Arremonops* sparrows (GBSP: Green-backed Sparrow, *A. chloronotus*; BSSP: Black-striped Sparrow, *Arremonops A. conirostris*; and TOSP: Tocuyo Sparrow, *A. tocuyensis*), between taxa in the Olive Sparrow complex, and between allopatric populations (Gulf, Pacific, Chiapas, Central America, and Peninsula). Hedges' *g* scores were calculated as pairwise distances between component factor scores summarizing the variation in eight acoustic measurements.

and 1.13 for PC2, whereas, at the allopatric population level, the average Hedges' *g* value was 1.00 for PC1 and 1.35 for PC2.

At the taxonomic group level, the results of pairwise comparisons suggest that, for the first component, the divergence between *rufivirgatus* vs. *verticalis* was larger than the smallest divergence between any pairs of recognized species of *Arremonops* (Fig. 3A). For the second component, divergence between all pairwise taxonomic groups was larger than the smallest divergence between any pairs of recognized species of *Arremonops* (Fig. 3B). These results suggest that the acoustic divergence between some recognized taxonomic groups of Olive Sparrows is similar to or

larger than the magnitude of divergence between recognized *Arremonops* species (Table 4).

At the allopatric population level, our results suggest that, for the first component, the divergence between one pairwise allopatric population comparisons (i.e., Gulf vs. Peninsula) was greater than the smallest divergence between pairs of recognized species of *Arremonops* (Fig. 3A). For the second component, divergence between all 10 allopatric population comparisons was greater than the smallest divergence between pairs of recognized species of *Arremonops* (Fig. 3B). These results suggest that the divergence between some allopatric populations of Olive Sparrows is similar to or greater than the magnitude of divergence between pairs of recognized *Arremonops* species (Table 4).

DISCUSSION

Our analysis revealed substantial vocal variation throughout the geographic distribution of Olive Sparrows. We found significant differences in at least two of the three principal components at both geographical levels. Furthermore, our divergence analysis revealed that the vocal pairwise differences at both levels, in at least one of the principal components, were on the same order as or greater than the vocal divergence among pairs of recognized species in the genus *Arremonops*.

We found divergence in the structural characteristics of songs, despite previous suggestions of either acoustic similarity (Howell and Webb 1995) or moderate song distinctiveness between groups of Olive Sparrows (Boesman 2016). Our analysis also revealed evidence of significant vocal variation among allopatric populations. Specifically, we found significant acoustic differences among three allopatric populations in the *superciliosus* group (Pacific, Chiapas, and Central America). Divergence in acoustic traits has been found to occur widely among allopatric populations (e.g., Toews and Irwin 2008, Cadena and Cuervo 2010, Chaves et al. 2010, Sosa-López et al. 2013, Sosa-López and Mennill 2014, Ortiz-Ramírez et al. 2016, Sandoval et al. 2017). For example, geographic isolation of Wedge-tailed Sabrewings (*Campylopterus curvipennis*) driven by paleoclimatic historical events might explain the vocal, genetic, and morphological

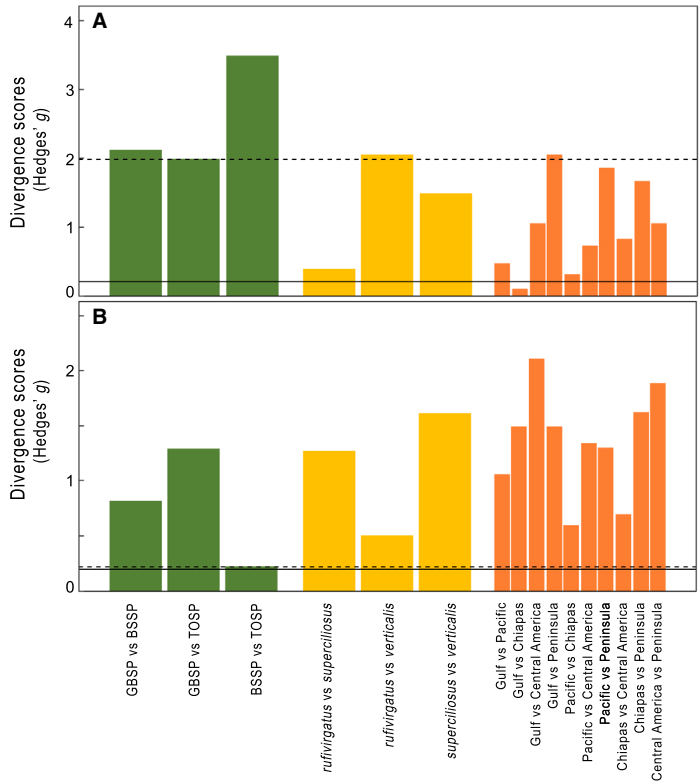


Fig. 3. Barplots showing divergent Hedges' g values among pairwise comparisons of *Arremonops* taxa by principal component: (A) first component and (B) second component. The three bars on the left show pairwise distances among recognized *Arremonops* species, including Green-backed Sparrows (*Arremonops chloronotus*, GBSP), Black-striped Sparrows (*Arremonops conirostris*, BSSP), and Tocuyo Sparrows (*Arremonops tocuyensis*, TOSP). Bars in the middle show pairwise distances among the three taxonomic groups (*rufivirgatus*, *superciliosus*, and *verticalis*) according to del Hoyo et al. (2016). Narrow bars on the right show pairwise distances among the five allopatric populations (Gulf, Pacific, Chiapas, Central America, and Peninsula). Dashed lines indicate the lowest divergence score between pairs of recognized *Arremonops* species for each component. Solid lines indicate a value of Hedges $g = 0.2$. Means of two groups with a $g < 0.2$ indicate that the difference is trivial, regardless of whether differences are statistically significant (Hedges and Olkin 1985). [Colour figure can be viewed at wileyonlinelibrary.com]

differentiation exhibited by allopatric populations (González et al. 2011). For Nightingale-Thrushes (*Catharus frantzii*), isolation events explain similar patterns of geographic variation in both vocal and genetic traits within the complex (Ortiz-Ramírez et al. 2016). These studies suggest that isolation in tandem with selective (e.g., natural selection and sexual selection) and/or non-selective (e.g., genetic drift and cultural drift) evolutionary forces may explain such variation in songs across geographic ranges. In support of the hypothesis that isolation is an important factor in the origin of acoustic diversity in bird song, our results revealed no significant

differences among sampled localities within allopatric populations, whereas allopatric populations were significantly different. Multiple factors drive song evolution. For instance, Tobias et al. (2010) suggested that habitat structure promotes song divergence, particularly in frequency-related traits, and has contributed to reproductive isolation in an Amazonian bird community. Variation in morphological traits could also explain patterns of acoustic variation. Among Darwin's finches, beak morphology shapes the fine structural characteristic of songs, affecting traits such as frequency and note rate (Podos 2001, Podos and Nowicki 2004, Tobias et al.

2010). Our analysis revealed marked variation in frequency (as described by the first and second component, Fig. 2D, 2) and song rate-related traits (as described by the third component, Fig. 2F) among allopatric populations of Olive Sparrows, suggesting that, as reported in other species of songbirds (Matessi et al. 2000, Podos 2001, Derryberry et al. 2012), habitat and morphology could be affecting song characteristics. However, our field observations suggest that the populations included in our study are in areas with similar habitat conditions (deciduous and semideciduous forest with thorny shrubs; Howell and Webb 1995), and there are no apparent differences in morphological traits. Future analyses incorporating morphological and habitat characteristics, as well as genetic analysis, are needed to understand the factors contributing to the evolution of song characteristics in the Olive Sparrow complex.

Our isolation-by-distance analysis suggests a relationship between acoustic divergence and geographic distance, with patterns of variation such as isolation-by-distance (where random changes in vocal traits could increase and accumulate linearly with distance as result of learning processes; Koetz et al. 2007) and the presence of regional song dialects. However, future studies should focus on microgeographic variation in the songs in each allopatric population, with detailed and continuous sampling of songs throughout their distribution.

Remsen (2005) suggested that allopatric populations should be treated as species under the Biological Species Concept if the degree of divergence is similar to or greater than that of taxa known to be reproductively isolated (Isler et al. 1998, Helbig et al. 2002). Although we lack information about whether the three taxonomic groups or the five allopatric populations are reproductively isolated, our results suggest that the extent of acoustic divergence in the songs among groups and allopatric populations of Olive Sparrows is similar to or greater than the differences between recognized *Arremonops* species. Thus, the marked vocal differences between groups and allopatric populations in our study suggest that birds could likely discriminate between songs from different populations, acting as a reproductive isolation barrier between populations (Matens 1996, Wilkins et al. 2013). However, we acknowledge the

small sample sizes of our congeneric samples and recommend caution in interpreting this result. Future genetic studies would help determine how divergent these taxa are, and experimental playback studies could be used to determine whether song divergence is perceived by individuals as a biologically relevant trait for species recognition. In conclusion, we found significant differences in the songs of Olive Sparrows across their geographic distribution. We argue that such variation could be the result of isolation working in tandem with selective and/or non-selective forces. Further, by comparing song differences in the Olive Sparrow complex to differences in songs among pairs of recognized species in the genus *Arremonops*, our study adds to the long-standing taxonomic debate and provides new insights concerning the origins of vocal diversity in the Olive Sparrow complex.

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SUPPORTING INFORMATION

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

Table S1. Recording localities for Olive Sparrows, Green-backed Sparrows, Black-striped Sparrows, and Tocuyo Sparrows showing the source of recordings, the taxonomic group, the identity of the allopatric population (for Olive Sparrows only), and the geographic coordinates. The total number songs included in the analyses are shown.

Table S2. Recordings used from libraries and private collections for the acoustic analysis of the Olive Sparrow complex and three other *Arremonops* species. Numbers refer to catalogue identity of each recording file in the analysis.

Table S3. Results of Bonferroni post hoc test for pairwise comparisons between groups

in the Olive Sparrow complex and between allopatric populations, showing the principal components with significant differences.

Fig. S1. Spectrograms showing within song variation in eight individuals of the different populations. We depict exemplars for allopatric populations of Olive Sparrows (A-E) and exemplar for the three species of the *Arremonops* (F-H) genus used in the acoustic divergence analysis. Each row shows three songs sang for a single individual: (A) Gulf population (RON_170340, RON_170341; Chavarrillo, Emiliano Zapata, Veracruz, Mexico), (B) Pacific population (RON_170131, RON_170353; Chamela, E.B., La Huerta, Jalisco, Mexico), (C) Chiapas population (RON_170259, RON_170260; Cañon del Sumidero, P.N., Tuxtla Gutiérrez, Chiapas, Mexico), (D) Central America population (ML_209299; Lomas de Barbudal, R.B., Liberia, Guanacaste, Costa Rica), (E) Peninsula population (RON_180213; MAPSA,

área de recuperación; Mérida; Yucatán; Mexico), (F) Green-backed Sparrow (XC219088; Bonampak, Chiapas, Mexico), (G) Black-striped Sparrow (XC273704; Mana Dulce, Cundinamarca, Colombia), and (H) Tocuyo Sparrow (XC353183; Rioacha, La Guajira, Colombia). Intra-individual variation shows changes in the number of elements or subtle variation in bandwidth of the song o sections.

Fig. S2. Spectrogram (A) and power spectrum (B) of an Olive Sparrow song generated with “frange” function in “warbleR,” showing some of the acoustic variables analyzed in our study. The power spectrum shows the distribution of amplitude as an average calculated over the entire signal. The dashed line marks a threshold of 15% of the amplitude used to identify the minimum and maximum point of the signal.