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Biology of Tropical Birds

Geographic variation of the song of the Belding's Yellowthroat *Geothlypis beldingi* in the Baja California Peninsula, Mexico

Variación geográfica del canto de *Geothlypis beldingi* en la península de Baja California, México

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ABSTRACT. Acoustic communication is essential for exchanging information between individuals of the same species. In birds, song plays a vital role in mate attraction and territory defense, and when it varies between populations, it can create barriers to gene flow and promote speciation. The Belding's Yellowthroat (*Geothlypis beldingi*) is an endemic bird of the Mexican state of Baja California Sur and is endangered because of declining populations. Its populations are small and highly fragmented, but knowledge of the effects of fragmentation on the ecology and behavior of this species, especially its songs, remains limited. Five allopatric populations along its distribution were studied to assess their acoustic geographic variation. Six acoustic variables from 31 individual songs were analyzed using two supervised classification and multivariate techniques. Our results revealed significant differences between the northern and southern populations, which are separated by approximately 350 km, but the relationship between geographic distance and song variation is limited and could be influenced by different factors. Our results suggest that population-level acoustic differentiation may be associated with the "archipelago-like" distribution of oases. This association with fragmentation should be corroborated by more phenotypic characters and genetic information to determine if there are two significant evolutionary units within this species or lineages at the species level.

RESUMEN. La comunicación acústica es esencial para el intercambio de información entre individuos de la misma especie. En las aves, el canto cumple un papel fundamental en la atracción de pareja y la defensa del territorio, y cuando varía entre poblaciones, puede generar barreras al flujo génico y promover la especiación. *Geothlypis beldingi* es una especie endémica del estado mexicano de Baja California Sur que se encuentra en peligro debido a la disminución de sus poblaciones. Sus poblaciones son pequeñas y altamente fragmentadas, pero el conocimiento de los efectos de la fragmentación sobre la ecología y el comportamiento de esta especie, especialmente en sus cantos, sigue siendo limitado. Se estudiaron cinco poblaciones alopátricas a lo largo de su distribución para evaluar la variación geográfica acústica. Se analizaron seis variables acústicas a partir de 31 cantos individuales utilizando dos técnicas de clasificación supervisada y análisis multivariados. Nuestros resultados revelaron diferencias significativas entre las poblaciones del norte y del sur, las cuales se encuentran separadas por aproximadamente 350 km; sin embargo, la relación entre la distancia geográfica y la variación del canto es limitada y podría estar influenciada por diferentes factores. Nuestros resultados sugieren que la diferenciación acústica a nivel poblacional podría estar asociada con la distribución de tipo "archipiélago" de los oasis. Esta asociación con la fragmentación debería ser corroborada mediante otros caracteres fenotípicos e información genética para determinar si existen dos unidades evolutivas significativas dentro de esta especie o linajes a nivel de especie.

Key Words: *conservation; differentiation; isolation; Parulidae; speciation*

INTRODUCTION

Acoustic communication is essential for birds, and song plays a fundamental role in both mate attraction and territory defense. Song also conveys information about individual identity and, in some cases, the age and sex of the signaler. In addition, song function and information content may vary between sexes and may vary among populations (Marler and Tamura 1962, Catchpole and Slater 2008). Geographic variation in song can arise from several interacting factors, including differences in habitat structure (Morton 1975), climatic conditions (Slabbekoorn and Smith 2002, Tubaro and Lijtmaer 2006, Patten and Pruett 2009), and geographic isolation among populations (Baker 1996, Lachlan et al. 2018). In the case of learned songs, cultural divergence allows vocalizations to accumulate differences between isolated populations through drift or localized learning traditions (Podos and Warren 2007). Over time, such divergence may hinder recognition between individuals from different populations, generating a reproductive barrier that can eventually lead to speciation (Grant and Grant 1996, Lachlan and Servedio 2004). Thus, song variation not only reflects adaptations to the environment but may also act as a direct driver of evolutionary processes, particularly in species with vocal learning and limited dispersal capacity (Price 2008, Lachlan et al. 2018).

Studying geographic variation in a species' song provides valuable information about its evolutionary trajectory (Slabbekoorn and Smith 2002, Podos and Warren 2007). Several studies show that geographically isolated populations may develop distinctive acoustic traits, and such changes can influence species recognition, even when genetic differentiation is low (Freeman

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and Montgomery 2017). For example, there is marked geographic variation in song structure among Hermit Thrush (*Catharus guttatus*) populations, which is associated with isolation, habitat differences, and cultural drift (Roach and Phillmore 2017). Likewise, evidence suggests that habitat selection and acoustic adaptation can contribute to the evolution of song differences and reproductive isolation when communication is shaped by local selective pressures (Patten et al. 2004). These cases demonstrate that the interplay among fragmentation, limited dispersal, and cultural transmission can have a substantial influence on the evolution of avian vocalizations.

Processes promoting vocal divergence are particularly relevant for species inhabiting naturally fragmented environments, such as the oases of the Baja California Peninsula. These oases function as small ecological islands, characterized by permanent water bodies and dense vegetation embedded within a desert matrix. This strong habitat discontinuity and limited connectivity can promote population differentiation (Grismer and McGuire 1993, Maya et al. 1997). Because dispersal among oases is likely restricted, populations may diverge behaviorally, morphologically, or acoustically in archipelagos or other fragmented systems. Within this context lies the Belding's Yellowthroat (*Geothlypis beldingi*), an endemic species classified as endangered (SEMARNAT 2010) and listed as Vulnerable by the IUCN Red List (IUCN 2021). Currently, two subspecies are recognized: *G. b. goldmani*, in the northern region of Baja California Sur, and *G. b. beldingi* in the south (Oberholser 1917). The most recent estimates of the species' total population are tentative. Still, the population estimates range between 650 and 1670 mature individuals (BirdLife International 2021), mainly distributed in San José del Cabo (SJ), San Ignacio (SI), La Purísima (PU), and to a lesser extent Comondú (CO) and San Bartolo (SB; Carmona et al. 2020, 2025). These abbreviations are used throughout the manuscript to refer to each population.

Despite its conservation importance, the biology and ecology of this species remain poorly understood, and even less is known about its vocal behavior. A previous study conducted in three oases (SI, CO, and SB) described aspects of the vocal structure of four individuals, focusing on the number and type of notes (Córdova-Rojas 2021). The song of male Belding's Yellowthroats is a relatively simple structure composed of a short phrase with several clear and modulated notes, which is consistent with the general pattern reported for other *Geothlypis* species, whose songs are typically brief, repetitive, and characterized by small repertoires. Females only make calls; they do not sing. Variation was observed among the three study populations, with SB showing the least similarity with the other two (SI and CO; Córdova-Rojas 2021). This preliminary evidence showed that the species' song exhibits detectable acoustic differentiation among populations, although it remains to be tested whether this divergence is due to the fragmented distribution of its habitat, to potentially short-distance cultural transmission, operating mainly within oases or among geographically close male populations.

The objective of this study was to analyze geographic variation in spectro-temporal characteristics of song across five populations. Regarding geographic differentiation and isolation-by-distance in the Belding's Yellowthroat song, we predict that geographic distance affects song dissimilarities among individuals from different oases. We also expect differences in the temporal and frequency structure of songs among oases, which we tested using both multivariate and univariate techniques at the oasis level and between northern and southern populations.

MATERIALS AND METHODS

Study area

Five oases in Baja California Sur were selected based on the requirements of the Belding's Yellowthroat, such as the presence of reed grass and cattail vegetation. The oasis habitat is characterized by mesic vegetation associated with permanent bodies of water, where palm groves (*Washingtonia robusta* and *Phoenix dactylifera*), reed grass (*Phragmites communis*), and cattail (*Typha dominguensis*) predominate, as well as riparian vegetation. Oases vary in area and vegetation composition. SI, PU, and CO oases are in the north of the region, while SB and SJ oases are situated in the south (Fig. 1). In SJ (1.4 km²), SB (0.59 km²), and SI (2.69 km²) sites, dense patches of reed grass predominate with reduced areas of cattail. In contrast, in PU (2.25 km²) and CO (1.49 km²), extensive reed grass patches are found adjacent to fruit orchards. The northern oases are separated by distances ranging from ~30 km (between PU and CO) to 147 km (between SI and PU), while in the southern oases, the distance is ~78 km (between SB and SJ). The separation between regions is considerable, with ~325 km (between CO and SB) and up to ~570 km between the most distant oases (SI and SJ).

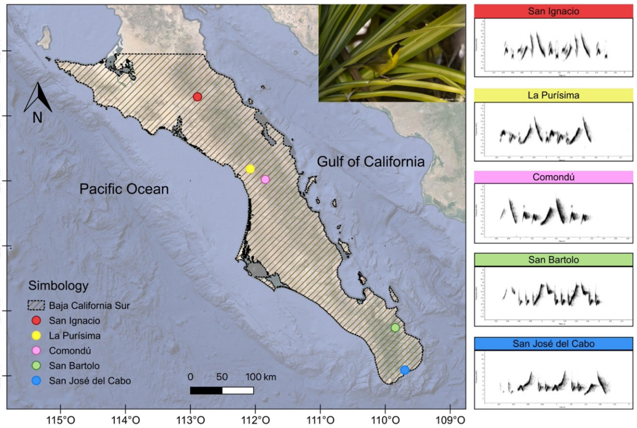
Song recording

Digital records of *G. beldingi* were obtained between April and May 2020, 2023, and 2024. The same person, IBCR, recorded all individuals. A Marantz model PMD620 (44.1 kHz, 16-bit) digital recorder, paired with a Sennheiser ME67 microphone, were used. The recording period was from 04:30 to 05:30 am (approximately 30 minutes to an hour before sunrise), as birds reach their peak vocal activity during this time, which decreases considerably after sunrise.

Recording analysis

For all birds, repertoire size was assessed as the number of distinct song types recorded. Most males produced two song types; however, for the present analysis only the most frequent song type per individual was considered, as the other type was emitted sporadically and in smaller numbers. Of the 155 songs we recorded, 31 belonged to individuals from the five populations (SI = 6, PU = 7, CO = 6, SB = 6, SJ = 6 individuals), with five songs recorded per individual. From all these songs, five from each population, those with the best quality, were selected for analysis. Individual birds were identified by their locations within territories, and persistent territorial song helped distinguish each bird. We selected records from five individuals from each population with the best quality, with minimal urban and ambient noise, and minimal overlap with other songs. Noise reduction was applied to the recordings, followed by low-pass and high-pass filtering to preserve the signal of interest. Finally, the recordings were normalized to -1 dB using Audacity 2.3.3 software. Once processed, the songs were analyzed with the Raven Pro 1.6 Software (Cornell Lab of Ornithology, Ithaca, NY, USA) in two ways: the temporal parameter (song duration) was measured directly in the waveform (Fig. 2b). For this, the visible noise below

Fig. 1. Sampling sites of Belding's Yellowthroat (*Geothlypis beldingi*) in Baja California Sur, Mexico, grouped into northern (San Ignacio, La Purísima, Comondú) and southern (San Bartolo, San José del Cabo) regions. Representative song spectrograms for each locality are shown (Hanning window, 90% overlap, 512 samples DFT).



the song was selected in the spectrogram and removed using the Filter Out Active Selection function (Ríos-Chelén et al. 2016). The threshold method was used for frequency measurements (Podos 1997, 2001, Ríos-Chelén et al. 2016), employing the power spectrum. This method is used to detect signals in a recording by standardizing measurements. A threshold of 15 dB was established, corresponding to a percentage of the maximum amplitude value detected in the signal. This threshold defined the parts of the signal suitable for analysis (Ríos-Chelén et al. 2016, Fernández-Gómez et al. 2020). The threshold value is arbitrary and depends on the quality of the recordings. Therefore, the minimum and maximum frequencies were defined as frequencies corresponding to the intersections with the left and right sides of the power spectrum curve (Fig. 2c), respectively, relative to the peak frequency (Ríos-Chelén et al. 2016). The parameters we measured for each song were the maximum frequency (Hz), minimum frequency (Hz), bandwidth (Hz), peak frequency (Hz), number of notes in the song, and duration of the song(s) (Hann window with 90% overlap, 11.6 ms resolution, and a 512 samples DFT), which represents a good compromise between frequency and duration resolution in a species with songs that include many short, quickly repeated notes that need to be identified.

Statistical analysis

Two supervised classification methods were used to determine whether the six selected acoustic variables could correctly classify *G. beldingi* populations: linear discriminant analysis (LDA) and random forest (RF) analysis. Additionally, a three-factor nested analysis of variance (ANOVA) was performed. For the LDA, the variables were first reduced to three dimensions using principal component analysis (PCA), with the resulting scores used as input. Locations were used as the dependent variable (explanatory variable), and scores were used as the independent variable. The percentage of correct classifications (TSS) for each group was calculated, and a confusion matrix was constructed. The RF analysis corroborated the population positioning based on acoustic variables. In this analysis, the two regions (north and south) were treated as the dependent variable, and our acoustic parameters were the independent variable. A confusion matrix was constructed, including an average error using the Gini index. For the nested ANOVA, the scores on the first three principal components from the PCA were used as dependent variables (one ANOVA per component, yielding three analyses). Populations were nested within groups (regions), and the individuals were nested within populations as categorical variables. Subsequently, a post-hoc Tukey test was performed. These analyses were conducted using R version 4.3.1 with the Ade4 package version 1.7-22 (Dray and Dufour 2007), MASS version 7.3 (Venables and Ripley 2002), and Random Forest version 4.7 (Liaw and Wiener 2002).

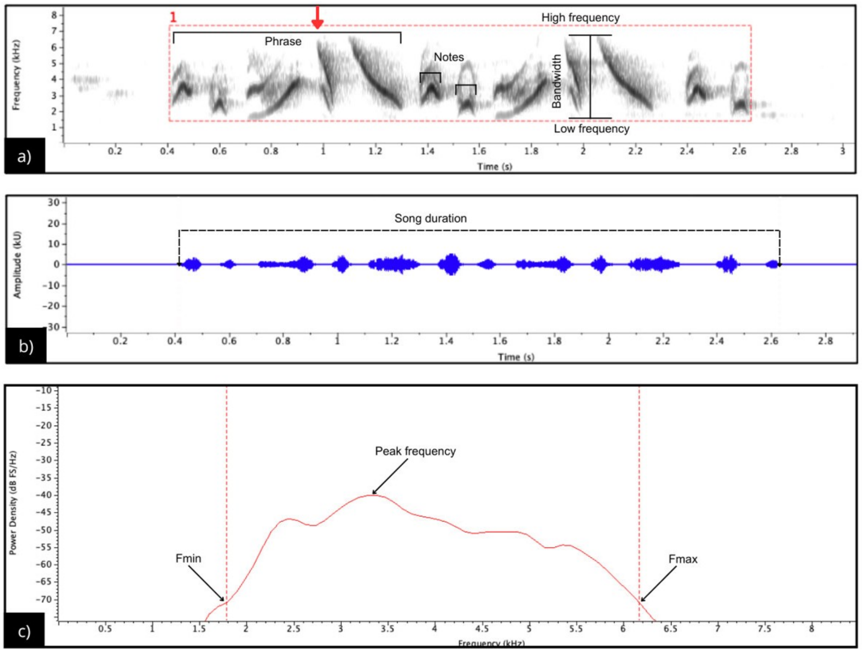
Using a Mantel test, we examined whether variation in *G. beldingi* song was influenced by geographic distance between individuals (isolation by distance). Two matrices were generated: one for acoustic distances and one for geographic distances. The first matrix was created by combining the scores of the first three principal components with the Euclidean distances calculated for each element. The second matrix contained geographical distances of the location points between individuals in kilometers. For this analysis, the Vegan package version 2.8-8 of the software in R was used (Oksanen et al. 2024).

To determine the song composition of *G. beldingi*, the different note types were identified visually by the same researcher (IBCR) and assigned a code for their identification. The composition of note types was subsequently analyzed using a Jaccard dissimilarity matrix constructed from presence/absence data for each note type per individual. To assess whether differences between regions were significant, a permutational analysis of variance (PERMANOVA) was performed. In addition, a principal coordinates analysis (PCoA) was performed to visualize clustering patterns among individuals in the multivariate space defined by the dissimilarity matrix, using the vegan package version 2.8-8 of software R (Oksanen et al. 2024).

RESULTS

The vocal behavior and social interactions of *G. beldingi* varied across the oases visited. In 2023, the vocal activity of birds in the oases of SI, PU, CO, and SB lasted approximately 30 minutes, while in SJ it lasted longer (60 minutes). However, in 2024, vocal activity differed, reducing the period from 30 to 20 minutes in the four oases mentioned above, while in SJ, the period was 40 minutes. *G. beldingi* sang within reed grass or cattail vegetation, avoiding open areas. Males are highly territorial and were usually observed accompanied by a female. An initial visual analysis of sonograms suggested that the song structure of populations from the northern and southern regions of Baja California Sur differed. The songs of northern birds (SI, PU, and CO) generally shared the same note types, although they differed in the way these notes were organized and in the number of elements. In SI, a note type we recorded was exclusive to this locality and absent in the other two (Fig. 2, red arrow). Additionally, the SI songs ended with two notes like those that introduce each phrase. In PU, songs were characterized by two similar phrases (Fig. 1). In contrast, in CO, the phrases exhibited a distinct structure compared to the other sites, resulting in shorter phrases with fewer notes (Table 1).

Fig. 2. Acoustic structure of *Geothlypis beldingi* song from San Ignacio: (a) spectrogram illustrating the temporal and frequency components used to measure song parameters (Hanning window, 90% overlap, 512 samples DFT). The song consists of two phrases of five notes each, followed by two terminal notes similar to introductory ones. A red arrow marks a note type observed only in songs from San Ignacio; (b) oscillogram showing how song duration measured; and (c) power spectrum indicating how minimum and maximum frequencies were determined using the threshold method.



The song of the Belding's Yellowthroat in the south of the Baja California Sur (SB and SJ) showed divergence from that in the northern region. In these populations, both the phrase structure and the type of notes differed from those in the northern area (Fig. 1). Two shared notes were identified: an ascending note at the beginning of the song and a descending note at the end of the song. Among southern populations, the song with the highest number of notes and the longest duration was that of the SJ population (Table 1). Despite this, birds from these two latter localities produced more notes per song than northern populations, which sang fewer notes and had longer intervals between them. Generally, the birds with the lowest minimum frequency were in the northern group, and those with the lowest maximum frequency were in the southern group (Table 1).

A total of 18 distinct note types were identified, with note type richness per individual being significantly higher in southern populations compared to northern ones. On average, northern individuals presented 4.89 ± 0.809 note types, while in the south, it was 6.08 ± 1.44 note types (see Fig. A1.1). PERMANOVA analysis revealed significant differences in note type composition between populations ($R^2 = 0.61$, $F = 46.56$, $p < 0.001$). These differences explained more than 60% of the total variation in repertoire structure between individuals. PCoA revealed a distinct separation across the northern and southern regions (see Fig. A1.2).

PCA's first three principal components explained 81.39% of the total song variance, indicating a continuum of variation (see Table A1.1) with some separation between northern and southern populations, particularly in the first component (see Figure A1.3). The variation in PCA across latitude and longitude indicates a particular geographic pattern (higher PC1 in both southern and northern populations; see Fig. A1.3). The LDA revealed a difference between the northern and southern regions, correctly classifying 76.77% of songs (see Table A1.2). Similarly, the RF model showed a 78.26% correct classification rate (see Table A1.3), indicating good predictive performance in distinguishing between the northern and southern regions. However, greater confusion was observed when classifying observations in the southern group, which could suggest that the acoustic variables analyzed exhibit greater similarity or overlap in this region (see Table A1.3). The most important variable for this classification was the minimum frequency, followed by the number of notes, maximum frequency, and peak frequency (see Fig. A1.4).

When testing for differences among regions, populations, and individuals using nested ANOVAs, we found significant variation in the first component, primarily driven by differences between regions, followed by differences among populations within regions and individuals within populations. This indicates distinct acoustic patterns in minimum and maximum frequencies, bandwidth, and number of notes among areas, populations, and individuals, as also identified by Random Forest analyses (see Tables A1.1 and A1.4). The second component was strongly correlated with song duration. Although no significant differences were found between regions in this component, significant differences were observed among populations within regions and among individuals within populations. Finally, there were substantial differences between regions and among

Table 1. Mean values (± SE) of acoustic variables measured from songs of Belding's Yellowthroat (*Geothlypis beldingi*) across five localities in the Baja California Sur: San Ignacio (SI), La Purísima (PU), Comondú (CO), San Bartolo (SB), and San José del Cabo (SJ). Variables include minimum frequency (Fmin, kHz), maximum frequency (Fmax, kHz), bandwidth (AB, kHz), peak frequency (FP, kHz), number of notes (NN) and song duration (s). "Bird number" refers to the number of individual males from which songs were recorded in each locality.

Variables	SI	PU	CO	SB	SJ
Bird number	6	7	6	6	6
Fmin	2059 ± 37.8	2058 ± 28.3	2115 ± 51.6	2427 ± 36.3	2489 ± 50.5
Fmax	5619 ± 66.5	5165 ± 59.4	5388 ± 111	5240 ± 80.2	5039 ± 145
AB	2263 ± 57.2	1712 ± 37.1	1884 ± 63.9	1981 ± 66.3	1772 ± 86.2
FP	3359 ± 58.6	3490 ± 39.6	3434 ± 52.3	3581 ± 100	3525 ± 64
NN	10.7 ± 0.43	11 ± 0.40	9.5 ± 0.51	13.5 ± 0.54	14.1 ± 0.39
Duration	1.90 ± 0.061	1.94 ± 0.067	1.62 ± 0.075	1.74 ± 0.056	1.91 ± 0.063

individuals within populations for the third component; however, no differences were observed among populations within regions, suggesting a variation related to the peak frequency (see Tables A1.1 and A1.4). With an isolation-by-distance analysis, the Mantel test showed a positive, albeit low, correlation between the acoustic characteristics of *G. beldingi* songs and the geographic distance between populations ($r = 0.19$, $p = 0.001$), suggesting that this relationship is limited.

DISCUSSION

Analysis of *Geothlypis beldingi* song composition showed marked differences between northern and southern populations, particularly in the diversity of note types. The clearest pattern supported by analyses is the geographic structuring of song; we found significant differences in note-type occurrence, minimum and maximum frequencies, and the number of notes, resulting in a consistent separation between the northern and southern regions. These results suggest that geographic distance and the fragmented "archipelago-like" distribution of oases reduce connectivity among populations and, as a consequence, may promote divergence in song characteristics independently of potential differences in population size, for which we lack information (see also Laiolo and Tella 2005, Benedict and Bowie 2009, Hamao et al. 2018, Yandell et al. 2018).

The PCA results support the geographic pattern of song divergence; the first component, linked to frequency parameters, showed significant differences between the northern and southern regions. Because these regions correspond to two recognized subspecies, *G. b. goldmani* in the north (larger bodied) and *G. b. beldingi* in the south (smaller bodied), part of the frequency divergence probably reflects morphological differences, as body size constrains the range of frequencies that a bird can produce (Shizuka et al. 2016, Roach and Phillmore 2017). The second component, associated with song duration, showed significant differences primarily at the local and individual levels, suggesting that temporal traits are more sensitive to microenvironmental factors or to learning between individuals within the same population. This pattern may also reflect that song duration is not under strong stabilizing selection, leading to greater flexibility and higher individual-level variability. In contrast, the third component, related to peak frequency, exhibited both regional and individual differences, indicating the combined influence of broad geographic divergence processes and intraindividual variation. Finally, Mantel analysis revealed a relatively low ($r = 0.19$), yet significantly positive, correlation between geographic distance and acoustic dissimilarity. This suggests that although distance contributes to song divergence, a significant portion of the variation remains unexplained.

A key ecological trait of *G. beldingi* is its limited dispersal capability, as it is considered a highly sedentary species (Rodríguez-Estrella et al. 1999). The species is closely associated with oases, where it inhabits reed grass (*Phragmites communis*) and cattail (*Typha dominguensis*) vegetation. These habitats are naturally discontinuous along the peninsula, with an approximate 350 km separation between the northern and southern regions. This spatial configuration restricts individual movements and significantly reduces opportunities for cultural exchange between populations. In this sense, oases function as small ecological islands in an arid environment, originating after the last glaciation (Ruiz-Campos et al. 2014). The discontinuity of these ecosystems reflects local processes that could be linked to broader dynamics of incipient differentiation.

Southern populations showed a greater variety of note types in Belding's Yellowthroat songs than northern ones. This pattern is consistent with expectations for fragmented systems, where restricted dispersal and local cultural transmission can promote divergence in song structure among populations. In other bird species, larger or less isolated populations have been shown to exhibit greater acoustic diversity, likely because of increased opportunities for cultural exchange (Laiolo and Tella 2005). However, not all populations face the same degree of isolation. Differences in oasis size, spatial isolation, and habitat continuity may influence both effective population size and the dynamics of cultural transmission. In smaller or more isolated populations, restricted dispersal can accentuate song divergence by limiting cultural flow among individuals. This isolation also influences population size and, consequently, the dynamics of cultural transmission, thereby favoring processes such as cultural drift (Slabbekoorn and Smith 2002, Lachlan and Slater 2003, Podos and Warren 2007, Williams et al. 2013). Under these conditions, song divergence and the loss or differentiation of repertoire elements are more likely to occur (Marler and Tamura 1962, Chilton et al. 2002, Päckert 2018, Hensel et al. 2022). Although demographic data are not currently available for each oasis, preventing a direct assessment of the relationship between population size, oasis area, and note type diversity, the observed pattern is consistent with theoretical and empirical expectations for culturally transmitted traits in fragmented landscapes.

A similar pattern may be observed in Comondú (northern), one of the smallest and possibly most isolated populations of *G. beldingi*, whose songs are characterized by fewer notes than neighboring populations (SI and PU). This pattern is consistent with reports in other species, in which small populations experience a "cultural bottleneck" in song transmission, leading to reduced variety and complexity (Laiolo and Tella 2007). Song divergence patterns associated with small populations and geographic isolation have been documented in other birds' species. In Darwin's finches of the Galápagos Islands, cultural differences in song have contributed to the emergence of reproductive barriers and to allopatric speciation processes (Grant and Grant 1996). In the White-crowned Sparrow (*Zonotrichia leucophrys*), the formation of regional dialects has been linked to population isolation. It is considered a mechanism that reinforces separation between populations (Lipshutz et al. 2017, Toews 2017). Similarly, the Olive Sparrow (*Arremonops rufivirgatus*) exhibits song divergence in allopatric populations, reinforcing that spatial isolation can have significant effects on acoustic divergence in the absence of geographic connectivity (Fernández-Gómez et al. 2020). Finally, studies of the Common Yellowthroat (*Geothlypis trichas*) have shown marked geographic variation in song related to geographic isolation (Bolus 2014). These examples highlight that limited dispersal not only influences genetic diversity but also cultural divergence in song, a process that may be occurring in *G. beldingi*.

Although the classification models (LDA and RF) showed low accuracy, likely because of the limited sample size, our findings suggest that an early stage of acoustic differentiation between populations is present. Multiple factors, including morphological traits, ecological conditions, and anthropogenic pressures, could influence this divergence. For example, bird body size has been documented to influence song variation; larger birds tend to produce lower frequencies (Martin et al. 2011, Bolus 2014, Ríos-Chelén et al. 2017), which is consistent with the described morphological divergence between northern and southern populations of *G. beldingi* (Oberholser 1917). Furthermore, habitat structure can modify song propagation, as high frequencies are attenuated more rapidly in denser environments (Morton 1975). Finally, anthropogenic pressure is a key factor for southern populations, such as San José del Cabo (Rodríguez-Estrella et al. 1999, Navarro et al. 2019), where urban noise can interfere with bird communication (León et al. 2014, Ríos-Chelén et al. 2017), generating adjustments in song frequencies to achieve effective communication (Francis et al. 2011, Derryberry et al. 2016, Phillips et al. 2020). In summary, this evidence suggests that the divergence of *G. belding*'s song could be modulated by morphological, environmental, and anthropogenic factors. Beyond the description of acoustic patterns, our results underscore the importance of considering habitat connectivity as a crucial element for the conservation of this Mexican endemic species. The loss or degradation of these environments not only threatens the viability of their populations but also provides a natural scenario to understand how limited dispersal in fragmented environments can drive processes of population differentiation and evolution.

CONCLUSIONS

Significant differences were found in the song frequencies of *G. beldingi* between the northern and southern regions of Baja California Sur. Geographic distance influenced the observed variation, but it may not be the only factor explaining it. Morphological and acoustic divergence between northern and southern populations suggests these groups may represent early stages of population differentiation but additional genetic and ecological evidence is needed to determine whether they constitute distinct evolutionary units. Genetic analyses would help clarify the evolutionary processes underlying the patterns observed here. This study represents the first step in understanding the geographic variation in the song of one of the most endangered endemic species in Baja California Sur. Conducting this research posed significant challenges because of limited knowledge about the species and its behavior. Despite these difficulties in the field, our findings lay the groundwork for future research to explore potential factors influencing variation in this species' song, such as environmental, reproductive, or anthropogenic factors.

Author Contributions:

IC: conceptualization, methodology, data curation, formal analysis, writing-original draft. PE: conceptualization, writing-review & editing. PL: resource, conceptualization, writing-review & editing. FG: conceptualization, methodology, writing-review & editing. OR: conceptualization, writing-review & editing. GA: Resource, funding acquisition, writing-review & editing.

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Data Availability:

All of the data collected are included in the document.

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Table A1.1. Principal component loadings for the first three components extracted from a PCA of six acoustic variables measured from songs of 31 male *Geothlypis beldingi*. These components summarize the major axes of acoustic variation among individuals.

Variables	PC1	PC2	PC3
Eigenvalues	2.68	1.26	1.09
Variance explained	44.72	21.08	18.19
Maximum frequency	0.7	0.2	-0.4
Minimum frequency	-0.8	-0.4	-0.2
Bandwidth	-0.7	-0.5	-0.1
Peak frequency	0.2	-0.2	-0.9
Number of notes	0.7	-0.6	0.1
Duration of the song	0.5	-0.7	0.3

Table A1.2. Linear Discriminant Analysis (LDA) confusion matrix showing classification accuracy of individual songs into northern and southern populations of *Geothlypis beldingi* based on six acoustic variables.

	Northern	Southern
Northern	79.20%	20.79%
Southern	27.77 %	72.22%

Table A1.3. Random Forest confusion matrix showing classification performance for northern and southern populations of *Geothlypis beldingi* using six acoustic variables.

	Northern	Southern	Class error
Northern	76.47%	23.52%	0.089
Southern	16.66 %	83.33%	0.166

Table A1.4. Results of a nested ANOVA partitioning acoustics variation (based on the first three PCA components among regions (north and south), populations, and individuals of *Geothlypis beldingi*.

Components	Df	Sum sq	Mean sq	F value	*P*
CP1[1]					
Region	1	72.52	72.52	143.43	<0.001
Population	3	32.99	11	21.75	<0.001
Individual	27	207.75	7.69	15.22	<0.001
Residuals	123	62.19	0.51		
CP2[2]					
Region	1	1.68	1.685	2.078	0.152
Population	3	21.65	7.217	8.901	<0.001
Individual	27	94.64	3.505	4.323	<0.001
Residuals	123	99.72	0.811		
CP3[3]					
Region	1	11.64	11.636	19.443	<0.001
Population	3	3.62	1.208	2.019	0.115
Individual	27	74.96	2.776	4.639	<0.001
Residuals	123	73.61	0.598		

[1] Component associated with minimum frequency, maximum frequency, bandwidth, and number of notes.

[2] Component associated with the duration of the song.

[3] Component associated with a peak frequency.

Figure A1.1. Number of note types by region. Northern populations showed lower note type diversity, whereas southern populations displayed a broader variety of note types.

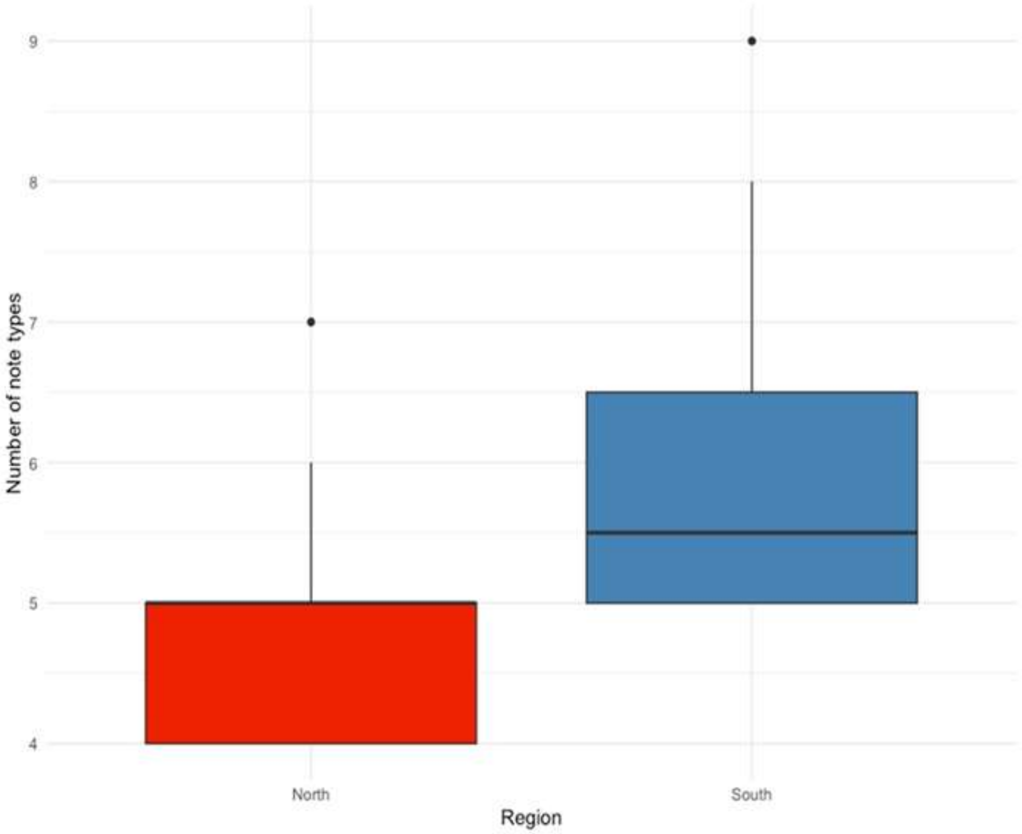

Figure A1.2. Principal Coordinates Analysis (PCoA) using Jaccard dissimilarity among populations. PERMANOVA results show significant regional separation ($R^2 = 0.61$, $p = 0.001$). Each point represents pairwise acoustic dissimilarities.

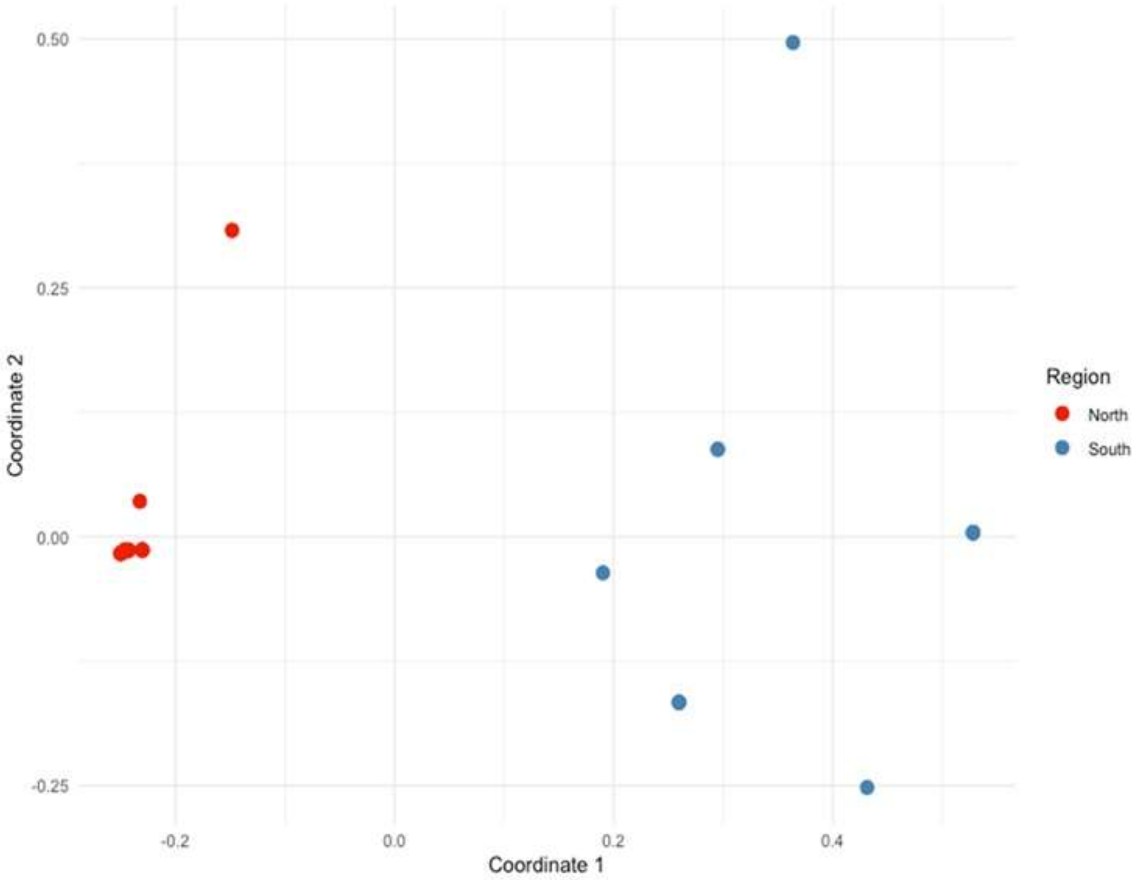

Figure A1.3. Principal Component Analysis (PCA) projection illustrating the acoustic space defined by three principal axes, explaining 81.39% of the total variation. Each point corresponds to the songs from one of the 31 individuals across five populations (North: San Ignacio, La Purísima, and Comondú; South: San Bartolo, San José del Cabo).

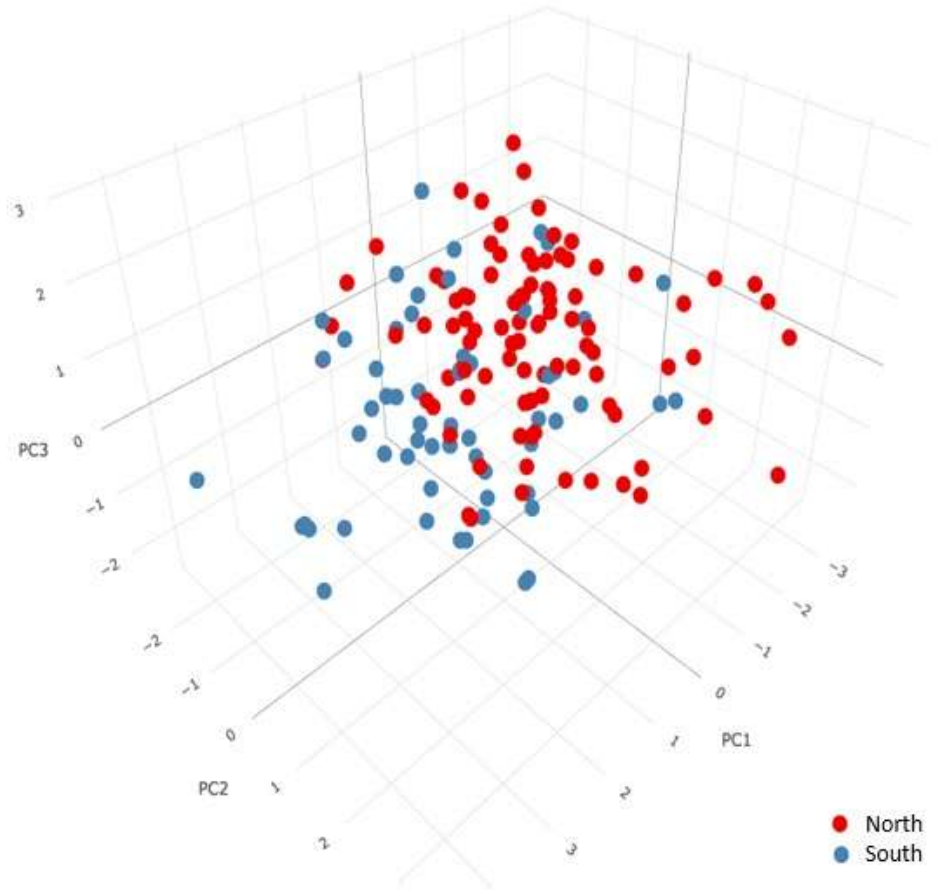

Figure A1.4. Importance of acoustic variables in the Random Forest classification of northern and southern populations of *Geothlypis beldingi*, based on the Gini index. Minimum frequency (Fmin) is the strongest predictor. Variables: Fmin = minimum frequency (kHz), NN = number of notes, Fmax = maximum frequency (kHz), FP = peak frequency (kHz), duration of the song (s), and AB = bandwidth (kHz).