GEOGRAPHIC VARIATION IN GOLDEN-CHEEKED WARBLER SONG CHARACTERISTICS ACROSS THEIR BREEDING RANGE IN CENTRAL TEXAS

A Thesis

by

DREW S. FINN

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Roel R. Lopez
Co-Chair of Committee,	Ashley M. Long
Committee Member,	Fred E. Smeins
Head of Department,	Kirk Winemiller

August 2020

Major Subject: Wildlife and Fisheries Sciences

ABSTRACT

Many taxa exhibit geographic variation in acoustic signals, which can lead to reproductive isolation and divergence among populations. Geographic variation in the acoustic signals of wood warblers is well-documented, and may be related to habitat characteristics, geographic isolation, and cultural drift. Furthermore, many wood warblers sing two song types that may be driven by inter- and intra-sexual selection. The golden-cheeked warbler (*Setophaga chrysoparia*; hereafter warbler) is a federally endangered Neotropical wood warbler that nests exclusively in Central Texas. Previous studies have indicated that golden-cheeked warblers use a two-category song system similar to other wood warbler species, and suggested that first category ("A") and second category ("B") songs exhibit different patterns of variation. Using warbler songs recorded in 2012, 2017, and 2018 (n = 171 individuals), I examined geographic variation in A and B song characteristics across the species' breeding range. I used frequency, time, and structure related song metrics to quantify and compare patterns of geographic variation in and between song types. A songs were more similar in form and less variable than B songs, supporting the idea that first and second category songs of wood warblers are driven by inter- and intra-sexual selection, respectively. I found different patterns of geographic variation in A and B songs and hypothesized that variation in A songs may be related to habitat characteristics, and variation in B songs may be related to geographic isolation and cultural drift. The results of my study further our understanding of the characteristics and variation of wood warbler song and song categories, and could be used in conjunction with other data (e.g., genetic) to help inform conservation planning for the golden-cheeked warbler.

DEDICATION

In loving memory of Ivan.

ACKNOWLEDGEMENTS

I would like to thank my committee co-chairs, Drs. Roel Lopez and Ashley Long, and my committee member, Dr. Fred Smeins, for their guidance, support, and expertise. I also thank Dr. Michael Morrison, Dr. Heather Mathewson, and all the graduate students and field technicians who planted the seeds of this research and collected song recordings. I also thank Drs. Melanie Colón and Andrea Montalvo for their feedback and assistance in navigating graduate school and the thesis process, and Shannon Longoria for being an amazing travel buddy and field technician.

I thank Texas Parks and Wildlife, Fort Hood, Joint Base San Antonio, The Nature Conservancy, Big Springs Ranch for Children, High Hope Ranch, Lower Colorado River Authority, and many private landowners for allowing data to be collected on their properties. I also thank the Macaulay Library at the Cornell Lab of Ornithology for providing access to historical song recordings.

Finally, I would like to send a huge thank you out to all of my friends and family, especially my husband, Spencer Collins, for their encouragement and emotional support.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Professor Roel R. Lopez [co-advisor] of the Department of Wildlife and Fisheries Sciences at Texas A&M University, Assistant Professor Ashley M. Long [co-advisor] of the School of Renewable Natural Resources at Louisiana State University and graduate faculty with the Department of Wildlife and Fisheries Sciences at Texas A&M University, and Professor Fred E. Smeins of the Department of Ecosystem Science and Management at Texas A&M University. All work for the thesis was completed by the student under the advisement of Assistant Professor, Dr. Ashley M. Long.

Funding Sources

This graduate study was financially supported by the Texas Ecological Laboratory (Ecolab) Program managed by Braun & Gresham, PLLC.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Many taxa exhibit geographic variation in acoustic signals, including insects (Simmons et al. 2001; Ferreira and Ferguson 2002; Etges et al. 2006), fish (Fine 1978), anurans (Snyder and Jameson 1965; Ryan 1986; Platz and Forester 1988), birds (Marler and Tamura 1962; Janes and Ryker 2011; Domínguez et al. 2016; Billings 2018), and mammals (Van Parijs et al. 2000; Campbell et al. 2010). Because acoustic signals can influence mate choice, resource defense, and species recognition, these variations can lead to reproductive isolation and divergence among populations (Liou and Price 1994; Ptacek 2000; Irwin et al. 2001; Lachlan and Servedio 2004). As such, understanding patterns of geographic variation in acoustic signals can provide insight into current or future population structure (e.g., MacDougall-Shackleton and MacDougall-Shackleton 2001; Irwin et al. 2008) and help inform management decisions for species of conservation concern.

Geographic variation in acoustic signals is most well-documented for songbirds and has been linked to habitat characteristics (Goretskaia et al. 2018; Luttrell and Lohr 2018), geographic isolation (Kroodsma et al. 1999), and cultural drift (Irwin et al. 2008), among other factors. Transmission properties of acoustic signals can vary among vegetation types (e.g., open grasslands vs. closed-canopy forests), which can lead to differentiation of song characteristics for species that utilize habitats with different structural components (Morton 1975; Slabbekoorn 2004). In addition, habitat fragmentation can create islands of geographically isolated populations leading to reduced song transmission and divergence of acoustic signals (Pérez-Granados et al. 2016; Hart et al. 2018). Because most songbirds learn vocalizations from nearby conspecifics, geographic variation in acoustic signals may also occur as a result of cultural drift, defined as variation in the relative frequency of different cultural elements over time (e.g., Lemon 1975; Schook et al. 2008). However, more often, divergence of acoustic signals is a complex process, and a combination of these factors may operate at different spatial scales and with varying levels of influence based on the unique circumstances faced by a species and its populations to produce variation in acoustic signals (e.g., Roach and Phillmore 2017; Yandell et al. 2018).

The presence of geographic acoustic signal variation within a songbird species can carry evolutionary significance if individuals learn vocalizations soon after fledging and adults use acoustic signals as cues for assortative mating (Marler and Tamura 1962; Nottebohm 1969). Geographic patterns of variation would then reflect the underlying population structure of the species, and could provide clues as to where genetic differentiation might arise. Monitoring population structure and the extent of genetic differentiation within a species is especially important when managing species of conservation concern. Thus, determining where the acoustic signals of priority species diverge may elucidate sources of future genetic differentiation that could be targeted for management.

Geographic variation in the acoustic signals of wood warblers is well-documented (Janes and Ryker 2006; Bolus 2014), and many wood warblers sing two song types that are used in different contexts ("first category" and "second category" songs; Spector 1992). Male wood warblers with two song types tend to sing first category songs early in the breeding season and in the presence of females, and second category songs later in the breeding season and when interacting with other males (Spector 1992). Second category songs tend to exhibit more variation than first category songs, which suggests that variation within first and second category songs may be driven by inter- and intra-sexual selection (i.e., selective pressures relating to members of the opposite and the same sex), respectively (Kroodsma 1981). Furthermore, evidence suggests that first category songs are learned during the hatching year, while second category song learning can happen over multiple years, as the similarity of second category songs of neighboring adults tends to increase over time (Byers and Kroodsma 1992). To examine variation of songs within wood warbler species, researchers have used both qualitative characteristics of song form that are readily distinguishable by eye using spectrograms (e.g., number and form of song elements) as well as quantitative measurements of characteristics such as frequency and duration. For example, Janes and Ryker (2006) investigated geographic variation in first category songs of hermit warblers (*Setophaga occidentalis*). In addition to obvious differences discernable by eye using spectrograms, they found that characteristics such as maximum and minimum frequencies, duration, and frequency range (i.e., bandwidth) helped to describe the variation observed among areas with different dialects.

The golden-cheeked warbler (*S. chrysoparia*; hereafter warbler) is a federally endangered Neotropical wood warbler that nests exclusively in Central Texas (Ladd and Gass 2020). The U.S. Fish and Wildlife Service (USFWS) listed the warbler as endangered in 1990 due to habitat loss and fragmentation within the Edwards Plateau and Cross Timbers and Prairies ecoregions of Texas (USFWS 1990). The recovery plan for the warbler (USFWS 1992) established eight recovery regions across the warbler's breeding range with a goal of maintaining at least one viable population in each. The USFWS delineated these recovery regions based on geologic, vegetation, and watershed boundaries rather than warbler population structure due to lack of knowledge regarding gene flow across the warbler's breeding range at the time.

Lindsay et al. (2008) and Athrey et al. (2011) have since used molecular markers to examine genetic structure across the warbler's breeding range. These studies found that genetic differentiation has increased over time (Athrey et al. 2011) and that the current level of gene flow is insufficient to prevent genetic differentiation (Lindsay et al. 2008). However, both studies concluded there is little evidence that warblers exhibit genetic differentiation across their breeding range (Lindsay et al. 2008; Athrey et al. 2011). Regardless, several studies have noted geographic variation in warbler responses across the breeding range, including foraging behavior (Smith-Hicks et al. 2016), vegetation used by warblers for nesting (Long et al. 2016), reproductive success (Campomizzi et al. 2012), and warbler occurrence (Collier et al. 2012). Analyzing genetic data is the most common approach to examining population structure and provides valuable information that can assist with species recovery (e.g., Buchholz-Sørensen and Vella 2016; Li et al. 2016; Szczecińska et al. 2016). However, understanding spatial variation in warbler song characteristics may elucidate behavioral aspects of warbler population structure and help inform conservation planning for this species.

A common strategy used to examine geographic variation in birds is to assign individuals to geographic regions and then compare responses among regions. Researchers have defined regions in several ways, including using location-based terms related to specific latitude and longitude data (Marler and Tamura 1962; Domínguez et al. 2016), ecoregions (Karanth et al. 2006; Campomizzi et al. 2012), and recovery regions (Roberts et al. 2011; Drever et al. 2015), among others. As previously mentioned, the USFWS established eight recovery regions (hereafter USFWS regions) across the warbler's breeding range based on landscape features rather than warbler population structure (USFWS 1992). In addition, Hatfield et al. (2012) provided three logical, though not biologically-based, recovery region definitions (hereafter Hatfield regions) for this species. Studies that quantify geographic variation in warbler behavioral responses among these different management regions may help identify whether they are biologically significant to the species.

Previous studies have indicated that golden-cheeked warblers use a song system similar to other wood warbler species. Bolsinger (2000) recorded the songs of a focal group of warblers on Fort Hood, Texas across two breeding seasons (1993–1994) and found that they used a two-category song system. Warblers tended to sing first category songs (hereafter "A" songs) early in the breeding season and when near females, and second category songs (hereafter "B" songs) later in the breeding season and near the edges of territories (Bolsinger 2000). Furthermore, all individuals sang a single A song subtype, while most sang two or more distinct B song subtypes (Bolsinger 2000). Bolsinger (1997) also recorded warblers outside of Fort Hood at 12 locations spanning the breeding range and found that, similar to second category songs of other wood warbler species, warbler B songs were more variable than A songs. In 2009, Leonard et al. (2010) recorded warblers in Bexar County, Texas singing a novel B song subtype that had not been reported by Bolsinger (1997), which suggested that warbler B songs may also vary over time. New studies spanning the breeding range of the warbler could help to determine the full extent of geographic and temporal variation in both song types.

Warbler A and B songs differ in complexity (i.e., elements per song), duration, and frequency of modal intensity; A songs are less complex, shorter, and higher in frequency than B songs (Bolsinger 2000). Bolsinger (1997) termed the three elements of the A song the introductory sequence, the buzz, and the terminal note; the introductory sequence consists of several short, repeated syllables, the buzz is a single longer note, and the terminal note is higher in frequency than the rest of the song. As stated above, B songs are much more variable, even within individuals, and may also vary over time (Bolsinger 1997; Leonard et al. 2010). Bolsinger (1997; 2000) also described a "C" song, but was unsure if it represented a third category of songs or if it was a B song subtype. Bolsinger (1997) examined individual and geographic variation within song types through visual inspection of spectrograms so distinguished variation based on qualitative differences in form rather than quantitatively through statistical analysis of song metrics. Additionally, researchers investigating the effects of road construction noise on warblers in two different regions of the warbler's breeding range noted that B songs differed between the two regions (M. L. Morrison, unpublished data), but did not pursue formal analyses at that time. Studies that measure and statistically compare elements within song types could build on previous observations of variation in warbler song and quantitatively describe the differences in frequency and duration that are visible on spectrograms.

In this study, I examined geographic variation in warbler song characteristics by collecting new recordings across both USFWS and Hatfield regions (Figure 1), which simultaneously represent the latitudinal and longitudinal locations of the warbler across its breeding range in Central Texas. I tested the null hypothesis that there is no variation in the form and characteristics of warbler A and B songs across the breeding range. However, based on previous knowledge of warbler song types, form, and characteristics (Bolsinger 1997; Leonard et al. 2010; M. L. Morrison, unpublished data), I expected to find variation in both A and B songs, but greater variability in the form and characteristics of B songs. I predicted that I would find multiple B song subtypes, and that variation in both A and B song characteristics would align more closely with Hatfield regions than USFWS regions. I did not expect to find a relationship between variation in A song characteristics and geographic distance, but I expected to find a positive relationship between variation in B song characteristics and geographic distance (Kroodsma 1981; Byers and Kroodsma 1992).

Thus, my study objectives were to:

1. Quantify variation in frequency, time, and structure related characteristics of A and B songs among USFWS and Hatfield regions;
2. Classify A and B songs into subtypes, if present;
3. Identify geographic patterns of A and B song characteristics across the breeding range regardless of subtype designations;
4. Determine whether variation in A and B song characteristics aligns more closely with USFWS or Hatfield regions; and
5. Examine the relationships between variation in A and B song characteristics and geographic distance.

The results of my research further our understanding of the characteristics and variation of wood warbler song and song categories and could help inform recovery efforts for the golden-cheeked warbler.

METHODS

Study Sites

I used recordings of warbler songs collected in 2012, 2017, and 2018 from 25 study sites across the warbler's breeding range in Central Texas (Figure 1): Possum Kingdom State Park (PKSP), Palo Pinto Mountains State Park (PPMSP), Dinosaur Valley State Park (DVSP), High Hope Ranch (HHR), Meridian State Park (MSP), two sites at Fort Hood (FHN and FHS), Colorado Bend State Park (CBSP), Canyon of the Eagles (COTE), Longhorn Cavern State Park (LCSP), Pedernales Falls State Park (PFSP), Barton Creek Habitat Preserve (BCHP), a private property in Travis County (Travis), Guadalupe River State Park (GRSP), Joint Base San Antonio-Camp Bullis (JBSA-BUL), Government Canyon State Natural Area (GCSNA), Hill Country State Natural Area (HCSNA), South Llano River State Park (SLRSP), Kerr Wildlife Management Area (KWMA), a private property in Kerr County (Kerr), Big Springs Ranch for Children (BSRC), Garner State Park (GSP), two private properties in Edwards County (Edwards1 and Edwards2), and Kickapoo Cavern State Park (KCSP).

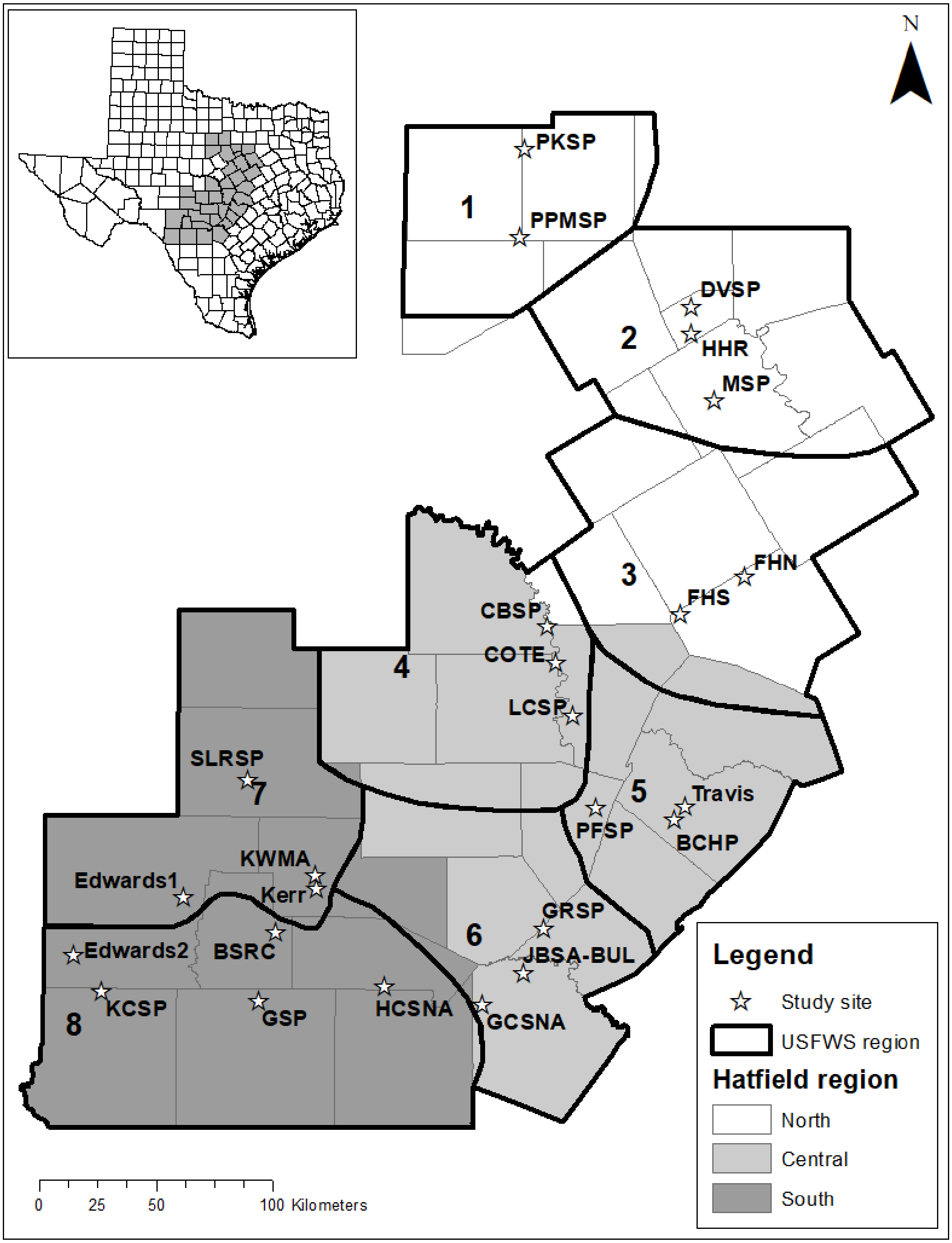

Figure 1. Golden-cheeked warbler (*Setophaga chrysoparia*; hereafter warbler) recovery regions (USFWS 1992), recovery regions proposed by Hatfield et al. (2012), and study sites sampled in 2012, 2017, and 2018 to examine geographic variation in warbler song characteristics.

Warbler habitat within the species' breeding range is characterized by mature oak-juniper (*Quercus-Juniperus*) woodland (Ladd and Gass 2020); however, plant species composition varies regionally by climate (Griffith et al. 2007). Mean annual precipitation ranges from 55–85 cm, and declines from east to west (NOAA 2014). Mean annual temperature ranges from 18.5–20°C, and declines from south to north (NOAA 2014). Ashe juniper (*J. ashei*) is a vital component of warbler breeding habitat, as warblers use strips of its bark to construct their nests (Ladd and Gass 2020). A combination of Ashe juniper and oaks provide important foraging, nesting, and roosting sites (Ladd and Gass 2020), but the predominant oak species within warbler breeding habitat varies by region (Diamond 1997; Campbell 2003; Ladd and Gass 2020). Warbler breeding habitat is fragmented, with smaller and more fragmented patches occurring within the northernmost portion of the breeding range (Collier et al. 2012).

Song Recording Collection and Analysis

From mid-March to mid-June 2012, 2017, and 2018, trained observers and I recorded warbler songs at the 25 study sites listed above using Sony IC digital voice recorders (model ICD-BX112). We attempted to record individuals for 30 minutes and to stay within 20 m of their locations while recording, and we noted the Global Positioning System (GPS) coordinates where we recorded our song data. We collected recordings from the majority of study sites during one sampling event to ensure that we did not record the same individual more than once. If we sampled a study site more than once, we attempted to record unique individuals by avoiding areas where we had already recorded warblers (adult male warblers exhibit high site fidelity and retain relatively small and distinct territories over the course of the breeding season; Ladd and Gass 2020), or we identified individuals using unique color band combinations fitted on warblers from concurrent studies (USFWS Threatened and Endangered Species permit TE32917C-1; USGS banding permit #24126).

I then categorized each recorded song as A or B and retained 3–10 songs per individual per song type for subsequent analyses. For individuals with >10 recorded A or B songs, I randomly selected 10 songs per song type for further use to even sample sizes among individuals. I used SonoBird 1.6.5 (DNDesign, Arcata, CA, USA) to measure the duration (s), peak frequency (kHz), average lower frequency (kHz), average upper frequency (kHz), and average bandwidth (kHz) for three segments of the A song and five segments of the B song, and I counted the number of notes in the first segment of A songs and the fourth segment of B songs. I divided the number of notes in the first segment of A songs and the fourth segment of B songs by the duration of their respective segments to determine notes per second for the first segment of A songs and the fourth segment of B songs. I additionally calculated the difference in the peak frequency (kHz), average lower frequency (kHz), and average upper frequency (kHz) between segments 3 and 2 of A songs and between adjacent segments of B songs. Finally, I used SonoBird 1.6.5 (DNDesign, Arcata, CA, USA) to measure the duration (s), peak frequency (kHz), and time to peak frequency (s) of entire songs. I also visually examined song spectrograms and classified them into subtypes, if present.

Statistical Analyses

I averaged each A and B song metric within individuals to reduce A and B song datasets to one value per metric per individual. I then calculated the mean and 95% confidence interval (95% CI) for each A and B song metric per Hatfield and USFWS region, and used analysis of variance (ANOVA) followed by Tukey's honestly significant difference tests (Tukey's HSD) to identify differences between regions for each metric (Zar 1999). I used a false-discovery-rate adjustment to correct for multiple testing within song types ($\alpha = 0.05$; Benjamini and Hochberg 1995). To reduce the number of metrics for subsequent multivariate tests and avoid issues of collinearity (Dormann et al. 2012), I selected 10 representative metrics per song type that were not calculated from other metrics. I then used function *vifstep* in the R package usdm (Naimi et al. 2014; R Core Team 2019) with the variance inflation factor (VIF) threshold set to 5 to ensure that none of the representative metrics were highly correlated with each other per song type. The reduced sets of metrics for both A and B songs did not conform to multivariate normality, so I used the R package npmv (Burchett et al. 2017; R Core Team 2019) to conduct nonparametric multivariate analysis of variance (MANOVA) and post-hoc tests to identify multivariate differences among regions.

I conducted K-means cluster analyses (Lloyd 1982) using all A song metrics and 26 B song metrics to test whether my subtype classifications were accurate, to identify geographic patterns in A and B songs, and to determine how the clusters aligned with Hatfield and USFWS regions. I used the silhouette and elbow methods to determine the optimal number of clusters for both A and B song cluster analyses (Rousseeuw 1987; Jain 2010). I then performed FDAs (Hastie et al. 1994) to determine the relative ability of Hatfield and USFWS regions to explain variation in A and B songs. FDA is a nonparametric approach to discriminant function analysis (DFA; Poulsen and French 2004), a classification method that uses multiple predictor variables to assign individuals into categorical groups with the least amount of error, which I used to determine whether Hatfield or USFWS regions could be identified with greater accuracy. Prior to analyses, I reduced the number of A song metrics to 4 and the number of B song metrics to 5 because DFA requires the sample size of the smallest group to exceed the number of predictor variables (Poulsen and French 2004). These 4 metrics for A songs (segment 1 average bandwidth, segment 2 duration, segment 2 average bandwidth, and segment 3 duration) and 5 metrics for B songs (segment 2 duration, segment 2 average bandwidth, segment 3 duration, segment 4 peak frequency, and segment 4 notes) were the simplest, least correlated sets of metrics representative of each song type. I used FDA instead of linear discriminant analysis (LDA) or quadratic discriminant analysis (QDA) because the reduced sets of metrics for A and B songs did not conform to multivariate normality. I used function *fda* with the bruto method from the R package mda (Hastie and Tibshirani 2017; R Core Team 2019) for all FDAs.

Finally, I used linear regression to examine the relationships between variation in A and B song characteristics and geographic distance. I first created dissimilarity matrices using means per USFWS region for the 4 A and 5 B song metrics described for FDA above. I then calculated the geographic distances between each pair of study sites using the centroids of my study sites per USFWS region. I plotted the dissimilarity values for A and B songs in relation to geographic distance, determined whether the relationships were statistically significant at $\alpha = 0.05$, and calculated coefficients of determination (R^2) for each relationship (Zar 1999).

RESULTS

I analyzed 638 A songs from 84 warblers and 798 B songs from 106 warblers. I report sample sizes, means, and 95% confidence intervals for each A song metric per Hatfield region and for a subset of B song metrics per Hatfield and USFWS region in the Appendix. Due to small sample sizes, I did not calculate descriptive statistics for or use ANOVAs and nonparametric MANOVAs to compare A song metrics among USFWS regions, I excluded segment 1 from my analyses of B songs among Hatfield regions, and I excluded Region 1 and segments 1 and 5 from my analyses of B songs among USFWS regions. I present the results for all ANOVAs in the Appendix, but to briefly summarize, I found statistically significant differences in 16 of 23 A song metrics and 25 of 34 B song metrics among Hatfield regions, and 17 of 26 B song metrics among USFWS regions. The results of my nonparametric MANOVAs indicated that A songs (ANOVA-type test value $F_{13, 463} = 10.27$, $P < 0.01$) and B songs (ANOVA-type test value $F_{13, 601} = 7.02$, $P < 0.01$) varied among Hatfield regions. I also found that B songs varied among USFWS regions (ANOVA-type test value $F_{37, 507} = 3.73$, $P < 0.01$). For A songs, post-hoc tests revealed that the South Hatfield region was significantly different than both the North and Central Hatfield regions, and that the North and Central Hatfield regions were not significantly different from each other. B songs were significantly different between each pair of Hatfield regions, and I found four groups with similar B song characteristics across USFWS regions (Table 1).

Table 1. Groupings of similar golden-cheeked warbler (*Setophaga chrysoparia*) B songs (averaged within individuals) recorded in 2012, 2017, and 2018 across current recovery regions in Central Texas (USFWS 1992) as determined by nonparametric MANOVA post-hoc tests.

USFWS region	Group A	Group B	Group C	Group D
2	X			
3	X	X		
4	X	X	X	
5	X	X	X	
6		X		
7			X	
8				X

Within song types, A songs were more similar in form than B songs (Figure 2). All A songs began with an opening segment consisting of several short notes followed by a second segment consisting of either 1 (n = 595) or 2 (n = 43) longer note(s); I only detected A songs with a second segment consisting of 2 notes at GCSNA, HCSNA, and KWMA. The majority of A songs (n = 615) consisted of three segments with a single higher-pitched note as the third segment; the rest (n = 23) consisted of two segments as described above. Due to similarity in the form of A songs, I did not split them into subtypes or conduct cluster analyses to test whether my subtype classifications would be accurate, but similar to Bolsinger (1997), I found that the third segment of most A songs (n = 499) decreased in frequency over the duration of the segment (see segment 3 of Figure 2C); I only detected A songs without this characteristic at the northern and southwestern extremes of the breeding range (i.e., at PKSP, PPMSP, SLRSP, BSRC, GSP, Edwards1, Edwards2, and KCSP).

B songs consisted of two to five segments, and were sometimes missing opening and/or closing segments. I was able to distinguish three B song subtypes based on differences in form and labeled them B North, B Central, and B South (Figure 2). The main difference between the three subtypes occurred in segment 4, as can be seen in Figure 2. Additionally, the B North and B Central subtypes were usually missing opening segments. I only detected the B North subtype at PPMSP, while the B Central and B South subtypes were more widespread (Table 2). I then used three K-means clusters to test whether my subtype classifications were accurate, and found general concordance with my classifications (Table 3). All B songs labeled B North were clustered together, 93% of B songs labeled B Central were clustered together, and 99% of B songs labeled B South were clustered together.

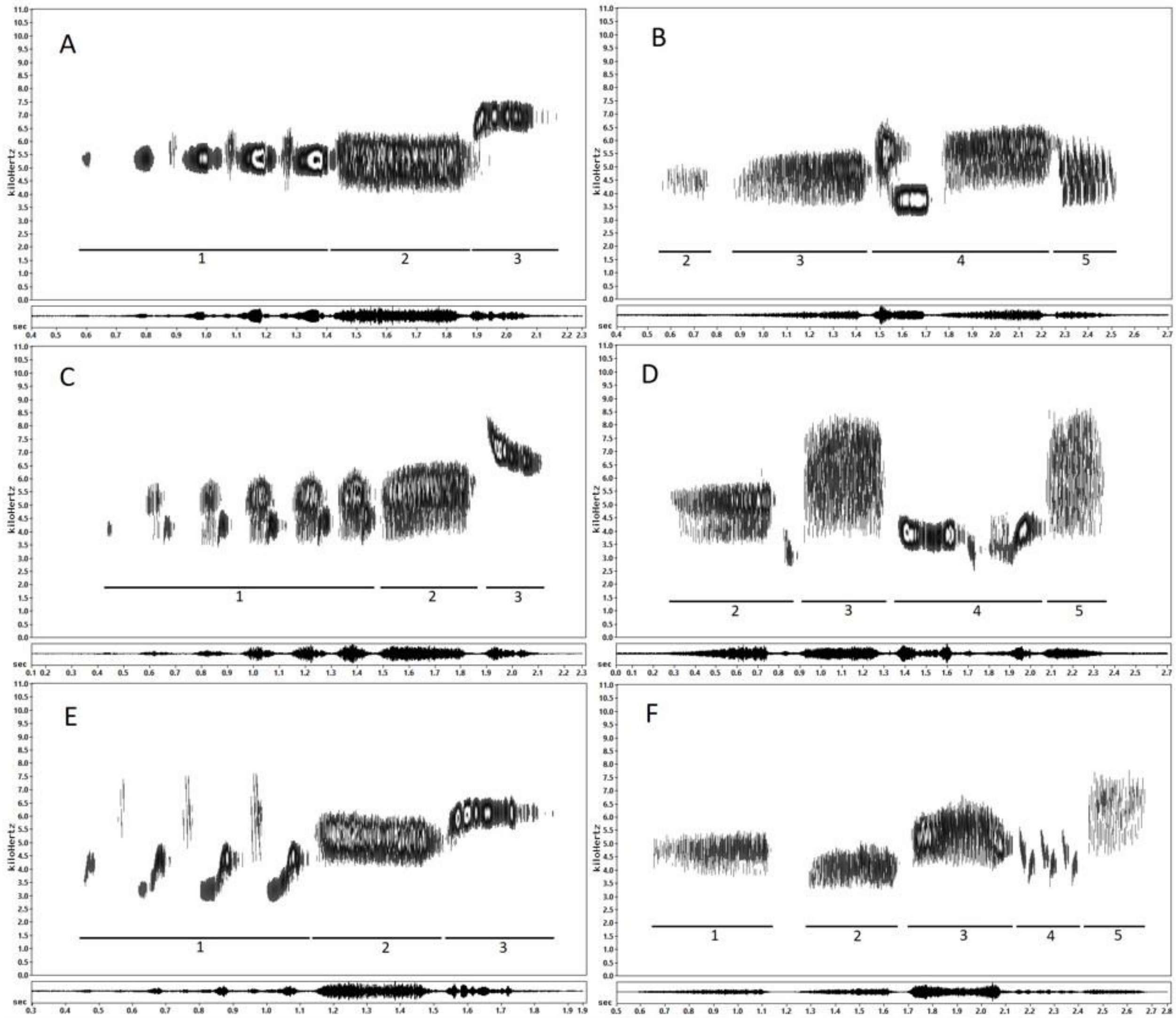

Figure 2. Spectrograms of golden-cheeked warbler (*Setophaga chrysoparia*) A songs recorded in the North (A), Central (C), and South (E) and B songs recorded in the North (B), Central (D), and South (F) recovery regions proposed by Hatfield et al. (2012) in 2018. B is representative of the B North subtype, D is representative of the B Central subtype, and F is representative of the B South subtype.

Table 2. Number of golden-cheeked warbler (*Setophaga chrysoparia*) B songs qualitatively assigned to each B song subtype per region; songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas.

Region	B songs analyzed	B songs labeled B North	B songs labeled B Central	B songs labeled B South
Hatfield region				
North	202	38	145	19
Central	324	0	285	39
South	272	0	73	199
USFWS region				
1	61	38	23	0
2	73	0	63	10
3	68	0	59	9
4	77	0	77	0
5	142	0	124	18
6	105	0	84	21
7	155	0	62	93
8	117	0	11	106

Table 3. Number of golden-cheeked warbler (*Setophaga chrysoparia*) B songs qualitatively assigned to each subtype and their categorizations as determined by K-means cluster analysis; songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, and bold numbers indicate which cluster contained the majority of songs per B song subtype.

B song subtype	n songs	n songs in Cluster 1	n songs in Cluster 2	n songs in Cluster 3
B North	26	0	0	**26**
B Central	499	34	**465**	0
B South	251	**249**	2	0

I then used K-means cluster analyses to identify geographic patterns in A and B songs and to determine how the clusters aligned with Hatfield and USFWS regions. I used the silhouette and elbow methods to determine the optimal number of clusters, and found that the optimal number of clusters for A songs was two, and for B songs was two or three. When using two clusters, a majority of songs from the North and Central Hatfield regions were assigned to one cluster, while a majority of songs from the South Hatfield region were assigned to the other (Table 4). Two-cluster analysis among USFWS regions yielded similar results with the exception of B songs in Region 1, as the B North subtype was clustered with the B South subtype (Table 4). Results were similar when using three clusters, with the B North subtype separated out as the third cluster (Table 5).

For both A and B songs, FDA identified Hatfield regions with greater accuracy than USFWS regions. For A songs, percent correct classification per Hatfield region ranged from 43–76% and overall percent correct classification was 64%, while percent correct classification per USFWS region ranged from 0–100% and overall percent correct classification was 51% (Table 6). For B songs, percent correct classification per Hatfield region ranged from 44–70% and overall percent correct classification was 60%, while percent correct classification per USFWS region ranged from 0–85% and overall percent correct classification was 31% (Table 7).

I found no relationship between A song dissimilarity and geographic distance ($R^2 = 0.01$, $F_{1, 26} = 1.35$, $P = 0.26$; Figure 3), and a positive relationship between B song dissimilarity and geographic distance ($R^2 = 0.17$, $F_{1, 26} = 6.57$, $P = 0.02$; Figure 4).

Table 4. Number of golden-cheeked warbler (*Setophaga chrysoparia*) A and B songs analyzed then averaged within individuals and assigned to each cluster per region using two K-means clusters; songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, and bold numbers indicate which cluster contained the majority of songs per region.

Region	A songs	A songs in Cluster 1	A songs in Cluster 2	B songs	B songs in Cluster 1	B songs in Cluster 2
Hatfield region						
North	28	**24**	4	27	**20**	7
Central	34	**31**	3	43	**36**	7
South	19	2	**17**	33	10	**23**
USFWS region						
1	8	**8**	0	6	3	3
2	5	**4**	1	11	**9**	2
3	15	**12**	3	10	**8**	2
4	8	**7**	1	10	**9**	1
5	14	**13**	1	20	**18**	2
6	12	**11**	1	13	**9**	4
7	7	2	**5**	18	8	**10**
8	12	0	**12**	15	2	**13**

Table 5. Number of golden-cheeked warbler (*Setophaga chrysoparia*) B songs analyzed then averaged within individuals and assigned to each cluster per region using three K-means clusters; songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, and bold numbers indicate which cluster contained the majority of songs per region.

Region	B songs	B songs in Cluster 1	B songs in Cluster 2	B songs in Cluster 3
Hatfield region				
North	27	**20**	4	3
Central	43	**36**	7	0
South	33	10	**23**	0
USFWS region				
1	6	3	0	3
2	11	**9**	2	0
3	10	**8**	2	0
4	10	**9**	1	0
5	20	**18**	2	0
6	13	**9**	4	0
7	18	8	**10**	0
8	15	2	**13**	0

Table 6. Percent correct classifications of golden-cheeked warbler (*Setophaga chrysoparia*) A songs (averaged within individuals) per region using flexible discriminant analysis; songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas.

Region		Percent correct classification per region	Overall percent correct classification
Hatfield	North	43	64
	Central	76	
	South	74	
USFWS	1	100	51
	2	0	
	3	67	
	4	0	
	5	36	
	6	67	
	7	57	
	8	50	

Table 7. Percent correct classifications of golden-cheeked warbler (*Setophaga chrysoparia*) B songs (averaged within individuals) per region using flexible discriminant analysis; songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas.

Region		Percent correct classification per region	Overall percent correct classification
Hatfield	North	44	60
	Central	70	
	South	61	
USFWS	1	67	31
	2	0	
	3	0	
	4	0	
	5	85	
	6	31	
	7	0	
	8	47	

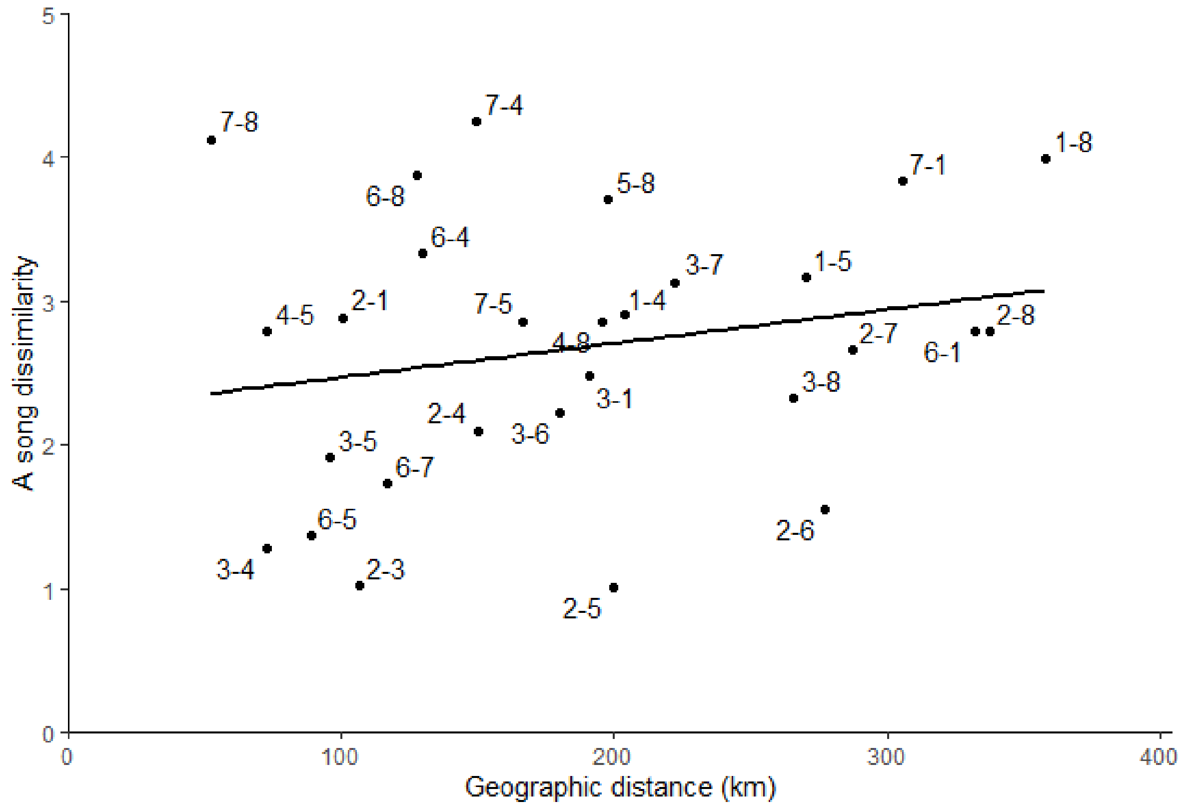

Figure 3. Golden-cheeked warbler (*Setophaga chrysoparia*) A song dissimilarity versus geographic distance between pairs of current recovery regions (USFWS 1992); songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, points are labeled by pairs of recovery regions, and the line is the regression of song dissimilarity on geographic distance.

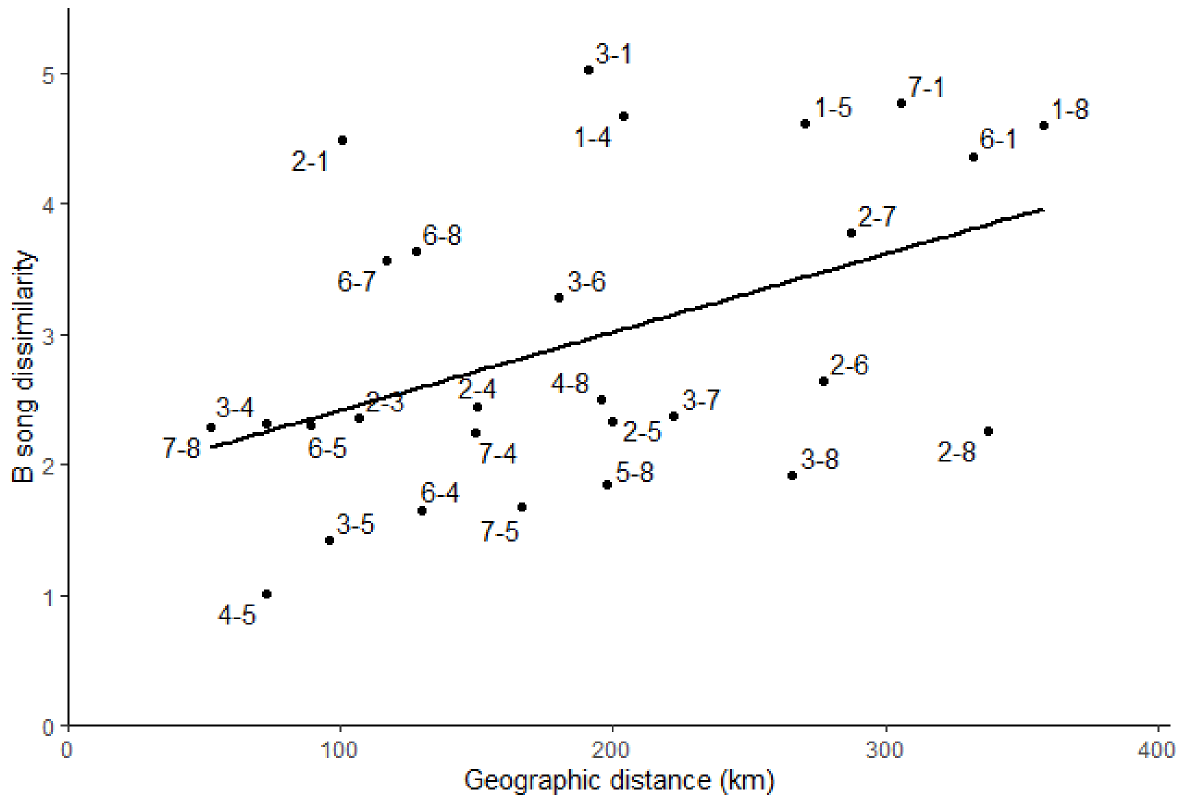

Figure 4. Golden-cheeked warbler (*Setophaga chrysoparia*) B song dissimilarity versus geographic distance between pairs of current recovery regions (USFWS 1992); songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, points are labeled by pairs of recovery regions, and the line is the regression of song dissimilarity on geographic distance.

DISCUSSION AND SUMMARY

As predicted, I found geographic variation in both song types of the golden-cheeked warbler across its breeding range in Central Texas. A songs from the North and Central Hatfield regions were generally more similar to each other than to A songs from the South Hatfield region—A songs from the South Hatfield region had fewer notes in the first segment and were lower in frequency than A songs from the North and Central Hatfield regions. B songs were much more variable than A songs both within and among Hatfield and USFWS regions, and I detected several individuals singing more than one B song subtype. My results are consistent with Bolsinger (1997), and because warbler A songs were more similar in form and less variable than B songs, support the idea that first and second category songs of wood warblers are driven by inter- and intra-sexual selection, respectively (Kroodsma 1981). However, the existence of geographic patterns of variation in A songs, and to a lesser extent, B songs, suggests that inter- and intra-sexual selection are not the only mechanisms at work.

As found for other species (e.g., Goretskaia et al. 2018; Luttrell and Lohr 2018), geographic patterns of variation in A songs may be related to habitat characteristics. Though I did not measure habitat characteristics as part of my study, researchers have noted variation in shrub and tree species composition, percent canopy cover, and patch size across the warbler's breeding range (Groce et al. 2010). Oak-juniper woodland in the southwestern portion of the breeding range (analogous to the South Hatfield region or USFWS regions 7 and 8) tends to have lower overall percent canopy cover within patches compared to other locations. According to Morton's (1975) acoustic adaptation hypothesis, signals are adapted to maximize their propagation through the habitat in which they are produced, thus acoustic signals propagated in closed habitats should be lower in frequency than those in more open habitats. As such, one might expect A songs in the South Hatfield region to be higher in frequency relative to regions with higher percent canopy cover within patches, but I found the opposite. It may be more important for A songs in the North and Central Hatfield regions to propagate through open habitats from one patch to another rather than through a single patch, as patch sizes tend to be smaller and more fragmented in these regions; it is possible that higher frequency songs in the North and Central Hatfield regions allow males to more readily attract females located in adjacent patches. In this way, habitat characteristics may act in concert with intersexual selection to influence variation in A songs. While geographic isolation and cultural drift can also explain geographic variation in acoustic signals (e.g., Hart et al. 2018; Schook et al. 2008), these factors may not be as important as habitat characteristics in explaining patterns of variation in A songs because males likely learn A songs during their hatching year (Byers and Kroodsma 1992) and at least some males may disperse a considerable distance from their natal sites as adults (Jetté et al. 1998; City of Austin and Travis County 2019; see below).

Geographic isolation and cultural drift may be more important than habitat characteristics in explaining patterns of variation in B songs, as some studies suggest that wood warblers may learn aspects of second category songs as adults (e.g., Byers and Kroodsma 1992). While I found no relationship between variation in A song characteristics and geographic distance, I did find a positive relationship between variation in B song characteristics and geographic distance. Male warblers tend to have high site fidelity as adults, but we have limited information regarding potential dispersal of juvenile males from their natal sites. Jetté et al. (1998) found that the average dispersal distance of 25 hatch year (HY) males at Fort Hood was approximately 4 km, but in 2019, a male that was uniquely color-banded as an HY in 2018 at JBSA-BUL was resighted approximately 134 km away in Travis County, Texas (City of Austin and Travis

County 2019). If male warblers disperse from their natal sites but, as adults, breed in the same general area each year, my results suggest that golden-cheeked warblers may learn the B songs of their neighbors as adults. The dominance of B song subtypes in different regions, and specifically the presence of the B North subtype at a single study site, also supports this hypothesis and may represent geographic isolation acting in concert with cultural drift, as patch size and occupancy decline with increasing latitude (Collier at al. 2012).

Regardless of any geographic patterns, if variation in A and B songs is driven by inter- and intra-sexual selection and A songs are only learned as HYs, one would expect B songs to exhibit more variation than A songs over time. Byers et al. (2010) found this to be the case for chestnut-sided warblers, and Leonard et al. (2010) provided evidence that this may be true for golden-cheeked warblers as well, though Leonard et al. (2010) only examined this phenomenon in warblers that occurred in one county and focused their formal analyses toward B songs. I did not investigate temporal variation in song characteristics as part of this study, but following up on my analyses of geographic patterns, I visually compared spectrograms from my study to spectrograms presented by Bolsinger (1997) and Leonard et al. (2010). Spectrograms of A songs from research conducted at the same study sites over time appeared similar (Appendix). However, most of the B songs reported in Bolsinger (1997) appeared similar in form to the B North subtype from my study, a subtype I only detected at PPMSP (Appendix). Furthermore, the B Central subtype I observed seemed similar to the novel B song subtype reported by Leonard et al. (2010). Formal analyses investigating variation in warbler song characteristics over time would help to quantify the patterns visible between spectrograms from different studies.

After I addressed my objectives, I also visually examined spectrograms of Bolsinger's (1997) C songs, and I believe that they likely represented a B song subtype rather than a third category of songs. Both Bolsinger's (1997) B and C songs contain elements similar to the most widespread B song from my study, the B Central subtype (see Figure 2 and Appendix). Bolsinger's (1997) C songs may be functionally equivalent to the B South subtype sung by individuals in my study whose "preferred" B song subtype was B Central. I considered the B South subtype sung by these individuals to be more similar in form to the dominant B song from the southwestern portion of the breeding range than to the B Central subtype (see Figure 2 and Appendix), hence my classification of them as "B South." Additionally, Bolsinger (1997) did not report a B song that resembled the B South subtype from my study. Perhaps different B song subtypes arise from copying errors and innovation in song learning which are then passed on to neighbors and spread across the breeding range (e.g., Janes and Ryker 2013). However, formal analyses examining variation in warbler B song form and characteristics over time are needed to further investigate patterns of cultural evolution in warbler songs.

I used FDAs to determine the relative ability of Hatfield and USFWS regions to explain variation in A and B songs, and found that Hatfield regions better explained variation in both. Although overall classifications using Hatfield regions were still somewhat low (64% for A songs and 60% for B songs), Hatfield regions outperformed USFWS regions, showing that variation in both A and B songs aligned more closely with Hatfield regions than USFWS regions. This could have implications for the delineation of management units that are biologically significant to the species if used in conjunction with other analyses (e.g., genetic and/or population viability analyses [PVA]). Hatfield et al. (2012) proposed recovery regions based on PVA estimates in conjunction with a desire to simplify the current recovery region boundaries. They showed that maintaining three populations of 3,000 breeding pairs results in a 99.9936% chance of the species persisting over 100 years. Other studies (e.g., Long et al. 2016;

Smith-Hicks et al. 2016) have found geographic variation in warbler responses across the breeding range, and maintaining viable populations in the three recovery regions proposed by Hatfield et al. (2012) may help preserve genetic and ecological diversity, a common goal for listed species.

Although previous genetic studies found little evidence that warblers exhibit genetic differentiation across their breeding range, researchers found that genetic differentiation has increased over time (Athrey et al. 2011) and that the current level of gene flow is insufficient to prevent genetic differentiation (Lindsay et al. 2008). New studies may reveal that genetic differentiation has continued to increase over time and that some groups of warblers now represent distinct populations. Research relating variation in A and B song characteristics to genetic distance could reveal the degree to which A and B songs reflect underlying genetic structure. Based on my results, I recommend including study sites at the northern and southwestern extremes of the breeding range (e.g., at PPMSP and KCSP) in genetic analyses or any other studies examining warbler-habitat relationships. A songs from the South Hatfield region were different than A songs from the rest of the breeding range, and B songs from USFWS Region 8 were different than B songs from the rest of the breeding range. Though my sample sizes for USFWS Region 1 prevented me from including this region in analyses comparing USFWS regions, I detected a B song subtype only present in this region, and the smallest and most fragmented habitat patches in the warbler’s breeding range occur in the north.

The results of my study show that golden-cheeked warblers exhibit geographic variation in song characteristics across their breeding range in Central Texas, and that patterns of variation differ between song types. Mine is the first study to quantify variation in golden-cheeked warbler song characteristics of both A and B songs using statistical analyses, and could be used in conjunction with other data to help inform conservation planning for this species. My results further our understanding of the characteristics and variation of wood warbler song and song categories, and contribute to our knowledge of songbird communication.

LITERATURE CITED

Athrey, G., D. L. Lindsay, R. F. Lance, and P. L. Leberg. 2011. Crumbling diversity: comparison of historical archived and contemporary natural populations indicate reduced genetic diversity and increasing genetic differentiation in the golden-cheeked warbler. Conservation Genetics 12:1345–1355.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society 57:289–300.

Billings, A. C. 2018. The low-frequency acoustic structure of mobbing calls differs across habitat types in three passerine families. Animal Behaviour 138:39–49.

Bolsinger, J. S. 1997. Patterns of use and variation in the songs of the golden-cheeked warbler (*Dendroica chrysoparia*). Thesis, University of Massachusetts Amherst, Amherst, Massachusetts, USA.

Bolsinger, J. S. 2000. Use of two song categories by golden-cheeked warblers. Condor 102:539–552.

Bolus, R. T. 2014. Geographic variation in songs of the common yellowthroat. Auk 131:175–185.

Buchholz-Sørensen, M., and A. Vella. 2016. Population structure, genetic diversity, effective population size, demographic history and regional connectivity patterns of the endangered dusky grouper, *Epinephelus marginatus* (Teleostei: Serranidae), within Malta’s Fisheries Management Zone. PLOS ONE 11:e0159864.

Burchett, W. W., A. R. Ellis, S. W. Harrar, and A. C. Bathke. 2017. Nonparametric inference for multivariate data: the R package npmv. Journal of Statistical Software 76(4):1–18.

Byers, B. E., and D. E. Kroodsma. 1992. Development of two song categories by chestnut-sided warblers. Animal Behaviour 44:799–810.

Byers, B. E., K. L. Belinsky, and R. A. Bentley. 2010. Independent cultural evolution of two song traditions in the chestnut-sided warbler. American Naturalist 176:476–489.

Campbell, L. 2003. Endangered and threatened animals of Texas: their life history and management. Texas Parks and Wildlife Department, Austin, Texas, USA.

Campbell, P., B. Pasch, J. L. Pino, O. L. Crino, M. Phillips, and S. M. Phelps. 2010. Geographic variation in the songs of neotropical singing mice: testing the relative importance of drift and local adaptation. Evolution 64:1955–1972.

Campomizzi, A. J., S. L. Farrell, T. M. McFarland, H. A. Mathewson, M. L. Morrison, and R. N. Wilkins. 2012. Species conservation at a broad spatial scale: reproductive success of golden-cheeked warblers across their breeding range. Wildlife Society Bulletin 36:440–449.

City of Austin and Travis County. 2019. 2019 annual report: golden-cheeked warbler (*Setophaga chrysoparia*) monitoring program Balcones Canyonlands Preserve. Austin Water Conservation Division and Travis County Department of Transportation and Natural Resources, Natural Resources and Environmental Quality Division, Balcones Canyonlands Preserve, Austin, Texas, USA.

Collier, B. A., J. E. Groce, M. L. Morrison, J. C. Newnam, A. J. Campomizzi, S. L. Farrell, H. A. Mathewson, R. T. Snelgrove, R. J. Carroll, and R. N. Wilkins. 2012. Predicting patch occupancy in fragmented landscapes at the rangewide scale for an endangered species: an example of an American warbler. Diversity and Distributions 18:158–167.

Diamond, D. D. 1997. An old-growth definition for western juniper woodlands: Texas Ashe juniper dominated or codominated communities. U.S. Department of Agriculture, Forest Service, Southern Research Station, Asheville, North Carolina, USA.

Domínguez, M., J. C. Reboreda, and B. Mahler. 2016. Effects of fragmentation and hybridization on geographical patterns of song variation in the endangered yellow cardinal *Gubernatrix cristata*. Ibis 158:738–746.

Dormann, C. F., J. Elith, S. Bacher, C. Buchmann, G. Carl, G. Carré, J. R. García Marquéz, B. Gruber, B. Lafourcade, P. J. Leitão, T. Münkemüller, C. McClean, P. E. Osborne, B. Reineking, B. Schröder, A. K. Skidmore, D. Zurell, and S. Lautenbach. 2012. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36:27–46.

Drever, M. C., L. W. Gyug, J. Nielsen, A. K. Stuart-Smith, I. P. Ohanjanian, and K. Martin. 2015. Using field data and GIS-derived variables to model occurrence of Williamson's sapsucker nesting habitat at multiple spatial scales. PLOS ONE 10:e0130849.

Etges, W. J., K. F. Over, C. C. De Oliveira, and M. G. Ritchie. 2006. Inheritance of courtship song variation among geographically isolated populations of *Drosophila mojavensis*. Animal Behaviour 71:1205–1214.

Ferreira, M., and J. W. H. Ferguson. 2002. Geographic variation in the calling song of the field cricket *Gryllus bimaculatus* (Orthoptera: Gryllidae) and its relevance to mate recognition and mate choice. Journal of Zoology 257:163–170.

Fine, M. L. 1978. Seasonal and geographical variation of the mating call of the oyster toadfish *Opsanus tau* L. Oecologia 36:45–57.

Goretskaia, M. I., I. R. Beme, D. V. Popova, N. Amos, K. L. Buchanan, P. Sunnucks, and A. Pavlova. 2018. Song parameters of the fuscous honeyeater *Lichenostomus fuscus* correlate with habitat characteristics in fragmented landscapes. Journal of Avian Biology 49:e01493.

Griffith, G., S. Bryce, J. Omernik, and A. Rogers. 2007. Ecoregions of Texas. Report prepared for the Texas Commission on Environmental Quality, Austin, Texas, USA.

Groce, J. E., H. A. Mathewson, M. L. Morrison, and R. N. Wilkins. 2010. Scientific evaluation for the 5-year status review of the golden-cheeked warbler. Institute of Renewable Natural Resources, Texas A&M University, College Station, Texas, USA.

Hart, P. J., E. Sebastián-González, A. Tanimoto, A. Thompson, T. Speetjecs, M. Hopkins, and M. Atencio-Picado. 2018. Birdsong characteristics are related to fragment size in a neotropical forest. Animal Behaviour 137:45–52.

Hastie, T., and R. Tibshirani. 2017. mda: mixture and flexible discriminant analysis. R package version 0.4-10. https://CRAN.R-project.org/package=mda.

Hastie, T., R. Tibshirani, and A. Buja. 1994. Flexible discriminant analysis by optimal scoring. Journal of the American Statistical Association 89:1255–1270.

Hatfield, J. S., F. W. Weckerly, and A. Duarte. 2012. Shifting foundations and metrics for golden-cheeked warbler recovery. Wildlife Society Bulletin 36:415–422.

Irwin, D. E., S. Bensch, and T. D. Price. 2001. Speciation in a ring. Nature 409:333–337.

Irwin, D. E., M. P. Thimgan, and J. H. Irwin. 2008. Call divergence is correlated with geographic and genetic distance in greenish warblers (*Phylloscopus trochiloides*): a strong role for stochasticity in signal evolution? Journal of Evolutionary Biology 21:435–448.

Jain, A. K. 2010. Data clustering: 50 years beyond K-means. Pattern Recognition Letters 31:651–666.

Janes, S. W., and L. Ryker. 2006. Singing of hermit warblers: dialects of type I songs. Condor 108:336–347.

Janes, S. W., and L. Ryker. 2011. Geographic variation in type I songs of black-throated gray warblers. Wilson Journal of Ornithology 123:339–346.

Janes, S. W., and L. Ryker. 2013. Rapid change in a type I song dialect of hermit warblers (*Setophaga occidentalis*). Auk 130:30–35.

Jetté, L. A., T. J. Hayden, and J. D. Cornelius. 1998. Demographics of the golden-cheeked warbler (*Dendroica chrysoparia*) on Fort Hood, Texas. U.S. Army Corps of Engineers Technical Report 98/52.

Karanth, K. K., J. D. Nichols, J. R. Sauer, and J. E. Hines. 2006. Comparative dynamics of avian communities across edges and interiors of North American ecoregions. Journal of Biogeography 33:674–682.

Kroodsma, D. E. 1981. Geographical variation and functions of song types in warblers (Parulidae). Auk 98:743–751.

Kroodsma, D. E., B. E. Byers, S. L. Halkin, C. Hill, D. Minis, J. R. Bolsinger, J. Dawson, E. Donelan, J. Farrington, F. B. Gill, P. Houlihan, D. Innes, G. Keller, L. Macaulay, C. A. Marantz, J. Ortiz, P. K. Stoddard, and K. Wilda. 1999. Geographic variation in black-capped chickadee songs and singing behavior. Auk 116:387–402.

Lachlan, R. F., and M. R. Servedio. 2004. Song learning accelerates allopatric speciation. Evolution 58:2049–2063.

Ladd, C., and L. Gass. 2020. Golden-cheeked warbler (*Setophaga chrysoparia*), version 1.0. *In* P. G. Rodelwald, editor. Birds of the World. Cornell Lab of Ornithology, Ithaca, New York, USA.

Lemon, R. E. 1975. How birds develop song dialects. Condor 77:385–406.

Leonard, W. J., J. Neal, and R. Ratnam. 2010. Variation of type B song in the endangered golden-cheeked warbler (*Dendroica chrysoparia*). Wilson Journal of Ornithology 122:777–780.

Li, Y., S. J. B. Cooper, M. L. Lancaster, J. G. Packer, and S. M. Carthew. 2016. Comparative population genetic structure of the endangered southern brown bandicoot, *Isoodon obesulus*, in fragmented landscapes of southern Australia. PLOS ONE 11:e0152850.

Lindsay, D. L., K. R. Barr, R. F. Lance, S. A. Tweddale, T. J. Hayden, and P. L. Leberg. 2008. Habitat fragmentation and genetic diversity of an endangered, migratory songbird, the golden-cheeked warbler (*Dendroica chrysoparia*). Molecular Ecology 17:2122–2133.

Liou, L. W., and T. D. Price. 1994. Speciation by reinforcement of premating isolation. Evolution 48:1451–1459.

Lloyd, S. P. 1982. Least squares quantization in PCM. IEEE Transactions on Information Theory 28:129–137.

Long, A. M., J. C. Newnam, M. R. Colón, K. N. Smith-Hicks, and M. L. Morrison. 2016. Characteristics of vegetation used by golden-cheeked warblers in Central Texas. Southeastern Naturalist 15:153–161.

Luttrell, S. A. M., and B. Lohr. 2018. Geographic variation in call structure, likelihood, and call-song associations across subspecies boundaries, migratory patterns, and habitat types in the marsh wren (*Cistothorus palustris*). Auk 135:127–151.

MacDougall-Shackleton, E. A., and S. A. MacDougall-Shackleton. 2001. Cultural and genetic evolution in mountain white-crowned sparrows: song dialects are associated with population structure. Evolution 55:2568–2575.

Marler, P., and M. Tamura. 1962. Song "dialects" in three populations of white-crowned sparrows. Condor 64:368–377.

Morton, E. S. 1975. Ecological sources of selection on avian sounds. American Naturalist 109:17–34.

Naimi, B., N. A. S. Hamm, T. A. Groen, A. K. Skidmore, and A. G. Toxopeus. 2014. Where is positional uncertainty a problem for species distribution modelling? Ecography 37:191–203.

NOAA. 2014. National Climatic Data Center Reports. http://www.ncdc.noaa.gov/. Accessed 5 January 2015.

Nottebohm, F. 1969. The song of the chingolo, *Zonotrichia capensis*, in Argentina: description and evaluation of a system of dialects. Condor 71:299–315.

Pérez-Granados, C., T. Osiejuk, and G. M. López-Iborra. 2016. Habitat fragmentation effects and variations in repertoire size and degree of song sharing among close Dupont's lark *Chersophilus duponti* populations. Journal of Ornithology 157:471–482.

Platz, J. E., and D. C. Forester. 1988. Geographic variation in mating call among the four subspecies of the chorus frog: *Pseudacris triseriata* (Wied). Copeia 1988:1062–1066.

Poulsen, J., and A. French. 2004. Discriminant function analysis (DA). San Francisco State University, San Francisco, California, USA.

Ptacek, M. B. 2000. The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. Behavioural Processes 51:111–134.

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Roach, S. P., and L. S. Phillmore. 2017. Geographic variation in song structure in the hermit thrush (*Catharus guttatus*). Auk 134:612–626.

Roberts, D. G., J. Baker, and C. Perrin. 2011. Population genetic structure of the endangered eastern bristlebird, *Dasyornis brachypterus*; implications for conservation. Conservation Genetics 12:1075–1085.

Rousseeuw, P. J. 1987. Silhouettes: a graphical aid to the interpretation and validation of cluster analysis. Journal of Computational and Applied Mathematics 20:53–65.

Ryan, M. J. 1986. Neuroanatomy influences speciation rates among anurans. Proceedings of the National Academy of Sciences 83:1379–1382.

Schook, D. M., M. D. Collins, W. E. Jensen, P. J. Williams, N. E. Bader, and T. H. Parker. 2008. Geographic patterns of song similarity in the dickcissel (*Spiza americana*). Auk 125:953–964.

Simmons, L. W., M. Zuk, and J. T. Rotenberry. 2001. Geographic variation in female preference functions and male songs of the field cricket *Teleogryllus oceanicus*. Evolution 55:1386–1394.

Slabbekoorn, H. 2004. Singing in the wild: the ecology of birdsong. Pages 178–205 *in* P. Marler and H. Slabbekoorn, editors. Nature’s Music: The Science of Birdsong. Elsevier Academic Press, San Diego, California, USA.

Smith-Hicks, K. N., J. C. Newnam, M. R. Colón, A. M. Long, and M. L. Morrison. 2016. Golden-cheeked warbler behavior in relation to vegetation characteristics across their breeding range. American Midland Naturalist 176:81–94.

Snyder, W. F., and D. L. Jameson. 1965. Multivariate geographic variation of mating call in populations of the Pacific tree frog (*Hyla regilla*). Copeia 1965:129–142.

Spector, D. A. 1992. Wood-warbler song systems: a review of paruline singing behaviors. Current Ornithology 9:199–238.

Szczecińska, M., G. Sramko, K. Wołosz, and J. Sawicki. 2016. Genetic diversity and population structure of the rare and endangered plant species *Pulsatilla patens (L.) Mill* in east central Europe. PLOS ONE 11:e0151730.

USFWS. 1990. Endangered and threatened wildlife and plants; final rule to list the golden-cheeked warbler as endangered. Federal Register 55:53153–53160.

USFWS. 1992. Golden-cheeked warbler (*Dendroica chrysoparia*) recovery plan. USFWS, Albuquerque, New Mexico, USA.

Van Parijs, S. M., G. D. Hastie, and P. M. Thompson. 2000. Individual and geographical variation in display behaviour of male harbour seals in Scotland. Animal Behaviour 59:559–568.

Yandell, D. D., W. M. Hochachka, S. Pruett-Jones, M. S. Webster, and E. I. Greig. 2018. Geographic patterns of song variation in four species of *Malurus* fairy-wrens. Journal of Avian Biology 49:e01446.

Zar, J. H. 1999. Biostatistical analysis. Fourth Edition. Prentice Hall, Upper Saddle River, New Jersey, USA.

APPENDIX

Table A-1. Sample sizes, means, and 95% confidence intervals for golden-cheeked warbler (*Setophaga chrysoparia*) A song metrics (averaged within individuals) per recovery region proposed by Hatfield et al. (2012); songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, metrics that were significantly different among regions are starred, different shades of gray represent significantly different regions, and if two regions were significantly different and the third was not, the third is presented with a border.

		Segment 1						
Hatfield region	*n*	Duration (s)*	Peak frequency (kHz)*	Average lower frequency (kHz)*	Average upper frequency (kHz)	Average bandwidth (kHz)	Notes*	Notes s^{-1}
North	29	0.83 (0.76–0.91)	5.03 (4.93–5.13)	4.06 (3.88–4.25)	5.95 (5.88–6.03)	1.89 (1.70–2.08)	5.34 (4.90–5.78)	5.99 (5.75–6.23)
Central	35	0.81 (0.74–0.88)	5.02 (4.94–5.10)	4.03 (3.91–4.14)	6.11 (6.06–6.15)	2.08 (1.98–2.18)	5.72 (5.36–6.07)	6.55 (6.18–6.92)
South	20	0.68 (0.62–0.74)	4.50 (4.37–4.62)	3.63 (3.43–3.83)	5.95 (5.60–6.31)	2.32 (1.87–2.78)	4.38 (4.02–4.74)	6.06 (5.57–6.54)

		Segment 2				
Hatfield region	*n*	Duration (s)*	Peak frequency (kHz)*	Average lower frequency (kHz)*	Average upper frequency (kHz)*	Average bandwidth (kHz)*
North	29	0.44 (0.41–0.46)	5.69 (5.58–5.79)	4.33 (4.26–4.39)	6.47 (6.38–6.57)	2.15 (2.09–2.21)
Central	35	0.45 (0.43–0.48)	5.75 (5.66–5.83)	4.29 (4.22–4.35)	6.54 (6.47–6.62)	2.26 (2.18–2.34)
South	20	0.52 (0.44–0.61)	5.38 (5.26–5.50)	4.16 (4.03–4.28)	6.16 (6.03–6.29)	2.01 (1.84–2.17)

		Segment 3				
Hatfield region	*n*	Duration (s)	Peak frequency (kHz)*	Average lower frequency (kHz)*	Average upper frequency (kHz)*	Average bandwidth (kHz)*
North	28	0.25 (0.23–0.26)	6.89 (6.79–6.99)	6.07 (5.95–6.19)	7.79 (7.62–7.96)	1.72 (1.52–1.92)
Central	34	0.23 (0.22–0.25)	6.93 (6.87–6.99)	6.21 (6.16–6.26)	7.70 (7.61–7.79)	1.49 (1.41–1.57)
South	19	0.24 (0.22–0.26)	6.41 (6.27–6.54)	5.79 (5.68–5.90)	7.00 (6.82–7.18)	1.21 (1.08–1.33)

		Song			Difference in segments 3 and 2			
Hatfield region	*n*	Duration (s)	Peak frequency (kHz)	Time to peak frequency (s)	*n*	Peak frequency (kHz)*	Average lower frequency (kHz)*	Average upper frequency (kHz)*
North	29	1.53 (1.44–1.62)	6.02 (5.81–6.22)	1.11 (1.01–1.20)	28	1.19 (1.07–1.30)	1.73 (1.61–1.85)	1.30 (1.13–1.46)
Central	35	1.52 (1.46–1.58)	6.07 (5.84–6.30)	1.11 (1.04–1.17)	34	1.19 (1.10–1.27)	1.92 (1.86–1.99)	1.15 (1.06–1.24)
South	20	1.46 (1.38–1.53)	5.67 (5.47–5.87)	1.10 (1.03–1.17)	19	1.00 (0.91–1.10)	1.60 (1.45–1.75)	0.81 (0.70–0.92)

Table A-2. Sample sizes, means, and 95% confidence intervals for 22 of 34 golden-cheeked warbler (*Setophaga chrysoparia*) B song metrics (averaged within individuals) per recovery region proposed by Hatfield et al. (2012); songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, metrics that were significantly different among regions are starred, different shades of gray represent significantly different regions, and if two regions were significantly different and the third was not, the third is presented with a border.

Segment 2						
Hatfield region	*n*	Duration (s)*	Peak frequency (kHz)*	Average lower frequency (kHz)	Average upper frequency (kHz)*	Average bandwidth (kHz)
North	27	0.55 (0.48–0.62)	4.86 (4.76–4.96)	3.77 (3.55–3.99)	5.53 (5.43–5.62)	1.76 (1.57–1.95)
Central	43	0.62 (0.59–0.64)	4.71 (4.62–4.80)	3.59 (3.48–3.70)	5.37 (5.30–5.44)	1.78 (1.67–1.89)
South	33	0.54 (0.51–0.56)	4.58 (4.46–4.70)	3.59 (3.48–3.69)	5.20 (5.09–5.31)	1.61 (1.51–1.72)

Segment 3						
Hatfield region	*n*	Duration (s)*	Peak frequency (kHz)*	Average lower frequency (kHz)	Average upper frequency (kHz)*	Average bandwidth (kHz)*
North	29	0.48 (0.45–0.52)	5.77 (5.58–5.96)	4.28 (4.16–4.40)	6.89 (6.62–7.15)	2.61 (2.39–2.82)
Central	44	0.47 (0.44–0.49)	5.80 (5.66–5.94)	4.22 (4.16–4.28)	7.07 (6.93–7.22)	2.86 (2.71–3.01)
South	33	0.40 (0.39–0.42)	5.45 (5.29–5.61)	4.29 (4.19–4.39)	6.59 (6.40–6.78)	2.30 (2.11–2.50)

Segment 4								
Hatfield region	*n*	Duration (s)*	Peak frequency (kHz)*	Average lower frequency (kHz)*	Average upper frequency (kHz)*	Average bandwidth (kHz)*	Notes	Notes s^{-1}*
North	29	0.63 (0.54–0.72)	4.48 (4.28–4.68)	3.39 (3.27–3.51)	5.23 (4.98–5.49)	1.84 (1.60–2.08)	2.86 (2.35–3.38)	5.26 (3.90–6.62)
Central	44	0.67 (0.61–0.73)	3.99 (3.94–4.05)	3.13 (3.08–3.18)	4.70 (4.60–4.80)	1.57 (1.45–1.69)	3.51 (3.18–3.85)	5.41 (4.86–5.97)
South	33	0.47 (0.38–0.55)	4.18 (4.09–4.27)	3.15 (3.06–3.24)	5.02 (4.88–5.17)	1.87 (1.71–2.03)	3.40 (2.97–3.82)	8.68 (7.15–10.21)

Segment 5						
Hatfield region	*n*	Duration (s)*	Peak frequency (kHz)*	Average lower frequency (kHz)*	Average upper frequency (kHz)*	Average bandwidth (kHz)
North	16	0.26 (0.23–0.29)	5.12 (4.90–5.35)	3.40 (3.22–3.58)	6.53 (6.08–6.98)	3.13 (2.55–3.72)
Central	24	0.28 (0.26–0.30)	5.19 (4.94–5.44)	3.65 (3.41–3.89)	6.53 (6.27–6.78)	2.88 (2.53–3.23)
South	24	0.23 (0.21–0.26)	5.91 (5.68–6.14)	4.10 (3.86–4.34)	7.11 (6.82–7.40)	3.01 (2.64–3.37)

Table A-3. Sample sizes, means, and 95% confidence intervals for 12 of 34 golden-cheeked warbler (*Setophaga chrysoparia*) B song metrics (averaged within individuals) per recovery region proposed by Hatfield et al. (2012); songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, metrics that were significantly different among regions are starred, different shades of gray represent significantly different regions, and if two regions were significantly different and the third was not, the third is presented with a border.

		Song		
Hatfield region	*n*	Duration (s)	Peak frequency (kHz)*	Time to peak frequency (s)
North	29	1.89 (1.78–2.00)	5.13 (4.90–5.35)	1.13 (1.03–1.22)
Central	44	1.98 (1.89–2.07)	4.66 (4.45–4.87)	1.21 (1.13–1.30)
South	33	1.94 (1.87–2.01)	4.85 (4.69–5.01)	1.22 (1.17–1.27)
		Difference in segments 3 and 2		
Hatfield region	*n*	Peak frequency (kHz)	Average lower frequency (kHz)	Average upper frequency (kHz)*
North	27	0.96 (0.82–1.10)	0.54 (0.34–0.74)	1.45 (1.21–1.69)
Central	43	1.10 (0.97–1.24)	0.63 (0.50–0.76)	1.72 (1.60–1.85)
South	33	0.87 (0.73–1.01)	0.70 (0.57–0.83)	1.39 (1.25–1.53)
		Difference in segments 4 and 3		
Hatfield region	*n*	Peak frequency (kHz)*	Average lower frequency (kHz)*	Average upper frequency (kHz)*
North	29	-1.29 (-1.61–-0.97)	-0.89 (-1.06–-0.71)	-1.65 (-2.12–-1.18)
Central	44	-1.81 (-1.95–-1.67)	-1.09 (-1.16–-1.02)	-2.37 (-2.57–-2.18)
South	33	-1.27 (-1.46–-1.08)	-1.14 (-1.27–-1.00)	-1.57 (-1.84–-1.30)
		Difference in segments 5 and 4		
Hatfield region	*n*	Peak frequency (kHz)*	Average lower frequency (kHz)*	Average upper frequency (kHz)*
North	16	0.40 (-0.07–0.86)	-0.04 (-0.29–0.20)	0.98 (0.21–1.76)
Central	24	1.25 (0.97–1.53)	0.56 (0.33–0.79)	1.85 (1.57–2.12)
South	24	1.76 (1.54–1.99)	0.91 (0.66–1.16)	2.19 (1.97–2.41)

Table A-4. Sample sizes, means, and 95% confidence intervals for 15 of 26 golden-cheeked warbler (*Setophaga chrysoparia*) B song metrics (averaged within individuals) per current recovery region (USFWS 1992); songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, and metrics that were significantly different among regions are starred.

		Segment 2				
USFWS region	*n*	Duration (s)*	Peak frequency (kHz)*	Average lower frequency (kHz)	Average upper frequency (kHz)*	Average bandwidth (kHz)
2	11	0.57 (0.53–0.61)	4.90 (4.69–5.11)	3.68 (3.25–4.12)	5.56 (5.35–5.76)	1.87 (1.50–2.25)
3	10	0.63 (0.62–0.65)	4.87 (4.70–5.05)	3.90 (3.49–4.30)	5.50 (5.34–5.66)	1.60 (1.32–1.89)
4	10	0.63 (0.58–0.68)	4.82 (4.59–5.05)	3.67 (3.38–3.95)	5.46 (5.27–5.65)	1.79 (1.45–2.14)
5	20	0.61 (0.57–0.65)	4.73 (4.60–4.85)	3.65 (3.49–3.82)	5.35 (5.25–5.45)	1.69 (1.55–1.84)
6	13	0.62 (0.57–0.67)	4.59 (4.42–4.76)	3.43 (3.26–3.61)	5.33 (5.21–5.45)	1.90 (1.70–2.09)
7	18	0.56 (0.54–0.59)	4.54 (4.36–4.73)	3.62 (3.45–3.78)	5.17 (4.98–5.36)	1.55 (1.37–1.74)
8	15	0.50 (0.47–0.54)	4.63 (4.47–4.80)	3.56 (3.42–3.70)	5.24 (5.11–5.37)	1.68 (1.60–1.77)

		Segment 3				
USFWS region	*n*	Duration (s)*	Peak frequency (kHz)*	Average lower frequency (kHz)	Average upper frequency (kHz)*	Average bandwidth (kHz)*
2	11	0.46 (0.43–0.50)	5.87 (5.54–6.21)	4.42 (4.20–4.64)	7.04 (6.75–7.33)	2.62 (2.36–2.88)
3	10	0.45 (0.42–0.48)	5.92 (5.71–6.12)	4.33 (4.16–4.50)	7.15 (6.84–7.47)	2.82 (2.50–3.14)
4	10	0.45 (0.41–0.48)	5.90 (5.68–6.12)	4.27 (4.15–4.40)	7.16 (6.81–7.50)	2.88 (2.48–3.28)
5	21	0.44 (0.41–0.48)	5.90 (5.66–6.13)	4.21 (4.10–4.31)	7.14 (6.92–7.37)	2.94 (2.74–3.13)
6	13	0.52 (0.46–0.58)	5.58 (5.33–5.82)	4.20 (4.08–4.31)	6.90 (6.61–7.19)	2.70 (2.37–3.04)
7	18	0.41 (0.38–0.44)	5.51 (5.27–5.75)	4.25 (4.09–4.41)	6.74 (6.43–7.05)	2.49 (2.18–2.81)
8	15	0.39 (0.37–0.42)	5.38 (5.15–5.61)	4.34 (4.23–4.45)	6.42 (6.23–6.61)	2.08 (1.89–2.26)

		Segment 4				
USFWS region	*n*	Duration (s)*	Peak frequency (kHz)*	Average lower frequency (kHz)	Average upper frequency (kHz)*	Average bandwidth (kHz)*
2	11	0.58 (0.45–0.72)	4.26 (4.09–4.42)	3.33 (3.19–3.47)	4.84 (4.63–5.04)	1.51 (1.23–1.78)
3	10	0.61 (0.37–0.86)	4.21 (4.02–4.40)	3.32 (3.09–3.55)	4.95 (4.78–5.13)	1.64 (1.33–1.95)
4	10	0.64 (0.50–0.79)	3.98 (3.85–4.12)	3.14 (3.04–3.24)	4.82 (4.44–5.21)	1.69 (1.27–2.10)
5	21	0.67 (0.57–0.77)	4.04 (3.96–4.13)	3.16 (3.08–3.25)	4.67 (4.55–4.78)	1.51 (1.34–1.67)
6	13	0.68 (0.58–0.78)	3.92 (3.85–3.99)	3.08 (2.99–3.17)	4.65 (4.53–4.77)	1.57 (1.43–1.71)
7	18	0.55 (0.42–0.68)	4.13 (3.98–4.28)	3.17 (3.05–3.30)	4.95 (4.73–5.18)	1.78 (1.57–1.99)
8	15	0.37 (0.26–0.47)	4.24 (4.13–4.35)	3.13 (2.98–3.27)	5.11 (4.92–5.30)	1.98 (1.73–2.23)

Table A-5. Sample sizes, means, and 95% confidence intervals for 11 of 26 golden-cheeked warbler (*Setophaga chrysoparia*) B song metrics (averaged within individuals) per current recovery region (USFWS 1992); songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, and metrics that were significantly different among regions are starred.

USFWS	Segment 4		
region	*n*	Notes*	Notes s^{-1}*
2	11	2.30 (1.61–3.00)	3.86 (3.01–4.70)
3	10	2.62 (1.72–3.53)	6.59 (2.61–10.57)
4	10	3.66 (2.42–4.90)	5.50 (3.83–7.18)
5	21	3.34 (2.85–3.82)	5.07 (4.33–5.82)
6	13	3.69 (3.46–3.91)	5.90 (4.86–6.93)
7	18	3.94 (3.33–4.55)	8.26 (6.75–9.78)
8	15	2.74 (2.31–3.17)	9.17 (6.10–12.25)

	Song			
USFWS region	*n*	Duration (s)	Peak frequency (kHz)*	Time to peak frequency (s)
2	11	1.85 (1.65–2.05)	5.04 (4.52–5.56)	1.06 (0.94–1.18)
3	10	1.96 (1.69–2.24)	5.19 (4.86–5.52)	1.16 (0.95–1.37)
4	10	1.88 (1.68–2.08)	4.82 (4.34–5.30)	1.19 (1.00–1.38)
5	21	1.94 (1.78–2.10)	4.61 (4.26–4.97)	1.17 (1.03–1.31)
6	13	2.13 (1.99–2.26)	4.61 (4.30–4.92)	1.31 (1.17–1.45)
7	18	1.99 (1.88–2.09)	4.62 (4.41–4.82)	1.23 (1.15–1.32)
8	15	1.89 (1.79–1.98)	5.13 (4.96–5.31)	1.20 (1.13–1.27)

	Difference in segments 3 and 2			
USFWS region	*n*	Peak frequency (kHz)	Average lower frequency (kHz)	Average upper frequency (kHz)*
2	11	0.97 (0.80–1.14)	0.74 (0.42–1.05)	1.48 (1.27–1.69)
3	10	1.05 (0.85–1.25)	0.43 (0.03–0.83)	1.65 (1.38–1.93)
4	10	1.08 (0.90–1.26)	0.61 (0.39–0.83)	1.70 (1.43–1.96)
5	20	1.18 (0.95–1.42)	0.56 (0.33–0.78)	1.84 (1.66–2.02)
6	13	0.99 (0.72–1.26)	0.76 (0.52–1.01)	1.57 (1.29–1.85)
7	18	0.96 (0.76–1.17)	0.63 (0.43–0.84)	1.57 (1.37–1.77)
8	15	0.75 (0.55–0.95)	0.78 (0.62–0.95)	1.18 (1.05–1.31)

	Difference in segments 4 and 3			
USFWS region	*n*	Peak frequency (kHz)*	Average lower frequency (kHz)	Average upper frequency (kHz)*
2	11	-1.62 (-1.90–-1.34)	-1.09 (-1.33–-0.84)	-2.20 (-2.52–-1.88)
3	10	-1.71 (-1.93–-1.48)	-1.01 (-1.25–-0.78)	-2.20 (-2.57–-1.83)
4	10	-1.92 (-2.15–-1.69)	-1.13 (-1.28–-0.99)	-2.33 (-2.87–-1.79)
5	21	-1.86 (-2.10–-1.61)	-1.05 (-1.18–-0.92)	-2.47 (-2.77–-2.18)
6	13	-1.66 (-1.90–-1.42)	-1.11 (-1.23–-0.99)	-2.25 (-2.61–-1.89)
7	18	-1.37 (-1.65–-1.10)	-1.07 (-1.26–-0.88)	-1.79 (-2.21–-1.36)
8	15	-1.14 (-1.42–-0.87)	-1.21 (-1.41–-1.01)	-1.31 (-1.60–-1.02)

Table A-6. Results of ANOVAs comparing golden-cheeked warbler (*Setophaga chrysoparia*) A song metrics (averaged within individuals) among recovery regions proposed by Hatfield et al. (2012); songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, 16 of 23 metrics were significantly different after correcting for multiple testing (Benjamini-Hochberg false discovery rate, α = 0.05), and metrics are ranked from lowest to highest *P* value with significant values in bold.

Rank	Metric	*n*	*F*	*P*
1	Segment 3 peak frequency	81	34.35	**<0.001**
2	Segment 3 average upper frequency	81	33.17	**<0.001**
3	Segment 1 peak frequency	84	33.05	**<0.001**
4	Segment 3 average lower frequency	81	19.73	**<0.001**
5	Segment 2 average upper frequency	84	16.92	**<0.001**
6	Segment 2 peak frequency	84	13.60	**<0.001**
7	Difference in segments 3 and 2 average upper frequency	81	13.59	**<0.001**
8	Segment 3 average bandwidth	81	11.20	**<0.001**
9	Segment 1 notes	84	11.02	**<0.001**
10	Difference in segments 3 and 2 average lower frequency	81	9.88	**<0.001**
11	Segment 1 average lower frequency	84	7.59	**0.001**
12	Segment 2 average bandwidth	84	6.86	**0.002**
13	Segment 2 duration	84	4.75	**0.011**
14	Segment 1 duration	84	4.60	**0.013**
15	Segment 2 average lower frequency	84	4.37	**0.016**
16	Difference in segments 3 and 2 peak frequency	81	3.77	**0.027**
17	Song peak frequency	84	3.39	0.039
18	Segment 1 notes per second	84	3.33	0.041
19	Segment 1 average bandwidth	84	3.27	0.043
20	Segment 1 average upper frequency	84	1.55	0.218
21	Song duration	84	0.95	0.390
22	Segment 3 duration	81	0.81	0.449
23	Time to peak frequency of song	84	0.02	0.985

Table A-7. Results of ANOVAs comparing golden-cheeked warbler (*Setophaga chrysoparia*) B song metrics (averaged within individuals) among recovery regions proposed by Hatfield et al. (2012); songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, 25 of 34 metrics were significantly different after correcting for multiple testing (Benjamini-Hochberg false discovery rate, α = 0.05), and metrics are ranked from lowest to highest *P* value with significant values in bold.

Rank	Metric	*n*	*F*	*P*
1	Difference in segments 5 and 4 peak frequency	64	19.60	**<0.001**
2	Segment 4 peak frequency	106	18.95	**<0.001**
3	Difference in segments 5 and 4 average lower frequency	64	14.61	**<0.001**
4	Segment 5 peak frequency	64	14.36	**<0.001**
5	Segment 4 average upper frequency	106	12.24	**<0.001**
6	Segment 4 notes per second	106	11.79	**<0.001**
7	Segment 2 average upper frequency	103	11.42	**<0.001**
8	Segment 4 average lower frequency	106	11.25	**<0.001**
9	Segment 3 average bandwidth	106	10.18	**<0.001**
10	Difference in segments 4 and 3 peak frequency	106	9.87	**<0.001**
11	Difference in segments 4 and 3 average upper frequency	106	9.87	**<0.001**
12	Segment 5 average lower frequency	64	9.53	**<0.001**
13	Difference in segments 5 and 4 average upper frequency	64	9.12	**<0.001**
14	Segment 3 duration	106	8.65	**<0.001**
15	Segment 4 duration	106	8.32	**<0.001**
16	Segment 3 average upper frequency	106	6.83	**0.002**
17	Segment 2 peak frequency	103	6.50	**0.002**
18	Segment 3 peak frequency	106	6.09	**0.003**
19	Segment 2 duration	103	5.80	**0.004**
20	Song peak frequency	106	5.51	**0.005**
21	Difference in segments 3 and 2 average upper frequency	103	5.45	**0.006**
22	Segment 5 average upper frequency	64	5.10	**0.009**
23	Segment 4 average bandwidth	106	4.71	**0.011**
24	Difference in segments 4 and 3 average lower frequency	106	4.35	**0.015**
25	Segment 5 duration	64	3.81	**0.028**
26	Difference in segments 3 and 2 peak frequency	103	3.16	0.047
27	Segment 4 notes	106	2.70	0.072
28	Segment 2 average lower frequency	103	2.01	0.140
29	Segment 2 average bandwidth	103	2.01	0.140
30	Time to peak frequency of song	106	1.51	0.225
31	Difference in segments 3 and 2 average lower frequency	103	1.04	0.375
32	Song duration	106	0.94	0.394
33	Segment 3 average lower frequency	106	0.88	0.416
34	Segment 5 average bandwidth	64	0.37	0.690

Table A-8. Results of ANOVAs comparing golden-cheeked warbler (*Setophaga chrysoparia*) B song metrics (averaged within individuals) among current recovery regions (USFWS 1992); songs were recorded in 2012, 2017, and 2018 across the breeding range of the golden-cheeked warbler in Central Texas, 17 of 26 metrics were significantly different after correcting for multiple testing (Benjamini-Hochberg false discovery rate, α = 0.05), and metrics are ranked from lowest to highest *P* value with significant values in bold.

Rank	Metric	*n*	*F*	*P*
1	Segment 2 duration	97	6.35	**<0.001**
2	Difference in segments 4 and 3 average upper frequency	98	5.85	**<0.001**
3	Segment 3 average bandwidth	98	5.55	**<0.001**
4	Difference in segments 4 and 3 peak frequency	98	5.06	**<0.001**
5	Segment 3 duration	98	5.03	**<0.001**
6	Difference in segments 3 and 2 average upper frequency	97	4.83	**<0.001**
7	Segment 3 average upper frequency	98	4.68	**<0.001**
8	Segment 4 peak frequency	98	4.42	**0.001**
9	Segment 4 notes per second	98	4.41	**0.001**
10	Segment 4 notes	98	4.21	**0.001**
11	Segment 3 peak frequency	98	3.77	**0.002**
12	Segment 2 average upper frequency	97	3.65	**0.003**
13	Segment 4 average upper frequency	98	3.48	**0.004**
14	Segment 4 duration	98	3.38	**0.005**
15	Segment 2 peak frequency	97	2.84	**0.014**
16	Song peak frequency	98	2.69	**0.019**
17	Segment 4 average bandwidth	98	2.57	**0.024**
18	Segment 4 average lower frequency	98	2.10	0.061
19	Difference in segments 3 and 2 peak frequency	97	1.86	0.096
20	Segment 2 average bandwidth	97	1.70	0.130
21	Segment 2 average lower frequency	97	1.42	0.217
22	Segment 3 average lower frequency	98	1.41	0.219
23	Song duration	98	1.40	0.224
24	Time to peak frequency of song	98	1.31	0.260
25	Difference in segments 3 and 2 average lower frequency	97	1.08	0.380
26	Difference in segments 4 and 3 average lower frequency	98	0.59	0.738

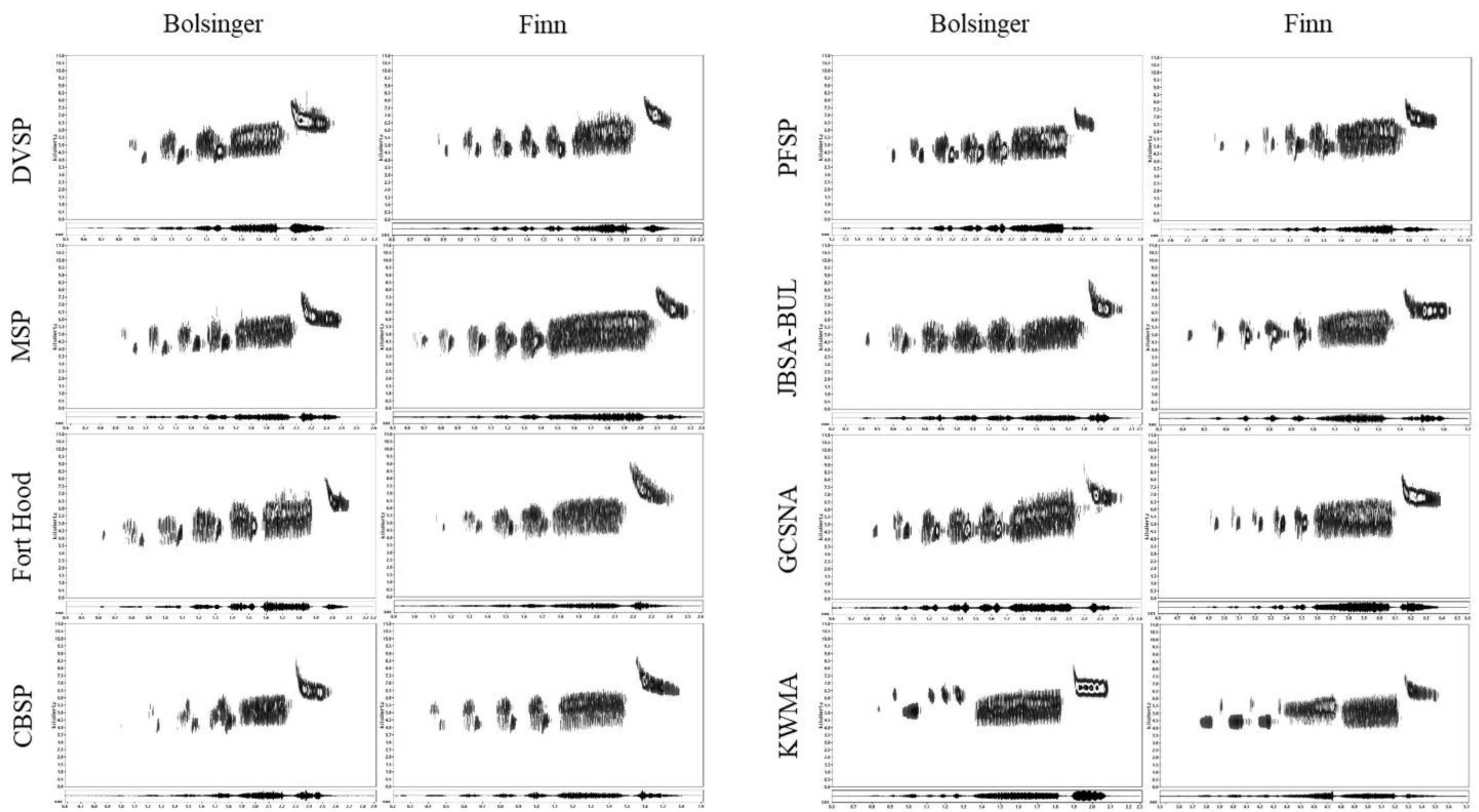

Figure A-1. Spectrograms of golden-cheeked warbler (*Setophaga chrysoparia*) A songs recorded at Dinosaur Valley State Park (DVSP), Meridian State Park (MSP), Fort Hood, Colorado Bend State Park (CBSP), Pedernales Falls State Park (PFSP), Joint Base San Antonio-Camp Bullis (JBSA-BUL), Government Canyon State Natural Area (GCSNA), and Kerr Wildlife Management Area (KWMA) in 1994 and 1995 (Bolsinger) and 2012, 2017, and 2018 (Finn). I used the following recordings from the Macaulay Library at the Cornell Lab of Ornithology to create spectrograms representing A songs from 1994 and 1995: ML109473, ML109583, ML109639, ML109696, ML109753, ML109771, ML109870, and ML110138.

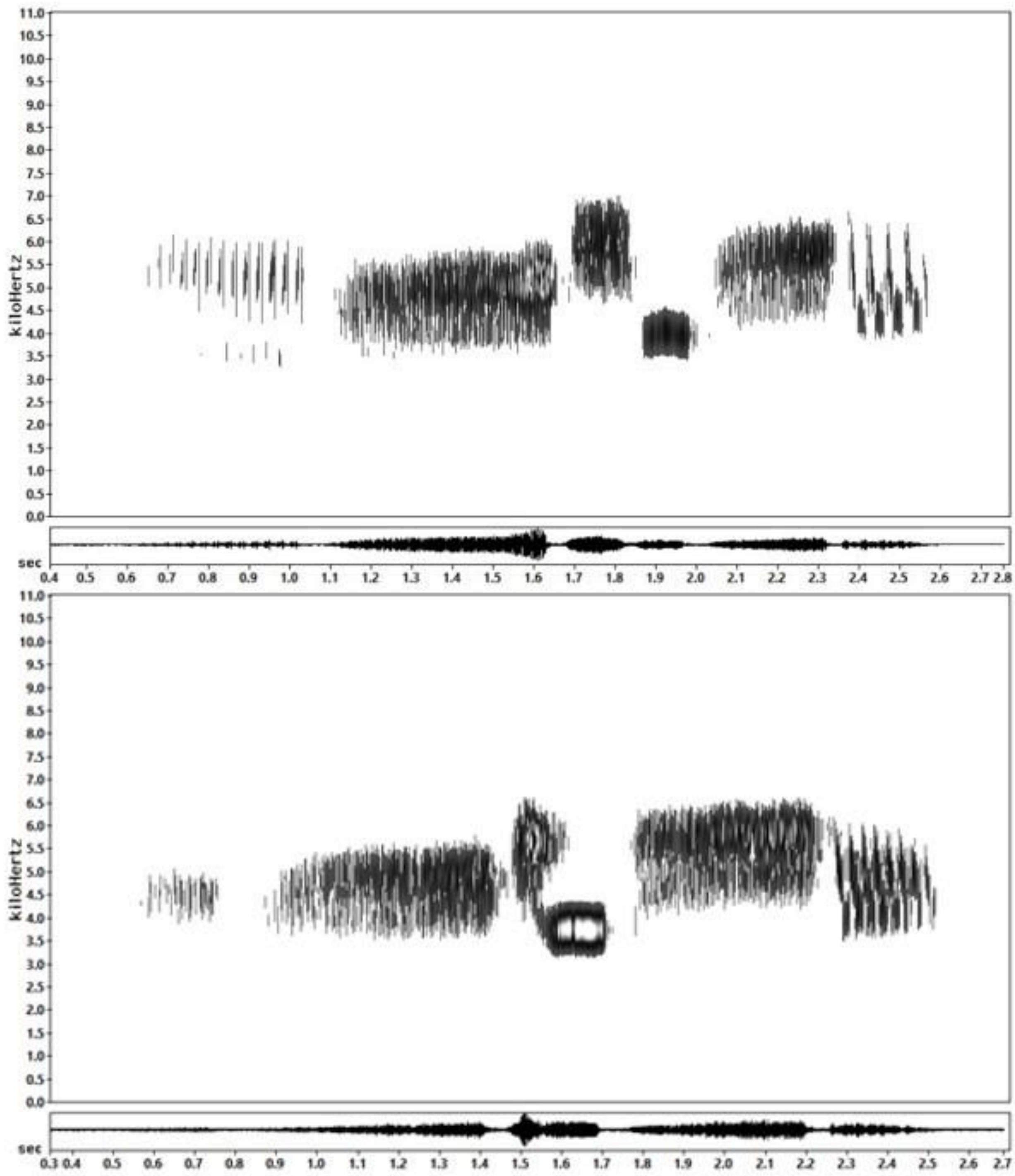

Figure A-2. Spectrograms of golden-cheeked warbler (*Setophaga chrysoparia*) B songs recorded at Dinosaur Valley State Park in 1994 (top; ML109768) and Palo Pinto Mountains State Park in 2018 (bottom).

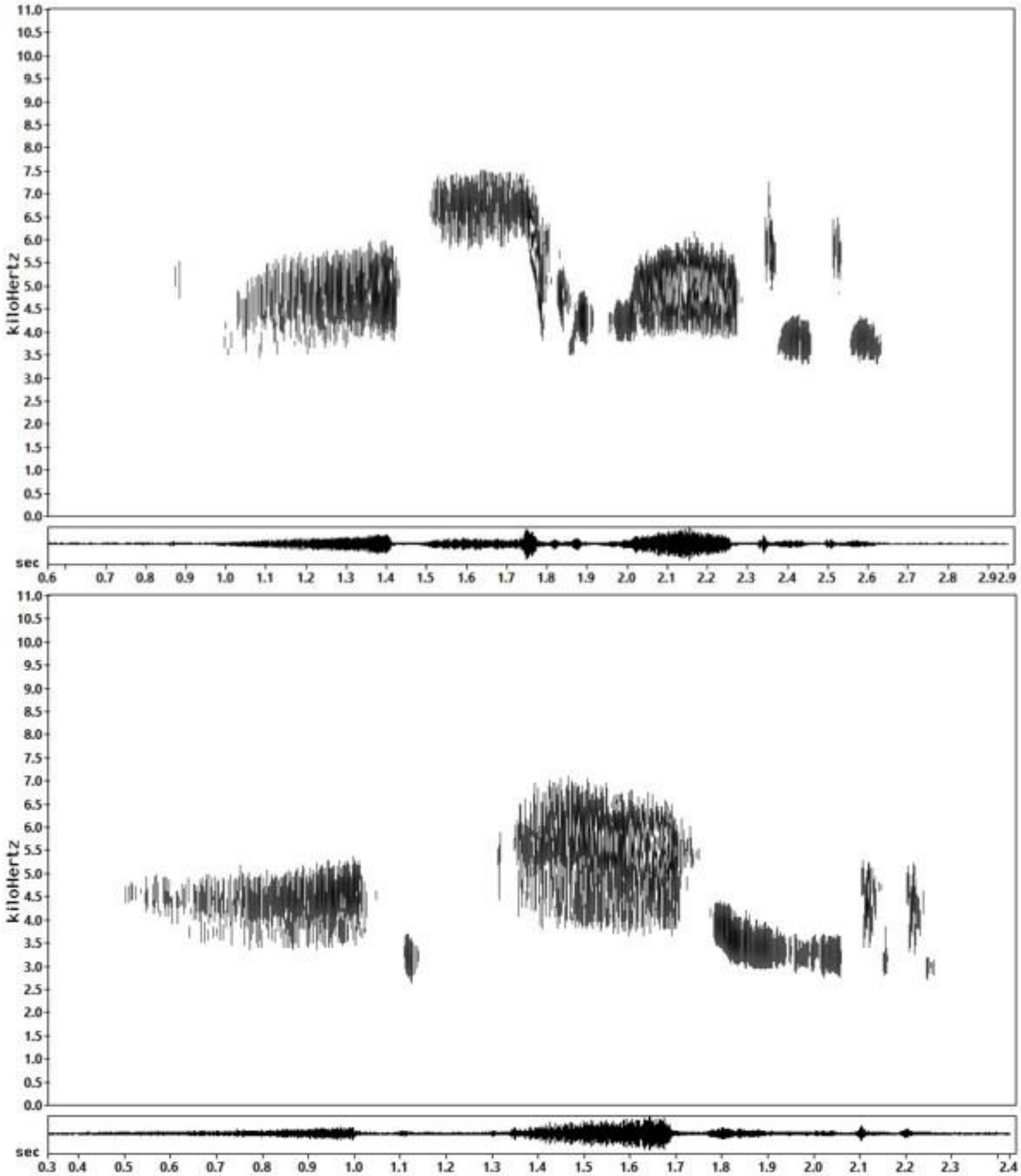

Figure A-3. Spectrograms of golden-cheeked warbler (*Setophaga chrysoparia*) songs recorded at Fort Hood in 1994 (top; Bolsinger's [1997] C song; ML109479) and in 2018 (bottom; B Central subtype).

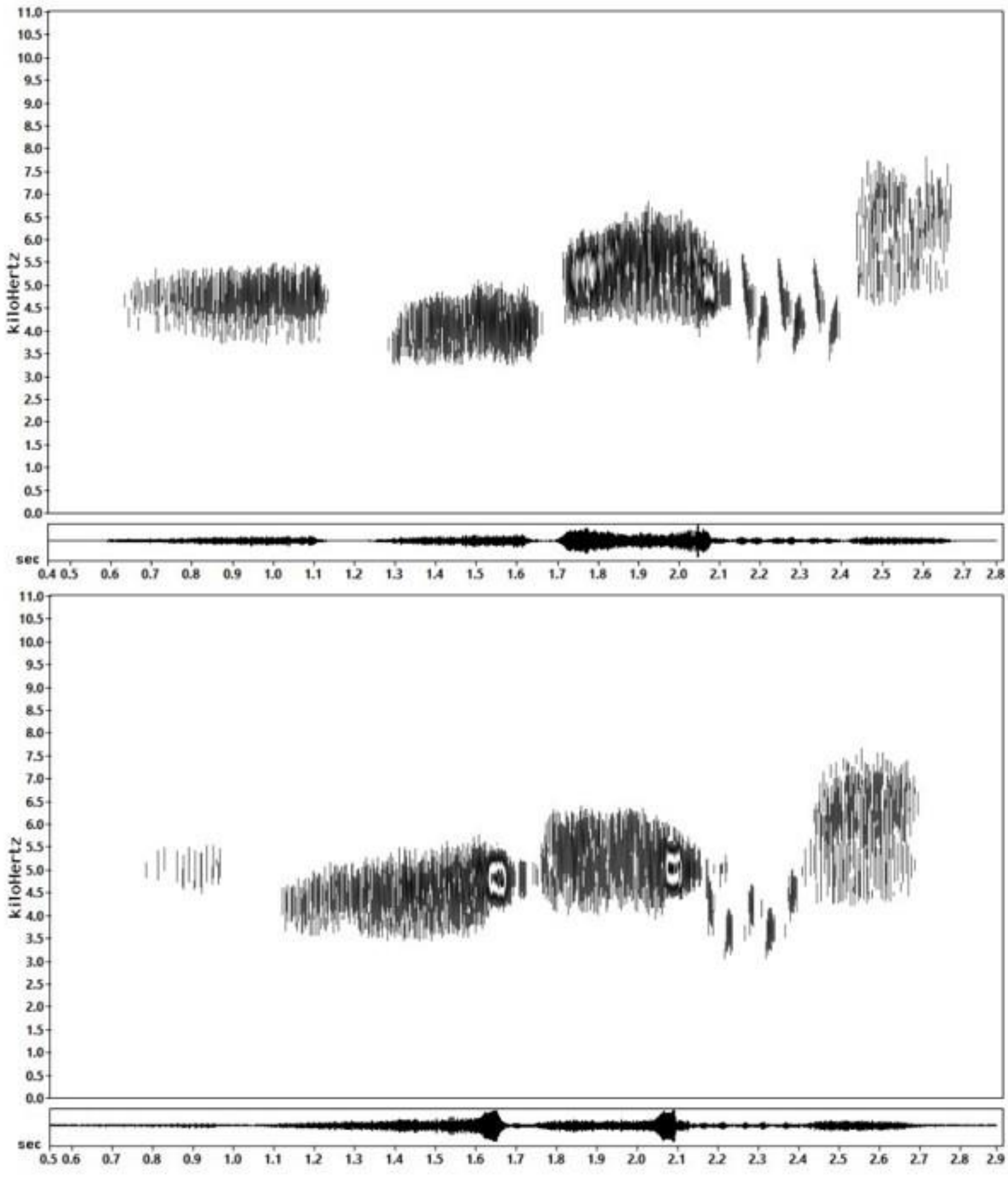

Figure A-4. Spectrograms of golden-cheeked warbler (*Setophaga chrysoparia*) B songs labeled B South recorded at a private property in Edwards County in 2018 (top) and Joint Base San Antonio-Camp Bullis in 2017 (bottom).