Geographic variation in song structure in the Hermit Thrush (Catharus guttatus)

Authors: Roach, Sean P., and Phillmore, Leslie S.

Source: The Auk, 134(3) : 612-626

Published By: American Ornithological Society

URL: https://doi.org/10.1642/AUK-16-222.1

The Auk
Ornithological Advances
AmericanOrnithology.org

Volume 134, 2017, pp. 612–626
DOI: 10.1642/AUK-16-222.1

RESEARCH ARTICLE

Geographic variation in song structure in the Hermit Thrush (*Catharus guttatus*)

Sean P. Roach* and Leslie S. Phillmore

Department of Psychology and Neuroscience, Dalhousie University, Halifax, Nova Scotia, Canada
* Corresponding author: seanroach@gmail.com

Submitted October 26, 2016; Accepted March 9, 2017; Published May 24, 2017

ABSTRACT

Although the Hermit Thrush (*Catharus guttatus*) exhibits distinctive morphological and genetic differences across its breeding range (Alvarado et al. 2014), comparatively little is known about geographic variation in song structure and whether song variation corresponds with those morphological and genetic differences. To address this, we collected recordings from throughout the species' breeding range and assessed frequency and temporal characteristics of song structure. Eastern and western birds differed dramatically with respect to song structure, most notably with respect to introductory note frequencies: eastern birds exhibited a larger range of introductory note frequencies than western birds. They also differed with respect to temporal characteristics: western birds sang longer introductory notes, but shorter post-introductory portions, than eastern birds. Numerous differences in structure were also detected within each lineage. For example, within the western lineage, introductory note frequencies were much higher in the Western Lowland group that occupies the Pacific coast compared to the inland Western Mountain group. There were also some differences seen within those groups; for example, in the western Canadian subspecies *C. g. euborius* recordings from high altitudes (>1900 m in the Canadian Rockies) were structurally distinct from those recorded at lower altitudes. Highlighting the distinctive geographic differences in song structure, a discriminant function analysis using song structure characteristics classified recordings into their geographic groups with 97.0% accuracy. This array of geographic song differences, taking place at multiple levels, suggests that Hermit Thrush song has been shaped by a variety of forces, ranging from large-scale geological events to factors such as habitat differences and cultural drift that operate on a smaller geographic scale (i.e. within subspecies).

Keywords: Hermit Thrush, *Catharus guttatus*, geographic variation, birdsong, evolution

Variación geográfica en la estructura del canto de *Catharus guttatus*

RESUMEN

Aunque *Catharus guttatus* exhibe diferencias morfológicas y genéticas distintivas a lo largo de su área de reproducción (Alvarado et al., 2014), se sabe relativamente poco sobre la variación geográfica en la estructura del canto y si la variación en el canto corresponde con las diferencias genéticas y morfológicas. Para investigar esto recolectamos grabaciones a lo largo de área de reproducción de la especie y determinamos la frecuencia y las características temporales de la estructura de su canto. Las aves del oriente y el occidente fueron dramáticamente diferentes con respecto a la estructura de su canto, notablemente con respecto a la frecuencia de las notas introductorias: las aves del oriente mostraron un mayor rango de frecuencias en sus notas introductorias que las aves del occidente. También fueron diferentes con respecto a las características temporales: las aves del occidente tienen cantos con notas introductorias más largas, pero con porciones posteriores a la introducción más cortas que las aves del oriente. También se detectaron numerosas diferencias en la estructura del canto al interior de cada linaje. Por ejemplo, en el linaje occidental, las frecuencias de las notas introductorias fueron mucho más altas en el grupo de tierras bajas que ocupa la costa pacífica, comparadas con el grupo de alta montaña. También hubo algunas diferencias observadas al interior de esos grupos; por ejemplo, las grabaciones poblaciones de grandes elevaciones (>1900 m en las Montañas Rocallosas canadienses) de la subespecie del occidente de Canadá, *C. g. euborius*, fueron estructuralmente diferentes de las grabaciones de bajas elevaciones. Un análisis de función discriminante usando las características de estructura del canto clasificó las grabaciones en sus respectivos grupos geográficos con 97% de precisión, lo cual resalta las diferencias geográficas en la estructura del canto. Este conjunto de diferencias geográficas en el canto, que se hace evidente a múltiples niveles, sugiere que el canto de *C. guttatus* ha sido moldeado por una variedad de fuerzas evolutivas, desde eventos geológicos de gran escala hasta factores como diferencias en el hábitat y deriva cultural que operan en una escala geográfica más pequeña (i.e. al interior de las subespecies).

Palabras clave: cantos de aves, *Catharus guttatus,* evolución, variación geográfica

INTRODUCTION

While many songbird species produce songs that are consistent in structure throughout most or all of their ranges, songs of other species can vary substantially depending on location. Because song plays a vitally important role in songbird behavior, both in territorial defense and in mate attraction (Catchpole and Slater 2008), geographic song variation can influence the evolutionary trajectory of species. For example, sufficient differences in song between populations can lead to lack of conspecific recognition, contributing to reproductive isolation followed by speciation (e.g., Irwin et al. 2001). Nonetheless, geographic song variation is most often a by-product of evolutionary processes (Podos and Warren 2007). Regardless of whether song variation is a driving force or a consequence of reproductive isolation and similar events, understanding how and why song varies across a species' range can provide valuable insights into its evolutionary history (Slabbekoorn and Smith 2002, Podos and Warren 2007).

Geographic divergence in song structure can develop through a variety of mechanisms. Such divergence can be a secondary consequence of physical adaptations to ecological conditions. For example, differences in beak morphology among Darwin's finches represent an adaptation to optimize feeding, but changes in beak shape have also contributed to differences in song structure (Huber and Podos 2006). Selection on body size affects the physical apparatus of song production (e.g., syrinx size), which in turn influences the sound frequencies that a bird can produce (Ryan and Brenowitz 1985, Bertelli and Tubaro 2002), with some species showing a similar negative association between body size and frequency bandwidth (Mason and Burns 2015).

Variation in song structure may arise not only as a by-product of other adaptations to the environment but also as a direct adaptation. As the acoustic adaptation hypothesis states, the potential of the habitat itself to attenuate and degrade vocal signals can lead to selection for song structure characteristics that minimize interference and maximize signal fidelity (Wiley and Richards 1978, Slabbekoorn 2004). As a result, habitat structure can influence amplitude, temporal, and frequency characteristics of song structure (e.g., Wiley 1991). In addition to habitat structure, song characteristics may also be influenced by environmental sounds, including other natural sounds (e.g., insects, rainfall; Slabbekoorn 2004) or noise associated with human activity (Slabbekoorn and den Boer-Visser 2006).

Song divergence may also arise via cultural drift, wherein errors that occur during song learning persist within populations, accumulating and contributing to geographic variation (Slater 1989, Lachlan and Servedio 2004). Geographic song variation can also be caused by a number of other factors, including sexual selection (Irwin et al. 2001), differences in perch height (Lemon et al. 1981), and territory size (Calder 1990). It is important to note that these mechanisms of song variation (i.e. physical adaptation, environmental adaptation, and cultural transmission) are not mutually exclusive and may act in concert. For example, song structure differences among Darwin's finches have been linked both to changes in beak morphology via natural selection (Podos and Nowicki 2004, Huber and Podos 2006) and to cultural drift (Grant and Grant 1996).

In recent years, research on geographic song variation has provided valuable insights into the histories and systematics of thrushes in the genus *Catharus*. For example, in the Gray-cheeked Thrush (*C. minimus*), the structural song differences between *Catharus minimus bicknelli* and other *C. minimus* subspecies was one of the main factors in establishing the former as its own species (Bicknell's Thrush [*C. bicknelli*]; Ouellet 1993). Researchers have also examined geographic song variation in Swainson's Thrush (*C. ustulatus*), in the context of understanding the migratory routes and genetic divergence of its 2 major geographic groups (Ruegg et al. 2006a). However, relatively little is known about geographic song variation in another, closely related species, the Hermit Thrush (*C. guttatus*). Males sing songs composed of a single introductory note followed by a complex post-introductory series of notes (Figure 1). Individuals have repertoires of 6–12 song types, which they deliver with immediate variety (never repeating the same song type twice) and in a semi-random order (Roach et al. 2012). Existing research suggests that song-type sharing between neighbors occurs either at a very low rate or not at all (Jones 2005, Roach et al. 2012).

The Hermit Thrush has a large breeding range that spans most of Canada, as well as much of the western and northeastern United States (Dellinger et al. 2012). There are ~12 subspecies, which have been divided into 3 major geographic groups on the basis of breeding location (Dellinger et al. 2012): Northern, which covers eastern North America and spans across Canada into central British Columbia and central Alaska; Western Lowland, which stretches along the Pacific coast from Alaska to Baja California; and Western Mountain, which comprises the

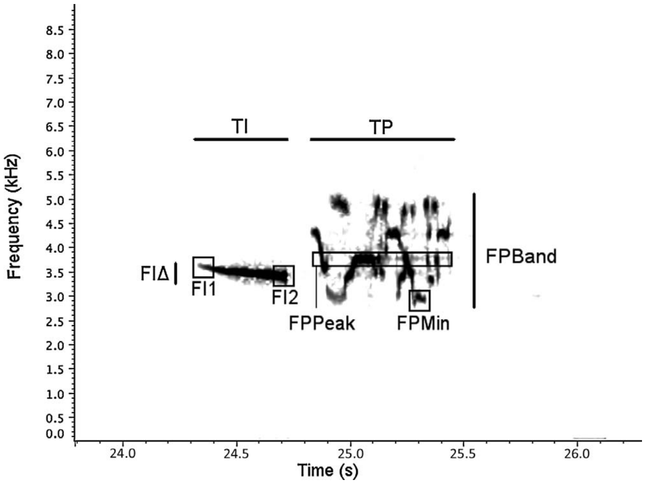

FIGURE 1. Spectrogram of a Hermit Thrush song type, illustrating the acoustic song variables measured. FI1 and FI2 are the frequency at the beginning and end of the introductory note, and FIΔ is the absolute difference between them. With respect to the post-introductory portion, FPMin is the minimum frequency, FPBand is the bandwidth (the difference between the maximum and minimum frequencies), and FPPeak is the frequency of highest energy. TI and TP represent the duration of the introductory note and post-introductory portion, respectively.

mountain ranges of the western United States (e.g., Rocky Mountains, Sierra Nevada) (Figure 2).

In terms of geographic song differences, Rivers and Kroodsma (2000) found extensive frequency and temporal differences between populations of Hermit Thrush in Arizona (*C. g. auduboni,* a Western Mountain subspecies) and New England (*C. g. faxoni,* a Northern subspecies). On a smaller scale, Roach et al. (2012) found more restricted acoustic differences related to duration of song elements between 2 *C. g. faxoni* populations located in Nova Scotia and Maine. However, little is known about overall geographic variation in Hermit Thrush song structure, and Rivers and Kroodsma (2000) identified this as a priority for future research.

Alvarado et al. (2014) recently used geolocators in combination with genetic and morphological data to examine migratory patterns in Hermit Thrush. They found a high degree of genetic divergence between western (corresponding to the Western Mountain and Western Lowland groups) and eastern (corresponding to the Northern group) birds, spanning a migratory divide similar in location to that reported in Swainson's Thrush (Ruegg and Smith 2002, Ruegg et al. 2006a) and likely originating from a glacial separation event one million years ago. The genetic divergence seen in Hermit Thrush, reminiscent of other boreal superspecies complexes (groups of newly separated yet highly similar species) that were separated by the same glaciation event, is sufficiently large to warrant a provisional split into 2 separate species (Kerr et al. 2007, Topp et al. 2013). Though the possibility of speciation is intriguing, further study is required, especially in the hybrid zone that lies between the eastern and western lineages (Alvarado et al. 2014).

The objective of this study was to provide, for the first time, a thorough overview of geographic variation in Hermit Thrush song structure. Recordings of songs from throughout the Hermit Thrush breeding range were collected from a variety of sources and analyzed with respect to song structure. Principal component and discriminant function analyses were used in order to assess the degree of divergence in song structure across Hermit Thrush major geographic groups. Given the potential of habitat structure to shape song structure, we were also interested in how ecological and climatic differences correspond to observed differences in song structure. Information on how song varies geographically in this species has the potential to inform the currently unclear picture of its subspecies designations and, more significantly, to relate song structure to the genetic and morphological patterns associated with the observed migratory divide (Alvarado et al. 2014). Such integration of information about genetics, morphology, and song structure will improve our understanding of the forces shaping the evolution of the species and its taxonomic subgroups.

METHODS

Recordings

Recordings, each representing a single vocalizing Hermit Thrush, were collected from the Cornell Lab of Ornithology's Macaulay Library (macaulaylibrary.org), xeno-canto (www.xeno-canto.org), Michigan State University's Avian Vocalization Center (avocet.zoology.msu.edu), and the Ohio State Borror Laboratory of Bioacoustics (blb.osu.edu), as well as a number of independent recordists. Recording dates ranged from 1951 to 2015. In cases where the exact GPS coordinates or altitude information was not provided for a given recording, that information was obtained by inputting location information reported by the recordist into Google Earth. The recordings varied substantially in quality, including compressed MP3 files from xeno-canto and from some of the independent recordists. Although compression of recordings can influence the measurement of acoustic variables (e.g., Medina-Garcia et al. 2015), this variation in quality did not influence our findings: rerunning the MANCOVA described below with compressed MP3 recordings excluded from the analysis did not alter our results, nor did running the same MANCOVA with recording quality (compression or no compression) treated as a covariate.

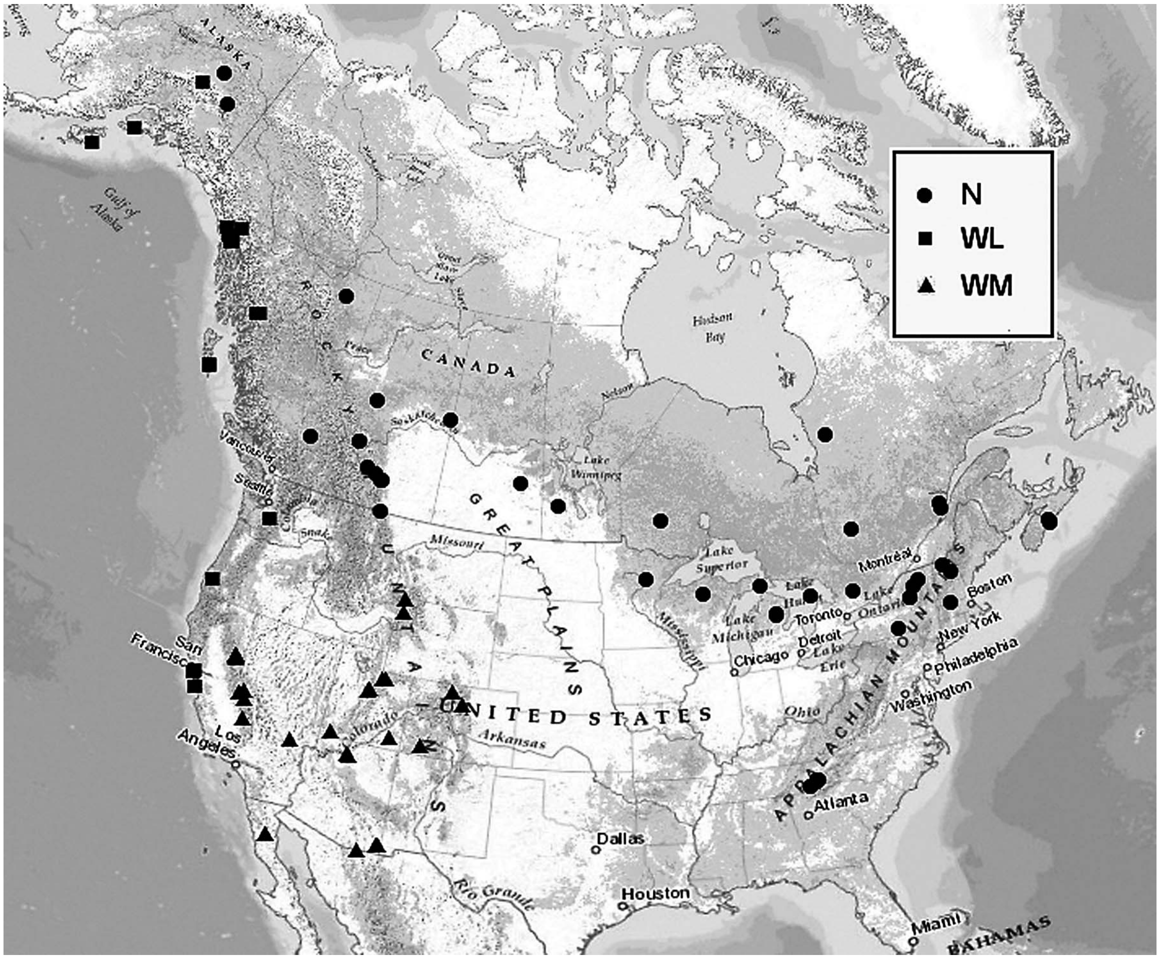

FIGURE 2. Map showing the locations of recordings analyzed in this study, divided into their major geographic groups of subspecies: Northern (N; $n = 45$), Western Lowland (WL; $n = 23$), and Western Mountain (WM; $n = 32$). Some symbols may be obscured in cases where separate recordings of a different individual have the same or similar coordinates. Geographic group affiliation was determined using GPS coordinates, based on the descriptions of the ranges for the subspecies making up the groups, provided by Dellinger et al. (2012).

Given the lack of evidence for song-type sharing in Hermit Thrush (Jones 2005, Roach et al. 2012), we were able to use visual inspection of song spectrograms to examine recordings made in close proximity to each other in order to eliminate any duplicate recordings of individuals. The resulting 101 recordings of unique individuals were classified into major geographic group (Northern, Western Lowland, and Western Mountain) and subspecies based on recording location using the detailed geography-based range descriptions of the subspecies making up the groups provided by Dellinger et al. (2012).

Spectrographic analysis of song structure was conducted using RavenPro 1.4 (www.birds.cornell.edu/raven) (Hann window size 1000 FFT). For each song type within a recording, we measured 8 acoustic variables associated with frequency and duration: frequency at the start (FI1) and end (FI2) of the introductory note, the absolute change in frequency from the start to the end of the introductory note (FIΔ), the minimum (FPMin) and peak energy (FPPeak) frequencies of the post-introductory portion, the bandwidth of the post-introductory portion (difference between maximum and minimum frequency; FPBand), and the durations of the introductory note (TI) and the post-introductory portion (TP) (Figure 1).

In the interest of comparing structure among individuals, and to avoid issues related to pseudoreplication, we calculated each variable's mean across all song types within a recording. However, it was also important to account for the fact that there is substantial variation within individual Hermit Thrush repertoires with respect to frequency characteristics. For example, in the Northern subspecies *C. g. faxoni*, the introductory note frequencies of each individual's song types typically span 3,000 Hz or more (Roach et al. 2012). To capture such variation and avoid losing information, we calculated not just the mean but also the maximum and minimum frequency for the song types in each recording. With mean values for all 8 acoustic variables and maximum and minimum values for the 6 frequency-related variables, there were a total of 20 acoustic variables used in the analyses.

Statistical Analyses

Because of the uncertainty regarding Hermit Thrush subspecies designations based on genetic information, statistical analyses were conducted mainly on the level of the 3 major geographic groups (Northern, Western Mountain, and Western Lowland). Analyses were completed in IBM SPSS Statistics 22 with an α value of 0.05, unless otherwise noted.

To reduce the number of variables and examine how they contributed to song structure variation, we conducted a principal component analysis (PCA) based on the correlation matrix of the 20 variables. Principal components with eigenvalues greater than 1.00 were used in subsequent analyses. One of the resulting principal components (PCs) lacked within-class normality, as assessed by examining the data's residuals, and could not be made normal by transformation (with log, ln, or square root). Thus, the principal component data did not the meet the multivariate normality assumption of the planned analyses (MANCOVA and discriminant function analysis). One multivariate outlier was identified based on examination of both the raw data and of plots showing residuals of regression for the principal components. When the principal component analysis was rerun with the remaining 100 individuals, the non-normal distribution was still present.

Because the PC data did not meet the requirements for MANCOVA and discriminant function analysis, data were first examined using a classification method that does not require a normal distribution, *k*-nearest neighbor analysis, which classifies cases into groups based on the group membership of the most similar other cases. It correctly predicted the group membership of 89% (89/100) of individuals. Among the 11 misclassified individuals were 5 of the 6 individuals belonging to *C. g. slevini*, a Western Lowland subspecies. Similarly, the algorithm misclassified 5 of the 6 members of the Northern subspecies *C. g. euborius* that were recorded at high altitudes (>1,900 m) in the Canadian Rockies (7 other *C. g. euborius* were recorded at lower altitudes (<1,500 m) in the surrounding areas). On the basis of these consistent misclassifications, the 100 birds were reclassified into 5 groups: Northern ($n = 39$ individuals), Western Lowland ($n = 17$), Western Mountain ($n = 32$), high-altitude *C. g. euborius* (consisting of the individuals recorded >1,900 m; $n = 6$), and *C. g. slevini* (consisting of the members of this subspecies; $n = 6$). This new classification exhibited within-class normality and was therefore used for the subsequent analyses.

Differences in song structure across these 5 groups were assessed via MANCOVA using the principal components that resulted from the PCA. Because the sampling period of the recordings was so large (1951–2015), year of recording was treated as a covariate. Between-group differences were examined using Bonferroni-corrected post-hoc comparisons. In order to test whether individuals could be accurately classified on the basis of song structure, we then conducted a discriminant function analysis (DFA) using the same principal components. Geographic group was used as the grouping variable, with the five-group classification described above.

To assess how ecological factors may have influenced song variation, we used Mantel and partial Mantel tests to measure correlations among matrices representing geographic distance (the distance between recording sites), ecological distance (how different recording sites are with respect to climatic variables), and song distances (how different recordings are from each other with respect to song structure) between recordings. For ecological distance, we used climate data related to various aspects of rainfall and temperature; such data have been used by other studies of geographic song variation (e.g., Ruegg et al. 2006b, Wei et al. 2015) in order to provide a broad picture of environmental effects on habitat. Climatic data representing the period 1950–2000, which corresponds to this study's sampling period, were downloaded from http://worldclim.org, and 19 climatic variables representing temperature and precipitation data were extracted using ArcGIS (Esri, Redlands, California, USA) for each recording site at a resolution of ~1 km^2. A principal component analysis based on the correlation matrix was conducted in order to reduce the number of climatic variables. A matrix of the Euclidean ecological distances between each pair of recordings was calculated in SPSS using the principal components with eigenvalues over 1.00; likewise, a matrix of Euclidean song distances was calculated from the first 2 discriminant functions resulting from the discriminant function analysis. Using the longitude and latitude of each recording site, the package fields in the statistical analysis program R was used to calculate a matrix containing the great circle distance (the shortest route over the earth's surface between any 2 points) between each pair of recording locations.

The analysis program PASSaGE 2 (www.passagesoftware.net/) was used to conduct Mantel tests testing for correlations between song distance and each of geographic and ecological distance, thus measuring the degree to which song differences varied in concert with geographic location and ecological factors. We also conducted partial Mantel tests, wherein a correlation between 2 matrices is calculated while the influence of a third is controlled.

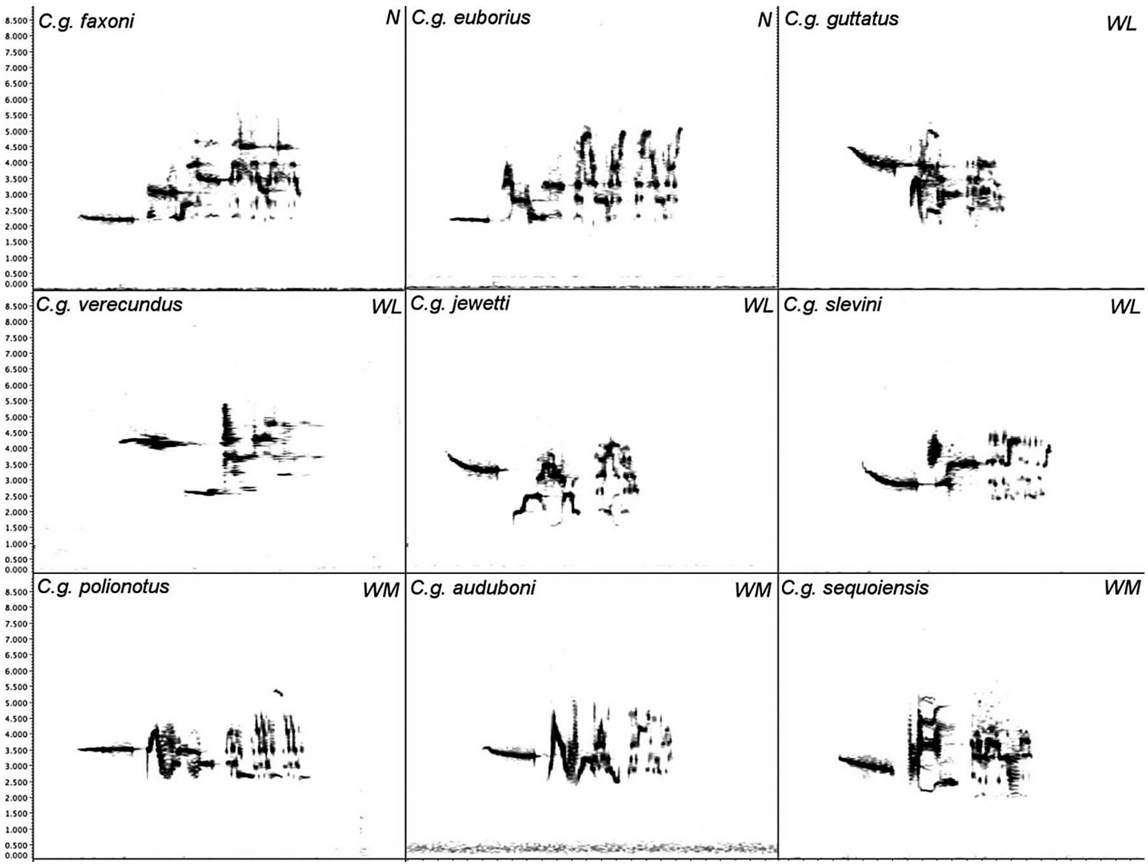

FIGURE 3. Spectrograms showing example song types from subspecies within the Northern (N), Western Lowland (WL), and Western Mountain (WM) groups.

RESULTS

Principal Component Analysis

Analyses included recordings of 100 individuals, containing 873 song types (Figure 2). Examples of songs from each subspecies included are shown in Figure 3. A principal component analysis conducted to reduce the initial 20 acoustic variables generated 5 principal components with eigenvalues greater than 1.00 (see Table 1 for factor loadings). Together, these principal components explained 79.57% of the variation observed in the original 20 variables. The raw data for the 20 acoustic variables, classified by geographic group, are summarized in Appendix Table 3.

Comparison across Geographic Groups

A MANCOVA using the above-noted principal components revealed an overall difference in song structure across the 5 groups (Pillai's Trace = 2.026, $F = 19.087$, $p < 0.001$) while controlling for year of recording. There were significant differences by group with respect to PC1 ($F = 342.563$, df = 4 and 100, $p < 0.001$), PC2 ($F = 23.568$, df = 4 and 100, $p < 0.001$), PC3 ($F = 8.311$, df = 4 and 100, $p < 0.001$), and PC4 ($F = 7.732$, df = 4 and 100, $p < 0.001$), but not for PC5 ($F = 2.058$, df = 4 and 100, $p = 0.07$).

Bonferroni-corrected post-hoc comparisons between groups revealed that PC1, which had high loadings for the mean and minimum values of introductory note frequency (FI1 and FI2) and absolute frequency change within the introductory note (FIΔ) (see Table 1), was higher in the Western Lowland group than the Western Mountain group ($p < 0.001$), and higher in the Western Mountain group than the Northern group ($p < 0.001$) (Figure 4A). The introductory note frequencies of these groups occupied dramatically different ranges of values, as can be seen by plotting the introductory note frequencies of all song types measured (Figure 5). Specifically, the introductory note frequencies of Northern birds spanned a large range (approximately 1,700–5,000 Hz in terms of FI1), while those of Western Lowland (approximately 4,000–5,500 Hz), and Western Mountain (approximately 3,000–4,000 Hz) birds occupied frequency ranges that were smaller, but distinct from each other (Figure 4A). Figure 5 also demonstrates the differences in frequency change within the introductory notes (FIΔ), the magnitude

TABLE 1. Factor loadings of acoustic variables for principal components with eigenvalues >1. High factor loadings are in bold.

Variable	PC1	PC2	PC3	PC4	PC5
FI1$_{\text{Mean}}$	**0.956**	0.136	0.185	0.076	−0.080
FI1$_{\text{Max}}$	0.275	**0.664**	0.539	0.158	−0.011
FI1$_{\text{Min}}$	**0.950**	−0.168	−0.037	0.079	−0.112
FI2$_{\text{Mean}}$	**0.925**	0.176	0.238	0.134	−0.086
FI2$_{\text{Max}}$	0.484	**0.614**	0.488	0.131	0.006
FI2$_{\text{Min}}$	**0.934**	−0.167	−0.012	0.125	−0.130
FIΔ_{Mean}	**0.833**	−0.128	−0.245	−0.302	0.168
FIΔ_{Max}	**0.698**	−0.084	−0.194	−0.383	0.257
FIΔ_{Min}	0.542	−0.080	−0.423	−0.032	0.195
FPMin$_{\text{Mean}}$	−0.201	**0.773**	−0.030	0.484	0.105
FPMin$_{\text{Max}}$	−0.309	**0.861**	−0.058	0.144	−0.041
FPMin$_{\text{Min}}$	−0.085	0.111	−0.281	**0.812**	0.281
FPBand$_{\text{Mean}}$	−0.056	0.214	**0.907**	−0.106	0.163
FPBand$_{\text{Max}}$	−0.050	0.061	**0.795**	−0.061	0.185
FPBand$_{\text{Min}}$	0.012	0.137	**0.765**	−0.163	0.013
FPPeak$_{\text{Mean}}$	−0.060	**0.781**	0.419	−0.004	0.262
FPPeak$_{\text{Max}}$	−0.068	**0.852**	0.166	−0.239	0.137
FPPeak$_{\text{Min}}$	0.289	0.123	0.429	0.149	**0.594**
TI$_{\text{Mean}}$	0.190	−0.144	−0.121	−0.119	**−0.680**
TP$_{\text{Mean}}$	−0.483	0.064	0.502	0.214	0.034
Eigenvalue	5.94	5.44	2.30	1.20	1.03
Variance explained (%)	29.68	27.23	11.53	5.99	5.15

of which is reflected by the distance from the line that represents no frequency change (slope = 1): FIΔ was largest in Western Lowland birds, smaller in Western Mountain birds, and very small in the Northern group. With respect to PC1, high-altitude *C. g. euborius* birds were intermediate between Western Lowland and Western Mountain individuals, while *C. g. slevini* birds were most similar to the Western Mountain group.

For both PC2 and PC3 (Figures 4B and 4C), most strongly associated with post-introductory frequency measures (the mean and maximum values for FPMin and FPPeak, as well as all FPBand values; Table 1), the Northern group was higher than the Western Mountain group (PC2: $p < 0.001$; PC3: $p < 0.001$), as was the Western Lowland group (PC2: $p < 0.001$; PC3: $p = 0.003$). The Northern and Western Lowland groups did not differ from each other. High-altitude members of *C. g. euborius* had lower PC2 values (FPMin and FPeak) than Northern ($p = 0.002$) and Western Lowland birds ($p = 0.003$), and did not differ from any groups with respect to PC3 (FBand). *C. g. slevini* birds were lower than Northern ($p = 0.003$) and Western Lowland birds ($p = 0.005$) with respect to PC3, but similar to those groups with respect to PC2 (Figures 4B and 4C). Values for PC4 (Figure 4D), associated with minimum post-introductory peak frequency (FPPeak$_{\text{Min}}$) and introductory note duration (TI), were significantly lower in *C. g. slevini* compared to the other groups ($p <$ 0.002 for each); the other groups did not differ from each other on PC4.

Because neither of the temporal characteristics loaded highly on PCs that explained substantial variation in the original variables, each characteristic was assessed separately via ANCOVA, with year of recording treated as a covariate. Duration of both the introductory note (TI) and post-introductory song portion (TP) differed significantly across the groups after controlling for year of recording (TI: $F = 12.433$, df = 4 and 95, $p < 0.001$; TP: $F = 9.406$, df = 4 and 95, $p < 0.001$) (Figure 6). Bonferroni-corrected post-hoc comparisons revealed that introductory note duration was longer in Western Mountain birds compared to Northern birds; in contrast, post-introductory note song duration was greater in Northern birds than in Western Lowland and Western Mountain birds.

Discriminant Function Analysis

A discriminant function analysis (DFA) was conducted using the 5 principal components and with geographic group (Northern, Western Lowland, and Western Mountain, plus *C. g. slevini* and high-altitude *C. g. euborius* in their own groups) as the grouping variable further demonstrated that song structure was distinctly different across the groups: it correctly classified 97.0% (97/100) of the individuals (Figure 7). The 3 incorrectly classified individuals included a *C. g. slevini* bird misclassified as Western Mountain, a Western Mountain bird misclassified as *C. g. slevini*, and a Western Lowland bird misclassified as high-altitude *C. g. euborius* (see filled-in symbols in Figure 7).

The position of group centroids and the distribution of individuals indicated that song structures across the 3 major geographic groups (Northern, Western Lowland, and Western Mountain) were highly distinct from each other, with Northern and Western Lowland song structure more similar to each other than either was to the Western Mountain group (Figure 7). *C. g. slevini* song structure was similar to that of the Western Mountain group (this similarity accounted for 2 of the 3 misclassifications), while song structure in high-altitude *C. g. euborius* was intermediate between the Western Lowland and Western Mountain groups.

Relationships between Song Structure, Geographic Distance, and Ecological Factors

To examine possible connections between ecology and song structure, we conducted Mantel tests designed to measure the correlations between differences in song structure, geographic distance, and ecology (assessed using 19 climatic variables; Appendix Table 4) across individuals and their recording locations. A principal component analysis using the climatic variables generated 4 PCs with eigenvalues above 1.00, which combined to

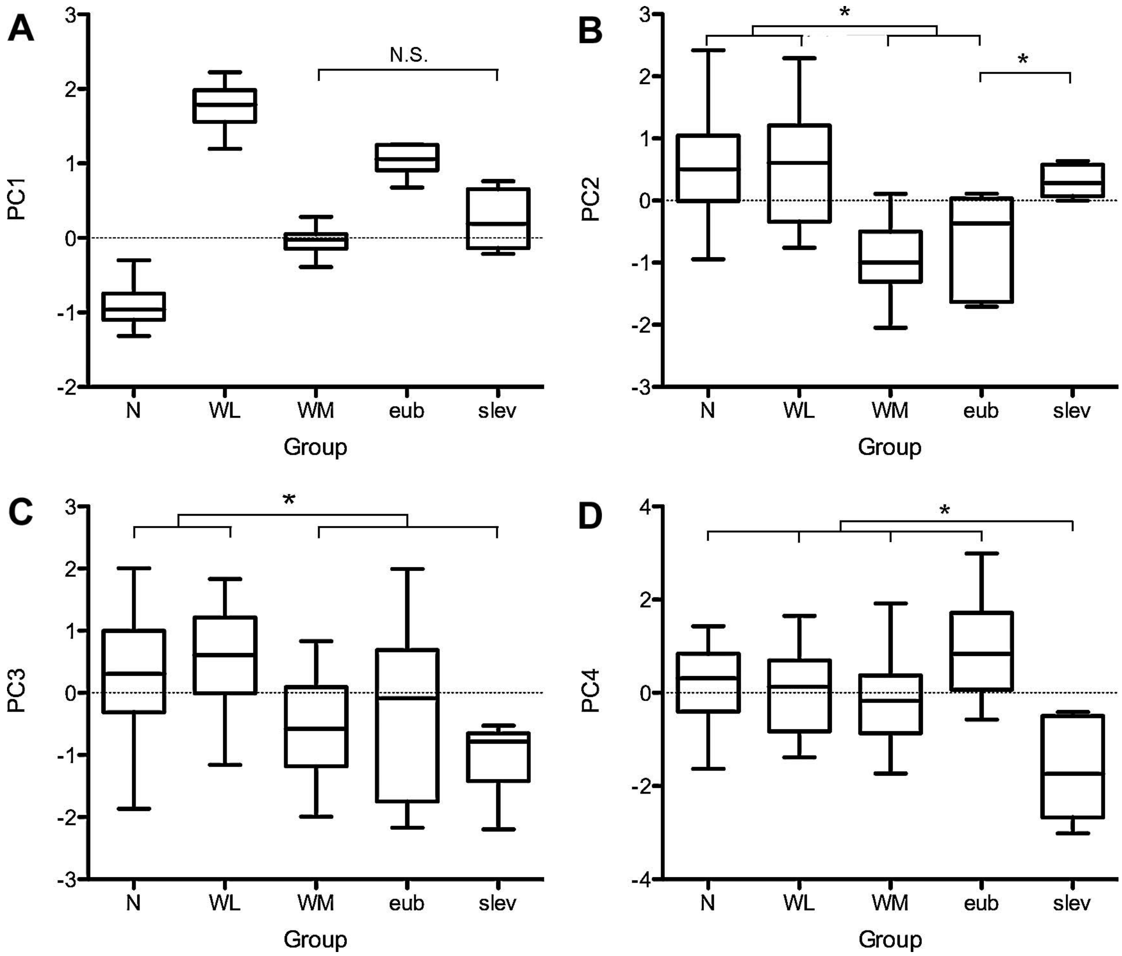

FIGURE 4. Boxplots showing group data for PCs 1–4 (**A**–**D**). Significant differences between groups (Bonferroni-corrected post-hoc comparisons; $\alpha = 0.05$) are indicated by asterisks (*); for PC1, all between-group differences were significant except for one (marked by "N.S.").

explain 91.20% of the variation across recording sites (Appendix Table 4).

The results of the Mantel tests are summarized in Table 2. Across all recordings, song distance was strongly correlated with both geographic distance and ecological distance, even when partial Mantel tests were used to compare song distance to each of those variables while excluding the influence of the other. In the Northern group, there was a significant correlation between song distance and geographic distance, which

TABLE 2. Summary of the associations between song distance and each of geographic distance and ecological distance, as measured by Mantel tests. Bold formatting indicates that the association remained significant after excluding the influence of the third factor (e.g., controlling for geographic distance in the relationship between song distance and ecological distance) via Partial Mantel test. "N.S." indicates that the Mantel test did not indicate a significant association.

	Geographic distance		Ecological distance	
	Correlation	Significance	Correlation	Significance
All recordings	**$r = 0.583$**	**$p < 0.001$**	**$r = 0.342$**	**$p < 0.001$**
Northern	**$r = 0.187$**	**$p = 0.01$**	$r = 0.209$	$p = 0.001$
Northern (no high-altitude *C. g. euborius*)	$r = 0.058$	N.S.	$r = 0.076$	N.S.
Western Lowland	**$r = 0.827$**	**$p < 0.001$**	$r = 0.695$	$p < 0.001$
Western Lowland (no *C. g. slevini*)	$r = 0.548$	$p = 0.002$	$r = 0.264$	N.S.
Western Mountain	$r = -0.008$	N.S.	$r = -0.050$	N.S.

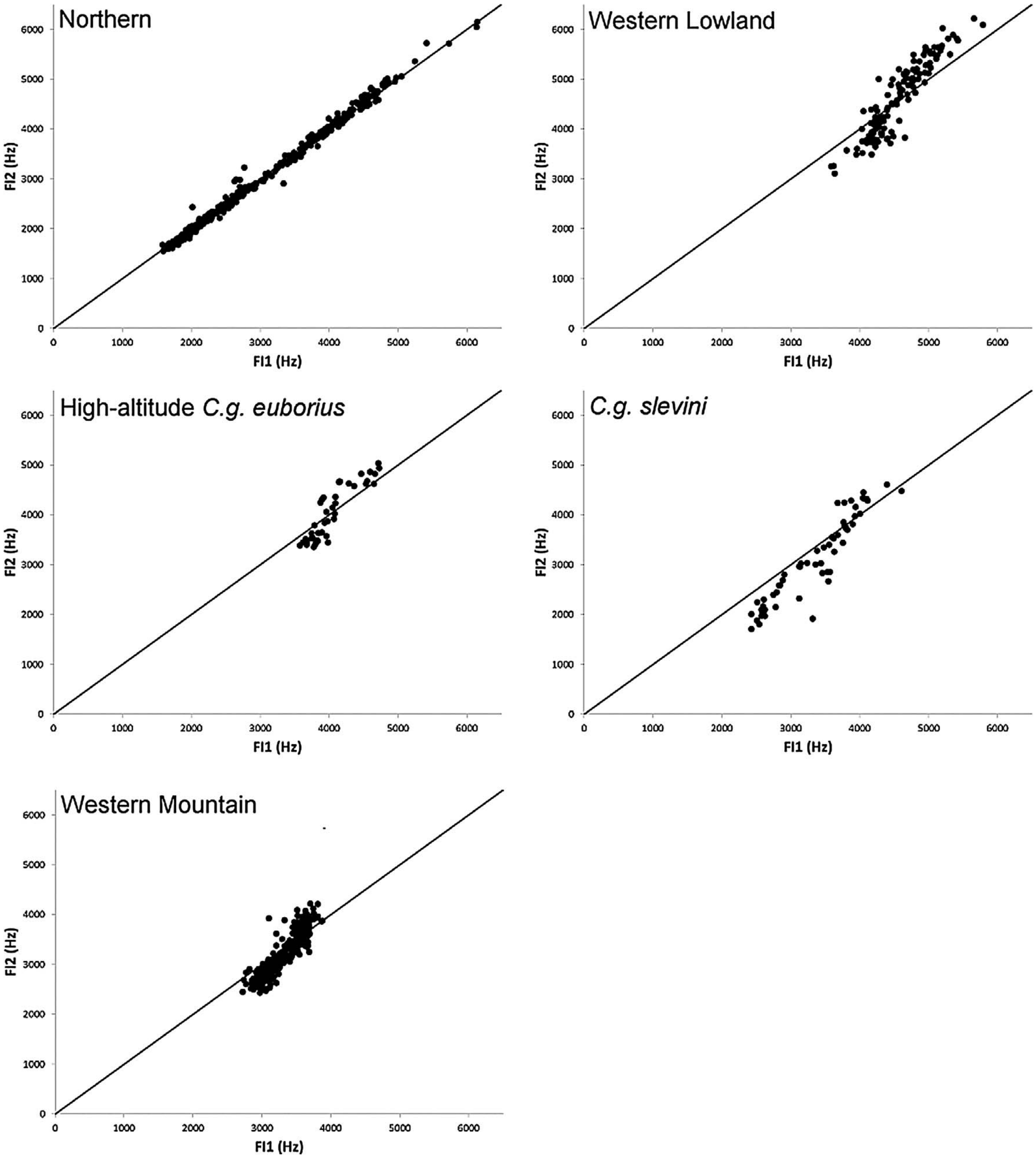

FIGURE 5. Scatterplots showing FI1 and FI2 for all song types of individuals from the Northern ($n = 369$ song types), Western Lowland ($n = 129$), and Western Mountain ($n = 270$) groups. Song types from high-altitude (>1,900 m) *C. g. euborius* individuals from the Northern group ($n = 45$) and birds from *C. g. slevini* ($n = 60$) are shown separately. Diagonal lines indicate where FI1 and FI2 would be equal, representing no change in frequency within the introductory note (FI$\Delta = 0$); thus, distance from the lines reflect the magnitude of FIΔ.

remained after controlling for ecological distance; there was also a significant correlation between song distance and ecological distance, although it did not remain after controlling for geographic distance. When high-altitude *C. g. euborius* birds were excluded from the Northern group, Mantel tests revealed no significant associations. In the Western Lowland group, song distance was correlated with both geographic distance and ecological distance; as with the Northern group, only the association between song distance and geographic distance remained significant following partial Mantel tests. Exclusion of *C. g. slevini* reduced the strengths of the associations observed in the Western Lowland group. Song distance was correlated with neither geographic nor ecological distance within the Western Mountain group.

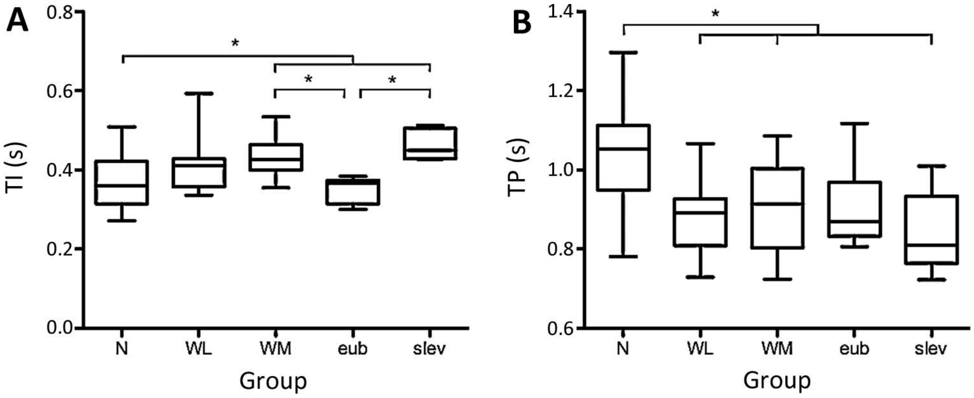

FIGURE 6. Boxplots showing group data for duration of the introductory note (**A**) and the post-introductory song portion (**B**). Significant differences between groups (Bonferroni-corrected post-hoc comparisons; $\alpha = 0.05$) are indicated by asterisks (*).

DISCUSSION

We found numerous differences in song structure among the 3 major geographic groups (Northern, Western Lowland, and Western Mountain). Analysis also revealed that there were 2 subgroups, *C. g. slevini* and high-altitude members of *C. g. euborius,* whose songs differed from their parent groups (Western Lowland and Northern, respectively). Confirming the differences among groups, a discriminant function analysis based on 20 acoustic variables assigned individual birds to their respective geographic categories with a high level of accuracy (97.0%).

The most striking difference in song structure across groups related to introductory note frequency: the introductory notes of Northern birds covered a large frequency range that spanned ~4,000 Hz, whereas those of western birds spanned a range that did not exceed 1,500 Hz. Among the western birds, Western Lowland introductory notes occupying a higher and somewhat larger range

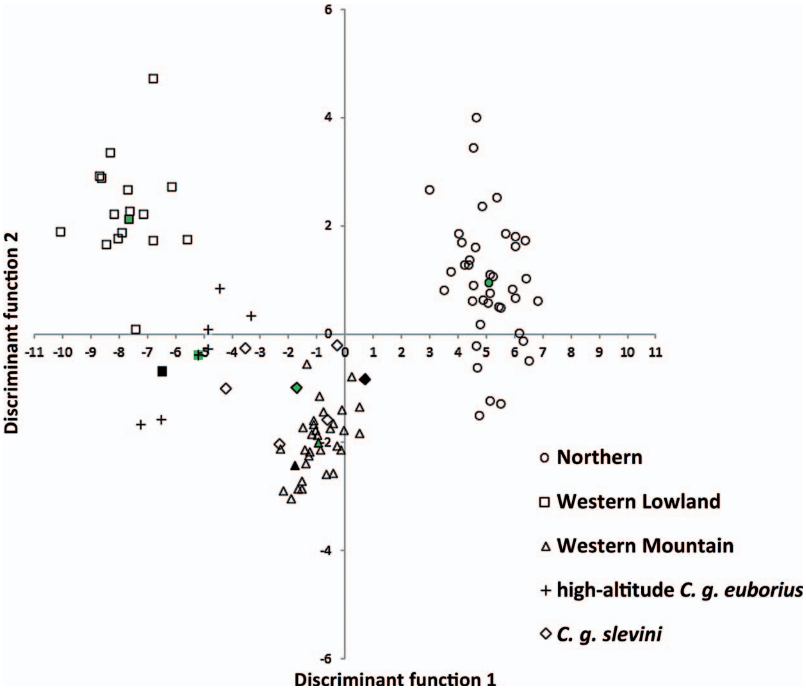

FIGURE 7. Plot of discriminant functions 1 and 2 representing results of discriminant function analysis. Symbols represent group affiliation; empty symbols represent correctly classified birds, black-colored symbols represent misclassified birds, and green-colored symbols represent group centroids.

of frequencies than those of Western Mountain birds. Post-introductory song frequencies were higher and spanned a larger bandwidth in the Northern and Western Lowland groups compared to the Western Mountain groups. Temporal characteristics also differed across groups: Western Lowland and Western Mountain songs had longer introductory notes, but shorter post-introductory portions, than Northern songs.

Evolutionary Divergence of Major Geographic Groups

Some of the observed variation in Hermit Thrush song structure likely dates back to separation of Hermit Thrush into 2 lineages, as instigated by the emergence of ice sheets during the Pleistocene era. Genetically, Hermit Thrush can be divided into 2 distinct groups representing eastern (stretching west to eastern British Columbia, roughly corresponding to the Northern group) and western (corresponding to the Western Lowland/Western Mountain groups) populations (Kerr et al. 2007, Topp et al. 2013, Alvarado et al. 2014) that also differ from each other morphologically (mainly with respect to body size; Alvarado et al. 2014). Overall differences in song structure align with this eastern–western division, as there were distinct differences between the Northern and Western Lowland/Western Mountain groups for both frequency (e.g., introductory note frequency) and temporal characteristics (i.e. duration of the introductory and post-introductory parts of the song).

Some of the song structure differences within the western lineage (i.e. between the Western Lowland and Western Mountain groups) may be related in part to further separation events. In examining the evolutionary histories of boreal superspecies, Weir and Schluter (2004) pointed not just to the above-noted separation event one million years ago but also to a second one, which involved more southern glacial advances and took place around 700,000 years ago. For such superspecies complexes, this second period of separation led to division of the western groups into so-called Pacific Coast and Rocky Mountain fragments, which geographically align with this study's Western Lowland and Western Mountain groups, respectively. While there is no evidence of further genetic divergence within the western lineage of Hermit Thrush, it is possible that this second separation event contributed to differences in song structure between the 2 western groups. Any song divergence that accumulated (e.g., via cultural drift) may have been maintained in part by the group's different migration patterns: Western Lowland birds travel along the Pacific coast, while Western Mountain birds are altitudinal migrants that winter in valleys near their Rocky Mountain breeding grounds (Dellinger et al. 2012).

Frequency differences between the 2 western groups may relate in part to body size, which has been negatively correlated with song frequency in a number of songbird species (Ryan and Brenowitz 1985). The frequency of both introductory and post-introductory song components was higher in Western Lowland birds, a group reported by Aldrich (1968) to contain the smallest of all Hermit Thrush subspecies, than in the Western Mountain group, which includes the largest subspecies. This variation in body size may also contribute to the comparatively larger bandwidth of the post-introductory portion of song in Western Lowland birds, as bandwidth has also been negatively correlated with body size in some species (e.g., Mason and Burns 2015).

Role of Altitude and Habitat

Overall, song structure was relatively consistent within each of the 3 major geographic groups. One interesting exception involved high-altitude members of the Northern subspecies *C. g. euborius* located in the Canadian Rockies. While a portion of *C. g. euborius* birds sang songs that were similar to the rest of the Northern group, song structure in birds at high elevations (1,910–2,275 m) was unlike that of other Northern birds and intermediate between Western Lowland and Western Mountain song structure, as indicated by their DFA misclassification (Figure 7). Compared to the Northern group, these birds had songs with higher introductory note frequencies and lower post-introductory frequencies. Such elevation-related differences in song structure have been documented in some other songbirds, such as the Gray-breasted Wood-Wren (*Henicorhina leucophrys*) (Caro et al. 2013).

These differences may relate in part to habitat structure at higher elevations. Compared to the high tree density at lower elevations in the Rockies, the habitat around 2,000 m is more open, with trees giving way to shrubs like mountain heathers as the subalpine forest transitions toward the alpine tundra (Luckman 1990). Particularly for the introductory note, which may function in long-range communication and therefore be more subject to the habitat's effects on sound transmission, this open habitat may have led to comparatively higher frequencies, as predicted by the acoustic adaptation hypothesis (Wiley and Richards 1978). Due to the limited number and coverage of the recording sites examined here, it remains unclear whether song structure changes gradually with increasing elevation, as in other species (e.g., Caro et al. 2013), or more abruptly. Recording individuals at various altitudes along this elevation gradient, possibly in conjunction with playback experiments to examine responsiveness to different song forms, will clarify the nature and significance of song structure variation in this part of the species range.

Birds from *C. g. slevini*, a Western Lowland subspecies, were also frequently misclassified by the DFA, indicating that their song structure differs from their parent geographic group. Both the introductory and post-introductory portions of the song were lower in frequency among *C. g. slevini* individuals compared to other Western Lowland birds. These differences may also relate to habitat. While most Western Lowland birds occupy an ecoregion (Marine West Coast Forests) characterized by very high levels of precipitation, *C. g. slevini* occupy a comparatively dry ecoregion (Mediterranean California) along the Pacific coast, where precipitation is seasonal and overall lower than in other Western Lowland habitats. It may be that the higher song frequencies among most Western Lowland birds reflects the influence of sound interference, in the form of constant rainfall (see Slabbekoorn 2004), a selection pressure that would be less prevalent for *C. g. slevini*. It is also possible that the more open habitats within the Mediterranean California ecoregion contribute to the lower song frequencies observed in *C. g. slevini*.

Comparison of song structure and ecological characteristics using Mantel tests revealed a positive correlation between differences in song structure and differences in habitat. This association suggests that environmental factors drive song variation to some degree. However, although ecological differences likely contribute in some cases (as in the cases of *C. g. euborius* and *C. g. slevini* described above), the overall differences among the main geographic groups argue against a dominant role for habitat. For example, birds in the Western Mountain group, which occupy open, high-altitude habitats, sang at the lowest frequencies—opposite of what would be predicted by the acoustic adaptation hypothesis (Wiley and Richards 1978). It may be that the association between environment and song structure is somewhat spurious: other factors such as cultural drift or morphological differences may covary with ecological differences and play a larger role in song divergence. Future work that examines morphology, genetics, and song structure in concert may help to resolve this uncertainty.

Independent Divergence of Song Components

Our analysis suggests that each of the 2 components of Hermit Thrush song, the introductory note and the post-introductory portion, have been shaped somewhat independently and subjected to a different mix of influences. If the 2 components were shaped by the same factors and in the same way, we would expect the differences among groups to be similar for both parts of the song. Instead, the patterns of geographic divergence are quite different for each of the components. For example, introductory note frequencies were highest in Western Lowland birds and lowest in Northern birds, and occupied dramatically different ranges of the spectrum; in contrast, post-introductory frequencies of Western Lowland and Northern birds were similar to each other and higher than those of Western Mountain birds. With respect to temporal characteristics, Western Lowland and Western Mountain birds had the longest introductory notes, whereas Northern birds possessed the longest post-introductory song durations.

This disconnect may be partly a result of the structurally simple introductory note and more complex post-introductory portion serving different functions. Research in other species has shown that whistles of consistent frequency, such as the introductory note, are more resistant to degradation than other types of song elements (Brown and Handford 2000) and their transmission may even be aided by forest reverberations (Slabbekoorn et al. 2002). In Blackbirds (*Turdus merula*) (Dabelsteen and Pederson 1993, Dabelsteen et al. 1993) and Eastern Towhees (*Pipilo erythrophthalmus*) (Richards 1981), the introductory song portion, which in each case is structurally simple and therefore relatively resistant to degradation by the environment, is important for both long-range communication and conspecific recognition. In contrast to those introductory portions, more complex parts of Blackbird and Eastern Towhee songs, which are more vulnerable to degradation, are thought to serve close-range functions related to individual recognition. Since Hermit Thrush have a comparable song structure to the above species, with a whistled introductory note and complex post-introductory portion, it may be that a similar functional division exists, with the introductory note serving in long-range species recognition (possibly directed mainly at females) and the rest of the song more associated with short-range individual recognition by neighboring males.

In partial support of this idea, research in another thrush species, the Veery (*Catharus fuscescens*), suggests such a role for its introductory note. Playback experiments with Veeries indicate that the introductory note is not essential for eliciting territorial responses (MacNally et al. 1986), and male Veeries often use songs lacking introductory notes in close-range territorial interactions with other males (Belinsky et al. 2015). Like Veeries, Hermit Thrush males often sing songs lacking an introductory note in response to territorial intrusions (Roach 2016). If the introductory portion functions primarily for long-range signaling in Hermit Thrush, it may be subject to unique influences, such as sexual selection. Further playback studies, in combination with transmission experiments, will provide further information on the respective roles of the introductory and post-introductory portions of Hermit Thrush song. This, in turn, will help in understanding the forces that have shaped each part of the signal.

Conclusion

In summary, this study has documented reliable differences in song structure across the breeding range of Hermit Thrush. Overall, the observed patterns of geographic song variation in Hermit Thrush are likely the result of a complex mix of factors and selection pressures that have influenced song structure at various points during the species' evolutionary history. Broadly, the most distinctive differences between the Northern and the Western Lowland/Western Mountain groups (e.g., introductory note frequency) likely date back to the separation of Hermit Thrush into eastern and western lineages, and arise via genetic differences and cultural drift. Song divergence within the western lineage may also have resulted from cultural drift, maintained by differences in migration and overwintering between the Western Lowland and Western Mountain groups; it is also possible that secondary consequences of natural selection's effects upon body size have contributed to spectral differences between these groups. Selection for optimal sound transmission in different habitats has also likely contributed to song variation, especially in the case of within-group variation (i.e. *C. g. slevini* and high-altitude *C. g. euborius*).

The large-scale song differences between eastern and western lineages parallel established genetic and morphological differences in some respects, although variation within and between the geographic groups highlight more extensive influences on song structure. Future work that examines song structure, morphology, and genetics in concert—especially in areas of interest such as the hybrid zone identified by Alvarado et al. (2014) and the Canadian Rockies—will help to clarify the evolutionary history of Hermit Thrush as well as the forces that have shaped its song over that span. Such information has the potential to add to our overall understanding of how song structure variation contributes to speciation and associated processes in songbirds.

ACKNOWLEDGMENTS

We thank Jeffrey MacLeod for assistance with field recording and the following for providing recordings: Cornell Lab of Ornithology's Macaulay Library, xeno-canto, Michigan State University's Avian Vocalization Center, the Ohio State Borror Laboratory of Bioacoustics, M. Ross Lein, Allison Nelson, and Dena Emmerson.

Funding statement: This research was supported by an NSERC Discovery Grant to LSP (326869-2012); the agency did not have input into the content nor was their approval required before submission of the manuscript.

Ethics statement: This research was conducted in compliance with the Canadian Council of Animal Care guidelines (11-044).

Author contributions: SPR formulated the research questions, collected and analyzed data, and wrote the paper. LSP supervised research and edited the paper.

LITERATURE CITED

Aldrich, J. W. (1968). Population characteristics and nomenclature of the Hermit Thrush. In Proceedings of the United States National Museum Vol. 124, No. 3637. Smithsonian Institution, Washington, DC.

Alvarado, A. H., T. L. Fuller, and T. B. Smith (2014). Integrative tracking methods elucidate the evolutionary dynamics of a migratory divide. Ecology and Evolution 4:3456–3469.

Belinsky, K. L., C. E. Nemes, and K. A. Schmidt (2015). Two novel vocalizations are used by Veeries (*Catharus fuscescens*) during agonistic interactions. PLOS One 10:e0120933. doi:10.1371/journal.pone.0120933

Bertelli, S., and P. L. Tubaro (2002). Body mass and habitat correlates of song structure in a primitive group of birds. Biological Journal of the Linnaean Society 77:423–430.

Brown, T. J., and P. Handford (2000). Sound design for vocalizations: Quality in the woods, consistency in the fields. The Condor 102:81–92.

Calder, W. A. I. (1990). The scaling of sound output and territory size: Are they matched? Ecology 71:1801–1816.

Caro, L. M., P. C. Caycedo-Rosales, R. C. K. Bowie, H. Slabbekoorn, and C. D. Cadena (2013). Ecological speciation along an elevational gradient in a tropical passerine bird? Journal of Evolutionary Biology 26:357–374.

Catchpole, C. K., and P. J. Slater (2008). Bird Song: Biological Themes and Variations, 2nd edition. Cambridge University Press, Cambridge, UK.

Dabelsteen, T., O. N. Larsen, and S. B. Pedersen (1993). Habitat-induced degradation of sound signals: Quantifying the effects of communication sounds and bird location on blur ratio, excess attenuation, and signal-to-noise ratio in Blackbird song. The Journal of the Acoustical Society of America 93:2206–2220.

Dabelsteen, T., and S. B. Pedersen (1993). Song-based species discrimination and behaviour assessment by female Blackbirds, *Turdus merula*. Animal Behaviour 45:759–771.

Dellinger, R., P. B. Wood, P. W. Jones, and T. M. Donovan (2012). Hermit Thrush (*Catharus guttatus*). In The Birds of North America Online (A. Poole, Editor). Cornell Lab of Ornithology, Ithaca, New York, USA. doi: 10.2173/bna.261

Grant, B. R., and P. R. Grant (1996). Cultural inheritance of song and its role in the evolution of Darwin's finches. Evolution 50: 2471–2487.

Huber, S. K., and J. Podos (2006). Beak morphology and song features covary in a population of Darwin's finches (*Geospiza fortis*). Biological Journal of the Linnaean Society 88:489–498.

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

Jones, D. F. (2005). Voice-printing the Hermit Thrush (*Catharus guttatus*). Canadian Acoustics 34:14–15.

Kerr, K. C., M. Y. Stoeckle, C. J. Dove, L. A. Weigt, C. M. Francis, and P. D. Herbert (2007). Comprehensive DNA barcode coverage of North American birds. Molecular Ecology Notes 7:535–543.

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

Lemon, R. E., J. Struger, M. J. Lechowicz, and R. F. Norman (1981). Song features and singing heights of American warblers: Maximization or optimization of distance? The Journal of the Acoustical Society of America 69:1169–1176.

Luckman, B. H. (1990). Mountain areas and global change: A view from the Canadian Rockies. Mountain Research and Development 10:183–195.

MacNally, R. C., D. M. Weary, R. E. Lemon, and L. Lefebvre (1986). Species recognition by song in the Veery (*Catharus fuscescens*: Aves). Ethology 71:125–139.

Mason, N. A., and K. J. Burns (2015). The effect of habitat and body size on the evolution of vocal displays in Thraupidae (tanagers), the largest family of songbirds. Biological Journal of the Linnaean Society 114:538–551.

Medina-Garcia, A., M. Araya-Salas, and T. F. Wright (2015). Does vocal learning accelerate acoustic diversification? Evolution of contact calls in Neotropical Parrots. Journal of Evolutionary Biology 28:1782–1792.

Ouellet, H. (1993). Bicknell's Thrush: Taxonomic status and distribution. The Wilson Bulletin 105:545–572.

Podos, J., and S. Nowicki (2004). Beaks, adaptation, and vocal evolution in Darwin's finches. BioScience 54:501–510.

Podos, J., and S. P. Warren (2007). The evolution of geographic variation in birdsong. Advances in the Study of Behavior 37: 403–458.

Richards, D. G. (1981). Alerting and message components in songs of Rufous-sided Towhees. Behaviour 76:223–249.

Rivers, J. W., and D. E. Kroodsma (2000). Singing behavior of the Hermit Thrush. Journal of Field Ornithology 71:467–471.

Roach, S. P. (2016). Aspects of Singing Behaviour and Song Perception in Two North American Forest Songbirds, Black-capped Chickadee (*Poecile atricapillus)* and Hermit Thrush (*Catharus guttatus*). Ph.D. dissertation, Dalhousie University, Halifax, NS, Canada.

Roach, S. P., L. Johnson, and L. S. Phillmore (2012). Repertoire composition and singing behaviour in two eastern populations of the Hermit Thrush (*Catharus guttatus*). Bioacoustics 21:1–14.

Ruegg, K. C., R. J. Hijmans, and C. Moritz (2006a). Climate change and the origin of migratory pathways in the Swainson's Thrush, *Catharus ustulatus*. Journal of Biogeography 33:1172–1182.

Ruegg, K., H. Slabbekoorn, S. Clegg, and T. B. Smith (2006b). Divergence in mating signals correlates with ecological variation in the migratory songbird, Swainson's Thrush (*Catharus ustulatus*). Molecular Ecology 15:3147–3156.

Ruegg, K. C., and T. B. Smith (2002). Not as the crow flies: A historical explanation for circuitous migration in Swainson's Thrush (*Catharus ustulatus*). Proceedings of the Royal Society B 269:1375–1381.

Ryan, M. J., and E. A. Brenowitz (1985). The role of body size, phylogeny, and ambient noise in the evolution of bird song. The American Naturalist 126:87–100.

Slabbekoorn, H. (2004). Habitat-dependent ambient noise: Consistent spectral profiles in two African forest types. The Journal of the Acoustical Society of America 116:3727–3733.

Slabbekoorn, H., and A. den Boer-Visser (2006). Cities change the songs of birds. Current Biology 16:2326–2331.

Slabbekoorn, H., J. Ellers, and T. B. Smith (2002). Bird song and sound transmission: The benefits of reverberations. The Condor 104:564–573.

Slabbekoorn, H., and T. B. Smith (2002). Bird song, ecology and speciation. Philosophical Transactions of the Royal Society of London Series B 357:493–503.

Slater, P. J. B. (1989). Song learning: Causes and consequences. Ethology Ecology and Evolution 1:19–46.

Topp, C. M., C. L. Pruett, K. G. McCracken, and K. Winker (2013). How migratory thrushes conquered northern North America: A comparative phylogeography approach. PeerJ 1:e206.

Wei, C., C. Jia, L. Dong, D. Wang, C. Xia, Y. Zhang, and W. Liang (2015). Geographic variation in the calls of the Common Cuckoo (*Cuculus canorus*): Isolation by distance and divergence among subspecies. Journal of Ornithology 156:533–542.

Weir, J. T., and D. Schluter (2004). Ice sheets promote speciation in boreal birds. Proceedings of the Royal Society B 271:1881–1887.

Wiley, R. H. (1991). Associations of song properties with habitats for territorial oscine birds of eastern North America. The American Naturalist 4:973–993.

Wiley, R. H., and D. G. Richards (1978). Physical constraints on acoustic communication in atmosphere: Implications for evolution of animal vocalizations. Behavioral Ecology and Sociobiology 3:69–94.

APPENDIX TABLE 3. Mean ± standard deviation for each of the 20 acoustic variables within each geographic group. The unit for all frequency variables is hertz (Hz); the unit for the temporal variables (TI and TP) is seconds (s).

Variable	Northern	Western Lowland	Western Mountain	High-altitude *C. g. euborius*	*C. g. slevini*
FI1$_{\mathrm{Mean}}$	3032.2 ± 214.7	4564.6 ± 211.6	3319.7 ± 98.6	4029.6 ± 121.6	3339.8 ± 345.8
FI1$_{\mathrm{Max}}$	4581.2 ± 498.5	5048.5 ± 316.7	3647.6 ± 143.8	4367.0 ± 304.4	4139.0 ± 245.7
FI1$_{\mathrm{Min}}$	1768.9 ± 118.8	3780.3 ± 187.8	2730.3 ± 125.4	3529.6 ± 48.3	2314.1 ± 536.8
FI2$_{\mathrm{Mean}}$	3031.2 ± 222.7	4597.5 ± 234.9	3241.6 ± 102.4	4020.0 ± 146.3	3109.7 ± 390.8
FI2$_{\mathrm{Max}}$	4671.7 ± 502.7	5585.5 ± 330.8	3886.5 ± 164.6	4737.3 ± 233.9	4383.1 ± 126.3
FI2$_{\mathrm{Min}}$	1753.3 ± 124.3	3686.4 ± 185.4	2660.2 ± 138.3	3429.7 ± 57.8	2091.0 ± 581.0
FIΔ_{Mean}	59.3 ± 24.6	330.0 ± 47.7	207.5 ± 52.7	255.2 ± 92.2	348.5 ± 152.2
FIΔ_{Max}	166.6 ± 103.4	607.4 ± 100.9	411.5 ± 138.8	408.5 ± 109.3	743.9 ± 347.5
FIΔ_{Min}	9.1 ± 11.6	65.9 ± 51.5	43.1 ± 36.8	89.1 ± 80.7	64.8 ± 70.0
FPMin$_{\mathrm{Mean}}$	2691.2 ± 215.4	2497.2 ± 314.1	2247.2 ± 263.2	2304.4 ± 306.3	2279.7 ± 109.6
FPMin$_{\mathrm{Max}}$	4185.9 ± 367.0	3772.6 ± 608.7	3435.5 ± 378.4	3304.9 ± 409.0	3722.7 ± 233.6
FPMin$_{\mathrm{Min}}$	1496.3 ± 178.6	1492.5 ± 204.0	1465.6 ± 233.5	1640.6 ± 306.9	1412.6 ± 269.6
FPBand$_{\mathrm{Mean}}$	3159.6 ± 393.6	3149.6 ± 319.7	2631.1 ± 346.8	2659.2 ± 526.6	2651.8 ± 229.5
FPBand$_{\mathrm{Max}}$	4138.0 ± 744.4	4098.3 ± 538.3	3474.7 ± 570.6	3523.3 ± 929.5	3352.5 ± 566.4
FPBand$_{\mathrm{Min}}$	2330.6 ± 401.1	2402.7 ± 283.5	2011.9 ± 368.9	1914.4 ± 680.6	2027.3 ± 149.7
FPPeak$_{\mathrm{Mean}}$	3945.5 ± 270.6	4024.4 ± 241.5	3370.5 ± 157.7	3617.9 ± 215.1	3791.2 ± 241.1
FPPeak$_{\mathrm{Max}}$	5590.6 ± 695.4	5536.5 ± 757.8	4549.6 ± 389.7	4615.9 ± 564.2	5457.5 ± 343.1
FPPeak$_{\mathrm{Min}}$	2743.8 ± 274.8	2964.9 ± 281.1	2580.5 ± 240.4	2805.9 ± 455.1	2791.3 ±208.9
TI$_{\mathrm{Mean}}$	0.369 ± 0.066	0.413 ± 0.067	0.430 ± 0.047	0.351 ± 0.033	0.461 ± 0.037
TP$_{\mathrm{Mean}}$	1.038 ± 0.116	0.879 ± 0.094	0.901 ± 0.105	0.904 ± 0.112	0.840 ± 0.103

APPENDIX TABLE 4. Bioclimatic variables and their factor loadings in principal component analysis.

Bioclimatic variable	PCClim1	PCClim2	PCClim3	PCClim4
Annual Mean Temperature	**0.724**	0.191	0.000	**0.643**
Mean Diurnal Range (Max Temp − Mean Temp)	0.132	**−0.801**	−0.296	0.216
Isothermality ((Var2/Var7)*100)	**0.788**	−0.199	−0.444	0.135
Temperature Seasonality (Standard deviation * 100)	**−0.893**	−0.300	0.136	0.153
Max Temp (Warmest Month)	0.105	−0.501	−0.107	**0.799**
Min Temp (Coldest Month)	**0.884**	0.372	−0.078	0.172
Temperature Annual Range (Var5–Var6)	**−0.760**	−0.552	0.027	0.185
Mean Temp (Wettest Quarter)	−0.584	0.090	−0.064	**0.633**
Mean Temp (Driest Quarter)	**0.930**	0.129	0.017	−0.044
Mean Temp (Warmest Quarter)	0.090	−0.078	0.114	0.951
Mean Temp (Coldest Quarter)	**0.910**	0.255	−0.100	0.253
Annual Precipitation	0.310	**0.759**	0.530	−0.005
Precipitation (Wettest Month)	0.384	**0.885**	0.101	−0.019
Precipitation (Driest Month)	0.027	0.297	**0.942**	−0.011
Precipitation Seasonality (Coefficient of variation)	0.257	0.285	**−0.863**	0.114
Precipitation (Wettest Quarter)	0.385	**0.877**	0.122	−0.012
Precipitation (Driest Quarter)	0.053	0.328	**0.935**	−0.005
Precipitation (Warmest Quarter)	−0.286	0.319	**0.804**	0.199
Precipitation (Coldest Quarter)	0.582	**0.716**	0.121	−0.060
Variance explained (%)	42.47	26.97	14.24	7.53