

RESEARCH ARTICLE

Song type variations of Louisiana Waterthrush (*Parkesia motacilla*) and their geographic distributions

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Abstract

Louisiana Waterthrush (*Parkesia motacilla*) is a familiar singer in the Western Hemisphere family *Parulidae*, yet apparent geographic variations in its song and potentially related causal mechanisms have not received detailed examination in previously published studies. Here, we analyzed song pattern variations of 651 Louisiana Waterthrush singers in audio spectrogram recordings obtained from our field work and publicly accessible bioacoustics archives. Visual and auditory assessment of the introductory note sequence of each song identified three distinct song types (A, B, and C) and most of the songs were assigned to one of these types. Linear Discriminant Analysis and Random Forest methods were used to verify the assignments and showed strong agreement for Type A with slightly less agreement on Types B and C. User error rates (proportion of the Linear Discriminant Analysis classifications that were incorrect) were low for Types A and B, and somewhat higher for Type C, while producer error rates (proportion of the song type for which the Linear Discriminant Analysis was incorrect) were somewhat higher for Types A and C than the minimal levels achieved for Type B. Our findings confirmed that most between-individual variation was in the number of notes and note sequence duration while most within-individual variation resulted from the percent of downstrokes. The location of each singer was plotted on a map of the breeding range and results suggested the song types have large-scale discrete geographic distributions that co-occur in some regions but not range-wide. Evaluation of the distributions provided tentative support for a hypothesis that two of the song types may independently exhibit congruence with the geographic extent of Pleistocene glacial boundaries and the third song type may be distinguished by a lack of congruence, but further investigation is needed to elucidate whether the song variations represent subpopulations with three separate evolutionary histories.

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Introduction

The Louisiana Waterthrush (*Parkesia motacilla*) is unique among *Parulidae* as the only bird in the southeastern United States that breeds exclusively along forested streams [1]; prime habitat consists of narrow linear corridor territories along uncontaminated headwater streams in unfragmented mature forest with open understory and an optimal percentage of 30–69% deciduous species [2]. It is common for both males and females to return to their same territory in consecutive years [3]. Most foraging time (88.0–90.4%) is spent in or beside the stream and song is delivered from a favored perch above the stream or from the ground while foraging, and at times also in flight during territorial disputes [3]. The extent to which males discriminate between "familiar and unfamiliar" songs is not known [3]. Males sing vigorously upon arrival on the breeding grounds but after females arrive and are paired with males, singing sharply declines [3]. Eaton [4] reported rarely hearing the "advertising song" from the time of pair formation until incubation started around 3–4 weeks later [3], with reinstated song never delivered as often as prenuptial singing. Males sing infrequently during the incubation and early nestling phases, and nestlings may on occasion also be exposed to female song [3]. Several females have been observed singing a version of male song that is recognizable but has a less complicated and softer delivery and apparently functions to summon the male during incubation recess [3]. Males at the latitude of Ithaca, New York sang occasionally in late June and early July while tending semi-independent juveniles and had a resurgence of song in the first half of August after their molt was complete just prior to their early departure for wintering grounds [4]. The dependency period for fledglings lasts 3–4 weeks [4] after which some but not all may begin to wander from the natal area to varying degrees. Two juveniles banded by Eaton [4], were documented 2.0 km and 4.8 km from their natal areas just over a month after leaving the nest.

Geographic variation in songbird vocalizations has been documented in a large number of species [5, 6] and can be the consequence of various factors [7]. Imitative learning promotes geographic variation when cultural transmission of songs between generations allows shared novel vocalization patterns in song structure to arise through inaccurate learning and appearance of new components [8–10]. Nevertheless, variation in vocalizations is not unrestrained. Instead of copying the range of sounds to which they might be exposed, juvenile oscines preferentially learn the vocalizations characteristic of their species [11] or subspecies [12], and variations that arise during song evolution may be constrained or redirected by genetically based mechanisms such as physiological song production abilities [7]. Variation may also be limited within small colonizing or isolated populations that arise from genetically depauperate founder populations such as those on islands or in recently deglaciated areas populated from glacial refugia [13–16]. Populations that become geographically isolated undergo random cultural drift [17], and the songs of isolates may ultimately evolve to the extent that conspecifics in other populations alter their responses. This can lead to reproductive isolation [18] as was found with Australia's Chowchilla (*Orthonyx spaldingii*) whose song structure has large-scale geographic variations in bandwidth and frequency peaks attributed to cultural drift during isolation in Pleistocene refugia [19].

A combination of multiple evolutionary processes may influence geographic song variation as was observed in the bandwidth and internote duration variances between eastern and western Common Yellowthroats (*Geothlypis trichas*) by Bolus [20], who noted sexual selection can be a contributor that reinforces changes in song structure. Although nearly a third of all female oscines are not known to sing [21], they must nonetheless learn their parental song type alongside their male siblings in order to recognize and select a conspecific mate [22, 23]. Song dialects, defined by Mundinger [24] as variation between song forms with loosely discrete

boundaries, can be distinguished by both males and females of White-crowned Sparrow (*Zonotrichia leucophrys*) subspecies *nuttalli* [25]. Kroodsma [26] suggested certain populations with songs that differ discontinuously may possess differing evolutionary histories as has been demonstrated by the closely related and phenotypically similar taxonomic pairs of Marsh Wrens (*Cistothorus palustris plesius* and *C. p. iliacus*) [26, 27] and Eastern and Western meadowlarks (*Sturnella magna* and *S. neglecta*) [28, 29]. In regions of recontact, singing eastern and western Marsh Wrens and Eastern and Western meadowlarks evoke territorial defense from both respective male congeners despite their contrasting songs, yet females of both pairs of taxa reserve receptivity responses solely for singers of their parental song type [26, 28]. The importance of female discrimination was demonstrated in a study of sympatric Eastern and Western meadowlarks [30] in which the absence of song convergence was attributed to selection in order to oppose the establishment of a single song type and avoid interspecific matings in the area of sympatry.

Song divergence has been correlated with geographic variation in numerous avian subpopulations [20, 31–33]. It has been assumed that Louisiana Waterthrush exhibits no phenotypic geographic variation [3] in contrast with sister species Northern Waterthrush (*Parkesia noveboracensis*) [34], although Mattsson et al. [3] proposed subpopulations could potentially arise from discontinuous waterways on breeding grounds. A lack of connectivity between some riparian corridors may limit dispersal as suggested by Mattsson [1] for Louisiana Waterthrush, and by Machtans for other forest songbirds [35]. Geographic discontinuities in song structure can occur in species that have a pattern of limited dispersal and learn relatively simple repertoires in the natal region during the pre-dispersal period [10], the latter characteristic having been documented in Louisiana Waterthrush [3, 4].

Studies have revealed that artifactual adaptations to Pleistocene glaciations still endure today in some Neotropical migrants as exemplified by the unnecessarily circuitous migratory pathway that the Swainson's Thrush (*Catharus ustulatus*) has retained [36]. The ~2.6 million year Pleistocene Epoch [37] not only likely affected heritable traits, but also determined the contemporary distributions of many avian species [38–40] as repeated cycles of advancing and retreating glacial ice [41] fragmented and redistributed vegetation types [42] such as on the Great Plains where habitat transitioned between forests and grasslands [43]. Range expansions and recolonizations across newly-deglaciated habitat during ~20,000-year interglacial periods were succeeded by glacial intrusions that separated and shifted populations into discrete refugia for ~100,000-year periods [44]. East of the Rocky Mountains across what is presently Louisiana Waterthrush's breeding range [45], nearly all of the Laurentide Ice Sheet reached maximum extent during the earlier pre-Illinoian Stage glaciations, whereas only the central and eastern portions of the ice sheet reached commensurately extensive glacial maxima during the later Illinoian Stage, and only the eastern portion of the ice sheet reached a comparable extent during the Last Glacial Maximum (LGM) of the most recent Wisconsinan Stage [41, 46]. During major glaciations, high-altitude periglacial permafrost in the Appalachian Mountains [47, 48] would have adversely affected habitability adjacent to the ice sheet. Periglacial permafrost conditions at lower elevations directly west of the Appalachians were restricted to a narrow band which allowed temperate forest vegetation to exist close to the ice margin, but farther west, permafrost conditions along the western edge of the ice sheet covered a wider expanse during the LGM [42, 49, 50].

Pleistocene conditions appear to have contributed to intraspecific separations in dozens of avian species [51]. Glacial vicariance impacted many terrestrial and aquatic taxa in the southeastern United States [52–55] during periods of glacial maxima as the ice sheet at maximum extent adjoined the Mississippi Embayment alluvial landscape [56, 57] and divided southeastern refugia into eastern and western locations referred to as Appalachian and Ozark centers of

endemism by Strange and Burr [58]. The isolating combination of ice sheet and Embayment's habitat barrier produced divergent lineages of North American salamanders [59, 60] and stream dwelling "highland fishes" [58, 61], all of which share aquatic invertebrate prey sources with Louisiana Waterthrush and may themselves be included in its diet [3]. The Embayment's bisection of the distributions of some *Parulidae* species is evident in the current breeding ranges of Louisiana Waterthrush, Prairie Warbler (*Setophaga discolor*), and Worm-eating Warbler (*Helmitheros vermivorum*) [45].

As a result of observing Louisiana Waterthrush song pattern variations locally, we accessed publicly available bioacoustic archival recordings to inventory audio spectrograms from across the species' entire breeding range. In this study, we analyzed the song types identified in personal and public recordings, plotted their geographic distributions, assessed conceivable explanations for the configuration of the distributions, and evaluated each song type's distribution for potential correlation with Pleistocene glacial conditions.

Materials and methods

Song structure and song type characterization

The repertoire of the Louisiana Waterthrush is limited to a single song which is deemed to be its primary song when delivered without the extended song conclusion that is appended occasionally [62]. Each male's song is unique and individually recognizable [62, 63]. Variation in the pattern of introductory notes sung by some individuals has been noted [64]. Typical primary songs (Fig 1) commence with a repetitive series of introductory notes (Introductory

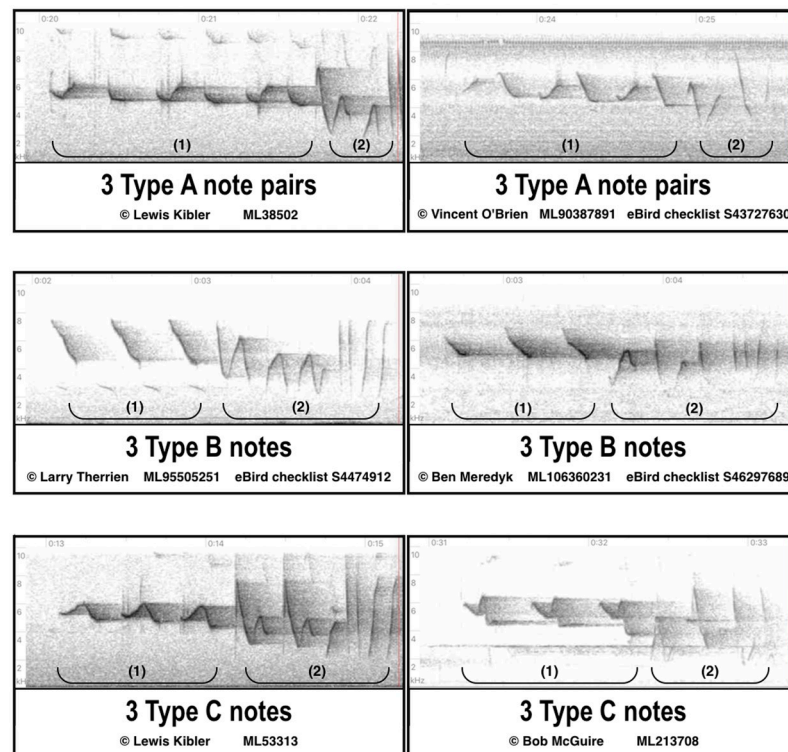


Fig 1. Spectrogram examples of the A, B, and C note types. Two examples of each note type have been selected from S1 Appendix. The analyzed introductory (1) and unanalyzed secondary (2) note sequences are designated by brackets; song type is determined solely by the introductory note sequence. Type A is comprised of alternating upstroke and downstroke note pairs, Type B employs downstrokes only, and Type C notes undulate both upwards and downwards.

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Note Sequence, INS) and culminate in a phrase of non-repetitive notes (Secondary Note Sequence, SNS) [64]. The extended song conclusion can be appended to the primary song [65] singly or in repetition, is usually employed during territorial defense by males that are most frequently paired [64], and when included, is thought to perform the same functions that some *Parulidae* species achieve by using a repertoire of more than one song [65, 66].

To investigate the range-wide distributions of the song type variations we had encountered in field work, the entirety of personal and public submissions of Louisiana Waterthrush audio recordings at Macaulay Library [67] and xeno-canto [68] from 1957 through 2018 was inventoried, and 820 spectrograms containing 4000+ songs were obtained. Visual and auditory examination of note shapes and sequences in the spectrograms allowed for identification of recordings shared by counter singing individuals whose songs required separate evaluation, and elimination of multiple recordings of a single singer submitted from the same location. Also eliminated were those that lacked sufficient quality for measurement, a few from wintering grounds south of the United States, and several misidentified species that were disclosed and corrected. The resulting database (S1 Appendix) contained 3833 songs of 651 individual singers. For spectrograms containing more than one song, the RANDBETWEEN function in Microsoft Excel was used to select one song per singer at random. A “note” was defined as a continuous trace on a spectrogram and only the INS notes that initiate the primary song were evaluated (Fig 1). Raven Lite 2.0 sound analysis software (Cornell Laboratory of Ornithology) was utilized to measure the INS in each of the 651 songs for the seven following variables: number of notes (“Notes”), note sequence duration (“Duration”), duration per note (“Dur_note”), percent of duration spent singing the downstroke portions of notes (“Percent_down”), minimum frequency (Min_freq”), maximum frequency (“Max_freq”), and frequency span (“Freq_span”). We emphasize that all measurements of song attributes were done by a single observer. Furthermore, these attributes are either simple to measure (e.g., number of notes) or are automatically measured by the Raven software (e.g., duration and frequency). Thus, they are readily repeatable and not subject to significant human judgment errors. The SNS phrase of notes that follows the INS and terminates the primary song was distinguished from the INS by its miscellany of notes that extend to lower minimum frequencies than the minimum frequencies of the more uniformly patterned INS notes. SNS patterns shared regionally by multiple singers provided an additional aid for distinguishing the SNS from the INS, and the SNS portion of the song was helpful for differentiating singers with nearly identical INS patterns. Although we were intrigued by the multitude of SNS patterns and their distributions relative to the INS, it was not feasible in this study to analyze the complex assemblage of abstruse note shapes comprising the poorly understood SNS part of the primary song. The extended song conclusion that is appended inconsistently to the primary song was also excluded from analysis. Some singers uttered either a single call note or a brief “grace note” preceding the INS delivery but many singers did not employ these notes that often were too abbreviated to yield sufficiently measurable data. Some individuals sang INS notes with faint marks stacked vertically above the notes in the spectrogram image but these marks were presumed to indicate overtone frequencies so were not evaluated. After careful visual and auditory examination of each spectrogram, the note shapes in the INS of each song were assigned to one of the three song types (Fig 1), or in cases where assignment was unclear, categorized as Unassigned.

Statistical analyses

Our goal was to identify and describe key characteristics of Louisiana Waterthrush songs, confirm the accuracy of our subjective classification of three song types, and understand which (if

any) of seven song features were good predictors of song type. We used descriptive statistics (mean + SD) to characterize seven quantitative measures of song type. We then used Linear Discriminant Analysis (LDA) [69] to classify song type on the basis of the seven measures. For this analysis, we eliminated frequency span because it was strongly correlated with measures of minimum and maximum frequency, was highly variable, and was generally uninformative with respect to song type. Because the six remaining variables had different scales, we standardized them ($SD = 1$ for all of them) and then plotted them for visual inspection. All analyses were run using the standardized data, which then are depicted in the relevant figures in our results. We did not convert back to the original scale for each variable when presenting the results. This plot helped us interpret the influence of each of the six predictors of song type on a two-dimensional plot of song types for this analysis. For example, observations with a high value of Notes (above the mean) and a low value of Duration (below the mean) will be plotted in the bottom left of the LDA scores plot (e.g., in the direction of Notes and opposite the direction of Duration). Similarly, variables like Max_freq and Min_freq that are close to the origin (0,0), even after rescaling, have little impact on the position of the observations in the LDA scores plot. This was also confirmed with the use of a Random Forest (RF) analysis [70], which is similar to the LDA but allows interactions between measures. Lastly, we used multiple song measures as described above under “Song structure and song type characterization” to describe the intra- and inter-song variation in a sample of Louisiana Waterthrush spectrograms. We fit a variance-covariance model to the data where individual was a random variable and there were replicates within each individual. We used the Intra-Class Correlation (ICC), which is the group variance divided by total variance, to assess the proportion of variation that was due to within- and between-individual variation. All analyses were run using Program R. (R Core Team 2018) and for the random forest we specified 1000 trees with two variables at each split.

Map of assigned singer locations with glacial environment

Recording locations were represented on a map of the eastern United States on Google My Maps and National Institute of Statistics and Geography map data (https://www.google.com/maps/d/u/0/viewer?mid=1PAkxNYqk_7iRslxEd57mgqR38u0&ll=40.669561163969384%2C-83.80830025&z=5) with color-coded pin markers placed at described locales or latitude and longitude coordinates provided by recordists. The 11 singers recorded in Florida are presumed migrants but were retained to contribute additional songs for analysis. Pleistocene glacial maximum boundaries were indicated on the map with gray screens that depicted the pre-Illinoian and Illinoian major glacial stages and the Wisconsinan LGM stage was represented with a black outline; map sources [42, 47, 71–74] encompassed both general and detailed illustrations to most accurately delineate former glacial boundaries for comparison to contemporary Louisiana Waterthrush locations. The high-altitude periglacial permafrost region in the Appalachian Mountains adjacent to the glacial boundary [47] was also depicted with a gray screen, the Wisconsinan LGM periglacial extent was depicted with a white line [49, 50], and the Mississippi Embayment region [75] that bisected suitable habitat in tandem with the ice sheet was portrayed with a gray outline.

Results

We measured 3833 Louisiana Waterthrush songs representing 651 individual singers (S1 Appendix) in this study. Visual/auditory assignments placed 88.33% of the songs into one of three song type groups designated as Type A (72; 11.06%), Type B (404; 62.06%), or Type C

Table 1. Summary statistics for measures of Louisiana Waterthrush song types.

Type	n	Notes	Duration	Dur_note	Pct_down	Min_freq	Max_freq	Freq_span
A	72	4.40 (1.27)	1.03 (0.25)	0.24 (0.05)	30 (9)	4019 (273)	6056 (481)	2037 (478)
B	404	2.81 (0.58)	0.77 (0.20)	0.27 (0.04)	53 (9)	4442 (230)	6853 (455)	2411 (441)
C	99	2.86 (0.61)	0.84 (0.20)	0.30 (0.05)	33 (9)	4248 (259)	6373 (451)	2124 (465)
Un	76	3.67 (1.28)	0.94 (0.25)	0.27 (0.05)	37 (11)	4187 (423)	6443 (583)	2256 (571)

Numbers are means (SD) and are given for the seven quantitative measures of each song.

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(99; 15.21%). The remaining 11.67% were categorized as Unassigned ("Un": Table 1) and were omitted from the analyses depicted below in Figs 2–5 and Tables 2 and 3.

Song type classification

For this analysis we used a subset of 651 songs, one per individual, for which we measured seven attributes (Table 1). The LDA coefficients varied across the six included variables (Fig 2) and helped interpret a plot of song type on the two LDA axes (Fig 3). Type A songs have above average values for Notes and below average values for Duration; the other four variables had

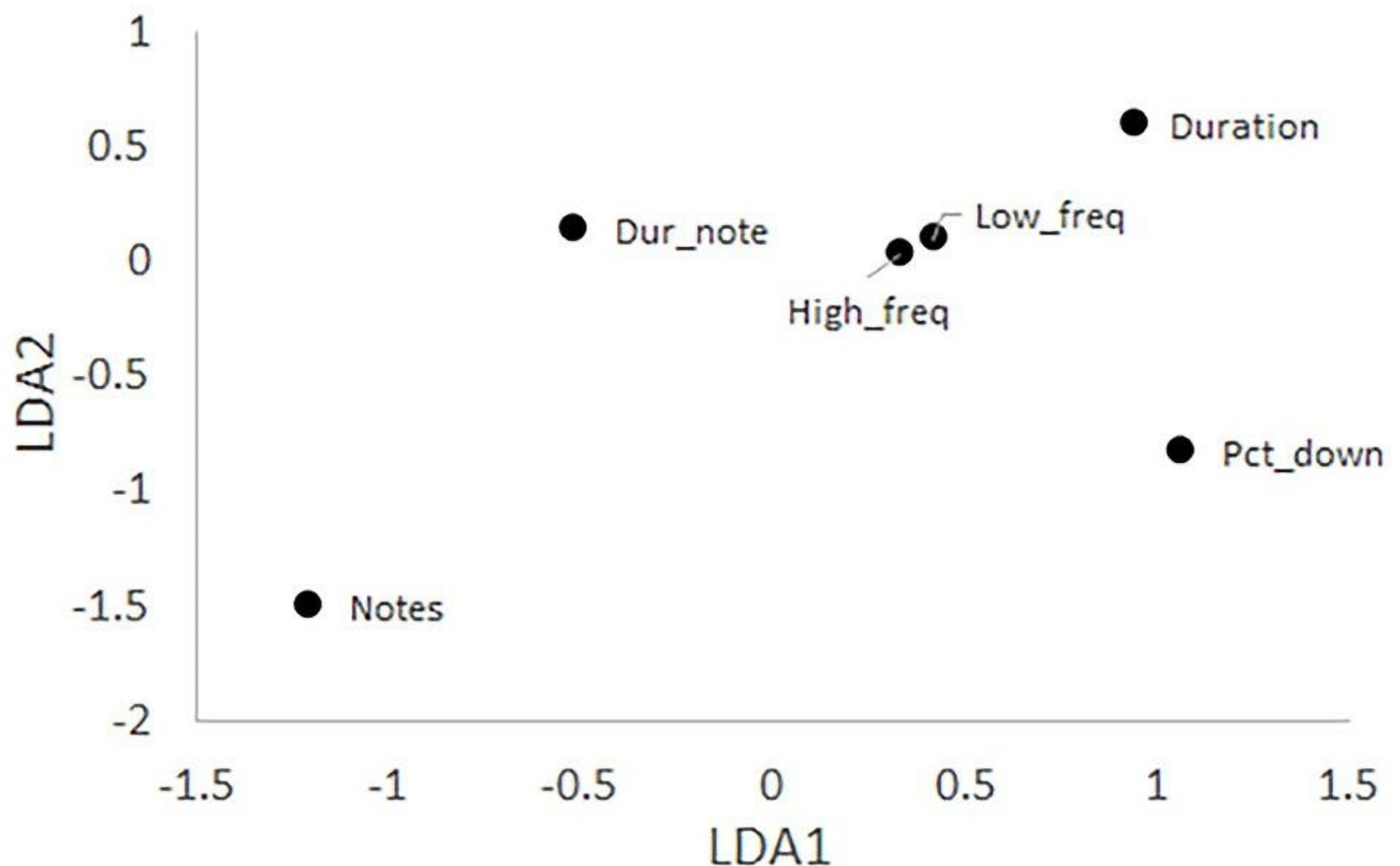


Fig 2. Plot showing the scaling coefficients of the Linear Discriminant Analysis of Louisiana Waterthrush song types. The six predictors of song type are arranged around the overall scaled mean (0,0) to indicate their relative influences. The six variables plotted are the number of notes (Notes), note sequence duration (Duration), the duration per note (Dur_note), the percent of duration spent singing the downstroke portions of notes (Pct_down), the minimum frequency (Min_freq), and the maximum frequency (Max_freq). Variables farthest from the scaled mean (Notes and Duration) have the greatest influence.

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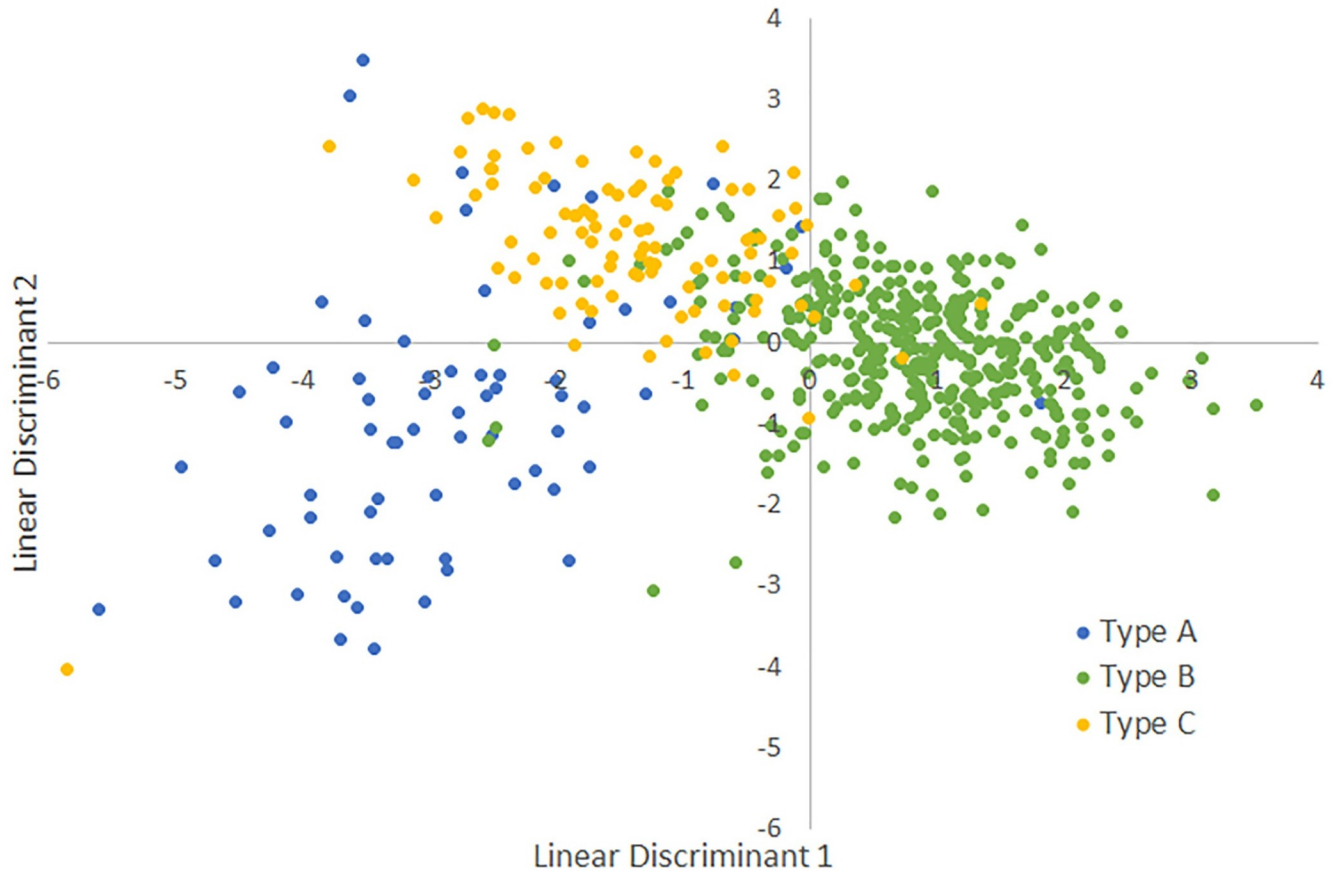


Fig 3. Diagram showing assigned song types (A, B, and C) for Louisiana Waterthrushes 1957–2018. The two axes are interpreted using the information in Fig 2 and the Linear Discriminant Analysis equations from the text.

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little role in pulling Type A songs away from the other song types (Fig 3). The confusion matrix for three song types (Table 2) showed that the model performed well with most misclassifications occurring between Types B and C. The user error rates (proportion of the LDA classifications that were incorrect) were <10% for Types A and B, but 26% for Type C. The producer error rates (proportion of the song type for which the LDA was incorrect) were >25% for Types A and C, but were <5% for Type B. The LDA equations were:

$$\text{LDA1} = -1.369 * \text{Notes} + 4.212 * \text{Duration} - 11.69 * \text{Dur_note} + 0.07852 * \text{Pct_down} + 0.001499 * \text{Min_freq} + 0.0006093 * \text{Max_freq} - 10.379$$

$$\text{LDA2} = -1.710 * \text{Notes} + 2.690 * \text{Duration} + 3.290 * \text{Dur_note} - 0.06055 * \text{Pct_down} + 0.0003522 * \text{Min_freq} + 0.00006326 * \text{Max_freq} + 2.937.$$

A comparison with results from a RF analysis confirmed that the two approaches yielded similar results (Table 3). Finally, we plotted misclassifications between the assigned song type and both the LDA classification (Fig 4) and the Discriminant Function (DF) classification (Fig 5) to better indicate where these methods showed disagreement.

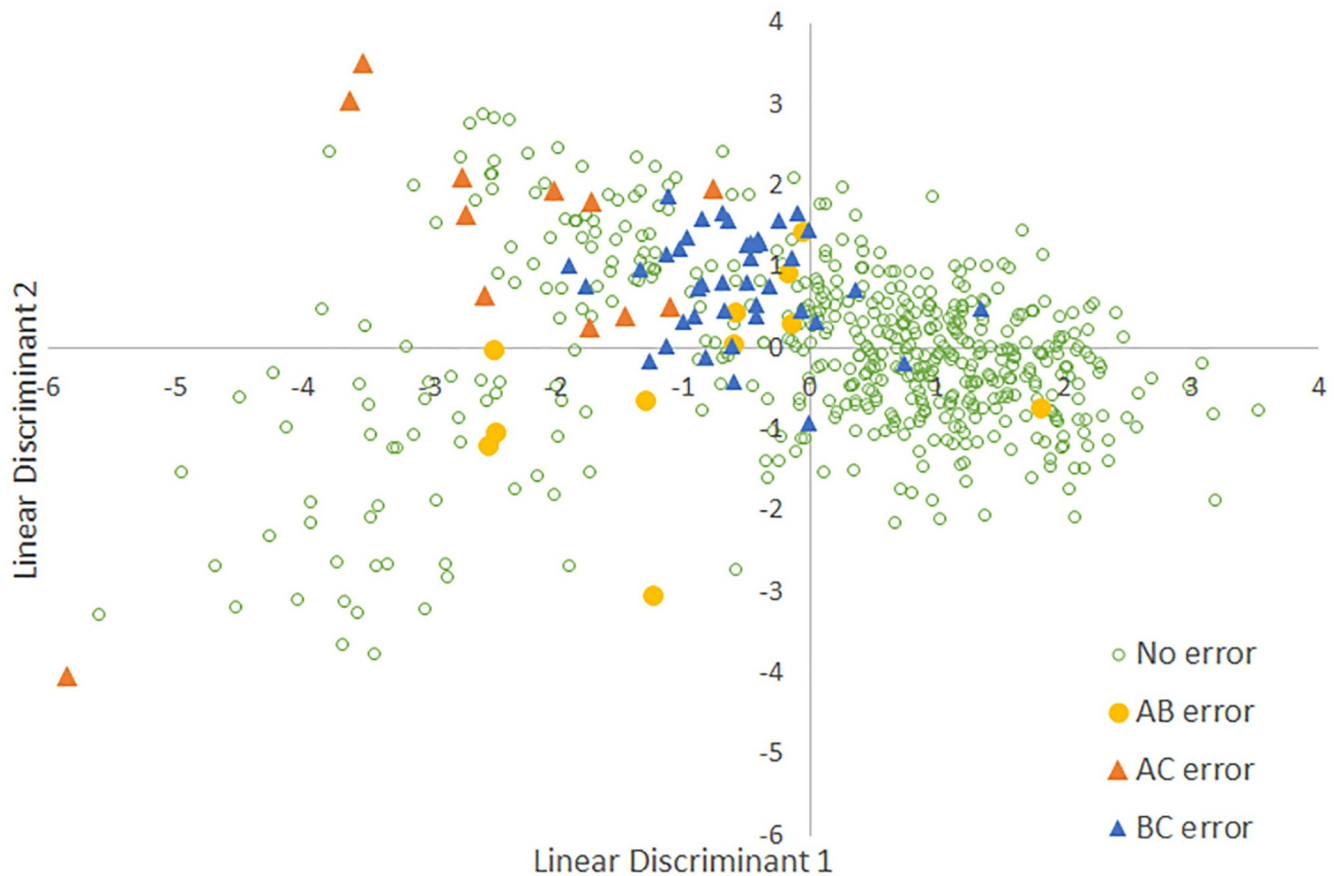


Fig 4. Errors between manual song type assignments and song type assigned by the Linear Discriminant Analysis. “No error” indicates song types with a match between assigned song type and Linear Discriminant Analysis assignment. “AB error” indicates song types that were classified by one method as A and by the other method as B. “AC error” and “BC error” map errors between those respective pairs of song types.

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Within- and between-song variation

To assess the contribution of within- and between-song variation, we used a subset of the 651 singers listed in the [S1 Appendix](#) comprised of all singers with 10 or more recorded songs per spectrogram for a total of 87 individuals with 1583 measured songs (11 Type A, 52 Type B, 15 Type C, and 9 Unassigned type). The mean number of measured songs per individual was 18 (SD = 7.8, range was 10 to 32). Most variation was between individuals ([Table 4](#)) with all ICC > 0.82. For the duration per note and the three frequency measures there was almost no variation either within or between song types.

Unassigned songs

Songs with INS note shapes or patterns that were not readily assignable as Type A, B, or C comprised 11.67% (76) of the total and were designated as Unassigned. The INS of the Unassigned songs sorted into three subcategories: “mixed types”, with notes incorporated from two or in one example three types (38; 5.83%), “equivocal”, which were open to more than one interpretation (30; 4.61%), and “anomalous”, involving note shapes or patterns atypical of Louisiana Waterthrush (8; 1.23%) ([Fig 6](#)). The geographic distribution of “mixed types” songs was nearly range-wide, although when displayed on the map concurrently with the A, B, and C types, 30 out of 38 “mixed types” singers occurred in areas where two or three types were

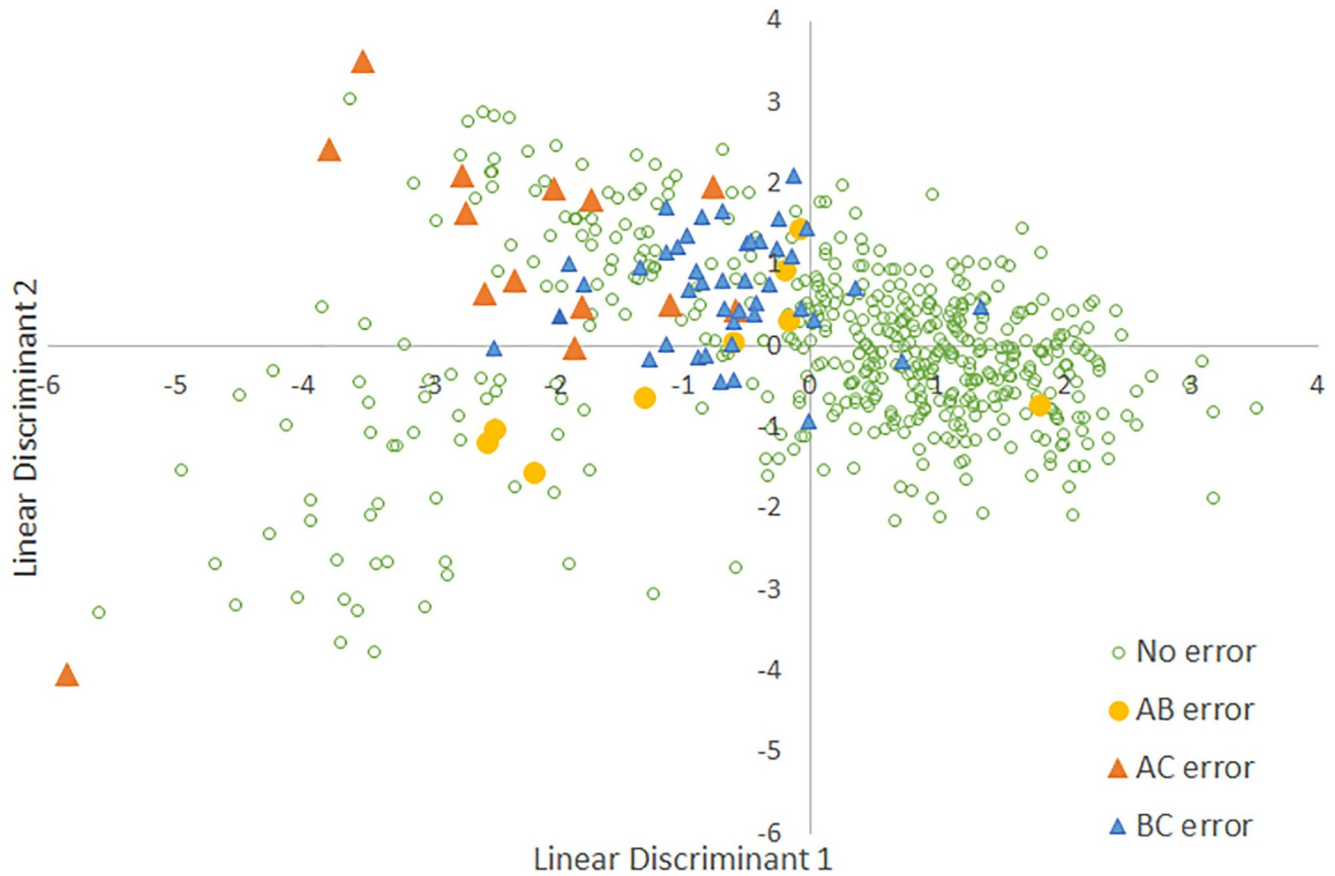


Fig 5. Errors between manual song type assignments and song type assigned by the discriminant function. “No error” indicates song types with a match between assigned song type and discriminant function assignment. “AB error” indicates song types that were classified by one method as A and by the other method as B. “AC error” and “BC error” map errors between those respective pairs of song types.

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present whose INS notes corresponded to notes employed by “mixed types” singers in the vicinity (https://www.google.com/maps/d/u/0/viewer?mid=1PAkxNYqk_7iRslxEd57mgqR38u0&ll=40.669561163969384%2C-83.80830025&z=5). Singers of the undetermined “equivocal” songs had a distribution that conformed with the distributions of Types A and C and were absent in most of the LGM deglaciated zone where Type B predominated. At least half of the “equivocal” songs had INS patterns that included what appeared to be

Table 2. Confusion matrix for Louisiana Waterthrush song types from a Linear Discriminant Analysis of seven song attributes. Columns are the Linear Discriminant Analysis classes. The numbers away from those in bold on the diagonal represent the misclassifications.

	Type A	Type B	Type C	Producer error
Type A	54	7	11	25.0%
Type B	4	386	14	4.5%
Type C	1	27	71	28.3%
User error	8.5%	8.1%	26.0%	

The user error is calculated by column and is $1 - (\# \text{ correct}/\text{total})$. The producer error is the same but is calculated for rows.

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Table 3. Comparison of results of linear discriminant and random forest analyses for Louisiana Waterthrush song types. For birds classified as Type A (left third of panel), 52 individuals were classified as Type A by both analyses, 6 individuals each as Types B and C by both analyses, and fewer individuals were assigned different song types by each analysis (those numbers off the diagonal). This is repeated in the middle and right panels for individuals classified as Types B and C. The numbers away from those in bold on the diagonal in each panel represent the inconsistent classifications between the two methods.

Style	Type A			Type B			Type C		
	A	B	C	A	B	C	A	B	C
Type A	52	1	1	2	1	1	1	0	0
Type B	0	6	1	0	381	5	0	23	4
Type C	4	1	6	0	4	10	4	6	61

Rows are the Linear Discriminant Analysis assignments and columns are the Random Forest assignments.

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variations on one or more Type A note pairs but these note pairs lacked the symmetry of alternating upstrokes and downstrokes typically seen in the Type A note pair pattern and so were left as Unassigned. The eight “anomalous” singers mostly occurred in the eastern part of the range and consisted of five singers with INS patterns that were a rapid series of abbreviated upstrokes, and three singers that lacked variation between the minimum frequencies of the INS and SNS notes in possible examples of SNS notes substituted for the INS series.

Song type distributions and comparisons with glacial environment

Markers plotted on the map to denote the song locales showed Types A, B, and C had potentially discrete geographic distributions that overlapped in some but not all areas (Figs 7 and 8). A depiction of Pleistocene glacial maxima overlaid on the map indicated all three types were distributed widely across previously unglaciated areas of the current breeding range with the exception of the Mississippi Embayment where occurrence was limited to Crowley’s Ridge (Fig 7). The majority of Type B singers (219; 54.2%) were located in the deglaciated zone of the LGM where that type was exclusively ubiquitous. Conversely, occurrences in the LGM deglaciated zone were rare for Type A (2; 2.8%) and minimal for Type C (20; 20.2%) with most of those occurrences recorded near the East Coast (Table 5, Fig 7). Type A singers had notable populations in the Maryland region and Southern Appalachians and were almost entirely absent between the Appalachians and the Embayment but predominated west of the Embayment in the region that remained ice-free during the LGM. Type A was the only song type found along the southwestern edge of the breeding range with the exception of two Type B singers at the range’s southwest terminus. Type C singers occurred primarily east of the Embayment with some located in the LGM deglaciated zone, but the majority were on the

Table 4. Variance components and Intra-Class Correlation (ICC) for within- and between-song variation in Louisiana Waterthrushes.

Song attribute	Between individuals	Within individual	ICC
Notes	1.030000	0.078400	0.929
Duration	0.041700	0.008920	0.824
Dur_note	0.001810	0.000300	0.858
Pct_down	0.017800	0.139000	0.928
Min_freq	0.000014	0.009840	0.934
Max_freq	0.000031	0.000151	0.954
Freq_span	0.000022	0.000219	0.909

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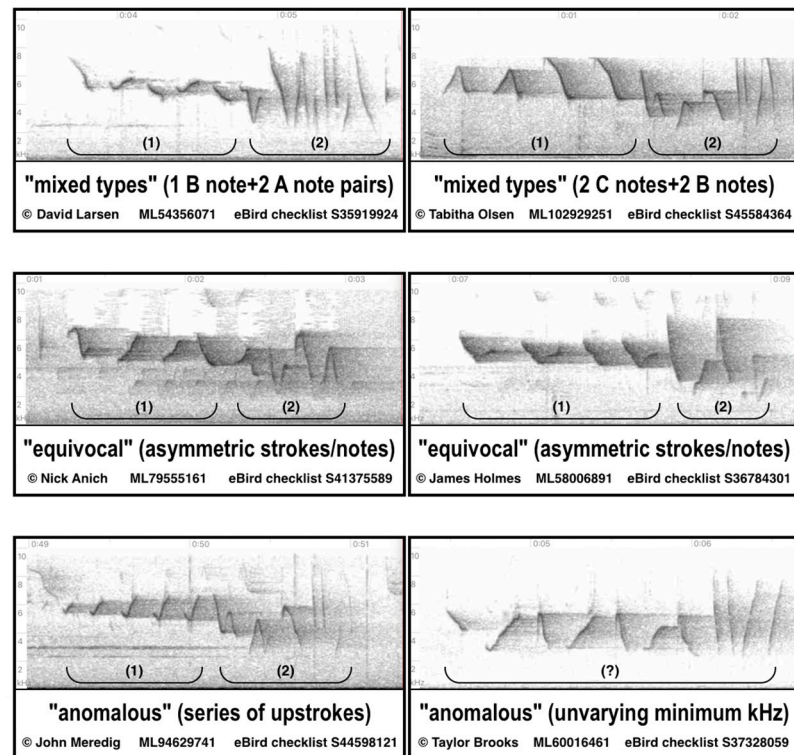


Fig 6. Spectrogram examples of unassigned songs. Two examples of each of the three subcategories of songs not readily assignable as Type A, B, or C have been selected from [S1 Appendix](#). The analyzed introductory (1) and unanalyzed secondary (2) note sequences are designated by brackets; classifications are determined solely by the introductory note sequence when practicable.

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unglaciated side of or abutting the Illinoian glacial maximum boundary, and this song type was the only type that occurred directly northeast of the Embayment. A few Type C singers were also observed west of the Embayment where their limited distribution more closely paralleled the distribution of Type A than Type B.

Discussion

The INS portion of most Louisiana Waterthrush songs consisted of a repetitive series of one of three note shape types that were assigned as Type A, B, or C. Statistical analyses supported assignments obtained from visual and auditory assessment and identified strong predictors of each type. Within the broader structure of each type's note shapes, there was considerable variance between individual singers in note sizes and dimensions. This individuality within the species' songs allowed for a multitude of singers to be identified and catalogued from the burgeoning supply of publicly available audio spectrograms, thus offering a largely untapped opportunity to study lineages and dispersal tendencies through unique song variations that are presumably culturally transmitted between generations. Although the number of notes delivered in the INS may fluctuate, there is strong implication that Louisiana Waterthrushes do not modify their INS patterns over time. Many spectrograms ([S1 Appendix](#)) showed identical Louisiana Waterthrush INS patterns recorded on the same territory in consecutive years indicative of returning singers that exhibited no significant changes in structure from the previous year unlike some other species such as Indigo Bunting (*Passerina cyanea*) [76]. INS patterns that

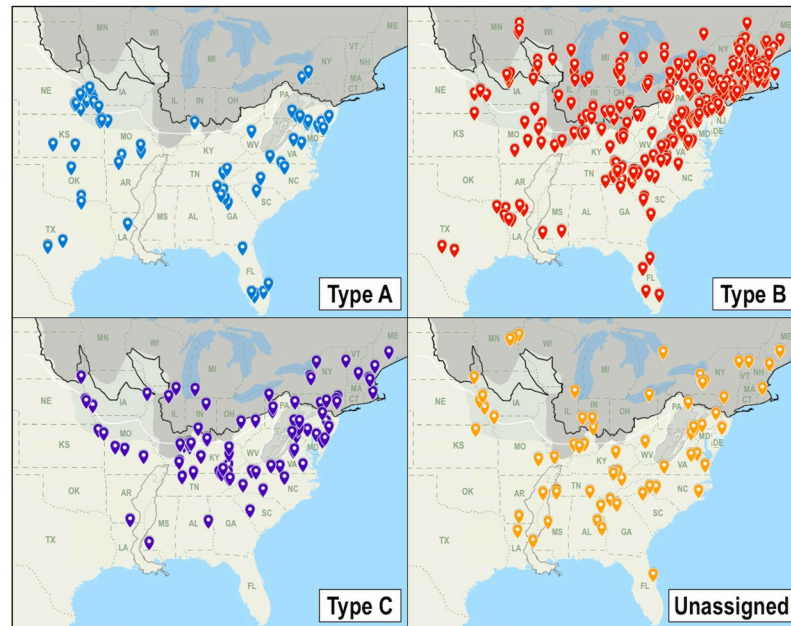


Fig 7. Mapped locations of all analyzed singers displayed by category with glacial environment. Comparison of the distributions of the four designated categories with former glacial conditions. Pre-Illinoian glacial maximum is represented by a light gray screen, Illinoian glacial maximum and high-altitude periglacial permafrost are represented by dark gray screens, the Last Glacial Maximum boundary is indicated by a black line and accompanying permafrost boundary by a white line, and a gray outline represents the Mississippi Embayment.

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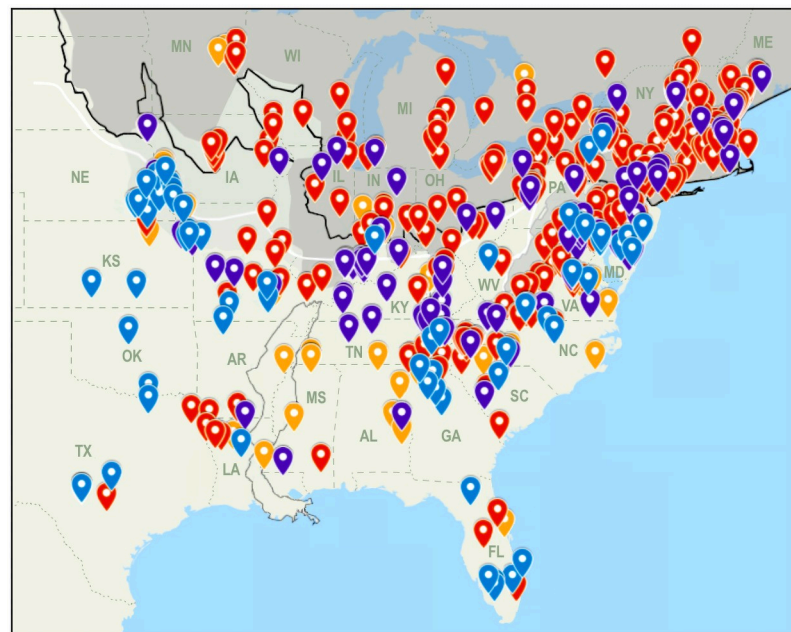


Fig 8. Mapped locations of all analyzed singers displayed concurrently with glacial environment. Song type sympatry and former glacial conditions. Pre-Illinoian glacial maximum is represented with a light gray screen, Illinoian glacial maximum and high-altitude periglacial permafrost are represented by dark gray screens, the Last Glacial Maximum boundary is indicated by a black line and accompanying permafrost boundary by a white line, and a gray outline represents the Mississippi Embayment.

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Table 5. Percentage of each song type distributed north and south of the Last Glacial Maximum boundary.

Song Type	North	%	South	%
A (N = 72)	2	2.8	70	97.2
B (N = 404)	219	54.2	185	45.8
C (N = 99)	20	20.2	79	79.8

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were consistent from year to year were observed in all three Louisiana Waterthrush song types as well as in an Unassigned singer in [S1 Appendix](#) whose uniquely “anomalous” song pattern was recorded on the same territory in three consecutive years (https://macaulaylibrary.org/asset/96116561#_ga=2.151583545.1271760166.1542289459-1614378356.1522970868). Each Unassigned singer delivered an individually unique combination and pattern of INS notes with a level of consistency between repetitions on par with singers of Types A, B, and C songs. Two singers recorded in July and three singers recorded in August (e.g. Type B singer in [S1 Appendix](#): <https://ebird.org/checklist/S3874893>) were considered to be potential examples of juvenile singing because they sang recognizable song patterns with INS note shapes that were markedly inconsistent yet still assignable to type.

Distinct INS patterns consisting of a single uniform song type may possibly be maintained by female selection similar to dialect recognition in female White-throated Sparrows [25] or what is seen between the taxonomic pairs of eastern and western Marsh Wrens and Eastern and Western meadowlarks when the sympatric occurrence of those phenotypically similar congeners results in females reserving receptivity for singers of their parental song type while singing males evoke territorial defense from both taxa despite their contrasting songs [26, 27]. Regarding female Louisiana Waterthrush INS discrimination, we note here an observation of a “mixed types” singer that was defending a territory on which juveniles were present, indicative of a singer with a non-uniform INS having successfully attracted a female who may or may not have shared the same “mixed types” lineage. Limited habitat at that locale accommodated only one male rival who sang a corresponding “mixed types” song; this finding of adjacent singers that shared a unique “mixed types” song pattern suggests a male had returned to his natal stream to vie for territory with a related male. While some shared song patterns (INS + SNS) within the song types were found to occur a considerable distance apart, with the greatest distance noted in a shared Type B pattern found in the deglaciated zone 906 km (563 mi) apart, shared song patterns of all the types were also commonly found on adjacent territories or streams, suggesting that as with the matching “mixed types” neighbors, dispersal may be limited at times, a trait known to contribute to geographic discontinuities in song structure [10].

Some insight into the assembly of Louisiana Waterthrush song was provided by examining the INS of “mixed types” singers in the Unassigned category. It is informative that most were recorded in the vicinity of two or three neighboring song types whose INS notes corresponded to INS notes in the “mixed types” songs nearby. (Figs 7 and 8; https://www.google.com/maps/d/u/0/viewer?mid=1PAkxNYqk_7iRslxEd57mgqR38u0&ll=40.669561163969384%2C-83.80830025&z=5). The parsimonious explanation for the origin of these songs with a mixture of types in the INS is a scenario in which juveniles were exposed to adult males singing different song types on adjacent territories during a crucial learning period. In personal observations ([S1 Appendix](#)), adjacent territories were most frequently defended by males that shared the same INS type, but it was not uncommon for males of differing INS types to also vigorously defend adjacent territories. The low percentage (5.83%) of total songs analyzed that have a mixture of types in the INS is perhaps unexpected given the prevalent sympatry of the three song types. Careful inspection of the spectrograms indicated several instances of counter

singing males with differing song types but the presence of more than one type on a stream typically did not result in any known neighboring "mixed types" singers. This observation leads to questions relating to song convergence: why is there a paucity of songs with INS patterns comprised of more than a single type, and why hasn't exposure to the different types sung by neighbors over the course of time resulted in more Louisiana Waterthrush singers having incorporated various combinations of INS note types as the "mixed types" singers have done? Perhaps this can be attributed to female preference for INS uniformity.

The widespread and often overlapping geographic distributions of three statistically separable variations in INS type raise other intriguing questions. For example, what factors could have contributed to the distributional differences between them? Latitudinal differences place Type B's distribution northernmost, Type A's distribution extending northwards the least, and Type C's as intermediate between Types A and B. Although the three distributions seem indistinctly discrete when cursorily examined, the addition of glacial maxima boundaries to the map in conjunction with the song type distributions (Figs 7 and 8) reveals possible correlations that may offer clues to each type's evolutionary history. Differences in the distributions become more pronounced upon examination of the notable presence and absence each type has in different parts of the breeding range: Type A is the only type found along the far western edge with the exception of two Type B singers at the southern extreme, yet Type A's distribution abruptly ends where the nearby LGM deglaciated zone begins. Type B conversely has a large numerical majority in the deglaciated zone, yet it is oddly absent in the Delaware region where the other two types abound. Type C is the only type found immediately northeast of the Embayment, yet it has a meager presence southwest of the Embayment.

Type B's predominance across the region that was most recently covered by glacial ice on what would have been newly available habitat following the recession of the LGM could have resulted from "localized expansion" from a northerly glacial refugium, a postglacial migration scenario proposed for a diversity of upper Midwestern species by Li et al. [77]. Type A's preponderance on the west edge of the breeding range may indicate that type similarly expanded westward across that same deglaciated zone during an earlier interglacial period, and subsequently was isolated as a subpopulation in the western region during convergence of a glaciation consistent with the Illinoian glacial maximum with the Embayment that facilitated a regionalized expansion of Type A at a time when forest habitat would have prevailed in parts of the Great Plains. The comparatively small Type A subset's disproportionate representation west of the Embayment on previously unglaciated terrain and in the area that has not been glaciated since pre-Illinoian episodes is puzzling when contrasted with its near absence in the adjacent LGM deglaciated zone predominated by Type B (Fig 7). The literature has no indication of heterogeneity in the specialized riparian breeding habitat utilized by Louisiana Waterthrushes and so it is unlikely the discernible demarcation between the distributions of Type A and Type B can be attributed to differences in the present-day habitat of those contiguous parts of the range. Alternatively, a heritable adaptation involving aversion to previously glaciated areas akin to what is seen in Swainson's Thrush [36] could feasibly account for the difference in the distributions if such an adaptation is exhibited by Type A but not shared by Type B occupants of the deglaciated zone. This rationale for Type A's broad-ranging distributional congruency with former LGM glacial conditions could similarly explain the disproportionate abundance (35) of Type C singers between the east side of the Embayment and the Appalachians which occur on the previously unglaciated side of and about the Illinoian glacial maximum boundary line, with only a few (5) observed in the deglaciated zone across that same longitudinal span (Fig 7). Although Type C's distribution largely parallels the distribution of Type A in that large majorities of each occur in the unglaciated region or the region that has not been glaciated since the earlier pre-Illinoian episodes, Type C is not as consistent as Type

A in its distributional mirroring of glacial maxima, and a portion (20.2%) of Type C singers also occurs alongside Type B singers in the LGM deglaciated zone.

Geographic distribution is not the only attribute in which Type C displays overlap with Types A and B. Of the three song types, Type A's INS frequencies extend the lowest on the kHz spectrum and consist of the lowest average minimum and maximum frequencies, Type B's INS frequencies extend the highest on the song type spectrum and present the highest minimum and maximum average frequencies, and Type C's INS minimum and maximum frequency measures are intermediate between the song types (Table 1). Although the three INS types have similar frequency spans, their separation in minimum and maximum frequencies and large-scale distributions may bear some resemblance to the large-scale geographic variations in frequency peaks and span of Chowchilla songs that resulted from isolation in Pleistocene refugia [19]. Origins of the INS variations in Louisiana Waterthrush are obscure. We were unable to detect any clinal patterns in the INS that might indicate a drift-like process had led to the three variations. The initial notes of the SNS are often shared locally and so perhaps that portion of the song is a regionally tailored adjunct to the broader INS distributions, but because the INS patterns of the three types are widespread rather than restricted to localized occurrence, results of this study do not readily support pattern variance specifically in the INS portion of the song having arisen on non-contiguous locally isolated streams, an isolating mechanism suggested by Mattsson et al. for possible discontinuous subpopulations [3].

Molecular evidence studied by Avise and Walker [51] suggests many avian intraspecific populations date to Pleistocene separations. The aforementioned merger of the glacial maximum with the Embayment would have provided an isolation event [57] in which a Louisiana Waterthrush Ozark Type A subpopulation's INS pattern could have potentially diverged from an Appalachian Type C subpopulation's INS pattern, and there is evidence of additional Pleistocene isolating scenarios in that region that provided theoretically favorable conditions for INS variation to arise. Genetic research in the eastern United States within Louisiana Waterthrush's breeding range has established that northerly refugia were maintained in the northern Appalachians region and in the upper Midwest in areas that gave rise to variations in multiple taxa and harbored potentially suitable deciduous habitat. A Pleistocene refugium has been indicated for the sugar maple tree species *Acer saccharum* in the Northeast and a subsequent postglacial migration route from this refugium proposed from the periglacial areas of the Pennsylvania region [78] near a congregation of Type A and C singers. Another refugium in the Midwest, where Type B currently predominates, sustained an unglaciated keyhole of habitat in the Driftless Area in the contiguous corners of Minnesota, Wisconsin, Illinois, and Iowa, where several species of mammals, deciduous plants, and amphibians survived [79–83]. The herbaceous vine *Smilax* sp. survived the LGM in a northern refugium in that area, resulting in an isolated population in the Driftless Area separated from other populations east of the Embayment [77].

After evaluating the mapped distributions of the three identified Louisiana Waterthrush song types, we propose that Types A and C exhibit a preference for previously unglaciated areas and areas that have not been glaciated since pre-Illinoian episodes, and that the preponderance of Type B dissimilarly occurs in areas that were most recently glaciated. We hypothesize that geographic relationships between INS variations and former Pleistocene glacial boundaries in separate parts of the breeding range could represent contemporary patterns in the type distributions that are indicative of separate evolutionary lineages in three subpopulations shaped either wholly or in part by glaciation-related isolation events. Although this hypothesis may offer a reasonable explanation for the differing song types and their distributions, support is inconclusive. We hope this initial exploration of the topic will spur further investigation into potential geographic congruency between the INS portion of the songs and

former glacial limits, and also into whether the distributions of the SNS portion of the songs represent smaller-scale dialects.

Supporting information

S1 Appendix. Locations, dates, and seven measures of the 651 analyzed Louisiana Waterthrush singers.

(XLSX)

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References

1. Mattsson BJ. Louisiana Waterthrush ecology and conservation in the Georgia Piedmont. 2006. PhD thesis, University of Georgia, Athens, Georgia, U.S.A.
2. Prosser DJ, Brooks RP. A verified Habitat Suitability Index for the Louisiana Waterthrush. *J Field Ornithol.* 1998; 69:288–298.
3. Mattsson BJ, Master TL, Mulvihill RS, Robinson WD. Louisiana Waterthrush (*Parkesia motacilla*), version 1.0. In: *Birds of the World*, Poole AF editor. Ithaca, NY: Cornell Lab of Ornithology 2020. doi.org/10.2173/bow.louwat.01
4. Eaton SW. A life history study of the Louisiana Waterthrush. *Wilson Bull.* 1958; 70:211–36.
5. Marler P., Tamura M. Song "Dialects" in Three Populations of White-Crowned Sparrows. *Condor* 1962; 64:368–77.
6. Krebs JR, Kroodsma DE. Repertoires and geographical variation in bird song. *Adv Study Behav.* 1980; 11:143–77.

7. Podos J, Huber SK, Taft B. Bird song: The interface of evolution and mechanism. *Annual Reviews in Ecological Evolutionary Systems* 2004; 35:55–87.
8. Marler P. Bird song and mate selection. In: *Animal Sounds and Communication*, Lanyon WE, Tavolga WN, editors. Washington DC: American Institute of Biological Science; 1960. pp. 348–367.
9. Slabbekoorn H, Smith TB. Bird song, ecology and speciation. *Philos Trans R Soc Lond, B, Biol Sci.* 2002; 357:493–503. <https://doi.org/10.1098/rstb.2001.1056> PMID: [12028787](https://pubmed.ncbi.nlm.nih.gov/12028787/)
10. Podos J, Warren PS. The Evolution of Geographic Variation in Birdsong. *Adv Study Behav.* 2007; 37:403–58. [https://doi.org/10.1016/S0065-3454\(07\)37009-5](https://doi.org/10.1016/S0065-3454(07)37009-5)
11. Remsen J V Jr. Pattern, process, and rigor meet classification. *Auk* 2005; 122:403–13.
12. Nelson DA. A preference for own-subspecies' song guides vocal learning in a songbird. *Proc Natl Acad Sci U S A* 2000; 97:13,348–53.
13. Thielcke G. On the origin of divergence of learned signals (songs) in isolated populations. *Ibis* 1973; 115:511–6.
14. Galbreath KK, Cook JA. Genetic consequences of Pleistocene glaciations for the tundra vole (*Microtus oeconomus*) in Beringia. *Mol Ecol.* 2004; 13:135–148. <https://doi.org/10.1046/j.1365-294x.2004.02026.x> PMID: [14653795](https://pubmed.ncbi.nlm.nih.gov/14653795/)
15. Xing XY, Alstrom P, Yang XJ, Lei FM. Recent northward range expansion promotes song evolution in a passerine bird, the Light-vented Bulbul. *J Evol Biol.* 2013; 26:867–877. <https://doi.org/10.1111/jeb.12101> PMID: [23438018](https://pubmed.ncbi.nlm.nih.gov/23438018/)
16. Lynch M. Random Drift, Uniform Selection, and the Degree of Population Differentiation. *Evolution* 1986; 40:640–3. <https://doi.org/10.1111/j.1558-5646.1986.tb00515.x> PMID: [28556342](https://pubmed.ncbi.nlm.nih.gov/28556342/)
17. Irwin DE. Song variation in avian ring species. *Evolution* 2000; 54:998–1010. <https://doi.org/10.1111/j.0014-3820.2000.tb00099.x> PMID: [10937272](https://pubmed.ncbi.nlm.nih.gov/10937272/)
18. Koetz AH, Westcott DA, Congdon BC. Geographical variation in song frequency and structure: the effects of vicariant isolation, habitat type and body size. *Anim Behav.* 2007; 74:1573–83. doi.org/10.1016/j.anbehav.2007.03.022.
19. Baker MC, Baker MSA, Baker EM. Rapid evolution of a novel song and an increase in repertoire size in an island population of an Australian songbird. *Ibis* 2003; 145:465–471. doi.org/10.1046/j.1474-919X.2003.00190.x.
20. Bolus RT. Geographic variation in songs of the Common Yellowthroat. *Auk* 2014; 131:175–185. doi.org/10.1642/AUK-12-187.1.
21. Odom KJ, Hall ML, Riebel K, Orland K, Langmore NE. Female song is widespread and ancestral in songbirds. *Nat Commun.* 2014; 5:3379. <https://doi.org/10.1038/ncomms4379> PMID: [24594930](https://pubmed.ncbi.nlm.nih.gov/24594930/)
22. Borror DJ. Intraspecific variation in passerine bird songs. *Wilson Bull.* 1961; 73:57–78.
23. Kroodsma DE. Geographical variation and functions of song types in warblers (*Parulidae*). *Auk* 1981; 98:743–51.
24. Mundinger PC. Microgeographic and macrogeographic variation in the acquired vocalizations of birds. In: *Acoustic communication in birds*, Kroodsma DE, Miller EH, editors. New York: Academic Press; 1982. Vol 2, pp 147–208.
25. Milligan MM, Verner J. Inter-population song dialect discrimination in the White-crowned Sparrow. *Condor* 1971; 73:208–13.
26. Kroodsma DE. Two North American song populations of the Marsh Wren reach distributional limits in the central Great Plains. *Condor* 1989; 91:332–40.
27. Kroodsma DE. Two species of Marsh Wren (*Cistothorus palustris*) in Nebraska? *Nebraska Bird Review* 1988; 56:40–2.
28. Lanyon WE. *The Comparative Biology of the Meadowlarks (Sturnella) in Wisconsin*. Cambridge, Massachusetts: Publications of the Nuttall Ornithological Club, No. 1; 1957.
29. Barker FK, Vandergon AJ, Lanyon SM. Assessment of species limits among yellow-breasted meadowlarks (*Sturnella* spp.) using mitochondrial and sex-linked markers. *Auk* 2008; 125:869–79.
30. Ordal JM. Effect of Sympatry on meadowlark vocalizations. *Condor* 1974; 78:100–1.
31. Eilers J, Slabbekoorn H. Song divergence and male dispersal among bird populations: a spatially explicit model testing the role of vocal learning. *Anim Behav.* 2003; 65:671–681. doi.org/10.1006/anbe.2003.2081.
32. González C, Ornelas JF, Gutiérrez-Rodríguez C. Selection and geographic isolation influence hummingbird speciation: genetic, acoustic and morphological divergence in the wedge-tailed sabrewing (*Campylopterus curvipennis*). *BMC Evol Biol.* 2011; 11: 38. <https://doi.org/10.1186/1471-2148-11-38> PMID: [21299905](https://pubmed.ncbi.nlm.nih.gov/21299905/)

33. Shizuka D, Lein MR, Chilton G. Range-wide patterns of geographic variation in songs of Golden-crowned Sparrows (*Zonotrichia atricapilla*). *Auk* 2016; 133:520–9.
34. Whitaker DM., Eaton SW. Northern Waterthrush (*Parkesia noveboracensis*), version 1.0. In: *Birds of the World*, Poole AF editor. Ithaca, NY: Cornell Lab of Ornithology 2020. doi.org/10.2173/bow.norwat.01.
35. Machtans CS, Villard M-A, Hannon SJ. Use of riparian buffer strips as movement corridors by forest birds. *Conserv Biol.* 1996; 10:1366–79.
36. Ruegg KC, Hijmans RJ, Moritz C. Climate change and the origin of migratory pathways in the Swainson's thrush, *Catharus ustulatus*. *J Biogeogr* 2006; 33:1172–82.
37. Da J, Zhang YG, Li G, Meng X, Junfeng J. Low CO2 levels of the entire Pleistocene epoch. *Nat Commun.* 2019; 10, 4342. <https://doi.org/10.1038/s41467-019-12357-5> PMID: 31554805
38. Mengel RM. The probable history of species formation in some northern Wood Warblers (*Parulidae*). *Living Bird* 1964; 3:9–43.
39. Bermingham E, Rohwer S, Freeman S, Wood C. Vicariance biogeography in the Pleistocene and speciation in North American wood warblers: a test of Mengel's model. *Proc Natl Acad Sci U S A.* 1992; 15:89: 6624–28. <https://doi.org/10.1073/pnas.89.14.6624> PMID: 11607307
40. Cabanne GS, Calderon LP, Arias NT, Flores P, Pessoa R, d'Horta F et al. Effects of Pleistocene climate changes on species ranges and evolutionary processes in the Neotropical Atlantic Forest. *Biol J Linn Soc Lond.* 2016; 119:856–72.
41. Mickelson DM, Colgan PM. The southern Laurentide Ice Sheet in the United States In: *Quaternary History of the United States*, Gillespie AR, Porter S. editors. International Quaternary Association Special Volume for 2003 International Meeting in Reno, Nevada, pp 1–16.
42. Loehle C. Predicting Pleistocene climate from vegetation in North America. *Clim Change* 2007; 94:233–45
43. Wisely SM, Statham MJ, Fleischer RC. Pleistocene Refugia and Holocene Expansion of a Grassland-Dependent Species, the Black-Footed Ferret (*Mustela nigripes*). *J Mammal* 2008; 89:87–96. doi.org/10.1644/07-MAMM-A-077.1.
44. Huybers P, Wunsch C. Obliquity pacing of the late Pleistocene glacial terminations. *Nature* 2005; 434:491–94. <https://doi.org/10.1038/nature03401> PMID: 15791252
45. Dunn JL, Garrett KL. *A Field Guide to the Warblers of North America*. Boston and New York: Houghton Mifflin Company 1997.
46. Reinertsen DL. Guide to the geology of the Galena area: Jo Daviess County, Illinois, Lafayette County, Wisconsin. Illinois State Geological Survey/Fieldtrip guidebook No. 1992D 1992.
47. Péwé TL. The periglacial environment of the United States in late Wisconsin time. In: *Late Quaternary environments of the United States, Volume 1, The late Pleistocene*. Wright HE Jr. editor. Minneapolis, MN: University of Minnesota Press, 1993. pp 157–89.
48. French HM, Millar SWS. Permafrost at the time of the Last Glacial Maximum (LGM) in North America. *Boreas* 2014; 43:667–77. <https://doi.org/10.1111/bor.12036>
49. Vandenberghe J, French HM, Gorbunov A, Marchenko S, Velichko AA, Jin H, et al. Last Permafrost Maximum (LPM) map of the Northern Hemisphere: permafrost extent and mean annual air temperatures, 25–17 ka BP. *Boreas* 2014; 43:652–66. <https://doi.org/10.1111/bor.12070> ISSN 0300-9483.
50. Miller CE Jr. The Late Pleistocene-Early Holocene in Central Pennsylvania. Map p. 4 in *Pennsylvania Geology* 2019; 49 (2):3–13. <http://docs.dcnr.pa.gov/cs/groups/public/documents/document/v49no2.pdf>.
51. Avise JC, Walker DE. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. *Proceedings of the Royal Society B.* <https://doi.org/10.1098/rspb.1998.0317> PMID: 9569664
52. Wiley EO, Mayden RL. Species and speciation in phylogenetic systematics, with examples from the North American fish fauna. *Annals of the Missouri Botanical Garden* 1985; 72:596–635.
53. Gill FB, Mostrom AM, Mack AL. Speciation in North American chickadees: I. Patterns of mtDNA genetic divergence. *Evolution* 1993; 47:195–212. <https://doi.org/10.1111/j.1558-5646.1993.tb01210.x> PMID: 28568102
54. Inoue K, Monroe EM, Elderkin CL, Berg DJ. Phylogeographic and population genetic analyses reveal Pleistocene isolation followed by high gene flow in a wide-ranging but endangered freshwater mussel. *Heredity* 2014; 112:282–90. <https://doi.org/10.1038/hdy.2013.104> PMID: 24149656
55. Thesing BD, Noyes RD, Starkey DE, Shepard DB. Pleistocene climatic fluctuations explain the disjunct distribution and complex phylogeographic structure of the Southern Red-backed Salamander, *Plethodon serratus*. *Evol Ecol.* 2015; 30:89–104. <https://doi.org/10.1007/s10682-015-9794-3>

56. Cushing EM, Boswell EH, Hosman RL. General Geology of the Mississippi Embayment. USGS Professional Paper 448-B 1964. 28 p.
57. Van Arsdale RB, Cox RT. The Mississippi's Curious Origins. Scientific American January 2007.
58. Strange RM, Burr BM. Intraspecific phylogeography of North American Highland fishes: A test of the Pleistocene vicariance hypothesis. *Evolution* 1997; 51:885–97. <https://doi.org/10.1111/j.1558-5646.1997.tb03670.x> PMID: 28568567
59. Highton R, Webster TP. Geographic protein variation and divergence in populations of the salamander *Plethodon cinereus*. *Evolution* 1976; 30:33–45. <https://doi.org/10.1111/j.1558-5646.1976.tb00879.x> PMID: 28565041
60. Zamudio KR, Savage WK. Historical isolation, range expansion, and secondary contact of two highly divergent mitochondrial lineages in spotted salamanders (*Ambystoma maculatum*). *Evolution* 2003; 57: 1631–52. <https://doi.org/10.1554/02-342> PMID: 12940367
61. Near TJ, Page LM, Mayden RL. Intraspecific phylogeography of *Percina evides* (Percidae: Etheostominae): an additional test of the Central Highlands pre-Pleistocene vicariance hypothesis. *Molecular Ecology* 2001; 10:2235–40. <https://doi.org/10.1046/j.1365-294x.2001.01362.x> PMID: 11555265
62. Craig RJ. Comparative ecology of the Louisiana and Northern Waterthrushes. Ph.D. Thesis, Univ. of Connecticut, Storrs, 1981.
63. Smith WJ, Smith AM. Information about behaviour provided by Louisiana Waterthrush, *Seiurus motacilla* (Parulinae), songs. *Anim Behav.* 1996; 51:785–99.
64. Pieplow N. Peterson Field Guide to bird sounds of Eastern North America. Boston, MA and New York (NY): Houghton Mifflin Harcourt. 2017.
65. Spector DA. Wood warbler song systems: a review of Paruline singing behaviors. *Current Ornithol.* 1992; 9:199–238.
66. Bolsinger JS. Use of two song categories by Golden-cheeked Warblers. *Condor* 2000; 102:539–52.
67. Macaulay Sound Library, Cornell Laboratory of Ornithology. <http://macaulaylibrary.org>.
68. xeno-canto. Sharing bird songs from around the world. <http://www.xeno-canto.org/species/Parkesia-motacilla>.
69. Fisher RA. The use of multiple measurements in taxonomic problems. *Annals of Eugenics* 1936; 7:179–188.
70. Ho TK. Random decision forests. Pages 278–282 in Proceedings of the 3rd International Conference on Document Analysis and Recognition, Montreal, QC, 14–16 August 1995.
71. Sanders JE, Merguerian C. The glacial geology of New York City and vicinity, In: The Geology of Staten Island, New York, Field guide and proceedings, editor Benimoff AI. The Geological Association of New Jersey, XI Annual Meeting, 1994. 296 p.
72. Sevon WD, Fleeger GM, Shepps VC. Pennsylvania and the ice age. Harrisburg, PA: Pennsylvania Geological Survey Education Series 6, 1999.
73. Boulding JR. Pre-Illinoian Glacial Deposits in Indiana (2): Are Glacial Till and Outwash West of the Knobstone Escarpment in Brown County Pre-Illinoian? BSW Working Paper 2, Version 1.0. 2018.
74. Berggren, DJ, and Killey MM. Quaternary Glaciations in Illinois. Illinois State Geologic Survey, Geonote #3.
75. Gray HH, Letsinger SL. A history of glacial boundaries in Indiana: Indiana Geological Survey Special Report 71, 2011.
76. Payne RB. Indigo Bunting (*Passerina cyanea*), version 1.0. 2020. In Birds of the World (Poole A. F., Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. <https://doi.org/10.2173/bow.indbun.01>
77. Li P., Li M., Shi Y., Zhao Y., Wan Y., Fu C., et al. 2013. Phylogeography of North American herbaceous Smilax (Smilacaceae): Combined AFLP and cpDNA data support a northern refugium in the Driftless Area†. *Am. J. Botany* 100: 801–814. <https://doi.org/10.3732/ajb.1200250> Citations: 10
78. Vargas-Rodriguez YL, Platte WJ, Urbatsch LE, Foltz DW. Large scale patterns of genetic variation and differentiation in sugar maple from tropical Central America to temperate North America. *BMC Evol Biol.* 15:1–14.
79. Jaramillo-Correa J-P, Beaulieu J, Bosquet J. Variation in mitochondrial DNA reveals multiple distant glacial refugia in black spruce (*Picea mariana*), a transcontinental North American conifer. *Mol Ecol.* 2004; 13:2735–47. <https://doi.org/10.1111/j.1365-294X.2004.02258.x> PMID: 15315685
80. Rowe KC, Heske EJ, Paige KN. Comparative phylogeography of eastern chipmunks and white-footed mice in relation to the individualistic nature of species. *Mol Ecol.* 2006; 15:4003–20. <https://doi.org/10.1111/j.1365-294X.2006.03063.x> PMID: 17054499

81. Godbout J, Jaramillo-Correa JP, Beaulieu J, Bosquet J. A mitochondrial DNA minisatellite reveals the postglacial history of jack pine (*Pinus banksiana*), a broad-range North American conifer. *Mol Ecol.* 2005; 14:3497–3512. <https://doi.org/10.1111/j.1365-294X.2005.02674.x> PMID: 16156818
82. McLachlan JS, Clark JS, Manos PS. Molecular indicators of tree migration capacity under rapid climate change. *Ecology* 2005; 86:2088–98. <https://doi.org/10.1890/04-1036>.
83. Lee-Yaw J., Irwin JT, Green DM. Postglacial range expansion from northern refugia by the wood frog, *Rana sylvatica*. *Mol Ecol.* 2008. 17:867–84. <https://doi.org/10.1111/j.1365-294X.2007.03611.x> PMID: 18179428