Identifying subspecies of Warbling Vireo through spectrogram analysis of birdsong

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Abstract

Based on recent evidence of genetic differentiation in the Alberta contact zone of the Warbling Vireo, an ~85km stretch of land in Barrhead County, we sought to classify the Eastern subspecies (*Vireo gilvus gilvus*) and Western subspecies (*Vireo gilvus swainsoni*) through bioacoustic analysis. Our lab collected data from the open-source sound recording database, Xeno-canto, to identify qualitative and quantitative differences in subspecies song of Warbling Vireos outside of Alberta. A companion project then used this information to determine where in Alberta, eastern and western subspecies were found. I used the software RavenLite to annotate sound spectrograms of Warbling Vireo (hereafter, WAVI) songs across 126 locations in North America. Results revealed significant variation in song characteristic bench markers — such as song length, mean frequency, and number of notes — between the *gilvus* and *swainsoni* subspecies, as well as a third subspecies *Vireo gilvus brewsteri*.

Introduction

The taxonomic limits of the Warbling Vireo species, a subject of study known as the 'Warbling Vireo Complex', have been long standing subjects of debate. A recent study conducted across the Alberta contact zone suggests that the Eastern form (*V. g. gilvus*) and Western form (*V. g. swainsoni*) are genetically differentiated taxa (Lovell, 2021). This confirms anecdotal and quantitative sound data found in an earlier study in Colorado (Floyd, 2014).

From a conservation perspective, establishing clear taxonomic limits is vital to conservation of species. Some studies have gone so far as to contend the biological species concept; one study published by Cambridge University online proposes that the formal nomenclature (deriving *subspecies* from the more 'biological', *species*) can hinder the breadth and depth of data commonly accessed by conservation experts (Hazevoet, 1996). When data is aggregated, or 'lumped', conservation biologists may have an inaccurate representation of subspecies fitness — resulting in inefficient conservation strategies.

An approach to subspeciation through the phylogenetic species concept rather than the biological species concept is heightened when working with a cryptic species, such as the WAVI (**Figure 1**). For example, the results of a study on the response of some WAVI's to a parasitic cowbird egg was varied — however, when data was disaggregated it became clear that all Western population samples had accepted the egg compared to only 8 out of 24 Eastern population samples (Sealy et al., 2000). Given these behavioral differences, our lab set out to assess if the proposed subspeciation of the Warbling Vireo observed through genetic and behavioral differences could also be seen through bioacoustic analysis, or, the phylogenetic study of birdsong. This method of data analysis is particularly important for establishing boundary limits as we can analyze song patterns in dissimilar regions, allowing for greater distinction within a cryptic species without having to do genetic analysis.

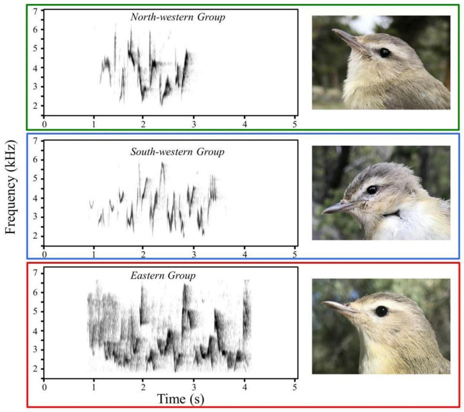

Figure 1. Carpenter, A. M. (2021) [Bioacoustic representation of Warbling Vireo subspecies' pictured next to a spectrogram of their birdsong. The subspecies', in descending order, are as follows: *gilvus swainsoni*, *gilvus brewsteri*, and *gilvus gilvus*.] BioRxiv. https://doi.org/10.1101/2021.07.12.452121

Methods

The Xeno-canto online database contains over 400,000+ sound files (2018), moreover, it aims to classify all taxa rather than all species (Troncoso, 2020). This makes it an ideal data source for bioacoustic subspecies analysis. Our lab downloaded WAVI sound files from over 126 locations across the species' range from the Xeno-canto database in descending order of quality: beginning with quality A, and ending with quality B. This created a highly varied data set of sound files in locations across North America for song characteristic annotation.

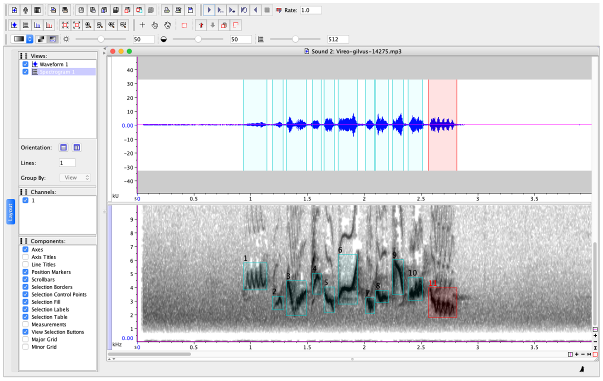

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Figure 2. Screen Capture of RavenLite software. Above, the waveform highlights where sound (notes) begin and end. Below, the spectrogram visualizes bird song; annotation boxes are visible on both screens. In this image both y-axes represent sound in Hz as a function of time in seconds.

Birdsongs acquired via active acoustics from the Xeno-canto database were then annotated through RavenLite, a sound visualization software. This program, developed by The K. Lisa Yang Center for Conservation Bioacoustics (CCB) visualizes sound through spectrogram and waveform (Spencer, 2010). The program allows the user to annotate individual notes in a song, which is then used to generate a selection table containing information about note length, lowest and highest frequencies, note start and end time, and song length in seconds (**Figure 2**). We used the statistical software R, along with the R package *tuneR* to measure the mean frequency and bandwidth of each note. Then, for each full song consisting of several notes, we measured various derived features: number of notes, frequency of the last note, standard deviation of note mean frequencies, and frequency excursion (the amount that consecutive notes change in frequency).

Results

Of the 50 'A' quality recordings pulled from Xeno-canto, only 1 was discarded due to significant tailings that impeded annotation (2%). 48 'B' quality recordings were then pulled, all of which were capable of annotation despite imperfect sound collection, notably 'white noise' due to bodies of water such as creeks and streams, as well as overlapping bird songs.

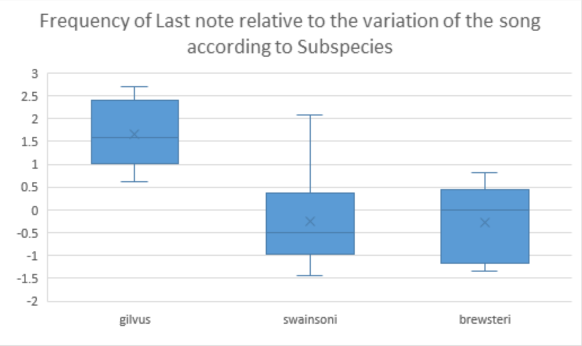

Figure 3. The relative endnote frequency of different WAVI subspecies. The Eastern *gilvus* tends towards higher relative endnote frequencies whereas *swainsoni* and *brewsteri* dip slightly.

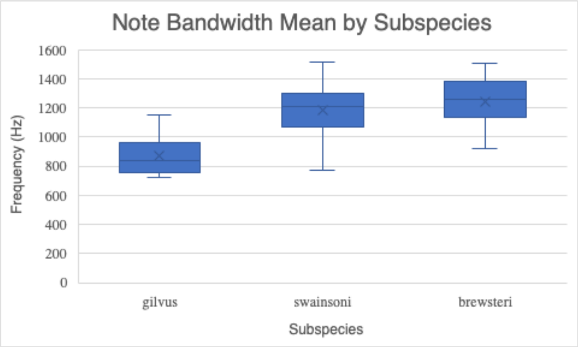

Figure 4. Average frequency variations in a single song note relative to subspecies. Here, *brewsteri* demonstrates the highest average degree of variation in note bandwidth whereas *swainsoni* has the highest degree of variation from the mean.

Following the annotation process, clear distinctions were made between Eastern and Western WAVI birdsong. One of the most notable features of the Eastern song was a higher end note frequency relative to mean song variation frequency, whereas the Western song end notes were on average maintaining the song variation frequency (**Figure 3**). This confirms the anecdotal evidence regarding end note frequencies of the different subspecies found in the earlier Colorado study. Another characteristic of bird song is note frequency bandwidth, which was measured in four WAVI sample varieties: *gilvus gilvus*, *gilvus swainsoni*, and *gilvus brewsteri*. Note bandwidth essentially describes the range of frequencies present within a single note, measured as the highest frequency minus the lowest frequency. Note the similarities in *brewsteri* and *gilvus* subspecies data relative to variation around the mean in *swainsoni*'s data (**Figure 4**); note bandwidth mean in *swainsoni* birdsong has, by far, the greatest degree of variability.

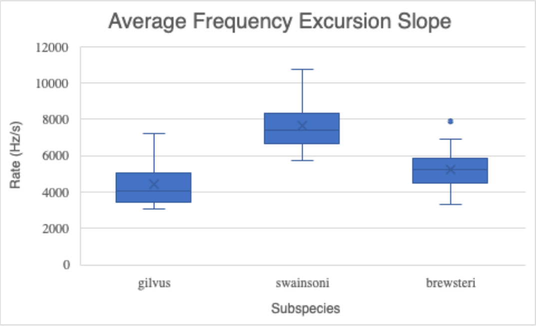

Figure 5. The frequency change per unit of time (frequency excursion) by subspecies. This value allows us to measure how fast the bird is changing their syrinx in order to sing the song (R. Hedley, personal communication, July 30 2021).

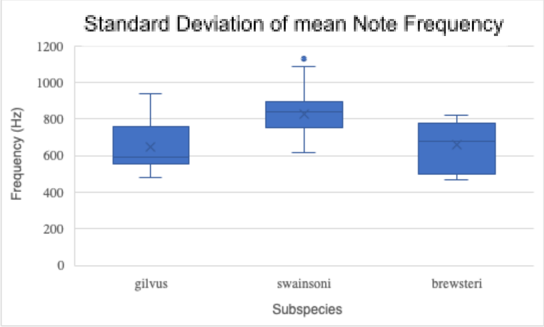

Figure 6. Standard deviation of mean note frequency measures the overall variability in the frequency of notes. In this sample *swainsoni* has the highest mean note frequency, ergo it's song fluctuates the most in frequency relative to *gilvus* and *brewsteri* samples.

A pattern we observed across all characteristics is the tendency of consecutive notes in *swainsoni* songs to vary a lot in frequency (as indicated by higher frequency excursion values shown in **Figure 5**) in most bioacoustic measurements taken. **Figures 5** and **6** serve to visually illustrate the trends we discovered in the data as a whole; notice the significant jumps in *swainsoni* bird song relative to the eastern *gilvus* and even the more southern *brewsteri* subspecies. *Swainsoni* consistently demonstrated elevated averages relative to the *gilvus* and *brewsteri* samples, notably in average note bandwidth (**Figure 4**) and standard mean frequency (**Figure 6**). However, the *gilvus* birdsong averaged higher on mean notes per song, song length, note rate per second, and average frequency of last note relative to the variation of the song. A more exhaustive summary of the results collected for song characteristics, relative to subspecies, is located in Table 1.

Table 1. *Benchmark characteristics measured in birdsong analysis of WAVI subspecies'.*

Subspecies	**Mean Average Notes / Song**	**Song Length (s)**	**Mean Note Length (s)**	**Note Rate (Notes/s)**	**Percent Gaps* (%)**
Gilvus	14.71	2.252	0.1281	6.595	0.1585
Swainsoni	12.39	1.982	0.1274	6.269	0.2052
Brewsteri	11.91	2.151	0.1405	5.467	0.2386

Subspecies	**Average Mean Frequency (Hz)**	**Standard Mean Frequency (Hz)**	**| *Average frequency change* | (Hz)**	**Average FE** Slope Mean (Hz/s)**	**Mean Bandwidth (Hz)**
Gilvus	3295	644.9	666.1	4434	871.9
Swainsoni	3753	829.5	1181	7669	1182
Brewsteri	3532	659.1	960.1	5253	1241

**Proportion of song that is silence between notes.*

***Frequency Excursion*

Discussion

Certain bioacoustic benchmarks proved to be diagnostic for the WAVI subspecies samples studied across this report. Most notably, average endnote frequency relative to the variation of the song was consistently higher in the *Vireo gilvus gilvus* than in any other subspecies; this supported anecdotal evidence that the Eastern WAVI subspecies, on average, could be recognized by a higher pitched ending note. As well, the *swainsoni* subspecies consistently demonstrated the highest variations around the mean in most benchmarks. Further research into *swainsoni*'s position and habitat relative to the *gilvus* and *brewsteri* may be needed in order to better identify bioacoustic benchmarks for this group.

Overall, the data we collected helps qualify some bioacoustic characteristics that distinguish subspecies of the Warbling Vireo, however the results are not diagnostic for the species as a whole due to insufficient data. Current bioacoustic evidence of WAVI song is largely qualitative, which results in the process of retrieving quantitative data from anecdotal records. Additional subspecies such as *Vireo gilvus brewsteri* and unknown populations within the contact zone hindered this data collection, as anecdotal evidence often doesn't consider parameters such as variation around the mean, or, the propensity for two subspecies to share the same bioacoustic qualities. It was for this reason that the research we conducted during this program required a trained ear to observe qualitative and some quantitative aspects of WAVI song, such as fluidity, pitch, and song length.

It is important to note that the Vireo is an oscine species, ergo "a good warbling vireo may learn the wrong song." (Floyd, 2014). This characteristic makes it very difficult to attribute sound bioacoustic characteristics to a subspecies, as even the distinctly high pitched ending note of an Eastern WAVI is within the variation around the mean for our Western WAVI data samples. Previous studies on bioacoustic analysis in establishing taxa in birds have also noted the limitations of audio recordings in establishing taxonomic limits for the same reason; the most pertinent effect this had on our research was the sound distance, or distance travelled. The species' preference for creek locations significantly obscured many sound files, and with the Eastern subspecies' tendencies to begin softer, many annotations may miss the first few song notes (R. Hedley, personal communication, July

2021). We responded to this problem through a revision process, where the annotations were reviewed by the undergraduate researcher in our lab. Of the 170 annotations (ongoing) that were reviewed and then sampled, >6 required correction (~3.5%). The most frequent error was in annotating the number of notes in the song, specifically where notes appeared to be touching but where the waveform revealed two distinct notes. We thus noted that the manual extraction could be improved in future studies.

Our results could greatly improve the breadth of data available in WAVI species monitoring, notably the ability of future researchers to distinguish *gilvus* and *swainsoni* subspecies by ear. Although bioacoustic data sampling has it's hindrances, the measurements we gathered show great promise in the phylogenetic identification of a cryptic species. Our lab determined that examining bioacoustic characteristics of WAVI subspecies' could have notable impacts on the conservation of the species as a whole.

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References

Carpenter, A. M., Graham, B. A., Spellman, G. M., Klicka, J., & Burg, T. M. (2021). *Genetic, bioacoustic and morphological analyses reveal cryptic speciation in the warbling vireo complex (Vireo gilvus: Vireonidae: Passeriformes)* [Unpublished doctoral dissertation]. University of Lethbridge. https://doi.org/10.1101/2021.07.12.452121

Floyd, T. (2014). Documentation by sound spectrogram of a cryptic taxon, *Vireo g. gilvus*, in Boulder County, Colorado. *Western Birds*, *45*(1), 57-70.

Hazevoet, C. J. (1996). Conservation and species lists: Taxonomic neglect promotes the extinction of endemic birds, as exemplified by taxa from eastern Atlantic islands. *Bird Conservation International, 6*(2), 181-196. https://doi.org/10.1017/S0959270900003063

Lovell, S. F., Lein, M. R., & Rogers, S. M. (2021). Cryptic speciation in the Warbling Vireo (*Vireo gilvus*). *Ornithology, 138*(1). https://doi.org/10.1093/ornithology/ukaa071

Sealy, S. G., Banks, A. J., & Chace, J. F. (2000). Two subspecies of Warbling Vireo differ in their responses to cowbird eggs. *Western Birds, 31*(3), 190-194.

Spencer, A. (2010). *Editing sounds in RavenLite.* EARBIRDING. http://earbirding.com/blog/archives/1624

Troncoso, A. O. (2020). *Bird identification apps: Q&a with Mario Lasseck.* Xeno-canto. https://www.xeno-canto.org/article/250