

Quantitative song variety is weakly linked to genotype in a hybridizing chickadee population

The prevalence of vocal communication in birds has led researchers to investigate how vocalizations evolve across many avian taxa. Of particular interest are the Passeri (songbirds), a group within the order Passeriformes which develop their songs according to both an innate neural template and auditory input from nearby singing adult birds of the same species. The well-demonstrated importance of postnatal learning for normal song development in the Passeri has raised the question of how much genetic background constrains the songs of Passeri birds. The project for which this funding was awarded sought to discern whether *song variety*, a continuously-distributed alternative measure to the more commonly used repertoire size of categorical song types, has a genetic basis in Black-capped Chickadees (*Poecile atricapillus*) and Carolina Chickadees (*Poecile carolinensis*). We chose this study system because Black-capped and Carolina Chickadees form narrow hybrid zones across their range (Figure 1) in which both genetic and vocal admixture occur and can be measured. In a hybridizing population of Black-capped and Carolina Chickadees in Henry County, Missouri, we found that genetic ancestry was somewhat, though not conclusively, predictive of a multidimensional measure of song variety, but poorly predictive of one-dimensional song variety (Figure 5). Our results suggest that in hybrid zones, genetically Carolina chickadee-like birds may sing with more variation in several acoustic parameters than Black-capped chickadee-like birds, which is reflective of those species' singing tendencies outside of the hybrid zone. Our findings contrast with results of other studies in this hybrid zone which have detected virtually no relationship between genetic ancestry and song measurements.

Data for this study were collected in Henry County, Missouri from 2021-2023. Chickadees in probable hybrid zone localities were captured using mist-nets and an audio playback lure, after which a blood sample and body measurements were taken and plastic colored bands were applied to the tarsi (ankles) for future relocation. Additional genetic data from outside the hybrid zone were acquired from KU Museum of Natural History. During the breeding seasons of 2022 and 2023, high volumes of songs were recorded from relocated color-banded singing chickadees using a shotgun microphone mounted in a parabolic dish. To determine genetic ancestry of hybrid zone chickadees, DNA was extracted from blood samples and analyzed for the presence of 10 species-diagnostic genetic markers, and ancestry scores were generated from these data using the program STRUCTURE (0 being pure Black-capped Chickadee and 1 being pure Carolina chickadee) (Figure 2). Acoustic measurements in the frequency (pitch) and time domain were extracted computationally from the chickadee song recordings, and the resulting dataset was reduced in dimensionality using principal component analysis (PCA) and plotted in two and three dimensions to create multidimensional *acoustic spaces* in which each individual chickadee's songs were distributed spatially (Figure 3). We then generated song variety scores for each chickadee for which at least 100 songs were recorded by quantifying how much n -dimensional space each individual's songs occupied within the total acoustic space (Figure 4). Linear regressions were run to test how well genetic ancestry predicts

song variety in each of one-, two-, and three-dimensional spaces (Figure 5).

The funds awarded by the Missouri Birding Society were imperative in the completion of data collection for this project, namely by defraying travel and lodging expenses in the field and costs of reagents for molecular work. We give wholehearted thanks to the Missouri Birding Society for choosing to invest in this study.

The funded study was a master’s thesis project done at Missouri State University in the lab of Dr. Jay McEntee with the help of several MSU graduate and undergraduate students in the field. A manuscript of this study is in preparation for submission to the journal *Ornithology*.

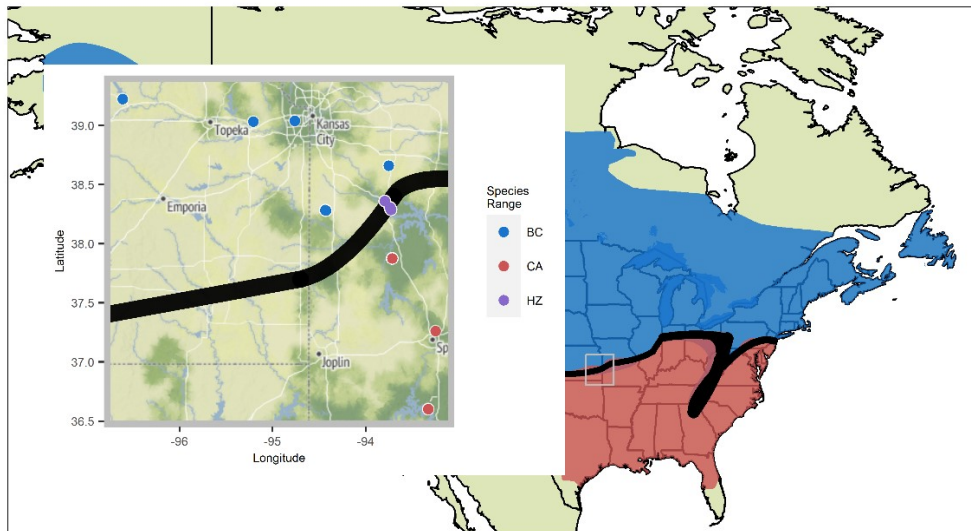


Figure 1. Approximate ranges of Black-capped (blue polygon) and Carolina (red polygon) chickadees, and all sampling localities (gray box). Range polygon data provided by IUCN Red List (<https://www.iucnredlist.org/resources/spatial-data-download>).

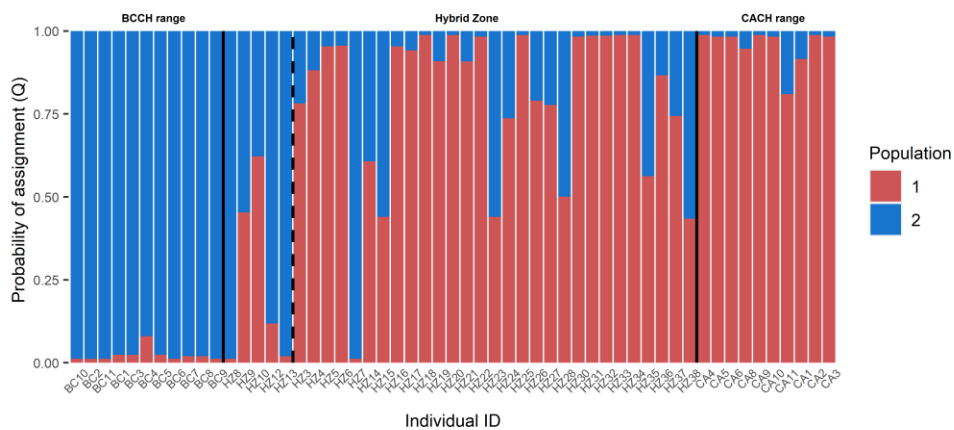


Figure 3

Figure 2. Results of STRUCTURE analysis showing probabilities of assignment Q to each of $K=2$ populations for each individual sampled. Population 1 corresponds to Carolina chickadee ancestry and Population 2 (blue) corresponds to Black-capped chickadee ancestry. The dashed line separates the two hybrid zone sampling sites.

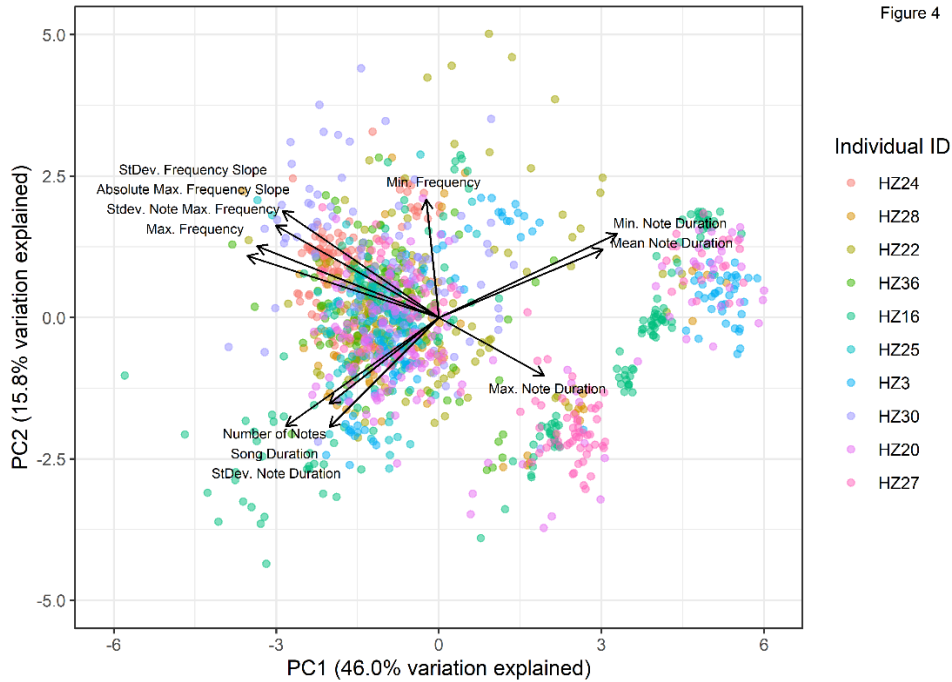


Figure 4

Figure 3. The two-dimensional acoustic space resulting from applying PCA to the acoustic dataset (1235 songs across 10 individuals) and plotting the first two principal components. Each point represents a single song and each color represents an individual chickadee.

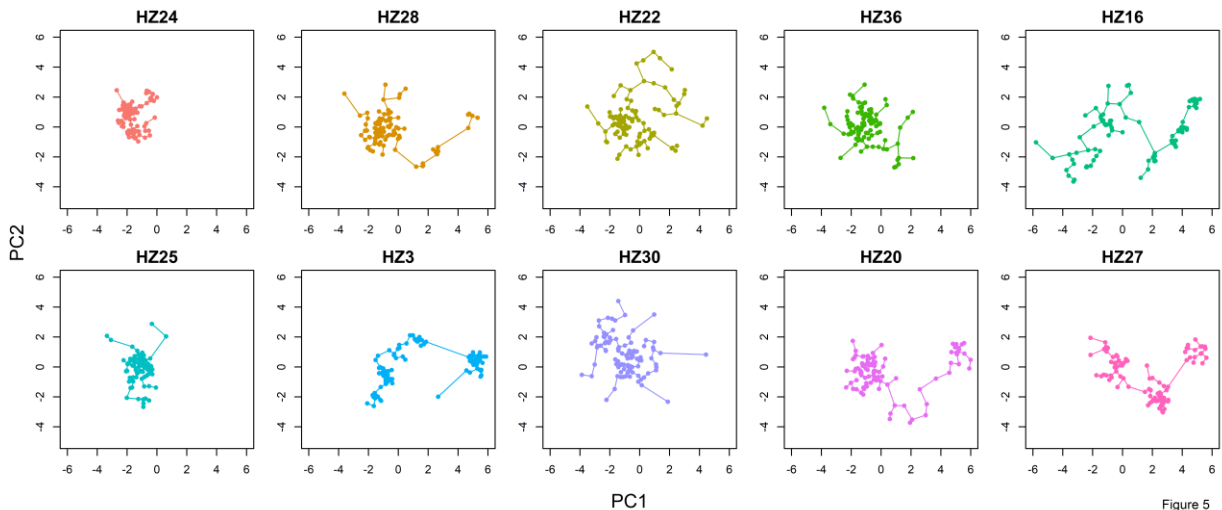


Figure 5

Figure 4. Plots of two-dimensional minimum spanning trees for each of the 10 fully-recorded chickadees. Edge weights of minimum spanning trees were summed to generate two-dimensional song variety scores for each individual.

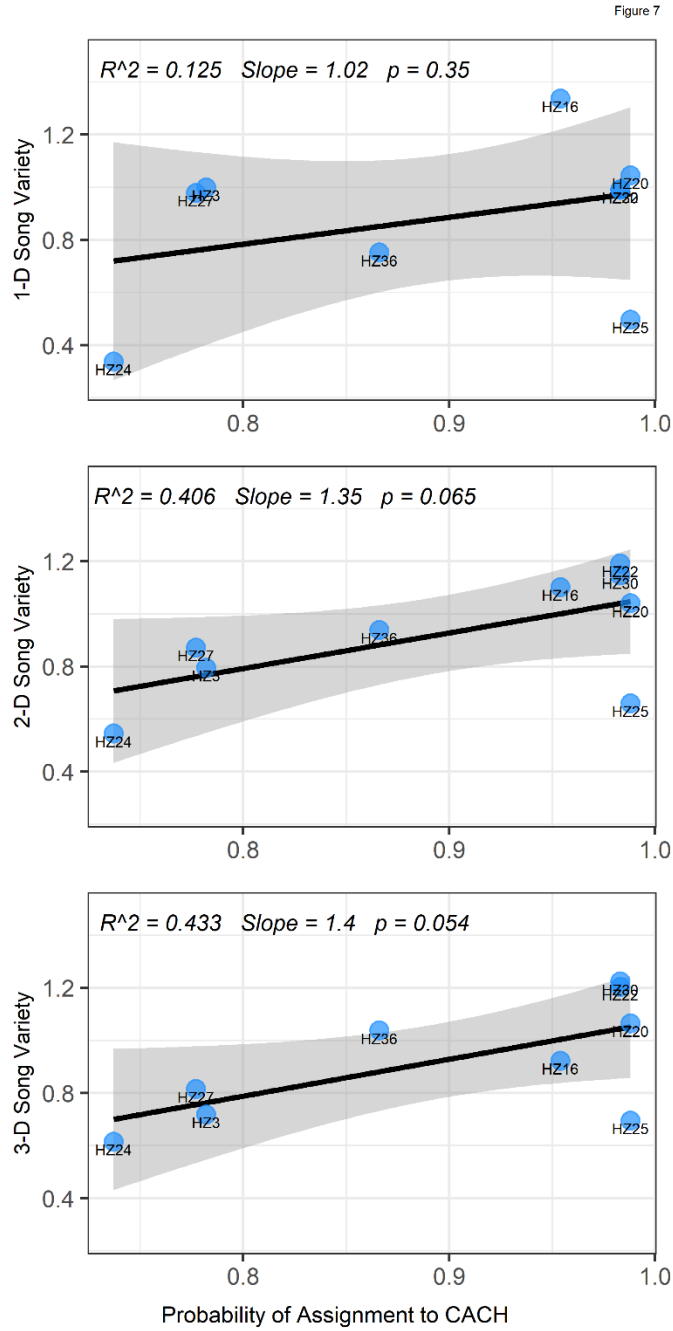


Figure 5. Linear regressions modeling the relationship between chickadee genetic ancestry (probability of assignment to the Carolina chickadee genetic cluster) and song variety values in one-dimensional, two-dimensional, and three-dimensional acoustic spaces.