Dawn Chorus Male Song Patterns in Relation to Ancestry in the Black-capped Chickadee × Carolina Chickadee Hybrid Zone

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Signals used in mate choice and intermale competition can deliver important information about the genetic quality of the sender, often serving as prezygotic barriers to hybridization. Our research aims to assess the extent to which song, as an acoustic mating signal, can reliably indicate a male's ancestry, as well as the ways in which signal learning can become muddled in hybrid zones. I analyzed data from 2016 – 2019 involving Poecile atricapillus (Black-capped Chickadee) and P. carolinensis (Carolina Chickadee) and their hybrids at Hawk Mountain, Pennsylvania. Species-diagnostic SNP genotypes provided information about ancestry for each male, while autonomous acoustic recording units yielded samples of dawn chorus male repertoires mostly prior to incubation. The sample of males (N = 18) comprised 10% Black-capped Chickadees, 50% Carolina Chickadees, and 40% hybrids. Repertoires ranged from only Black-capped Chickadee songs to a mixture of Black-capped and Carolina chickadee songs, sometimes including new, unique hybrid songs that did not fall under either category; no observed repertoires comprised solely Carolina Chickadee songs. Song patterns did not actively reflect genetic identity in the hybrid zone: dawn song repertoire characteristics, in both song participation and repertoire composition, did not correlate with the proportion of Carolina Chickadee alleles. This supports previous studies involving acoustic signaling in this chickadee hybrid zone, which have found that the genetic introgression of Carolina Chickadee alleles in the population does not coincide with a simultaneous cultural change in song repertoires towards Carolina Chickadee songs. Instead, Black-capped Chickadee songs can dominate the acoustic culture for years, even after Carolina Chickadee alleles become predominant as the hybrid zone moves northward. These findings support the hypothesis that learning environment and neural template, more so than ancestry alone, shapes song repertoires of individual chickadees, which could affect mating patterns and hybridization dynamics.

Introduction

Acoustic signals and vocal learning

Animals use acoustic signals for a variety of reasons, including species recognition, mate choice, and resource defense. Individuals usually direct these acoustic signals towards members of their own species, also known as conspecifics (1), and in return can recognize conspecifics by their signals. This species recognition is essential in within-species communication and can shape social dynamics (2). Due to their unique character, acoustic signals can also distinguish species that may otherwise be indistinguishable by morphological traits.

Many vertebrates are able to engage in auditory learning, or the ability to associate perceived sounds. While the vocalizations of most vertebrates are fixed and cannot be learned from their environment (3), some groups can learn their vocalizations from environmental stimuli. The ability to acquire vocalizations through imitation has evolved separately in three avian groups (parrots, hummingbirds, and songbirds) and four mammalian groups (humans, bats, cetaceans, and elephants) (3). It is theorized that these two categories of vocal learners may have each descended from a

vocal-learning common ancestor, or that related species lost their ability to learn vocalizations in this way over time (4). According to Erich Jarvis, a neurobiologist that studies the neural pathways of vocalization, the two main driving forces for the evolution of vocal learning are mating preference and environmental adaptation (3). Vocal learners have been shown to use their vocal variability to attract mates, as song variability in groups like songbirds can be stimulatory in several cases (5, 6). As a result, birds that can produce more variable vocalizations will likely be selected for in this way. In addition, individuals that can vary their vocalizations based on their environment may have another advantage. Most species have fixed vocalizations that travel best in specific environments; for example, species that live in environments with dense vegetation tend to produce vocalizations with lower frequencies because longer wavelengths travel better through leaves and branches that can scatter shorter wavelengths (7). Species that can change their vocalizations within a lifetime or several generations may have a long-term advantage in community communication in their versatility to environmental change.

For birds and humans, vocal learning occurs during two distinct phases: the perceptual phase and

the production phase (8). During the perceptual phase, songbirds specifically develop a neural template from the vocalizations of a "tutor" (often the parents or neighbors of the individual). In turn, this provides the framework for males' ability to produce such vocalizations later in life (9). For females, vocal learning often leads to sexual imprinting in early life stages, which in turn dictates mate choice later in life (10). This imprinting also occurs in two stages: sexual preference is established in an early acquisition phase, then this preference is linked to sexual behavior and stabilized in a consolidation stage (11).

<u>Acoustic signals in speciation and hybridization</u>

Acoustic signals and their mechanisms often have an important role in species recognition and mate choice, and thus can have important impacts on population dynamics. Acoustic signals often serve as precopulatory barriers for different species. If individuals of two separate species learn and imprint on only conspecific vocalizations early in life, they will likely only produce conspecific vocalizations and select conspecific mates in their adulthood. This constitutes behavioral reproductive isolation, which creates the conditions for speciation to occur (12).

While in many cases acoustic signals and vocal learning often serve as precopulatory barriers to hybridization, song can facilitate interbreeding where territories overlap. When two or more species share the same territory, juveniles of one species may sexually misimprint on the vocalizations of a neighboring species. Many studies have shown that animals can "eavesdrop" on neighboring vocalizations, which in turn can lead to the modification of sexual preferences and vocalizations (13-15). If an individual produces the learned vocalizations of another species, they could be selected for in mate choice by both unsuspecting interspecifics and misimprinted conspecifics. In a similar way, individuals that sexually misimprint on the vocalizations of another species have the potential to choose a mate of that species in adulthood. As a result, hybridization can be facilitated by the cross-learning of vocalizations in shared territories.

<u>The role of song in mate choice and hybridization in parids</u>

Many studies investigating acoustic signals and vocal learning center around the complex and diverse songs of songbirds (oscines). Oscines differ from suboscines, their sister group in the order Passeriformes, in both their morphology and song learning abilities (3). Mainly, oscines are vocal learners, while suboscines are not. The songs and calls of songbirds play critical roles in

their communication and mate selection. For example, many species of songbirds participate in a dawn chorus, where territorial male songbirds produce a high rate and variability of songs that in turn mediates social relationships between males, as well as informs mating decisions of females (16). There is even more variation in species-level ability of songbirds to learn, produce, and imprint on intraspecific and interspecific songs based on the characteristics of their neural templates. Songbirds with "narrow" neural templates have strong preferences for learning conspecific songs, while songbirds with "broader" neural templates can learn a greater variety of songs (3). As a result, songbirds with broader neural templates may be more likely to misimprint on the songs of another species, which in turn can facilitate hybridization.

These trends continue within the family Paridae, which includes tits, titmice, and chickadees. Studies have shown that dawn repertoires in tits play a vital role in extrapair copulations (during which hybridization may occur), with male song behavior attributes promptly repelling rival males and attracting females (17, 18). Several species of tits, including Black-capped Chickadees (19) and Great Tits (20) also engage in more extrapair behaviors at dawn. A more recent study suggests, however, that this relationship is much more complicated, with male song potentially influencing male and female receiver movements and mating decisions differently at different times of day and periods within the mating season (21). As such, many aspects of behavioral responses to male singing are still not well understood, but certainly influence mating decisions, and thus species dynamics. Numerous species of parids, like many songbirds, are also able to misimprint on the songs of other species. One study involving several species of tits demonstrated that cross-fostered birds would imprint on the signals of their foster parents rather than those of their own species (10). The extent to which certain parids can learn and produce the songs of other species depends on their neural template.

<u>Black-capped and Carolina chickadee hybridization in Pennsylvania</u>

In the eastern United States, two species of chickadees, Black-capped Chickadees (P. atricapillus; hereafter, BCCH) and Carolina Chickadees (*P. carolinensis*; CACH), hybridize along a narrow strip of land in which their ranges overlap from New Jersey to Kansas (Fig. 1). The hybrid zone, which stretches from 30–50 km in width (22), has moved north at a rate of 1 km per year, with the CACH range steadily encroaching northward (23). Mobile hybrid zones such as this often involve dynamic shifts in genetic

and behavioral composition (24), which are apparent in the introgression of CACH genes and traits along this hybrid zone. The resulting CACH genes in the population do not introgress at the same rate as their songs, which lag behind the spaces that they colonize (25). Eventually, BCCH songs and calls are replaced by CACH songs and calls (26). Chickadee song, much like other aforementioned acoustic signals, likely influence the hybridization process (25). CACH have been shown to have a broad neural template (25, 27), possessing the ability to sing multiple kinds of song, as well as the songs of other species like BCCH. While BCCH can modulate the frequency and structure of their own songs (28), their song production remains quite uniform and restricted to their own species across their range (29). Despite several post-zygotic hybridization barriers, including decreased hatching success and cognitive impairments (30, 31), hybridization continues to occur.
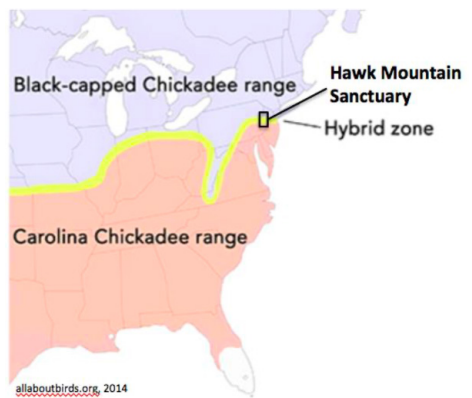
Figure 1. The Black-capped and Carolina Chickadee hybrid zone exists where their ranges overlap. Hawk Mountain Sanctuary (HMS) currently is situated within this hybrid zone.

Hypotheses and predictions

This study aims to determine the extent to which ancestry affects song production and ability in the Black-capped and Carolina Chickadee hybrid zone at Hawk Mountain Sanctuary. I have developed two alternative hypotheses for this question. My first hypothesis states that neural templates have diverged in CACH and BCCH during the over 3 million years that they have evolved independently, resulting in a more open neural template in Carolina Chickadees than Black-capped Chickadees. If this is the case, I predict that male Black-capped Chickadee repertoires will be limited to mostly—if not entirely—BCCH song, while Carolina Chickadee repertoires will contain CACH, BCCH, and potentially aberrant (HYCH) songs. Conversely, it may be true that chickadees of all species have the same neural template, and their social surroundings alone influence the songs

they develop and produce. If this alternative hypothesis is true, I expect that the song production characteristics and interspecific singing abilities of Black-capped, Carolina, and hybrid chickadee males will not vary based on their ancestry.

Methods

Study Site

I studied chickadee hybridization at Hawk Mountain Sanctuary (HMS) in Kempton, Pennsylvania (40.65°N, - 76.00°W). This site is located within the chickadee hybrid zone (23, 30). As of 2021, Dr. Curry's lab had approximately 200 artificial snags across the site, yielding about 30 snags containing active chickadee nests every season.

Autonomous recording

I analyzed data collected in 2016–2019 from autonomous recording units (ARUs), which are small devices used to automatically record acoustic data at a scheduled time. The ARUs (model SM2+, Wildlife Acoustics, Maynard, MA) were programmed to record the chickadee dawn chorus each day, beginning 1 hour before sunrise and recording for 2 hours. Each ARU used the time of sunrise each morning to automatically calculate and adjust the recording start time. Observers secured the ARUs to a tree in close proximity to an active nesting snag; they then left the device in the same position for multiple days to capture the songs of the male chickadee present at the nest. Members of the Curry Lab sampled chickadee song behavior from April to June, during which the chickadee breeding season results in the most singing behavior (32).

Song categorization and analysis

I analyzed dawn chorus recordings using Raven Pro v1.6 (Cornell Lab of Ornithology) to create sonograms for the visualization and analysis of acoustic data. I sorted chickadee songs into three categories: BCCH, CACH, and HYCH. At HMS, BCCH sing their common *fee-bee* song, while CACH sing—as their primary song—a higher-pitched *see-bee-see-bay*. These songs are easily distinguishable on the sonograms created by Raven (Fig. 2). While CACH are capable of singing several variant songs (33, 34), I only observed the aforementioned primary song in my recordings. HYCH songs most often appear as intermediates between BCCH and CACH song in pitch and structure, varying widely between individuals. To date, the Curry lab has not created further categories for HYCH song
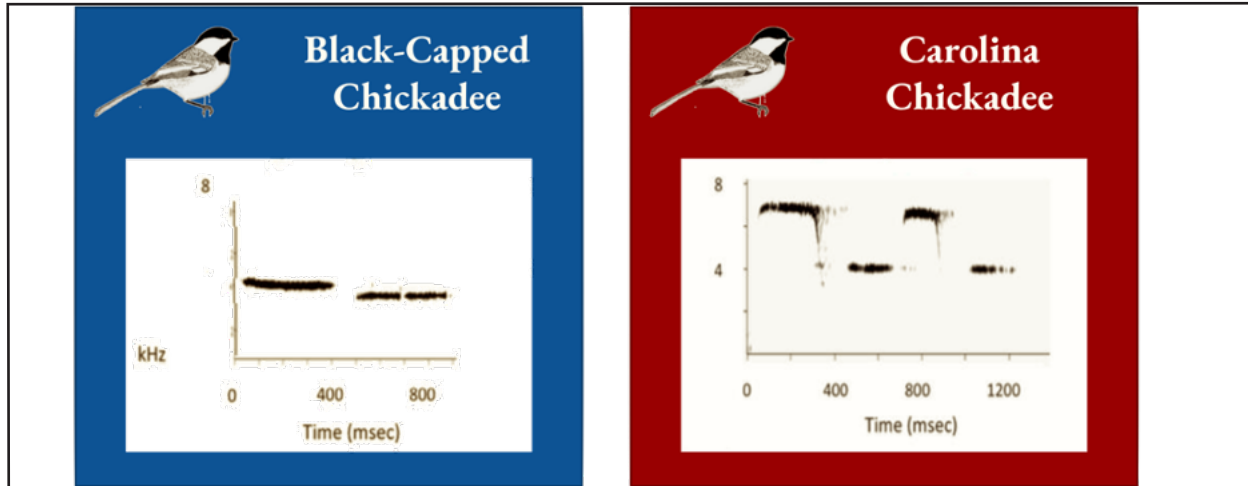
Figure 2. Sonograms of chickadee songs. Each sonogram shows pitch as a function of time.

due to this variation; while there is certainly room for investigation regarding the relationship between genetic identity and HYCH-specific song parameters, such an inquiry would fall outside the scope of this study. For this reason, I marked all intermediate songs as HYCH.

Genetic analysis

I made use of genetic data collected by members of Dr. Curry's lab in previous years from males that provided acoustic data for my study, as well as data from the males present at Hawk Mountain from 2016 to 2019 for more robust population composition estimates. Using blood samples obtained from these males in the field, lab members including Adam Driver, Burton, and Taylor Heuermann used the methods described in McQuillan et al. to determine the genotype of each individual using eight to ten separate loci (35). They then calculated the hybrid index score (%CACH) of each male, which represents the quantity of CACH alleles averaged across the loci. I then used the values obtained by running the hybrid index score of each male through the program STRUCTURE (36) to calculate the genetic probability CACH of the individual. When generally categorizing the individual males, I chose to create three categories with a 20% cutoff on each end of the CACH probability scores. I considered males scored 0 - 0.20 to be "pure" BCCH, while I considered males scored 0.80 to 1.00 to be "pure" CACH. Individuals with scores between 0.20 and 0.80 were considered to be hybrids. These methods of categorization differ from some recent publications involving ancestry in this hybrid zone, particularly in McQuillan et al. (31) and Huynh and Rice (37). While this approach did cause a few more-admixed males to be counted as BCCH or CACH, using this broader

definition allowed me to create more conservative estimates of species-level differences between Black-capped and Carolina chickadees by creating more robust sample sizes on each end of the spectrum.
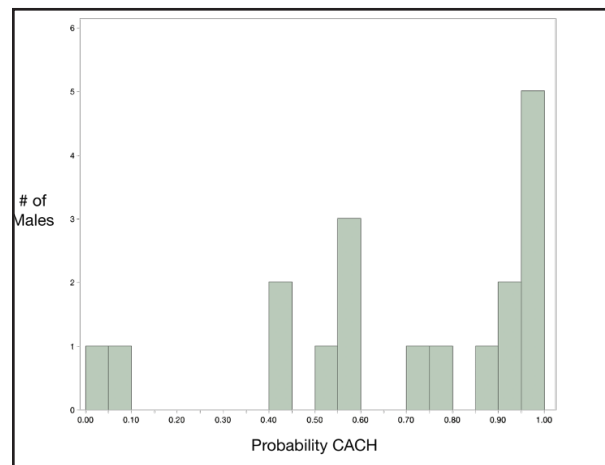
Figure 3. Frequency distribution of ancestry genotypes for 18 sampled males.

Results

Sample coverage

I sampled the songs of 18 unique males over the span of 4 years. Three males were present for 2 years; I included these males in each year's sample individually. I obtained 33 dawn chorus recordings from these males, analyzing a total of 6210 songs. Each recording contained an average of 194 ± 174 SD songs and ranged from 42 to 877 songs. Sample dates across all years ranged from 27 April (roughly coincident with

egg-laying) to 9 June (nestling stage).

### Ancestry of sampled males and overall male population

Males involved in this study ranged from pure BCCH to pure CACH, but most males had more CACH ancestry than not (Fig. 3).

When broken into categories based on STRUCTURE scores within 20% of each end, the 18 sampled males comprised 10% Black-capped Chickadees, 50% Carolina Chickadees, and 40% hybrids. Ancestry scores (ProbabilityCACH) averaged 0.682 ± 0.31 SD. Mean ancestry did not vary among years in 2016 – 2019 ($F_{3,17} = 0.75$, $p = 0.54$; Fig. 4).

Sampled males also generally represented the overall male ancestry at Hawk Mountain between 2016 and 2019 (Fig. 5). When analyzing 102 unique males from a broader sample of male genetic data between 2016 and 2019, ancestry also did not vary among the years ($F_{3,122} = 0.29$, $p = 0.83$).
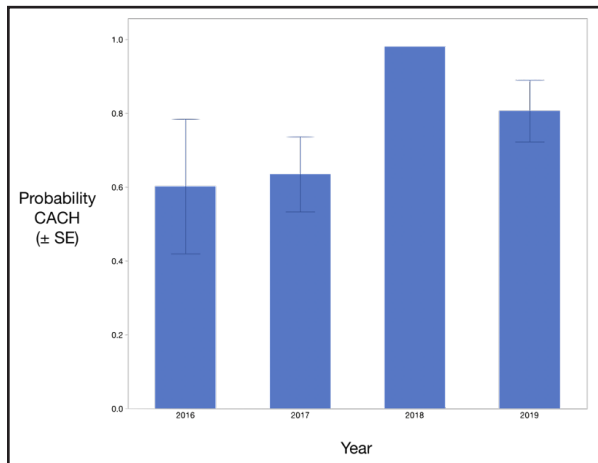
Figure 4. Average yearly CACH genetic probability of HM sampled males (N=18).
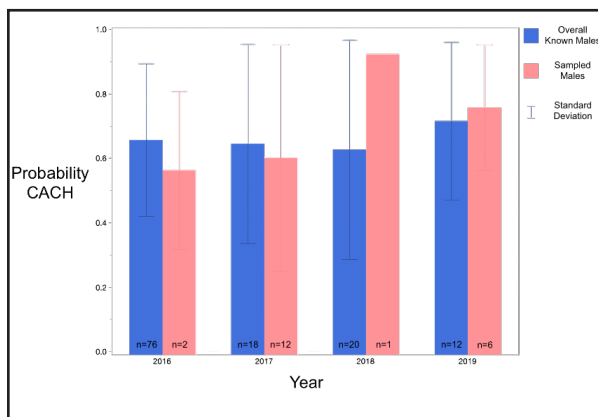
Figure 5. Average yearly CACH genetic probability of HM sampled males in both the larger overall and sampled groups.
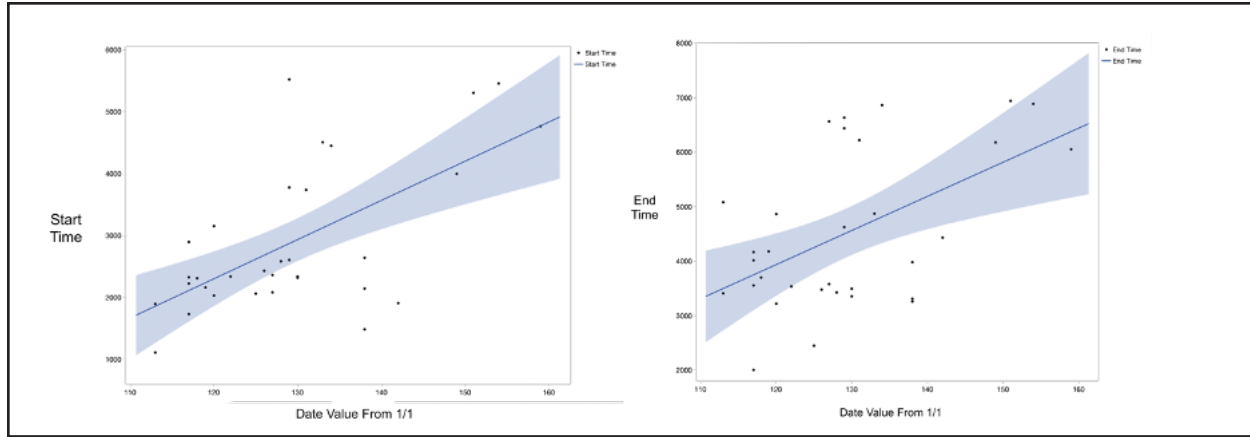
Dawn chorus participation

Song production characteristics varied on an individual basis. Of the 6210 sampled songs, chickadees produced 3912 (63%) within the first 1 hour of recording. On average, the chickadees sang for a 26.5 min $\pm$ 19.49 SD window (the interval between first and last song) during each dawn chorus (range $=$ 10.3 – 42.0 min).

Breeding season progression did affect some song participation characteristics. Sample date, as it relates to point in the breeding season, had no effect on the number of songs produced ($F_{1,30} = 19.40$, $p = 0.20$) or the span of time spent singing ($F_{1,30} = 0.002$, $p = 0.96$). As sample date increased, however, chickadees both started singing later in the morning ($R^2 = 0.39$, $F_{1,30} = 19.40$, $p < 0.0001$) and finished singing later ($R^2 = 0.27$, $F_{1,30} = 1.73$, $p < 0.0021$; Fig. 6).
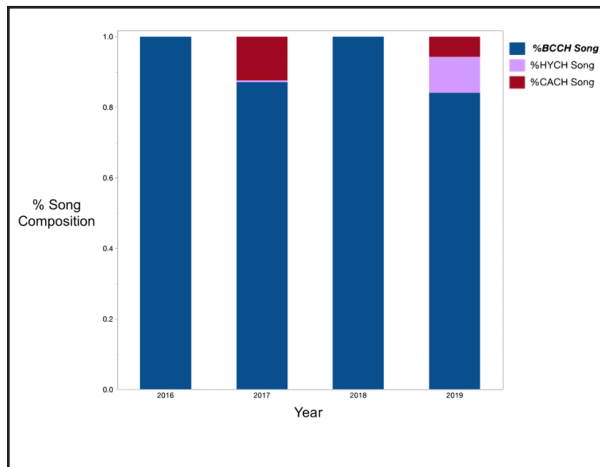
Dawn chorus male repertoire patterns

The 33 total sampled dawn chorus recordings contained all three categories of song: BCCH, CACH, and hybrid song. Overall, BCCH songs dominated the repertoires of sampled males, constituting 91.9% of total recorded songs from 2016 to 2019. Males also produced CACH (4.8%) and aberrant (3.3%) songs during that time period. Each year, BCCH song consisted greater than 80% of the songs produced during the dawn chorus at Hawk Mountain (Fig. 7).

Individually, 14 (78%) of the 18 unique sampled males produced only BCCH song. All male repertoires included BCCH song; 3 of the 4 males who produced non-BCCH song produced both CACH and aberrant (HYCH) song, with only one male producing a mix of BCCH and CACH songs only. In this way, 100% of the males that produced HYCH song also produced CACH song in their repertoires. The repertoires of the three males that were present for two years remained fairly consistent. Two of the three two-year males, who both were present in consecutive breeding seasons, produced only BCCH song in both years that they were sampled for. The third male, who appeared in 2017 and again in 2019, sang all three song types in 2017, but only produced BCCH and CACH songs in 2019. When considering males on a bird-year basis (N $=$ 21), 15 (71%) of the 21 sampled males produced only BCCH song. All male repertoires included BCCH song; 6 of 21 individuals also included aberrant (HYCH) or CACH songs in their dawn chorus. 3 of these 6 individuals produced only BCCH and CACH songs, while the other 3 produced all three song types.

Figure 6. Average dawn song start and end times as a function of sample date.
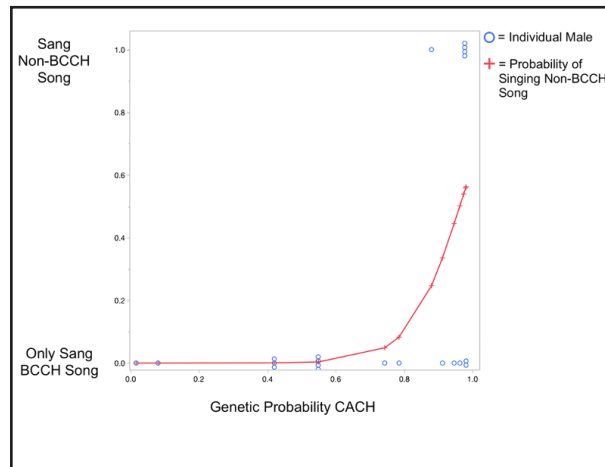

**Figure 7.** Percentages of song composition in total dawn chorus songs by year.

Figure 8. Male song ability in relation to male ancestry. Each blue circle represents individual males, showing whether they were able to sing non-BCCH song or only BCCH song. The red symbols and connecting line show the logistic regression line of fit, which indicates a positive relationship between higher CACH ancestry and non-BCCH song ability.

Singing behaviors as a function of ancestry

Male participatory song patterns in the hybrid zone did not vary with ancestry. Male ancestry had no effect on song production characteristics such as start time ($F_{1,19} = 0.26$, p = 0.62), end time ($F_{1,19} = 0.53$ p = 0.48), duration ($F_{1,19} = 0.18$, p = 0.68), and total song count ($F_{1,19} = 0.46$, p = 0.51).

Male ancestry did influence male song ability without affecting song proportions. Assessing males categorically, 100% of hybrids and 50% of CACH males produced only BCCH song, and the repertoires of 80% of all sampled CACH males were made up of more than half BCCH songs. BCCH males did not produce any song types other than BCCH song. There was no relationship between ancestry and the proportions of BCCH song ($F_{1,19} = 3.14$, p = 0.09), CACH song ($F_{1,19} = 3.68$, p = 0.07), or HYCH song ($F_{1,19} = 0.49$, p = 0.49)

that males sang during the dawn chorus. Nevertheless, the probability of a male singing any non-BCCH song increased with increasing male CACH genetic probability ($\chi^2 = 8.47$, df = 1, p = 0.0036; Fig. 8): BCCH-like males were unable to produce aberrant and CACH songs, but genetically CACH-like males were able to produce CACH, aberrant (HYCH), and BCCH songs.

Finally, I also wanted to confirm that the various characteristics of my sampled males did not impact the likelihood of sampling certain song types, considering that perhaps the males with more robust dawn chorus song participation might reveal CACH and HYCH song more easily than a "quieter" male. The probability of a male singing non-BCCH song had a weak negative

relationship with the average number of songs he produced (logistic regression, $\chi^2 = 3.63$, df = 1, p = 0.06; Fig. 9), and it did not change with the duration of time that he sang (logistic regression, $\chi^2 = 1.29$, df = 1, p = 0.26).
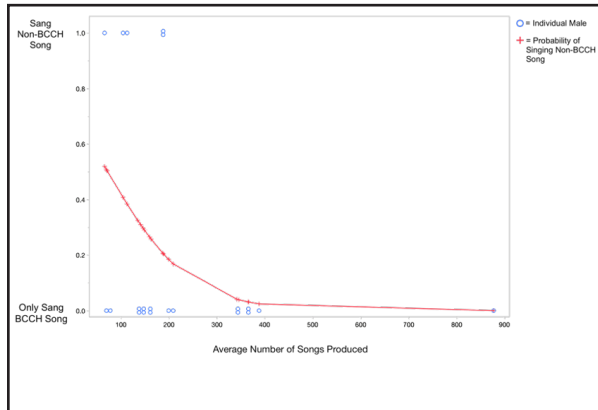
Figure 9. Male song ability in relation to average number of songs produced. Each blue circle represents individual males, showing whether they were able to sing non-BCCH song or only BCCH song. The red symbols and connecting line show the logistic regression line of fit, which indicates a potential, weak negative relationship between higher number of songs and non-BCCH song ability.

Discussion

Male ancestry did not strictly determine song patterns in the hybrid zone, as song type proportions did not correlate closely with male genetic identity. At the same time, as genetic probability of CACH ancestry increased, the ability to sing CACH and aberrant song increased. From 2016 to 2019, the population of males at Hawk Mountain Sanctuary consisted on average of greater than 50% CACH ancestry, meaning that a good number of males were genetically CACH-like, and thus should have been able to produce CACH and aberrant song.

Despite this, BCCH song was by far the most produced song during dawn chorus. The lack of correlation between male ancestry and song production, when understood in the context of the relationship between male ancestry and song ability, supports the idea that song misimprinting and cross-species, heterospecific song learning ability may be drivers for hybridization at the site, while simultaneously supporting the idea that Black-capped and Carolina chickadees have differential interspecific song-learning abilities.

One potential explanation for the persistence of BCCH song in progressed hybrid zone sites containing predominantly CACH-like chickadees is the facilitation of male song misimprinting by a more broad CACH

neural template. As Carolina Chickadees move northward into areas dominated by Black-capped Chickadee males, young Carolina Chickadees could feasibly misimprint on the songs of neighboring BCCH males, gaining the ability to reproduce BCCH, CACH, or aberrant songs later in life. These misimprinted CACH males may then be able to attract and mate with BCCH females by producing BCCH song, facilitating the introgression of CACH genes but maintaining the acoustic culture of their BCCH predecessors. In this way, male ancestry is likely affecting male repertoires at Hawk Mountain not by determining the repertoire content or proportion of each song type, but instead by determining the neural template, and thus interspecific song-learning ability of each male. As the chickadee population at Hawk Mountain becomes more CACH-like, I predict that the dominant BCCH acoustic culture will most powerfully influence male repertoires and persist for many years to come. Eventually, however, CACH song will creep into the acoustic culture as the echo of BCCH song slowly fades out, long after BCCH-like chickadees are no longer present.

Male repertoire patterns, ability, and ancestry in Southeastern Pennsylvania hybrid zone

Previous studies within the overall study system spanning the southeastern Pennsylvania hybrid zone, such as that of Katherine Monroe (38) and Stephanie Wright (39), have found this lack of a relationship between male repertoire and ancestry in Black-capped and Carolina Chickadees. In particular, Wright documented the song dynamics of this chickadee hybrid zone at a more southern study site: Nolde Forest (previously within the chickadee hybrid zone, now exclusively genetically CACH) experienced this same ancestry-song lag, with a population of almost entirely "pure" Carolina chickadees producing BCCH songs as late as 2015 (40). Past studies have demonstrated the early stages of this trend at Hawk Mountain as well; CACH genes were first detected at the site in 2001 (25), but non-BCCH song was not detected until 2011 (39). Most recently, the senior thesis work conducted in 2017 by Monroe (38) demonstrated this ancestry-song lag during both spontaneous dawn chorus bouts and playback experiments at Hawk Mountain Sanctuary.

My study, in the context of these previous projects, supports similar conclusions about the relationships between song patterns, ability, and ancestry in this hybrid zone site, but it does so with the help of more extensive genetic and temporal datasets. Monroe did make use of the same McQuillan et al. (35) genetic methods that I employed, while focusing on this dynamic within the scope of a single breeding season

in 2017. In my study, I was able to incorporate four breeding seasons, allowing me to consider yearly changes in ancestry and song patterns. I did not find such changes, as the ancestry distribution in 2017 was relatively similar to those in 2016, 2018, and 2019. Ancestry data from both previous and future breeding seasons may be necessary to observe significant CACH genetic introgression. This could be seen in future iterations of this study, as my dataset will likely expand even further into the 2020 and 2021 breeding seasons, as well as years prior to 2016, as I work towards the publication of this study. In a similar way, while Wright's dissertation analyzed more than a decade of acoustic recordings, our current genetic methods are more precise in their calculations of ancestry, giving me a clearer view of the genetic identity of my sampled males. In this way, my project uses these two foundational studies to answer larger questions about the relationship between song and hybridization at our site.

Finally, our sample size could introduce some error in this study. We were only able to analyze data to date from a single bird in our 2018 ARU samples, and from just two birds in our 2016 samples. During these years, many of the placed acoustic recording units did not capture chickadee songs at a volume that would indicate the singing male was present at the associated snag, with many samples not containing chickadee songs at all or had defective microphones. For this reason, many of the studied males are from the 2017 and 2019 breeding seasons. Despite this, our sample size is relatively robust considering the challenges posed by a hybridizing population with many unsuccessful nests, relatively unpredictable weather conditions, rotating equipment, and technical difficulties.

Complications and possible refinements

The Curry Lab owns a limited number of ARUs, which we must rotate between active nests every few days to ensure the collection of some data for every nest. Between accounting for heavy rain (which makes recordings inaudible), inactive or failed nests, and some pairs beginning late in the breeding season, it can be a logistical challenge to obtain robust dawn chorus data from each active nest.

A potential source of error lies in my assumption that each male in my dawn chorus recordings is the same male associated with the snag near which we placed the ARU. Without visually confirming the banded male each morning when observing the dawn chorus, I cannot be certain of his identity. I do, however, have a relatively strong confidence of my

assumption; male chickadees, who are more territorial during the breeding season, should perform their dawn choruses in the territory where they roosted overnight, meaning that each male would be singing close to his respective snag (32). There are some rare instances, however, where error could still be introduced, mainly if an identified male dies and is replaced by a new, unidentified mating partner at his snag without our notice.

Song-ancestry dynamics and hybridization in Black-capped, Carolina, and other chickadees

This disconnect between CACH ancestry and song presence has been seen along other tracts of the Black-capped and Carolina chickadee hybrid zone outside of Pennsylvania. Across Virginia, West Virginia, and Missouri transects of the BCCH × CACH hybrid zone, vocal admixture lagged far behind Carolina Chickadee genetic introgression (41), which supported the conclusion that song could not reliably indicate the extent of hybridization. Sattler et al. emphasized that this lag could be attributed to the nature of vocal learning in both Black-capped and Carolina Chickadees, as well as Carolina Chickadee's greater ability to learn heterospecific song than Black-capped Chickadees (42). My results were consistent with the findings of Sattler et al. (41), evidencing this mismatch between ancestry and song.

When considering the relationship between song and ancestry in other hybridizing chickadee species, there is certainly more research to be done. Most recently, evidence suggests the hybridization of Black-capped Chickadees, Mountain Chickadees (*Poecile gambeli*), Chestnut-backed Chickadees (*P. rufescens*) and Boreal Chickadees (*P. hudsonicus*) (this excludes the specific hybridization of Chestnut-backed and Mountain chickadees, for which no evidence was found), but does not explore song as a mechanism of this genetic admixture (43). It is important to note that song would not likely influence hybridization involving Chestnut-backed and Boreal Chickadees, as these two species in the brown-backed clade do not produce whistled songs like other Poecile (44, 45). It is important to consider, however, that calls as acoustic signals could influence species dynamics in hybrid zones containing these species, and this should certainly be investigated. In a closer look at the hybridization between Black-capped and Mountain chickadees in northwestern Canada, their hybridization could be facilitated by differences in traits such as dominance, with Black-capped Chickadee males being found to be socially dominant to Mountain Chickadee males (46). Song-ancestry relationships in chickadee

hybrid zones are largely unknown in this way and require further investigation.

Song and hybridization in tits and songbirds

Studies dealing with this relationship in the hybridization of other tit species have even more nuanced results. In a Tufted and Black-crested titmouse hybrid zone, the strength of song as an isolating mechanism varied based on the time since contact for two distinct areas within that hybrid zone (47). Taking these findings into consideration for understanding mechanisms of Black-capped and Carolina Chickadee hybridization, perhaps the song-ancestry lag we have observed in the southeastern Pennsylvania hybrid zone may be more or less pronounced than those in other areas along the hybrid zone band that continues west. Future studies could certainly benefit from attempting to quantify the magnitude of this lag and comparing across north/south points of contact for this hybrid zone.

Research involving song-ancestry relatedness in songbird hybridization turns out similarly varying results. A hybrid zone between MacGillivray's and Mourning warblers has a very similar lack of relationship between ancestry and song to my findings here, leading to conclusions that heterospecific song learning could be promoting their hybridization (48). When conversely looking at the effects of hybridization on song patterns, song convergence in Melodious Warblers (*Hippolais polyglottal*) and Icterine Warblers (*H. icterina*) appears to be influenced by both genetic and cultural transmission (49).

In summary: the jury is out when it comes to understanding the overall mechanisms of song in hybridization and vice versa. There is much to be learned about song patterns as both a mechanism and product of hybridization not just in Black-capped and Carolina Chickadees, but in chickadees, tits, and songbirds as a whole.

Future directions

This study further supports the existence of a lag between song and ancestry in a chickadee hybrid zone, but certainly leaves room for further inquiry. These findings provide the foundation for future projects to explore song-ancestry relationships along the Black-capped and Carolina Chickadee hybrid zone, pursue projects involving playback experiments in conjunction with dawn chorus data, as well as investigate more closely the genes associated with neural template development, and thus song learning abilities, in the two species. Recent genetic work has constructed the entire reference genome for Black-capped Chickadees, allowing for student inquiries into this topic (50).

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Mentor

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Dr. Robert Curry is a Professor in Villanova's Department of Biology (Ph.D. Michigan, 1987). Since 1991, he has taught courses on ecology, conservation biology, animal behavior, and tropical ecology, as well as a genealogy course for non-science majors. His research focuses on behavioral and evolutionary ecology, especially regarding hybridization in chickadees (songbirds). Other studies concern a uniquely herbivorous spider and island-endemic Neotropical birds. Dr. Curry has served as President of the Wilson Ornithological Society and on the Ornithological Council's Board of Directors. In 2019, he received Villanova's Outstanding Faculty Mentor Teaching Award and the Wilson Society's Margaret Morse Nice Medal.