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THE SONG REMAINS THE SAME:
AN ANALYSIS OF AGE-RELATED CHANGES IN SONG STRUCTURE AND SONG USE IN MALE AND FEMALE RUFOUS-AND-WHITE WRENS

by

LINCOLN MARCUS SAVI

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The song remains the same: an analysis of age-related changes in song structure and song use in male and female Rufous-and-white Wrens

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Declaration of Co-Authorship

I hereby declare that this thesis incorporates material that is the result of joint research, as follows:

I am the sole author of chapters 1 and 3, and the principal author of chapter 2. Chapter 2 was conducted under the supervision of Dan Mennill (University of Windsor), who contributed to the design of the study, data analyses, and writing of the chapter, as well as financial and logistical support throughout the process of the research, in the field in Costa Rica, and in the lab in Windsor. In all cases, the key ideas, primary contributions, experimental designs, data analyses and interpretations were performed by myself, with input from my co-author.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contributions of other researchers to my thesis, and I have obtained written permission from my co-author to include the above materials in my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

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Abstract

Bird song is one of the most well studied animal communication systems, and yet there are many features left to be explored. In particular, recent studies have revealed subtle ontogenetic changes in the voice of birds after reaching adulthood. Furthermore, recent literature reviews have highlighted the long-overlooked importance of female song, and its parallels to male song in terms of complexity and function. In this thesis, I review the current literature on the topics of song ontogeny, crystallized song, and song as a signal of age. Then, I explore the potential for song to signal an animal's age through a longitudinal study of a Neotropical songbird: the Rufous-and-white Wren. My analyses relied on 15 years of recordings of male and female Rufous-and-white Wren singing behaviour, collected during a long-term study of the behavioural ecology of a population living in northwestern Costa Rica. Focusing on the post-crystallization songs of the longest-lived 15 male and female Rufous-and-white Wrens in this dataset, I analyzed two aspects of song ontogeny: spectro-temporal variation and variation in repertoire use over each birds' adult lifetime. My results reveal that most of the variation in wren song was not explained by differences in age, although there were a few variables that did vary with the age of the singer: pause lengths in the introductory portion of the song decreased with age for both sexes, and frequency of the song's terminal note increased with age for females. I also note that female songs were sung at higher frequencies and wider bandwidths than male's songs. I discuss the implications of my findings and I highlight directions of future research in this field with specific predictions for how age-related changes in crystallized song might function in birds.

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Chapter 1: General Introduction

Introduction

Animals produce vocal signals that serve diverse functions including mate attraction, resource defense, and group cohesion (Bradbury & Vehrencamp 2011). Birds have been at the forefront of vocal communication research given the complexity of their vocal signals and the similarity of their vocal development to that of humans (Doupe & Kuhl 1999). Until recently, however, published research has been largely ignorant of female bird song. Once thought to be rare, female song is now estimated to be present in more than half of all surveyed songbird species (Odom et al. 2014; Webb et al. 2016). Furthermore, phylogenetic reconstruction demonstrates that both sexes sang in the ancestor of modern songbirds (Odom et al. 2014). Consequently, most of what we know about birdsong is biased by the fact that we have overwhelmingly focused on male songbirds(Odom & Benedict 2018). Through a long-term study of Rufous-and-white Wrens in Costa Rica, Dan Mennill and his students have collected 15 years of song recording data that includes both male and female singers. For my master's thesis, I explore the song ontogeny of two aspects of crystallized songs of male and female Rufous-and-white Wrens: fine spectro-temporal structure and repertoire-use patterns. In this General Introduction, I review the relevant concepts that are important for the data chapter that follows.

Bird song

Birds are a diverse group of animals that exhibit the most complex vocalizations in the animal kingdom (Mann et al. 2006). Second to human speech, the songs of songbirds (clade: Passeri) are likely the most well-studied animal vocalizations. Songs were once generally defined as the complex vocalizations produced by males during the breeding season (Catchpole & Slater 2008). This definition, as with much of ornithological research, shows a temperate zone bias (Stutchbury & Morton 2008). Indeed, songs in the temperate zone tend to be produced by males during the breeding season, typically during the spring and summer (Catchpole & Slater 2008). However, with increased research in the tropics, we now know that female song is common and widespread in the tropics, and is even recognized as more prevalent in the temperate zone than previously thought (Odom & Benedict 2018). There is evidence that females also modulate their singing behaviour similarly to males (Topp & Mennill 2008; Vondrasek 2006). The emerging concept of bird song, therefore, is that song can be considered a mating signal produced frequently during the breeding season, but not necessarily by males alone.

Song acts as a species-specific signal that serves two main ecological functions: mate attraction and territory defense (Catchpole & Slater 2008). Song performance is thought to advertise singer quality either through characteristics that are challenging to produce (such as high song output or rapid frequency-modulations; Hoi-Leitner et al. 1995; Podos 1997), or characteristics that that are developmentally challenging to learn (such as large song repertoires; Nowicki et al. 2002). These aspects of male song are often reinforced through female preference (reviewed in Nowicki & Searcy 2004). The aspects of song that are linked to quality or status can vary between species. Where one species may exhibit aspects of song as an apparent quality signal, others may not (reviewed in Gil & Gahr 2002). Although these patterns are reasonably well established for male song, it is unclear whether many patterns of quality-linked signals also exist in female song (MacDougall-Shackleton & Spencer 2012).

In diverse bird species, animals sing songs during interactive signaling exchanges, including agonistic encounters where birds can escalate or de-escalate aggressive behaviours by varying their patterns of song delivery (Todt & Naguib 2000). Various systems of aggression escalation and de-escalation have been described, including song-type matching, frequency matching, overlapping, or amplitude adjustment (reviewed by Searcy & Beecher 2009). Through such variation, the pattern of song delivery can serve as a signal of aggressive intent and thereby help birds navigate disputes over resources. Receivers listening to, and interacting with, the songs of conspecific animals can adjust their own behaviour accordingly based on the information perceived from the song. Whether that be modulating aggressive behaviour based on the perceived threat-level presented by a singer (Searcy & Beecher 2009) or modulating mating behaviours if the singer is a desirable mate (as in Hinde & Steel 1976).

Song learning

Birds in the order Passeriformes fall into two groups based on their vocal development: the oscine birds (clade: Passeri), or true songbirds, learn their songs from vocal tutors (reviewed in Catchpole & Slater 2008) whereas suboscines (parvorder: Tyranni) develop their songs innately (Touchton et al. 2014) even if raised in isolation or experimentally deafened early in development (Kroodsma & Konishi 1991). Oscine songbirds are one of several clades of animals to independently evolve vocal learning (Nowicki & Searcy 2014) which requires a vocal tutor to produce species typical vocalizations (Thorpe 1958).

Oscine songbirds can be further classified based on the timing of vocal learning in life: as either closed-ended or open-ended learners. Open-ended vocal learners, such as Budgerigars, *Melopsittacus undulates*, or European Starlings, *Sturnus vulgaris*, are capable of learning new vocalizations throughout their lives (Eens et al. 1992; Farabaugh et al. 1994). Alternatively, in closed-ended vocal learners, such as Song Sparrows, *Melospiza melodia*, or White-crowned Sparrows, *Zonotrichia leucophrys*, the learning process consists of a sensitive period early in life where the animal is most receptive to learning vocalizations. In closed-ended-learning birds, this period typically occurs within the first year of life (Marler 1970; Marler & Peters 1987). During this time the bird must hear its species' typical song and then, through auditory feedback, practice and perfect this song. After this time, the birds' songs become crystallized and the birds are seemingly incapable of learning new vocalizations (reviewed in Marler 1997). Closed-ended learning is often assumed to be the learning strategy of most songbirds. However, rarely is there sufficient evidence to conclude this, as it only takes one example to show that a bird is capable of learning beyond the sensitive period, but long-term evidence is required to conclude that it cannot (Beecher & Brenowitz 2005).

Song repertoires

In most species of songbirds adults learn a single version of their song, but in many species adults learn a repertoire of two or more versions of their species-typical song (MacDougall-Shackleton 1997). There are several proposed functions of vocal repertoires (reviewed in MacDougall-Shackleton 1997). One hypothesis is that large repertoires are honest signals of quality because developmental stress can reduce a bird's adult repertoire size, as demonstrated in Song Sparrows (Schmidt et al. 2013). Furthermore, a large repertoire may be more effective at repelling territorial intruders, and this has been demonstrated through loudspeaker playback experiments in Great Tits, *Parus Major* (Krebs 1978), and Red-winged Blackbirds, *Agelaius phoeniceus* (Yasukawa 1981). Females also show preferences for males with larger song repertoires in several species (Searcy 1992). However, female preference for large repertoires is not universal across songbirds (Byers & Kroodsma 2009). An alternative hypothesis is that song repertoires allow for increased complexity of communication because a song repertoire allows singers to select which signals they are sending (Molles 2006); some song types or some song delivery strategies might be more effective than others at signalling aggression or singer quality (as in Wood Warblers, family: Parulidae; Spector 1992). Understanding the intrasexual and intersexual functions of song repertoires, particularly in animals where both sexes sing, is an important and active area of investigation

Song as a signal of age

Older males are often of higher quality than younger males for three main reasons: differential survival of individuals with different phenotypes may result in animals with higher survivability in older age classes (reviewed in Forslund & Pärt 1995); older males may be a product of their own “good genes” that have allowed them to attain their old age; and older males may have acquired more experience rearing offspring and foraging by living longer (Martin 1995). Females of several animal species show preferences for mating with older males (Brooks & Kemp 2001), and in other species, females are known to benefit from larger clutches and enhanced parental effort by pairing with older males (e.g. Schubert et al. 2007). This could benefit the female both directly by providing her with a better territory and more resources, and indirectly by increasing her fitness if older mates produce better or more offspring.

Given song is an important quality assessment and advertisement tool, one might suspect that aspects of song might be associated with age. In open-ended song learners, repertoire size is often associated with age (Garamszegi et al. 2007; Kiefer et al. 2006). This should not come as a surprise because open-ended song learners, by definition, can add to their song repertoire throughout their lives, increasing its size over time. However, closed-ended song learners provide an interesting study system for how vocal behaviour changes with age since these birds are constrained by the fixed repertoire they learned at a young age; any age-related changes in their singing should be behavioural, such as song-type choice or song output, or performance based, as subtle spectro-temporal differences in song-type delivery. There is evidence that high performance song requires auditory feedback and practice in order to maintain (Leonardo & Konishi 1999). It is therefore possible that an older bird's increased experience and practice singing could also increase its ability to sing (Sakata & Vehrencamp 2012), although careful longitudinal studies of the ontogeny of vocal development in closed-ended learners are needed to corroborate this idea.

Historical research on closed-ended learners' song ontogeny typically focused on the first two years of life, to describe the time-limited learning process. Very few longitudinal studies exist that aim to determine how song performance varies at a longer time scale, such as through adult life, after song crystallization (Kipper & Kiefer 2010; Poesel & Nelson 2015). In the few studies that have looked at variation in crystalized song through adult birds' lives, a few different patterns of variation have emerged. In Java Sparrows, *Lonchura oryzivora*, males sing longer songs at an increased tempo with increased age (Ota & Soma 2014). In Banded Wrens, *Thryophilus pleurostictus*, males increase the consistency of their trill notes as they age (Vehrencamp et al. 2013). In Great Tits, males increase song consistency from a young age to a medium age, but decrease again from a medium to old age, providing the first evidence of senescence of birdsong (Rivera-Gutierrez et al. 2012). Therefore, in these three species of closed-ended learning songbird, song structure does vary with age, and may potentially serve as a signal of age. Yet, this pattern is not universal. Dark-eyed Juncos, *Junco hyemalis*, show no difference in song delivery with age (Cardoso et al.

2012), and White-crowned Sparrows show no change in consistency and a decrease in percentage peak performance with increased age (Poesel & Nelson 2015).

Signals of age within male song may be important in intersexual communication during mate choice, but may also be important in intrasexual communication during territory defence. Several playback studies have shown that male birds react more strongly to songs of older versus younger conspecific males. In Black Redstarts, *Phoenicurus ochruros*, playback subjects responded more quickly to songs of adult males versus subadult males (Cucco & Malacarne 1999). In Banded Wrens, playback subjects spent more time near speakers broadcasting first-year versus third-year male songs, and also produced more vocalizations near the first-year speaker (de Kort et al. 2009). In White-crowned Sparrows, first-year playback subjects flew closer to a speaker playing songs of first-year males versus songs from older males. Conversely, older-than-first-year playback subjects had closer approaches to speakers broadcasting songs from older birds versus songs from first-year males (Poesel & Nelson 2012). Therefore, age-related differences in crystallized song appears to be biologically relevant to males when they engage in territorial interactions, at least in the few species of closed-ended learners where this has been investigated.

A notable absence from all previous research on ontogenetic changes in song, is any rigorous attention to female song. A recent, extensive meta-analysis of over 85 studies on ontogenetic changes in song variation revealed that only two studies included female singers (Kipper & Kiefer 2010) The authors of this meta-analysis argued that more long-term studies are needed, especially of female song, to better understand the development and function of sexually selected signals such as song (Kipper & Kiefer 2010). More research is needed on female song in general to better understand the function and evolution of song and how these differ between sexes (Odom & Benedict 2018). Research on ontogenetic changes in crystallized song has been a male-centric study area that would benefit from the inclusion of female song research (MacDougall-Shackleton & Spencer 2012; Odom & Benedict 2018; Riebel et al. 2005). As with male song, female song may also signal important aspects of quality that might change with age (Hall & Langmore 2017).

Study species

My master's thesis research focuses on ontogenetic changes in the song of male and female Rufous-and-white Wrens. These birds are non-migratory Neotropical songbirds found in several types of habitat in Central and northwestern South America. These birds form socially monogamous mated pairs, and males and females sing both solo songs, and loosely coordinated duets to defend territories. Males typically have a repertoire of 10.8 unique song types, and females have a repertoire of 8.5 song types (Mennill & Vehrencamp 2005). Rufous-and-white Wren repertoire size does not appear to be under directional sexual selection, given that the males only advertise a subset of their repertoire when they are unpaired (Hennin et al. 2009). Visually, males and females are monomorphic although males are slightly larger than females (Valderrama et al. 2007). Our long-term study population of wild Rufous-and-white Wrens lives in the humid dry forest habitats in Sector Santa Rosa of the Guanacaste Conservation Area of northwestern Costa Rica (Figure 1.1). Here the birds' territories are arranged in small neighbourhoods of adjacent territories where the birds can sometimes be observed countersinging with their territorial neighbours. Given that both sexes sing (see examples in (Figure 1.2), and the birds often persist in our population for several years, this study system is suitable for longitudinal research of sex differences and ontogenetic change in crystalized song.

In the following data chapter of my thesis, I explore ontogenetic variation in two aspects of Rufous-and-white Wren singing behaviour: song-type choice and spectro-temporal song structure. I test the hypothesis that song changes with increased age thereby acting as a signal of age. I was motivated by the deficiency in female song research and the lack of longitudinal studies necessary for describing broader patterns the ontogeny of crystallized song in male and female songbirds.

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Figures

Figure 1.1: Map of Costa Rica with Sector Santa Rosa highlighted in yellow and magnified on the left. A red dot indicates the position of my study site within Sector Santa Rosa.

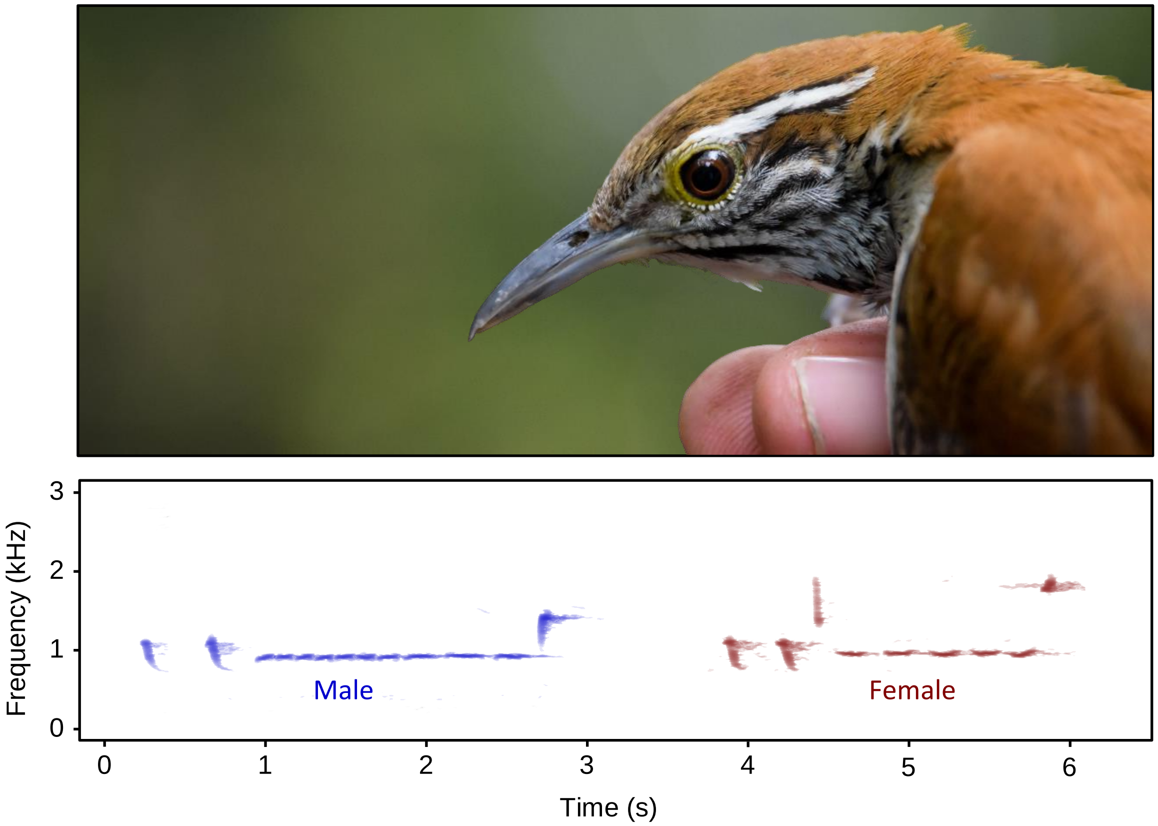

Figure 1.2: Photograph of a Rufous-and-white Wren and a spectrogram of matched song types from male and female individuals.

Chapter 2: The song remains the same: an analysis of age-related changes in song structure and song use in male and female Rufous-and-white Wrens

Chapter Summary

Older animals often make better mates or more threatening rivals due to their increased experience. For some songbirds, some performance-based characteristics of male songs vary with age, creating the potential for song to serve as a signal of age. Both rival males and prospecting females may stand to benefit if they can assess another animal's age on the basis of their voice. In the present study we trace two aspects of singing behaviour, song-type choice and song performance, through ontogeny in 15 male and 15 female Rufous-and-white Wrens, *Thryophilus rufalbus*, over the entirety of their adult lifetimes. We recorded song-type choices and measured spectro-temporal characteristics of songs from each individual bird for every year of its adult life. We found that repertoire use, and most spectro-temporal song variables did not change significantly with age. Interestingly, two aspects of song did change with age: the average pause lengths of the introductory section of the birds' songs decreased, and females, but not males, increased the frequency of their terminal syllables as they aged. We also found pronounced sex differences in many aspects of measured song but not in repertoire-use patterns. Our results suggest that, overall, songs and song delivery do not change with age in adult Rufous-and-white Wrens, although these two differences suggest that subtle aspects of song might reveal age differences, especially for females. Female song was once thought to be rare, but it is of recent research interest because it is now known to be widespread and common in tropical songbird species. We provide evidence for the potential of female songs to be signalling age-related changes that might be meaningful to other conspecific animals in

territorial or mating interactions, an idea that up until now, has mainly focused on male birds. For Rufous-and-white Wrens, more research is needed to determine whether the subtle differences in age that we describe are biologically relevant.

Introduction

Many animal behaviours change through ontogeny such that older individuals behave differently than younger individuals. For example, the visual signals of Long-tailed Manakins, *Chiroxiphia linearus*, take five years to mature (Doucet et al. 2007); the echolocation signals of Big Brown Bats, *Eptesicus fuscus*, differ between adults and juveniles (Masters et al. 1995); and the olfactory signals of Eurasian Otter, *Lutra lutra*, vary between adult and juvenile age classes (Kean et al. 2011). These age-linked signals can allow for receivers to assess differences between signallers and adjust their own behaviours accordingly, whether that be altering aggression if the signaller is a perceived rival, or modifying courtship behaviours if the signaller is a potential mate.

Birds provide a popular model system for studying vocal signals and animal quality. Birdsong is a socially selected signal of quality, that is important in both intrasexual interactions, such as territory defense, and in intersexual interactions, such as mate attraction (Catchpole and Slater 2008). Song is thought to function as a performance of skill and vigour that advertises aspects of singer quality (Byers et al. 2010) because song can be neuro-muscularly challenging to perform (Suthers et al. 1999), and variation in song performance can elicit costly responses from conspecific animals (Ballentine et al. 2004; de Kort et al. 2009). In song learning species, including oscine songbirds and hummingbirds, the sensitive period for song learning early in life coincides with major neuronal and muscular development (Brenowitz & Beecher 2005) wherein developmental stress can explain much of the inter-individual variation in adult song performance (Nowicki et al. 1998). Developmental stress can negatively impact song learning resulting in limited song performance or smaller song repertoires (Spencer et al. 2003). Therefore, birds' vocal signals vary over early stages of live, and may reveal aspects of the singer's quality or their developmental history.

Previous studies of bird song ontogeny have focused on males, and developmental changes early in life. This body of research shows that there are diverse song-learning strategies, where some species develop new songs only early in life, while others develop new songs annually (reviewed in Beecher & Brenowitz 2005). The most common strategy is closed-ended learning: wherein vocal learning is understood to occur only early in life, and song becomes crystallized and less variable at the onset of adulthood (Brenowitz & Beecher 2005). Although we have a well-developed understanding of vocal changes up to the crystallization of adult song, less attention has been given to intra-individual variation in song performance after crystallization through the entirety of birds' adult lives. This type of study is rare because it requires a large longitudinal dataset of repeated recordings of the same marked animals. Yet this type of study is important because song performance might change over the course of a bird's life to reflect changes in quality associated with age.

Song plasticity beyond crystallization in closed-ended learners has been described in several songbird species but species differ in which song traits vary across adulthood (Kipper & Kiefer 2010). Some birds change certain aspects of their songs between years (e.g., the trills of Banded Wrens, *Thryophilus pleurostictus,* increase in consistency with age; Vehrencamp et al. 2012), while others change different aspects (e.g., the trill length of Superb Fairy-wrens, *Malurus cyaneus*, increases with age; Dalziell & Cockburn 2008). There is experimental evidence that these changes are biologically relevant and can affect the behaviours of conspecific animals. Banded Wrens, for example, are more attentive and cautious when presented with simulated territory invasions by older individuals (de Kort et al. 2009). More longitudinal studies that examine multiple characteristics of crystallized song are needed to determine if patterns of plasticity in post-crystallized song exist between closely related species.

Why would it be advantageous for an animal to advertise its age based on features of its song? Older birds have been shown to be better mates or more competitive rivals (Angelier et al. 2007). Older birds have been exposed to more threats to survival, thereby revealing high survivability (reviewed in Forslund & Pärt 1995). Consequently, older birds are indicative of their own good genes that have allowed them to thrive in their environment and attain an older age (Kokko 1998). Furthermore, older birds have increased life experience as a result of their age, which can be important in tasks such as foraging or raising offspring (Martin 1995). Older birds' songs or singing behaviour might vary with age (Kipper & Kiefer 2010), especially if an older animal's ability to secure food resources increases its singing ability (Yamada & Soma 2016). Based on these ideas, we propose the hypothesis that after crystallization, birds' song may change with age, thereby serving as a signal of age that will be of interest as a social cue to conspecific animals.

An animal could reveal its age through ontogenetic changes in the fine-structure of its song (e.g., de Kort et al. 2009; Ota & Soma 2014), but it could also reveal its age by changing the way it uses its vocal repertoire to emphasize specific songs. Song repertoires allow some insight into developmental conditions because smaller song repertoires have been associated with developmental stress (Spencer & MacDougall-Shackleton 2011), and larger repertoires are more attractive to females in some species (e.g.,Sedge Warblers, *Acrocephalus schoenobaenus*, Catchpole et al. 1984, and Zebra Finches, *Taeniopygia guttata,* Collins 2007). Repertoire size varies considerably across songbird species, however, from the single-song repertoires of the Savannah Sparrows ,*Passerculus sandwichensis*, to the thousand-song repertoires of the Brown Thrasher, *Toxostoma rufum*, with most species possessing repertoires of one to ten songs (MacDougall-Shackleton 1997). Yet large repertoire size does not appear to be a target of sexual selection in all songbird species. Some evidence suggests that song repertoires arose from selection on increased signalling system complexity rather than repertoire size (reviewed in Byers & Kroodsma 2009). A study on Rufous-and-white Wrens, *Thryophilus rufalbus,* found that males used their repertoires differently when they were paired with females versus when they were bachelors. Furthermore, the males in this study emphasized a subset of songs from their repertoire with particular characteristics, such as songs with a wide terminal syllable bandwidth, when they were bachelors rather than emphasizing total repertoire size (Hennin et al. 2009). This supports the idea that repertoires might be more important in increased signalling-system complexity rather than simply advertising the ability of an animal to learn many songs. Documenting relative use of song types through life might uncover interesting patterns if birds emphasize song types in their repertoires with specific characteristics as they age.

Birdsong research has heavily focused on male song, and female song has been historically overlooked (Odom & Benedict 2018). It was long believed that males were the sole singers in most species of songbirds likely due to a temperate bias of ornithological research (Stutchbury & Morton 2008). However, increased research in the tropics has revealed that female song is much more prevalent than once believed, with more than half of surveyed songbird species exhibiting songs from both sexes (Odom et al. 2014; Webb et al. 2016). An extensive review on age-related change in song (Kipper & Kiefer 2010) found only two studies that explored these changes in female singers. These studies found that older female Alpine Accentors, *Prunella collaris,* have increased song complexity (Langmore et al. 1996) and that female European Starlings, *Sturnus vulgaris*: an open-ended song learner, have varying levels of repertoire turnover and tend to decrease their repertoire size with age (Pavlova et al. 2010). We are aware of only one other study that explored age-related changes in female song since the publication of Pavlova et al. (2010), which found that female Superb Starlings, *Lamprotornis superbus*, an open-ended song learner, increase their song diversity with age (Keen et al. 2016). Female song is now believed to be the ancestral state of songbirds (Odom et al. 2014) and therefore deserves far more research attention to understand the evolution and function of song (Odom & Benedict 2018).

In this study, we collected song recordings from a population of wild Rufous-and-white Wrens over a 15-year period to explore two aspects of ontogenetic change in song: structural changes in song, and song-type use frequency. Rufous-and-white Wrens are Neotropical resident songbirds that form monogamous reproductive pairs and defend territories together year-round (Douglas et al. 2012; Topp & Mennill 2008). Both males and females sing in this species, and often combine their songs into loosely co-ordinated duets. Males have relatively large repertoires of 10.8 ± 0.7 song types and females have slightly smaller repertoires of 8.4 ± 1.2 song types (Mennill & Vehrencamp 2005). Using a decade-and-a-half of acoustic recordings, we test the hypothesis that birds' songs and singing behaviour serve as a signal of age. We explore this in two aspects of vocal ontogeny: structural changes in individual song types through adult life, and repertoire-use patterns over the bird's adult lifetime. Importantly, we test this in both males and females. To our knowledge, this is the first study to explore post-crystallization song plasticity of a female tropical bird. Our approach includes many spectro-temporal variables to best determine which aspects of song, if any, change with increased age.

Methods

Study species and field techniques

We collected recordings of male and female Rufous-and-white Wrens in Sector Santa Rosa of the Guanacaste Conservation Area in northwestern Costa Rica (10°51' N, 85°36' W) every year between 2003 and 2017. Birds in this study population live year-round in patches of humid late-successional Neotropical dry forest. We monitored the birds' activity daily between approximately early April until early July, a period of the year that coincides with the transition from dry to wet season, an increase in singing, and the onset of breeding activity (Topp & Mennill 2008). We made notes of breeding activity, territory location, while we collected acoustic recordings of the birds' singing behaviour. We attempted to capture all unbanded Rufous-and-white Wrens with mist nets. We took standardized body measurements of each bird captured and gave each individual a unique combination of three coloured leg bands, and one numbered aluminum leg band so that we could visually identify individuals in the field. We determined sex by the presence of reproductive structures (brood patches in females) as well as singing and incubation behaviour. Males and females sing sexually dimorphic songs; females sing at higher sound frequencies and far less often than males (Mennill & Vehrencamp 2005). Females are also the sole egg incubator in this species.

We collected song recordings both actively through focal recordings taken by recordists in the field, and passively using automated recording units in order to collect a larger sampling of birds' songs and singing behaviour. We collected focal recordings during the dawn chorus between approximately 04:45 and 07:00 CST using a Sennheiser MKH70 or ME67/K6 microphone and a Marantz PMD-660 or PMD-661 digital recorder (44.1kHz sampling rate, 16-bit accuracy, WAVE format). In most focal recordings, we confirmed the identity of the recorded bird by its leg bands. In the few cases where we were unable to visually identify the birds, we confirmed the animal's identity based on comparison to previous recordings collected in the same location.

We collected passive recordings with Wildlife Acoustics Song Meter automated recorders strapped to trees near the centre of Rufous-and-white Wren territories. In this species, all-purpose breeding territories are large (1000-13000m^2; Osmun & Mennill 2011) so there is a low likelihood of misidentifying focal birds as neighbours when analysing the recordings. We programmed the automated recorders to record daily, from 04:45 until at least 11:00 CST. We later confirmed all bird identities in both focal and passive recordings by comparing the recorded song spectrograms to known birds' individual repertoires. Rufous-and-white Wren repertoires are individually distinctive, and even song types that are shared between two individuals can be differentiated based on subtle time and frequency characteristics (Harris et al. 2016).

Acoustic analysis

We selected 30 birds (15 males and 15 females) to analyze in our study. These 30 birds represent the longest-lived across the 15 years of studying this population. These birds were studied for at least four years, with the oldest bird being studied for nine years. In this subset of birds, males lived longer than females, with the average male living for 5.6 years and females living for 4.3 years.

We manually clipped songs from our focal and automated recordings using spectrograms generated in Syrinx-PC (John Burt, Seattle, Washington, USA). We scanned through recordings and isolated songs that had a high signal-to-noise ratio and were not overlapped by other animal vocalizations. We saved the isolated songs as separate WAV files. We measured three to five examples, limited by availability of good quality recordings, of each song type in each bird's repertoire, up to a maximum repertoire size of 15, for each year the birds were alive.

To quantify variation in male and female Rufous-and-white Wren song structure, we measured 22 spectro-temporal features summarized in Figure 2.1. To measure these characteristics and minimize subjectivity, we used the automated parametric measurements tool in Avisoft SASLab Pro (Avisoft Bioacoustics, Berlin, Germany) to measure the time and peak frequency at the beginning, end, and every 5ms interval in between, of every note in the songs. We resampled all songs to 8kHz, which is more than double the maximum frequency of Rufous-and-white Wren song, to maximize spectral resolution in AviSoft (as in Graham et al. 2018a). We filtered the sound files at 500Hz to remove low frequency noise, and we generated high-resolution spectrograms (settings: transform size: 1024Hz; overlap: 98.43%; effective resolution of 8Hz / 4ms; window: Hamming). Occasionally, we manually removed any heterospecific or conspecific animal noise using the standard eraser cursor in AviSoft before subjecting the sound clips to the parametric measurements tool.

We measured 2727 songs from 15 different males (average songs measured per male: 181.8; range: 88 to 330; average sampled repertoire size per male: 10.33; range: 7 to 13). We measured an average of 36.35 songs per year for each male. We measured 636 songs from 15 different females (average songs measured per female: 42.4; range: 16 to 73; average sampled repertoire size per female: 5.2; range: 2 to 8).

To quantify variation in male and female repertoire use over lifetime, we counted every song type sung by each of the 30 birds in every sound recording available throughout their adult lives (i.e. the high-quality recordings used for measuring spectro-temporal features plus a huge library of additional songs). To account for differences in time each bird was recorded, we converted our counts to proportions of total songs sung per year. We then calculated repertoire-use dissimilarity scores of every year compared to the bird's first year, for each individual bird (adapted from Sandoval et al. 2014). We generated these scores by calculating Bray-Curtis dissimilarity scores for each year compared to the first year for each of the 30 birds. This resulted in a numerical representation of dissimilarity in both the number of different song types sung, and their relative frequency, on a scale of zero to one; a low score (near zero) indicates that birds used their repertoires identically between years, and a high score (near one) indicates complete dissimilarity. These scores were based on an average of 541.6 songs per year and individual for males (range: 30 to 3437 songs) and 56.6 songs per year and individual for females (range: 3 to 282 songs) so we feel we have an extensive sample of annual repertoire use. The smaller sample of female songs arises because females sing less than males; a previous study suggests that , even for females, this level of sampling should be sufficient to accurately estimate repertoire size over the course of several years (Harris et al. 2016).

Statistical analyses

All statistical analyses were performed in R 3.4.4 (R Core Team 2018, Vienna, Austria). For our analysis of acoustic differences in song structure, we fit a series of 18 models based on our measured song variables. Sixteen of these were linear mixed models (‘lmer’ function, R package: lme4) with response variables including: the length of the introduction, trill, and terminus; element rate of the introduction, and trill; duty cycle of the introduction, and trill; average pause length of the introduction, trill, and entire song; average dominant frequency, and bandwidth for the introduction, trill, and terminus. Since the lmer function does not generate p-values we used the ‘Anova’ function (R package: car) to generate p-values for our linear mixed models. The other two song variables, number of introductory and trill notes, were fitted by generalized linear mixed models (‘glmer’ function, R package: lme4) because these response variables were integer count data. We excluded the other four measured variables from analysis (total song length, total song bandwidth, average dominant frequency of total song, and number of terminal notes) because they were either highly correlated with other variables (e.g. total song length is directly correlated with the length of the other song sections) or showed no variation (i.e. all songs only had one terminal note). All 18 models included age, sex and their interaction as fixed effects; and individual, and song type as random effects. We included the age-sex interaction term in all models in order to explore the possibility that male and female songs may experience different selection pressures (Price 2015) and thus might vary differently through ontogeny. Recent investigations have revealed that male and female songs may show very different patterns in use (Brunton et al. 2016; Cain et al. 2015), further justifying the importance of including this interaction term. We used a Holm-Bonferroni correction (Holm 1979) to determine significance of observed patterns, given we conducted 18 tests for age and sex differences across our measured song variables. Although Bonferroni correction is a matter of debate (Forstmeier et al. 2017; Nakagawa 2004) we felt it was appropriate to apply a Holm-Bonferroni correction given that we were conducting tests of the same hypothesis using 18 different acoustic variables. The Holm-Bonferroni is less conservative than a typical Bonferroni correction and has been used in other animal behaviour studies (e.g., Li et al. 2016; Miles & Fuxjager 2018; White et al. 2017).

For our analysis of repertoire-use differences between years of life, we used the 'lmer' function (R package: lme4) to run a linear mixed model with: age, sex, and the interaction between sex and age as fixed effects; individual and year as random effects; and repertoire-use dissimilarity as the response variable. P-values were generated by the 'Anova' function (R package: car).

Results

Song structure variation

Rufous-and-white Wrens showed consistent song structure across their adult lives (Table 2.1). Sixteen different spectro-temporal variables showed no structural change with age (Table 2.1). Two variables did show subtle changes with age. First, both males and females exhibited a decrease in the average pause length of the introductory section of their song (Figure 2.2). The differences between years were subtle, and no two particular years could be differentiated based on this feature, but overall, a statistically significant amount of the variation in introductory section pause length was explained by age. Second, female song showed an increase in the average dominant frequency of the terminal sections of their songs as they aged, whereas the average dominant frequency of the terminal sections in males was consistent across their lifespan (Figure 2.3). Again, the differences for females were subtle, and no two particular years could be differentiated based on this feature, but a statistically significant amount of the variation was explained by age (Table 2.1).

Rufous-and-white Wrens showed pronounced sex differences in several aspects of their song structure (Table 2.1). Males sang longer introductory and trill sections than females; males had increased duty cycle in their introductory and trill sections; and males sang introductory, trill, and terminal sections at lower frequencies and with narrower bandwidths than females (Table 2.1). No other aspect of song structure varied with sex. These results match a prior short-term investigation of sex-differences in Rufous-and-white Wren singing behaviour (Mennill & Vehrencamp 2005), and reveal that these differences persist regardless of age.

Repertoire use variation

Rufous-and-white Wrens showed differences in repertoire use between years (Bray-Curtis Dissimilarity score mean: 0.38 ± 0.144) whereby song-type choice frequency appears to change from year-to-year (Figure 2.4). In spite of variation from one year to the next, these differences did not appear to be influenced by age of the singer (model estimate ± SE: -0.013 ± 0.012, p = 0.18, Figure 2.5). The apparent increase in variance with age in Figure 2.5 is the result of decreasing sample size in oldest age classes and it is not likely a biologically relevant phenomenon.

Rufous-and-white Wrens also showed no significant sex differences in repertoire-use patterns (model estimate ± SE: 0.006 ± 0.074, p = 0.53, Figure 2.5) We also did not observe a sex-age interaction in repertoire use (model estimate ± SE: 0.004 ± 0.017, p = 0.80, Figure 2.5).

Discussion

A decade-and-a-half of recordings of the songs of free-living Rufous-and-white Wrens in Costa Rica, combined with detailed analyses of the 15 longest-lived males and females, do not provide compelling support for the hypothesis that song acts as a signal of age. Rufous-and-white Wrens did not exhibit any striking patterns of age-related change in most spectro-temporal aspects of song or repertoire-use patterns, such as those described in other songbird species (Kipper & Kiefer 2010; Vehrencamp et al. 2013). We described age-related patterns of change in two song features: average dominant frequency of the terminal section in females, and average pause length in the introductory section for both males and females. Yet these differences were subtle and did not facilitate ready discrimination between birds of different ages. Our analyses accounted for different song types and repeated measures across individuals, so we are confident the patterns we observed were within individuals and not between individuals or song types. Our analyses provide evidence of pronounced sex differences acoustic features of Rufous-and-white Wren songs, but not in repertoire-use similarity. We discuss the implications of these findings below.

Song variation by age

Only two aspects of Rufous-and-white Wren song showed variation with age. The first was that females, but not males, showed an increase in average dominant frequency of their terminal syllables. A song's dominant frequency is an aspect of song that has the potential to be a performance constraint (Podos et al. 2016): the terminal notes of Rufous-and-white Wren songs are often the highest frequency portion of the song, and it is possible that high-pitched terminal sections of female songs are more difficult to produce due to the large frequency shift from previous song elements. If this is difficult to produce, this characteristic of song has the potential to signal female age just as highly consistent song notes are understood to be difficult to produce (Sakata & Vehrencamp 2012) and are understood to be a signal of age in Banded Wrens (Vehrencamp et al. 2013). However, the differences in frequency between subsequent years was very subtle (an average increase of 40.24 Hz per year), and therefore further research is needed to determine if the observed pattern is biologically relevant.

The second aspect of song that varied with age was average pause length in the introductory section. In both males and females, average pause length decreased very slightly yet significantly with age. This difference in length is very subtle (an average decrease of just 0.002 s per year) and the birds can produce much shorter pauses between notes elsewhere within the same song, such as in the trill (see Figure 2.1 for an example). Another paper that found that silent intervals decrease with age in Java Sparrows, *Lonchura oryzivora* (Ota & Soma 2014) but this change in pause lengths also coincided with an increase in song tempo. We found no age-related change in song element rate and therefore we suspect decreasing pause lengths are not biologically relevant, or that they are the side-effect of some other aspect of song production, such as amount of breath required to produce notes later in the song. Furthermore, the average lifespan of Rufous-and-white Wrens in our long-term dataset is less than three years (Woodworth et al. 2018; D. Mennill unpublished data), and neither of the statistically significant acoustic differences are particularly different across a bird's first three years (Figure 2.2, Figure 2.3), suggesting that these signals of age would have limited utility.

We did not find that any other measured song variables changed with age. The lack of changes in song features with age came as a surprise, since congeneric Banded Wrens, have been shown to increase their trill note consistency as they age (de Kort et al. 2009; Vehrencamp et al. 2013). Although we did not test for trill note consistency directly (the trill notes of Rufous-and-white Wrens are not frequency modulated like the trills of Banded Wrens), we did not detect any changes in trills that might hint at age-graded changes in trill consistency, such as changes in average pause length or bandwidth of the trill. Banded Wren trills have a broad bandwidth (Molles & Vehrencamp 1999) whereas Rufous-and-white Wren trills are more tonal (Mennill & Vehrencamp 2005). Therefore, Rufous-and-white Wren trills are less likely to be nearing a performance constraint than Banded Wrens, which would make the performance of these vocalizations a good signal of an individual's age or quality (Podos 1997; Podos et al. 2009, 2016).

Our data support our long-held supposition that Rufous-and-white Wrens are closed-ended learners that develop their repertoire during the first year of their life (Graham et al. 2018b; Harris et al. 2016). The vast majority of songbirds are assumed to be closed-ended learners (Beecher & Brenowitz 2005; Brenowitz & Beecher 2005) and our data show that Rufous-and-white Wrens fit this mould, as predicted. If the wrens were adding new song types to their repertoires, we would have expected repertoire-use dissimilarity to increase with age, and we would have expected to encounter new song types as birds age, as has been observed in open-ended species such as Clay-coloured Thrushes, *Turdus grayi* (Vargas-Castro et al. 2015), Common Nightingales, *Luscinia megarhynchos* (Kiefer et al. 2006), and Sedge Warblers, *Acrocephalus schoenobaenus* (Nicholson et al. 2007). We did not detect either pattern, nor did we find that the song types in the birds' repertoires changed consistently in the number of syllables through life, consistent with patterns expected in a closed-ended learner.

We found that Rufous-and-white Wrens changed their song-type choices between years, but the variation in repertoire use was not explained by age. Further investigation is required to determine whether year-to-year variation in song-type choice coincides with a social or ecological function, such as songtype matching interactions (MacDougall-Shackleton 1997; Todt & Naguib 2000) or neighbourhood-level influence of song choice (see Beecher et al. 1996; Foote et al. 2008; Trillo & Vehrencamp 2005), or if annual song-type choices are simply selected at random. Future investigations that explore whether song-type choice is driven by interactions between neighbours may resolve the patterns in song type choice we quantified here. We provide evidence that Rufous-and-white wrens are a typical closed-ended song learner with a post-crystallization song repertoire of a fixed size, consistent with other authors' assumptions of closed-ended learning in other wren species in the genus *Thryophilus*, including Banded Wrens (Vehrencamp et al. 2013), or Niceforo's Wrens, *Thryophilus nicefori* (Valderrama et al. 2007).

Song variation by sex

Female Rufous-and-white Wrens sang their songs at a higher pitch than males. This is consistent with previous studies in this species that found females to sing songs at higher frequencies, especially the trill and terminal sections (Mennill & Vehrencamp 2005). Our results provide evidence that all three sections of song are sung at a higher frequency in females than in males. Rufous-and-white Wren females also sang all three sections of their songs at a higher bandwidth than males. Model estimates (± SE) include introductory section bandwidth at 178 ± 58Hz higher, trill bandwidth at 16 ± 5Hz higher, and terminal section bandwidth at 194 ± 72Hz higher in females than in males. Across most songbirds, including Rufous-and-white Wrens, males have larger body sizes than females (Kissner et al. 2003; Mennill & Vehrencamp 2005). Given that large bodied animals can more easily produce low-frequency sounds (Bradbury & Vehrencamp 2011;

Fletcher 2004), the sexual dimorphism we uncovered in vocal frequency may be a reflection of differences in body size between the sexes (similar to Linhart & Fuchs 2015).

Rufous-and-white Wrens showed no significant sex differences in annual repertoire-use patterns. Both males and females showed considerable variation in song-type choices between years. However, this variation was not significantly different between the sexes. A previous study showed that pairs of male and female Rufous-and-white wrens exhibit similar spatial patterns of territorial singing behaviour (Osmun & Mennill 2011). Taken with this, we provide some evidence that males and females also share similarities in how they use their repertoires (i.e. in relative song-type proportions sung) over the course of a breeding season. However, our analysis only reveals patterns in repertoire use on a whole-breeding-season timescale and not a shorter timescale such as patterns of song-types use and delivery at different breeding stages. Female Rufous-and-white Wrens sing far less than males (Mennill & Vehrencamp 2005). It is possible that an analysis of singing behaviour on a shorter timescale could reveal other sex differences in song-type delivery such as song-type switching rates or vocal output at different breeding stages (as observed in female New Zealand Bellbirds, *Anthornis melanura*; Brunton et al. 2016).

Conclusions and Future Directions

Through quantitative analysis of 15 years of data, focusing in the longest-lived male and females in a long-term colour-marked study population, we found pronounced sex differences in Rufous-and-white Wren songs, but little evidence of age differences. Our results suggest that, as expected, Rufous-and-white Wrens are closed-ended learners, where songs become fixed at the onset of adulthood and show remarkably little variation over time, even in the longest-lived animals in our study population. Age differences in female song are of particular interest to behavioural ecologists because our understanding of female song and its function are relatively poor in comparison to male song (Odom & Benedict 2018). Recent studies have found some evidence for a link between female song delivery and fitness in a few songbird species (Brunton et al. 2016; Cain et al. 2015; Keen et al. 2016) and this correlation between song and reproductive fitness may be linked by a third variable such as age (Hall & Langmore 2017). For Rufous-and-white Wrens, it remains unclear if female song provides any useful indication of age. Further research is necessary to determine if the patterns of age-related change we describe here are discernible by the birds, and if some other aspect of song delivery such as song output is correlated with fitness or age.

Our investigation can be distinguished from previous studies for relying on such a large longitudinal dataset that includes both male and female songs. Most acoustic studies of age variation have focused on the first few years of life (Kipper & Kiefer 2010), and only on the songs of males (Odom et al. 2014; Riebel et al. 2005; Webb et al. 2016). The importance of female song research was highlighted by the authors of a recent meta-analysis of bird song studies with a specific request for songbird researchers to document female song (Odom & Benedict 2018). Overall, we reveal that males and females in a resident tropical bird show differences between sexes but very similar patterns of overall consistency in acoustic features of song across their adult lives.

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Tables

Table 2.1: Summary of 18 mixed models with 18 different response variables corresponding to our measured Rufous-and-white Wren song elements. 16 models were linear mixed models (LMM) and 2 were generalized linear mixed models (GLMM). Emboldened p-values represent significant interactions after Holm-Bonferroni correction.

Measured Song Elements (LMM)			
	Estimate ± SE	**t value**	**p value**
Length of Introduction (s)			
Intercept	0.696 ± 0.100	6.96	
Sex (Male)	0.185 ± 0.048	3.82	**<0.0001**
Age	0.006 ± 0.008	0.73	0.86
Sex:Age Interaction	-0.006 ± 0.009	-0.71	0.48
Length of Trill (s)			
Intercept	0.856 ± 0.064	13.38	
Sex (Male)	0.203 ± 0.047	4.36	**<0.0001**
Age	-0.017 ± 0.007	-2.306	0.11
Sex:Age Interaction	0.014 ± 0.008	1.87	0.06
Length of Terminal Section (s)			
Intercept	0.157 ± 0.041	3.83	
Sex (Male)	-0.0026 ± 0.019	-0.14	0.035
Age	0.0057 ± 0.0024	2.40	0.77
Sex:Age Interaction	-0.0048 ± 0.0025	-1.92	0.55
Element Rate of Introduction (n/s)			
Intercept	4.752 ± 0.406	11.70	
Sex (Male)	-0.894 ± 0.456	-1.96	0.11
Age	-0.062 ± 0.073	-0.85	0.43
Sex:Age Interaction	0.091 ± 0.077	1.18	0.24
Element Rate of Trill (n/s)			
Intercept	10.026 ± 1.152	8.71	
Sex (Male)	-0.524 ± 0.439	-1.63	0.73
Age	-0.112 ± 0.063	-1.45	0.67
Sex:Age Interaction	0.137 ± 0.067	2.30	0.041
Duty Cycle of Introduction (s)			
Intercept	0.326 ± 0.053	6.09	
Sex (Male)	0.086 ± 0.024	3.66	**0.00046**
Age	0.0055 ± 0.004	1.36	0.66
Sex:Age Interaction	-0.0056 ± 0.004	-1.29	0.19

Duty Cycle of Trill (s)			
Intercept	0.464 ± 0.043	10.72	
Sex (Male)	0.123 ± 0.027	4.49	**<0.0001**
Age	-0.011 ± 0.005	-2.35	0.44
Sex:Age Interaction	0.011 ± 0.005	2.22	0.026
Average Pause Length of Introduction (s)			
Intercept	0.204 ± 0.018	11.16	
Sex (Male)	0.019 ± 0.010	1.83	0.022
Age	-0.002 ± 0.002	-1.41	**0.00081**
Sex:Age Interaction	0.0006 ± 0.002	0.31	0.75
Average Pause Length of Trill (s)			
Intercept	0.070 ± 0.011	6.62	
Sex (Male)	-0.0012 ± 0.004	-0.28	0.30
Age	0.0007 ± 0.0008	0.97	0.73
Sex:Age Interaction	-0.0009 ± 0.0008	-1.16	0.24
Average Pause Length of Full Song (s)			
Intercept	0.112 ± 0.011	10.42	
Sex (Male)	-0.005 ± 0.004	-1.104	0.17
Age	-0.0005 ± 0.0007	-0.73	0.0088
Sex:Age Interaction	-0.0001 ± 0.0008	-0.17	0.86
Average Dominant Frequency of Introduction (Hz)			
Intercept	1188.09 ± 54.42	21.83	
Sex (Male)	-187.57 ± 31.106	-6.03	**<0.0001**
Age	3.48 ± 3.58	0.97	0.11
Sex:Age Interaction	-1.77 ± 3.80	-0.47	0.64
Average Dominant Frequency of Trill (Hz)			
Intercept	1042.56 ± 26.12	39.91	
Sex (Male)	-122.59 ± 20.78	-5.90	**<0.0001**
Age	-0.50 ± 1.86	-0.27	0.07
Sex:Age Interaction	1.81 ± 1.96	0.92	0.36
Average Dominant Frequency of Terminal Section (Hz)			
Intercept	1916.67 ± 139.81	13.71	
Sex (Male)	-129.76 ± 68.93	-1.88	**<0.0001**
Age	38.81 ± 12.58	3.08	0.65
Sex:Age Interaction	-40.24 ± 11.70	-3.44	**0.00058**
Bandwidth of Introduction (Hz)			
Intercept	577.17 ± 106.05	5.44	
Sex (Male)	-178.44 ± 58.28	-3.06	**0.0001**
Age	15.81 ± 9.54	1.66	0.046
Sex:Age Interaction	-10.54 ± 10.15	-1.04	0.30

Bandwidth of Trill (Hz)			
Intercept	67.67 ± 6.42	10.64	
Sex (Male)	-16.17 ± 5.13	-3.32	**<0.0001**
Age	0.670 ± 1.16	0.20	0.53
Sex:Age Interaction	-0.388 ± 1.25	-0.30	0.76
Bandwidth of Terminal Section (Hz)			
Intercept	491.48 ± 93.89	5.23	
Sex (Male)	-194.31 ± 72.06	-2.70	**0.0024**
Age	-1.59 ± 9.84	-0.161	0.36
Sex:Age Interaction	-1.62 ± 10.45	-0.155	0.88
Count based Song Measurements (GLMM)			
	Estimate ± SE	**z value**	**p value**
Number of Introductory Notes			
Intercept	1.045 ± 0.107	9.74	
Sex (Male)	0.094 ± 0.079	1.19	0.24
Age	-0.002 ± 0.018	-0.097	0.92
Sex:Age Interaction	0.010 ± 0.019	0.52	0.60
Number of Trill Notes			
Intercept	2.032 ± 0.132	15.35	
Sex (Male)	0.164 ± 0.057	2.88	0.004
Age	-0.031 ± 0.011	-2.73	0.006
Sex:Age Interaction	0.031 ± 0.012	2.57	0.01

Figures

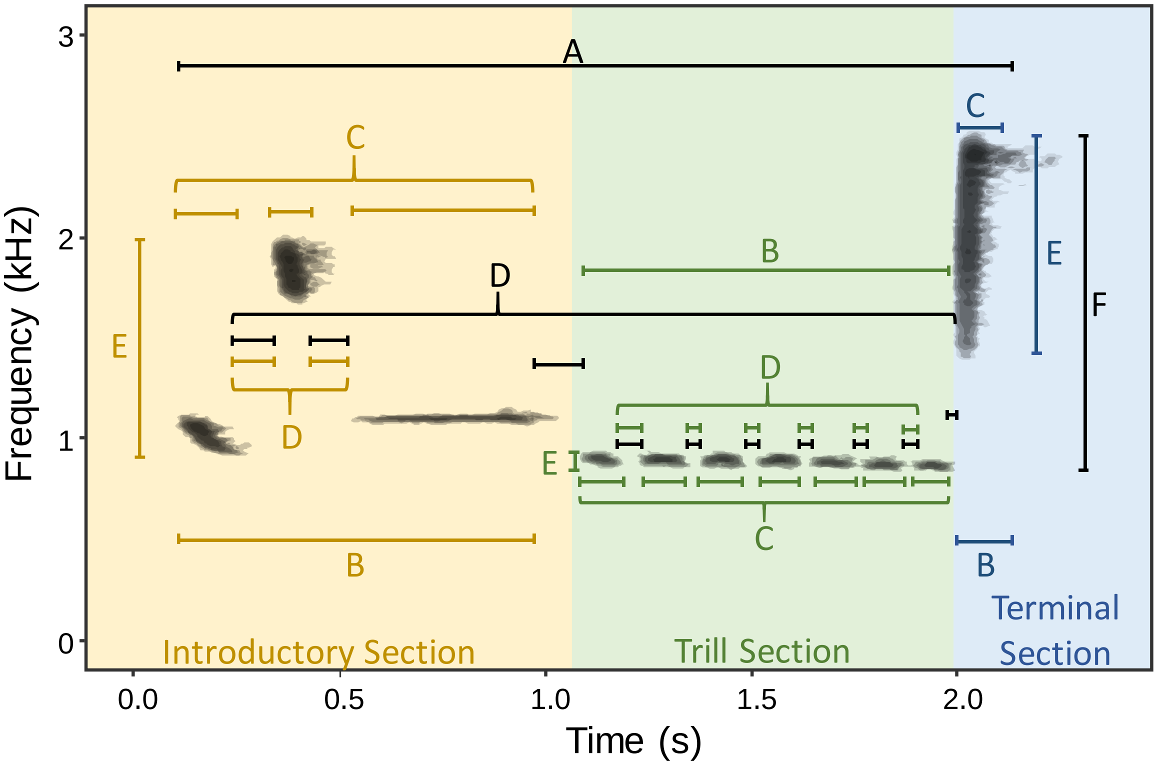

Figure 2.1: Sound spectrogram of Rufous-and-white Wren song showing the spectro-temporal measurements taken for the three sections of the song: the introductory section(yellow), trill section (green), and terminal the section (blue). Measurements included total song length (A), length of each song section (B), number of syllables for each song section (C), duty cycle for introductory and trill sections (C), element rate of introductory and trill sections (C divided by B), average pause length of introductory and trill sections (D) and the whole song (D), bandwidth and average frequency at maximum amplitude of each section (E) and of the entire song (F).

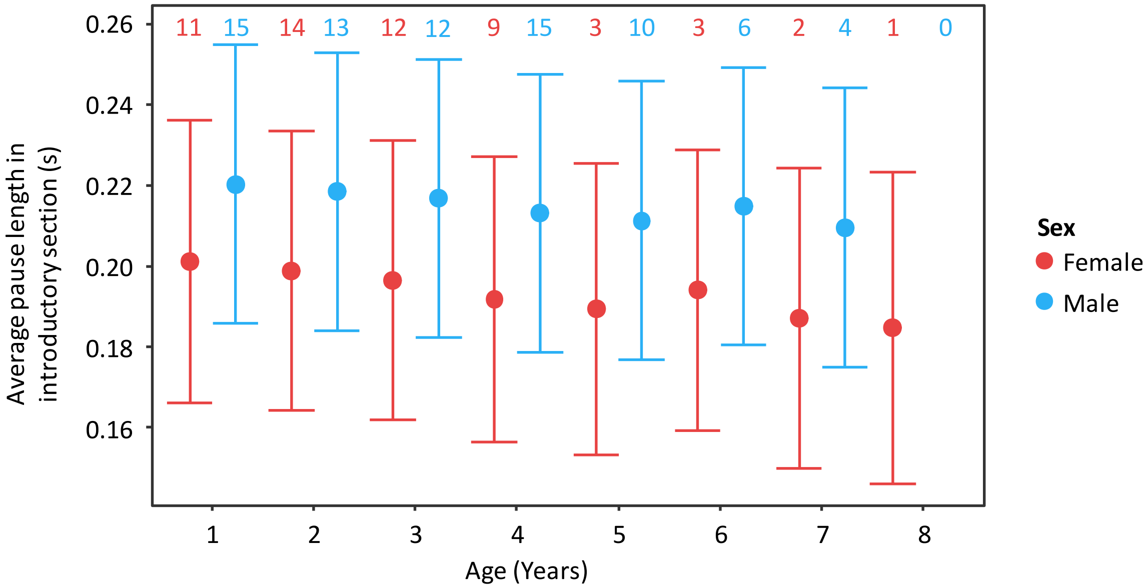

Figure 2.2: Two-way interaction bar-plot of sex and age on average pause length in the introductory section of adult Rufous-and-white Wren songs. Points represent means and error bars represent 95% confidence intervals around the mean. The number of individuals in each age class is displayed above each point.

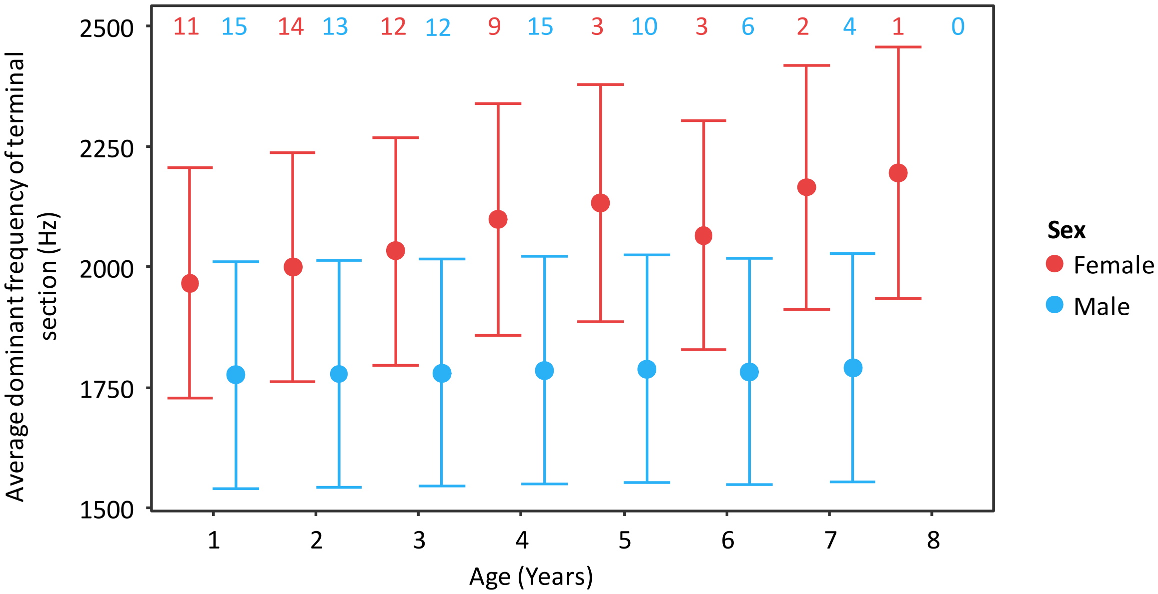

Figure 2.3: Two-way interaction bar-plot of sex and age on average dominant frequency of the terminal section of adult Rufous-and-white Wren songs. Points represent means and error bars represent 95% confidence intervals around the mean. The number of individuals in each age class is displayed above each point.

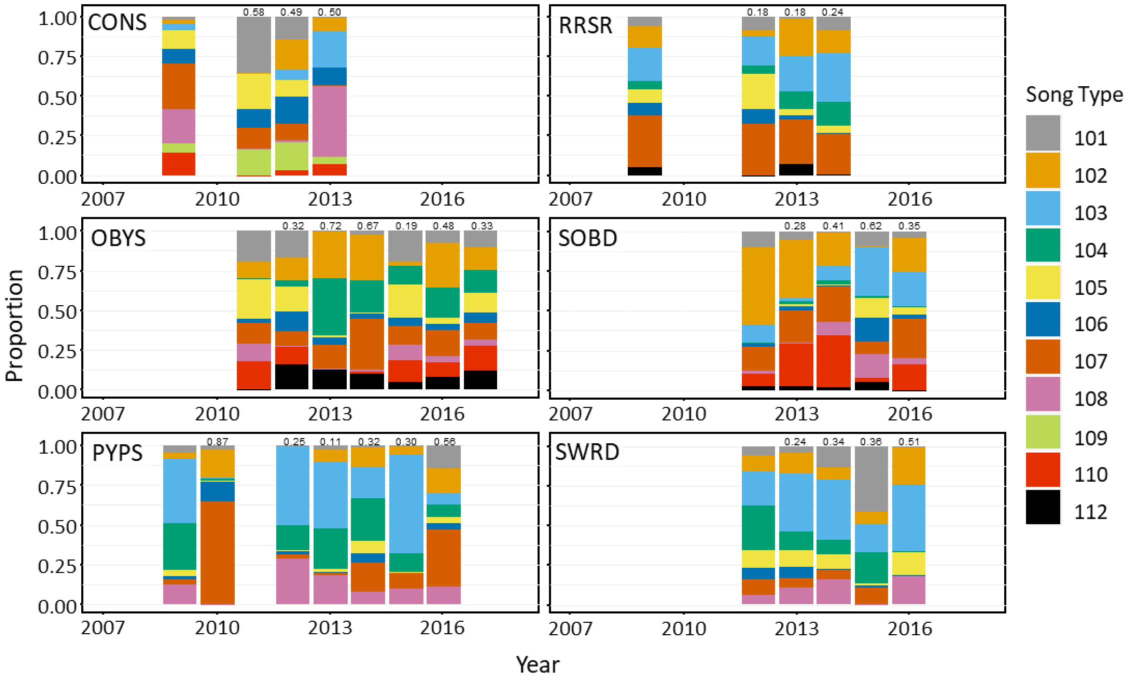

Figure 2.4: Individual per-year proportion of total song types uttered by a subset of six different male Rufous-and-white Wrens over their recorded lifetimes. Each song type is uniquely coloured. Male identification codes are in the top left of each plot. Bray-Curtis dissimilarity from first recorded year are above each bar. Scores are on a scale from 0 being identical and 1 being completely dissimilar. These six males were chosen for this figure because they had overlapping lifespans.

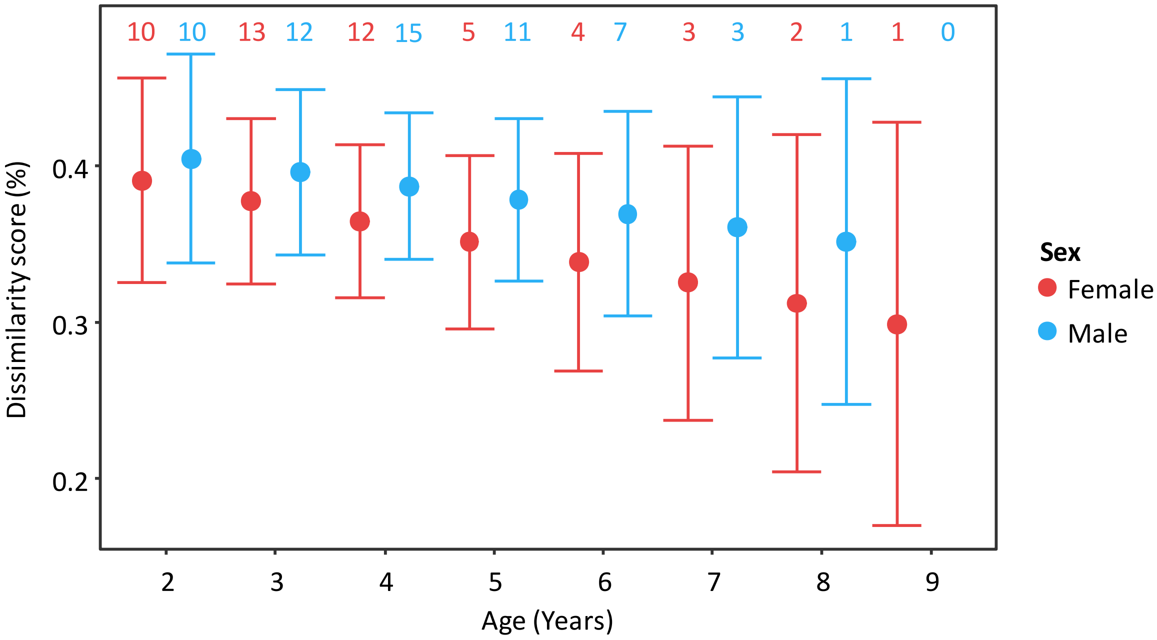

Figure 2.5: Two-way interaction bar-plot of sex and age on annual Rufous-and-white Wren repertoire-use dissimilarity. Bray-Curtis dissimilarity scores were calculated by comparing presence-absence of song types and the relative proportion that each of the song types were sung in year one, versus all other years. Points represent means and error bars represent 95% confidence intervals around the mean. The number of individuals in each age class is displayed above each point. Increasing variance in the oldest four age classes corresponds to decreasing sample sized in those age classes.

Chapter 3: General Discussion

Summary

Several decades of research on animal vocal behaviour have revealed many complexities in the communication systems of songbirds, including functional aspects of song, song learning strategies, and song delivery strategies. Yet there are still many poorly studied areas of research in this field, including the two areas that are foci of this thesis: the ecology and evolution of songs in female songbirds, and the form and function of post-crystallization ontogenetic changes in adult song. In both cases, more research is needed before syntheses can be conducted to reveal widespread patterns of sex-based variation or age-based variation in bird vocal behaviour. This is especially true when considering female song. Most of what we know about song is based on the songs of males (Catchpole & Slater 2008). Although recent years have seen an increasing interest in female song (Langmore 1998; Odom et al. 2014; Riebel et al. 2005), including an explicit call for more detailed studies across a broader taxonomic group (Odom & Benedict 2018), it is unclear if patterns observed in male songs are also present in female song. Similarly, most of what we know about the ontogeny of crystallized birdsong is limited to early in life. Only few longitudinal studies exist that attempt to describe changes in crystallized song throughout the lifetime of the singer. It is unclear if birds that show changes in their songs later in life are common, or an abnormality, just as it is unclear whether post-crystallization variation in song structure provide meaningful information in many species (Kipper & Kiefer 2010).

In the data chapter of this thesis, Chapter 2, I conducted a longitudinal analysis of two aspects of the singing behaviour of male and female Rufous-and-white Wrens over their lifetimes. Exploiting a dataset arising from one of the longest colour-marked studies of tropical songbirds, I measured variation in the songs and singing behaviour throughout the lives of the oldest 15 male and 15 female birds in our study population. I quantified both spectro-temporal features of songs, and birds' repertoire-use variation over lifetime by counting annual song-type use of each year in the birds' lives. My results suggest that Rufous-and-white Wren songs remain largely unchanged after crystallization: 16 out of 18 features failed to show age-related variation. Interestingly, two features of song did vary with age: average pause length of the introductory section of male and female songs decreased with age, and terminal syllable frequency increased in female songs only. Neither a bird's age or a bird's sex explained variation in repertoire-use patterns between years. I also describe two sex differences in Rufous-and-white Wren song: female songs are of higher frequency and bandwidth than males in all three sections of their songs. Taken together, this research shows that song structure and singing behaviour do not show pronounced age-related variation in male or female Rufous-and-white Wrens. Analyses of this sort are exceptionally rare: very few studies of tropical animals persist over such an extended timescale. Future behavioural research is needed to determine if the age differences – or lack thereof – are meaningful to the birds themselves. I outline ways this can be accomplished in the section that follows.

Future directions

The results of my longitudinal analyses reveal that there are very few changes with age in the adult lives of Rufous-and-white Wrens, although two spectro-temporal features do show subtle changes with age. To better understand whether Rufous-and-white Wrens could communicate age through their song, I believe an important next step is to conduct a series of playback experiments to test whether males and females react differently to songs based on the age and sex of the singer, and to determine whether the patterns of age-related change described in this thesis are biologically relevant.

The first proposed playback investigation would address the question: Can Rufous-and-white Wrens differentiate songs based on the age of the singer? I propose a playback experiment to test whether Rufous-and-white Wrens react differently to songs produced by older or younger individuals and the effect of sex on these reactions, using the recordings of known-age animals from the longitudinal dataset I used in this thesis. Following the experimental design of de Kort et al. (2009), this experiment would consist of exposing territorial pairs of Rufous-and-white Wrens to two trials of concurrent sound-stimuli pairs. One trial would contain a pair of male stimuli and the other would contain a pair of female stimuli. The stimuli pairs would consist of Rufous-and-white Wren songs of the same song type and from the same individual, but one stimulus would be recorded from early in the singer's life (e.g. first breeding year) and the other from later (e.g. third breeding year). Any significant differences in response of the playback subject, such as differences in latency of approach, or time spent near the speaker, would be attributed to the sex and age of the singer simulated by playback. If the features that showed significant differences in Chapter 2 of my thesis are meaningful to the birds, I predict that Rufous-and-white Wrens would respond differently to the young versus old stimuli. More specifically, I predict that female subjects would show a stronger response (i.e. quicker approach and more time spent near the speaker) to older females than younger females because if older females are of higher quality, they should be a perceived as a greater threat. I predict focal females would have a stronger response to older males than younger males because if older males are of higher quality, they should be perceived as a more desirable mate for extra-pair copulations. Similarly, I predict that males would respond stronger to older males and females because if older individuals are of higher quality, then older males should be perceived as more threatening rivals and older females should be perceived as more desirable mates for extra-pair copulations.

The second proposed playback investigation is designed to understand if the age-related changes in song I described in Chapter 2 are biologically relevant. I propose a similar playback experiment but in this experiment the stimuli pairs are Rufous-and-white Wren songs that contain artificially enhanced song features to emphasize the patterns we observed, including shortened or elongated introductory pause lengths and increased or decreased female terminal syllable frequency. These songs should be modified artificially to minimize the number of other undesired differences between stimuli, an approach that has proven fruitful in an examination of a sister taxon, the

Banded Wren (de Kort et al. 2009). One experimental treatment should have paired stimuli containing song characteristics within the natural range observed in the wrens and the other treatment should test the characteristics beyond the natural range. For example: a pair of stimuli, one with introductory pause lengths shorter than observed in the wild, and the other with pause lengths longer than observed in the wild. I predict that if shorter introductory pause lengths are a signal of age-related quality, that birds will have stronger reactions (i.e. faster approach and more time spent near the speaker) to the song-stimuli with shortened introductory pause lengths, and even stronger to those shortened beyond the natural range observed. If higher female terminal-note frequency signals age-related quality, I predict that birds will react more strongly to stimuli with artificially increased terminal-note frequency and even stronger reactions to those increased beyond the natural range observed. If there are no differences in reactions to the pairs of stimuli, then this would suggest that the patterns I described in chapter two are not biologically relevant, and unlikely to signal age-related quality changes in Rufous-and-white Wrens.

Abandoned study

The originally proposed version of this thesis included a second data chapter that focused on the influence of neighbouring Rufous-and-white Wrens on individuals' repertoire use through communication-network-based interactions. I was unable to complete that chapter within the timeline of my master's degree due to the extensive time commitment required for Chapter 2. Yet the ideas behind the abandoned chapter are relevant to the work contained in Chapter 2 and thus I think it is still valuable to discuss the abandoned data chapter for future researchers to consider.

One proposed function of song repertoires is to increase the ability of singers to match neighbours' songs (Beecher et al. 1996). This can benefit birds in two ways: first, it allows singing birds to appear more familiar to territory holders and thus decrease aggression received when prospecting new territories near territorial neighbours; second, it increases birds' ability to partake in song-type matching interactions to mediate aggressive interactions (MacDougall-Shackleton 1997). If Rufous-and-white Wren song repertoires are important for song-type matching with neighbours, then the song-type choices of individuals within a neighbourhood should both influence and be influenced by other individuals within that neighbourhood. Since Rufous-and-white Wren repertoires are individually distinctive (Harris et al. 2016; Mennill & Vehrencamp 2005), the introduction of an individual bird to the neighbourhood, either through birth or immigration, should influence the relative song-type use of the neighbourhood residents by nature of the newcomers' unique repertoire. Furthermore, immigrants (*sensu* Graham et al. 2018) provide an interesting focal subject if their repertoire use was documented prior to immigration because they might experience a complete change in communication network, composed of dissimilar-sounding individuals from other populations. I predict that these individuals' song-type choices would be most influenced by the song types of neighbours because they would experience the greatest change in communication network. Conversely, I predict that the song-type choices of pre-existing individuals of the neighbourhood would be less influenced by a single newcomer because of the comparatively small changes in their communication network.

In Chapter 2, I showed that variation in annual song-type choices were not explained by age or sex. However, my analysis did not account for interactions with, proximity to, or number of, territorial neighbours. The neighbourhood of each singer, comprised of individuals that may change from one year to the next, could influence song-type choices of a focal bird. Changes in neighbourhood composition may therefore explain some of the variation in repertoire use that sex and age failed to account for in Chapter 2. A future investigation that carefully quantifies the similarity between an animal's vocal repertoire and the vocal repertoire of its neighbours from one year to the next, would be a valuable area for further investigation.

Conclusion

I present data in this thesis that advance our understanding of female song, and post-crystallization changes in song structure of adult birds. Given that the ancestor of modern songbirds featured both male and female songs (Odom et al. 2014), then female song deserves far more research attention than it has previously been paid. I use song ontogeny as an avenue to explore female and male song at the same time because it is understudied in both sexes. The research I presented here will help future researchers determine if larger patterns of crystallized-song ontogeny exist across species and families. While Rufous-and-white Wren songs only showed little, if any, change with age, this result contributes to a growing field of knowledge on plasticity of crystallized song and female song in general.

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