RE-INVENTING AN OLD HIT: SONG EVOLUTION WITHIN AND BETWEEN POPULATIONS OF WHITE-THROATED SPARROWS

by

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ABSTRACT

Cultural evolution of birdsong occurs when songs change over generations and such changes can spread directionally or via drift within and between populations. The objective of this thesis was to document the emergence and spread of a novel song variant, the Modulated-Doublet, within and between populations of White-throated Sparrows. I analysed songs recorded in a Prince George population between 2015-2020 to document song shift within the population. I also analysed songs from public song archives to document song transition on a continental-scale between 2014-2020. I found the Modulated-Doublet started as a rare variant but became the predominant variant within our population over a very short time. At the continental scale, the Modulated-Doublet emerged simultaneously and spread synchronously in multiple breeding populations and migratory routes, replacing the previous variant in those populations. This rapid spread at both scales suggests it is driven by several transmission biases; however further investigation is needed to determine their nature.

RÉSUMÉ

L'évolution culturelle du chant des oiseaux se produit lorsque la structure ou la composition d’un chant change au fil des générations. Ces changements peuvent ensuite se propager de manière directionnelle ou aléatoire au sein d’une population, mais aussi d’une population à l’autre. L'objectif de ce mémoire est de documenter l'émergence et la propagation d'un nouveau variant de chant, le Doublet-Modulé (Modulated-Doublet) chez le bruant à gorge blanche au sein d’une population locale ainsi qu’entre populations, à l’échelle continentale. J'ai analysé les chants de mâles enregistrés dans une population de Prince George entre 2015-2020 pour documenter le changement du chant au sein de la population. J'ai également analysé des chants provenant d'archives publiques de chants pour documenter la transition à l'échelle continentale entre 2014-2020. J'ai démontré qu’au sein de la population de Prince George, le Doublet-Modulé était un variant rare en 2015, mais est devenu le variant prédominant au fil du temps. À l'échelle continentale, le Doublet-Modulé a émergé simultanément et s'est répandu de façon parallèle dans de multiples populations sur les aires de reproduction et les voies migratoires, remplaçant le variant précédent dans ces populations. Cette propagation rapide à l’échelle locale et continentale suggère que plusieurs facteurs favorisent la transmission et l’adoption du Doublet-Modulé, cependant, des recherches supplémentaires sont nécessaires pour déterminer la nature de ces facteurs.

PREFACE

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The design and execution of research protocols and data analysis were directed by S. Chartier, as such, the general introduction and the final chapter of this thesis are written in first person singular. However, the two data chapters which constitute the two independent studies of this thesis, are written in first person plural to acknowledge the contributions of collaborators.

Chapter 2: Chartier SL, Ramsay SM and Otter KA. 2021. Within-population Song Evolution in White-throated Sparrows (*Zonotrichia albicollis*). Submitted to Behavioural Ecology April 9th 2021 (BEHECO-2021-0172).

Chapter 3: Chartier SL, Ramsay SM and Otter KA. 2021. Between-population Song Evolution in White-throated Sparrows (*Zonotrichia albicollis).*

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Chapter 1: General Introduction

Songbirds (Order: Passeriformes, suborder: Passeri) use songs and calls to communicate. Calls are usually short and simple vocalizations used primarily to warn against predators (alarm calls), maintain group cohesion (contact calls) or to beg for or announce food (Marler 2004). Comparatively, songs are typically longer and more complex and used primarily by males to defend territories from other males and to attract mates (Collins 2004). Some song elements and singing behaviours can be energetically costly to produce while others can reflect the body size, experience and local adaptation of a bird. As a result, songs can be used by both males and females as indicators to assess potential of the singer as either a rival male or a potential mate. For example, during male-male conflicts, males use both song structure and how another male sings (e.g., matching or overlapping the songs of rivals) to assess the competitive ability and aggressiveness of its rival, and they use this information to escalate or de-escalate conflicts accordingly (Collins 2004; Searcy and Beecher 2009). Females will similarly assess potential mates using their song as an indicator of male quality. For example, females of several species preferentially pair with males that sing with higher song rates (i.e., sing a greater number of songs over a given period of time) (Wasserman and Cigliano 1991; Houtman 1992; Collins 2004; Nolan and Hill 2004). As song rates are often limited by relative food availability, elevated song rates can indicate which males have higher resource-holding potential; such males may be of sufficient condition to secure territories with high resource levels (Houtman 1992; Collins 2004; Murphy et al. 2008; Ritschard and Brumm 2012). Females benefit from selecting such males, as high-resource territories may provide opportunities for better parental care. Further, the genetic attributes that allowed males to attain high resource-holding potential could be transferred to their offspring (Collins 2004).

1.1. Song learning

Oscine songbirds are one of the three groups of birds that learn their vocalizations (although there is some evidence of song learning in other groups, such as suboscines) (Hultsch and Todt 2004). Repertoire size or number of songs learned and produced by a bird varies between species; some species such as White-throated Sparrows (*Zonotrichia albicollis*) typically only sing one song type, whereas species like Brown Thrashers (*Toxostoma rufum*) and Common Nightingales (*Luscinia megarhynchos*) can have repertoires of hundreds to thousands of different songs (Borror and Gunn 1965; Hultsch and Todt 2004; Beecher and Brenowitz 2005).

Juvenile birds learn these songs during a sensitive period, in which they are highly attuned to begin memorizing the songs they are exposed to (Hultsch and Todt 2004; Jarvis 2004; Beecher and Brenowitz 2005). Depending on the species, the sensitive period typically begins soon after young birds fledge from the nest and this acquisition period can vary from a few months to a year to a lifetime in certain species (Hultsch and Todt 2004; Beecher and Brenowitz 2005). Species in which song learning is age-limited are traditionally referred to as close-ended learners, and the song(s) the bird sings will typically crystallize (become fixed) by the start of their first breeding season. Other species can learn new songs throughout their lifetime, and are known as open-ended learners. However, there is growing evidence that this dichotomous classification of birds into close-ended or open-ended learners is overly simplistic - even closed-ended learners may be able to modify their songs later in life (Hultsch and Todt 2004; Beecher and Brenowitz 2005). Juvenile songbirds typically learn their songs by listening to and copying adult tutor birds; lab studies showed that deafened birds or birds raised in isolation produced abnormal songs, suggesting that songs are not completely innate and birds require tutors from which to copy in order to learn their songs (Konishi 1964; Marler and Tamura 1964; Hultsch and Todt 2004; Beecher and Brenowitz 2005). Juveniles are also predisposed to learning conspecific songs over heterospecific songs, using specific song elements as acoustic cues to preferentially learn species-typical songs (Marler and Peters 1977; 1988; Soha and Marler 2000; Hultsch and Todt 2004; Beecher and Brenowitz 2005). The degree of fidelity with which birds copy their song also varies between species; some perfectly imitate their tutors (imitation), others may copy parts of their tutors' songs with some variations (improvisation) and finally, some will learn songs that still follow the species-typical patterns, but will have no relation to the song of their tutor (innovation) (Hultsch and Todt 2004; Beecher and Brenowitz 2005).

Song development can be divided into three phases. First, young birds start by singing subsongs, which are highly variable, incomplete songs (Hultsch and Todt 2004). Juveniles practice these songs, comparing them and attempting to match them to songs memorized earlier in life (Brainard and Doupe 2000; Jarvis 2004). Subsongs eventually become more complex plastic songs, which contain more syllables and notes and have a more stereotyped structure resembling the species-typical structure of the song. Repertoire size may also increase at this stage, as individual, repeated songs become more recognizable (Hultsch and Todt 2004). Eventually, birds crystallize on individual song types and a specific repertoire; this song crystallization occurs when songs are no longer variable in their structure (Hultsch and Todt 2004). In some species, juvenile birds will initially overproduce songs, learning and producing more songs than the typical adult repertoire size (Nelson 1992; Nelson 2000). Once they have settled on their first breeding territory, their song repertoire decreases through attrition and they settle only on songs that most closely match their neighbour's (Nelson 1992; Nelson 2000; Beecher and Brenowitz

2005). Since songs are indicators of familiarity, and behaviours such as song matching are often used in male-male interactions, this selective attrition is hypothesized to facilitate counter-singing between neighbouring males (Nelson 1992; Marler 1997; Nelson 2000; Hultsch and Todt 2004; Beecher and Brenowitz 2005; Mennill et al. 2018).

1.2. The songbird brain and song learning and production

Song learning and production in songbirds are regulated by the same hormone-regulated neural network, with different clusters of neurons (i.e., brain nuclei) being responsible for each (Jarvis 2004; Beecher and Brenowitz 2005). Forebrain nuclei such as the lMAN and area X appear to play a role in song learning and development, but not song production (Bottjer et al. 1984; Scharff and Nottebohm 1991; Jarvis 2004). The higher vocal centre (HVC), another brain nucleus, connects to the muscles of the syrinx via two other brain nuclei, the robust nucleus of the arcopallium (RA) and the tracheosyringeal portion of the hypoglossal nucleus (nXIIts). Since the syrinx is the structure that produces songs in birds, the HVC, RA and nXIIts are considered to be some of the main brain nuclei responsible for song production (Nottebohm et al. 1976; Jarvis 2004). Brain nuclei are sequentially activated and gene activity changes as the bird progresses from song memorization to song production and crystallization and as its brain develops (Clayton 1997). For example, gene expression of several molecules such as NO synthase and synelfin increases early during song learning in some brain nuclei and early-phase genes are believed to play a role either in song memorization or in brain development (Clayton 1997; Jin and Clayton 1997). As song development progresses, these early gene products decrease significantly while other brain nuclei are activated and the expression of different genes increases; these gene products, in turn, are believed to be linked to song production and crystallization (Clayton 1997).

1.3. Song variation over time and space

Songs are culturally transmitted traits; most juvenile songbirds learn their song by imitating adult tutor birds and songs are passed from one generation to the next. As a result, juveniles tend to adopt songs that are similar to neighbouring birds from which they learned their songs (Kroodsma 2004; Podos and Warren 2007). This can lead to the emergence of regional song dialects as birds converge on local song variants within a population. Alternatively, local dialects can also emerge and be maintained if new males preferentially adopt songs resembling their neighbour's, even if they were originally born in (and exposed to) songs from other regions but dispersed to novel areas during their first breeding season (Kroodsma 2004; Beecher and Brenowitz 2005; Podos and Warren 2007). A well-studied example of regional song variants occurs in White-crowned Sparrow (*Zonotrichia leucophrys*) populations along the west coast of North America. Populations of both sedentary and migratory subspecies have clear, distinct song dialects; song patterns are constant within populations but differ significantly from other populations, with differences between populations mainly found in the note complexes and terminal trills of the song [e.g., Marin County (CA) – Marler et Tamura 1962, Puget sound (WA & BC) – Baptista 1977, Sierra Nevada (CA) – Orejuela and Morton 1975].

Regional song variants can change in structure and note composition and can evolve over time through cultural evolution (Podos and Warren 2007; Aplin 2019; Whiten 2019). Novel variants can emerge in an individual as a result of copying errors or innovations during song learning. If that individual is used as a tutor, these variants can then be adopted by others and spread within populations (Podos and Warren 2007; Aplin 2019). These song transitions or shifts can also occur between populations if birds emigrate between populations, or integrate songs from tutors in locations shared between different breeding populations (e.g., wintering grounds, migration stopovers) (Owen et al. 2019; Otter et al. 2020). Evolution of regional song variants can occur in a drift-like pattern or can be driven by selective forces (Boyd and Richerson 1985; Trainer 1989; Byers et al. 2010; Whiten 2019).

Shifts can occur via unbiased transmission if novel variants are functionally equal and provide no fitness advantage or disadvantage to the singer (Boyd and Richerson 1985, Podos and Warren 2007, Byers et al. 2010; Whiten 2019). This type of transmission results in a random and drift-like pattern of emergence and spread of novel variants and is often observed in small and isolated populations. Due to the small number of adult tutor birds present, juveniles have a higher probability of being exposed to a tutor singing the novel variant and adopt it by chance alone (Podos and Warren 2007).

Song evolution, both within and between populations, can also be directional if driven by selective pressures or transmission biases that favour males adopting a particular song variant over another. If the advantage is biased towards the locally-common song variant, this can result in song variants remaining stable over time and space. However, if selection favours novel variants, it could result in rapid turnover of the locally-common song following the emergence of novel variants. Boyd and Richerson (1985) identified three general classes of biased transmission that result in the transmission of culture in humans, and these have since also been used and adapted to explain the transmission and evolution of song variants as they also reflect learning biases in songbirds (e.g., Trainer 1989, Byers et al. 2010).

The first class of bias is a "direct bias", where an individual learns a cultural variant because the variant itself is more attractive than other variants (Boyd and Richerson 1985). In birds, these occur as "content-based" biases, where the structure or syntax of particular variants invoke a learning preference, leading males to preferentially adopt these variants (Byers et al 2010). This phenomenon is observed during song learning; juveniles of many species tend to adopt songs containing species-specific elements or structures over songs missing these elements (Marler and Peters 1977; 1988; Soha and Marler 2000). Some lab studies also showed that birds could still produce subsongs containing species-specific elements even when raised without song tutors (e.g., Marler and Tamura 1964; Marler and Sherman 1983, Shackleton and Ratcliffe 1993). Together, this suggests the existence of an innate species-specific template and learning bias favouring the adoption of species-specific elements.

The second class of transmission biases is an "indirect" bias or model-based transmission bias, where individuals will learn the cultural variant displayed by the most successful individuals, using the success of an individual as a proxy to determine which variant would be more advantageous to adopt (Boyd and Richerson 1985). In songbirds, this would mean that males would preferentially learn song variants sung by males that are more dominant or reproductively successful. In some populations, model-based biased transmission can lead to rapid turn-overs of song variants, especially in species where song is an indicator of dominance. In such cases, the song variant upon which males converge would depend on which individual is dominant at the time. Additionally, as the novel variant spreads and becomes common, it would no longer be a good indicator of which male is more successful. As a result, you would expect novel variants to emerge frequently, spread for some time, but themselves be replaced by other novel variants before they spread to ubiquity (Trainer 1989; Noad et al. 2000; Podos and Warren 2007; Garland et al. 2011). This has been suggested as the mechanism behind the rapid song changes observed in populations of Yellow-rumped Caciques (*Cacicus cela*) in Panama. Most songs changed from one breeding season to the next such that they were not recognizable as a song type from the previous year and males all adopted the same changes (Trainer 1989). Females often use songs as indicators of male quality (Collins 2004) and consequently, female preference for certain songs and song elements could also drive model-based transmission bias. For example, it is hypothesized that in some populations, females prefer males singing familiar or local songs if this indicates they carry locally-adapted genes. Under the local adaptation hypothesis, males learn songs of their natal region, and also return to these regions to breed – singing the local song variant would honestly indicate you possess any genes associated with local adaptations (Podos and Warren 2007). This would reinforce local dialects to become fixed over time. Similarly, model-based transmission bias could also favour the stability of a variant within a geographic area even if not associated with fixation of locally-adapted genes. If males crystallize on local song variants post-settlement, then these variants can become stable over time even if new males adopting them hatched and were reared in different areas. This type of convergence on the local song of established neighbours may occur if it facilitates better ability to counter-sing with local males (Beecher and Brenowitz 2005), and is predicted under the social facilitation hypothesis of dialect formation (Podos and Warren 2007). Both means of converging on local song variants can be driven by a model-based biased transmission mechanism.

The last class of transmission bias is a frequency-dependent bias, where individuals will preferentially learn the most common variant in the population (Boyd and Richerson 1985; Byers et al 2010). For male songbirds, it can be advantageous to learn the most common variant to facilitate counter-singing interactions (Marler 1997; Nelson 2000; Hultsch and Todt 2004; Beecher and Brenowitz 2005; Searcy and Beecher 2009; Mennill et al. 2018) as predicted by the social facilitation hypothesis of dialect formation (Podos and Warren 2007; Byers et al. 2010).

1.3.1. Song variation in small and isolated populations

Size and continuity of a population can affect song variation within population. Variation and song complexity may decrease for example, in isolated island populations where founder effects are hypothesized to cause a decrease in the number of song and syllable types in comparison to mainland populations. (e.g., Baker 1996). However, in other cases, small isolated populations display more variation in song than large contiguous populations (e.g., Kroodsma et al. 1999; Gammon 2007). Within large, contiguous populations, juveniles are under more selective pressure to conform and perfectly match the common song in order to counter-sing more effectively with neighbours (Kroodsma et al. 1999; Gammon 2007). However, in smaller and isolated populations, juveniles may have fewer neighbours or encounter fewer birds during their song learning and crystallization phase. As a result, mechanisms that would normally facilitate conformity are missing, increasing the chances of juveniles settling on songs with more novel elements (Kroodsma et al. 1999; Gammon 2007). If these peripheral populations also have low immigration/emigration from the main populations, songs of isolated populations may diverge away from common songs in the main population over time (Kroodsma et al. 1999). This has been documented in populations of Black-capped Chickadees (*Poecile atricapillus*) living in isolated habitat patches in Colorado. There is a median of two males living in each patch and these males exhibit more variation both within their own songs and between songs of different individuals than compared to contiguous populations across the rest of the species' range (Gammon 2007). Similar phenomenon is observed in peripheral and isolated populations of Black-capped Chickadee on Nantucket Island (Massachusetts) and in habitat patches in Oregon and Washington States – as chickadees are non-migratory and short-distance dispersers, individuals from these spatially isolated populations seldom disperse into the larger contiguous populations of Black-capped Chickadees across the northern US and Canada (Kroodsma et al. 1999). Additionally, small populations can also facilitate the spread of novelty between individuals. Juveniles have a higher chance of encountering adult tutor birds singing novel variants and using them as tutors. In comparison, an adult bird singing a rare or novel variant in a large population might get drowned out, decreasing the chances of juveniles copying these songs (Podos and Warren 2007).

Song acquisition and the spread of novel song variants within White-throated Sparrows (*Zonotrichia albicollis*) have been the focus of several recent studies (Ramsay and Otter 2015; Zimmerman et al. 2016; Otter et al. 2020). Rather than males conforming to local song variants, these studies show that novel song variants emerge and spread among males both within and between populations. The objectives of my thesis were to document the spread of a novel variant, to examine the mechanisms that may facilitate song shifts in this species, and to investigate whether selective pressures reinforce these shifts. To begin, I first turn to describing this species and the context in which I will investigate these ideas.

1.4. Study species and study system.

1.4.1. Study species

White-throated Sparrows (*Zonotrichia albicollis*) are small songbirds that breed throughout the boreal forest of North America. There are two plumage morphs found in White-throated Sparrows, which occur in both males and females (Falls and Kopachena 2020). The two distinct morphs result from a chromosomal inversion; individuals that possess two recessive, non-inverted chromosomes are tan-striped while heterogeneous individuals are white-striped (Throneycroft 1975). White-striped birds have contrasting white and black striped crowns with bright yellow lores, a bright white throat patch and a uniformly grey chest (Figure 1.1.a). Tan-striped birds are overall duller than white-striped birds; they have brown and grey striped crowns with duller, sometimes completely absent, yellow lores, duller throat patches and brown streaks on the chest (Figure 1.1.b) (Falls and Kopachena 2020). Plumage morphs are also associated with differences in behaviour; white-striped birds tend to be more dominant and aggressive than tan-striped birds (Falls and Kopachena 1993). Additionally, white-striped males tend to sing more and provide less parental care than tan-striped males (Knapton and Falls 1983). White-throated Sparrows engage in negative assortative mating where breeding pairs typically consist of one tan-striped individual and one white-striped individual (98.2% of breeding pairs surveyed - Hedrick et al. 2018, 92.9 % of breeding pairs surveyed - Throneycroft 1975, 98% of breeding pairs surveyed - Knapton and Falls 1983.).

Figure 1. 1 There are two distinct plumage morphs in White-throated Sparrows; a - White-striped morph (Photo Credit: Scott Ramsay 2017) and b - Tan-striped morph (Photo Credit: John Benson 2015).

White-throated sparrows breed throughout the boreal forests of Canada, predominately East of the Rocky Mountains and in the north-eastern and great lakes area of the United States (Falls and Kopachena 2020). Starting in the 1940s, a localized, breeding population established itself west of the Rocky Mountains, in central British Columbia (Munro 1949) and has been expanding to the southwest over the past half century (Falls and Kopachena 2020). Males arrive on breeding grounds starting in late-March (in the southernmost part of their breeding range) to mid-May (in the northernmost parts). Females typically arrive one to two weeks after the males, and pair formation occurs within a few days of female arrival. Nest building starts within two weeks of females arriving and usually takes between two to six days, and females lay their first egg usually at completion of the nest. Females start incubating after the last egg is laid and incubate 11 to 14 days before eggs start to hatch. Nestlings stay in the nest for seven to 12 days, and parents will care for the fledglings for at least an additional two weeks. White-throated Sparrows may also have a second brood, particularly in the southern parts of the breeding range where spring arrival dates are earlier (Falls and Kopachena 2020). Birds leave breeding grounds starting in late-August/September in the northernmost part of their breeding range to November in the southernmost parts and arrive on wintering grounds from mid-September to November (Falls and Kopachena 2020; *pers. comm. KA Otter*). Birds breeding in the Prairies and north-western Ontario overwinter in the south-central and south-eastern United States and birds breeding in eastern Canada overwinter in the south-eastern US and along the central- and southern-Atlantic coast (Mazerolle et al. 2005; Falls and Kopachena 2020). Birds breeding in British Columbia overwinter both in California and in the south-central United States (Otter et al. 2020).

1.4.2. White-throated Sparrow Song

1.4.2.1. Song structure – Introductory phrase

White-throated Sparrows typically sing a single song type consisting of two parts – an introductory phrase and an ending phrase of repeating strophes (Figure 1.2). Songs are comprised of whistled pure-tone notes ranging in pitch from 1.5 to 6.6 kHz (Borror and Gunn 1965; Lemon and Harris 1974; Falls and Kopachena 2020). There are four main types of introductory phrases which are based on the direction of change in pitch from one note to the next. Birds typically sing an introductory phrase of three whistled notes that are either: ascending (Ascending Intro - Figure 1.3. a); descending (Descending Intro – Figure 1.3. b); constant in pitch (Monotone Intro – Figure 1.3. c); or, increase in pitch from the first note to the second note and decrease from the second note to the subsequent notes (Up-Down Intro – Figure 1.3. d) (Lemon and Harris 1974, *pers. obs. S.Chartier*). The number and type of notes in the introductory phrase as well as the absolute pitch of the song varies between individuals within populations but introductory phrases do not appear to vary based on geographical location or population (Borror and Gunn 1965; Lemon and Harris 1974). Most birds sing only one introductory phrase type, but some can learn and sing up to two types (Borror and Gunn 1965;

Lemon and Harris 1974; *pers. obs. S.Chartier*). The introductory phrase has been hypothesized to play a role in both individual and species recognition. Males have been shown to respond to playbacks containing only introductory phrases suggesting that this phrase alone contains enough information for species identification (Brooks and Falls 1975). The presence of pure whistle tones and the change in pitch (i.e., descending, ascending, constant or ascending/descending) are suggested to be used in species recognition as they are common structural components found in every individual (Brooks and Falls 1975). Similarly, pitch ratios or the ratio of the highest frequency to the lowest frequency during the major pitch change of the introductory phrase could also play a role in species recognition. Hurly et al. (1991) showed that pitch ratio between the first and second introductory note in ascending songs and the second and third note in descending songs was constant between individuals and that males responded more strongly to playbacks of ascending songs with normal pitch ratios than playbacks with altered pitch ratios (Hurly et al. 1990; Hurly et al. 1992). Absolute pitch of the song, however, has been suggested to play a role in individual recognition as opposed to species recognition, as it varies between individuals (Brooks and Falls 1975; Hurly et al. 1991).

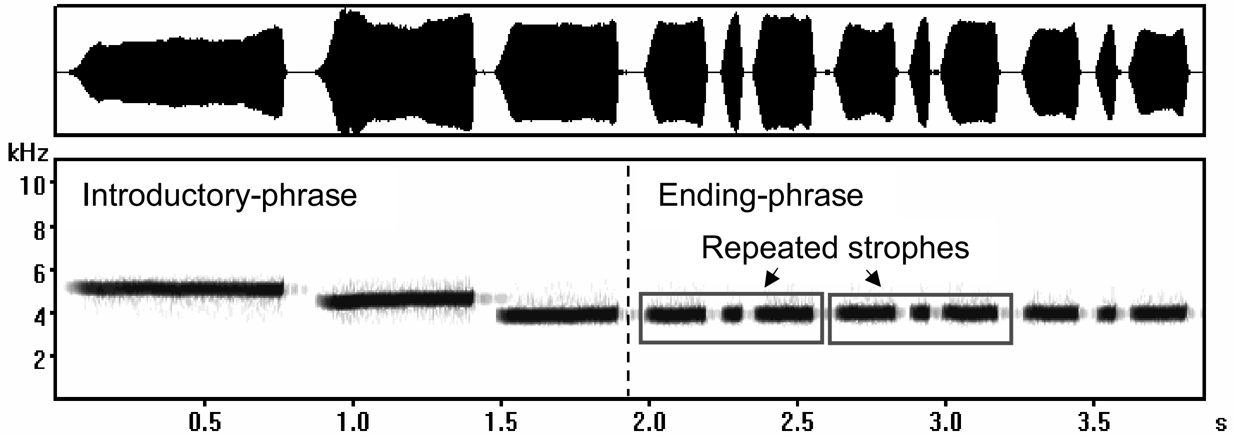

Figure 1. 2 General structure of a White-throated Sparrow song. Song is composed of an introductory phrase of whistled notes followed by an ending phrase of repeating strophes

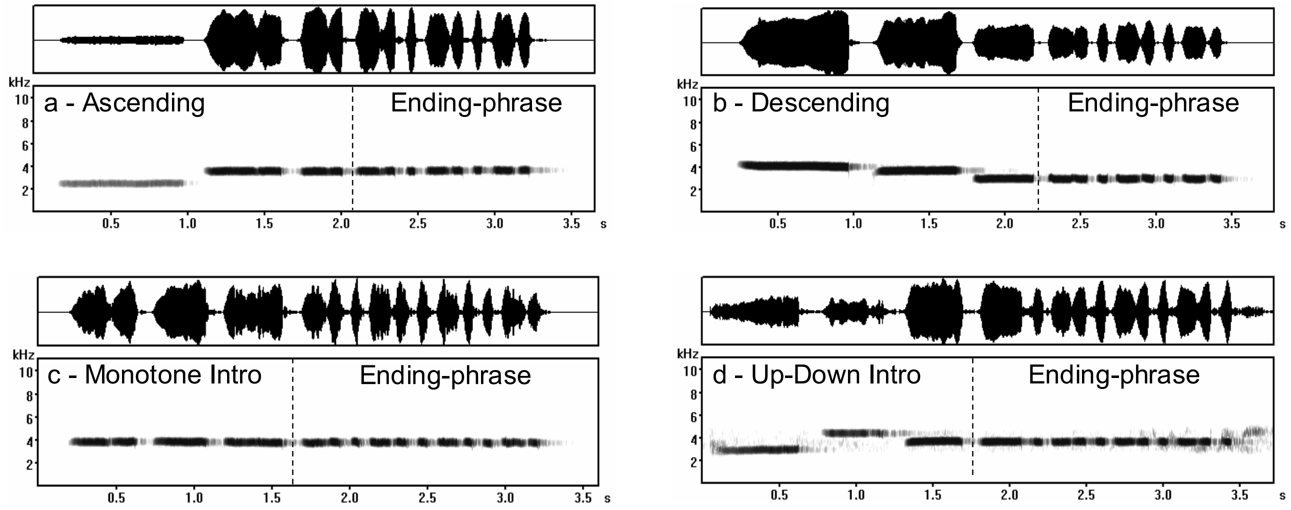

Figure 1. 3 Four main types of introductory phrases found in White-throated Sparrows. Introductory phrases vary in the direction of pitch change from ascending (a), descending (b), monotone (c) to a combination of ascending/descending notes (d).

1.4.2.2. Song structure – Ending-phrase

The ending-phrase, composed of strophes (repeating phrases of the same note pattern), is less variable with regards to note composition between individuals than the introductory phrase (Borror and Gunn 1965). There is, however, evidence showing that the ending-phrase changes through cultural evolution, with most males within a population converging to a single variant. Prior to 1990, White-throated Sparrows typically sang songs ending in repeating strophes of three notes or **Triplet-ending songs** throughout their range (Figure 1.4. a) (Borror and Gunn 1965). During this initial continent-wide survey, Borror and Gunn (1965) also noted a song variant consisting of a two-note repeating phrase (**Doublet-ending song**), but this variant was rare (less than 2% of recorded songs) and widely scattered across the continent. Some time prior to 1999, though, this Doublet-ending song variant spread within the small peripheral population in central British Columbia (BC) and became the sole local song variant (Figure 1.4. b; Ramsay and Otter 2015; Otter et al. 2020). Following its emergence in central BC however, the Doublet-ending song subsequently spread eastward across Canada and has been recorded as far as Québec in 2019, replacing the Triplet-ending variant as it spread (Otter et al. 2020). The ending-phrase has been hypothesized to play a role in sexual selection. Females have been shown to prefer males singing songs with ending-phrases as opposed to males singing songs with only introductory notes, suggesting females pay attention to the ending phrase of a song (Wasserman and Cigliano 1991). Conversely, males react similarly to playbacks of songs with and without ending phrases, as well as to playback songs with different ending-phrase regardless of the predominant variant in their population (Brooks and Falls 1975; Ramsay and Otter 2015), suggesting the ending-phrase type does not influence male-male interactions. As a result, Otter et al. (2020) suggested that if female sparrows are attentive to ending-phrases and show preference for certain type of endings, males would benefit from adopting these variants. This would suggest the large-scale transition in song in this species may be driven by an indirect transmission bias, where males preferentially copy or converge upon the songs of the males that receive the most female attention.

1.4.3. Study System

More recently, a second novel variant has emerged – a doublet-ending song with a pronounced amplitude modulation in the first note (S1) of the repeating two-note strophe (Figure 1.4. c). This **Modulated-Doublet** was first recorded in 2015 in our localized population in central British Columbia and has been spreading locally since then. The amplitude modulation in the S1 note of the doublet creates an audible pulse, giving the impression that the S1 note is divided into two short, rapid notes. For clarity, we will refer to the original Doublet-ending songs that lack this modulation as the "Unmodulated-Doublets" to distinguish them from the Modulated-Doublet songs. The objective of this study was to document the emergence and spread of this novel variant both within and between populations. More specifically, the objective of Chapter 2 was to characterize the Modulated-Doublet and how it differs from the Unmodulated-Doublet and to determine whether the Modulated-Doublet was spreading within a local population over time. Further, we examined whether the modulation was becoming more pronounced (i.e., a greater decrease in volume relative to the peak amplitude of the S1 note) between years. Finally, we investigated whether individual birds showed an increased amplitude modulation between years, to understand whether juveniles and/or adults drive song shifts in White-throated Sparrows. The objective of Chapter 3 was to determine whether the Modulated-Doublet was spreading between populations, over a continental scale and to determine the origin and the patterns of its spread. Otter et al. (2020) have previously documented the emergence and spread of the Unmodulated-Doublet in White-throated Sparrows, on both a population and a continental scale over a 20-year period. However, sampling was not continuous during the 20-year period, and therefore, some information such as the origin, rate and pattern of spread are not complete. The emergence of a novel variant, the Modulated-Doublet, around 2015 in central British Columbia, is an opportunity to follow a novel variant from the first few years following its emergence to complete spread within a local population (Chapter 2) and its initial spread on a larger, continental scale (Chapter 3). White-throated Sparrows are a particularly good species to observe this kind of phenomenon, as they tend to only have one song type, and the ending phrase varies little both within and between populations. As a result, if a novel song type starts spreading, it would completely replace the previous song type.

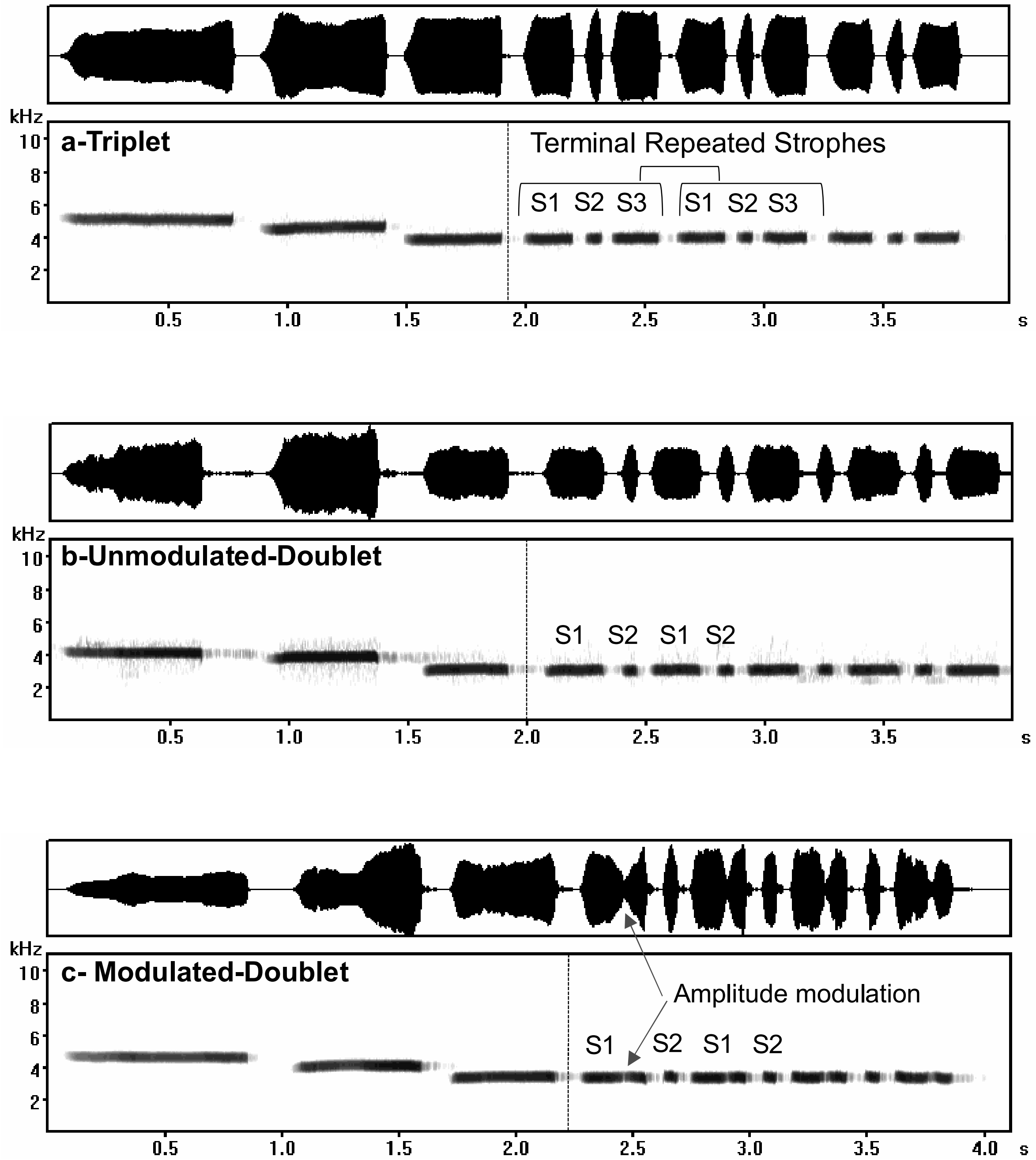

Figure 1. 4 Typical song variants in White-throated Sparrows. Songs start with an introductory phrase of whistled notes followed by terminal repeated strophes. The Triplet (a) ends in terminal repeated strophes of three notes, the Unmodulated-Doublet (b) ends in repeated strophes of two, unmodulated notes and the Modulated-Doublet (c) ends in repeated strophes of two notes, with an amplitude modulation in the S1 note.

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Chapter 2: Within-population song evolution in White-throated Sparrows (*Zonotrichia albicollis*).

2.1. Abstract

Cultural evolution in birdsong occurs as a result of innovation or copying errors during song learning and results in individuals singing novel songs. These novel variants can spread within populations if there is a predisposition (i.e., a transmission bias) for males to adopt the novel song. We describe the emergence, spread and evolution of a novel song variant within a localized population of White-throated Sparrows (*Zonotrichia albicollis*) in Prince George, British Columbia. Birds in this population have been known to sing a whistled song ending in repeated strophes of two solid notes (doublet-ending song) since the early 2000s. In 2015, we documented the first detection of a novel song variant, the Modulated-Doublet, which consists of a doublet-ending song with a pronounced amplitude modulation in the middle of the first repeating terminal note. We banded and recorded birds from 2015 to 2020 and classified songs using an audio test and spectrographic analysis. The proportion of birds singing the Modulated-Doublet increased over time while the proportion singing the Unmodulated-Doublet decreased. Additionally, the modulation in the first repeating terminal note became more pronounced over time. Individual males also seemed to be modifying their song, becoming more modulated over successive years. The spread of the Modulated-Doublet mimics previously recorded song transitions (from triplet-ending to doublet-ending song) within other populations of White-throated Sparrows, however this song shift appears to be occurring at a faster rate, suggesting that it may be driven by several transmission biases.

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2.2. Introduction

In most oscine songbirds, juveniles learn their songs during their first year, by listening to and copying adult tutor birds. This cultural transmission of song can lead to the emergence of local dialects or song variants if birds either return to natal areas to breed, or preferentially adopt songs resembling their neighbour's if they disperse to new areas during their first breeding season (Kroodsma 2004; Beecher and Brenowitz 2005; Podos and Warren 2007). These regional song variants can change over time through cultural evolution; novel innovations or errors in copying tutors can create new variants in individuals, which are then spread to other individuals who also used the same tutors (Gammon 2007; Podos and Warren 2007). This can result in shifts over time in common songs within populations. It could also result in shifts between populations if birds emigrate between populations, or integrate songs from tutors in locations shared between different breeding populations (e.g., wintering grounds, migration stopovers). This can lead to song spread within or between populations via drift, or could also lead to directional shifts in song if selection pressures favour the adoption of some variants over others (Trainer 1989; Noad et al. 2000; Podos and Warren 2007; Byers et al. 2010; Garland et al. 2011; Owen et al. 2019; Whiten 2019; Otter et al. 2020). Such song transitions have been documented in both bird (Trainer 1989; Byers et al. 2010; Janes and Ryker 2013; Otter et al. 2020) and whale song (Noad et al. 2000; Garland et al. 2011).

The transition of a population to a novel song variant depends on the timing of song acquisition and selection in juvenile birds. Lab and field studies on sparrows suggest that some juvenile birds memorize multiple songs during their first natal summer and overproduce songs when initially returning from migration for their first breeding season. As the breeding season progresses, repertoire size decreases as birds crystallize on songs that most closely match their neighbour's; this selective attrition of song types to those shared or similar to neighbours likely facilitates counter-singing interactions (Nelson 1992; Marler 1997; Nelson 2000; Collins 2004; Beecher and Brenowitz 2005; Mennill et al. 2018). In the context of song transition, song overproduction followed by selective attrition would result in an initial slow rate of transition, since a novel song would rarely be reinforced initially when first-year birds return to breeding grounds in spring, as few established birds would be expected to sing the novel variant. As the proportion of birds singing the novel variant increases, first-year birds have a higher chance of being re-exposed to the novel variant and adopting it during the selective attrition process; transition to the novel variant then accelerates, becoming exponential once a certain threshold of birds singing the novel variant is reached (Mennill et al. 2018; Otter et al. 2020).

Cultural evolution of song on a continent-wide scale has been documented in White-throated Sparrows (*Zonotrichia albicollis*). White-throated Sparrow song typically begins with an introductory phrase of whistled notes followed by an ending phrase composed of repeating strophes of three notes (Triplet-ending song; see Chapter 1, Figure 1.4. a.). Prior to 2000, a novel variant, the Unmodulated-Doublet song, emerged in a localized population in central British Columbia, and is composed of the same introductory phrase but followed by repeating terminal strophes of two notes (Figure 1.4. b.). The Unmodulated-Doublet variant has since spread throughout the breeding population east of the Rocky Mountains, replacing the Triplet-ending song as far as Quebec (Ramsay and Otter 2015, Zimmerman et al. 2016, Otter et al. 2020). Otter et al. (2020) showed that within one eastern population of sparrows (Algonquin Park, Ontario), the Unmodulated-Doublet remained rare (sung by less than 5% of the population) for four years after first being recorded in 2005, followed by a slow increase in proportion to 22% in 2014. From 2014-2017, however, the doublet-ending variant rapidly spread to 50% of the population. The initial slow transition followed by an exponential jump, suggests that the Unmodulated-Doublet song needed to reach a certain threshold in a population in order for the population to rapidly and completely transition. In White-throated Sparrows, eastern juvenile birds were most likely exposed to tutors singing the Unmodulated-Doublet songs on wintering grounds shared with western populations but may only have started to adopt the doublet-ending variant in larger numbers if they also heard it reinforced upon returning to their breeding ground (Otter et al. 2020).

Recently, a modified version of the Doublet-ending song has emerged in western populations, creating an audibly-distinct third variant, the Modulated-Doublet. The Modulated-Doublet was first documented in 2015 in Prince George, BC and has been spreading locally. Like other doublet-ending songs, the Modulated-Doublet ends in repeating strophes of two notes, however there is a noticeable decrease in amplitude in the middle of the first repeated note followed by an increase in amplitude back to the original volume (i.e., an amplitude modulation in the S1 note, Figure 1.4. c.). This modulation is lacking in the originally-documented doublet-ending songs (Borror and Gunn 1965, Ramsay and Otter 2015). The objective of this study was to document the emergence and spread of the Modulated-Doublet in a localized population of White-throated Sparrows in central British Columbia. We also compared the pattern of this shift with the previous shift from Triplets to Unmodulated-Doublets documented by Otter et al. (2020). Specifically, we examined whether the proportion of birds singing the Modulated-Doublet song has increased. We looked at the rate of spread to see if there was a pattern of initial slow transmission of the Modulated-Doublet song followed by a rapid transition once a certain threshold was reached. Finally, we also investigated whether individual birds showed increased modulation of the amplitude of their S1 over time.

2.3. Methods

All protocols involving the use of birds were carried out in accordance with the Canadian Council on Animal Care standards and approved by the Animal Care and Use Committee of the University of Northern British Columbia (UNBC Protocols, 2013-01, 2017-03 and 2020-04) and all bird banding was done under CWS Master Banding Permit #10940.

2.3.1. Recordings

The main study site was located in the southern part of Prince George, British Columbia (53.842581°, -122.763964°). The site covered approximately 170 ha of vegetation surrounding a decommissioned open-pit gravel mine, and was bisected by a forest service road and large power transmission line. The vegetation was composed predominantly of young trembling aspen (*Populus tremuloides*) groves, lodgepole pine (*Pinus contorta*), hybrid white spruce (*Picea engelmannii* × *glauca*) and shrubby edge habitat composed mainly of willows (*Salix spp*) surrounding numerous small open meadow patches. We also recorded and banded birds within a 1 km radius of the campus of the University of Northern British Columbia (UNBC). Habitat around the University was composed predominantly of mature Douglas-fir (*Pseudotsuga menziesii*) and trembling aspen forest. White-throated Sparrows occurred along the transition of these mature forests and the land cleared for the university grounds. This study includes recordings of White-throated Sparrows from these two sites beginning in 2015. In 2016, we first noticed a male singing the Modulated-Doublet. A small number of novel song variants emerge each year in our study population, but we noticed the proportion of males singing this variant appeared to increase in 2017/2018, so we increased sampling effort in 2019/2020 to document the transition. We also included recordings made in 2015 as part of another study, to see if males were already singing the Modulated-Doublet prior to being first noticed in 2016. In 2015, White-throated Sparrows were recorded but not marked; however, from 2016 to 2020, sparrows were both recorded and colour banded. We recorded and banded birds each year following their initial return from migration (ranging from 7th to 29th May each year) through territory establishment and pairing (1st – 8th June). Each year, we surveyed for singing males and used playbacks to induce singing if males were not singing spontaneously. For each bird, we recorded ten complete songs - i.e., songs containing both the introductory and ending phrases - noting the time, location, band combination and song introductory phrase type. We used songs recorded from colour-banded birds to compare songs of individual males across years. All 2019 and 2020 recordings were made using Marantz PMD611MKIII recorders and Sennheiser ME67 microphones. Historical recordings (2015-2018) were made with Marantz PMD661 and Zoom H4n Pro recorders and Sennheiser ME67 and Audio Technica ATB815 microphones. All songs were recorded at 16bit/44.1kHz sampling frequency.

2.3.2. Song Analysis

For each bird, we selected up to five songs to measure. We selected songs with: the best signal to noise ratios; the least amount of background noise; and, which were not overlapped by other birds or miscellaneous noises. If all ten songs recorded from an individual were of equal quality, we selected every other song. We excluded birds that did not sing an ending phrase and any birds that did not have any clear recordings for analysis. Using Avisoft SASLab Pro v.5.2.13 (Specht

2019), we filtered out sounds above and below the song's maximum and minimum frequency and standardized the volume of every song so that the peak amplitude of the first S1 note was -20 dB (+/- 2dB). We then used the bound reticule cursor and the sound envelope curve feature in Avisoft SASLab Pro (Specht 2019) to measure amplitude of the waveform. For each song, we measured the lowest amplitude (trough) relative to the peak amplitudes before and after the trough for the first S1 note (Figure 2.1.) using the waveform to select where in the note these high and low points occurred. We then averaged the two peaks and calculated the peak-to-trough ratio for the song ($dB_{trough}/dB_{average\ peak}$). If the S1 note did not have a modulation, we measured the amplitude at the beginning, end, and at the two-thirds mark of the first S1 note. The two-thirds point was chosen, as this is approximately the location of the trough in the S1 note for songs that contained some form of modulation. For each individual, we repeated the same procedure for the remaining songs and calculated the overall average peak-to-trough ratio across all songs for that individual. For some individuals, the first S1 note was not modulated or was less modulated than the following S1 notes (see Figure 2.2. for example). In cases where the first S1 note was not representative of the overall song, we standardized the volume of the second S1 note and measured the peak-to-trough ratio of that note. We then averaged the ratios of the first and second S1 notes to obtain the amplitude ratio for that individual.

We recorded a total of 39 birds in 2015, 24 in 2016, 16 in 2017, 23 in 2018, 70 in 2019 and 103 in 2020. We only included complete male songs in the analysis.

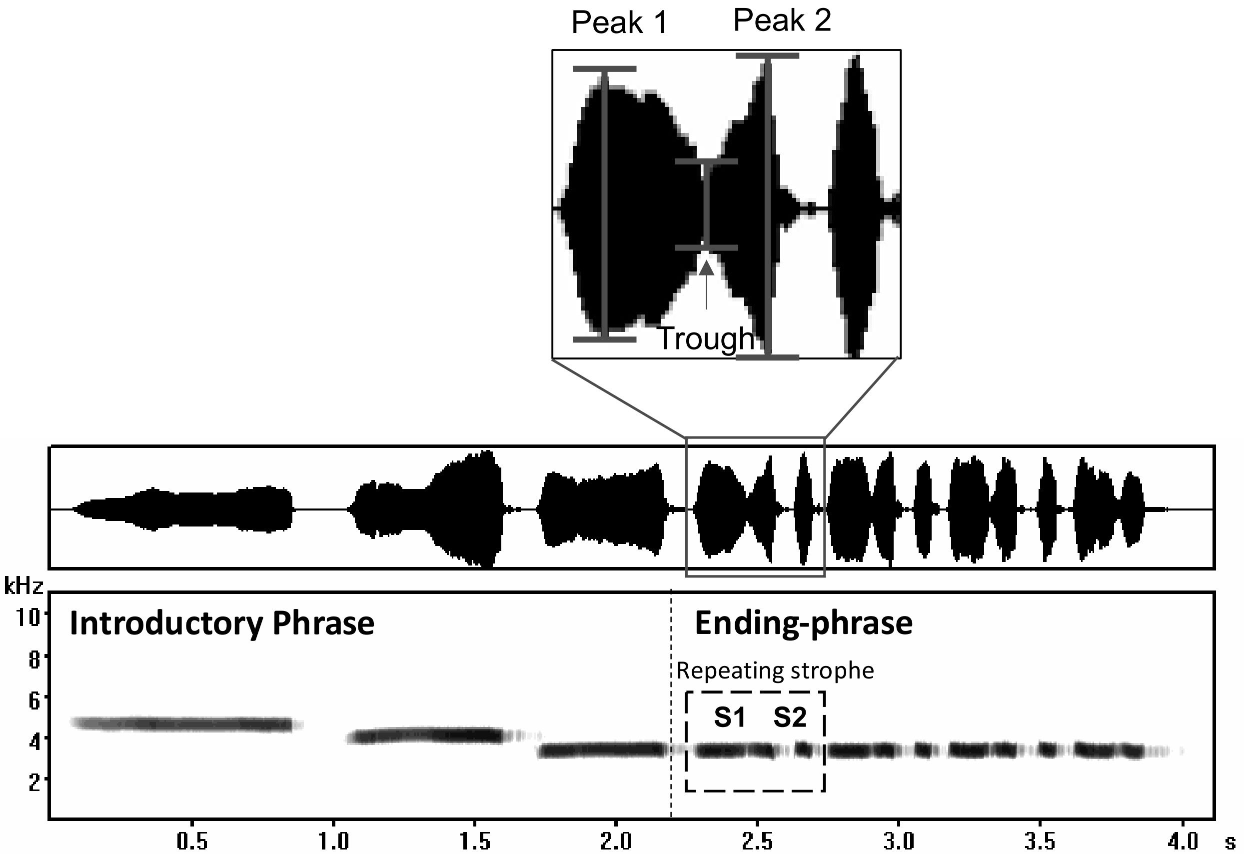

Figure 2. 1 S1 and S2 notes, with peaks and trough of the S1 note highlighted.

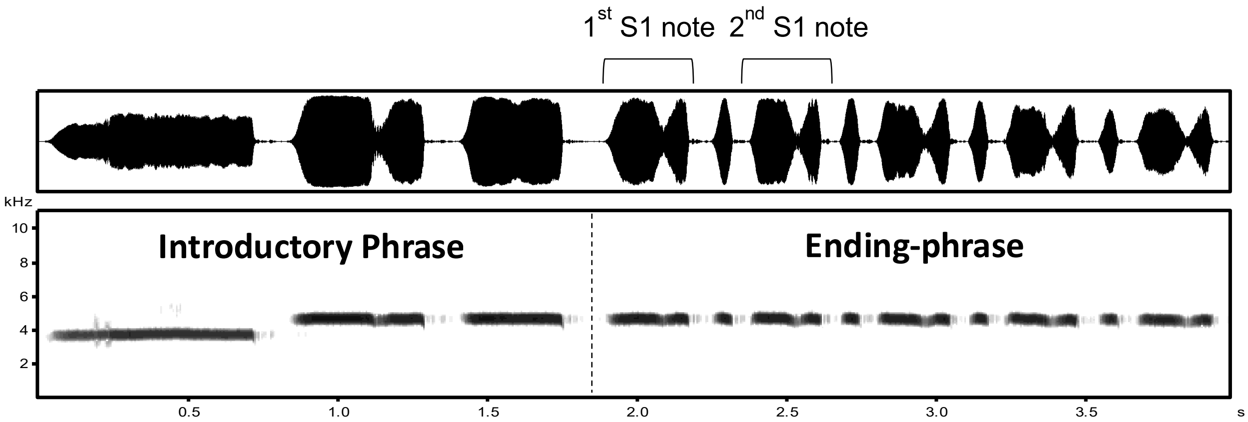

Figure 2. 2 For males producing songs where the modulation increases with later strophe repeats, such as this, we averaged the dB_{trough}/dB_{peak} ratio for the first two S1 notes.

2.3.3. Classifying songs

Birds that had an average amplitude ratio ≤ 0.6 (i.e., the amplitude of the trough was only 60% the amplitude of the peak amplitudes at the start and end of the song) were classified by human observers as Modulated-Doublets while birds with an average ratio ≥ 0.8 were classified as Unmodulated-Doublet songs. This distinction was based upon auditory classification of song exemplars by four observers familiar with White-throated Sparrow song whom we asked to initially categorize; there was consensus amongst observers that stimuli with amplitude ratios ≤ 0.6 had clearly audible modulation, but those ≥ 0.8 lacked modulation. Initial classification of stimuli suggested greater variability in categorization for stimuli with ratios between 0.6 and 0.8, so we used an independent audio test to categorize these songs.

The audio test was conducted in January 2020. This test consisted of seven ornithologists, who were trained using exemplar Modulated-Doublet song endings (ten songs with amplitude ratios ≤ 0.6) and Unmodulated-Doublet endings (ten songs with amplitude ratios ≥ 0.82). Participants were allowed up to ten minutes to listen to these exemplar endings during training. Following training, the classifiers were played an ending phrase (composed of two repeats, with exception of six songs that had only one repeat) for 121 birds whose amplitude ratio of at least one of the two first S1 notes fell between 0.6 and 0.8. For each bird, we selected a song that was most representative of its normal ending-type (i.e., closest to the individual's average amplitude ratio). This ending was played to the classifiers, asking them to categorize the song as "Modulated-Doublet" or "Unmodulated-Doublet". This was repeated for each song with an S1 within the amplitude ratio range of 0.6 - 0.8, so that all seven classifiers categorized all test songs (N=121). All songs in the audio test were recorded during 2015 to 2019, but classifiers were not given information as to the year or location of the recorded songs they were categorizing. Classifiers could listen to an ending up to three times and were allowed to go back over the training set of songs halfway through the audio-test. The songs were officially categorized as either Modulated-

Doublet or Unmodulated-Doublet songs based on agreement of at least five of seven classifiers. If there was not a clear consensus (e.g., <5 classifiers agreeing), the song was categorized as ambiguous. During these classification trials, we included eight control songs with amplitude ratios below 0.6 or above 0.8 – these control stimuli were from different males than those initially used to train the observers, and were interspersed throughout the other tests songs the classifiers were categorizing. We only considered the classifications of participants who correctly categorized at least 6/8 (75%) of embedded control stimuli during their trials.

These classifications are based on human perception and do not necessarily represent how birds perceive the modulation and distinguish between songs. Further investigation would be required to determine how and when birds perceive a difference in the amplitude modulation. The aim of this classification was to find the transition point between song variants, as perceived by humans, to document the song shift occurring in the study population. We chose classification by multiple observers as it allowed for dichotomous classification of songs based on cross-agreement of observers, insuring repeatability of the classification between observers and reducing perception biases that would be created if classification was made by only one observer.

2.3.4. Statistical Analyses

2.3.4.1. Logistic regression – Transition point between song variants

All statistical analyses were performed in R v.4.0.2 (R Core Team 2020) and all plots were made using base R or ggplot2 (Wickham 2016). Using the songs classified by participants, we fitted a logistic regression to identify the range of amplitude ratios where an Unmodulated-Doublet song audibly transitions to a Modulated-Doublet. To identify this transition point, we only used songs that participants classified unambiguously as either an Unmodulated-Doublet or Modulated-Doublet. For this analysis, we only included songs from an individual male in the first year it was recorded, so that all classified songs were from different males. Occasionally, individual males will sing two song types with different introductory phrases (approximate three to four birds in the population every year – *personal observation S Chartier*). For such males, we included only a single song type per male, using the song variant that included the longest ending phrase as in most cases, only one of the two song types used by these bilingual males contained complete ending phrases (i.e., contained at least one terminal strophe), so we included that song variant in the analysis. However, in rarer cases where males sang complete ending strophes in both song types (n = 2), the ending variants were typically similar in measured amplitude ratio. To avoid biasing by selecting one of the two song types for testing, we excluded these males.

We then used the logistic regression model to predict the classification of uncategorized songs recorded in 2020 as either Unmodulated-Doublets or Modulated-Doublets. Again, we excluded returning males and bilingual males with two complete song types from the 2020 test set.

2.3.4.2. Shift in song variant over time in the study population

We performed a log-likelihood ratio (G-test) of independence, with Williams's correction for small sample size, to compare the number of males singing each of the two song variants each year over five years of recordings (2015-2019). We included males whose songs were classified as Unmodulated-Doublet, Modulated-Doublets and ambiguous each year. To satisfy the assumption of independence for this analysis, we only included songs from the first year an individual male was recorded. Further, we excluded bilingual birds who had two song types that included ending strophes.

2.3.4.3. Change in the degree of S1 modulation over time in the study population

We performed a Kruskal Wallis test to investigate whether the mean amplitude ratio of S1 notes in songs changed amongst males in the population over time, from 2015 to 2020. Again, we only included songs from individual males (identified by banding) for the first year in which they were recorded, and we excluded bilingual males. In 2020, we recorded three males without being able to visually confirm whether or not they were banded. Since their banding status was unknown, we excluded these birds from this analysis. We then performed pairwise comparison of the different years using Wilcoxon rank-sum tests with a Bonferroni-adjusted critical alpha value of 0.0033 to account for multiple comparisons. We used a Bonferroni-adjusted critical alpha to be consistent with the non-parametric tests used rather than Tukey's HSD or Ryan's test which assume normal distribution (Tukey's HSD) and homogeneity of variance (Tukey's HSD and Ryan's test). Finally, we also calculated the average drop in amplitude ratio between each year.

2.3.4.4. Change in modulation ratio of individual birds.

To determine whether individuals shifted their songs between years, becoming more modulated over time, we performed a paired, two-tailed Wilcoxon test to compare the amplitude ratios of banded birds that were recorded in two different years (N=22). For every bird, we compared the amplitude ratio of its first recording year to its second recording year. To see if change in modulation of returning males was comparable to the change in the overall population, we compared the change in modulation within males to the overall change across males within the population between years.

2.3.4.5. Other song variants

Every year, a few males in the population sing ending types other than Unmodulated-Doublet or Modulated-Doublet songs (*pers. obs. S.Chartier*). Such song variants constitute potential opportunities for adoption and song spread. We calculated the proportion of males singing these other variants for each year, and whether there was a directional pattern of change between subsequent years. As with other analyses, we only used songs of individual males during the first year they were recorded.

2.4. Results

2.4.1. Audio Test

Overall, 22 songs had modulation ratios ≤ 0.6 and were automatically categorized as Modulated-Doublets while 10 songs had modulation ratios ≥ 0.8 and were categorized as Unmodulated-Doublets. Additionally, 121 songs had ratios between 0.6 and 0.8, so were classified using the audio test; 34 were classified by the volunteers as Unmodulated-Doublets, and 51 were classified as Modulated-Doublets. There was no clear consensus on the remaining 34 songs, so they were considered ambiguous for analysis purposes. One Unmodulated-Doublet control song (ratio 0.87) had no clear consensus among classifiers; we believe this was because the song had a reversed ending (i.e., the ending started with the shorter S2 note followed by the longer S1 note) which changes the cadence of the song, rendering classification difficult. As a result, we excluded any reversed-ending songs from the analysis (three songs) as classification of these songs likely does not reflect their level of modulation.

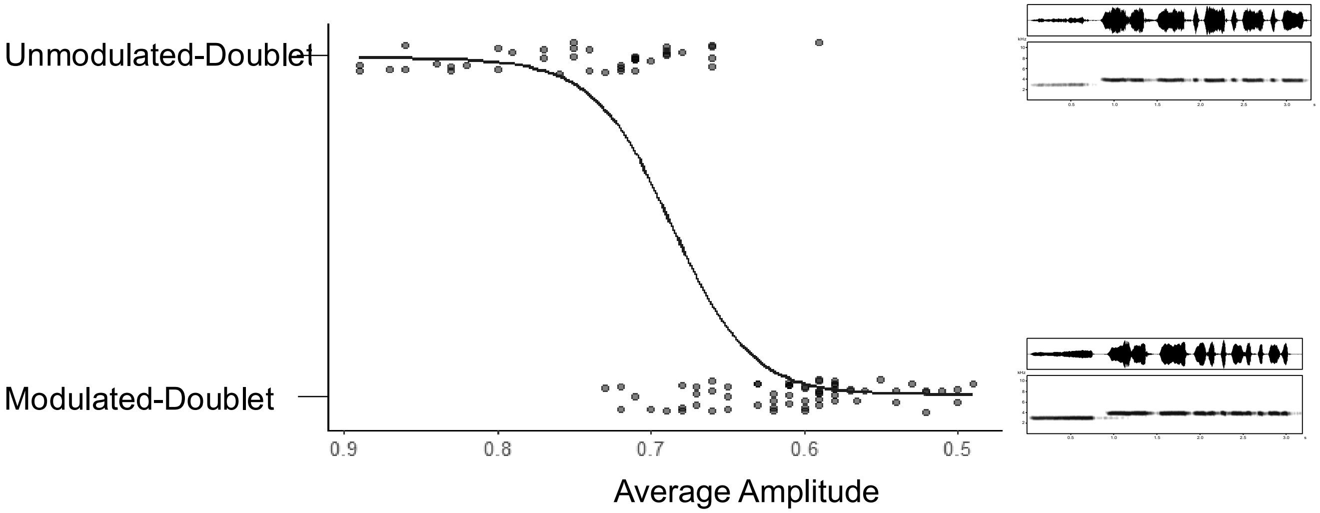

Figure 2. 3 Logistic regression graph showing the transition of Unmodulated-Doublets to Modulated-Doublets based on average amplitude ratio of the S1 note. Note that the X-axis is reversed to highlight the transition of Unmodulated-ending to Modulated-ending songs. Spectrograms of an Unmodulated-Doublet song and a Modulated-Doublet song shown on graph.

2.4.2. Transition point between variants

The average amplitude ratio of the first S1 note contributed significantly (Logistic regression: coefficient= 38.89, P< 0,0001; Figure 2.3) to the classification of a song as being either an Unmodulated-Doublet or a Modulated-Doublet song. There is a sharp transition from Unmodulated-Doublet songs to Modulated-Doublet songs when the S1 notes have an average amplitude ratio between 0.75 and 0.65 (Figure 2.3).

2.4.3. Shift in ending type in the study population

We compared the ending type (Unmodulated-Doublet, Modulated-Doublet, or ambiguous) of birds at our study sites across years (2015-2019), using the classifications from participants of the audio test. The proportion of birds singing unmodulated, modulated and ambiguous songs shifted over time (Gtest: G= 41.74, df= 8, P< 0.0001) with Unmodulated-Doublet songs decreasing and Modulated-Doublets increasing in proportion (Figure 2.4). Figure 2.4 also includes proportions from 2020. We used the logistic regression model to predict the classification of these songs as Unmodulated- or Modulated-Doublets. Since there was no ambiguous category due to the use of the logistic regression model for classification, the data for 2020 was not included in the G-test.

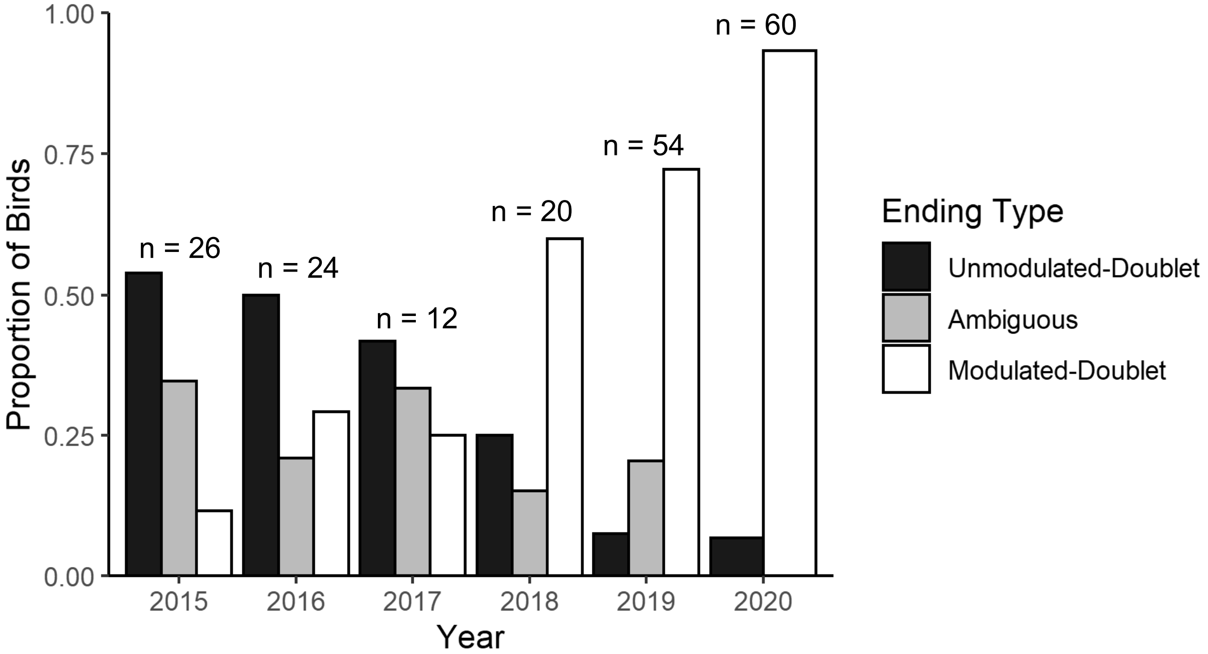

Figure 2. 4 Comparison of the proportion of birds singing Unmodulated-Doublets, Modulated-Doublets and songs classified as ambiguous from 2015 to 2020. 2020 songs were classified using the logistic regression model as Unmodulated-Doublet or Modulated-Doublet. Sample size (n) is specified for each year.

2.4.4. Modulation becoming more pronounced among males over time

The average amplitude ratio of modulated songs differed across years (Kruskal Wallis test: χ^2= 67.14, df= 5, P < 0.0001, Figure 2.5). Amplitude ratios in 2020 were significantly more pronounced than all other years; 2015 (Wilcoxon tests with Bonferroni-adjusted critical alpha value of 0.0033: W= 1448, P< 0.00001), 2016 (W= 1250, P< 0.00001), 2017 (W= 573, P= 0.001), 2018 (W= 913, P= 0.0005), 2019 (W= 2296, P= 0.0001). Additionally, two birds in 2020 had a modulation so pronounced, that the S1 note was completely broken into two notes, creating a triplet-ending song; however, this triplet-ending song has a different cadence than the current eastern triplet song (Figure 2.6). Amplitude ratios in 2019 were also significantly more pronounced than in 2015 (Wilcoxon test with Bonferroni-adjusted critical alpha value of 0.0033: W= 1196, P< 0.00001) and notably, but not significantly with Bonferonni-adjustement, more pronounced than 2016 (W= 913, P = 0.004). Amplitude ratios in 2018 were also smaller than in 2015 (Wilcoxon test with Bonferroni-adjusted critical alpha value of 0.0033: W= 436, P= 0.00004).

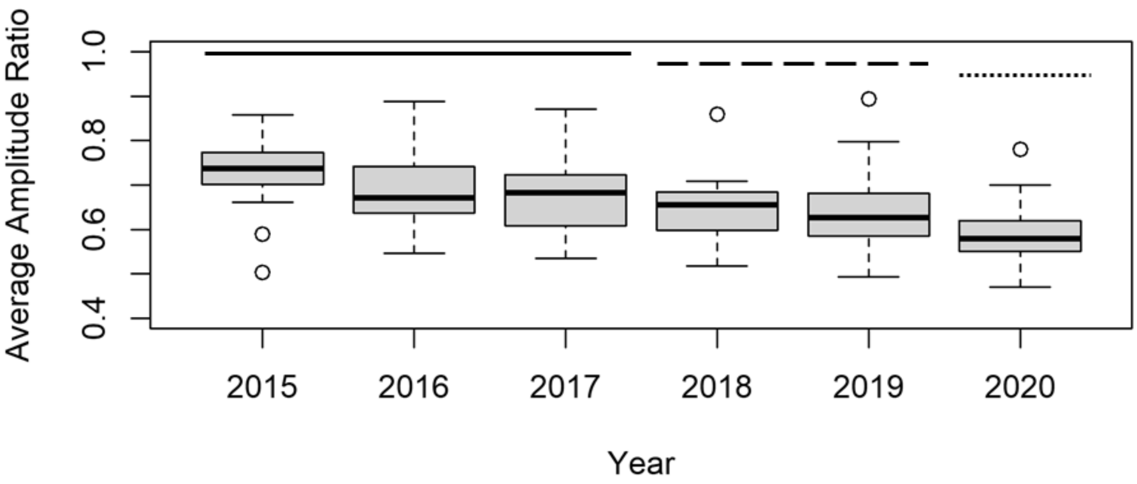

Figure 2. 5 Boxplot comparing the average amplitude ratio of the S1 note in songs recorded annually from 2015 to 2020. The modulation in song becomes more pronounced (smaller Amplitude Ratio) in 2018-2020. The lines above the boxplots indicate post-hoc comparison differences. The average amplitude ratios in 2015-2016-2017 are not significantly different, the average amplitude ratios in 2018 and 2019 are significantly different than 2015 but not from each other and the average amplitude ratios in 2020 are significantly different from all other years.

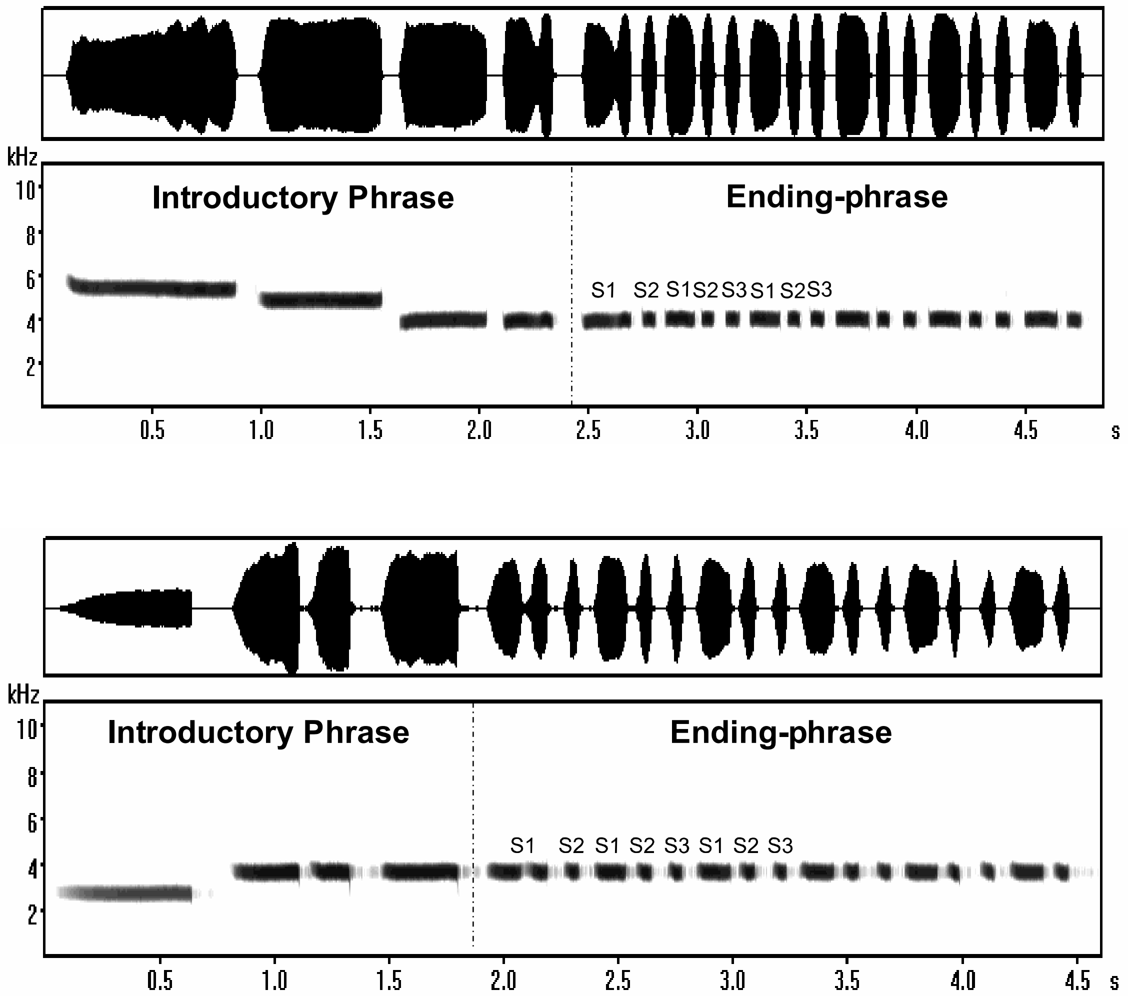

Figure 2. 6 Examples of songs where the S1 notes have been broken into two, creating a novel triplet-ending song. In both examples, the first S1 note is not fully broken but subsequent S1 notes are. Songs taken from two different males recorded in 2020.

2.4.5. Change in the modulation ratio of returning birds

Overall, 22 birds returned to the study sites for at least two years and had recordings clear enough for analysis. Overall, returning birds had smaller amplitude ratios in their second recording year than in their first, indicating the modulation in the S1 note became more pronounced between years for the majority of males recorded (Wilcoxon ranked test: V= 213.5, P= 0.005 – Figure 2.7). The change in modulation within returning males (Mean= 0.058, St. Dev= 0.084) was comparable to the change across males within the population between years

(Mean= 0.029, St. Dev= 0.014). Given the steep perceptible transition from Unmodulated- to Modulated-Doublet (Figure 2.3, between 0.75 to 0.65) and that the overall change in the population between years was 0.029, a shift of 0.03 in the amplitude ratio of a returning male could represent an audibly perceptible difference. We, therefore, classified males by whether the change in their amplitude ratios between years was: a decrease of 0.03 or greater (pronounced increase in modulation); an increase of 0.03 or greater (reduction in modulation between years); or an increase/decrease in modulation that was less than 0.03 (minor change). Of the 22 males, 15 (68%) had a modulation that became noticeably more pronounced (the amplitude ratio decreased by ≥ 0.03), only two had less pronounced modulation (the amplitude ratio increased by ≥ 0.03), and the remaining five had little discernible change of modulation (the amplitude ratio changed by ≤ 0.03 change in their amplitude ratio between years).

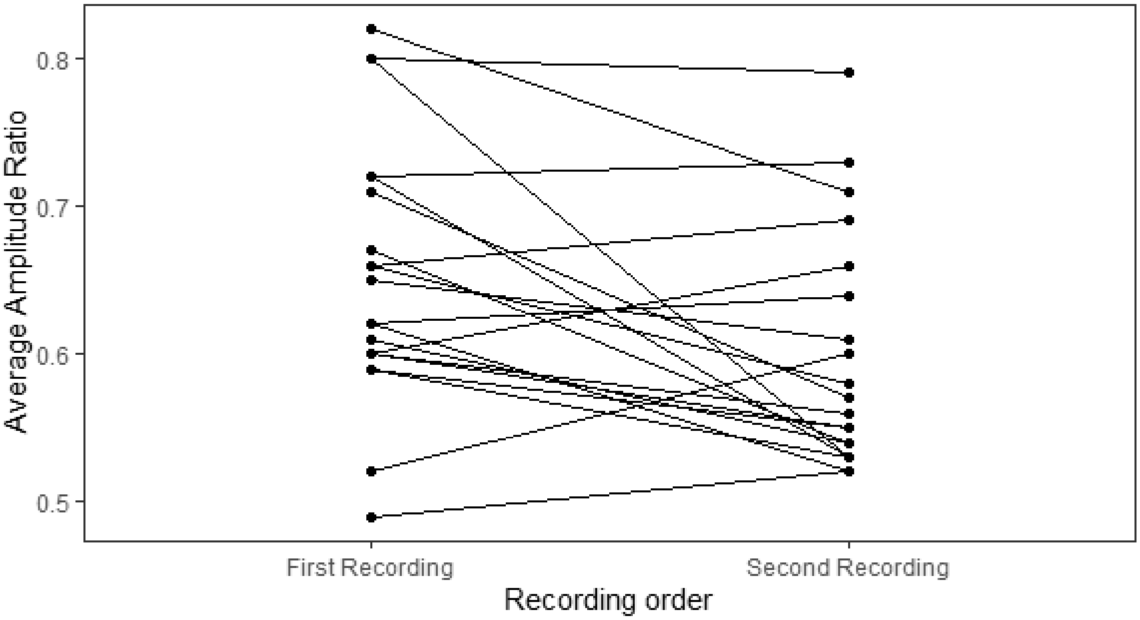

Figure 2. 7 Paired plot comparing the average amplitude ratio of individual birds during their first and second recording year.

2.4.6. Other variants

Of over 225 individual males that were recorded, 16 males sang a song variant other than Modulated-Doublets or Unmodulated-Doublets. These primarily consisted of variants on singlet-ending songs (a repeating single S1-like note): four males sang Unmodulated-Singlets (Fig 2.8.a) and 7 sang Modulated-Singlets (Fig. 2.8.b). Five other males sang a unique ending strophe-type not sung by any other males (see Fig. 2.8.c for example). The proportion of males singing other variants per year were: 2015 (1/29), 2017 (2/14), 2019 (9/64) and 2020 (4/75). No unique variants were recorded in 2016 and 2018. There was no consistent pattern of change in the proportions of any of these individual song variants between years.

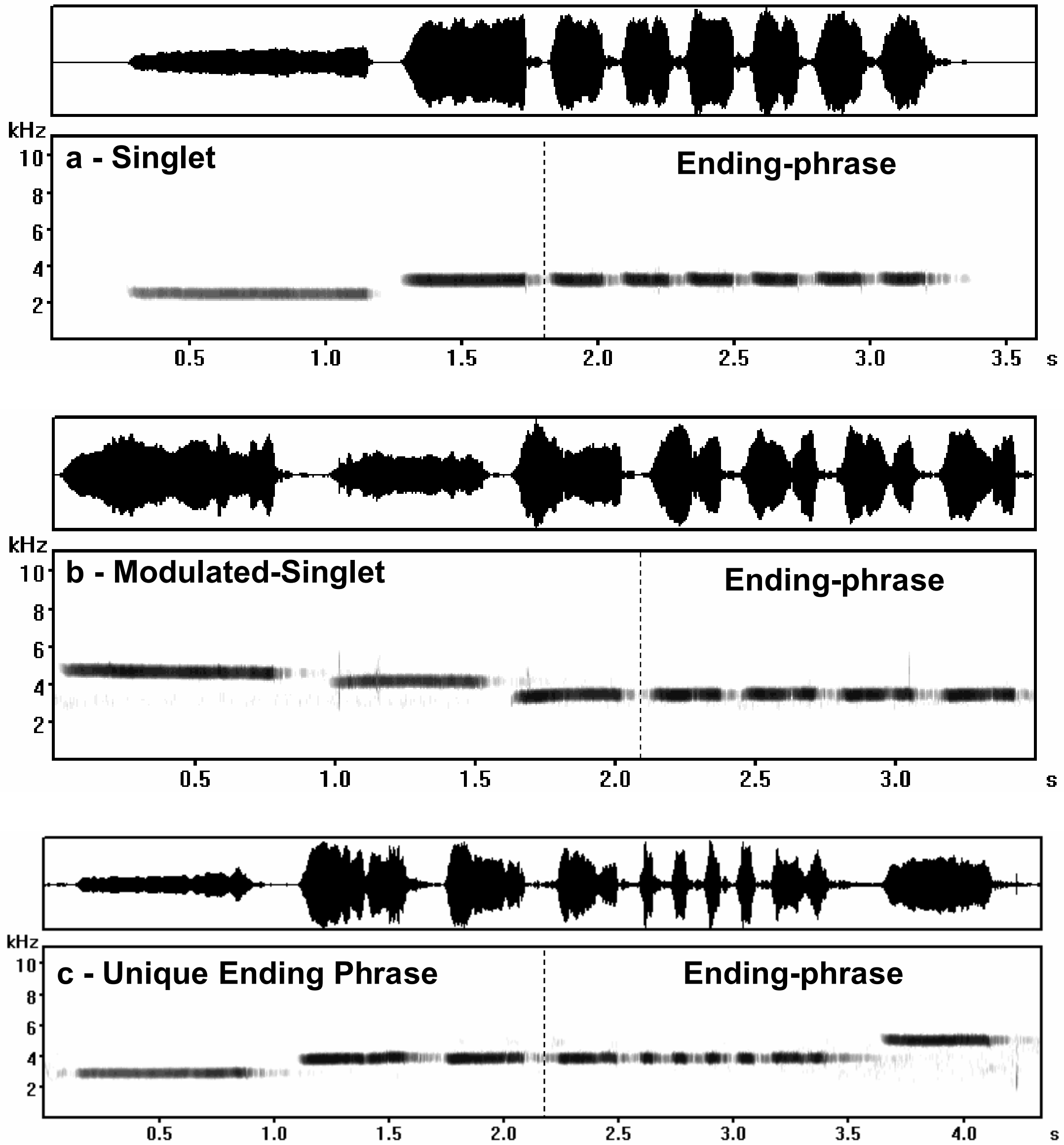

Figure 2. 8 Spectrograms of an Unmodulated-Singlet (a), Modulated-Singlet (b) and a unique ending variant (c).

2.5. Discussion

Our study population transitioned from the Unmodulated-Doublet song to the Modulated-Doublet song during our 6-year study period. The proportion of males singing the Modulated-Doublet increased between years while the proportion of males singing the Unmodulated-Doublet song decreased. Additionally, the modulation became more pronounced over the years.

Several factors appear to contribute to this increasing modulation: more males adopted modulated songs each year; the songs males adopted had more pronounced modulation; and returning males appeared to increase the modulation in their songs between subsequent years.

This rapid spread within a population mimics the shift over time from triplet-ending songs to doublet-ending songs in Alberta and Algonquin Park, Ontario documented by Otter et al. (2020). It also mirrors other studies that have documented subtle transitions in within-population song variants that accumulate over time, such as occurred in Chestnut-sided warbler (*Setophaga pensylvanica* - Byers et al. 2010), Hermit warblers (*Setophaga occidentalis* – Janes and Ryker 2013), Bellbirds (*Procnias spp.*- Kroodsma et al. 2013), and Yellow-rumped Caciques (*Cacicus cela* - Trainer 1989). This may reflect learning biases, such as model-based transmission bias, whereby birds preferentially adopt songs that are produced by more dominant or reproductively successful males, or content-based transmission biases, whereby particular aspects of the song — such as its cadence or acoustic structure — invokes a learning preference (Boyd and Richerson 1985, Byers et al. 2010).

2.5.1. Model-based biased transmission

Song transition in White-throated Sparrows may be partially driven by a model-based transmission bias, as females could possibly show a preference for novel song endings. In White-throated Sparrows, the structure of the ending-phrase of songs does not impede male-male interactions. When played Triplet versus Unmodulated-Doublet songs, previous studies have shown that males varied very little in their response to either song type, regardless of the dominant locally-occurring song variant (Ramsay and Otter 2015). This suggests that males do not necessarily differentiate ending phrase of songs during counter-singing interactions, so advantage of one variant over the other in male-male signalling may not be the driver of rapid adoption of novel song variants in White-throated Sparrows.

An alternate mechanism that may drive shifts in song variants is female preference. Female White-throated Sparrows prefer playback of songs that include terminal strophes compared to songs with only introductory notes (Wasserman and Cigliano 1991). This suggests that females are attentive to the ending phrases of the song and could choose a mate based on the ending type of their song. If females do select males singing the novel variants, the reproductive success of males would increase by adopting these variants and copying other males singing novel songs. This model-based transmission bias mechanism would predict the rapid spread of novel variants such as the Unmodulated-Doublet in the Algonquin population (Otter et al. 2020) and the Modulated-Doublet within the Prince George population (this study). Byers et al. (2010) described a similar example of a model-based transmission bias in a population of Chestnut-sided Warblers, where over a 19 year-period, one new type of accented-ending song emerged, one disappeared and three remained stable. In Chestnut-sided Warblers, accented-ending songs are used for mate attraction; the authors argued it may be advantageous for males to converge on few song types if songs are used by females to compare males. Similarly, in humpback whales, cycles of novel song emergence and spread have recently been documented in populations of the central south Pacific. Since humpback whale song is suspected to be used in sexual selection; if either females prefer novelty or if novelty reflects male status, it would be advantageous for a male to adopt novel song variants (Noad et al. 2000; Garland et al. 2011).

One potential flaw of invoking female preference for novel song variants in a model-based transmission bias is that as a song becomes common in the population, it would not necessarily remain novel. This could create patterns of novel variants appearing, spreading, but themselves then being replaced by other variants before they fully replace old variants. However, model-based transmission bias may achieve this pattern if song transition is also reinforced by other drivers.

2.5.2. Content-based biased transmission

Content-based biased transmission could also explain, in part, song transitions observed in White-throated Sparrows, as not all novel or rare song variants increased in frequency in our study population. Most years within our study population, there are a few males that sing rare variants (e.g., Unmodulated and Modulated-Singlets, Figure 2.8. a and Figure 2.8. b) or unique variants (Figure 2.8. c). Many of these variants, however, do not appear to spread, as their proportions in the population remain low over time. This suggests that there is an “acceptable” template or specific acoustic cue needed in the ending-type for a song to be copied by others. This phenomenon is observed more generally in song learning, where most species will preferentially copy the songs of conspecifics or songs with species-specific cues (Hultsch and Todt 2004; Beecher and Brenowitz 2005). In White-crowned Sparrows for example, birds will preferentially learn songs that start with a species-specific introductory whistle over songs missing the whistle, regardless of the rest of the song (Soha and Marler 2000). Similarly, Song Sparrows (Marler and Peters 1988) use song syntax, song syllables and note structures to select which songs to learn, and Swamp Sparrows will learn songs containing Swamp Sparrow syllables even if the temporal pattern of the song does not match the typical Swamp Sparrow song pattern (Marler and Peters 1977). Additionally, birds raised in isolation are still able to produce subsongs that contain song elements from the species' typical song, suggesting innate, species-specific templates (e.g., Marler and Sherman 1983, Shackleton and Ratcliffe 1993). These cues or templates could be lacking in the rare and unique ending variants in White-throated Sparrows, preventing other individuals from copying those variants. Since songs are used for species identification by both male and female birds, differing from the species-specific template could hinder the success of a male in attracting mates and/or defending territories.

Cycles of cultural evolution observed in White-throated Sparrows show that this species predominantly sings triplet-ending songs, suggesting this might be the species-specific template which could favour the adoption of triplet-like songs over other novel or unique variants. Historic recordings show that White-throated Sparrows in the 1950s-60s sang triplet-ending songs across their continental breeding range (Borror and Gunn 1965). However, these differed from the current eastern triplet as their ending had significantly shorter strophe lengths, with shorter inter-note intervals (i.e., interval between the S1, S2 and S3 notes) and shorter S1 and S3 notes (Zimmerman et al. 2016). Strophe lengths may have increased gradually over time, resulting in the current "longer" triplet-ending strophes in eastern populations. Some time prior to 1990, western birds dropped the third note (S3) from the ending strophes and increased the length of their first two notes (S1 and S2). This resulted in doublet-ending songs with strophe lengths similar to historic triplet-ending songs (Zimmerman et al. 2016). After nearly 15 years of monitoring this population, in which time only Unmodulated-Doublet-ending songs were evident, we observed the emergence of doublet-ending songs with an amplitude modulation in the S1 note in 2015. This modulation creates the auditory impression of a double sound pulse within the single note, and when combined with the shorter S2 note has a distinctive 3-pulse cadence. Content-based biased transmission could possibly explain the modulation then becoming more pronounced with each year, to the point of two males in 2020 having songs where the modulation is so pronounced, it fully separates the S1 into two notes. As a result, these two males recorded in 2020 were singing a triplet-ending song with a novel cadence pattern, the first triplet-ending song to be recorded in the population in nearly 20 years that this region has been sampled. This mechanism could also possibly explain why the transition to the Modulated-Doublet appears to have happened more quickly than the transition to the Unmodulated-Doublet in similar sized populations (i.e., around five years in the PG population to transition to the Modulated-Doublet versus 13 years for 50% of the population to transition to the doublet in Algonquin). It is possible the modulation within the S1 note results in the Modulated-Doublets resembling the acoustic structure of the species-typical triplet-ending songs that have historically been more common in White-throated Sparrows. Since the Modulated-Doublet is both a novel variant, and fits the species-typical triplet pattern, both content-based and model-based biased transmission could be driving song transition and favouring the adoption of the Modulated-Doublet over singlets, unique variants and Unmodulated-Doublets. Conversely, the previous transition from the Triplet to Unmodulated-Doublet song documented by Otter et al. (2020) may have been primarily driven by a model-based bias as the Unmodulated-Doublet song, while having the same strophe lengths and similar cadence, differs more strongly from the species typical triplet-ending pattern and would possibly counter the birds' content-based learning bias.

2.5.3. Role of juveniles, adults and wintering grounds in song transition

Transition rates could also be different between populations breeding West of the Rocky Mountains and in the main eastern breeding population. Otter et al. (2020) suggested that juveniles may adopt novel variants when these variants are heard and reinforced on both their breeding and wintering grounds. Wintering grounds in California appear to be used by birds from the western-most portion of their breeding range, and are less densely populated than eastern wintering grounds (Falls and Kopachena 2020; Otter et al. 2020). If a novel song variant emerges in western breeding or wintering populations, there is a higher probability that birds will hear the novel, rarer variant on both their breeding and wintering grounds. Conversely, wintering grounds east of the Rocky Mountains are shared by both western and eastern breeding populations (Falls and Kopachena 2020; Otter et al. 2020), reducing chances of re-exposure initially, as novel, rare variants would be drowned out in the larger population. This would also be consistent with the idea that song transition follows an exponential increase; starting slowly until a certain threshold in the proportion of birds singing novel variants is reached, after which, the rate of transition increases rapidly. In the Prince George population, from 2015 to 2017, the proportion of birds singing the Modulated-Doublet increased to 25% (3/12), however this proportion increased to 93% (56/60) in 2020, only three years later (Figure 2.4). Juveniles adopting novel song variants may drive the rapid spread if song transition is largely dependent on overproduction and selective attrition during song learning. Returning adult males in our population however, also appeared to be able to modify the ending of their songs and make the amplitude modulation more pronounced over time. However, due to small sample size and the variability between the males, we are unable to tell if older adult males have contributed to the drop in amplitude or if they are simply adjusting to keep pace with the overall change in amplitude modulation driven by younger males as they reach maturity.

2.5.4. Conclusion

Our study documented the emergence and spread of a novel song variant, the Modulated-Doublet, in a localized population of White-throated Sparrows, mimicking previous song transitions within populations of White-throated Sparrows (Otter et al. 2020) and other species (Trainer 1989, Byers et al. 2010, Janes and Ryker 2013, Kroodsma et al. 2013). This within-population adoption of Modulated-Doublet songs could also represent a new wave of larger-scale geographic spread of song types. Since the transition to the Modulated-Doublet song could be driven by both a model-based and a content-based bias, a continent-wide transition may occur at a faster rate than the previous transition from Triplet songs to the Unmodulated-Doublets (Otter et al. 2020). Additionally, Modulated-Doublets may progressively become a novel triplet-ending song as seen in two birds in 2020, continuing the cycles of cultural evolution documented in White-throated Sparrows over the years.

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Chapter 3: Song evolution and spread between populations in White-throated Sparrows

3.1 Abstract

Song shifts can occur over larger geographical scales when novel variants spread between populations. Songs can spread widely when different populations converge on shared locations where they encounter variants from tutors originating from other regions. Various transmission biases may also drive the spread of novel variants over larger scales. Given its rapid spread within our local study population, we investigated if the Modulated-Doublet was also spreading between populations. We analysed song recordings from public sound archives taken in May to July 2014-2020 and compared the pattern of spread to the previous spread of the Unmodulated-Doublet. We found that the Modulated-Doublet emerged simultaneously and spread in parallel in multiple migratory routes and breeding populations of western and central US and Canada, largely replacing the Unmodulated-Doublet in those areas. This differs from the eastward, wave-like movement of song previously suggested, however, recent increases in use of public archive platforms could have allowed us to more thoroughly document the pattern of early spread. Our results support the hypothesis proposed by Otter et al (2020) that White-throated Sparrows encounter and adopt novel variants in shared locations, as many of the breeding populations where the Modulated-Doublet appeared simultaneously share common wintering grounds and migratory routes.

3.2 Introduction

Cultural evolution describes the change in learned behaviours and traditions within a population over time (Whiten 2019). Such changes have been documented in the songs of both whale (Noad et al. 2000; Garland et al. 2011) and songbird populations (Trainer 1989; Byers et al. 2010; Janes and Ryker 2013; Otter et al. 2020), where vocalizations changed in structure and/or note composition over time. Since songs are learned in these taxa, cultural evolution can occur as a result of innovation or copying errors made by an individual during the song learning process, which then spread to others who use these individuals as tutors (Podos and Warren 2007). Novel song variants can also spread widely if members of different populations converge on common locations, such as migratory stop-overs or wintering grounds, during the song acquisition period and encounter tutors singing variants from other regions (e.g., Owen et al. 2019; Otter et al. 2020). Song shifts over large geographic scales may be driven by within-species transmission or learning biases that act as selective pressures and favour the adoption of novel song variants over previously entrenched variants, especially in populations where the novel variant is initially rare (Noad et al. 2000; Garland et al. 2011; Otter et al. 2020)

Such song transition over a large-geographic scale was documented in White-throated Sparrows (*Zonotrichia albicollis*). White-throated Sparrows typically sing songs beginning with an introductory phrase of whistled notes and ending in repeated phrases (strophes) of several notes. Prior to 1990, songs ending in repeated strophes of three notes (See Chapter 1, Figure 1.4. a.) – Triplet-ending songs – were ubiquitous throughout the geographic range of the White-throated Sparrows across Canada and the northern United States (Borror and Gunn 1965). Between 1990 and 2000, a novel variant - the Unmodulated-Doublet - a song ending in repeated strophes of two notes (Figure 1.4. b.) – emerged in northcentral British Columbia (CAN) and spread eastward, replacing the Triplet-Ending song (Ramsay and Otter 2015; Otter et al. 2020). The Unmodulated-Doublet first moved through the Prairies starting in the early 2000s, Ontario starting in 2005 and was recorded as far east as Quebec in 2019 and became the predominant song variant in these populations (Otter et al. 2020). Some birds breeding in British Columbia migrate over the Rocky Mountains and follow the Central and Mississippi flyways down to the Texas wintering grounds, which are shared with populations breeding in the Prairies and north-western Ontario (Mazerolle et al. 2005; Otter et al. 2020). Otter et al. (2020) hypothesized that birds breeding east of the Rocky Mountains encountered and learned Unmodulated-Doublets on these shared wintering grounds, allowing the rapid eastward spread of Unmodulated-Doublet songs.

Recently, we documented the emergence and spread of a second novel doublet-ending song variant within a population of White-throated Sparrows in central British Columbia (Chapter 2). The Modulated-Doublet also ends in repeated strophes of two notes but with a pronounced amplitude modulation in the first repeated note (Figure 1.4. c.). From 2015 to 2020, the proportion of birds singing the Modulated-Doublet in this population went from 12% (3/25) to 93% (56/60). Due to this rapid song transition within a population, the objective of this chapter was to investigate if the Modulated-Doublet is also spreading between populations and to map its early spread at a larger, continental scale, during both migration and the breeding season. We observed the rate and pattern (origin and direction) of spread of the Modulated-Doublet and compared this pattern with the spread of the Unmodulated-Doublet, in order to understand where novelty arises in this species and how it spreads between populations. To document this spread, we re-analysed and expanded the dataset of recordings obtained from public sound archives to determine whether doublet-ending songs recorded between 2014 and 2020 showed indications of containing amplitude modulations to track the larger-scale spread of this song variant over time.

3.3. Methods

3.3.1. Classification

Two trained ornithologists classified White-throated Sparrow songs taken from the sound archives Xeno-Canto (https://www.xeno-canto.org/, n = 71) and the Macaulay Library (https://www.macaulaylibrary.org/, n = 556) (S.Chartier 2014-2019, K. Legeard 2020). Additionally, we classified four recordings that were sent directly to us by the general public. This dataset includes posted songs originally classified as either doublet-ending or triplet-ending used in the analysis of Otter et al. (2020), but does not include personal recordings used in that study. Otter et al. (2020) noted only whether songs were doublet vs triplet-ending, but did not assess for the presence of a note-modulation in doublet-ending songs.

We only classified songs that were recorded from April through July in each year from 2014 to 2020, as this represents the transition period from wintering grounds, migration and early establishment (April-May) to established territories and breeding (June-July). Classification was made both by ear (by listening to the audio-file and comparing to exemplars of Triplet-ending, Unmodulated Doublet-ending and Modulated Doublet-ending songs) and by observation of the associated spectrograms on Xeno-Canto and the Macaulay Library, or with Avisoft SASLab Pro v.5.2.13 (Specht 2019) for recordings that were sent directly to us. Songs were classified based on their ending-phrase as Triplet-ending – ending in repeated strophes of three notes -, Unmodulated-Doublets, - ending in repeated strophes of two notes without any prominent within-note amplitude modulation -, or Modulated-Doublets – ending in repeated strophes of two notes, with an amplitude modulation in the first repeated note (see Chapter 1, Figure 1.4. for spectrogram examples). To be classified as a Modulated-Doublet, a song needed to have an audibly distinguishable modulation. While the Unmodulated-Doublet has a repeated cadence pattern of a long note followed by a short note (DAA-da, DAA-da), the Modulated doublet has a rapid three-pulse cadence with slurring of the pulse in the first note (DaA-da, DaA-da). However, the cadence of Modulated-Doublets is also different from eastern Triplets where the full triplet-ending song has a cadence of three clearly separated notes (DAA-da-DAA, DAA-da-DAA). If a song was ambiguous, two other trained ornithologists would also listen to and classify the song. The song was then classified based on agreement of at least 2 of 3 classifiers. We acknowledge that even using the agreement of the majority, there is still a possible auditory bias caused by classifying songs by ear. However, we chose classification by ear because songs could not always be downloaded and imported into a song analysis software for measuring.

To decrease the chance of bias through auditory classification, we only classified recordings that contained complete songs, i.e., songs with both an introductory and ending phrase. Additionally, we only classified songs with ending phrases composed of at least two repeated strophes to be able to distinguish between doublet and triplet-ending songs. We did not classify songs for which we could not audibly distinguish the ending-phrase clearly due to the poor quality of the recording or due to overlap with background noise or other birds – all assessed songs had to be of sufficient signal-to-noise ratio that auditory classification could be confirmed with online spectrograms of the songs. We also did not classify songs with endings that did not contain clear repeating strophes - this often occurs with songs recorded on wintering grounds, and sometimes during migration and early breeding season (Falls and Kopachena 2020). Such songs are also characteristic of uncrystallized juvenile song or female songs (Falls and Kopachena 2020). For each classified song, we noted the date and location of the recording, name of the recordist, ending type, as well as introductory notes pattern. Introductory notes are typically three whistled notes that: start at a low pitch and ascend in pitch (Ascending); begin with high-pitched note and descend in pitch (Descending); begin with a low-pitched note, followed by a high-pitched, then mid-pitched note (Up Down); or all notes of the introductory phrase are sung at the same pitch (Monotone). Some recordings were uploaded by the recordist to both Xeno-Canto and Macaulay library, and in such cases, we created only one entry in our database for the bird but noted down both recording numbers for reference. If a recordist submitted more than one recording of a White-throated Sparrow from the same location, date and time, we verified that the recordings did not contain the same individual bird by comparing variation in pitch and absolute pitch in introductory note patterns. As male White-throated Sparrows typically sing only a single song type (Borror and Gunn 1965), and males within populations vary widely in the type of introductory phrase pattern (Borror and Gunn 1965; Lemon and Harris 1974), we considered recordings separate males if they had differing introductory phrases. Some recordings contained multiple males on one recording; in such cases, we created an entry for each distinct and classifiable male.

3.3.2. Mapping in R

Maps were created in R (v 4.0.3; R Core Team 2020) as described in Otter et al. (2020) using rnaturalearth (South 2017) and patchwork (Pedersen 2020) to assemble the figures. For the maps, we only included recordings from April to July from 2014 to 2020 (n = 583). This date range includes spring migration (April-May) and most of the breeding season (June-July). This timing coincides with males starting to sing clear and stereotyped songs and also includes the period during which males sing generally the most (Falls and Kopachena 2020). We further divided these recordings and created two sets of maps, one representing April-May (n = 396; Figure 3.1) and one representing June-July (n = 187; Figure 3.2) to distinguish if Modulated-Doublets recorded in locations east of the Rocky Mountains represent birds passing these regions on migration to their western breeding ground (April-May) or if they were birds that were singing the Modulated-Doublet on their breeding grounds (June-July). For both sets of maps, we combined 2014 and 2015 into one map due to the small number of recordings for those years. We set the transparency of Modulated-Doublet points to 0.7 and Unmodulated-Doublet and Triplet-ending points to 0.5 in order to make the Modulated-Doublet more apparent in cases of overlap with other points and to highlight the spread of the Modulated-Doublet.

3.4. Results

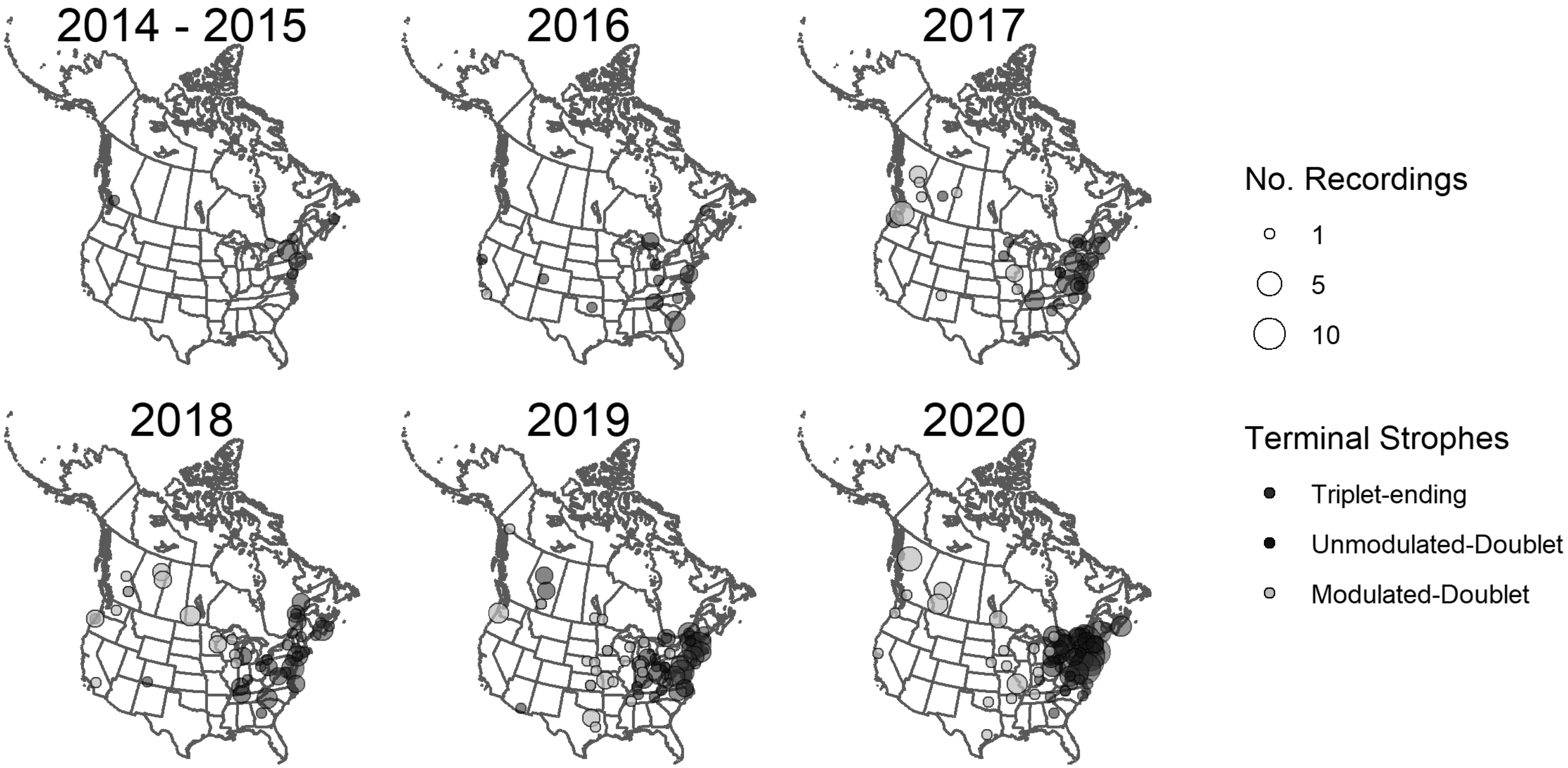

Figure 3. 1 Observations of birds singing during spring migration and early settlement, in April and May 2014 – 2020. Modulated-Doublets were first recorded in California (2016) and Central United States (2017) wintering grounds and migratory routes and are becoming more dominant along the Pacific and Mississippi Flyways. Darker yellow circles indicates that a Modulated-Doublet observation overlaps an Unmodulated-Doublet observation.

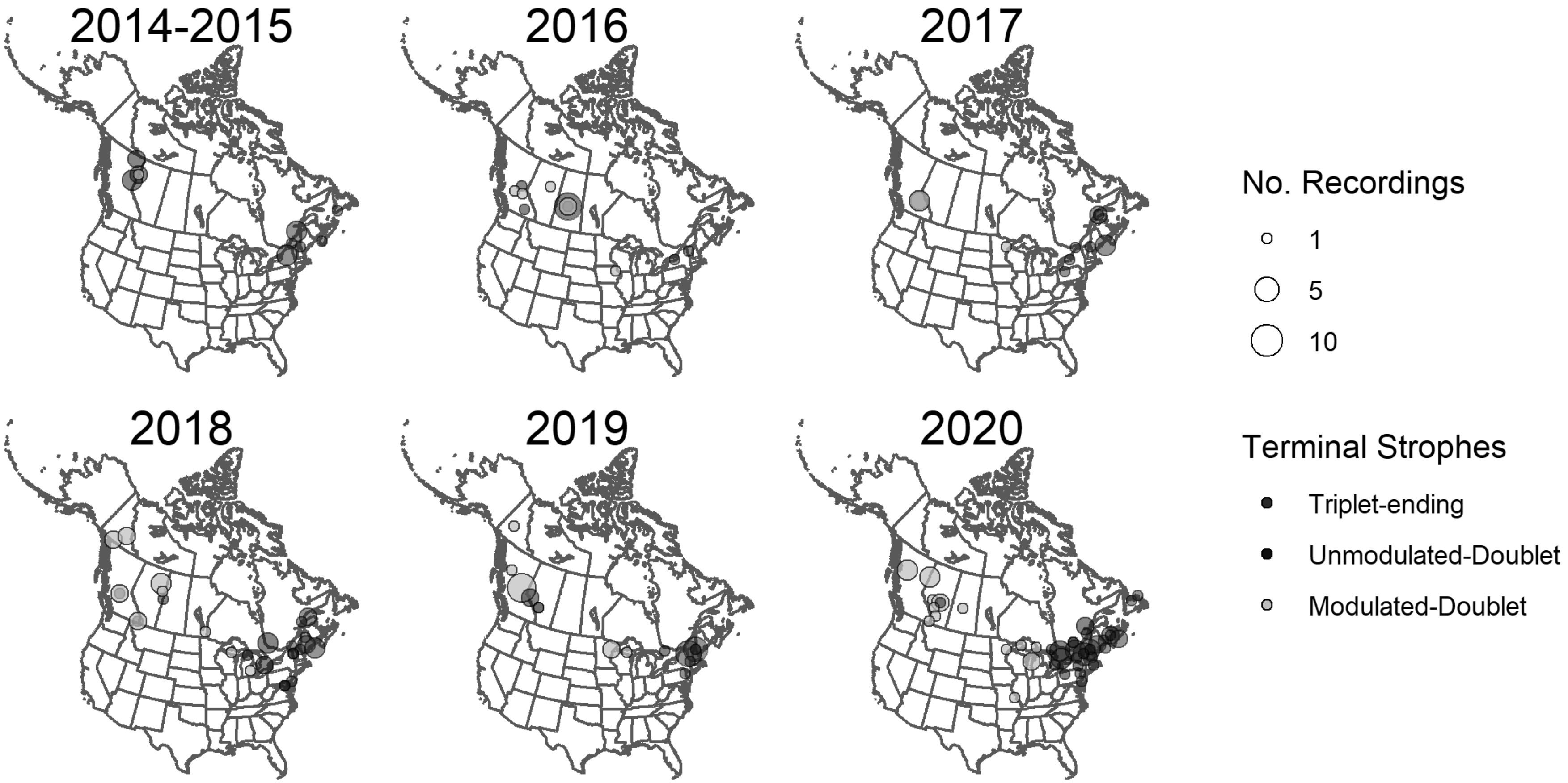

Figure 3. 2 Observations of birds singing on breeding grounds in June and July 2014-2020. Modulated-Doublets appeared simultaneously in several breeding populations in British Columbia, Alberta and Saskatchewan and replaced the Unmodulated-Doublet. Beginning in 2017-2018, Modulated-Doublets were recorded in populations surrounding the Great-Lakes. Darker yellow circles indicate that a Modulated-Doublet observation overlaps an Unmodulated-Doublet observation.

Of the 583 classified males mapped, 396 were recorded in April-May and 187 were recorded in June-July. For April-May, there were a total of 207 males singing Triplet-ending songs, 101 singing Unmodulated-Doublet ending songs and 88 singing Modulated-Doublet ending songs across all years. For June-July, there were 76 males singing Triplet-ending songs, 52 singing Unmodulated-Doublets and 59 singing Modulated-Doublets across all years.

The first instance of a Modulated-Doublet recorded during migration was in Californian wintering grounds in 2016 (Figure 3.1) and the first instance on breeding grounds was in 2014 in north-eastern British Columbia (Figure 3.2). From 2017 to 2020, we observed an increase in the number of birds reported singing Modulated-Doublets on wintering grounds and migratory routes of both Western and Central United States during migration (Figure 3.1). We observed a simultaneous emergence of the Modulated-Doublet in several breeding populations across the Western Provinces (British Columbia, Alberta, Saskatchewan) in 2014-2016 and in the Great-Lakes area starting in 2017 (Figure 3.2). In 2020, birds on the East coast still predominately sing Triplet-ending songs but some individuals along the coast are starting to sing Unmodulated-Doublets both during migration and breeding period (Figure 3.1 and 3.2).

3.5. Discussion

Our results show that the Modulated-Doublet emerged simultaneously and spread in parallel in the Pacific, Central and Mississippi migratory routes and in breeding populations of British Columbia, Alberta and Saskatchewan between 2014 and 2017. Modulated-Doublet observations increased concurrently in those regions over a period of 6 years, largely replacing the

Unmodulated-Doublet in those populations. We also observed the Modulated-Doublet appearing in breeding populations surrounding the Great-Lakes starting in 2017-2018.

In previous analyses of song shifts among White-throated Sparrows, evidence suggested doublet-ending songs originated in the westernmost part of the White-throated Sparrow range and spread eastward, replacing the ubiquitous Triplet-ending song (Otter et al. 2020). Part of the population breeding in central British Columbia (BC) migrates over the Rockies and overwinters in southcentral US but some birds are also known to follow the Pacific flyway and overwinter in California (Otter et al. 2020). California wintering grounds are thought to consist primarily of birds breeding on the western edge of the species range, making this wintering population isolated from the main breeding populations over much of Canada's Prairie and Eastern Provinces (Mazerolle et al. 2005; Otter et al. 2020). Peripheral and isolated populations can facilitate the emergence of novel songs; songs of these populations may evolve separately and deviate from the common song structure due to isolation and lack of influence and immigration from the main population (Kroodsma et al. 1999). Additionally, California wintering grounds are known to be less densely populated than Central and Eastern wintering grounds (Falls and Kopachena 2020). Small population size can facilitate the spread of novel variants within a population, as there is a higher chance that individuals singing novel songs are used by juvenile birds as tutors (Podos and Warren 2007). This would suggest that novel song may be more likely to emerge on California wintering grounds and/or peripheral and localized breeding populations in British Columbia. It may spread eastward, as these central BC breeding grounds are also occupied by individuals who traverse the Rocky Mountains and overwinter in the south-central US alongside birds from more central breeding populations.

Otter et al. (2020) suggested that doublet-ending song variants may have spread eastward in a wave-like fashion, replacing the triplet-ending songs. However, this wave-like spread may have been an artifact of the sampling regime available over this earlier study. Much of the recordings used to document the emergence of the doublet-ending song in western populations were recordings by the authors made from 2000-2010 that were heavily biased to western regions, and more broad-scale coverage was only available from 2010 onwards with the wide use of online sound libraries and citizen-science recordists. As the initial detection of the Modulated-Doublet song variant occurred after the expansion of public sound archives, it allowed us to more thoroughly document the pattern of early spread of a novel variant. Rather than spreading as a wave, the novel Modulated-Doublet seemed to emerge simultaneously and spread synchronously in multiple populations of the West and Prairies regions of the breeding range, as opposed to spreading sequentially between populations. Many of the breeding populations to which the song simultaneously spread share both migratory routes and wintering grounds in southcentral US (Mazerolle 2005; Otter et al. 2020). This would strongly suggest that the source of song variants exposure is on shared wintering grounds (or at least on shared migratory routes). Our study matches findings of others that White-throated Sparrows sing on migration and wintering grounds (Saunders 1947; 1948; Otter et al. 2020; Falls and Kopachena 2020); these shared locations could be where birds are introduced to and adopt novel song variants causing the parallel spread across a vast geographical area when birds migrate back north to breed. A similar mechanism was proposed to explain the spread of novel songs in humpback whale (*Megaptera novaeangliae*) populations in the central-south Pacific Ocean. Multiple new songs were recorded over an 11 year-period and spread between populations, with males converging on the novel song type and replacing the previous song types (Noad et al. 2000; Garland et al 2011). Males are possibly encountering and adopting novel songs on migration stop-overs shared by multiple populations (Owen et al. 2019). This may also explain why novel variants in White-throated Sparrows appear to be slower to spread to population of Eastern and Atlantic Canada and United States; birds breeding in the Atlantic and eastern Provinces migrate and overwinter on the Atlantic Coast with little overlap with populations wintering in south-central US (Mazerolle et al. 2005), decreasing the likelihood of being exposed to variants occurring in these central wintering grounds. Eastern wintering grounds are also more densely populated (Falls and Kopachena 2020) and novel songs may be drowned out and less likely to be picked up by juveniles. Our study shows these east-coast wintering grounds are still largely dominated with birds singing the original triplet-ending songs.

Sparrow species are known to overproduce songs as juveniles, but when returning to breeding grounds for the first time, they crystallize only the song that most closely matches their neighbour's song to facilitate counter-singing interactions (Nelson 1992; Marler 1997; Nelson 2000; Collins 2004; Beecher and Brenowitz 2005; Mennill et al. 2018). In large populations, this would result in most juvenile birds settling on the most common song type in the population and novel songs would not be likely to spread (Kroodsma et al. 1999, Gammon 2005). Simultaneous emergence and spread of the Modulated-Doublet in more densely populated central populations, suggest a strong selection for birds to adopt the Modulated-Doublet over the previous and more common variant. We previously suggested that the spread of novel songs in White-throated Sparrows could be driven by two transmission biases; a model-based bias where males preferentially learn the songs of the most successful males and a content-based bias, where a preexisting learning bias favours the adoption of songs following a certain species-specific structure (Boyd and Richerson 1985, Byers et al. 2010, Chapter 2). In White-throated Sparrows, females prefer songs that include the ending phrases over songs that only include the introductory phrase (Wasserman and Cigliano 1991). This suggests that females may be attentive to the terminal song phrases, and may be selecting males singing particular variants. If this preference includes novel song variants, males would gain an advantage to copy other males singing novel songs. However, not all documented novel song variants have spread in populations of White-throated Sparrows (Chapter 2) and both the Unmodulated- and Modulated-Doublets continued to spread even when they became the most common variant in a population, suggesting a second selective force (Otter et al. 2020; Chapter 2). Additionally, we previously showed that transition to the Modulated-Doublet appeared to occur faster within a population than the previous transition to the Unmodulated-Doublet. We hypothesized that there might be an additional learning bias which favours the adoption of songs closer in syncopation structure to the Triplet-ending song, which has historically been the most common ending type in this species (Borror and Gunn 1965; Zimmerman et al. 2016; Otter et al. 2020, Chapter 2). However, the modulation of Modulated-Doublet may create a structure that is closer to the species-specific triplet-ending song, as the double-pulse in the first syllable coupled with the second note of the doublet produces a “three-pulse” sound. This gives the impression that the song ends in repeated strophes of three notes. The transmission bias driving the spread of the Modulate-Doublet may have a stronger content-based bias component compared to the spread of the Unmodulated-Doublet, making the shift to the Modulated-Doublet faster within and potentially between populations.

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Chapter 4: Conclusions and further directions

The objective of this thesis was to document the emergence and spread of a novel song variant, the Modulated-Doublet, both within and between populations of White-throated Sparrows. I found that within our study population in Prince George, British Columbia, the proportion of males singing the Modulated-Doublet increased from 12% to 93% from 2015 to 2020. Additionally, the average modulation became more pronounced and returning males appeared to be able to modify their modulation between successive years, making it more pronounced over time. On a continental scale, the Modulated-Doublet appeared to emerge simultaneously and spread in parallel in breeding populations of British Columbia, Alberta and Saskatchewan, and on both Pacific and Central migratory routes. Modulated-Doublets are also becoming the predominant variant in these populations and appear to be replacing the Unmodulated-Doublet. At both the population and continental scales, the spread of the Modulated-Doublet appears faster than the previous continent-wide song shift of the Unmodulated-Doublet (Otter et al. 2020), suggesting the spread may be driven by multiple and stronger transmission biases.

On a continental scale, I expected the Modulated-Doublet to have emerged in the westernmost part of the White-throated Sparrow range and spread eastward, similarly to the spread of the Unmodulated-Doublet (Otter et al. 2020) and because isolated populations can favour the emergence and adoption of novel song elements (e.g., Kroodsma et al. 1999; Gammon 2007). As such, I predicted that novel song variants would be more likely to emerge either on California wintering grounds which are thought to be used only by birds breeding in the westernmost part of the range or in localized populations breeding in British Columbia. However, the data suggests that the Modulated-Doublet emerged simultaneously and is spreading in parallel in breeding populations of British Columbia and the Prairies and along the Pacific, Central and part of the Mississippi migratory flyways. The previous eastward movement of the Unmodulated-Doublet could result from the nature of the data used by Otter et al. (2020). Public sound libraries were not readily available or used in the early 2000s, and the authors used primarily personal recordings from western Canada to document the spread of the Unmodulated-Doublet from 2000 to 2010. As a result, simultaneous emergences on a larger scale could have been missed at the time. Alternatively, I could have missed an isolated emergence of the Modulated-Doublet in British Columbia since there were less recordings available on public sound archives for the earlier years of this study. However, given that we first documented the Modulated-Doublet in 2015 in our study population (Chapter 2) and found this variant spread rapidly over three years in both western and central parts of the range (Chapter 3), we most likely did capture the early pattern of song transition of the Modulated-Doublet. This pattern of spread provides further support to the hypothesis proposed by Otter et al. (2020) that White-throated Sparrows acquire novel variants on shared wintering grounds and migratory routes since central migratory routes and southcentral breeding grounds are shared by BC and Prairies breeding populations, where the emergence occurred simultaneously.

On a broader scale, if males do acquire songs during migration, it could imply that large-scale song shifts are more likely to occur in populations of migratory species than in non-migratory species. Migratory species have more opportunities to encounter novel songs from other breeding populations in shared locations such as migratory routes or wintering grounds. If those novel songs tap into a transmission bias, birds could preferentially adopt and transplant them when migrating back to various breeding grounds. Such a mechanism would predict that novel songs that invoke internal transmission biases within birds could spread rapidly among populations. In contrast, if a novel song emerges in a sedentary population, there are less opportunities for birds from other populations to encounter this song. Attractive novel songs could spread within the population, causing song shifts at a local scale, but with limited emigration and immigration of birds from other populations, song shifts would remain constrained to the population.

This research is part of a long-term study documenting cultural evolution in the song endings of White-throated Sparrows. This is the second, large-scale song shift documented in this species, following the spread of the Unmodulated-Doublet from central BC to Québec over a 20-year period (< 2000 to 2019) (Ramsay and Otter 2015; Otter et al. 2020). Zimmerman et al. (2016) also showed evidence that Triplet-ending songs in the 1960s were significantly different than the current Triplet-ending song produced by eastern birds. This suggests that other transitions in the song may have occurred over this period, but went unnoticed because they were subtle changes over time within a single song variant class (e.g., evolution of the structure within the triplet-ending songs) rather than involving a more noticeable shift in song variant class (triplet-ending vs doublet-ending). Finally, during this study, the modulation became more pronounced over time in our study population, to the point where two birds had a modulation so pronounced, it broke the S1 note and created songs ending in repeated strophes of three separate notes. This may indicate that the current Modulated-Doublet is a transition from a doublet-ending song back to a triplet-ending song, albeit it with a novel syncopation to the triplet compared to that still common in eastern North America. Together, this suggests that White-throated Sparrows go through continuous and large-scale cycles of song evolution.

Song shifts have previously been documented within several songbird populations e.g. (Byers et al. 2010; Janes and Ryker 2013; Trainer 1989) but rarely documented between populations or over larger scales (Whiten et al. 2019; but see Garland et al. (2011) for a large-scale song shift documented in Humpback whales). This study offers an example of a shift at both the local and continental scale and provides insight on the rate and patterns of spread at both scales and the mechanism of song transmission between populations. Directional shifts are often hypothesized to be driven by transmission biases (Boyd and Richerson 1985; Trainer 1989; Podos and Warren 2007; Byers et al. 2010; Garland et al. 2011). Similarly, I suggested song transitions in White-throated Sparrows are likely driven in part by a content-based bias, since not all novel songs spread and most songs that were historically predominant resemble triplet-ending songs in structure. In addition, since females may pay attention to song endings (Wasserman and Cigliano 1991), song transitions in this species could also be driven by female preference for novel songs. However, female preference needs to be tested to see if it does promote song shifts in White-throated Sparrows.

4.1. Further directions

4.1.1. Testing female preference for novel songs

Given both the rapid spread of novel song ending variants in White-throated Sparrows and previous evidence that females are attentive to the terminal strophes of the song (Wasserman and Cigliano 1991), I hypothesized that song shifts in this species are in part driven by female preference for certain types of novel songs. Further research could test this by conducting playback studies in mate choice chambers or aviaries and compare female responses to

Unmodulated-Doublets versus Modulated-Doublets. Mate choice experiments typically consist of presenting a female with two contrasting males (for phenotypical preferences) or two different songs and noting any behaviours that suggest a preference for one variant over the other. This could consist of differences in precopulatory displays, approach of male or speaker, time spent with each male, etc. (e.g., Searcy 1982; Wasserman and Cigliano 1991; Ballentine and Hill 2003). To test for female preference, an aviary could be divided into three chambers: a middle "neutral" chamber and two chambers at opposing ends of the aviary, each containing a 3-D White-throated Sparrow model and a speaker (for a similar design, see Grava et al. 2012). Females would be placed in the central chamber and the separate speakers would alternately broadcast a Modulated-Doublet vs an Unmodulated-Doublet or Triplet-ending song. An observer would record any pre-copulatory behaviour, approach to either speaker and time spent in each chamber and compare female response to both songs. Females from populations that predominately sing Unmodulated-Doublet or Triplet-ending songs should be selected for these trials, for the Modulated-Doublet to be considered novel to them. In addition, other artificial songs containing novel endings (e.g., "quartet-ending songs" – songs ending in repeated strophes of four notes) could be made and broadcasted to females to understand if females exhibit a preference for all novelty or if it is constrained to some song elements or structures.

An alternate approach to testing female preference would be the use of operant chambers (e.g., Riebel and Slater 1998; Kato et al. 2010). Captive females could be placed in chambers that had motion-activated perches – perches fitted with light sensors so that when the female lands on the perch she activates a speaker broadcasting a particular song variant. Females could be trained to activate song perches in the pre-trial training, obtaining a food reward whenever they activate the signal. Initially, the two perches would contain the same song, and perhaps just the introductory phrases of the song so as not to bias the female's choices. Once trained, the songs broadcast from the two perches could have the ending phrases added for the variants under investigation – e.g., a Modulated-doublet versus an Unmodulated-doublet, or doublet-ending vs triplet-ending. If females are equally rewarded whichever song perch is activated, yet there is a clear preference for the females to activate a perch with a particular song variant, it might indicate a preference by the female for that song type.

4.1.2. Spread of the Modulated-Doublet in breeding populations of central Canada

Our results suggest that the Modulated-Doublet emerged simultaneously and spread in parallel among breeding populations of British Columbia, Saskatchewan and Alberta. However, recordings taken from citizen science databases are not equally distributed over the landscape and over time, thus data could have gaps and emergence events could have been missed in some areas. There could also be biases in citizen-scientist data collection that songs that are atypical be more likely to be recorded and posted. Additionally, continent-scale maps show us where Modulated-Doublets have been recorded but not necessarily how quickly song transitions are occurring within local populations. Song shifts have been recorded previously within a BC population (Prince George – Chapter 2) and in an eastern interior population (Algonquin, ON – Otter et al. 2020). Obtaining recordings from the past five to ten years from a central population (Alberta, Saskatchewan, Manitoba) could confirm whether the Modulated-Doublet emerged simultaneously over a large geographical scale. Moreover, these populations share wintering grounds with both western (BC) and eastern populations (Mazerolle 2005; Otter et al. 2020); these data could be used to compare the rate of song transition in central populations to both western and eastern populations and confirm the importance and the role of shared migratory routes in the spread of novel variants.

4.1.3. Role of juveniles and adults in the spread of novel variants

I showed that returning males appeared to be able to modify their song to make their amplitude modulation more pronounced over time. However, due to small sample size and variability between males, I could not confirm that adult males contributed to the overall amplitude drop observed between years in our population. To get a better understanding of the roles of both juveniles and adult males in driving song shifts, further studies could compare the modulation of songs of Second-Year birds to songs of After Second-Year birds for each year and between years in our study population.

In addition, both Otter et al. (2020) and this thesis (Chapter 2) showed that initial rate of song transition within population is slow but becomes exponential once a certain threshold of birds singing the novel variant is reached. Both studies suggested this may be due to juveniles overproducing song (i.e., sing multiple song types) when they first return from migration and only settle on the novel song if it is reinforced on the breeding ground (i.e., selective attrition). To test if song transition is dependent on overproduction and selective attrition during song learning, we could record individual Second-Year birds multiple times during the breeding season and compare the variability in their song when they first returned from migration to the variability in their song later in the breeding season.

4.2. Conclusions

In this study, I documented a song shift in White-throated Sparrows at both the local and continental scale. I showed that the Modulated-Doublet went from being a rare song variant to becoming the predominant variant in our study population, with the modulation becoming progressively more pronounced over time. I also collected evidence that returning males were able to modify their song over time, however, whether returning males contribute to song transitions or if song transitions are mainly driven by juveniles remains unclear. Finally, I showed that the Modulated-Doublet is also spreading on a continental scale. My work builds on previous work by Ramsay and Otter (2015), Zimmerman et al. (2016) and Otter et al. (2020) who have been documenting song evolution in White-throated Sparrows for over 20 years. This study provides an example of a large-scale song shift which are rarely documented in the literature and offers insight on how song transitions occur both within and between populations of songbirds. Further research is needed to understand what drives large-scale song transitions in White-throated Sparrows. Additionally, long term monitoring should continue to determine if the Modulated-Doublet is a transition to a novel triplet-ending song, as suggested by two birds recorded in 2020 and to further document and understand the mechanisms behind cycles of song evolution in White-throated Sparrows.

4.3. References

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