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Alana Dawn Demko
University of Windsor

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THE FUNCTION AND EVOLUTION OF VOCAL AND VISUAL SIGNALS IN A NEOTROPICAL SONGBIRD, THE RUFOUS-CAPPED WARBLER (*BASILEUTERUS RUFIFRONS*)

by

Alana Dawn Demko

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University of Windsor

Windsor, Ontario, Canada

The function and evolution of vocal and visual signals in a Neotropical songbird, the Rufous-capped Warbler (*Basileuterus rufifrons*)

by

Alana Demko

APPROVED BY:

____
L. Benedict, External Examiner
University of Northern Colorado

____
C. Semeniuk
Great Lakes Institute for Environmental Research

____
S. Doucet
Department of Biological Sciences

____
D. Higgs
Department of Biological Sciences

____
D. Mennill, Advisor
Department of Biological Sciences

December 6, 2018

Declaration of Co-Authorship / Previous Publication

I. Co-Authorship

I hereby declare that this thesis incorporates material that is result of joint research, as follows: I am the sole author of Chapter 1 (General Introduction) and Chapter 7 (General Discussion). I am the primary author of Chapters 2–6, and my advisor, Dr. Daniel Mennill, is a co-author on all five of these chapters. Chapter 5 is co-authored by Dr. Stéphanie Doucet, and Chapters 5–6 are co-authored by Dr. José Roberto Sosa López. For all chapters, I developed the key ideas and experimental design, collected and analyzed the data, interpreted the data, and wrote the manuscripts. My co-authors Dr. Mennill, Dr. Doucet, and Dr. Sosa López provided logistical and financial support for data collection, and contributed to experimental design, data analysis and interpretation, and editing of manuscripts.

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

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II. Previous Publication

This thesis includes three original papers that have been previously published for publication in peer-reviewed journals, as follows:

Thesis Chapter	Publication title/full citation	Publication status
Chapter 2	Rufous-capped Warblers *Basileuterus rufifrons* show seasonal, temporal, and annual variation in song use	Published in IBIS
Chapter 3	Nest description and nesting behavior of the Rufous-capped Warbler (*Basileuterus rufifrons*)	Published in Ornitología Neotropical
Chapter 4	Male and female signaling behavior varies seasonally during territorial interactions in a tropical songbird	Published in Behavioral Ecology and Sociobiology

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Abstract

Animals communicate using multiple signaling modalities, with vocal and visual signals being the most prevalent in birds. The responses of animals to signal divergence among populations, along with the extent of divergence itself, may promote reproductive isolation and potentially speciation. Research on mating signal divergence, and experimental tests of responses to divergent signals, will expand our understanding of the mechanisms of reproductive isolation. In my dissertation, I investigated the form and function of vocal and visual signals in the Rufous-capped Warbler (*Basileuterus rufifrons*), a Neotropical resident songbird with complex song and bright plumage. I described male vocal behaviour in this species, and experimentally tested whether males and females showed seasonal variation in vocal behaviour during conspecific territorial interactions. I quantified range-wide variation in plumage, song, and morphology, and I experimentally tested whether two sympatric subspecies responded differently to each other's songs. I found that male Rufous-capped Warblers have large, complex repertoires, and vary their singing behaviour depending on the season, time of day, and presence of conspecifics. I found that female Rufous-capped Warblers sing, which is the first report of female song in this species. Females used both songs and calls to contribute to joint territory defence, but they approached playback and sang most often in the non-breeding season. My results suggest that Rufous-capped Warblers comprise two distinct species, the northern, white-bellied *B. rufifrons* and southern, yellow-bellied *B. delattrii*, based on phenotypic differences between them and low responses to heterotypic signals by two divergent subspecies living in sympatry. My research provides insight into the vocal behaviour of tropical resident wood-warbler species, and adds to the growing number of studies showing that female signals function in territory defence in tropical songbirds. My findings also support a revision of the Rufous-capped

Warbler's taxonomic status, thus refining our understanding of biodiversity in wood-warblers and Neotropical animals.

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Chapter 1: General Introduction

Introduction

Animals use multiple signaling modalities to communicate, including visual, acoustic, tactile, and chemosensory, and these signals play vital roles in animal survival and reproduction. Birds primarily communicate using acoustic signals such as songs and calls, and visual signals such as bright plumage colours and elaborate mating displays (Bradbury & Vehrencamp 2011). In birds, many acoustic and visual signals are sexually-selected traits (Searcy & Andersson 1986), which may contribute to reproductive isolation between populations, and potentially to speciation (Coyne & Orr 2004; Price 2008). In order to understand the role of these signals in promoting reproductive isolation, however, it is important to quantify patterns of signal divergence, and to understand how animals respond to divergent signals (e.g., Grant & Grant 2002; Uy et al. 2009; Dingle et al. 2010). In my dissertation, I examine acoustic signals and, to a lesser degree, visual signals to explore signal divergence in a tropical songbird.

Acoustic signals may evolve through ecological selection, sexual selection, drift, or a combination of all three processes (Wilkins et al. 2013). Through ecological selection, natural selection on morphological features (e.g., bill size) influences the structure of signals (e.g., Podos 2001; Benkman 2003; Aleixandre et al. 2013). Through sexual selection, signal divergence between populations corresponds to mate choice and competition for rivals (e.g., Patten et al. 2004; Danner et al. 2011). Through drift, signal divergence gradually increases between populations over time (e.g., Byers et al. 2010) and geographic distance (e.g., Irwin et al. 2008; Campbell et al. 2010). Although our understanding of signal divergence is largely focused on male signals, female signals may also evolve through social selection, which encompasses competition among conspecifics for resources including, but not exclusive to, mates (West-Eberhard 1983; Tobias et al. 2012).

The goal of my dissertation is to examine the function and evolution of acoustic and visual signals in a common yet little-studied Neotropical resident songbird, the Rufous-capped Warbler (*Basileuterus rufifrons*). Males and females of this species share conspicuous signals, including complex song and bright plumage, and I describe the structure and function of these traits for the first time in this dissertation. It is not known if these signals have similar functions in the two sexes, or if signal function also varies seasonally in these year-round territorial birds. Rufous-capped Warbler populations also vary geographically in plumage and song, and this variation broadly coincides with habitat differences related to vegetation and altitude (Howell & Webb 1995; Curson 2010). The extent of phenotypic differences between populations, and the responses of the warblers themselves to these signals, have not yet been quantified, despite the value of this information in resolving long-disputed taxonomic relationships within this clade (Ridgway 1902; Todd 1929; Monroe 1968; Howell & Webb 1995).

Vocal behaviour in songbirds and warblers

The oscine songbirds (i.e. Suborder Passeri of the Order Passeriformes) are among the few animal groups that learn their vocalizations from conspecifics (Beecher & Brenowitz 2005; Catchpole & Slater 2008), and they are therefore known for their highly diverse and complex songs. Males in most songbird species have repertoires of multiple song types (MacDougall-Shackleton 1997), and vary their patterns of song delivery depending on factors such as breeding status (Staicer 1996b), time of day (Nelson & Croner 1991), level of aggressive motivation (Järvi et al. 1980; Trillo & Vehrencamp 2005; Van Dongen 2006), and presence of particular conspecific neighbours (Beecher et al. 2000).

The Family Parulidae (commonly known as the 'wood-warblers') is a diverse family of over 100 New World songbird species. Among the wood-warblers that have been studied to date, many temperate-breeding migrant species, and at least one tropical resident species, Adelaide's Warbler (*Setophaga adelaidae*; Staicer 1996a), have two distinct subsets of songs in their repertoires. These 'song categories' or 'singing modes' vary structurally and in context of use (Spector 1992). The first category (Type I or A songs) are primarily used by unpaired males during the daytime and are often simpler in structure, whereas the second category (Type II or B songs) are primarily used by paired males, especially in the dawn chorus, and are often more complex in structure (Spector 1992; Staicer 1996a). First category songs were traditionally thought to function in male-female communication, and second category songs in male-male interactions (Kroodsma et al. 1989), although recent experimental work suggests that both categories may instead deliver different messages to conspecifics of both sexes (Beebee 2004). Although repertoire structure and use in the Parulidae is relatively well-studied in species of primarily migratory genera (e.g., *Setophaga*, *Vermivora*), our knowledge of song structure and singing behaviour in tropical resident genera is comparatively sparse (Spector 1992; Staicer 1996a). Furthermore, several tropical species in the genera *Basileuterus*, *Myioborus*, and *Myiothlypis* reportedly exhibit female song and male-female duets (although female song is likely under-reported in both migratory and resident wood-warblers; Spector 1992; Taff et al. 2012).

Female signals

Studies of the structure and function of elaborate animal signals have largely focused on males, whereas elaborate signals in females have received less research attention (Langmore 1998). Recent research suggests that female signals in birds are subject to similar selection pressures as male signals, but may be more strongly shaped by social selection (e.g., Tobias et al. 2011). In particular, females of many tropical bird species have bright plumage and complex songs equivalent to those of males (Stutchbury & Morton 2001). Many aspects of tropical species' behaviour, including the presence of female song, remain understudied (Stutchbury & Morton 2001), although female song has recently been acknowledged to be less rare than previously assumed, and also to be the ancestral state in songbirds (Odom et al. 2014). Female song may serve multiple functions including mutual mate attraction (Langmore et al. 1996; Tobias et al. 2011), territory defence (Krieg & Getty 2016), within-pair communication (Hall et al. 2015), or female-female competition (Langmore & Davies 1997; Cain & Langmore 2015).

Signal divergence and receiver response

The description of geographic variation in song and plumage features between closely-related taxa is a critical component of accurate taxonomic classification (Tobias et al. 2010). Although Neotropical bird diversity is extensive (Milá et al. 2012; Freile et al. 2014), we surely underestimate this diversity due to the lack of studies analyzing multiple phenotypic traits (e.g., morphology, plumage, and vocalizations) and molecular genetics (e.g., Cadena & Cuervo 2010; González et al. 2011; Caro et al. 2013; Sandoval et al. 2017). In particular, many Neotropical species and subspecies were first classified prior to the use of modern audio recording equipment and spectrophotometric analysis, and thus classification was based on morphological measurements and subjective assessments of plumage and voice differences (Ridgway 1902; Monroe 1968). Indeed, an increasing number of cryptic bird species (i.e. species with indistinguishable morphology and plumage) are now described based on acoustic divergence (e.g., Toews & Irwin 2008; O'Neill et al. 2011; Hosner et al. 2013). Since plumage and song are sexually-selected mating signals which could contribute to reproductive isolation (Coyne & Orr 2004; Price 2008), it is critical to quantify range-wide variation in these signals to accurately describe taxonomic relationships.

To investigate the contribution of signal divergence to reproductive isolation between populations, it is useful to examine not only the degree of signal difference, but also whether animals respond differently to the signals (Seddon & Tobias 2010; Hudson & Price 2014). Although responses to signal divergence often align with phenotypic and genetic divergence (e.g., Mendelson & Shaw 2005; Uy et al. 2009; Caro et al. 2013; Greig et al. 2015), animals may also show strong discrimination between similar signals (e.g., Grant & Grant 2002; Tobias & Seddon 2009; Grace & Shaw 2012) or discriminate little between apparently divergent signals (e.g., Gee 2005; Dingle et al. 2010). Additionally, character displacement, where animals show greater signal divergence or discrimination in sympatric compared to allopatric populations, may reduce hybridization in sympatry when hybrids are less fit (Gerhardt 2013; Hudson & Price 2014). Learning of signals, or learned preferences for signals, may also enhance or reduce reproductive isolation between populations (Verzijden et al. 2012). Learning can enhance reproductive isolation when animals learn to discriminate against heterotypic signals (e.g., Grant & Grant 2002; Seddon & Tobias 2010), but it can reduce isolation when animals instead learn the signals of a sympatric species or subspecies (e.g., McEntee et al. 2016; Kenyon et al. 2017). Experimental studies using playback of vocal signals (e.g., Grant & Grant 2002; Lemmon 2009; Grace & Shaw 2012) are useful to compare responses of animals to vocal variation between sympatric and allopatric populations, and to assess whether processes such as character displacement and learning are acting to enhance or reduce reproductive isolation.

Genus Basileuterus

The genus *Basileuterus* includes 11 Neotropical resident wood-warbler species distributed in Mexico, Central America, and South America (Curson 2010; Lovette et al. 2010; Chesser et al. 2016). Although several species are locally common or geographically widespread, few detailed studies exist of their natural history and behaviour (reviewed in Cox & Martin 2009). All *Basileuterus* species are sexually monochromatic, and some live as pairs on year-round territories (Curson 2010). *Basileuterus* warblers build dome-shaped nests with a side entrance, and females reportedly assume all nest-building and incubation duties (Stiles & Skutch 1989; Curson 2010), although descriptions of nesting behaviour, and even the nests themselves, exist for only four *Basileuterus* species (Skutch 1967; Greeney et al. 2005; Cox & Martin 2009).

No detailed analyses of vocal behaviour exist of any *Basileuterus* species, or of any species in two related tropical wood-warbler genera, *Myioborus* and *Myiothlypis* (Spector 1992). Female song is reported in only one *Basileuterus* species, the Three-striped Warbler (*B. tristriatus*: Spector 1992; Donegan 2014). However, given the prevalence of female song in tropical songbird species overall (Odom et al. 2014) and the lack of detailed behavioural studies in these genera, it is likely that female song exists in other congeners. Since repertoire structure coincides with phylogenetic relationships in some wood-warblers (e.g., *Setophaga*: Spector 1992; Wiley et al. 1994; Staicer 1996a) but not in others (e.g., *Cardellina*: Ammon & Gilbert 1999; Demko et al. 2013; *Geothlypis*: Byers 2015), detailed vocal behaviour studies of multiple species within each genus are needed to compare male and female repertoire structure and use at the genus and family level (e.g., Price & Lanyon 2004; Price 2009; Mason et al. 2017).

A recent phylogenetic analysis outlines generic relationships within the Family Parulidae (Lovette et al. 2010), and yet taxonomic classification is less well-defined at the species level. Species-level relationships have recently been revised within two *Basileuterus* clades. *B. culicivorus* and *B. hypoleucus* were grouped into one species based on molecular genetic analyses indicating monophyly (Vilaça & Santos 2010). *B. tristriatus* was split into three species based on allopatric distributions, genetic divergence, and vocal differences of Central and South American populations (Gutiérrez-Pinto et al. 2012; Donegan 2014).

The Rufous-capped Warbler

The Rufous-capped Warbler (*Basileuterus rufifrons*) is a widespread Neotropical resident warbler found from southern Arizona to southern Colombia. The taxonomic relationships between subspecies of Rufous-capped Warbler is a matter of historical controversy (Ridgway 1902; Todd 1929; Monroe 1968; Howell & Webb 1995). There are eight currently-recognized subspecies which differ considerably in plumage and vocalizations, but can be broadly categorized into two groups based on their distributions and phenotypic characteristics (Curson 2010; Figure 1.1). The northern *rufifrons* group (4 subspecies: *B. r. caudatus*, *dugesi*, *jouyi*, and *rufifrons*) lives in dry scrub habitats in southern Arizona, Mexico, and western Guatemala, has a white belly contrasting with the yellow throat and breast, and has repetitive songs containing few syllable types. The southern *delattrii* group (3 subspecies: *B. r. actuosus*, *delattrii*, and *mesochrysus*) lives in semi-open habitats and tropical dry forest in southeastern Mexico, Central America, and Colombia and Venezuela, has solid yellow underparts, and has songs containing many frequency-modulated syllable types (Howell & Webb 1995; Curson 2010). The final subspecies, *B. r. salvini*, which lives in the Atlantic lowland region of southern Mexico and northern Central America, has intermediate plumage between the *delattrii* and *rufifrons* groups, including a pale yellow or mixed white-yellow belly. Although the vocalizations of *B. r. salvini* are not well-described, its songs and calls appear to be similar to those of the neighbouring subspecies *B. r. rufifrons*, with which it reportedly intergrades (Howell & Webb 1995). Furthermore, *B. r. delattrii* and *B. r. rufifrons* are sympatric in southern Mexico at the limits of their respective ranges (Howell & Webb 1995), although the extent of phenotypic intergradation and competitive territorial interactions between the two subspecies remains undocumented. Although the *delattrii* and *rufifrons* groups were historically split into two species based on phenotypic differences (Todd 1929), the intermediate characteristics of *B. r. salvini*, and the possible intergradation between *B. r. rufifrons* and both *B. r. delattrii* and *B. r. salvini*, resulted in all subspecies being lumped taxonomically in subsequent analyses (Monroe 1968). No comprehensive phenotypic or genetic analyses have yet been conducted to resolve the taxonomic status of this clade (Curson 2010).

Dissertation overview

In my dissertation, I will describe the vocal behaviour of Rufous-capped Warblers, using both observational and experimental data to examine sex-specific and seasonal variation in vocal signal use during territorial interactions. I will also quantify range-wide phenotypic variation by using field and museum data to compare plumage, song, and morphology between Rufous-capped Warbler subspecies. Finally, I will conduct playback experiments to assess divergence in receiver response to vocal signals in two sympatric and potentially hybridizing subspecies in southern Mexico.

In Chapter 2, I will investigate the vocal behaviour of males in a colour-banded population of Rufous-capped Warblers (*B. r. delattrii*) in Costa Rica. I will test the hypotheses that males show repertoire specialization by using song categories or singing modes, and by using particular song types during conspecific interactions. This chapter is published in the journal *IBIS.* In Chapter 3, I will describe the nest structure and nesting biology of *B. r. delattrii*, using focal observations and video monitoring of nests with colour-banded adults during the nest-building, incubation, and nestling periods. This chapter is published in the journal *Ornitología Neotropical.* In Chapter 4, I will use a stereo playback experiment to test how male and female *B. r. delattrii* use vocal signals during territorial interactions, and to examine whether vocal signal use varies seasonally. I will also provide the first description of female song structure and use in this species. This chapter is published in the journal *Behavioral Ecology and*

Sociobiology. In Chapter 5, I will use data from field and museum specimens and online natural sound libraries to assess taxonomic relationships between subspecies based on morphology, plumage, and vocal differences, focusing on three divergent subspecies found in southern Mexico: *B. r. delattrii*, *B. r. rufifrons*, and *B. r. salvini*. In Chapter 6, I will experimentally test whether warblers' responses to the songs of two southern Mexican subspecies, *B. r. delattrii* and *B. r. rufifrons*, differ between sympatric and allopatric populations, and if these response differences potentially contribute to reproductive isolation between the two subspecies.

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Figures

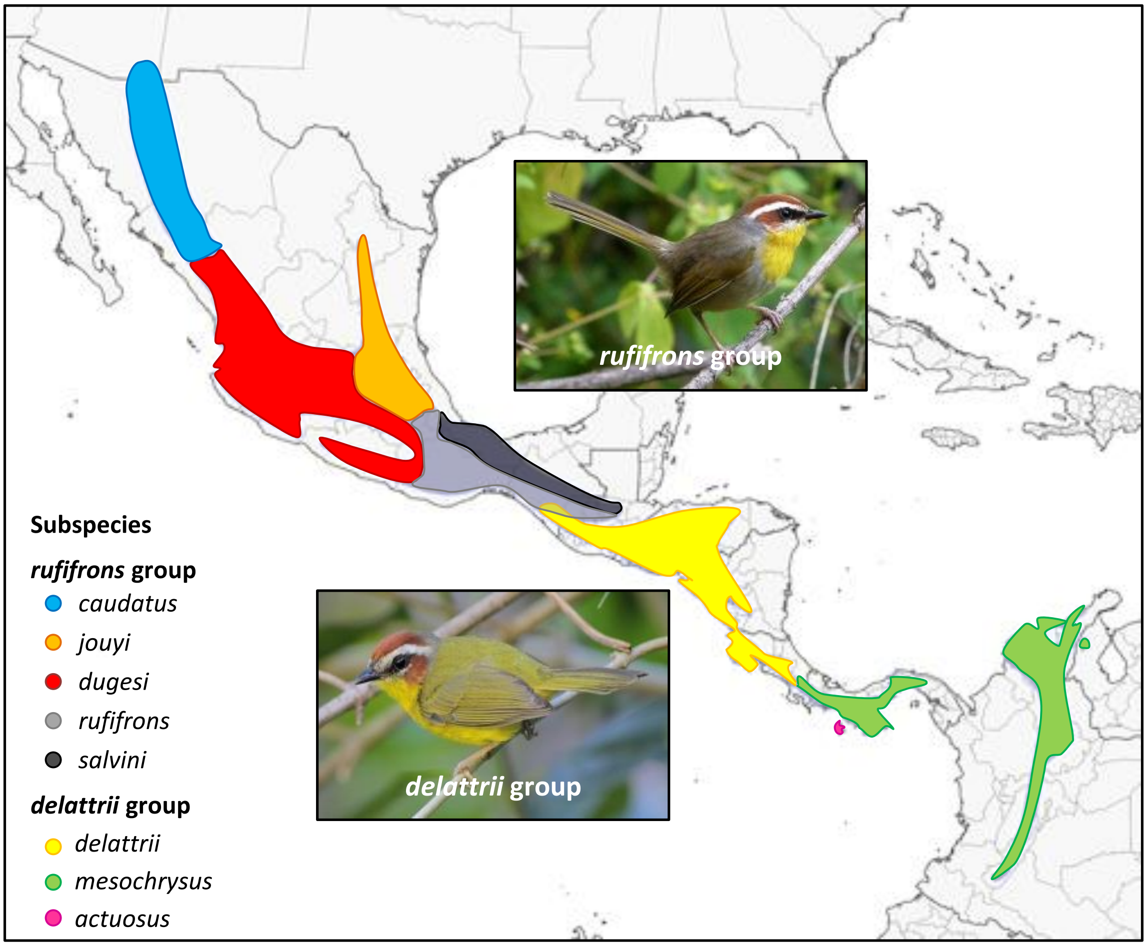

Figure 1.1. Range distributions of the eight recognized Rufous-capped Warbler subspecies. The *rufifrons* group (*B. r. caudatus*, *jouyi*, *dugesi*, and *rufifrons*) are found in Arizona, Mexico, and western Guatemala, whereas the *delattrii* group (*B. r. delattrii*, *mesochrysus*, and *actuosus*) live in southern Mexico, Central America, and South America. The intermediate subspecies *B. r. salvini* (black shading) lives in the lowland Atlantic region of southern Mexico and western Guatemala. *B. r. rufifrons* (grey shading) and *B. r. delattrii* (yellow shading) are sympatric in southern Chiapas, Mexico.

Chapter 2: Rufous-capped Warblers *Basileuterus rufifrons* show seasonal, temporal, and annual variation in song use

*****This chapter is the outcome of joint research with D. Mennill.

Chapter Summary

In the majority of songbird species, males have repertoires of multiple song types used for mate attraction and territory defence. The wood-warblers (Family Parulidae) are a diverse family of songbirds in which males of many migratory species use different song types or patterns of song delivery (known as ‘singing modes’) depending on context. The vocal behaviour of most tropical resident warblers remains undescribed, although these species differ ecologically and behaviourally from migratory species, and may therefore differ in their vocal behaviour. We test whether male Rufous-capped Warblers *Basileuterus rufifrons* use distinct singing modes by examining song structure and context-dependent variation in their songs. We recorded multiple song bouts from 50 male warblers in a Costa Rican population over three years to describe seasonal, diel, and annual variation in song structure and vocal behaviour. We found that Rufous-capped Warbler songs are complex, with many syllable types shared both within and between males’ repertoires. Males varied their song output depending on context: they sang long songs at a high rate at dawn and during the breeding season, but sang shortened songs in the presence of a vocalizing female mate. Unlike many migratory species, Rufous-capped Warblers do not appear to have different singing modes; they did not change the song variants used or the pattern of song delivery according to time of day, season, or female vocal activity. Our research provides the first detailed vocal analysis of any *Basileuterus* warbler species, and enhances our understanding of the evolution of repertoire specialization in tropical resident songbirds.

Introduction

The songs of birds are multipurpose signals that serve diverse functions including mate attraction and territory defence (Catchpole & Slater 2008). In over 70% of songbird species, males have repertoires of multiple song types that may serve specialized functions (MacDougall-Shackleton 1997, Catchpole & Slater 2008). Many species vary their use of particular song types or syllable types depending on breeding status, time of day, or level of aggressive motivation (e.g., Järvi et al. 1980; Nelson & Croner 1991; Kunc et al. 2005). Birds can also adjust their vocal behaviour depending on context by using different patterns of song delivery (e.g., Wiley et al. 1994; Trillo & Vehrencamp 2005) or varying their vocal output through changes in song rate (e.g., Benedict et al. 2012; Szymkowiak & Kuczyński 2016) or song length (e.g., Nelson & Poesel 2011). The evolution of specialized song types or vocal behaviour may be constrained by phylogeny (Mann et al. 2009), although female choice, male-male competition, and cultural drift likely drive repertoire diversity (Byers & Kroodsma 2009, Price 2013). Detailed vocal descriptions provide valuable insight into the evolution of acoustic communication and lay critical groundwork for comparative studies between closely-related species (e.g., Price & Lanyon 2004; Mann et al. 2009; Mason et al. 2017).

In many territorial bird species with repertoires of multiple song types, males share song types with neighbouring males and use them during territorial interactions (e.g., Beecher et al. 2000b; Vehrencamp et al. 2007; Camacho-Schlenker et al. 2011). Collectively, studies of such species suggest that song sharing is important for territory establishment and ongoing territory defence. For example, male Song Sparrows *Melospiza melodia* match song types with neighbours during male-male territorial interactions (Beecher et al. 2000b) and males with more shared songs have longer territory tenure (Beecher et al. 2000a). Males of many species use their repertoires differently across years through differential repertoire use or even learning new songs, in order to better match the song types or syllables used by their neighbours (e.g., McGregor & Krebs 1989; Payne & Payne 1993; Lemon et al. 1994; Vargas-Castro et al. 2012, 2015). Within and across species, song sharing is more prevalent in resident populations or short-distance migrants with high return rates than in long-distance migrants (Handley & Nelson 2005; Yoon et al. 2013), although some migratory populations show high sharing (Foote & Barber 2007). While much research has explored repertoire use during the breeding season in temperate songbird populations, little is known about seasonal and annual variation in the vocal behaviour of tropical resident species.

Tropical birds exhibit life-history traits which differ from those in temperate birds, including widespread year-round territoriality and long-term pair bonds, and these differences may influence the structure and function of their vocalizations (Stutchbury & Morton 2001). In tropical resident birds, singing for mate attraction and territory defence is not confined to the breeding season (Fedy & Stutchbury 2005; Topp & Mennill 2008; Tobias et al. 2011; Odom et al. 2017), as it is in most temperate species (Catchpole & Slater 2008). Although detailed vocal analyses are few, studies of tropical resident songbirds have revealed complex behaviours such as song-matching with neighbouring males during territorial interactions (e.g.,Vehrencamp et al. 2007; Price & Yuan 2011), song sharing combined with annual variation in song type use (Vargas-Castro et al. 2012, 2015), and use of different patterns of song delivery depending on context (e.g., Staicer 1996a; Molles & Vehrencamp 1999).

The wood-warblers (Family Parulidae) are a diverse family that includes both resident and migratory species, and studies of the vocalizations of some migratory wood-warblers have revealed an interesting system of vocal behaviour. Males in the genera *Setophaga*, *Mniotilta*, and *Vermivora* use two distinct subsets of songs or patterns of song delivery, known as 'singing modes', which vary in structure and context of use (Spector 1992). Type I songs are primarily used during day singing or male-female interactions and are often delivered in repeat mode (i.e. eventual variety, or repetition of one song type), whereas Type II songs are used during dawn singing or male-male interactions and are typically delivered in serial mode (i.e. immediate variety, or switching between multiple song types; Wiley et al. 1994; Staicer et al. 2006). Males of some migratory warbler species share more song types with neighbours than non-neighbours (Lemon et al. 1994; Beebee 2002; Janes & Ryker 2006; Demko et al. 2016), and add or drop songs from their repertoires between years depending on their use by neighbours (Lemon et al. 1994; Demko et al. 2016). According to the latest phylogenetic classification of the Parulidae, related species tend to share similar vocal behaviour (Spector 1992; Lovette et al. 2010), including both temperate and tropical *Setophaga* warblers (Spector 1992, Staicer 1996a). However, recent work on the genera *Cardellina* (Ammon & Gilbert 1999; Demko et al. 2013) and *Geothlypis* (Byers 2015) shows that congeners can exhibit different vocal behaviour.

Rufous-capped Warblers *Basileuterus rufifrons* are common tropical resident warblers that defend year-round territories (Contreras-González et al. 2010; Curson 2010). As with other *Basileuterus* warblers, their vocal repertoire is undescribed; temporal, seasonal, and annual variation in song type use or patterns of song delivery remains undocumented. The objective of our study is to test hypotheses related to repertoire structure and singing behaviour by describing song structure and vocal activity of a Costa Rican population of the subspecies *B. r. delattrii*. To test whether males have two singing modes, as do many temperate warblers, we investigated repertoire size and organization, and seasonal and temporal variation in use of different song types or patterns of song delivery. Based on the hypothesis that male Rufous-capped Warblers use two singing modes, we predicted that they would use specific song types or patterns of song delivery during particular seasons (non-breeding and breeding) or times of day (dawn and daytime; Spector 1992). We also investigated the potential role of repertoire sharing in mediating conspecific interactions by analyzing repertoire sharing between individuals, and annual variation in use of specific song types and syllables in the repertoire. Based on the hypothesis that songs are important in mediating conspecific interactions for Rufous-capped Warblers, we predicted that males should share more songs with neighbours than non-neighbours, and that use of specific song types and syllables should change across years (Lemon et al. 1994; Demko et al. 2016). We also briefly describe non-song vocalizations (calls). Our research is the first quantitative vocal analysis of any species in the tropical resident genera *Basileuterus*, *Myioborus*, or *Myiothlypis*, and provides a foundation for understanding the evolution of repertoire specialization in this group of tropical resident songbirds.

Methods

Study site and data collection

From April to July of 2013 to 2015, we studied a colour-banded population of Rufous-capped Warblers in Sector Santa Rosa, Área de Conservación Guanacaste, northwestern Costa Rica (10°51’N, 85°36’30”W). The warblers are common residents in second-growth and mature forest areas of this tropical dry forest. The time period of data collection coincided with the end of the dry (non-breeding) season (December to mid-May) and the beginning of the rainy (breeding) season (mid-May to November; Campos & Fedigan 2013). Over three field seasons, we sampled 50 focal males (2013: *n* = 14; 2014: *n* = 26; 2015: *n* = 31); 13 males were sampled in two consecutive years, and four males were sampled in all three years. We collected dawn and daytime recordings of territorial males using a Marantz PMD660 digital recorder, Sennheiser ME62 omnidirectional microphone, and Telinga parabola. At least once per season, we collected continuous dawn chorus recordings for each male, beginning at the male's first song of the morning (approx. 04:45 CST) until the end of the period of continuous song that we considered to be the dawn chorus (approx. 06:00). We collected 30–60 min daytime recordings between 06:30 and 12:00 approximately weekly from each male.

We captured focal birds in mist-nets using conspecific playback, and gave each bird a unique combination of colour-bands in order to identify individuals and differentiate between the sexes (Rufous-capped Warblers are sexually monomorphic). During the pre-breeding and breeding season (late April to July), we sexed birds in the hand by observing a cloacal protuberance for males or a brood patch for females. When we could not determine the bird's sex based on these characteristics, we instead determined sex based on their behaviour during territorial observations. Only males sing a continuous dawn chorus bout, and females are the primary nest-builders and sole incubators (Stiles & Skutch 1989; Chapter 3). Forty-six of the 50 focal males were colour-banded. Since the warblers have individually-distinctive song repertoires, we were able to confirm the identity of the four unbanded males by obtaining at least two recordings of a male warbler with a similar song repertoire on the same territory within the same year.

Repertoire size and song organization

Using Syrinx-PC sound analysis software (J. Burt, Seattle, WA), we annotated all male focal recordings and classified vocalizations at several levels of organization. We defined a syllable as the smallest continuous trace on a spectrogram (Catchpole & Slater 2008). We categorized each syllable based on its shape, duration, and frequency bandwidth, and thus produced a syllable catalogue for each focal male and a syllable pool of all syllable types observed in all males in the population (Byers 1995). A. Demko classified all syllables; to ensure objectivity, a second observer classified syllables for 50 songs and obtained >95% agreement in classification using two inter-observer reliability scores (Chapter 4). We defined a 'song variant' as a unique sequence of syllables in the same order, excluding consecutive repetitions of the same syllable type within a song. More generally, a 'song' was a syllable sequence separated by at least 0.5 s from another syllable sequence.

We measured several variables in order to describe song and repertoire organization for all 50 males. First, we calculated the total number of syllables per song variant, and the number of different syllable types per song variant (Rendall & Kaluthota 2013). We then used these values to calculate a syllable diversity index, which is the ratio of the number of different syllable types to the total number of syllables (Gil & Slater 2000; dos Santos et al. 2016). We calculated this index for each song variant, and then calculated an average of these values across all of a male's recorded song variants to obtain an average syllable diversity score for each male. A value of 1 indicates that every syllable within a song is used only once, whereas a value close to 0 indicates that syllable types are frequently repeated within a song. We measured syllable repertoire size and song variant repertoire size, and then used Kendall's *T* correlations to test whether these measures were correlated with the number of songs recorded (Podos et al. 1992; Gil & Slater 2000).

Our recordings revealed that Rufous-capped Warbler songs are complex, and not visually classifiable into song types (i.e. song variants that share similar sequences of syllables) in contrast to many other warbler species (e.g., Byers 1995, Staicer 1996a). Therefore, we used similarity indices and cluster analyses to quantify the degree of within-male repertoire organization, in order to determine whether a male's song variants can be objectively grouped into song types (Podos et al. 1992; Gil & Slater 2000). We used the Jaccard's distance adjusted for differences in song length to compare the number of syllable types shared between each of a male's song variants. We used the 'designdist' function in the 'vegan' R package (Oksanen et al. 2017) to calculate pairwise Jaccard's distances between all of a male's song variants to produce a distance matrix (e.g., MacDougall-Shackleton et al. 2009; Sosa-López & Mennill 2013). The adjusted Jaccard's coefficient, S_j (adj) was calculated as:

$$S_j\,(\mathrm{adj}) = c\,/((a + b + c) - d) \tag{1}$$

For two songs, X and Y, a was the number of unique syllables found in song X, but not in song Y; b was the number of unique syllables found in song Y, but not in song X; c was the number of shared syllables in songs X and Y; and d was the difference between the number of syllables in songs X and Y. We then used the 'pvclust' package (Suzuki & Shimodaira 2015) to test for the presence of clusters (song types) using hierarchical cluster analysis with average linkage. Cut-off values were selected for each cluster based on a boot-strapping analysis which assesses the probability ($\alpha = 0.05$) of obtaining a specific cluster compared to randomized groupings based on 1000 replications.

Seasonal and temporal variation in song use

To test whether male Rufous-capped Warblers use distinct singing modes, we calculated song rate (number of songs/min), number of song variants used, syllable diversity index, and song duration for multiple 20-song bouts per male (total number of bouts: $n = 141$, number of males: $n = 44$, number of bouts per male: 1–12). A song bout was a sequence of consecutive recorded songs separated by < 30 s (dos Santos et al. 2016). Song rate and song duration are standard measures of song output, and are known to differ between singing modes and times of day in other warbler species (e.g., Spector 1991; Staicer et al. 2006; Price & Crawford 2013). To calculate the relative occurrence of unique song variants within a bout, we calculated a Shannon-Wiener index using the 'vegan' R package (Oksanen et al. 2017). This index is often used to measure species richness in ecological communities, and is also useful for calculating the relative occurrence of commonly and rarely used song variants within a bout (Molles & Vehrencamp 1999). To assess whether Rufous-capped Warblers used different patterns of song delivery (eventual or immediate variety), we calculated the switching rate within song bouts, which is the number of transitions between different song variants divided by the total possible number of transitions (the number of songs in the bout minus 1). A switching rate of 1 indicates that each successive song variant in a bout is different (i.e. high-switching serial mode), whereas a switching rate of 0 indicates that each successive song variant is the same (i.e. low-switching repeat mode; Molles & Vehrencamp 1999). We were interested in whether female vocal activity influenced male singing behaviour, so when we recorded female calls in the background of the male focal recording during a song bout, we counted the female as 'vocal' and otherwise counted her as 'silent'. It is likely that females also attend to male vocalizations even when not vocalizing themselves. However, since the behaviour of females was more difficult to monitor when they were silent, we confined our analysis to vocalizing females. Since males of warbler species with two song categories use primarily Type II singing during the dawn chorus (Spector 1992), we also calculated the percentage of song variants that focal males used during both periods, compared to those used exclusively at dawn, for a subset of males (*n* = 15) with at least 50 songs recorded in each period in any given year.

We used linear mixed models to test the influence of season (non-breeding season vs. breeding season), time of day (dawn vs. day), whether the female vocalized near the male (vocal vs. silent), and the interaction effect between season and time of day on each of the response variables. In this analysis, we used season, time of day, season × time interaction, and female vocal activity as fixed effects, and bird identity as a random effect. We used likelihood ratio tests to estimate *P*-values of fixed effects, and ran post-hoc tests to quantify all significant effects using R's 'multcomp' package (Hothorn et al. 2017). For song switching rate, we applied the arcsine transformation to the response variable to meet the assumptions of linear mixed model analysis.

Syllable sharing

Since male Rufous-capped Warbler repertoires consist of song variants comprised of combinations of syllables rather than discrete song types (Figure 2.1), we quantified repertoire sharing at the level of the syllable. We calculated the adjusted Jaccard's similarity index to compare the presence or absence of specific syllable types in the syllable repertoires of all males to one another (MacDougall-Shackleton et al. 2009; Sosa-López & Mennill 2013). Using the pairwise sharing coefficients, we calculated the proportion of between-male syllable sharing within the study population. We then tested for the presence of clusters (groups of males with similar syllable repertoires) using hierarchical cluster analysis with average linkage, followed by boot-strapping analysis at $\alpha = 0.05$ with 10 000 replications. We ran separate analyses for 2013 ($n = 14$), 2014 ($n = 26$), and 2015 ($n = 31$).

Annual variation in song use

To document annual variation in repertoire use by focal males, we analyzed syllable repertoires and song bouts from all males with 2–3 years of available song recordings (n = 17). For each male, we measured whether specific song variants or syllables were added or dropped across years within the male's observed repertoire. This could indicate differential use of the existing song repertoire based on the presence or absence of specific neighbouring males that share those song types (e.g., Payne & Payne 1993; Nordby et al. 2007) or learning of new songs by males across years (e.g., Vargas-Castro et al. 2015). To quantify the proportion of song variants and syllables changed in a given male's repertoire between years, we calculated the Sørensen-Dice index (Eriksen et al. 2011; Vargas-Castro et al. 2015):

$$SD = 1 - (2N_s / (R_1 + R_2)) \tag{2}$$

In this formula, N_s is the number of song variants or syllables used in both years, R_1 is the repertoire size in year 1, and R_2 is the repertoire size in year 2. A value of 0 indicates that the repertoires were the same between years, and a value of 1 indicates that the entire repertoire composition changed between years. We converted the values to percentages in the Results. We conducted all statistical analyses using R v.3.4.3 (R Development Core Team 2017).

Non-song vocalizations and female song

In addition to male songs, we sampled calls produced by Rufous-capped Warblers on our focal recordings, as describing calls is a critical first step towards quantifying sex-specific differences in call structure and function (Digby et al. 2013; Benedict & Krakauer 2013). Owing to a small number of available high-quality call recordings from known-sex individuals, we provide a qualitative description of 'chip' call types recorded from male and female Rufous-capped Warblers during territorial interactions. We also recorded rare instances of female song during our focal recording collection and qualitatively describe these vocalizations.

Results

Male repertoire size and song organization

Our analyses revealed that male Rufous-capped Warblers have complex vocal repertoires comprised of many song variants produced from a small pool of syllables. On average, males had repertoires of 181 ± 166 song variants (range: 22–820) and 42 ± 9 syllable types (range: 26–64; $n = 50$). There was little syllable repetition within songs; the average within-male syllable diversity was 0.78 ± 0.06 (range: 0.63–0.90). Since the song variant repertoire size was strongly correlated with the number of songs recorded (Kendall's $T = 0.63$, $P < 0.001$, $n = 50$) which varied considerably between males (range: 62–2491), we chose a subset of males that had been recorded extensively (> 500 songs; $n = 24$) to accurately describe the relationship between aspects of repertoire complexity. There was a significant positive correlation between song variant repertoire size and the number of songs recorded ($T = 0.32$, $P = 0.03$, $n = 24$), although the syllable repertoire size was not significantly correlated with the number of songs ($T = 0.11$, $P = 0.47$, $n = 24$). Males with larger syllable repertoires did not have larger song variant repertoires, as syllable and song variant repertoire sizes were not significantly correlated ($T = 0.18$, $P = 0.22$, $n = 24$). Rufous-capped Warbler repertoires were not objectively classifiable into song types (i.e. groups of song variants that share similar sequences of syllables). Repertoire size estimates based on shared syllable types ranged from 1–60 song types (mean ± SD = 14.7 ± 14.3), and these categories were not consistent with visual classification of song variants based on syllable similarity.

Seasonal and temporal variation in song use

Male Rufous-capped Warblers showed seasonal and temporal variation in song rate during active singing bouts. For song rate, there was a significant season × time of day interaction (Table 2.1). During the non-breeding season, males (*n* = 44) sang at a similar rate during dawn and daytime singing (estimate = -0.11 ± 0.97, *t* = -0.1, *P* = 0.91; Figure 2.2A), but during the breeding season, males sang at a significantly higher rate at dawn than during the day (estimate = 2.72 ± 0.37, *t* = 7.4, *P* < 0.001; Figure 2.2B). Female vocal activity did not have a significant effect on male song rate (Table 2.1).

Song duration varied according to season, time of day, and female vocal activity. For song duration, there was a significant season × time of day interaction (Table 2.2). During the non-breeding season, songs were of similar length during both dawn and daytime singing (estimate = -0.03 ± 0.11, *t* = -0.3, *P* = 0.75; Figure 2.3A), but during the breeding season, males sang significantly longer songs at dawn than during the day (estimate = 0.20 ± 0.04, *t* = 4.8, *P* < 0.001; Figure 2.3B). Males also sang longer songs overall during the breeding season than during the non-breeding season (estimate = -0.31 ± 0.05, *z* = -5.7, *P* < 0.0001). Female vocal activity had a significant effect on male song duration (Table 2.2); males sang shorter songs when the female was vocalizing compared to when she was silent (estimate = -0.11 ± 0.05, *z* = -2.13, *P* = 0.03; Table 2.3).

The switching rate between song variants changed significantly with time of day (likelihood ratio test: $\chi^2_1 = 3.85$, *P* = 0.05; Table 2.S1). Switching rates were higher during dawn singing compared to daytime singing (estimate = -0.05 ± 0.02, *z* = -2.2, *P* = 0.03), although switching rates were high (mean > 0.90; Table 2.3) during both time periods. Neither song variant diversity (Shannon-Wiener index), syllable diversity, nor the number of song variants per bout varied significantly with season, time of day, or female vocal activity (Tables 2.3, 2.S2–2.S4). Males used many song variants exclusively during dawn or daytime singing; males (n = 15 with the most songs recorded) used 77.3 ± 11.2% (range: 50–90%) of their song variants exclusively in dawn or daytime singing within a given year.

Syllable sharing

Males shared many syllable types with neighbours. In a cluster analysis of syllable similarity across all three years, 49 of the 50 focal males grouped into 3–5 clusters of males with similar syllable repertoires (Figure 2.4). Average syllable sharing between all males in the population was low (mean ± SD: S_j (adj) = 0.11 ± 0.09), but was highly variable across pairs of males (pairwise range: 0.01–0.90). Males shared twice as many syllables with other males within the same cluster (S_j (adj) = 0.16 ± 0.13; within-group range: 0.15–0.44; pairwise range: 0.02–0.90) than with males in different clusters (S_j (adj) = 0.08 ± 0.04; pairwise range: 0.01–0.26). Within years, males in nearby locations at the study site had similar syllable repertoires. In 2015, there were three exceptions: males 92, 93, and 94 grouped vocally with males outside of their geographical area (Figure 2.4). Male 92 had no immediate neighbours among the other focal birds in the study population; males 93 and 94 in the IQ Trail area both claimed their territories in mid-June and likely moved there from other locations where they would have been exposed to different syllable and song types. Most males present in more than one year remained in the same cluster across years (Figure 2.4).

Annual variation in song use

Males varied both the syllable types and song variants used in their repertoires between years. Males (n = 17) changed 79.8 ± 17.0% (range: 42.6–100%) of their song variants between seasons. They changed relatively few syllable types, however, showing only 8.4 ± 9.3% turnover of syllables between seasons (range: 0–45%). Nearly half (mean ± SD = 40.6 ± 22.5%) of the syllables that males changed between seasons were 'rare' syllables that occurred in only 1–2 annotated songs in any given year. The percentage of rare syllables present in all of a male's changed syllables varied considerably between individuals (range: 0–83.3%), and may reflect variation in sampling effort (i.e. rare syllables are more likely to be missed in a smaller sample of songs).

Non-song vocalizations

Male and female Rufous-capped Warblers produced call notes during a variety of conspecific interactions. Both sexes produced at least six different 'chip' call variants (Figure 2.5A) during territorial interactions with other warblers and during foraging activities, especially during the non-breeding season. Females (*n* = 17) also gave 'chip' calls when vocalizing near a singing male partner (Figure 2.5A). Both sexes also produced high-pitched, short-duration alarm calls during perceived threats to their nest site or fledglings (Figure 2.5B).

Female song

Female Rufous-capped Warblers sing rarely. In our three-year study, we recorded confirmed spontaneous song from a banded female only once, on a dawn chorus recording from the start of the breeding season on June 4, 2015. This was the day after the first major rain event of the season, and the first day when we observed female warblers nest-building that year. The singing female had recently paired with a new mate, her former neighbour, after May 26. In a concurrent playback study, we found that female Rufous-capped Warblers occasionally sing in response to conspecific playback of male songs and female calls (Chapter 4). The female songs produced spontaneously during the current study were similar in structure to the songs females produce in response to playback, in that they were shorter with fewer syllables and syllable types than male songs (Chapter 4).

Discussion

Male Rufous-capped Warblers have large, complex repertoires of song variants produced from a small pool of approximately 45 syllables. In support of the hypothesis that Rufous-capped Warbler songs are important in mediating conspecific interactions, our results suggest that males may use shared songs and syllables during these interactions, given that they share more syllable types with neighbours than non-neighbours. They also use the same syllables across years, but they change the particular song variants used annually. Rufous-capped Warblers also have complex vocal behaviour: they vary song structure and use according to season, time of day, and social context. In particular, males sing at the highest rates with the longest songs during the breeding season dawn chorus, but sing shortened songs in the presence of a vocalizing female. Although switching rates between songs were higher at dawn than during the day, males switch frequently between songs at all times of day. We did not find support for the hypothesis that Rufous-capped Warblers use two singing modes; in contrast to many wood-warblers, this species sings with immediate variety and high within-song complexity irrespective of time of day, breeding status, or female vocal activity. The vocal behaviour of Rufous-capped Warblers, which display complex and variable repertoires without apparent singing modes, differs from other warbler species studied to date (e.g., Spector 1992; Demko et al. 2013), but is similar to other songbird species with complex multi-syllable repertoires (e.g., Willow Warblers *Phylloscopus trochilus*: Gil & Slater 2000; House Wrens *Troglodytes aedon*: Rendall & Kaluthota 2013).

We found that although Rufous-capped Warblers do not have two distinct singing modes, their complex repertoires differ in structure and use from other wood-warbler species (reviewed in Spector 1992). Warblers with two singing modes typically only sing structurally complex songs with immediate variety in Type II singing (e.g., Byers 1995); in warblers with a single singing mode, males generally sing a single, stereotyped primary song type in most contexts (Lein 1981, Ritchison 1995). Apart from Rufous-capped Warblers, the only known single-mode singers with complex songs are three tropical *Geothlypis* species, which have elaborate songs in contrast to their other tropical resident and migratory congeners (Byers 2015). Interestingly, many comparative studies in songbirds to date suggest that breeders in higher-latitude or seasonally variable habitats, rather than tropical breeders, have more elaborate songs, perhaps owing to heightened sexual selection pressures (e.g., Botero et al. 2009; Weir & Wheatcroft 2011; Kaluthota et al. 2016; Xing et al. 2017). Further detailed work on the vocal behaviour of the tropical resident genera *Basileuterus*, *Myioborus*, and *Myiothlypis* is necessary to discern large-scale patterns in repertoire use in the Family Parulidae.

Males show the highest song output during the breeding season dawn chorus, when they increase both song rate and song duration. This pattern is similar to that observed for many migratory warbler species (Spector 1992; Staicer et al. 1996) and the well-studied tropical resident Adelaide's Warbler *Setophaga adelaidae*, which sings a dawn chorus only during the breeding season even though it is territorial year-round (Staicer 1996b). In many songbird species, dawn chorus singing serves to defend territories against rival males (e.g., Liu 2004; Amrhein & Erne 2006; Foote et al. 2011). This is a likely function of the dawn chorus in Rufous-capped Warblers as well, since they appear to be more exclusively territorial in the breeding season. During the non-breeding season, we occasionally observed non-territorial adult warblers on a focal pair's territory, but never during the breeding season (A. Demko pers. obs.). High song output, particularly high song rate, may also be a male signal to social or extra-pair female mates (e.g., Cockburn et al. 2009). In several species, males with higher dawn song rates within a population show reproductive benefits: they obtain a mate earlier (Hofstad et al. 2002; Murphy et al. 2008) or have female mates who lay eggs earlier (Poesel et al. 2001). Furthermore, male Field Sparrows *Spizella pusilla* sing at the highest rates at dawn during the incubation and nestling periods (Zhang et al. 2015), suggesting that high song rate in this species is a signal to other males and possibly extra-pair females. Further analyses examining seasonal variation in song output in individual warblers of known breeding status would be valuable to determine the function of the increased song rate and song length we observed during the dawn chorus.

Male Rufous-capped Warblers sing shorter songs when their female partner is vocalizing nearby. This finding corresponds with a recent hypothesis suggesting that short, simple songs are directed to females, and better allow them to detect and compare the quality of male vocalizations (Price 2013). Indeed, use of short or stereotyped songs near females is documented for other songbird species with both single-song repertoires (Nelson & Poesel 2011) and specialized singing modes (Ficken & Ficken 1967; Kroodsma et al. 1989). Shortened songs may be directed signals to stimulate the female during her fertile period, and to leave the nest during the incubation period, as shorter songs are thought to draw less attention from predators or rivals (Nelson & Poesel 2011). However, males also use long, complex songs at times when both male and female conspecifics would be listening, such as during the dawn chorus. Therefore, variation in specific song components (e.g., song duration) may convey different messages to male and female conspecifics depending on context of use (e.g., Molles 2006). Future work comparing responses of both sexes to different patterns of song delivery and song durations would be useful to determine the intended receivers and social function of each signal component.

The finding that males in our population share many syllable types with their neighbours suggests that using particular song or syllable types is beneficial for territory defense during male-male interactions (Beecher & Brenowitz 2005). In many songbird species, males use shared song types or series of syllables to interact with conspecific neighbours during territorial boundary disputes (e.g., Beecher et al. 2000b; Anderson et al. 2005; Vehrencamp et al. 2007; Price & Yuan 2011). This is likely in Rufous-capped Warblers as well, since pairs establish and defend territories year-round using their songs (Chapter 4), and share the same neighbours over multiple years (this study). Since female Rufous-capped Warblers also sing during conspecific interactions, use of shared songs may also be important in male-female interactions. In Banded Wrens *Thryophilus pleurostictus*, a species with female singing behaviour similar to that of Rufous-capped Warblers (i.e. females sing shorter, less complex songs than males and do not sing coordinated duets), female song is primarily used in male-female communication between pair members and in territory defence (Hall et al. 2015). Further experimental work could clarify whether Rufous-capped Warblers, and other year-round resident tropical species with long-term territory tenure across years, use particular matching songs or syllables during both intrasexual and intersexual conspecific interactions (e.g., Beecher et al. 2000b; Vehrencamp et al. 2007).

The large annual turnover in the specific song variants used by male Rufous-capped Warblers suggests benefits to changing songs over time. One explanation is that specific song variants convey messages to other individuals in the population, and that presence or absence of those individuals across seasons or years may drive annual song turnover. In many songbirds, males can add or drop songs or syllables in order to more closely match the repertoires of neighbouring males (McGregor & Krebs 1989; Lemon et al. 1994; Nicholson et al. 2007; Demko et al. 2016). Another explanation for the observed pattern of song sharing is cultural drift, whereby males vary their repertoire use over time, based on copying of specific syllables or variants in the population (e.g., Byers et al. 2010). In Rufous-capped Warblers, an analysis of countersinging bouts between neighbouring males would be necessary to evaluate whether males use matching songs during vocal interactions, and whether arrival or departure of specific neighbours affects whether a focal male uses particular song variants in a given year.

Our study revealed that Rufous-capped Warblers have complex repertoires comprised of a finite number of syllables that males can recombine to produce a large number of song variants, or unique sequences of syllables. Syllables, rather than whole songs, appear to be the fundamental learned unit of the repertoire. Males share many syllable types with neighbours and change the song variants and syllables used across years, suggesting that both sharing and annual song variation are important signals to conspecifics. Males vary their singing behaviour primarily by increasing song rate and song duration during the breeding season dawn chorus, perhaps as a strategy to defend territories from other males, and by decreasing song duration near female mates, perhaps as an advertisement signal. Our results suggest that in contrast to other migratory and tropical resident warblers studied to date, Rufous-capped Warblers have complex repertoires and vocal behaviour without apparent singing modes. Our research thus contributes towards comparative studies of repertoire specialization across the Family Parulidae. Our study will also inform a comparison of vocalizations and vocal behaviour with a northern subspecies of Rufous-capped Warbler *B. r. rufifrons*, which has distinct vocalizations, plumage patterns, and territorial behaviour from the southern *B. r. delattrii* (Curson 2010, Chapter 5).

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Tables

Table 2.1. Linear mixed model and post-hoc comparison results of variation in song rate of male Rufous-capped Warblers (*n* = 44) according to season, time of day, and female vocal activity.

Song rate			
Full LMM	χ^2	df	*P* value
Time of day	**37.67**	**1**	**<0.001**
Season	**8.92**	**1**	**0.003**
Female vocal activity	0.57	1	0.45
Time of day × Season	**7.28**	**1**	**0.007**
Post-hoc comparisons	Estimate ± SE	*t* value	*P* value
Breeding season			
Dawn-Day	**2.72 ± 0.37**	**7.4**	**<0.001**
Non-breeding season			
Dawn-Day	-0.11 ± 0.97	-0.1	0.91

Table 2.2. Linear mixed model and post-hoc comparison results of variation in song duration of male Rufous-capped Warblers (*n* = 44) according to season, time of day, and female vocal activity. Significant effects are highlighted in bold.

Song duration			
Full LMM	χ^2	df	*P* value
Time of day	**16.68**	**1**	**<0.001**
Season	**24.77**	**1**	**<0.001**
Female vocal activity	**4.62**	**1**	**0.03**
Time of day × Season	**3.90**	**1**	**0.05**
Post-hoc comparisons	Estimate ± SE	*t* value	*P* value
Breeding season			
Dawn-Day	**0.20 ± 0.04**	**4.8**	**<0.001**
Non-breeding season			
Dawn-Day	-0.03 ± 0.11	-0.32	0.75

Table 2.3. Comparison of acoustic variables measured from 20-song bouts (*n* = 141) of male Rufous-capped Warblers (*n* = 44 males). Data are summarized relative to time of day, season, and female vocal activity; all values are mean ± SD. The sample sizes below each category indicate the number of bouts analyzed.

	Pre-breeding season		Breeding season		Female vocal activity	
Acoustic variable	Dawn (*n* = 22)	Day (*n* = 5)	Dawn (*n* = 68)	Day (*n* = 46)	Vocal (*n* = 26)	Silent (*n* = 115)
Song rate (songs/min)	8.33 ± 2.30	8.40 ± 2.41	10.36 ± 2.27	7.59 ± 1.46	8.38 ± 2.00	9.23 ± 2.46
Song duration (s)	2.09 ± 0.24	2.11 ± 0.20	2.44 ± 0.31	2.27 ± 0.28	2.16 ± 0.25	2.36 ± 0.32
Number of song variants	14.05 ± 3.47	15.00 ± 3.54	13.84 ± 3.83	14.07 ± 3.46	14.04 ± 3.88	13.97 ± 3.57
Song variant diversity	2.44 ± 0.41	2.54 ± 0.40	2.46 ± 0.38	2.47 ± 0.37	2.43 ± 0.45	2.47 ± 0.36
Switching rate	0.97 ± 0.07	0.94 ± 0.07	0.97 ± 0.05	0.95 ± 0.07	0.94 ± 0.09	0.96 ± 0.05
Syllable diversity	0.80 ± 0.08	0.82 ± 0.10	0.79 ± 0.07	0.80 ± 0.07	0.81 ± 0.08	0.79 ± 0.07

Figures

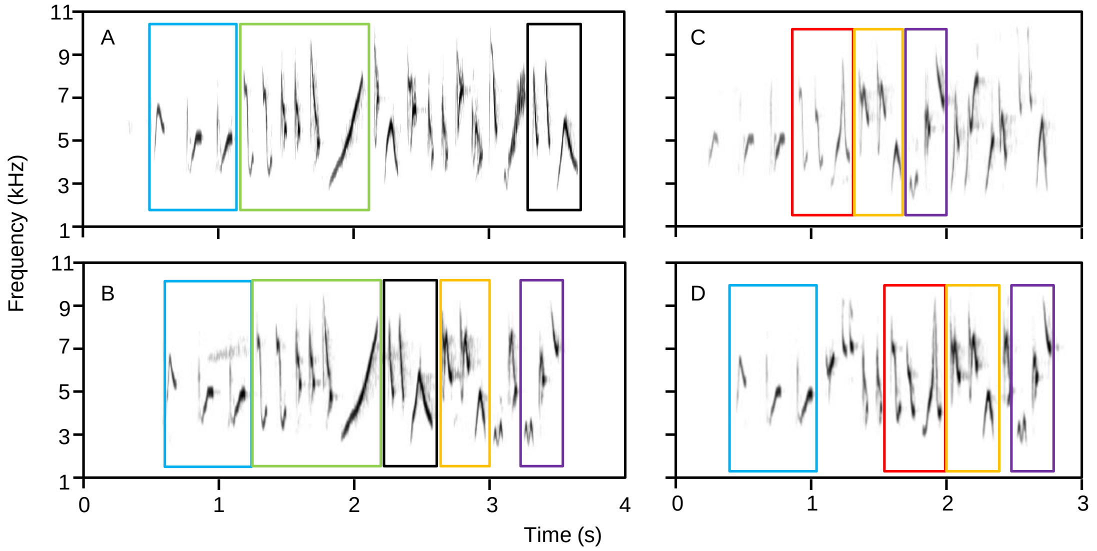

Figure 2.1. Sound spectrograms depicting examples of four song variants from one male Rufous-capped Warbler. This male used each syllable type in multiple song variants. For example, he used the same three-syllable group (in black box) at end of song A and middle of song B.

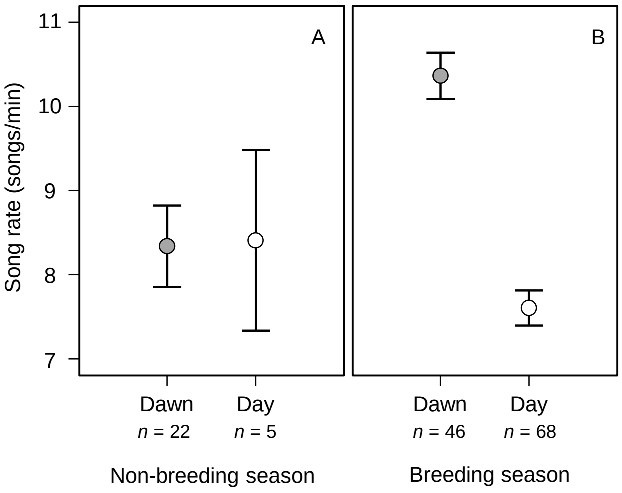

Figure 2.2. Male Rufous-capped Warblers (n = 44) sang at similar song rates during dawn and daytime singing in the non-breeding season (A), but sang at significantly higher rates during dawn singing than during the day during the breeding season (B). Error bars represent standard error around the mean. Sample sizes below bars indicate the number of song bouts analyzed.

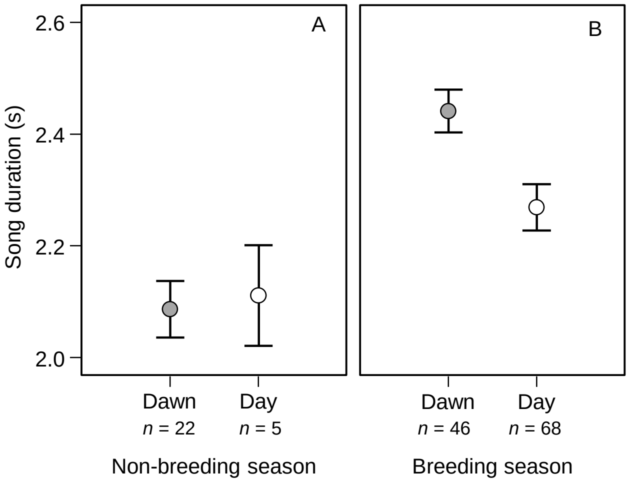

Figure 2.3. Male Rufous-capped Warblers (*n* = 44) sang songs of similar duration during dawn and daytime singing in the non-breeding season (A), but sang significantly longer songs at dawn than during the day during the breeding season (B). Error bars represent standard error around the mean. Sample sizes below bars indicate the number of song bouts analyzed.

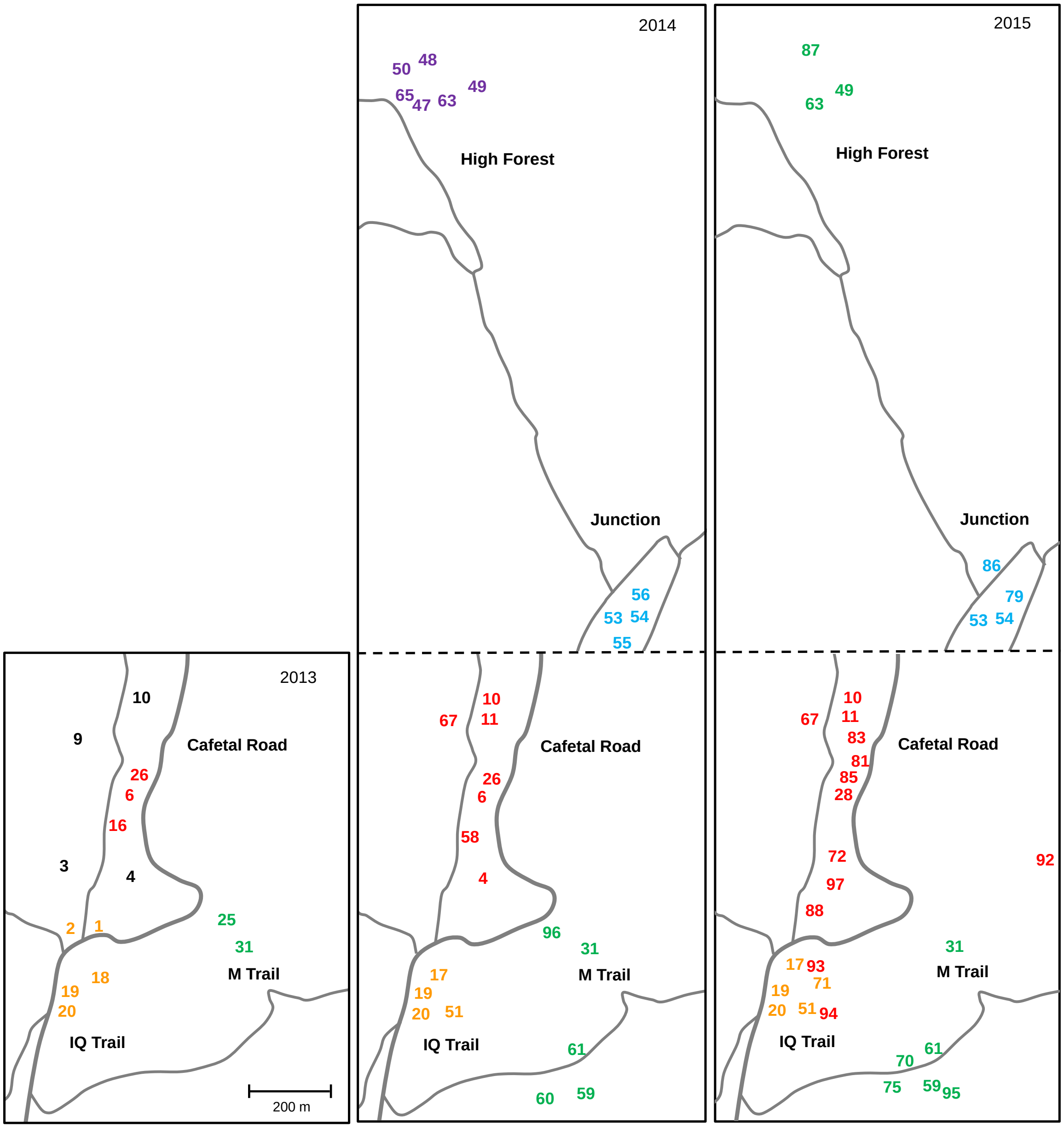

Figure 2.4. Male Rufous-capped Warblers clustered into 3–5 groups in 2013–2015 according to syllable repertoire similarity. Each group consisted of neighbouring males from the same location at the study site (High Forest, Cafetal Road, Junction, M Trail, and IQ Trail), except for birds 92 (M Trail) and 93-94 (IQ Trail) in 2015. Numbers represent individual males (2013: *n* = 14; 2014: *n* = 26; 2015: *n* = 31), and colours indicate cluster membership. Breaks in 2014 and 2015 maps indicate gaps without monitored territories; the distance between the north end of the bottom map and south end of the top map is approximately 650 m.

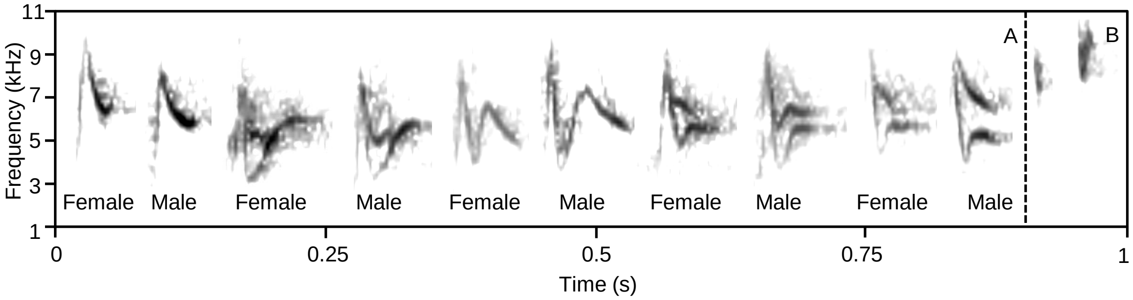

Figure 2.5. Examples of Rufous-capped Warbler ‘chip’ calls produced by both sexes during territorial interactions and foraging (A), and an alarm call used by both sexes during perceived threats to the nest site or fledglings (B). Calls in (A) were recorded from different individual males and females and represent our best-quality recordings; calls in (B) are from individuals of unknown sex.

Supplementary Material for Chapter 2

Table 2.S1. Linear mixed model results of variation in switching rate of male Rufous-capped Warblers (*n* = 44) according to season, time of day, and female vocal activity. Significant effects are highlighted in bold.

Switching rate			
Full LMM	χ^2	df	*P* value
Time of day	**3.85**	**1**	**0.05**
Season	2.18	1	0.14
Female vocal activity	1.22	1	0.27
Time of day × Season	0.57	1	0.45

Table 2.S2. Linear mixed model results of variation in song variant diversity index of male Rufous-capped Warblers (*n* = 44) according to season, time of day, and female vocal activity. No effects were significant.

Song variant diversity index			
Full LMM	χ^2	df	*P* value
Time of day	0.06	1	0.81
Season	1.05	1	0.31
Female vocal activity	0.02	1	0.88
Time of day × Season	1.98	1	0.16

Table 2.S3. Linear mixed model results of variation in syllable diversity index of male Rufous-capped Warblers (*n* = 44) according to season, time of day, and female vocal activity. No effects were significant.

Syllable diversity index			
Full LMM	χ^2	df	*P* value
Time of day	0.44	1	0.51
Season	0.06	1	0.81
Female vocal activity	0.19	1	0.67
Time of day × Season	0.57	1	0.45

Table 2.S4. Linear mixed model results of variation in number of song variants used by male Rufous-capped Warblers (*n* = 44) according to season, time of day, and female vocal activity. No effects were significant.

Number of song variants			
Full LMM	χ^2	df	*P* value
Time of day	0.19	1	0.67
Season	2.52	1	0.11
Female vocal activity	0.60	1	0.44
Time of day × Season	1.80	1	0.18

Chapter 3: Nest description and nesting behaviour of the Rufous-capped Warbler (*Basileuterus rufifrons*)

*****This chapter is the outcome of joint research with D. Mennill.

Chapter Summary

The breeding biology of most tropical resident bird species is little-documented, particularly for sexually monomorphic species in which sex-specific contributions to nest-building and nestling provisioning are challenging to quantify. We describe nest structure and adult behaviour throughout the nesting cycle for a colour-banded population of Rufous-capped Warblers (*Basileuterus rufifrons*), a widespread sexually monomorphic tropical resident songbird. Using focal observations of 11 nests, combined with video monitoring during the incubation and nestling periods, we provide new information on this species' nesting behaviour, particularly sex-specific parental contributions to nest-building, incubation, and nestling care. All nests were dome-shaped structures with a side entrance constructed of grasses, twigs, and leaves, as is typical for *Basileuterus* warblers. Females were the primary nest-builders, although we also observed male-assisted nest-building at one nest, which is a rare behaviour in wood-warblers. Females were the sole incubators, and the incubation period in this population was 13–14 days. Video recordings confirmed that males and females made equivalent contributions to nestling feeding and nest sanitation. Our study provides detailed observations of both male and female Rufous-capped Warblers during all stages of the nesting cycle, and thus improves our understanding of nesting behaviour in this common yet little-studied species.

Introduction

The breeding biology and nesting behaviour of tropical resident bird species have received little study compared to their temperate counterparts (Stutchbury & Morton 2001). Although nests are described for many tropical species, detailed observations on male and female behaviour during different nesting stages are uncommon. This is especially true for sexually monomorphic species, where the contributions of males and females are more difficult to observe without a colour-banded population (Cox & Martin 2009, Sandoval & Mennill 2012). This is the case for the Family Parulidae (New World wood-warblers), in which life-history descriptions of species in the monomorphic tropical resident genera *Basileuterus*, *Myioborus*, and *Myiothlypis* are scarce (reviewed in Cox & Martin 2009). All *Basileuterus* warblers studied to date have similar dome-shaped nests with a side entrance, built on or near the ground in a slope or under a shrub for concealment and protection from heavy rains (e.g., Rowley 1962, Skutch 1967, Greeney et al. 2005, Cox & Martin 2009). However, descriptions of nesting behaviour, including nest-building, incubation, and parental provisioning of nestlings, are largely based on observations of unmarked individuals of unconfirmed sex.

The Rufous-capped Warbler (*Basileuterus rufifrons*) is a widely-distributed resident songbird found from southern Arizona to northern South America. This species lives in a variety of semi-open habitats and dry forest from 0–3000 m a.s.l. (Contreras-González et al. 2010, Curson 2010). Although pairs are territorial year-round, they breed seasonally during the rainy season (Stiles & Skutch 1989). There are two primary subspecies groups: the white-bellied *rufifrons* group of Mexico and western Guatemala, which inhabits arid scrub and semi-open montane habitats; and the yellow-bellied *delattrii* group of southeastern Mexico, Central and South America, which lives in dry forest and humid semi-open habitats (Howell & Webb 1995).

There are previous nest descriptions of both subspecies groups. For the *rufifrons* group in Mexico, there are opportunistic observations of one nest in Jalisco (Zimmerman & Harry 1951), one nest in Oaxaca (Rowley 1966), and three nests in Morelos (Rowley 1962). For the *delattrii* group in Costa Rica, there is a description of one nest with eggs (Cherrie 1892), and detailed observations of five nests (two with eggs, three with nestlings), along with incubation and nestling watches on one nest each (Skutch 1967). Only the female incubates eggs and broods nestlings (Skutch 1967) but both parents feed nestlings (Zimmerman & Harry 1951, Skutch 1967).

In this study, we describe the nests, nestlings, and nesting behaviour of Rufous-capped Warblers, based on data collected from *B. r. delattrii* in northwestern Costa Rica. In particular, we provide the first detailed record of sex-specific nesting behaviour using a colour-banded population. We observed nests during the nest-building, incubation, and nestling periods, and we also collected video recordings of selected nests during the incubation and nestling periods.

Methods

We collected data from mid-May to early July 2013–2015 during the Rufous-capped Warbler breeding seasons at Sector Santa Rosa, Área de Conservación Guanacaste, northwestern Costa Rica (10°51’N, 85°36’30”W; 300 m a.s.l.). This site is a Neotropical dry forest with both second-growth and mature stands, where the warblers are common year-round residents in both habitat types. In this population, birds breed only during the rainy season, which typically begins in May of each year. We captured and colour-banded male and female warblers as part of a separate study on the territorial behaviour of this species; either one or both adults was colour-banded for each nesting pair included in this study. We sexed birds based on the presence of secondary sexual characteristics when captured (cloacal protuberance for males and brood patch for females) and behaviour during focal observations (only males sing prolonged spontaneous song bouts).

Nest structure

We monitored 11 nests: one nest in 2013, four nests in 2014, and six nests in 2015. We located nests by following females carrying nesting material (*n* = 10) or adults carrying food to nestlings (*n* = 1). We monitored the number of eggs and nestlings weekly until fledging by conducting 20-min nest watches, after which time we approached the nest to check contents if the female was not on the nest. We did not flush females off nests to check nest contents, to minimize disturbance to incubating females. We colour-banded and weighed nestlings (*n* = 14) from five nests (1–4 young per nest) in all cases where the young survived to 8–10 days old. We were not able to confirm fledging dates for these nests, because fledging occurred after our field expeditions had concluded. We estimate that the nestling period is approximately 12 days, since Skutch (1967) reported a 12-day nestling period for two nests in another Costa Rican population, and 10-day-old nestlings in our study population were highly mobile during and after banding (A. Demko pers. obs.).

We collected measurements of the nest and surrounding environment for 10 nests. One nest still contained small (i.e., 1–2-day old) nestlings at the end of our field expedition, so we did not remove the nestlings to measure the nest. We measured the inner height and width of the nest entrance (in mm), the horizontal depth of the nest from the entrance to the back wall (in mm), and the amount of tree canopy cover (estimated within 5%). We also described the overall nest structure and construction materials of all nests, and measured the length and width of two eggs from an abandoned nest and described their colour pattern.

Breeding behaviour

In 2014, we collected video recordings of two warbler nests. Each recording session was an approximately 4-hour continuous recording between 06:30–11:00 CST; we placed video cameras on tripods 7–10 m away from the nest to avoid interference with normal parental behaviour. We recorded Nest 3 during both the incubation and nestling periods, and Nest 4 during the nestling period only, for a total of 4 h 23 min during incubation and 8 h 4 min during the nestling period. We annotated the videos using VLC Media Player (v. 2.2.6). We identified adults by recording their colour-bands whenever possible and noted the timing and length of each adult visit to the nest. For both the incubation and nestling periods, we calculated nest attentiveness (i.e., percentage of total time spent by adults on or at the nest). For the nestling period only, we also observed adult behaviour during nest visits (e.g., carrying food or fecal sacs). We found most nests during the nest-building stage, so we also described nest-building activity by both males and females during 30–60 min observation periods upon nest discovery.

Results and Discussion

Nest structure and location

All of the Rufous-capped Warbler nests we studied were dome-shaped structures with a covered top and a side entrance, and were located on or near the ground (Table 3.1). The outer structure of the nests was constructed of fine grasses topped with dead leaves (*n* = 5; Figure 3.1A), fine grasses (*n* = 3; Figure 3.1B), small twigs (*n* = 2; Figure 3.1C), or large grasses (*n* = 1; Figure 3.1D), and all nests were lined with fine grasses. Nests measured on average 45 x 63 x 84 mm (height x width x depth), and canopy cover above nests was 66 ± 27% (mean ± SD; range: 25–90%; *n* = 10). Most nests were located on a slope, either below leaf litter and small shrubs (*n*

= 7) or below large rocks (*n* = 2); two nests in areas with flat terrain were located on the ground in a hummock (*n* = 1) or below a shrub (*n* = 1). The nest structure and placement of Rufous-capped Warbler nests at our study site were consistent with other accounts of *Basileuterus* nests (Cox & Martin 2009) and with previous reports of this species' nests (Cherrie 1892, Zimmerman & Harry 1951, Rowley 1962, Skutch 1967).

Clutch size and egg description

Clutch sizes were 3–4 eggs (*n* = 6 confirmed completed clutches; Table 3.1). Five nests were abandoned or depredated before clutch completion (*n* = 4) or were not observed during incubation (*n* = 1). We did not collect data on the frequency or timing of egg laying, although other accounts suggest that females of this species lay one egg per day (Skutch 1967). We measured two eggs: they measured 18.6 x 13.5 mm and 18.0 x 13.5 mm, and were pinkish-white with brown speckles concentrated at the wider end of the egg. Clutch size and egg descriptions are consistent with other published accounts for this species (Cherrie 1892; Rowley 1962, 1966; Skutch 1967) and other *Basileuterus* warblers (Cox & Martin 2009).

Nest-building behaviour

We found 10 nests during the nest-building stage and one nest during the nestling stage. We found the first nests of each breeding season (*n* = 9) within two days of the first major rain event of that year (22 May 2013, 10 May 2014, and 3 June 2015), whereas we located re-nests (*n* = 2) after depredation or nest abandonment up to 32 days after the first rain. Both re-nests were newly constructed; we did not observe warblers re-using a previous nest structure. Females were the primary nest builders, as is reported for other temperate and tropical wood-warbler species generally (Kendeigh 1945, Skutch 1954) and Rufous-capped Warblers specifically

(Skutch 1967). During observation periods, females made many trips to the nest, bringing nest material (e.g., grasses, twigs, leaves). The females were largely silent while nest-building, but occasionally uttered soft ‘chip’ calls when their male mates were nearby. While females were building the nest, males at 10 of 11 nests sang or produced ‘chip’ calls 3–10 m away from the female, and three males accompanied the female while she collected nest materials.

We observed one male, whose sex we confirmed based on his colour-bands, bringing twigs to the nest twice and entering the nest once. Nest-building by males is rare in wood-warblers (Kendeigh 1945, Skutch 1954), and ours is the first report of this behaviour in Rufous-capped Warblers (Zimmerman & Harry 1951, Rowley 1962, Skutch 1967). The temperate-breeding Louisiana Waterthrush (*Parkesia motacilla*; Mattsson et al. 2009) and the tropical resident Buff-rumped Warbler (*Myiothlypis fulvicauda*; Skutch 1954) are the only known wood-warbler species where males and females contribute equally to nest-building (Table 3.2). Occasional male-assisted nest-building, such as we observed, is reported for 12 other temperate-breeding and two tropical resident warbler species, in which some males carry material to the nest but contribute much less than females (Table 3.2). We suggest that careful observation of other tropical resident warblers may reveal that male nest-building is more prevalent in this family than previously reported.

Incubation and nestling provisioning behaviour

Our observations during incubation and nestling periods are consistent with other reports in this species, in that only the female appears to incubate and brood nestlings, whereas both parents contribute equally to nestling provisioning (Skutch 1967). The incubation period, calculated as the time elapsed from the last egg laid to the first egg hatched, was 13 days for four nests, and 13–14 days for a fifth nest. We collected a video recording of Nest 3 on 27 June

2014 from 06:41–11:04 CST, when the female was incubating four eggs. The female visited the nest twice for an average of 0.5 visits per hour and total nest attentiveness of 78.7%. The two incubation bouts were 70.4 min and ≥116.5 min; the total time of the second bout was unknown since the female was still on the nest at the end of the recording. The male was not detected at or near the nest during this video.

Our observations point to a slightly longer incubation bout length and a higher level of nest attentiveness than the observations of Skutch (1967). He reported average morning incubation bouts of 50.6 min, with the longest being 70 min, and nest attentiveness of 65.7%. These differences could be related to the time of the incubation period, although it was likely comparable between the two studies, since our nest was observed late in incubation (day 9 of 13–14) and Skutch's was "well advanced in incubation" (Skutch 1967). Since these differences could also be owing to low sample sizes, further observations at multiple intervals across the incubation stage with a large sample of nests would be useful to investigate patterns of within-species variability in incubation activity.

Nestling mass at 8–10 days old was 9.2 ± 1.1 g (range: 8–10.5 g; $n = 10$). Although we could not estimate the exact age of the nestlings because eggs hatched over a 2-day period, the least-developed nestlings (estimated to be 8–9 days old) had open eyes, fully feathered heads, large pin feathers on the wings and tail, and narrow yellow feather tracts down the sides of the bare belly (Figure 3.2A-B). The most-developed nestlings (estimated to be 9–10 days old) had fully feathered wings with buffy wing bars, and wide yellow feather tracts covering most of the belly (Figure 3.2C-D).

We collected a video recording of Nest 4 on 3 June 2014 from 06:36–10:37 CST, when it contained four nestlings that were 1–2 days old. Adults visited the nest 11 times for an average of 2.4 visits per hour, with average visit length of 7.4 ± 5.6 min (mean ± SD; range: 0.4–19.7 min) and total nest attentiveness of 34.3%. Female visits (*n* = 4) were 7.4 ± 4.6 min (mean ± SD; range: 2.9–12.7 min), and the female was also on the nest for the first 5.3 min of recording. The six remaining visits were made by unknown adults whose colour-bands were not visible on the video. During 5 of 10 visits, an adult brought food items to the nestlings; because of the video quality, we could not identify any specific food items during these visits.

We collected a video recording of Nest 3 on 6 July 2014 from 06:39–10:42 CST, when it contained four nestlings of 5–6 days old. Adults visited the nest 26 times for an average of 6.4 visits per hour. For the 21 visits of known length, the average length was 2.6 ± 5.2 min (mean ± SD; range: 0.1–18.7 min). The total nest attentiveness was 39.9%; on seven occasions, the male and female were both present at the nest at the same time. We confirmed the bird's sex for 19 visits. Male visits (*n* = 12) were all short: 0.2 ± 0.2 min (mean ± SD; range: 0.1–0.7 min). Female visits (*n* = 7) were more variable in length: 7.2 ± 7.2 min (mean ± SD; range: 0.5–18.7 min). During five of these seven visits, the female entered the nest for >5 min to brood the nestlings. During 23 of 26 visits, both adults made confirmed food deliveries to nestlings, including green caterpillars (*n* = 3 visits) and brown caterpillars (*n* = 1 visit; Figure 3.3). Both adults also contributed to nest sanitation by carrying fecal sacs away from the nest after feeding young (*n* = 6 visits: 4 female, 1 male, 1 unknown adult). Overall, these video recordings suggest that males and females made a similar number of visits to feed nestlings, but female visits were longer because females often entered the nest for several minutes after feeding to brood nestlings rather than leaving immediately.

Conclusion

Our study provides new information on the nests and nesting behaviour of Rufous-capped Warblers, particularly incubation times and sex-specific parental contributions to nest-building and nestling provisioning. Our work also advances our knowledge of breeding behaviour in a little-studied genus of tropical resident wood-warblers. Published nest descriptions exist for only four of the eleven *Basileuterus* species: Rufous-capped Warbler, Three-striped Warbler (*B. tristriatus*: Greeney et al. 2005, Cox & Martin 2009), Golden-crowned Warbler (*B. culicivorus*), and Black-cheeked Warbler (*B. melanogenys*: Skutch 1967). Natural history information on the remaining species, most of which have restricted distributions, will be useful not only to compare temperate and tropical wood-warbler breeding biology, but also to focus conservation efforts for rare species using further data on their habitat and nest site requirements.

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Tables

Table 3.1. Nest structure and contents of 11 Rufous-capped Warbler (Basileuterus rufifrons) nests found during the 2013–2015 breeding seasons at Sector Santa Rosa, Guanacaste, Costa Rica. A blank space indicates measurement not collected; an asterisk (*) indicates unconfirmed clutch size or nestling number.

Nest	Primary material	Location	Nest width (mm)	Nest height (mm)	Nest depth (mm)	Cover (%)	Clutch size	No. of young	Final status
1	small twigs	on flat ground; below shrub				25	3	3	nestlings
2	fine grasses, dead leaves	on slope; below leaf litter	65	45	90	70	1	0	failed
3	fine grasses	in side of bank; below rocks	60	45	75	90	4	4	nestlings
4	fine grasses, dead leaves	on slope; below leaf litter	80	50	95	90	4	4	nestlings
5	fine grasses, dead leaves	on flat ground; in hummock	70	60	95	90	2	0	failed
6	fine grasses	on slope; below shrub	43	30	84		*	0	failed
7	small twigs	on slope; base of two rocks	66	38	85		3–4*	3	nestlings
8	fine grasses	on slope; below leaf litter	59	46	74	65	3	3	nestlings
9	fine grasses, dead leaves	on slope; below leaf litter	75	48	95	35	2–3*	2–3*	nestlings
10	large grasses	on slope; below leaf litter	46	32	75		*	0	failed
11	fine grasses, dead leaves	on slope; below leaf litter	65	56	75		3	3	nestlings

Table 3.2. List of wood-warbler species (Family Parulidae) with reported frequent (males contribute equally to females) or occasional (males contribute rarely) male-assisted nest-building.

Species	Breeding range	Reference
Frequent male nest-building		
Louisiana Waterthrush (*Parkesia motacilla*)	temperate	Mattsson et al. 2009
Buff-rumped Warbler (*Myiothlypis fulvicauda*)	tropical	Skutch 1954
Occasional male nest-building		
Blue-winged Warbler (*Vermivora cyanoptera*)	temperate	Gill et al. 2001
Prothonotary Warbler (*Protonotaria citrea*)	temperate	Petit 1999
Cerulean Warbler (*Setophaga cerulea*)	temperate	Boves & Buehler 2012
Northern Parula (*Setophaga americana*)	temperate	Moldenhauer & Regelski 2012
Magnolia Warbler (*Setophaga magnolia*)	temperate	Dunn & Hall 2010
Pine Warbler (*Setophaga pinus*)	temperate	Rodewald et al. 2013
Yellow-rumped Warbler (*Setophaga coronata*)	temperate	Hunt & Flaspohler 1998
Yellow-throated Warbler (*Setophaga dominica*)	temperate	McKay & Hall 2012
Golden-cheeked Warbler (*Setophaga chrysoparia*)	temperate	Ladd & Gass 1999
Black-throated Green Warbler (*Setophaga virens*)	temperate	Morse & Poole 2005
Black-throated Blue Warbler (*Setophaga caerulescens*)	temperate	Holmes et al. 2017
Canada Warbler (*Cardellina canadensis*)	temperate	Reitsma et al. 2009
Tropical Parula (*Setophaga pitiayumi*)	tropical	Regelski & Moldenhauer 2012
Slate-throated Redstart (*Myioborus miniatus*)	tropical	Skutch 1954
Rufous-capped Warbler (*Basileuterus rufifrons*)	tropical	this study

Figures

Figure 3.1. Photographs of Rufous-capped Warbler (*Basileuterus rufifrons*) nests found at Sector Santa Rosa, Guanacaste, Costa Rica from April-June 2013–2015. Nests were dome-shaped with a side entrance and were constructed from different materials: (A) Nest 9 constructed of fine grasses topped with dead leaves; (B) Nest 8 constructed of fine grasses; (C) Nest 1 constructed of small twigs; and (D) Nest 10 constructed of large grasses. Photographs taken by A. Demko.

Figure 3.2. Photographs of Rufous-capped Warbler (*Basileuterus rufifrons*) nestlings at Sector Santa Rosa, Guanacaste, Costa Rica: at age 8–9 days old on 6 July 2015 from Nest 11 (A: ventral view; B: dorsal view) and 9–10 days old on 2 July 2015 from Nest 7 (C: ventral view; D: dorsal view). Older nestlings had more extensive feathering on the wings, back, and belly than did younger nestlings. Photographs taken by A. Demko.

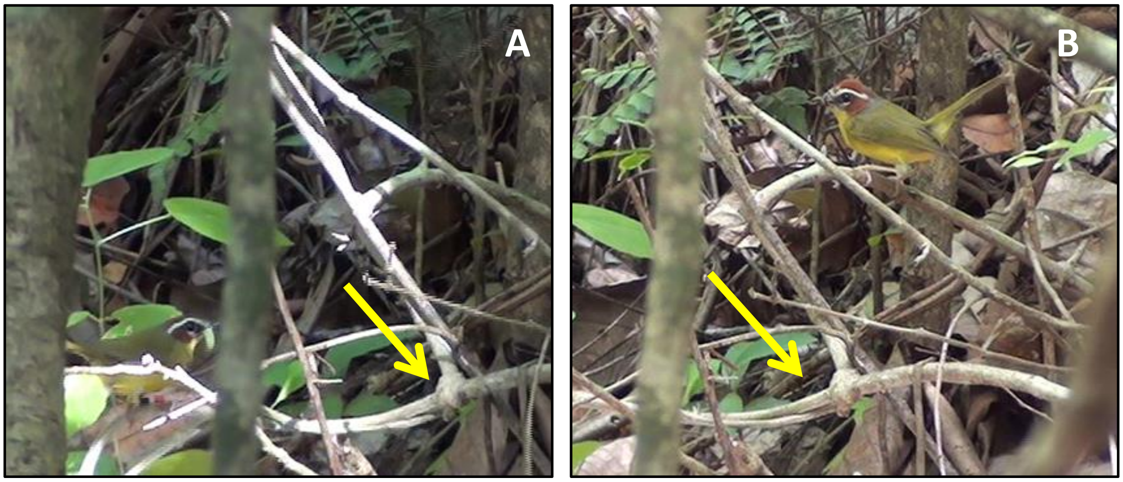

Figure 3.3. Photographic stills taken from a video recording of (A) a banded male and (B) an unbanded female Rufous-capped Warbler (*Basileuterus rufifrons*) delivering food items to 5–6 day old nestlings on 6 July 2014 at Sector Santa Rosa, Guanacaste, Costa Rica. Nest entrance is partially obscured by branches; yellow arrows indicate the nest location on the photographs.

Chapter 4: Male and female signaling behaviour varies seasonally during territorial interactions in a tropical songbird

*****This chapter is the outcome of joint research with D. Mennill.

Chapter Summary

In many tropical birds, both sexes use conspicuous vocal signals during territorial interactions. Although a growing number of studies examine male and female signals in the context of coordinated vocal duets, the use of vocal signals by both sexes in non-duetting species is poorly documented, even though these species are more numerous than duetting species. Furthermore, few studies of tropical non-duetting species test for seasonal variation in signaling behaviour. We studied season-specific and sex-specific variation in signaling behaviour of a tropical resident songbird, the Rufous-capped Warbler (*Basileuterus rufifrons*), by conducting a playback experiment where we simulated conspecific territorial intruders producing three types of vocalizations (male songs, female calls, or a ‘pair’ with simultaneous male songs and female calls) and a heterospecific control. We repeated playback during the pre-breeding and breeding seasons. Response intensity to playback varied with season and sex of the focal birds. During the pre-breeding season, both sexes showed strong physical approach responses and vocal responses to all conspecific intrusions, especially paired intrusions. During the breeding season, males responded strongly to all conspecific treatments, whereas females showed little response. Although females primarily used calls in response to conspecific playback, many females also sang, especially during the non-breeding season. Our results therefore suggest that both male and female signals are used for shared territory defence, but that the contributions of each sex to territory defence vary seasonally. Our results also contribute to our understanding of the evolution of combined male and female signaling during territory defence.

Introduction

Many animals use conspicuous vocal and visual signals to communicate, including complex vocalizations and brightly coloured ornaments. Across taxa, these signals are known to function in mate attraction, competition for mates, and defence of territorial resources (Bradbury & Vehrencamp 2011). Traditionally, sexual selection on males to attract and compete for mates was thought to be the primary mechanism driving the evolution of conspicuous traits (reviewed in Kraaijeveld et al. 2007; Tobias et al. 2012). In many tropical birds, however, both males and females produce similar vocal and visual signals, and both sexes defend territories throughout the year (Stutchbury & Morton 2001). Furthermore, recent analyses show that male ornaments in dimorphic species are not necessarily sexually selected (Candolin & Tukiainen 2015). An alternative view suggests that female signals, including vocalizations and bright colouration, function in competition among conspecifics for territorial resources (West-Eberhard 1983; Tobias et al. 2012).

Most research on female signaling in birds has focused on duetting species, where both members of a breeding pair sing in a coordinated manner (Hall 2004; Dahlin & Benedict 2014). When duets are played to territorial pairs, birds often show strong coordinated responses to territorial intrusions by producing more duets and staying close together (e.g., Hall and Peters 2008; Mennill and Vehrencamp 2008; Benedict 2010; Dahlin and Wright 2012; Koloff and Mennill 2013). A growing body of evidence suggests that duets are multifunctional signals, and that birds use duets primarily in cooperative territory defence, mate-guarding, and maintaining contact between pair members (reviewed in Douglas and Mennill 2010; Dahlin and Benedict 2014; Tobias et al. 2016). In contrast, the function of female vocal signals in non-duetting species has received little study, in spite of the fact that 84% of all bird species do not produce duets (Tobias et al. 2016). Many non-duetting tropical resident species also defend shared territories year-round (e.g., Freed 1987). Females of these species often use calls only (e.g., Adelaide's Warblers, *Setophaga adelaidae*; Staicer 1996) or both calls and songs that are not produced as duets (e.g., Banded Wrens, *Thryophilus pleurostictus*; Hall et al. 2015) during territorial interactions.

In territorial resident birds that breed seasonally, both male and female signals might serve different functions across the seasons (e.g., Fedy and Stutchbury 2005; Gill et al. 2007). Most studies have focused on temperate zone species where territory defence is performed primarily by males during the breeding season (reviewed in Catchpole and Slater 2008). In tropical resident species, however, both sexes may be involved in territory defence and these behaviours may persist throughout the year (Stutchbury & Morton 2001). Previous studies conducted on tropical resident duetting birds during both the breeding and non-breeding seasons indicate that territorial responses are season- and sex-specific (Fedy & Stutchbury 2005; Gill et al. 2007, 2008; Odom et al. 2017). In White-bellied Antbirds (*Myrmeciza longipes*), both males and females show high levels of aggression to intruders and respond more intensely during the non-breeding season, providing evidence that both sexes contribute to shared resource defence (Fedy & Stutchbury 2005). In Buff-breasted Wrens (*Cantorchilus leucotis*), both sexes show high aggression to rival females and pairs in both seasons, suggesting both territory defence and female-female competitive functions for their signals (Gill et al. 2007, 2008). Remarkably few studies have quantified seasonal variation in vocalizations of males versus females for resident, non-duetting bird species (Logue 2005). Furthermore, although both duetting and non-duetting species often use calls for territory defence (e.g., Staicer 1996; Neudorf and Tarof 1998), few playback studies have presented both songs and calls to territorial pairs to compare conspecific responses to these signals (Sandoval et al. 2013).

In this investigation, we studied Rufous-capped Warblers (*Basileuterus rufifrons*), non-duetting tropical resident songbirds with conspicuous male and female vocal signals, using an experimental approach to evaluate whether these animals exhibit season- and sex-specific variation in signal use. We evaluated season- and sex-specific variation in territorial behaviour in the context of three non-mutually-exclusive hypotheses of signal function: (1) territory defence; (2) intrasexual competition for mates; and (3) mate attraction (Kraaijeveld et al. 2007; Tobias et al. 2011). If birds use their signals primarily for territory defence, we predicted that male and female vocal signals would be used throughout the year in response to territorial intrusions, and we predicted that both sexes would respond strongly to both intersexual and intrasexual conspecific intruder signals (Tobias et al. 2011; Dowling & Webster 2016). Conversely, if birds use their signals primarily for intrasexual competition for mates, we predicted that these signals would be used more frequently during the breeding season, and that males and females would both respond more strongly to intrasexual versus intersexual signals (Tobias et al. 2011). Finally, if birds use their signals primarily in mate attraction, we predicted they would be used more at the start of the breeding season, and that males and females would respond more strongly to intersexual versus intrasexual signals (Tobias et al. 2011).

Methods

Study species

Rufous-capped Warblers are tropical resident songbirds distributed from southern Arizona and northern Mexico to northern Colombia and Venezuela, from sea level up to 3000 m elevation (Curson 2010). They are common foliage-gleaning insectivores in semi-open habitats such as second-growth forest and shade coffee plantations (Perfecto et al. 2004; Jedlicka et al.

2006; Morrison et al. 2010; Morrison & Lindell 2011) as well as mature Neotropical dry forests (Stiles & Skutch 1989). Pairs typically defend territories year-round (Jedlicka et al. 2006), but may also participate in mixed-species foraging flocks during the non-breeding season (Hutto 1988). Both sexes have bright, sexually monomorphic plumage (Curson 2010). In our study population, males sing regularly and females sing only rarely (Chapter 2), but both males and females produce 'chip' calls (Figure 4.1; females produce these calls more often than males) and pair members often vocalize simultaneously (i.e., the male produces songs or calls while the female produces calls). Females appear to produce calls in response to their partner's songs, a behaviour observed in other tropical resident warbler species (Staicer 1996).

Playback design

We conducted playback experiments from April to June 2015 in Sector Santa Rosa of the Área de Conservación Guanacaste in northwestern Costa Rica (10°51'N, 85°36'30"W). Santa Rosa is characterized by Neotropical dry forest habitat with a pronounced dry season from approximately December to May (the onset of the rainy season varies from late April until early June), and a rainy season from approximately May to November. Rufous-capped Warblers begin breeding activities at the start of the rainy season (Stiles & Skutch 1989). We therefore conducted pre-breeding season trials on territorial pairs of warblers at the end of the dry season (April 28–May 22; *n* = 25 pairs received playback in the pre-breeding season; the rainy season began on June 3 in 2015), and breeding season trials at the beginning of the rainy season (June 8–23; *n* = 21 pairs received playback in the breeding season). We conducted all trials from 06:30–11:00 CST, a time window that coincides with the morning period of peak vocal activity, while avoiding overlap with the dawn chorus when males typically sing at a higher rate (Chapter 2).

We used a stereo playback design, broadcasting male and female vocalizations from separate speakers, to provide a natural simulation of a territorial intrusion by two individuals (reviewed in Douglas and Mennill 2010). We presented three treatments of conspecific vocalizations to pairs of territorial warblers to simulate different numbers and sexes of intruders: (1) Male treatment, of a male song from one speaker; (2) Female treatment, of a female call from one speaker; and (3) Pair treatment, of a male song and female call broadcast simultaneously from two separate speakers. In addition, we presented (4) a one-speaker Control treatment, of male vocalizations of Long-tailed Manakins (*Chiroxiphia linearis*), a sympatric non-competitor bird species that is common at our study site. Each pair received one treatment on each of four successive days using a factorial design with randomly-selected order of stimulus presentation. Each set of treatments was repeated for each pair in both the pre-breeding and breeding seasons. To minimize time-of-day effects on response strength, trials for each pair took place within 30 min of the same start time across days.

We tested the same pairs during both the pre-breeding and breeding periods, whenever possible, to account for individual variation in response (as in Gill et al. 2007; Akçay et al. 2014); 16 of 25 subject pairs received playback in both periods. For the remaining five pairs, a different male defended a given territory during the pre-breeding and breeding periods. In seven pairs tested, the female was not banded, so it is possible that the female changed between periods and we did not detect this change. We captured one or both pair members using mist-nets and conspecific playback, and gave each bird a unique colour-band combination to allow for individual identification during trials, since both sexes look alike. We waited at least 10 days between the capture date and start of playback trials for all birds, and used different conspecific stimuli from the playback trials while luring birds into mist nets, thereby minimizing the effect of previous experience on playback response. We determined the sex of individuals based on the presence of a cloacal protuberance for males (from April 15 through July) and a brood patch for females (in June and July). We also confirmed the sex of each bird behaviourally during territorial observations, based on continuous song during the dawn chorus for males, and observations of nest-building and incubation activity for females.

We placed two speakers (model: FoxPro Scorpion TX200) 5 m apart and 1 m above the ground near the focal pair's territory centre (as in Mennill 2006; Koloff and Mennill 2013); this allowed us to simulate a pair of birds engaging in a territorial intrusion. We chose the speaker locations based on 60-min territorial observation periods of each pair in early April. We placed small pieces of flagging tape at 1 m and 5 m distances away from each speaker to aid the two observers in estimating the distance of birds from the speakers during trials. All stimuli were broadcast at 88 dB(A) SPL (amplitude measured at 1 m from the speaker with a Casella CEL–240 sound level meter; Casella CEL Inc., Buffalo, NY, USA). This amplitude is a natural level for warbler vocalizations (e.g., Hof and Hazlett 2010) and comparable to the natural volume of Rufous-capped Warbler vocalizations heard in the field.

Trials consisted of a 5 min playback period followed by a 5 min post-playback observation period. During each trial, two observers sat together 15–20 m away from the playback speakers and recorded the trial with a Marantz PMD660 digital recorder and an Audiotechnica AT8015 directional microphone. Two observers were necessary to accurately track the behaviour of the male and female of each pair separately during the playback trials. Each observer dictated the horizontal and vertical distance of one bird from the speaker and other physical behaviours. We included any pair's response in our analysis as long as one or both individuals approached within 10 m of either speaker during the 5 min playback. For the conspecific treatments, if there was no response during this 5 min period and the focal birds were not seen or heard in the territory, we assumed that they did not detect the playback (territories can be >100 m across; A. Demko pers. obs.). We then waited 2 min and played the 5 min stimulus again. If there was still no response, or if a neighbour also responded to the playback by approaching within 10 m or interacting with the focal birds during the trial, we repeated the trial on the following day. If there was still no response on the second day, we considered the pair to be non-responsive to that treatment. To calculate the proportion of responses by males and females to each playback treatment, we used only the final trial conducted for a specific treatment type and pair of birds.

We recorded both physical and vocal responses from males and females separately during each trial. We analyzed the following four physical approach responses: (1) number of flights over each speaker; (2) closest approach to speaker (m); (3) latency to approach within 10 m of the speaker; and (4) time spent within 10 m of the speaker. We analyzed the following four vocal responses: (1) number of songs; (2) number of calls; (3) latency to first song; and (4) latency to first call. For trials where males sang at least one song, we also tested whether song duration and number of syllables per song differed by treatment or season. We did not statistically compare female song duration or number of syllables per song by treatment or season because of the small available sample size. These response measures have been used in other playback studies on songbirds and are associated with strong territorial responses (e.g., Akçay et al. 2013; Hof and Podos 2013). Since identification of colour-banded individuals was central to our study and the playback stimuli were audibly distinct to the observers, we were unable to use blinded methods for data collection.

Playback stimuli

We created playback stimuli from high-quality recordings of male songs and female calls we collected from colour-banded male and female Rufous-capped Warblers from the study population in 2013–2014. We used male songs and female calls because they were the most common vocalizations recorded from each sex during naturally-occurring conspecific territorial interactions (A. Demko pers. obs.). We used Audition 3.0 software (Adobe, San Jose, CA, USA) to filter recordings with a high pass filter of 1000 Hz and to normalize the amplitude of the final playback files to -1 dB. All stimuli were from birds with territories at least 200 m away from the playback subject's territory, and therefore presumably unfamiliar to the focal birds prior to the playback experiment. We used a different set of playback stimuli for each pair whenever possible to avoid pseudoreplication (McGregor 1992), although we were limited by the number of high-quality recordings of confirmed-identity animals. For male stimuli (n = 20), we used four different song types from each male, presented alternately at a natural daytime song rate of 6 songs/min. Since males typically alternate between song types during daytime song bouts (Chapter 2), the use of multiple song types per bird was more natural than repeating only one song type. For female stimuli (n = 9), we used one 'chip' call repeated at a natural call rate of 15 calls/min. We calculated natural song and call rates from a subset of daytime 2013–2014 focal recordings from Santa Rosa (n = 10 each for males and females). We constructed pair stimuli using one male and one female stimulus file broadcast from separate speakers, which simulated the vocal behaviour of Rufous-capped Warbler pairs observed during naturally occurring territorial interactions (A. Demko pers. obs.). For control stimuli (n = 15), we used Long-tailed Manakin songs we recorded from Santa Rosa, broadcast at a rate of 9 songs/min. We used the same stimuli for each pair during both seasons to ensure that variation in response strength across seasons was unrelated to differences in acoustic properties of the playback stimuli used. In the cases where partnerships changed on a given territory between seasons, we used the same stimuli previously used at that territory.

Although both male and female Rufous-capped Warblers produce ‘chip’ calls, sex-specific differences in the frequency of these calls and the behavioural context of their use should allow sex identification of the caller by territorial pairs during the Female and Pair treatments. To confirm sex-specificity of ‘chip’ calls, we conducted a paired comparison of structural variation in this call type between 10 mated warbler pairs, and found significant sex differences (Figure 4.1). Male calls had a minimum frequency that was on average, 604 Hz lower than the minimum frequency of females (mean ± SD males: 3237 ± 455 Hz, females: 3841 ± 626 Hz; paired *t*-test, $t_9 = 3.55$, $P = 0.006$), although neither maximum frequency ($t_9 = 1.44$, $P = 0.18$) nor note duration ($t_9 = -0.15$, $P = 0.89$) differed between the sexes. Songs and calls are known to convey different messages across bird species (Catchpole & Slater 2008), and therefore it is possible that the type of vocalization (song versus call) is confounded with the sex of the treatment type (male versus female). Therefore, different responses to the treatment types should be interpreted primarily in the context of seasonal effects rather than sex-specific effects, pending further studies comparing responses to the same vocalizations across sexes (e.g., testing responses to male vs. female songs and male vs. female calls).

Male versus female song comparison

After discovering that females sang in response to playback stimuli (see Results), we compared male and female Rufous-capped Warbler songs. We measured songs from 10 males (10 songs each randomly selected from the pool of high-quality spontaneous songs available from focal recordings) and 10 females (1–18 songs each from the best-quality songs available on playback recordings). The males and females selected for this analysis were 10 of the mated pairs tested in our study. We measured seven acoustic variables on each song: song duration, number of syllables per song, number of syllable types, syllable diversity (number of different syllable types divided by the total number of syllables per song), maximum frequency, and minimum frequency. We defined a syllable as the smallest continuous trace on a spectrogram comprising a song (Catchpole & Slater 2008), and we defined a syllable type as a syllable with specific spectro-temporal properties that is different from other such syllables. A. Demko identified all syllable types based on their frequency, shape, and duration by visual comparison of spectrograms. A second observer naive to the sex and identity of the individual birds repeated the syllable classification for a subset of 50 songs (35 male and 15 female), and we then compared the scores of both observers using two inter-observer reliability coefficients modified from Illes (2015):

$$\text{(1)} \qquad \text{Score 1} = 1 - ((a/b)/b)$$

$$\text{(2)} \qquad \text{Score 2} = 1 - ((c\text{-}b)_{\text{absolute value}}/b)$$

a was the absolute value of the mean difference between A. Demko's and the second observer's syllable count for each song; b was the mean of A. Demko's syllable type count per song; and c was the mean of the second observer's syllable type count per song. Both observers had high consistency in syllable classification, with similarity scores of 99.6% for Score 1, and 97.1% for Score 2.

We then calculated average values within individual birds to produce a single value for each variable, and ran two-sample t-tests to compare these variables between sexes. We collected frequency measurements in Avisoft-SASLab Pro (version 5.2.09; R. Specht, Berlin, Germany) from the power spectrum of each song at a threshold amplitude of -20 dB from the peak amplitude. This method is the recommended method in order to standardize measurements across recordings with differing signal-to-noise ratios (Zollinger et al. 2012; Brumm et al. 2017). Power spectra were produced with a Hann window with FFT size of 512 and frequency resolution of 62.5 Hz. We collected temporal measurements manually from spectrograms in Raven Pro v. 1.4 (Cornell Laboratory of Ornithology, Ithaca, NY) with the following settings: Hann spectrogram window with FFT size of 512, time resolution of 2.9 ms, and frequency resolution of 86.1 Hz.

Analyses

We used Syrinx PC software (J. Burt, Seattle, WA, USA) to annotate the observers' narration of physical responses by the birds, as well as the vocalizations produced by the birds, on the recordings for each playback session, producing a time-stamped record of the behaviours and vocalizations of each playback subject. We analyzed physical approach responses and vocal responses separately, in order to quantify differences in use of visual and vocal signals in this species. Since many of the response variables were correlated, we used Principal Components Analysis (without factor rotation) on the extracted variables to produce uncorrelated composite variables (McGregor 1992). For males, the distance of closest approach was log-transformed prior to analysis to improve linear relationships between the response variables according to the assumptions of principal components analysis (Quinn & Keough 2002). To incorporate the two-speaker Pair treatment in this analysis, we used the strongest response of each individual to either speaker for each physical response variable. Since male and female speakers were only 5 m apart, we considered the target area for stimulus detection to be similar for the one-speaker and two-speaker treatments, particularly in relation to the large territories of our study species.

For physical approach responses, the first principal component (PC1) explained 73.0% of the overall variation for males, and 75.7% for females. For both sexes, the PC1 loadings corresponded to a shorter latency to approach, a closer distance of approach, more time spent within 10 m of the speakers, and more flights over the speakers (Table 4.1); we refer to this PC1 score as “physical approach response”. For vocal responses, PC1 explained 51.1% of the overall variation for males, and 63.4% for females. For males and females, the PC1 loadings corresponded to a greater number of songs and calls produced, and a shorter latency to first song and first call; we refer to this PC1 score as “vocal response”. PC2 explained 40.7% of the variation in males and 27.3% of the variation in females. For both sexes, PC2 loadings corresponded to more calls and longer latency to first song, as well as fewer songs and a longer latency to first call (Table 4.2).

Using the PC1 scores as the response variables for both physical approach and vocal responses, we ran linear mixed models using the ‘lme4’ package in R (Bates et al. 2015). We ran models separately for males and females with fixed effects of playback treatment (four levels: Male, Female, Pair, or Control) and season (two levels: pre-breeding or breeding), and pair identity as a random effect to account for repeated sampling of the same individuals. We initially included a fixed effect of treatment presentation order, but this effect was non-significant (all $P > 0.09$) so we excluded it from final analyses. To estimate *P*-values for fixed effects, we conducted likelihood ratio tests comparing models with each combination of additive and interactive fixed effects. To quantify significant fixed effects, we conducted post-hoc analyses using the ‘glht’ function in the ‘multcomp’ package in R (Hothorn et al. 2017).

To account for multiple comparisons in *t*-tests and post-hoc tests, we used the false discovery rate correction with an adjusted α value based on the number of comparisons used in the test (Benjamini & Hochberg 1995). We conducted all analyses using R v.3.3.1 (R Development Core Team 2016). Values are presented as mean ± SE unless otherwise specified.

Results

Overall playback response

Both sexes of Rufous-capped Warbler responded to playback of conspecific songs and calls by approaching the speakers and vocalizing, and response intensity varied with season and sex of the focal bird. In the pre-breeding season, both males and females responded (i.e. approached within 10 m of the playback) to the majority of conspecific treatments (males: 95%, females: 73%). Both sexes had similar response rates to Pair and Female treatments (Chi-squared test: Pair: $\chi^2_1 = 2.4$, $P = 0.12$; Female: $\chi^2_1 = 1.9$, $P = 0.17$; Figure 4.2), although females responded less often than males to the Male treatment ($\chi^2_1 = 5.2$, $P = 0.02$). In the breeding season, males responded to the majority of conspecific treatments (78% overall), whereas females responded little (29% overall; Pair: $\chi^2_1 = 11.8$, $P < 0.001$; Female: $\chi^2_1 = 7.7$, $P = 0.005$; Male: $\chi^2_1 = 6.10$, $P = 0.01$; Figure 4.2). Both sexes responded little to the heterospecific Control stimuli (Figure 4.2).

Physical approach responses

Males showed strong physical approach responses to all conspecific treatments during both seasons, showing the strongest response to the Pair treatments (Figure 4.3A-B). There were significant effects of treatment (likelihood ratio test: $\chi^2_3 = 111.8$, $P < 0.001$) and season ($\chi^2_1 = 17.0$, $P < 0.001$) on responses to playback for males. Males responded more strongly to all conspecific treatments than to the Control (post-hoc tests: all $P < 0.001$; Table 4.3A). They responded significantly more to Pair than Female treatments (mean ± SE: estimate = 0.63 ± 0.21, $z = 2.9$, $P = 0.02$), although responses did not differ between Pair and Male treatments (estimate = 0.47 ± 0.21, $z = 2.2$, $P = 0.12$) or Male and Female treatments (estimate = 0.16 ± 0.21, $z = 0.7$, P

= 0.88). There was no significant interaction effect between treatment and season (likelihood ratio test: treatment × season: $\chi^2_3 = 7.3$, $P = 0.06$), indicating that males showed similar responses to each treatment type during both seasons (Figure 4.3A-B).

Females showed strong physical approach responses to conspecific treatments during the pre-breeding season, but responded very little during the breeding season (Figure 4.3C-D). There were significant effects of treatment (likelihood ratio test: $\chi^2_3 = 41.2$, $P < 0.001$) and season ($\chi^2_1 = 22.8$, $P < 0.001$) on responses to playback for females. There was also a significant interaction between treatment and season ($\chi^2_3 = 11.1$, $P = 0.01$), indicating that females differed significantly in their response strength to each treatment type across seasons. In the pre-breeding season, females responded more strongly to all conspecific treatments than to the Control treatment (all $P < 0.001$; Table 4.3A, Figure 4.3C). They responded significantly more to Pair than Male treatments (estimate = -1.25 ± 0.41, $t = -3.1$, $P = 0.01$)**,** although responses did not differ between Pair and Female treatments (estimate = -0.89 ± 0.41, $t = -2.2$, $P = 0.14$) or Male and Female treatments (estimate = 0.36 ± 0.41, $t = 0.9$, $P = 0.82$). In contrast, during the breeding season, physical approach responses were uniformly low, and did not differ significantly between conspecific treatments and the Control treatment (all $P > 0.05$; Table 4.3B, Figure 4.3D).

Vocal responses

Males showed strong vocal responses to all conspecific treatments during both seasons, but the relative response strength to each treatment type differed seasonally (Figure 4.4A-B). For vocal responses, there was a significant interaction between treatment and season (likelihood ratio test: $\chi^2_3 = 10.2$, $P = 0.02$). In the pre-breeding season, males had a stronger vocal response to all conspecific treatments than to the Control (all $P < 0.001$; Table 4.4A). They responded more to Pair than to Female treatments (estimate = -1.03 ± 0.24, t = -4.3, $P < 0.001$), although the vocal response during Pair and Male treatments (estimate = -0.41 ± 0.24, t = -1.7, P = 0.31; Figure 4.4A) and Male and Female treatments (estimate = -0.62 ± 0.24, t = -2.5, P = 0.05) did not differ. During the breeding season, males had a stronger vocal response to conspecific treatments than to the Control treatment (all $P < 0.001$; Table 4.4A), although vocal response did not differ among conspecific treatments (all $P > 0.05$; Table 4.4A, Figure 4.4B).

Male song duration differed significantly by season (likelihood ratio test: $\chi^2_1 = 22.8$, $P < 0.001$) but not by treatment type ($\chi^2_2 = 0.95$, $P = 0.62$). Males sang significantly longer songs in the breeding season (mean ± SD = 2.1 ± 0.4 s) than in the non-breeding season (mean ± SD = 1.7 ± 0.4 s; post-hoc comparison estimate = -0.35 ± 0.07, z = -5.0, $P < 0.001$). The number of syllables per song also differed significantly by season (likelihood ratio test: $\chi^2_1 = 23.6$, $P < 0.001$) but not by treatment type ($\chi^2_2 = 0.96$, $P = 0.62$). Males sang songs with significantly more syllables in the breeding season (mean ± SD = 15.9 ± 3.9) than in the non-breeding season (mean ± SD = 12.5 ± 3.7; post-hoc comparison estimate = - 3.39 ± 0.68, z = -5.0, $P < 0.001$).

Females showed strong vocal responses during all conspecific treatments during the pre-breeding season, but responded little during the breeding season (Figure 4.4C-D). For female vocal responses, there was a significant interaction between treatment and season (likelihood ratio test: $\chi^2_3 = 18.3$, $P < 0.001$). In the pre-breeding season, females responded more strongly to all conspecific treatments than the Control treatment (all $P \leq 0.001$; Table 4.4B). They also responded more to Pair versus Male treatments (estimate = 1.08 ± 0.34, t = 3.1, P = 0.01) and Pair versus Female treatments (estimate = 1.21 ± 0.34, t = 3.5, P = 0.003), although their vocal responses to Male versus Female treatments did not differ (estimate = 0.13 ± 0.35, t = 0.4, P = 0.98; Figure 4.4C). During the breeding season, female vocal responses were low, and did not differ significantly between conspecific treatments and the Control treatment (all $P > 0.05$; Table 4.4B, Figure 4.4D).

Female song

We recorded female song, previously undocumented in Rufous-capped Warblers, during many conspecific playback trials. Over one-third of females in the study (36%) sang at least one song in response to playback, with female song recorded during 20% of pre-breeding and 6% of breeding season trials. Female songs were similar to male songs in minimum frequency and syllable structure (Table 4.5, Figure 4.5). Female songs, however, were shorter, had a higher maximum frequency and broader bandwidth, contained fewer syllables and syllable types, and had a lower syllable diversity than male songs (Table 4.5).

Discussion

Recent studies comparing male and female signaling in diverse animal taxa suggest that conspicuous signals are not only used by both sexes to attract and compete for mates, but also to compete with conspecifics for territories (e.g., Robinson and Kruuk 2007; Watson and Simmons 2010; Tobias et al. 2011; Cain and Langmore 2015; Tibbetts et al. 2015). In Rufous-capped Warblers, a tropical resident songbird, we found both season- and sex-specific responses to simulated territorial intrusions. In the breeding season, males were more responsive than females to all conspecific treatments. This pattern of response resembles that of temperate bird species (Catchpole & Slater 2008) and other tropical resident species in that males responded more strongly than females during the breeding season (e.g., Busch et al. 2004). In the pre-breeding season, however, responses were more equal between the sexes; at this time of year, both male and female Rufous-capped Warblers responded strongly to all conspecific intruders, as has been observed in previous studies of tropical birds (e.g., Fedy and Stutchbury 2005; Gill et al. 2007, 2008). Males also sang longer songs overall during all conspecific treatments in the breeding season compared to the pre-breeding season. The strong seasonal variation in response to territorial intrusions by Rufous-capped Warblers highlights the importance of conducting behavioural studies across different seasons and breeding stages to gain a more thorough understanding of signal function in tropical resident animals.

During the pre-breeding season, both male and female Rufous-capped Warblers showed strong physical approach and vocal responses to all conspecific treatments, and responded most strongly to the Pair treatment. Our results suggest that shared territory defence is an important function of this species' vocal signals (Tobias et al. 2011; Dowling & Webster 2016). Other studies on tropical resident songbirds have also found strong responses by both sexes to paired intrusions during the non-breeding season (Gill et al. 2007, 2008) and stronger responses overall during the non-breeding season than the breeding season (Fedy & Stutchbury 2005). Defence of territorial and food resources may be critical during the non-breeding season, particularly at our Neotropical dry forest study site, which experiences an extended dry non-breeding season when food resources for warblers are especially scarce. Similarly, ecological resource defence during the non-breeding season is a proposed function of female signaling in Stripe-headed Sparrows (*Peucaea ruficauda*) at the same field site (Illes 2015) and White-bellied Antbirds at another site with a comparable climate (Fedy & Stutchbury 2005). Higher population density during the non-breeding season related to scarcity or patchiness of food resources could also increase the intensity of territorial defence behaviour during this period (e.g., Wicklund and Village 1992). Furthermore, we conducted our study during the pre-breeding season, when intensity of territory and mate defence may be greater than earlier in the non-breeding season, so it could be useful to conduct further studies well outside of the breeding season in order to gain a deeper understanding of any differences between non-breeding and pre-breeding territorial behaviour (e.g., Odom et al. 2017). Regardless of the drivers of territory defence during the non-breeding and pre-breeding periods, both male and female Rufous-capped Warblers likely use vocal signals to defend territorial resources from conspecifics.

During the breeding season, only males showed a strong response to conspecific intruders by vocalizing and approaching the speakers. This pattern of response was also found in the tropical resident Rufous-collared Sparrow (*Zonotrichia capensis*), in which males responded more strongly than females to intruders of both sexes during the breeding season (Busch et al. 2004). The low female response we observed may be related to breeding stage, because 75% (6 of 8) of females with monitored nests in our study were nest-building, egg-laying, or incubating during the trial period, and females are the primary nest-builders and sole incubators in this species (Stiles and Skutch 1989; Chapter 3). Our results therefore refute the intrasexual mate competition hypothesis, since males responded strongly and females responded very little towards all intruders during the breeding season, rather than showing stronger same-sex responses. Although female Rufous-capped Warblers contributed less to territory defence during the breeding season than did males, further studies examining territorial behaviour across multiple breeding stages will be useful for comparing seasonal roles of both sexes in territory defence (e.g., Dowling and Webster 2016).

Male Rufous-capped Warblers showed strong responses to all conspecific treatments during the breeding season, whereas they responded less to the Female treatment than to Pair and Male treatments during the pre-breeding season. Since the focal males were all paired and many of their female mates were incubating on nests during the breeding season, it is possible that males were responding strongly to all conspecific intruders in the absence of territory defence by their mates during the breeding season. Conversely, males may have been signaling more to the playback-simulated female during the breeding season. Our finding that males sang longer songs with more syllables during the breeding season than during the non-breeding season suggests that they modify their singing behaviour depending on the breeding status of their mate. In many songbird species, males increase song complexity during their female mate's pre-fertile or fertile period (e.g., Ballentine et al. 2003; Zhang et al. 2015). Another explanation is that males approached and vocalized more to females in order to solicit extra-pair copulations. Rufous-capped Warblers in our study population live at high densities, suggesting that extra-pair mating may occur at high rates. Longer songs are also associated with increased extra-pair paternity and decreased loss of within-pair paternity for males in at least one other songbird species (Willow Warblers, *Phylloscopus trochilus*; Gil et al. 2007). However, any such interpretations should be made cautiously, because extra-pair paternity rates are currently unknown for Rufous-capped Warblers (Macedo et al. 2008). Although the results of our study do not support the mate attraction hypothesis for male vocal signals, future detailed vocal analyses will investigate how male Rufous-capped Warblers modify their song structure and singing behaviour relative to breeding status.

Our study was the first to document female song in Rufous-capped Warblers. Although calls were the most common vocalization used by females in response to playback, over one-third of females in our study population also produced song during playback trials. Female song is now known to be more common in birds than previously thought (Odom et al. 2014), and was likely overlooked in the past in species such as the Rufous-capped Warbler in which both sexes look alike and male and female songs are similar in acoustic structure. A recent comparative analysis of female trait evolution in the Parulidae revealed that female song likely evolved independently in different genera, suggesting that it may serve different functions across species (Najar & Benedict 2015). Occasional female song early in the breeding season is reported for at least 13 temperate zone parulid warbler species (e.g., Taff et al. 2012; Matthews et al. 2016). Pair bond formation is the proposed function in at least four temperate-breeding species: Wilson's Warbler (*Cardellina pusilla*; Gilbert and Carroll 1999), Common Yellowthroat (*Geothlypis trichas*; Taff et al. 2012), Prothonotary Warbler (*Protonotaria citrea*; Matthews et al. 2016), and Prairie Warbler (*Setophaga discolor*; Nolan 1978), and is also a possible function of female song in Rufous-capped Warblers. Territory defence is a more likely function of female song in our study species, as in duets produced by other tropical species (e.g., Hall and Peters 2008; Benedict 2010; Dahlin and Wright 2012; Koloff and Mennill 2013) and non-duet female songs used by temperate and tropical songbirds (Hall et al. 2015; Krieg & Getty 2016). We documented the majority of female song during the Pair treatment in the pre-breeding season, to which both males and females responded strongly by vocalizing at high rates and approaching the speakers closely. Furthermore, females sang in our study population in other situations where a territory defence function is likely: (1) in response to continuous playback of conspecific songs and calls during capture attempts; (2) spontaneous song on the territory when unaccompanied by the male; and (3) during the dawn chorus in the first week of the breeding season (Chapter 2). Further studies testing seasonal responses of both male and female Rufous-capped Warblers to female song playback would be useful to thoroughly investigate the function of this signal.

Our study revealed seasonal variation in the responses of Rufous-capped Warblers to territorial intrusions, wherein pairs typically responded together during the pre-breeding season and males typically responded alone in the breeding season. We found that males primarily used song during shared territory defence against both single and paired conspecific intruders, whereas females primarily used calls. The similarity of this response pattern to other temperate warbler species is consistent with the proposed temperate origins of the Family Parulidae and other related families (reviewed in Barker et al. 2015), and provides important evidence for further investigation into the evolution of duetting behaviour and female song in this clade. We also found that males and females may make different use of similar signals, such as song, depending on season or breeding status. Male Rufous-capped Warblers used song to defend territories during both seasons, whereas females primarily used song during the non-breeding season. Indeed, shared male and female signals in other species, such as complex song in Superb Fairy-wrens (*Malurus cyaneus*; Cain and Langmore 2015) and bill colour in American Goldfinches (*Spinus tristis*; Murphy et al. 2014), are also used differently by males and females. Species-specific levels of female competition may also affect female signaling behaviour (Colombelli-Négrel 2016), although this idea requires further investigation in our study species and other tropical resident species. Overall, our research provides support for the shared territory defence hypothesis for both male and female Rufous-capped Warblers. In addition to paralleling results of conspecific intrusion studies on other tropical species (e.g., Fedy and Stutchbury 2005; Gill et al. 2007, 2008), our study demonstrates that seasonality influences the territorial behaviour of both sexes in a year-round resident songbird. It also highlights that females of a non-duetting tropical songbird participate in shared territory defence as effectively as duetting species, by using vocal signals such as calls and songs not produced as duets. Future studies incorporating playback of multiple types of male and female vocalizations (such as male calls and female songs), or using visual models to experimentally test responses to visual signals, will be useful to further understand the relative importance of vocal and visual signals, and seasonal variation in use of such signals, in the territorial behaviour of tropical animals.

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Tables

Table 4.1. Summary of correlations between physical approach response variables for first principal component in two separate principal components analyses, one for male responses to playback, and one for female responses to playback.

	Male PC1	Female PC1
Eigenvalue	2.92	3.03
Percentage of variation (%)	73.0	75.7
Latency to approach within 10 m	-0.55	-0.55
Closest approach	-0.53	-0.52
Time spent within 10 m	0.55	0.54
Number of flights over speaker	0.34	0.37

Table 4.2. Summary of correlations between vocal response variables for first and second principal components in two separate principal components analyses, one for male responses to playback, and one for female responses to playback.

	Male PC1	Male PC2	Female PC1	Female PC2
Eigenvalue	2.05	1.63	2.54	1.09
Percentage of variation (%)	51.1	40.7	63.4	27.3
Number of songs	0.62	-0.33	-0.46	0.59
Number of calls	0.19	0.70	-0.51	-0.48
Latency to first song	-0.65	0.24	0.53	-0.42
Latency to first call	-0.40	-0.58	0.50	0.49

Table 4.3. Linear mixed model and post-hoc comparisons results of physical approach response (PC1) for male (A) and female (B) Rufous-capped Warblers in response to playback. Significant effects (after false discovery rate correction) are highlighted in bold.

A. Male physical approach response (PC1)			
Full LMM	χ^2	df	*P* value
Treatment	**111.8**	**3**	**<0.001**
Season	**17.0**	**1**	**<0.001**
Treatment × Season	7.3	3	0.06
Post-hoc comparisons	Estimate ± SE	*z* value	*P* value
Both seasons			
Control – Female	**1.66 ± 0.21**	**7.7**	**<0.001**
Control – Male	**1.82 ± 0.21**	**8.5**	**<0.001**
Control – Pair	**2.29 ± 0.21**	**10.7**	**<0.001**
Female – Male	0.16 ± 0.21	0.7	0.88
Female – Pair	**0.63 ± 0.21**	**2.9**	**0.02**
Male – Pair	0.47 ± 0.21	2.2	0.12
B. Female physical approach response (PC1)			
Full LMM	χ^2	df	*P* value
Treatment	**41.2**	**3**	**<0.001**
Season	**22.8**	**1**	**<0.001**
Treatment × Season	**11.1**	**3**	**0.01**
Post-hoc comparisons	Estimate ± SE	*t* value	*P* value
Breeding season			
Control – Female	-0.90 ± 0.44	-2.0	0.17
Control – Male	-1.14 ± 0.44	-2.6	0.05
Control – Pair	-0.97 ± 0.44	-2.2	0.13
Female – Male	-0.25 ± 0.44	-0.6	0.95
Female – Pair	-0.07 ± 0.44	-0.2	1.0
Male – Pair	0.18 ± 0.44	0.4	0.98
Pre-breeding season			
Control – Female	**-2.01 ± 0.41**	**-4.9**	**<0.001**
Control – Male	**-1.65 ± 0.41**	**-4.0**	**<0.001**
Control – Pair	**-2.90 ± 0.41**	**-7.1**	**<0.001**
Female – Male	0.36 ± 0.41	0.9	0.82
Female – Pair	-0.89 ± 0.41	-2.2	0.14
Male – Pair	**-1.25 ± 0.41**	**-3.1**	**0.01**

Table 4.4. Linear mixed model and post-hoc comparisons results of male (A) and female (B) vocal responses (PC1) of Rufous-capped Warblers to playback. Significant effects (after false discovery rate correction) are highlighted in bold.

A. Male vocal response (PC1)			
Full LMM	χ^2	df	*P* value
Treatment	**115.7**	**3**	**<0.001**
Season	**9.5**	**1**	**0.002**
Treatment × Season	**10.2**	**3**	**0.02**
Post-hoc comparisons	Estimate ± SE	*t* value	*P* value
Breeding season			
Control – Female	**-1.34 ± 0.26**	**-5.1**	**<0.001**
Control – Male	**-1.37 ± 0.26**	**-5.3**	**<0.001**
Control – Pair	**-1.41 ± 0.26**	**-5.4**	**<0.001**
Female – Male	-0.03 ± 0.26	-0.1	1.0
Female – Pair	-0.07 ± 0.26	-0.3	0.99
Male – Pair	-0.04 ± 0.26	-0.2	1.0
Pre-breeding season			
Control – Female	**-1.31 ± 0.24**	**-5.4**	**<0.001**
Control – Male	**-1.93 ± 0.24**	**-8.0**	**<0.001**
Control – Pair	**-2.34 ± 0.24**	**-9.8**	**<0.001**
Female – Male	-0.62 ± 0.24	-2.5	0.05
Female – Pair	**-1.03 ± 0.24**	**-4.3**	**<0.001**
Male – Pair	-0.41 ± 0.24	-1.7	0.31
B. Female vocal response (PC1)			
Full LMM	χ^2	df	*P* value
Treatment	**35.4**	**3**	**<0.001**
Season	**21.1**	**1**	**<0.001**
Treatment × Season	**18.3**	**3**	**<0.001**
Post-hoc comparisons	Estimate ± SE	*t* value	*P* value
Breeding season			
Control – Female	0.72 ± 0.37	1.9	0.22
Control – Male	0.74 ± 0.37	2.0	0.19
Control – Pair	0.47 ± 0.37	1.3	0.58
Female – Male	0.03 ± 0.37	0.1	1.0
Female – Pair	-0.25 ± 0.37	-0.7	0.91
Male – Pair	-0.27 ± 0.37	-0.7	0.88
Pre-breeding season			
Control – Female	**1.44 ± 0.35**	**4.1**	**<0.001**
Control – Male	**1.57 ± 0.35**	**4.5**	**<0.001**
Control – Pair	**2.65 ± 0.34**	**7.7**	**<0.001**
Female – Male	0.13 ± 0.35	0.4	0.98
Female – Pair	**1.21 ± 0.34**	**3.5**	**0.003**
Male – Pair	**1.08 ± 0.34**	**3.1**	**0.01**

Table 4.5. Comparison of seven acoustic variables of male and female Rufous-capped Warbler songs. All values are mean ± SD, and significant t-test results are highlighted in bold.

Acoustic variable	Males (n = 10)	Females (n = 10)	*t* value	*P* value
Maximum frequency (kHz)	**8.06 ± 0.23**	**8.70 ± 0.34**	**4.97**	**<0.001**
Minimum frequency (kHz)	3.28 ± 0.17	3.23 ± 0.32	-0.44	0.67
Bandwidth (kHz)	**4.77 ± 0.29**	**5.47 ± 0.40**	**4.45**	**<0.001**
Song duration (s)	**2.37 ± 0.26**	**1.26± 0.28**	**-9.18**	**<0.001**
Total number of syllables	**17.57 ± 2.73**	**7.23 ± 1.83**	**-9.96**	**<0.001**
Number of syllable types	**14.24 ± 2.53**	**4.81 ± 1.64**	**-9.88**	**<0.001**
Syllable diversity	**0.81 ± 0.05**	**0.68 ± 0.16**	**-2.31**	**0.04**

Figures

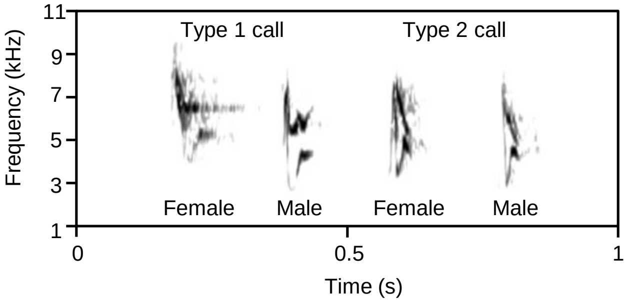

Figure 4.1. Sound spectrograms of male and female Rufous-capped Warbler ‘chip’ calls of two types. Male and female calls are similar in structure, but male calls have a lower average minimum frequency than female calls.

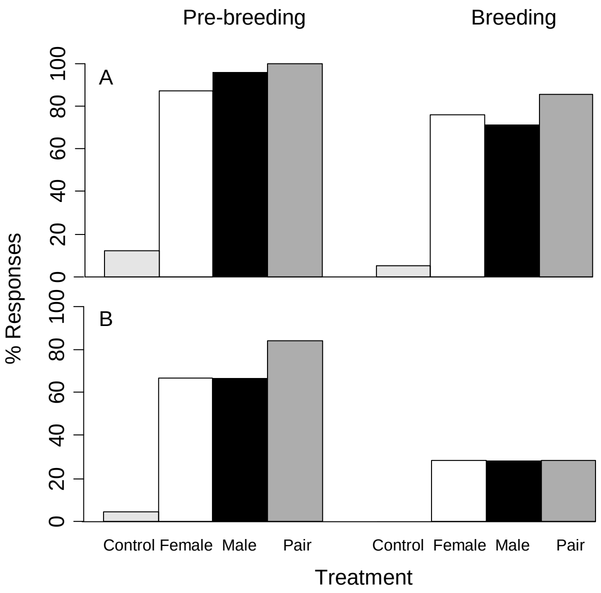

Figure 4.2. The responses of Rufous-capped Warbler males (A) and females (B) to four playback treatments (heterospecific control, conspecific female calls, conspecific male songs, or both conspecific female calls and male songs) during the pre-breeding season (left) and the breeding season (right). Males responded to all conspecific treatments during both seasons, while females responded primarily in the pre-breeding season.

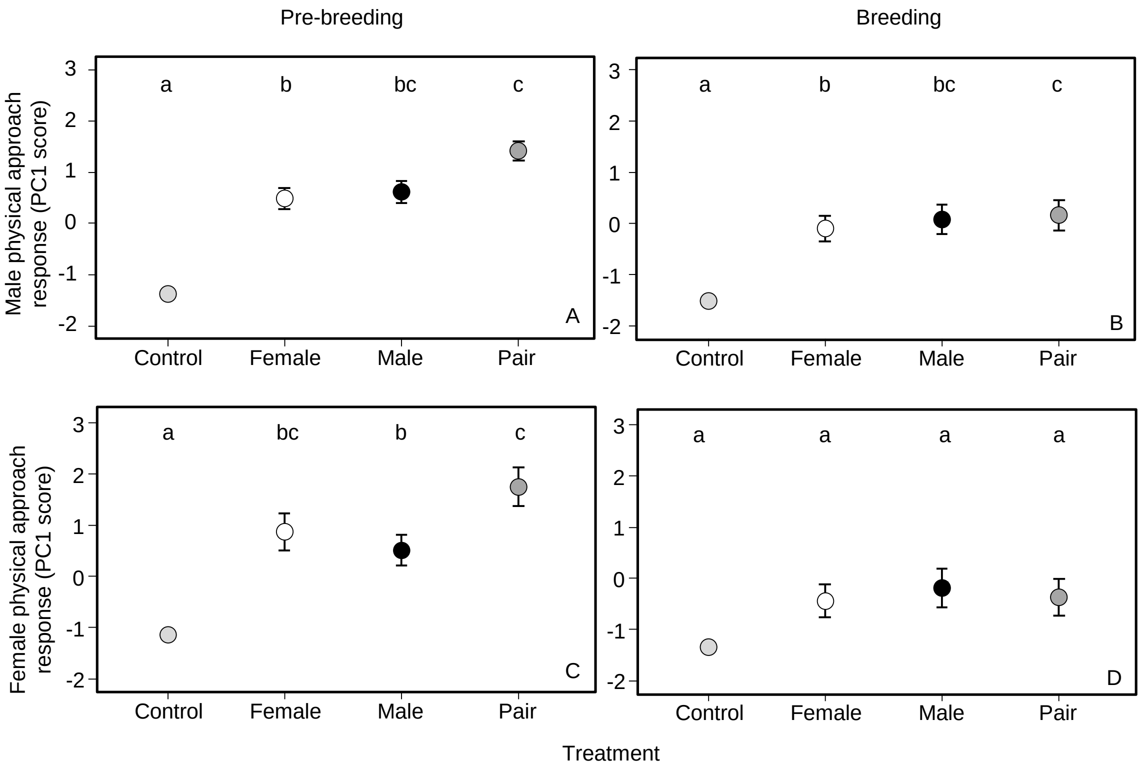

Figure 4.3. Male Rufous-capped Warblers responded more strongly to Pair treatments than to Female treatments during both the pre-breeding (A) and breeding (B) seasons. Females responded more strongly to Pair treatments than to Male treatments during the pre-breeding season (C) and responded weakly to all conspecific treatments during the breeding season (D). A higher PC 1 score indicates stronger response intensity to playback. Error bars indicate mean ± SE of PC 1 scores, and letters above bars denote statistical significance.

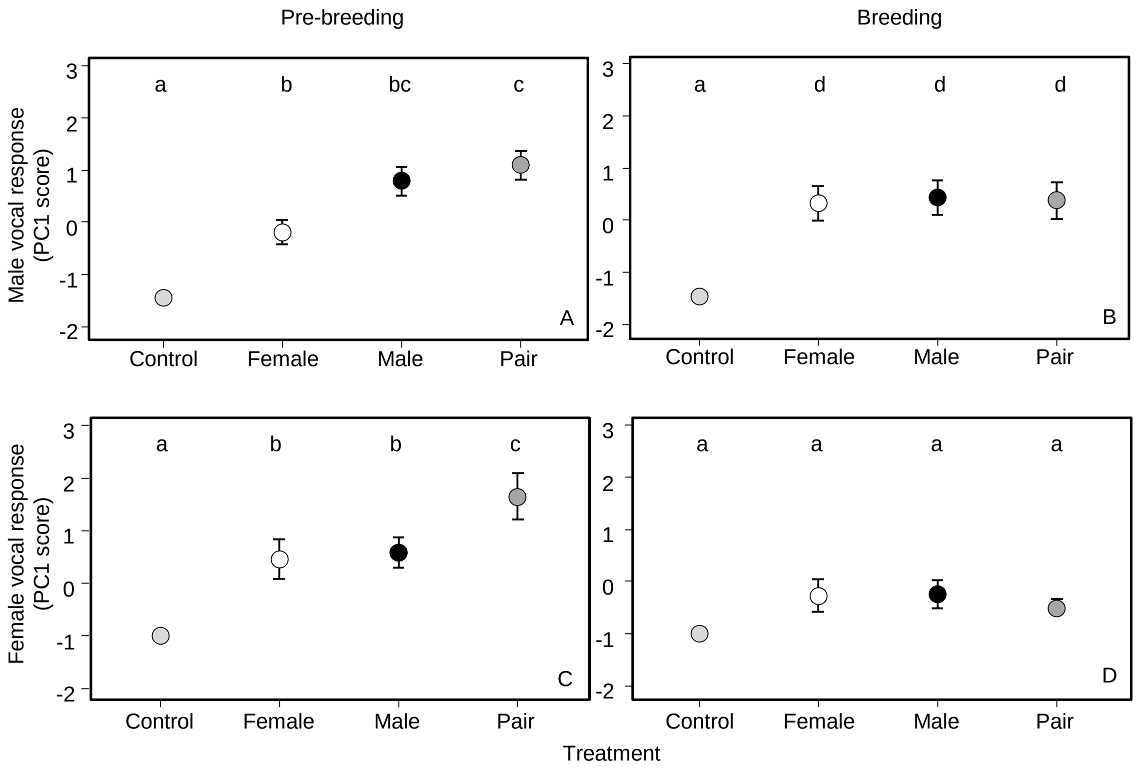

Figure 4.4. Males had stronger vocal responses to Pair treatments than to Female treatments during the pre-breeding season (A), but responded strongly to all conspecific treatments during the breeding season (B). Females had a stronger vocal response to Pair than to Male and Female treatments during the pre-breeding season (C), but responded little to all conspecific treatments during the breeding season (D). A higher PC 1 score indicates stronger response intensity to playback. Error bars indicate mean ± SE of PC 1 scores, and letters above bars denote statistical significance.

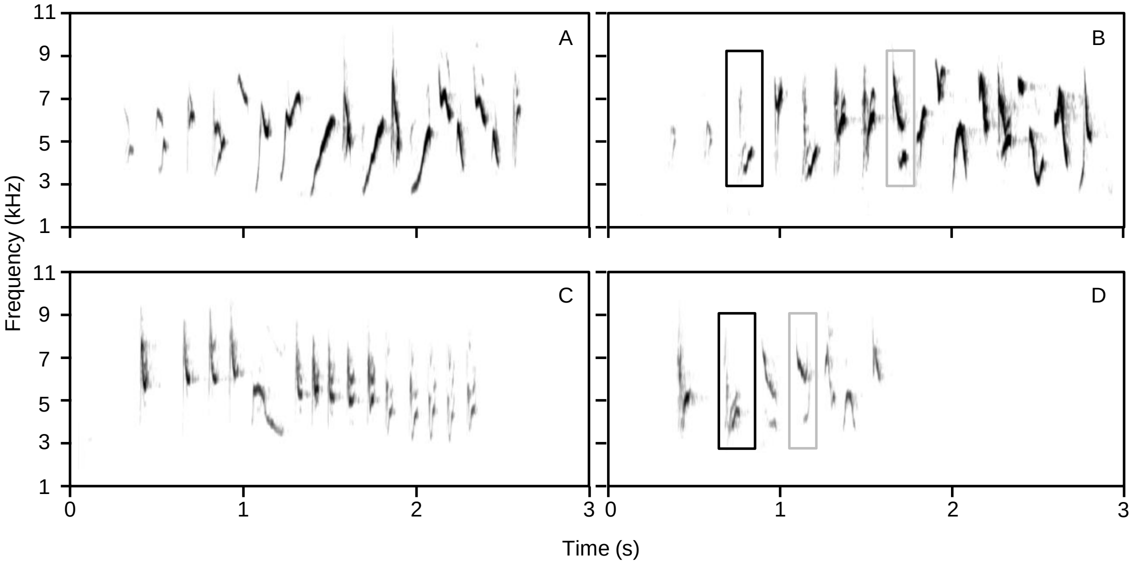

Figure 4.5. Sound spectrograms of male (A-B) and female (C-D) Rufous-capped Warbler songs from two mated pairs (A/C and B/D) in Santa Rosa, Costa Rica. Male and female songs share a similar syllable structure and organization. Examples of syllable types shared by males and females are enclosed in boxes for male song (B) and female song (D).

Chapter 5: Divergence in plumage, voice, and morphology indicates two species groups of Rufous-capped Warblers (*Basileuterus rufifrons*)

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*****This chapter is the outcome of joint research with D. Mennill, S. Doucet, and J. R. Sosa-López.

Chapter Summary

Neotropical biodiversity is considerable, but it is likely underestimated owing to historical gaps in our sampling effort and our focus on morphological features of animals rather than divergence in their mating signals and behaviour. Recent multi-trait analyses incorporating morphological, plumage, and vocal data, combined with molecular analyses, allow more accurate descriptions of tropical diversity. We present a comprehensive study of morphological, plumage, and vocal variation in the Neotropical resident Rufous-capped Warbler (*Basileuterus rufifrons*). This species' taxonomic status is controversial because the *B. r. salvini* subspecies is intermediate in plumage between neighbouring *B. r. delattrii* and *B. r. rufifrons*, and because *delattrii* and *rufifrons* also reportedly hybridize in a zone of sympatry. Using morphological and spectral plumage measurements of field and museum specimens, and vocalizations from field recordings and natural sound libraries, we compared phenotypes of all eight recognized subspecies. We found that *B. r. delattrii* and *B. r. rufifrons* differ significantly in morphology and plumage, whereas *B. r. salvini* is similar to *B. r. rufifrons* rather than being intermediate for most plumage features. Vocalizations fall into two distinct groups, *delattrii* and *rufifrons-salvini*; these two groups differ in multiple acoustic characteristics with no overlap even between individuals from the *delattrii-rufifrons* zone of sympatry. Our results suggest that Rufous-capped Warblers comprise two distinct groups, Rufous-capped Warblers (*B. r. rufifrons* and *salvini* as well as *B. r. caudatus*, *dugesi*, and *jouyi*) and Chestnut-capped Warblers (*B. r. delattrii* as well as *B. r. actuosus* and *mesochrysus*), and provide evidence for a revision of the species' current taxonomic status. Future genomic analysis of samples from multiple sites in Mexico and Central America will further refine our assessment of range-wide phenotypic and genetic divergence in this species complex.

Introduction

The Neotropics exhibit high biodiversity, with a wide variety of habitats promoting local adaptation and reproductive isolation (Pérez-Emán 2005; Cadena et al. 2007; Smith et al. 2014). Although biodiversity remains greatly underestimated in tropical species (Milá et al. 2012; Freile et al. 2014), recent advances in the use of multi-trait phenotypic analyses in systematics research are revealing previously undocumented variation (e.g., Cadena & Cuervo 2010; González et al. 2011; Caro et al. 2013; Sandoval et al. 2017). The resulting taxonomic refinements allow researchers to more accurately describe tropical diversity and focus conservation efforts (e.g., Haig & D'Elia 2010).

Although Neotropical birds are well-studied compared to other taxa, particularly non-vertebrates, many historical subspecies descriptions were based on specimens collected prior to the use of audio recordings of vocalizations and spectrophotometric analyses of plumage colour, and therefore morphology and subjective impressions of plumage colouration were the primary traits used for classification (e.g., Ridgway 1902; Monroe 1968). Since birdsong is a sexually-selected mating signal (Catchpole & Slater 2008), song divergence between populations may promote reproductive isolation even when morphology and plumage vary little (Toews & Irwin 2008; Dingle et al. 2010). In fact, many cryptic species in both temperate and tropical regions have recently been described based largely upon vocal differences (e.g., Toews & Irwin 2008; O'Neill et al. 2011; Hosner et al. 2013). However, a lack of range-wide data on vocalizations, particularly from hybrid or contact zones, means that taxonomic relationships remain unclear even for widespread species (e.g., González et al. 2011). In order to assess the degree of reproductive isolation between closely-related populations, researchers should therefore compare phenotypes both within and outside secondary contact or hybrid zones. Gradual or clinal variation would indicate limited reproductive isolation between groups, whereas more abrupt changes would indicate strong isolation (e.g., the groups overlap in range but do not interbreed; Toews & Irwin 2008).

The Rufous-capped Warbler (*Basileuterus rufifrons*) is a common resident warbler species distributed in Arizona, Mexico, Central America, and South America whose taxonomic status has long been disputed (Ridgway 1902; Todd 1929) because of considerable variation in plumage and vocalizations throughout its range (Curson 2010). Eight subspecies are recognized, seven of which fall into two plumage-based groups: (1) the northern, white-bellied *rufifrons* group of southern Arizona, Mexico, and western Guatemala (*B. r. caudatus*, *dugesi*, *jouyi*, and *rufifrons*); and (2) the southern, yellow-bellied *delattrii* group of southeastern Mexico, Central America, and Colombia and Venezuela (*B. r. actuosus*, *delattrii*, and *mesochrysus*; Curson 2010; Figure 5.1). Songs of both males and females also differ between these two groups; *rufifrons* group songs have trilled, repetitive syllables, whereas *delattrii* group songs have varied, frequency-modulated syllables (Howell & Webb 1995; Curson 2010; Chapter 2). The eighth subspecies, *B. r. salvini,* found in the lowland Atlantic area of southern Mexico and northern Central America, is intermediate between the *delattrii* and *rufifrons* groups: it has a pale yellow or mixed white-yellow belly, although other features such as crown colour resemble the *rufifrons* group (Friedmann et al. 1957; Curson 2010). However, comparisons of *B. r. salvini* vocalizations to those of the *delattrii* and *rufifrons* groups are contradictory (Monroe 1968; Howell & Webb 1995), and no previous analyses have quantified vocal differences between any Rufous-capped Warbler subspecies. Furthermore, the *delattrii* group, *rufifrons* group, and *B. r. salvini* differ broadly in their habitat preferences. Whereas birds in the *delattrii* group inhabit dry to semi-humid deciduous forest (e.g., tropical dry forest) and second-growth habitat (e.g., coffee plantations) between 0–1500 m a.s.l., birds in the *rufifrons* group live in dry scrub, semi-open, and second-growth habitats between 0–3000 m a.s.l., most commonly at mid- to high-elevation (>1000 m a.s.l.), and *B. r. salvini* live in semi-open and second-growth lowland habitats (0–500 m a.s.l.; Curson 2010). Based on the disputed status of *B. r. salvini*, some authorities have historically lumped all subspecies together (Ridgway 1902; Monroe 1968), whereas others have split them into the Rufous-capped Warbler *B. rufifrons* (i.e. "the *rufifrons* group" and *B. r. salvini*) and the Chestnut-capped Warbler *B. delattrii* (i.e. "the *delattrii* group"; Todd 1929; Howell & Webb 1995).

Over the past century, the Rufous-capped Warbler taxon has been repeatedly split and lumped with the addition of new data throughout the species' range. The first range-wide classification of this clade (Ridgway 1902) considered all subspecies to be conspecific because of the similar belly and upperpart colouration of the *delattrii* group and *B. r. salvini.* However, subsequent work classified the *delattrii* group as a separate species from the *rufifrons* group and *B. r. salvini* based on the *delattrii* group's positive wing-tail ratio (wing longer than tail), all-yellow breast and belly, chestnut crown and auricular, and absence of a white lower auricular patch (Todd 1929; Figure 5.2). All subspecies were once again grouped together based on the work of Monroe (1968), who combined *B. r. delattrii* and *B. r. salvini* because of reported hybridization between the two subspecies in eastern Guatemala, El Salvador, and Honduras. Despite stating that "there are no apparent differences in song pattern, ethology, or morphology" between *B. r. delattrii* and *B. r. salvini*, Monroe (1968) supplied no supporting information on the vocalizations or behaviour of either subspecies. In their comprehensive field guide to Mexican birds, Howell & Webb (1995) favoured a species split. They argued that: (1) *B. r. salvini* are not found in El Salvador and Honduras, so they could not interbreed with *B. r. delattrii* there; (2) there are no confirmed hybrids between *B. r. delattrii* and either *B. r. rufifrons* or *B. r. salvini*; and (3) plumage features (e.g., belly, crown, and auricular colour) and song are similar between *B. r. rufifrons* and *B. r. salvini* whereas both differ markedly from *B. r. delattrii.* They also reported that *B. r. delattrii* and *B. r. rufifrons* are sympatric in southeastern Mexico and western Guatemala (Howell & Webb 1995), although the presence of hybridization in this contact zone needs confirmation.

Our objective was to conduct a comprehensive study of Rufous-capped Warbler morphological, plumage, and vocal variation in order to resolve the taxonomic status of this clade. Our main focus was to assess whether *B. r. salvini* is intermediate between, or more similar to either, the *delattrii* and *rufifrons* groups; and to compare individual birds' phenotypes in the *B. r. delattrii* and *B. r. rufifrons* contact zone to evaluate the presence and extent of hybridization. Using audio recordings of vocalizations and spectrophotometric measurements of plumage colour collected throughout this species' range, we sought to re-assess taxonomic relationships within the Rufous-capped Warbler clade.

Methods

Morphology

We measured Rufous-capped Warbler specimens from 11 natural history museums. We aimed to sample both sexes evenly within geographical regions, and included only adult birds of known sex in our final analyses (*n* = 261 males and 180 females). We also included morphological field data collected from 250 live birds (*n* = 195 males and 55 females) in Costa Rica and southern Mexico from 2013–2017. The same observer (A. Demko) collected all field and museum measurements. A comparison between field and museum data showed that the majority of measurements did not differ significantly between the two sampling methods (Supplementary Methods 1; Table 5.S1). Although our field and museum data set spanned the entire range distribution of the species and all documented subspecies, our primary focus was on southern Mexico and northern Central America (Guatemala, Honduras, and El Salvador), the region of reported sympatry and hybridization between *B. r. delattrii*, *B. r. rufifrons*, and *B. r. salvini* (Monroe 1968; Howell & Webb 1995).

For each individual, we measured six standard morphological traits (e.g., Cadena & Cuervo 2010; Halley et al. 2017): wing chord (unflattened wing length), tail length, tarsus length, bill length (nares to tip), and bill depth and bill width (both measured at anterior end of nares). We measured wing and tail to the nearest 0.5 mm with a wing ruler, and tarsus and bill to the nearest 0.05 mm with dial calipers. For each sex separately, we ran MANOVA and Discriminant Function Analysis (DFA) to assess whether individuals could be reliably grouped into subspecies and plumage groups, and used the leave-one-out cross-validation (LOOCV) method to determine the most diagnostic morphometric variables (e.g., Sandoval et al. 2017). We analyzed data using three classifications: (1) all subspecies divided into three plumage-based groups (yellow-bellied *delattrii* group, white-bellied *rufifrons* group, and *B. r. salvini*); (2) three extensively-sampled subspecies with sympatric or parapatric distributions in southern Mexico (*B. r. delattrii*, *B. r. rufifrons*, and *B. r. salvini*); and (3) all eight subspecies separately. To compare each plumage group and subspecies, we ran ANOVA and diagnosability index tests for the most diagnostic traits in each DFA (Patten & Unitt 2002; Sandoval et al. 2017). The diagnosability test was done at the 75% level; positive values of this index indicate that the trait is diagnosable between groups, whereas negative values indicate that the trait is not diagnosable (Patten & Unitt 2002). We also ran ANOVA comparing the wing-tail ratio (wing length minus tail length) between groups, because of previous reports of subspecific variation in this trait wherein the *rufifrons* group have shorter wings than tails and the *delattrii* group have longer wings than tails (Todd 1929).

Plumage

To compare overall plumage patterns across individuals, we took digital photographs of the dorsal, ventral, and left and right sides of each museum specimen (*n* = 261 males and 180 females). Using these photographs, we measured two plumage traits varying between subspecies. The first trait was the extent of white in the auricular; birds had either a white patch below the eye extending to the cheek, or a small white spot below the eye. The second trait was the extent of yellow plumage coverage on the breast and belly, calculated by dividing the total length from chin to lower edge of the yellow breast patch by the total body length from chin to cloaca measured using the measurement tool in GIMP2 photo editor. We scored specimens with an entirely yellow breast and belly as 1 (100%) for this trait.

We collected five spectral reflectance measurements from each of 10 plumage patches per specimen: belly, breast, crown, mantle (upperparts), nape, rump, shoulder, tail, throat, and wing. We then averaged the five measurements within a plumage patch to obtain one measurement per patch for each individual. In cases where a particular plumage patch was damaged or missing on a specimen (e.g., missing tail feathers), we excluded that measurement from the analysis. We collected plumage reflectance measurements using an Ocean Optics USB 2000 reflectance spectrometer and PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, FL, USA). The fiber-optic probe was fitted with a rubber tip to maintain the probe at a fixed distance of ~5 mm from the surface being measured, perpendicular to the surface, and to exclude external light. We calibrated the readings using a Spectralon white standard (Ocean Optics, Dunedin, FL, USA) after every 10 specimens measured.

We ran Principal Components Analysis (PCA) with the correlation matrix to produce composite response variables, using as original variables the mean reflectance values in 10 nm bins between 300–700 nm (Montgomerie 2006). Given the absence of UV reflectance in the pigments of the crown, we ran two PCAs: one for the crown patch only, and the other for the remaining nine plumage patches. We excluded individuals labelled as juveniles on the museum tags, and those with visibly juvenile plumage (e.g., buffy underpart colour). We therefore included only adult birds of known sex in our final analyses (*n* = 261 males and 180 females for the all-subspecies analysis; and *n* = 183 males and 136 females for the three-subspecies analysis). We ran separate PCAs for the all-subspecies and three-subspecies analyses, and included all principal components (PCs) with eigenvalues >1 in subsequent analyses. We describe the most influential PCs below for the all-subspecies analysis. PC loadings and eigenvalues were similar for the three-subspecies analysis, and are described in Supplementary Methods 2.

For the crown patch, PC1 accounted for 79.5% of the variation (eigenvalue: 32.6) with negative loadings across the 300–700 nm range, and was negatively associated with brightness. PC2 accounted for 15.1% of the variation (eigenvalue: 6.2) with negative loadings from 300–510 nm and increasingly positive loadings from 520–700 nm, and was positively associated with redness. PC3 represented 4.4% of the variation (eigenvalue: 1.5) with positive loadings from 390–590 nm and low negative loadings at 300–380 nm and 600–700 nm; PC3 was positively associated with yellow and green reflectance (Figure 5.S1).

For the other nine patches, PC1 accounted for 84.1% of the variation (eigenvalue: 34.5) with negative loadings across the 300–700 nm range, and was negatively associated with brightness. PC2 accounted for 11.3% of the variation (eigenvalue: 4.7) with positive loadings from 370–500 nm and negative loadings at 310–360 and 510–700 nm. PC2 was associated with carotenoid level, wherein negative scores corresponded to a high carotenoid component to the patch (i.e. yellow) and positive scores indicated a lack of carotenoids (i.e. white or grey). PC3 accounted for 4.1% of the variation (eigenvalue: 1.7) with positive loadings from 410–700 nm and negative loadings from 300–400 nm, and was negatively associated with UV reflectance (Figure 5.S2).

Using the ‘lme4’ package in R (Bates et al. 2015), we ran general linear models using PC1, PC2, and PC3 for each plumage patch as response variables, and subspecies, sex, and subspecies × sex interaction as fixed effects. Since the age of museum specimens ranged over more than 100 years (range: 1893–2009) and since reduced reflectance, especially in the UV part of the spectrum, is reported for older museum specimens of other warbler species (McNett & Marchetti 2005), we also included specimen collection year as a covariate in the models. We conducted likelihood ratio tests to estimate *P*-values for each independent variable, and conducted post-hoc analyses for all significant effects using the ‘glht’ function in R’s ‘multcomp’ package (Hothorn et al. 2017). We conducted all statistical analyses using R v.3.4.3 (R Development Core Team 2017).

Songs

We measured songs obtained from digital recordings of singing males and females from our own field recordings collected in Mexico and Costa Rica, supplemented with recordings from the University of Florida and the Cornell Lab of Ornithology’s Macaulay Library. We focused only on high-quality songs in this analysis (i.e. those with a high signal-to-noise ratio and minimal overlapping background noise). Our sample size was 405 songs from 126 males (37 *delattrii* group, 75 *rufifrons* group, and 14 *B. r. salvini*) and 18 females (3 *delattrii* group, 14 *rufifrons* group, and 1 *B. r. salvini*). We measured 1–12 songs per individual; within individuals, each song measured was a different variant (i.e. unique sequence of syllable types) to account for intra-individual song variation. We included 1–16 individuals from each sampling location.

Using Adobe Audition 3.0 (Adobe, San Jose, CA, USA), we high-pass filtered all songs at 1 kHz to remove background noise, and spot-filtered background noise (e.g., vocalizations of other animals) overlapping with the frequency range of Rufous-capped Warbler vocalizations using the lasso selection tool. Using Avisoft-SASLab Pro (version 5.2.09; R. Specht, Berlin, Germany), we measured 14 acoustic variables commonly used for vocal comparisons of closely-related avian taxa (e.g., Toews & Irwin 2008; Cadena & Cuervo 2010; Mason et al. 2014; Kenyon et al. 2017). For whole songs, we measured minimum frequency, maximum frequency, frequency bandwidth, peak frequency, total number of syllables, number of different syllable types, song duration, syllable production rate (total number of syllables/song duration), and syllable versatility. Syllable versatility is the ratio of the number of different syllable types to the total number of syllables, and is useful for quantifying the repetition rate of syllables within a song (Gil & Slater 2000). For individual syllables, we measured minimum frequency, maximum frequency, peak frequency, and duration, and calculated mean values across all syllables within a song.

We used the automatic parameter measurement tool in Avisoft to measure both song- and syllable-specific variables, except for song peak frequency, directly from spectrograms. The spectrogram window settings we used were FFT size of 512, time resolution of 1.5 ms, and frequency resolution of 43 Hz; these settings optimized both frequency and temporal resolution across the spectral range of the songs. To standardize measurements across songs, we measured all variables at a threshold amplitude of -20 dB from the peak amplitude. In rare cases where specific syllables could not be measured accurately at the -20 dB threshold (e.g., faint introductory syllables), we either adjusted the threshold amplitude to measure those syllables, or measured them manually on the spectrograms using on-screen cursors. We measured song peak frequency from power spectra with a Hann window, FFT size of 512, and frequency resolution of 62.5 Hz.

To compare acoustic variation between male songs for each subspecies, we first calculated mean values of each variable within individuals to produce a single data point for each individual. We ran separate ANOVA for each variable to assess subspecies differences, and then conducted PCA with a correlation matrix to produce composite response variables based on all 14 spectro-temporal variables (e.g., Toews & Irwin 2008; Mason et al. 2014). The first three PCs had eigenvalues >1 and accounted for 54.1%, 16.1%, and 8.8% of the total variation, respectively (Table 5.S2). We ran ANOVA to compare PC scores between the *delattrii* group, *rufifrons* group, and *B. r. salvini*. To assess whether individual songs could be accurately grouped according to our pre-defined subspecies categories, we used MANOVA and DFA with LOOCV (e.g., Cadena & Cuervo 2010; Halley et al. 2017; Kenyon et al. 2017). We ran DFA using a subset of eight non-collinear variables (song duration, song minimum frequency, song maximum frequency, peak frequency, number of syllables, number of syllable types, syllable production rate, and syllable versatility).

To assess sex-specific differences in the *rufifrons* group and *B. r. salvini* songs, we ran *t*-tests to compare male and female songs for each variable. We excluded females from the previous analyses owing to small sample sizes of female songs. For multiple comparisons, we applied the Benjamini-Hochberg false discovery rate α-value correction (Benjamini & Hochberg 1995).

Results

Morphology

The morphology of the *delattrii* and *rufifrons* groups showed significant separation in several characters. Furthermore, *B. r. salvini* was similar to the *rufifrons* group, rather than being intermediate between the *delattrii* and *rufifrons* groups. For males, the three groups differed as a whole according to MANOVA (Wilk's $\Lambda = 0.22$, $F_{2,\,371} = 67.8$, $P < 0.001$). The most diagnostic traits in our discriminant analysis were wing and tail length, which together correctly classified 82.9% of individuals. Classification was accurate for both the *delattrii* (119 of 127 correct, or 93.7%) and *rufifrons* groups (175 of 190 correct, or 92.1%). However, only 28.1% (16 of 57) of *B. r. salvini* were correctly assigned; the majority of incorrect assignments (39 of 57, or 68.4%) were to the *rufifrons* group. Wing length was significantly different across all three groups ($F_{2,\,453} = 259.3$, $P < 0.001$; Figure 5.3A), whereas tail length ($F_{2,\,444} = 45.6$, $P < 0.001$) was longer for the *rufifrons* group (Tukey's $P < 0.001$) than the other two groups (Tukey's $P = 0.14$).

For females, the three groups also differed as a whole according to MANOVA (Wilk's $\Lambda = 0.29$, $F_{2,\,184} = 25.3$, $P < 0.001$). The most diagnostic trait by discriminant analysis was wing length, which correctly classified 68.4% of individuals. Correct classification rates were high for both the *delattrii* (62 of 75 correct, or 82.7%) and *rufifrons* groups (54 of 73 correct, or 74.0%), but low for *B. r. salvini* (12 of 39 correct, or 30.8%; 27 of 39 (69.2%) of individuals were classified as *rufifrons* group. Wing length ($F_{2,\,231} = 117.2$, $P < 0.001$; Figure 5.3B) and tail length ($F_{2,\,219} = 15.0$, $P < 0.001$) were both significantly different across all three groups. For both sexes, the only diagnosable trait was wing length between the *delattrii* group and *B. r. salvini* (males: $D_{ds} = 0.63$; females: $D_{ds} = 0.19$); wing length was longer for the *delattrii* group than *B. r. salvini* (Table 5.1).

The wing-tail ratio was significantly different between all three groups (ANOVA, males: $F_{2,444} = 381.1$, $P < 0.001$; females: $F_{2,219} = 96.8$, $P < 0.001$), with *B. r. salvini* being intermediate between the *delattrii* and *rufifrons* groups. However, the mean value was negative for both the *rufifrons* group and *B. r. salvini* (wing shorter than tail) and positive for the *delattrii* group (wing longer than tail; Table 5.1; Figure 5.3C-D).

Plumage

Overall pattern.—Rufous-capped Warbler subspecies in the same plumage-based group shared similar facial and underpart colour patterns as reported in previous descriptions (Ridgway 1902; Todd 1929). All *rufifrons* group subspecies and *B. r. salvini* had a white auricular patch below the eye extending to the cheek. In contrast, all *delattrii* group subspecies had only a small white spot below the eye (Figure 5.2). All *rufifrons* group subspecies had yellow breast patches extending less than 50% of the body length, and no individuals had entirely yellow bellies. In contrast, all individuals from *delattrii* group subspecies had all-yellow breasts and bellies. Finally, *B. r. salvini* were intermediate in the extent of yellow coverage on the breast and belly; the mean percentage of yellow was 66.2% for males and 64.3% for females, although 27.7% of *B. r. salvini* individuals had entirely yellow bellies (Table 5.S3).

All-subspecies analysis.—Rufous-capped Warblers differed between the subspecies groups in all 10 measured plumage patches (Table 5.S6). The crowns of the *rufifrons* group and *B. r. salvini* were rufous (red-orange), and those of the *delattrii* group were chestnut (red-brown; Table 5.2). The *rufifrons* group and *B. r. salvini* had significantly brighter (lower PC1 score; ANCOVA $F_{2,432} = 17.3$, $P < 0.001$) and yellower (higher PC3 score; ANCOVA $F_{2,432} = 11.7$, $P < 0.001$) crowns than the *delattrii* group (Figure 5.4A), whereas the *rufifrons* group had significantly redder crowns (higher PC2 score; ANCOVA $F_{2, 432} = 3.9$, $P = 0.02$) than the *delattrii* group and *B. r. salvini* (Tables 5.S6–5.S7).

The throats and upper breasts of the *rufifrons* group and *B. r. salvini* were lemon yellow. However, their lower breast and belly colours differed, with the *rufifrons* group being brownish-white and *B. r. salvini* mixed white-yellow to pale yellow. In contrast, the *delattrii* group had golden yellow throats, breasts, and bellies (Table 5.2). Yellow colour of the belly (PC2; ANCOVA $F_{2, 432} = 286.0$, $P < 0.001$) differed across all three groups, with the *delattrii* group being the most yellow, *B. r. salvini* intermediate, and the *rufifrons* group the least yellow (Figure 5.4B). The *rufifrons* group had brighter bellies (lower PC1 score; ANCOVA $F_{2, 432} = 3.5$, $P = 0.03$) and less yellow breasts (higher PC2 score; ANCOVA $F_{2, 432} = 38.0$, $P < 0.001$; Figure 5.4C) than the *delattrii* group and *B. r. salvini.* However, *delattrii* and *rufifrons* groups had brighter breasts (lower PC1 score; ANCOVA $F_{2, 432} = 5.9$, $P = 0.003$) than *B. r. salvini*. The throat patch showed no significant group differences in brightness (PC1; ANCOVA $F_{2, 432} = 0.6$, $P = 0.52$) although yellow hue differed significantly across all three groups where *B. r. salvini* were yellowest, *rufifrons* group intermediate, and *delattrii* group least yellow (PC2; ANCOVA $F_{2, 432} = 5.4$, $P = 0.005$; Table 5.S7).

The mantle colour varied from olive in the *delattrii* group to olive-grey in the *rufifrons* group, with *B. r. salvini* being an intermediate olive to olive-grey (Table 5.2). The *rufifrons* group and *B. r. salvini* had significantly brighter mantles compared to the *delattrii* group (lower PC1 score; ANCOVA $F_{2, 432} = 6.2$, $P = 0.002$). However, mantle colour differed significantly across all three groups, with the *delattrii* group being yellowest, *B. r. salvini* intermediate, and the *rufifrons* group least yellow (lower PC2 score; ANCOVA $F_{2, 432} = 43.6$, $P < 0.001$; Table 5.S7).

The napes of the *rufifrons* group and *B. r. salvini* were grey, compared to olive-grey in the *delattrii* group (Table 5.2). The nape patch was significantly brighter (lower PC1 score;

ANCOVA $F_{2, 432} = 19.7$, $P < 0.001$; Figure 5.4D) and less yellow (higher PC2 score; ANCOVA $F_{2, 432} = 29.7$, $P < 0.001$) in the *rufifrons* group and *B. r. salvini* than in the *delattrii* group.

The rumps and shoulders of the *delattrii* group and *B. r. salvini* were olive, compared to olive-grey in the *rufifrons* group (Table 5.2). All three groups differed in rump colour, with the *delattrii* group yellowest, *B. r. salvini* intermediate, and the *rufifrons* group least yellow (lower PC2 score; ANCOVA $F_{2, 432} = 26.1$, $P < 0.001$), although they did not differ in rump brightness (PC1; ANCOVA $F_{2, 432} = 2.5$, $P = 0.08$; Table 5.S7). The groups did not differ in shoulder patch brightness (PC1; ANCOVA $F_{2, 432} = 0.2$, $P = 0.79$), but all groups differed in yellow colour, with *B. r. salvini* yellowest, the *delattrii* group intermediate, and the *rufifrons* group least yellow (PC2; ANCOVA $F_{2, 432} = 10.4$, $P < 0.001$).

The tail and wing patches were dusky grey in all individuals, with yellow-green feather borders in the *rufifrons* group and *B. r. salvini*, and olive borders in the *delattrii* group (Table 5.2). For the tail, all three groups differed in yellow colour, with *B. r. salvini* yellowest, the *rufifrons* group intermediate, and the *delattrii* group least yellow (PC2; ANCOVA $F_{2, 426} = 5.4$, $P = 0.005$); the subspecies did not differ in brightness (PC1; ANCOVA $F_{2, 426} = 0.6$, $P = 0.55$). For the wing, *B. r. salvini* were significantly yellower (lower PC2 score; ANCOVA $F_{2, 432} = 17.1$, $P < 0.001$) than the *delattrii* group whereas the *rufifrons* group were intermediate; all three groups had similar brightness (PC1; ANCOVA $F_{2, 432} = 0.6$, $P = 0.54$; Table 5.S7).

For belly, breast, mantle, rump, shoulder, tail, throat, and wing, the *delattrii* group had significantly greater UV reflectance (lower PC3 score; ANCOVA all $P < 0.05$) than the *rufifrons* group and *B. r. salvini.* For nape, PC3 did not differ between subspecies (ANCOVA all $P > 0.05$; Table 5.S7).

Rufous-capped Warbler plumage differed significantly between the sexes in four plumage patches: belly, crown, mantle, and nape (Table 5.S6). For the crown, all three subspecies groups showed sexual dichromatism in PC3; males had less yellow crowns (lower PC3 score; ANCOVA $F_{1, 432} = 7.6$, $P = 0.006$) than females (Figure 5.4A). There was a subspecies × sex interaction where *delattrii* group males had significantly brighter napes (lower PC1 score; ANCOVA $F_{2, 432} = 5.1$, $P = 0.006$) than *delattrii* group females (Figure 5.4D). Males had brighter (lower PC1 score; ANCOVA $F_{1, 432} = 4.7$, $P = 0.03$) and less yellow mantles (higher PC2 score; ANCOVA $F_{1, 432} = 4.1$, $P = 0.04$) than females. Males showed more UV reflectance than females in the belly (lower PC3 score; ANCOVA $F_{1, 432} = 6.4$, $P = 0.01$) and mantle (ANCOVA $F_{1, 432} = 7.4$, $P = 0.007$). These effects were largely driven by sex differences in *B. r. salvini*, as the *delattrii* and *rufifrons* groups did not differ between the sexes for these plumage patches (Table 5.S7).

Three-subspecies analysis.—Rufous-capped Warblers differed between subspecies in all 10 measured patches (Table 5.S8). Overall, *B. r. rufifrons* and *B. r. salvini* were similar to one another and differed from *B. r. delattrii*, although this general pattern varied by plumage patch. The crowns of *B. r. rufifrons* and *B. r. salvini* were rufous (red-orange), and those of *B. r. delattrii* were chestnut (red-brown; Table 5.2). The crowns of *B. r. rufifrons* and *B. r. salvini* were significantly brighter (lower PC1 score; ANCOVA $F_{2, 306} = 20.1$, $P < 0.001$) and yellower (higher PC3 score; ANCOVA $F_{2, 306} = 4.7$, $P = 0.01$) than those of *B. r. delattrii*. The crowns of *B. r. salvini* were redder than those of *B. r. delattrii* (higher PC2 score; ANCOVA $F_{2, 306} = 4.7$, $P = 0.01$), while *B. r. rufifrons* were intermediate between the two (Tables 5.S8–5.S9).

The breast and belly colour overlapped between *B. r. delattrii* and *B. r. salvini*, because many *B. r. salvini* had both white and yellow in these patches. Yellow colour of the belly (PC2; ANCOVA $F_{2, 306} = 160.8$, $P < 0.001$) differed across all three subspecies, with *B. r. delattrii* being the yellowest, *B. r. salvini* intermediate, and *B. r. rufifrons* the least yellow. Compared to *B. r. delattrii* and *B. r. salvini*, *B. r. rufifrons* had less yellow breasts (higher PC2 score; ANCOVA $F_{2, 306} = 24.2$, $P < 0.001$) and brighter bellies (lower PC1 score; ANCOVA $F_{2, 306} = 3.5$, $P = 0.03$). However, *B. r. delattrii* had brighter breasts (lower PC1 score; ANCOVA $F_{2, 306} = 5.4$, $P = 0.005$) than *B. r. rufifrons* and *B. r. salvini*. The throat patch showed no subspecies differences in brightness (PC1; ANCOVA $F_{2, 306} = 0.1$, $P = 0.89$) or yellow hue (PC2; ANCOVA $F_{2, 306} = 2.6$, $P = 0.08$; Table 5.S9).

Mantle colour varied from olive in *B. r. delattrii* to olive-grey in *B. r. rufifrons*, with *B. r. salvini* being olive to olive-grey (Table 5.2). The mantles of *B. r. rufifrons* were significantly brighter than those of *B. r. delattrii* (lower PC1 score; ANCOVA $F_{2, 306} = 4.7$, $P = 0.01$), whereas *B. r. salvini* were intermediate between the other two subspecies. Mantle colour (PC2) was similar between *B. r. delattrii* and *B. r. salvini*, which both had significantly yellower mantles than *B. r. rufifrons* (lower PC2 score; ANCOVA $F_{2, 306} = 16.0$, $P < 0.001$; Table 5.S9).

The nape patch was grey in *B. r. rufifrons* and *B. r. salvini*, compared to olive-grey in *B. r. delattrii* (Table 5.2). *B. r. rufifrons* and *B. r. salvini* had significantly brighter (lower PC1 score; ANCOVA $F_{2, 306} = 17.0$, $P < 0.001$) and less yellow (higher PC2 score; ANCOVA $F_{2, 306} = 29.5$, $P < 0.001$) napes than *B. r. delattrii*.

The rumps and shoulders of *B. r. delattrii* and *B. r. salvini* were olive, compared to olive-grey in *B. r. rufifrons* (Table 5.2). Therefore, *B. r. delattrii* and *B. r. salvini* both had yellower rumps (lower PC2 score; ANCOVA $F_{2, 306} = 9.4$, $P = 0.001$) than *B. r. rufifrons*; the three subspecies did not differ in rump brightness (PC1; ANCOVA $F_{2, 306} = 1.6$, $P = 0.21$; Table 5.S9). The shoulder patch did not differ between subspecies in brightness (PC1; ANCOVA $F_{2, 306} = 0.5$, $P = 0.62$), but *B. r. salvini* were significantly more yellow (lower PC2 score; ANCOVA $F_{2, 306} = 5.3$, $P = 0.005$) than *B. r. delattrii* and *B. r. rufifrons*.

The tail and wing patches were dusky grey in all individuals, with yellow-green feather borders in *B. r. rufifrons* and *B. r. salvini*, and olive borders in *B. r. delattrii* (Table 5.2). The tails and wings of *B. r. rufifrons* and *B. r. salvini* were significantly yellower than *B. r. delattrii* (lower PC2 score; tail: ANCOVA $F_{2, 301} = 7.6$, $P < 0.001$; wing: ANCOVA $F_{2, 306} = 22.9$, $P < 0.001$). All three subspecies had similar brightness in both tail (PC1; ANCOVA $F_{2,\,301} = 0.7$, $P = 0.48$) and wing patches (PC1; ANCOVA $F_{2,\,306} = 1.2$, $P = 0.30$; Table 5.S9). For all patches other than crown, *B. r. delattrii* had significantly greater UV reflectance (lower PC3 score; ANCOVA all $P < 0.05$) than *B. r. rufifrons* and *B. r. salvini* (Table 5.S9).

Rufous-capped Warblers differed significantly between the sexes in five plumage patches: crown, nape, rump, shoulder, and wing (Table 5.S8). All three subspecies were sexually dichromatic for crown PC3, with males having less yellow reflectance in the crown (lower PC3 score; ANCOVA $F_{1,\,306} = 12.2$, $P < 0.001$) than females. For the nape, rump, and shoulder patches, *B. r. delattrii* were sexually dichromatic, but *B. r. rufifrons* and *B. r. salvini* were not. There were subspecies × sex interactions wherein *B. r. delattrii* males had significantly brighter napes (lower PC1 score; ANCOVA $F_{2,\,306} = 5.5$, $P = 0.005$) and rumps (ANCOVA $F_{2,\,306} = 3.8$, $P = 0.02$) than *B. r. delattrii* females. There were also significant sex effects where males had brighter shoulders (lower PC1 score; ANCOVA $F_{1,\,306} = 4.2$, $P = 0.04$), less yellow napes (higher PC2 score; ANCOVA $F_{1,\,306} = 4.0$, $P = 0.05$) and rumps (ANCOVA $F_{1,\,306} = 8.1$, $P = 0.005$), more UV-reflective rumps (lower PC3 score; ANCOVA $F_{1,\,306} = 10.6$, $P = 0.001$) and napes (ANCOVA $F_{2,\,306} = 8.3$, $P = 0.004$), and yellower wings (lower PC2 score; ANCOVA $F_{1,\,306} = 6.1$, $P = 0.01$) than females. These effects were largely driven by sex differences in *B. r. delattrii*; males had less yellow rumps, more UV-reflective napes and rumps, and yellower wings than females, whereas male and female *B. r. rufifrons* and *B. r. salvini* did not show sex differences (Table 5.S9).

For both the all-subspecies and three-subspecies analyses, there were specimen age effects (i.e. significant collection year covariate) for several plumage patches. For both analyses, there was a UV reflectance component (PC3) year effect for all plumage patches; a yellow colour (PC2) year effect for the tail and throat, and a red colour (PC2) year effect for the crown (PC2; Tables 5.S6, 5.S8). For the all-subspecies analysis, there was a year effect of brightness (PC1) for the belly, breast, crown, nape, throat, and wing (Table 5.S6). For the three-subspecies analysis, there was a year effect of brightness for the belly, breast, nape, shoulder, and wing (Table 5.S8).

Songs

Male songs.—Rufous-capped Warbler songs differed significantly in both acoustic structure and organization between subspecies, with the *delattrii* group being well-differentiated from the *rufifrons* group and *B. r. salvini* (Figure 5.5). For 13 of 14 measured variables, the *delattrii* group differed significantly from the *rufifrons* group and *B. r. salvini*, which were similar to each other; the final variable (syllable peak frequency) differed significantly across all three groups (Table 5.3). PC1 differed significantly between all three groups (ANOVA: $F_{2,\,123} = 395.6$, $P < 0.001$), and corresponded well with spectro-temporal features distinguishing the *delattrii* group songs from the *rufifrons* group and *B. r. salvini* songs (Figure 5.6). Positive loadings corresponded to song duration, number of syllables, and syllable production rate, whereas negative loadings corresponded to all other variables (Table 5.S2). Therefore, songs with positive PC1 scores (the *rufifrons* group and *B. r. salvini*; Figure 5.5A, C, and E) were longer and faster-paced, had more syllables but less variety of syllable types, were lower in frequency at both the song and syllable level, and had shorter, narrower-bandwidth syllables. Songs with negative PC1 scores (the *delattrii* group; Figure 5.5B, D, and F) were shorter and slower-paced, had fewer syllables but more variety of syllable types, were higher in frequency at both the song and syllable level, and had longer, wider-bandwidth syllables. PC2 and PC3 did not differ significantly between subspecies (ANOVA PC2: $F_{2,\,123} = 0.5$, $P = 0.62$; PC3: $F_{2,\,123} = 0.9$, $P = 0.41$), and were not as readily interpretable as PC1 in terms of acoustic structure.

Discriminant analysis results also showed that the *delattrii* group songs differed from the *rufifrons* group and *B. r. salvini* songs. Songs differed significantly between subspecies according to MANOVA (Wilk's $\Lambda = 0.06$, $F_{16, 232} = 46.9$, $P < 0.001$). The most diagnostic trait according to discriminant analysis was the syllable versatility index, which correctly classified 88.1% of individuals. The majority of the *delattrii* group songs (97.3%, or 36 of 37) were assigned to the correct group. All the *rufifrons* group (75 of 75) and *B. r. salvini* (14 of 14) songs were classified as *rufifrons* group.

Male and female song comparison.—Male and female *rufifrons* group and *B. r. salvini* songs differed significantly in 9 of the 14 measured acoustic variables (Table 5.4). Compared to male songs, female songs were shorter with higher maximum frequency and bandwidth. Syllables of female songs were also shorter, with higher maximum frequency, peak frequency, and bandwidth than male syllables. Although female songs had fewer syllables overall than male songs, they had a greater variety of syllable types (i.e. higher syllable diversity index). Male and female songs shared similar song and syllable minimum frequency, song peak frequency, number of syllable types, and syllable production rate (Figure 5.7). In contrast to female *delattrii* group songs, which had similar syllable types as male songs (Chapter 2), female *rufifrons* group syllables were structurally distinct from those of males (Figure 5.7).

Discussion

We found considerable differences between the *delattrii* and *rufifrons* groups in morphology, song, and many plumage features, based on data collected from live birds, museum specimens, and archived sound recordings sampled across the range of Rufous-capped Warblers. Our analyses also show that *B. r. salvini* are similar to the *rufifrons* group rather than being intermediate between *delattrii* and *rufifrons*. These patterns suggest that Rufous-capped Warblers comprise two distinct groups, *delattrii* and *rufifrons-salvini*, and supports a revision of the species' current taxonomic status. Our results align with historical taxonomic groupings based on morphology and plumage colour (Todd 1929) and recent evidence based on field observations (Howell & Webb 1995) rather than the current American Ornithological Society classification as a single species (Monroe 1968). In addition, the song differences between the *delattrii* and *rufifrons-salvini* subspecies groups described for the first time in our study provide further evidence of divergence between the two groups. We therefore propose that the taxonomy of the Rufous-capped Warbler clade should be revised to account for these differences in plumage, morphology, and voice.

Morphology

Overall, our morphological analyses suggested that the *delattrii* group was distinct from the *rufifrons* group and *B. r. salvini*, providing support for their separation into two groups. Interestingly, the groups also differed in overall body shape as well as size; for example, although the *delattrii* group had the longest wings of the three groups, the *rufifrons* group had the longest tails. Our wing-tail ratio measurements corroborate those of Todd (1929), who also noted that all *rufifrons* group subspecies and *B. r. salvini* have a negative wing-tail ratio (wing shorter than tail) and all *delattrii* group subspecies have a positive wing-tail ratio (wing longer than tail). Furthermore, our discriminant analysis suggested that *B. r. delattrii* is a well-defined group, and that *B. r. rufifrons* and *B. r. salvini* are not well-defined as separate subspecies but instead group together. The discriminant analysis of all eight subspecies further corroborated that the *rufifrons* group and the *delattrii* group represent two distinct units, as most of the incorrect assignments for a given subspecies were to another subspecies within the same plumage-based group.

Our morphological analyses did reveal some overlap between subspecies, since despite significant differences in all morphological measurements, the only diagnosable trait was the difference in wing length between the *delattrii* group and *B. r. salvini.* This overlap in morphology between groups may be owing to the similar overall size of all *Basileuterus*, which are among the larger members of the Family Parulidae (Curson 2010). Although not explicitly tested in our study, subspecies or populations may also be locally adapted to specific diets, which could explain why traits related to foraging behaviour (e.g., bill size) varied widely within and between subspecies (e.g., Benkman 2003; Francis & Guralnick 2010; Riyahi et al. 2013; Aleixandre et al. 2013). Another possibility is that morphological variation is a an adaptation to variation in temperature or other climatic variables; a separate study testing the validity of ecogeographical rules using the same museum data set found that across all subspecies, Rufous-capped Warblers living in cooler regions had longer wings and tails than those living in warmer regions (Bastien 2018). Further studies could assess these relationships within each subspecies, and in particular, address whether the unique morphological features of *B. r. salvini*, such as short wings, are in fact ecological adaptations to their habitat.

Plumage

We found that plumage colour varied more between Rufous-capped Warbler subspecies than did morphology or song, as separate colour patches showed different patterns of similarity between subspecies. Although *delattrii* group plumage was more distinct overall from that of *rufifrons* group and *B. r. salvini*, *B. r. salvini* were intermediate between the *delattrii* and *rufifrons* groups in some patches, notably belly colour. Belly colour was highly variable between subspecies, being golden yellow in the *delattrii* group, brownish-white in the *rufifrons* group, and mixed yellow-white to entirely pale yellow in *B. r. salvini*. Both the *delattrii* group and *B. r. salvini*, which have yellow breasts and olive- to olive-green mantles and rumps, differed in the colour of these traits compared to the *rufifrons* group, which have white breasts and olive-grey mantles and rumps (Table 5.2).

Still, plumage variation between subspecies largely mirrored the pattern found for morphology and song, in that the *rufifrons* group and *B. r. salvini* were similar while both differed from the *delattrii* group. Most notably, UV reflectance was highest in all measured plumage patches in the *delattrii* group. In the subspecies group comparison, the *rufifrons* group and *B. r. salvini* had brighter and yellower crowns, brighter but less yellow napes, and brighter mantles than the *delattrii* group. In the comparison of the three subspecies with adjoining ranges, additional differences were apparent: *B. r. rufifrons* and *B. r. salvini* also had duller breasts and yellower wings and tails than *B. r. delattrii*. Previous Rufous-capped Warbler taxonomic comparisons qualitatively described plumage colours and classified each subspecies based on their geographic ranges and subtle colour variation relative to the reference subspecies *B. r. delattrii* and *B. r. rufifrons* (Table 5.2; Ridgway 1902; Todd 1929; Howell & Webb 1995). Our analyses used quantitative spectrophotometric analyses to confirm the overall differences and similarities in plumage characteristics described by earlier researchers, while providing new insights into colour variation not visible to humans (e.g., UV reflectance).

The large variation in plumage colouration we observed could result from ecological selection, wherein plumage colour evolves to either maximize conspicuousness or crypsis relative to the environment (e.g., Endler & Théry 1996; Doucet et al. 2007; Simpson & McGraw 2018). All three subspecies groups have particular habitat preferences; although all Rufous-capped Warblers prefer semi-open habitats, the *rufifrons* group prefer dry, open, high-elevation sites above 1000 m a.s.l., the *delattrii* group prefer dry to semi-humid, forested, low-elevation sites below 1000 m a.s.l., and *B. r. salvini* prefer humid, open, low-elevation sites below 500 m a.s.l. In the forested habitat of the *delattrii* group, olive-green upperparts would be more cryptic, whereas a deep red crown and yellow breast, which are potentially sexually-selected signals, would be more conspicuous (e.g., Doucet et al. 2007; Simpson & McGraw 2018). Furthermore, female mating preferences may result in plumage features such as a yellow *B. r. delattrii* belly introgressing into adjacent *B. r. rufifrons* populations to produce intermediate *B. r. salvini* plumage. A similar scenario occurred when yellow collar colour introgressed into white-collared populations across a hybrid zone between Golden-collared (*Manacus vitellinus*) and White-collared Manakins (*M. candei*; Stein & Uy 2006). Since no confirmed hybrids exist between *B. r. delattrii* and *B. r. salvini*, however (Todd 1929; Howell & Webb 1995), genetic analyses will be necessary to assess the presence and extent of introgression of plumage traits across subspecies.

An intriguing finding of our plumage analysis was that although Rufous-capped Warblers appear sexually monochromatic to humans, several plumage patches are in fact sexually dichromatic. Overall, our findings coincide with two studies based on spectral reflectance data, one of 166 North American passerine species, and the other of 376 species in the cardinal and tanager clades, in which over 90% of the putatively monochromatic species were actually dichromatic based on avian visual systems (Eaton 2007; Burns & Shultz 2012). We found that the *delattrii* group, *rufifrons* group, and *B. r. salvini* females all had yellower (i.e. less red or chestnut) crowns than males within the same group. Several sex-specific differences were found only in the *delattrii* group, providing further evidence that the *delattrii* group is distinct from the *rufifrons* group and *B. r. salvini.* This sexual dichromatism suggests that the crown, and possibly the nape in the *delattrii* group, is a signal used in mate choice or sex recognition. Mate choice experiments using visual models of warblers with varying crown colours (e.g., Baldassarre et al. 2013) may be useful to further explore the role of these plumage patches as mating signals. Further research on little-studied monochromatic tropical species, particularly bright monochromatic species like the Rufous-capped Warbler, will likely reveal other cases of cryptic sexual dichromatism.

Songs

Male Rufous-capped Warbler songs separated distinctly into two groups: *delattrii* and *rufifrons-salvini*. These vocal differences aligned with morphological and plumage differences, suggesting that *B. r. salvini* are conspecific with *B. r. rufifrons* (Todd 1929; Howell & Webb 1995) rather than being similar to *B. r. delattrii* or intermediate between *B. r. delattrii* and *B. r. rufifrons* (Ridgway 1902; Monroe 1968). Songs of the *delattrii* group were shorter, slower-paced, and higher in frequency, with fewer syllables but more variety of syllable types, and longer, wider-bandwidth syllables. In contrast, the *rufifrons* group and *B. r. salvini* songs were longer, faster-paced, and lower in frequency, with more syllables but less variety of syllable types, and had shorter, narrower-bandwidth syllables. Songs are used to attract mates and defend territories (Catchpole & Slater 2008), and may therefore act as a strong isolating barrier even between closely-related taxa without large morphological or plumage variation (e.g., Toews & Irwin 2008; Dingle et al. 2010). Indeed, a playback study in a sympatric zone between *B. r. delattrii* and *B. r. rufifrons* in southern Mexico (Chapter 6) showed that warblers of both subspecies responded more strongly to own-subspecies than other-subspecies male songs, suggesting that song could serve as an isolating barrier between subspecies (e.g., Irwin et al. 2001; Dingle et al. 2010; Benites et al. 2015). Observations in the contact zone also suggested that *B. r. delattrii* and *B. r. rufifrons* territories overlapped without inter-subspecies aggression, and that the warblers mated assortatively, as we never observed mixed pairs. Further evidence for vocal divergence in sympatry is that within the subset of male songs measured from the sympatric site (*B. r. delattrii*: $n = 9$; *B. r. rufifrons*: $n = 9$), all fit within the average range of PC1 values for their subspecies (Figure 5.6). Ongoing genetic analyses will assess whether gene flow is ongoing between the two subspecies in sympatry.

The marked vocal divergence we observed between the *delattrii* and *rufifrons-salvini* groups could result from the effects of habitat structure on sound transmission (Wilkins et al. 2013). The Acoustic Adaptation Hypothesis states that animal acoustic signals have evolved to transmit optimally in an animal's habitat, since both vegetation features (Morton 1975) and ambient noise levels in the habitat (Slabbekoorn & Smith 2002) may affect signal transmission. In Rufous-capped Warblers, the *rufifrons* group and *B. r. salvini*, which live primarily in open habitats, have many rapid trilled syllables in their songs, whereas the *delattrii* group, which prefer forested habitats, have slower-paced songs with larger inter-note intervals. Both types of songs would be expected to transmit well in their respective environments according to the Acoustic Adaptation Hypothesis (Morton 1975). In Rufous-collared Sparrows (*Zonotrichia capensis*), a similar pattern of vocal variation occurs wherein songs from open-habitat populations are lower-frequency, narrower-bandwidth, and contain longer, faster trills than songs from forest-dwelling populations (Handford & Lougheed 1991). However, the Acoustic Adaptation Hypothesis is not universally supported across all Rufous-collared Sparrow populations (Kopuchian et al. 2004; Lijtmaer & Tubaro 2007) and there is little evidence of local song adaptation in other species where song varies little structurally across populations (e.g., Rufous-and-white Wrens *Thryophilus rufalbus*; Graham et al. 2017). Similarly, it is likely in Rufous-capped Warblers that acoustic adaptation is not the sole explanation for population-level vocal differences, since other song features, such as frequency, are not consistent with the Acoustic Adaptation Hypothesis relative to each habitat type (Morton 1975). A small study comparing habitat features of *B. r. delattrii* and *B. r. rufifrons* territories within and outside the Mexican sympatric zone showed that vegetation (e.g., number of trees, canopy cover) did not differ significantly between the two subspecies' territories within the contact zone, but did differ between subspecies in allopatric sites (Vargas-Herrera et al. 2017). Since songs are similar within Rufous-capped Warbler subspecies in both sympatric and allopatric areas, further work is needed to determine if other site-specific factors (e.g., ambient noise) may influence song structure (e.g., Slabbekoorn & Smith 2002). Additionally, our sample of songs was concentrated in southern Mexico and Costa Rica, so further recordings from sites throughout the Rufous-capped Warbler's range, coupled with vegetation sampling, sound transmission experiments, and ambient noise measurements at the same sites, could clarify whether acoustic adaptation plays a role in shaping song structure in this species.

Vocal divergence between Rufous-capped Warbler subspecies was also apparent in female songs of both the *delattrii* and *rufifrons* groups, which differed from one another while being structurally similar to their male counterparts. Female *delattrii* songs, described in a previous study (Chapter 4), were shorter, with fewer syllables and syllable types, and higher maximum frequency than male songs. They appear to serve a territory defence function in intense interactions such as playback-simulated conspecific territorial intrusions by pairs (Chapter 4), yet are rarely used spontaneously (Chapter 2). Female *rufifrons* group songs, described in this study, were also shorter, with fewer syllables and higher maximum frequency than male songs. The syllables themselves were also shorter, and of higher maximum frequency and larger bandwidth. Interestingly, female singing behaviour of the two subspecies may also differ: *B. r. rufifrons* and *B. r. salvini* females appeared to produce more spontaneous songs in response to their male partner's songs during focal recordings (A. Demko pers. obs.) and sang more frequently in response to playback during an experimental study than did *B. r. delattrii* females (Chapter 6). A detailed study of singing behaviour based on extensive focal recordings would be useful to investigate the function of female song in the *rufifrons* group. The *B. r.*

rufifrons and *B. r. salvini* pairs included in this study were only recorded once each for 10–15 min, compared to multiple 30–60 min recordings per pair for the *delattrii* population we studied (Chapter 2). Given that female song is common yet poorly-studied in tropical songbird species (Stutchbury & Morton 2001; Odom & Benedict 2018), and that the *delattrii* and *rufifrons* groups appear to be vocally distinct based on our analyses, further investigations of singing behaviour in both groups would be informative to explore song evolution in the Family Parulidae.

Conclusion

Our research revealed that the *rufifrons* group and *B. r. salvini* are well-differentiated from the *delattrii* group in plumage and song, and to a lesser extent in morphology. The low responses to other-subspecies song and apparent lack of inter-subspecies territoriality and hybridization in a sympatric zone between *B. r. delattrii* and *B. r. rufifrons* in southern Mexico further suggests reproductive isolation between the two groups (Chapter 6). We therefore propose that the *delattrii* group be elevated to full species status separately from the *rufifrons* group. We also suggest that *B. r. salvini* be classified with the *rufifrons* group, given *B. r. salvini*'s similarity to *B. r. rufifrons* in song, plumage, and morphology. The Rufous-capped Warbler (*Basileuterus rufifrons*) would therefore comprise the five northern subspecies (*B. r. caudatus*, *dugesi*, *jouyi*, *rufifrons*, and *salvini*), and the Chestnut-capped Warbler (*Basileuterus delattrii*) would comprise the three southern subspecies (*B. d. actuosus*, *delattrii*, and *mesochrysus*). Ongoing genomic analysis of samples from the *B. r. delattrii* and *B. r. rufifrons* contact zone and other sites throughout the Rufous-capped Warbler's range in Mexico and Central America will compare patterns of range-wide phenotypic and genetic divergence, and further refine the taxonomic classification proposed here.

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Tables

Table 5.1. Morphological measurements of male and female Rufous-capped Warblers according to subspecies group (*delattrii* group, *rufifrons* group, and *B. r. salvini*). Values in bold indicate measurements with significant differences between groups.

Males	*delattrii* group (*n* = 176)	*rufifrons* group (*n* = 211)	*salvini* (*n* = 69)
Wing length (mm)	57.0 ± 2.2	52.8 ± 2.3	50.8 ± 1.7
Tail length (mm)	53.2 ± 3.3	55.9 ± 3.0	52.6 ± 2.0
Tarsus (mm)	20.7 ± 0.8	20.8 ± 0.7	20.5 ± 0.8
Bill length (mm)	7.3 ± 0.4	6.9 ± 0.4	7.2 ± 0.3
Bill depth (mm)	3.9 ± 0.3	3.8 ± 0.2	3.9 ± 0.2
Bill width (mm)	3.8 ± 0.4	3.6 ± 0.3	3.6 ± 0.2
Wing-tail ratio (mm)	3.8 ± 2.7	-3.0 ± 2.1	-1.8 ± 2.1
% yellow underparts	100 ± 0.0	47.7 ± 4.6	66.2 ± 22.1
Females	*delattrii* group (*n* = 102)	*rufifrons* group (*n* = 87)	*salvini* (*n* = 46)
Wing length (mm)	54.5 ± 2.4	50.8 ± 2.3	49.2 ± 1.5
Tail length (mm)	51.7 ± 3.4	53.4 ± 3.2	50.3 ± 2.0
Tarsus (mm)	20.3 ± 0.9	20.5 ± 0.7	20.3 ± 0.7
Bill length (mm)	7.3 ± 0.4	6.8 ± 0.3	7.1 ± 0.3
Bill depth (mm)	3.9 ± 0.3	3.7 ± 0.2	3.8 ± 0.1
Bill width (mm)	3.8 ± 0.4	3.5 ± 0.3	3.6 ± 0.2
Wing-tail ratio (mm)	2.8 ± 2.8	-2.6 ± 2.6	-1.1 ± 2.1
% yellow underparts	100 ± 0.0	47.7 ± 5.1	64.3 ± 21.0

Table 5.2. Plumage colours by body region in *B. r. delattrii*, *B. r. rufifrons*, and *B. r. salvini* subspecies according to Ridgway (1902); Todd (1929); Howell & Webb (1995); and this study.

Body region	*B. r. delattrii*	*B. r. rufifrons*	*B. r. salvini*
Crown	chestnut (red-brown)	rufous (red-orange)	rufous (red-orange)
Lower auricular	chestnut	white	white
White spot below eye	present	absent	absent
Nape	olive-grey	grey	grey
Mantle (upperparts)	olive	olive-grey	olive-grey to olive
Rump, shoulder	olive	olive-grey	olive
Throat, upper breast	golden yellow	lemon yellow	lemon yellow
Lower breast, belly	golden yellow	brownish-white	mixed white-yellow to pale yellow
Wing, tail	dusky, olive border	dusky, yellow-green border	dusky, yellow-green border

Table 5.3. Vocal measurements of male Rufous-capped Warbler songs by subspecies group (*delattrii* group, *rufifrons* group, and *B. r. salvini*). All variables were significantly different between *delattrii* group and both *rufifrons* group and *B. r. salvini*, aside from syllable peak frequency which was significantly different across all three groups.

Vocal variable	*delattrii* (*n* = 37)	*rufifrons* (*n* = 75)	*salvini* (*n* = 14)
Song duration (s)	**2.24 ± 0.37**	2.77 ± 0.85	3.04 ± 1.06
Song maximum frequency (Hz)	**8906.0 ± 436.3**	7518.5 ± 638.8	7460.2 ± 640.5
Song minimum frequency (Hz)	**3292.1 ± 291.6**	2647.3 ± 313.8	2759.5 ± 191.0
Song bandwidth (Hz)	**4844.4 ± 710.6**	5624.2 ± 723.4	5624.2 ± 723.4
Song peak frequency (Hz)	**5907.1 ± 384.3**	5416.7 ± 555.0	5270.5 ± 532.5
Syllable duration (s)	**0.08 ± 0.02**	0.06 ± 0.01	0.06 ± 0.01
Syllable maximum frequency (Hz)	**7289.7 ± 383.2**	5913.5 ± 325.6	5709.0 ± 297.1
Syllable minimum frequency (Hz)	**4659.5 ± 237.8**	3951.8 ± 271.4	3832.2 ± 253.2
Syllable bandwidth (Hz)	**2630.2 ± 473.8**	1961.7 ± 343.5	1876.7 ± 290.4
Syllable peak frequency (Hz)	**5892.6 ± 223.7**	**4949.9 ± 299.3**	**4653.5 ± 231.4**
Number of syllables	**15.60 ± 3.40**	26.73 ± 9.27	29.96 ± 11.64
Number of syllable types	**10.94 ± 3.56**	6.05 ± 1.83	5.80 ± 1.15
Syllable versatility index	**0.69 ± 0.11**	0.24 ± 0.05	0.21 ± 0.06
Syllable production rate (syllables/s)	**6.92 ± 0.82**	9.65 ± 1.30	9.78 ± 1.17

Table 5.4. Vocal measurements of male and female *rufifrons* group and *B. r. salvini* songs. Male and female songs differed significantly in 9 of the 14 measured variables. Significant differences are highlighted in bold.

Vocal variable	Males (n = 89)	Females (n = 15)	*t* value	*P* value
Song duration (s)	**2.82 ± 0.89**	**1.90 ± 0.80**	**-4.05**	**<0.001**
Song maximum frequency (Hz)	**7509.3 ± 635.7**	**8315.7 ± 594.7**	**4.81**	**<0.001**
Song minimum frequency (Hz)	2665.0 ± 299.8	2691.5 ± 287.1	0.33	0.75
Song bandwidth (Hz)	**4844.4 ± 710.6**	**5624.2 ± 723.4**	**3.87**	**0.001**
Song peak frequency (Hz)	5393.7 ± 551.2	5597.0 ± 520.9	1.39	0.18
Syllable duration (s)	**0.06 ± 0.01**	**0.05 ± 0.01**	**-6.78**	**<0.001**
Syllable maximum frequency (Hz)	**5881.3 ± 328.3**	**6589.2 ± 387.5**	**6.68**	**<0.001**
Syllable minimum frequency (Hz)	3933.0 ± 270.8	3967.7 ± 482.9	0.27	0.79
Syllable bandwidth (Hz)	**1948.4 ± 335.6**	**2621.6 ± 645.0**	**3.95**	**0.001**
Syllable peak frequency (Hz)	**4903.3 ± 308.3**	**5562.1 ± 520.0**	**4.77**	**<0.001**
Number of syllables	**27.24 ± 9.68**	**18.23 ± 8.00**	**-3.91**	**<0.001**
Number of syllable types	6.01 ± 1.74	5.22 ± 2.24	-1.31	0.21
Syllable versatility index	**0.23 ± 0.05**	**0.29 ± 0.08**	**2.75**	**0.01**
Syllable production rate (syllables/s)	9.67 ± 1.28	9.56 ± 0.91	-0.38	0.71

Figures

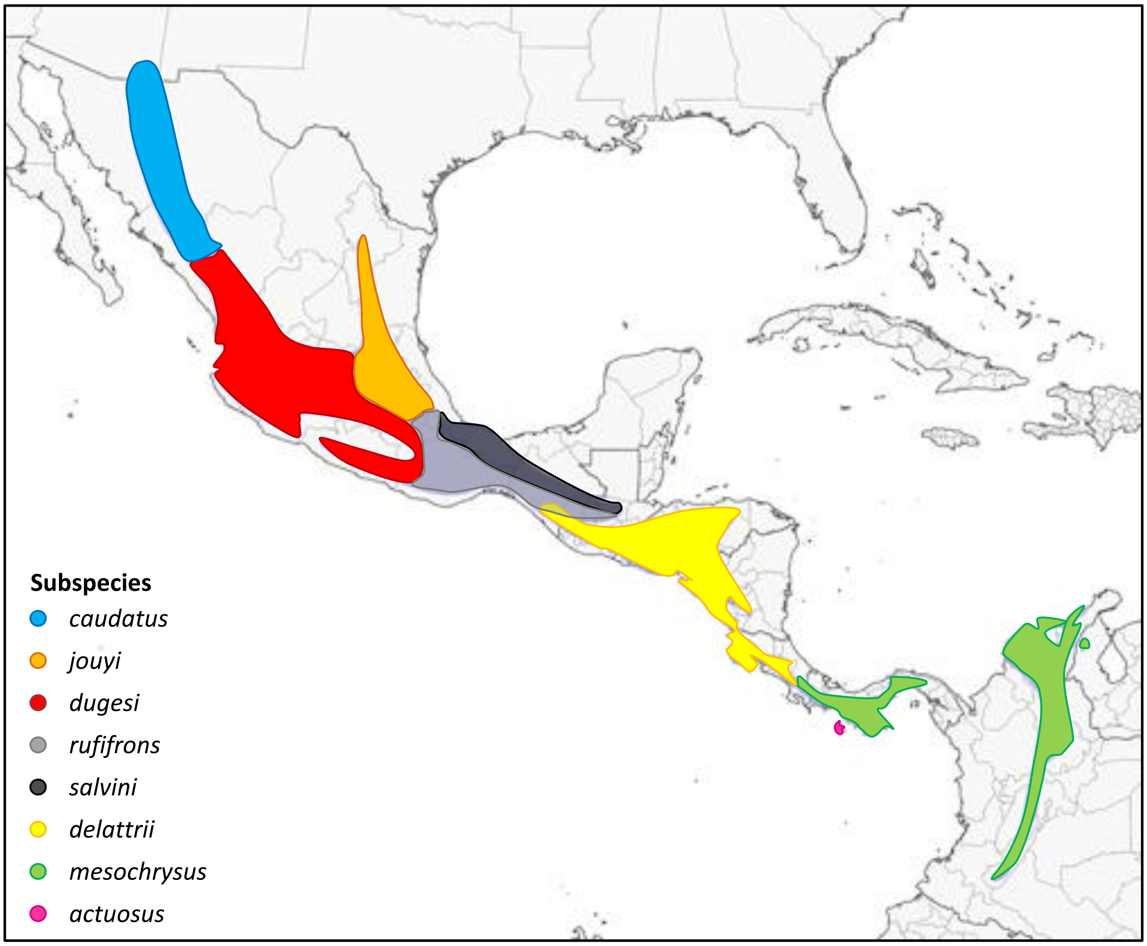

Figure 5.1. Range distributions of the eight recognized Rufous-capped Warbler subspecies. White-bellied *B. r. rufifrons* (grey shading) and yellow-bellied *B. r. delattrii* (yellow shading) are sympatric in southern Chiapas, Mexico. The range of intermediate-plumaged *B. r. salvini* (black shading) adjoins that of *B. r. rufifrons*, but does not overlap with that of *B. r. delattrii*.

Figure 5.2. Photographs of adult Rufous-capped Warblers (*Basileuterus rufifrons*) of three subspecies found in southern Mexico: (A-B) *B. r. rufifrons*, Motozintla, Chiapas; (C-D) *B. r. rufifrons*, Finca La Victoria, Chiapas; (E-F) *B. r. salvini*, Estación de Biología Los Tuxtlas, Veracruz; (G-H) *B. r. delattrii*, Finca La Victoria, Chiapas, and (I-J) *B. r. delattrii*, Mapastepec, Chiapas. *B. r. rufifrons* (A-D) have a rufous crown and auricular, a large white auricular patch, and a white belly contrasting with the yellow throat. *B. r. delattrii* (G-J) have a chestnut crown and auricular, a small white spot below the eye, and a yellow throat and belly. *B. r. salvini* (E-F) have similar head colouration as *B. r. rufifrons*, but have extensive yellow streaking on the white belly.

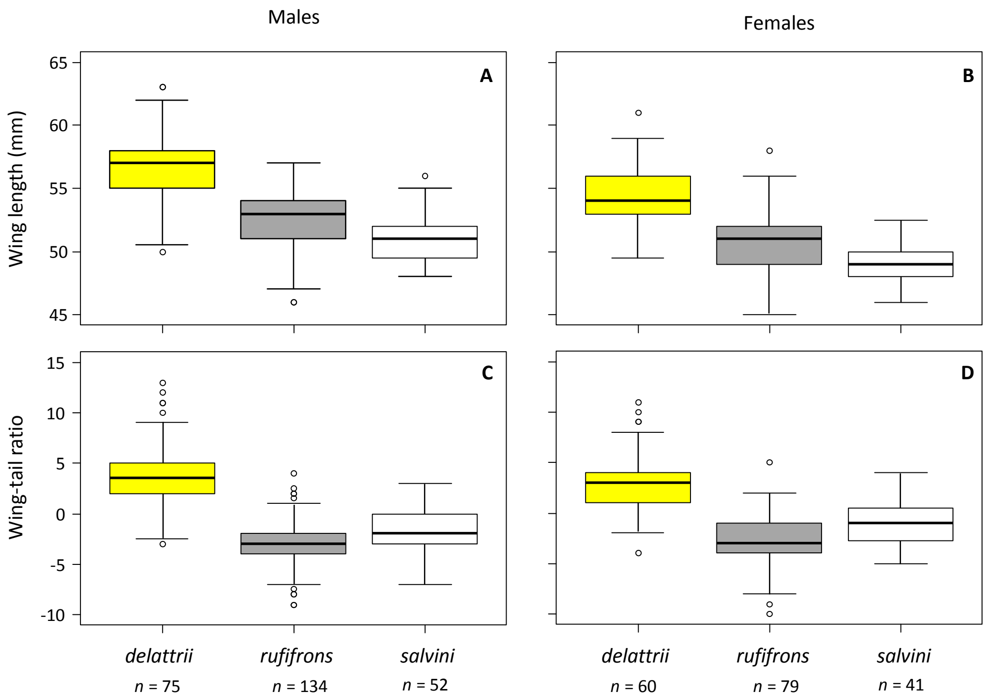

Figure 5.3. (A) Male and (B) female Rufous-capped Warblers showed significant differences in wing length between the *delattrii* group, *rufifrons* group, and *B. r. salvini*, with the *delattrii* group having the longest wings and *B. r. salvini* the shortest wings. (C) Males and (D) females also differed in the wing-tail ratio (wing length minus tail length), with the *delattrii* group having a positive wing-tail ratio (wing longer than tail) and both the *rufifrons* group and *B. r. salvini* having a negative wing-tail ratio (wing shorter than tail).

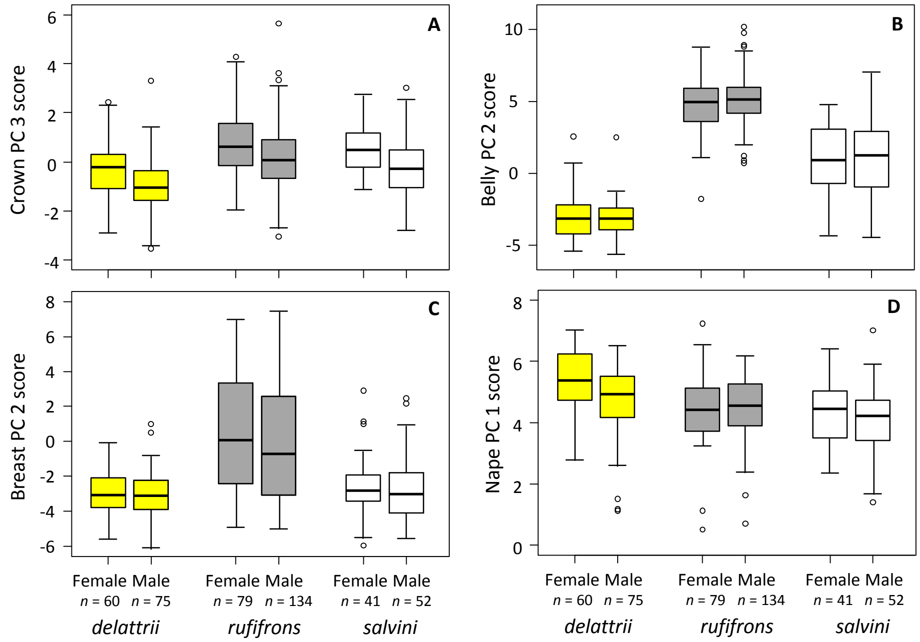

Figure 5.4. Rufous-capped Warblers showed subspecies and sex differences in plumage reflectance spectra between the *delattrii* group, *rufifrons* group, and *B. r. salvini*. (A) Males had less yellow crowns (lower PC3 score) than females for all three groups. (B) The belly colour was yellowest for the *delattrii* group (lower PC2 score), intermediate for *B. r. salvini*, and least yellow for the *rufifrons* group. (C) The breast was yellower for the *delattrii* group and *B. r. salvini* (lower PC2 score) than for the *rufifrons* group. (D) Males from the *delattrii* group had brighter napes (lower PC1 score) than *delattrii* group females, although both male and female *rufifrons* group and *B. r. salvini* had brighter napes than *delattrii* group birds.

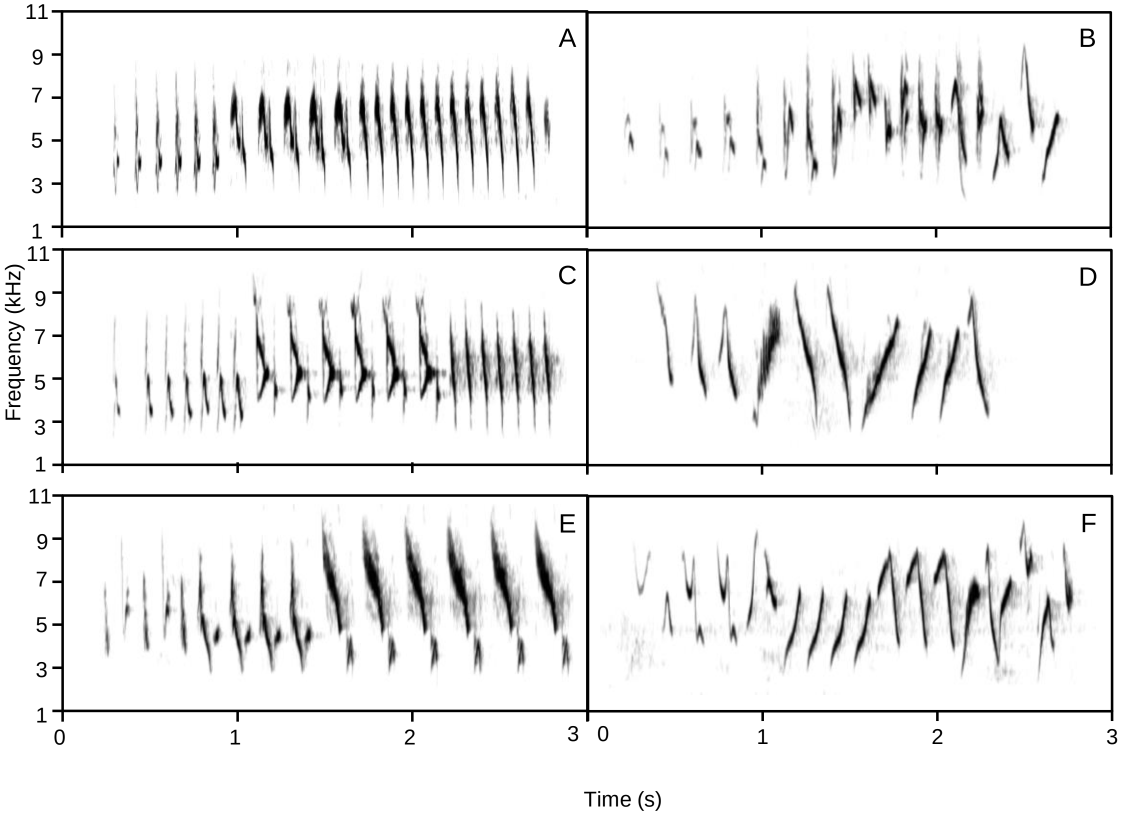

Figure 5.5. Song spectrograms of Rufous-capped Warbler male songs: (A) allopatric *B. r. rufifrons* (Yagul, Oaxaca, Mexico); (B) allopatric *B. r. delattrii* (Santa Rosa, Guanacaste, Costa Rica); (C) allopatric *B. r. salvini* (Estación de Biología Los Tuxtlas, Veracruz, Mexico); (D) allopatric *B. r. delattrii* (Mapastepec, Chiapas, Mexico); (E) sympatric *B. r. rufifrons* (Finca La Victoria, Chiapas, Mexico); and (F) sympatric *B. r. delattrii* (Finca La Victoria). Songs of *rufifrons* group (A and E) and *B. r. salvini* (C) are structurally similar to one another, and differ from *delattrii* group songs (B, D, and F). Songs from sympatric *B. r. rufifrons* (E) and *B. r. delattrii* (F) resemble allopatric songs of their own subspecies rather than being intermediate or mixed.

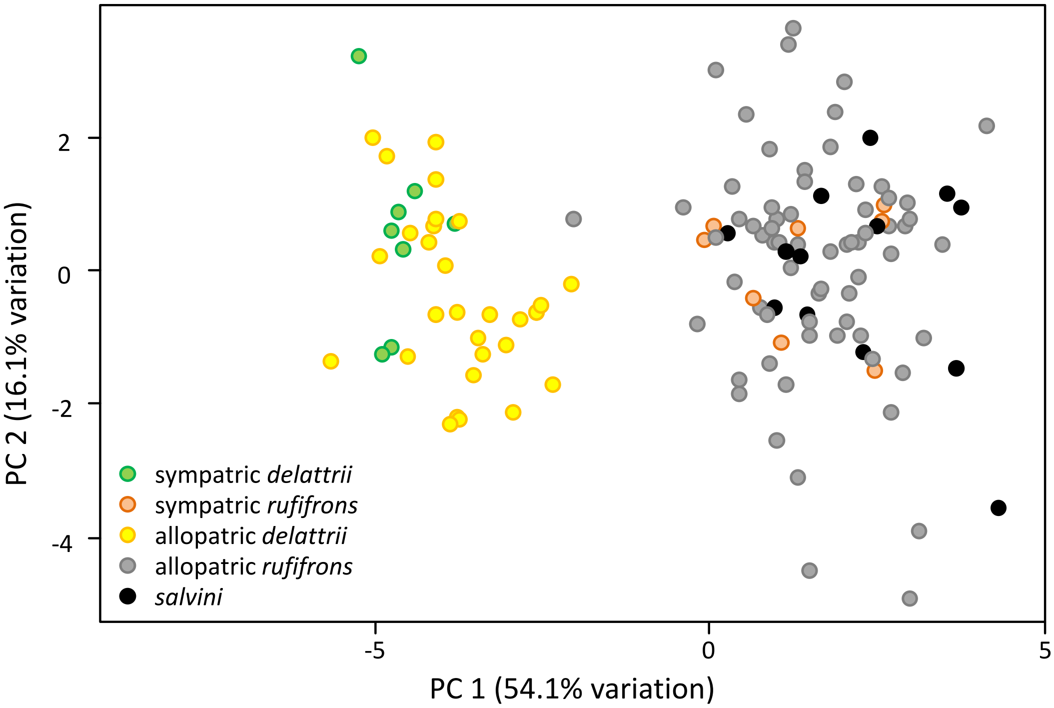

Figure 5.6. Male songs of the *delattrii* group were distinct from the *rufifrons* group and *B. r. salvini* based on PC1 scores. Individuals of both sympatric *B. r. delattrii* (green dots) and *B. r. rufifrons* (orange dots) fell within the average range of PC1 scores for their subspecies in allopatry, whereas PC2 scores did not differ significantly between subspecies.

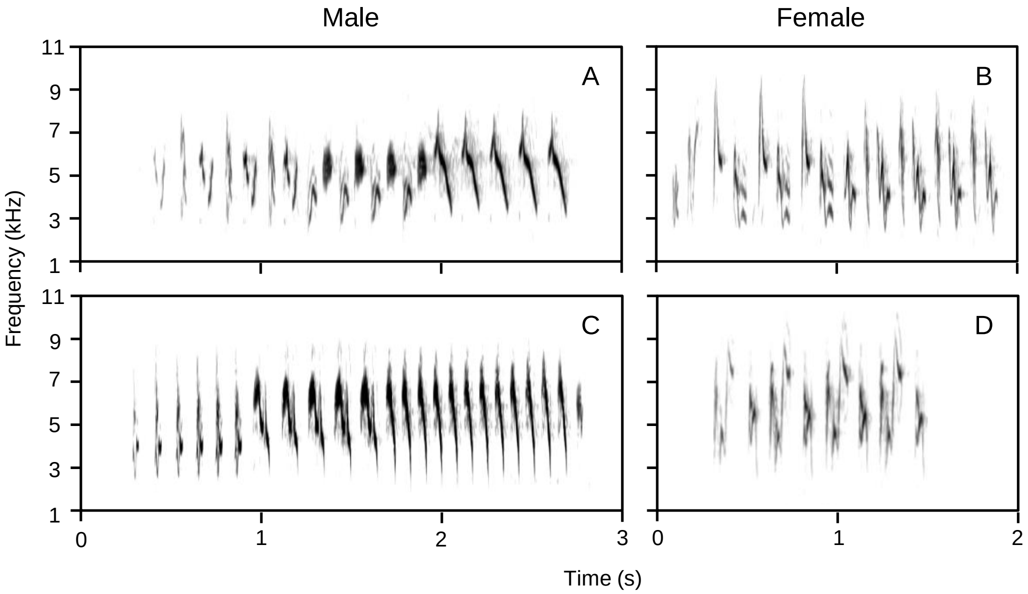

Figure 5.7. Song spectrograms of *B. r. rufifrons* male and female songs from (A-B) Motozintla, Chiapas, Mexico and (C-D) Yagul, Oaxaca, Mexico. Female songs and syllables are shorter, have higher maximum and peak frequency, and larger bandwidth than male songs.

Supplementary Material for Chapter 5

Supplementary Methods 1: Comparison between morphological measurements collected from live birds in the field and museum specimens of *B. r. delattrii*, *B. r. rufifrons*, and *B. r. salvini.*

The mean values of the majority of morphological measurements were similar between field and museum specimens of all three subspecies. For *B. r. delattrii*, males were significantly larger in field compared to museum specimens for wing length, tail length, tarsus, and bill width in males, and for tarsus and bill width in females. For *B. r. rufifrons*, males were significantly larger in field compared to museum specimens for wing length, tail length, tarsus, and bill width in males, and for wing length, tail length, and tarsus in females. For *B. r. salvini*, males were significantly larger in field compared to museum specimens for tarsus and bill width in males, and for bill width in females; however, wing length in *B. r. salvini* females was significantly smaller in field compared to museum specimens. All other comparisons between field and museum specimens were not significantly different (Table 5.S1).

Supplementary Methods 2: Description of principal components of plumage reflectance PCAs for the three-subspecies analysis.

Three-subspecies analysis.—In the PCA with only the three most widely-sampled subspecies, the crown patch PC1 accounted for 80.1% of the variation (eigenvalue: 32.8) with negative loadings across the 300–700 nm range, and was negatively associated with brightness. PC2 accounted for 15.0% of the variation (eigenvalue: 6.1) with negative loadings from 300–510 nm and positive loadings from 520–700 nm, and was positively associated with redness. PC3 represented 4.4% of the variation (eigenvalue: 1.5) with positive loadings from 390–600 nm and negative loadings at 300–380 nm and 610–700 nm. PC3 was positively associated with yellow and green reflectance (Figure 5.S1).

For the other nine patches, PC1 accounted for 84.0% of the variation (eigenvalue: 34.4) with negative loadings across the 300–700 nm range, and was negatively associated with brightness. PC2 accounted for 11.6% of the variation (eigenvalue: 4.7) with positive loadings from 370–500 nm and negative loadings at 320–360 and 510–700 nm. PC2 was associated with carotenoid levels, wherein negative scores corresponded to a high carotenoid component to the patch (i.e. yellow) and positive scores indicated a lack of carotenoids (i.e. white or grey). PC3 accounted for 4.0% of the variation (eigenvalue: 1.7) with positive loadings from 410–700 nm and negative loadings from 300–400 nm, and was negatively associated with UV reflectance (Figure 5.S2).

Supplementary Results: Results of morphological analyses comparing three subspecies (*B. r. delattrii*, *B. r. rufifrons*, and *B. r. salvini*) and all eight subspecies of Rufous-capped Warbler.

Three-subspecies analysis.—The *B. r. delattrii* and *B. r. rufifrons* subspecies showed considerable morphological separation, whereas *B. r. salvini* was similar to *B. r. rufifrons*. For males, the three subspecies differed as a whole according to MANOVA (Wilk's Λ = 0.20, $F_{2,\,311}$ = 62.4, $P < 0.001$). The most diagnostic traits according to discriminant analysis were wing and tail length; these two characters correctly classified 79.9% of individuals. Classification accuracy was high for *B. r. delattrii* (102 of 110 correct, or 92.7%) and *B. r. rufifrons* (124 of 147 correct, or 84.4%). However, only 43.9% (25 of 57) of *B. r. salvini* were correctly assigned; the majority of incorrect assignments (30 of 57, or 52.6%) were to *B. r. rufifrons*. Both wing length ($F_{2,\,374}$ = 220.4, $P < 0.001$) and tail length ($F_{2,\,365}$ = 305.9, $P < 0.001$) were significantly different across all three subspecies.

For females, the three subspecies differed as a whole according to MANOVA (Wilk's Λ = 0.27, $F_{2,\,146}$ = 21.5, $P < 0.001$). The most diagnostic traits were also wing and tail length, which correctly classified 73.8% of individuals. As with males, 91.8% (56 of 61) of *B. r. delattrii* were classified correctly, whereas *B. r. rufifrons* and *B. r. salvini* overlapped considerably. For *B. r. rufifrons*, 65.3% (32 of 49) were assigned to *B. r. rufifrons* and 20.4% (10 of 49) to *B. r. salvini*. For *B. r. salvini*, 35.9% (14 of 39) were assigned to *B. r. rufifrons* and 56.4% (22 of 39) to *B. r. salvini.* Wing length was significantly different across all three subspecies ($F_{2,\,182}$ = 120.9, $P <$ 0.001). Tail length ($F_{2,\,174}$ = 11.4, $P < 0.001$) was similar for *B. r. delattrii* and *B. r. rufifrons* (Tukey's $P = 0.93$), but shorter for *B. r. salvini* than for either *B. r. delattrii* or *B. r. rufifrons* (Tukey's $P < 0.001$). For both sexes, the only diagnosable trait was wing length between *B. r. delattrii* and *B. r. salvini* (males: $D_{ds} = 0.56$; females: $D_{ds} = 0.20$), with wing length being longer for *B. r. delattrii* than *B. r. salvini* (Table 5.S3).

The wing-tail ratio was significantly different between all three subspecies (ANOVA; males: $F_{2,\,365} = 305.9$, $P < 0.001$; females: $F_{2,\,174} = 67.6$, $P < 0.001$); however, the mean value was negative for both *B. r. rufifrons* and *B. r. salvini* (wing shorter than tail) and positive for *B. r. delattrii* (wing longer than tail; Table 5.S3).

Eight-subspecies analysis.—Classification accuracy by discriminant analysis varied considerably across subspecies for both sexes, although most incorrect assignments were to a subspecies within the same plumage-based group, and *B. r. salvini* grouped with *B. r. rufifrons*. For males, the subspecies differed as a whole according to MANOVA (Wilk's $\Lambda = 0.13$, $F_{7,\,366} = 22.4$, $P < 0.001$). The most diagnostic traits according to discriminant analysis were wing and tail length, which correctly classified 67.6% of individuals. For the yellow-bellied subspecies, the correct classification rate was high for *B. r. delattrii* (90.9%), but low for *B. r. actuosus* (60%) and *B. r. mesochrysus* (16.7%). However, all incorrectly assigned *B. r. actuosus* and *B. r. mesochrysus* grouped with another yellow-bellied subspecies. For the white-bellied subspecies, only *B. r. rufifrons* had a high correct classification rate (84.4%); 0% of *B. r. caudatus*, *B. r. dugesi*, and *B. r. jouyi* were assigned correctly, with most individuals classified as *B. r. rufifrons* or *B. r. salvini*. Classification accuracy of *B. r. salvini* was low (42.1%), with the majority of remaining individuals (52.6%) assigned to *B. r. rufifrons* (Table 5.S4).

In females, the subspecies differed as a whole according to MANOVA (Wilk's $\Lambda = 0.13$, $F_{6,\,179} = 12.7$, $P < 0.001$). The most diagnostic traits were wing and tail length, which correctly classified 64.5% of individuals. For the yellow-bellied subspecies, the correct classification rate was high for *B. r. delattrii* (88.7%) and *B. r. mesochrysus* (77.8%). Although classification accuracy was low for *B. r. actuosus* (50%), all incorrectly assigned *B. r. actuosus* grouped with another yellow-bellied subspecies, *B. r. delattrii*. For the white-bellied subspecies, *B. r. rufifrons* (65.3%) had the highest correct classification rates. 22.2% of *B. r. jouyi* and 0% of *B. r. dugesi* were assigned correctly, with most incorrectly-assigned individuals grouping with *B. r. rufifrons* or *B. r. salvini.* Classification accuracy was also low for *B. r. salvini* (56.4%), with the majority of remaining individuals grouping with *B. r. rufifrons* (35.9%; Table 5.S4).

Table 5.S1. Comparison between field and museum-collected morphological measurements from males and females of three extensively-sampled subspecies, *B. r. delattrii*, *B. r. rufifrons*, and *B. r. salvini*. Significant comparisons are highlighted in bold; values are mean ± SD.

Measurement	Field	Museum	*t* value	*P* value
B. r. delattrii				
Males (*n* = 151)				
Wing length (mm)	**57.5 ± 1.8**	**55.5 ± 2.3**	**5.53**	**<0.001**
Tail length (mm)	**54.5 ± 3.2**	**52.7 ± 2.8**	**3.59**	**<0.001**
Tarsus (mm)	**21.0 ± 0.6**	**20.0 ± 0.7**	**8.28**	**<0.001**
Bill length (mm)	7.2 ± 0.4	7.2 ± 0.4	-0.06	0.95
Bill depth (mm)	3.8 ± 0.2	3.9 ± 0.2	-1.75	0.08
Bill width (mm)	**3.8 ± 0.4**	**3.7 ± 0.3**	**2.88**	**0.004**
Females (*n* = 84)				
Wing length (mm)	54.4 ± 1.8	54.5 ± 2.6	-0.27	0.79
Tail length (mm)	52.3 ± 3.2	52.8 ± 2.9	-0.84	0.41
Tarsus (mm)	**20.8 ± 0.7**	**19.7 ± 0.8**	**6.96**	**<0.001**
Bill length (mm)	7.2 ± 0.4	7.1 ± 0.3	0.69	0.49
Bill depth (mm)	3.8 ± 0.3	3.8 ± 0.2	-0.98	0.33
Bill width (mm)	**3.8 ± 0.4**	**3.6 ± 0.2**	**2.89**	**0.006**
B. r. rufifrons				
Males (*n* = 157)				
Wing length (mm)	**54.2 ± 1.6**	**52.2 ± 2.4**	**6.16**	**<0.001**
Tail length (mm)	**58.0 ± 2.2**	**54.3 ± 2.4**	**9.92**	**<0.001**
Tarsus (mm)	**21.2 ± 0.6**	**20.6 ± 0.6**	**5.46**	**<0.001**
Bill length (mm)	6.9 ± 0.3	7.0 ± 0.4	-1.51	0.13
Bill depth (mm)	3.9 ± 0.2	3.8 ± 0.2	0.93	0.35
Bill width (mm)	**3.7 ± 0.2**	**3.5 ± 0.2**	**4.89**	**<0.001**
Females (*n* = 56)				
Wing length (mm)	**51.3 ± 1.0**	**50.2 ± 2.2**	**2.15**	**0.04**
Tail length (mm)	**56.1 ± 3.3**	**52.1 ± 2.4**	**3.26**	**0.01**
Tarsus (mm)	**21.1 ± 0.5**	**20.5 ± 0.7**	**2.61**	**0.02**
Bill length (mm)	6.8 ± 0.3	6.9 ± 0.3	-0.51	0.62
Bill depth (mm)	3.7 ± 0.2	3.7 ± 0.2	0.35	0.73
Bill width (mm)	3.6 ± 0.3	3.5 ± 0.3	0.94	0.37

Measurement	Field	Museum	*t* value	*P* value
B. r. salvini				
Males (*n* = 69)				
Wing length (mm)	50.3 ± 1.3	50.9 ± 1.8	-1.53	0.13
Tail length (mm)	53.3 ± 1.7	52.4 ± 2.1	1.86	0.07
Tarsus (mm)	**20.8 ± 0.7**	**20.3 ± 0.8**	**2.66**	**0.01**
Bill length (mm)	7.3 ± 0.3	7.1 ± 0.3	1.77	0.08
Bill depth (mm)	4.0 ± 0.1	3.9 ± 0.2	1.92	0.06
Bill width (mm)	**3.8 ± 0.2**	**3.5 ± 0.2**	**4.18**	**<0.001**
Females (*n* = 46)				
Wing length (mm)	**47.8 ± 1.1**	**49.4 ± 1.4**	**-2.98**	**0.03**
Tail length (mm)	51.4 ± 1.8	50.2 ± 2.0	1.24	0.28
Tarsus (mm)	20.8 ± 0.5	20.3 ± 0.7	2.07	0.08
Bill length (mm)	7.0 ± 0.2	7.1 ± 0.3	-0.83	0.44
Bill depth (mm)	3.8 ± 0.1	3.6 ± 0.1	0.71	0.51
Bill width (mm)	**3.8 ± 0.1**	**3.6 ± 0.2**	**2.46**	**0.05**

Table 5.S2. Summary of factor loadings of 14 acoustic variables for first three principal components in a PCA. PC1 describes differences between the *delattrii* and *rufifrons-salvini* group songs. The most influential loadings have scores > 0.27 or < -0.27 and are highlighted in bold.

	PC1	PC2	PC3
Eigenvalue	7.58	2.25	1.23
Percentage of variation (%)	54.1	16.1	8.8
Song duration	0.14	**-0.44**	**0.38**
Song maximum frequency	**-0.30**	-0.26	0.14
Song minimum frequency	-0.26	0.11	-0.26
Song bandwidth	-0.21	**-0.36**	**0.31**
Song peak frequency	-0.19	-0.03	-0.09
Syllable duration	-0.25	0.26	**0.46**
Syllable maximum frequency	**-0.35**	0.00	0.08
Syllable minimum frequency	**-0.28**	-0.24	**-0.31**
Syllable bandwidth	**-0.28**	0.21	**0.39**
Syllable peak frequency	**-0.33**	-0.08	-0.13
Number of syllables	0.23	**-0.44**	0.21
Number of syllable types	-0.21	**-0.42**	-0.22
Syllable versatility index	**-0.32**	-0.09	-0.20
Syllable production rate	**0.29**	-0.19	-0.25

Table 5.S3. Morphological measurements of male and female Rufous-capped Warblers according to subspecies (*B. r. delattrii*, *B. r. rufifrons*, and *B. r. salvini*). All measurements were significantly different between groups.

Males	*delattrii* (*n* = 151)	*rufifrons* (*n* = 157)	*salvini* (*n* = 69)
Wing length (mm)	56.9 ± 2.2	53.2 ± 2.3	50.8 ± 1.7
Tail length (mm)	53.9 ± 3.2	56.1 ± 3.0	52.6 ± 2.0
Tarsus (mm)	20.6 ± 0.8	20.9 ± 0.7	20.5 ± 0.8
Bill length (mm)	7.2 ± 0.4	7.0 ± 0.3	7.2 ± 0.3
Bill depth (mm)	3.8 ± 0.2	3.9 ± 0.2	3.9 ± 0.2
Bill width (mm)	3.7 ± 0.3	3.6 ± 0.3	3.6 ± 0.2
Wing-tail ratio (mm)	3.0 ± 2.2	-2.9 ± 2.0	-1.8 ± 2.1
% yellow underparts	100 ± 0.0	48.3 ± 4.6	66.2 ± 22.1
Females	*delattrii* (*n* = 84)	*rufifrons* (*n* = 56)	*salvini* (*n* = 46)
Wing length (mm)	54.5 ± 2.2	50.3 ± 2.1	49.2 ± 1.5
Tail length (mm)	52.6 ± 3.1	52.7 ± 2.9	50.3 ± 2.0
Tarsus (mm)	20.2 ± 0.9	20.6 ± 0.7	20.3 ± 0.7
Bill length (mm)	7.2 ± 0.3	6.9 ± 0.3	7.1 ± 0.3
Bill depth (mm)	3.8 ± 0.3	3.7 ± 0.2	3.8 ± 0.1
Bill width (mm)	3.7 ± 0.3	3.6 ± 0.3	3.6 ± 0.2
Wing-tail ratio (mm)	2.0 ± 2.2	-2.5 ± 2.5	-1.1 ± 2.1
% yellow underparts	100 ± 0.0	48.2 ± 5.9	64.3 ± 21.0

Table 5.S4. Discriminant analysis classification accuracy (%) of male and female Rufous-capped Warblers according to subspecies. Row headers indicate discriminant analysis subspecies classification and column headers indicate original subspecies classification.

	rufifrons group					*delattrii* group		
Males	*caudatus* (*n* = 3)	*dugesi* (*n* = 29)	*jouyi* (*n* = 11)	*rufifrons* (*n* = 147)	*salvini* (*n* = 57)	*delattrii* (*n* = 110)	*mesochrysus* (*n* = 12)	*actuosus* (*n* = 5)
caudatus	0	0	0	0	0	0	0	0
dugesi	0	0	9.1	0	1.8	0	0	0
jouyi	0	0	0	0	0	0	0	0
rufifrons	100	82.8	72.7	84.4	52.6	5.5	0	0
salvini	0	17.2	9.1	10.9	42.1	1.8	0	0
delattrii	0	0	9.1	4.8	3.5	90.9	58.3	40.0
mesochrysus	0	0	0	0	0	1.8	16.7	0
actuosus	0	0	0	0	0	0	25.0	60.0
Females	*caudatus* (*n* = 0)	*dugesi* (*n* = 14)	*jouyi* (*n* = 9)	*rufifrons* (*n* = 49)	*salvini* (*n* = 39)	*delattrii* (*n* = 62)	*mesochrysus* (*n* = 9)	*actuosus* (*n* = 4)
caudatus	–	–	–	–	–	–	–	–
dugesi	–	0	0	0	0	0	0	0
jouyi	–	0	22.2	2.0	0	1.6	0	0
rufifrons	–	71.4	55.6	65.3	35.9	4.8	0	0
salvini	–	14.3	0	20.4	65.4	4.8	11.1	0
delattrii	–	14.3	22.2	12.2	7.7	88.7	11.1	50.0
mesochrysus	–	0	0	0	0	0	77.8	0
actuosus	–	0	0	0	0	0	0	50.0

Table 5.S5. Morphological measurements (mean ± SD) of male and female Rufous-capped Warblers according to subspecies.

	rufifrons group					*delattrii* group		
Males	*caudatus* (*n* = 4)	*dugesi* (*n* = 33)	*jouyi* (*n* = 17)	*rufifrons* (*n* = 157)	*salvini* (*n* = 69)	*delattrii* (*n* = 151)	*mesochrysus* (*n* = 16)	*actuosus* (*n* = 9)
Wing length (mm)	52.0 ± 2.7	51.7 ± 1.8	52.1 ± 2.8	53.2 ± 2.3	50.8 ± 1.7	56.6 ± 2.2	57.0 ± 2.7	59.1 ± 2.0
Tail length (mm)	54.8 ± 2.2	55.1 ± 2.5	55.4 ± 3.4	56.1 ± 3.0	52.6 ± 2.0	53.9 ± 3.2	49.4 ± 3.0	51.9 ± 2.1
Tarsus (mm)	20.2 ± 0.3	20.2 ± 0.7	20.8 ± 0.8	20.9 ± 0.7	20.5 ± 0.8	20.6 ± 0.8	20.2 ± 0.7	21.3 ± 0.5
Bill length (mm)	6.7 ± 0.2	6.7 ± 0.3	7.0 ± 0.4	7.0 ± 0.3	7.2 ± 0.3	7.2 ± 0.4	7.6 ± 0.3	8.1 ± 0.4
Bill depth (mm)	3.6 ± 0.2	3.7 ± 0.2	3.8 ± 0.1	3.9 ± 0.2	3.9 ± 0.2	3.8 ± 0.2	4.1 ± 0.1	4.5 ± 0.2
Bill width (mm)	3.3 ± 0.2	3.4 ± 0.2	3.6 ± 0.1	3.6 ± 0.3	3.6 ± 0.2	3.7 ± 0.3	4.1 ± 0.2	4.3 ± 0.2
Wing-tail ratio (mm)	-2.8 ± 2.4	-3.4 ± 1.8	-3.2 ± 3.0	-2.9 ± 2.0	-1.8 ± 2.1	3.0 ± 2.2	7.5 ± 2.6	7.2 ± 2.8
% yellow underparts	48.1 ± 7.3	46.6 ± 4.2	47.0 ± 4.6	48.3 ± 4.6	66.2 ± 22.1	100 ± 0.0	100 ± 0.0	100 ± 0.0
Females	*caudatus* (*n* = 2)	*dugesi* (*n* = 17)	*jouyi* (*n* = 12)	*rufifrons* (*n* = 56)	*salvini* (*n* = 46)	*delattrii* (*n* = 84)	*mesochrysus* (*n* = 13)	*actuosus* (*n* = 5)
Wing length (mm)	49.0 ± 0.0	51.1 ± 2.0	52.6 ± 2.8	50.3 ± 2.1	49.2 ± 1.5	54.5 ± 2.2	54.2 ± 2.6	56.4 ± 3.1
Tail length (mm)	54.0 ± NA	54.1 ± 3.1	55.3 ± 3.8	52.8 ± 2.9	50.3 ± 2.0	52.6 ± 3.1	47.1 ± 1.7	50.6 ± 1.8
Tarsus (mm)	20.5 ± 0.7	20.2 ± 0.6	20.7 ± 0.7	20.6 ± 0.7	20.3 ± 0.7	20.2 ± 0.9	20.7 ± 0.6	21.1 ± 0.6
Bill length (mm)	6.5 ± 0.1	6.7 ± 0.3	6.9 ± 0.3	6.9 ± 0.3	7.1 ± 0.3	7.2 ± 0.3	7.7 ± 0.3	8.3 ± 0.2
Bill depth (mm)	3.8 ± 0.0	3.7 ± 0.3	3.8 ± 0.3	3.7 ± 0.2	3.8 ± 0.1	3.8 ± 0.3	4.0 ± 0.2	4.3 ± 0.1
Bill width (mm)	3.3 ± 0.0	3.3 ± 0.2	3.6 ± 0.3	3.6 ± 0.3	3.6 ± 0.2	3.7 ± 0.3	4.0 ± 0.3	4.5 ± 0.2
Wing-tail ratio (mm)	-5.0 ± NA	-2.9 ± 3.0	-2.8 ± 3.1	-2.5 ± 2.5	-1.1 ± 2.1	2.0 ± 2.2	6.7 ± 2.8	5.8 ± 2.9
% yellow underparts	44.7 ± 1.2	46.6 ± 3.9	47.9 ± 3.2	48.2 ± 6.0	64.3 ± 21.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

Table 5.S6. Linear mixed model results of plumage colour analysis for Rufous-capped Warblers in *delattrii* group, *rufifrons* group, and *B. r. salvini*. Values in bold indicate significant effects.

		PC1 (brightness)			PC2 (yellow)			PC3 (UV)		
		F	df	*P*	*F*	df	*P*	*F*	df	*P*
Belly	Subspecies	**3.5**	**2, 432**	**0.03**	**286.0**	**2, 432**	**<0.001**	**36.7**	**2, 432**	**<0.001**
	Sex	0.0	1, 432	0.93	0.1	1, 432	0.70	**6.4**	**1, 432**	**0.01**
	Subspp × Sex	1.3	2, 432	0.27	0.8	2, 432	0.46	2.2	2, 432	0.12
	Year	**31.8**	**1, 432**	**<0.001**	0.0	1, 432	0.97	**171.8**	**1, 432**	**<0.001**
Breast	Subspecies	**5.9**	**2, 432**	**0.003**	**38.0**	**2, 432**	**<0.001**	**21.2**	**2, 432**	**<0.001**
	Sex	0.0	1, 432	0.90	0.1	1, 432	0.75	0.8	1, 432	0.38
	Subspp × Sex	0.4	2, 432	0.64	0.4	2, 432	0.65	0.8	2, 432	0.44
	Year	**37.0**	**1, 432**	**<0.001**	3.0	1, 432	0.08	**148.6**	**1, 432**	**<0.001**
Mantle	Subspecies	**6.2**	**2, 432**	**0.002**	**43.6**	**2, 432**	**<0.001**	**6.0**	**2, 432**	**0.003**
	Sex	**4.7**	**1, 432**	**0.03**	**4.1**	**1, 432**	**0.04**	**7.4**	**1, 432**	**0.007**
	Subspp × Sex	0.9	2, 432	0.41	1.3	2, 432	0.27	1.9	2, 432	0.14
	Year	1.1	1, 432	0.30	2.2	1, 432	0.14	**23.5**	**1, 432**	**<0.001**
Nape	Subspecies	**19.7**	**2, 432**	**<0.001**	**29.7**	**2, 432**	**<0.001**	1.3	2, 432	0.28
	Sex	1.1	1, 432	0.29	1.3	1, 432	0.25	2.2	1, 432	0.14
	Subspp × Sex	**5.1**	**2, 432**	**0.006**	2.0	2, 432	0.13	2.0	2, 432	0.13
	Year	**10.7**	**1, 432**	**0.001**	2.2	1, 432	0.14	**58.5**	**1, 432**	**<0.001**
Rump	Subspecies	2.5	2, 432	0.08	**26.1**	**2, 432**	**<0.001**	**4.1**	**2, 432**	**0.02**
	Sex	0.1	1, 432	0.74	0.3	1, 432	0.61	1.5	1, 432	0.22
	Subspp × Sex	2.3	2, 432	0.10	0.9	2, 432	0.39	0.8	2, 432	0.45
	Year	0.2	1, 432	0.66	0.0	1, 432	0.89	**31.0**	**1, 432**	**<0.001**
Shoulder	Subspecies	0.2	2, 432	0.79	**10.4**	**2, 432**	**<0.001**	**14.0**	**2, 432**	**<0.001**
	Sex	0.8	1, 432	0.36	0.1	1, 432	0.71	3.2	1, 432	0.07
	Subspp × Sex	0.0	2, 432	0.98	0.2	2, 432	0.83	0.3	2, 432	0.71
	Year	1.9	1, 432	0.17	0.0	1, 432	0.90	**56.6**	**1, 432**	**<0.001**
Tail	Subspecies	0.6	2, 426	0.55	**5.4**	**2, 426**	**0.005**	**6.9**	**2, 426**	**0.001**
	Sex	0.3	1, 426	0.57	0.1	1, 426	0.74	2.5	1, 426	0.12
	Subspp × Sex	0.1	2, 426	0.89	0.3	2, 426	0.73	1.1	2, 426	0.35
	Year	0.1	1, 426	0.77	**6.5**	**1, 426**	**0.01**	**33.1**	**1, 426**	**<0.001**
Throat	Subspecies	0.6	2, 432	0.52	**5.4**	**2, 432**	**0.005**	**6.1**	**2, 432**	**0.003**
	Sex	0.0	1, 432	0.86	0.1	1, 432	0.75	0.2	1, 432	0.67
	Subspp × Sex	0.2	2, 432	0.82	0.2	2, 432	0.83	0.4	2, 432	0.65
	Year	**5.6**	**1, 432**	**0.02**	**25.6**	**1, 432**	**<0.001**	**60.3**	**1, 432**	**<0.001**
Wing	Subspecies	0.6	2, 432	0.54	**17.1**	**2, 432**	**<0.001**	**21.0**	**2, 432**	**<0.001**
	Sex	0.3	1, 432	0.56	1.1	1, 432	0.29	3.0	1, 432	0.09
	Subspp × Sex	0.1	2, 432	0.90	0.3	2, 432	0.71	1.4	2, 432	0.24
	Year	**9.8**	**1, 432**	**0.002**	1.8	1, 432	0.18	**20.3**	**1, 432**	**<0.001**

		PC1 (brightness)			PC2 (red)			PC3 (yellow)		
		F	df	*P*	*F*	df	*P*	*F*	df	*P*
Crown	Subspecies	**17.3**	**2, 432**	**<0.001**	**3.9**	**2, 432**	**0.02**	**11.7**	**2, 432**	**<0.001**
	Sex	0.6	1, 432	0.44	1.2	1, 432	0.27	**7.6**	**1, 432**	**0.006**
	Subspp × Sex	0.7	2, 432	0.50	2.2	2, 432	0.11	0.1	2, 432	0.91
	Year	**4.1**	**1, 432**	**0.04**	**13.6**	**1, 432**	**<0.001**	**7.4**	**1, 432**	**0.007**

Table 5.S7. Subspecies and sex differences in plumage colouration between *delattrii* group (yellow), *rufifrons* group (white), and *B. r. salvini* Rufous-capped Warblers. Significant Tukey's post-hoc test results are highlighted in bold; values for subspecies × sex interactions are provided when significant for that PC score.

		PC1			PC2			PC3		
		Est. ± SE	*t*	*P*	Est. ± SE	*t*	*P*	Est. ± SE	*t*	*P*
Belly	Subspecies									
	white-*salvini*	**-1.84 ± 0.48**	**-3.8**	**<0.001**	**3.82 ± 0.23**	**16.8**	**<0.001**	0.40 ± 0.22	1.8	0.16
	yellow-*salvini*	-0.05 ± 0.52	-0.1	1.00	**-4.13 ± 0.25**	**-16.8**	**<0.001**	**-1.95 ± 0.24**	**-8.2**	**<0.001**
	white-yellow	**1.79 ± 0.43**	**4.2**	**<0.001**	**-7.95 ± 0.20**	**-39.5**	**<0.001**	**-2.36 ± 0.20**	**-12.1**	**<0.001**
Breast	Subspecies									
	white-*salvini*	**-1.07 ± 0.39**	**-2.7**	**0.02**	**2.87 ± 0.32**	**9.1**	**<0.001**	0.17 ± 0.20	0.8	0.67
	yellow-*salvini*	**-1.65 ± 0.43**	**-3.9**	**<0.001**	-0.25 ± 0.34	-0.7	0.74	**-1.35 ± 0.22**	**-6.2**	**<0.001**
	white-yellow	-0.58 ± 0.35	-1.7	0.22	**-3.12 ± 0.28**	**-11.1**	**<0.001**	**-1.52 ± 0.18**	**-8.5**	**<0.001**
Crown	Subspecies									
	white-*salvini*	-0.23 ± 0.64	-0.4	0.93	0.05 ± 0.31	0.2	0.99	0.21 ± 0.16	1.4	0.37
	yellow-*salvini*	**5.40 ± 0.69**	**7.8**	**<0.001**	-0.62 ± 0.33	-1.9	0.15	**-0.83 ± 0.17**	**-4.9**	**<0.001**
	white-yellow	**5.63 ± 0.56**	**10.0**	**<0.001**	**-0.67 ± 0.27**	**-2.5**	**0.04**	**-1.05 ± 0.14**	**-7.5**	**<0.001**
Mantle	Subspecies									
	white-*salvini*	-0.15 ± 0.13	-1.2	0.48	**0.52 ± 0.06**	**9.4**	**<0.001**	0.04 ± 0.08	0.4	0.90
	yellow-*salvini*	**0.39 ± 0.14**	**2.8**	**0.02**	**-0.14 ± 0.06**	**-2.4**	**0.05**	**-0.31 ± 0.09**	**-3.6**	**0.001**
	white-yellow	**0.54 ± 0.11**	**4.7**	**<0.001**	**-0.66 ± 0.05**	**-13.5**	**<0.001**	**-0.34 ± 0.07**	**-4.9**	**<0.001**
Nape	Subspecies									
	white-*salvini*	0.26 ± 0.13	2.0	0.11	0.03 ± 0.05	0.7	0.77	0.05 ± 0.09	0.6	0.84
	yellow-*salvini*	**0.80 ± 0.14**	**5.7**	**<0.001**	**-0.36 ± 0.05**	**-7.1**	**<0.001**	**-0.25 ± 0.09**	**-2.7**	**0.02**
	white-yellow	**0.53 ± 0.11**	**4.7**	**<0.001**	**-0.39 ± 0.04**	**-9.5**	**<0.001**	**-0.30 ± 0.08**	**-3.9**	**<0.001**
	Subspp × sex									
	yellow M-F	**0.61 ± 0.18**	**3.4**	**<0.001**						
	white M-F	-0.07 ± 0.15	-0.5	0.64						
	salvini M-F	0.20 ± 0.21	0.9	0.36						
Rump	Subspecies									
	white-*salvini*	-0.32 ± 0.21	-1.5	0.27	**0.61 ± 0.10**	**6.1**	**<0.001**	-0.01 ± 0.14	-0.1	1.00
	yellow-*salvini*	-0.09 ± 0.23	-0.4	0.92	**-0.28 ± 0.11**	**-2.6**	**0.02**	**-0.72 ± 0.16**	**-4.6**	**<0.001**
	white-yellow	0.23 ± 0.19	1.3	0.42	**-0.89 ± 0.09**	**-10.1**	**<0.001**	**-0.71 ± 0.13**	**-5.6**	**<0.001**
Shoulder	Subspecies									
	white-*salvini*	0.11 ± 0.14	0.8	0.71	**0.36 ± 0.05**	**6.8**	**<0.001**	0.15 ± 0.09	1.7	0.20
	yellow-*salvini*	-0.02 ± 0.15	-0.1	0.99	**0.15 ± 0.06**	**2.7**	**0.02**	**-0.50 ± 0.10**	**-5.2**	**<0.001**
	white-yellow	-0.14 ± 0.13	-1.1	0.53	**-0.20 ± 0.05**	**-4.5**	**<0.001**	**-0.66 ± 0.08**	**-8.3**	**<0.001**
Tail	Subspecies									
	white-*salvini*	0.12 ± 0.14	0.8	0.67	**0.13 ± 0.04**	**3.2**	**0.004**	0.02 ± 0.08	0.3	0.95
	yellow-*salvini*	0.16 ± 0.15	1.1	0.52	**0.23 ± 0.04**	**5.4**	**<0.001**	**-0.40 ± 0.08**	**-4.7**	**<0.001**
	white-yellow	0.05 ± 0.12	0.4	0.93	**0.10 ± 0.04**	**2.9**	**0.01**	**-0.42 ± 0.07**	**-6.1**	**<0.001**
Throat	Subspecies									
	white-*salvini*	-0.38 ± 0.42	-0.9	0.64	**0.52 ± 0.20**	**2.6**	**0.02**	0.13 ± 0.20	0.7	0.79
	yellow-*salvini*	-0.58 ± 0.46	-1.3	0.41	**1.03 ± 0.22**	**4.8**	**<0.001**	**-0.88 ± 0.22**	**-4.0**	**<0.001**
	white-yellow	-0.21 ± 0.37	-0.6	0.85	**0.51 ± 0.18**	**2.9**	**0.01**	**-1.02 ± 0.18**	**-5.6**	**<0.001**
Wing	Subspecies									
	white-*salvini*	0.15 ± 0.17	0.9	0.65	**0.21 ± 0.05**	**4.6**	**<0.001**	0.04 ± 0.06	0.6	0.80
	yellow-*salvini*	0.38 ± 0.18	2.1	0.10	**0.40 ± 0.05**	**8.0**	**<0.001**	**-0.46 ± 0.07**	**-6.6**	**<0.001**
	white-yellow	0.23 ± 0.15	1.5	0.27	**0.19 ± 0.04**	**4.6**	**<0.001**	**-0.50 ± 0.06**	**-8.8**	**<0.001**

Table 5.S8. Linear mixed model results of plumage colour analysis for *B. r. delattrii*, *B. r. rufifrons*, and *B. r. salvini* subspecies of Rufous-capped Warbler. Values in bold indicate significant effects.

		PC1 (brightness)			PC2 (yellow)			PC3 (UV)		
		F	df	*P*	*F*	df	*P*	*F*	df	*P*
Belly	Subspecies	**3.5**	**2, 306**	**0.03**	**160.8**	**2, 306**	**<0.001**	**33.6**	**2, 306**	**<0.001**
	Sex	0.2	1, 306	0.69	0.0	1, 306	0.92	3.4	1, 306	0.07
	Subspp × Sex	0.6	2, 306	0.57	0.4	2, 306	0.66	1.7	2, 306	0.18
	Year	**26.8**	**1, 306**	**<0.001**	0.2	1, 306	0.67	**113.5**	**1, 306**	**<0.001**
Breast	Subspecies	**5.4**	**2, 306**	**0.005**	**24.2**	**2, 306**	**<0.001**	**16.6**	**2, 306**	**<0.001**
	Sex	2.7	1, 306	0.10	0.0	1, 306	0.87	0.1	1, 306	0.83
	Subspp × Sex	0.6	2, 306	0.55	0.0	2, 306	0.97	0.3	2, 306	0.72
	Year	**23.9**	**1, 306**	**<0.001**	0.5	1, 306	0.48	**104.3**	**1, 306**	**<0.001**
Mantle	Subspecies	**4.7**	**2, 306**	**0.01**	**16.0**	**2, 306**	**<0.001**	**7.2**	**2, 306**	**<0.001**
	Sex	1.8	1, 306	0.18	0.1	1, 306	0.72	2.2	1, 306	0.14
	Subspp × Sex	1.5	2, 306	0.22	1.3	2, 306	0.28	2.0	2, 306	0.13
	Year	3.1	1, 306	0.08	0.1	1, 306	0.78	**18.8**	**1, 306**	**<0.001**
Nape	Subspecies	**17.0**	**2, 306**	**<0.001**	**29.5**	**2, 306**	**<0.001**	1.6	2, 306	0.21
	Sex	**12.3**	**1, 306**	**<0.001**	**4.0**	**1, 306**	**0.05**	**8.3**	**1, 306**	**0.004**
	Subspp × Sex	**5.5**	**2, 306**	**0.005**	2.1	2, 306	0.12	2.9	2, 306	0.06
	Year	**8.6**	**1, 306**	**0.004**	2.5	1, 306	0.12	**31.9**	**1, 306**	**<0.001**
Rump	Subspecies	1.6	2, 306	0.21	**9.4**	**2, 306**	**0.001**	**3.9**	**2, 306**	**0.02**
	Sex	**14.2**	**1, 306**	**<0.001**	**8.1**	**1, 306**	**0.005**	**10.6**	**1, 306**	**0.001**
	Subspp × Sex	**3.8**	**2, 306**	**0.02**	1.7	2, 306	0.19	1.8	2, 306	0.17
	Year	0.0	1, 306	0.95	0.2	1, 306	0.64	**14.7**	**1, 306**	**<0.001**
Shoulder	Subspecies	0.5	2, 306	0.62	**5.3**	**2, 306**	**0.005**	**6.5**	**2, 306**	**0.002**
	Sex	**4.2**	**1, 306**	**0.04**	0.0	1, 306	0.94	2.4	1, 306	0.13
	Subspp × Sex	0.4	2, 306	0.70	0.1	2, 306	0.90	0.2	2, 306	0.82
	Year	**6.1**	**1, 306**	**0.01**	2.5	1, 306	0.12	**45.6**	**1, 306**	**<0.001**
Tail	Subspecies	0.7	2, 301	0.48	**7.6**	**2, 301**	**<0.001**	**6.1**	**2, 301**	**0.002**
	Sex	1.5	1, 301	0.22	0.0	1, 301	0.86	1.3	1, 301	0.26
	Subspp × Sex	0.1	2, 301	0.87	0.4	2, 301	0.69	0.8	2, 301	0.46
	Year	0.1	1, 301	0.72	**10.3**	**1, 301**	**0.002**	**31.9**	**1, 301**	**<0.001**
Throat	Subspecies	0.1	2, 306	0.89	2.6	2, 306	0.08	**4.1**	**2, 306**	**0.02**
	Sex	0.2	1, 306	0.63	0.0	1, 306	0.83	0.0	1, 306	0.85
	Subspp × Sex	0.1	2, 306	0.95	0.2	2, 306	0.86	0.5	2, 306	0.60
	Year	3.2	1, 306	0.07	**19.2**	**1, 306**	**<0.001**	**40.2**	**1, 306**	**<0.001**
Wing	Subspecies	1.2	2, 306	0.30	**22.9**	**2, 306**	**<0.001**	**13.2**	**2, 306**	**<0.001**
	Sex	0.0	1, 306	0.88	**6.1**	**1, 306**	**0.01**	0.0	1, 306	0.88
	Subspp × Sex	0.1	2, 306	0.94	0.7	2, 306	0.48	0.9	2, 306	0.40
	Year	**4.2**	**1, 306**	**0.04**	0.2	1, 306	0.62	**22.2**	**1, 306**	**<0.001**

		PC1 (brightness)			PC2 (red)			PC3 (yellow)		
		F	df	*P*	*F*	df	*P*	*F*	df	*P*
Crown	Subspecies	**20.1**	**2, 306**	**<0.001**	**4.7**	**2, 306**	**0.01**	**4.7**	**2, 306**	**0.01**
	Sex	0.0	1, 306	0.99	1.1	1, 306	0.30	**12.2**	**1, 306**	**<0.001**
	Subspp × Sex	0.2	2, 306	0.79	2.5	2, 306	0.08	0.1	2, 306	0.86
	Year	0.0	1, 306	0.84	**8.9**	**1, 306**	**0.003**	3.4	1, 306	0.07

Table 5.S9. Subspecies and sex differences in plumage colouration between *B. r. delattrii*, *B. r. rufifrons*, and *B. r. salvini* subspecies of Rufous-capped Warbler. Significant Tukey's post-hoc test results are highlighted in bold; values for subspecies × sex interactions are provided when significant for that PC score.

		PC1			PC2			PC3		
		Est. ± SE	*t*	*P*	Est. ± SE	*t*	*P*	Est. ± SE	*t*	*P*
Belly	Subspecies									
	delattrii-rufifrons	**-2.20 ± 0.54**	**-4.0**	**<0.001**	**8.18 ± 0.28**	**29.3**	**<0.001**	**2.77 ± 0.24**	**11.7**	**<0.001**
	delattrii-salvini	-0.19 ± 0.59	-0.3	0.94	**4.43 ± 0.30**	**14.8**	**<0.001**	**2.60 ± 0.25**	**10.2**	**<0.001**
	rufifrons-salvini	**2.01 ± 0.54**	**3.7**	**<0.001**	**-3.74 ± 0.28**	**-13.5**	**<0.001**	-0.18 ± 0.24	-0.8	0.73
Breast	Subspecies									
	delattrii-rufifrons	**1.03 ± 0.42**	**2.4**	**0.04**	**3.09 ± 0.32**	**9.7**	**<0.001**	**1.62 ± 0.21**	**7.6**	**<0.001**
	delattrii-salvini	**1.84 ± 0.45**	**4.1**	**<0.001**	0.48 ± 0.34	1.4	0.34	**1.62 ± 0.23**	**7.0**	**<0.001**
	rufifrons-salvini	0.81 ± 0.42	1.9	0.13	**-2.61 ± 0.32**	**-8.2**	**<0.001**	0.00 ± 0.21	0.0	1.00
Crown	Subspecies									
	delattrii-rufifrons	**-6.39 ± 0.67**	**-9.5**	**<0.001**	0.75 ± 0.34	2.2	0.07	**0.73 ± 0.17**	**4.4**	**<0.001**
	delattrii-salvini	**-6.58 ± 0.73**	**-9.1**	**<0.001**	**1.04 ± 0.36**	**2.9**	**0.01**	**0.80 ± 0.18**	**4.5**	**<0.001**
	rufifrons-salvini	-0.19 ± 0.67	-0.3	0.96	0.29 ± 0.33	0.9	0.67	0.07 ± 0.17	0.4	0.90
Mantle	Subspecies									
	delattrii-rufifrons	**-0.45 ± 0.14**	**-3.2**	**0.005**	**0.47 ± 0.07**	**7.1**	**<0.001**	**0.34 ± 0.08**	**4.2**	**<0.001**
	delattrii-salvini	-0.29 ± 0.15	-1.9	0.14	0.06 ± 0.07	0.8	0.71	**0.43 ± 0.09**	**4.9**	**<0.001**
	rufifrons-salvini	0.16 ± 0.14	1.1	0.50	**-0.41 ± 0.07**	**-6.2**	**<0.001**	0.09 ± 0.08	1.1	0.53
Nape	Subspecies									
	delattrii-rufifrons	**-0.51 ± 0.15**	**-3.5**	**0.002**	**0.50 ± 0.06**	**8.9**	**<0.001**	**0.34 ± 0.09**	**3.8**	**<0.001**
	delattrii-salvini	**-0.85 ± 0.16**	**-5.4**	**<0.001**	**0.49 ± 0.06**	**7.9**	**<0.001**	**0.38 ± 0.10**	**3.9**	**<0.001**
	rufifrons-salvini	-0.33 ± 0.15	-2.3	0.06	-0.02 ± 0.06	-0.3	0.94	0.04 ± 0.09	0.4	0.91
	Subspecies × sex									
	delattrii M-F	**0.70 ± 0.22**	**3.2**	**0.002**						
	rufifrons M-F	-0.17 ± 0.19	-0.9	0.37						
	salvini M-F	0.20 ± 0.22	0.9	0.36						
Rump	Subspecies									
	delattrii-rufifrons	-0.05 ± 0.24	-0.2	0.98	**0.67 ± 0.13**	**5.2**	**<0.001**	**0.81 ± 0.15**	**5.3**	**<0.001**
	delattrii-salvini	0.27 ± 0.26	1.0	0.55	0.14 ± 0.14	1.0	0.57	**0.96 ± 0.16**	**5.9**	**<0.001**
	rufifrons-salvini	0.32 ± 0.24	1.3	0.39	**-0.53 ± 0.13**	**-4.2**	**<0.001**	0.16 ± 0.15	1.0	0.56
	Subspecies × sex									
	delattrii M-F	**1.38 ± 0.36**	**3.8**	**<0.001**						
	rufifrons M-F	0.23 ± 0.32	0.7	0.48						
	salvini M-F	0.11 ± 0.36	0.3	0.76						
Shoulder	Subspecies									
	delattrii-rufifrons	0.20 ± 0.16	1.3	0.42	0.11 ± 0.06	1.8	0.16	**0.55 ± 0.09**	**5.9**	**<0.001**
	delattrii-salvini	0.00 ± 0.17	0.0	1.00	**-0.17 ± 0.06**	**-2.8**	**0.02**	**0.50 ± 0.10**	**4.9**	**<0.001**
	rufifrons-salvini	-0.21 ± 0.16	-1.3	0.40	**-0.28 ± 0.06**	**-4.8**	**<0.001**	-0.05 ± 0.09	-0.6	0.84
Tail	Subspecies									
	delattrii-rufifrons	0.13 ± 0.16	0.8	0.71	**-0.24 ± 0.05**	**-4.8**	**<0.001**	**0.45 ± 0.08**	**5.4**	**<0.001**
	delattrii-salvini	-0.14 ± 0.17	-0.8	0.69	**-0.29 ± 0.05**	**-5.4**	**<0.001**	**0.45 ± 0.09**	**5.0**	**<0.001**
	rufifrons-salvini	-0.27 ± 0.16	-1.7	0.22	-0.05 ± 0.05	-1.0	0.58	0.00 ± 0.08	0.0	1.00
Throat	Subspecies									
	delattrii-rufifrons	0.23 ± 0.46	0.5	0.87	-0.03 ± 0.23	-0.1	0.99	**1.05 ± 0.21**	**4.9**	**<0.001**
	delattrii-salvini	0.29 ± 0.50	0.6	0.83	**-0.64 ± 0.25**	**-2.6**	**0.03**	**1.02 ± 0.23**	**4.4**	**<0.001**
	rufifrons-salvini	0.06 ± 0.46	0.1	0.99	**-0.61 ± 0.23**	**-2.6**	**0.02**	-0.04 ± 0.21	-0.2	0.98
Wing	Subspecies									
	delattrii-rufifrons	-0.03 ± 0.19	-0.2	0.99	**-0.36 ± 0.05**	**-7.3**	**<0.001**	**0.50 ± 0.07**	**7.1**	**<0.001**
	delattrii-salvini	-0.45 ± 0.20	-2.2	0.07	**-0.44 ± 0.05**	**-8.2**	**<0.001**	**0.46 ± 0.08**	**6.2**	**<0.001**
	rufifrons-salvini	-0.42 ± 0.19	-2.2	0.07	-0.07 ± 0.05	-1.5	0.30	-0.03 ± 0.07	-0.5	0.87

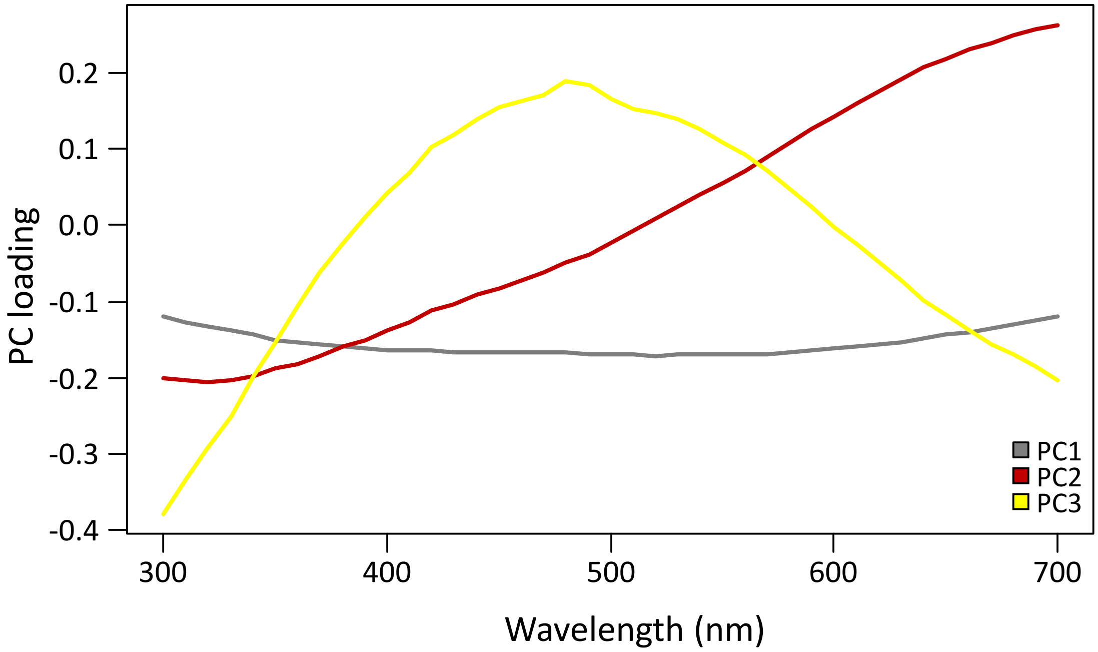

Figure 5.S1. Summary of factor loadings for first three principal components in a PCA describing reflectance of the crown patch. Original variables are mean reflectance values grouped into 10 nm bins across the avian visual spectrum (300–700 nm). PC1 (grey line) represents brightness, PC2 (red line) represents amount of red reflectance, and PC3 (yellow line) represents amount of yellow reflectance.

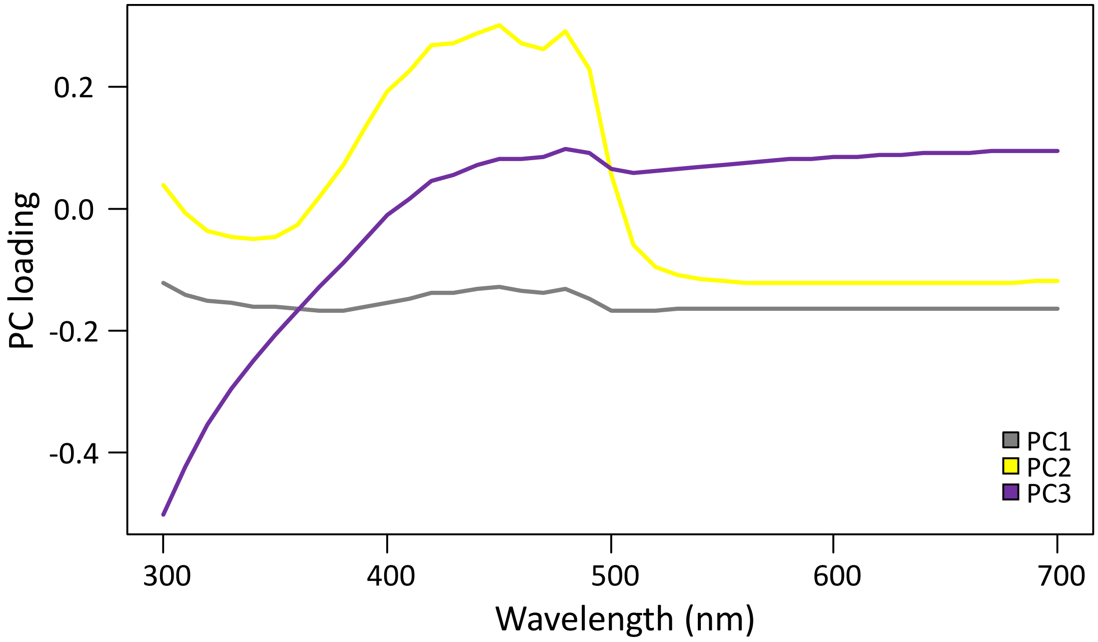

Figure 5.S2. Summary of factor loadings for first three principal components in a PCA describing reflectance of all plumage patches except for the crown. Original variables are mean reflectance values grouped into 10 nm bins across the avian visual spectrum (300–700 nm). PC1 (grey line) represents brightness, PC2 (yellow line) represents amount of yellow reflectance, and PC3 (purple line) represents amount of UV reflectance.

Chapter 6: Receiver response to song divergence in a Neotropical songbird

*This chapter is the outcome of joint research with D. Mennill and J. R. Sosa-López.

Chapter Summary

When animal mating signals diverge between populations, reproductive isolation and speciation may occur. Variation in animals' responses to these signals may reveal whether differences in perception contribute to behavioural differences between populations. We tested whether signal divergence influences receiver responses to playback in the Rufous-capped Warbler (*Basileuterus rufifrons*), a Neotropical resident songbird with a contact zone between two divergent subspecies, *B. r. delattrii* and *B. r. rufifrons*, in southern Mexico. We presented allopatric and sympatric pairs of warblers with playback simulating a territorial male rival of each subspecies. We found that song acts as a premating isolating barrier between subspecies. Warblers responded more strongly to playback of their own subspecies than the other subspecies when we conducted playback to *delattrii* and *rufifrons* living in sympatry. However, *delattrii* living in allopatry responded strongly to playback of both subspecies, suggesting possible reproductive character displacement. Our research demonstrates that *delattrii* and *rufifrons* discriminate between each other's songs, suggesting that song is an isolating mechanism, and adds to the growing literature on receiver response to vocal signal divergence in closely-related sympatric and allopatric animal populations.

Introduction

Signal divergence between animal populations promotes premating reproductive isolation and speciation (Price & Bouvier 2002; Coyne & Orr 2004), and occurs through ecological selection, sexual selection, and cultural or genetic drift (Wilkins et al. 2013). Investigating the mechanisms of signal divergence solely by examining patterns of phenotypic and genetic divergence may be inconclusive. In some populations, divergence in mating signals such as bright plumage and elaborate song mirrors genetic differentiation (Mendelson & Shaw 2005; Uy et al. 2009; Caro et al. 2013; Greig et al. 2015). Other closely-related taxa may show strong phenotypic differentiation and discrimination despite little genetic difference (e.g., Mason & Taylor 2015; Benites et al. 2015), or discriminate strongly between phenotypically similar signals (e.g., Grant & Grant 2002; Tobias & Seddon 2009; Grace & Shaw 2012). Therefore, it is critical to examine not only the extent of divergence, but also the strength of discrimination or response to trait divergence by the animals themselves (Seddon & Tobias 2010; Hudson & Price 2014). In animals using acoustic and visual modalities to communicate, experimental studies using playback of vocal signals (e.g., Grant & Grant 2002; Lemmon 2009; Grace & Shaw 2012) and presentation of visual models (e.g., Gabor & Ryan 2001; Mays & Hopper 2004; Hick et al. 2016) are useful tests of response to signal variation.

Although signal divergence between allopatric populations is often considered an important indicator of reproductive isolation (Coyne & Orr 2004), the extent of signal divergence in closely-related allopatric populations does not always reflect the degree of reproductive isolation (Hudson & Price 2014). Instead, reproductive character displacement (i.e. greater signal divergence, heightened discrimination, or both) when closely-related populations come into secondary contact may maintain premating isolation through reinforcement when selection against hybrids is strong (Gerhardt 2013; Hudson & Price 2014). This pattern has been observed in taxa including insects (e.g., Jang & Gerhardt 2006; Grace & Shaw 2012), fishes (e.g., Gabor & Ryan 2001), and anurans (e.g., Lemmon 2009). In birds, male Black-crested Titmice (*Baeolophus atricristatus*) and Tufted Titmice (*B. bicolor*) showed stronger song discrimination in an older hybrid zone because of reduced hybrid fitness (Curry & Patten 2016). In Collared Flycatchers (*Ficedula albicollis*) and Pied Flycatchers (*F. hypoleuca*), plumage and song diverged more in sympatry than in allopatry and corresponded to female preferences, minimizing undesirable interspecific mating (Sætre et al. 1997; Haavie et al. 2004). A similar situation can occur in parapatric (adjoining) populations which potentially hybridize; in two Grey-breasted Wood-wren (*Henicorhina leucophrys*) subspecies, males in parapatric populations had more acoustically different songs and stronger song discrimination than males in allopatric populations (Dingle et al. 2010). In these cases, reinforcement promotes assortative mating thereby limiting hybridization, although not all contact zones show this pattern (Wilkins et al. 2018).

Birdsong is a widely studied territory defence and mating signal (Catchpole & Slater 2008). Related populations that compete for resources (e.g., food, nesting sites) may respond strongly to heterotypic songs of competitors even when they are reproductively isolated (Martin & Martin 2001; Tobias & Seddon 2009; Jankowski et al. 2010; Freeman 2016). When one closely-related species or subspecies replaces another along an ecological gradient, competitive interactions can promote between-population discrimination upon secondary contact (e.g., Jankowski et al. 2010; Caro et al. 2013; Freeman et al. 2016). To establish whether different responses to song between closely-related sympatric populations are related to mate attraction or resource defence, it is important to confirm whether gene flow is ongoing. If gene flow is absent, competition for territorial resources, rather than mates, likely explains heightened responses to heterotypic signals (reviewed in Lipshutz 2018).

Differences in receiver perception of signals, whether learned or innate, are expected to influence signal divergence (Endler & Basolo 1998; Verzijden et al. 2012). Learned discrimination between particular signal features may influence response strength even if the signals diverge little structurally (Grant & Grant 2002; Gee 2005; Seddon & Tobias 2010; Grace & Shaw 2012), and may also result in asymmetric discrimination between different populations (e.g., Colbeck et al. 2010; Dingle et al. 2010). Different populations may vary in their innate sensory sensitivity to specific signal features (e.g., frequency), further enhancing reproductive isolation in combination with learned discrimination (Dingle et al. 2010; McEntee 2014). However, learning can reduce reproductive isolation when animals learn signals from neighbouring individuals of a related species or subspecies (e.g., McEntee et al. 2016; Kenyon et al. 2017). For instance, in a young hybrid zone, male Pied Flycatchers sang mixed songs containing elements copied from Collared Flycatcher neighbours, resulting in increased hybridization (Haavie et al. 2004). These examples underscore the importance of quantifying both innate and learned components of discrimination in order to examine their contribution to reproductive isolation.

The Rufous-capped Warbler (*Basileuterus rufifrons*) is an ideal species in which to study receiver response to signal divergence between populations. This common resident warbler of Mexico, Central America, and northern South America shows pronounced geographic variation in vocal and visual signal phenotypes. The eight recognized subspecies fall into two groups that differ in plumage and voice: (1) the northern, white-bellied *rufifrons* group of Mexico and western Guatemala, and (2) the southern, yellow-bellied *delattrii* group of southeastern Mexico and Central and South America (Curson 2010; Figure 6.1). The current taxonomy recognizes a single species based on the existence of an intermediate-plumaged subspecies, *B. r. salvini*, in the Atlantic lowland region of southern Mexico, and reported hybridization between white-bellied *B. r. rufifrons* and yellow-bellied *B. r. delattrii* in a narrow zone of sympatry in southern Mexico and Guatemala (Monroe 1968; Curson 2010; Figure 6.1). However, other authorities split the two groups into separate species based on their highly divergent plumage and songs (Todd 1929; Howell & Webb 1995). The two groups also differ in their habitat preferences; the white-bellied group lives in arid scrub and pine-oak from 1000–3000 m a.s.l., whereas the yellow-bellied group inhabits semi-open humid habitats and tropical dry forest from 0–1500 m a.s.l. (Curson 2010). Given these phenotypic differences, this system is ideal for testing variation in receiver response to vocal signals between populations.

The objective of our research was to experimentally test whether song divergence between phenotypically divergent Rufous-capped Warbler subspecies contributes to behavioural differences in response to these vocal signals. We aimed to determine if vocal divergence influences reproductive isolation between two Rufous-capped Warbler subspecies with a narrow contact zone, *B. r. rufifrons* and *B. r. delattrii*. We predicted that if males compete between subspecies for mates and territories, males in both sympatry and allopatry should respond equally strongly to both subspecies. If males do not compete between subspecies for mates and territories, males in both sympatry and allopatry should respond strongly to their own subspecies and weakly to the other subspecies. If song is a reproductively isolating barrier between subspecies, and if males of each subspecies also compete for territories when they occur in sympatry, males in sympatry should respond strongly to both subspecies, whereas males in allopatry should respond strongly to their own subspecies and weakly to the other subspecies. If reproductive character displacement occurs between the two subspecies, males in sympatry should respond strongly to their own subspecies and weakly to the other subspecies, whereas males in allopatry should respond strongly to both subspecies. If aggression levels or innate perceptual sensitivity differ between subspecies, we expected an asymmetric response between the subspecies (e.g., Colbeck et al. 2010; Dingle et al. 2010).

Methods

Study species and location

We conducted our study in southeastern Chiapas, Mexico, where *delattrii* are found in humid forest at low elevation (200–800 m a.s.l.) and *rufifrons* live in montane scrub habitat at high elevation (1200–3000 m a.s.l.; Howell & Webb 1995). In this region, *delattrii* and *rufifrons* co-occur in humid, semi-open habitats (e.g., coffee plantations) at mid–elevation (800–1200 m), but appear to share territories, mate assortatively, and have distinct plumage and songs (A. Demko and J. R. Sosa-López pers. obs.). We experimentally tested the importance of vocal signals for within-subspecies discrimination by presenting territorial pairs of *delattrii* and *rufifrons* with song playback of both subspecies (e.g., Uy et al. 2009; Greig et al. 2015). We conducted our playback study at three localities at the start of the breeding (rainy) season: (1) allopatric *delattrii* near Mapastepec, Chiapas (15.34°N, 92.52°W; elev. 450 m), a humid montane deciduous forest (May 25–31, 2017); (2) allopatric *rufifrons* near Motozintla, Chiapas (15.38°N, 92.27°W; elev. 1700 m), an arid montane pine-oak scrub (June 8–9, 2017); and (3) sympatric *delattrii* and sympatric *rufifrons* at Finca La Victoria, Chiapas (15.29°N, 92.42°W; elev. 1000 m), a humid shade coffee plantation (May 8–21, 2017; Figure 6.2).

Playback design

We captured warblers on their territories using mist-nets and song playback, and we banded each animal with a unique colour-band combination for individual identification. We banded both the female and the male owners in 5 territories; in 23 territories we captured the male only; and in 18 territories both birds were unbanded. For unbanded pairs, we verified the territory holder identity by comparing the unique song types used by the males during each trial. Our analysis included territories of 15 allopatric *delattrii*, 6 allopatric *rufifrons,* 17 sympatric *delattrii*, and 8 sympatric *rufifrons* (Table 6.1).

We presented three treatments to each pair, one on each successive day: (1) male *delattrii* song; (2) male *rufifrons* song; and (3) control of male song of a sympatric non-competitor species, the Banded Wren (*Thryophilus pleurostictus*). Thus, each focal bird received own-subspecies, other-subspecies, and heterospecific stimuli. We broadcast stimuli from a FoxPro Scorpion TX200 speaker placed 1 m above the ground near the centre of the warblers' territory. We estimated the territory centre based on capture locations and 30-min territorial observations of the territory holders prior to the experiment; males typically sang from the same perch during the dawn chorus, which we presumed to be near their territory centre. We broadcast stimuli at 90 dB(A) SPL measured at 1 m from the speaker using a Casella CEL–240 sound level meter (Casella CEL Inc., Buffalo, NY, USA). This amplitude approximates the natural volume of Rufous-capped Warbler broadcast songs and the amplitude used in other warbler song playback studies (e.g., Hof & Hazlett 2010).

Trials consisted of a 5-min pre-playback, 5-min playback, and 2-min post-playback period. We ran all trials between 07:00–12:30 CDT, which is the period of peak daily vocal activity in this species, excluding the early-morning dawn chorus when males typically sing spontaneously at a high rate (Chapter 2). One or two observers sat 15–20 m away from the speaker to record the birds' responses and describe their behaviour. We used a Marantz PMD660 digital recorder and Audiotechnica AT8015 directional microphone to record all playback trials. During the trials, the observer(s) dictated the horizontal and vertical distance of each bird from the playback, and described other activities related to aggressive territorial responses (e.g., flights over the speaker).

We considered a response to be a trial where at least one warbler of the focal pair approached within 10 m of the playback speaker during the 5-min playback period (e.g., Gill et al. 2007; Jankowski et al. 2010). If both the male and female responded, we recorded all response data for each individual separately, rather than pooling all responses together. We repeated the 5-min playback in a new location within the pair's territory on the following day if there was no response to the own-subspecies stimulus on the first attempt. We used this protocol because we expected all birds to respond territorially to the song of their own subspecies, and thus assumed that the first location chosen was not near the actual territory centre. If there was still no response on the second attempt, we did not include that territory's data in the analysis. We also repeated any trials on the following day if a neighbour of the same subspecies also approached within 10 m of the playback during the 5-min playback period, or if another animal species approached the playback at the same time as the focal bird. At the sympatric site, we did not repeat the trial if a warbler from the other subspecies also responded, since the territories of the two subspecies frequently overlapped (A. Demko pers. obs.). When territories overlapped between the two subspecies, we identified the focal subspecies based on capture locations of individuals and dawn singing observations. In all cases where we repeated a trial, we used only the second, successful trial in our analyses. We ensured that neighbouring warbler pairs used in the experiment did not receive the same playback stimuli to avoid familiarity with particular song types.

Playback stimuli

We used our own field recordings collected under natural or playback-induced conditions in Mexico in 2016 and 2017 to produce all playback stimuli. Since Rufous-capped Warblers use similar song types in natural and playback-induced conditions (Chapter 4), we considered songs from both types of recordings to be equivalent and we chose the highest-quality recordings available to produce playback stimuli. Our investigation focused on the overall responses of each subspecies to each other rather than their responses to the local population, so birds at all three sites received only non-local song playback of both subspecies (modified from Dingle et al. 2010). Furthermore, since we expected warblers at the sympatric site to be more familiar with and potentially more responsive to local song of both subspecies, the use of non-local songs at all sites permitted a more conservative approach to data analysis. Birds at each site received the conspecific stimuli indicated in Table 6.1. To produce heterospecific stimuli, we recorded Banded Wren songs opportunistically from five individual males at Mapastepec, Motozintla, and Parque Nacional Cañon del Sumidero, Chiapas.

Each playback stimulus consisted of a single song type recorded from one individual male, repeated at a natural daytime song rate of 6 songs/min (Chapter 4). We prepared stimuli by filtering each song with a 1000 Hz high-pass filter, editing out background noise around the song using the lasso selection tool, and normalizing the amplitude of the final playback files to -1 dB using Adobe Audition 3.0 software (Adobe, San Jose, CA, USA). We produced the following number of different stimuli for each subspecies and location: Finca La Victoria *rufifrons* (*n* = 6); Finca La Victoria *delattrii* (*n* = 9); Motozintla *rufifrons* (*n* = 9); Mapastepec *delattrii* (*n* = 11); and Banded Wren (*n* = 5).

Analysis

We annotated recordings of playback trials using Syrinx PC (J. Burt, Seattle, WA, USA), focusing on the observers' description of the birds' physical responses to the playback, and all songs and calls produced by each warbler during the trials. We recorded the following response measures for each focal male and female: (1) closest distance of approach to speaker in m; (2) latency to approach within 10 m of speaker; (3) time spent within 10 m of speaker; (4) number of songs given; and (5) song duration in seconds (calculated by averaging the song duration of all songs recorded during the playback trial). We used Principal Components Analysis to create uncorrelated response variables (McGregor 1992); we first log-transformed the latency to approach and time within 10 m variables to meet the assumptions of PCA (Quinn & Keough 2002). PC1 (eigenvalue: 2.92) represented 58.3% of the total variation in response strength, and was the only influential PC (i.e. eigenvalue >1). Factor loadings for PC1 corresponded to physical and vocal approach responses of warblers to playback; a positive PC1 score corresponded to a stronger response, including closer approach distance, shorter latency to approach, more time spent near the speaker, more songs sung, and longer songs produced (Table 6.2). We then ran linear mixed models with PC1 as the response variable; playback treatment (*delattrii*, *rufifrons*, or control), site (allopatric *delattrii*, sympatric *delattrii*, allopatric *rufifrons*, or sympatric *rufifrons*), and treatment × site interaction as fixed effects; and bird identity and playback stimulus file as random effects (Greig et al. 2015). We included an order effect in the initial models, but as this was non-significant ($\chi^2_1 = 0.2$, $P = 0.67$), we excluded it from the final models. We conducted likelihood ratio tests to estimate fixed effects *P*-values, and conducted separate post-hoc analyses for each site using the 'glht' function in the 'multcomp' package in R (Hothorn et al. 2017). Data met LMM assumptions aside from the presence of three outliers; we elected to retain the outliers in our final analysis, because they represented actual responses of pairs to playback, and a separate analysis with the outliers removed yielded the same significant effects and post-hoc test results. We conducted all statistical analyses using R v.3.4.3 (R Development Core Team 2017).

Results

Male responses to playback

Male Rufous-capped Warblers at all sites showed high response rates to playback; 88–100% of individuals responded (i.e. approached within 10 m of the speaker) during own-subspecies trials and 20–87% during other-subspecies trials. Only sympatric *delattrii* responded to significantly more own-subspecies than other-subspecies playback trials (Fisher's exact test, $P < 0.001$). Sympatric *rufifrons*, allopatric *delattrii*, and allopatric *rufifrons* did not differ significantly in the proportion of responses to own- and other-subspecies playback (Fisher's exact test, all $P > 0.10$; Figure 6.3). Although some males responded to control trials (0–43% by site; Figure 6.3), response strength (PC1) was greater to conspecific trials than to control trials at all sites (Figure 6.4). A significant treatment main effect ($\chi^2_2 = 16.6$, $P < 0.001$) and treatment × site interaction (likelihood ratio test: $\chi^2_6 = 39.0$, $P < 0.001$) indicated that responses to each treatment type varied between sites (Table 6.3).

The sympatric and allopatric sites showed different patterns of response strength to playback. Sympatric *delattrii* and *rufifrons* both discriminated between the two subspecies (Figure 6.4). Sympatric *delattrii* males responded significantly more strongly to *delattrii* playback than to either *rufifrons* (estimate = 2.18 ± 0.47, $t = 4.6$, $P < 0.001$) or control playback (estimate = -2.34 ± 0.50, $t = -4.7$, $P < 0.001$), whereas their responses to *rufifrons* and control playback did not differ (estimate = -0.16 ± 0.50, *t* = -0.3, *P* = 0.94). Sympatric *rufifrons* males responded significantly more strongly to *rufifrons* playback than to either *delattrii* (estimate = -2.34 ± 0.66, *t* = -3.6, *P* = 0.002) or control playback (estimate = -2.58 ± 0.67, *t* = -3.8, *P* < 0.001), whereas their responses to *delattrii* and control playback did not differ (estimate = -0.24 ± 0.69, *t* = -0.3, *P* = 0.94; Table 6.3).

In contrast to the sympatric sites, allopatric *delattrii* males did not discriminate between the two subspecies; instead, they responded significantly more strongly to both *delattrii* (estimate = -2.64 ± 0.52, *t* = -5.1, *P* < 0.001) and *rufifrons* playback (estimate = -2.02 ± 0.54, *t* = -3.8, *P* < 0.001) than to the control, whereas their responses to *delattrii* and *rufifrons* playback did not differ (estimate = 0.62 ± 0.51, *t* = 1.2, *P* = 0.45; Figure 6.4). In allopatric *rufifrons*, males showed a trend towards discrimination between subspecies; they responded more strongly to *rufifrons* playback than to *delattrii* (estimate = -1.88 ± 0.94, *t* = -2.0, *P* = 0.12) or control (estimate = -3.07 ± 1.44, *t* = -2.1, *P* = 0.09; Table 6.3), although neither comparison was significant. The observed trend only suggests discrimination between subspecies, since a low sample size at this site precluded accurate statistical analysis.

At the sympatric site, an opposite-subspecies neighbour sometimes responded to playback when the focal bird did not respond (e.g., during a *rufifrons* playback treatment on a *delattrii* territory, a neighbouring *rufifrons* sometimes responded). Some *rufifrons* individuals responded to off-territory playback of both *rufifrons* (6 of 17 trials) and *delattrii* playback (2 of 17 trials). During both of these *delattrii* trials and 1 of the 6 *rufifrons* trials, the *delattrii* territory holder also responded to the playback. Only one *delattrii* individual responded to *delattrii* playback (1 of 8 trials) on a *rufifrons* territory. Two of the *rufifrons* intruders were confirmed as neighbours of the focal *delattrii* male based on their colour band combinations. The remaining unbanded off-territory responders were presumed to be males based on the song types they sang on the playback recording, as both *delattrii* and *rufifrons* males sing acoustically distinct songs from females (Chapter 5).

Female responses to playback

Female Rufous-capped Warblers also occasionally responded to playback along with their male partners. Despite lower sample sizes for *rufifrons* than *delattrii* territories, results suggested that female *rufifrons* showed stronger responses to playback than did female *delattrii*. Female *rufifrons* responded in 33% (1 of 3) of *rufifrons* trials and 20% (1 of 5) of *delattrii* trials at the allopatric site, and 25% (2 of 8) of *rufifrons* trials at the sympatric site. All four responding *rufifrons* females sang in response to the playback, indicating a more intense response. Female *delattrii* responded in 13.3% (2 of 15) of *delattrii* trials and 6.7% (1 of 15) of *rufifrons* trials at the allopatric site, and 5.9% (1 of 17) of *delattrii* trials and 6.2% (1 of 16) of control trials at the sympatric site. Only 1 of 5 responding *delattrii* females sang in response to playback.

Discussion

Our results showed differential responses between Rufous-capped Warbler subspecies to each other’s songs, where sympatric *delattrii* and *rufifrons* responded more strongly to own-subspecies than other-subspecies song playback. Both the high responses to own-subspecies playback and the low responses to other-subspecies playback in sympatry indicate that the two subspecies are not interspecifically territorial. Weaker discrimination between the two subspecies’ songs at the allopatric *delattrii* site compared to the sympatric site also suggests that reproductive character displacement may be enhancing discrimination in sympatry. We also found potential evidence that *delattrii* and *rufifrons* differ in aggression levels. In sympatry, *rufifrons* males appeared to be more aggressive; although less numerous than *delattrii*, they responded more to other-subspecies playback than did *delattrii.* In allopatry, however, *delattrii* appeared to be more aggressive since males responded strongly to both subspecies (e.g., Hick et al. 2016).

One explanation for the weak other-subspecies responses in sympatry is historical premating behavioural isolation, where populations which have diverged over a long time period and come into secondary contact recently do not recognize one another as potential mates or competitors (Uy et al. 2018). This is possible for Rufous-capped Warblers, since both subspecies co-exist on territories in sympatry, and sing in adjacent trees without apparent antagonism (A. Demko pers. obs.). A similar pattern was found for two related species of African tinkerbirds (*Pogoniulus bilineatus* and *P. subsulphureus*), which are not interspecifically territorial and thus respond less to heterospecifics in sympatry than in allopatry (Kirschel et al. 2009). Another potential explanation is that selection against hybrids may be occurring in the contact zone, and promotes stronger discrimination against heterotypic signals in sympatry than in allopatry through reproductive character displacement (Gerhardt 2013; Uy et al. 2018). This is the case for Collared and Pied Flycatchers, which have come into secondary contact following historical isolation in allopatry. In those species, hybrid females are sterile and hybrid males have lower pairing success, favouring character displacement of both song and plumage in sympatry to avoid interspecific mating (Sætre & Sæther 2010). This scenario seems plausible for Rufous-capped Warblers as well, since sympatric *delattrii* and *rufifrons* showed stronger discrimination against other-subspecies playback than did allopatric *delattrii.* Although songs did not differ acoustically within each subspecies between sympatric and allopatric sites (Chapter 5), enhanced discrimination in sympatry is still an important component of reproductive character displacement (e.g., Kirschel et al. 2009; Dingle et al. 2010). Furthermore, no apparent hybrids were found at our sympatric site, and all warblers we observed there (over 40 *delattrii* and 25 *rufifrons* pairs) mated assortatively. The apparent absence of hybrids in the contact zone suggests either complete reproductive isolation or selection against hybrids. Ongoing molecular analyses will assess the degree and timing of genetic divergence between *delattrii* and *rufifrons*, and determine whether hybridization has occurred historically or is ongoing in the contact zone. Furthermore, tests of female choice for these divergent signals (e.g., Sætre et al. 1997; Jang & Gerhardt 2006) in Rufous-capped Warblers would be useful to assess the contribution of reproductive character displacement to heightened discrimination in sympatry.

Allopatric *delattrii* responded strongly to playback of both subspecies; 87% of *delattrii* pairs responded to both *delattrii* and *rufifrons* songs. Interestingly, a separate playback study on *delattrii* in Central America also found strong responses to both subspecies; in that study, 60% of pairs (total *n* = 15) responded to songs of both local *delattrii* and allopatric *B. r. caudatus*, a northern Mexican subspecies with similar songs and plumage as *rufifrons* (Freeman & Montgomery 2017). Therefore, one explanation for the differing responses of *delattrii* and *rufifrons* is that *delattrii* have a broader acoustic perceptual sensitivity than *rufifrons*. Studies comparing other closely-related songbird species show that strong heterotypic responses can occur in reproductively isolated populations when birds exhibit innate perceptual sensitivities to particular acoustic features (Dingle et al. 2010; McEntee 2014) or respond preferentially to songs which are acoustically similar to their own (Sosa-López et al. 2016). An analysis comparing acoustic features of *delattrii* and *rufifrons* songs showed that *delattrii* songs have a larger average syllable bandwidth than *rufifrons* songs and that the frequency ranges of the two subspecies overlap (Chapter 5), suggesting that *delattrii* may be sensitive to a wider frequency range than *rufifrons*.

Allopatric *delattrii* may also have responded strongly to *rufifrons* because they are behaviourally dominant and therefore more aggressive towards intruders. Overall, *delattrii* are larger than *rufifrons* and are more numerous at the sympatric site; both characteristics are associated with dominance in other bird species (Freshwater et al. 2014; Hick et al. 2016). However, the dominance of *delattrii* remains inconclusive, because *rufifrons* responses were stronger in some respects than those of *delattrii*; for example, *rufifrons* used more female song and had more out-of-territory aggressive responses to playback in sympatry. A study that measures aggression more directly, such as by using visual models which birds could potentially attack, would allow comparison of the intensity of aggressive behaviours of both *delattrii* and *rufifrons* during territorial interactions (e.g., Greig et al. 2015; Hick et al. 2016).

It is also possible that *delattrii* at our allopatric site had prior exposure to *rufifrons* during the non-breeding season, resulting in a heightened response to their songs. Although Rufous-capped Warblers are year-round residents throughout their range, individuals are more mobile during the non-breeding season, and could potentially encounter one another if either subspecies moves altitudinally at any time during the year. The *rufifrons* subspecies occurs at higher elevations (generally above 1000 m a.s.l.) adjacent to the allopatric *delattrii* site (450 m a.s.l.), and Rufous-capped Warbler habitat is continuous between the two locations according to historical records. Given that both subspecies responded less to one another in sympatry, where they are in continuous contact, this explanation appears unlikely. However, year-round surveys of Rufous-capped Warbler distribution in locations where altitudinal movements are possible would be useful to determine if dispersal and inter-subspecies contact occur in the non-breeding season.

Another potential factor influencing responses is song variation between source populations used to produce playback stimuli. The *delattrii* and *rufifrons* songs used as playback at the allopatric sites were all recorded from the sympatric site, so if any sympatric *rufifrons* used hybrid or mixed songs (i.e. songs with *delattrii*-type syllables), those songs could potentially provoke a strong response in allopatric *delattrii* (e.g., Qvarnström et al. 2006). We do not believe this to be an important confounding factor in our study, because our analysis of acoustic structure comparing *delattrii* and *rufifrons* songs revealed that the two subspecies' songs had significantly distinct spectro-temporal characteristics (Chapter 5). Furthermore, all recordings analyzed from the sympatric site were within the average acoustic range for their given subspecies, and we found no mixed songs containing syllable types of both subspecies. However, our playback study only included one sympatric and one allopatric site for each subspecies for logistical reasons, resulting in small sample sizes, especially for *rufifrons*. Since other studies comparing multiple allopatric populations have found within-species or subspecies response differences across sites (e.g., Gabor & Ryan 2001; McEntee 2014), further research at more *delattrii* and *rufifrons* sites would be valuable to assess whether response differences between subspecies are innate (e.g., different perceptual sensitivity) or learned (e.g., learned aggressive responses).

We found that females also discriminate between the two subspecies, and that *delattrii* and *rufifrons* females may differ in their aggression levels. In particular, *rufifrons* females responded more frequently than *delattrii* females to playback, and showed intense behavioural responses such as singing. In both subspecies, female choice for specific song characteristics or heightened female discrimination between signals could therefore result in lower other-subspecies responses and assortative mating. Indeed, females of many species show stronger responses than males to local-population songs, indicating a preference and heightened discrimination for those signals (Seddon & Tobias 2010; Danner et al. 2011; Wheatcroft & Qvarnström 2017). In Rufous-capped Warblers, playback experiments comparing female responses to songs of local and non-local own- and other-subspecies populations would be useful to investigate the influence of vocal geographic variation on female mate choice (Jang & Gerhardt 2006; Danner et al. 2011).

The fact that *delattrii* and *rufifrons* differ in their habitat preferences and potentially in their aggression levels should be considered when assessing response differences between subspecies. If the low-elevation species (i.e. *delattrii*) is more dominant, anthropogenic and climate-change related effects, including habitat alteration and temperature increases, may cause the low-elevation species to expand its range upslope, thus restricting the range of the high-elevation species (e.g., Jankowski et al. 2010; Freeman & Montgomery 2016; Freeman et al. 2016). If the high-elevation species (i.e. *rufifrons*) is more dominant, elevational replacement can still occur if habitat changes favour the lower-elevation species' occupation of higher-elevation habitat (e.g., Barve & Dhondt 2017) or the expansion of the higher-elevation species' habitat downslope (e.g., through increased severity of droughts). In Rufous-capped Warblers, our sympatric study site was in a humid forest zone cleared to produce a semi-open shade coffee plantation. Rufous-capped Warbler territory characteristics in the contact zone are more similar in vegetation density and canopy cover to allopatric *delattrii* territories than allopatric *rufifrons* territories (Vargas-Herrera et al. 2017), so it is probable that *rufifrons* have moved downslope from their native habitat to the contact zone. Since *delattrii* are more numerous in the contact zone, and the habitat there is more similar to native *delattrii* habitat, this suggests that the contact zone may be sub-optimal *rufifrons* habitat. Further experimental work could test whether one subspecies is more dominant, and if so, whether this relationship varies between sympatric populations which have been in contact for differing time periods. Such data would allow researchers to understand potential effects of climate and habitat change on the population dynamics and range distributions of *delattrii* and *rufifrons*.

Our research suggests that song is an important trait promoting premating reproductive isolation between the *delattrii* and *rufifrons* subspecies of Rufous-capped Warbler, since birds preferentially responded to their own subspecies in sympatry. The strong discrimination between own- and other-subspecies songs in sympatry suggests that reproductive character displacement may be contributing to divergence between the two subspecies. In contrast, the strong response of allopatric *delattrii* to both subspecies may indicate a wider range of perceptual sensitivity or higher aggression levels in *delattrii* compared to *rufifrons*, although further experimental studies incorporating additional allopatric populations and visual signals would be useful to explore these ideas (e.g., Gabor & Ryan 2001; McEntee 2014; Hick et al. 2016). Overall, our work adds to the growing number of studies examining receiver response differences to learned signal divergence between bird populations, and the importance of vocal signal divergence as a premating isolating barrier in secondary contact zones.

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Tables

Table 6.1. Playback locations and origin of Rufous-capped Warbler stimuli used to produce playback. All populations received non-local stimuli.

Playback location	Subspecies	Range	Number of territories	Origin of *rufifrons* stimulus	Origin of *delattrii* stimulus
Finca La Victoria	*delattrii*	sympatric	17	Motozintla	Mapastepec
Finca La Victoria	*rufifrons*	sympatric	8	Motozintla	Mapastepec
Mapastepec	*delattrii*	allopatric	15	Finca La Victoria	Finca La Victoria
Motozintla	*rufifrons*	allopatric	6	Finca La Victoria	Finca La Victoria

Table 6.2. Summary of correlations between five physical and vocal response variables for first three principal components. PC1 represents response strength of male Rufous-capped Warblers to playback; neither PC2 nor PC3 differed significantly between treatments or sites.

	PC1	PC2	PC3
Eigenvalue	2.92	0.86	0.70
Percentage of variation (%)	58.3	17.2	14.0
Number of songs	0.49	0.38	-0.24
Song duration	0.45	0.48	-0.35
Closest approach distance	-0.46	0.16	-0.61
Time within 10 m	0.50	-0.19	0.35
Latency to approach to 10 m	-0.31	0.75	0.57

Table 6.3. Linear mixed model and post-hoc comparison results of physical and vocal approach (PC1) of male Rufous-capped Warblers in response to playback. Significant effects are highlighted in bold.

Full LMM	χ^2	df	*P* value
Treatment	**16.6**	**2**	**<0.001**
Site	7.4	3	0.06
Treatment × Site	**39.0**	**6**	**<0.001**
Post-hoc comparisons	Estimate ± SE	*t* value	*P* value
Sympatric *delattrii*			
control – *delattrii*	**-2.34 ± 0.50**	**-4.7**	**<0.001**
control – *rufifrons*	-0.16 ± 0.50	-0.3	0.94
delattrii – rufifrons	**2.18 ± 0.47**	**4.6**	**<0.001**
Sympatric *rufifrons*			
control – *delattrii*	-0.24 ± 0.69	-0.3	0.94
control – *rufifrons*	**-2.58 ± 0.67**	**-3.8**	**<0.001**
delattrii – rufifrons	**-2.34 ± 0.66**	**-3.6**	**0.002**
Allopatric *delattrii*			
control – *delattrii*	**-2.64 ± 0.52**	**-5.1**	**<0.001**
control – *rufifrons*	**-2.02 ± 0.54**	**-3.8**	**<0.001**
delattrii – rufifrons	0.62 ± 0.51	1.2	0.45
Allopatric *rufifrons*			
control – *delattrii*	-1.19 ± 1.35	-0.9	0.65
control – *rufifrons*	-3.07 ± 1.44	-2.1	0.09
delattrii – rufifrons	-1.88 ± 0.94	-2.0	0.12

Figures

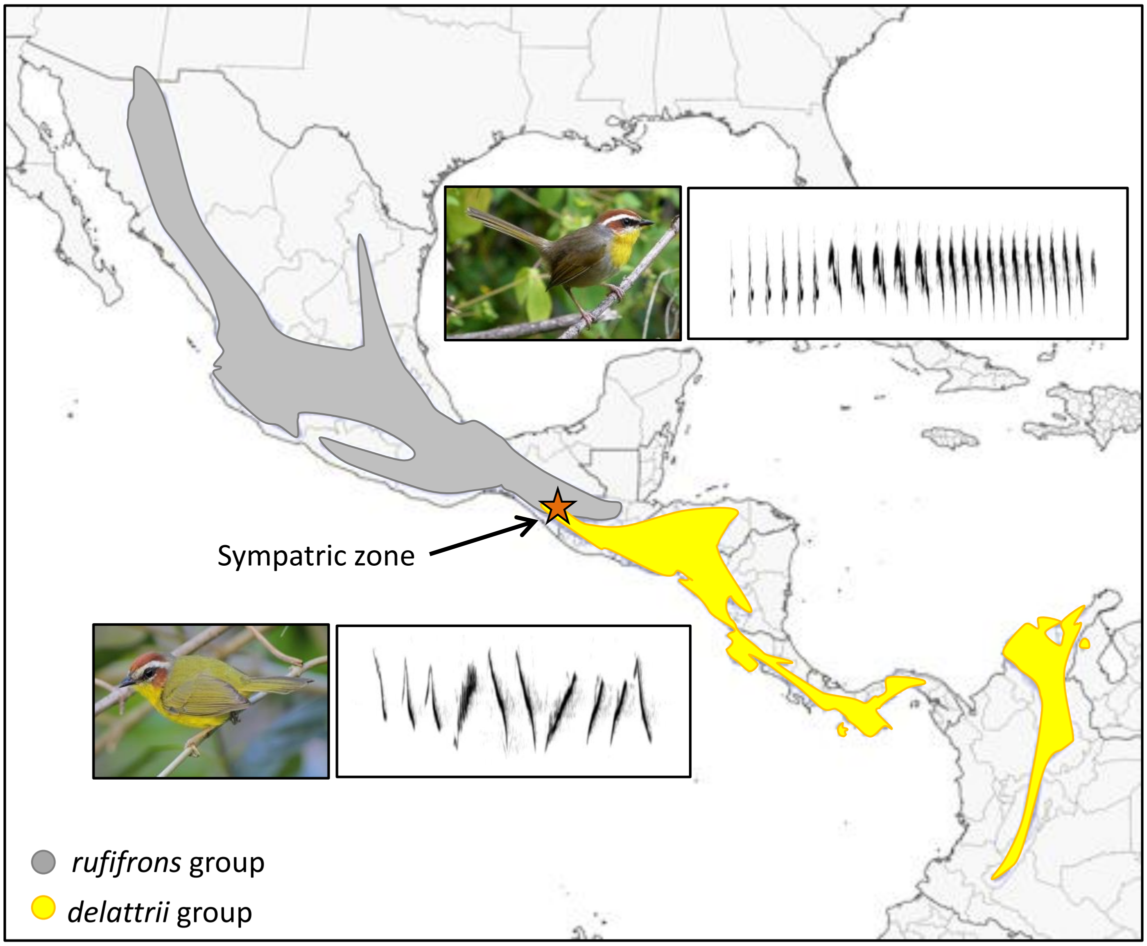

Figure 6.1. Map of Rufous-capped Warbler (*Basileuterus rufifrons*) range distribution. The white-bellied *rufifrons* group (grey shading) distributes from southern Arizona to western Guatemala, while the yellow-bellied *delattrii* group (yellow shading) distributes from southeastern Mexico through Central and South America. Two subspecies from these groups, northern *B. r. rufifrons* and southern *B. r. delattrii*, live in sympatry at mid-elevation sites in southern Mexico (orange star).

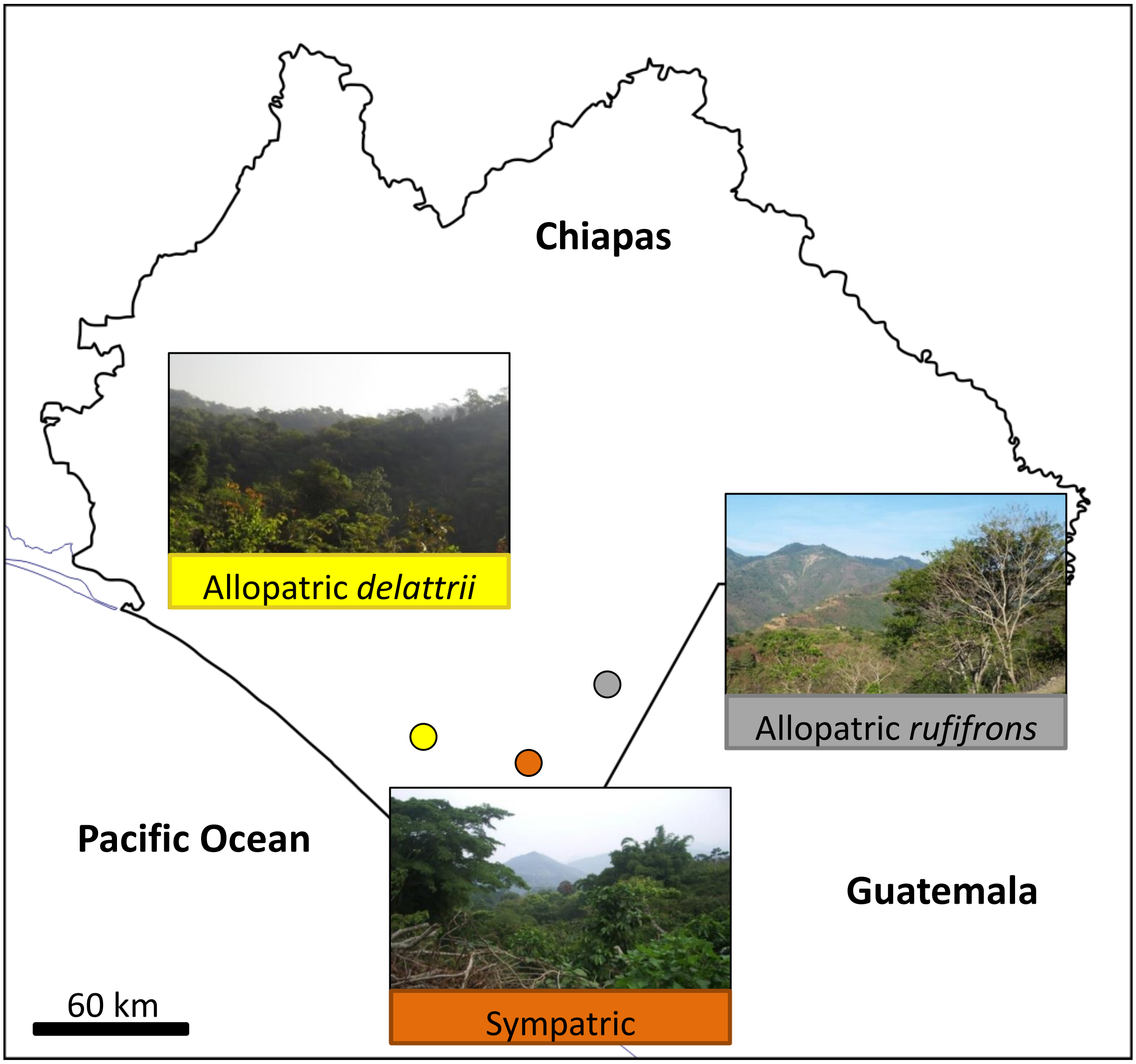

Figure 6.2. Locations of sympatric and allopatric Rufous-capped Warbler study sites in southern Chiapas, Mexico. The sympatric site is approximately 30 km away from each allopatric site; the two allopatric sites are approximately 60 km apart.

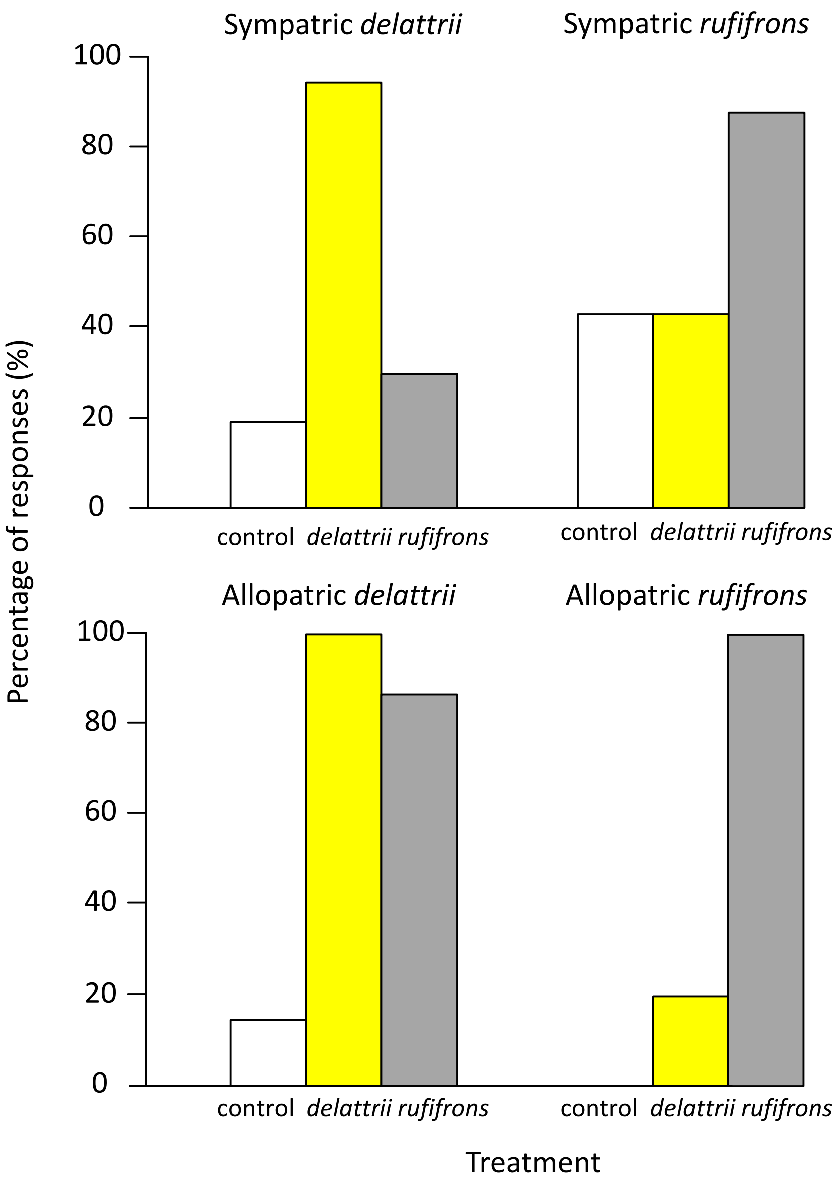

Figure 6.3. Sympatric *delattrii* responded more often (i.e. approached within 10 m) to own-subspecies than other-subspecies playback, whereas sympatric *rufifrons*, allopatric *delattrii*, and allopatric *rufifrons* did not differ significantly in number of responses to own-subspecies and other-subspecies playback.

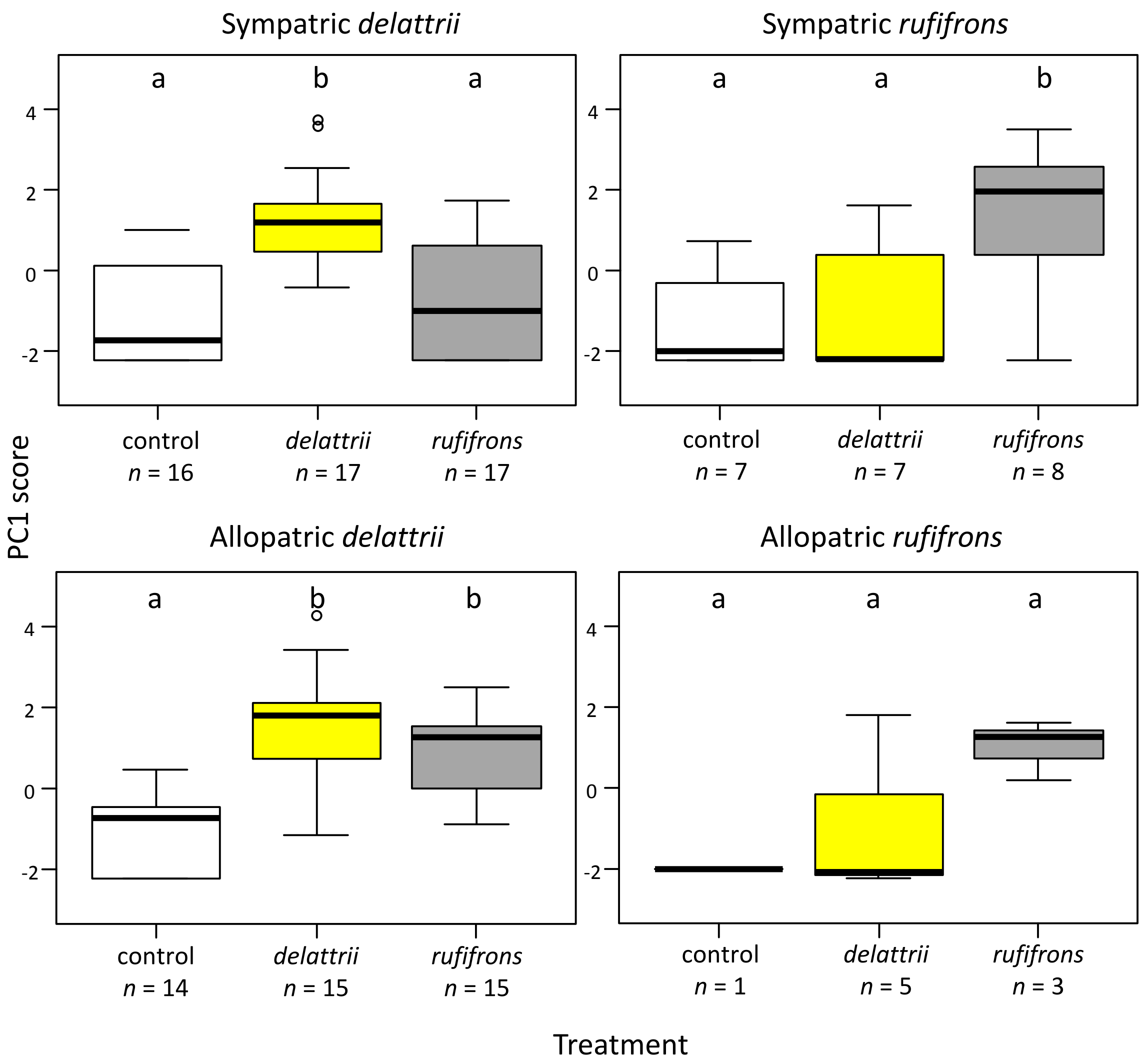

Figure 6.4. Sympatric *delattrii* and *rufifrons* responded more strongly to own-subspecies than other-subspecies playback, whereas allopatric *delattrii* responded strongly to both own-subspecies and other-subspecies playback. Letters above boxplots indicate significant post-hoc test results.

Chapter 7: General Discussion

Introduction

In my dissertation, I investigated the vocal behaviour of a Neotropical resident songbird, the Rufous-capped Warbler (*Basileuterus rufifrons*), using both observational data from focal recordings and experimental data from playback studies. I assessed whether phenotypic variation contributes to reproductive isolation by comparing plumage, song, and morphology between subspecies, and by conducting a playback experiment to test for differences in responses to divergent vocal signals in two sympatric subspecies. Overall, my research provided new insight into the vocal behaviour and breeding biology of tropical wood-warblers, including the first quantitative analysis of both male and female song in the genus *Basileuterus* (Chapters 2 and 4), and a description of sex-specific roles in parental care by Rufous-capped Warblers during each nesting stage (Chapter 3). My work also revealed that Rufous-capped Warblers likely comprise two phenotypically distinct groups (Chapter 5) which correspond to the historical taxonomic classification of this clade as two species (Todd 1929). In particular, song structure differed significantly between the two groups (Chapter 5), and reduced responses to heterotypic songs in sympatry by both *B. r. delattrii* and *B. r. rufifrons* indicate that vocal differences may promote reproductive isolation between them (Chapter 6).

My work provides new information on season- and sex-specific variation in breeding and territorial behaviour, which are poorly-studied aspects of the ecology of tropical resident birds (Stutchbury & Morton 2001). My research showed both sexes of Rufous-capped Warblers contributed more equally to territory defence than is reported in studies of migratory wood-warblers. Both sexes regularly produced songs and calls, and closely approached playback simulating conspecific territorial intrusions (Chapter 4). Female Rufous-capped Warblers responded more strongly to playback during the non-breeding season than during the breeding season, similarly to other tropical year-round resident songbird species (e.g., Fedy & Stutchbury 2005; Gill et al. 2007, 2008). This suggests that female songs and calls that are produced outside the context of duets likely serve a territory defence function, as do female signals used as part of duets in many other tropical resident species (reviewed in Dahlin & Benedict 2014; Tobias et al. 2016). My findings also reveal that social selection on competition for resources may contribute to female signal evolution (e.g., Tobias et al. 2011).

My research improves our understanding of vocal behaviour in tropical resident wood-warblers*.* Rufous-capped Warblers appear to differ in their vocal behaviour from other migratory and tropical resident warbler species; males have large, complex repertoires of song variants that they use interchangeably, rather than two distinct song categories or singing modes (e.g., Wiley et al. 1994; Staicer 1996) or one primary song type (e.g., Lein 1981; Ritchison 1995). My findings therefore suggest that tropical resident warblers have diverse vocal behaviours similar to those of migratory species (Spector 1992). At least one tropical resident species in the genus *Setophaga*, the Adelaide's Warbler (*S. adelaidae*), uses two song categories like its migratory congeners (Staicer 1996). Males of at least one species in the genus *Geothlypis*, the Gray-crowned Yellowthroat (*G. poliocephala*), have multiple song types, while its other tropical congeners use only a single song type (Byers 2015). Although female song has been anecdotally reported for several tropical resident warblers (Spector 1992; Medina 2015), my study is the first to document female song in the Rufous-capped Warbler, making it only the second *Basileuterus* species with known female song (see Donegan 2014). Overall, this research highlights that even widespread, common tropical resident species such as the Rufous-capped Warbler warrant further study of their ecology and vocal behaviour.

The morphological, vocal, and plumage differentiation between Rufous-capped Warbler subspecies strongly suggests that they represent two species, the northern rufous-capped, white-bellied group (suggested name: Rufous-capped Warbler, or *B. rufifrons*) and the southern chestnut-capped, yellow-bellied group (suggested name: Chestnut-capped Warbler, or *B. delattrii*), as originally named by both historical (Todd 1929) and recent sources (Howell & Webb 1995). Notably, I established that the reportedly intermediate subspecies *B. r. salvini* is more phenotypically similar to *B. r. rufifrons* than to *B. r. delattrii*. In addition to providing a more accurate description of taxonomic relationships within the genus *Basileuterus*, my work provides an incentive for further studies on the ecology and vocal behaviour of both the *delattrii* and *rufifrons* groups. Although the breeding biology and behaviour of the *delattrii* group is relatively well-documented (Skutch 1967; Chapters 2–4), the *rufifrons* group remains little-studied apart from a handful of nest descriptions (Zimmerman & Harry 1951; Rowley 1962, 1966) and this study's analysis of song structure (Chapter 5). Furthermore, the two groups appear to be reproductively isolated by song, since both *B. r. delattrii* and *B. r. rufifrons* responded little to heterotypic playback in sympatry (Chapter 6). My work adds to a growing number of studies demonstrating that vocal signal divergence may contribute to reproductive isolation between closely-related bird populations (Sætre et al. 1997; Kirschel et al. 2009; Dingle et al. 2010).

Future directions: Male vocal behaviour

My research on male repertoire structure and singing behaviour (Chapter 2) revealed that male *B. r. delattrii* have large, variable repertoires and show seasonal variation in repertoire use. The function of both of these traits warrants further examination. The large repertoire sizes and highly complex songs of this species suggest that these traits may be under selection, and perhaps even subject to different selective pressures (Searcy & Andersson 1986; Price & Lanyon 2004; Handley & Nelson 2005). In order to explore repertoire function in Rufous-capped Warblers, future studies should examine whether repertoire size and complexity are associated with measures of male quality, such as territory tenure, survival, or lifetime reproductive success (e.g., Buchanan & Catchpole 1997; Beecher et al. 2000a; Reid et al. 2005; Nicholson et al. 2007), and with female choice (e.g., Nolan & Hill 2004). In order to investigate the function of seasonal variation, future researchers should conduct a longitudinal analysis of song use in focal males of known breeding status. Since song rate and song length increased in Rufous-capped Warblers during the breeding season and the dawn chorus, it would be useful to examine whether these two traits vary predictably according to specific breeding stages (e.g., egg-laying, incubation), times of day, or other contexts (e.g., Nelson & Poesel 2011; Zhang et al. 2015).

An intriguing aspect of Rufous-capped Warbler singing behaviour is that males showed annual turnover in song and syllable types. In other songbird species, males add or drop song types or syllables from their repertoires depending on whether those vocalizations are also used by conspecific neighbours (e.g., Lemon et al. 1994; Nicholson et al. 2007; Demko et al. 2016). Since Rufous-capped Warbler pairs hold year-round territories and may retain the same territories for at least three years (Chapter 2), song sharing could facilitate communication between particular neighbours, as in other temperate (e.g., Beecher et al. 2000b) and tropical resident species (e.g., Vehrencamp et al. 2007, 2014). Future research should therefore explore whether changes in male song or syllables types are associated with changes in territorial neighbours, and whether factors such as age or territory fidelity may influence annual song type turnover. If shared song types are important for mediating male-male social interactions, males with longer territory tenure would be expected to share more songs or syllables with neighbours (e.g., Beecher et al. 2000a), and males should alter their repertoire composition as neighbours change in order to share more songs with them (e.g., Lemon et al. 1994; Nicholson et al. 2007).

Future directions: Female vocal behaviour

The finding that female Rufous-capped Warblers sing opens the door for further studies of the form and function of female song in this species and other tropical resident warblers. In *B. r. delattrii*, female song was infrequent, and females did not appear to form coordinated duets with males (Chapter 4). My experimental study on male and female signaling behaviour during territorial interactions suggested that *B. r. delattrii* females use song in joint territory defence (Chapter 4). However, other documented functions of female song in other species, such as mate guarding and female-female competition, were not thoroughly explored in that study. Further experimental tests using female songs as playback stimuli could further test those hypotheses by determining whether warblers respond differently to the songs produced by each sex (Dowling & Webster 2016; Krieg & Getty 2016) and whether responses vary seasonally or according to other contexts such as breeding status (Baptista et al. 1993; Dowling & Webster 2016).

In *B. r. rufifrons*, females sang frequently and formed duets with their male partners by partially overlapping songs with them (Chapter 6; A. Demko pers. obs.). The majority of detailed studies of duetting behaviour in Neotropical passerine birds thus far have been conducted in wrens, sparrows, icterids (blackbirds and orioles), and antbirds (reviewed in Dahlin & Benedict 2014). In Adelaide's Warbler, the only tropical wood-warbler whose duets are described, observational data supported the territory defence hypothesis of duet function, since pairs sang duets most often after fights with conspecific neighbours (Medina 2015). Since both sexes of *B. r. rufifrons* have multiple song types in their repertoires, future studies should examine whether this species adheres to a duet code, where males and females preferentially sing particular song types in sequence (e.g., Mennill & Vehrencamp 2005; Logue 2006; Rivera-Cáceres et al. 2016).

Using this information, researchers could then measure whether coordination of both song timing and song type use during duets varies depending on length of the pair bond (Hall & Magrath 2007; Rivera-Cáceres et al. 2016), or other contexts and possible functions (e.g., territory defence, mate guarding).

Another fruitful area for future research would be to explore the presence and frequency of use of female song (e.g., occasional song, coordinated song with males) in other tropical wood-warbler species. Comparative studies of the evolution of male and female traits in the Family Parulidae (e.g., Najar & Benedict 2015; Simpson et al. 2015) could incorporate female song use into future analyses. A recent comparative analysis of the evolution of plumage dichromatism in the Parulidae suggested that social selection maintains the ancestral state of monochromatism in tropical resident species, while natural selection drives a shift to dichromatism in migratory species (Simpson et al. 2015). Since female song is proposed to be the ancestral state in songbirds (Odom et al. 2014), a more complete documentation of female song across warbler species could allow researchers to analyze whether similar selective pressures explain female song evolution in wood-warblers.

Future directions: Signal divergence and responses

Future research should examine the potential role of habitat structure and climate in shaping both acoustic and visual signals. Songs, plumage, and morphology varied considerably between Rufous-capped Warbler subspecies, and these differences corresponded broadly with habitat characteristics (dry, open, high-elevation habitat in the northern part of their range; semi-humid, forested, low- to mid-elevation habitat in the southern part of their range; Curson 2010). In fact, a parallel Honours thesis study using the museum data set revealed that latitude and temperature were correlated with plumage colouration and morphological differences across the Rufous-capped Warbler's range (Bastien 2018), and that *B. r. delattrii* and *B. r. rufifrons* living in allopatry in southern Mexico used different habitat types (Vargas-Herrera et al. 2017). To build on these findings, future research could test the Acoustic Adaptation Hypothesis by comparing detailed vegetation measurements and sound transmission studies to confirm whether variation in song structure correlates with habitat features (e.g., Handford & Lougheed 1991; Graham et al. 2017).

The results of the experiment I designed to study subspecies recognition suggested that Rufous-capped Warblers may exhibit character displacement in sympatry (Chapter 6). This hypothesis should be tested using female choice experiments to assess whether females prefer songs of different subspecies from both local and non-local populations (Danner et al. 2011). Genomic analyses would also provide an invaluable contribution to our understanding of this phenomenon for several reasons: (1) to confirm the extent and timing of divergence between Rufous-capped Warbler subspecies; (2) to assess the extent of historical and current hybridization between sympatric *B. r. delattrii* and *B. r. rufifrons*; and (3) to investigate whether genetic divergence corresponds to phenotypic divergence. This research is currently underway using blood samples collected during the course of the studies presented in Chapters 5 and 6.

Conclusion

My dissertation research provides insight into the function of both male and female vocal signals in tropical year-round resident animals, and highlights that both female songs and calls are used in territory defence. It also provides a foundation for comparative analyses of vocal trait evolution in the Family Parulidae, particularly with respect to male repertoire specialization and the presence and elaboration of female ornamentation. My dissertation research also provides support for a revised taxonomy of Rufous-capped Warblers according to phenotypic divergence in multiple traits. It also reveals that vocal signal divergence is potentially an important factor in maintaining reproductive isolation between closely-related sympatric taxa, and demonstrates that the vocal and visual signals of animals play important roles in the processes of behavioural isolation and speciation.

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Vita Auctoris

NAME: Alana Dawn Demko

PLACE OF BIRTH: Saanichton, British Columbia, Canada

YEAR OF BIRTH: 1983

EDUCATION: Sir John Franklin High School, Yellowknife, Northwest Territories, Canada
(1996–2000)

University of Victoria, Victoria, British Columbia, Canada
Bachelor of Science
(2000–2005)

Dalhousie University, Halifax, Nova Scotia, Canada
Master of Science
(2009–2012)

University of Windsor, Windsor, Ontario, Canada
Doctor of Philosophy
(2013–2018)