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Vocal behaviour, geographic variation, and the evolution of song in Troglodytes wrens

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VOCAL BEHAVIOUR, GEOGRAPHIC VARIATION, AND THE EVOLUTION OF SONG IN *TROGLODYTES* WRENS

by

JOSÉ ROBERTO SOSA LÓPEZ

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the Degree of Doctor of Philosophy at the
University of Windsor

Windsor, Ontario, Canada

Vocal behaviour, geographic variation, and the evolution of song in Troglodytes wrens

by

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January 21, 2014

Declaration of Co-Authorship / Previous Publication

I. Co-Authorship Declaration

I declare that this thesis incorporates material that is result of joint research, as follows: I am the sole author of the General Introduction, Chapter 1, and the General Discussion, Chapter 7. I am the principal author of the manuscripts that comprise the five data chapters: Chapters 2 through 6. All five data chapters are co-authored with my supervisor, Dr. Daniel Mennill. In all five instances, the key ideas are mine, and the experimental design, experimental execution, data analysis, interpretation, and writing were performed primarily by me. Dr. Daniel Mennill contributed to experimental design, data analysis, interpretation, and the writing and editing of the manuscripts, as well as providing financial and logistical assistance for the research described in all five data chapters.

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 my co-author to include the above material in my thesis.

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

II. Declaration of Previous Publication

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

Thesis Chapter	Publication title/full citation	Publication status
Chapter 2	Vocal behaviour of the island-endemic Cozumel Wren (*Troglodytes aedon beani*): Song structure, repertoires, and song sharing	Published in Journal of Ornithology
Chapter 3	The vocal behaviour of the Brown-throated Wren (*Troglodytes brunneicollis*): song structure, repertoires, sharing, syntax, and diel variation	Published in Journal of Ornithology
Chapter 4	Continent-wide patterns of divergence in acoustic and morphological traits in the House Wren species complex	In press in the journal Auk: Ornithological Advances

Chapters 2 and 3 are both published in the Journal of Ornithology and are formatted for that journal. Chapter 4 is accepted for publication in the journal Auk: Ornithological Advances and is formatted for that journal. Chapters 5 and 6 are written in manuscript format for future submission.

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Abstract

Animal acoustic signals are important for mate attraction, resource defense, and species recognition. When vocalizations diverge between closely related groups they can play a key role in speciation. Therefore it is important for biologists to describe the vocalizations of wild animals, to document geographic differences in acoustic signals, and to evaluate the relationship between acoustic variation, genetic variation, and ecological variation. In this dissertation, I study the songs of New World wrens in the genus *Troglodytes*, small territorial songbirds distributed throughout the Americas, including several oceanic islands, that are well known for their loud, complex songs. I conducted observational and experimental studies of *Troglodytes* wrens in the field, and morphometric studies of museum specimens. In chapter 1, I provide background information on the topics in the subsequent chapters. In chapters 2 and 3, I provide the first empirical description of the vocal behaviour of two species of *Troglodytes* wrens from Mexico: Brown-throated Wrens and Cozumel Wrens. In chapter 4, I quantify geographic variation in songs of wrens within the House Wren species complex—a group of birds noted for taxonomic controversies—demonstrating that several subspecies show acoustic differentiation on par with many species. In chapter 5, I show that patterns of geographic variation in vocalizations predict genetic divergence, and that the fine structural characteristics of *Troglodytes* songs, as well as birds' responses to playback of those songs, are associated with phylogenetic relatedness. In chapter 6, I show that songs vary at a continental scale and that several aspects of wren vocalizations are closely associated with both phylogenetic differentiation and ecological variation. Taken together, my results suggest that songs of *Troglodytes* wrens exhibit substantial geographic variation, that they encode species identity that is used to distinguish conspecific rivals, and that they show evidence of adaptation to

ecological features. My research stands as an example of how acoustic signals can be use for resolving taxonomic problems. My findings enhance our understanding of how acoustic diversity originated and how fine structural characteristics are constrained, adapted, and maintained through evolutionary lineages.

Dedication

Al amor de mi vida, Pam

A mis padres, Conchita y Federico

A mis hermanas, Clau y Pau

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Chapter 1

General Introduction

Introduction

Animal acoustic signals play an important role in resource defense, mate attraction, and species recognition (Catchpole and Slater 2008, Bradbury and Vehrencamp 2011). The variety of contexts in which animals produce acoustic signals suggest that these traits are under forces of both ecological and sexual selection (Coyne and Orr 2004, Wilkins et al. 2013). Divergence in acoustic signals of closely related taxa could promote genetic differentiation through assortative mating or by limiting options for dispersal, and thereby play a role in speciation (e.g. Slabbekoorn and Smith 2002, Seddon 2005, Ruegg et al. 2006). The relationship between acoustic divergence and speciation highlights the importance of describing the vocal behaviours of wild animals, documenting patterns of geographic variation in acoustic signals, and evaluating the relationship between variation in acoustic signals, genetic differentiation, and ecological patterns (Wilkins et al. 2013).

In this dissertation I study acoustic variation and evolution in the songs of New World wrens in the genus *Troglodytes*. This genus ranks among the most widely distributed taxa in the Americas (Brewer 2001). It comprises species concentrated primarily in the tropics, inhabiting a broad diversity of habitats, with many populations living on islands (Kroodsma and Brewer 2005). In spite of a recent increase in research on the genus *Troglodytes* (e.g. Johnson 1998, Hejl 2002, Sosa 2011, Bowers et al. 2012, Campagna et al. 2012, Sosa López et al. 2012, Toews and Irwin 2012, Farwell and Marzluff 2013, Rendall and Kaluthota 2013), the lack of behavioural and genetic information has led to controversy regarding relationships and species limits inside the group (Brumfield and Capparella 1996, Martínez Gómez et al. 2005, Rice et al. 1999).

This dissertation was motivated by my interest in animal communication, evolutionary processes, natural history, and the complex vocalizations of *Troglodytes* wrens. My goal was to apply these interests towards developing a better understanding of *Troglodytes* wrens, and towards advancing our understanding of the evolution of animal vocalizations. By describing the songs of species whose vocal behaviour has never been quantified before, I sought to provide the groundwork for comparative analyses, and to better understand populations restricted to neotropical islands, which face significant anthropogenic stress (Kroodsma et al. 1996). By comparing songs between different taxa within the genus *Troglodytes*, I sought to better understand taxonomic subdivisions within this group. By studying variation in song alongside morphological features, ecological features, and birds' responses to playback, I sought to understand the evolution of complex songs and sexually selected traits. In this General Introduction, I provide a brief summary of the topics that are addressed in this dissertation, a short description of the natural history of the organisms I studied, and an overview of the five data chapters that follow.

Vocal behaviour in birds

Bird vocalizations are the most complex and well-studied acoustic signals in the animal kingdom (Hultsch and Todt 2004; Marler and Slabbekoorn 2004). Avian vocal behaviours differ enormously between species. For instance, vocalizations can vary in fine structural characteristics (e.g. the frequency and or pitch and the length of song components), in syllable composition (e.g. the diversity of elements within songs), in the rules that govern the position and order of the syllables within songs (i.e. vocal syntax), in repertoire patterns (e.g. how many variants of a song each animal produces), in patterns of repertoire delivery (e.g. how often song types are repeated before an animal changes to a different type), in daily vocal activity (e.g. how song output changes throughout the day), and in syllable and song sharing (e.g. are songs or song components common among the repertoires of nearby animals) among other features (Catchpole 1976, van Horne 1995, Molles and Vehrencamp 1999, Wright and Dahlin 2007, Valderrama et al. 2008, Camacho-Schlenker et al. 2011, Price and Yuan 2011, Price 2013, Price and Crawford 2013).

In addition, bird vocalizations may vary with the mode of acquisition (i.e. whether young birds inherit songs genetically from their parents, or whether vocal tutoring and vocal learning is involved in the development of normal adult song), and with the form of selection they experience (e.g. adaptation to local habitats, female choice, performance constraints by physical features of the body, etc.; Catchpole and Slater 2008). Understanding the variation in acoustic traits is not only important to gain insight into their function, but to understand how they evolve and the ecological factors that influence them. A comparative approach, where songs and vocal behaviour are studied in related animals with different ecologies, has proven to be a particularly fruitful approach for evaluating the behaviour, ecology, and evolution of animal vocal signals (Price et al. 2011). Comparative studies of variation in bird songs have taught us that closely related species often have more similar songs than distantly related species (e.g. Price and Lanyon 2002, Valderrama et al. 2007, Price 2009, Sosa López et al. 2013a, 2013b); that habitat and climate differences are associated with acoustic differences (Slabbekoorn and Smith 2002, Seddon 2005, Ruegg et al. 2006, Dingle et al. 2008); and that birds often exhibit vocal dialects (e.g. Marler and Tamura 1962, McGregor 1980, Koetz-Trowse et al. 2012).

Geographic variation in bird songs

The complex vocalizations of birds often vary among closely related species (e.g. Valderrama et al. 2007, Toews and Irwin 2008) and even between populations of the same species (e.g. Odom and Mennill 2012; Sosa López et al. 2013a, 2013b). Such variation in acoustic traits is highly informative to taxonomists and can be used for delimiting species boundaries. Understanding species boundaries is crucial to correctly estimate species diversity, particularly because the biodiversity of many groups of birds is thought to be underestimated at a global scale (Wilson 2003). The necessity for a better understanding in species boundaries is particularly important in the tropics, where the number of avian lineages is thought to be much greater than currently recognized (Milá et al. 2012). For instance, research on two physically identical subspecies of Grey-breasted Wood-Wrens (*Henicorhina l. leucophrys* and *H. l. hilaris*) demonstrates that these subspecies are genetically and acoustically divergent (Dingle et al. 2008). The two subspecies live in habitats in Ecuador with different ambient noise profiles and different vegetation density, suggesting that acoustic adaptation to different habitats may be a principal factor in song divergence, and providing support for a contribution of song divergence in reproductive isolation (Dingle et al. 2008). Thus, quantification of acoustic variation is important to further elucidate the factors involved in the evolution of complex signals by providing insights into the mechanisms that promote and maintain patterns of biological diversity (Endler 1977, Helbig et al. 1996, Irwin et al. 2001, Päckert et al. 2003, Toews and Irwin 2008).

Acoustic recognition and phylogenetic relatedness

It is not uncommon to find that acoustic signals vary with phylogenetic relationships, in birds and in other animals (e.g. Ryan and Rand 1999, Johnson et al. 2000, de Kort and ten Cate 2001, Price and Lanyon 2002, Ord and Martins 2006, Farnsworth and Lovette 2008). An association between acoustic signals and phylogenetic relatedness provides evidence that acoustic traits exhibit phylogenetically conserved components (i.e. components that are shared in common between closely related taxa as a result of their common evolutionary origins; Wilkins et al. 2013). Although phylogenetic patterns in acoustic variation between related taxa have been documented in many groups of animals, we have a poor understanding of whether the perception of evolutionarily conservative components is biologically relevant to animals (Irwin and Price 1999, Grether 2011, Mendelson and Shaw 2012). Do closely related species perceive phylogenetically conserved components of other species' acoustic signals as similar to the analogous components of their own species' acoustic signals? To date, very few studies have used a comparative approach to address this question.

Two exceptions come from studies of doves (de Kort and ten Cate 2001) and frogs (Ryan and Rand 2001, Ryan et al. 2003). In a study of two species of African turtle dove (*Streptopelia roseogrisea* and *S. vinacea*), de Kort and ten Cate (2001) conducted a playback experiment where vocalizations were played back to both conspecific and heterospecific animals. They demonstrated that the intensity of birds' responses to playback decreased with genetic distance, suggesting that despite divergence in acoustic signals (due to speciation), dove vocalizations still contain information that elicits behavioural responses (de Kort and ten Cate 2001). Their findings therefore provide evidence for phylogenetic constraints on perception (de Kort and ten Cate 2001). In a laboratory-based study of tungara frogs, Ryan and Rand (2001) showed that stimuli designed to mimic the calls of ancestors elicit responses from female frogs. Further, they showed that the strength of female response to heterospecific stimuli was associated with the acoustic divergence between the conspecific and heterospecific signals (Ryan et al. 2003). Experimental studies evaluating whether animals perceive conserved traits in acoustic signals are needed in other taxa. Notably, such an investigation has never been conducted in the song-learning oscine songbirds, where acoustic divergence can arise not only due to genetic differences, but also to cultural differences between populations.

Factors affecting song evolution

Diverse selection pressures promote acoustic divergence between populations including ecological selection, sexual selection, and drift (Podos et al. 2004a, Wilkins et al. 2013). In terms of ecological selection, the acoustic adaptation hypothesis (Morton 1975, Ryan and Brenowitz 1985) predicts that interspecific differences in animal vocal signals will be associated with physical features of the habitat, community composition, and ambient noise, all of which can shape the way that a signal transmits between signaler and receiver (Brumm and Naguib 2009, Tobias et al. 2010). The competition for acoustic space hypothesis (Marler 1960) predicts that communities with fewer species, such as island avifaunas, will have relaxed competition between animals for the finite time-and-frequency range available for acoustic signals (Marler 1960, Nelson and Marler 1990; Luther 2009). Ecological selection can also promote acoustic divergence when selection acts on morphological traits related to acoustic communication. An excellent example is the correlated evolution of beak morphology and song characteristics of Darwin’s Finches; the structural adaptations of beaks, driven by variation in seed hardness, has given rise to acoustic differences in songs (Schluter et al. 1985, Podos 2001, Podos et al. 2004b).

In terms of sexual selection, acoustic divergence is expected to arise when females from different populations exhibit different mating preferences, such as variation in females’ preferences for local signals or elaborate signals, or when males from different populations use different strategies during aggressive signaling interactions over access to resources (Wilkins et al. 2013). For instance, the relationship between testes mass and acoustic traits in the songs of the fairywrens, emuwrens, and grasswrens (family Maluridae; a group of oscine songbirds distributed throughout Australia and New Guinea) suggest that variation in songs is likely the product of sexual selection (Greig et al. 2013). Another example comes from Hawaiian swordtail crickets (genus: *Laupala*) where populations exhibit divergent pulse rates in their mating calls (Mendelson and Shaw 2005); a playback study in *Laupala cerasina* demonstrated that females discriminate between male pulse rates, preferring local males (Grace and Shaw 2012).

In terms of drift, vocalizations are expected to evolve due to random variation in morphological features associated with sound production (e.g. changes in beak size or the morphology of the syrinx), which can lead to divergence between acoustic signals (Podos 2001, Podos and Nowicki 2004, Podos et al. 2004a). Some animals learn to vocalize by learning from nearby adults. Learned vocalizations are observed in a variety of taxa, including humans, bats, and whales, and within three separate orders of birds: songbirds (order: Passeriformes; suborder: Passeri), hummingbirds (order: Apodiformes; family: Trochillidae), and parrots (order: Psittaciformes; Jarvis 2004). For such animals, cultural drift may also occur where imperfect copying of adult acoustic signals gives rise to vocalizations that deviate between generations. These deviations have been called "cultural mutations," a name that reflects the fact that these learned components of songs may become more common in subsequent generations through the process of learned, cultural transmission (Podos et al. 2004a).

Describing the evolution of animal acoustic signals requires a multi-faceted understanding of ecological selection, sexual selection, and drift. Together, these forces shape the acoustic limits within which an animal's acoustic signal can potentially fluctuate (Wilkins et al. 2013).

The genus *Troglodytes*

My research focuses on the songs of wrens in the genus *Troglodytes* (see Table 1.1). *Troglodytes* wrens are small territorial songbirds characterized by cryptic brown plumage colouration (Brewer 2001). They are socially monogamous, mostly insectivorous, and widely known for producing loud and highly complex songs (Kroodsma and Brewer 2005). *Troglodytes* wrens are concentrated in the New World (only one species, the Eurasian Wren, is found in the Old World; Brewer 2001). They are distributed from the far north of North America to the southernmost parts of South America. Eleven New World species are recognized by many taxonomists (American Ornithologists' Union 1998; Chesser et al. 2012, 2013; Remsen et al. 2013), but the genus *Troglodytes* is well known for taxonomic controversies; recent molecular analyses and bioacoustic analyses are reshaping our understanding of diversity within the genus (Chaves et al. 2014). My research focuses heavily on five taxa within the genus *Troglodytes*. Below I provide a brief description of each of these animals, including their geographic distribution, appearance, and natural history.

Cozumel Wren

Cozumel Wrens (*T. beani*) are endemic to Cozumel Island, in the Mexican Caribbean Sea off the northeastern coast of the Yucatan Peninsula. Males and females exhibit monomorphic plumage: the head is greyish-brown, the back and rump are reddish-brown, the wings and tail are brownish-red with dark bars, and the throat and underparts are buffy-white (Brewer 2001). Male Cozumel Wrens produce complex songs that begin with harsh scolding notes, breaking into a "short rich warble", sounding similar to Southern House Wrens (*Troglodytes musculus*), but "fuller and richer" (Brewer 2001; Kroodsma and Brewer 2005). Their voice has never been studied quantitatively, with descriptions existing only in field guides. They nest in tree cavities (JRSL pers. obs.), but the nest and eggs have never been described (Brewer 2001). Many aspects of their natural history are unknown. For example, there is little information available about their feeding behaviour (Brewer 2001); observations I collected during this research suggest that Cozumel Wrens feed on small insects and even scorpions (JRSL pers. obs.). The taxonomic status of Cozumel Wrens is controversial, and they are sometimes placed within the House Wren complex (i.e. *T. aedon beani*), but have also been suggested to be an independent species (i.e. *T. beani*).

Brown-throated Wren

Brown-throated Wrens (*T. brunneicollis*) are found throughout the mountains of Mexico, as far north as southern Arizona in the United States. Similar to other *Troglodytes* wrens, male and female Brown-throated Wrens exhibit monomorphic plumage: the head is dark greyish-brown, the back is brown and the rump is greyish-brown, the wings and tail are brown with dark bars, and the throat is grayish-brown fading to darker brown in the chest and reddish-brown in the flanks with dark bars (Brewer 2001). Males produce complex songs described by Kroodsma and Brewer (2005) as a “bubbling, cascading series of trill and rattles”. As in Cozumel Wrens, there has been no quantitative description of the vocalizations of Brown-throated Wrens. Brown-throated Wrens nest in cavities, but the nest and eggs have not been described (Brewer 2001). Similar to Cozumel Wrens, their taxonomic status is controversial, and they are sometimes placed within the House Wren complex (i.e. *T. aedon brunneicollis*) and other times considered to be an independent species (i.e. *T. brunneicollis*), with three subspecies in three different mountain ranges of Mexico (Brewer 2001, Kroodsma and Brewer 2005).

Socorro Wren

Socorro Wrens (*T. sissonii*) are restricted to Socorro Island, one of the four volcanic islands of the Revillagigedo Archipelago, Mexico, approximately 500 km west of the Pacific coast of the Baja Peninsula. Males and females exhibit monomorphic plumage and are notably paler than other *Troglodytes* wrens: the head and back are brown, the rump is warmer brown, the wings and tail are blackish-grey with dark bars, the throat and chest are white, and the flanks are brown (Brewer 2001). Males produce variable songs that start with gruff notes, breaking into short, slightly scratchy warbles, often finishing with a “rich chortle” (Brewer and Kroodsma 2005). Socorro Wrens feed on insects and small invertebrates, and observations indicate that they are also cavity nesters (Brewer 2001, Sosa López 2011, JRSL pers. obs.). As in many *Troglodytes* wrens isolated to a remote island, little is known about the natural history of this species, including descriptions of their vocalizations, nests, eggs, and feeding behaviours (Brewer 2001, Sosa López 2011). The taxonomic status of this species is controversial. Socorro wrens were formerly considered a species within the genus *Thryomanes*, but experienced ornithologists and a recent phylogenetic analysis classify this species within the genus *Troglodytes* (Brewer 2001, Martínez Gómez et al. 2005).

Clarion Wren

Clarion Wrens (*T. tanneri*) are restricted to Clarion Island, one of the four volcanic islands of the Revillagigedo Archipelago, Mexico, 300 km west of Socorro Island and 800 km west of the Baja peninsula. Males and females exhibit monomorphic plumage: the head is blackish-brown, the back is greyish-brown, the rump is reddish-brown, the wings and tail are greyish-brown with light dark bars, and the throat and underparts are buffy-white, becoming darker in the lower flanks (Brewer 2001). Male Clarion Wrens produce variable songs, beginning with gruff notes and continuing as a “warble” (Brewer and Kroodsma 2005). As with many other *Troglodytes* wrens, their voice has not been described quantitatively. The nests, eggs, and breeding behaviour of Clarion Wrens had never been described, but I recently described them using data that I collected during my dissertation field studies (Sosa López et al. 2012). I found that Clarion Wrens are cavity nesters, using diverse substrates like pockets of volcanic rock, as well as cavities in anthropogenic equipment such as metal and rubber tubes, buildings, boats, and vehicles (Sosa López et al. 2012). I found that females lay four eggs per clutch and that both males and females provide parental care (Sosa López et al. 2012). Clarion Wrens are sometimes treated as a race of House Wren (i.e. *T. aedon* Brewer 2001), but more often as an independent species.

House Wren

House Wrens (*T. aedon*) may have the broadest distribution range of any native passerine in the New World. They are found from Canada to the southernmost part of South America, including several islands in the West Indies (Johnson 1998). Males and females exhibit monomorphic plumage: the head, back, rump, wings and tail are uniform brownish gray, the throat and underparts are light grey, and the flanks, wings and tail have dark bars (Brewer 2001). Males have large repertoires composed of complex songs described by Kroodsma and Brewer (2005) as a “bubbling, cascading series of complex phrases”. Their songs have been described in quantitative detail, most recently by Rendall and Kaluthota (2013). House Wrens may be one of the most studied passerine species in North America, and detailed information has been collected on their vocal behaviour, migration patterns, habitat preferences, breeding behaviour, and demography (reviewed in Johnson 1998).

The House Wren is the wren with the longest-standing taxonomic controversy. This group of birds is often referred to as the “House Wren complex” because the diversity within the group is extensive, and the boundaries between taxa are very poorly resolved. According to the American Ornithologists’ Union (1998), the House Wren complex comprises 30 subspecies, although the number of recognized subspecies varies among taxonomic authorities (e.g. Brewer 2001, Navarro-Sigüenza and Peterson 2004, Kroodsma and Brewer 2005). Based on slight morphological and geographical differences, *T. aedon* subspecies can be separated into five main groups (e.g. American Ornithologists’ Union 1998; see Chapter 3 for details). The recent and well-supported molecular phylogeny by Chaves et al. (2014), however, has provided a breakthrough in our understanding of the House Wren complex. Chaves et al. (2014) document more than 40 independent evolutionary units within the genus *Troglodytes*, and confirm that our historical understanding of the diversity within the genus *Troglodytes* in general, and *Troglodytes aedon* specifically, is grossly underestimated. The new phylogeny demonstrates that the genus has undergone a recent adaptive radiation, with ancestral mountain *Troglodytes* wrens rapidly evolving into multiple lineages and fulfilling new lowland niches. A large portion of the radiation occurred within *T. aedon*, with ancestors colonizing lowlands in the Eastern United States (where birds are presently recognized as *T. a. parkmanii*) and south towards the Neotropics and South America (where birds are presently recognized as *T. a. musculus*, or just *T. musculus*, and all its variants).

With this new phylogenetic information (Chaves et al. 2014), combined with existing descriptions of the ecology and habitat characteristics of different *Troglodytes* wren species (e.g.

Stotz et al 1996, Brewer 2001, Kroodsma and Brewer 2005), and a growing number of descriptive analyses and comparisons of the vocal characteristics (e.g. Rendall and Kaluthota 2013), the *Troglodytes* wrens are well-suited for exploring the relationship between acoustic variation, phylogenetic history, and ecological selection.

Dissertation overview

In this dissertation, I describe the vocalizations and vocal behaviour of *Troglodytes* wrens, I explore the extent of acoustic diversity across the genus, I evaluate wrens' perception of this diversity, and I examine how vocal traits are constrained, adapted, and maintained through evolutionary lineages using a phylogenetic framework.

In chapters 2 and 3, I provide the first detailed analysis of the vocal behaviour of two species of *Troglodytes* wrens. In chapter 2 I describe the vocal behaviour of Cozumel Wrens; I describe the fine structural characteristics of the songs, assess repertoire size, and explore patterns of repertoire organization and song sharing. This chapter is also published in the *Journal of Ornithology*. In chapter 3 I describe the vocal behaviour of Brown-throated Wrens; I document the fine structural characteristics of the songs, assess repertoire size, quantify repertoire sharing among neighbours, describe how song sharing varies with distance, and explore whether this species uses syntactical rules for creating their songs. This chapter is also published in the *Journal of Ornithology*.

In chapter 4, I assess the variation in both acoustic and morphometric traits within the House Wren complex, comparing patterns of variation to currently recognized subspecies boundaries. I first compare songs and morphology among eight subspecies within the House

Wren complex. I then use variation in male song to assess divergence between House Wren subspecies. I compare variation between subspecies to variation across seven currently recognized *Troglodytes* species. This chapter is in press at the journal *Auk: Ornithological Advances*.

In chapter 5, I use a playback experiment to study how *Troglodytes* wrens respond to the mating signals of conspecific and allopatric, congeneric animals. Focusing on four species of *Troglodytes* wrens, I assess physical and vocal responses to simulated territorial intruders. I ask whether acoustic divergence correlates with genetic distance; whether wrens respond differently to conspecific versus congeneric acoustic signals; whether wrens respond with similar intensity to allopatric congeneric treatments; and whether acoustic similarity between the subject's songs and the playback stimulus predicts the strength of behavioural responses to playback. Furthermore, I ask whether phylogenetic distance predicts behavioural responses.

In chapter 6, I analyze the variation in the fine structural characteristics of the *Troglodytes* wren songs and compare this variation to the wrens' phylogenetic relationships, morphological features, and ecological characteristics. First, I assess the phylogenetic signal in wren vocalizations. Then, I compare acoustic variation in wren songs to morphological measurements that I collected at museums, and to patterns in the wrens' ecological characteristics that I extracted from the literature, including habitat, foraging stratum, elevation, and distribution patterns.

The research that I present in this dissertation combines observational studies of free-living organisms, analyses of morphological features and acoustical traits from museum collections, and experimental analyses that control for different factors affecting vocal production.

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Table 1.1. Summary of the *Troglodytes* wrens studied in this dissertation.

Common Name	Latin name	Distribution	Taxonomic Note
Troglodytes wrens studied in detail in this dissertation:			
House Wren	*T. aedon*	Lowland forests and scrub of North and South America	Often split into *T. aedon* (northern house wrens) and *T. musculus* (southern house wrens)
Cozumel Wren	*T. beani*	Cozumel Island in the Caribbean Sea	Sometimes considered a subspecies of *T. aedon*
Brown-throated Wren	*T. brunneicollis*	Montane forests of Mexico and southern Arizona	Sometimes considered a subspecies of *T. aedon*
Socorro Wren	*T. sissonii*	Socorro Island in the Pacific ocean	Formerly grouped with the genus *Thryomanes*
Clarion Wren	*T. tanneri*	Clarion Island in the Pacific ocean	
Troglodytes wrens studied for comparison in this dissertation:			
Cobb's Wren	*T. cobbi*	Malvinas-Falkland Islands in the Atlantic Ocean	Formerly grouped with T. *aedon*
Winter Wren	*T. hiemalis*	Lowland forests throughout North America	Formerly grouped with *T. pacificus*
Santa Marta Wren	*T. monticola*	Montane forests of Colombia	
Ochraceous Wren	*T. ochraceus*	Montane forests of Costa Rica and Panama	
Pacific Wren	*T. pacificus*	Montane forests in northwestern North America	Formerly grouped with *T. hiamalis*
Rufous-browed Wren	*T. rufocilliatus*	Montane forests from Mexico to El Salvador	
Tepui Wren	*T. rufulus*	Montane forests of Venezuela and Brazil	
Mountain Wren	*T. solstitialis*	Montane forests of South America from Venezuela to Argentina	

Chapter 2

Vocal behaviour of the island-endemic Cozumel Wren (*Troglodytes aedon beani*): song structure, repertoires, and song sharing[*]

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

Chapter summary

Documenting the diversity of vocal behaviour across different avian taxa is key to understanding the ecology and evolution of complex behaviours. Unique to Cozumel Island in the Mexican Caribbean Sea, the Cozumel Wren (*Troglodytes aedon beani*) provides an opportunity to investigate how isolation influences complex cultural traits. Most aspects of the biology, natural history, and taxonomy of Cozumel Wrens are unknown. In an attempt to better understand the Cozumel Wren's biology, we provide the first description of the songs and the vocal behaviour of this island-endemic bird. Based on more than 700 h of recordings, including more than 36,000 songs, we describe the fine structural characteristics of male Cozumel Wrens songs, and explore patterns of repertoire organization and song sharing. Cozumel Wrens sing songs composed of highly variable syllables, with prominent trills at the end of each song. Each bird has a limited repertoire of songs, which they create by recombining a restricted number of syllable and trill types. They repeat a song type several times before switching to a different one, with some variation in the number of times they repeat specific elements. Cozumel Wrens share more song types with neighbours than distant individuals. Syllable sharing, however, is equivalent between neighbours and distant individuals. Our results provide important data for future research on the ecology, evolution, and behaviour of this island-endemic songbird, and for helping to clarify the taxonomic status of Cozumel Wrens.

Introduction

The diversity in vocal behaviour across the songbirds provides a rich source of material for biologists interested in the ecology and evolution of complex cultural traits (Catchpole and Slater 2008). To understand variation in these complex characters, it is necessary to document and describe the vocal behaviour and song structure for each species (e.g. Mann et al. 2009). Quantitative research on avian vocal behaviour also helps to inform taxonomists, conservation biologists, and wildlife managers interested in characterizing and protecting biodiversity, particularly in the tropics, where biodiversity is poorly surveyed and under increasing anthropogenic threat (Kroodsma et al. 1996; Tubaro 1999).

In this study, we analyze the vocal behaviour of Cozumel Wrens (*Troglodytes aedon beani*). These small, active birds are restricted to Cozumel Island in the Caribbean Sea off the northeastern coast of the Yucatan Peninsula in Mexico (Figure 2.1). Cozumel Wrens are common in the forest habitat on this island, wherever there are cavities suitable for nesting (Sosa López, pers. obs.), and they adapt well to low-density human settlement (Brewer 2001; Kroodsma and Brewer 2005). Despite their restricted distribution, they do not hold a threatened status (Brewer 2001; Kroodsma and Brewer 2005). The isolated, island-endemic status of Cozumel Wrens makes them an interesting subject for studying vocal behaviour.

Animals that live on islands often express different traits compared to their mainland counterparts (Mirsky 1976; Baptista and Johnson 1982; Catchpole and Komdeur 1993; Päckert and Martens 2004; Baker 2006; Baker et al. 2006). For the complex songs of birds, this is manifest in multiple ways, where island-living birds sometimes exhibit patterns that contrast with mainland-living species. Some island populations have songs with a simple and highly variable structure (e.g. *Certhia familiaris*, Baptista and Johnson 1982); some have unusually small repertoire sizes (e.g. *Meliphaga virescens*, Baker

1996); some have large repertoire sizes and sing novel songs unknown on the mainland (e.g. Gerygone fusca, Baker et al. 2003); and others have songs with simpler structures, but with larger repertoires (e.g. *Acrocephalus sechellensis*, Catchpole and Komdeur 1993). Due to the complex vocalizations of the wrens—a group that exhibits some of the most complex voices of any organism on Earth (Van Horne 1995; Mann et al. 2006, 2009)—research on island-living wrens provides an intriguing opportunity to understand how isolation influences complex cultural traits.

Most aspects of the biology of Cozumel Wrens, including their vocal behaviour, are undescribed. A few anecdotal descriptions of their vocalizations exist. Some authors suggest that Cozumel Wren songs are similar to Northern House Wren and Southern House Wren songs, but ‘‘fuller’’ or ‘‘richer’’, and without trills (Howell and Webb 1995; Brewer 2001; Kroodsma and Brewer 2005). The lack of behavioural data is not restricted to Cozumel Wrens; among the ten recognized species in the genus *Troglodytes* in North and South America (American Ornithologists’ Union 1998; Banks et al. 2006; Chesser et al. 2010; Remsen et al. 2013), quantitative descriptions exist for only Northern House Wrens (*T. aedon*) and Pacific Wrens (*T. pacificus*; Platt and Ficken 1987; Van Horne 1995). The dearth of quantitative studies of *Troglodytes* vocal behaviour may be due to their elaborate songs (Platt and Ficken 1987; Kroodsma 1980; Van Horne 1995). For example, male Northern House Wrens attach different introductory notes to songs, presenting challenges to attempts to classify song types (Platt and Ficken 1987; Johnson 1998), while Pacific Wrens create new song types continuously, so estimating an exact song repertoire size is very difficult (Van Horne 1995). Thus, it is unclear whether *Troglodytes* wrens have fixed song repertoires, or whether they use a repertoire of syllables to create their variable and complex songs, or whether both types of repertoire are evident in different species.

Like their songs, the taxonomy of Cozumel Wrens is enigmatic. Cozumel Wrens are thought to belong to a group known as the House Wren complex, together with Northern House Wrens (*T. aedon*), Southern House Wrens (*T. a. musculus*), Brown-throated Wrens (*T. a. brunneicollis*), and wrens restricted to Lesser Antilles islands (American Ornithologists' Union 1998). Cozumel Wrens share morphological similarities with Southern House Wrens (the taxon understood to be their closest mainland relative), but there are marked differences in size and colour: Cozumel Wrens are larger and their plumage is lighter in colour (Howell and Webb 1995; Navarro-Sigüenza and Peterson 2004). These morphological differences have raised questions regarding whether Cozumel Wrens should be considered full species (Howell and Webb 1995; Stotz et al. 1996; Navarro-Sigüenza and Peterson 2004). The lack of behavioural and genetic data limits our ability to answer these questions. Thus, a detailed study of the Cozumel Wren's song structure and vocal behaviour can inform their taxonomic classification.

Our study is the first to provide a detailed bioacoustic analysis of the song and vocal behaviour of Cozumel Wrens. Based on two years of field research, we describe the fine structural characteristics of Cozumel Wren songs. We estimate both song repertoire size and syllable repertoire size, and we assess repertoire sharing among neighbours, testing whether geographic distance is related to song and syllable sharing.

Methods

Study area

Cozumel Island is located in the Mexican Caribbean Sea, 18 km off the northeastern coast of the Yucatan Peninsula (Figure 2.1). The island is 53 km long and 16 km wide, covering an area of approximately 470 km^2. The terrain is flat, with an average elevation of 5 m above sea level and a highest peak of 15 m (Muckelbauer 1990). The vegetation is dominated by tropical semi-deciduous forest, low tropical deciduous forest, mangroves, and coastal dune vegetation (Romero-Nájera et al. 2007).

Field techniques

We recorded and observed Cozumel Wrens from May 19 to 31, 2010, and from May 17 to 31, 2011. Both recording periods fell during the breeding season, and wrens were actively singing and engaged in reproductive activities throughout both field expeditions. In total, we followed the vocal behaviour of 23 males: 9 males recorded only in 2010, 12 males recorded only in 2011, and 2 males recorded in both 2010 and 2011. We recorded wrens at four locations on the island (Figure 2.1): (1) one bird north of the ‘‘San Miguel de Cozumel’’ (20°320 N, 86°550 W; recorded in 2010); (2) two birds 6 km north of kilometre 5.5 of ‘‘Carretera Transversal’’ (20°300 N, 86°510 W; recorded in 2011); (3) 18 birds at ‘‘Pueblo Fantasma’’ (20°270 N, 86°570W; recorded in 2010 and 2011); and (4) two birds near the southwestern edge of the island (20°180N, 86°580 W; recorded in 2010).

To distinguish individuals, we caught birds using mist nets and banded them with a unique combination of coloured leg bands. We assigned sex by inspecting the cloacal protuberance or brood patch. We banded 16 birds: 14 males and two females. Of the 14 banded males, at least 5 were paired: 1 was observed with an unbanded partner who was incubating eggs, and he was observed bringing food to the nest, helping to clean the nest, and later attending to fledglings; a second male was also observed with a partner (a banded female) who was incubating eggs, and this male was also observed bringing food to the nest; a third male was observed with a partner (a banded female) and fledglings; the two remaining males were observed affiliating with an unbanded partner. Our observations of the banded males confirmed that they remained within their territories for the duration of our field study, and were even found in the same territory between years (all three of the banded males that were present in 2010 and 2011 occupied the same territory in both years). Therefore, we distinguished the unbanded birds based on their territory position. Our observations of the unbanded males confirmed that at least two of those males were paired: one was observed with a partner and fledglings, and the other was observed affiliating with a partner. In summary, of the 23 males included in this study, we are certain that 7 were paired, and 4 of these males were observed to have a nest or to have fledglings. We were not able to determine the pairing status or breeding stage of the remaining males due to the challenges of observing birds through the thick vegetation at the study site, but we suspect that all birds were paired and at similar stages of breeding.

Sound recording

To ensure rigorous sampling of repertoires of songs, and to guarantee a high number of recording hours in the field, we used two complementary recording techniques. First, we collected focal recordings where a recordist followed the male around his territory between 0500 and 1100 hours and between 1500 and 1700 hours (CST), identifying the bird by its colour bands whenever possible. We followed one or two birds per day. Focal recordings were collected using three sets of equipment: (1) a Marantz PDM660 digital recorder (WAV format; 44.1 kHz; 16 bits) with a Sennheiser MHK67 shotgun microphone; (2) a Marantz PMD660 digital recorder (WAV format; 44.1 kHz; 16 bits) with a Telinga parabola and a Sennheiser ME62/K6 omnidirectional microphone; or (3) a Nagra Ares-BB+ digital recorder (WAV format; 48 kHz; 16 bits) with a Telinga parabola and a Stereo Pro 6 Telinga microphone.

Second, we recorded birds using autonomous digital recorders, which allowed us to collect a long, continuous recording in each bird's territory from 0400 to 2000 hours. These autonomous recordings minimized any disturbance to the birds' behaviour caused by the presence of the human recordist, and generated longer periods of recordings to facilitate thorough calculations of repertoire size and quantitative descriptions of diel variation in vocal behaviour. These recordings were collected using song meters (Wildlife Acoustics; model SM1 and SM2; WAV format; 44.1 kHz; 16 bits; see details in Mennill et al. 2012). One autonomous recorder was placed in the area where the male spent most of the time singing during a preceding focal recording. Our behavioural observations of Cozumel Wrens, and other *Troglodytes* species, confirmed that these wrens are territorial and that territories are well defined, with little or no overlap between them. Therefore, we are confident that our autonomous recordings reliably sampled the targeted individuals.

We collected recordings in every male's territory for an average recording period of 4.5 ± 0.6 days (here, and hereafter, values are shown as the mean ± SE), with a range of 1–11 days. Altogether, we collected a total of 700.5 h of recordings (22.5 h of focal recordings plus 678 h of autonomous recordings) with an average of 30.4 ± 6.9 h per territory (range 0.1–96.7 h). Within these recordings, we sampled a total of 36,271 songs (1,577 ± 424.2 per male; range 6–7,943; *n* = 23).

Definitions

Following Catchpole and Slater (2008), we defined a ‘‘syllable’’ as the basic unit in Cozumel Wren songs; each syllable can be composed of one to several ‘‘elements’’, which we defined as a distinct continuous tracing on a sound spectrogram. We defined a ‘‘trill’’ as a section of the song composed of a series of the same syllable repeated three or more times. We defined a ‘‘song’’ as a long, complex vocalization containing a series of syllables with no silent gaps of ≥ 1 s (silent gaps within songs were always much smaller). We defined ‘‘song repertoire size’’ as the total number of unique song types recorded from each bird. We defined ‘‘syllable repertoire size’’ as the total number of unique syllable types and trill types recorded by each bird.

Song and syllable classification

Songs of Cozumel Wrens are composed of two sections. The introductory section is composed of a variety of low amplitude and broadband noisy, nasal, harmonic, or tonal sounds; the terminal section is composed of a variety of mostly tonal sounds, and typically begins with the first trill in the song (Figure 2.2). After scrutinizing all of the songs recorded, we classified seven types of syllables and six types of trills (Figure 2.3). We classified three categories of broadband and nasal sounds: *waah* syllables were variable, broadband noisy sounds (Figure 2.3a); *snarl* syllables were nasal sounds with narrowly spaced harmonic stacks that were less than 0.2 s in length (Figure 2.3b); and *mew* syllables were sounds with widely spaced harmonic stacks that were longer than 0.2 s (Figure 2.3c).

Frequency-modulated tonal syllables were sounds depicted in the spectrogram as a clear and continuous line with no overtones (Figure 2.3d–g). We classified four different categories of tonal syllables: frequency modulated syllables with frequencies below 4 kHz, which were quite variable (Figure 2.3d); short downslurred syllables with a wide bandwidth and a length shorter than 0.1 s (Figure 2.3e); slower downslurred syllables that were longer than 0.1 s (Figure 2.3f); and upslurred syllables with a frequency modulation occurring at the highest point (Figure 2.3g).

We classified six different trills based on the shape and sound of the syllables, regardless of the number of times that the syllable was repeated (Figure 2.3h–m): trills with relatively narrowband chevron-shaped syllables below 4 kHz (Figure 2.3h); trills with quick downslurred syllables (Figure 2.3i); trills with broadband upslurred syllables ending in a chevron shape (Figure 2.3j); trills with upslurred syllables that reached a plateau at the end of each syllable (Figure 2.3k); trills with double elements made up of downslurred broadband syllables alternated with short low elements (e.g. Figure 2.3l); and trills with downslurred syllables below 4 kHz, where sometimes the first syllable was longer and of higher frequency (Figure 2.3m). In some instances, wrens added 1–3 extra syllables to the end of a trill, as shown in Figure 2.2e, f, but we still considered these to be the same trill type. Series of repeated *waah*, snarl or mew syllables were rare and were not considered trills.

We classified songs as being of the same type if they shared the same mew and tonal syllables in the introductory section and the same trill type in the terminal section, as well as the order of the syllables and trills (Figure 2.2). We used these three song components (*mew* syllables, tonal syllables, and trills) to define similar songs because they are relatively stereotyped, they are a prominent feature of the songs, and they are found across birds. We did not include frequency-modulated syllables below 4 kHz when classifying song types, because these syllables showed high variability (e.g. Figure 2.3d). When songs had the same mew, tonal, and trill syllables, but included additional different syllables, we considered them to belong to the same song type (e.g. Figure 2.2d–f).

Repertoire size assessment

To assess song repertoire size, we annotated all of our recordings in Syrinx-PC (J. Burt, Seattle, WA, USA) using the time and frequency cursors to highlight every recorded song in our focal and automated recordings. Each new song was added to a digital catalogue, where we noted the identity of the singing bird and the song type. We followed the same procedure to assess syllable repertoire by highlighting each syllable and trill in each song. Each new category of syllable or trill was given a unique number and added to a digital catalogue, where we noted the identity of the singing bird and the syllable type or trill type. We annotated syllables and trills using the catalogue of song types obtained in the song repertoire analysis. These analyses were carried out using spectrograms with a 512-point fast Fourier transform (FFT) and a Blackman window.

We tested whether our sampling of Cozumel Wren song repertoires was exhaustive by plotting the number of changes in song type recorded against the total number of song types detected (i.e. song type changes were counted chronologically from the start to the end of an individual's recording). Similarly, we tested whether our sampling of syllable and trill repertoires was exhaustive by plotting the number of song types recorded against the total number of syllable and trill types detected (Baker 1996; Catchpole and Slater 2008).

Song and syllable sharing

In many bird species with repertoires, males share some of their songs, or song subcomponents, with neighbours (e.g. Vehrencamp 1999). We assessed the proportion of songs, as well as syllables and trills, shared between pairs of males by calculating a sharing coefficient (McGregor and Krebs 1982; Vehrencamp 1999; Molles and Vehrencamp 1999). We calculated both song sharing indices and syllable/trill sharing indices as:

$$S = 2Ns/(R1 + R2)$$

Where Ns is the number of shared songs or syllables and trills, and R1 + R2 is the total repertoire size of the two males. A value of S = 0 indicates that two males share no songs or syllables/trills, and a value of S = 1 indicates that all songs or syllables/trills are shared by both individuals.

We tested whether song and syllable/trill sharing indices varied with geographic distance by conducting Mantel tests. Mantel tests were performed using 10,000 permutations in the Passage 2 software package (Rosenberg and Anderson 2011). The geographic distance matrix was calculated using the coordinates at the approximate center of every male's territory, based on handheld GPS data collected during focal recordings in the field. We calculated the pairwise distances between the territories of every pair of males using the Geographic Distance Matrix Generator V.1.2.3 software package (American Museum of Natural History, Chicago, IL, USA).

Song structure

We quantified the fine structural characteristics of Cozumel Wren songs in terms of frequency, time, and syllable and trill types. For this analysis, we randomly selected up to five single exemplars of different song types from each male's catalogue. In total, we measured 86 songs from 23 males for this analysis (3.9 ± 0.26 song types per male). Each song was saved into a separate sound file with at least 0.5 s of silence at the beginning and the end of the song. Then, we filtered the sound files with a 1-kHz high-pass filter and normalized songs to a peak amplitude of -1 dB using the Audition software package (Adobe Systems, San Jose, CA, USA). We then created a spectrogram for every song using a 1024-point FFT, 93.75 % overlap, a Blackman window, 22 Hz frequency resolution, and 2.9 ms temporal resolution in SASLab Pro (version 5.2.04; AviSoft; R. Sprecht, Berlin, Germany). We quantified a total of seven fine structural variables for each song: (1) song length (in s); (2) minimum frequency (in Hz); (3) maximum frequency (in Hz); (4) bandwidth (in Hz); (5) total number of syllables in the song; (6) number of unique syllable types; and (7) number of trills. Measurements were performed using the automatic parameter measurement feature in Avisoft-SasLab Pro, with a threshold setting of -20 dB and a hold time of 10 ms. Automatic detection always identified the start and the end of a trill, but it could not always distinguish the first syllables of the introductory section due their low amplitude. In these cases, we selected the onset of the introductory section manually, by looking at the first syllables on the spectrogram. We calculated an average value for all structural measurements for each male.

Results

Song structure

Cozumel Wren songs are composed of two sections, an introductory section with diverse types of syllables that start at a low amplitude and crescendo, and a terminal section with a trill (Figure 2.2). Songs had an average length of 1.6 ± 0.04 s (range 1.3–2.0, n = 23), a minimum frequency of 1,650.5 ± 56.1 Hz (range: 1,090–2,133), a maximum frequency of 6,704 ± 117 Hz (range 5,643–7,793), and a bandwidth of 4,050 ± 187 Hz (range 2,998–6,350). Songs had 14.1 ± 0.4 syllables and trills (range 10.6–18.5) with 3.9 ± 0.1 different syllable types (range 3.0–5.2) and 1.2 ± 0.06 different trill types (range 0–3) per song. During 28 days of field research over 2 years, and based on more than 22.5 h of focal recordings, we only recorded males singing; we only observed female Cozumel Wrens producing calls, not songs.

Song and syllable repertoire size

Cozumel Wrens repeat the same song type many times before switching to a new song type. However, when repeating the same song type, males alter the number of times they repeat specific syllables that constitute the song type, suggesting that Cozumel Wrens sing with a pattern that is intermediate between ‘‘eventual’’ and ‘‘immediate’’ variety. Inspection of the repertoire asymptotic curves for song types showed that 10 of the 23 Cozumel Wrens recorded reached the asymptotic line (all 10 were recorded in Pueblo Fantasma; Figure 2.4a). Using data from birds that reached the asymptotic curve, we found a total of 50 different song types among all individuals analyzed. Individually, birds had an average repertoire size of 18.7 ± 0.6 song types (range 15–23; n = 10). For the remaining males, we recorded between 2 and 16 song types and none reached an asymptote. The total number of songs analyzed for males that reached the asymptote was on average 3,302.3 (range 1,240–7,943), and the number of songs analyzed for males that did not reach the asymptote was 249.8 (range 6–671).

We found that Cozumel Wrens had an average syllable and trill repertoire of 12.8 ± 0.1 (range 12–13, n = 10; Figure 2.4b). All birds had *waah*, *snarl*, and *mew* syllables in their repertoires. On average, the birds had a repertoire of 3.9 ± 0.1 tonal syllables (range 3–4, n = 10) and 5.9 ± 0.1 trill types (range 5–6, n = 10). Most *waah*, *snarl*, *mew*, and tonal syllables occurred in the introductory sections of songs, although tonal syllables also occurred in the terminal sections of songs, sometimes between trills and at the end of the song (Figure 2.2).

Repertoire comparison between years

There were two Cozumel Wrens recorded in both 2010 and 2011. We found only subtle differences in song repertoire size between years. The song repertoire sizes for the two birds in 2010 and 2011 were 19 and 12 song types (bird TAB 03), and 17 and 14 song types (bird TAB 05), respectively. When both 2010 and 2011 data were plotted together, the song repertoire sizes were 20 and 18 song types, respectively (Figure 2.4a). Both of the birds recorded had the same pool of syllable and trill types in both years. The asymptote lines for the song repertoire and the syllable and trill repertoire showed similar patterns when both years were computed either separately or together, and in both cases the males reached an asymptotic curve (Figure 2.4). In 2011, the birds repeated 57.8 % (i.e. 11 of 19 song types for TAB 03) and 76.4 % (i.e. 13 of 17 song types for TAB 05) of the song types that were recorded in 2010.

Song and syllable sharing

We evaluated sharing between eight males recorded in 2011 for which we were confident that we had recorded most of their repertoires based on their asymptote curves. The distance between the centers of the territories of the tested males was, on average, 600.1 ± 124.3 m (range 102.8–1,388.6 m; $n = 8$). These wrens shared, on average, 60 % of their song repertoires (song sharing index 0.6 ± 0.03; range 0.4–0.7; see examples in the top two rows of Figure 2.2) and 90% of their syllable and trill repertoires (syllable/trill sharing index 0.9 ± 0.01; range 0.8–1). Mantel tests revealed a significant relationship between song-type sharing and geographic distance ($r = -0.3$; one tailed $P= 0.03$; $n = 8$). Conversely, the syllable and trill sharing index showed no pattern with geographic distance ($r= -0.1$; one tailed $P = 0.1$).

Discussion

Cozumel Wrens sing complex songs that are a conspicuous component of the soundscape of Cozumel Island, Mexico. Males sing the same song type many times before switching to a new song type, subtly varying the number of times they repeat specific elements during successive repeats of the same song type. Songs are composed of four different categories of syllables with a prominent trill near the end of the song. Cozumel Wren males have fixed repertoires of songs, and of syllables and trills. They create their songs by combining a restricted number of syllable and trill types that are shared among most individuals in the population. Song-type sharing between males decreases with distance, whereas syllable and trill sharing between males is consistently high, even for non-neighbours.

Cozumel Wrens sing, on average, 18 song types, and song type repertoires are finite and quantifiable. Every song type comprises, on average, 4 different syllable types and 1 trill type from a repertoire of approximately 13 syllable and trill types. These song characteristics are similar to those of mainland Northern House Wrens, where a restricted number of song types are built based on a restricted number of syllable types (Platt and Ficken 1987). Anecdotal evidence from our observations of two birds recorded in two consecutive years supports the idea of fixed repertoires of songs, syllables, and trills; in 2011, these birds used the same pool of syllable and trill types and most of the song types recorded in 2010, showing little change between years. During song bouts, Cozumel Wren males sing the same song type many times before switching to a new song type. During successive renditions of the same song type, however, males alter the number of times they repeat specific elements that constitute the song type, suggesting that Cozumel Wrens sing with a style that is somewhat intermediate between ‘‘eventual’’ and ‘‘immediate’’ variety. This style of song, which adds versatility to song bouts, is a behaviour observed previously in Northern House Wrens (Platt and Ficken 1987).

Interestingly, given the number of syllables and trills in a Cozumel Wren’s repertoire, it would be possible for these birds to build a larger song repertoire than they actually manifest. We found that the Cozumel Wrens have a restricted number of discrete songs that are stereotyped in syllable and trill composition and length. It is possible that the occurrence of songs that are stereotyped in both syllable and trill composition and length arises due to the use of syntactic rules where syllable and trill types are produced in non-random order. Such a pattern has been seen in other bird species (e.g. Leger 2005; Wright and Dahlin 2007; Ivanitskii et al. 2012). We are not aware of any study conclusively demonstrating the use of syntactic rules in members of the family Troglodytidae. However, previous studies confirm that some compositional rules are important in wren song (Van Horne 1995; Holland et al. 2000; Camacho-Schlenker et al. 2011). This is an important area for further investigation.

Stereotyped songs and high degrees of sharing can arise due to learning and imitating strategies (Kroodsma 2004). Our results show that Cozumel Wrens combine syllables to create song types that are shared only at a local level. Local song sharing is known to occur when birds copy the details of songs from their parents and neighbouring adults, a behaviour known as imitative learning (Kroodsma et al. 2002; Kroodsma 2004). Our results that reveal high local sharing of song types are consistent with the idea of imitative learning in Cozumel Wrens. Furthermore, it is thought that one of the consequences of imitative learning is the formation of dialects (Kroodsma 2004). Dialects—groups of nearby animals that share more acoustic similarity to each other than to different groups—have been shown to occur in several songbird species, including some members of the Troglodytidae family (Verner 1975; Morton 1987; Wegrzyn and Leniowski 2010; Camacho-Schlenker et al. 2011).

We found that syllable and trill types of the Cozumel Wren are widespread across Cozumel Island. A common library of syllables and trills are used to build songs that vary geographically around the island. Similar patterns have been reported in Swamp Sparrows (*Melospiza georgiana*, Marler and Pickert 1984) and Indigo and Lazuli Buntings (*Passerina cyanea* and *P. amoena*; Baker and Boylan 1995). Baker and Boylan (1995) suggest that syllables could be the fundamental unit of cultural evolution in species where syllables are widely distributed among populations and are stable in their acoustic traits across generations. Further research across space and time in Cozumel Wrens could help us to understand the relation- ship between songs and syllables, and their significance in the evolution of cultural traits.

Several prior investigations have reported differences in vocal behaviour between island and mainland bird populations (e.g. Baker 2006; Baker et al. 2006; Aleixandre et al. 2013; Potvin 2013). Our observations suggest that there are also significant vocal differences between Cozumel Wrens and continental populations of Northern and Southern House Wrens (Sosa López and Mennill, unpublished data). Several bioacoustic studies on members of the family Troglodytidae have shown that vocal characters are useful as taxonomic characters (e.g. *Hylorchilus navai* vs. *H. sumichrasti*, Gómez de Silva 1997; *Thryothorus nicefori* vs. *T. rufalbus*, Valderrama et al. 2007; *Troglodytes pacificus* vs. *T. hiemalis*, Toews and Irwin 2008, 2012; across the *Thryothorus* wrens, Mann et al. 2009; *Henicorhina l. leucophrys* vs. *H. l. hilaris*, Dingle et al. 2010); including the recent recognition of Cobb's Wren (*T. cobbi*) as a full species (Campagna et al. 2012; Remsen et al. 2013). A careful and detailed comparison performed across the geographic range of the House Wren complex is needed to improve the taxonomy of Cozumel Wrens.

What is the function of song in Cozumel Wrens? Prior observational and experimental studies of Northern House Wrens demonstrate that male song is important in both mate attraction and territory defence (Johnson and Kermott 1991; Johnson and Searcy 1996; Cramer 2013). Our observations of Cozumel Wrens suggest the same functions are at play. During the early morning, all males performed their singing activities from a single perch area, repeating their loud songs for an extended period; later in the morning, males moved around to produce song in punctuated bouts throughout their territory.

We conducted playback experiments as part of a separate investigation, and found that territorial male Cozumel Wrens reacted aggressively when conspecific songs were played, approaching the loudspeaker and singing at a heightened rate (Sosa López and Mennill, unpublished data). These patterns are consistent with a territorial function of Cozumel Wren song. In order to understand whether Cozumel Wren song also plays a role in mate attraction, future research should focus on male singing behaviour early in the breeding season, prior to pairing, and on the link between male song and female choice. We speculate that songs in Cozumel Wrens play a role in both mate attraction and territory defence, as is common in many other songbirds (Catchpole and Slater 2008).

Our study is the first to provide a detailed study of the vocal behaviour of Cozumel Wrens. They sing complex songs made up of variable syllables and trills. Each bird has a limited repertoire of stereotyped songs, which they create by recombining a restricted number of syllable and trill types. Our results provide behavioural information for ornithologists to better understand the relationship between Cozumel Wrens and other *Troglodytes* wrens. Our results also provide a foundation for future research on the ecology, evolution, and behaviour of Cozumel Wrens and island-living animals.

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Figures

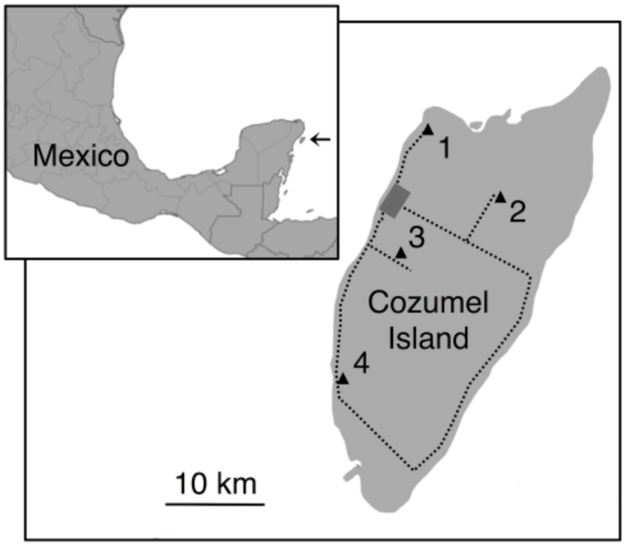

Figure 2.1. Map of Cozumel Island, Mexico, with four triangles showing the locations where recordings of Cozumel Wrens were collected during this two-year field study: (1) north of San Miguel de Cozumel, (2) 6 km north of kilometre 5.5 of Carretera Transversal, (3) Pueblo Fantasma, and (4) near the southwestern edge of the island. Map at upper left shows the location of Cozumel Island, 18 km off the eastern coast of the Yucatan Peninsula

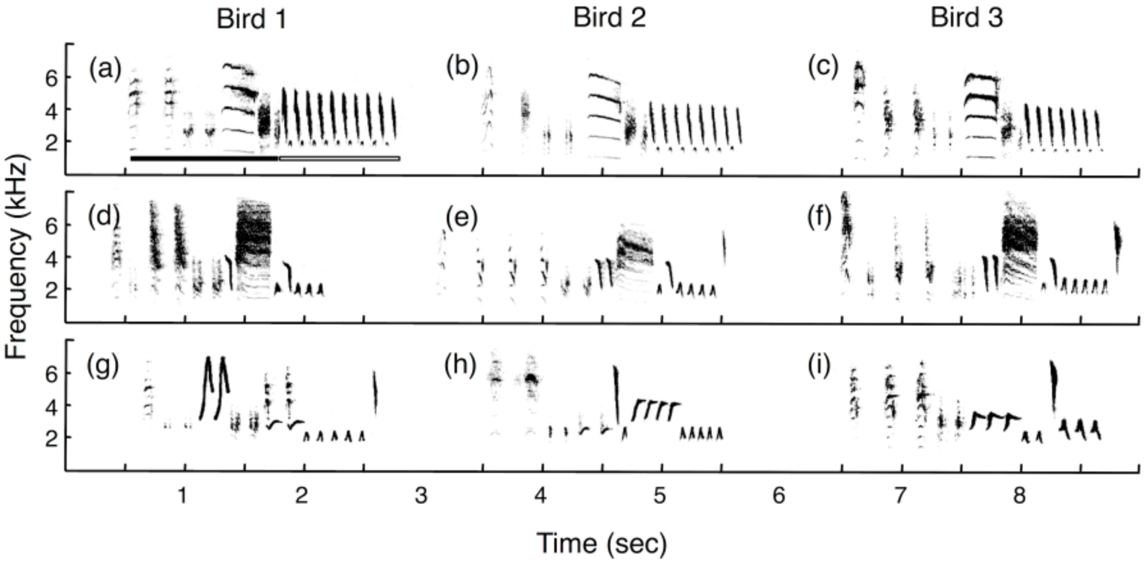

Figure 2.2. Sound spectrograms showing three songs for each of three male Cozumel Wrens (a–g). Each column shows a subset of the repertoire for three different males (three songs per male). Songs are arranged to show song types that are shared in common between the three males in examples a–c and d–f, whereas non-matched songs are shown for g–i. For song a, the solid bar shows the introductory section of the song, and the open bar shows the terminal, trilled section of the song

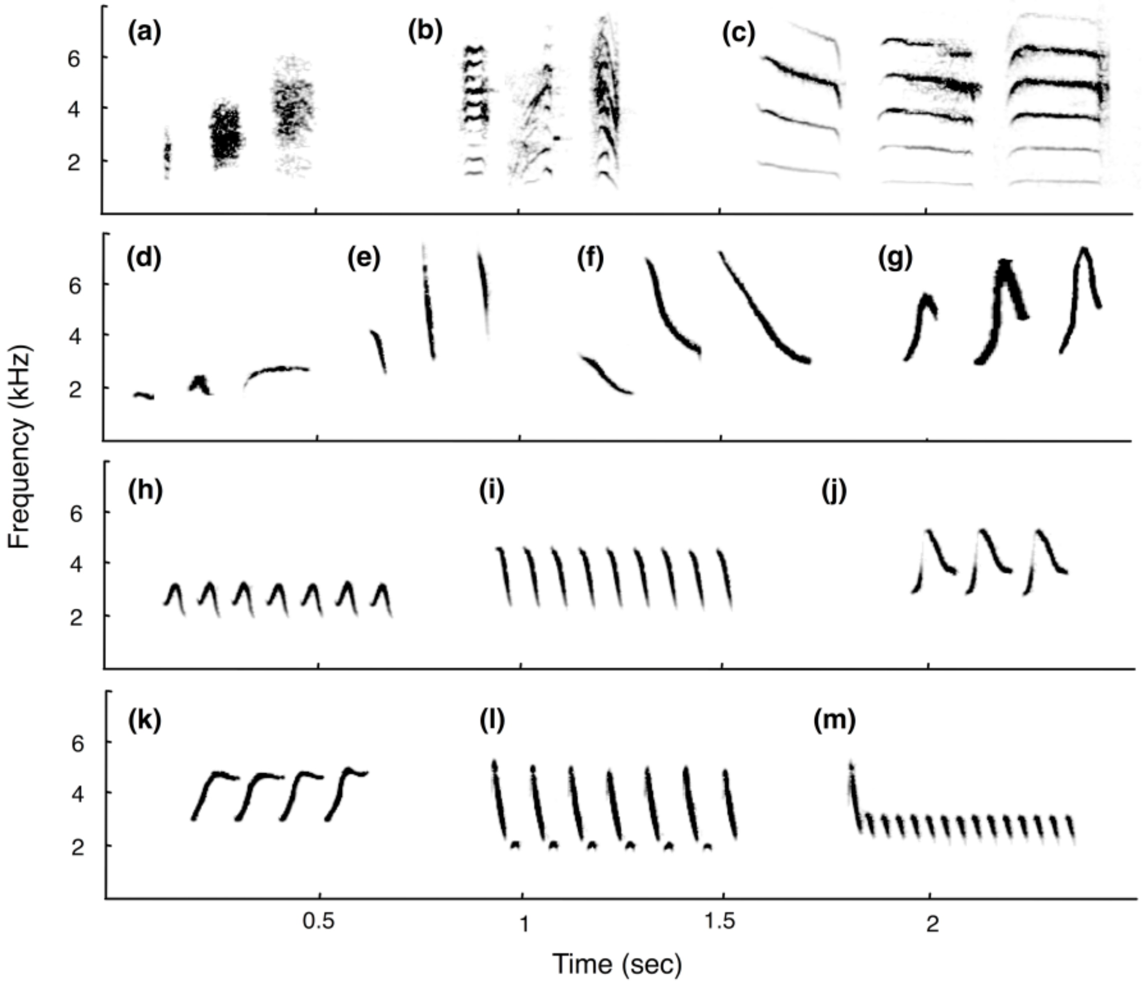

Figure 2.3. Catalogue of sound spectrograms showing examples of the different syllable and trill types produced by male Cozumel Wrens: (a) *waah* syllables, (b) *snarl* syllables, (c) *mew* syllables, (d–g) frequency-modulated tonal syllables, and (h–m) trills. The spectrograms depict three examples for each syllable type and one example for each trill type

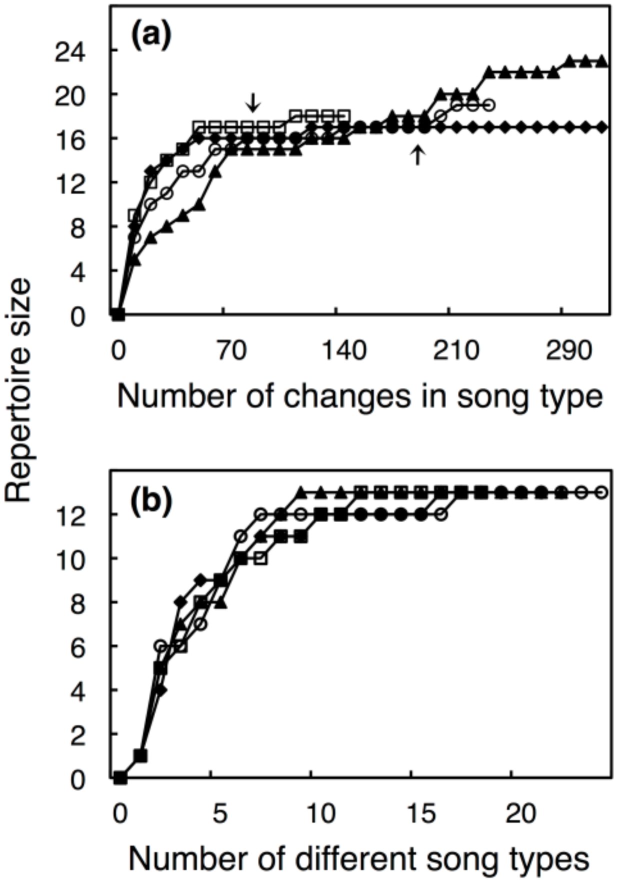

Figure 2.4. Repertoire asymptote curves for (a) song types and (b) syllable and trill types of Cozumel Wrens. For both songs types and syllable and trill types, Cozumel Wrens have a fixed repertoire size given sufficient sampling. Examples of asymptotic curves are shown for four birds. Asymptotic curves with filled symbols correspond to two birds recorded in 2010 only. Asymptotic curves with open symbols show data for birds recorded in both years of the study; arrows indicate changes between years for those birds recorded in 2010 and 2011

Chapter 3

The vocal behaviour of the Brown-throated Wren (*Troglodytes brunneicollis*): song structure, repertoires, sharing, syntax, and diel variation[*]

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

Chapter summary

Empirical descriptions of vocal behaviour are important for understanding avian biology. In this study, we provide the first detailed analysis of the vocal behaviour of the Brown-throated Wren (*Troglodytes brunneicollis*), a neotropical songbird found in oak forests in the highlands of Mexico and the southwestern United States. We quantify repertoire sharing among neighbours, we describe how sharing varies with distance, and we explore whether this species uses syntactical rules for creating their songs. Our analyses reveal that Brown-throated Wrens have complex songs and simple calls. They sing with eventual variety, repeating songs many times before switching to a new song type. Males combine syllables into phrases to create songs. We show that song repertoire size is not fixed; birds recombine their syllables to produce highly variable song types. Brown-throated Wrens sing with high vocal output after sunrise and song activity declines throughout the morning. Our results show that the peak in vocal output corresponds with low song type diversity, whereas periods of low song activity (i.e. mid-day) are associated with more frequent changes in song type. Song sharing shows no variation with distance among our sampled individuals. We divide the syllables in Brown-throated Wren songs into 13 categories; birds sing some syllables more frequently than others, and some syllables are more likely to be found at the beginning, middle, or end of the song. Transitions between syllable categories deviate significantly from random chance, and most males analyzed follow similar patterns of syllable transitions, revealing syntactical structure. This research, which provides the first empirical study of Brown-throated Wren song, expands our knowledge of the behaviour of this poorly-studied taxon, and contributes insight into the organization and composition of song in tropical birds.

Introduction

Bird vocalizations are some of the most complex and well-studied acoustic signals in the animal kingdom (Hultsch and Todt 2004; Marler and Slabbekoorn 2004). They can be analyzed at several levels of organization, from the simplest units of notes or syllables, to higher levels of the composition of an entire song, to the highest levels of organization manifested in song repertoires and patterns of song delivery (Catchpole and Slater 2008). Bird species differ enormously in the variety of song styles and vocal organization. Traits that vary between avian taxa include the fine structural characteristics of songs and calls, the relative abundance of syllable types in the song (syllable composition), repertoires, daily vocal activity, syllables and song sharing, and rules that govern the position and order of the syllables within songs (e.g. Catchpole 1976; Kroodsma 1977; van Horne 1995; Molles and Vehrencamp 1999; Wright and Dahlin 2007; Valderrama et al. 2008; Camacho-Schlenker et al. 2011).

The description of bioacoustic traits is important because songs vary with the mode of acquisition (e.g. cultural or genetic inheritance) or with the form of selection they experience (e.g. habitat constrains, performance constraints, female choice, etc; Catchpole and Slater 2008). To understand how these traits evolved and gain insight into their function, it is necessary to document and describe the precise vocal behaviour for multiple taxa and evaluate them using a comparative approach. Careful descriptions of acoustic variation provide a foundation for detailed research on the influence of selection on bird songs and song transmission between generations (Lynch 1996; Podos et al. 2004).

In this study, we provide the first comprehensive account of the vocal behaviour of the Brown-throated Wren (*Troglodytes brunneicollis*). Brown-throated Wrens inhabit most of the mountains of Mexico and the extreme south of Arizona in the United States. They are found in open areas and forest edges of humid pine-oak forest and cloud forest (Howell and Webb 1995). They are a sedentary species, with occasional, limited movements between adjacent forest patches (Watson 2003). Many authors treat the Brown-throated Wren as a full species with two to three subspecies based on appearance, distribution, and habitat (Howell and Webb 1995; Brewer and MacKay 2001; del Hoyo 2005): *brunneicollis* from San Luis Potosi and Hidalgo south to the northern mountains of Oaxaca; *chooni* form southern Arizona south to central Mexico; and *nitidus* isolated in the mountains of Sierra Madre del Sur of Guerrero and Oaxaca. Other authors consider Brown-throated Wrens to be a subspecies of the House Wren (*Troglodytes aedon*; AOU 1998). Species limits in the House Wren complex, however, are controversial (Brumfield and Capparella 1996; Rice at al. 1999; Martínez Gómez et al. 2005). Whether Brown-throated Wrens are best understood as a distinct species or a subspecies of the House Wren requires further investigation (Brumfield and Capparella 1996; Rice et al. 1999; Martínez Gómez et al. 2005; Klicka *unpublished data*). A quantitative description of the vocal behaviour of the Brown-throated Wren would provide a useful point of comparison for a better understanding of their taxonomy, yet their voices have never been analyzed.

We recorded Brown-throated Wrens in southern Mexico and conducted detailed bioacoustics analyses of their vocalizations. Our goals were: (1) to provide a description of the fine structural characteristics of the song and calls of Brown-throated Wrens, (2) to assess the repertoire characteristics in terms of both song types and syllable categories, and (3) to quantify how song output varies with time of day. We also sought to investigate (4) whether song-sharing varies with geographic distance, and (5) whether they deliver syllables in non-random order to create songs. We were motivated to conduct this investigation for several reasons. First, we wished to provide a careful bioacoustic description of this taxon’s songs and singing behaviour and thereby facilitate comparisons to House Wrens, allowing us to explore the hypothesis that these taxa are distinct species (Howell and Webb 1995; Brewer and MacKay 2001; del Hoyo 2005). Second, we were interested in exploring repertoire sharing in Brown-throated

Wrens, a behaviour thought to be important in intra-sexual interactions in other songbirds (e.g. Beecher et al. 2000; Todt and Naguib 2000). Third, we sought to explore whether repertoire size may be the principal target of selection in Brown-throated Wrens, as predicted by the Repertoire Size Hypothesis, which states that males should sing all the components of their repertoire with similar frequency, in order to best showcase their repertoire size (Lapierre et al. 2011). Finally, we wanted to test whether Brown-throated Wrens deliver syllables in a non-random order, exhibiting syntax and defined as the set of rules that govern the temporal arrangement of syllables and phrases (Hultsch and Todt 2004).

Methods

We studied Brown-throated Wrens at San Mateo Rio Hondo, Oaxaca, Mexico (16°8'24" N, -96°26'26" W) from 3 to 17 May 2010 and from 5 to 17 June 2011. Both recording periods fell during the peak of the breeding season, when the recorded birds were building nests and or rearing young. During both recording periods, wrens were actively singing. During two field expeditions, we studied birds in 27 different breeding territories. In 2010 we studied Brown-throated Wrens in nine different territories. We caught at least one individual per territory using mist nets, banded them with a unique combination of colour bands, and determined sex by inspection of the cloacal protuberance and brood patch. In 2011 we studied Brown-throated Wrens in 20 territories, including two territories where birds had been caught and banded in 2010, and 18 additional territories where we caught and banded at least one of the resident birds. Our analyses are based on the detailed recordings of males in seven territories monitored in 2010 as well as two territories recorded in both 2010 and 2011, and complemented by additional observations of males from the 18 additional territories in 2011.

To ensure rigorous sampling of the repertoires of songs and to guarantee a high number of hours recorded in the field, we used two complementary recording techniques. First, we conducted focal recordings where a recordist followed the male and female as they traveled around their territory between 0600 and 1100 h and between 1500 and 1800 h, identifying the bird by its colour bands whenever possible. Second, we recorded birds using autonomous digital recorders which allowed us to collect long, continuous recording in each bird's territory, thereby minimizing human disturbance and generating longer periods of recordings to provide more thorough estimations of repertoire size. We chose the position for the autonomous recorders at the end of our focal recording sessions, placing them near a song perch where the male had performed his first song bout of the day and where he spent most of the time during the two-hour focal recording. Our observations suggest that these birds inhabit the same area over extended periods, and perform songs from the same or similar perches every morning (see Results below). Our observations of interactions between neighbouring males suggest that they are highly territorial birds with defined territorial boundaries; when two neighbouring males engaged in counter-singing behaviour, they did not cross the boundaries of their territories. Based on these observations, we are confident that our autonomous recording devices recorded the target birds, and not birds in adjacent territories.

Our observations showed that Brown-throated Wrens occupied relatively large territories, where the widest dimension was 82.0 ± 13.5 m (mean ± SE; *n* = 15; calculated as the maximum distance between the farthest points where a wren was detected). Territories had irregular shapes and usually did not overlap neighbours' territories (6 of 9 birds with neighbours had small regions of overlap at territory peripheries). Centers of the studied territories were separated by an average distance of 244.5 m (range: 28.6–438.7 m).

Focal recordings were collected using three sets of equipment: a Marantz PMD660 with either a Sennheiser MHK67 shotgun microphone or a Telinga parabola with a Sennheiser K6/ME62 omnidirectional microphone, and a Nagra Ares-BB+ with a Telinga parabola and a Stereo Pro 6 Telinga microphone. Recordings were saved in WAV format at 44.1 kHz/16 bits and 48 kHz/24 bits, respectively. Autonomous recordings were collected using Wildlife Acoustics Song Meters (model SM1 and SM2), which include built-in, stereo microphones (see Mennill et al. 2012); recordings were saved in WAV format at 44.1 kHz/16 bits. We recorded males on every territory for a period of 4.2 ± 2.4 days. Altogether, we collected a total of 255.5 hours of recordings (7.7 hours of focal recordings, and 247.8 hours of continuous autonomous recordings) with an average of 28.3 hours per territory in the nine territories recorded in 2010, or 2010 and 2011 (range: 9.2–79.75 h).

Previous studies on House Wrens (*T. aedon*) and Winter Wrens (*T. Troglodytes*; Kroodsma 1977, Platt and Ficken 1987; van Horne 1995) suggest that *Troglodytes* wrens have very large repertoires and complex singing behaviour. Extremely long field recordings are required to rigorously sample each animal's behavioural repertoire (Catchpole and Slater 2008). Consequently, in this study we chose to maximize the recording time of a small number of birds, rather than collecting shorter recordings of many birds.

Bioacoustic definitions and classification

Definitions

Brown-throated Wrens exhibit similar patterns to other wren species in their songs and singing behaviour; therefore we used the previously established criteria as a guideline to define syllables, phrases, songs, song repertoires, bouts of songs, and calls (van Horse 1995; Mennill and Vehrencamp

2005; Valderrama et al. 2008). We defined a “syllable” as the unit in the song of Brown-throated Wrens. Syllables can be composed by one or more elements (i.e. one or more continuous tracings on a sound spectrogram; as in Catchpole and Slater 2008). We identified “syllable categories” by shape, using temporal and frequency characteristics to distinguish different categories (Platt and Ficken 1987; van Horne 1995); syllable categories were established *a priori* (details below). We defined a “phrase” as a combination of syllables in stereotyped order that were common across different birds or different vocalization bouts from the same bird (Catchpole and Slater 2008). For purposes of this study, we differentiate songs and calls based on their structure, rather than their function. We defined a “song” as long, complex vocalizations produced by males and containing a series of syllables with no gaps of ≥ 1 sec; we defined a “call” as a shorter, simpler vocalization delivered by both sexes (Catchpole and Slater 2008). The distinction between the study birds’ complex songs and simple calls was obvious in our recordings (see Figs 1 and 3). We defined “song repertoire” as the total number of song types recorded from each bird (Catchpole and Slater 2008). We defined a “bout of songs” as a period of singing activity with short gaps between songs. Typically, a song bout included one song type repeated at a regular pace. We considered a bout to end when a bird stopped singing, or when a bird changed to a different song type (following Borror 1956; Morton 1987). We defined a “trill” as a series of identical syllables, repeated three or more times in rapid succession within a song.

Song, syllable and call classification

After scrutinizing all songs recorded, we assigned all syllables into 13 categories based on the length of the syllable, its harmonic structure, and the number of inflection points in frequency-modulated syllables (Figure 3.2). Syllables with harmonic structure were classified in two categories: “*snarl* syllables” were nasal sounds with narrowly spaced harmonic stacks less than 0.1 s length (e.g. Figure 3.2a); and "*mew* syllables" were sounds with widely spaced harmonic stacks, longer than 0.2 s (e.g. Figure 3.2b). Frequency-modulated syllables were sounds depicted in the spectrogram as a clear and continuous line with no overtones. We classified all syllables based on the number of elements and frequency modulations or inflections: one-element syllable having one inflection (e.g. Figure 3.2c), two inflections (e.g. Figure 3.2d), and three or more inflections (e.g. Figure 3.2e); and two-element syllables having one inflection (e.g. Figure 3.2f), two inflections (e.g. Figure 3.2g), and three or more inflections (e.g. Figure 3.2h). We classified syllables with more rapid frequency modulations in four categories: syllables with a slow modulations (e.g. Figure 3.2i), syllables composed of a section with slow modulations and a pure tonal section (e.g. Figure 3.2j), syllables composed of a section with rapid modulations and a pure tonal section (e.g. Figure 3.2k), and syllables made of rapid modulations (e.g. Figure 3.2l). We classified syllables without inflections with one or two elements in a single category, all of which were short syllables (e.g. Figure 3.2m). Acoustic signals produced by Brown-throated Wrens are very complex, and they may produce more than 13 syllable categories; our classification scheme is conservative and provided us an objective, repeatable method for studying the syllables in this species' complex songs.

We classified song types on the basis of being composed of the same categories of syllables in the same order (following Mennill and Vehrencamp 2005; Valderrama et al. 2008). Two songs were considered as the same type if they shared ≥ 75% of the same syllables. In the classification we considered the syllable category in a trill, but we did not take in account the number of repetitions of that syllable (i.e. a trill with four repetitions of a particular category of syllable was treated the same as a trill with five repetitions of that category of syllable).

We provided a classification and description of the different call types that Brown-throated Wrens produced during our field study. We classified call types based on qualitative traits like frequency modulation, presence or absence of harmonics, number of elements conforming the calls, and length. We also noted the context in which calls were performed.

Acoustic analyses

Fine structural characteristics of the songs

We described the fine structure of the song of the Brown-throated Wren in terms of frequency and time measurements. We measured six fine structural variables for each song: (1) song length (in seconds, measured from the beginning of the first syllable to the end of the last syllable in the song, using the waveform as guideline to define the start and end of a syllable), (2) number of syllables, (3) number of trills, (4) maximum frequency (the highest frequency with energy in the song; in Hz), and (5) minimum frequency (the lowest frequency in the song; in Hz). Measurements were extracted from spectrograms visually, using Syrinx-PC (J. Burt, Seattle, WA). Sound spectrograms were created using a 512 point fast Fourier transform (FFT; Blackman window setting), resulting in approximately 43 Hz frequency resolution and 4 msec time resolution.

Song repertoire

To assess song repertoire size, we used the time and frequency cursors in Syrinx-PC to highlight each song for each bird. Each new song was given a unique number and added to a digital catalogue, where we noted the identity of the singing bird and the song type. To assess song repertoire size, we plotted the cumulative number of song types detected against the number changes in song for every male (as described in Catchpole and Slater 2008; Valderrama et al. 2008). Brown-throated Wrens sing with eventual variety, where a song type is repeated many times before switching to another song type. Hence, we used number of changes in song type to estimate song repertoire because short samples could under-estimate the actual repertoire size when using total number of songs (Catchpole and Slater 2008). We considered ourselves to have recorded the full repertoire of a male when the number of syllables or songs reached an asymptotic line (i.e. the line became horizontal). Two of the 9 birds recorded in 2010 were still alive in 2011. For these birds, the analyses of song repertoire included both sets of data from 2010 and 2011.

Diel variation

To study diel variation in vocal behaviour, we calculated the total number of vocalizations, the total number of songs, and the total number of calls per hour, as well as the different number of song types sung per hour, for those birds with at least one continuous day of recording. Our analyses started at 0500 h (sunrise occurred at 0605) and ended at 1900 h (sunset occurred at 1915). Our field observations suggest that calls are not sex-specific and thus overall rate of calling was calculated on a per–pair basis. We tested whether vocal output differed with time of day by performing a nonparametric Friedman test using PASW statistics (version 18.0; Chicago, IL, USA).

Song sharing and sharing-by-distance

Assessing sharing is a challenge given the high variability in Brown-throated Wren songs, where syllables are added or deleted between subsequent songs (a behaviour also seen in other birds, such as some populations of Song Sparrows, *Melospiza melodia*; Lapierre et al. 2011). We followed previously established methods for comparing these variable songs to estimate the degree to which two song types matched (Lapierre et al. 2011). Using the catalogue of syllables in figure 2, and following a similar approach used to assess song repertoire, we scanned each song type within each male's repertoire for the presence of each syllable category. We chose songs with clear recordings, and with non overlapping sounds that could obscure the categorization.

We assessed sharing between all pair-wise combinations of song types using the Jaccard's coefficient of similarity:

$$J_{AB} = c / (a + b + c - d)$$

Here, c is the number of syllables common to song types A and B; a is the number of syllables present in song type A but not song type B; b is the number of syllables present in song type B but not song type A; and d is the absolute value of the difference in number of syllables in song types A and B. We computed a single sharing coefficient by calculating an average of all coefficient values between two males (*neighbour sharing coefficient*).We also calculated the degree to which a male shared syllables between songs within his own repertoire by computing all coefficient values between his different songs (*within male sharing coefficient*).

To understand the relationship between sharing and the physical distance between birds' territories, we performed a Mantel test (Quinn and Keough 2002). All matrices created for the Mantel test had pair-wise comparisons of every bird with every other bird. First, we created a matrix with *neighbour sharing coefficient* values. Then, we created a matrix with the actual distance between the centers of the birds' territories. The sharing matrix of *neighbour sharing coefficients* was created using Jaccard's coefficient of similarity (explained above). Mantel tests were performed using 10000 permutations in PASSaGE (version 2; Rosenberg and Anderson 2011). Distances between territories were calculated using the geographic coordinates at the centers of the territories with the program Geographic Distance Matrix Generator (version 1.2.3; New York, NY, USA; Ersts 2011), based on tracking of birds during collection of focal recordings. Sharing coefficients were calculated using PASW statistics.

Temporal arrangement and syllable transitions

We conducted three analyses of the temporal pattern of Brown-throated Wren singing behaviour using classified syllables in previous analysis. First, we investigated whether birds preferentially produced some syllable categories more often than others by analyzing the relative frequency distribution of all syllable categories by bird. We ran a separate test for each of our nine most intensively-recorded males.

Secondly, we analyzed whether the temporal arrangement of syllables within a song were given at random by analyzing the relative frequency distribution of the relative position of each syllable category within a song. We ran a separate test for every syllable category across all songs and individuals. We ran chi-square goodness-of-fit tests using relative values. Our data met the assumptions for these tests (see Krebs 1999).

Third, we tested whether syllable-to-syllable transitions within songs of Brown-throated Wrens deviated from random using lag sequential analysis (as in Wright and Dahlin 2007). Following Bakeman and Quera (2012), we calculated the probabilities of transitions between the observed syllable (first behaviour; row) and the following syllable (second behaviour; column) within the strings of specified events (a lag, or in this case a song), for each bird. We performed the analysis at two levels: at lag +1 measuring the frequency of transitions between syllables that were immediately adjacent within songs, and at lag +2 measuring the frequency of transitions between syllables with one intermediate syllable. We performed lag sequential analysis using the software SDIS-GSEQ (version 5.1).

We analyzed whether individuals had similar patterns of transitions between syllables by calculating the Bray-Curtis distance among all individuals. Bray-Curtis coefficients take into account abundant transitions, and ignores missing transitions between two individuals, providing a distance value between 0 and 1, with 1 indicating that two samples are identical (Krebs 1999). We calculated Bray-Curtis distance using PAST (version 2.17b; Hammer at al. 2001). Throughout, values are presented as means ±SE. All tests are two-tailed with a significance threshold of $P = 0.05$.

Results

Songs

We collected recordings of the vocal behaviour of Brown-throated Wrens in southern Mexico, including observations of birds in 27 territories, with detailed recordings of birds in nine territories. We only recorded males singing; during two field expeditions, we never encountered a female singing.

Songs of Brown-throated Wrens are, on average, 2.2 ± 0.1 s long and have 17.1 ± 0.6 syllables. Songs typically have 1.4 ± 0.01 occurrences of trills, and a bandwidth ranging from a minimum of 2259 ± 31 Hz to a maximum of 8117 ± 125 Hz (minimum and maximum are the average across all measured song types).

Brown-throated Wrens sing with eventual variety, where a song is repeated many times before switching to a new song type. Bouts of songs include 35.2 ± 20.1 songs. Within song bouts, songs are separated by silent gaps of 6.2 ± 2.2 sec (based on measurements of the first bout of a morning for $n = 9$ males).

Brown-throated Wrens compose songs by combining syllables. Songs often contain syllables given in stereotyped order, so that particular strings of syllables (“phrases”) were found frequently in different song types within the repertoire of a given bird, or across the repertoires of different birds. Birds varied where they inserted or substituted these phrases in their songs, producing complex patterns of repetition (see Figure 1 for examples). Males often initiated a bout of songs by delivering an unusually long song followed by a short gap, and then continuing the remainder of the bout with shorter songs. Often the long, introductory song comprised two song types in rapid succession or one song type repeated twice with no break (e.g. AB…B B B B; or AA…A A A A).

Calls

Both females and males produced a variety of tonal and atonal calls. We recorded four types of calls (Figure 3.3; Table 3.1). The *whistle* call is a slow downward-modulated call, usually given as two notes with the first higher than the second. Both notes have rapid frequency modulations at the start (Figure 3.3a). This was the most common call; we recorded 936 *whistle* calls produced by 6 pairs. We observed both sexes producing this call, and we recorded this call at all times of the day, although it seemed more common in the afternoon. Interestingly, we heard this call particularly often when following pairs with fledglings, but never during interactions between neighbours. These observations suggest that the *whistle* call may be a contact call.

The *seee* call is comprised of very short high frequency notes (Figure 3b). We recorded 105 *seee* calls produced by 4 pairs. We believe the *seee* call is given in aggressive interactions because often males exposed to playbacks of their own species' song answered using these calls (JRSL unpubl. data). Although we never observed a female producing this call, we are not confident that males are the only sex that produces it.

We also recorded additional, varied calls that we were not able to quantify in detail, and for which the context is unclear. Many of these calls were harsh and unmelodic sounds, as is common for many wrens, including harsh broadband sounds (Figures 3c and 3d). Occasionally, we observed one *whistle* call immediately preceding these harsh calls. We recorded such sounds on 33 occasions by 5 pairs. In addition, we recorded a broadband, downwards-modulated call (Figure 3.3e) on four occasions by 2 pairs.

Song repertoires

On average, we analyzed 911.8 ± 570.5 songs and 60.1 ± 8.7 changes in song type per male (n = 9). The two males that we recorded in both years were recorded for additional periods of 47.5 ± 17.6 hours in the second year, annotating an additional 618.5 ± 229.8 songs per male. Inspection of the repertoire asymptote curves (Figure 3.4) showed that none of the asymptotic curves reached a plateau or showed any sign of leveling off in spite of our extensive recording, including those birds recorded in both years. This suggests that Brown-throated Wrens do not have a fixed song repertoire. We identified, on average, 54.4 ± 10 song types per bird (range: 16–112; n = 9).

Diel variation

Brown-throated Wrens started to sing around sunrise, with an average start time of 0640 h (range: 0630 to 0703 h, n = 9 birds recorded in 2010). Song rates declined slowly during the morning and persisted at lower levels for the remainder of the day. In general, males initiated their first song bout of the morning at specific perches and moved around their territories, giving short flights and singing continuously as they moved around their territories. We monitored 12 males for at least two sequential days; all males chose the same perch to deliver their first song of the morning on subsequent days.

Vocal output varied with time of day (Figure 3.5; analysis based on 7 males recorded extensively in 2010). In general, the highest peak of vocal activity fell between 0600 and 0900 h, when all types of vocalizations were considered together. Song output varied significantly with time of day, with the highest levels of activity occurring in the morning, after dawn, and a peak between 0600 and 0900 h (χ^2 = 59.5, df = 13, $p < 0.001$; Figure 3.5a); song output remained low throughout the late morning and afternoon, with no sign of an increase at dusk. We found an interesting pattern in how often birds changed song types across the day, where birds changed song types more frequently (in relation with the total number of songs per hour) between 1100 and 1400 h (Friedman test: χ^2 = 24.4, df = 13, p = 0.02, Figure 3.5b). Thus, our results show that the peak in song rate is related with the use of a low number of song types sung repeatedly, whereas a low rate of songs coincides with more frequent changes in song type. Calls were produced much less frequently than songs at all times of day. Calls showed significant variation across the day with a peak at 0700 h and three peaks of similar intensity at 0900, 01400 and 1700 h (χ^2 = 29.4, df = 13, p = 0.006; Figure 3.5c).

Sharing and sharing-by-distance

We based our analysis of song sharing on nine males that we recorded extensively in territories that were in close proximity to one another. On average, we analyzed 23.3 ± 4.5 song types per bird (range: 4–42; n = 9), and classified an average of 521.8 ± 105.7 syllables per bird (range: 89–995; n = 9). The mean *within male sharing coefficient* (0.57 ± 0.04; range: 0.52–0.64, n = 9) did not differ from the mean *neighbour sharing coefficient* (0.55 ± 0.03; range: 0.47–0.60, n = 36; pair-wise comparisons, Kruscal-Wallis test: 0.77, df = 1, p = 0.3). In other words, two song types produced by a single male shared the same amount of syllable categories compare to two song types sung by two different birds. We found no relationship between the physical distance separating male territories and their *neighbour sharing coefficients* (Mantel test: r = -0.2, P = 0.1; n = 9). Figure 3.1 shows multiple examples of syllable sharing between birds.

Temporal arrangement and syllable transitions

The chi-square analysis revealed that Brown-throated Wrens produce some syllable categories more often than others (χ^2 range = 56.2–211.8, df = 12, p < 0.001; see Figure 3.S1 in the Appendix). The chi-square goodness-of-fit tests also showed that the relative position of different syllable categories within songs are not homogenous, with some syllables more likely to occur at a particular position within the song (χ^2 range = 5.4–194.5, df = 9, p < 0.025; see Figure 3.S2 in the Appendix).

Transition probabilities between syllables deviated from chance for all nine birds; this was true for both lag +1 and lag +2 sequential analyses, except for lag +2 analysis of bird TB08 (see Table 3.S1 in Appendix). The distance analysis between males showed an average index of 0.56 (range: 0.32–0.74) for lag +1 analysis, and 0.54 (range: 0.30–0.69) for lag +2 analysis. These results suggest that at least 50% of abundant transitions are common among all individuals.

Discussion

Male Brown-throated Wrens produce elaborate, complex songs and simple calls. Males sing with eventual variety, and have an apparently unlimited repertoire of song types. Brown-throated Wrens sing with the highest levels of output right after sunrise. Singing behaviour varies with time of day, in terms of the number of songs, calls, and repetition of song types. In terms of syllable composition, the difference between two song types produced by a single male are as different to two songs produced by different males. There is no relationship between song sharing and geographic distance. Birds show a predilection to overproduce some syllables and underproduce others. The contribution of particular syllables to songs is non-random, where specific syllables are more prone to occur at the beginning, middle, or end of the song. Transitions between syllables are not given at random and all males analyzed followed similar patterns of transition between syllables. This first quantitative description of the vocal behaviour of Brown-throated Wrens shows that these birds have complex and interesting patterns of vocal behaviour.

Brown-throated Wren songs share similar features to House Wren songs from Wisconsin, US (*T. aedon aedon*): in both taxa, songs are composed of highly frequency-modulated syllables, often including trills; song length and frequency are similar (Platt and Ficken 1987); and songs are repeated several times before switching to a new song type (Kroodsma 1977). Our results, however, show that Brown-throated Wrens differ from House Wrens in some acoustic traits. For example, House Wrens song repertoire is fixed (males have on average repertoire of 36 song types; Platt and Ficken 1987), whereas our analyses suggest that Brown-throated Wren repertoires are not fixed, or are much larger than 36 song types. Prior studies reveal that House Wren singing behaviour varies across the breeding cycle, and future comparisons between House Wrens and Brown-throated Wrens will be improved by controlling for breeding stage (Johnson and Kermott 1991).

Brown-throated Wren and House Wren songs show similarity with other closely related wrens' songs. Bewick's Wrens (*Thryomanes bewickii*) and Winter Wrens also compose songs by combining syllables in stereotyped order, creating phrases that are often found repeatedly in the repertoire of a given bird (Kroodsma 1977, Kroodsma 1980, van Horne 1995). Birds insert or substitute these phrases at the beginning, middle, or end of the song, suggesting that they consider these phrases the basic building blocks of song construction (Kroodsma 1977; Figure 3.1). However, unlike House Wrens and Bewick's Wrens, where phrases are composed by less variable syllables resulting in intermediate complexity, Brown-throated Wrens and Winter Wrens compose highly complex phrases with more variable syllables (Kroodsma 1977, Kroodsma 1980, van Horne 1995). The structural features we described here for Brown-throated Wrens are intermediate between Winter Wrens and House Wrens. Interestingly, Martínez Gómez et al. (2005) suggest that Brown-throated Wrens may be basal within the House Wren complex (but see Brumfield and Capparella 1996). Further detailed comparisons are needed in order to understand whether the vocal differences that we present here match the results of recent molecular studies showing that Brown-throated Wrens and House Wrens differ genetically (Rice et al. 1999; Martínez Gómez et al. 2005; but see Brumfield and Capparella 1996).

Empirical evidence suggests that high variability in song repertoire size is related to sexual selection through female choice (i.e. Repertoire Size Hypothesis; Catchpole 1976). The Repertoire Size Hypothesis predicts that if repertoire size is the principal target of selection, then males should sing all the components of their repertoire with similar frequency, in order to best showcase their repertoire size (Lapierre et al. 2011). This strategy would decrease the chances of a listener underestimating the repertoire size of a singer (Lapierre et al. 2011). In contrast to this prediction, our results show that Brown-throated Wrens do not use all the syllables with similar frequency; certain syllable categories are over-produced, and some syllable categories are produced rarely. Therefore, our results do not provide support for the Repertoire Size Hypothesis. Alternatively, intra-sexual interactions may drive patterns of song repertoire delivery in birds (e.g. Beecher et al. 2000; Todt and Naguib 2000). Shared signals may be used for communicating different levels of threat, either escalating or de-escalating a conflict, explaining complex patterns of repertoire use during intra-sexual interaction (Beecher and Campbell 2005). Winter Wrens from Europe share most of their repertoire with neighbours when distances between territories are small (up to 500 m, similar to the distances in our study), but the pattern does not hold when territories are located at more than 500 m (Camacho-Schlenker et al. 2011). Given that our analyses focus on nearby territorial males, we do not know whether sharing decreases with larger geographic distances in Brown-throated Wrens in a similar fashion. Further research is needed to clarify our understanding of the importance of song sharing during countersigning interactions in Brown-throated Wrens, and future playback studies may be particularly insightful to test whether males match components of playback stimuli.

The pattern of diel variation in Brown-throated Wren vocal output is consistent with the dawn chorus behaviour known in many bird species (Staicer et al. 1996), with a peak at the beginning of the day and decreasing as the day progresses. We found that the peak in song rate is related with low song type diversity, whereas a low rate of songs coincides with more frequent changes in song type. Interestingly, our field observations suggest that the peak in vocal activity, which coincides with infrequent changes in song type, occurred after sunrise when males were signing from exposed perches with few movements through their territories; in contrast, periods of low vocal activity, which coincides with frequent changes in song type, occurred throughout the afternoon when most of the interactions among territory owners and neighbours were observed. In several species of songbirds (e.g. Spector 1991, 1992) males sing with little variety early in the morning, and much greater variety during the day; this difference corresponds to apparent differences in context and function, where early morning songs are given with an emphasis on attracting females and daytime songs are given in interactions with other males, where song-type matching may be especially important (Todt and Naguib 2000).

Our recordings reveal that Brown-throated Wrens deliver some syllables more frequently than others, and that some syllables are given preferentially at specific positions within songs. This suggests that Brown-throated Wrens deliver syllables in a non-random order, providing evidence for the idea that this species has vocal syntax. Syntax is defined as the set of rules that govern the temporal arrangement of syllables and phrases (Hultsch and Todt 2004). Evidence for syntactical rules in *Troglodytes* wrens have been presented in two other studies. The first is a descriptive analysis by van Horse (1995) of North American Winter Wrens, showing a non-random temporal arrangement of syllables within songs and transition probabilities differing from expected values. The second comes from an experimental playback study conducted by Holland et al. (2000) showing that European Winter Wrens are able to discriminate between songs with typical syntax versus modified syntax. This type of syntax, known as combinatorial syntax (Leger 2005) has also been reported in nonpasserine birds (e.g. Blue-throated Hummingbird, *Lampornis clemenciae*; Ficken et al. 2000), suboscine birds (e.g. Flammulated Attila, *Attila flammulatus*; Leger 2005), and other oscine birds (e.g. Bengalese Finches, *Lonchura striata*; Okanoya and Yamaguchi 1997).

Empirical studies have shown that song types are the salient unit of interaction for birds (e.g. song-type matching in Song Sparrows, Beecher et al. 2000). However, a study by Kroodsma (1977) suggested the idea that phrases are the basic building blocks of song production in some members of the Troglodytidae family. There are at least two lines of empirical evidence suggesting that phrases are the functional units of song production (reviewed in Suthers 2004). First, empirical evidence suggests that the avian brain stores song information as syllables or packages of syllables (Hultsch and Todt 1989, Suthers 2004), and that muscular movements and the respiratory system are programmed to respond synchronously when producing these components of song. Thus, in complex singers, animals may learn strings of syllables (phrases) as a unit, and then use these phrases to compose their song repertoire. Second, when repertoires are large, receivers are unlikely to count entire repertoires and may rely on other cues, such as phrase detection probabilities (Garamszegi et al. 2005). The fact that Brown-throated Wrens combine syllables in stereotyped order, creating particular strings of syllables into phrases (see Figure 3.1. for examples of sharing phrases), supports the idea that phrases, rather than whole songs, are the basic building blocks of song production.

In summary, our study provides the first comprehensive description of the vocal behaviour of the Brown-throated Wren. We show that this species has a non-fixed song repertoire, and that this species combines syllables to create phrases, using particular rules for syllable placement, creating highly complex songs. These findings are valuable additions to the variety of song organization strategies already known, but also provide a baseline for further comparisons with other *Trolgodytes* wrens for a better understanding of their taxonomy.

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Tables

Table 3.1. Description of the fine structural characteristics (mean ± SE) of four call types of Brown-throated Wrens

Call type	Length (s)	High frequency (Hz)	Low frequency (Hz)	Bandwidth (Hz)
Whistle call	0.57 ± 0.04	5610 ± 66	3219 ± 93	2391 ± 139
Seee call	0.28 ± 0.06	10436 ± 385	7110 ± 900	3326 ± 695
Harsh call	1.24 ± 0.3	7208 ± 679	2528 ± 411	4680 ± 1001
Downward series call	0.55 ± 0.1	10634 ± 1114	3678 ± 1409	6956 ± 2523

Figures

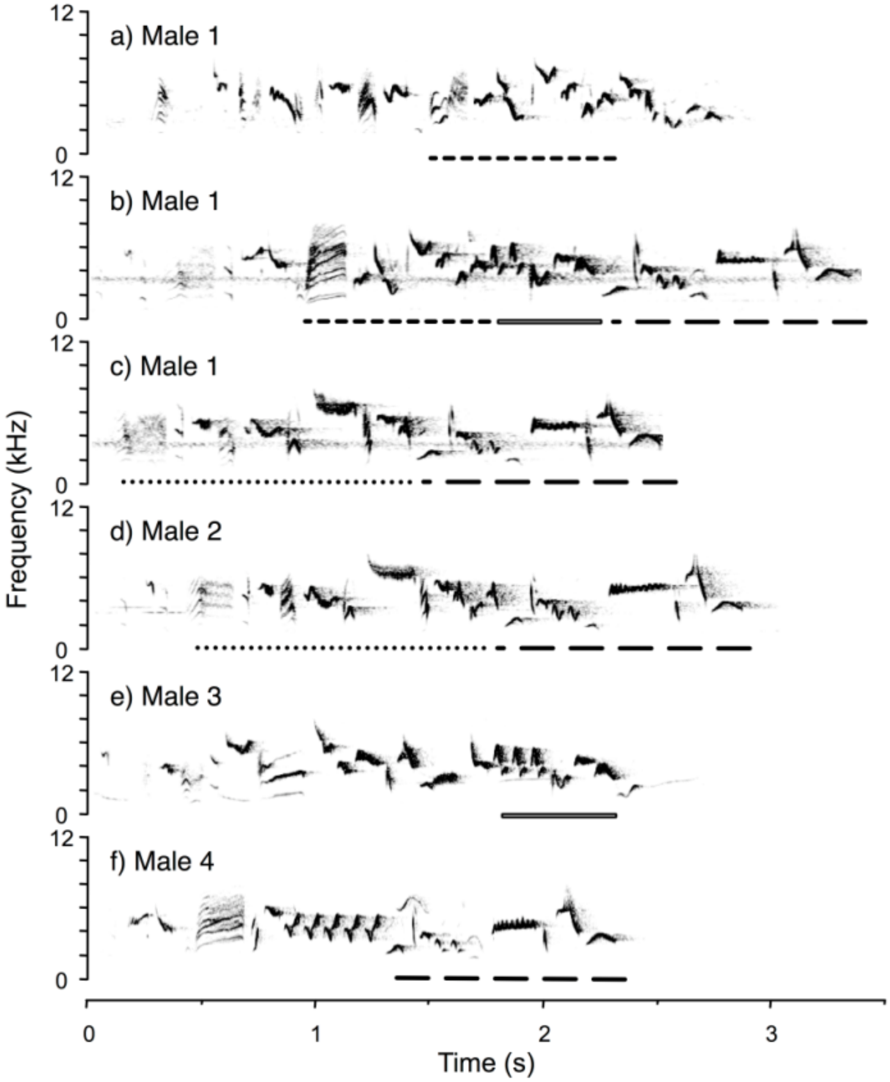

Figure 3.1. Sound spectrograms depicting songs recorded from five male Brown-throated Wrens. Underlined sections highlight some of the phrases that are shared within males (a–c, g–h) and between males (a–h). (c-d) A song type shared between two males

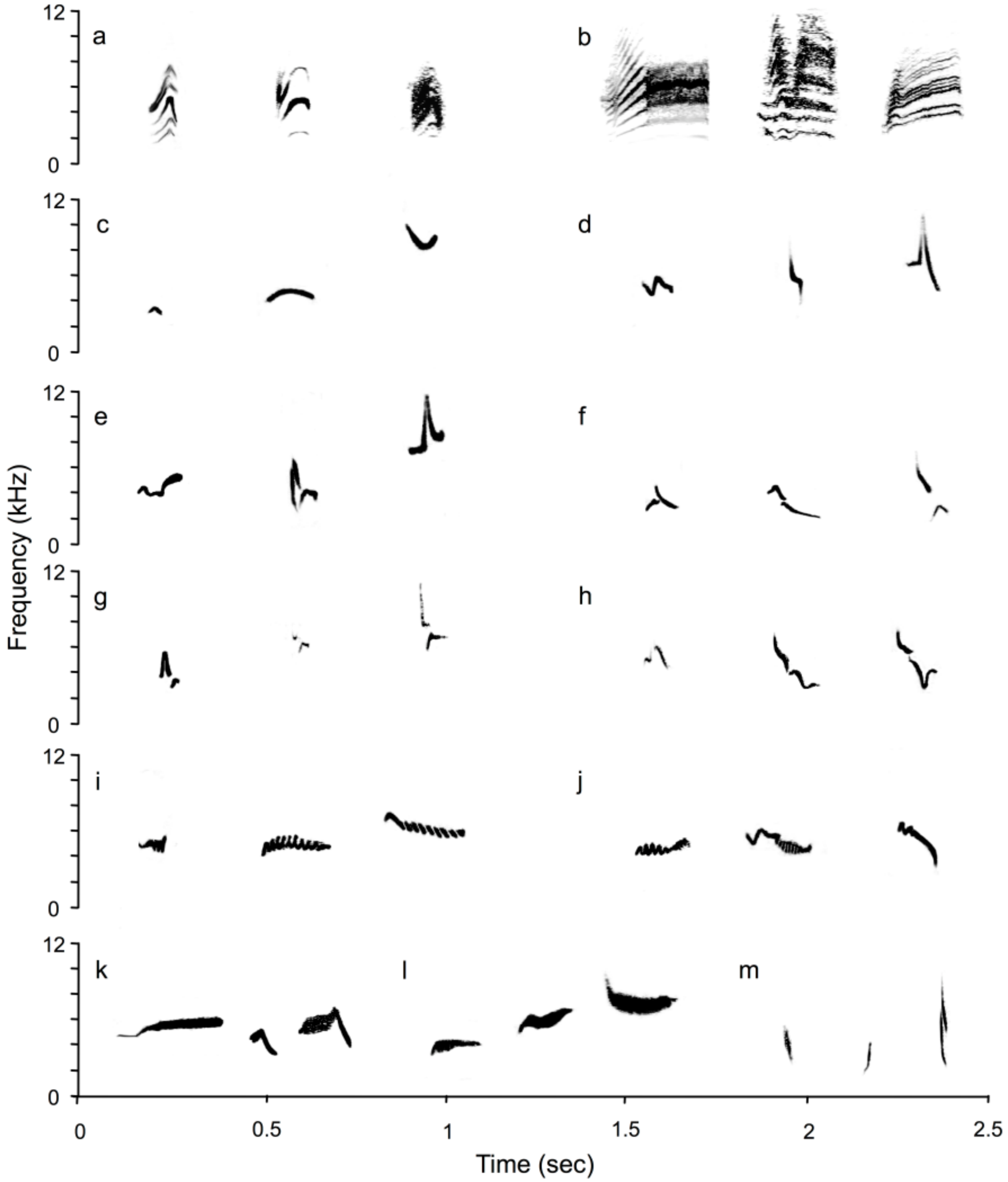

Figure 3.2. Sound spectrograms of the syllable catalogue of Brown-throated Wrens comprising 13 different syllable categories. Letters refer to the syllable identity

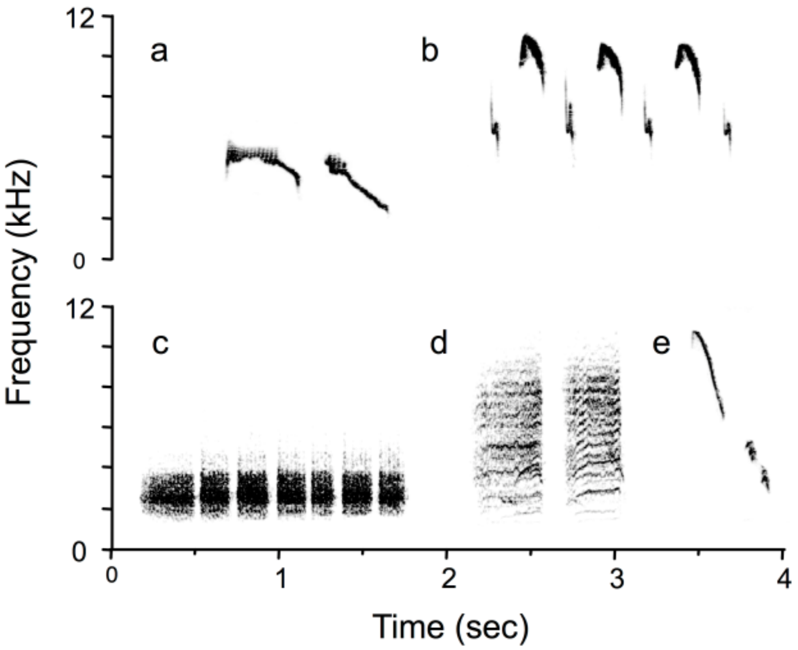

Figure 3.3. Sound spectrograms of the calls of the Brown-throated Wren. (a) A two-element whistle call; (b) a series of seee calls; (c-d) two examples of harsh calls; and (e) a downward series call

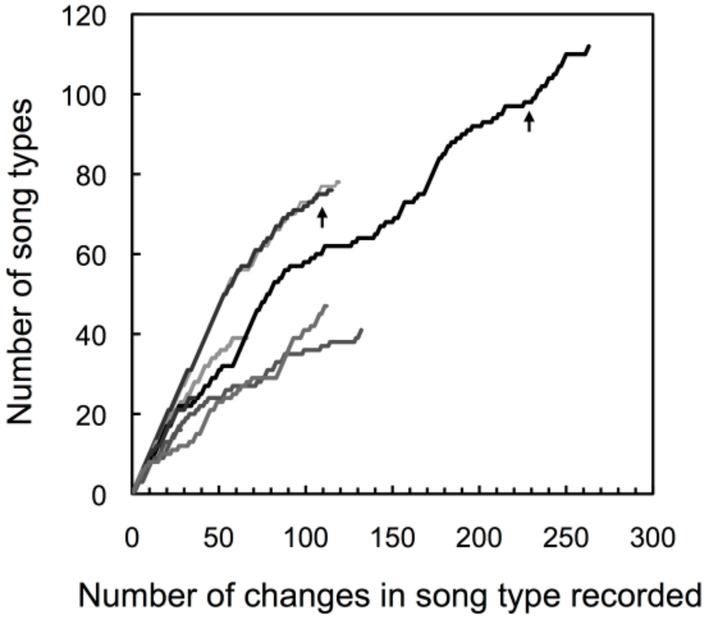

Figure 3.4. Repertoire asymptote accumulation curves showing the number of unique song types based on recording effort for nine male Brown-throated Wrens. The graph shows the cumulative song repertoire size plotted against the number of changes in song type recorded. Arrows show the repertoire asymptote accumulation curves for birds that were recorded in both 2010 and 2011

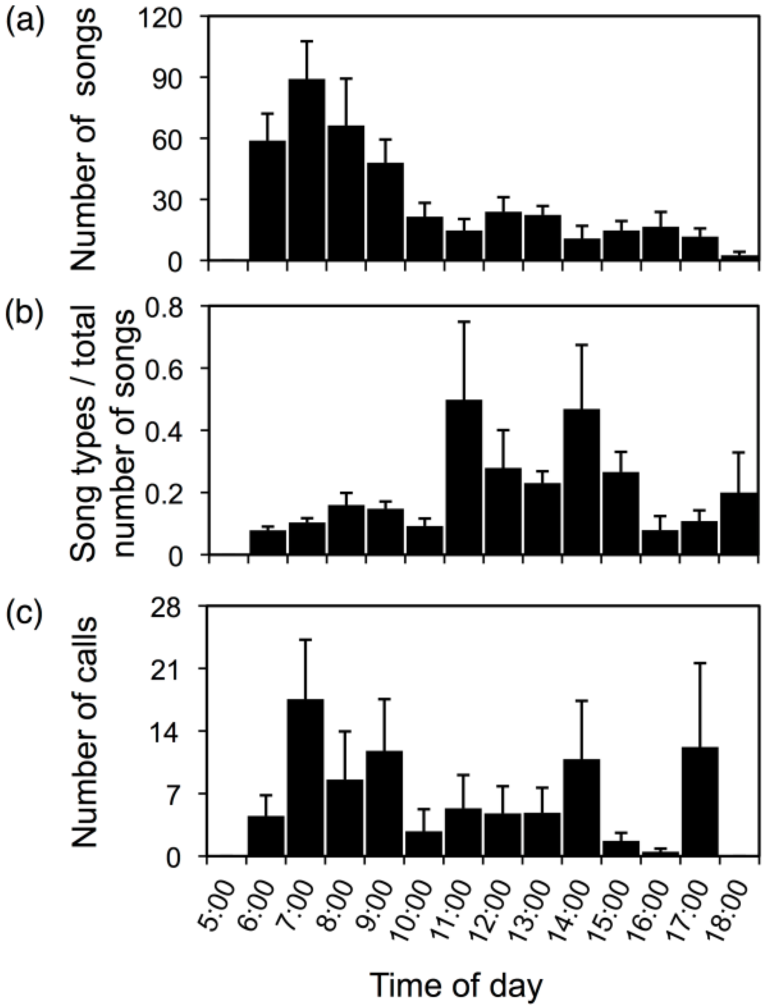

Figure 3.5. Vocal output of Brown-throated Wrens varies with time of day. (a) Mean number of songs per hour; (b) mean number of different song types divided by the number of songs sung per hour; and (c) mean number of calls per hour. Error bars show standard error. Sunrise occurred at 0605, and sunset at 1915

Chapter 4

Continent-wide patterns of divergence in acoustic and morphological traits in the House Wren species complex[*]

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

Chapter summary

Phenotypic traits are important for assessing differences between populations, especially in groups where taxonomy is poorly resolved. The House Wren complex is one such group that presents extensive taxonomic controversy and is thought to comprise many independent evolutionary units. Although the songs and morphological features of House Wrens show extensive variation, differences between populations have not been quantified. In this study, we assess variation in acoustic and morphometric traits within this complex, and compare patterns of variation to currently recognized subspecies boundaries. First, we compare songs and morphology among eight recognized House Wren subspecies (*Troglodytes a. aedon, T. a. parkmanii, T. a. cahooni, T. a. brunneicollis, T. a. nitidus, T. a. musculus, T. a. beani, T. a. rufescens*), controlling for significant effects of latitude. Second, we use variation in male song—a trait with an important role in mate choice and male-male competition—to assess divergence between House Wren subspecies. We compare variation between subspecies to variation across seven currently recognized *Troglodytes* species (*T. hiemalis, T. pacificus, T. tanneri, T. sissonii, T. cobbi, T. rufociliatus*, *T. ochraceus*). Our results, based on broad sampling of songs (*n* = 786) and morphological traits (*n* = 401) from 609 locations throughout the Americas, show that most of the subspecies examined diverge in song, morphology, or both. In addition, we show that the acoustic differences between subspecies are similar to, and in some instances greater than, the divergence between pairs of currently recognized species. Our study suggests that at least four allopatric subspecies—*T. a. nitidus, T. a. musculus, T. a. beani*, and *T. a. rufescens*—are likely different species, and identifies many other vocally and morphologically differentiated subspecies that may result in new species upon further detailed genetic analysis.

Introduction

Biologists are faced with the difficult task of estimating biological biodiversity. Current inferences of species diversity in many groups are likely underestimated (Wilson 2003). For instance, the number of avian lineages in the tropics is thought to be greater than currently recognized (Milá et al. 2012). Furthermore, recent findings of new bird species (e.g. Lara et al. 2012, Seeholzer et al. 2012, Hosner et al. 2013) along with revisions to the taxonomic status of many other species (e.g. Chesser et al. 2012, 2013), clearly indicate that that further research in this field is still required (Brumfield 2012).

One of the principal challenges for biologists when assessing diversity is to draw boundaries between species. This challenge is overcome by documenting phenotypic and genetic variation of organisms across geographic regions (Nyári 2007). The use of informative traits is crucial for delimiting species boundaries. Avian acoustic signals are important because they play a direct role in mate choice, male-male competition, and species recognition in many taxa (Catchpole and Slater 2008, Wilkins et al. 2013), thereby acting as pre-mating isolation barriers. It is not surprising, therefore, that research focusing on song as an important phenotype has produced significant insight into avian taxonomy (e.g. Toews and Irwin 2008, Alström et al. 2011, Campagna et al. 2012, Lara et al. 2012, Sosa López et al. 2013).

With well-known historical taxonomic problems and a distribution that includes most parts of the Americas (Figure 4.1), the House Wren complex (*Troglodytes aedon*) stands out as an ideal group to explore vocal geographic variation and its taxonomic implications. The American Ornithologists' Union (1998) currently recognizes 30 House Wren subspecies within this complex, however the number of subspecies varies among taxonomic authorities (e.g. Brewer 2001, Navarro-Sigüenza and Peterson 2004, Kroodsma and Brewer 2005, Clements et al. 2012, Gill and Donsker 2013). Several authorities agree that all subspecies can be clustered into five main groups on the basis of slight morphological and geographical differences (e.g. American Ornithologists' Union 1998, Clements et al. 2012). (1) The "aedon group" includes two subspecies: *T. a. aedon* in southeastern Canada and eastern United States, and *T. a. parkmanii* from southwestern Canada and central and western United States to Baja California, Mexico. (2) The "brunneicollis group" includes three subspecies: *T. a. cahooni* from the mountains of southern Arizona south to central Mexico, *T. a. brunneicollis* from the mountains of northeastern Mexico South of the mountains of Sierra Madre del Sur of Oaxaca, and *T. a. nitidus* in the mountains of Zempoaltepec, Oaxaca. (3) The "musculus group" includes 20 subspecies, populating most areas from central Mexico south to Tierra del Fuego, with some subspecies restricted to islands. (4) The "martinicensis group" includes six subspecies, each restricted to their own island in the Lesser Antilles: *T. a. guadeloupensis* in Guadeloupe, *T. a. martinicensis* in Martinique (probably extinct), *T. a. mesoleucus* in St. Lucia, *T. a. musicus* in St. Vincent and Grenada, and *T. a. rufescens* in Dominica. (5) The "beani group" includes only the subspecies *T. a beani* and is restricted to Cozumel Island in the Yucatan Peninsula, Mexico. Some taxonomic authorities treat most of these groups as full species. For example, Howell and Webb (1995) recognize the brunneicollis group and the beani group as full species, and both Navarro-Sigüenza and Peterson (2004), and Kroodsma and Brewer (2005) recognize the aedon group, brunneicollis group, musculus group, and beani group as full species. Clearly, there is little agreement on whether the major groups within this complex should be considered different species.

A series of recent genetic studies suggest that the aedon group, brunneicollis group, and musculus group have independent evolutionary trajectories (Brumfield and Capparella 1996, Rice et al. 1999, Martínez Gómez et al. 2005, see also Mann et al. 2006, Campagna et al. 2012) For instance, Brumfield and Capparella (1996) provide genetic data suggesting three or more distinct lineages—the aedon group, brunneicollis group, and musculus group—and place the brunneicollis group and the aedon group in the same clade, with the musculus group as the sister taxon. Rice et al. (1999) and Martínez Gómez et al. (2005) also support three distinct lineages, however, they placed the brunneicollis group as the sister taxon. These studies used different subspecies of the brunneicollis group in their analysis; Brumfield and Capparella (1996) used *T. a. cahooni,* whereas Rice et al. (1999) and Martínez Gómez et al. (2005) used *T. a. nitidus*. Interestingly, *T. a. cahooni* is thought to be sympatric with *T. a. aedon* in southern Arizona, while *T. a. nitidus* has an allopatric distribution, isolated in the mountains of Zempoaltepec, Oaxaca (Kroodsma and Brewer 2005). In another study, Campagna et al. (2012) suggested the existence of significant genetic differences within the aedon group, between *T. a. aedon* (eastern Canada) and *T. a. parkmanii* (western Canada), placing *T. a. aedon* and subspecies of the musculus group in the same clade, and *T. a. parkmanii* as the sister taxon. There is a lack of consensus as to whether the five currently recognized groups represent independent lineages, obscuring the taxonomy of this species complex.

Groups of *Troglodytes* that are restricted to islands (i.e. the beani group and martinicensis group) have received less attention than their mainland counterparts. Several authors suggest that the island taxa within this complex are likely to be distinct species based on their isolated distribution and on morphological differences in size as well as colour (Navarro-Sigüenza and Peterson 2004, Kroodsma and Brewer 2005). Recent research on the Falkland Islands suggests that this is true for Cobb's Wrens (*T. cobbi*; Campagna et al. 2012, Chesser et al. 2013, Remsen et al. 2013).

Together, these previous investigations reveal complicated relationships within the House Wren complex, and suggest the existence of several species, even within some of the five main groups. The vocalizations of House Wrens are known to exhibit substantial variation among these subspecies, but no study to date has empirically quantified the extent of bioacoustic variation (Johnson 1998). Similarly, morphometric variables are also suspected to vary in the House Wren complex, but no study has yet quantified this variation (Brewer 2001). A lack of behavioural and morphological data, and poor knowledge of genetic relationships, limits our ability to answer critical questions about the taxonomy of this group.

In this study, we assess geographic variation in acoustic and morphological traits across subspecies of the House Wren complex. Our first objective was to assess whether differences in phenotypic traits correspond to recognized subspecies of House Wrens. Our motivation was to validate House Wren subspecies divisions using both fine structural characteristics of male song as well as morphology. Our second objective was to understand the extent of song diversification between subspecies in the House Wren complex and provide a relative measure of song diversification to help improve the taxonomic classification of this group. To this end, we assessed the bioacoustic differences between subspecies of House Wrens that have an ambiguous taxonomic status, and compared the magnitude of these differences to that seen between currently recognized *Troglodytes* species.

Methods

Subspecies analyzed

In this study, we classified all recordings and morphological samples by subspecies using the taxonomy proposed by Kroodsma and Brewer (2005). We obtained acoustic recordings and morphological samples for eight subspecies: *T. a. aedon, T. a. parkmanii, T. a. cahooni, T. a. brunneicollis, T. a. nitidus, T. a. musculus, T. a. beani,* and *T. a. rufescens.* We pooled together all our acoustic and morphological data corresponding to the musculus group under the category of *T. a. musculus*, because of the lack of data on subspecies boundaries within this South American group. We also pooled together two possible subspecies recordings (but not morphological data) obtained from the Lesser Antilles into *T. a. rufescens*, because we did not have information on the island where these recordings were collected (for recording details see Table 4.S1 in the Appendix). This lack of geographic resolution in the Lesser Antilles is not ideal, but we felt it was important to include these recordings in our analysis because remarkably few recordings exist for these birds, and their taxonomic status is of great importance from a conservation perspective.

The distributions of some taxa within the complex are thought to overlap. For example, *T. a. parkmanii* and *T. a. cahooni* overlap in Southern Arizona (American Ornithologists' Union 1998, Kroodsma and Brewer 2005). For these groups, we only used recordings acquired during the breeding season to avoid confusing northern migrants with resident southern birds. To further avoid any mismatch in subspecies identification, recordings made above 1600 m elevation were considered to be from *T. a. cahooni*, whereas recordings recorded below this altitude were considered to be from *T. a. parkmanii* (Brewer 2001).

Acoustic analysis

Our sampling approach involved directly collecting recordings during field expeditions, and gathering existing recordings from 16 natural sound libraries and private collections (see Appendix Table 4.S1 and S2 for details). We collected recordings directly using three sets of equipment: a Marantz PMD660 digital recorder with a Sennheiser MHK67 shotgun microphone (recordings collected in WAV format; 44.1 kHz; 16 bits); a Marantz PMD660 digital recorder with a Telinga parabola with a Sennheiser ME62/K6 omnidirectional microphone (recordings collected in WAV format; 44.1 kHz; 16 bits); or a Nagra Ares-BB+

digital recorder with a Telinga parabola with a Stereo Pro 6 Telinga microphone (recordings collected in WAV format; 48 kHz; 16 bits).

We carefully scrutinized the recordings provided to us from libraries and private collections to avoid including more than one recording from the same individual. There were three instances in which we excluded recordings from our analysis: when multiple recordings clearly came from the same individual; when the identity of the bird in the recording was unclear and recordings were collected less than 1 hour apart; and when recordings were made on the same day but did not specify the recording time.

The recordings used in the analysis contained between 1 and 60 songs from the same individual. We randomly selected one song from each recording by generating a sequence of random numbers and matching the numbers with the number of the song in the recording. Each selected song was extracted and saved into a separate sound file with at least 0.5 s of silence at the beginning and the end. We created a spectrogram for every song using a 1024 point fast Fourier transform (FFT), 93.75% overlap, Blackman window, 22 Hz frequency resolution, and 2.9 ms temporal resolution. We applied a 1 kHz high-pass filter and measured all fine structural characteristics of the songs using AviSoft-SASLab Pro (version 5.2.04; R. Sprecht, Berlin, Germany).

We conducted the measurements using the automatic parameter measurements tool in Avisoft-SASLab Pro, thereby minimizing human subjectivity in collecting acoustic measurements. We detected the start and end of each element in the song by using a separation threshold of -25 dB relative to the maximum amplitude of the element; we distinguished separate elements when the amplitude dropped below the -25 dB threshold for ≥ 5 ms. Frequency variables were calculated using a threshold setting of -20 dB relative to the song's peak amplitude (see Figures 4.S1A, B in the Appendix), including all the peaks exceeding the threshold. Measurements based on the power spectrum, such as entropy (see below), were derived from the average spectrum across an entire element. *Troglodytes* songs start with a series of low amplitude introductory elements, and the threshold of automatic detection could not always detect these very quiet elements. In these cases, we selected the onset of the introductory section manually by looking at the first element on the spectrogram.

We quantified a total of 15 fine-structural features (depicted in Figure 4.S1 in the Appendix). The first set of measurements was conducted at the level of the individual element. (1) Element length (s): the average duration of each element within the song. (2) Inter-element interval (s): the average of the length of the silent space between elements, calculated as the average time from the end of the preceding element to the start of the current element for all the elements across the entire song. (3) Mean maximum frequency (kHz): the average of the maximum frequency of all elements within the song. (4) Mean bandwidth (kHz): the average of the bandwidth of every element within the song, calculated as the difference between the lowest (minimum frequency) and highest frequency (maximum frequency) for each element. (5) Mean peak frequency (kHz): the average of the peak frequency of all elements within the song (peak frequency was determined as the frequency with the highest amplitude in the power spectrum for each element). (6) Mean entropy: the average of the entropy measurement for each element within the song. (Note that the entropy parameter is a measure of the randomness of the sound with values ranging from 0 to 1; pure-tone elements have values close to 0 and noisy sounds have values close to 1). The second set of measurements was conducted at the level of the song. (7) Song length (s): the duration from the beginning of the first element to the end of the last element in the song. (8) Number of elements: total number of elements detected within the song. (9) Number of trills: we defined trill as a section of the song composed of a series of identical syllables repeated three or more times in a row (as in Catchpole and Slater, 2008, syllables can be composed of one or more elements, i.e. one or more continuous tracings on a sound spectrogram). (10) Minimum frequency (kHz): the lowest frequency with amplitude delimited by the threshold from the power spectrum of each element, and across the entire song. (11) Maximum frequency (kHz): the highest frequency with amplitude delimited by the threshold from the power spectrum of each element, and across the entire song. (12) Peak frequency shifts per second: number of times that the frequency peak switched between a value above and below 5.0 kHz, from one element to the next, sequentially across the entire song. We chose a threshold of 5.0 kHz because this was the mid-point between the mean minimum and mean maximum frequencies; we counted the number of switches and divided them by the song length. This is similar to the variable "transitions per second" used by Toews and Irwin (2008) and Campagna et al. (2012). The third set of measurements was conducted to describe the variation in frequency and entropy between elements in a song. (13) Standard deviation in maximum frequency (kHz). (14) Standard deviation in bandwidth (kHz). (15) Standard deviation in entropy.

Statistical analysis of acoustic data

To reduce the number of variables for analysis, and to avoid multicollinearity among variables in our analysis, we performed a principal component analysis (PCA), with Varimax rotation, on the acoustic measurements outlined above. The analysis resulted in five principal component factors with Eignevalues greater than one that together explained 74.8 % of the total variation in the original 15 acoustic variables. The first factor was strongly associated with maximum frequency, standard deviation in maximum frequency, standard deviation in bandwidth, and standard deviation in entropy; the second factor was strongly associated with mean maximum frequency, mean peak frequency, and peak frequency shifts per second; the third factor was strongly associated with song length, number of elements, and number of trills; the fourth factor was strongly associated with mean bandwidth and mean entropy; and the fifth factor was strongly associated with minimum frequency, element length, and inter-element interval (Table 4.1; detail of the correlation matrix is given in Table 4.S3 in the Appendix).

We then performed one-way analyses of covariance (ANCOVAs) to test whether subspecies differed from each other in the fine-structural characteristics of their songs (summarized by the five principal component factors), while controlling for variation in latitude (as a proxy for distance). In each analysis, we included one of the five principal component factors (Table 4.1) as the dependent variable. We used subspecies (i.e. *T. a. aedon, T. a. parkmanii, T. a. cahooni, T. a. brunneicollis, T. a. nitidus, T. a. musculus, T. a. beani,* and *T. a. rufescens*) as a fixed factor in the model, and latitude was entered as covariate. We then performed post-hoc tests between pairs of subspecies using sequential Bonferroni-adjusted correction for multiple comparisons ($\alpha < 0.05$; Holm 1979, Rice 1989). The second and fifth principal component factors were Log10 transformed prior to the analysis to improve normality (Quinn and Keough 2002).

Morphological analysis

Morphological characteristics like body size and beak shape are related to foreging substrate, feeding strategies and prey size (Miles et al. 1987, Carrascal et al. 1990), and therefore they are expected to reflect local adaptation to ecology, especially in isolated populations such islands (Ricklefs and Bermingham 2007). We gathered morphological data during field expeditions, and from specimens preserved in three museum collections: The American Museum of Natural History in New York, The Field Museum of Natural History in Chicago, and Museo de Zoología “Alfonso L. Herrera” in Mexico City (see

Tables 4.S4 and 4.S5 in the Appendix). Following Pyle (1997), we measured seven morphological characters: wing chord, and tail length at 1 mm accuracy, and tarsus length, exposed culmen length, culmen length, bill depth, and bill width at 0.1 mm accuracy. We then applied the same series of statistical analyses that were used for the acoustic analysis. First, we reduced the number of variables using a principal component analysis with Varimax rotation. The analysis resulted in two principal component factors with Eigenvalues greater than 1 that together explained 68.2 % of the total variation in the seven morphological variables. The first factor was strongly associated with tarsus length, exposed culmen length, culmen length, bill depth, and bill width; and the second factor was strongly associated with wing chord and tail length (Table 4.2; details of the correlation matrix is given in Table 4.S6 in the Appendix).

We then performed one-way ANCOVA analyses to test whether subspecies differ from each other based on morphological characters, while controlling for variation in latitude. In each analysis, we included one of the two principal component factors as the dependent variable (Table 4.2). We used subspecies (i.e. *T. a. aedon, T. a. parkmanii, T. a. cahooni, T. a. brunneicollis, T. a. nitidus, T. a. musculus, T. a. beani,* and *T. a. rufescens*) as fixed factor in the model, and latitude was entered as covariate. We then performed a post-hoc test between pairs of subspecies using sequential Bonferroni correction for multiple comparisons.

For both the song and morphology ANCOVA, residuals were normally distributed and all other assumptions were satisfied (Quinn and Keough 2002), except for homogeneity of regression slopes in the fifth acoustic factor and in the first morphological factor. The robustness of ANCOVA to deviation of homogeneity of regression slopes increases with sample size (Hamilton 1976), and owing to our large sample size, we consider our analysis to be robust to the violation of this assumption for these two factors.

Acoustic divergence

We performed an additional principal component analysis on all acoustic measurements, this time including the same eight House Wren subspecies in our original analysis, but adding measurements of the songs of seven recognized species: Cobb's Wren (*T. cobbi*), Clarion Wren (*T. tanneri*), Socorro Wren (*T. sisonnii*), Rufous-browed Wren (*T. rufocilliatus*), Ochraceous Wren (*T. ochraceus*), Winter Wren (*T. hiemalis*), and Pacific Wren (*T. pacificus*). The analysis resulted in four principal component factors with Eigenvalues greater than 1 that together explained 78.5 % of the total variation in the original 15 acoustic variables. The first factor was strongly associated with song length, minimum frequency, element length, number of elements, number of trills, mean bandwidth, and mean entropy; the second factor was strongly associated with maximum frequency, standard deviation in maximum frequency, standard deviation in bandwidth, and standard deviation in entropy; the third factor was strongly associated with mean maximum frequency, mean peak frequency, and peak frequency shifts per second; and the fourth factor was strongly associated with inter-element interval (Table 4.3).

We calculated acoustic divergence scores as the pair-wise distance between principal component factors for different wren taxa. To account for both the distance between group means as well as within-group variance, we used Cohen's *d* scores in this analysis, providing a more accurate estimate of divergence (Toews and Irwin 2008). We calculated Cohen's *d* as the difference between the two groups' mean principal component factor scores divided by the pooled standard deviation (Cohen 1992). We calculated these acoustic divergence scores between each of the eight House Wren subspecies and all other House Wren subspecies (e.g. *T. a. aedon* vs. all other House Wren subspecies pooled together, etc.). For comparison, we also calculated acoustic divergence scores between pairs of recognized species of *Troglodytes* wrens (e.g. *T. sissonii* vs. *T. tanneri*), selecting pairs of species that are known to be closely related: *T. ochraceus* vs. *T. rufocilliatus* (Martínez Gómez et al. 2005); *T. pacificus* vs. *T. hiemalis* (Toews and Irwin 2008); *T. cobbi* vs. *T. musculus* (Campagna et al. 2012); and *T. sissonii* vs. *T. tanneri* (two species restricted to adjacent islands off Mexico's Baja coast). We conducted this comparison for each of the four principal component factors that summarize variation in acoustic features.

All statistical analyses were carried out using PASW statistics (version 18.0; Chicago, IL, United States).

Results

We measured geographic variation in the songs and morphology of House Wrens across 609 different sites, comprising most of the geographic distribution of this species complex in North and South America. We gathered a total of 1065 recordings from different sources, and selected 786 recordings for analysis from different individuals (Figure 4.1; Tables 4.S1 and 4.S2 in the Appendix). Of the 786 recordings, 573 correspond to recordings of eight subspecies within the House Wren complex included in this study: *T. a. aedon* (*n* = 54), *T. a. parkmanii* (*n* = 103), *T. a. cahooni* (*n* = 45), *T. a. brunneicollis* (*n* = 14), *T. a. nitidus* (*n* = 24), *T. a. musculus* (n = 281), *T. a. beani* (*n* = 40), and *T. a. rufescens* (*n* = 12). The remaining 213 recordings correspond to the seven recognized species in the House Wren complex that are included in this study: *T. cobbi* (*n* = 12), *T. rufocilliatus* (*n* = 26), *T. sissonii* (*n* = 30), *and T. tanneri* (*n* = 41), *T. ochraceus* (*n* = 3), *T. hiemalis* (n = 65), and *T. pacificus* (n = 36).

For the morphological analysis, we gathered morphometric data from 401 *Troglodytes* skins, all from adult male specimens, corresponding to *T. a. aedon* (*n* = 19), *T. a. beani* (*n* = 12), *T. a. brunneicollis* (*n* = 10), *T. a. cahooni* (*n* = 32), *T. a. musculus* (*n* = 279), *T. a. nitidus* (*n* = 11), *T. a. parkmanii* (*n* = 29), and *T. a. rufescens* (*n* = 9; Tables 4.S4 and 4.S5 in the Appendix).

Song analysis

Song differed significantly between the currently recognized subspecies for all five principal component factors (Table 4.4). Descriptively, we found that both *T. a. parkmanii*, in United States and Canada, and *T. a. beani*, on Cozumel Island, have songs with higher scores for the first principal component factor, related to higher maximum frequencies, and larger variation in maximum frequencies, bandwidth, and entropy (Figure 4.2). North American subspecies (*T. a. aedon*, *T. a.parkmanii, T. a. cahooni, T. a. brunneicollis,* and *T. a. nitidus*) and *T. a. rufescens*, in Dominica, have songs with higher scores for the second principal component factor, related to higher mean maximum and peak frequencies, and higher numbers of shifts in peak frequency (Figure 4.2A). *T. a. aedon*, in the United States and Canada, and *T. a. rufescens*, in Dominica, have songs with higher scores for the third principal component factor, related to longer song lengths and higher numbers of elements and trills (Figure 4.2B). *T. a. rufescens*, in Dominica, have songs with higher scores for the fourth principal component factor, related to higher mean bandwidth and mean entropy (Figure 4.2C). *T. a. brunneicollis* and *T. a. nitidus,* in Mexico, *T. a. musculus,* in South America, *T. a. beani,* in Cozumel Island, and *T. a. rufescens*, in Dominica, have songs with highest scores for the fifth principal component factor, related to longer element duration and inter-element interval, and lower minimum frequencies (Figure 4.2D).

Variation in two of five acoustic principal component factors had a significant association with latitude (Table 4.4). Values of the third factor, related to song length and number of elements and trills, decreased significantly with latitude, from North to South (Figure 4.3A). Values of the fifth factor, related to element duration, inter-element interval, and minimum frequency, increased significantly with latitude reaching the highest values in Central America, and then decreasing towards South America (Figure 4.3B).

Post-hoc tests between subspecies following the ANCOVA on acoustic traits showed that *T. a. aedon, T. a. parkmanii. T. a. cahooni,* *T. a. beani*, *T. a. musculus* and *T. a. rufescens* were significantly different from each other and from all other subspecies, whereas differences in song between *T. a. nitidus* vs. *T. a. brunneicollis* were non-significant (Table 4.5).

Morphological analysis.—Morphological traits differed significantly between subspecies for the two principal component factors (Table 4.4). Descriptively, we found that *T. a. beani*, from Cozumel Island, has higher scores for the first principal component factor, related to longer tarsus length and beak characteristics, than other subspecies (Figure 4.4). North American subspecies (*T. a. aedon, T. a. parkmanii, T. a. cahooni, T. a. brunneicollis*, and *T. a. nitidus*) and *T. a. beani*, from Cozumel Island, have higher scores for the second principal component factor, related to longer wings and tails, compared to *T. a. musculus* and *T. a. rufescens* (Figure 4.4).

Variation in both morphological principal component factors showed a significant association with latitude (Table 4.4). Values of the first factor, related to tarsus length and beak morphology, increased significantly with latitude, reaching the highest values in Central America, and then decreasing towards South America (Figure 4.3C). Values of the second factor, related to wing and tail size, decreased significantly with latitude from north to south (Figure 4.3D).

The post-hoc tests following the ANCOVA on morphological traits showed that *T. a. parkmanii* and *T. a. beani* were significantly different from each other and all other subspecies in all pair-wise comparisons, whereas morphological differences between *T. a. aedon* vs *T. a. cahooni, T. a. aedon* vs *T. a. brunneicollis, T. a. cahooni* vs *T. a. brunneicollis, T. a. nitidus* vs *T. a. musculus, T. a. nitidus* vs *T. a. rufescens,* and *T. a. musculus* vs *T. a. rufescens* were significant for some comparisons and non-significant for others (Table 4.5).

Acoustic divergence

Average divergence scores between the songs of each subspecies and all other subspecies of House Wrens were substantial. For factor 1 we calculated an acoustic divergence score of 0.4 (range: 0.2 – 0.6); for factor 2: 0.5 (range: 0.3 – 1.0); for factor 3: 0.9 (range: 0.6 – 2.2); and for factor 4: 1.1 (range: 0.7 – 1.4; Figure 4.5). Average divergence scores between pairs of closely related species were similar for factor 1: 0.4 (range: 0.2 – 0.7); for factor 2: 0.6 (range: 0.1 – 1.1); for factor 3: 1.9 (range: 1.2 – 2.9); and for factor 4: 0.5 (range: 0.2 – 1.1; based on pair-wise comparisons of *T. cobbi* vs*. T. a. musculus, T. tanneri* vs*. T. sissonii, T. ochraseus* vs*. T. rufocilliatus,* and *T. hiemalis* vs*. T. pacificus*). Our results suggest that acoustic divergence between pairs of subspecies of House Wrens was on the same order as acoustic divergence between recognized species for factor 1 (summarizing variation in element length, mean bandwidth, mean entropy, song length, number of elements and trills, and minimum frequency), factor 2 (summarizing variation in maximum frequency, variation in maximum frequency, variation bandwidth, and variation in entropy), and factor 4 (summarizing variation in inter-element interval). In contrast, divergence within subspecies was less pronounced for factor 3 (summarizing variation in mean maximum frequency, mean peak frequency, and peak frequency shifts per second) for our subspecies-level comparisons relative to the species-level comparisons (Figure 4.5; see Table 4.S7 in the Appendix for full Cohen's *d* values for effect sizes of acoustic divergence).

Discussion

Based on acoustic data from 768 individuals from 373 different locations throughout the Western Hemisphere, as well as morphological data from 401 individuals from 236 different locations, we quantified acoustic and morphological variation among subspecies of the House Wren complex. Our results reveal marked differences between all subspecies of House Wrens after controlling for latitude (as a proxy for distance), showing that it is possible to distinguish between them using acoustic traits, morphological traits, or both. Our acoustic analysis showed that vocal divergence between many subspecies was comparable to, or stronger than, the vocal divergence between pairs of currently recognized *Troglodytes* species. Together these results suggest that at least four allopatric subspecies—*T. a. nitidus* (so called "Zempoaltepec Wrens"), *T. a. musculus* (so called "Southern House Wrens"), *T. a. beani* (so called "Cozumel Wrens"), and *T. a. rufescens* (so called "Dominica House Wrens")—may merit species status. In addition, our results shed light into the patterns of acoustic and morphological variation within the *Troglodytes aedon* complex, and have important implications for the taxonomy of this complex.

We found that most pairs of allopatric subspecies included in our analysis (e.g. *T. a. aedon* and *T. a beani*) have distinctive features to their songs. Divergent acoustic traits in allopatric populations have been reported to occur in many other bird species, with isolation as the most parsimonious explanation for this pattern (e.g. Vázquez-Miranda et al. 2009, González et al. 2011, Aleixandre et al. 2013, Sosa López et al. 2013, Campagna et al. 2012). For example, Cobb's Wrens (a close relative of House Wrens restricted to the Falkland Islands) are acoustically and genetically different from their continental counterparts (i.e. *T. a. musculus*, Campagna et al. 2012), as well as being morphologically different (Woods 1993). Moreover, experimental studies using playback have confirmed that divergent acoustic signals elicit different behavioural reactions in allopatric populations (e.g. de Kort and ten Cate 2001, Kirschel et al. 2009, Danner et al. 2011), supporting the idea that songs play a role in reproductive isolation.

Our morphological analysis shows that, despite general similarities, there are also significant differences between most pairs of allopatric subspecies (i.e. *T. a. nitidus,* and *T. a. beani)*. The general tendency of latitudinal increase in body size combined with a decrease in beak size in some subspecies of House Wrens (lowland subspecies*: T. a. aedon* and *T. a. parkmanii*, vs. highland subspecies: *T. a. cahooni, T. a. brunneicollis,* and *T. a. nitidus*; Figure 4.3), suggests that selection may be driving morphological divergence along latitudinal gradients (McCormack and Smith 2008, Milá et al 2010). Conversely, large beaks, such as those observed in *T. a. beani* on Cozumel Island, may be the result of relaxed competition for resources (Scott et al. 2003), a factor thought to drive divergence in beak size in island bird species (Boag and Grant 1984, Aleixandre et al. 2013). Other factors, such as drift, however, are also known to be related to divergence in morphological traits; future comparative studies can provide further insight into whether these factors are involved in morphological trait evolution in House Wrens.

Our results suggest that the subspecies of House Wrens with overlapping or abutting distributions have different songs (i.e. *T. a. aedon* vs. *T. a. parkmanii, T. a. parkmanii* vs. *T. a. cahooni*, and *T. a. cahooni* vs. *T. a. brunneicollis*), and morphology (i.e. *T. a. aedon* vs. *T. a. parkmanii* and *T. a. parkmanii vs T. a. cahooni*). Whether variation between sympatric populations represents extremes of a continuum, or whether there is a secondary contact zone between them, is still an open question. For instance, vocal divergence in populations with sympatric distribution has been reported in several studies and has often mirrored differences from genetic or playback analyses (e.g. Dingle et al. 2008, Toews and Irwin 2008, Vázquez-Miranda et al. 2009, Dingle et al. 2010, Sosa López et al. 2013). Determining the presence of a secondary contact zone is challenging, particularly in a group like the House Wrens, which exhibit very subtle phenotypic variation that might aid in differentiating multiple forms (as in Toews and Irwin 2008, for example). Future genetic analyses and playback studies will provide deeper insight into the differences between these taxa in both allopatry and sympatry, and the consequences of the acoustic differences with regards to species recognition.

Taxonomically, should some of these bioacoustically divergent groups of House Wrens be considered different species? Our data show that the allopatric subspecies *T. a. nitidus*, *T. a. musculus*, *T. a. beani*, and *T. a. rufescens* can be differentiated from each other acoustically, and from the rest of the subspecies; thus they may well be treated as different species under the phylogenetic concept (Nixon and Wheeler 1990). On the other hand, the biological species concept requires reproductive isolation between populations for the diagnosis of species (Mayr 1963), and further genetic analysis and playback experiments could help to clarify whether complete isolation exists between these taxa. In addition to being acoustically different from one another, we found that the allopatric *T. a. nitidus, T. a. musculus*, *T. a. beani*, and *T. a. rufescens* are as different from their counterparts as pairs of recognized *Troglodytes* wrens, adding support to the hypothesis that they represent reproductively isolated lineages. Whether sympatric subspecies represent independent evolutionary lineages is still an open question, and further genetic analysis is needed before making a clear taxonomic assessment.

An alternative explanation for the results of the acoustic divergence analysis (Figure 4.5) is that some of the recognized species that we used in this study may are incorrectly identified as species, and might be better understood as subspecies. We consider this alternative to be unlikely for several reasons. There is established acoustic and genetic evidense for two pairs of species used in our analysis that show that these pairs of taxa are indeed different species (*T. pacificus vs. T. hiemalis*, Towens an Irwin 2008; and *T. musculus vs. T. cobbi*, Campagna et al. 2012). In addition, other studies have found patterns of vocal divergence between allopatric species in the family suggesting more than one evolutionary unit occurring within species complex. These results have been later confirmed by genetic studies (see for example, *Hylorchilus navai vs H. sumichrasti*, Gómez de Silva 1997; *Thryothorus nicefori vs T. rufalbus*, Valderrama et al. 2007; *Henicorhina l. leucophrys vs H. l. hilaris*, Dingle et al. 2010).

While we lack genetic data to make a rigorous assessment of taxonomic status, it is relevant that many of these subspecies live in allopatry, and that they have distinctive acoustic traits. We do not intend to encourage species definitions based on phenotypic dissimilarity (Moritz and Cicero 2004), but instead we conclude that our data strongly suggest that the current taxonomy underestimates the real diversity within the House Wren complex. We believe that future genetic studies will distinguish some of the currently-recognized subspecies as full species. We encourage further taxonomic examination of both island populations and sympatric populations in the House Wren complex.

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Tables

Table 4.1. Loadings of the first five principal component factors summarizing 15 acoustic variables measured from 573 individuals of different subspecies of *Troglodytes aedon*. Eigenvalues and the percentage of variation explained are presented for each component, and variables with the strongest loading are shown in bold font.

	Factor 1[a]	Factor 2[a]	Factor 3[a]	Factor 4[a]	Factor 5[a]
Eigenvalues	3.17	2.75	2.02	1.81	1.45
Variance explained (%)	21.1	18.3	13.5	12.1	9.6
Factor loadings:					
Element length (s)	-0.16	0.33	0.06	-0.34	**0.42**
Inter-element interval (s)	0.02	-0.17	-0.18	-0.04	**0.8**
Mean maximum frequency (kHz)	0.28	**0.82**	0.03	0.42	0.03
Mean bandwidth (kHz)	0.3	0.35	-0.03	**0.77**	0.23
Mean peak frequency (kHz)	0.12	**0.9**	0.07	0.07	-0.04
Mean entropy	0.18	0.14	0.03	**0.83**	-0.13
Song length (s)	0.12	0.13	**0.79**	0.09	0.39
Number of elements	0.08	0.19	**0.89**	-0.01	-0.16
Number of trills	0.02	-0.07	**0.72**	-0.05	-0.18
Minimum frequency (kHz)	-0.05	0.42	-0.06	-0.33	**-0.53**
Maximum frequency (kHz)	**0.76**	0.42	0.16	0.16	-0.03
Peak frequency shifts per second	0.11	**0.74**	0.12	0.1	-0.2
Standard deviation in maximum frequency (kHz)	**0.92**	0.21	0.04	0.07	0.02
Standard deviation in bandwidth (kHz)	**0.91**	0.08	0.02	0.24	0.13
Standard deviation in entropy	**0.81**	-0.08	0.07	0.12	-0.12

[a] Principal component analysis was based on the correlation matrix shown in Table 4.S3 in the Appendix. Components with Eigenvalues > 1 were extracted. Factor scores were calculated using the regression method. The hypothesis that the correlation matrix contained only zero correlations was rejected (Bartlett's test: $\chi^2 = 6{,}066.9$, df = 105, $p < 0.001$).

Table 4.2. Loadings of the first two principal component factors summarizing seven morphological variables measured from 401 wren skins of different subspecies of *Troglodytes aedon*. Eigenvalues and the percentage of variation explained also are presented for each component. Bold font indicates factors with strong contributions to each principal component score.

	Factor 1[a]	Factor 2[a]
Eigenvalues	3.40	1.37
Variance explained (%)	48.6	19.6
Factor loadings:		
Wing chord	0.40	**0.75**
Tail length	-0.24	**0.81**
Tarsus length	**0.70**	0.27
Exposed culmen length	**0.86**	0.12
Culmen length	**0.86**	0.11
Bill depth	**0.76**	-0.11
Bill width	**0.78**	-0.18

[a] Principal component analysis was based on the correlation matrix shown in Table 4.S6 in the Appendix. Components with Eigenvalues > 1 were extracted. Factor scores were calculated using the regression method. The hypothesis that the correlation matrix contained only zero correlations was rejected (Bartlett's test: χ^2 = 1,316.5, df = 21, p < 0.001).

Table 4.3. Loadings of the first four principal component factors summarizing 15 acoustic variables measured from 786 *Troglodytes* wren songs, including subspecies of *Troglodytes aedon* and current recognized *Troglodytes* species. Eigenvalues and the percentage of variation explained also are presented for each component. Bold font indicates factors with strong contributions to each principal component score.

	Factor 1[a]	Factor 2[a]	Factor 3[a]	Factor 4[a]
Eigenvalues	4.21	3.47	2.89	1.15
Variance explained (%)	28.1	23.1	19.2	7.7
Factor loadings:				
Element length (s)	**0.83**	-0.29	0.28	0.01
Inter-element interval (s)	-0.11	-0.04	-0.18	**0.88**
Mean maximum frequency (kHz)	0.04	0.24	**0.94**	0.05
Mean bandwidth (kHz)	**-0.60**	0.48	0.32	0.31
Mean peak frequency (kHz)	0.38	-0.06	**0.84**	-0.09
Mean entropy	**-0.63**	0.43	0.16	0.17
Song length (s)	**0.87**	-0.11	0.29	0.15
Number of elements	**0.87**	-0.11	0.29	-0.11
Number of trills	**0.75**	0.03	0.04	-0.14
Minimum frequency (kHz)	**0.55**	-0.26	0.33	-0.32
Maximum frequency (kHz)	0.03	**0.77**	0.44	-0.09
Peak frequency shifts per second	0.16	0.04	**0.70**	-0.34
Standard deviation in maximum frequency (kHz)	-0.08	**0.91**	0.09	-0.04
Standard deviation in bandwidth (kHz)	-0.32	**0.89**	0.00	0.11
Standard deviation in entropy	-0.23	**0.80**	-0.17	-0.02

[a] Principal component analysis was based on the correlation matrix. Components with Eigenvalues > 1 were extracted. Factor scores were calculated using the regression method. The hypothesis that the correlation matrix contained only zero correlations was rejected (Bartlett's test: $\chi^2 = 12{,}329.8$, df = 105, $p < 0.001$).

Table 4.4. Summary of ANCOVA results for differences between *Troglodytes aedon* subspecies in both acoustical (n = 573) and morphological (n = 401) traits, using latitude as a covariate.

Dependent variables	Model	F	df	P	η^2	R^2 *adj.*
Acoustic Analysis						
Factor 1	Overall model	4.6	8	< 0.001	0.06	0.04
	Subspecies	4.8	7	< 0.001	0.05	
	Latitude	0.7	1	0.3	0.001	
Factor 2	Overall model	55.9	8	< 0.001	0.4	0.43
	Subspecies	35.9	7	< 0.001	0.3	
	Latitude	0.3	1	0.5	0.001	
Factor 3	Overall model	16.7	8	< 0.001	0.1	0.18
	Subspecies	11.6	7	< 0.001	0.1	
	Latitude	8.7	1	0.003	0.01	
Factor 4	Overall model	5.0	8	< 0.001	0.07	0.05
	Subspecies	6.1	7	< 0.001	0.07	
	Latitude	0.005	1	0.9	< 0.001	
Factor 5	Overall model	30.8	8	< 0.001	0.3	0.29
	Subspecies	30.8	7	< 0.001	0.2	
	Latitude	31.1	1	< 0.001	0.05	
Morphological Analysis						
Factor 1	Overall model	69.5	8	< 0.001	0.5	0.57
	Subspecies	76.4	7	< 0.001	0.5	
	Latitude	182.3	1	< 0.001	0.3	
Factor 2	Overall model	16.4	8	< 0.001	0.2	0.23
	Subspecies	10.8	7	< 0.001	0.1	
	Latitude	112.3	1	< 0.001	0.2	

Table 4.5. Results of post-hoc pairwise comparisons of divergence between subspecies of *Troglodytes aedon*. Pairs of subspecies were compared for both acoustic and morphological divergence, and the principal component factors that showed significant differences following sequential Bonferrioni correction are shown.

Subspecies	Acoustic differences?	Morphological differences?	Acoustic factors	Morphological factors
Sympatric pairs of taxa				
T. a. aedon vs *T. a. parkmanii*	Yes	Yes	Factor 3	Factor 1, 2
T. a. parkmanii vs T. a. cahooni	Yes	Yes	Factor 4, 5	Factor 1
T. a. cahooni vs T. a. brunneicollis	Yes	No	Factor 5	
Allopatric pairs of taxa				
T. a. aedon vs T. a. cahooni	Yes	No	Factor 3	
T. a. aedon vs T. a. brunneicollis	Yes	No	Factor 5	
T. a. aedon vs T. a. nitidus	Yes	Yes	Factor 5	Factor 1
T. a. aedon vs T. a. musculus	Yes	Yes	Factor 2, 3, 5	Factor 1
T. a. aedon vs T. a. beani	Yes	Yes	Factor 2, 3, 5	Factor 1
T. a. aedon vs T. a. rufescens	Yes	Yes	Factor 5	Factor 1
T. a. parkmanii vs T. a. brunneicollis	Yes	Yes	Factor 1, 4, 5	Factor 1
T. a. parkmanii vs T. a. nitidus	Yes	Yes	Factor 4, 5	Factor 1, 2
T. a. parkmanii vs T. a. musculus	Yes	Yes	Factor 2, 5	Factor 1, 2
T. a. parkmanii vs T. a. beani	Yes	Yes	Factor 2, 3, 5	Factor 1
T. a. parkmanii vs T. a. rufescens	Yes	Yes	Factor 5	Factor 1, 2
T. a. cahooni vs T. a. nitidus	Yes	Yes	Factor 5	Factor 1
T. a. cahooni vs T. a. musculus	Yes	Yes	Factor 2, 5	Factor 1, 2
T. a. cahooni vs T. a. beani	Yes	Yes	Factor 1, 2, 5	Factor 1
T. a. cahooni vs T. a. rufescens	Yes	Yes	Factor 3, 4, 5	Factor 1, 2
T. a. brunneicollis vs T. a. nitidus	No	Yes		Factor 1
T. a. brunneicollis vs T. a. musculus	Yes	Yes	Factor 1, 2	Factor 1
T. a. brunneicollis vs T. a. beani	Yes	Yes	Factor 1, 2, 3	Factor 1
T. a. brunneicollis vs T. a. rufescens	Yes	Yes	Factor 4	Factor 1
T. a. nitidus vs T. a. musculus	Yes	No	Factor 2, 4	
T. a. nitidus vs T. a. beani	Yes	Yes	Factor 2, 3	Factor 1, 2
T. a. nitidus vs T. a. rufescens	Yes	No	Factor 4	
T. a. musculus vs T. a. beani	Yes	Yes	Factor 2, 3	Factor 1
T. a. musculus vs T. a. rufescens	Yes	No	Factor 2	
T. a. beani vs T. a. rufescens	Yes	Yes	Factor 2, 3	Factor 1, 2

Figures


Eight *Troglodytes aedon* subspecies:
Western House Wren (*T. a. parkmanii*)
Eastern House Wren (*T. a. aedon*)
Cahoon's Wren (*T. a. cahooni*)
Brown-throated Wren (*T. a. brunneicollis*)
Zampoaltepec Wren (*T. a. nitidus*)
Cozumel Wren (*T. a. beani*)
Dominica House Wren (*T. a. rufescens*)
Southern House Wren (*T. a. musculus*)
T. a. parkmanii
T. a. aedon
T. a. brunneicollis
T. a. cahooni
T. a. beani
T. a. nitidus
T. a. rufescens
T. a. musculus
Seven comparison *Troglodytes* species:
■ Pacific Wren (*T. pacificus*)
▲ Winter Wren (*T. hiemalis*)
⬟ Clarion Wren (*T. tanneri*)
⬢ Socorro Wren (*T. sisonnii*)
★ Rufous-browed Wren (*T. rufocilliatus*)
● Ochraceous Wren (*T. ochraceus*)
◆ Cobb's Wren (*T. cobbi*)
13
11
9
7
5
3
1
0
0.5
1
1.5
2
2.5

Figure 4.1. (Previous page) Map of North and South America showing the recording locations for the songs of eight House Wren subspecies (*T. aedon*, open circles) and seven comparison species (filled symbols). The dark grey shaded area shows the breeding season distribution of *T. aedon*. Dashed lines indicate approximate boundaries between continental House Wren subspecies. The spectrograms at right depict one example of a male song from each of the eight subspecies analyzed in this study. The spectrograms at left depict one example of a male song from each of the seven species that were analyzed for comparison. For all spectrograms, the x-axis tick marks show increments of 0.5 seconds (note that the time axis varies between spectrograms, to maximize display area), and y-axis tick marks show increments of 1 kHz from 1 kHz to 13 kHz.

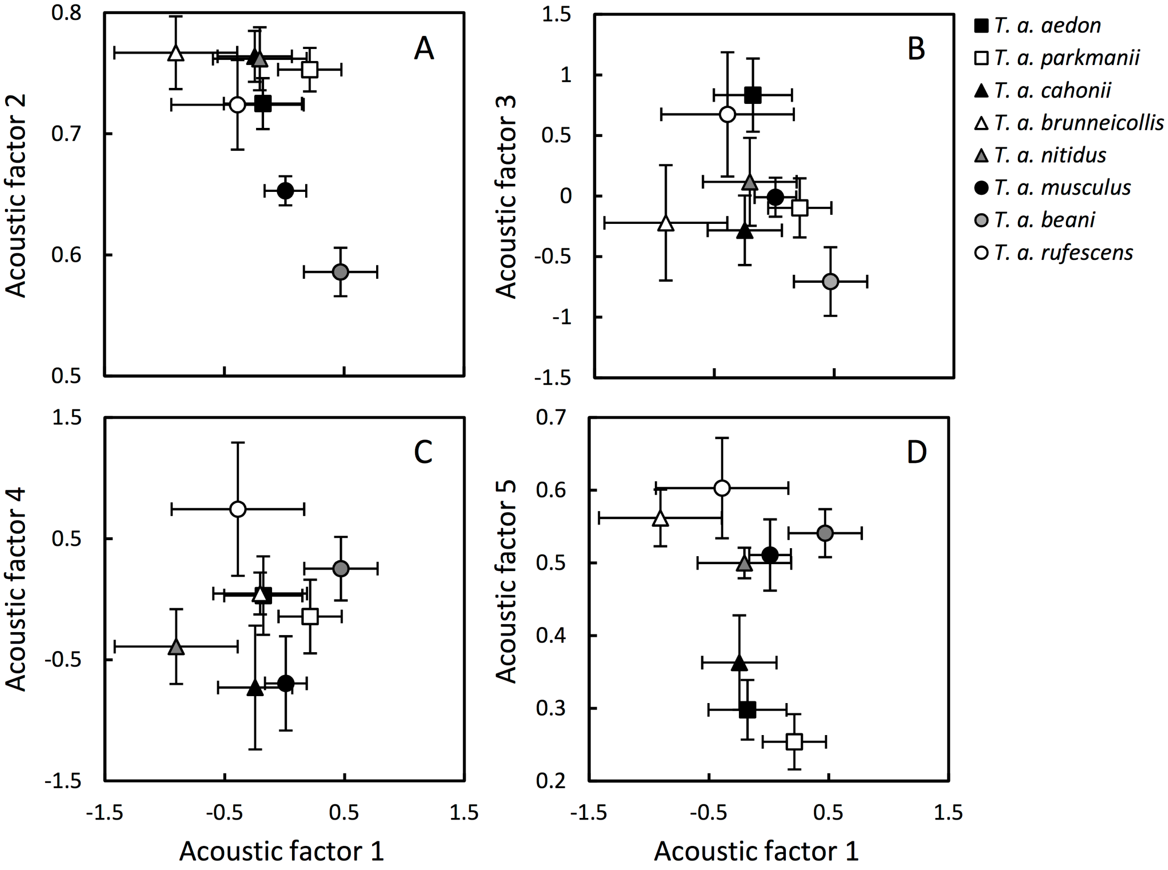

Figure 4.2. Acoustic variation between subspecies of House Wrens described by principal component factors that summarize variation in acoustic features of male songs. The first principal component factor is plotted against the second (A), third (B), fourth (C), and fifth (D). Points correspond to adjusted means after controlling for latitude. Bars indicate 95% confidence intervals.

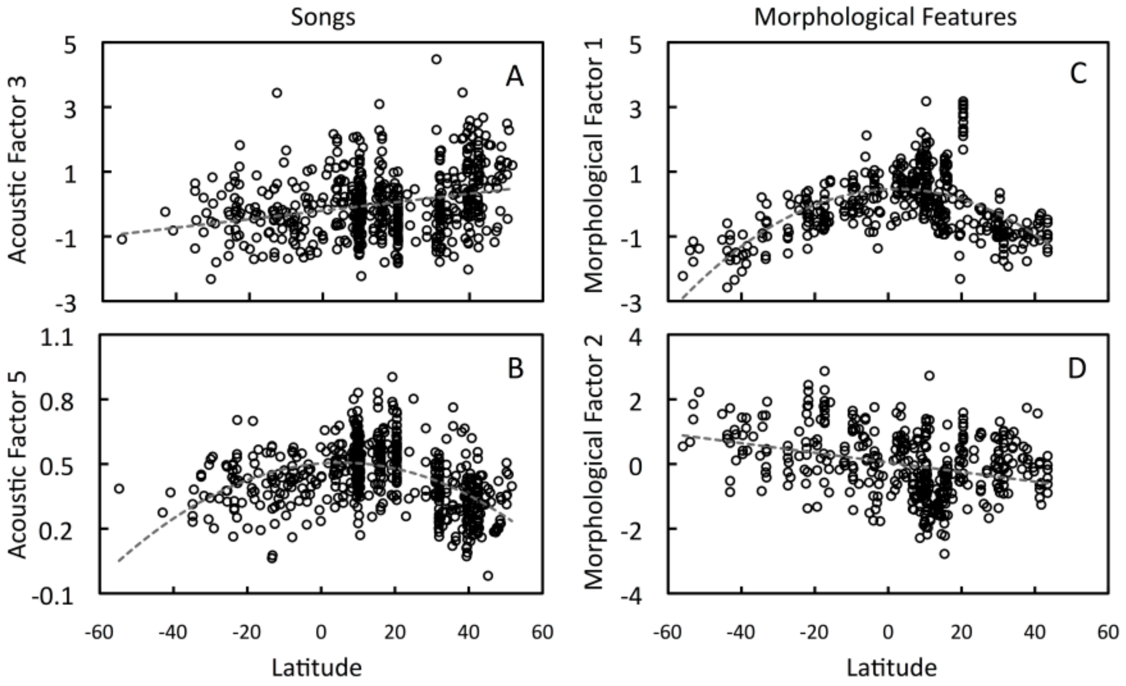

Figure 4.3. Songs and morphological features vary with latitude in subspecies of House Wrens. (A) Values of the third acoustic principal component factor, summarizing variation in song length, number of elements, and number of trills, decreases from north to south (linear regression: adjusted $R^2 = 0.07$, $F_{1,567} = 46.5$, $P < 0.001$). (B) Values of the fifth acoustic principal component factor, summarizing variation in element length, inter-element interval, and minimum frequency (cubic regression: adjusted $R^2 = 0.2$, $F_{3,556} = 68.8$, $P < 0.001$), and (C) values of the first morphological principal component factor, summarizing variation in tarsus length, exposed culmen length, culmen length, bill depth, and bill width (cubic regression: adjusted $R^2 = 0.3$, $F_{3,397} = 83.5$, $P < 0.001$), follow a similar pattern, with a increase in values from north to south, reaching the maximum values in Central America and then decreasing towards South America. (D) Values of the second morphological principal component factor, summarizing wing chord and tail length, decrease from north to south (linear regression: adjusted $R^2 = 0.1$, $F_{1,399} = 47.7$, $P < 0.001$).

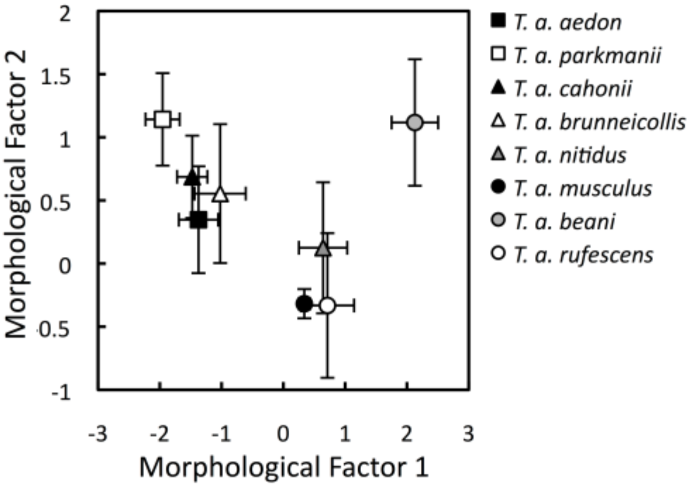

Figure 4.4. Morphological variation between subspecies of House Wrens described by principal component factors summarizing variation in morphological traits between the first two component factors. Points correspond to adjusted means after controlling for latitude. Bars indicate 95% confidence intervals.

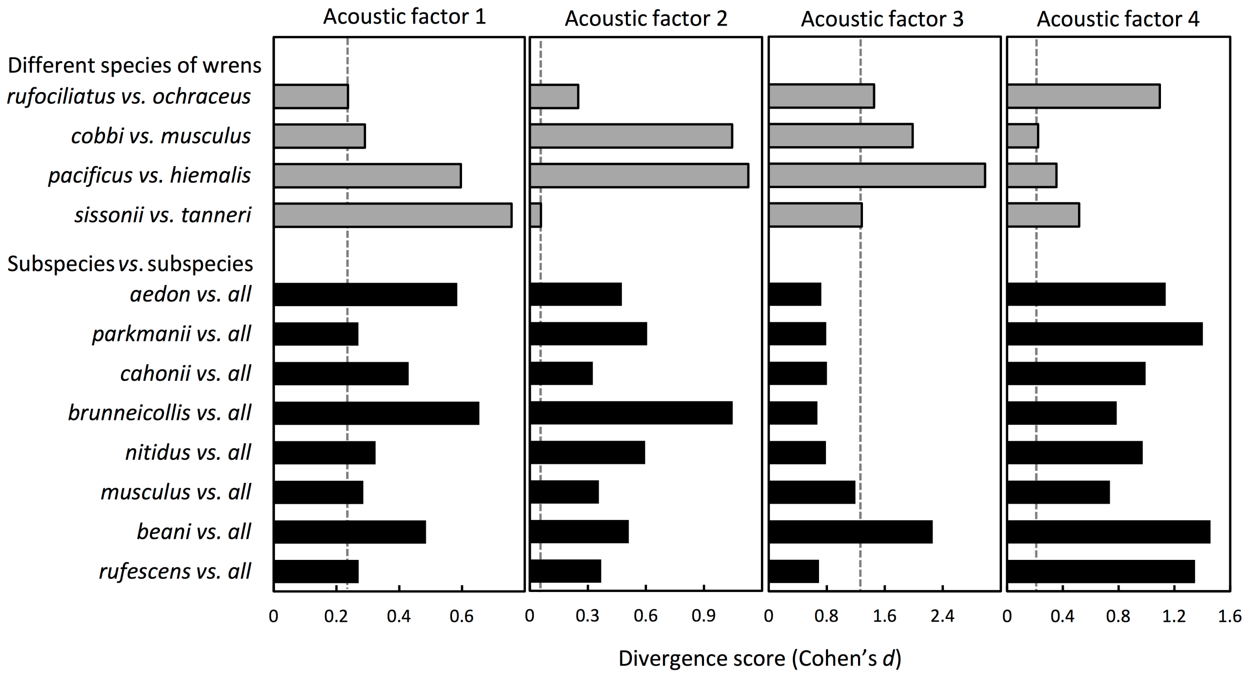

Figure 4.5. Graphs depicting divergence scores between pairs of closely related *Troglodytes* species (top) and between each subspecies of *Troglodytes* aedon and all other subspecies (bottom) for the first four principal component factors describing variation in acoustic features of males. Dashed lines indicate the lowest divergence score between pairs of closely-related *Troglodytes* wren species. Divergence scores are expressed as the difference in the two groups' means divided by the pooled standard deviations (i.e. Cohen's d).

Chapter 5

Responses to playback in four *Troglodytes* wren species are related to acoustic divergence and phylogenetic relatedness[*]

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

Chapter summary

Animals use acoustic signals to defend resources against rivals and attract breeding partners. As with many biological traits, acoustic signals reflect ancestry; closely related species often have more similar signals than do distantly related species. Whether this similarity in acoustic signals is biologically relevant to animals is poorly understood. We conducted a playback experiment to measure the physical and vocal responses of male songbirds to the songs of both conspecific and allopatric-congeneric animals that varied in their acoustic similarity and genetic similarity. Our subjects were territorial males of four species of neotropical *Troglodytes* wrens: Brown-throated Wrens *T. brunneicollis*, Cozumel Wrens *T. beani*, Clarion Wrens *T. tanneri*, and Socorro Wrens *T. sissonii*. Our results indicate that (1) acoustic divergence correlates with genetic distance; (2) wrens respond more strongly to playback of conspecific versus congeneric acoustic signals; (3) wrens respond with similar intensity to playback of different allopatric congeneric treatments; (4) acoustic similarity between subjects' songs and playback stimuli predicts the strength of behavioural responses to playback; and (5) phylogenetic distance between the subject species and playback stimulus species predicts behavioural responses. Collectively, these results demonstrate that the song similarity between wren species decreases with phylogenetic distance, and reveal that male *Troglodytes* wrens perceive divergence in fine structural characteristics of songs.

Introduction

Animal acoustic signals are important in resource defense and mate attraction, and consequently these traits are under strong forces of ecological and sexual selection (Coyne and Orr 2004, Wilkins et al. 2013). Differences in the acoustic signals of closely-related taxa have been studied in diverse animals, enhancing our understanding of the evolution of animal communication (Ryan 1986, Wilkins et al. 2013). Many investigations have revealed that variation in acoustic signals is consistent with phylogenetic relationships (e.g. Ryan and Rand 1999, Johnson et al. 2000, de Kort and ten Cate 2001, Price and Lanyon 2002, Ord and Martins 2006, Farnsworth and Lovette 2008), suggesting that acoustic signals have phylogenetically conserved components. However, the evolutionary processes that drive animal perception of phylogenetically conserved components of acoustic signals are not as well studied (Irwin and Price 1999). Whether closely related species perceive the phylogenetically conserved traits in acoustic signals remains poorly understood (Irwin and Price 1999, Grether 2011, Mendelson and Shaw 2012).

When two closely related species live in sympatry they may compete for limited resources. The ensuing acoustic competition may encourage divergence in acoustic signals—as well as receivers' sensitivity to those signals—leading to character displacement (Brown and Wilson 1956, Kirschel et al. 2009, Dingle et al. 2010). In allopatry, however, interspecific interactions between closely related species are eliminated, and consequently there is no such selection for divergence in acoustic signals or receiver sensitivity to those signals (Ryan and Rand 1999, de Kort and ten Cate 2001). Nonetheless, closely related species living in allopatry often exhibit divergent acoustic signals, possibly as a result of acoustic adaptation to different environments, sexual selection, or drift (Wilkins et al. 2013). Interestingly, several studies report that playback of allopatric-heterospecific signals still elicit behavioural responses; this behaviour is known as “false recognition” and it suggests that receivers are sensitive to phylogenetically constrained components of acoustic signals (e.g. Nevo and Capranica 1985, Coyne and Orr 1989, Ryan and Rand 1993, de Kort and ten Cate 2001, Ryan et al. 2003).

Animal signals are thought to elicit behavioural responses from receivers based on a minimum threshold of species-specific information (Emlen 1972, Höbel and Gerhardt 2003; but see Seyfarth et al. 1980, Evans et al 1993, Macedonia and Evans 1993). The amount of species-specific information contained in an acoustic signal may affect the intensity of the receiver’s response (Derriberry 2007, Parker et al. 2010, Danner et al. 2011). One important question in the study of receiver behaviour is whether individuals perceive variation in phylogenetically constrained signals (de Kort and ten Cate 2001). If receivers vary the intensity of their response with the amount of species-specific information contained in an acoustic signal, and if the amount of species-specific information varies with phylogenetic relatedness, we should expected animals to respond to the signals of both conspecific and heterospecific animals and we should expect response intensity to decrease with genetic distance. Under this scenario, animals are expected to falsely recognize signals of allopatric-congeners (de Kort and ten Cate 2001, Ryan and Rand 2003). Alternatively, receivers may perfectly discriminate between conspecific and allopatric-congener acoustic signals, especially if selection favours perception of acoustic traits that differ between species (e.g. Nelson and Marler 1989). Under this scenario, animals are expected discriminate between conspecifics and allopatric-congeners and will not falsely recognize signals of allopatric-congeners as rivals (de Kort and ten Cate 2001, Ryan and Rand 2003).

In this study, we investigate receiver responses to conspecific and allopatric-congeneric acoustic signals. We performed a series of playback experiments to territorial males of four species of neotropical wrens in the genus *Troglodytes*: (1) Brown-throated Wrens *T. brunneicollis* in the highlands of southern

Mexico; (2) Cozumel Wrens *T. beani* on Cozumel Island in the Caribbean Sea; (3) Socorro Wrens *T. sissonii* on Socorro Island in the Pacific Ocean; and (4) Clarion Wrens *T. tanneri* on Clarion Island in the Pacific Ocean. We used the songs of allopatric-congeneric species as stimuli, involving multiple *Troglodytes* species, and embracing a wide range of acoustic variation and phylogenetic relatedness (Figure 5.1). Although the songs of all wrens in the genus *Troglodytes* share similar characteristics overall, our previous findings indicate that the songs of all species included in this study are significantly different in their fine structural acoustic features (Chapter 4). We played back songs to territorial male wrens of each species, including conspecific songs, allopatric-congeneric songs, and songs of an unrelated bird as a control stimulus. We then measured the vocal and physical behaviour of the territorial subjects to these playback treatments. Our analysis has four parts:

(1) In the first part of this study, we compare the acoustic divergence between eight species of *Troglodytes* wrens (the four playback subject species and four additional species used as stimuli) and we assess the association between acoustic divergence and phylogenetic distance. An association between acoustic divergence and phylogenetic distance would indicate the presence of phylogenetically conserved traits in *Troglodytes* songs (de Kort and ten Cate 2001).

(2) In the second part of this study, we ask whether *Troglodytes* wren responses to playback vary between conspecific versus congeneric songs. If wrens falsely recognize signals of allopatric-congeners, we predicted that wrens should respond more vigorously towards conspecific stimuli, with low response levels directed at playback simulating an unrelated species (i.e. the control stimulus), and with intermediate or high response levels directed at playback simulating a congeneric species. Conversely, if wrens do not falsely recognize signals of allopatric congeners, we predicted that wrens should respond

more vigorously towards conspecific stimuli, with the low response levels directed at playback simulating both congeneric species as well as an unrelated species (i.e. the control stimulus).

(3) In the third part of the study, we ask whether *Troglodytes* wrens' responses to playback are influenced by acoustic similarity. We assess the acoustic divergence between the song of the subjects and the playback stimuli, and test for an association between acoustic similarity and intensity of response to playback. If the amount of species-specific information contained in the songs of *Troglodytes* wrens elicits a proportional response in the receiver, we predicted that playback response intensity would correspond to acoustic similarity, with stronger responses towards similar stimuli and decreasing intensities of response towards dissimilar stimuli. Conversely, if wrens perfectly discriminate between conspecific and allopatric-congeneric acoustic signals, we predicted that wrens would show strong responses towards similar conspecific stimuli, and weak responses towards allopatric-congeneric stimuli.

(4) In the fourth and final part of the study, we explore the association between *Troglodytes* wren responses to playback and the degree of genetic relatedness between the subject species and the stimulus species. We estimate the phylogenetic relatedness between the subject species and the stimulus species as the amount of divergence between the two lineages based on a recent, well-supported phylogenetic analysis (Chaves et al. 2014). An association between the intensity of playback responses and the phylogenetic distance between subject and stimulus species would indicate the presence of phylogenetic constraints in perceptual mechanisms (de Kort and ten Cate 2001).

This investigation sheds light on a poorly studied area in the evolution of animal communication. Very few studies have attempted to address whether behavioural responses to signals and phylogenetic relationships are associated; existing data come from experimental analysis of frogs (Ryan et al. 2003), and non-learning bird species (i.e. doves; de Kort and ten Cate 2001). By studying the responses to conspecific and congeneric signals in allopatric species, we eliminated both the effects of interspecific competition (e.g. Grant and Grant 2010, Amézquita et al. 2011), and the role of previous experience in differential responses (Grant and Grant 1997). In addition, our study provides auto-replication by testing four different related species. Experiments evaluating whether animals perceive differences in signals that exhibit a phylogenetic signature are needed in order to understand how species-specific signals are perceived.

Methods

Study species and locations

The distribution of *Troglogytes* wrens in the New World ranges from the far north of North America, in Alaska and northern Canada, to the southern tip of South American, in Chile and Argentina, with a centre of diversity in the tropics (Brewer 2001). We conducted playback experiments to four *Troglodytes* wren species: Brown-throated Wrens (*T. brunneicollis*; specifically, we studied the subspecies *T. b. nitidus* which may be a separate species from the other two subspecies of Brown-throated wren; Chaves et al. 2014), in the mountains of southwestern Mexico in Guerrero and Oaxaca; Socorro Wrens (*T. sissonii*) and Clarion Wrens (*T. tanneri*), on the deep Pacific oceanic islands Socorro Island and Clarion Island in the Archipelago of Revillagigedo in Mexico; and Cozumel Wrens (*T. beani*) on Cozumel Island off the northeastern coast of the Yucatan Peninsula in Quintana Roo, Mexico. These four wren species are allopatric with respect to all of their congeners (Kroodsma and Brewer 2005). The number of species within the genus *Troglodytes* has been debated for many years (Howell and Webb 1995, American Ornithologists' Union 1998, Navarro-Sigüenza and Peterson 2004, Kroodsma and Brewer 2005, Chapter 4). In this study, we followed the taxonomic system used by Kroodsma and Brewer (2005) that suggests the existence of 11 species within the genus. A recent genetic study suggests the existence of 42 independent evolutionary lineages (Chaves et al. 2014). Although the number of species within this genus is still under debate, genetic and acoustic data (Campagna et al. 2012, Chaves et al. 2014, Chapter 4) confirm that our four focal wren species are indeed different species (see also Brumfield and Capparella 1996, Rice et al. 1999, Martínez Gómez et al. 2005; Figure 5.1).

We studied Brown-throated Wrens in San Mateo Rio Hondo, Oaxaca, Mexico (6° 07'N, -96° 56'W) from June 9 to 15, 2011; Cozumel Wrens on Cozumel Island, Quintana Roo, Mexico (20° 24'N, -87° 00'W) from May 25 to 30, 2011; Socorro Wrens on Socorro Island, Revillagigedo Archipelago, Colima, Mexico (18° 46', -110° 57'W) from August 7 to 17, 2011; and Clarion Wrens on Clarion Island, Revillagigedo Archipelago, Colima, Mexico (18° 20'N, -114° 44'W) from December 10 to 19, 2011. Our playback experiments involved a total of 61 male subjects (15 Brown-throated Wrens, 16 Cozumel Wrens, 16 Clarion Wren, and 14 Socorro Wrens). To distinguish individuals in the field, we caught most experimental males using mist nets and banded them with a unique combination of coloured leg bands; we distinguished between the unbanded males on the basis of their territory position. We conducted playback experiments during the breeding season in all four species. We confirmed the birds' breeding status on the basis of observing brood patches or cloacal protuberances when banding birds, or observing nest building or nestling feeding.

Playback design

We used playback to simulate the acoustic signals of both conspecific and congeneric wrens. Our playback experiment consisted of five or six treatments for each subject, as summarized in Table 5.1. Treatment A was conspecific song, treatments B through E were congeneric songs, and treatment F was a heterospecific control. Congeneric treatment songs were: Brown-throated Wren, Cozumel Wren, Clarion Wren, Socorro Wren, Pacific Wren (*T. pacificus*), Rufous-browed Wren (*T. rufociliatus*), Southern House Wren (*T. musculus intermedius*), and Western House Wren (*T. aedon parkmanii*). The heterospecific control treatment for all subjects was Black Catbird (*Melanoptila glabrirostris*). Our intention was to present subjects with stimuli from multiple allopatric congeners that varied in their degree of acoustic divergence and genetic divergence. Therefore, each subject species received a slightly different set of congeneric species (Table 5.1). For each subject species we included stimuli from the main two mainland groups of *Troglodytes* wrens (Western House Wren from North America and Southern House Wren from Central and South America), as well as species that are possible sister species (Table 5.1). Black Catbirds, in the Mimidae family, are not closely related to the Troglodytidae family (Barker 2004, Lovette et al. 2012), thus we assumed Balck Catbirds to equally related to all four *Troglodytes* species. Black Catbirds are also allopatric to three of the four species. Although Cozumel Wrens live in sympatry with Black Catbirds, they do not respond to vocalizations of the later species.

Every subject received the six playback treatments during a 63-min playback trial. The first 3-min was a pre-playback silent period. The experimental period was divided into six sections, each with a 2-min stimulus followed by 8-min of silence. During the playback and the first 3-min of silence following the playback, we assessed the response of subjects; we treated the following 5-min as a recovery period before the next stimulus was broadcast. Our preliminary observations in the field indicated to us that 5-min recovery periods were sufficient for wrens to cease interacting with the playback loudspeaker and return to their pre-playback activities. All four species received six treatments, except for Cozumel Wren which received five treatments (see table 1), and therefore playback trials were 53-min for this species.

Stimuli were obtained from personal field recordings by JRSL and from the Macaulay Library of Natural Sounds (ML) and the Borror Laboratory of Bioacoustics (BLB). Each stimulus consisted of a single exemplar of a given song type (approximately 3 sec length) repeated every 7 sec for 2 min. Song rates of the stimuli were held constant across all treatments to all four species. Each focal animal received playback of stimuli recorded from a different individual, although some stimuli were used more than once in playback to different species (e.g. one male Socorro Wren may have been used for a conspecific stimulus to a different Socorro Wren, and a congeneric stimulus to a Clarion Wren, etc.). We avoided the use of neighbouring males' songs as stimuli and did not test adjacent males on the same or subsequent day. We normalized the amplitude of the playback stimuli at -1 dB, so that amplitude was held constant across all treatments and trials, and filtered them using 1 kHz high-pass filter in Adobe Audition (v2.0, Adobe, San Jose, CA, U.S.A.). In choosing the order of the presentation of treatments within trials, we followed a Latin square design (Ott and Longnecker 2001).

In total, we used 166 different birds' songs across nine species for creating stimuli (total number of songs per species: Black Catbird = 18, Brown-throated Wren = 18, Clarion Wren = 20, Cozumel Wren = 16, Pacific Wren = 15, Rufous-browed Wren = 15, Socorro Wren = 28, Southern House Wren = 18, and Western House Wren = 18).

Playback experiments

We positioned a loudspeaker (Foxpro Scorpion model X1-A) near the centre of the territory of the experimental subject at a height of 1.8 m. To facilitate distance estimation of the responding male to the loudspeaker, we marked each cardinal direction at 1 m, 5 m, 10 m, and 20 m from the loudspeaker. We standardized the speaker amplitude at -90 dB measured at 1 m form the speaker using a RadioShack sound level meter (model 33-2055), which resulted in wren songs that appeared to be broadcast at a natural amplitude based on our comparison to live birds in the field. We recorded the behaviour of the territorial males as they responded to playback using a Marantz PMD660 digital recorder (WAV format; 44.1 kHz; 16 bits) with two microphones, each connected to a different channel; an observer quietly dictated the subjects' behaviour into a Sennheiser MHK67 shotgun microphone in one channel, and a Telinga parabola with a Sennheiser K6/ME62 omnidirectional microphone was used to record the songs of the subject and the loudspeaker in another channel. A field assistant helped to quantify the behaviour of the subjects as they responded to playback. The field assistant was not familiar with the songs of the species being broadcast in the experiment, and was blind to the order of the stimuli. Both observers were positioned at distances of 20 to 25 m from the speaker.

We quantified the subjects' responses to playback in terms of vocal responses and physical responses. We quantified three aspects of male vocal responses: (1) total number of songs produced in response to playback; (2) number of songs in the longest bout of song produced by the subject (we define a bout of songs as the series of songs delivered by the focal bird with an inter-song interval not greater than 10 sec); and (3) latency from the start of playback to the subject's first song (in seconds). We quantified four aspects of male physical responses: (4) number of passes over the speaker; (5) closest approach to the speaker (in meters); (6) latency to the first movement towards the speaker (in seconds); and (7) time spent within 10 meters of the speaker.

We reduced the number of response variables by performing a principal component analysis using the response data from all four species (*n* = 61 subjects). We conducted the principal component analysis using the four physical response variables and the three vocal response variables. We used Varimax rotation and selected principal component factors with eigenvalues ≥ 1, resulting in two factors that together explained 68.9% of the total variation present in the measurements. The first principal component factor, related to physical responses, explained 37.7% of the total variation; the second principal component factor, related to vocal responses, explained 31.2% of the variation (Table 5.2).

Acoustic similarity between stimulus and subject species

To investigate whether acoustic similarity had an effect on the response of an individual, we calculated acoustic similarity measurements, comparing the fine structural details of the birds' songs in multi-dimensional space. The recordings for assessing acoustic distance were collected and analyzed following the methods described in Chapter 4. We measured acoustic properties of one song from each of the 166 stimuli used in the playback experiments. In addition, we included songs of 135 individuals of the four subject species, including most of the birds in our study populations that we recorded during field expeditions and from libraries of natural sounds (number of additional songs per species: Brown-throated Wren = 24, Cozumel Wren = 40, Clarion Wren = 41, Socorro Wren = 30). We created high quality spectrograms of these 301 songs (1024 FFT, 93.75% overlap, Blackman window, 22 Hz frequency resolution, and 2.9 ms temporal resolution, using 1 kHz high-pass filter), and measured 15 fine structural characteristics of the songs using the automatic parameter measurements tool in AviSoft-SASLab Pro (version 5.2.04; R. Sprecht, Berlin, Germany). We measured: (1) song length (s), (2) minimum frequency (kHz), (3) maximum frequency (kHz), (4) element length (sec), (5) number of elements, (6) number of trills, (7) silence (sec), (8) mean maximum frequency (kHz), (9) mean bandwidth (kHz), (10) mean peak frequency (kHz), (11) mean entropy, (12) peak frequency shifts per second, (13) standard deviation in maximum frequency (kHz), (14) standard deviation in bandwidth (kHz), and (15) standard deviation in entropy (for details and a spectrographic example, see Figure 4.S1 in the Appendix).

We reduced the number of fine structural characteristic measurements by performing a principal component analysis, with Varimax rotation, on the 15 acoustic measurements outlined above. We selected all principal components with eigenvalues ≥ 1 and extracted four component factors that together explained 76.8% of the total variation of the original 15 acoustic variables (Table 5.3). The first factor explained 21.7% of the variation and was related to minimum frequency, silence, mean maximum frequency, mean peak frequency and peak frequency shifts per second; the second factor explained 20.1% of the variation and was related to song length, element length, number of element, and number of trills; the third factor explained 19.4% of the variation and was related to maximum frequency, standard deviation in maximum frequency, standard deviation in bandwidth, and standard deviation in entropy; and the fourth factor explained 15.5% of the variation and was related to mean bandwidth, and mean entropy. We plotted each song on these four axes, and assessed acoustic similarity as the Euclidean distance between each stimulus (a total of 166 stimuli) and the centroid of the cluster for each subject species (calculated on the basis of the 135 subject species' recordings).

Genetic distance between stimulus and subject species

To calculate genetic distances between *Troglodytes* species, we used the phylogenetic hypothesis proposed by Chaves et al. (2014). Chaves et al. (2014) documented the existence of 42 lineages within the genus *Troglodytes*, representing genetically and geographically independent evolutionary units (i.e. phylogroups; Avise et al. 1998). This molecular analysis was based on a thorough geographical sampling that included three mitochondrial and eight nuclear markers, and featured a high level of phylogenetic support (>95% maximum likelihood bootstrap values; Chaves et al. 2014). We calculated the genetic distance between different species of *Troglodytes* as the genetic divergences between pairs of lineages

(Paradis 2012), by analyzing the topology and metrics of the phylogenetic tree reported in Figure 5.2. in Chaves et al. (2014). Using TreeSnatcher Plus software (Laubach et al. 2012), we calibrated our measurements using the scale provided in the figure, and then generated a file with information on the tree structure and branch lengths; we used this file to calculate pairwise patristic distances (i.e. distance along the horizontal branches of the shortest path connecting two species; Paradis 2012) in the R package adephylo (version 1.1-5; Jombart and Dray 2008, Paradis 2012, R Core Team 2013).

Statistical analyses

To test whether divergence in songs of *Troglodytes* wrens is related to genetic distance, we performed a Mantel test using matrices representing acoustic and genetic differences between species. The matrices had pairwise divergence values for the eight *Troglodytes* species under analysis: Brown-throated Wren, Clarion Wren, Cozumel Wren, Pacific Wren, Rufous-browed Wren, Socorro Wren, Southern House Wren, and Western House Wren. We created the acoustic matrix by calculating the Euclidean distance on the basis of the average value for each species of the four principal component factors summarizing acoustic measurements described above. We created the genetic matrix by calculating the pairwise patristic distance between species as described above. We computed Mantel test using the software PASSaGE (version 2; Rosenberg and Anderson 2011) with 10,000 randomizations.

To test whether wrens responded differently to conspecific and congeneric signals, we evaluated wrens' responses to conspecific and congeneric signals using linear mixed models. We ran a separate analysis for each of the four subject species. We included the two factors summarizing vocal and physical responses as dependent variables. We included treatment species as well as playback order as fixed effects. Individual identity was included as a subject variable with random effects to account for the fact that stimuli were presented repeatedly to the same subjects. We performed subsequent post hoc pairwise tests between conspecific playbacks and congeneric playbacks, using Bonferroni correction for multiple comparisons.

To test whether the birds' responses to playback varied with the acoustic similarity between the subject and the playback stimulus, we used linear mixed models to evaluate the response data for all four species. We included the two factors summarizing vocal and physical responses as dependent variables. We included as fixed effects the four subject species, and the interaction between subject species and acoustic distance. We included the acoustic distance between the subject species' song and the stimulus song as a covariate. We included individual identity as a subject variable with random effects. The control treatment (i.e. Black Catbird) was not included in this analysis because of the low response levels we observed during control treatments (see results), which could generate a false significant association between behavioural responses and acoustic similarity in the linear mixed model.

To test whether the birds' responses to playback varied with the subject species' phylogenetic relatedness to the stimulus species, we used linear mixed models to evaluate the response data for all four species. We included the two factors summarizing vocal and physical responses as dependent variables. We included as fixed effects the four subject species, as well as the interaction between subject species and genetic distance. We included the genetic distance between the stimulus species and subject species as a covariate. We included individual identity as a subject variable with random effects. As in the previous analysis, we did not include the control treatment (i.e. Black Catbird) in this analysis.

All linear mixed models were performed using restricted maximum likelihood method for estimating fixed effects. To improve linearity in the association between responses variables, acoustic distance, and genetic distance, we square-root transformed acoustic distances and exponential transformed genetic distances. Residuals were normally distributed. Statistical analyses were performed in PASW statistics (version 18.0; Chicago, IL, United States).

Results

Does divergence in Troglodytes songs vary with genetic divergence?

We investigated whether divergence in *Troglodytes* songs increases with increasing genetic distance. Based on a comparison that included both acoustic divergence and phylogenetic distance scores for 8 species of *Troglodytes* wrens, a Mantel test showed a strong significant relationship between acoustic divergence and genetic distance (r = 0.9, p = 0.001; Figure 5.1).

Responses to playback

We performed playback to 61 male *Troglodytes* wrens of four different species at four different sites in Central America: 15 Brown-throated Wrens, 16 Cozumel Wrens, 16 Clarion Wrens, and 14 Socorro Wrens. Males showed strong responses to many playback treatments, often approaching the loudspeaker and singing. In general, physical responses varied with treatments, acoustic distance, and genetic distance, with strong responses towards conspecific stimuli, and weaker responses towards congeneric species and unrelated species.

Do wrens respond differently to conspecific and congeneric signals?

Brown-throated Wrens showed significant variation across treatments in both their physical responses ($F_{5,\ 10.2}$ = 11.4, p = 0.001; Figure 5.2A), and vocal responses ($F_{5,\ 14.7}$ = 3.3, p = 0.03; Figure 5.2B). Post hoc tests revealed significant differences in physical and vocal responses between treatments. Physical responses varied along a gradient, with the strongest responses to conspecific playback and playback of Western House Wrens, the weakest responses to control stimuli (Black Catbirds), and intermediate values for the remaining three conspecific stimuli (Figure 5.2A). Similarly, vocal responses varied along a gradient, with the most vocal responses to conspecific treatment, the least vocal responses to the control species (Black Catbirds), and intermediate intensities of response to the four congeners (Figure 5.2B).

Cozumel Wrens showed significant variation across treatments in both physical responses ($F_{4,\ 19.5}$ = 10.7, p < 0.001; Figure 5.2C) and vocal responses ($F_{4,\ 23.9}$ = 9.2, p < 0.001; Figure 5.2D). Post hoc tests revealed significant differences in physical and vocal responses between treatments. Physical responses were significantly stronger in response to conspecific playback than all other treatments (Figure 2C). Vocal responses varied along a gradient, with the strongest responses to conspecific playback and weakest responses to the control species (Black Catbirds), and intermediate intensities in response to the three congeners (Figure 2D).

Clarion Wrens showed significant variation across treatments in physical responses ($F_{5,\ 15.8}$ = 23.1, p < 0.001; Figure 5.2E), but not in vocal responses ($F_{5,\ 20.5}$ = 0.6, p = 0.6; Figure 5.2F). Post hoc tests revealed significantly stronger physical responses to conspecific treatments than all of the other treatments (Figure 5.2E).

Socorro Wrens also showed significant variation across treatments in physical responses ($F_{5,\ 16.2}$ = 7.2, p = 0.001; Figure 5.2G), but not in vocal responses ($F_{5,\ 12.4}$ = 0.5, p = 0.7; Figure 5.2H). Post hoc tests revealed that physical response varied along a gradient, with the strongest responses to conspecific playback and weakest responses to the control species (Black Catbirds) and intermediate intensities of response to the four congeners (Figure 5.2G).

The subjects' physical and vocal responses showed no significant variation in the order of playback treatments for Cozumel Wrens, Clarion Wrens, Socorro Wrens, and Brown-throated Wrens (physical responses: F range = 0.9 – 1.4, p > 0.2; vocal responses: F range = 0.1 – 0.9, p > 0.4), except for the Brown-throated Wren physical responses, which showed a significant effect of the order of the treatments ($F_{5,\ 24.5}$ = 3.6, p = 0.01); for this species, post hoc tests revealed that birds exhibited weaker physical responses to the treatments presented in fourth position compared to the seventh position; all other treatments did not vary with order.

Did playback responses vary with acoustic similarity?

We tested whether there was a significant relationship between behavioural responses to playback and acoustic similarity between the subject species' songs and the playback stimuli. *Troglodytes* wrens' physical responses showed a significant association with acoustic distance ($F_{1,\ 212.9}$ = 20.1, p < 0.001; Figure 5.3); birds showed stronger responses towards the most similar stimuli, and decreasing with the lowest response levels to most dissimilar stimuli. Vocal responses, in contrast, showed no significant association with acoustic distance ($F_{1,\ 194.8}$ = 2.6, p = 0.1). This analysis, including acoustic divergence, revealed no significant differences in the physical responses between the four species of subjects ($F_{3,\ 190.9}$ = 1.6, p = 0.1), and the interaction between species and acoustic distance was not significant ($F_{3,\ 149.2}$ =

0.2, $p = 0.8$). Vocal responses, however, varied between the four species of subjects ($F_{3,\ 156.3} = 4.1$, $p = 0.007$), with Cozumel Wrens showing the highest responses to treatments, Brown-throated Wrens and Socorro Wrens showing intermediate responses, and Clarion Wrens showing the lowest responses. The interaction between species and acoustic distance was not significant ($F_{3,\ 135.7} = 1.1$, $p = 0.3$).

Did playback responses vary with phylogenetic distance?

We tested whether there was a significant relationship between behavioural responses to playback and the genetic distance between the subject species and the stimulus species. We found that both physical and vocal responses showed a significant association with genetic distance (physical responses: $F_{1,\ 90.4} = 101.5$, $p < 0.001$; vocal responses: $F_{1,\ 85.1} = 17.8$, $p < 0.001$; Figure 5.4), with stronger responses towards stimuli of genetically similar species, and decreasing with the lowest response levels towards stimuli of genetically dissimilar species. This analysis, including genetic distance, showed differences in the physical responses between the four species of subjects ($F_{3,\ 124.3} = 8.7$, $p < 0.001$), with Cozumel Wrens showing stronger responses to treatments compared to the rest of the subject species. The interaction between subject species and genetic distance was not significant for physical responses ($F_{3,\ 84.2} = 1.6$, $p = 0.1$). Vocal responses did not differ between the four species of subjects ($F_{3,\ 108.9} = 1.9$, $p = 0.1$), and the interaction between subject species and genetic distance was significant for vocal responses ($F_{3,\ 72.8} = 4.6$, $p = 0.005$).

Discussion

Acoustic divergence between eight *Troglodytes* wren species correlates with phylogenetic distance. To study birds' perception of acoustic divergence, we investigated the behavioural responses of four species of *Troglodytes* wrens to playback of conspecific and allopatric-congeneric songs. Our results demonstrate that wrens responded more strongly to playback of conspecific songs than to allopatric-congeneric songs, that wrens showed generally similar responses to different species of allopatric congeners, and that wrens responded weakly to playback of an unrelated heterospecific control. In spite of similar responses to the different allopatric-congeneric playback species' songs, our analyses showed a significant association between acoustic similarity between the stimuli and the subject species and the intensity of the subjects' behavioural responses. Furthermore, our analyses revealed that the phylogenetic distance between the subject and playback species predicted the intensity of the behavioural responses. Collectively, our results suggest that song divergence increases with genetic distance, that males of four species of *Troglodytes* wren respond to differences between conspecific and allopatric-heterospecific animals, that they perceive fine structural differences in acoustic signals and respond more strongly to similar stimuli, and that phylogenetic constraints are evident in wren's perception of the songs of rivals.

Several studies have suggested that behavioural discrimination between conspecific and hetersopecific signals is a widespread behaviour across diverse animal taxa (e.g. Ryan and Rand 1993, de Kort and ten Cate 2001, Amézquita et al. 2011). Our results provide strong evidence that this pattern holds true for Cozumel Wrens and Clarion Wrens, which both showed statistically higher intensities of response to conspecific playback than congeneric playback, and to a lesser degree for Brown-throated Wrens and Socorro Wrens, which showed their highest intensities of response to conspecific playback but these differences were not significantly higher than some congeneric treatments. Our results demonstrate that *Troglodytes* wren songs contain species-specific features that encode species identity, and that birds use this information to distinguish conspecific rivals.

Whether or not wrens were able to discriminate between congeneric playbacks, the significant association between intensity of response and acoustic distance in all four species suggests that the songs of allopatric congeners contain sufficient information to trigger territorial responses (Emlen 1972, Höbel and Gerhardt 2003, Ryan et al. 2003). When the amount of species-specific information varies with the degree of phylogenetic relatedness, signals may elicit responses that are proportional to the genetic distance between species (e.g. de Kort and ten Cate 2001, Ryan and Rand 2003). In a study of doves, de Kort and ten Cate (2001) suggested that the relationship between behavioural responses and phylogenetic relatedness could be the result of either similarity in signals or similarity in perceptual mechanisms. In our study, the association between behavioural responses and acoustic distance suggests that *Troglodytes* wrens may respond based on the similarity between stimulus species' song and the receiver's species' song. Whether perceptual mechanisms are constrained by phylogenetic relationships is still unclear, but studies of frogs suggest that phylogenetic relationships have an important influence on animal perception (Ryan et al. 2001). In some cases, animals might respond to the signals of closely related congeners because of previous experience, such as during aggressive interactions while competing for similar resources (Grant and Grant 2010, Amézquita et al. 2011). In our experiment, we ruled out this effect by studying allopatric species; given the isolated geographic distribution of our four study species, we can be confident that our subjects had not previously encountered the heterospecific stimuli we presented to them in this experiment. Therefore, our results point to evolutionary mechanisms as important forces influencing both signals and receivers' sensitivity between species (Ryan and Rand 1993).

In line with several studies that report false recognition in animals (i.e. behavioural responses to playback of allopatric-heterospecific signals; Nevo and Capranica 1985, Coyne and Orr 1989, Ryan and Rand 1993, de Kort and ten Cate 2001, Ryan et al. 2003), our results demonstrate that *Troglodytes* wrens also respond aggressively to the acoustic signals of allopatric-congeneric animals. A possible explanation for false recognition in allopatric species is provided by Ryan et al. (2003). They suggest that organisms may recognize signals through the process of generalization, where subjects associate specific traits in common between similar signals, and the similarity between signals should predict the strength of the response (Shepard 1987, Ryan et al. 2003). Our findings that response intensity is affected by the similarity between the subject species' songs and the playback stimuli, and that *Troglodytes* wrens respond to unfamiliar allopatric-congeneric signals, support the idea that recognition might be influenced by generalization, as has been hypothesized to occur in frogs and other songbirds (Ryan et al. 2001, Ryan et al. 2003, Derryberry 2011). Birds may generalize by using their "auditory template" (a neuronal representation defining the characteristic of a song in a given species; Catchpole and Slater 2008), and comparing the features of playback stimuli to their auditory template. In this case, the strength of the response should be associated with the similarity between the auditory template and the external stimuli. Describing the auditory template in birds is a challenging task; however, studies in frogs using signals modified in the laboratory demonstrate that it is possible (e.g. Amézquita et al. 2011).

In conclusion, we analyzed whether behavioural responses to allopatric-congeneric songs are predicted by acoustic distance and phylogenetic relatedness in *Troglodytes* wrens. We showed that birds respond most strongly to playback of conspecific song, and that intensity of response varies with both the acoustic similarity between the playback subject's song and the playback-simulated species song as well as the phylogenetic distance between the species. We provide auto-replication by testing the same hypothesis in four related species distributed throughout different parts of the neotropics. Our investigation is one of very few to focus on phylogenetic differences in perception, rather than phylogenetic differences in signals themselves, and our findings suggest that wrens perceive fine structural variation in acoustic signals.

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Tables

Table 5.1. The playback treatments given to four different species of *Troglodytes* wrens.

Treatments		Subject species			
		Cozumel Wren	Brown-throated Wren	Socorro Wren	Clarion Wren
A	Conspecific	Cozumel wren	Brown-throated Wren	Socorro Wren	Clarion Wren
B	Congeneric 1	Western House Wren	Western House Wren	Western House Wren	Western House Wren
C	Congeneric 2	Southern House Wren	Southern House Wren	Southern House Wren	Southern House Wren
D	Congeneric 3	Socorro Wren	Rufous-browed Wren	Clarion Wren	Socorro Wren
E	Congeneric 4	NA*	Pacific Wren	Brown-throated Wren	Brown-throated Wren
F	Control	Black Catbird	Black Catbird	Black Catbird	Black Catbird

*Cozumel wrens received only three heterospecific treatments, whereas the other species received four heterospecific treatments.

Table 5.2. Details of the principal components analysis performed on physical and acoustic responses of four *Troglodytes* species recorded during the playback of 349 stimuli. Eigenvalues and the percentage of variation explained are presented for each component, and variables with the strongest loading are shown in bold font.

Variables*	Factor 1	Factor 2
Number of songs	-0.28	**0.90**
Number of songs in the longest bout	-0.03	**0.90**
Latency to first song	0.41	**-0.63**
Number of crosses	**-0.66**	0.11
Closest approach	**0.81**	-0.25
Latency to first movement	**0.74**	-0.22
Time within 10 meters	**-0.85**	0.14
Eigenvalues	2.6	2.1
Variance explained (%)	37.7	31.2

*PCA was computed using the correlation matrix and factor scores were extracted using regression method; we assessed sampling adequacy using Bartlett's test and rejected the hypothesis that the correlation matrix contained only zero correlations ($\chi^2 = 1182$, df = 21, $p < 0.001$; Budaev 2010).

Table 5.3. Details of the principal components analysis performed to reduce 15 fine structural characteristics of 301 stimuli songs used to investigate whether acoustic similarity had an effect on the response of an individual. Eigenvalues and the percentage of variation explained are presented for each component, and variables with the strongest loading are shown in bold font.

Variable*	Factor 1	Factor 2	Factor 3	Factor 4
Song length (sec)	0.02	**0.85**	-0.04	-0.07
Minimum frequency (kHz)	**0.58**	0.37	-0.20	-0.36
Maximum frequency (kHz)	0.48	0.13	0.64	0.28
Element length (sec)	0.12	**0.80**	-0.19	-0.21
Number of elements	0.35	**0.81**	-0.08	-0.04
Number of trills	0.15	**0.72**	-0.01	0.01
Silence (sec)	**-0.66**	0.03	-0.07	-0.15
Mean maximum frequency (kHz)	**0.78**	0.24	0.04	0.48
Mean bandwidth (kHz)	0.10	-0.07	0.15	**0.95**
Mean peak frequency (kHz)	**0.86**	0.33	-0.08	0.01
Mean entropy	0.07	-0.19	0.18	**0.90**
Peak frequency shifts per second	**0.83**	0.13	0.05	-0.07
Standard deviation in maximum frequency (kHz)	0.07	-0.03	**0.91**	0.05
Standard deviation in bandwidth (kHz)	-0.04	-0.16	**0.90**	0.24
Standard deviation in entropy	-0.12	-0.17	**0.82**	0.03
Eigenvalues	3.2	3.0	2.9	2.3
Variance explained (%)	21.7	20.1	19.4	15.5

*PCA was computed using the correlation matrix, and factor loadings were extracted using regression method; we assessed sampling adequacy using Bartlett's test and rejected the hypothesis that the correlation matrix contained only zero correlations ($\chi 2$ = 4090.5, df = 105, p < 0.001; Budaev 2010).

Figures

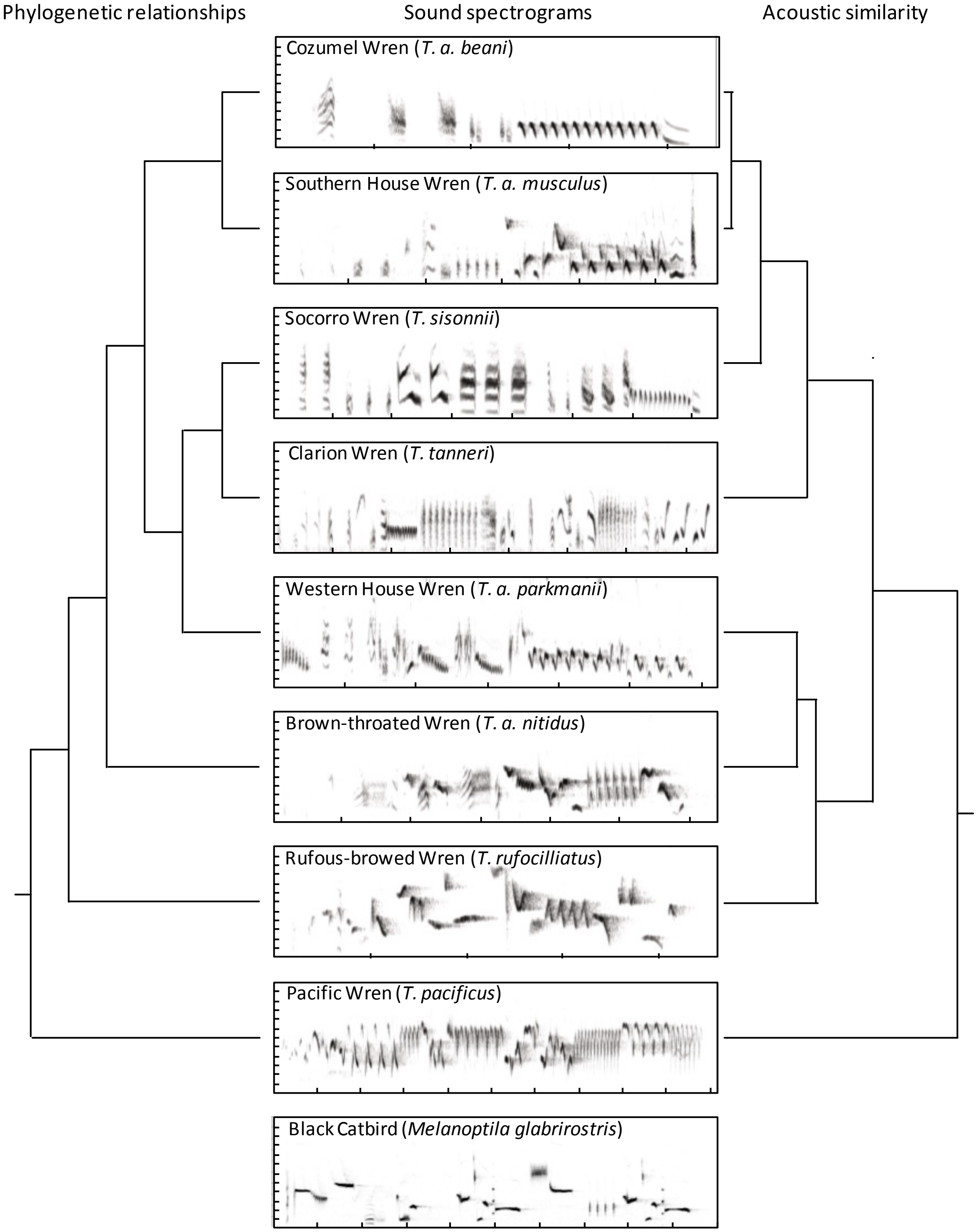
Phylogenetic relationships
Sound spectrograms
Acoustic similarity
Cozumel Wren (*T. a. beani*)
Southern House Wren (*T. a. musculus*)
Socorro Wren (*T. sisonnii*)
Clarion Wren (*T. tanneri*)
Western House Wren (*T. a. parkmanii*)
Brown-throated Wren (*T. a. nitidus*)
Rufous-browed Wren (*T. rufocilliatus*)
Pacific Wren (*T. pacificus*)
Black Catbird (*Melanoptila glabrirostris*)

Figure 5.1. (Previous page) Phylogenetic relationships among *Troglodytes* wrens and sound spectrograms depicting typical songs of the subject species in this study, and the species used as stimuli. At left a phylogenetic tree shows the phylogenetic relationships among *Troglodytes* wrens (data from Chaves et al. 2014); at right a dendrogram shows the acoustic similarity among *Troglodytes* wrens based on the mean similarity values calculated in this study. The dendrogram of acoustic distance was calculated based on the Euclidean distances of the first four principal component factors summarizing acoustic measurements, and using within-groups average linkage cluster method. Note that branches in both the phylogenetic tree and the dendrogram only show relationships and similarity between *Troglodytes* wrens, respectively, and not the actual phylogenetic distances or acoustic distances. For all spectrograms, the x-axis tick marks show increments of 0.5 seconds (note that the time axis varies between spectrograms, to maximize display area), and y-axis tick marks show increments of 1 kHz from 1 kHz to 13 kHz.

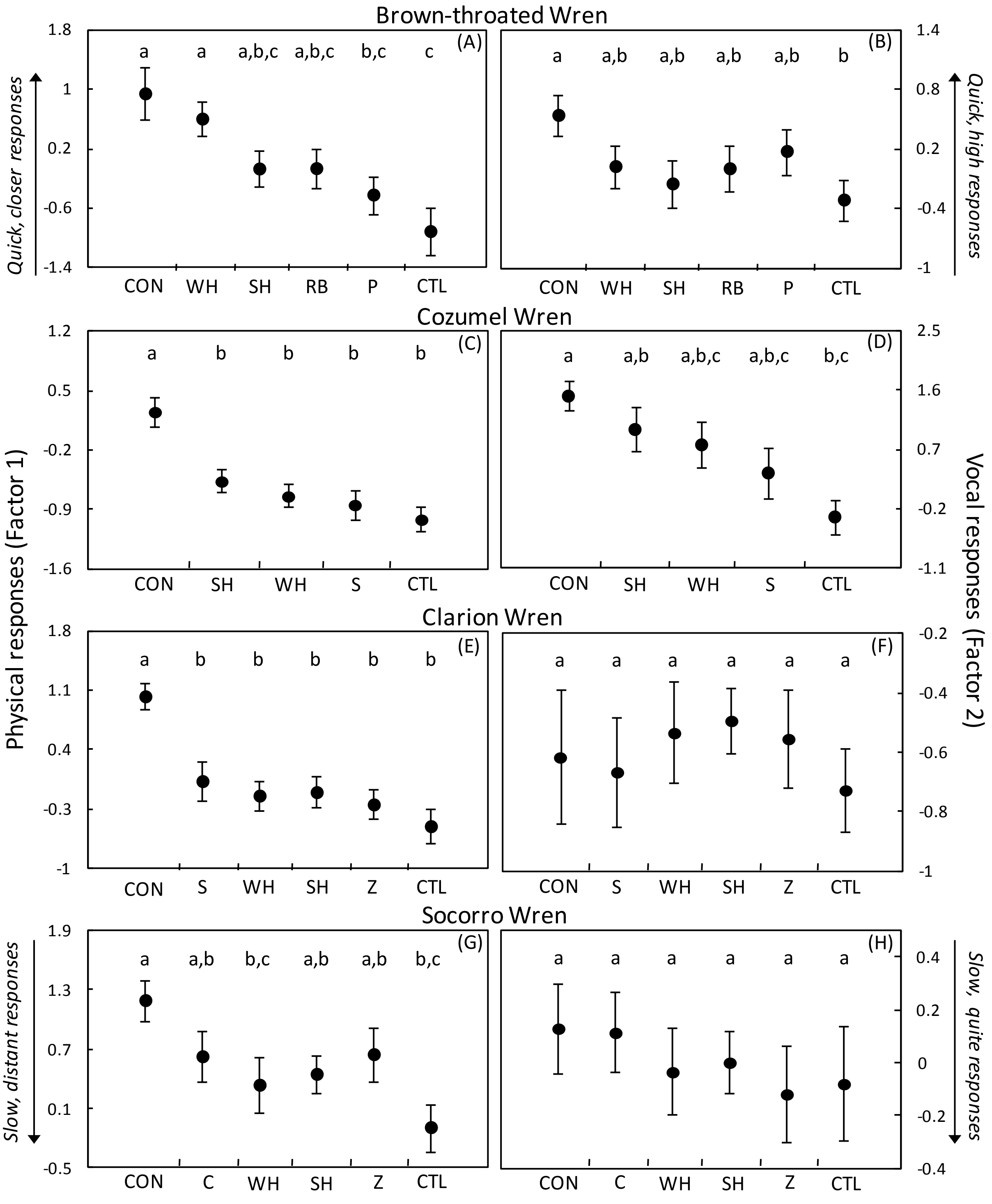
Brown-throated Wren
(A)
a a a,b,c a,b,c b,c c
1.8
1
0.2
-0.6
-1.4
CON WH SH RB P CTL
Quick, closer responses
(B)
a a,b a,b a,b a,b b
1.4
0.8
0.2
-0.4
-1
CON WH SH RB P CTL
Quick, high responses
Cozumel Wren
(C)
a b b b b
1.2
0.5
-0.2
-0.9
-1.6
CON SH WH S CTL
(D)
a a,b a,b,c a,b,c b,c
2.5
1.6
0.7
-0.2
-1.1
CON SH WH S CTL
Physical responses (Factor 1)
Vocal responses (Factor 2)
Clarion Wren
(E)
a b b b b b
1.8
1.1
0.4
-0.3
-1
CON S WH SH Z CTL
(F)
a a a a a a
-0.2
-0.4
-0.6
-0.8
-1
CON S WH SH Z CTL
Socorro Wren
(G)
a a,b b,c a,b a,b b,c
1.9
1.3
0.7
0.1
-0.5
CON C WH SH Z CTL
Slow, distant responses
(H)
a a a a a a
0.4
0.2
0
-0.2
-0.4
CON C WH SH Z CTL
Slow, quite responses

Figure 5.2. (Previous page) Physical (left) and vocal (right) responses of to playback of allopatric-congeneric songs by Brown-throated Wrens (A,B), Cozumel Wrens (C,D), Clarion Wrens (E,F), and Socorro Wrens (G,H). Playback stimuli were conspecific (CON), Southern House Wrens (SH), Western House Wrens (WH), Socorro Wrens (S), Clarion Wrens (C), Brown-throated Wrens (Z), and Black Catbirds as a control stimulus (CTL). Data are shown as means ± SE. Lowercase letters indicate the results of the post hoc test using Bonferroni correction for multiple comparisons; levels connected by the same letter are not significantly different.

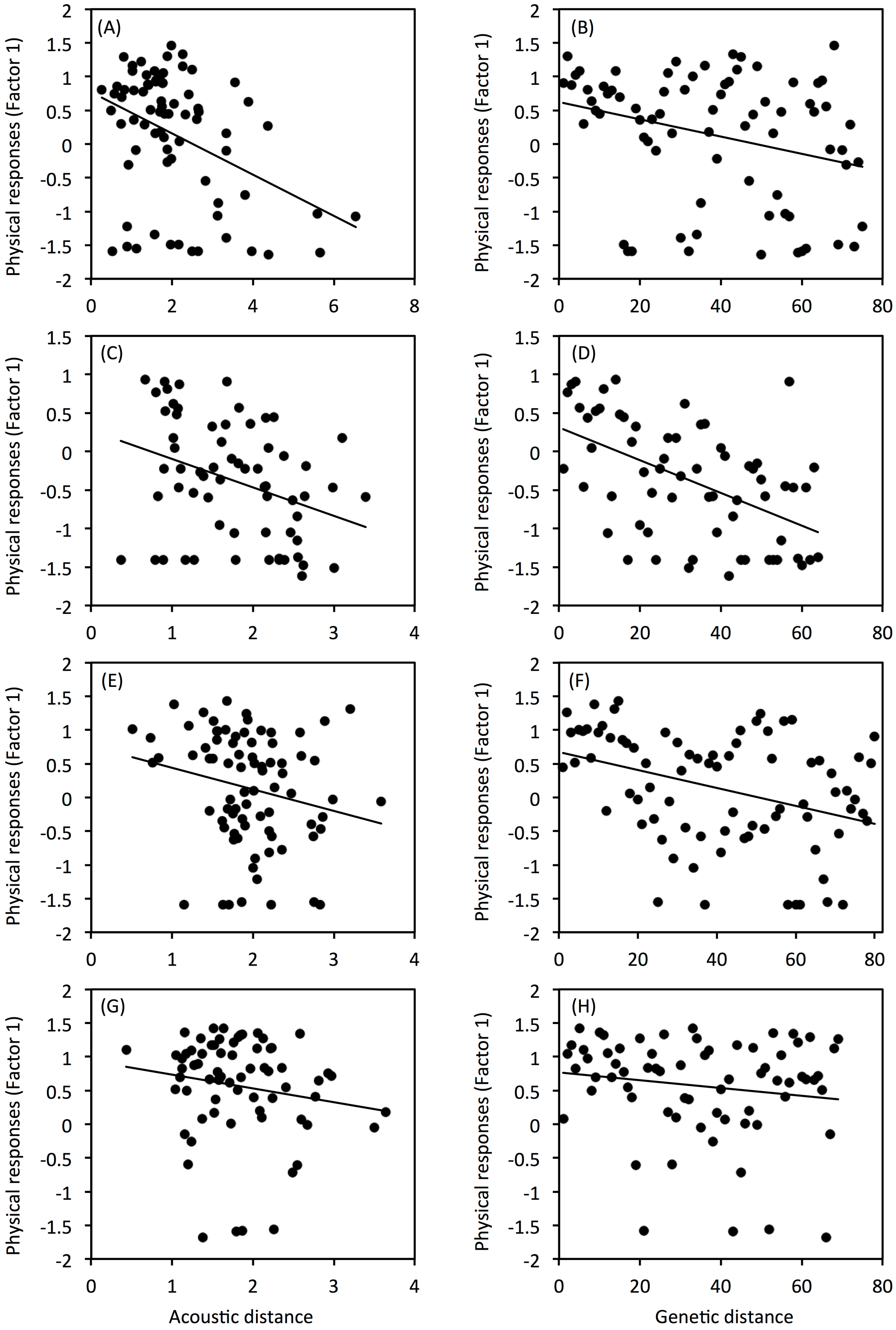
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
Physical responses (Factor 1)
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
0
1
2
3
4
6
8
20
40
60
80
Acoustic distance
Genetic distance

Figure 5.3. (Previous page) Physical responses to playback of allopatric-congeneric songs by Brown-throated Wrens (A,B), Cozumel Wrens (C,D), Clarion Wrens (E,F), and Socorro Wrens (G,H) are predicted by the acoustic divergence (left) and genetic relatedness (right) of the stimuli to the subject species. Physical response is a principal component score summarizing variation in multiple measures of the birds' playback responses.

Chapter 6

The influence of phylogeny, morphology, and ecology on the evolution of songs in *Troglodytes* wrens[*]

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

Chapter Summary

Vocalizations play a central role in mate choice for many animals, and when vocalizations diverge between closely related groups they can play an important role in speciation. By studying variation in acoustic features of animal vocalizations, and comparing this to genetic variation and ecological variation, we can gain a more comprehensive understanding of the evolution of animal communication. In this study, we explore variation in the fine structural characteristics of the songs of wrens in the genus *Troglodytes* and compare this variation to the wrens' phylogenetic relationships, morphological features, and ecological characteristics. We analyze the songs of 38 lineages of wrens at continental and island sites throughout North, Central, and South America. Using an established molecular phylogeny, we assess the phylogenetic signal in wren vocalizations. We compare acoustic variation in wren songs to morphological measurements we collected at museums, including tarsus length and beak length, and to the wrens' ecological characteristics, including habitat, foraging stratum, elevation, and distribution patterns. Our results suggest that closely related *Trolgodytes* wrens tend to resemble each other, exhibiting phylogenetic signal in their acoustic traits.Our results show that temporal properties of *Troglodytes* songs are correlated with ecological characteristics including the strata where wrens forage, the annual precipitation in wren habitats, and whether wrens occupy islands or mainland sites. Wrens found in relatively densely vegetated habitats sing longer songs; wrens inhabiting areas with high annual precipitation and wrens inhabiting islands sing songs with longer inter-element intervals. Morphological features, in contrast, had no influence on acoustic variation in *Troglodytes* wrens. Our study sheds light on the factors influencing acoustic diversity and the ways that fine structural characteristics of bird songs vary in related species.

Introduction

Animal vocalizations play a fundamental role in evolution (Wilkins et al. 2013). Divergence in vocalizations has been shown to have a dramatic impact on mate choice, resource defence, and species recognition (Slabbekoorn 2004). For instance, it is common for closely related species to show acoustic divergence in their vocalizations (e.g. Otte 1989, Littlejohn 1999, Rendell et al. 1999, Valderrama et al. 2007, Toews and Irwin 2008), affecting species recognition and mate choice (e.g. Irwin et al. 2001, Braune et al. 2008, Podos 2010, Mendelson and Shaw 2012). Thus, acoustic divergence is thought to play a key role in fostering genetic differentiation through assortative mating or by limiting options for dispersal (e.g. Slabbekoorn and Smith 2002, Seddon 2005, Ruegg et al. 2006). Documenting patterns of acoustic geographic variation between closely related taxa, and understanding the relationship between acoustic, genetic, and ecological factors, enhances our understanding of how selection drives and maintains acoustic diversity in living organisms (Wilkins et al. 2013).

Acoustic divergence between populations may arise through ecological selection, sexual selection, and drift (Podos et al. 2004a, Wilkins et al. 2013). Under ecological selection, the acoustic adaptation hypothesis (Morton 1975, Ryan and Brenowitz 1985) predicts that interspecific differences will be associated with physical features of the habitat, and ambient noise. The competition for acoustic space hypothesis (Marler 1960) predicts that communities with fewer species, such as island avifaunas, will have relaxed competition for acoustic space (defined as a multi-dimensional range of acoustic parameters within which a taxon’s acoustic signal can evolve; Marler 1960, Nelson and Marler 1990; Luther 2009). Acoustic divergence through ecological selection is expected to occur when natural selection acts directly on acoustic signals (e.g. specialization in echolocation frequencies for different prey sizes in bats; Jones 1997), or indirectly when natural selection acts on morphological traits related to acoustic communication (e.g. song evolution is influenced by beak adaptation to seed size in Darwin Finches, *Geospiza spp.*; Schluter et al. 1985, Podos 2001, Podos et al. 2004b). Under sexual selection, acoustic divergence is expected to arise when females exhibit different mating preferences. For example, acoustic divergence may arise due to females' preferences for local signals or elaborate signals (discussed in Wilkins et al. 2003). Under drift, vocalizations may evolve due to random variation in morphological features associated with sound production, thus driving the evolution of acoustic signals by influence acoustic performance (Podos 2001, Podos and Nowicki 2004, Podos et al. 2004a). In animals that learn to vocalize through tutoring by adults, acoustic signals are also expected to evolve by cultural drift. Imperfect copying of tutors' acoustic traits leads to differences in acoustic signals between generations, and these so called "cultural mutations" may become more common in subsequent generations (Campbell et al. 2010). Understanding the evolution of animal acoustic signals requires a multi-faceted assessment of these factors, which together determine the acoustic limits within which an animal's acoustic signal can potentially fluctuate.

In this study, we investigate the evolution of acoustic signals in New World *Troglodytes* wrens. Similar to other members of the Troglodytidae family, these birds are primarily insectivorous, they are highly active, they are characterized by cryptic brown plumage colouration, and they produce loud, complex songs (Brewer 2001). They are distributed from Alaska and northern Canada to the southern tip of Chile and Argentina, including several oceanic islands. Within North, Central, and South America, many authorities recognize 11 species in the genus *Troglodytes* (i.e. *T. aedon, T. tanneri, T. sissonii, T. rufocilliatus, T. solstitialis, T. ochraceus, T. monticola, T. rufulus, T. pacificus, T. hiemalis, and T. cobbi*; American Ornithologists' Union 1998; Chesser et al. 2012, 2013; Remsen et al. 2013). A recent well-supported phylogenetic analysis (Chaves et al. 2014), however, documented many independent evolutionary units within the genus that are currently unrecognized, suggesting that the genetic diversity in this genus has been grossly underestimated. Importantly, the new molecular phylogeny reveals that the ancestral *Troglodytes* wrens, which presumably occupied high elevations, have undergone a recent rapid diversification of lineages fulfilling new lowland niches, giving rise to 42 independent evolutionary units of New World *Troglodytes* wrens (Chaves et al. 2014). Of particular note, a large radiation has occurred within *Troglodytes aedon* (the so called "House Wren complex"), with ancestors colonizing lowlands in Eastern United States (presently recognized as *T. a. parkmanii*) and south towards the Neotropics and South America (presently recognized as *T. a. musculus* and all its variants, including the Lesser Antilles forms, and *T. a. beani* isolated to Cozumel Island), resulting in 29 independent evolutionary units within the House Wren complex (Chaves et al. 2014). Furthermore, the highland group, *T. solstitialis*, appears to be a polytypic species with multiple lineages (Chaves et al. 2014). Different taxa of *Troglodytes* wrens are now found in a broad variety of habitats, from isolated islands deep in the Pacific Ocean, to mainland tropical lowland forests, to cloud forests and the timberline in Central and South America (Kroodsma and Brewer 2005).

With a compelling new molecular phylogeny (Chaves et al. 2014), several summaries of the ecology and distribution of populations (e.g. Stotz et al 1996, Brewer 2001, Kroodsma and Brewer 2005), and a growing number of descriptive analyses and comparisons of the voices of many species (e.g. Chapter 2; Chapter 3; Chapter 4; Rendall and Kaluthota 2013), the *Troglodytes* wrens are well-suited for exploring the effects of phylogenetic history, and ecological selection on the evolution of acoustic signals. In this study, we explore variation in the fine structural features of *Troglodytes* wren songs, focusing on the effects of variation in genetics, morphological features, and ecological characteristics. We begin by asking whether the fine structural characteristics of the songs, morphological features, and habitat characteristics are correlated with the phylogenetic relatedness among *Troglodytes* lineages, by assessing the phylogenetic signal of each trait (phylogenetic signal is defined as the tendency of closely related lineages to resemble each other in phenotype; Blomberg et al. 2003). If these traits exhibit a strong phylogenetic signal, we expect to observe similar traits across closely related *Troglodytes* wrens, and decreasing similarity with increasing phylogenetic distance (Romer 1993, Losos 2008). Conversely, if these traits exhibit a weak phylogenetic signal, we expect to observe highly variable traits among closely related *Troglodytes* wrens, or convergence in traits of distantly related species (Kamilar and Cooper 2013). For instance, a strong phylogenetic signal is expected under neutral evolution by genetic drift, whereas a weak phylogenetic signal is expected in traits under selection pressures in an adaptive radiation, due to the tendency of organisms to rapidly adapt to fill new niches (Kamilar and Cooper 2013). Assessing phylogenetic signals in morphology and habitat is worthwhile for interpreting phylogenetic signals in acoustic traits (Kamilar and Cooper 2013).

Next, we ask whether morphological features or ecological characteristics, or a combination of both, explain variation in the fine structural characteristics of *Troglodytes* songs. For instance, acoustic divergence may ocurr as result of the relationship between body size and song frequencies, which is expected due the association between mass and the size of the syringeal membranes (Nowicki and Marler 1988). Under this scenario, we expect that lineages of *Troglodytes* wrens with large bodies would produce songs with low frequencies (Wallschläger 1980, Ryan and Brenowitz 1985, Podos 1997, Palacios and Tubaro 2000, Martin et al. 2011). For habitat characteristics, based on the acoustic adaptation hypothesis, we expected that wrens living in habitats with dense vegetation would produce longer, more tonal songs with less frequency variation and lower frequencies (because longer, more tonal, and lower songs should experience less degradation in dense vegetation); and that silent intervals between song components would be longer (because more spaced elements should be less degraded by reverberation in dense vegetation; Wiley and Richards 1982, Ryan and Brenowitz 1985, Handford and Lougheed 1991, Dingle at al. 2008, Tobias et al. 2010). Furthermore, if variation in the fine structural characteristics of wren song is influenced by the number of species competing for acoustic space, we expected that island-living wrens would use a broader acoustic space than their continental counterparts, including broader frequency ranges and longer songs, due to a relaxed competition for acoustic space (Lack and Southern 1949, Marler 1960, Baptista and Johnson 1982, Nelson and Marler 1990, Baker et al. 2003). By contrasting the effects of phylogeny, morphological features, and ecological characteristics, we hope to develop a better understanding of how acoustic diversity originated and how fine structural characteristics are constrained, adapted, or maintained through evolutionary lineages.

Methods

Study species and phylogenetic information

Our understanding of species limits and the taxonomy within the genus *Troglodytes* has changed over time. Recent genetic studies (Chaves et al. 2014), combined with morphological and behavioural studies (Campagna et al. 2012, Chapter 4), strongly suggest that species diversity within the genus is underestimated (Brumfield and Capparella 1996, Rice et al. 1999, Martínez Gómez et al. 2005, see also Mann et al. 2006). Based on a thorough geographical sampling, and genetic analyses that included three mitochondrial and eight nuclear markers, Chaves et al. (2014) documented the existence of at least 42 lineages within the genus, representing genetically and geographically independent evolutionary units (i.e. phylogroups; Avise et al. 1998). Their analysis featured a high level of phylogenetic support (>95% maximum likelihood bootstrap values), and therefore we focus our genetic analyses on the results of Chaves et al. (2014).

For our comparative investigation we conducted analyses using a Bayesian tree based on the molecular phylogeny of Chaves et al. (2014); the tree included mitochondrial sequences of 2555 bp of all recognized species (including *Thryorchilus browni*, which was formerly considered a species within *Troglodytes*; Bangs 1902; and excluding the critically endangered *T. monticola*, about which little is known), and mitochondrial and nuclear sequences of 7333 bp of most subspecies in the House Wren complex (Chaves et al. 2014). To test the influence of phylogeny, morphology, and ecology, we analyzed the topology of the phylogenetic tree reported in figures 1 and 2 of Chaves et al. (2014), recreating the tree and measuring branch lengths in TreeSnatcher Plus software (Laubach et al. 2012). This software allowed us to extract information on branch lengths from the phylogenetic tree for use in our subsequent comparative analyses.

Recordings and measurement of acoustic features

We gathered a library of wren songs from our own recordings collected during field expeditions, and from existing recordings in 16 sound libraries and private collections. Our library included 1065 recordings used in a previous study that compared acoustic variation in House Wrens to other *Troglodytes* species (Chapter 4), and an additional 51 recordings that included *T. solstitialis*, *T. rufulus*, and *Thryorchilus browni*. Recording techniques are described in Chapter 4. We avoided including more than one recording from the same individual by excluding recordings from our analysis when multiple recordings came from the same individual; when the identity of the bird in the recording was unclear and recordings were collected less than 1 hour apart; and when recordings were made on the same location and day but did not specify the recording time.

To match acoustic data with genetic data, we selected acoustic recordings that matched the sampling location for genetic samples in Chaves et al. (2014), matching both the species identity and the provinces or states where samples were collected. We gave special consideration to the continuity of the geographic area where samples were collected, matching samples that were gathered in the same mountain chain, or in the same lowland area if nearby areas featured large rivers or lakes (Table 6.1). With this approach, we were able to successfully match recordings from our archive with genetic data for 38 of the 42 lineages (including *Thryorchilus browni*) from Chaves et al. (2014).

After excluding recordings that did not match the genetic sampling localities, our analyses included 726 recordings from different individuals, with an average of 19±3.8 (mean ± SE) recordings per lineage (Table 6.1). Most recordings included multiple songs. We selected one song from each recording for our analyses by generating sequences of numbers that corresponded to the order of the high-quality songs within a recording (i.e. those with minimal background noise), and we then picked one song based on a random number generator. We extracted each selected song and saved it into a separate sound file in WAV format (at either 44.1 kHz/16 bits or 48 kHz/16 bits).

We analyzed the acoustic characteristics of each of the 726 songs by creating sound spectrograms in AviSoft-SASLab Pro (version 5.2.04; R. Sprecht, Berlin, Germany). We generated spectrograms with the following settings: 1024 point fast Fourier transform (FFT), 93.75% overlap, Blackman window (frequency resolution: 22 Hz; temporal resolution: 2.9 ms). We exclude low-frequency background noise from all recordings by applying a 1 kHz high-pass filter. We measured fine structural characteristics of each song using the automatic parameter measurement tool of AviSoft-SASLab Pro, thereby minimizing subjectivity in our measurements. We detected the start and end of each element in the song by using a separation threshold of -25 dB relative to the maximum amplitude of the element;

we distinguished separate elements when the amplitude dropped below the -25 dB threshold for ≥ 5 ms. Frequency variables were calculated using a threshold setting of -20 dB relative to the song's peak amplitude, including all the peaks exceeding the threshold. Measurements based on the power spectrum, such as entropy (see below), were derived from the average spectrum across an entire element. Many *Troglodytes* songs start with a series of low amplitude introductory elements, and the threshold of automatic detection could not always detect these very quiet elements. In these cases, we selected the onset of the introductory section manually by looking at the first element on the spectrogram.

We measured a total of 15 fine-structural song characteristics (as in Chapter 4). (1) Element length (s): the average duration of each element within the song. (2) Inter-element interval (s): the average of the length of the silent space between elements, calculated as the average time from the end of the preceding element to the start of the current element for all the elements across the entire song. (3) Mean maximum frequency (kHz): the average of the maximum frequency of all elements within the song. (4) Mean bandwidth (kHz): the average of the bandwidth of every element within the song, calculated as the difference between the lowest (minimum frequency) and highest frequency (maximum frequency) for each element. (5) Mean peak frequency (kHz): the average of the peak frequency of all elements within the song (peak frequency was determined as the frequency with the highest amplitude in the power spectrum for each element). (6) Mean entropy: the average of the entropy measurement for each element within the song. (Note that the entropy parameter is a measure of the randomness of the sound with values ranging from 0 to 1; pure-tone elements have values close to 0 and noisy sounds have values close to 1). (7) Song length (s): the duration from the beginning of the first element to the end of the last element in the song. (8) Number of elements: total number of elements detected within the song. (9) Number of trills: we defined trill as a section of the song composed of a series of identical syllables repeated three or more times in a row (as in Catchpole and Slater, 2008, syllables can be composed of one or more elements, i.e. one or more continuous tracings on a sound spectrogram). (10) Minimum frequency (kHz): the lowest frequency with amplitude delimited by the threshold from the power spectrum of each element, and across the entire song. (11) Maximum frequency (kHz): the highest frequency with amplitude delimited by the threshold from the power spectrum of each element, and across the entire song. (12) Peak frequency shifts per second: number of times that the frequency peak switched between a value above and below 5.0 kHz, from one element to the next, sequentially across the entire song. We chose a threshold of 5.0 kHz because this was the mid-point between the mean minimum and mean maximum frequencies; we counted the number of switches and divided them by the song length. This is similar to the variable "transitions per second" used by Toews and Irwin (2008) and Campagna et al. (2012). (13) Standard deviation in maximum frequency (kHz). (14) Standard deviation in bandwidth (kHz). (15) Standard deviation in entropy. (A graphical representation of these measurements can be found in Figure 4.S1 in the Appendix section for Chapter 4).

Analysis of acoustic variables

To select acoustic variables for analysis, we began by reducing the number of acoustic variables by performing a principal component analysis on the 15 acoustic measurements using Varimax rotation and Kaiser Normalization, and extracted factors with Eigenvalues greater than one. This analysis resulted in five factors that correspond to variation in frequencies between elements within songs (factor 1), the duration of songs and song elements (factor 2), the frequency of elements within songs (factor 3), the tonality of elements within songs (factor 4), and the silent spaces between elements in songs (factor 5). Together these factors explained 82.2% of the variance of the original dataset (Table 6.2). Rather than analyzing the abstract principal component factors, we conducted our analyses on the variable with the highest factor loading score for each of the five principal component factors. The variables with the highest factor loading scores are also the variables with the highest correlation values within factors (Table 6.3). Our final selection included the following five acoustic variables, each of which corresponded to one of the five principal components: standard deviation in maximum frequency (factor 1), song length (factor 2), mean maximum frequency (factor 3), mean bandwidth (factor 4), and inter-element interval (factor 5). We were unable to normalize the variable "number of song elements" (the variable with the highest loading score in factor 2); thus, we selected the variable song length, which had the second highest in loading. For each of these five representative acoustic variables, we calculated the average value for each of the 38 lineages (Table 6.1).

Analysis of morphological variables

Both body size and beak morphology are known to influence acoustic features of bird songs (Podos 1997, Palacios and Tubaro 2000). Body size has a negative relationship with song frequency (e.g. Wallschläger 1980, Martin et al. 2011, Potvin 2013). This relationship arises because of a correlation between mass and the size of the syringeal membranes (Nowicki and Marler 1988). In similar fashion, beak morphology is correlated with the suprasyringeal tract, influencing frequency structure (Palacios and Tubaro 2000). Also, there is a trade-off between bite force and speed of movement of beaks, generating a negative relationship between beak size and the number of notes a bird can produce per second (Podos 1997). We measured tarsus length (as a proxy for body size) and beak length of 930 individuals. We collected measurements during field expeditions and from specimens preserved in three museum collections: the American Museum of Natural History in New York, the Field Museum of Natural History in Chicago, and the Museo de Zoología "Alfonso L. Herrera" in Mexico City (see Chapter 4 for full details on morphological analysis). Measurements were taken following Pyle (1997). We selected morphological measurements for inclusion in our analysis following the procedure described above for the acoustic variables, resulting in measurements from 539 individuals with an average of 12.8±1.3 individuals per lineage (Tables 6.1). Our matching procedure resulted in 36 lineages with morphological data. All 36 lineages were also represented in the acoustic dataset, but for 2 of the 38 lineages in the acoustic dataset we did not have access to specimens for measurement (*T. solstitialis* from Manabi, Ecuador; and *T. musculus* from Neuquen, Argentina; Chaves at al. 2013).

Analysis of ecological variables

We classified the ecological characteristics of each lineage based on five features: habitat, foraging stratum, elevation, precipitation, and distribution pattern. We based our classification on species descriptions of neotropical birds from Stotz et al. (1996). Additional ecological information was extracted from Brewer (2001), and Kroodsma and Brewer (2005; Table 6.1).

For habitat, we categorized each lineage broadly as occupying either "closed habitat" (tropical deciduous forest, tropical lowland evergreen forest, elfin forest, montane evergreen forest, pine-oak forest, coniferous forest, and secondary-growth forest; the same category as "forest" in Stotz et al. 1996) or "open habitat" (*Troglodytes* wrens under this classification are mostly associated to secondary-growth scrub, but it includes others such as arid low forest scrub, arid montane scrub, semihumid/humid mountane scrub, and cerrado; the same category as "nonforest" in Stotz et al. 1996). This classification system, developed by Stotz et al. (1996), allowed us to distinguish between dense vegetation structure (i.e. closed habitat) and open vegetation structure (i.e. open habitat); these differences in vegetation are expected to have an influence on sound transmission (Handford and Lougheed 1991, Boncoraglio and Saino 2007).

For foraging stratum, we categorized each lineage as foraging primarily at "understory" or "midstory" strata, following the variable "strata" by Stotz et al. (1996; no wrens in this group are understood to forage in the upperstory). In the genus *Troglodytes*, wrens that forage in the midstory (e.g. *T. ochraceus, T. rufocilliatus, T. solstitialis*) are exclusively found in montane habitats, restricted to elfin forest, montane evergreen forest, and pine-oak forest but not usually in secondary growth. Wrens that forage in the understory (e.g. the House Wren complex, *T. aedon*) are exclusively restricted to lowland habitats and are typically associated to scrubby secondary growth and even anthropogenically modified habitat (Stotz et al. 1996). Other studies have found that understory vegetation is denser than midstory vegetation (e.g. Seddon 2005). Our observations, however, suggest that *Troglodytes* wrens that tend to forage in understory (e.g. *T. a. aedon*, *T. musculus intermedius*, *T. tanneri*, and *T. sissoni*) are found in secondary growth and anthropogenically modified habitats that are more similar to open habitats with less dense vegetation. These *Troglodytes* wrens also tend to sing from exposed perches above the secondary growth vegetation, which should impose fewer obstacles to acoustic transmission (Ryan and Brenowitz 1985).

For elevation, we classified wrens according to the elevation at which each species is most common, following the variable "center of abundance" by Stotz et al. (1996). We classified *Troglodytes* wrens' elevation distribution as "lowland" (areas lower than 500 m; typically in the tropics; the same category as "lower tropical" in Stotz et al. 1996), or "montane" (mountains of moderate elevation, 1600-2600 m, and high elevation, above 2600 m). This classification corresponds with the phylogenetic ancestral state reconstruction based on elevation profiles by Chaves et al. (2014), thus allowing us to test whether the recent expansion into lowland habitats was associated with changes in wren song.

For precipitation, we calculated annual precipitation for each recording location using the Worldclim database. We used the variable Bio12, a sum of monthly precipitation estimates, extracting data from a Worlclim grid with a resolution of 2.5 arc-minutes in the software DIVA-GIS 7.5 (approximately 5 km spatial resolution; Hijmans at al. 2001; Table 6.1). We then calculated the average annual precipitation values for each lineage. Precipitation stands as a proxy for environmental variation (Hijmans et al. 2005) and precipitation is positively correlated with habitat features such as vegetation density (Carling and Thomassen 2012). Vegetation density affects sound transmission, and different types of sounds should evolve in particularly dense habitats versus less dense habitats (Morton 1975, Wiley and Richards 1982, Ryan and Brenowitz 1985, Tubaro and Lijtmaer 2006).

For distribution pattern, we classified whether each lineage is found on islands or continental mainland. This classification is important because island avifaunas generally have lower species richness compared to corresponding mainland continental areas (Stotz et al. 1996). As such, this variable allowed us to assess the effects of relaxed competition in the songs of *Troglodytes* wrens (Lack and Southern 1949, Marler 1960, Luther 2009).

Comparative method

We quantified the phylogenetic signals of the acoustic, morphological, and ecological features to determine whether these traits vary with phylogenetic relatedness between *Troglodytes* lineages. We estimated the phylogenetic signal in each trait using Blomberg's K, where K values fluctuate from 0 to infinity. Blomberg's K tests the null hypothesis of the pattern of similarity among close relatives using a randomization procedure (Blomberg et al. 2003). Values of $K = 1$ indicates that traits evolve in a Brownian motion, which is expected when trait divergence among taxa increases linearly with time (Diniz-Filho et al. 2012, Paradis 2012). Values of $K < 1$ indicate a tendency for a given trait to be independent with respect to phylogenetic relationships, suggesting that closely related taxa are less similar than expected under Brownian motion evolution. Values of $K > 1$ indicate a tendency for a given trait to be dependent with respect to phylogenetic relationships, suggesting that closely related taxa are more similar than expected under Brownian motion evolution (Paradis 2012, Kamilar and Cooper 2013). Thus, traits with $K < 1$ are described as having a weak phylogenetic signal which is likely cuased by a departure from Brownian motion evolution due to adaptive evolution uncorrelated with the phylogeny, and traints with $K > 1$ are described as having a strong phylogenetic signal. K-values that do not differ statistically from zero indicate that traits are no more similar than if they were placed randomly on the tree (see Blomberg et al. 2003 for discussion). We tested for statistical significance of our phylogenetic signal calculations using the randomization procedure (Blomberg's *K* using 999 randomizations) featured in the R package picante (v. 1.6-1; Kembel et al. 2010).

We then tested whether the acoustic features of *Troglodytes* songs showed a relationship with morphological, ecological, and habitat features. For this analysis, we used phylogenetic generalized least squares (PGLS). By using PGLS, we were able to weight the data from each lineage in a linear model using Pagel's λ (Freckleton et al. 2002, Paradis 2012). Pagel's λ measures phylogenetic dependence and varies from 0 to 1, where $\lambda = 0$ indicates no influence of phylogeny; $\lambda < 1$ indicates that the relation between a trait and phylogeny is not as strong as expected under a Brownian motion evolution; and $\lambda = 1$ indicates phylogenetic dependence (Pagel 1999).

Bolmberg's *K,* unlike Pagel's λ, is not restricted in the range of phylogenetic signal that can detect (Münkemüller et al. 2012). Blomberg's *K* can detect higher values (e.g. *K* >1) indicating stronger trait similarity between related species than expected under Brownian motion model (Diniz-Filho et al. 2012, Münkemüller et al. 2012). Thus, we use Blomberg's K to test for phylogenetic signal and report the strength of the deviation from the Brownian mition evolution model, wheras Pagel's λ is used in the PGLS to control for phylogenetic effects.

We corrected for multiple comparisons by using Bonferroni adjustment by dividing the overall alpha value of 0.05 by the number of comparisons for each of our hypotheses. To improve normality and linearity, we reciprocally-transformed (i.e. 1/x) song length and tarsus length, and Log-transformed mean maximum frequency (Whitlock and Schuter 2009). We estimated phylogenetic signal and computed PGLS in R packages picante (version 1.6-1; Kembel et al. 2010) and ape (version 3.0-11, Paradis et al. 2004, Paradis 2012, R Core Team 2013), respectively. All other statistical analyses were carried out using PASW statistics (version 18.0; Chicago, IL, United States).

Results

We analyzed the songs from 726 *Troglodytes* wrens and the morphological features of 539 wrens sampled throughout the range of the genus in North, Central, and South America. We matched the acoustic data for 38 lineages and morphological data for 36 lineages with phylogenetic distance measurements from Chaves et al. (2014) and with ecological information for each lineage from existing publications and databases.

Phylogenetic signals of acoustic, morphological, and ecological traits

Four of the five acoustic traits that we measured showed a significant phylogenetic signal (range for Blomberg's K: 0.2–1.6, all $p < 0.03$; Table 6.4). Song length and mean bandwidth showed a strong phylogenetic signal, while mean maximum frequency and inter-element interval showed a weak phylogenetic signal (Table 6.4). The only acoustic variable that did not show a significant phylogenetic signal was standard deviation in maximum frequency (K: 0.3, $P = 0.07$).

There was no phylogenetic signal in the two morphological traits we measured: tarsus size (a proxy for body size; $K = 0.1$, $p = 0.40$) and beak size ($K = 0.4$, $p = 0.28$; Table 6.4).

Most ecological variables showed a significant phylogenetic signal (K range: 0.2–1.5, all $p < 0.02$; Table 6.4). Foraging stratum and elevation showed a strong phylogenetic signal, and annual precipitation and habitat showed a weak phylogenetic signal (Table 6.4). The only ecological variable that did not show a significant phylogenetic signal was distribution pattern (i.e. island versus mainland; K: 0.1, $p = 0.63$). Together, these results suggest that divergence in most features were more similar between closely related *Troglodytes* than expected by chance. The strength of association between the phylogeny and acoustic association among close related *Troglodytes* varies from weak (i.e. $K < 1$ suggesting that traits among close related taxa are more divergent than expected under Brownian motion evolution) to strong (i.e. $K > 1$ suggesting that traits among close related taxa are less divergent than expected under Brownian motion evolution).

Morphological and ecological relationships with acoustic variation

Morphological features showed several associations with fine structural characteristics of wren songs. Our results revealed a strong significant inverse association between inter-element interval and tarsus length (PGLS: $F_{1,34} = 15.9$, $p = 0.0003$; Figure 6.1a), suggesting that birds with longer tarsi (i.e. larger birds) sing songs with smaller inter-element intervals. Two morphological features showed relationships that did not retain significance following correction for multiple comparisons: standard deviation in maximum frequencies and tarsus length ($F_{1,34} = 5.2$, $p = 0.02$; Figure 6.1b), and inter-element interval and beak length ($F_{1,34} = 7.7$, $p = 0.008$; Figure 6.1c). These patterns suggest the possibility that wrens with small tarsi have low variation in maximum frequencies, and that birds with larger beaks have large inter-element intervals in their songs (Table 6.5). All other associations with morphological traits analyzed were not significant ($F_{1,34} = 0.02$–2.9, $p > 0.09$; Table 6.5).

Ecological characteristics showed several significant associations with the fine structural characteristics of wren songs. We found a strong significant association between song length and foraging stratum ($F_{1,36} = 13.2$, $p = 0.0009$; Figure 6.2a), suggesting that wrens foraging in midstory levels have longer songs than wrens foraging at understory levels. We found a significant association between inter-element interval and annual precipitation ($F_{1,36} = 10.9$, $p = 0.002$; Figure 6.2b), suggesting that wrens in environments with high annual precipitation have songs with longer inter-element intervals; and inter-element interval and distribution pattern ($F_{1,36} = 10.6$, $p = 0.008$; Figure 6.2c), suggesting that birds inhabiting islands have longer inter-element intervals in their songs than continental birds. Two ecological characteristics showed a relationship that did not retain significance following correction for multiple comparisons: standard deviation in maximum frequencies and habitat ($F_{1,36} = 4.6$, $p = 0.03$; Figure 6.2d), and mean maximum frequency and foraging stratum ($F_{1,36} = 10.1$, $p = 0.003$; Figure 6.2e).

These patterns suggest the possibility that wrens inhabiting forested habitats have low variation in maximum frequencies between song elements, and wrens that forage in midstory levels have songs with higher maximum frequencies (Table 6.5). All other relationships with ecological traits analyzed were not significant ($F_{1,36}$ = 0.001–10.1, $p > 0.1$; Table 6.5).

Morphological features and ecological characteristics showed no significant associations with each other ($F_{1,34}$ = 0.0–4.4, $p > 0.04$; Table 6.5), although they showed two relationships that were significant before, but not after, correction for multiple comparisons: tarsus and habitat ($F_{1,34}$ = 4.2, p = 0.04) and tarsus and foraging stratum ($F_{1,34}$ = 4.4, p = 0.04). These patterns suggest the possibility that wrens with longer tarsi (i.e. larger birds) inhabit closed forests and forage at midstory levels.

Discussion

We explored the association between the fine structural characteristics of *Troglodytes* wren songs and the birds' phylogenetic relatedness, morphological features, and ecological characteristics. Our results, based on extensive sampling throughout North, Central, and South America, reveal a strong phylogenetic signal in song length, song bandwidth, foraging stratum, and elevation; a weak phylogenetic signal in maximum frequency, inter-element interval, annual precipitation, and habitat; and no phylogenetic signal in standard deviation in maximum frequency, tarsus length, beak length, and distribution pattern (island versus mainland). Analyses that control for phylogeny show associations between *Troglodytes* wren song traits and climatic conditions, the strata at which birds forage, and whether birds are found on islands or mainland. Specifically, we found that wrens that forage in midstory tend to have longer songs compared to those foraging in understory levels; and that wrens inhabiting localities with high annual precipitation, and wrens restricted to islands, are likely to have longer inter-element intervals.

Morphological traits show no significant relationships with song traits. Overall, our analyses reveal complex patterns in the evolution of song in *Troglodytes* wrens, where both phylogeny and ecology are reflected in the divergence of acoustic traits.

Phylogenetic signal in acoustic and ecological features

We found a strong phylogenetic signal in two acoustic traits, duration of songs and song bandwidth, and two ecological features, the stratum at which wrens forage (midstory versus understory), and elevation (montane versus lowland). These results indicate that these traits and features are more similar among closely related species than expected under the Brownian motion model of evolution (Gingras et al. 2013), and suggest that these traits and features have evolve gradualy within *Troglodytes* wrens (Kamilar and Cooper 2013). In a previous investigation, we showed that acoustic divergence in songs increases with divergence in phylogenetic distance in eight species of *Troglodytes* wrens (chapter 5), supporting the idea that wren songs have phylogenetically conserved components. The relationship between phylogeny and ecological similarity that we present here (shown by the strong phylogenetic signal in foraging stratum and elevation), on the other hand, suggests that these wrens may exhibit phylogenetic niche conservatism, which is expected to occur when closely related species are ecologically similar (Losos 2008). Whether the similarity in the songs of some wrens arises primarily because of shared ancestry, or shared environments, is an important area for future studies.

We found weak phylogenetic signal in variation in the frequency of elements within songs (i.e. mean maximum frequency), the silent spaces between elements in songs (i.e. inter-element interval), and some ecological traits (i.e. annual precipitation, and habitat). Values of $K < 1$ indicates that closely related taxa are less similar than expected under Brownian motion evolution, suggesting that selection may influence variation of these traits. In line with this, the PGLS analysis shows that there is a relationship between inter-element interval, annual precipitation, and distribution pattern suggesting selection forces are acting on inter-element interval. On the other hand, frequency characteristics of songs are known to be associated with vegetation characteristics of the animal's habitat as well as body size (Morton 1975, Wallschläger 1980). Althpugh the PGLS analysis shows no significan relationship between these traits, the results suggest a tendency in these associations. It is likely that proxy variables such as tarsus length (which we used as a proxy for body size) may have only a weak association with body size, thereby obscuring the results.

We found no phylogenetic signal in variation in frequencies between elements within songs (i.e. standard deviation in maximum frequency), morphological traits (i.e. tarsus and beak length), and some ecological traits (i.e. distribution pattern). Similar results indicating phylogenetic independence in acoustic traits have been shown in the fairy-wrens, emu-wrens, and grass-wrens (family Maluridea; Greig et al. 2013). Under an adaptive radiation scenario, a lack of phylogenetic signal is expected due to the fact that closely related species have diversified into different niches creating rapid trait differentiation, thus producing a mismatch between phylogeny and traits (Kamilar and Cooper 2013). According to Chaves et al. (2014), ancestral montane *Troglodytes* wrens have undergone a recent rapid diversification, expanding from montane to lowland niches, resulting in several independent lineages within the House Wren group. The rapid colonization of new habitats may have fostered new adaptations in morphological traits to divergent environments. Thus, non phylogenetic signal is probably due to adaptation to divergent environments in closely related wrens that have recently undergone adaptive radiation in the *Troglodytes* wrens (Kamilar and Cooper 2013).

Song and morphology

Owing to an allometric relationship between body mass and the size of the membranes in the syrinx, larger birds are expected to sing songs with lower frequencies (Nowicki and Marler 1988). This expectation has been borne out in several studies, both at the species level (e.g. Montezuma Oropendolas, *Psarocolius montezuma*; Price et al. 2006) and across multiple taxa (e.g. songbirds in the order Passeriformes; Wallschläger 1980, Martin et al. 2011, Grieg et al. 2013). Contrary to our expectation, our data showed no relationship between body size and song frequency measurements, and therefore provided no support for the morphological constraint hypothesis (Ryan and Brenowitz 1985). Other studies in species of the Troglodytidae family (e.g. Sosa López et al. 2013) also have failed to find a relationship between body size and frequency.

Song and ecological characteristics

Birds singing in dense habitats with high attenuation and reverberation properties should produce longer songs in order to maximise sound propagation, and they should produce songs with longer inter-element intervals to minimize signal masking through reverberation (Morton 1975, Ryan and Brenowitz 1985, Handford and Lougheed 1991). We found an association between acoustic variables representing song length and foraging stratum, and between the length of silent intervals and annual precipitation, suggesting that that wrens foraging in midstory levels of vegetation have longer songs than those foraging in understory levels, and wrens inhabiting areas with high annual precipitation have songs with greater silent space between elements. Based on a meta-analysis conducted across several groups of birds, Boncoraglio and Saino (2007) concluded that habitat has a significant effect on peak frequency, and little or no effect on other frequency and temporal variables. Our results, however, provide support for the acoustic adaptation hypothesis in multiple frequency and temporal variables. Our findings are also consistent with studies reporting a relationship between dense vegetation and attenuation, or dense vegetation and reverberation, or both, in warblers (Badyaev and Leaf 1997), flycatchers (van Dongen and Mulder 2006), between two subspecies of *Henicorhina leucophrys* (Dingle et al. 2008), and communities of Amazonian bird species (Tobias et al. 2010).

Avifaunal communities with lower species richness, such as those on islands, may exhibit released competition for acoustic space among bird species (Lack and Southern 1949; Marler 1960; Baptista and Johnson 1982; Nelso and Marler 1990; Baker 1996, 2006; Baker et al. 2003, 2006). Our results showed the expected association, revealing that wrens inhabiting islands have longer silent spaces between elements in a song. One possible explanation for this result is that wrens found on islands may tend to increase the space between the elements within a song due to a relaxed competition for acoustic space. To our knowledge, no study to date has reported a similar pattern. Alternatively, the longer inter-element intervals in island-living wrens' songs may reflect the dense vegetation of the mostly-tropical island habitats, as suggested by the association between annual precipitation and inter-element interval. However, we believe that this alternative explanation is unlikely since two of the four island lineages included in this study do not feature especially dense vegetation (e.g. Clarion Island and Dominica, both of which provide home to wrens living in scrub forest habitat). Thus, our results suggest that the presence of silent spaces between elements in a song is likely to be affected not only by habitat characteristics, but by whether wrens are restricted to islands or inhabit continental mainland.

Morphology and ecology

Morphological adaptations are expected to occur in species that have undergone adaptive radiation (Kamilar and Cooper 2013). As a consequence, indirect changes in song structure may be expected to arise after morphological traits adapt to divergent environments (Seddon 2005). The most famous example of this comes from studies of Medium Ground-finches (*Geospiza fortis*) in the Galapagos Islands, where adaptation to different seed hardness gave rise to acoustic differences in finch song (Schluter et al. 1985, Podos 2001, Podos et al. 2004b). If similar forces were at play for *Troglodytes* wrens, we expected to observe variation in acoustic traits as a result of morphological adaptations to new niches (Chaves et al. 2014). Although our results show no clear relationship between morphological traits and ecological variables after applying Bonferroni correction, they suggest a trend between morphological traits, habitat characteristics, and foraging stratum. Specifically, our findings show that wrens living in forests, and those foraging at midstory levels, tend to have longer tarsi, suggesting that larger wrens are found in montane habitats. This trend is in line with the general rule indicating that organisms are usually bigger in colder environments (Atkinson and Sibly 1997), and is an association found across several bird taxa (Ashton 2002). It is likely that the broad ecological classifications that we used in our analyses do not provide sufficiently specific information about the different lowland habitats where radiation has occurred, and this may obscure effects that habitat and other ecological factors could have on song structure. Further analysis may help to better understand the relationship between these factors by focusing on the specific ecological conditions where each lineage of *Troglodytes* wren is found, particularly in lowland wrens that have recently undergone such an extensive radiation. In addition, our results suggest that the high phylogenetic signal in song length may be a byproduct of its correlation with foraging stratum. This association suggests that the variation in song length may arise as a result of variation in habitat and not necessarily through shared ancestry, whereas foraging behaviour is likely to be a product of shared ancestry. Our findings also suggest that the low phylogenetic signal and the correlation between inter-element interval and annual precipitation is expected to be the result of the recent rapid diversification thought to have occurred in *Troglodytes* wrens (Chaves et al. 2014), fostering the expansion to new niches and creating song divergence through acoustic habitat adaptation (Kamilar and Cooper 2013).

Conclusion

In this study we show that some of the fine structural characteristics of songs, morphological characteristics, and ecological features of *Troglodytes* wrens exhibit a phylogenetic signal, where signal strength varies between traits. It is likely that acoustic traits, morphological characteristics, and ecological features in *Troglodytes* wrens have undergone complex evolutionary processes showing evidence of phylogenetically conserved characteristics, but also traits evolving at high rates producing large differences between recently diverged taxa (Kamilar and Cooper 2013). We suggest that that the great variability in morphological characteristics and most ecological features observed in closely related *Troglodytes* wrens is likely the result of the recent adaptive radiation that this group has undergone (Losos 2008, Chaves et al. 2014). Variation in some acoustic traits, such as song length and mean bandwidth, and some ecological features, such as foraging stratum and elevation, appear to be conserved traits (Kamilar and Cooper 2013). We suggest that variation in temporal properties of *Troglodytes* songs are explained by habitat, and that variation in temporal properties wren songs restricted to islands are likely to be explained by the release of acoustic competition. Conversely, our analyses provide weak support or no support for the association between morphological features and song frequencies, and variation in acoustic signals as a byproduct of morphological adaptation to habitat. We suggest that the variation observed in temporal characteristics of *Troglodytes* songs is likely the result of acoustic adaptation to habitat, and release from acoustic competition. Together, these findings help to expand our understanding of the factors influencing diversity in acoustic traits, and provide evidence supporting the idea that fine structural characteristics of bird songs are adapted and maintained through evolutionary time.

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Table 6.1. List of species and lineages included in the analysis of song, phylogeny, morphology, and ecology. Average raw values and sample sizes are shown for acoustic, morphological, and ecological variables. Acronyms: Continent (Cont); Island (Is); Lowland (Low); Montane (Mt).

Species	Lineage[a]	N_{song}	SD max. freq.	Song length	Mean max. freq.	Mean band.	Inter-element interval	N_{morph}	Tarsus	Beak	Annual ppt.	Habitat[b]	Stratum[b]	Distribution	Elevation[b]
T. aedon	aedon_USA_E	60	1613.2	2.5	5778.3	2846.1	0.04	19	17.1	12.1	1011.4	Open	Under	Cont	Low
T. aedon	aedon_USA_W	105	1795.2	2.4	6112.9	3113.7	0.03	29	16.9	12.2	553.2	Closed	Under	Cont	Mt
T. beani	beani_MEX_Cozumel	43	1643.7	2.1	4629.8	2660.8	0.07	12	20.2	17.4	1380.9	Closed	Under	Is	Low
T. brunneicollis	brunneicollis_MEX_Guer	23	1395.9	3.5	5782.0	2189.2	0.05	11	20.3	14.3	1505.0	Closed	Under	Cont	Mt
T. brunneicollis	brunneicollis_MEX_Morel	24	1275.9	5.5	5733.3	2758.0	0.04	10	18.2	12.8	1040.2	Closed	Under	Cont	Mt
T. hiemalis	hiemalis	65	1150.6	2.2	5600.0	1001.5	0.04	19	16.7	10.9	1174.7	Closed	Under	Cont	Low
T. musculus	martinicensis_TRI_Trin	3	1339.6	1.9	5031.5	2758.3	0.06	16	18.8	15.4	2110.3	Closed	Under	Is	Low
T. musculus	musculus_ARG_BAires	1	1815.0	1.9	4800.0	2358.4	0.04	6	17.5	13.3	1026.0	Open	Under	Cont	Low
T. musculus	musculus_ARG_Misiones	18	1636.6	1.8	5033.2	2762.3	0.04	7	17.0	13.5	1506.6	Open	Under	Cont	Low
T. musculus	musculus_ARG_Neuquen	4	1454.2	2.3	5144.4	2729.9	0.03				297.8	Open	Under	Cont	Low
T. musculus	musculus_BOL_Cochabam	7	1297.3	2.2	4987.7	2564.6	0.04	22	18.3	13.5	612.7	Open	Under	Cont	Low
T. musculus	musculus_BRA_Amapa	7	1740.4	3.0	5084.5	2934.3	0.05	13	18.3	13.4	1821.9	Open	Under	Cont	Low
T. musculus	musculus_DOM_Dom	12	1449.9	1.9	6005.7	3230.7	0.07	19	18.4	14.9	2788.8	Open	Under	Is	Low
T. musculus	musculus_ECU_Loja	11	1963.5	2.0	5532.7	3005.6	0.04	3	17.8	13.3	1510.8	Open	Under	Cont	Low
T. musculus	musculus_GUA_Retalhuleu	5	1565.6	2.3	4649.4	2362.3	0.05	23	17.3	12.9	1921.2	Open	Under	Cont	Low
T. musculus	musculus_MEX_Veracruz	10	1656.8	2.1	5178.0	3017.1	0.05	2	17.1	13.2	2034.5	Open	Under	Cont	Low
T. musculus	musculus_NIC_AtlNorte	71	1705.4	2.7	5435.7	3163.8	0.06	21	17.8	13.2	2554.9	Open	Under	Cont	Low
T. musculus	musculus_PAN_Veraguas	7	1545.0	1.9	5102.1	2947.7	0.08	14	18.5	14.6	2989.7	Open	Under	Cont	Low
T. musculus	musculus_PER_Arequipa	3	1634.8	1.9	5254.7	2565.6	0.05	7	18.2	13.6	35.0	Open	Under	Cont	Low
T. musculus	musculus_PER_Junin	5	1428.4	2.3	5188.3	2554.3	0.05	1	18.8	13.0	978.2	Open	Under	Cont	Low
T. musculus	musculus_PER_Lima	1	1569.0	2.7	5189.2	2734.8	0.04	5	18.2	13.5	23.0	Open	Under	Cont	Low
T. musculus	musculus_PER_Pasco	1	1291.5	2.0	5127.8	2461.6	0.04	9	18.5	13.6	1111.0	Open	Under	Cont	Low
T. musculus	musculus_PER_Puno	5	1680.9	1.9	5546.8	2651.8	0.03	1	18.4	14.5	758.0	Open	Under	Cont	Low
T. musculus	musculus_PER_SanMartin	4	1577.4	2.1	5521.1	2683.2	0.04	13	18.5	14.2	1102.8	Open	Under	Cont	Low
T. musculus	musculus_VEN_Sucre	26	1584.7	2.1	5082.3	2618.7	0.05	17	18.4	14.0	1300.3	Open	Under	Cont	Low
T. ochraceus	ocharceus_PAN_Cocle	10	1316.2	5.3	6992.5	2106.0	0.03	15	17.2	13.3	2771.5	Closed	Mid	Cont	Mt
T. pacificus	pacificus	33	1208.9	1.8	7165.4	2216.5	0.03	22	17.5	10.9	1637.0	Closed	Under	Cont	Mt
T. ruficiliatus	rufociliatus	32	1582.9	2.6	6623.9	2052.8	0.05	25	17.8	12.1	1656.3	Closed	Mid	Cont	Mt
T. rufulus	rufulus_VEN_Bolivar	1	1123.4	2.2	6117.1	1820.4	0.04	21	21.2	14.9	1580.0	Closed	Under	Cont	Mt
T. sissoni	sissonii	38	1424.9	1.8	5377.6	3064.8	0.06	33	20.2	15.3	342.2	Closed	Under	Is	Low
T. solstitialis	solstitialis_BOL_SantaCruzuz	3	1759.9	1.5	6940.3	2038.5	0.05	12	18.3	12.6	760.7	Closed	Mid	Cont	Mt
T. solstitialis	solstitialis_ECU_Carchi	9	1030.8	1.5	7891.0	2065.2	0.04	8	18.6	12.7	2113.2	Closed	Mid	Cont	Mt
T. solstitialis	solstitialis_ECU_Manabi	4	1223.6	1.5	7981.7	2397.1	0.03				566.0	Closed	Mid	Cont	Low
T. solstitialis	solstitialis_PER_Cajamar	11	1450.3	1.9	8133.7	2407.0	0.03	3	18.8	12.5	817.3	Closed	Mid	Cont	Mt
T. solstitialis	solstitialis_PER_Cuzco	8	1154.4	1.7	8569.1	1783.6	0.04	9	18.0	13.4	1010.5	Closed	Mid	Cont	Mt
T. solstitialis	solstitialis_VEN_Merida	8	1396.6	2.3	6569.3	1876.0	0.04	7	18.1	13.0	963.3	Closed	Mid	Cont	Mt
T. tanneri	tanneri	41	1475.5	6.7	6149.4	3873.3	0.05	8	19.2	16.3	113.0	Open	Under	Is	Low
Thryorchilus browni	browni_CRI_Cartago	7	1146.7	2.5	5238.0	1301.4	0.08	11	21.0	12.7	2555.0	Closed	Under	Cont	Mt

[a] Designations based on Chaves et al. (2014); [b] Following Stotz et al. (1996)

Table 6.2. Loading scores of the first five principal component factors summarizing 15 acoustic variables measured from 725 individuals of different lineages of *Troglodytes* wrens. Eigenvalues and the percentage of variation explained are presented for each factor, and variables with the strongest loading are shown in bold. The variables that were selected for analysis to represent each of the factors are shown with an asterisk.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Eigenvalues	3.01	2.90	2.88	2.27	1.19
Variance explained (%)	20.50	19.30	19.20	15.10	7.90
Factor loadings:					
Standard deviation in maximum frequency (kHz)*	**0.94**	-0.05	0.07	0.11	0.00
Standard deviation in bandwidth (kHz)	**0.85**	-0.19	-0.04	0.36	0.07
Standard deviation in entropy	**0.77**	-0.16	-0.22	0.24	-0.04
Maximum frequency (kHz)	**0.75**	0.02	0.52	0.10	-0.08
Number of elements	-0.12	**0.89**	0.20	-0.22	-0.18
Song length (s)*	-0.10	**0.87**	0.11	-0.10	0.18
Number of trills	-0.02	**0.74**	-0.11	-0.08	-0.34
Element length (s)	-0.27	**0.71**	0.33	-0.39	0.08
Mean maximum frequency (kHz)*	0.13	0.03	**0.95**	0.19	-0.03
Mean peak frequency (kHz)	-0.06	0.13	**0.92**	-0.21	-0.06
Minimum frequency (kHz)	-0.18	0.09	**0.61**	-0.53	-0.18
Peak frequency shifts per second	0.09	0.29	**0.47**	0.06	-0.33
Mean bandwidth (kHz)*	0.32	-0.20	0.09	**0.88**	0.08
Mean entropy	0.25	-0.26	-0.04	**0.85**	-0.06
Inter-element interval (s)*	0.00	-0.07	-0.18	0.05	**0.92**

Principal component analysis was computed using the correlation matrix. Components with Eigenvalues > 1 before rotation were extracted. Factor scores were calculated using the regression method. The hypothesis that the correlation matrix contained only zero correlations was rejected (Bartlett's test: Chi-square = 12,369.46, *df* = 105, $p < 0.001$).

Table 6.3. Correlation between the 15 fine structural characteristics describing the *Troglodytes* songs. Bold numbers indicate correlation values between variables within factors. Order of variables corresponds to the order of the factors extracted from the principal component analysis. The variables that were selected for analysis to represent each of the factors are shown with an asterisk.

	SD in max. frequency (Factor 1)	SD in bandwidth (Factor 1)	SD in entropy (Factor 1)	Max. freq. (Factor 1)	No. elements (Factor 2)	Song length (Factor 2)	Number of trills (Factor 2)	Element length (Factor 2)	Mean max. freq. (Factor 3)	Mean peak freq. (Factor 3)	Min. freq. (Factor 3)	Peak freq. shifts second (Factor 4)	Mean band-width (Factor 4)	Mean entropy (Factor 5)
SD in max. freq.*														
SD in bandwidth	**0.80**													
SD in entropy	**0.65**	**0.75**												
Maximum freq.	**0.74**	**0.61**	**0.37**											
No. elements	-0.17	-0.37	-0.30	0.02										
Song length*	-0.15	-0.28	-0.24	-0.02	**0.78**									
Number of trills	-0.07	-0.21	-0.13	-0.02	**0.66**	**0.44**								
Element length	-0.30	-0.47	-0.47	-0.08	**0.80**	**0.70**	**0.43**							
Mean maximum frequency*	0.19	0.14	-0.04	0.59	0.15	0.07	-0.03	0.21						
Mean peak frequency	-0.04	-0.17	-0.26	0.3	0.35	0.22	0.08	0.47	**0.86**					
Minimum frequency	-0.18	-0.43	-0.30	0.11	0.37	0.19	0.19	0.49	**0.46**	**0.66**				
Peak freq. shifts per second	0.1	-0.01	-0.07	0.28	0.36	0.18	0.14	0.25	0.40	0.43	0.16			
Mean bandwidth*	0.43	0.64	0.43	0.37	-0.40	-0.29	-0.23	-0.52	0.28	-0.18	-0.46	**-0.02**		
Mean entropy	0.33	0.51	0.54	0.24	-0.42	-0.33	-0.22	-0.58	0.13	-0.26	-0.39	**-0.11**	**0.82**	
Inter-element interval*	-0.01	0.06	0.03	-0.17	-0.27	-0.01	-0.22	-0.10	-0.20	-0.24	-0.26	-0.28	0.10	0.02

Table 6.4. Phylogenetic signals in acoustic features, morphological features, and ecological characteristics.

Variable	Blomberg's *K**	*P*
Acoustic features:		
Standard deviation in maximum frequency	0.3	0.07
Song length	1.6	0.001
Mean maximum frequency	0.5	0.001
Mean bandwidth	1.1	0.001
Inter-element interval	0.2	0.03
Morphological features:		
Tarsus	0.1	0.40
Beak length	0.4	0.28
Ecological characteristics:		
Annual precipitation	0.2	0.02
Habitat	0.5	0.007
Foraging stratum	1.5	0.001
Distribution pattern	0.1	0.63
Elevation	1.4	0.001

*Blomberg's *K* was estimated based on 999 randomizations.

Table 6.5. Relationships between the fine structural characteristics of *Troglodytes* wren songs and morphological features and ecological characteristics.

Variable	Predictor	Pagel's λ	Slope	*F*	*df*	*P*[a]
Song vs. Morphological features						
SD in maximum frequency	Tarsus	0.5	23908.6	5.2	34	**0.02**
SD in maximum frequency	Beak length	0.6	-1036.8	1.3	34	0.26
Song length	Tarsus	1.0	2.8	0.7	34	0.42
Song length	Beak length	1.0	0.0	0.0	34	0.86
Mean maximum frequency	Tarsus	0.9	12.9	2.9	34	0.09
Mean maximum frequency	Beak length	0.9	-0.9	2.0	34	0.17
Mean bandwidth	Tarsus	0.9	23927.4	1.5	34	0.23
Mean bandwidth	Beak length	0.9	593.4	0.1	34	0.72
Inter-element interval	Tarsus	0.9	-17.9	15.9	34	**0.0003***
Inter-element interval	Beak length	0.8	1.1	7.7	34	**0.008**
Song vs. Ecological Characteristics						
SD in maximum frequency	Annual precipitation	0.6	0.0	0.4	36	0.52
SD in maximum frequency	Habitat	0.3	-166.4	4.6	36	**0.03**
SD in maximum frequency	Foraging stratum	0.6	41.7	0.1	36	0.75
SD in maximum frequency	Elevation	0.6	-29.2	0.1	36	0.77
SD in maximum frequency	Distribution pattern	0.6	110.5	1.4	36	0.24
Song length	Annual precipitation	1.0	0.0	2.7	36	0.11
Song length	Habitat	1.0	0.0	0.1	36	0.80
Song length	Foraging stratum	1.0	0.2	13.2	36	**0.0009***
Song length	Elevation	1.0	-0.1	1.9	36	0.17
Song length	Distribution pattern	1.0	0.0	0.1	36	0.80
Mean maximum frequency	Annual precipitation	0.9	0.0	0.0	36	0.97
Mean maximum frequency	Habitat	0.9	-0.1	2.5	36	0.13
Mean maximum frequency	Foraging stratum	0.7	0.3	10.1	36	**0.003**
Mean maximum frequency	Elevation	0.8	0.1	1.5	36	0.23
Mean maximum frequency	Distribution pattern	0.9	0.1	0.7	36	0.41
Mean bandwidth	Annual precipitation	0.9	0.0	0.1	36	0.71
Mean bandwidth	Habitat	0.9	-243.2	2.3	36	0.14
Mean bandwidth	Foraging stratum	0.9	-18.5	0.0	36	0.95
Mean bandwidth	Elevation	0.9	-185.6	0.7	36	0.42
Mean bandwidth	Distribution pattern	0.9	-225.2	1.8	36	0.71
Inter-element interval	Annual precipitation	0.7	0.0	11.0	36	**0.002***
Inter-element interval	Habitat	0.9	0.1	2.4	36	0.13
Inter-element interval	Foraging stratum	0.8	-0.1	1.5	36	0.23
Inter-element interval	Elevation	0.8	0.0	0.1	36	0.80
Inter-element interval	Distribution pattern	0.8	-0.1	10.6	36	**0.002***
Morphological Features vs. Ecological Characteristics						
Tarsus	Annual precipitation	0.6	0.0	0.0	36	0.84
Tarsus	Habitat	0.7	0.0	4.2	34	**0.04**
Tarsus	Elevation	0.6	0.0	0.0	34	0.87
Tarsus	Foraging stratum	0.8	0.0	4.4	34	**0.04**
Beak length	Annual precipitation	0.6	0.0	0.1	34	0.81
Beak length	Habitat	0.8	0.0	3.5	34	**0.07**
Beak length	Elevation	0.6	0.0	2.7	34	0.11
Beak length	Foraging stratum	0.7	0.0	1.9	34	0.17

[a] Bold *p*-values identify associations with significant effect ($p < 0.05$). Asterisks denote significant association after Bonferroni correction.

Figures

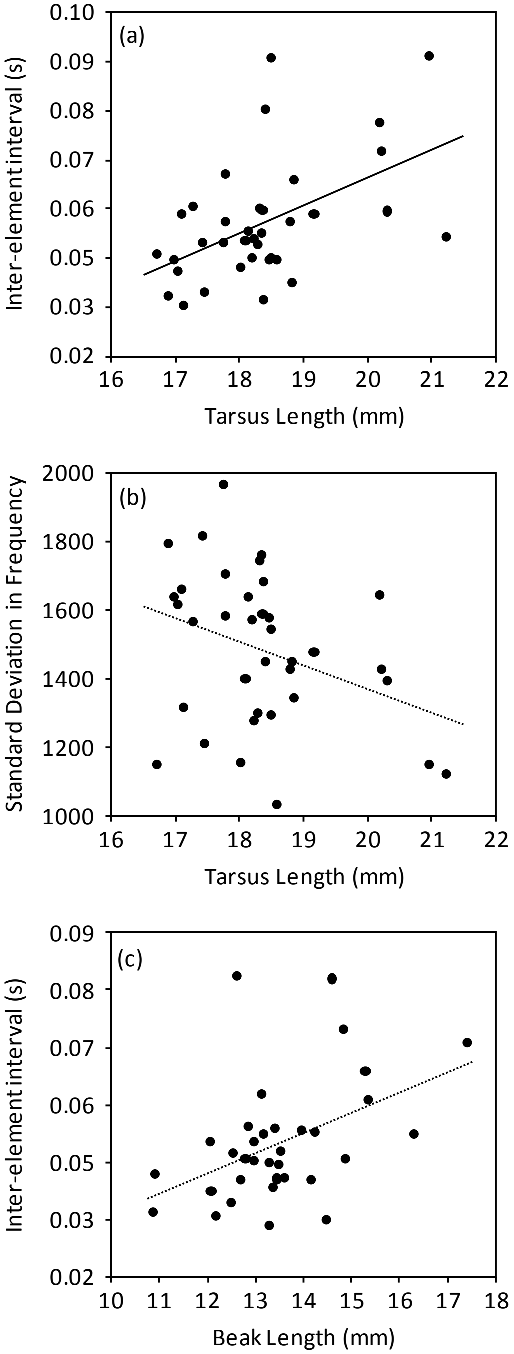

Figure 6.1. Scatter plots depicting relationships between morphological variables and fine structural acoustic features of the songs in 36 lineages of *Troglodytes* wrens throughout North, Central, and South America. (a) Wrens with longer tarsi (i.e. larger birds) sang songs with

significantly longer average inter-element intervals. (b) Wrens with shorter tarsi (i.e. smaller birds) sang songs with less variation in frequency measurements between song elements, although this pattern was not statistically significant following correction for multiple comparisons. (c) Wrens with longer beaks sang songs with longer inter-element intervals, although this pattern was not statistically significant following correction for multiple comparisons. Lines of best fit are shown, with solid lines showing significant relationships and dotted lines showing nonsignificant trends. Raw data are shown whereas normalized data were used in the phylogenetic least squares analyses (see text for details).

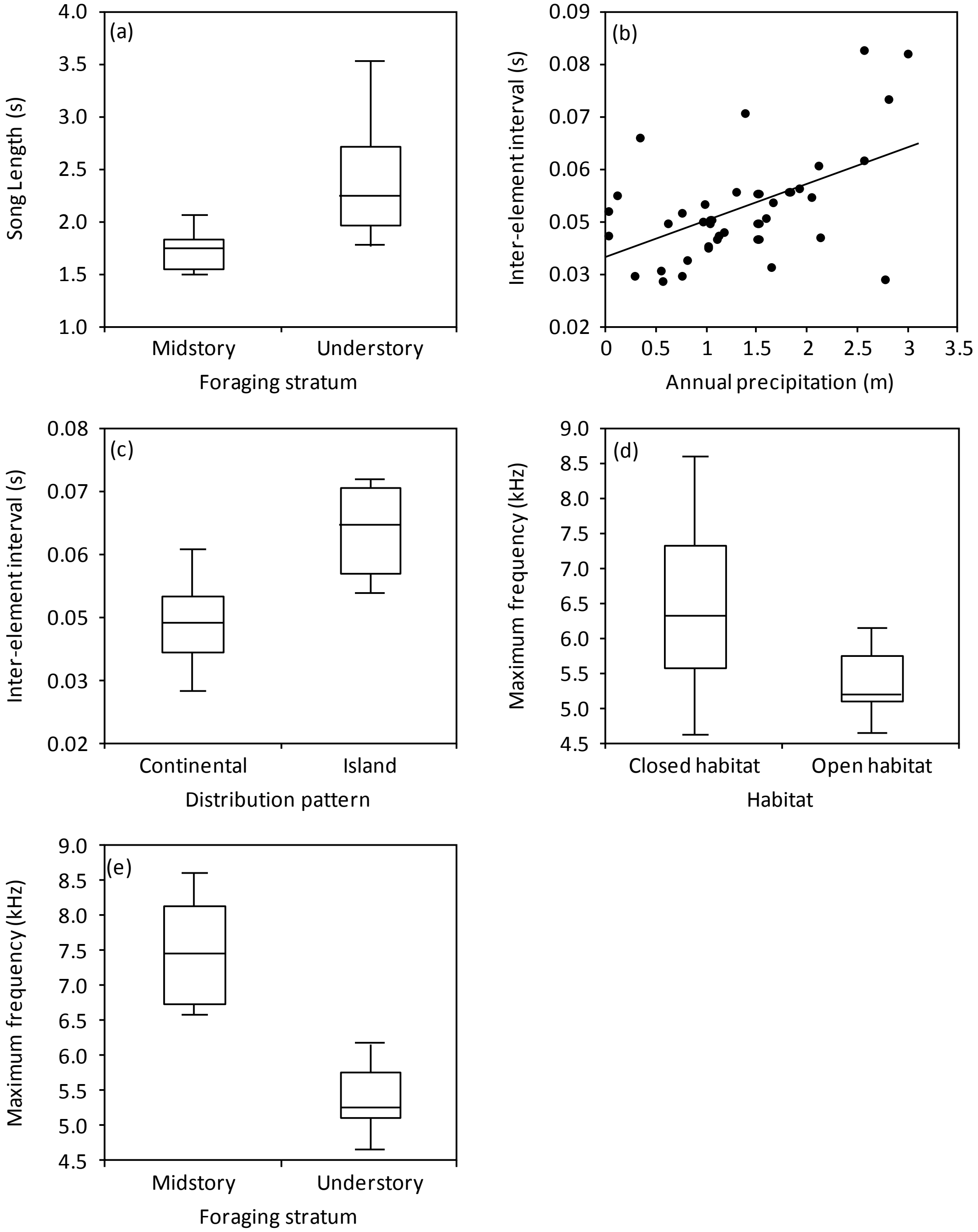

Figure 6.2. Scatter plots and box plots depicting relationships between ecological variables and fine structural acoustic features of the songs in 38 lineages of *Troglodytes* wrens throughout North, Central, and South America. (a) Wrens that forage in the understory sang significantly longer songs than wrens that forage in the midstory. (b) Wrens living in habitats with more

annual precipitation had significantly longer inter-element intervals; a line of best fit is shown. (c) Wrens living on islands sang songs with significantly longer inter-element intervals. (d) Wrens living in closed habitats sang songs with higher maximum frequencies than wrens living in open habitats, although this pattern was not statistically significant follow correction for multiple comparisons. (e) Wrens foraging in the midstory sang songs with higher maximum frequencies than wrens foraging in the understory, although this pattern was not statistically significant following correction for multiple comparisons. Box blots show the 10th, 25th, 50th, 75th, and 90th percentiles. Raw data are shown whereas normalized data were used in the phylogenetic least squares analyses (see text for details).

Chapter 7

General Discussion

Introduction

The idea that acoustic signals may be optimized for transmission through different habitats originated in the late 1940's (Lack and Southern 1949; Marler 1960), but was only tested rigorously in 1975 by Eugene Morton. Morton (1975) started a new line of investigation by showing that the time and frequency characteristics of bird songs are adapted for transmission through different types of habitat. Ten years later, Ryan and Brenowitz (1985) expanded Morton's research by revealing that acoustic signals vary not only with habitat characteristics, but also with body size, phylogenetic relatedness, and ambient noise. Since then, our understanding of acoustic signal evolution has expanded dramatically and numerous investigations have explored how diversity in acoustic signals has originated and been maintained. Despite the increasing number of studies of the influence of ecological, morphological, and phylogenetic factors on acoustic signals, many questions remain unanswered and much remains to be learned (Catchpole and Slater 2008). Studies that describe both the variation in animal acoustic signals and the way that animals perceive variation in signals are fundamental for developing a comprehensive understanding of the ecology and evolution of animal communication and the role that acoustic signals play in speciation (Wilkins et al. 2013).

Songbirds exhibit remarkable diversity in vocal behaviour and provide rich material for investigators interested in understanding the evolution of animal communication (Catchpole and Slater 2008). The vocalizations of songbirds are highly complex, comprising sets of characters rather than a simple trait, and each character can be limited by different costs and constraints (Gil and Gahr 2002). In order to understand the evolution of song it is necessary to document and describe the precise song structure present in each species (Langmore 2002, Hall 2004, Mann et al. 2009) and the variation in patterns of song production and song delivery (Todt and

Naguib 2000). Then, a second important step is to quantify the extent of acoustic variation within and between species (Catchpole and Slater 2008). Documenting vocal geographic variation allows scientists to associate variation in acoustic signals with phylogeny, morphological features, and ecological factors, providing the framework to identify the conditions that have led to the evolution of acoustic traits (e.g. Mann et al. 2009).

In this dissertation, I combined observational and experimental studies of free-living songbirds with measurements of museum specimens and archived recordings in a phylogenetic framework. I presented novel information on vocal behaviour, systematics, signal recognition, and acoustic signal evolution in *Troglodytes* wrens. Specifically, I provided the first detailed analysis of the vocal behaviour of two species of *Troglodytes* wrens from Mexico, one of which is an endemic species in the Caribbean Sea (chapters 2-3); I demonstrated that vocal diversity within the genus *Troglodytes* is extensive, further clarifying the taxonomic status of multiple subspecies within the House Wren complex (chapter 4); I showed that both the fine structural characteristics of *Troglodytes* songs and their perception are associated with phylogenetic relatedness (chapter 5); and I explored the evolutionary basis of diversification in acoustic signals (chapter 6). My research has important implications for the systematics of songbirds; the conservation of island endemic avian populations; and understanding the influence of isolation, habitat, morphology, and phylogenetic relatedness on acoustic signals. In this final dissertation chapter, I summarize the conclusion of each of the data chapters of my dissertation, I comment on the significance of my findings, and I provide suggestions for further investigations that would help to expand upon the research I have presented here.

Documenting vocal behaviour

My behavioural observations allowed me to provide the first empirical description of the vocal behaviour of two songbird species: the Cozumel Wren (*Troglodytes beani*) and the Brown-throated Wren (*Troglodytes brunneicollis*). In chapter 2, I showed that Cozumel Wrens sing songs composed of highly variable syllables, with prominent trills at the end of each song. Based on field observations collected over two years, I discovered that Cozumel Wrens have a limited repertoire of songs, which they create by recombining a restricted number of syllable and trill types. Cozumel Wrens repeat a song type several times before switching to a different one, with some variation in the number of times they repeat specific elements. I showed that Cozumel Wrens share more song types between neighbours than between distant individuals. I found that syllable sharing, however, was equivalent between neighbours and distant individuals. My detailed description of the singing behaviour of Cozumel Wrens facilitates future research on the ecology, evolution, and behaviour of this island-endemic songbird. Importantly, my research offers important evidence towards clarifying the controversial taxonomic status of Cozumel Wrens, suggesting that they sing substantially divergent songs from their mainland counterparts and supporting the hypothesis that they are a distinct species.

In chapter 3, I showed that Brown-throated Wrens produce complex songs and simple calls. My analyses demonstrated that individuals sing with eventual variety, repeating songs many times before switching to a new song type. I showed evidence that males combine syllables into phrases to create songs and that repertoire size is not fixed, as birds recombined their syllables to produce novel and highly variable song types. Brown-throated Wrens also sing with high vocal output after sunrise, and song activity then declines throughout the morning. In contrast to the pattern I showed for Cozumel Wrens, I found that the degree of song sharing in

Brown-throated Wrens exhibits no variation with distance among the individuals I sampled. Splitting the syllables of Brown-throated Wren songs into 13 categories, I found that birds sing some syllables more frequently than others. Moreover, I revealed that syllables are not sung at random; particular categories of syllable were more likely to be contributed to the beginning, middle, or end of the song. I showed that transitions between syllable categories deviate significantly from random chance, and most males analyzed follow similar patterns, revealing population-wide syntactical structure within their songs. These observations, which provide the first empirical study of Brown-throated Wren song, expand our knowledge of the behaviour of this poorly-studied taxon, and shed light into the organization and composition of song in tropical birds.

Evaluating acoustic divergence

In chapter 4, I focused on the diversity of singing behaviours within the House Wren complex. I measured songs from 786 individuals, and morphological traits from 401 individuals, from 609 locations throughout the Americas. I showed that most of the House Wren subspecies have diverged in song, morphology, or both. My results also showed that the acoustic differences between subspecies are similar to, and in some cases greater than, the divergence between pairs of currently recognized *Troglodytes* species. My research suggests that at least four allopatric subspecies—*T. a. nitidus*, *T. a. musculus*, *T. a. beani*, and *T. a. rufescens*—are likely to be different species, pending future genetic studies. Furthermore, my study sheds light into many other vocally and morphologically differentiated subspecies that will assist taxonomic authorities as they evaluate species boundaries in concert with future genetic analyses.

Phylogenetic implications of acoustic and behavioural variation

In chapter 5, I showed that acoustic divergence corresponds to phylogenetic distance across eight species of *Troglodytes* wrens. Then, by conducting a series of playback experiments to males of four species of *Troglodytes* wrens, I demonstrated that conspecific and allopatric-congeneric songs incite different intensities of response. Despite an overall similar intensity of males' responses to different allopatric-congeneric playback treatments, I showed that the acoustic similarity between the subject's songs and the playback stimuli predicts the strength of behavioural responses to playback. In addition, I revealed an association between phylogenetic distance and behavioural responses. Together, my findings indicated that phylogenetic divergence predicts acoustic divergence and that wrens perceive variation in songs. I concluded that the songs of *Troglodytes* wrens—and territorial males' perception of those songs—have phylogenetically constrained features.

In chapter 6, I showed that most acoustic features of *Troglodytes* wren songs exhibit a phylogenetic signal, suggesting that related Troglodytes have more similar traits than if traits were distributed randomly in the tree. I demonstrated that temporal properties of *Troglodytes* songs are correlated with ecological characteristics such as the strata where wrens forage, and the annual precipitation in wren habitats (a proxy for vegetation density), and whether wrens occupy island or mainland habitats. My results showed that wrens foraging in relatively dense vegetation sing longer songs, and wrens inhabiting areas with high annual precipitation sing songs with longer inter-element intervals. On the other hand, I showed that wrens inhabiting islands have longer inter-element intervals, supporting the predictions of the acoustic competition hypothesis. Further, my data reveal that morphological features had little influence on acoustic variation in *Troglodytes* wrens.

Conclusions and significance

Taken together, my dissertation research documents significant variation within the acoustic signals of *Troglodytes* wrens. The first chapters of my dissertation reveal that the songs in *Troglodytes* wrens are diverse, and that this diversity corresponds with subspecies boundaries. Further, vocal geographic variation predicts genetic divergence between *Troglotyes* wrens, and behavioural responses are predicted by both the fine structural characteristics of *Troglodytes* songs and phylogeny. These results suggest that *Troglodytes* wren songs encode species identity that is used to recognize conspecific rivals. In chapter 6 of my dissertation, I argue that temporal properties of *Troglodytes* songs vary with ecological characteristics at a continental scale, providing support for both the acoustic adaptation hypothesis and the acoustic competition hypothesis.

My research complements other studies describing avian vocal behaviour, and expands our understanding of the diversity of song organization strategies already known in birds (e.g. Kroodsma 1977, 1980; Platt and Ficken 1987; Spector 1991, 1992; van Horne 1995; Leger 2005, Valderrama et al. 2008). I provide new information regarding the taxonomy of *Troglodytes* wrens, with a special focus on island populations, which are under heightened levels of anthropogenic stress (e.g. Winarni and Jones 2012). Island-living birds have proven to be especially revealing subjects for evolutionary investigations (e.g. Grant 1998), and my research on island-living songbirds further expands our understanding of the evolution of sexually-selected traits on islands.

My findings are consistent with numerous studies that have documented a relationship between geographic isolation and divergence in acoustic traits (e.g. Dingle et al 2008, Vázquez-Miranda et al. 2009, González et al. 2011, Aleixandre et al. 2013, Sosa López et al. 2013, Campagna et al. 2012). My research offers one of only three examples in vertebrates, as far as I am aware, that shows that signals elicit responses that are proportional to the genetic distance between species; this pattern has been previously demonstrated only in two species of doves (de Kort and ten Cate 2001) and frogs (Ryan et al. 2003). In contrast to the general trend reported by Boncoraglio and Saino (2007) that habitat influences peak frequency of vocalizations, but not other frequency or temporal variables, my research provides support for the idea that both temporal variables and other frequency-related song traits vary with habitat and whether birds are found in islands. My findings thereby add to the growing body of literature that supports the acoustic adaptation hypothesis and the acoustic competition hypothesis (e.g. Lack and Southern 1949, Marler 1960, Badyaev and Leaf 1997, van Dongen and Mulder 2006, Dingle et al. 2008, Tobias et al. 2010).

Suggestions for future research

Further research in *Troglodytes* wrens should explore the diversity of vocal behaviour within this genus. Basic vocal descriptions for most taxa in this diverse genus are completely lacking. The recent molecular phylogeny presented by Chaves et al. (2014) suggests that there are 42 lineages within the genus, and yet we have detailed vocal descriptions for only four of these lineages (Cozumel Wrens, Chapter 2; Brown-throated Wrens, Chapter 3; House Wrens, Rendall and Kaluthota 2013; and Pacific Wrens, Toews and Irwin 2012). Recordings that I collected on Socorro Island and Clarion Island while conducting the experiment in Chapter 5 will give rise to two future descriptions of the vocal behaviour of these two species. This leaves at least 36 evolutionarily independent lineages in the genus *Troglodytes* in need of careful vocal study.

Current evidence suggests that House Wren singing behaviour varies with breeding stage and that male song is a context-dependent trait, serving different functions at different times of year (Johnson and Kermott 1991). Indeed, seasonal changes in vocalizations and vocal behaviour appear to be widespread in birds (e.g. Spector 1991, 1992; Topp and Mennill 2008; Tremain et al 2008; Koloff and Mennill 2013). Future studies that include long-term recordings could provide valuable information on the function of song in *Troglodytes* wrens throughout the year, and would help to establish whether the patterns I documented in Cozumel Wrens and Brown-throated Wrens hold true outside of the breeding season. Further, theory suggests that song repertoire size is related to sexual selection through female choice (i.e. the Repertoire Size Hypothesis; Catchpole 1976). Future studies should test whether repertoire size in *Troglodytes* wrens is associated with female choice; this might explain the diversity in repertoire sizes that I documented in Cozumel Wrens. Conversely, the high variability in song repertoire size present in Brown-throated Wrens, and the pattern I found that males do not emphasize the different components of their repertoire with similar frequency, suggest that other factors such as intra-sexual interactions may be driving patterns of song repertoire use. As such, future research should attempt to explore whether shared song elements may be used for communicating different levels of threat, such as escalating or de-escalating conflict during male-male interactions, explaining the complex patterns of repertoire use during intra-sexual interactions in this and other *Troglodytes* wrens (Beecher et al. 2000).

Future research on vocal geographic variation should attempt to document variation between sympatric populations, testing whether the differences in vocalizations that I report in this dissertation change abruptly or continuously between taxa when they co-occur. This task is challenging, particularly in species that exhibit little phenotypic variation, like wrens in general, and like the birds in the House Wren complex in particular (Brewer 2001). Such studies should combine song analysis, genetic studies, and experimental playbacks (e.g. Dingle et al. 2008, Toews and Irwin 2008, Chapter 5) in order to provide deeper understanding of divergence between these taxa.

In discussing the responses of wrens to playback of allopatric-conspecific songs, I argued that the playback subjects may have compared the playback stimuli to an internal “auditory template” (i.e. a neuronal representation of a song in a given species; Catchpole and Slater 2008). To better understand how songbirds evaluate the similarity between the songs of closely-related and distantly-related animals, future studies should study auditory templates. Describing the auditory templates in animals is a challenging task and has rarely been attempted (although see Marler 2004). Playback experiments to captive frogs, however, have used experimentally modified signals to assess the limits of frog perception (e.g. Amézquita et al. 2011); a similar approach may be feasible in wrens, which respond well to temporary captivity (e.g. Templeton et al. 2013), so that they could be assessed responding to a variety of modified signals.

Although a growing number of studies have documented ecological effects on acoustic signals, the relative importance of divergence in acoustic and ecological factors and speciation remains poorly known, as highlighted in a recent, high-profile review paper by Wilkins et al. (2013). To demonstrate that speciation is the result of ecological factors acting on acoustic divergence in *Troglodytes* wrens, future studies should try to test whether variation in songs is associated with assortative mating (Wilkins et al. 2013). Furthermore, I found a strong phylogenetic signal in two acoustic traits (song duration and song bandwidth) and two ecological features (the stratum at which wrens forage and elevation), suggesting that these traits may have followed a gradual evolution within *Troglodytes* wrens (Kamilar and Cooper 2013). This scenario is expected to occur under neutral evolution or drift (Felsenstein 1985). To investigate whether acoustic divergence is heavily influenced by drift, future investigations should compare neutral genetic distance and acoustic divergence (Wilkins et al. 2013). Future investigations of song evolution should also attempt to directly assess whether properties of the habitat (i.e. vegetation density, background noise) affect song transmission in *Troglodytes* wrens using playback experiments (e.g. Dingle et al 2008).

Conclusion

In conclusion, the findings in my dissertation expand our understanding of the evolution of animal acoustic signals. My dissertation research stands as an example of the complexity of animal acoustic signals and how these signals can be used to resolve taxonomic problems. My findings enhance our understanding of how vocal communication—a sexual trait—is influenced by geographic isolation, habitat, morphology, and phylogeny. My findings also shed light on how organisms perceive species-specific signals and the importance of phylogenetic conservatism in signal perception. Finally, my findings help to clarify our understanding of the taxonomy and diversity in the genus *Troglodytes*.

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Appendix

Supplementary Information Accompanying Chapter 3: The vocal behaviour of the Brown-throated Wren (*Troglodytes brunneicollis*): song structure, repertoires, sharing, syntax, and diel variation

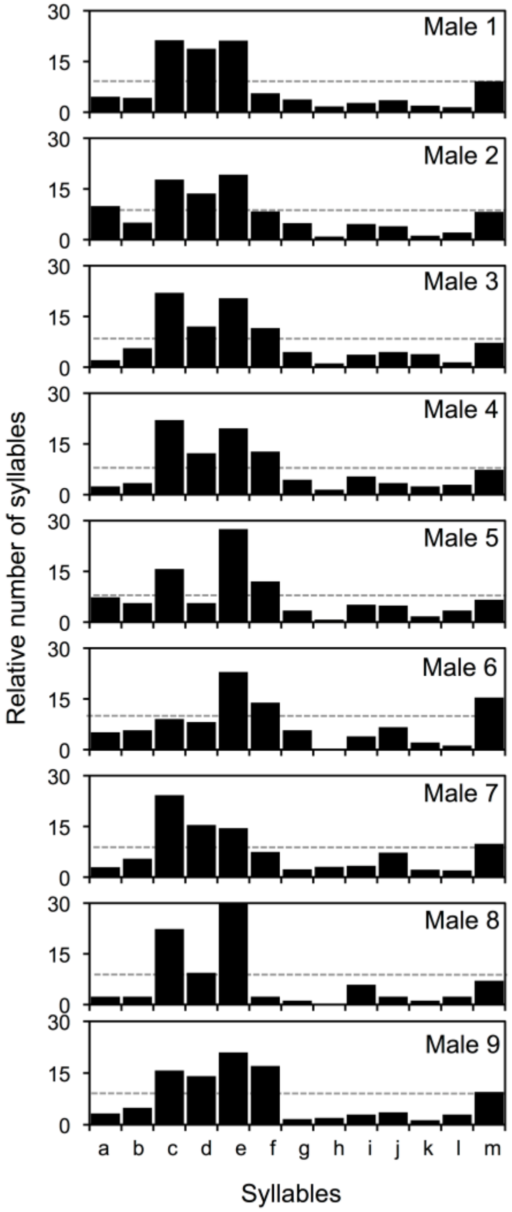

Figure 3.S1. Relative frequency distribution of the occurrence of syllable categories for nine Brown-throated Wrens. Some syllables were sung much more frequently than others. The dotted line shows the predicted frequency of each song syllable if all syllables were given equally often

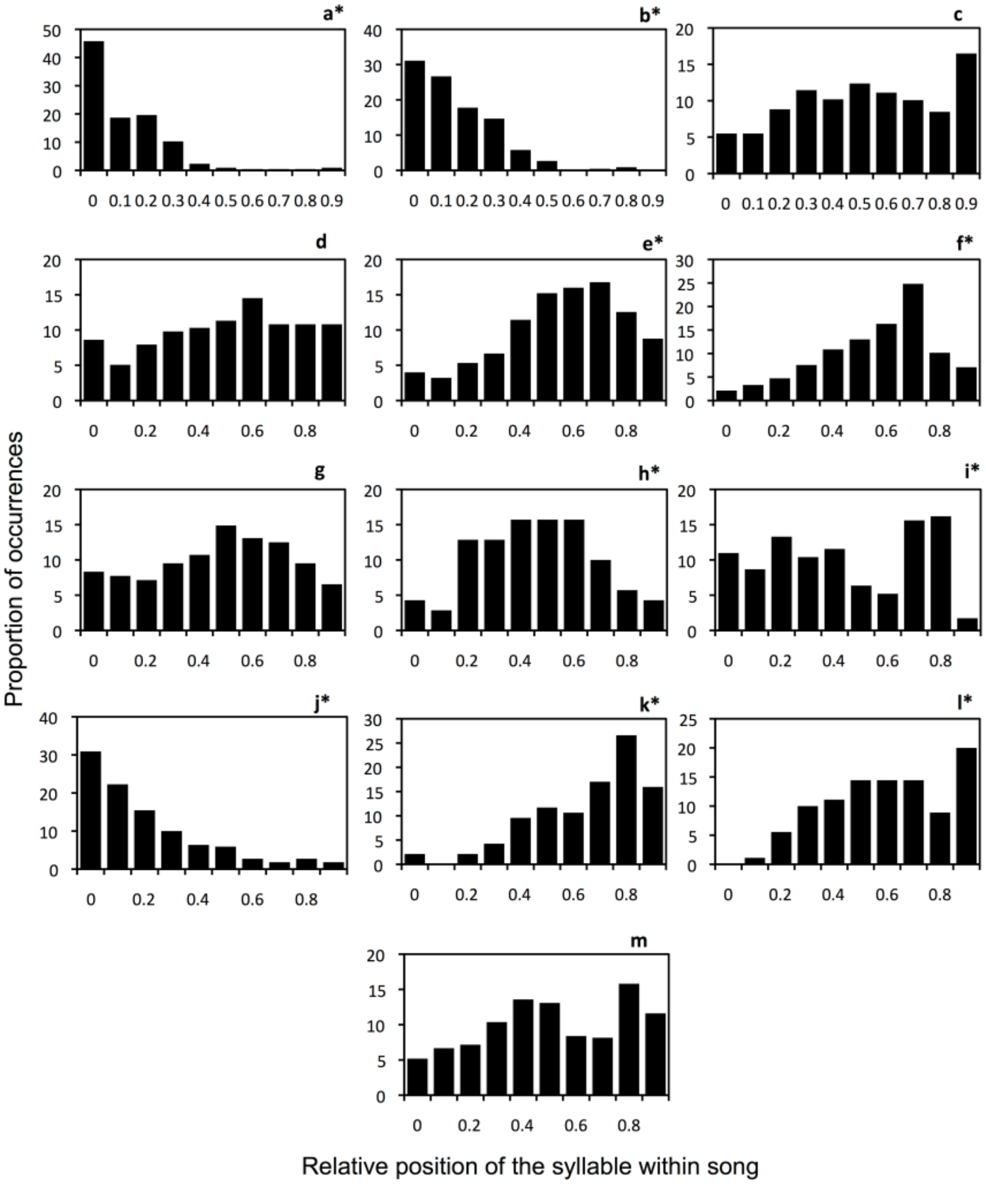

Figure 3.S2. Relative position of thirteen syllable categories in the songs of Brown-throated Wrens. Letters correspond to the syllable categories shown in Figure 3.2. of the main paper. Asterisks denote the nine syllables for which the chi-square tests showed statistically significant deviation from the null hypothesis that each syllable category was equally likely to occur at any point in the song

Table 3.S1. Results of lag sequential analysis used to evaluate whether Brown-throated Wren songs show evidence of syntactical rules. Values given are overall Pearson chi-square statistics for each of nine individuals for the transitions between syllables with lag +1 and lag +2

	Lag +1			Lag +2			
Bird identity	Pearson chi-square (χ^2)	df	*p*	Pearson chi-square (χ^2)	df	*p*	*n*
Male 1	582.28	144	<0.01	389.79	144	<0.01	42
Male 2	838.73	144	<0.01	713.20	169	<0.01	30
Male 3	534.59	144	<0.01	388.68	144	<0.01	30
Male 4	264.50	144	<0.01	213.56	144	<0.01	10
Male 5	575.40	144	<0.01	387.99	144	<0.01	19
Male 6	421.81	144	<0.01	323.25	144	<0.01	18
Male 7	692.44	144	<0.01	512.68	144	<0.01	42
Male 8	208.61	144	<0.01	128.93	144	0.81	4
Male 9	420.29	144	<0.01	334.99	144	<0.01	15

Supplementary Information Accompanying Chapter 4: Continent-wide patterns of divergence in acoustic and morphological traits in the House Wren species complex

Table 4.S1. Detailed information of the recordings used in the study from personal collections, published CDs, and libraries of natural sounds. The table shows species, group to which each recording belongs, country, province, GPS coordinates, and number of recordings.

Source[a,b]	Species[e]	Group[†]	Country	Province	Coordinates	Songs[c]
JRSL	*T. a. beani*	beani	Mexico	Quintana Roo	20° 24'N, -87° 00'W	33
	T. a. nitidus	brunneicollis	Mexico	Oaxaca	16° 07'N, -96° 56'W	24
	T. a. musculus	musculus	Costa Rica	Puntarenas	10° 16'N, -84° 47'W	3
	T. a. musculus	musculus	Argentina	Buenos Aires	-34° 29'N, -58° 32'W	1
	T. a. musculus	musculus	Argentina	Mendoza	-34° 49'N, -68° 30'W	3
	T. rufocilliatus		Mexico	Chiapas	15° 38'N, -92° 48'W	2
	T. rufocilliatus		Mexico	Chiapas	15° 39'N, -92° 48'W	4
	T. sissonii		Mexico	Colima	18° 46', -110° 57'W	9
	T. tanneri		Mexico	Colima	18° 20'N, -114° 44'W	11
LS	*T. a. musculus*	musculus	Costa Rica	Alajuela	10° 08'N, -84° 14'W	2
	T. a. musculus	musculus	Costa Rica	Cartago	09° 49’N, -83° 26’W	4
	T. a. musculus	musculus	Costa Rica	Heredia	10° 01'N, -84° 05'W	30
	T. a. musculus	musculus	Costa Rica	Heredia	10° 04'N, -84° 04'W	1
	T. a. musculus	musculus	Costa Rica	Heredia	10° 07'N, -84° 06'W	1
	T. a. musculus	musculus	Costa Rica	Puntarenas	09°01’ N, -83°01’W	1
	T. a. musculus	musculus	Costa Rica	Puntarenas	08°23'N, -83°18'W	1
	T. a. musculus	musculus	Costa Rica	Puntarenas	09°29'N, -84°02'W	1
	T. a. musculus	musculus	Costa Rica	San José	09° 54' N, -84°03'W	8
	T. a. musculus	musculus	Nicaragua	Matagalpa	13° 00'N, -84° 54'W	1
MG	*T. a. musculus*	musculus	Mexico	Veracruz	17° 58'N, -94° 31'W	1
MAS	*T. a. musculus*	musculus	Costa Rica	Puntarenas	08° 59'N, -082° 51'W	1
REW	*T. a. parkmanii*	aedon	Mexico	Baja California	30° 57'N, -115° 36'W	2
	T. a. parkmanii	aedon	Mexico	Baja California	30° 58'N, -115° 34'W	2
	T. a. parkmanii	aedon	Mexico	Baja California	30° 58'N, -115° 44'W	2
	T. a. parkmanii	aedon	Mexico	Baja California	31° 00'N, -115° 33'W	5
	T. a. parkmanii	aedon	Mexico	Baja California	32° 02'N, -115° 54'W	2
	T. a. cahooni	brunneicollis	Mexico	Sonora	28° 21'N, -109° 01'W	4
	T. a. cahooni	brunneicollis	USA	Arizona	31° 45'N, -109° 25'W	2
	T. a. cahooni	brunneicollis	USA	Arizona	31° 53'N, -109° 16'W	2
	T. a. cahooni	brunneicollis	USA	Arizona	31° 53'N, -109° 09'W	14
	T. a. cahooni	brunneicollis	USA	Arizona	31° 55'N, -109° 16'W	2
	T. a. cahooni	brunneicollis	USA	Arizona	31° 56'N, -109° 15'W	2
	T. a. cahooni	brunneicollis	USA	Arizona	31° 58'N, -109° 22'W	1
RW	*T. cobbi*		England	Falkland Islands	-51° 16'N, -60° 33'W	2
	T. cobbi		England	Falkland Islands	-51° 37'N, -57° 45'W	8
	T. cobbi		England	Falkland Islands	-51° 44'N, -59° 34'W	1
TC	*T. a. parkmanii*	aedon	USA	California	33° 57'N, -117° 23'W	2
	T. a. parkmanii	aedon	USA	California	33° 57'N, -117° 30'W	2
VW	*T. a. parkmanii*	aedon	USA	Colourado	40° 16'N, -103° 50'W	1
	T. a. brunneicollis	brunneicollis	Mexico	Mexico City	19° 18'N, -99° 18'W	1
	T. a. musculus	musculus	Argentina	ND	-54° 45'N, -68° 28'W	1
	T. a. musculus	musculus	Colombia	ND	-04° 11'N, -69° 56'W	1
	T. hiemalis		USA	North Carolina	35° 45'N, -82° 16'W	1
	T. rufocilliatus		Mexico	Chiapas	16° 43'N, -92° 41'W	1
	T. sissonii		Mexico	Colima	18° 46'N, -110° 57'W	1
	T. tanneri		Mexico	Colima	18° 20'N, -114° 44'W	1
BSC	*T. a. musculus*	musculus	Colombia	Nariño	1° 21'N, -77° 17'W	1
BSM	*T. a. brunneicollis*	brunneicollis	Mexico	Veracruz	19° 37'N, -97° 04'W	1
	T. a. musculus	musculus	Mexico	Veracruz	18° 49'N, -96° 54'W	1

ML[d]	*T. a. aedon*	aedon	Details available through ML metadata	37
	T. a. parkmanii	aedon	Details available through ML metadata	58
	T. a. cahooni	brunneicollis	Details available through ML metadata	13
	T. a. brunneicollis	brunneicollis	Details available through ML metadata	4
	T. a. musculus	musculus	Details available through ML metadata	136
	T. a. beani	beani	Details available through ML metadata	6
	T. a. rufescens	martinicensis	Details available through ML metadata	7
	T. a. rufescens or musicus	martinicensis	Details available through ML metadata	5
	T. cobbi		Details available through ML metadata	1
	T. hiemalis		Details available through ML metadata	64
	T. pacificus		Details available through ML metadata	36
	T. rufocilliatus		Details available through ML metadata	6
BLB[d]	*T. a. aedon*	aedon	Details available through BLB metadata	11
	T. a. parkmanii	aedon	Details available through BLB metadata	25
	T. a. cahooni	brunneicollis	Details available through BLB metadata	2
	T. a. musculus	musculus	Details available through BLB metadata	6
	T. sissonii		Details available through BLB metadata	6
FNJV[d]	*T. a. parkmanii*	aedon	Details available through FNJV metadata	1
	T. a. musculus	musculus	Details available through FNJV metadata	45
MZFC[d]	*T. a. cahooni*	brunneicollis	Details available through MZFC metadata	1
	T. sissonii		Details available through MZFC metadata	1
	T. tanneri		Details available through MZFC metadata	9
BSA[d]	*T. a. musculus*	musculus	Details available through BSA metadata	24
BSAM[d]	*T. aedon*	musculus	Details available through BSAM metadata	5
	T. rufocilliatus		Details available through BSAM metadata	10
FLMNH[d]	*T. a. aedon*	aedon	Details available through FLMNH metadata	6
	T. a. parkmanii	aedon	Details available through FLMNH metadata	1
	T. a. cahooni	brunneicollis	Details available through FLMNH metadata	2
	T. a. brunneicollis	brunneicollis	Details available through FLMNH metadata	8
	T. a. musculus	musculus	Details available through FLMNH metadata	2
	T. a. beani	beani	Details available through FLMNH metadata	1
	T. tanneri		Details available through FLMNH metadata	2
	T. ochraceus		Details available through FLMNH metadata	3
	T. rufocilliatus		Details available through FLMNH metadata	3

[a] Recordists' names and libraries: JRSL, José Roberto Sosa López; LS, Luis Sandoval; MG, Manuel Grosselet; MAS, Marcelo Araya Salas; REW, Richard E. Webster; RW, Robin Woods; TC, Tony Cellis, ML, Macaulay Library; BLB, Borror Laboratory of Bioacoustics; FNJV, Fonoteca Neotropical "Jacques Vielliard"; MZFC, Museo de Zoología "Alfonso L. Herrera"; BSA, Banco de Sonidos Animales; BSAM, Biblioteca de Sonidos Aves de México.

[b] Published CDs: VW, Voices of the Wren; BSC, A Guide to the Bird Sounds of the Colombian Andes; BSM, Bird Songs of Mexico, Veracruz: volume 1.

[c] Number of total recordings. For purposes of data presentation, we condensed the information showing one geographic coordinate for recordings that occurred in locations with similar degrees and minutes, but different seconds.

[d] We provide the catalogue number of the recordings gathered from libraries in Table A2.

[e] Subspecies designations used in this table are based on Kroodsma and Brewer (2005).

[f] According to the American Ornithologists' Union (1998).

Table 4.S2. Recordings from libraries used in the analysis of vocal divergence in the House Wren complex. Numbers refer to catalogue identity of each recording in the analysis.

Library and catalogue number
Macaulay Library (ML), Cornell University, United States
23, 9614, 9615, 9616, 9618, 9619, 9620, 9621, 9622, 9623, 9624, 9625, 9627, 9628, 9629, 9630, 9631, 9632, 9634, 9636, 9637, 9638, 9639, 9640, 9641, 9642, 9643, 9644, 9645, 9647, 9650, 9651, 9652, 9653, 9654, 9655, 9656, 9657, 9658, 9659, 9660, 9661, 9662, 9663, 9666, 9667, 9668, 9669, 9670, 9671, 9672, 9675, 9676, 9677, 9678, 9679, 9680, 9682, 9683, 9684, 9685, 9686, 9687, 9688, 9689, 9690, 9691, 9692, 9693, 9695, 9697, 10467, 11501, 11636, 12805, 13654, 17176, 17193, 17476, 18052, 18711, 19581, 19949, 20086, 21111, 21143, 21454, 21581, 21694, 22856, 22858, 22863, 23067, 23213, 23734, 23868, 24065, 24080, 24342, 25407, 26751, 26760, 27189, 27195, 27825, 28003, 29098, 29381, 30516, 30617, 31475, 32118, 32143, 32336, 32410, 32477, 33633, 33661, 33671, 33889, 33906, 33930, 34042, 34072, 35051, 35231, 35858, 36947, 36952, 37596, 37765, 37800, 37826, 38401, 38554, 38567, 39262, 39327, 39876, 40668, 41450, 42080, 42192, 42231, 42272, 44046, 44047, 44048, 44051, 44052, 44056, 44058, 44150, 44865, 44940, 45094, 45274, 45280, 45307, 49067, 49783, 50129, 50144, 50721, 50733, 50744, 51044, 52466, 52497, 52924, 53178, 53278, 53315, 53429, 54239, 55058, 55100, 55101, 55103, 55448, 56812, 56819, 56882, 56889, 56991, 62910, 63079, 63209, 63210, 63233, 63235, 63236, 63237, 63238, 63239, 63240, 63241, 63243, 63245, 63248, 63249, 63271, 63272, 63273, 63274, 63275, 63276, 63277, 63278, 63279, 63281, 63282, 63283, 63284, 63285, 63287, 63288, 63289, 63996, 66983, 66985, 66986, 66987, 66988, 66989, 66990, 66992, 66993, 66995, 66996, 66997, 66998, 66999, 67000, 67001, 67002, 67003, 67004, 67005, 67006, 67007, 67008, 67009, 71869, 71891, 73323, 73991, 76531, 76705, 78981, 79416, 79419, 79467, 79473, 80377, 80588, 80838, 80965, 80987, 81584, 82096, 82394, 82438, 82656, 82663, 84806, 86882, 88924, 90094, 90095, 90433, 92913, 93372, 93797, 94290, 94340, 95079, 100786, 100830, 100847, 100871, 100917, 101026, 102263, 103377, 104361, 105298, 105318, 105476, 105613, 105647, 105964, 105986, 106081, 106562, 106636, 106664, 106666, 106671, 106683, 106687, 106769, 106773, 106869, 106894, 107041, 107302, 107307, 107375, 107384, 107406, 108881, 109102, 109105, 109115, 109120, 109126, 109297, 110917, 110970, 111032, 111049, 111075, 111089, 111099, 112013, 112104, 112148, 112607, 114880, 115803, 115811, 117980, 118642, 118646, 118684, 118890, 121956, 126401, 126480, 127203, 127497, 127643, 129349, 129397, 129766, 133324, 133974, 133986, 134951, 136152, 136183, 136324, 136450, 136468, 136635, 138657, 139000, 140007, 140680, 140702, 140724, 140728, 146737, 147891, 148380, 161087, 163264, 163925, 163946, 163953, 163969, 164427
Borror Laboratory of Bioacoustics (BLB), The Ohio State University, USA
3435, 5187, 6542, 6549, 6571, 6584, 6604, 7685, 8195, 8620, 8820, 9145, 9359, 9696, 10167, 10771, 10794, 10820, 11370, 11481, 12416, 12497, 12528, 12550, 13029, 14122, 15715, 16057, 16463, 18438, 18445, 20293, 20474, 20983, 21000, 21001, 21002, 21024, 21047, 21594, 25905, 28403, 29141, 29456, 29578, 30824, 32092, 33471, 34118, 34992
Fonoteca Neotropical "Jacques Vielliard" (FNJV), Universidade de Campinas, Brazil
4510, 4474, 4475, 5716, 4479, 4483, 5720, 4476, 4477, 4489, 5724, 4487, 4490, 4478, 4484, 5721, 4491, 4480, 4481, 4482, 5717, 5718, 5719, 4492, 4486, 4470, 4471, 4472, 4494, 4488, 4520, 4485, 5722, 4498, 8274, 10430, 11982, 5734, 4497, 4499, 4511, 4502, 10446, 10488, 10782, 4518, 9735, 4516, 4495, 4503, 5730, 4496, 4504, 10012, 4507, 4508, 5748, 5753, 4500, 5737, 4512, 4517, 4513, 5749, 4501, 4519, 5727, 5739, 5740, 5732, 5723, 5733, 5752, 5746, 4514, 4515, 5728, 5707, 5708, 5709, 5726, 5711, 5712, 4493, 5735, 5736, 5725, 5715, 5713, 5741, 5747, 10013, 5729, 5714, 10457, 4736
Museo de Zoología "Alfonso L. Herrera" (MZFC), Universidad Nacional Autónoma de México, Mexico
001REV17, 002REV10, 002REV18, 003REV03, 003REV11, 004REV04, 005REV05, 007REV15, 008REV08, 008REV16, CLARI006, CLARI009, CLARI011, CLARI014, CLARI016, CLARI018, CLARI030, CLARI037, CLARI050, CLARI062, CLARI070, CLARI076, CLARI079, CLARI084, CLARI092, CLARI097, CLARI099, CLARI108, MO0587, SOCO1001, SOCO1003, SOCO1005, SOCO1011, SOCO1012, SOCO1022, SOCO1036, SOCO1038, SOCO1040, SOCO1055, SOCO1071, SOCO1100, SOCO1107
Banco de Sonidos Animales (BSA), Instituto Alexander von Humboldt, Colombia
3435, 5187, 6542, 6549, 6571, 6584, 6604, 7685, 8195, 8620, 8820, 9145, 9359, 9696, 10167, 10771, 10794, 10820, 11370, 11481, 12416, 12497, 12528, 12550, 13029, 14122, 15715, 16057, 16463, 18438, 18445, 20293, 20474, 20983, 21000, 21001, 21002, 21024, 21047, 21594, 25905, 28403, 29141, 29456, 29578, 30824, 32092, 33471, 34118, 34992
Biblioteca de Sonidos Aves de México (BSAM), INECOL, Mexico
90227_15, 090626_02, 90629_07, 090630_05, 90925_02, 90928_10, 91130_00, 100422_03, 100702_01, 100703_04, 110328_01, 110328_07, 110328_10, 110616_00, 9GOGF06318
Florida Museum of Natural History (FLMNH), University of Florida, USA
399, 702, 778, 1322, 3926, 4961, 5229, 11875, 12795, 15020, 18519, 19864, 19865, 19866, 19867, 20135, 21711, 21712, 24377, 24384

Table 4.S3. Correlation matrix of the 15 acoustic variables used to perform Principal Component Analysis. Significant correlationas at *p* = 0.05 are shown in bold font.

	Song length	Minimum frequency	Maximum frequency	Element length	Number of elements	Number of trills	Inter-interval element	Mean maximum frequency	Mean bandwidth	Mean peak frequency	Mean entropy	Peak frequency shifts per second	SD in maximum frequency	SD in bandwidth
Minimum frequency	**-0.11**													
Maximum frequency	**0.24**	0.05												
Element length	0.05	0.02	**-0.06**											
Number of elements	**0.67**	**0.08**	**0.28**	0.03										
Number of trills	**0.30**	**0.06**	**0.11**	0.02	**0.47**									
Silence	**0.21**	-0.25	**-0.13**	0.04	**-0.35**	**-0.18**								
Mean maximum frequency	**0.21**	**0.19**	**0.62**	0.04	**0.17**	-0.01	**-0.12**							
Mean bandwidth	**0.13**	**-0.21**	**0.50**	-0.02	0.02	-0.02	0.05	**0.71**						
Mean peak frequency	**0.21**	**0.32**	**0.44**	**0.07**	**0.21**	0.01	**-0.13**	**0.82**	**0.30**					
Mean entropy	**0.12**	-0.01	**0.30**	**-0.13**	**0.07**	-0.02	**-0.10**	**0.46**	**0.62**	**0.19**				
Peak frequency shifts per second	**0.11**	**0.16**	**0.38**	0.03	**0.30**	0.01	**-0.30**	**0.58**	**0.26**	**0.64**	**0.16**			
SD in maximum frequency	**0.15**	-0.01	**0.81**	**-0.12**	**0.14**	0.06	-0.01	**0.45**	**0.42**	**0.29**	**0.24**	**0.25**		
SD in bandwidth	**0.16**	**-0.21**	**0.74**	**-0.07**	**0.08**	0.01	0.01	**0.41**	**0.55**	**0.17**	**0.32**	**0.17**	**0.84**	
SD in entropy	**0.18**	0.01	**0.45**	**-0.16**	**0.13**	0.01	-0.03	**0.19**	**0.20**	**0.10**	**0.36**	**0.10**	**0.63**	**0.69**

Table 4.S4. Information of the birds measured for the morphological study caught during field work. The table shows subspecies, group to which every recording belongs, country, province, GPS coordinates, and number of individuals.

Subspecies[a]	Group[b]	Country	Province	Coordinates	Birds
T. aedon beani	beani	Mexico	Quintana Roo	20° 24'N, -87° 00'W	13
T. aedon nitidus	brunneicollis	Mexico	Oaxaca	16° 07'N, -96° 56'W	13

[a] According to Kroodsma and Brewer (2005).

[b] According to the American Ornithologists' Union (1998).

Table 4.S5. Catalogue number of skins used in the analysis of morphological variation in the House Wren complex.

Museum and catalogue number
American Museum of Natural History (AMNH)
39497, 39498, 39500, 55124, 55125, 58969, 58971, 58972, 60714, 68525, 70472, 75849, 78011, 78012, 78013, 79048, 79049, 86039, 101363, 102942, 105667, 105679, 107228, 108298, 108301, 109098, 109099, 109102, 112521, 118088, 118091, 118092, 122486, 122489, 122490, 122499, 122500, 124819, 133975, 136678, 136680, 144381, 144384, 147840, 152380, 164388, 164389, 166300, 166303, 166307, 166314, 172037, 172042, 172047, 174731, 174733, 175499, 177717, 182883, 185997, 230345, 230346, 230347, 235013, 247183, 248342, 254632, 254634, 325621, 327710, 327712, 327713, 374500, 388770, 391711, 391712, 391713, 391720, 391721, 395912, 395923, 395924, 395927, 395929, 395934, 395939, 395954, 395959, 395961, 502183, 502262, 502295, 502361, 502362, 502363, 502371, 502461, 502465, 502466, 502468, 502469, 502470, 502472, 502473, 502474, 502475, 502476, 502477, 502478, 706785, 706786, 757501, 757584, 757585, 757586, 757587, 757588, 757589, 757594, 757595, 757596, 757598, 757699, 775759, 775760, 775761, 781865, 781866, 785847, 788884, ND
Field Museum of Natural History (FMNH)
2548, 2549, 2551, 6338, 8337, 9393, 9400, 9402, 9403, 9407, 9408, 9409, 9411, 9412, 9413, 9418, 9420, 9421, 9424, 9427, 9429, 9453, 9454, 9455, 9456, 9458, 9460, 9462, 9463, 9464, 9467, 9469, 9470, 13380, 14947, 15026, 15027, 15030, 17907, 17912, 17916, 20374, 20376, 20377, 22112, 22113, 22115, 23223, 32431, 35069, 35070, 35071, 35072, 35074, 35075, 35079, 35080, 35082, 35083, 35084, 35679, 35680, 35682, 35687, 35689, 35690, 36194, 43638, 46680, 46682, 46683, 46684, 46686, 46687, 46688, 46689, 47624, 47625, 50177, 50192, 50193, 50194, 50195, 50196, 50200, 50202, 50205, 50211, 50212, 50213, 50215, 50216, 50217, 50219, 50220, 50221, 53042, 53044, 53556, 54089, 54093, 54094, 54095, 57545, 58993, 58996, 58997, 59869, 59871, 59872, 59873, 59874, 59875, 59876, 59877, 59878, 59880, 59881, 59882, 59883, 59884, 61964, 61966, 61970, 61971, 61973, 61974, 61975, 61977, 61979, 61980, 61981, 61982, 61984, 61985, 61990, 61992, 61993, 61996, 61997, 61999, 62000, 62001, 62002, 62003, 62006, 62714, 62715, 62716, 62717, 62718, 62721, 64669, 64670, 64672, 64821, 64822, 64824, 64825, 64827, 64829, 64830, 69814, 69815, 72489, 72493, 72495, 91740, 91741, 92251, 92252, 92253, 92255, 92257, 92260, 92262, 92931, 93872, 93874, 93875, 93876, 95305, 102649, 102650, 102651, 108526, 108528, 108529, 108530, 109838, 109839, 109840, 109841, 109842, 109843, 109844, 110202, 110973, 110974, 111584, 111585, 111586, 111713, 119234, 119238, 119240, 120728, 123948, 124800, 124801, 124802, 124804, 124805, 124806, 124807, 144724, 144730, 144733, 144734, 144741, 144743, 144749, 144750, 144752, 144753, 144779, 144790, 144794, 144795, 144796, 144797, 144798, 144805, 144806, 152177, 152632, 152633, 152634, 153962, 162098, 175177, 175178, 180816, 180817, 180818, 180819, 180820, 180821, 180822, 180823, 180828, 180830, 180833, 180834, 183447, 187342, 191020, 207496, 207497, 207501, 209063, 212481, 212482, 212483, 213819, 213850, 220456, 220457, 220458, 220459, 220461, 220463, 226764, 226765, 226768, 247285, 249093, 249881, 251384, 252194, 255729, 256870, 256872, 256873, 256874, 258199, 261689, 261690, 261691, 261692, 267492, 278669, 281388, 281389, 281391, 281394, 282217, 285048, 285049, 285050, 287415, 294448, 294453, 294454, 294455, 294457, 294460, 294461, 294462, 295423, 295424, 296330, 296331, 296332, 297682, 297683, 297987, 299298, 299302, 304966, 304971, 304972, 304976, 304981, 335330, 335331, 344730, 373619, 373623, 373624, 373626, 373627, 373629, 373632, 373633, 373635, 399757, 434085
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8160, 8161

Table 4.S6. Correlation matrix of the seven morphological variables used to perform the Principal Component Analysis. Significant correlationas at $p = 0.05$ are shown in bold font.

	Wing chord	Tail length	Tarsus length	Exposed bill culmen	Bill culmen	Bill depth
Tail length	**0.29**					
Tarsus length	**0.39**	0.01				
Exposed bill culmen	**0.40**	**-0.12**	**0.56**			
Bill culmen	**0.41**	**-0.14**	**0.49**	**0.85**		
Bill depth	**0.22**	**-0.16**	**0.44**	**0.50**	**0.50**	
Bill width	**0.13**	**-0.17**	**0.45**	**0.53**	**0.54**	**0.62**

Table 4.S7. Acoustic divergence scores (Cohen's *d* scores based on pair-wise comparisons between taxa's principal component factor scores summarizing variation in acoustic measurements) for songs of each subspecies versus all other subspecies of House Wrens, for pairs of subspecies with proximate geographic distributions, and for pairs of recognized *Troglodytes* species.

	Cohen's *d* scores			
	Factor 1	Factor 2	Factor 3	Factor 4
Subspecies				
aedon vs. all subspecies	0.58	0.48	0.73	1.14
parkmanii vs. all subspecies	0.27	0.61	0.79	1.40
cahooni vs. all subspecies	0.43	0.33	0.80	0.99
brunneicollis vs. all subspecies	0.66	1.05	0.67	0.79
nitidus vs. all subspecies	0.32	0.60	0.79	0.97
musculus vs. all subspecies	0.29	0.36	1.19	0.74
beani vs. all subspecies	0.49	0.51	2.26	1.46
rufescens vs. all subspecies	0.27	0.37	0.69	1.35
Geographically proximate subspecies				
aedon vs. parkmanii	0.54	0.21	0.58	0.36
parkmanii vs. cahooni	0.12	0.51	0.08	0.62
cahooni vs. brunneicollis	0.27	0.65	0.44	0.64
cahooni vs. nitidus	0.42	0.19	0.22	1.05
brunneicollis vs. nitidus	0.72	0.75	0.27	0.22
musculus vs. beani	0.40	0.38	0.91	0.92
musculus vs. rufescens	0.07	0.01	1.28	1.04
Recognized *Troglodytes* species				
rufocilliatus vs. ochraceus	0.24	0.25	1.45	1.09
cobbi vs. musculus	0.29	1.05	1.98	0.22
pacificus vs. hiemalis	0.60	1.13	2.98	0.35
sissonii vs. tanneri	0.76	0.06	1.28	0.52

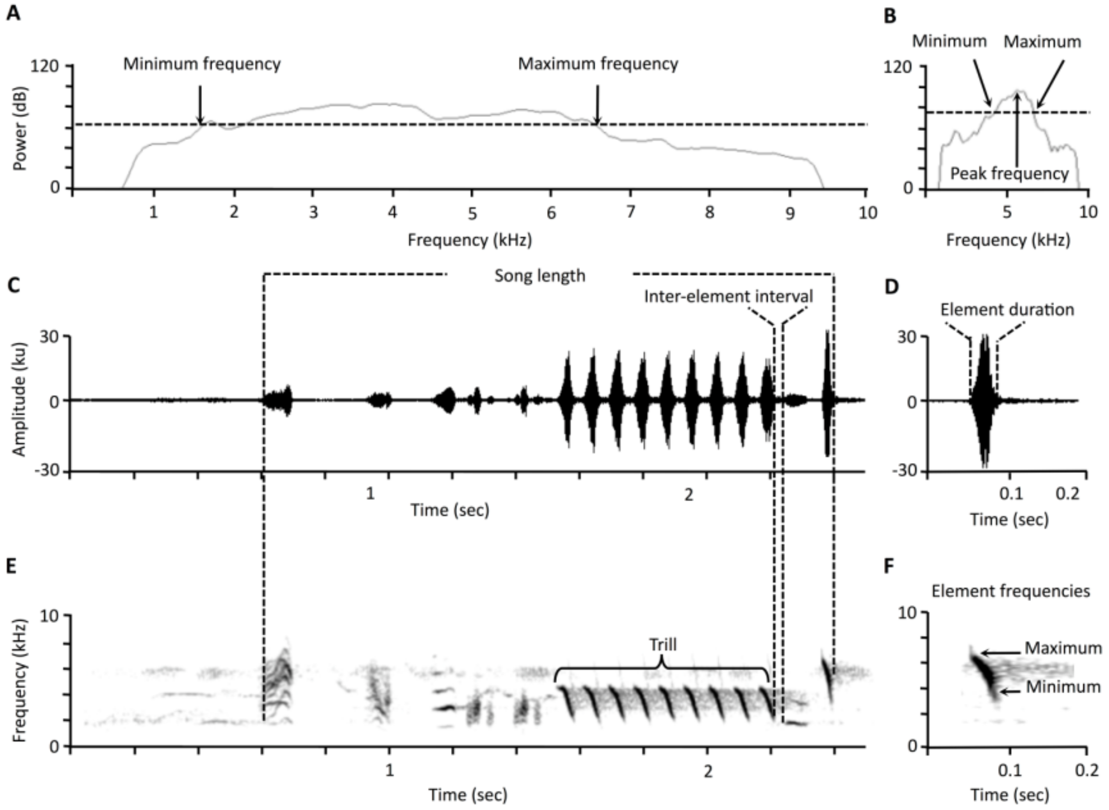

Figure 4.S1. Power spectra, waveforms, and sound spectrograms showing some of the vocal variables analyzed in this study. All subspecies six graphs are from a Cozumel Wren song. (A and B) power spectra, (C and D) waveforms, and (E and F) spectrograms for the same song (left) or element (right). Power spectra show the threshold of -20 dB relative to the peak frequency for (A) a song and (B) an element. Spectra also show the estimation of (A) the minimum and maximum frequency, (C and E) song length, inter-element interval and (E) trills in the song. (B and F) Minimum and maximum frequencies, and (D) duration for a given element are also shown.

Vita Auctoris

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	Master’s degree, Biology, Museo de Zoologia “Alfonso L. Herrera”, Facultad de Ciencias, Universidad Autonoma de Mexico (2004-2007)
	Doctor of Philosophy, Department of Biological Sciences, University of Windsor (2009-2014)
Awards	University of Windsor Department of Biological Sciences Graduate Excellence Award (2013)
	American Ornithologists’ Union Student Research Award (2012)
	Field Museum's visiting scholar research award (2011)
Publications:	Sosa López JR, Mennill D (2014) Continent-wide patterns of divergence in acoustic and morphological traits in the House Wren species complex. ***Auk: Ornithological Advances*** 131:41-54.
	Sosa López JR, Mennill D (2013) The vocal behaviour of the Brown-throated Wren (*Troglodytes brunneicollis*): song structure, repertoires, sharing, syntax, and diel variation. ***Journal of Ornithology*** online first.

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Conferences:

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