THE NATURE AND FUNCTION OF SONG DIVERSITY IN SOUTHERN HOUSE WRENS (*Troglodytes aedon chilensis*)

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DEDICATION

This thesis and all my academic achievements are dedicated to the memories of my parents, Georgina de Oliveira Barros (1953-2016) and Edvaldo Nascimento dos Santos (1952-2001), and to my brother, George Barros dos Santos.

ABSTRACT

This thesis focused on an austral population of House Wrens breeding in the south-temperate zone in Mendoza, Argentina. A description of song organization and complexity is provided for males in this population, and comparisons are made to song patterns reported for House Wrens in the north-temperate zone. Song patterns were remarkably similar between the two zones. Further analyses revealed significant correlations between metrics of song complexity and breeding success in the focal population of House Wrens in Argentina. The latter findings suggest that pressures of sexual selection have affected song evolution in this austral population of House Wrens in ways similar to reported sexually selected effects on song for north-temperate songbird species. This outcome is not well accommodated by current theory. These findings underscore how traditional theory concerning the evolution of song could be expanded through additional studies on South American populations and species, which have been understudied to date.

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CHAPTER 1

INTRODUCTION

1.1. SEXUAL SELECTION AND THE EVOLUTION OF BIRD SONG DIVERSITY

The songs of male songbirds can vary widely among species and even among different populations of the same species (Catchpole & Slater 2008). This variation can range from the single simple song of the Henslow's Sparrow, described as "one of the poorest vocal efforts of any bird" (Peterson 1947), to the highly elaborate repertoires of Brown Thrashers, where individual males can sing over 1,000 different songs (Kroodsma 2005). Such variation is believed to have arisen from differences in evolutionary pressures within and between species (Read & Weary 1992). However, as yet, we have only an incomplete understanding of the factors that lead to such dramatic variation in song patterns.

Understanding the evolution of elaborated bird song is in many ways analogous to the classic question of how the male peacock got its extravagant tail. In fact, we can see male bird song as an acoustic equivalent of the peacock's tail (Catchpole 1987). Phenomena like these perplexed Darwin himself because he was not able to explain the evolution of ornamented traits such as elaborate bird song using his classic mechanism of Natural Selection (Darwin 1859). Ornamented traits seemed to contradict his idea of Natural Selection because they did not seem to offer any survival advantage to their bearers. On the contrary, traits like the peacock's long and cumbersome tail, that are costly to maintain and hamper mobility, would seem to compromise their survival.

1.1.2. The mechanism of Sexual Selection

This problem pushed Darwin to further develop and introduce his theory of Sexual Selection, as an addendum to his mechanism of Natural Selection. In The Descent of Man and Selection in Relation to Sex, Darwin (1871) introduced his Theory of Sexual Selection to explain the evolution of dramatic traits (often, but not always, manifest in males rather than in females) that function in attracting mates and in competing with rivals for access to them. He proposed two specific mechanisms by which this might occur: inter-sexual selection, operating within sexes and involving traits that improved their bearer's ability to outcompete same-sex rivals for access to the opposite sex; and intra-sexual selection operating between sexes and involving traits that made their bearer's more appealing or attractive to members of the opposite sex.

Darwin specifically considered the songs of male birds to be a case in point and has having evolved under the pressure of Sexual Selection, ''*The diversity of the sounds, both vocal and instrumental, made by the males of many species during the breeding-season, and the diversity of the means for producing such sounds, are highly remarkable. We thus gain a high idea of their importance for sexual purposes...*'' (Darwin 1871). He argued that ornamented traits may be maintained and favored because of the advantages they provide in mating. "*Secondary sexual characters are more diversified and conspicuous in birds ... than in any other class of animals… The females are most excited by, or prefer pairing with, the more ornamented males, those that are the best songsters, or play the best antics.*" Of song in particular, he noted that "*They [males] charm the female by vocal or instrumental music of the most varied kinds.*" Darwin's emphasis here was on the role of song in inter-sexual selection or female choice. However, contemporary research also includes a focus on intra-sexual selection, or the way in which song can also function in competition between rival males.

1.1.3. Song diversity and male quality

About 150-years later, current research now hypothesizes that variable, or complex, songs are more attractive to females because they demand time and energy to be signaled and require high costs to be fully developed (Andersson 1994). Males who can sing more diverse songs demonstrate higher quality at multiple levels. For example, males that sing more complex songs have better cognitive abilities in Song Sparrows, *Melospiza melodia* (Pfaff et al. 2007), and better immune systems in Zebra Finches, *Taeniopygia guttata* (Boorget et al. 2008). Further, experimental studies in Song Sparrows (Nowicki et al. 1998; Spencer & MacDougall-Shackleton 2011) have shown that levels of song diversity can be traced to a male's early rearing environment, including the relative availability of food, which then affects the development of a variety of physiological and behavioral traits that can influence song production. Consequently, complex songs are thought to reliably signal superior male quality and development. Females that choose males that sing more diverse songs may then be mating with superior males and might obtain greater direct benefits from them, such as better parental care and a larger territory with more resources, or greater indirect benefits in the form of superior genes that improve offspring viability (Kirkpatrick & Ryan 1991). In turn, males that are able to produce songs with higher diversity, or complexity, might acquire multiple female mates or females of superior quality and have increased reproductive success as a result.

1.2. SONG DIVERSITY, MATING SYSTEMS AND MIGRATION

An important question naturally follows: if elaborate song evolved to signal superior male quality, then why do not all males sing elaborate songs? In other words, why is there variation in the degree of song elaboration between species and even within populations of the same species? Why are there species that sing only one simple song, while others sing so many?

One general hypothesis is that differences in song complexity are due to differences in the intensity of Sexual Selection, as might arise between populations that are polygynous and where males therefore mate with several females, compared to populations that are monogamous and mating opportunities for males are more limited (Kroodsma 1977; Read & Weary 1992; Collins et al. 2009). It is also proposed that sexual selection on song may be exaggerated in species, or populations, that are migratory because, by comparison to sedentary populations, males that must migrate to breeding grounds and have a relatively short breeding season therefore also have a comparatively short time in which to attract mates and breed, and females in turn have relatively little time to make mate choice decisions. Together, these factors may create more intense pressure on song for efficient mediation of mate competition and mate choice, favoring males that can readily signal their superior quality via elaborate song (Catchpole 1980).

One approach to evaluating this hypothesis has been to adopt a comparative approach and examine behavioral and ecological differences between species as they relate to correlated variation in the levels of song diversity they manifest. For example, Kroodsma (1977) studied nine species of temperate-zone wrens and found that the polygynous species among them had more complex songs than did the monogamous ones, supporting the hypothesis that song diversity varies with mating system. Similarly, Collins et al. (2009) compared two sedentary and two migratory populations of Blackcaps (*Sylvia Atricapilla*) and found that, in the migratory populations, males produced longer, female-directed warbles by comparison to the two sedentary populations, supporting the hypothesis that songs can be more variable or complex in migratory populations. Read & Weary (1992) undertook the broadest comparative study to date, using a database of 165 different passerine species. They reported that syllable repertoires were higher in polygynous species compared to monogamous ones, and that song repertoires were also larger in species that provide more parental care or that are migratory.

In contrast, however, other studies have failed to confirm that males from species (or populations) that are migratory and/or polygynous sing more complex songs. For example, Kroodsma & Verner (1987) measured song repertoire size in migratory and sedentary populations of Marsh Wren, *Cistothorus palustris*, breeding in five American states. They found mixed results concerning the relationship between migration and song diversity. Males from a migratory population breeding in eastern Washington had smaller song repertoires than males from sedentary populations from central Washington and California. At the same time, males from a migratory population breeding in New York had larger song repertoires than males from a sedentary population in North Carolina. In a different study, Ewert and Kroodsma (1994) reported that males from a sedentary population of Eastern Towhees (*Pipilo erythrophthalmus*) breeding in Florida had a mean of 8 song types, while males from a migratory population breeding in New York had a mean of only 3.5 song types. Peters et al. (2000) compared four measurements of song repertoire complexity in migratory and sedentary populations of Song Sparrows and found that males from sedentary populations actually exhibited more complex repertoires than males from migratory populations.

1.3. FEMALE CHOICE AND BREEDING SUCCESS

A second approach to understanding the evolution of male song diversity has involved more direct assessment of female preference for complex male song. Here, song diversity has generally been characterized in terms of repertoire size, which is calculated either as the number of different songs a male can sing or the number of different syllable types a male can produce (Podos et al. 1992; Ready & Weary 1992; Nowicki & Searcy 2004). Such measurements of repertoire size have been shown to vary dramatically from species to species, and even between different populations of the same species (Peters et al. 2000). Both field and laboratory experiments have shown that females prefer males with more complex songs.

For example, Howard (1974), studying a population of Northern Mockingbirds, *Mimus polyglottos*, found that females prefer males with larger song repertoire sizes. In male Great Reed-Warblers, *Acrocephalus arundinaceus*, male repertoire size is correlated with the number of female mates (Catchpole 1986), and with success in attracting extra-pair mates (Hasselquist et al. 1996). In Marsh Warblers, *Acrocephalus palustris*, male song diversity is associated with faster mate acquisition and larger clutches (Darolová et al. 2012). Similar results have been found in Song Sparrows (Hiebert et al. 1989), Sedge Warblers, *Acrocephalus schoenobaenus* (Buchanan & Catchpole 1997; Catchpole 1980), and Red-Winged Blackbirds, *Agelaius phoeniceus* (Yasukawa et al. 1980).

Laboratory experiments have likewise shown that females exhibit stronger responses in the form of solicitation displays for copulation when they are exposed to larger song repertoires in Song Sparrows (Searcy & Marler 1981; Searcy 1984), Great

Tits, *Parus major* (Baker et al. 1986), Sedge Warblers (Catchpole et al. 1984) and Great Reed-Warblers (Catchpole et al. 1986).

At the same time, however, a variety of studies have failed to provide evidence for reproductive advantages in males with more complex songs (reviewed in Byers & Kroodsma 2009), and there are no direct experimental data showing that females, under natural conditions, choose mates based on repertoire size (Kroodsma 2004).

One of the obstacles to a clear resolution of these outcomes is that laboratory and field studies of song diversity are probably capturing different phenomena due to research design differences. Studies conducted in laboratories, or otherwise controlled conditions, have been testing immediate female responses to playback stimuli simulating different sizes of male song repertoires, and have used this measure of female response as a proxy for female choice. However, it has been suggested that these findings may not be representative of female choice in free-living birds if subjects in laboratory experiments are deprived of additional social interactions and factors that ultimately might be important in mate choice in the wild (Byers & Kroodsma 2009).

Field studies involve a completely different set of proxy measures of female choice. Because it is hard to measure female choice directly in free-living birds, several indirect measures are often used instead (such as paring date, first egg date, harem size and extra-pair paternity). Results remain a bit equivocal even when only field studies are considered. For example, a recent meta-analysis conducted by Soma & Garamszegi (2011) investigated the link between song diversity and reproductive success in 42 field studies. Results suggested only a weakly significant association between song diversity and reproductive success. Overall, the current empirical evidence for a clear connection between increased male song diversity and improved breeding success is mixed.

1.4. SIGNIFICANCE

Ultimately, the aforementioned problems connect quite directly to the supreme problem that challenged Darwin, namely the origins and diversification of species, as he poetically penned in the closing lines of the Origin of Species: "*There is grandeur in this view of life … from so simple a beginning, endless forms most beautiful and most wonderful, have been, and are being, evolved.*" Despite Darwin's seminal insights concerning the evolutionary mechanisms of Natural Selection and Sexual Selection, we still have a fairly limited understanding of the mechanisms that promote the diversification of species. The study of bird song variation, however, offers a singular opportunity to help clarify how Sexual Selection might play a key role.

Sexual reproduction is generally preceded by elaborate courtship behaviors, where males and females identify potential mates of the same species through the exhibition of mating displays (acoustic, visual, chemical and/or tactile signals). In most cases, it is the males that exhibit such displays and females respond to them. The outcome of these interactions may lead, or not, to sexual reproduction, contingent upon on how females assess and respond to male displays. Because mating displays are species-specific (females generally will be attracted only to displays produced by conspecific males), they function as a premating isolating mechanism (Coyne & Orr 2004). In other words, mating displays reinforce sexual reproduction only among conspecifics and create barriers to mating between heterospecifics. Hence, mating displays might promote speciation as differences in them accumulate over time to the point where individuals no longer recognize one another as conspecifics appropriate as mates.

The possibility that mating displays might promote speciation could be especially likely where mating displays are acquired through a learning process, as song is in most songbirds. Indeed, it has long been hypothesized that bird song could facilitate speciation specifically *because* it is learned and is thus subject to the development of regional song dialects – song patterns that differ slightly between local populations. Subtle dialect variation could promote breeding among individuals within local populations but prevent breeding among individuals from different populations if females respond preferentially to the song displays of only local males (Marler & Tamura 1964; Price 1998; MacDougall-Shackelton & MacDougall-Shackelton 2001). Such biased mating within populations based on song patterns can, over time, lead to genetic differentiation between populations and ultimately to speciation. These ideas are intuitive, but have not been definitively demonstrated (Baptista & Trail 1992).

This thesis does not directly address the link between song diversity and speciation. Nevertheless, it addresses a number of key elements involved by providing a comprehensive characterization of the form and extent of song diversity in a species noted for complex song patterns, by examining the relationship between male song diversity and breeding success in this species, and by making some preliminary assessments of how variation in the migratory patterns, mating systems and life-history traits of different populations of this species may influence population variation in song patterns. In each of these ways, it may contribute to a fuller understanding of how sexual selection influences the evolution of song diversity, and ultimately then how that in turn might influence species diversification.

1.5. MODEL SPECIES

The research uses House Wrens as a model species and focuses on a population of House Wrens breeding in the south temperate zone of South America. House Wrens are a small species (10-12g) of territorial songbird, noted for their very broad distribution and habitat tolerance (Brewer & MacKay 2001; Johnson 2014). In fact, House Wrens have the widest distribution of any native songbird in the western hemisphere, reaching their northern limits in central Alberta, Canada and their southern limits in Tierra del Fuego, Argentina. Across this range, they show tremendous variation in ecology and life history. For example, populations in the north-temperate zone are mainly migratory, show high rates of social polygyny (15-35%: Johnson 2014) and extra-pair paternity (54% of broods and 25% of offspring: LaBarbera et al. 2010), and large clutch sizes (7-8 eggs). In contrast, populations in the south-temperate zone are non-migratory, exhibit lower levels of social polygyny (1–5%: Freed 1986; Llambías and Fernández 2009) and show only moderate rates of extra-pair fertilizations (33% of broods and 16% of offspring: LaBarbera et al. 2010) and smaller clutch sizes (4-5 eggs).

Traditionally, House Wrens across this broad range have been divided into three different species occurring in three broad geographic areas: the Northern House Wren, *Troglodytes aedon*, in North America; the Brown-throated Wren, *Troglodytes brunneicollis*, in Mexico; and the Southern House Wren, *Troglodytes musculus*, in Central and South America (Brumfield & Capparella 1996). However, currently, these types are all considered to be a part of a single species, *Troglodytes aedon,* with approximately 30-subspecies (Remsen et al. 2018).

Due to their broad distribution across the Americas, and their relative ubiquity where they occur, House Wrens are among the best-studied wild bird species, with various aspects of their biology and behavior the subject of more than 700 papers over several decades (Johnson 2014). Most of this literature, however, focuses on migratory populations of House Wrens breeding in the north-temperate zone of North America, and there is comparatively little work done on song, in particular.

Male and female House Wrens can both sing, but female songs are much less structured, common or conspicuous, at least in north temperate populations (Johnson & Kermott 1990; Krieg & Burnett 2017). In contrast, the song of male House Wrens is loud, conspicuous and delivered throughout the breeding season, although with considerable variation in the frequency and rate of singing at different stages in the breeding cycle (Platt & Ficken 1987; Cramer 2012; Rendall & Kaluthotha 2013). Singing rates are highest when males are unpaired and are prospecting for female mates. At this stage, males can sing up to 600 songs/hour. Rates of song delivery tend to decrease dramatically to less than 100 songs/hour after females commence laying eggs and begin incubating them, and again later when both males and females are feeding nestlings. However, singing rate can increase again if males lose their mate, or if they are seeking to attract a second mate. Songs also vary in amplitude throughout the breeding cycle (Platt & Ficken 1987; Johnson 2014; Rendall & Kaluthotha 2013). Unpaired males sing songs with higher amplitude when courting females. After pairing and egg-laying, they sing softly when interacting directly with their female mates, but can sing loudly again if defending territory, or trying to attract additional female mates (Platt & Ficken 1987; Johnson 2014; Rendall & Kaluthotha 2013).

Individual songs are typically 2–3 s in length and are delivered in protracted bouts, with short intervals between songs (2-5 s), and particular song types tend to be repeated several times before switching to a different type (i.e., eventual variety). Most songs include a softer introduction section, composed of syllables that are either broadband or tonal with several overtones, followed by a louder terminal section, composed of clear, tonal and frequency-modulated syllables (Rendall & Kaluthotha 2013). Rendall and Kaluthota (2013) reported very high levels of song diversity in a population of House Wrens breeding in the north temperate zone in Alberta, Canada (*Troglodytes aedon parkmannii*) that was comparable to levels of song diversity found in some of the most prolific singers (Kroodsma & Parker 1977). Rendall and Kaluthota (2013) also reported that individual males produced a large and mostly shared repertoire of 25 different syllable types that they then concatenated and recombined flexibly to produce even larger repertoires of distinct song types that could exceed 450 without reaching a plateau.

In summary, then, House Wrens are widely distributed, characterized by variable migratory patterns, mating systems, and life-histories, and are noted for exaggerated levels of song diversity. Hence, they represent an ideal species for further investigations into the evolution of song diversity and complexity, and the role of variable sexual selection. Given the dearth of research on House Wren populations in the Southern Hemisphere, and the almost complete absence of work on song patterns for the species in this region, there is a special opportunity to add to our understanding of song evolution in this species, and more broadly, through study of song in House Wrens populations in South America.

1.6. THESIS OBJECTIVES

This thesis has three specific objectives:

(1) To provide a detailed characterization of male song structure and organization for a sedentary population of southern House Wrens breeding in the temperate zone of South America in Mendoza Province, western Argentina (*Troglodytes aedon chilensis*) and provide some preliminary comparisons to a migratory population of House Wrens (*Troglodytes aedon parkmannii*) breeding in the temperate zone of North America in Alberta, Canada (Rendall and Kaluthota 2013).

(2) To examine the relationship between male song diversity and breeding success in the sedentary population of House Wrens studied in the temperate zone of South America in Western Argentina.

(3) To examine the extent to which documented relationships between song diversity and breeding success in the focal population of House Wrens studied in South America can be influenced by variation in the sample size of both song recordings and male subjects, an issue that often bedevils field investigations of such relationships.

1.7. CHAPTER OVERVIEW

Chapter 2 provides the first ever detailed characterization of the structure and organization of male song for a South American population of House Wrens (*Troglodytes aedon chilensis*). This was a critical first step that would allow more detailed analysis of the potential sexually selected functions of song diversity in this population in the following chapters. The description of song patterns was based on data collected during a field study conducted over two consecutive breeding seasons (2012 - 2013) in Mendoza Province, Argentina in the foothills of the Andes mountains. Data were collected from a sedentary (year-round resident) population of House Wrens, and included a total of 13,440 songs recorded from 18 males, each of which was captured and individually marked. Analysis of song recordings focused first on the general singing style of males in this population and the basics of how songs and song bouts were constructed and organized. Most songs were composed of two basic sections, an introduction section, which was a concatenation of multiple relatively soft but noisy or broad-band elements, and a terminal section, which was composed of louder and tonal, frequency-modulated elements. Because the syllables in the introduction section were relatively soft and difficult to identify clearly, subsequent detailed analysis of syllable structure concentrated on the terminal section of songs. A catalogue of all notes and syllable-types contained in the terminal section of songs is presented. Detailed analysis of song structure then involved quantifying syllable and song repertoires for individual males, and analyzing variability in the sequencing of syllables within songs and in the sequencing of songs within bouts using first-order transition matrices. Discussion in this chapter includes a comparison of the song patterns described for this population of southern House Wrens with those reported from an analogous study of a migratory population of House Wrens (*Troglodytes aedon parkmannii*) breeding in the temperate zone of North America in Alberta, Canada (Rendall & Kaluthota 2013).

Chapter 3 builds on the detailed characterization of song patterns provided in Chapter 2 to undertake analysis of the relationship between male song diversity and breeding success in the focal population of southern House Wrens in Mendoza Province, Argentina. This analysis tests the hypothesis that song diversity is currently subject to sexual selection. It addresses the specific question of whether males that sing with higher diversity also enjoy higher breeding success, evaluated using indirect measures of breeding success (e.g., dates of nest initiation and fledging; and number of eggs and fledglings produced). The sample for this analysis derives from the same field study conducted over two consecutive breeding seasons, and includes a sample of more than 33,000 songs from 26 males. Birds in this study, bred in nest boxes which made it possible to monitor nest productivity throughout the breeding season. Data on pairing, nest building, egg laying and fledglings were recorded on a daily basis. Four standard breeding variables were used to evaluate breeding success: *laying date* (the day in which the first egg was laid by the female mate); *fledging date* (the day chicks left the nest); *total number of eggs* (produced during the entire breeding season); and *total number of fledglings* (also for the entire breeding season). Song complexity was operationalized using six variables that captured variation at different organizational levels: *Normalized song repertoire size* (song repertoire size divided by the number of recordings); *Levenshtein distance* (degree of song diversity between consecutive songs); *Number of syllable types per song*; *Syllable type rate (*number of syllable types divided by song duration); and also the coefficients of variation of these last two variables. Multiple linear regression tests were then used to evaluate the relationships between the different measures of song complexity and the different breeding metrics. Discussion in this chapter includes broader implications of the findings for the evolution of song diversity in House Wrens.

Based on the positive relationships reported in Chapter 3 between male song diversity and breeding success, Chapter 4 examines the extent to which these results could be influenced by variation in the size of the samples of both song recordings and individual male subjects. A series of tests are presented that test whether the positive relationships between song diversity and breeding success observed with the full sample presented in Chapter 3 are retained with successively smaller samples of either song recordings, individual male subjects, or both. The rationale for these tests is twofold: 1.

to simulate the challenges faced by researchers testing and documenting clear relationships between song and breeding success from field studies where samples can be limited in size; and 2. to simulate the analogous sampling problem faced by females prospecting for a suitable male mate where the ability to reliably assess song diversity for a given male, which may be revealed only over a relatively protracted period of time, trades-off against the ability to make this same assessment for multiple males in order to compare among them. The analyses in this chapter start with a sample of 14,000 songs from each of 20 males, with a standard song recording sample for each (n=700 songs per male). Bootstrap linear regressions are then performed on successively smaller samples of either male subjects, song recordings, or both using two specific breeding metrics (*laying date* and *total number of eggs*) as dependent variables and two song metrics identified from Chapter 3 (*CV number of syllable types per song* and *song repertoire size*) as predictors. Discussion in this chapter focuses on the practical implications of the findings for the sampling problems faced by researchers evaluating relationships between the complexity of male breeding displays and their effects on breeding outcomes and the analogous sampling problem faced by females evaluating these same relationships as a guide to mate choice.

The final chapter, Chapter 5, provides a summary of the major findings of the work, considers some of the limitations of the work conducted, and suggests some opportunities for future work to clarify or expand on the results reported.

CHAPTER 2

The structure and organization of song in Southern House Wrens (*Troglodytes aedon chilensis*)*

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ABSTRACT

Studies of birdsong across very broad geographic scales, such as between the north temperate zone and the tropics, provide special opportunities to understand the role of variable ecologies, life histories and mating pressures on song structure and organization. The problem is typically studied through comparative, cross-species analyses because few species have such broad distributions to encompass both regions. The House Wren is an important exception, having the widest distribution of any native songbird in the Americas, from Canada to Tierra del Fuego. Across this range, they manifest considerable variation in life history, mating systems and migration, but there is no systematic research on corresponding song variation. Here we provide a first detailed characterization of song structure and organization for Southern House Wrens (*Troglodytes aedon chilensis*) in western Argentina and provide preliminary comparisons to Northern House Wrens. Songs of Southern House Wrens contained two distinct sections: an introduction of broadband noisy, or harmonic, notes followed by a louder terminal section of tonal, frequency-modulated syllables with a mean of seven syllables and three syllable types per song. The syllable repertoire was large (28), mostly shared and used to construct very large song repertoires (up to 170 song types with no evidence of a ceiling), but much smaller repertoires of commonly produced song types (24). Males tended to repeat song types many times before switching (eventual variety) but, at times, sang with immediate variety. Compared to Northern House Wrens, there were differences in the detailed form of some notes and syllables as well as in the relative emphasis of the softer introduction versus louder terminal section of songs. In broader patterns of song construction, organization, delivery, and the size of syllable and song

repertoires, the two populations were very similar. These patterns are discussed in light of differences in life history, mating and migration between them.

2.1. INTRODUCTION

A longstanding issue in the study of birdsong concerns variation in song patterns between populations. Such variation can arise from social processes related to the role of learning and cultural transmission in the cross-generational transmission of song, which, over time, can lead to divergence in song patterns between populations, traditionally referred to as song 'dialects' (Marler & Tamura 1962). Geographic, or dialect, variation can also arise through processes of ecological adaptation of song features to local habitat conditions and their associated acoustic transmission characteristics, which can differ between populations (Slabbekoorn & Smith 2002). Differences of either sort can occur even over very short distances, as documented for White-crowned sparrows in northern California (*Zonotrichia leucophrys*: MacDougall-Shackleton & MacDougall-Shackleton 2001) and Rufous-collared sparrows in Argentina (*Zonotrichia capensis*: Tubaro et al. 1993; Kapouchian et al. 2004). Because song often plays an important role in reproduction, disjunct populations might ultimately diverge sufficiently to represent different subspecies or even species, as elegantly documented for greenish warblers in central Asia (*Phylloscopus trocholoides*: Irwin 2000; Irwin et al. 2008).

There is currently considerable interest in patterns of song variation at exceptionally broad geographic scales, such as might characterize birds inhabiting the north temperate zone versus the tropics arising from very broad differences in ecology, life history and selection regimes in the two regions (reviewed in Podos 2013). For example, it has been proposed that the pressures of sexual selection on song might often be greater for migratory birds in the north temperate zone compared to sedentary birds in the tropics because of the relatively short breeding season in the former, which provides only a limited time window in which to compete for territories and find mates (Catchpole

1987). As a result, we might expect more elaborate or complex song patterns in north temperate birds for efficient mediation of these behaviors. There is some support for this proposal (e.g., Collins et al. 2009; Botero et al. 2009a; Weir and Wheatcraft 2011), but the relationships are far from definitive, and there are a variety of exceptions and confounding factors (Byers & Kroodsma 2009; Cardoso & Hu 2011; Soma & Garamszegi 2011). One complication is that most such broad scale studies necessarily involve comparisons across different species inhabiting either the temperate zone or the tropics because there are very few species with sufficiently broad distributions to encompass both regions and thereby allow more focused testing within a single species.

There are, however, some exceptions that can shed additional light on our understanding of latitudinal differences in song structure and singing behavior. For example, it has been suggested that differences in residency and neighbor stability between north temperate and tropical-to-south temperate Sedge Wrens (*Cistothorus platensis*) can select for different song development strategies, which in turn affects song sharing between neighbors (Kroodsma et al. 1999). The House Wren (*Troglodytes aedon*) is another exceptional species with an extremely broad distribution that is ideally suited to detailed investigation of geographic variation in song, but no systematic studies of tropical-south temperate populations have been conducted to date.

House Wrens have the widest latitudinal distribution of any native songbird in the western hemisphere (Brewer & MacKay 2001). They reach their northern limits in central Alberta, Canada (58°N) and their southern limits in Tierra del Fuego (55°S). Across this range, there is tremendous variation in ecology, behavior and life history. For example, populations in the north-temperate zone are mainly migratory, show appreciable rates of social and genetic polygyny, and have large clutch sizes (reviewed in

Johnson 2014). In contrast, populations in the south-temperate zone are sedentary, social polygyny is rare with only moderate rates of extra-pair fertilizations, and clutch sizes are small (Young 1996; Llambías 2009, 2012; Llambías & Fernandéz 2009; Ippi et al. 2012; Llambías et al. 2012, 2015; LaBarbera et al. 2010, 2012). Differences such as these in basic dimensions of life history, mating system and migration patterns are among the traits implicated in differences in song structure and complexity (Catchpole 1987; Read & Weary 1992; MacDougall-Shackleton 1997; Kroodsma et al. 1999; Collins et al. 2009; Irwin et al. 2008). As a result, there is considerable scope for variation in song patterns in House Wren populations across the Americas, and this represents a truly unprecedented opportunity to examine how variable ecology, life history, migration and mating systems influence song.

Previously, we provided a detailed description of song structure and organization for a population of Northern House Wrens breeding in southern Alberta, Canada (*Troglodytes aedon parkmannii*: Rendall & Kaluthota 2013). Here we provide a complementary study of song for Southern House Wrens from a population in western Argentina (*Troglodytes aedon chilensis*). This represents the first detailed account of song for the species in the southern hemisphere and is a critical first step toward allowing more detailed comparisons of population variation in this exceptionally widespread species group.

Given the many documented differences in life history and mating systems just noted, and following the proposal for latitudinal gradients in song elaboration and complexity, one would predict the songs of Southern House Wrens to be relatively simplified compared to those of their northern counterparts. Specifically, we might expect their songs to be shorter and to involve fewer elements and smaller song repertoires.

2.2. METHODS

2.2.1. Study site and subjects

Research was conducted on a population of Southern House Wrens at a long-term study site in Mendoza province, Argentina, near the town of Uspallata (32.62°S 69.36°W). The site is in a montane habitat (1850 m) in the foothills of the Andes where the breeding habitat for House Wrens is a plantation of White Poplar (*Populus alba*). To facilitate our broader research program on breeding biology, wooden nest boxes were provided and used by the birds (Llambías & Fernandéz 2009; Llambías 2012; Llambías et al. 2012). Since 2011, we have captured and marked individuals at this site with a numbered metal band and three color bands to facilitate long-term identification, and we have re-sighted marked individuals on their territories during the austral winter, suggesting that House Wrens are year-round residents at this site. The breeding season is from late October to mid-February. Maximum daytime temperatures range from 18 to 35 °C with little rainfall (mean annual precipitation = 134 mm; Carretero 2000).

The present study was conducted in the 2012 breeding season (October 2012–February 2013). An initial survey was conducted at the start of the breeding season to identify suitable males for song recording. Candidate males that were not yet marked were captured using mist nets and banded in the fashion just described and thereby added to the long-term sample. These males were then monitored daily for the remainder of the breeding season.

2.2.2. Song recording and analysis

Song recordings of marked males were made primarily in the early morning (5:00–10:00 a.m.) when males were most active and singing. Recordings were made with a digital recorder (Sound Devices 702) and Sennheiser microphone system (Sennheiser MKH 816) at distances as close as possible (5–10 m) without disturbing the birds.

Our complete recording sample involved 30 males. However, for some males, we had very few recordings. Hence, for subsequent detailed analysis, we limited the sample to males for which we had more than 200 songs. The final sample thus comprised a total of 13,440 songs from 18 males (Table 2.1). Sample sizes for these males also varied, reflecting natural differences in singing activity among males at different breeding stages and associated differences in sampling effort. Recordings were analyzed in PRAAT, version 5.3 (Boersma & Weenink 2015), to capitalize on its robust modules for signal segmentation, annotation and database export. The first step was to characterize the basic structure of song. Songs lasted for 1–3 s and tended to be delivered in bouts in which consecutive songs were separated by relatively short silent intervals (2–5 s). We used a silent interval of >=30 s to define different bouts of singing. The majority of songs were composed of two discrete sections, readily distinguished by ear and in spectrograms (Fig. 2.1). The opening section (labeled the introduction) was an arrangement of unstructured broadband notes that were either harsh (noisy) or tonal with multiple harmonic overtones, while the second section (labeled the terminal section) was comprised of well-structured, tonal and frequency-modulated notes. The introduction section was often notably softer than the terminal section. As a result, it was sometimes difficult to delineate clearly the structure of some notes in the introduction section.

After characterizing the basic components of songs and song bouts, the next step was to develop a catalog of notes and syllables. In this, we focused our efforts on the terminal section of songs where the structure of notes was very clear and easily delineated. Because this section of songs was also generally louder than the introduction, it also had greater potential to be heard by males and females in the wider area. Following conventional definitions and nomenclature, notes were defined as continuous signal traces in the song spectrogram, and syllables were defined as regularly grouped combinations of notes consistently produced together as a common unit. This conventional approach also mirrored our earlier analyses of song structure and organization for a population of Northern House Wrens (Rendall & Kaluthota 2013). We delineated different note and syllable types based on clear and constant differences in overall temporal-spectral shape that were repeatable across songs both within and between males. Songs were then segmented and their syllable content annotated. Subsequent detailed analysis involved quantifying the note, syllable and song repertoires of individual males. For each song, we also quantified the syllable and syllable type production rate and calculated the ratio between different syllable types and the total number of syllables (the Syllable Versatility Index, SVI; Gil & Slater 2000). Finally, we analyzed variability in the sequencing of syllables within songs and in the sequencing of songs within bouts using first-order transition matrices. Statistics were performed in SPSS, version 20 (SPSS, Chicago, IL).

2.3. RESULTS

2.3.1. General summary of singing, courtship and breeding behavior

Males sang at high rates at the start of the breeding season, often from relatively high perches. Early in the season, they delivered songs in long bouts of 30 min or more continuously. Males increased their singing rate even more when visited by females and showed additional signs of heightened arousal, chasing the female and wing-fluttering near her when she settled on a branch or inspected a specific nestbox. Additionally, male songs were interspersed with high-frequency ‘‘squeak’’ calls at such times (cf. Johnson & Kermott 1991). Most males continued to sing while their female partners completed nest building. However, they dramatically reduced their singing rate just before and during egg laying. During this stage, song bouts were usually much shorter with longer intervals between consecutive songs. Some males resumed singing when their mates had finished laying and began incubating eggs, but others only resumed singing just before, or shortly after, chicks from their first nest had fledged. Throughout the breeding season, males sang most often, and at higher rates, in the early morning, starting just before sunrise. They sang only infrequently in the afternoon or evening. Males tended to sing from specific locations within their territories, usually from the tree containing their nestbox or from a neighboring tree. Although males were provided with two nestboxes per territory, we did not observe any case of polygyny.

2.3.2. Global song structure

Most songs (97 %) contained both introduction and terminal sections, with only 2.5 % lacking a terminal section and the remaining 0.5 % lacking an introduction. Individual song duration averaged 2.11 s (±SD = 0.62 s), with the introduction generally longer (mean = 1.38 s ± SD = 0.54 s) than the terminal section (mean = 0.73 s ± SD =

0.25 s). Notes in the terminal section were organized as a sequence of discrete syllable types. Many syllable types were repeated within a song before switching to a different type, and some syllable types appeared again later within the same song. Songs contained an average of three syllable types (range 1–10) and seven syllables (range 1–26) produced at an average rate of 4.3 syllable types s^{-1} and 9.75 syllables s^{-1} (Table 2.1; Fig. 2.2). The relative syllable diversity of songs (SVI index) averaged 0.46. The interval between songs in a bout averaged 2.77 s (±SD = 2.27 s).

2.3.3. Note and syllable repertoires

A total of 15 different notes and 28 different syllable types were identified in the terminal section of songs (Fig. 2.3). The structure of notes and syllables was highly regular and consistent within and between individuals, showing no obvious intermediate forms (Fig. 2.4). Some syllable types were formed from only a single note, while others were composed of two or three notes (Fig. 2.3). The different syllable types varied in their position within songs, some syllables occurring primarily at the beginning or the end of songs, and others occurring primarily in the middle. Still others were more flexible and could occur in various positions (Fig. 2.5).

Syllable repertoires were strongly conserved across males, with individual males producing an average of 25 of the total 28 different syllable types (Table 2.1). Although they shared most of the syllable repertoire, males varied in their use of different syllable types. Some syllable types (e.g., C, I, P, Q, R) were produced and used frequently by all males, while others were used much less commonly (e.g., AB, D), and still others (e.g., K, W, R, T) were produced frequently by some males but rarely by others.

Figure 2.6 shows the time course over which syllable types were revealed in successive song recordings for each male. This figure shows that, for all males, syllable accumulation curves ultimately reached an asymptote typically within the first 500 songs recorded. However, the figure also shows some variation among males in the rate at which complete syllable repertoires were revealed. Some males revealed their full syllable repertoire within the first 200 songs, while others required up to 1000 songs to reveal an equal-sized repertoire of syllables.

2.3.4. Syllable transition patterns

For the song sample for each male, we constructed a syllable transition matrix to summarize the first order transitions between adjacent syllable types within songs. In this analysis, transitions between the same syllable type (i.e., repetitions of the same syllable type) were ignored. Some syllable transitions were very common (e.g., A, B; G–L; H–U; I, J; I–O; O–S), while other transitions were much rarer (e.g., A–I; I–K; B–L), and many possible syllable-type transitions never occurred at all. Furthermore, some syllable types transitioned to one of many other syllable types (e.g., C was followed by 20 different syllable types), while others were followed by only one or a few other syllable types (e.g., E was followed by only three different syllable types).

2.3.5. Song type repertoires

We defined different songs, or song types, conservatively as a unique sequence of syllable types, ignoring variation in the number of times a particular syllable type might be repeated in a given position within a song (Kroodsma 1977). If a syllable type was repeated in a different position in the song—that is, after the occurrence of other intervening syllables—this was counted as a distinct song type. Despite our conservative definition of song types, there was tremendous diversity in the song types recorded. The complete sample of 13,440 songs included 809 different song types. Males varied substantially in the size of their song type repertoires, from a low of 24 song types to a high of 170 (mean = 79; Table 2.1). However, much of this variation among males was almost certainly due to unequal sampling effort. This is captured in Fig. 2.7, which plots the time course over which new song types were introduced in successive recordings for each male. Males with relatively small song repertoires were also males with a smaller overall recording sample. These males would probably show a larger song repertoire with additional recordings, as Fig. 2.7 also shows that, for all males, new song types were introduced steadily and continuously, with no indication of an asymptote for any male, regardless of the size of the recording sample. Even for the male with the largest sample of recordings (UP466, n = 2051 songs) and one of the largest documented song repertoires (n = 143), there was no indication of reaching a ceiling.

Given the diversity of different song types sung by males, there was relatively little sharing of complete song types among them, defined conservatively as a complete matching of their syllable type sequences (irrespective of differences in syllable repetition). Of the total of 809 different song types produced, no single song type was shared by all males, and only 26 different song types (3.2 %) were shared by more than half. Indeed, only 178 different song types (22 % of the total) were produced by more than one male. The remainder (n = 631, or 78 %) were unique to particular males. On average, the number of males producing a particular song type was 1.78.

Notably, although males had large repertoires involving many unique song types, they sang most song types only rarely. Of the 809 song types in the sample, 390 (48.2 %)

were sung only once, and only 51 song types (6.1 %) were sung >50 times. Thus, individual repertoires of commonly produced song types were much smaller. To establish these, we defined a ‘‘commonly produced’’ song type as one that accounted for >1 % of a male’s song production. By this criterion, the repertoires of individual males were more consistent in size and averaged only 24 song types (range 17–34, Table 2.1). There was no relationship between the size of the repertoire of commonly produced song types for individual males and the number of song recordings for each (Pearson r = 0.351, P = 0.2027).

2.3.6. Song-type transitions

To further examine the relative diversity of songs within bouts, we constructed a song type transition matrix quantifying the first order transitions between song types in adjacent positions in the sequence of songs within a bout. This analysis was limited to a set of 24 song types that accounted for more than 1 % of the entire sample. Even limiting the analysis in this way, the matrix is too large to display, but it revealed that despite the large number of song type transitions that are possible only a few actually occurred. By far the most common ‘‘transition’’ between successive songs was to the same song type. Of the total 5245 transitions involving this set of 24 common song types, 76.7 % (n = 4023) were repetitions of the same song type. Only 22.3 % (n = 1222) involved switching to a different song type. Hence, successive songs in a bout tended to involve repetitions of the same song type, with only occasional switching to a different song type, and most such switches involved song types that were only minimally different in their syllable type makeup. As a result, over long bouts of singing, the syllable content of songs tended to change gradually.

At the same time, though, males sometimes sang with much greater short-term variety and with much more rapid switching between song types. These differences in singing style are illustrated in Fig. 2.8. This figure depicts first-order transitions between successive songs in a protracted bout of singing by two different males on two different days. The data were standardized as much as possible to facilitate comparisons such that each example involves a continuous bout of singing involving a similar number of total songs. The figures show that for each male there is a clear difference in singing style, with singing on one occasion involving production of a relatively large number of different song types with considerable switching among them, and on another occasion involving production of relatively few different song types with greater serial repetition of each.

2.4. DISCUSSION

The detailed patterns of song organization and singing style reported here for Southern House Wrens (SHOWR) were, in most respects, very similar to those reported recently for Northern House Wrens (NHOWR: Rendall & Kaluthota 2013). There were some differences between the two populations in the detailed form of individual notes and syllables (cf. Fig. 3 in Rendall & Kaluthota 2013). Differences at this level even over very short distances are not uncommon in the learned songs and calls of other species (e.g., Marler & Tamura 1962; Mundinger 1982; MacDougall-Shackleton & MacDougall-Shackleton 2001; Kapouchian et al. 2004); hence, it is not surprising to find note and syllable structure differences across the macro-geographic scale involved for NHOWR and SHOWR.

There were some other differences in song structure related to differences in the relative length and emphasis of the introduction versus terminal sections of song in the two groups and in the number of syllables and syllable types they contained (see Table 2.2). The introduction section of songs in SHOWR was longer relative to the terminal section, by comparison to NHOWR. As a consequence, the proportionately shorter terminal section of songs in SHOWR also contained fewer syllables and syllable types than the terminal section of songs in NHOWR. However, because the reduction in the number of elements contained in the terminal section of songs in SHOWR was not quite proportional to the reduction in the duration of this section, syllables and syllable types were actually delivered at slightly faster rates in SHOWR (Table 2.2).

Most of these differences were not large but nevertheless might be important and functional with respect to additional differences in life history and mating system between the two populations. For example, the longer terminal section of songs with a greater number of syllables and syllable types in NHOWR is consistent with the prediction of greater song elaboration or complexity in populations at higher latitudes with shorter breeding seasons (Catchpole 1987; Collins et al. 2009). It is also consistent with higher rates of polygyny and extra-pair fertilizations in NHOWR compared to SHOWR (LaBarbera et al. 2010, 2012; Llambías et al. 2012; Johnson 2014) and with the manner in which social polygyny can arise in the two groups. In NHOWR, social polygyny arises when a male attracts a second female to his territory. In contrast, in SHOWR social polygyny is rare but can arise when a monogamously paired male replaces a neighboring male and then breeds with the female resident there (Llambías 2012; Johnson 2014). Taken together, these patterns point to a possibly increased emphasis on female attraction in NHOWR and on territorial defense in SHOWR.

It is also possible that the softer introduction section of songs is absolutely longer in SHOWR because it reflects an increased emphasis on close communication and coordination with a female partner. Johnson and Kermott (1991) suggested previously that some aspects of male song in NHOWR functioned to coordinate close-range nest activities between male and female post-pairing. It might then be that the more protracted pair bonds between males and females in SHOWR select for some extension of the softer introduction section for close-range communication between them compared to the much louder terminal section, which has far greater broadcast potential. These possibilities remain speculative at this stage and require further study and experimentation.

In most other respects, the organization of songs and patterns of song delivery were very similar in the two populations. Thus, the note and syllable repertoires of the two populations were similar in size (NHOWR: 22 different notes and 27 syllable types; SHOWR: 15 different notes and 28 syllable types), and notes were organized into discrete syllable types in a similar fashion. Syllable types were then concatenated together into songs in very similar ways, showing similar patterns of serial repetition within songs (trills) and similar patterns of transition diversity and constraint. For example, in both populations there were clear biases in the positioning of particular syllable types within songs, some syllables occurring primarily only at the beginning, others only at the end and still others occurring more flexibly in different positions within songs (cf. Table 2.2 in Rendall & Kaluthota 2013). Likewise, there were similar patterns of flexibility and constraint on transitions between particular syllable types in the two populations, where transitions between some syllable types were quite common, others were much rarer, and many possible syllable type transitions never occurred at all.

Similarities in the organization of notes and syllables were paralleled by similarities at higher organizational levels as well. Thus, there was tremendous diversity in the song repertoires of both populations. In NHOWR, we recorded 996 different song types in a sample of 15,608 song recordings from 15 males, while in SHOWR we recorded proportionately similar numbers: 809 different song types in a sample of 13,440 song recordings from 18 males. The song repertoires of individual males were also similarly large in the two populations (NHOWR range 38–194 song types; SHOWR range 25–170 song types) and showed no evidence of reaching a ceiling with increasing numbers of recordings (cf. Fig. 7 in Rendall & Kaluthota 2013) and limited overlap, or sharing, of complete song types between males. The size of these song repertoires in both populations puts them at the very high end of the continuum for song diversity, alongside some other wren species (Sedge Wrens and Marsh Wrens, *Cistothorus palustris*: Kroodsma & Verner 1978; Kroodsma et al. 1999) and members of the Mimidae (e.g., Brown Thrashers, *Toxostoma rufum*, and possibly Gray Catbirds, *Dumetella carolinensis*: Boughey & Thompson 1981; Kroodsma 2005).

At the same time, males in both NHOWR and SHOWR had much smaller repertoires of commonly produced songs (defined identically as those sung more than 1 % of the time), which averaged exactly the same number (24 song types). Hence, in both populations, males tended to focus their singing on a much smaller number of song types than they were capable of producing.

Males in both populations also showed similar patterns of song delivery during protracted song bouts. Typically, males sang in a fairly monotonous fashion, repeating the same song type many times before switching and, when switching song types, doing so only gradually via incremental addition, deletion or substitution of one syllable type at a time. Hence, although males in both populations had very large observed song repertoires and a clear capacity for even larger song repertoires than were recorded, their typical singing style tended not to reveal this capacity for diversity but rather concealed it. We referred earlier to this juxtaposition for NHOWR as one of 'global diversity combined with short-term monotony' (Rendall & Kaluthota 2013), and it seems to apply similarly to SHOWR. It is important to note, however, that males sometimes showed a capacity for greater short-term diversity in their singing, at times producing a larger number of song types within a single protracted bout and switching among song types more frequently (illustrated for SHOWR in Fig. 2.8), and this capacity for greater short-term variety has also been noted previously for males in North America (Rendall & Kaluthota 2013; Platt & Ficken 1987). Hence, males in both populations seem to have a capacity for singing with either eventual or immediate variety. We speculated previously for NHOWR that this capacity might point to selection simultaneously for elements of both consistency and diversity in song performance. In fact, recent work on a species of Mimidae, the Tropical Mockingbird (*Mimus givlus*), supports this possibility (Botero et al. 2009b). Mockingbirds have very large and diverse song repertoires but nevertheless tend to repeat song themes over short intervals, and the consistency of repeated performance correlates with age, social status and reproductive success.

It is also possible that the use of different singing styles in House Wrens is associated with different breeding stages or target audiences (female mates versus rival males), as in the simple 'repeat' and more diverse 'serial' singing styles of American redstarts (*Setophaga ruticilla*: Lemon et al. 1993). For House Wrens, these possibilities remain speculative and are an important focus of future planned work.

Overall, the pattern of song construction in both NHOWR and SHOWR appears quite similar and based on a core repertoire of syllable types common to all males in a local population. These syllable types are used as the building blocks for songs, with song variety arising from the flexibility with which different syllable types are combined in song construction. The corollary is that song learning in this species probably does not entail learning entire songs, as complete songs, as typifies many other oscines (Catchpole & Slater 2008). Instead, it likely involves learning the repertoire of common syllable types and then constructing songs from them through an improvisation-like process as hypothesized for some other species with exceptionally large song repertoires (e.g., Northern Sedge Wrens, Brown Thrashers and Gray Catbirds, Kroodsma 2005). Notably, improvisation has also been proposed to account for large song repertoires in north temperate populations of closely related Sedge Wrens, while sedentary, populations of Sedge Wrens in the tropical-south temperate zone are proposed to learn their songs in more canonical fashion from adjacent neighbors (Kroodsma et al. 1999; Kroodsma 2005). This pattern points to an interesting possible difference in how the selective forces on song learning and repertoire size might differ in migratory and sedentary populations between Sedge Wrens and House Wrens.

That many of the features of song organization and singing style in SHOWR were so similar to those in NHOWR is a bit unexpected in light of the many documented differences in life history and mating system between them. This might suggest that some conventional metrics of song variability and complexity—such as song repertoire size—are not straightforwardly applied to all species (Byers & Kroodsma 2009; Cardoso & Hu 2011; Soma & Garamszegi 2011) or that effects are more likely to be found in more detailed features of song not yet measured (reviewed in Gil & Gahr 2002; Sakata &

Vehrencamp 2012; but see Cramer 2013a, b), and these too are a central focus of future work.

Nevertheless, the high degree of similarity between NHOWR and SHOWR in many features of song organization and structure bears on an additional important topic in geographic variation, namely the taxonomic status of different populations. Currently, NHOWR and SHOWR are classified as part of a single species (*Troglodytes aedon*; Remsen et al. 2013), but at times in the past they have been split into separate species. There has been renewed interest in the taxonomy of this broadly distributed species complex and new proposals to consider recognizing additional divisions based on certain detailed features of song (Sosa-Lopez & Mennill 2014). While some of the differences we found in the detailed form of notes and syllables between NHOWR and SHOWR might further substantiate this proposal, in the main, our analyses highlighted considerable similarity between the two at higher organizational levels including how songs are constructed from constituent notes and syllables; how those syllables are organized serially to produce songs; how many such songs males produce; how many songs they produce commonly; how many songs they share; and how songs are actively delivered during protracted singing bouts. Hence, at this point, it is perhaps still premature to make definitive proposals about taxonomic status for this broadly distributed species group based on the limited available song data.

2.5. ACKNOWLEDGMENTS

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Table 2.1. Summary statistics (counts or means ± SD) for the sample of recorded songs of 18 male House Wrens from a population breeding in western Argentina in 2012.

Bird ID	Number of bouts	Number of songs	Syllables song^{-1}	Syllable types songs^{-1}	SVI [a]	Syllables sec^{-1}	Syllable types sec^{-1}	Total syllable types	Total song types	Common song types [b]
UP211	85	711	6.9 ± 2.8	2.8 ± 1.0	0.43 ± 0.13	9.8 ± 2.7	4.1 ± 1.2	27	52	25
UP212	89	1458	7.5 ± 3.1	3.3 ± 1.4	0.46 ± 0.13	9.7 ± 2.6	4.4 ± 1.5	28	159	34
UP266	27	292	5.2 ± 2.0	2.7 ± 1.2	0.52 ± 0.11	8.8 ± 2.7	4.5 ± 1.5	20	33	22
UP403	45	223	7.3 ± 3.5	3.0 ± 1.4	0.44 ± 0.15	8.9 ± 2.7	3.9 ± 1.4	26	46	20
UP405	68	1481	6.5 ± 2.2	2.8 ± 0.7	0.46 ± 0.15	10.8 ± 2.9	4.8 ± 1.5	27	78	23
UP409	58	797	7.5 ± 3.5	3.0 ± 1.3	0.44 ± 0.16	9.7 ± 2.8	4.1 ± 1.3	28	170	29
UP411	151	1665	5.5 ± 2.8	2.6 ± 1.2	0.52 ± 0.15	8.8 ± 2.3	4.3 ± 1.0	28	114	24
UP414	51	336	7.4 ± 3.3	2.8 ± 1.0	0.41 ± 0.13	9.7 ± 3.1	3.8 ± 1.4	28	46	25
UP416	38	215	6.8 ± 3.7	2.6 ± 0.9	0.48 ± 0.22	10.5 ± 3.5	4.5 ± 1.6	19	25	17
UP417	46	483	7.9 ± 3.0	2.8 ± 0.9	0.39 ± 0.13	9.5 ± 3.0	3.5 ± 0.9	27	47	20
UP419	46	651	8.3 ± 4.4	3.3 ± 1.4	0.45 ± 0.16	9.2 ± 2.1	4.0 ± 1.1	27	118	18
UP422	41	613	6.1 ± 2.3	2.7 ± 1.0	0.45 ± 0.11	9.3 ± 2.4	4.1 ± 1.2	22	60	22
UP423	57	351	8.9 ± 2.6	3.7 ± 1.1	0.43 ± 0.10	10.9 ± 2.1	4.7 ± 1.5	22	38	20
UP424	22	230	9.8 ± 3.9	4.1 ± 1.8	0.44 ± 0.13	10.4 ± 2.7	4.5 ± 1.7	27	116	32
UP428	18	379	5.8 ± 2.5	2.8 ± 0.9	0.53 ± 0.15	9.2 ± 2.8	4.6 ± 1.1	22	44	25
UP438	70	1087	6.1 ± 2.4	2.6 ± 1.0	0.45 ± 0.12	9.7 ± 2.6	4.2 ± 1.3	26	78	20
UP466	123	2051	6.9 ± 3.0	3.0 ± 1.1	0.48 ± 0.17	9.3 ± 2.4	4.2 ± 1.1	28	143	26
UP475	32	417	8.0 ± 3.6	3.3 ± 1.4	0.44 ± 0.13	11.9 ± 3.1	5.0 ± 1.6	27	67	22
Mean			**7.13**	**3.00**	**0.46**	**9.75**	**4.30**	**25**	**79**	**24**

a Syllable Versatility Index (SVI): calculated by dividing the number of syllable types in a song by the number of syllables.

b Count is limited to types representing more than 1 % of a male's song or syllable production.

Table 2.2. Comparison of basic song features between Northern and Southern House Wrens.

Song feature	Southern House Wrens[a]	Northern House Wrens[b]
Song duration (s)	2.11	2.25
Introduction section (s)	1.42	0.92
Terminal section (s)	0.69	1.33
Syllables/song	7.13	10.5
Syllable types/song	3.0	3.9
SVI	0.43	0.39
Syllable production rate	9.75	8.0
Syllable type production rate	4.3	3.1
Syllable repertoire size	28	27
Song repertoire size	19-170 (79)	38-194 (109)
Common song type size	24	24

[a] This study

[b] From Rendall and Kaluthota (2013)

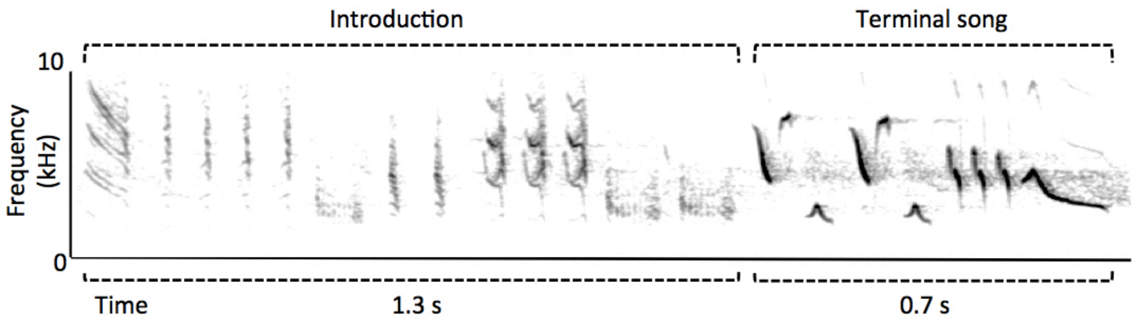

Figure 2.1. A representative song of a male House Wren illustrating the basic structural division of song into an introduction section composed of relatively low-amplitude broadband notes and a terminal section composed of a concatenation of higher amplitude and tonal, frequency-modulated notes organized into discrete syllable types.

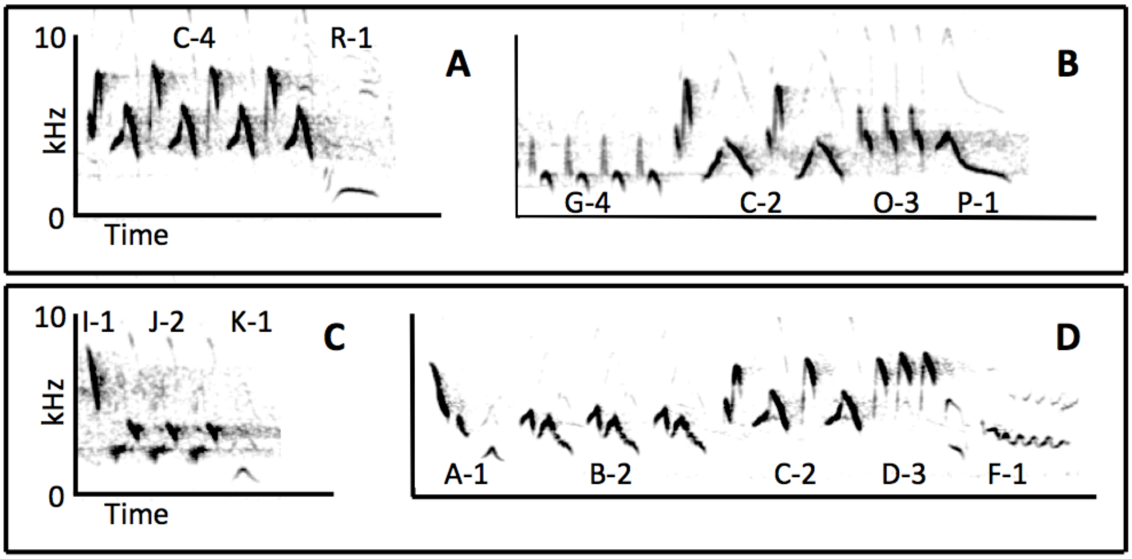

Figure 2.2. Four songs illustrating common variation in the number of different syllable types contained in the terminal section of songs.

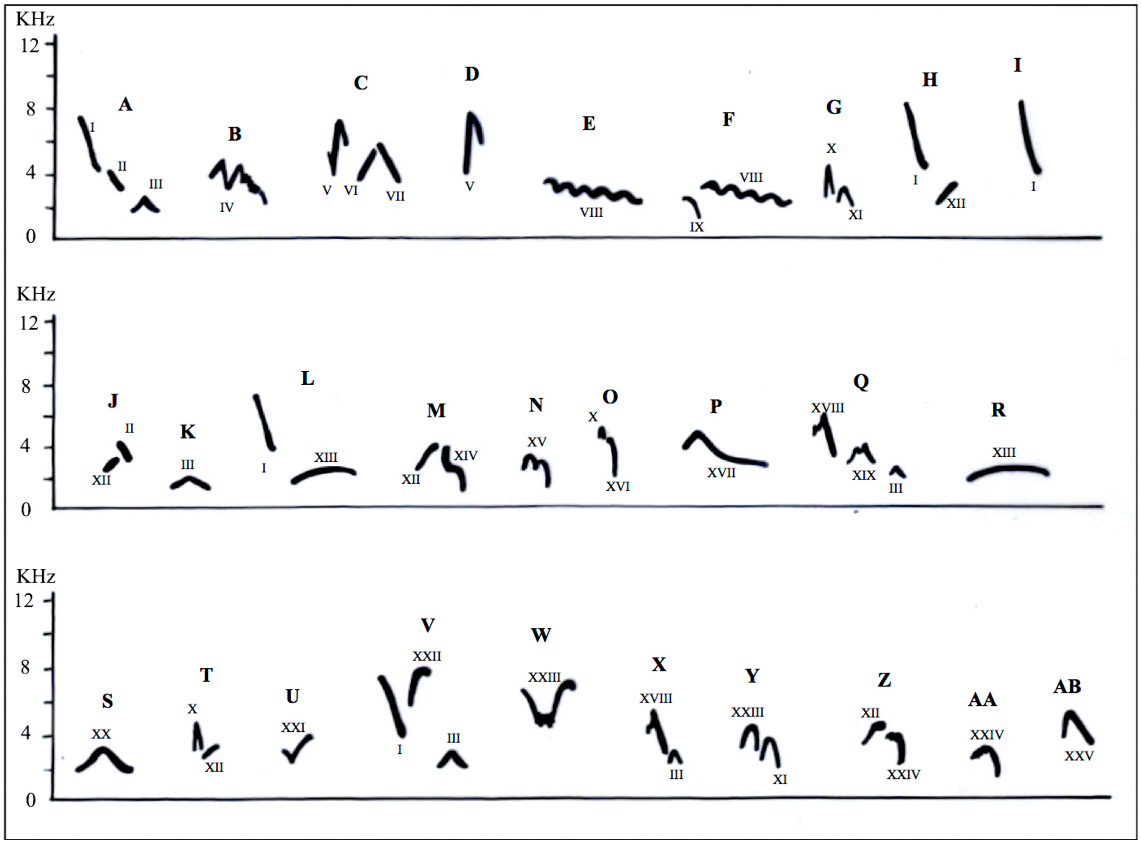

Figure 2.3. The note and syllable type repertoire for the study population based on the terminal section of songs. Notes are indicated by Roman numerals and syllable types by capitalized letters. Some notes appear in several different syllable types (e.g., I is used in syllables A, H, I, L and V), while other notes are unique to only one syllable type (e.g., XVII is used in syllable P).

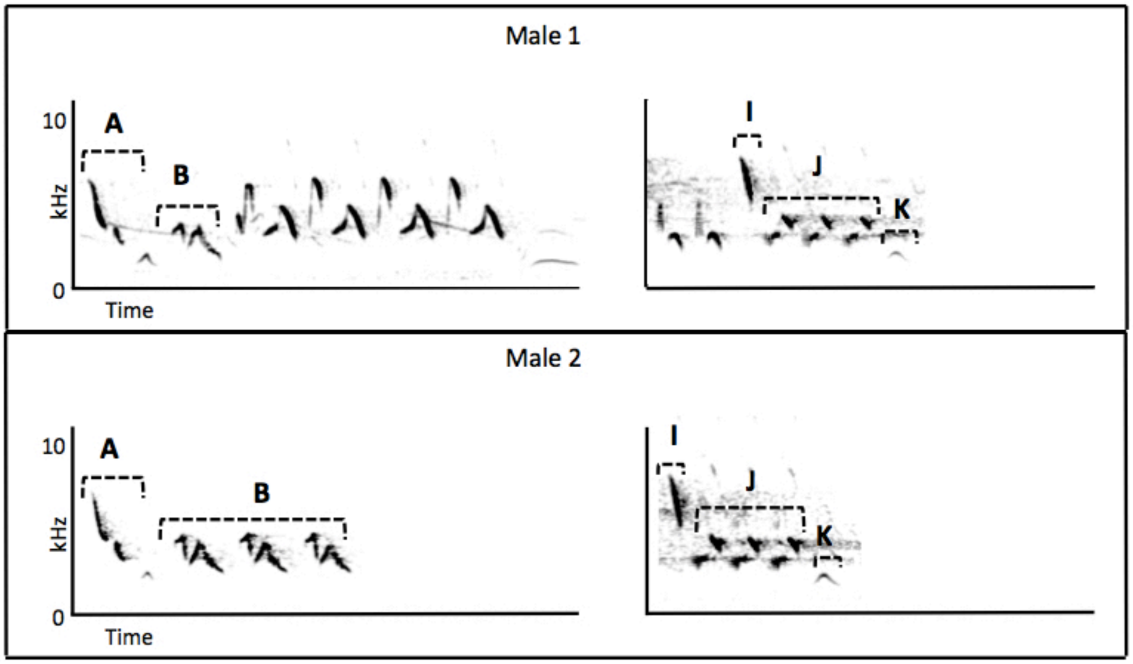

Figure 2.4. Two different songs from each of two different males. Syllable types common to songs used by both males are annotated to illustrate similarity in the forms of specific syllable types across males.

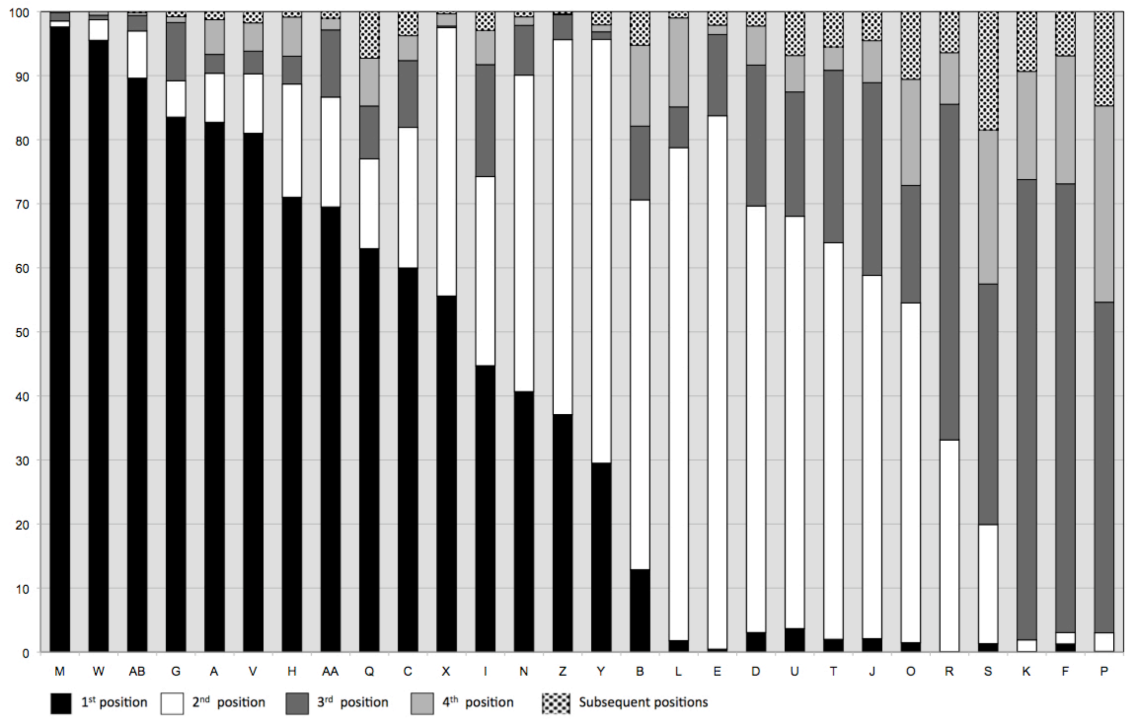

Figure 2.5. All syllable types in the study population's repertoire organized according to the frequency with which they occur in first, second, third, fourth and all other subsequent positions within songs.

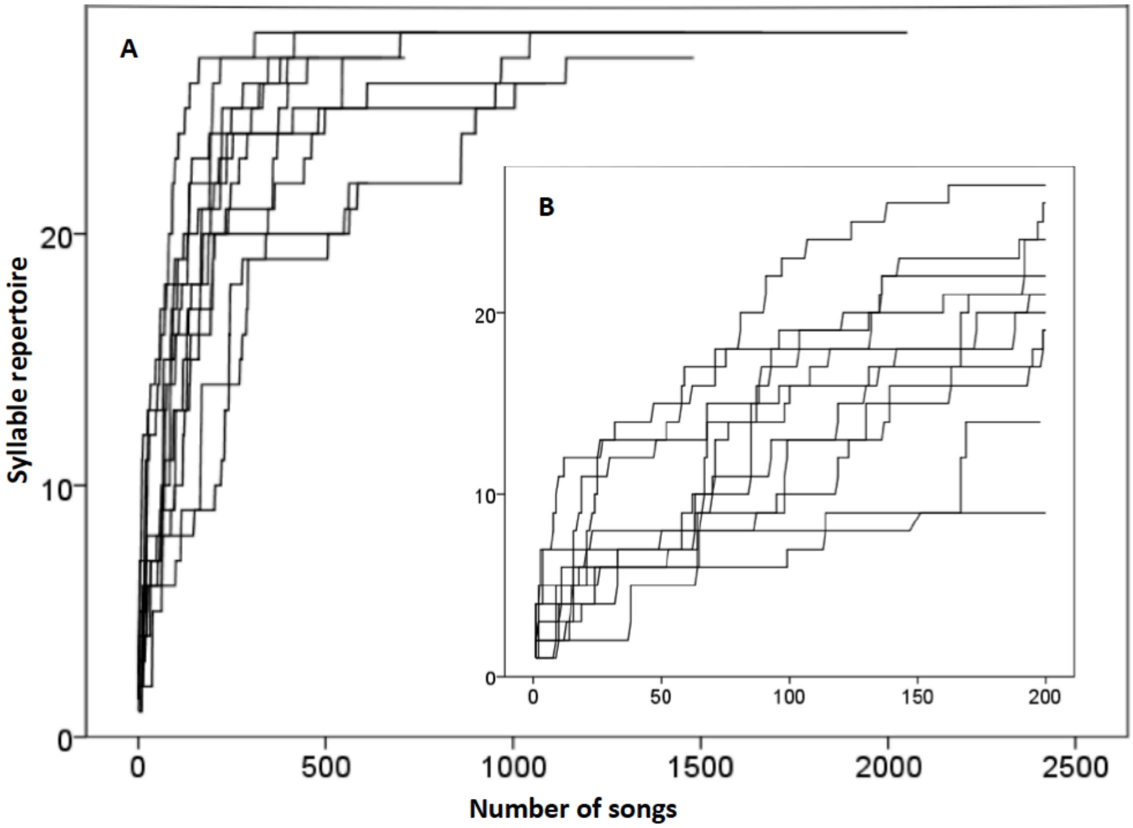

Figure 2.6. Syllable repertoire size and accumulation functions for a subsample of male House Wrens (n = 11). Each line represents a different male and shows the time course over which they introduced new syllable types into their songs across successive recordings. A Covers the entire recording sample and illustrates the clear asymptote in the syllable repertoire, while the inset B zooms in on the first 200 songs recorded for each male to better illustrate differences in the early accumulation functions between them.

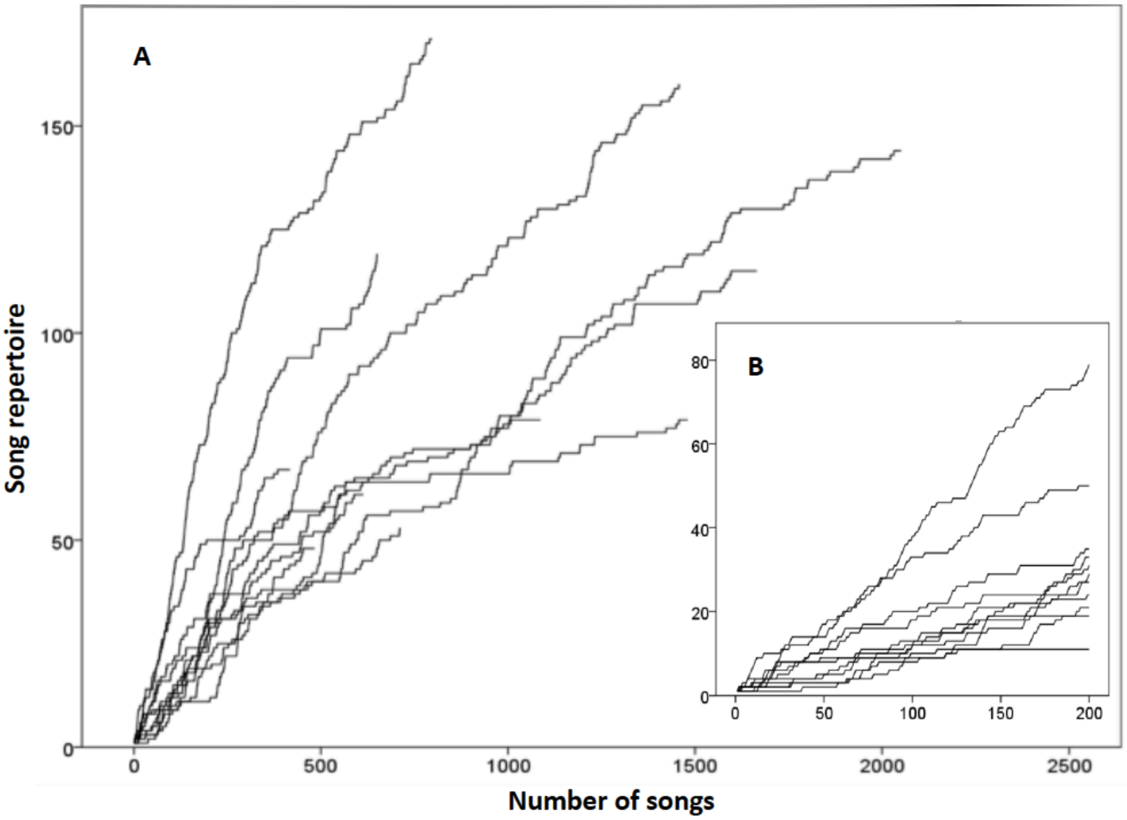

Figure 2.7. Song repertoire size and accumulation functions for subsample of male House Wrens (n = 11). Each line represents a different male and shows the time course over which males introduced new song types across successive recordings. Note that the accumulation functions show no sign of a plateau for any of the males regardless of the size of their recording sample. A Covers the entire recording sample and the inset B zooms in on the first 200 songs recorded for each male.

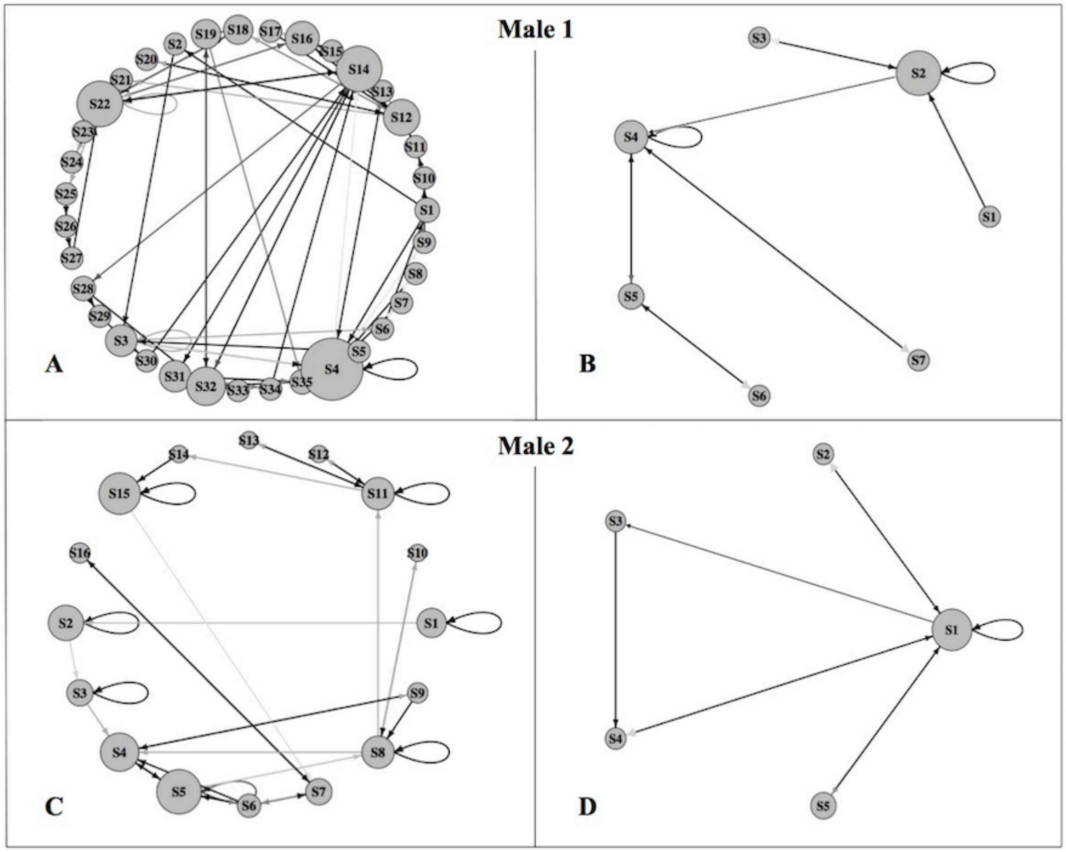

Figure 2.8. Diagrams illustrating variation in male singing styles. Each panel is constructed from a single bout of protracted singing from a particular male. The structure was developed using a custom R code implemented in the iGraph software package (Csardi and Nepusz 2006). It operates on the string of successive songs in a bout and the first-order transitions between them. Each circle represents a different song type, and differences in the size of the circles capture variation in the relative frequency with which the different song types were produced during that bout. Lines between circles represent song type switching, with the arrow indicating the direction of the transition and the darkness of the line signifying the relative frequency of that particular song type change. Looped connections (curved lines that loop back onto the same song type) indicate instances in which a song type is repeated in succession. The top two panels (A, B) correspond to two different bouts of singing from the same male (UP409) on two different days. They illustrate a clear difference in singing style: on one occasion a bout of 114 songs from this male involved production of a large number of different song types (35) and considerable switching among them; on another occasion, a similar bout of 81 songs involved production of a much smaller number of song types (7), many of which were therefore repeated frequently. The bottom two panels (C, D) capture a similar difference in singing style from a second male (UP466), once again based on single bouts of 75 and 98 songs, respectively, recorded on 2 different days. In this case, UP466 shows lower overall song diversity on both occasions, but greater diversity (16 song types) with more switching among song types on one day versus the other (5 song types).

CHAPTER 3

MALE SONG DIVERSITY AND ITS RELATION TO BREEDING SUCCESS IN SOUTHERN HOUSE WRENS (*Troglodytes aedon chilensis*)*

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ABSTRACT

Theory proposes an adaptive relationship between male song complexity, including large song repertoires, and improved breeding success. Evidence supporting these relationships exists but is sometimes mixed or weak. Here we provide a first comprehensive study of the relationship between male song diversity and breeding success in a non-migratory, austral population of house wrens (*Troglodytes aedon chilensis*) breeding in Mendoza, Argentina. During a two-year field study, we measured breeding success for a population of 62 males and recorded more than 34,000 songs from a subsample of 26 males. For the latter subsample, we tested for correlations between six measures of song diversity and four canonical measures of annual breeding success. Males that sang with greater overall syllable type diversity and that had larger song repertories paired with females that bred earlier and laid more eggs over the course of the breeding season. However, these males also showed lower levels of immediate song type diversity, as measured by the Levenshtein distance between successive songs. We discuss implications for the evolution of song complexity in this exceptionally widespread species and the selective mechanisms that might influence song complexity in resident populations in the Neotropics compared to migratory populations in the northern hemisphere.

3.1. INTRODUCTION

Darwin (1871) speculated about the evolution of elaborate male mating displays, specifically including elaborate male singing behavior. Subsequent research has largely affirmed Darwin's insights, and confirmed the sexually selected nature of birdsong (reviewed by Catchpole & Slater 2008). Contemporary theory now predicts that exaggerated, or complex, singing patterns are a reliable cue to male quality assessment by rivals and/or potential mates (Catchpole 1987, Andersson 1994, Catchpole & Slater 2008). Song complexity can, of course, encompass many dimensions of song, and one that has been a common focus of study has been the size of a male's repertoire of different songs. At the same time, the functional role of song complexity, including large song repertoires, can vary dramatically between species, and there therefore remains some uncertainty about the extent to which these features of song are consistently associated with increased breeding success (Byers & Kroodsma 2009; Soma & Garamszegi 2011).

For example, classic laboratory studies of song sparrows (*Melospiza melodia*) have shown that females experimentally presented with male song respond more strongly to those that are more variable, or indicative of a larger overall song repertoire (Searcy & Marler 1981; Searcy 1984). Some field studies have also found relationships between song complexity and specific male reproductive advantages. For instance, Catchpole (1980) and Buchanan & Catchpole (1997) reported that male sedge warblers (*Acrocephalus schoenobaenus*) with larger song repertoires succeeded in pairing earlier in the breeding season, while long-term studies in song sparrows (*Melospiza Melodia*) have likewise shown earlier laying dates, and also greater annual and lifetime breeding success for males with larger song repertoires (Hiebert et al. 1989; Reid et al. 2004).

Field studies of several other species have reported similar relationships between song complexity and proxy measures of breeding success or female choice, such as pairing date and first egg date (European starlings *Sturnus vulgaris*: Mountjoy & Lemon 1996; pied flycatchers *Ficedula hypoleuca*: Lampe & Epsmark 2003); extra-pair paternity (great reed warbler *Acrocephalus arundinaceus*: Hasselquist et al. 1996); and annual breeding success (eastern song sparrow *Melospiza melodia melodia*: Potvin et al. 2015).

At the same time, recent reviews have emphasized caution because some other studies have reported comparatively weak or no associations between song complexity and female choice or breeding success (Byers & Kroodsma 2009; Soma & Garamszegi 2011); or they have yielded contradictory results for the same species when studied in the field compared to the laboratory, suggesting that differences in research design can sometimes influence how data can be interpreted (red-winged blackbird: Yasukawa et al. 1980; Searcy 1988; great reed warbler: Catchpole 1986). For example, laboratory and other experimental studies have often involved testing immediate female responses to playback stimuli simulating male song repertoires of varying size, as a proxy of female mate choice, and have found greater responses to larger repertoires (Byers & Kroodsma 2009). However, it has been argued that these results might not be entirely representative of female choice in free-living birds because female subjects in laboratory experiments are often deprived of broader social interactions with potential mates that might normally influence mating decisions (Byers & Kroodsma 2009). Because it is hard to measure the behavioral dimensions of female choice directly in free-living birds, field studies typically use different measures of female choice, such as pairing date, first egg date, harem size and extra pair paternity, and in these studies the findings have not been so definitive. A recent meta-analysis of 42 field studies by Soma & Garamszegi (2011) revealed only a weak overall association between song complexity and reproductive success.

Here we address the relationship between male song complexity and breeding success in a neotropical population of southern house wrens in Argentina. We focus on an austral population of house wrens for several reasons. First, a great deal of what is known about the evolution of song and song complexity is based on migratory songbirds breeding in the northern hemisphere. By comparison, non-migratory, or sedentary, species breeding in the Neotropics have received much less attention (Kroodsma et al. 1996; Podos 2013), although there has been growing appreciation of the need to increase our focus on neotropical species to more fully document the suite of evolutionary pressures shaping song complexity and other dimensions of songbird behavior, biology and mating systems (Macedo & Machado 2013). Furthermore, southern house wrens are part of a larger species complex that is distributed widely across North, Central and South America. Currently, populations across this range are recognized as a single species (with many subspecies). In fact, house wrens have the widest latitudinal distribution of any native songbird in the Western Hemisphere (Brewer 2001; Dickinson & Christidis 2014). The very broad distribution of house wrens thus represents a special opportunity to examine latitudinal variation in song diversity within a single, very widely distributed species that is characterized by variation in other factors that are often thought to affect the intensity of sexual selection pressure on mating displays such as migratory pattern, breeding seasonality, and mating system. Finally, very recent work has documented high levels of song diversity in house wren populations in both North and South America. For example, a recent study of house wrens in southern Alberta, Canada (*Troglodytes aedon parkmannii*) found that individual males produced a large and mostly shared repertoire of 25 different syllable types that they then concatenated and recombined flexibly to produce even larger repertoires of distinct song types that could exceed 450 without approaching a ceiling (Rendall & Kaluthota 2013). A complementary study of house wrens in Mendoza, Argentina (*Troglodytes aedon chilensis*) documented a similarly large and shared repertoire of 28 different syllable types, once again used flexibly to produce very large individual song repertoires numbering in the hundreds with no evidence of a ceiling (Dos Santos et al. 2016).

Taken together, house wrens are well suited to further study of the factors influencing the evolution of song complexity. Past theorizing would suggest that males that sing with greater diversity, or complexity, or that produce larger repertoires of songs, will show higher breeding success (Catchpole & Slater 2008). This prediction is, however, derived largely from research on migratory species in the north temperate zone where relatively short breeding seasons and increased breeding synchrony, as well as higher rates of social polygyny and extra-pair paternity, are associated with increased sexual selection pressure and reliance on song to mediate male territoriality and female mate choice relative to resident species in the Neotropics (Stutchbury & Morton 1995; Bennet & Owens 2002; Collins et al. 2009). Hence, it is possible that the relationship between male song complexity and breeding success in resident, neotropical populations of house wrens might be relatively weak or absent if year-round residency and/or pairing reduces the reliance on song, compared to other factors, in mediating male territoriality or female mate choice.

3.2. METHODS

3.2.1. Study species

House wrens are a small (10-12 g) territorial passerine distributed broadly across the Americas (Brewer 2001; Johnson 2014). They reach their northern limits in central Alberta, Canada, and their southern limits in Tierra del Fuego, Argentina (Brumfield & Capparella 1996). Traditionally, house wrens have been divided into three major continental groups: the northern house wren in North America (*Troglodytes aedon*); the brown-throated wren in Mexico (*Troglodytes brunneicollis*); and the southern house wren in Central and South America (*Troglodytes musculus*, Brumfield & Capparella 1996). However, the most recent taxonomic assessment considers them all to be a single species, *Troglodytes aedon,* with approximately 30 subspecies (Remsen et al. 2017).

Across their broad range, house wrens show relatively modest differences in morphology and plumage (Brumfield & Capparella 1996), but are reported to have marked differences in migratory patterns and social mating systems. Social polygyny is more frequent in the predominately migratory populations of northern house wrens (5–40%; e.g. Johnson et al. 1993; Soukup & Thompson 1998; Poirier et al. 2004; Dubois et al. 2006) compared to resident populations of southern house wrens (1–5%; e.g. Freed 1986; Llambías & Fernández 2009). So too are rates of extra-pair paternity higher in northern house wrens (54% of broods and 25% of offspring) compared to southern house wrens (33% of broods and 16% of offspring: LaBarbera et al. 2010). Between-season divorce rates are also higher in migratory, north temperate populations (57.1–96.3%; Drilling & Thompson 1988; Poirier et al. 2003) compared to resident populations in the tropics (1%; Freed 1986) and the south temperate zone (35.1%; Carro et al. 2017).

3.2.2. Study site and subjects

We conducted research on a population of southern house wrens (*Troglodytes aedon chilensis*) breeding at a long-term study site near the town of Uspallata, Mendoza Province, Argentina (32.62°S, 69.36°W). The site is located in montane habitat (1850 m) in the foothills of the Andes Mountains where local daytime temperatures in summer range from 18 to 35 °C with little rainfall (mean annual precipitation = 134 mm; Carretero 2000). The study population breeds in a plantation of White Poplars (*Populus alba*) where the birds have been provided with wooden nest boxes for breeding similar to those used to study house wrens at other sites in Argentina (Llambías & Fernandéz 2009; Llambías 2012; Llambías et al. 2012). We have captured and monitored birds at this site since 2011 to facilitate individual identification using a numbered aluminium band and three coloured bands in unique combinations.

The breeding season at this latitude is from late October to mid-February. The present study was focused on two successive breeding seasons (2012/13 and 2013/14). At the start of the breeding season, we surveyed the local population to identify suitable pairs for focused study. Because our primary focus was on documenting and understanding the importance of male song complexity, our survey of suitable pairs concentrated on territorial males that were singing from a nest box where they had added nest material. Thereafter, we performed daily monitoring of these males, the females paired to them, and their associated nest boxes for the remainder of the breeding season.

3.2.3. Song recording

We recorded songs from marked birds on their territories and concentrated on the early morning hours (0500 to 1000 hours) when males were most active and singing. We confirmed the identity of males on their territories prior to beginning recording. We made recordings with a digital recorder (Sound Devices 702) and a shotgun microphone (Sennheiser MKH 816). In most cases, our recordings were made at distances of 5-10 m. We obtained recordings from a total of 44 males across the two breeding seasons (23 males in 2012; 21 males in 2013). For some males, the sample of songs was relatively small (< 300 songs). For purposes of more detailed analysis of song patterns, we limited the sample to males for which we had recorded more than 300 songs within a single breeding season. The resulting sample for use in detailed song analysis in this study comprised a total of 34,105 songs from 26 males (mean = 1,312 songs, SD ±820.6, range: 336 – 3,227). Each male was represented in only one of the two study years (see Supplementary Material for additional details of the song sample).

3.2.4. Song structure and organization

We provided a comprehensive characterization of the structure and organization of male song for this population in Dos Santos et al. (2016). Briefly, males typically sing in protracted bouts, with individual songs being 1-3 s in duration and separated by silent intervals of 2-5 s. Most songs have two discrete parts as illustrated in Figure 1: an introductory section composed of relatively unstructured notes that are either broadband and noisy in structure, or tonal with multiple harmonic overtones; followed by a main (or terminal) section that tends to be higher in amplitude and is composed of multiple different but discrete tonal and frequency-modulated notes. Further detailed characterization of song variation has focused on the latter terminal section of songs because it is louder and thus likely to be better transmitted to potential rivals and mates, and because it contains more structured and discrete elements that are readily identified and categorized.

The complete catalogue of note and syllable types for the terminal section of songs for this population involves 15 different notes that together constitute 28 different syllable types, each syllable type representing a consistent combination of 1-3 notes (for complete catalogue of note and syllable repertoire, see Figure 3 in Dos Santos et al. 2016). Individual males in our study population used 27 syllable types on average (SD ±2.1, range: 22 – 28, n = 26). Songs are composed of a concatenation of 1-5 different syllable types (mean = 3) with some syllable types repeated several times in succession (see Figure 3.1). Following conventional practices, song types are operationalized by their distinct syllable type sequences. Different song types, or variants, are defined conservatively as a unique syllable type sequence that ignores differences in the number of times a particular syllable type is repeated (for additional details, see Dos Santos et al. 2016).

3.2.5. Metrics of song complexity

For each male in the sample, we obtained six different measures of song diversity, or complexity, that collectively captured song variation at multiple organizational levels. The first two measures captured short-term variation within songs and were (1) *the number of different syllable types per song*; and (2) *the rate of syllable type production* (number of different syllable types/song duration). We used three different measures to capture variation occurring between songs. The first two of these were (3) *the coefficient of variation (CV) in the number of different syllable types per song*, and (4) *the coefficient of variation (CV) in the rate of syllable type production.* These two measures were calculated on the basis of each male’s entire recording sample. Hence, they capture global variation among all of the songs in a male's sample when considered as a collection. The third measure of between-song variation that we used was the *(5) Levenshtein distance* (Levenshtein 1966). This measure was used to capture variation between successive songs in the sequence of specific syllable types they contained (Gil & Slater 2000; Tougaard & Eriksen 2006).

The Levenshtein distance, sometimes also referred to as 'the edit distance', is a commonly used metric in linguistics, or related fields, that analyse strings of text or other characters. It quantifies the minimum number of edits that are required to convert one-character string, such as a word, into another using single-place deletions, insertions or replacements of individual characters. In this study, we used the Levenshtein distance to quantify the edit distance between successive song types, where we represented each song type as a letter string that reflected the identity of its constituent syllable types (see Figure 3.1 for an example of calculating the Levenshtein distance). In this way, we used the Levenshtein distance to quantify the simple edit distance between successive song types when information about syllable type repetition was not included.

The final measure of song complexity we used was another standard metric, namely (6) *the size of a male's song repertoire*. In our case, we did not use a simple tally of the number of song types that a male produced but rather normalized this total by dividing by the total number of songs recorded for that male. We specifically used this *normalized song repertoire*, rather than a simple count of repertoire size, because the total sample of song recordings varied considerably for different males. Hence, a simple tally of a male's overall song repertoire would be biased by the number of recordings obtained for that male and would thus likely yield a much higher total for males with larger recording samples. Our normalized measurement of song repertoire size eliminates this confound by dividing the song repertoire tally for each male by the number of songs recorded for that male. We made song annotations and performed acoustic analyses using PRAAT, version 5.3 (Boersma & Weenink 2012).

3.2.6. Breeding Success

To obtain standard measures of breeding activity, we monitored territories and nest boxes across the entire breeding season, and not just for the sample of males to be used in detailed song analysis (n = 26) but for the complete study population (n = 62). Our baseline monitoring involved checking nest boxes every other day during the morning hours (0600 – 1100) for evidence of any change in the status of the nest. During egg laying, and again close to the time of both hatching and fledging, we checked boxes daily for changes in egg and nestling status and number. We recorded the dates of landmark events in the breeding cycle for individual nests, including the dates of nest initiation (defined as the first appearance of lining in the nest cup which is the sole prerogative of females in this species), first egg laid, hatching and fledging. We used total number of eggs laid during the breeding season and total number of nestlings fledged as additional measures of breeding success. We considered males to have paired fully if their nest reached the egg stage, and we classified them as successful breeders if their nest fledged at least one nestling. For males that failed to pair, we considered total number of eggs laid and breeding success to be zero. Nest failure is attributable mostly to predation and interference during nest take-overs by grayish baywings (*Agelaioides badius*) and house sparrows (*Passer domesticus*).

3.2.7. Statistical analysis

Summary statistics and correlation coefficients for the song measures are presented in Table 3.1. There was no evidence of multi-collinearity among the song measures; hence, we did not attempt to reduce the dimensionality of this set of song variables in subsequent tests of the relationships between song complexity and breeding success, which we tested using multiple linear regressions. We performed separate multiple regressions for each of the four key breeding measures (laying date, fledging date, total number of eggs and total number of fledglings) as dependent variables with the six song diversity metrics as predictor variables. The data for laying date and fledging date met model assumptions for normally distributed residuals. Because the variable measuring the total number of eggs is a non-continuous count variable, we used a Poisson regression model. The variable for the total number of fledglings, also a count variable, contained a large number of zeroes (failed nests) and did not meet the assumptions for Poisson regression requiring that the mean equal the variance. As a consequence, we used a zero-inflated Poisson model instead. The latter is a model designed to deal with variables that entail count data with an over-representation of zero values (Lambert 1992).

We also tested the relationship between song complexity and breeding success using different types of generalized linear mixed models (GLMMs) depending on the distribution of the dependent variables. For the models involving laying date and fledging date as dependent variables, we developed GLMMs with identity-link and Gaussian error distribution. To analyze the relationship between song complexity and the total number of eggs laid during the season, we used a GLMM with a log-link and Poisson error distribution. Finally, for the model including the total number of fledglings produced during the season as the dependent variable, we used a GLMM with a log-link and negative binomial error distribution (to account for the excessive number of zeros). All four GLMMs included year (breeding season) as a random factor. We compared model fit between the fixed and mixed models using the Bayesian information criterion, BIC (Schwarz 1978; Aho et al. 2014).

Our multiple regression tests of the relationship between song complexity and the dates of egg laying and fledging were limited to first breeding attempts (for lay date) and first successful brood (for fledge date). Hence, these analyses do not include the dates of egg laying or fledging for subsequent nesting attempts because these would confound the analysis.

In contrast, for the analysis of *total number of eggs* and *total number of fledglings* produced, we used the seasonal totals for each pair, thus summing across all nesting attempts for a given male within a breeding season. For one male in the sample (405), we were unable to confirm with certainty the total of number of eggs laid because the nest was destroyed before egg laying was complete. For a second male (574), we were unable to confirm the total number of fledglings produced for the entire season because when our observations ended the nestlings were still in the nest. We have recorded the uncertainty for these two males in Table 3.2 using NA codes for *total number of eggs* and *total number of fledglings*, and these males were therefore also omitted from tests related to these two breeding metrics.

For the broader sample of males for which we had breeding data (but not song data) we also tested for relationships between laying date and breeding success, defined both as a dichotomous dependent variable (yes/no) and in terms of the total number of fledglings produced. In both cases, we used a GLMM with a logit link and binomial error distribution, including year as a random factor.

All statistics were performed in Stata 14 (StataCorp 2015) and all tests were performed two-tailed.

3.2.8. Ethical note

Research adhered to guidelines of the Canadian Council on Animal Care and was approved by the Animal Welfare Committee of the University of Lethbridge (AWC#1429). Local permits were granted by Secretaría de Medio Ambiente, Dirección de Recursos Naturales Renovables, Gobierno de Mendoza, Argentina (Res: 459).

3.3. RESULTS

3.3.1. General breeding patterns for the entire study population

The complete breeding sample for the two study years involved 62 males and a total of 76 nesting attempts. In both years, the date of first nest initiation was November 5 and the date of first egg laid was November 10. The breeding season length, calculated as the number of days between the first and last laying attempt in the population, was 72 in 2012 and 76 days in 2013. Across the two breeding seasons, 89.3% of nests initiated made it to the egg stage but only 41.3% successfully fledged young. Successful nests produced a mean of 4.66 eggs (SD ±0.79, n = 76, range: 2 – 7), and for those that successfully fledged young, a mean of 4.13 fledglings (SD ±0.88, n = 35, range: 2 – 5). There was no relationship between laying date and breeding success, whether characterized dichotomously (yes/no: GLMM, $\chi^2 = 0.28$, $P = 0.597$, n = 76) or in terms of the total number of fledglings produced (GLMM, $\chi^2 = 0.34$, $P = 0.842$, n = 76).

For further detailed song analysis, we used a subset of 26 males for which we had a sufficient sample of recordings. For this sample of 26 males, 22 (84.6%) paired and bred successfully to at least the egg stage (Supplementary Material). For females paired to this set of males, laying date ranged from 14-Nov to 13-Jan in 2012 and from 10-Nov to 21-Jan in 2013, while fledging date ranged from 19-Dec to 27-Jan in 2012 and from 11-Dec to 10-Feb in 2013. Mean clutch size was 4.86 eggs (SD ±0.89, n = 28, range: 2 – 7) and yielded an average 4.31 fledglings (SD ±0.95, n = 15, range: 2 – 5) per nest in successful attempts.

3.3.2. Song diversity and breeding success

The Bayesian Information Criterion for goodness of fit indicated that the fixed effects models of the relationship between song complexity and breeding success provided a better fit to the data than did the mixed effects models. Therefore, we present only the results for the fixed effects models.

Multiple linear regression of song variables against laying date revealed significant relationships for several song variables that accounted for 68% (adjusted R^2) of the variance in laying date ($F_{6,15} = 8.58$, $P < 0.01$). There were significant negative relationships between laying date and both *CV number of different syllable types per song* ($t = -5.22$, $P < 0.01$, Figure 3.2a) and *Normalized song repertoire size* ($t = -4.83$, $P < 0.01$, Figure 3.2b, Table 3.2a). Hence, females paired to males with greater variety in the number of syllable types in their songs and larger song repertoires produced eggs earlier in the season. In contrast, there was a positive relationship between laying date and *Levenshtein distance* ($t = 3.80$, $P < 0.01$), indicating that females paired to males with greater immediate song variety produced eggs later in the season.

Multiple linear regression of song variables against fledging date also identified significant relationships and accounted for 85% (adjusted R^2) of the variance in fledging date ($F_{6,5} = 11.47$, $P < 0.01$). There was a significant negative relationship between fledging date and *Normalized song repertoire size* ($t = -5.76$, $P < 0.01$, Figure 3.2c, Table 3.2a), indicating that females paired to males with larger song repertoires also fledged young earlier in the season. There were also relationships between fledging date and both *CV number of different syllable types per song (*$t = -2.46$, $P = 0.057$*)* and *Levenshtein distance* ($t = 2.33$, $P = 0.067$), but in this analysis these two relationships did not reach significance (Table 3.2a).

A Poisson multiple regression model revealed a positive relationship between total number of eggs and the *CV number of different syllable types per song* ($z = 3.04$, $P < 0.01$, Figure 3.2d, Table 3.2b). Females paired to males that sang with greater variety in the number of syllable types in their songs showed greater overall egg production across the breeding season and across multiple possible nesting attempts. However, this model accounted for only 10% of the variance in the total number of eggs ($\chi^2 = 14.61$, $P = 0.024$).

There were no significant relationships between the *total number of fledglings* and any of the song variables (zero-inflated Poisson multiple regression model: $\chi^2 = 6.59$, $P = 0.36$, Table 3.2b).

3.4. DISCUSSION

We found significant relationships between some standard measures of male song complexity and breeding success in southern house wrens. Specifically, we found that females paired to males that sang with greater syllable diversity and had larger song repertoires bred earlier and laid more eggs, where the latter breeding outcomes are generally associated with improved reproductive success through increased juvenile survival and recruitment (reviewed by Williams 2012). We did not find relationships between these same song measures and fledging success, but that is probably be due to the high rates of nest predation in the study population which yielded a much smaller sample, and comparatively weak power, for those tests.

We also found a significant relationship for the Levenshtein distance measuring short-term variation in the syllable type sequences of adjacent songs. The relationship ran counter to general expectations regarding song diversity in that males that were paired to females that bred earlier in the season sang with less (rather than more) short-term diversity. Although counter-intuitive on the surface, this outcome in fact corroborates the general singing style that is now well documented for house wrens (Kroodsma 1977; Platt & Ficken 1987; Johnson & Kermot 1991; Cramer 2013; Rendall & Kaluthota 2013; dos Santos et al 2016). Despite producing extremely large repertoires of different song types, male singing is relatively monotonous in the short-term and involves protracted repetition of the same song type many times before switching. As a result, the species singing style is characterized as involving ‘eventual’ rather than ‘immediate’ variety (Kroodsma 1977). Elsewhere (Rendall & Kaluthota 2013), we have discussed the possibility that this singing style, juxtaposing global diversity with short-term monotony, might represent a balance of selection pressures favouring both increased diversity, or complexity, but also an element of consistency in singing performance (reviewed in Sakata & Vehrencamp 2012). This mixed pattern of outcomes underscores a broader point that greater diversity, or complexity, is not an inevitable outcome of selection on song displays (Cardoso & Hu 2011).

Our findings have additional implications for theory concerning the evolution of birdsong. Historically, theory has been grounded in research on migratory species that breed in the north temperate zone, with much less known about the evolution of song patterns in non-migratory species breeding in the Neotropics (Kroodsma et al. 1996; Podos 2013). As a result, current theory proposes a general pattern of increased sexual selection pressure, and with it also greater song complexity, in migratory species in the north temperate zone compared to sedentary species in the tropics (Catchpole 1987; Collins et al. 2009). Following this hypothesis, it might be expected that the relationships between male song complexity and breeding success would be relatively weak or absent in the resident population of southern house wrens that we studied. The fact that they were not, requires some further theoretical elucidation, particularly in light of another recent, continent-wide study of song in house wrens (Kaluthota et al. 2016).

That latter study compared several basic elements of song performance and complexity in house wrens from 281 locations across North, Central and South America, and it included some of the same song features studied here (e.g., the number of elements and unique elements per song, the rate of element production, and the number and rate of trill production). Results confirmed that these basic measures of song complexity increased linearly from the equator northward to 52° North latitude in accordance with current theory. However, the study also revealed a novel pattern of latitudinal variation in which song complexity increased again at higher latitudes in the southern hemisphere as well. This novel latitudinal gradient suggests that, while pressures of sexual selection might often differ consistently between the tropics and north temperate regions, as predicted by current theory, they might also be similar and convergent among populations at latitudinal extremes in both hemispheres.

This potential for latitudinal convergence in selection pressures and outcomes has not been discussed in previous theorizing, but it might help to explain our current findings. In the Kaluthota et al. (2016) study, evidence for latitudinal convergence in song patterns first appears at approximately 30°-35° North and South (see Figures 3-5 in Kaluthota et al.), and the population of southern house wrens that is the focus of the present study is located in exactly this latitudinal band at 32.62°S. Hence, although this population of house wrens in Argentina is resident, it is possible that the breeding season length and synchrony, and thus the general forms and intensity of sexual selection, are actually convergent with those of many north temperate populations.

For example, one resulting prediction of such convergence would be that, in both northern and southern populations, males that sing with greater complexity have higher breeding success through female choice by obtaining additional mates or extra-pair matings. We did not observe polygyny in our focal population, and polygyny is likewise reported to be low-to-negligent in other populations of southern house wrens (1-5%: Freed 1986;Llambías & Fernández 2009), while rates of polygyny for northern house wrens can be many times higher (15–35%: Johnson 2014). Hence, selection favoring increased social polygyny seems unlikely to be an equally relevant driver of song complexity in both northern and southern house wrens. We were not able to measure extra-pair matings or paternity in the current study, but studies on other populations of southern house wrens have generally found rates of extra-pair paternity to be approximately half of those reported for northern house wrens (La Barbera et al. 2010). Nevertheless, the absolute levels of extra-pair paternity reported for southern house wrens are not trivial at 33% of broods and 16% of offspring. Hence, selection for extra-pair matings might be a more likely common driver of song complexity in both regions, and future song studies in this species must aim to measure and test this factor directly.

Alternatively, it is possible that elevated song complexity in southern house wrens is driven less by the courtship function of song and more by its function in male-male territory competition, if there is increased selection pressure on year-round territory competition and maintenance in resident, neotropical populations compared to migratory populations in the north temperate zone (Collins et al. 2009). We cannot either support or reject this possibility but are circumspect about its relevance specifically to house wrens because it is not obviously consistent with other details of their behavior. In house wrens, there are certainly instances of aggressive behavior and chasing between neighbouring males, affirming the general importance of territory competition and maintenance in this species. However, these interactions are seldom accompanied by singing by either the territory resident or the intruder. Furthermore, the broader singing behavior of male house wrens does not include obvious instances of counter-singing even from males in their respective territories; nor does it include evidence of song-matching that is hypothesized to be a conventional element of territory signalling between males (Vehrencamp 2001). In fact, although male house wrens in a local population share virtually all of the same syllable types, they share very few entire song types: a given song type is shared, on average, by only 1.6 and 1.8 males, respectively, in northern and southern house wrens (Rendall & Kaluthota 2013; Dos Santos et al. 2016). This low level of song sharing inherently limits the capacity for song-matching in the service of male territory signalling and seems to reflect a process of song learning that is focused on learning individual syllable types, rather than entire song types, with different song types being improvised from the stock of common syllable types. This pattern of song learning is different from many other species that do learn entire song types (cf. Nelson et al.

2001), but it is common to a few species that are noted also for producing very large song repertoires, including some other wren species and members of the *Mimidae* (Kroodsma & Verner 197; Boughey & Thompson 1981).

Another hypothesis for the correlation between elevated male song complexity and breeding success is that males that sing with greater complexity pair with females that are older, more experienced, or more fecund. This mechanism could in principle apply equally to both northern and southern populations, but it was first presented by Darwin (Darwin 1871) and further developed by Fisher (1958) as a mechanism to explain the development of elaborate male traits specifically in monogamous populations with limited potential for polygyny or extra-pair matings. The proposal is that females pairing earlier in the season have higher body condition, and thus are more fecund, than females that pair later. Hence, early-pairing females have a head-start in assessing and choosing males that exhibit greater song complexity (Kirkpatrick 1990). At the same time, it is possible that the males that sing with greater song complexity are themselves older males, such that the older, more fecund females pairing early in the season are selecting older, more experienced males. Previous studies of several other species have shown that male age can be associated with greater song diversity and higher reproductive success (e.g., Geslin et al. 2004; Kiefer et al. 2006). We do not have the requisite age data for males and females to be able to test these possibilities, but the pattern we observed in which early breeding females were differentially paired to males with more complex song and also produced more eggs over the breeding season is consistent with this hypothesis.

A final, non-exclusive alternative is that females paired to males that sing with greater song complexity invest more in offspring care and development (Burley 1986, 1988). This ‘differential allocation hypothesis’ proposes that females use male song complexity as a guide to male quality and thus also their own levels of post-pairing investment in reproduction, investing more when paired to (higher quality) males that sing with greater complexity. Once again, this mechanism could be relevant in both northern and southern populations; however, in southern populations it might be driven more by direct benefits from males (compared to indirect benefits) if the more limited polygyny potential and extra-pair mating opportunities in southern populations induces greater male investment in offspring care compared to their northern counterparts, and if females in turn adjust their investment in response to that of males (see review of invest-matching in Sheldon 2000). We plan to test this possibility directly for house wrens in future analyses using complementary data we have for both northern and southern house wrens on levels of male and female investment in nest-building, brooding and chick provisioning.

In summary, we have shown positive correlations between nest initiation and female fecundity and some but not all of the measures of male song complexity we investigated for a population of southern house wrens. Although we are not able to provide a definitive account of the specific functional pressures that account for these findings, they nevertheless contribute to ongoing investigations into the evolution of elaborate male song displays. Importantly, they also add to a growing literature concerning the nature and evolution of song in neotropical populations which have been relatively neglected historically (Macedo & Machado 2013), and they specifically underscore the potential for both similarities and differences between populations in the northern and southern hemispheres in patterns of song complexity and in the specific selective mechanisms that may have shaped them.

Acknowledgments

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Table 3.1. Summary statistics (mean ± standard deviation) and Spearman correlation coefficients for the song variables recorded from 26 male House Wrens breeding in Uspallata, Mendoza, Argentina in 2012 and 2013.

		Correlation Coefficients					
Song Variables (predictors)	**Mean (SD)**	**1**	**2**	**3**	**4**	**5**	**6**
1. *Number of different syllable types per song*	2.986 (0.275)	—					
2. *Rate of syllable type production*	4.375 (0.335)	0.34	—				
3. *CV number of different syllable types per song*	0.375 (0.061)	0.17	0.14	—			
4. *CV rate of syllable type production*	0.301 (0.044)	0.05	0.12	0.25	—		
5. *Levenshtein distance*	0.806 (0.355)	-0.02	0.02	0.56*	0.25	—	
6. *Normalized song repertoire size*	0.086 (0.032)	0.21	0.02	0.36	0.26	0.71*	—

*$P < 0.01$

Table 3.2a. Multiple linear regression models of breeding metrics on song variables.

	Breeding Metrics (Dependent Variables)							
	Laying Date (n = 22) ($F_{6,15}$ = 8.58, Adj. R^2 = 0.68, $P < 0.01$)				**Fledging Date** (n = 12) ($F_{6,5}$ = 11.47, Adj. R^2 = 0.85, $P < 0.01$)			
Song Variables (Predictors)	**Estimate (SE)**	**ω^2**	***t***	***P*-value**	**Estimate (SE)**	**ω^2**	***t***	***P*-value**
Number of different syllable types per song	13.08 (8.08)	0.09	1.62	0.126	19.41 (12.68)	0.18	1.53	0.186
Rate of syllable type production	3.14 (6.79)	0.00	0.46	0.650	-6.55 (7.29)	0.00	-0.90	0.410
CV number of different syllable types per song	-207.08 (39.67)	0.62	-5.22	**0.000**	-162.68 (66.15)	0.46	-2.46	**0.057**
CV rate of syllable type production	67.72 (49.46)	0.05	1.37	0.191	-66.88 (59.70)	0.04	-1.12	0.313
Levenshtein distance	36.11 (9.50)	0.46	3.80	**0.002**	28.79 (12.34)	0.43	2.33	0.067
Normalized song repertoire size	-463.62 (95.90)	0.59	-4.83	**0.000**	-516.30 (89.68)	0.84	-5.76	**0.002**

Table 3.2b. Multiple linear regression models of breeding metrics Total Number of Eggs and Total Number of Fledglings on song variables.

	Breeding Metrics (Dependent Variables)					
	Total Number of Eggs[1] (n = 25) (χ^2 = 14.81, Pseudo R^2 = 0.10, P = 0.022)			**Total Number of Fledglings**[2] (n = 24) (χ^2 = 6.59, P = 0.36)		
Song Variables (Predictors)	**Estimate (SE)**	***z***	***P*-value**	**Estimate (SE)**	***z***	***P*-value**
Number of syllable types per song	0.32 (0.37)	0.88	0.380	-0.28 (0.77)	-0.36	0.717
Rate of syllable type production	-0.09 (0.33)	-0.26	0.794	-0.05 (0.51)	-0.10	0.921
CV number of syllable types per song	5.94 (1.95)	3.04	**0.002**	5.32 (4.06)	1.31	0.190
CV rate of syllable type production	1.00 (2.13)	0.47	0.639	-1.32 (4.40)	-0.30	0.764
Levenshtein distance	-0.27 (0.43)	-0.62	0.534	-0.13 (0.81)	-0.16	0.874
Normalized song repertoire size	-1.67 (4.05)	-0.41	0.681	3.16 (6.64)	0.48	0.635

[1] Poisson multiple linear regression model.

[2] Zero-inflated poisson multiple linear regression model

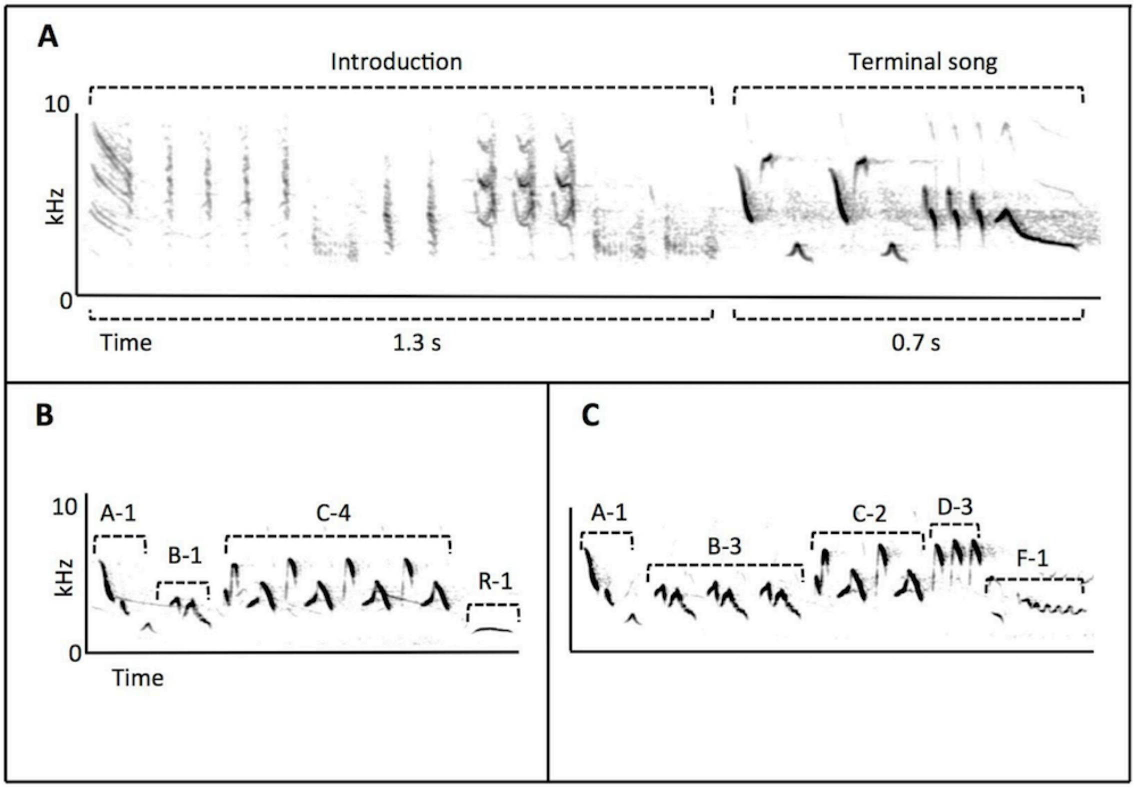

Figure 3.1. (A) A typical song from a southern house wren male depicting the major structural subdivision into an Introduction section composed of low-amplitude broadband notes, and a Terminal section composed of a sequence of higher-amplitude, tonal and frequency-modulated notes organized into discrete syllable types. Panels (B) and (C) show common forms of variation in the terminal section of songs in the number and specific identity of syllable types, as well as in different patterns of syllable type repetition. The number of syllable-type editing changes necessary to transform the song in panel (B) A-B-C-R into the song in panel (C) A-B-C-D-F would involve one substitution (D for R) and one addition (F). Hence, *the Levenshtein distance* between the two song types is 2.

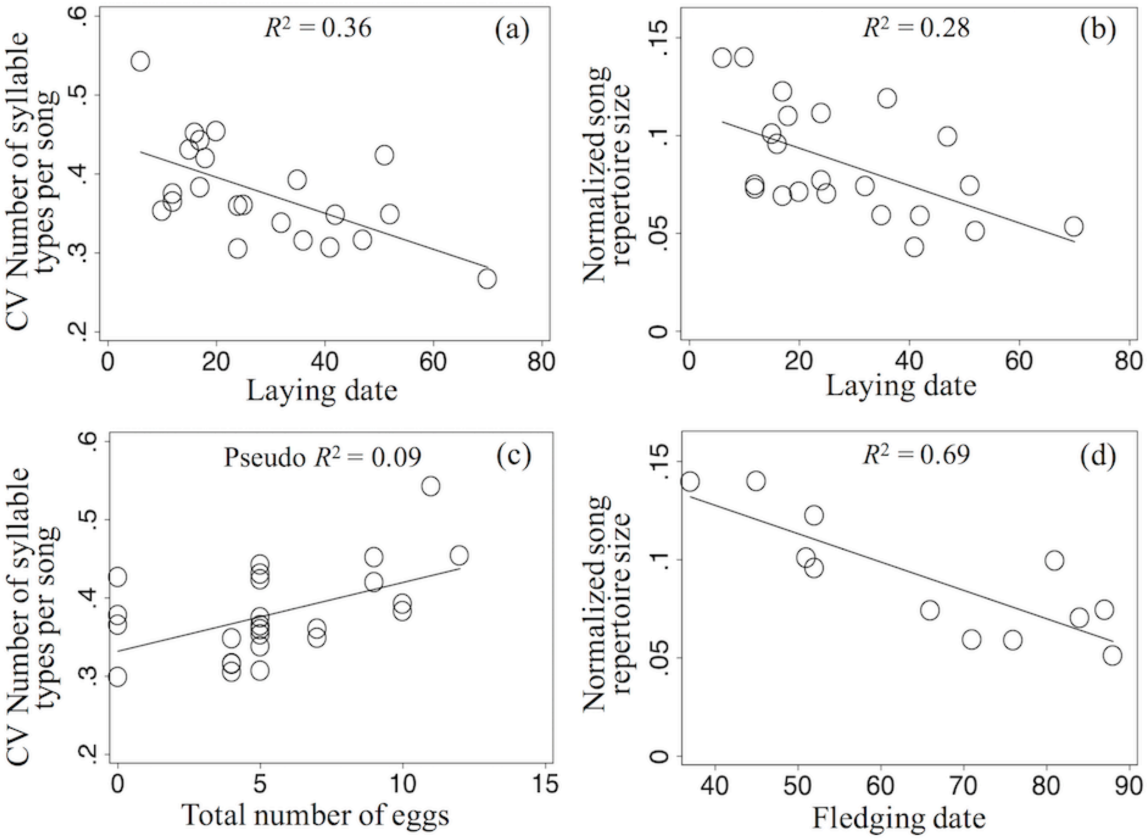

Figure 3.2. Regression relationships between the *CV number of different syllable types per song* and both *Laying date* (a) and *Total number of eggs* (c); and between *Normalized song repertoire size* and both *Laying date* (b) and *Fledging date* (d). All regressions are statistically significant (see text for detailed statistics).

Supplementary Material. Details of breeding metrics and number of song recordings for subsample of male house wrens (n = 26) used to compare song complexity and breeding success.

Bird ID	Season	First egg date	Fledging date	Total eggs*	Total fledglings*	Number of songs	Total syllable types
211	2012	16-Nov-12	-	5 (5)	0	711	27
212	2012	22-Nov-12	-	9 (5,4)	4 (0, 4)	1458	28
405	2012	13-Jan-13	-	N/A	0	1481	27
411	2012	21-Nov-12	-	5 (5)	0	1665	28
414	2012	14-Nov-12	19-Dec-12	5 (5)	5 (5)	336	28
417	2012	21-Dec-12	24-Jan-13	4 (4)	4 (4)	483	28
422	2012	-	-	0	0	613	22
423	2012	28-Nov-12	-	4 (4)	0	351	22
428	2012	10-Dec-12	-	4 (4)	0	379	22
438	2012	16-Dec-12	-	5 (5)	0	1087	26
466	2012	29-Nov-12	27-Jan-13	7 (5,2)	2 (0,2)	2051	28
475	2012	-	-	0	0	417	27
210	2013	16-Dec-13	19-Jan-14	4 (4)	4 (4)	2046	28
409	2013	21-Nov-13	26-Dec-13	10 (5,5)	4 (4,0)	703	27
410	2013	24-Nov-13	-	12 (6,6)	0	1904	27

419	2013	19-Nov-13	25-Dec-13	5 (5)	5 (5)	1519	28
504	2013	10-Nov-13	11-Dec-13	11 (5,6)	10 (5,5)	1368	28
557	2013	28-Nov-13	-	5 (5)	0	520	22
570	2013	25-Dec-13	30-Jan-14	5 (5)	5 (5)	3227	28
573	2013	06-Dec-13	09-Jan-14	5 (5)	5 (5)	1653	28
574	2013	09-Dec-13	14-Jan-14	10 (5,5)	N/A	2545	28
575	2013	15-Dec-13	-	5 (5)	0	2289	28
581	2013	20-Nov-13	26-Dec-13	9 (5,4)	8 (5,3)	701	25
585	2013	-	-	0	0	720	26
588	2013	-	-	0	0	2739	28
599	2013	26-Dec-13	31-Jan-14	7 (7)	3 (3)	1139	26

* First number represents seasonal total of eggs (or fledglings) summed across all nesting attempts, while numbers in brackets indicate the totals for individual nest attempts.

CHAPTER 4

THE EFFECTS OF VARIABLE SAMPLING ON THE RELATIONSHIP BETWEEN MALE SONG DIVERSITY AND BREEDING SUCCESS IN SOUTHERN HOUSE WRENS (*Troglodytes aedon chilensis*)

4.1. INTRODUCTION

Darwin (1871) introduced his theory of Sexual Selection to explain the evolution and maintenance of exaggerated male traits that function in attracting mates and competing with other males. He specifically considered the songs of male birds as a case in point, which he proposed had evolved under sexual selection pressure through female choice. According to this idea, song diversity or complexity is one of the aspects of male birdsong that females can potentially assess.

Song diversity has been used in the birdsong literature as an umbrella term that encompasses a broad number of song parameters. However, it is often characterized in terms of "repertoire size", which is calculated either as the number of different songs a male can sing or the number of syllable types a male can produce (Podos et al. 1992; Ready & Weary 1992; Nowicki & Searcy 2004). Such measures of repertoire size vary dramatically from species to species, and even between different populations of the same species (Peters et al. 2000). In some species, females prefer males that are able to sing more complex songs (reviewed in Nowicki & Searcy 2004). For example, Howard (1974), studying a population of Northern Mocking birds (*Mimus polyglottos*), found that females prefer males singing more complex songs. In male Great Reed-Warblers, male repertoire size is correlated with their harem size (Catchpole 1986) and with success in attracting extra-pair mates as well as offspring survival (Hasselquist et al. 1996). In Marsh Warblers (*Acrocephalus arundinaceus*), male song complexity is associated with faster mate acquisition and larger clutch sizes (Darlova et al. 2012). Similar results have been found in Song Sparrows (*Melospiza melodia*, Hiebert et al. 1989), Sedge Warblers *(Acrocephalus schoenobaenus*, Buchanan & Catchpole 1997; Catchpole 1980) and Red-Winged Blackbirds (*Agelaius phoeniceus*, Yasukawa et al. 1980).

Laboratory experiments have also shown female preference for more complex male songs in Song Sparrows (Searcy & Marler 1981; Searcy 1984), Great Tits (*Parus major*, Baker et al. 1986), Sedge Warblers (Catchpole et al. 1984) and Great Reed-Warblers (Catchpole et al. 1986).

At the same time, however, a variety of studies have failed to provide evidence for reproductive advantages in males with more complex songs (Byers & Kroodsma 2009; Soma & Garamszegi 2011). Some studies conducted in the field have failed to find effects of song diversity on female choice (Krebs et al. 1978); other studies have reported contradictory results for the same species studied in the field (Red-Winged Blackbird, Yasukawa et al. 1980; Great Reed Warbler, Catchpole 1986) and in the laboratory (Red-Winged Blackbird, Searcy 1988; Great Reed Warbler, Catchpole et al. 1986); while still other studies show female preference for simpler rather than more complex songs (Byers 2011). Byers and Kroodsma (2009) conducted a meta-analysis of existing studies and pointed out that the majority of studies that found positive correlations between song diversity and mate choice were experiments conducted in laboratories.

A disproportionately smaller number of field studies have reported effects of song diversity on proxy measurements of breeding success or female choice and this has been explained by some as a result of either species differences or differences in study design (Soma & Garamszegi 2011; Collins et al 2009). Byers & Kroodsma (2009) provided a different explanation related to insufficient statistical power, resulting from comparatively small sample sizes in field studies.

Here I investigate this proposal by Byers & Kroodsma (2009) by conducting a detailed investigation of sampling effects on the relationship between male song diversity and breeding success. This study extends the findings reported in Chapter 3 which documented significant relationships between male song diversity and breeding season for a population of House Wrens in Mendoza Province, Argentina.

4.2. METHODS

4.2.1. Study species

House Wrens are a small (10-12 g) territorial songbird species widely distributed across all the Americas. They have the widest distribution of any native songbird in the western hemisphere (Johnson 2014; Brewer & MacKay 2001). It spans from central Alberta, Canada, to Tierra del Fuego, Argentina (Brumfield & Capparella 1996). They show little morphological or plumage variation across this range (Brumfield & Capparella 1996), but have different mating systems and migratory behavior. House Wren populations breeding in North America are migratory and significantly polygynous (Johnson 2014), while they are sedentary and mainly monogamous where studied in Argentina (Llambías & Fernández 2009). House Wrens have been traditionally divided into three major continental groups: Northern House Wren in North America (*Troglodytes aedon*); Brown-throated Wren in Mexico (*Troglodytes brunneicollis*); and Southern House Wren in Central and South America (*Troglodytes musculus*, Brumfield & Capparella 1996). However, the most recent taxonomic assessment considers them all to be a single species, *Troglodytes aedon* (Remsen et al. 2018).

4.2.2. Study site

Research was conducted in Uspallata, Mendoza, Argentina (32.62°S, 69.36°W) on a Southern House Wren population (*Troglodytes aedon chilensis).* The research site is located in montane habitat (1850 m) in the foothills of the Andes Mountains where local daytime temperatures range from 18 to 35 °C with little rainfall (mean annual precipitation = 134 mm; Carretero 2000). The study population breeds in a White Poplars plantation (*Populus alba*) where the birds have been provided with wooden nest boxes for breeding (Llambías & Fernandéz 2009; Llambías 2012; Llambías et al. 2012). Birds have been captured and band-marked at this site since 2011. Individuals are marked with three coloured bands and a numbered aluminium band to facilitate identification. Marked birds have been re-sighted in the area during the austral winter, suggesting that House Wrens are year-round residents at this site.

The breeding season at this latitude is from late October to mid-February. The present study was focused on two successive breeding seasons (2012/13 and 2013/14). At the start of the breeding season, the local population was surveyed to identify suitable males for focused study, concentrating on males that had clearly settled on a territory. From this group, males not previously banded were captured using mist nests and banded as just described. Individual males were then monitored daily for the remainder of the breeding season.

4.2.3. Song recording

Songs were recorded from marked birds on their territories and were concentrated in the early morning hours (0500 to 1000 hours) when males were most active and singing. The identity of males on their territories was confirmed prior to beginning recording. Recordings were made with a digital recorder (Sound Devices 702) and a shotgun microphone (Sennheiser MKH 816). In most cases, recordings were made at distances of 5-10 m. Recordings were obtained from a total of 44 males across two breeding seasons (2012 and 2013), but for purposes of this study, the sample was limited to males from which we had recorded at least 700 songs. The resulting sample for use in this study thus comprised a total of 14,000 songs from 20 males (6 males recorded in 2012; 14 males recorded in 2013).

4.2.4. Song structure and organization

Male House Wren sing in long bouts. Individual songs have a duration of 1-3 s and are separated by silent intervals of 2-5 s. Most songs are comprised of an introductory section, composed of broadband or multiple harmonic notes, and a terminal section, comprised of tonal and frequency-modulated notes and syllables. In a previous study (Dos Santos et al. 2016), we catalogued the notes and syllable types present in the terminal section of songs for this population. The terminal section of songs is composed of 1-5 different syllable types (mean = 3). Some of the individual syllable types may be repeated multiple times. Song bouts were defined in a conservative and conventional manner, as a unique sequence of syllable types. Hence, this method ignores differences in the number of times a particular syllable type might be repeated. For additional details about the structure and organization of male song for this population, see Dos Santos et al (2016).

4.2.5. Sample size effects

To investigate the proposal of Byers & Kroodsma (2009) that small samples might often plague field studies ability to identify significant relationships between male song diversity and breeding success, I conducted a series of analyses to test the strength of significant relationships established in Chapter 3 between two metrics of female breeding success (*laying date* and *total number of eggs*) and two metrics of male song diversity (*CV number of syllable types per song* and *size of song repertoire*) using successively smaller samples of either song recordings, male subjects, or both.

I first selected from the complete sample of males those that bred successfully (and thus had relevant data for the key breeding metrics) and for which I had recorded at least 700 songs. I then limited the sample for each of these males to a standard total of 700 songs. The result was a set of 14,000 songs from 20 different males (6 males recorded in 2012 and 14 males recorded in 2013).

From the full sample of 700 songs for each of these males, I then generated three subsamples comprised of 100, 300, and 500 songs. Using each of these subsamples and the full sample, I calculated the two song metrics values (*CV number of syllable types per song* and *song repertoire size*) for each of the 20 different males and used them as predictors. I used two breeding metrics (*laying date* and *total number of eggs*) in separate models as dependent variables. I conducted two sets of bootstrap linear regression analyses (Efron 1979). The first set investigated the relationship between song diversity and breeding metrics using a constant sample of males and song metric values calculated from four different samples of songs successively smaller (namely 700, 500, 300 and 100 songs); while the second set of analyses used song metric values calculated from a constant sample of songs for each male but successively smaller samples of subjects. Following conventional methods for bootstrap regressions, subjects were drawn randomly from the original population with replacement. Iterations were repeated many times, each with a new random sample of subjects. Hence, some of the subjects may have appeared once, more than once, or did not appear at all in each iteration.

For all of the linear regression models, I used bootstrapping with 10,000 replicates to estimate a 95% confidence interval (CI), and the threshold sample size required for a statistical significant effect in individual regressions was evaluated using an alpha-level of 0.05. Statistics were performed in Stata 14 (StataCorp 2015) and all tests were performed two-tailed.

4.3. RESULTS

4.3.1. Sample size effects for laying date

From the complete set of 20 males, only 18 bred successfully to at least the egg stage and thus could provide data for analyses related to the date of first eggs (laying date). For females paired to this set of 18 males, laying date ranged from 16-Nov to 13-Jan in 2012 and from 10-Nov to 26-Dec in 2013.

4.3.1.1. Sample size: 700 songs

The threshold male sample size required for a significant negative relationship between *CV number of syllable types* and *laying date* was 14 subjects (n = 14, ß = -134.62, Bootstrap 95% CIs = -266.2, -3.0, *P* = 0.045; Figure 4.1; Table 4.1). There was no significant relationship between *song repertoire size* and *laying date* even when the analysis included the full sample of 18 males (n = 18, ß = -241.21, Bootstrap 95% CIs = -518.5, 36.1, *P* = 0.088; Table 4.2).

4.3.1.2. Sample size: 500 songs

Once again, the threshold male sample size required for a significant negative relationship between *CV number of syllable types* and *laying date* was 14 subjects (n =

14, ß = -132.60, Bootstrap 95% CIs = -256.9, -8.3, *P* = 0.037; Figure 4.1; Table 4.1) and there was no significant relationship between *song repertoire size* and *laying date* even using the full sample of 18 males (n = 18, ß = -187.00, Bootstrap 95% CIs = -454.4, 80.4, *P* = 0.171; Table 4.2).

4.3.1.3. Sample size: 300 songs

With only 300 songs used for each male in the analysis, there were no significant relationships for either *CV number of syllable types per song* (n = 18, ß = -135.49, Bootstrap 95% CIs = -348.6, 77.6, *P* = 0.213; Table 4.1) or *song repertoire size* (n = 18, ß = -49.03, Bootstrap 95% CIs = -177.3, 79.3, *P* = 0.454; Table 4.2). For both models, bootstrap confidence intervals (CIs) shrank progressively with each additional male introduced to the analysis, but failed to reach significance even with the full sample of 18 males included.

4.3.1.4. Sample size: 100 songs

Similarly, with only 100 songs used for each male, there were no significant relationships for either *CV number of syllable types per song* (n = 18, ß = 71.37, Bootstrap 95% CIs = -96.6, 239.3, *P* = 0.405; Table 4.1) or *song repertoire size* (n = 18, ß = -13.53, Bootstrap 95% CIs = -108.9, 81.9, *P* = 0.781; Table 4.2), even with all 18 males included in the sample.

4.3.2. Sample size effects: total number of eggs

These analyses used the full set of 20 males, including the two males who paired but whose females failed to produce eggs: the total number of eggs for these males was thus coded as ‘0’. Males paired to females whose nests reached the egg stage produced a mean of 6.5 (±2.66) eggs (range: 3 – 12) when summed across multiple breeding attempts within a season.

4.3.2.1. Sample size: 700 songs

The threshold male sample size required for a significant positive relationship between *CV number of syllable types* and *total number of eggs* was 13 (n = 13, ß = 27.79, Bootstrap 95% CIs = 0.4, 55.2, *P* = 0.047; Figure 4.2; Table 4.3), while that required for a significant positive relationship between *song repertoire size* and *total number of eggs* was 11 (n = 11, ß = 62.42, Bootstrap 95% CIs = 3.0, 121.8, *P* = 0.039; Figure 4.3; Table 4.4).

4.3.2.2. Sample size: 500 songs

The threshold male sample size required for a significant positive relationship between *CV number of syllable types* and *total number of eggs* was 9 (n = 9, ß = 29.04, Bootstrap 95% CIs = 0.5, 57.6, *P* = 0.046; Figure 4.2; Table 4.3) while that required for a significant positive relationship between *song repertoire size* and *total number of eggs* was 13 (n = 13, ß = 47.97, Bootstrap 95% CIs = 0.6, 95.4, *P* = 0.047; Figure 4.3; Table 4.4).

4.3.2.3. Sample size: 300 songs

With only 300 songs in the sample for each male, the threshold male sample size required for a significant positive relationship between *CV number of syllable types* and *total number of eggs* was 15 (n = 15, ß = 22.97, Bootstrap 95% CIs = 0.3, 45.7, *P* =

0.048; Figure 4.2; Table 4.3) but there was now no significant relationship for *song repertoire size* even using the full sample of 20 males (n = 20, ß = 28.42, Bootstrap 95% CIs = -9.1, 65.9, *P* = 0.137; Figure 4.3; Table 4.4).

4.3.2.4. Sample size: 100 songs

With only 100 songs used for each male, there were no significant relationships for either *CV number of syllable types per song* (n = 20, ß = 11.46, Bootstrap 95% CIs = -3.2, 26.1, *P* = 0.125; Figure 4.2; Table 4.3) or *song repertoire size* (n = 20, ß = 4.70, Bootstrap 95% CIs = -26.2, 35.6, *P* = 0.766; Figure 4.3; Table 4.4). For both models, bootstrap confidence intervals (CIs) shrank progressively with each additional male introduced to the analysis, but failed to reach significance even with the full sample of 20 males included.

4.4. DISCUSSION

Results of the previous chapter identified significant negative relationships between the date that a male's partner began laying eggs and both the size of the male's song repertoire and the CV of the number of syllable types in his songs, as well as a significant positive relationship between the latter variable and the total number of eggs produced by the male's partner across the breeding season. In this chapter, I tested the extent to which the identification of these effects hinges on the size of the samples used in statistical testing, as they relate either to the number of male subjects included or to the number of song recordings for each male.

Predictably, the strength of all of these relationships decreased as the sample sizes of either male subjects or song recordings was reduced. Ultimately, bootstrap linear regression models indicated that the threshold sample sizes required to obtain a significant effect for *CV number of syllable types per song* on female *laying date* was 14 male subjects and 500 song recordings for each; while the threshold sample sizes required to obtain a significant effect for *CV number of syllable types per song* on *total number of eggs* was 15 male subjects and 300 song recordings for each. No significant effects were obtained for *song repertoire size* on *laying date*, even using the full sample of male subjects (n=20) and the full sample of song recordings for each male (n=700), but a significant effect for *song repertoire size on* the *total number of eggs* was obtained with threshold sample sizes of 13 male subjects and 500 song recordings for each.

These findings are consistent in pointing to a minimum sample of 13-15 males with 300-500 song recordings for each in order to reveal significant relationships between core metrics of song diversity and standard measures of breeding success for the study population of southern House Wrens.

The potential implications of these findings are two-fold. First, they indicate that, consistent with the suggestion of Byers & Kroodsma (2009), small samples and resulting weak statistical power might be among the factors accounting for relatively few field studies reporting significant relationships between song diversity and breeding success. This possibility underscores the importance of sampling issues, particularly for field researchers. Field researchers are, of course, motivated to obtain adequate data samples in their studies; however, this priority competes with another, namely limitations in research time, labor and expense. Hence, the two priorities of adequate sample sizes and research expense inherently trade-off against each other and field researchers must be intentional in their efforts to strike an optimal balance between the two because low statistical power is the ultimate arbiter. Underpowered analyses inflate the probability of false negatives (Type-II error) – i.e., failing to detect effects that are in fact present which is a common problem in animal behavior research (Still 1992).

In the present work, I did not begin to detect significant relationships between song diversity and breeding metrics until the samples used in statistical tests included 300-500 songs per male and 13-15 male subjects. These are not exceptionally large samples, but nor are they trivially small. Although these particular sample sizes obviously cannot simply be generalized to studies of other species or questions, where larger samples may be required or smaller samples may prove adequate. Nevertheless, the general lesson is clear that special caution should be taken in planning appropriate sample sizes for field studies. This point may be particularly relevant when the research involves a species that is known to have relatively large repertoires of syllables or songs, or other singing patterns that are known to be relatively diverse or complex, to begin with.

The issue of adequate sampling is not exclusive to researchers, of course, but also characterizes the animals themselves. In this instance, females prospecting for a suitable male mate face a similar trade-off. Limitations of time, energy and opportunity mean that a female's ability to thoroughly evaluate the attributes of any given male inherently trade-offs against the ability to do so for each of many other males. Relatively little is yet known about how exactly male song complexity and vocal performance are evaluated by female birds and what sort of decision heuristics females adopt in these circumstances. However, a substantial number of theoretical studies on female sampling behavior and optimal mate choice decisions have been developed since a seminal publication by Janetos (1980). These models investigate the potential mate choice strategies females might employ when prospecting for mates (Janetos 1980; Parker 1983; Wittenberger

1983; Real 1990).

Benton and Evans (1998), using simulation models, reported that the correlation between a male trait and variables capturing proxy measurements of reproductive success or female choice dramatically increases as a function of the number of males sampled in a best-of-*n* strategy. The correlation rises as each additional male is evaluated by the female, reaching an asymptote above 7-10 males.

Some empirical studies have also been conducted (reviewed in Gibson & Langen 1996), but only a few have focused on songbirds. In a radio tracking study, Bensch & Hasselquist (1992) reported that unpaired Great Reed Warbler females visited an average of six males within a period of one to three days before pairing. Roth et al (2009), in a study investigating diel singing patterns in Nightingales (*Luscinia megarhynchos*), tracked radio-tagged females as they actively prospected for male mates. Although this was not the focus of their study, they reported that during the course of a night, a single female covered 5.8 km, visiting the territories of at least six different males and spent 5-35 minutes in each territory.

To my knowledge, no empirical research has yet combined female radio-tracking techniques with male song complexity analysis and breeding success data. However, such data could not only provide insight into how female birds actually sample and evaluate male song when prospecting for a mate but, in the process, could further illuminate the specific pressures most directly involved in shaping the evolution of song diversity and complexity.

In sum, the extent to which the identification of significant relationships between metrics of male song complexity and breeding success hinges on the size of the samples of both male subjects and song recordings, with non-trivial samples of each required to avoid inflated Type II errors. Such sampling issues are, of course, a well-known challenge for researchers of all kinds, including those focused on birdsong, though they are seldom explicitly tested as I have tried to do here. The birds, and other animals, we study confront exactly analogous challenges, a point that is, once again, widely appreciated in theory but that has been subject to relatively little empirical study despite promising significant insights into the evolutionary questions to which we crave answers.

Table 4.1. Bootstrap linear regression models (based on 10,000 bootstrap samples) of laying date on the coefficient of variation on the number of syllable types per song.

	700 Songs				500 Songs				300 Songs				100 Songs			
Number of subjects	Model B1	Bootstrap 95% CIs		P>|z|	Model B1	Bootstrap 95% CIs		P>|z|	Model B1	Bootstrap 95% CIs		P>|z|	Model B1	Bootstrap 95% CIs		P>|z|
—	-134.62	Lower	Upper	—	-132.60	Lower	Upper	—	-49.03	Lower	Upper	—	-13.53	Lower	Upper	—
18	—	-246.7	-22.5	**0.019**	—	-230.7	-34.5	**0.008**	—	-177.3	79.3	0.454	—	-108.9	81.9	0.781
17	—	-249.8	-19.5	**0.022**	—	-237.2	-28.0	**0.013**	—	-183.0	85.0	0.473	—	-112.4	85.4	0.789
16	—	-254.1	-15.1	**0.027**	—	-243.2	-22.0	**0.019**	—	-187.7	89.6	0.488	—	-115.5	88.4	0.795
15	—	-259.6	-9.6	**0.035**	—	-249.2	-16.0	**0.026**	—	-192.4	94.3	0.503	—	-119.5	92.4	0.802
14	—	-266.2	-3.0	**0.045**	—	-256.9	-8.3	**0.037**	—	-199.5	101.4	0.523	—	-123.5	96.4	0.809
13	—	-273.2	4.0	0.057	—	-266.4	1.1	0.052	—	-207.3	109.2	0.544	—	-129.1	102.0	0.818
12	—	-281.0	11.8	0.071	—	-278.5	13.3	0.075	—	-216.5	118.4	0.566	—	-135.1	108.1	0.827
11	—	-292.3	23.1	0.094	—	-293.8	28.6	0.107	—	-227.3	129.3	0.59	—	-141.8	114.8	0.836
10	—	-305.4	36.1	0.122	—	-316.1	50.9	0.157	—	-240.1	142.0	0.615	—	-149.7	122.6	0.846

Table 4.2. Bootstrap linear regression models (based on 10,000 bootstrap samples) of laying date on song repertoire size.

	700 Songs				500 Songs				300 Songs				100 Songs			
Number of subjects	Model B1	Bootstrap 95% CIs		P>|z|	Model B1	Bootstrap 95% CIs		P>|z|	Model B1	Bootstrap 95% CIs		P>|z|	Model B1	Bootstrap 95% CIs		P>|z|
—	-241.21	Lower	Upper	—	-187.00	Lower	Upper	—	-135.49	Lower	Upper	—	71.37	Lower	Upper	—
18	—	-518.5	36.1	0.088	—	-454.4	80.4	0.171	—	-348.6	77.6	0.213	—	-96.6	239.3	0.405
17	—	-532.5	50.0	0.105	—	-466.9	92.9	0.19	—	-362.0	91.0	0.241	—	-105.8	248.6	0.43
16	—	-545.7	63.3	0.12	—	-478.7	104.7	0.209	—	-373.3	102.4	0.264	—	-113.9	256.6	0.45
15	—	-559.8	77.3	0.138	—	-492.4	118.4	0.23	—	-385.0	114.0	0.287	—	-123.4	266.2	0.473
14	—	-576.2	93.8	0.158	—	-506.4	132.4	0.251	—	-397.4	126.4	0.311	—	-134.8	277.5	0.497
13	—	-612.6	130.2	0.203	—	-522.7	148.7	0.275	—	-411.3	140.3	0.336	—	-147.0	289.7	0.522
12	—	-642.9	160.4	0.239	—	-541.3	167.3	0.301	—	-430.1	159.1	0.367	—	-164.8	307.5	0.554
11	—	-666.0	183.6	0.266	—	-564.8	190.8	0.332	—	-454.0	183.1	0.404	—	-182.0	324.8	0.581
10	—	-697.7	215.3	0.3	—	-593.3	219.2	0.367	—	-483.7	212.7	0.446	—	-203.0	345.7	0.61

Table 4.3. Bootstrap linear regression models (based on 10,000 bootstrap samples) of total number of eggs on the coefficient of variation on the number of syllable types per song.

	700 Songs				**500 Songs**				**300 Songs**				**100 Songs**			
Number of subjects	**Model B1**	**Bootstrap 95% CIs**		**P>|z|**	**Model B1**	**Bootstrap 95% CIs**		**P>|z|**	**Model B1**	**Bootstrap 95% CIs**		**P>|z|**	**Model B1**	**Bootstrap 95% CIs**		**P>|z|**
—	27.79	Lower	Upper	—	29.04	Lower	Upper	—	22.97	Lower	Upper	—	11.46	Lower	Upper	—
20	—	7.0	48.6	**0.009**	—	14.6	43.5	**0.000**	—	3.7	42.2	**0.019**	—	-3.2	26.1	0.125
19	—	6.5	49.1	**0.011**	—	14.4	43.7	**0.000**	—	3.1	42.9	**0.024**	—	-3.7	26.6	0.139
18	—	5.8	49.8	**0.013**	—	13.7	44.4	**0.000**	—	2.6	43.4	**0.027**	—	-4.2	27.1	0.152
17	—	4.9	50.6	**0.017**	—	12.8	45.3	**0.000**	—	1.8	44.2	**0.034**	—	-4.9	27.8	0.169
16	—	4.0	51.6	**0.022**	—	12.0	46.1	**0.001**	—	1.1	44.9	**0.04**	—	-5.4	28.3	0.183
15	—	3.0	52.6	**0.028**	—	11.1	47.0	**0.002**	—	0.3	45.7	**0.048**	—	-6.1	29.0	0.201
14	—	1.8	53.8	**0.036**	—	9.9	48.2	**0.003**	—	-0.8	46.7	0.058	—	-6.8	29.7	0.219
13	—	0.4	55.2	**0.047**	—	8.6	49.5	**0.005**	—	-2.0	47.9	0.071	—	-7.7	30.6	0.241
12	—	-0.9	56.5	0.058	—	7.1	51.0	**0.009**	—	-3.0	49.0	0.083	—	-8.8	31.7	0.267
11	—	-2.6	58.2	0.073	—	5.3	52.8	**0.017**	—	-4.6	50.6	0.103	—	-10.0	32.9	0.295
10	—	-4.7	60.3	0.094	—	3.1	55.0	**0.028**	—	-6.5	52.4	0.126	—	-11.4	34.3	0.326
9	—	-7.1	62.6	0.118	—	0.5	57.6	**0.046**	—	-8.4	54.4	0.152	—	-13.1	36.0	0.36

Table 4.4. Bootstrap linear regression models (based on 10,000 bootstrap samples) of total number of eggs on song repertoire size.

	700 Songs				**500 Songs**				**300 Songs**				**100 Songs**			
Number of subjects	**Model B1**	**Bootstrap 95% CIs**		**P>|z|**	**Model B1**	**Bootstrap 95% CIs**		**P>|z|**	**Model B1**	**Bootstrap 95% CIs**		**P>|z|**	**Model B1**	**Bootstrap 95% CIs**		**P>|z|**
—	62.42	Lower	Upper	—	47.97	Lower	Upper	—	28.42	Lower	Upper	—	4.70	Lower	Upper	—
20	—	23.8	101.0	**0.002**	—	12.6	83.3	**0.008**	—	-9.1	65.9	0.137	—	-26.2	35.6	0.766
19	—	22.5	102.3	**0.002**	—	11.3	84.7	**0.01**	—	-11.1	67.9	0.158	—	-27.3	36.7	0.774
18	—	21.0	103.8	**0.003**	—	10.0	85.9	**0.013**	—	-12.5	69.3	0.173	—	-28.4	37.8	0.781
17	—	19.3	105.5	**0.005**	—	8.5	87.5	**0.017**	—	-14.0	70.8	0.189	—	-29.7	39.1	0.789
16	—	17.5	107.3	**0.006**	—	6.8	89.1	**0.022**	—	-15.9	72.8	0.209	—	-31.1	40.5	0.797
15	—	15.5	109.4	**0.009**	—	5.0	91.0	**0.029**	—	-17.8	74.7	0.228	—	-32.7	42.1	0.806
14	—	13.1	111.7	**0.013**	—	3.0	92.9	**0.036**	—	-20.0	76.9	0.25	—	-34.8	44.2	0.816
13	—	10.5	114.4	**0.018**	—	0.6	95.4	**0.047**	—	-22.2	79.0	0.271	—	-36.9	46.3	0.825
12	—	6.6	118.2	**0.028**	—	-2.5	98.4	0.062	—	-25.1	81.9	0.298	—	-39.3	48.7	0.834
11	—	3.0	121.8	**0.039**	—	-6.9	102.8	0.087	—	-28.7	85.6	0.33	—	-42.6	52.0	0.846
10	—	-2.1	126.9	0.058	—	-11.7	107.6	0.115	—	-33.1	89.9	0.365	—	-46.3	55.7	0.857
9	—	-8.0	132.8	0.082	—	-18.1	114.1	0.155	—	-37.9	94.7	0.401	—	-51.3	60.7	0.869

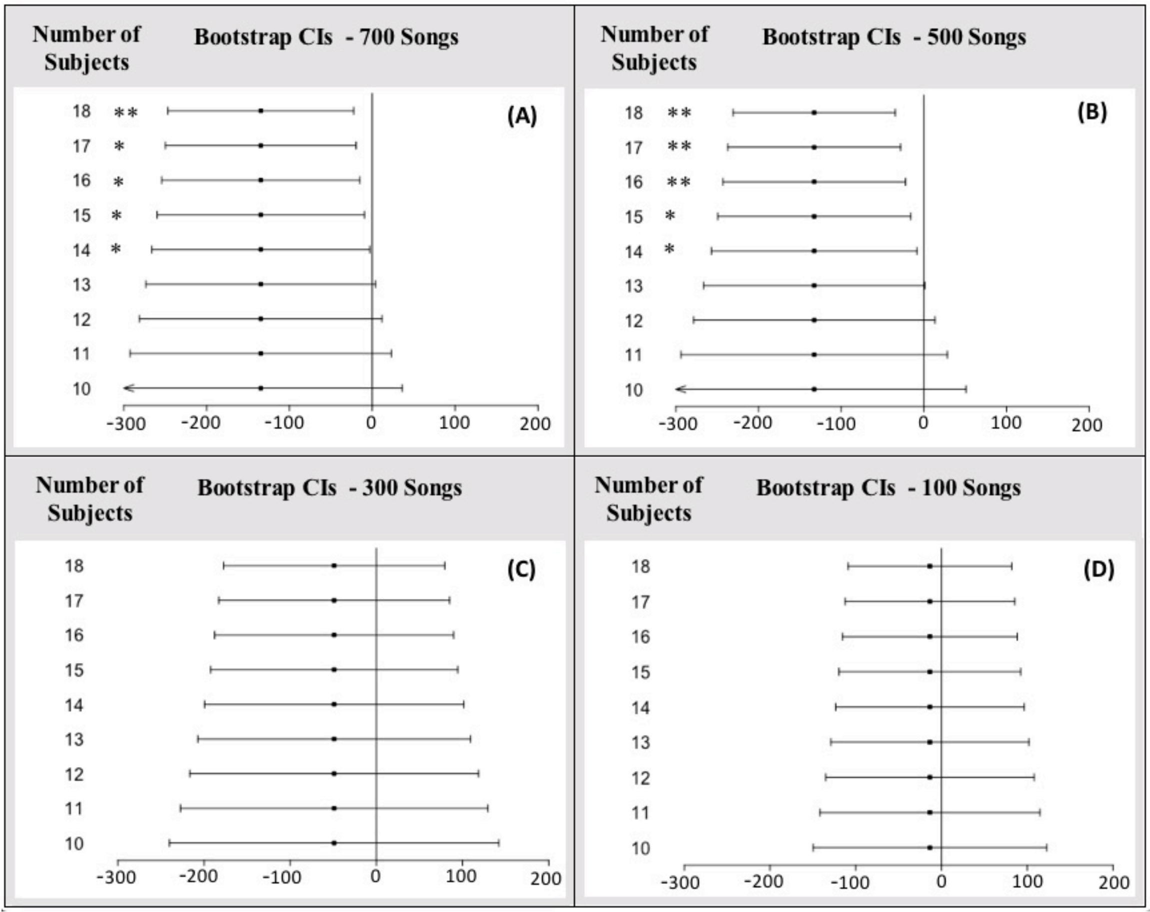

Figure 4.1. Bootstrap confidence intervals for linear regression models of laying date on the coefficient of variation on the number of syllable types per song. Confidence intervals vary as a function of number of subjects and sample sizes of 700 songs (A), 500 songs (B), 300 songs (C) and 100 songs (D). Horizontal lines that do not cross zero (vertical line) represent statistically significant results (* = P ≤ 0.05, ** = P ≤ 0.01 and *** = P ≤ 0.001).

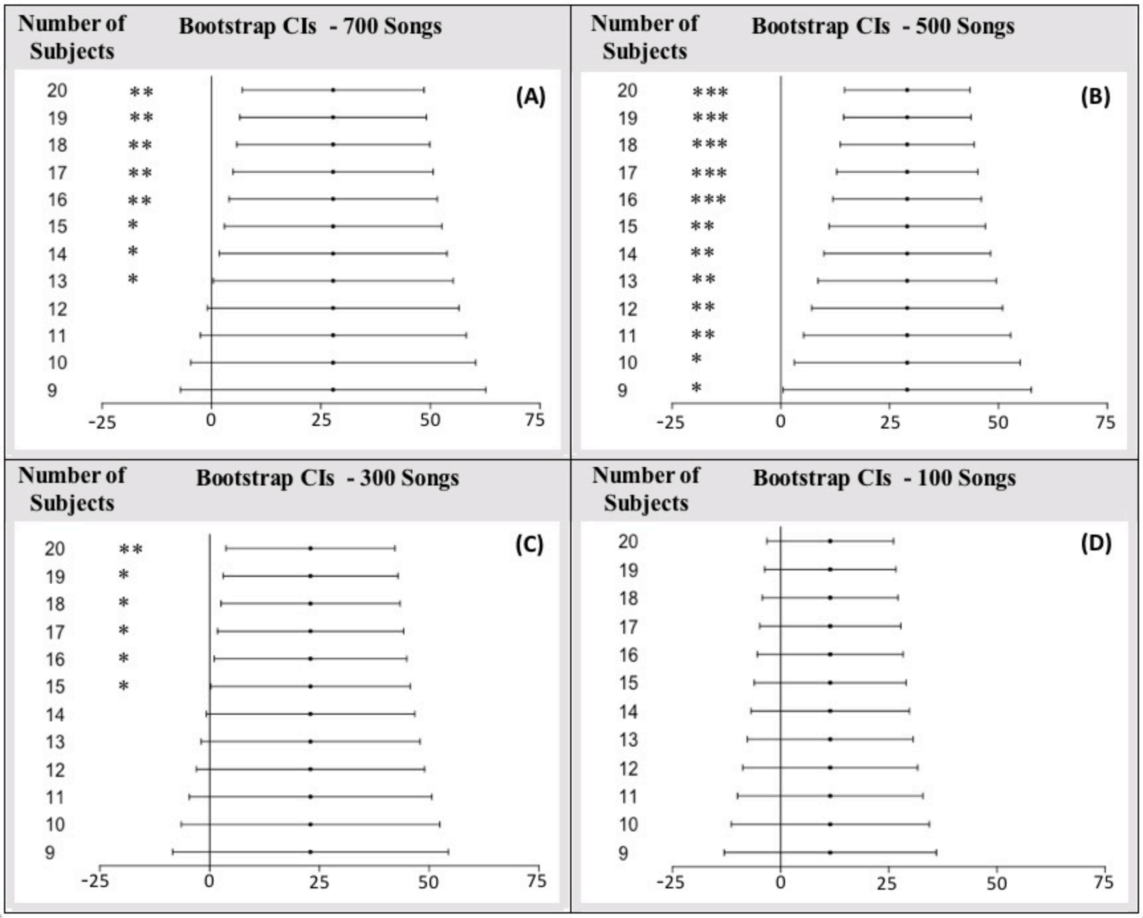

Figure 4.2. Bootstrap confidence intervals for linear regression models of total number of eggs on the coefficient of variation on the number of syllable types per song. Confidence intervals vary as a function of number of subjects and sample sizes of 700 songs (A), 500 songs (B), 300 songs (C) and 100 songs (D). Horizontal lines that do not cross zero (vertical line) represent statistically significant results (* = P ≤ 0.05, ** = P ≤ 0.01 and *** = P ≤ 0.001).

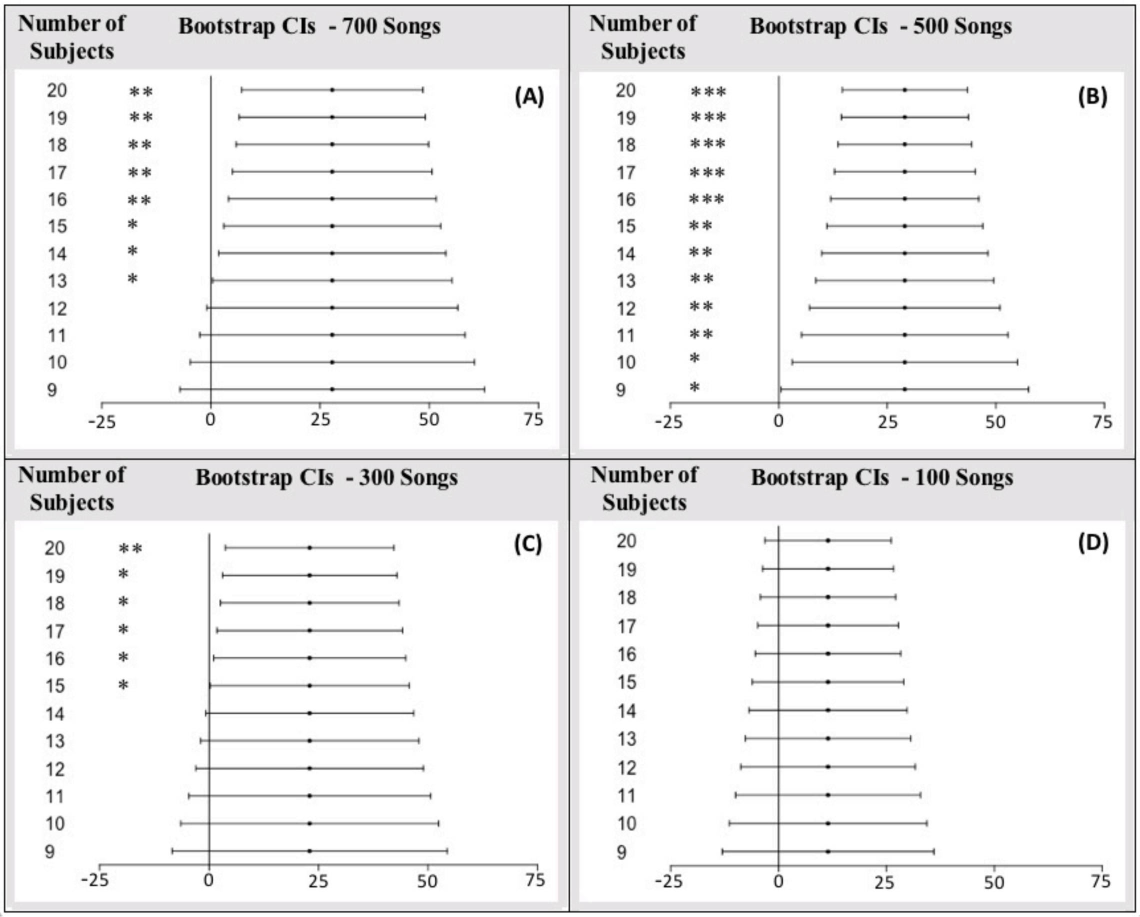

Figure 4.3. Bootstrap confidence intervals for linear regression models of total number of eggs on song repertoire size. Confidence intervals vary as a function of number of subjects and sample sizes of 700 songs (A), 500 songs (B), 300 songs (C) and 100 songs (D). Horizontal lines that do not cross zero (vertical line) represent statistically significant results (* = P ≤ 0.05, ** = P ≤ 0.01 and *** = P ≤ 0.001).

CHAPTER 5

GENERAL DISCUSSION

5.1. CHAPTER OVERVIEW

The initial goals of this thesis were first to provide a detailed description of the structure and organization of male song for a sedentary population of House Wrens, *Troglodytes aedon chilensis*, breeding in the temperate zone of South America (Chapter 2). This was a first important step to providing some preliminary comparisons to the song patterns reported for a migratory population of House Wren breeding in the temperate zone of North America, in Alberta, Canada (*T.a. parkmannii*: Rendall and Kaluthota 2013) to begin to address the role that variable migratory strategies, mating systems and life-histories might play in shaping variable song diversity within a single species, and, by extension, ultimately between species. A second, related goal was to then test the relationship between a variety of metrics of song diversity and breeding success in the study population of House Wrens in South America (Chapter 3), and to subsequently examine the extent to which observed relationships are affected by variable sampling effort (Chapter 4), a vexatious problem in field studies of birdsong.

In this chapter, I provide a general discussion of data patterns and ideas presented in these previous chapters. I summarize the main findings from a broader and integrative perspective, emphasizing some points not discussed in detail previously. In the process, I attempt to highlight the main contributions of the thesis, as well as its limitations, and outline some potential topics for future studies.

5.2. GENERAL SINGING PATTERNS

5.2.1. Singing output rate

The description of singing patterns, song structure and organization in Chapter 2 was based on field study of a population of House Wrens breeding in Uspallata, Mendoza, Argentina. Males in that population start singing early in the day and continue throughout the morning. The singing output is higher, and songs are louder, as long as males remain unpaired. Male singing rate drops dramatically after their mates' finish laying eggs. However, males can resume singing at higher rates if they become single again, as a consequence of divorce or death of their mate. This change in the singing output pattern is readily noticeable in the field. For example, during a song recording session in Uspallata, I suddenly heard what seemed to be a single male singing from a territory already occupied by male UP410 and an associated female who was already incubating eggs. On closer inspection, I noticed that it was actually male UP410 singing like an unpaired male. A short time later, I determined that his female mate had been predated.

It is also the case that the rate and amplitude of male singing can increase dramatically again if the male is courting additional females, but this pattern seems to be more prominent in northern House Wrens in accordance with the higher levels of social polygyny reported for North American populations.

5.2.2. Basic song structure and organization

Southern House Wrens sing in long bouts, in which sequences of consecutive songs are separated by silent intervals. Most songs are structured as two main parts; an introductory section comprised of relatively soft but harsh, broadband syllables; and a terminal section comprised of louder, tonal and frequency-modulated syllables. Males use a finite repertoire of syllable types, that are shared by all males in the population, but recombine them in different arrangements, thus producing a very large number of different song types that are mostly not shared. Different song types are generally produced with eventual variety, i.e. the same song type is repeated several types before switching to a new type. However, males can also sing with much greater immediate variety as illustrated in Figure 2.8 of Chapter 2. Nevertheless, the general pattern of singing with ‘global diversity but short-term monotony’ appears to be common to both southern and northern populations of House Wrens (cf. Rendall & Kaluthota 2013).

5.2.3. Are there temporal variations in song diversity across the breeding cycle?

As just noted, the rate of male singing can vary at different stages of the breeding cycle. A natural question, then, concerns the extent to which song diversity also varies across the breeding cycle. This might be a natural opportunity to test additional potential functions of song in House Wren beyond those related to attracting female mates. In some species, male song has been identified as functioning in the physiological stimulation of females throughout the reproductive cycle (Kroodsma & Byers, 1991). For example, increases in the singing rate of male Northern Mockingbirds (*Mimus polyglottos*) coincide with the initiation of nesting attempts (Logan, 1983). A related question is whether song diversity increases during the female’s fertile period (i.e., during egg laying), an idea known as ‘the fertility announcement hypothesis’. Forstmeier & Balsby (2002) found support for this hypothesis. They reported that male Dusky Warblers (*Phylloscopus fuscatus*) use more variable song types when their own females and also neighboring females are laying eggs.

5.2.3.1. Challenges to studying temporal variation in southern House Wren songs

One of the challenges to studying temporal variations in song diversity across the breeding cycle in House Wrens is the dramatic reduction in singing following egg laying because this severely constrains the collection of song samples. As a consequence, it hinders some analytical approaches that require testing variables that capture song diversity at a larger scale, such as song repertoire size or long-term variability in the number of different syllable types in songs. This is especially problematic for southern House Wrens, given that significant correlations between measures of song diversity and breeding required non-trivial samples of male subjects and song recordings (as reported in Chapter 4). Of course, tests for breeding stage variation in song diversity may not require equally large samples, but the data presented in Chapter 4 emphasize the need for caution in examining functional aspects of song diversity with small sample sizes. One way to overcome this limitation would be to develop new song measures that could capture song diversity on a shorter time scale, such as within song, and thus allow testing with smaller samples of song.

5.2.4. Age-related variation in song diversity

Another important dimension of temporal variation in song diversity is that which might be related to age. Previous studies have indicated that, in some species, age is related to variation in male song diversity. For example, in Common Nightingales, *Luscinia megarhynchos*, older males have larger song repertoire sizes than first year males (Kiefer et al. 2006). The population of southern House Wren males studied here generates songs from a common repertoire of 28 syllable-types. These syllable types are shared by all males in the population. What varies among males are the song types they create by recombining syllables into different song types. It is possible, then, that some of the variation observed in song repertoire size among males in the study population was related to age differences, and that older males are able to produce a greater number of novel song types from the common repertoire of syllable types shared by all males. This possibility remains untested but could be addressed relatively easily with year-over-year recordings of marked males, particularly if those males are of known age to begin with.

5.3. COMPARISONS WITH NORTHERN HOUSE WREN SONGS

5.3.1. Why are there so many similarities in the song patterns of northern and southern House Wrens?

Chapter 2 provides comparisons of basic song patterns found here for the study population of southern House Wrens breeding in Mendoza Province, Argentina, with those reported recently for a population of northern House Wrens breeding in Alberta, Canada (Rendall & Kaluthota 2013). Despite considerable differences in migratory strategy, social and genetic mating systems, and some other basic life-history dimensions reviewed previously, the general structure of and organization of songs is remarkably similar. This includes similarities in how individuals songs are constituted in two clearly distinct sections (an introduction and a terminal section); how they are constructed from recombinations of a similar number of syllable types that are also shared by all males in the population; how a common and finite syllable repertoire is nevertheless used flexibly to create very large repertoires of song types most of which are not shared by different males; and how song types tend to be repeated many times before switching and then generally change gradually over the course of protracted song bouts in a style referred to as ‘eventual variety’.

Collectively, these many obvious similarities pose an intriguing question: why do males from such disjunct populations characterized by differences in core features of behavior thought to influence song complexity (i.e., migratory strategy, mating system, and life-history patterns) nevertheless sing in such similar ways? This outcome is quite unexpected given traditional theoretical approaches to song diversity (Catchpole & Slater 2008; Collins et al. 2009). Northern populations of House Wrens are migratory and show high levels of both social and genetic polygyny and large clutch sizes; while southern populations are year-round residents, exhibit social monogamy and low levels of genetic polygyny, and have small clutch sizes. In theory, these different traits should lead to and or reflect differing levels of sexual selection pressures, and northern House Wrens would be predicted to show inflated levels of song complexity as a consequence.

One obvious answer to this question would be that there remain to be discovered important differences in song complexity between the two populations that were simply not captured in work to date. Certainly, the studies conducted thus far have investigated only a handful of basic metrics of song complexity. The metrics studied were ones commonly studied in birdsong research, and therefore naturally motivated; however, there may be many additional metrics of song complexity that could be added to the analyses and these may reveal differences consistent with current theoretical predictions.

It is, of course, also possible that the surprising similarities observed for male song in both northern and southern House Wrens should prompt us to consider alternative explanations or at least some variation on current theory.

5.3.2. Latitudinal variation in House Wren song diversity

The unexpected parallels observed in basic metrics of song complexity for northern and southern House Wrens prompted an additional study to investigate broader patterns of latitudinal variation in this exceptionally widely distributed species (Kaluthota et al. 2016). That study investigated variation in a number of very basic elements of song in male House Wrens from 298 locations across the Americas spanning 100º of latitude (52º N –55º S). The expressed goal was to examine broadscale geographic variation in song complexity potentially attributable to latitudinal variation in sexual selection pressures between sedentary populations inhabiting the tropics and migratory populations breeding in the temperate zone (Collins et al. 2009).

5.3.2.1. Thesis results fit into a broader latitudinal pattern

The results of this broader study revealed a wholly novel latitudinal gradient of song variation in which a number of basic elements of song performance and complexity, such as the number of elements per song and the rate of element production, decreased towards the equator and increased towards the poles in both hemispheres. Hence, not only were song patterns more complex in the temperate zone of the northern hemisphere compared to the tropics, as previously hypothesized (Collins et al. 2009; Catchpole & Slater 2008), but levels of song complexity in the temperate zone of the southern hemisphere converged on that observed in the temperate zone of the northern hemisphere. The novel finding suggests that pressures of sexual selection might be convergent in some respects among populations at latitudinal extremes in both hemispheres. This in turn prompts further questions about the mechanisms driving increased song diversity in southern populations of House Wrens, such as the one studied in this thesis, considering it is non-migratory and predominately social monogamous with lower levels of extra-pair paternity (La Barbera et al. 2010; Llambías et al. 2015).

5.3.3. What drives song diversity in southern populations?

One possibility is that, despite variation in levels of social and genetic polygyny between northern and southern populations of House Wrens, the time window available for breeding is similarly short at the latitudinal extremes in both hemispheres generating similarly intense pressure on song to mediate mate choice and male-competition. Hence, this pressure is, in fact, consistent in the two populations despite their other differences in mating system and life-history. Of course, other, non-exclusive explanations are also possible.

5.3.3.1. Darwin-Fisher hypothesis of sexual selection in monogamous birds

For example, it is possible that males who sing with greater complexity pair with older, more experienced females, or females who are otherwise more fecund. This idea was first presented by Darwin (Darwin 1871) and further developed by Fisher (1958) as a mechanism specifically to explain elaborate male traits in monogamous populations, where such trait elaboration otherwise would not be predicted based on variation in male access to female mates (i.e., reproductive skew). The Darwin-Fisher account proposes that females pairing earlier in the season have higher body condition, and thus are more fecund, than females that pair later. Hence, early-pairing females would have the advantage of first assessing and choosing among available male mates those that exhibit better quality through increased song complexity (Kirkpatrick 1990).

5.3.3.2. The Differential Allocation hypothesis

A third, non-exclusive hypothesis, is the differential allocation hypothesis (Burley 1986, 1988). This hypothesis proposes that females use male song complexity (vis a vis male quality) as a guide to adjust their own levels of post-pairing investment in reproduction, investing greater effort in offspring care and development when paired with males that exhibit greater song diversity.

Either of the latter two possibilities could be relevant for southern populations of House Wrens, and, for that matter could well apply also to northern populations. However, they might help to explain why similar levels of song diversity would be manifest in disjunct northern and southern populations that seem otherwise quite distinct on core traits such as migratory strategy and levels of social and genetic polygyny.

5.4. FUNCTIONAL ASPECTS OF SOUTHERN HOUSE WREN SONGS

5.4.1. Song diversity and breeding success

To further address the potential mechanisms influencing song diversity in southern populations, in particular, Chapter 3 examined the relationship between male song diversity and breeding success. As reviewed at the outset of this thesis, the connection between male song diversity and breeding success has been considered for a very long time (e.g., Darwin 1871); however, empirical evidence for a clear connection between increased song diversity, including large song repertoires in particular, and improved breeding success has been surprisingly mixed, especially for field studies (e.g., Byers & Kroodsma 2009; Soma & Garamszegi 2011). Results reported in Chapter 3 indicate that, for the focal population of southern House Wrens, there is a clear connection between the two: males with larger song repertoires (or greater complexity in other aspects of song) paired with females that bred earlier in the breeding season and produced a larger number of eggs over the course of the breeding season.

5.4.1.2. Focus on an austral population

To my knowledge, these represent the first detailed findings on the relationship between song diversity and breeding success in southern House Wrens. This outcome was possible only with the considerable initial investment in providing a comprehensive description of the patterns of song structure, organization and delivery as reported in Chapter 2, which highlights the importance to functional, hypothesis-driven research of careful initial naturalistic and descriptive research.

The findings in Chapter 3 are important also because most of our current understanding of bird song and behavior derives from research on migratory species and populations in the north temperate zone. Neotropical species and populations, by comparison, have not received similar attention (Kroodsma et al. 1996; Podos 2013), although the value of documenting songbird behavior, biology and mating systems in the southern hemisphere has been emphasized recently (Macedo & Machado 2013). This thesis therefore represents a useful step in this direction.

5.4.1.3. Parallels with other studies on song diversity and breeding success

The particular findings with respect to male song diversity and breeding success reported here for southern House Wrens accord well with results of previous field studies of some other species, including Marsh Warblers (*Acrocephalus palustris*, Bell et al. 2004) and Eastern Song Sparrows, *Melospiza melodia melodia* (Potvin et al. 2015).

However, some other field studies have failed to find significant correlations between standard metrics of male song diversity and breeding success, including Great Tits, *Parus major* (Krebs et al. 1978), Red-Winged Blackbird, *Agelaius phoeniceus* (Yasukawa et al. 1980), Snow Buntings, *Plectrophenax nivalis*, (Hofstad et al. 2002) and Blue Tits, *Cyanistes caeruleus* (Poesel et al. 2001).

These differences might in part simply reflect species differences. It should not be assumed that the pressures of sexual selection necessarily play themselves out the same way in all species and thus produce the same signalling outcomes (as noted in previous sections even for House Wrens). At the same time, differences in research design or sampling issues could also contribute to the contradictory results between studies and species.

5.4.2. Sampling effects

This issue of sampling effects was taken up directly in Chapter 4 where relationships established in Chapter 3 between male song diversity and breeding success were re-evaluated using successively smaller samples of male subjects or song recordings, or both. For the House Wrens studied here, significant relationships between male song diversity and breeding success metrics emerged only when the sample included a minimum of 14 subjects and a total of 7,000 songs (500 songs per male), which are not trivial samples. While the particular sample sizes identified here obviously cannot simply be generalized to other studies or species, the general outcomes underscore the importance of sampling issues when designing research to avoid insufficient statistical power that might lead to failing to detect effects that really are present, particularly for songbird species that have large syllable or song repertoires to begin with.

5.4.2.1. Female mate sampling

At the same time, these same sorts of sampling issues pertain to female songbirds searching for and selecting among prospective mates. As pointed out in theoretical studies on female sampling behavior and optimal mate choice decisions (Janetos 1980; Parker 1983; Wittenberger 1983; Real 1990), unpaired females must make similar choices concerning how many different males to sample and how much time to spend assessing their songs.

5.4.2.2. Female radio tracking studies

A potentially major step forward in this respect would be increased study of female sampling behavior using radio-tracking techniques (Roth et al. 2009). This line of research might offer significant new insights into the extent to which inter-sexual selection (female mate choice) in particular has influenced the evolution of elaborate male song patterns.

5.3. FEMALE BENEFITS

If female choice, in particular, has had a significant role in selecting for male song diversity in House Wrens, it is natural to ask whether females also benefit in some way from their choice of males with more elaborated songs. Song can, of course, also function in intrasexual selection, as a signal relevant in male-male competition for access either to territories or to potential female mates. However, the focus in this thesis has been on the potential function of song in attracting social mates, in part because naturalistic patterns of male behavior and song delivery throughout the breeding cycle strong indicate that this is a core function of song in House Wrens (not to the exclusion of an additional possible role in mediating male-male competition but certainly not secondary to that function either).

5.3.1. Indirect benefits

Females might benefit from choosing males with higher song diversity through the indirect benefits such males provide in their genetic contribution to the health and vigor of her offspring. Although such benefits could not be examined here, previous studies have indicated that song diversity can be associated with male quality, including better immunity and body condition in Song Sparrows, *Melospiza melodia* (Reid et al. 2005; Pfaff et al. 2007) and in Nightingales, *Luscinia megarhynchos* (Kipper et al. 2006). Song diversity might also reveal aspects of male phenotype affected by early environmental factors. For example, the effects of early developmental stress have been found to be correlated with song diversity (Nowicki et al. 2002) and experimental studies have shown that early environmental conditions, such as food availability, affect physiological and behavioral traits ultimately connected to adult song diversity (Nowicki et al. 1998; Spencer & MacDougall-Shackleton 2011).

5.3.2. Direct benefits

Females might also obtain direct benefits from males exhibiting increased song diversity if, for example, song is a reliable index of the quality of a male’s territory (Andersson 1994), or a reliable predictor of his subsequent investment in offspring. Both factors could be relevant to female House Wrens because males are centrally involved in provisioning chicks and because females (and males) rely on a limited supply of natural cavities for nesting, often including cavities in trees previously excavated by other species. Indeed, a recent study by Kaluthota and Rendall (2017) reported a number of significant nesting preferences in northern House Wrens, including a preference for trees with few cavities, and small cavity entrances oriented in particular cardinal directions and relatively low to the ground, all of which point to nesting decisions that involve trying to minimize nest competition and also nest failure from predation and unfavorable environmental factors. To date, the possible relationships between male song diversity and either territory quality or nestling provisioning have not been explicitly tested but are the focus of imminent future work.

5.4. GENERAL SUMMARY

In summary, this thesis has provided a detailed characterization of the general patterns of song structure, organization and delivery in a South American population of House Wrens (*Troglodytes aedon chilensis*), and has provided some additional basic comparisons to song patterns reported for House Wrens in North America (*Troglodytes aedon parkmannii*). For the first time, it has documented that increased levels of male song diversity are associated with increased measures of breeding success in House Wrens. These outcomes, obtained under naturalistic field conditions, have contributed to an important and often debated relationship between the complexity of male singing and core metrics of breeding success. The work has also helped to broaden the literature on the evolution of bird song by adding useful data for a South America population of songbird. In addition, the thesis has provided an analysis of how the relationships between male song diversity and breeding success are affected by sampling issues, a vexatious matter both for birdsong researchers trying to uncover such relationships and likely also the birds themselves trying to make ‘wise’ behavioral decisions. Overall, the results reported have strongly implicated a role for sexual selection in the elaborated song diversity manifest in male House Wrens. Further research is needed, however, to better delineate the potential roles of inter- versus intrasexual selection in this process, and to clarify a number of other important related issues not studied here.

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