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**THE VOCAL BEHAVIOUR AND REPRODUCTIVE STRATEGIES OF A
NEOTROPICAL DUETTING WREN**

by

SARAH BRYNN DOUGLAS

A Thesis
Submitted to the Faculty of Graduate Studies
through Biological Sciences
in Partial Fulfillment of the Requirements for
the Degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada

2011

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Declaration of Co-Authorship

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

I am sole author of Chapter 1 and the principal author of the two data chapters, Chapter 2 and Chapter 3, which are co-authored by Dr. Mennill and Dr. Heath. I acknowledge the valuable input of my supervisor Dr. Daniel Mennill, who funded this research, aided in research design, statistical analyses, writing, and who collected the historical data on which the thesis is based. I acknowledge the valuable input of Dr. Daniel Heath, who provided oversight and support for the molecular analyses in Chapter 2 and Chapter 3.

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

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I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has

not been submitted for a higher degree to any other University or Institution. The chapters are presented in manuscript format for peer-reviewed publication.

Abstract

In tropical bird species where both males and females sing, breeding partners may coordinate their songs in vocal duets. This thesis explores relationships between vocal duetting behaviour and reproductive activities in neotropical Rufous-and-white Wrens (*Thryothorus rufalbus*). In Chapter 2 I describe the previously unknown genetic mating strategy of this species. Extra-pair paternity accounted for two percent of nestlings in three of 51 broods. In Chapter 3 I test four hypotheses for duet function: the reproductive synchrony, paternity guarding, signalling quality, and signalling commitment hypotheses. I found little support for these hypotheses, although female duet output appears to signal willingness to invest in future reproductive activities. Previous studies demonstrate that duets play an important role in acoustic contact, territory defence, and mate guarding. Together with the findings of this thesis, the cumulative research on Rufous-and-white Wrens suggests duets play a role in other activities more so than in reproductive behaviour.

Dedication

For my G.P. Norman Matheson: the positive attitude you carried through life and your determination to overcome obstacles continues to inspire me in all that I do.

And for Ben: my best friend and most dedicated supporter.

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First and foremost, I thank my supervisor Dr. Dan Mennill. It has been a true pleasure to have the opportunity to work with him and discover academia under his expert guidance, support, and tireless encouragement. The passion he has for birds and their songs is incredibly inspiring. I thank him for granting me the privilege of working in Costa Rica on such a charismatic species, over three unforgettable field seasons, alongside a talented team of ornithologists and friends. This experience has far surpassed what I had hoped it would be, and I owe that to him.

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senses of humour. In the face of adversity always remember “I wish...!” statements... positivity will always get you through.

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Lastly, I thank my husband Ben, who is a true adventurer at heart. I thank him for accompanying me and supporting me on this exciting journey, and for helping me to learn so much about myself. I promise that someday we will go on an uneventful trip (vacation, maybe) to Costa Rica.

I look forward to my next adventure with him...

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Chapter 1: General introduction

Bird song

Animals communicate using a diverse array of signalling strategies through several modalities. Bird song, a form of acoustic signalling, is an especially well-studied system in the field of animal communication (Todt & Naguib 2000; Catchpole & Slater 2008). Numerous studies investigating the singing behaviour of male north-temperate songbirds (order: Passeriformes) during the breeding season have demonstrated that male song serves dual functions in mate attraction and in territory defence (reviewed in Catchpole & Slater 2008). Female song, on the other hand, has historically been investigated much less because of false assumptions that it is rare and functionless, compounded by a strong focus on temperate species in ornithological research (sometimes called “temperate zone bias”; Langmore 1998; Stutchbury & Morton 2001). As a consequence, we know comparatively little about the functions of female song, and whether female song has evolved via similar processes and under comparable selection pressures as male song (Langmore 1998; Stutchbury & Morton 2001; Slater & Mann 2004).

In many south-temperate and tropical bird species where both males and females sing, breeding partners may coordinate their songs into a complex vocal display called a duet (reviewed in Farabaugh 1982 and Hall 2004, 2009). The motivation for this thesis research arises out of an interest to explore the ecology and evolution of female song and vocal duets, as well as an interest to describe the poorly-studied voices and reproductive behaviours of duetting neotropical birds. In order to understand the ecology and evolution of female song in the tropics, we must develop a more comprehensive knowledge of tropical breeding systems and reproductive behaviours. This introductory chapter

provides a brief overview of background topics that give context to the research described in the subsequent data chapters.

Avian vocal duets

Duets occur when two birds, usually the male and female of a mated pair, vocalize together in a highly coordinated fashion (Farabaugh 1982; Hall 2004, 2009). These impressive acoustic displays have been ranked among the most complex behaviours in the animal kingdom by behavioural ecologists (Mann et al. 2003). Marked diversity exists among the 220 species known to perform duets, both taxonomically and in the structure of the male and female contributions (Farabaugh 1982; Hall 2009). Duetting behaviour is not well understood, in part because animals that engage in these displays are concentrated in dense tropical habitats where field research is logistically challenging and duetting animals are often difficult to observe (Mennill & Vehrencamp 2008).

Why two birds might combine their vocalizations into a duet instead of singing solo songs is perplexing. Numerous hypotheses have been proposed to explain the functions of avian duets (Farabaugh 1982; Hall 2004, 2009). The following four hypotheses centre on the idea that duets are a cooperative act between pair members with a common goal. (1) The *territory defence hypothesis* suggests that duets function to defend a territory or other resource from conspecifics (Seibt & Wickler 1977). This hypothesis for duet function has received much empirical support (reviewed in Logue 2005; Douglas & Mennill 2010). (2) The *pair-bond maintenance hypothesis* suggests that male and female partners duet with one another to strengthen and maintain their partnership (Armstrong 1963, Hall 2004, 2009). (3) The *contact maintenance hypothesis* suggests that duets facilitate localization and allow mates to maintain contact in

environments with dense foliage (Cobb 1897). (4) The *reproductive synchrony hypothesis* suggests that duets coordinate reproductive activities between pair members in preparation for breeding (Dilger 1953). If duets function in this fashion, duetting may be an especially important behavioural cue in species living in ecosystems with low seasonality (Kunkel 1974).

The traditional paradigm of duetting as a cooperative display is under scrutiny with recent evidence that pair members have independent (and sometimes contrasting) strategies for territory defence, and that participation in duets may arise out of intersexual conflict (Levin 1996; Hall 2004). The following hypotheses centre on the idea that duets are conflict-based. (5) The *mate guarding hypothesis* suggests that birds perform duets to advertise their partner's mated status and prevent same-sex rivals from pairing with their mate (Stokes & Williams 1968). (6) The *paternity guarding hypothesis* states that males perform duets during their female's fertile period to repel extra-pair males seeking copulations (Sonnenschein & Reyer 1983). In this case, the male is not only guarding his partner, but more specifically his paternity in his mate's clutch. (7) The *signalling quality hypothesis* suggests that an individual's duetting behaviour (e.g. timing of response to their partner's songs, or proportion of their partner's songs a bird answers) indicates some aspect of individual quality to their partner (Smith 1994). (8) Lastly, the *signalling commitment hypothesis* (Wickler 1980) suggests that the amount of effort an individual allocates to duetting with their partner could provide information about how willing they are to invest in the partnership. Investment may include parental care behaviours, territory defence, or other partner-related activities (Hall 2004). A number of additional, weakly

supported secondary hypotheses have been proposed for duet function (reviewed in Hall 2004), but are not directly relevant to this thesis and will not be discussed here.

The hypotheses for duet function are not mutually exclusive; duets are understood to be signals with varying interspecific (Hall 2004) and intraspecific functions, and several empirical studies provide direct evidence that they can serve multiple functions simultaneously (Sonnenschein & Reyer 1983; Grafe et al. 2004; Mennill & Vehrencamp 2008). Given that duets are multifunctional, it may be safe to assume that duets are not rooted exclusively in cooperation or conflict, but probably include elements of both (Hall 2004).

Avian mating systems and extra-pair paternity

The overwhelming majority (>90%) of bird species on Earth are socially monogamous, meaning that they develop a prolonged social partnership with a single individual of the opposite sex for the purposes of reproducing (Griffith et al. 2002). Before molecular techniques were used in behavioural research, monogamous species were believed to be both socially monogamous (i.e. affiliating with a single partner) and genetically monogamous (i.e. exclusively copulating with a single partner; Neudorf 2004). Strong evidence that social monogamy may not necessarily translate into genetic monogamy came from a pivotal study on the Red-winged Blackbird (*Agelaius phoeniceus*), where the social mates of vasectomized males continued to lay fertilized eggs, indicating that females were copulating with males other than their social partner (Bray et al. 1975). Since 1975, genetic markers have been used to determine the genetic mating strategy of approximately 150 bird species. True genetic monogamy (birds with no extrapair paternity) occurs in only 14% of surveyed passerines, while the remaining

86% follow a mixed reproductive strategy (Griffith et al. 2002). If we restrict our focus to socially monogamous species, across all birds studied to date, extra-pair young account for an average of 11.1% of nestlings, and 18.7% of broods (Griffiths et al. 2002). Among many possible benefits, females stand to increase their reproductive success by copulating with males with good genes or high genetic compatibility (Griffith et al. 2002).

The vast majority of studies exploring avian mating systems have been conducted on temperate breeding birds. Broad generalizations about avian mating systems based on these studies omit the majority of bird species, given the high avian biodiversity in tropical ecosystems (Stutchbury & Morton 2001). A recent review by Macedo et al. (2008) summarized extra-pair paternity rates from all known studies of tropical species ($n = 12$ species). Although researchers initially thought that tropical birds might show low incidence of extra-pair paternity compared to temperate species because of differences in life histories (e.g. Stutchbury & Morton 2001), extrapair paternity in tropical species appears to be comparable to rates in north-temperate birds (Macedo et al. 2008). Given the small subsample of tropical species involved in the analysis (the data characterize $<0.1\%$ of tropical terrestrial birds; Macedo et al. 2008) we must interpret these data with caution. Without more knowledge of the genetic mating strategies of tropical birds, our understanding of the factors contributing to interspecific variation in extra-pair paternity levels, as well as the importance of extra-pair paternity in sexual selection will be restricted (Macedo et al. 2008).

Duetting birds and extra-pair paternity

The link between duetting behaviour and extra-paternity has received very little attention in the literature. Because most duetting animals reside in tropical latitudes

(Farabaugh 1982), and the genetic mating strategy of most tropical birds is unknown (Macedo et al. 2008), it is difficult to assess the relationship between duetting behaviour and reproductive strategy. Extra-pair paternity rates have been investigated in only four tropical duetting species to date (Dusky Antbirds, *Cercomacra tyrannia*: Fleischer et al. 1997; Australian Magpie-larks, *Grallina cyanoleuca*: Hall & Magrath 2000; Buff-breasted Wrens, *Thryothorus leucotis*: Gill et al. 2005; Purple-crowned Fairy Wrens, *Malurus coronatus*; Kingma et al. 2009); extra-pair paternity appears to be low in these four species (2 to 4% of young; 3 to 6% of broods). Although more studies are needed, if extra-pair paternity rates are legitimately lower among duetting birds compared to non-duetting birds, ornithologists may need to re-evaluate the hypotheses for duet function in light of this fact (reviewed in Hall 2004, 2009).

Study species and study population

My thesis explores relationships between vocal duetting and reproductive behaviours in the Rufous-and-white Wren (*Thryothorus rufalbus*).¹ These resident neotropical songbirds are in the family Troglodytidae. Their range extends from Mexico, through Central America, and into northern Colombia and northwestern Venezuela (Stiles and Skutch 1989; Mann et al. 2009). Rufous-and-white Wrens are medium-sized passerines that are sexually monomorphic in plumage, but dimorphic in all body measurements, with males being larger than females (males: $25.8 \pm 0.4\text{g}$; females: $23.7 \pm 0.5\text{g}$; Mennill & Vehrencamp 2005). Adult birds have rufous plumage on the head, mantle, wings, and tail, and a bright white throat and underparts. The cheeks are heavily

¹ Ongoing molecular work suggests that the genus *Thryothorus* may be better understood as a group of four genera, in which case this species will be renamed *Thryophilus rufalbus*; see Mann et al. 2009. Given that the American Ornithologists' Union has yet to advocate this change, I maintain the traditional nomenclature of *Thryothorus* in this thesis.

streaked, and the wings, tail, and crissum are barred with black (Fig. 1a; Stiles & Skutch 1989). Five subspecies of Rufous-and-white Wrens are recognized by the American Ornithologists' Union. My thesis research involves subspecies *T. rufalbus castonanotus*. My study population is located in Sector Santa Rosa of the Area de Conservación Guanacaste (10°40'N, 85°30'W). Santa Rosa, a UNESCO world heritage site, is a mosaic of habitats; resident wrens inhabit a narrow ribbon of mature humid and late-successional regrowth forests in the reserve.

Though no published species account currently exists, long-term monitoring by Daniel Mennill and his students of a population in Sector Santa Rosa (2003 – present) suggests that Rufous-and-white Wrens are relatively long-lived passerines that form long-term social partnerships. As of 2010, the longest-surviving male in the study population was at least eight years old and the longest-surviving female was at least seven years old. As in many tropical ecosystems predation of adult birds is low, and thus survivorship is high (Johnston et al. 1997). In contrast to the predation patterns for adults, predation on nestlings is severe. White-faced Capuchin monkeys (*Cebus capucinus*), White-throated Magpie Jays (*Calocitta formosa*), and snakes depredate most eggs and nestlings in the study population. Following predation events, pairs attempt to re-nest, even if nest depredation occurs multiple times within a single breeding season (Topp & Mennill 2008).

Male and female Rufous-and-white Wrens defend territories against rival conspecifics and both sexes are involved in reproductive activities. Breeding commences in mid-May and coincides with the onset of the rainy season (range: May 9 to May 19; 2003-2010). Prior to the start of the rains, territory-holding males and females

cooperatively build nests. The nest of this species is a globular structure comprised of grass and black fungal rhizomes with a downward-sloping entrance tube (Fig. 1b; Stiles & Skutch 1989). Nests are usually built in acacia trees (*Acacia collinsii*) that host aggressive symbiotic ants (Stiles & Skutch 1989). At the start of the nest building period, males perform displays by perching on the potential nest site with a twig in their bill, or placing twigs on a potential site, until the female begins to add material. Nest building occurs in earnest in the late dry season. Females do not begin laying until the start of the rainy season, after which a single blue-green egg is laid daily until the clutch is complete (mean clutch size \pm SE 2003 - 2010: 3.97 ± 0.12 eggs, $n = 31$ clutches). Females are the sole incubator of eggs during the incubation period (Topp & Mennill 2008), which lasts 16 - 18 days (pers. obs.).

Both male and female Rufous-and-white Wrens provision nestlings. Much inter-pair variation exists in the partitioning of labour between males and females (pers. obs.). The nestling period lasts approximately 20 days (pers. obs). Once fledged from the nest, young birds remain on their natal territory for an extended period of time (based on observations in late July and August). Field observations suggest that female Rufous-and-white Wrens will initiate a second brood while fledged offspring from the first brood are cared for by the social father. We currently know little about fledgling dispersal. However, on a few occasions banded nestlings have been observed defending territories approximately 100 m to 5 km away from their natal territories. In 2009 we observed a female banded nestling breeding 100 m away from her natal territory, and in 2010 we observed a male banded nestling breeding 5 km away from his natal territory.

Rufous-and-white Wren song

Both male and female Rufous-and-white Wrens have a repertoire of song types which they may sing as solos, or combine into coordinated antiphonal and polyphonal duets (Fig. 2; Mennill & Vehrencamp 2005). Male repertoire sizes are larger than that of females; on average male repertoires consist of 10.8 ± 0.7 songs, while female repertoires consist of only 8.5 ± 0.7 songs (Mennill & Vehrencamp 2005). Both male and female songs typically begin with tonal introductory syllables immediately followed by a trill (repeated syllables), and end with a terminal note that is usually the highest frequency and amplitude portion of the song (Mennill & Vehrencamp 2005). Fine structural analysis of male and female songs by Mennill & Vehrencamp (2005) revealed significant differences between the sexes. Compared to female songs, male songs are lower in frequency in the trill and terminal syllables, and male trills contain more repeated elements (Fig. 2). Males are more vocal than females, and sing significantly more often than females during singing bouts, repeating their songs every 11.9 seconds, while females sing every 16.4 seconds (Mennill & Vehrencamp 2005). Males and females have divergent seasonal singing patterns: female song peaks in the pre-breeding season and then declines, whereas male song peaks at the start of the breeding season when females are fertile (Topp & Mennill 2008).

Rufous-and-white Wren duets occur when a paired male and female temporally coordinate their vocalizations within one second of each other (as defined by Mennill & Vehrencamp 2005). Both sexes create duets with their partner by responding to their partner's solo songs (Mennill & Vehrencamp 2005). The majority of duets are created by females responding to their partner (Mennill & Vehrencamp 2005). Duets are loosely

coordinated with substantial variation in the delay in onset between male and female songs (Mennill & Vehrencamp 2005). Duetting shows a similar seasonal pattern to female song, peaking at the onset of breeding when females are fertile and then declining throughout the remainder of the breeding season. During the female fertile period the majority of duets are created by males responding to their partner's solo songs. Males are most responsive to their partner's songs during her fertile period (Topp & Mennill 2008). A previous study has suggested that this may reflect a male's attempt to maintain paternity within his clutch (Topp & Mennill 2008), but the genetic mating strategy of this species is currently unknown.

Summary

This thesis explores the link between vocal communication and reproductive behaviours in a neotropical duetting wren. In Chapter 2, I describe the previously unknown genetic mating system of Rufous-and-white Wrens based on four polymorphic microsatellite markers, using a seven-year dataset. In Chapter 3, I explore relationships between duetting behaviour and reproductive behaviour in Rufous-and-white Wrens by testing predictions of four hypotheses for duet function (the reproductive synchrony, paternity guarding, signalling quality, and signalling commitment hypotheses), using an eight-year dataset. Additionally, I quantify male and female nest building and nestling provisioning behaviour, as the relative contribution of males and females to parental activities has rarely been quantified in socially monogamous neotropical birds. Lastly, I provide a brief summary of major results and implications stemming from this research, and highlight promising avenues for future investigations.

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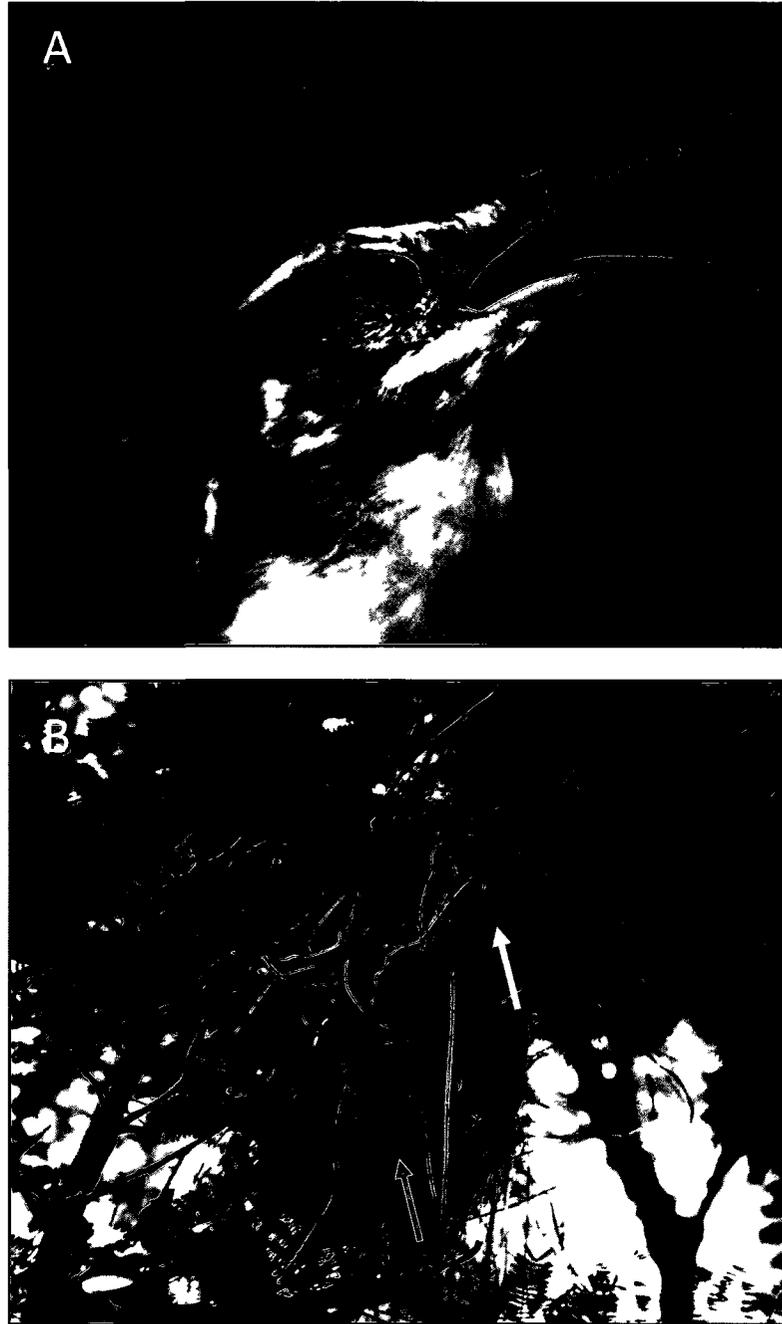


Figure 1.1. (A) An adult Rufous-and-white Wren (photo by Dale Morris; copyright Daniel Mennill). Males and females are similar in size and plumage colouration. (B) A Rufous-and-white Wren nest in an acacia tree (*Acacia collinsii*). The white arrow indicates the entrance to the nest, which is an elongated tunnel. The yellow arrow indicates the nest cup, where eggs are laid (photo by the author).

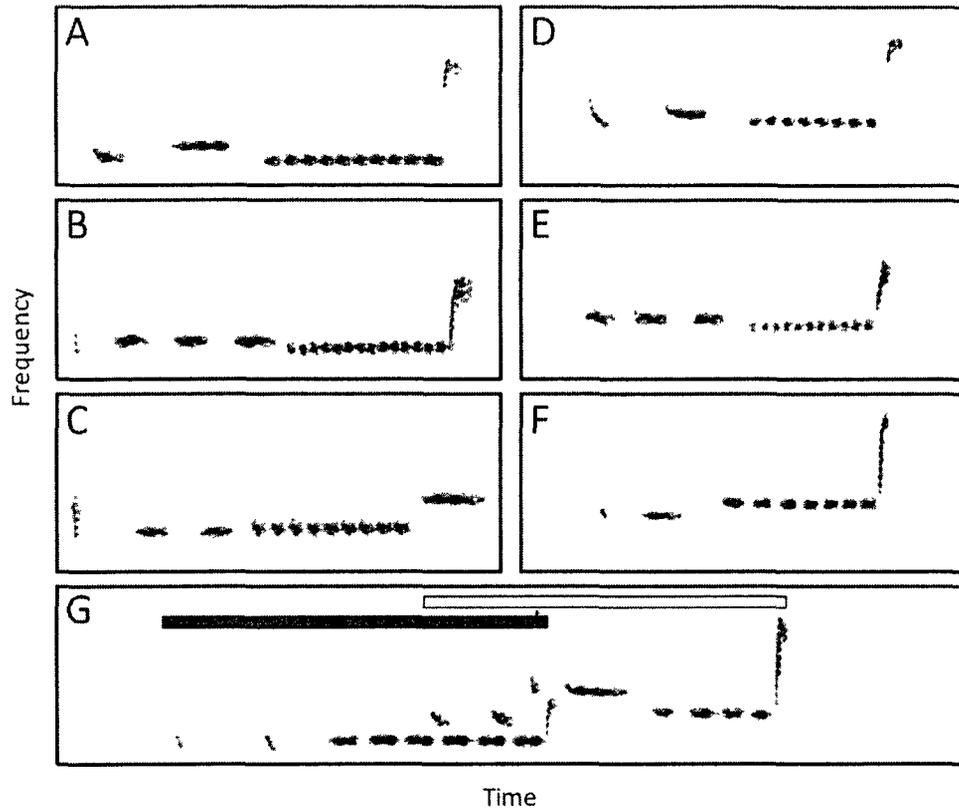


Figure 1.2. Sound spectrograms of Rufous-and-white songs. (A-C) Solo songs of a male. (D-F) Solo songs of a female. (G) A female-created duet, where the female (indicated by the white bar) responds to her partner's song by overlapping his vocalization (indicated by the black bar).

**Chapter 2: Low levels of extra-pair paternity in a duetting neotropical songbird, the
Rufous-and-white Wren***

*This study is the product of joint research.

Chapter synopsis

Molecular analyses have confirmed that most socially monogamous birds follow a mixed reproductive strategy. Most studies, however, have been conducted on north-temperate species; tropical species are grossly underrepresented despite the fact that most of Earth's biodiversity is concentrated in the tropics. In this study, I describe the genetic mating strategy of the Rufous-and-white Wren (*Thryothorus rufalbus*), a medium-sized, neotropical passerine that exhibits socially monogamous mating behaviour. Over eight years of studying a colour-banded population in Costa Rica, genetic data were collected from 51 broods for paternity analysis. Microsatellite analysis using four variable loci revealed that Rufous-and-white Wrens follow a mixed reproductive strategy, where extra-pair young are found in a small minority of broods. Three of 158 nestlings (2%) were the result of extra-pair fertilizations; three of the 51 broods (6%) contained a single nestling with allelic mismatches to its social father. I assigned paternity for two of the extra-pair nestlings to males in a territory adjacent to the cuckolded male. The extra-pair paternity estimates reported here are lower than the average reported across north-temperate socially monogamous passerines, but are comparable to rates seen in three closely related wrens, and to three other duetting tropical birds. This suggests that low levels of extra-pair paternity may be common among *Thryothorus* wrens and among duetting tropical birds in general.

Introduction

Birds have played a pivotal role as model systems for studies of sexual selection (Andersson 1994, Birkhead & Moller 1998). Since the introduction of molecular techniques to study avian mating systems over three decades ago, researchers have gained much insight into female mate choice and reproductive strategies. Studies using genetic markers have revealed that although 90% of bird species are socially monogamous, less than 14% of species surveyed are genetically monogamous, as a result of females following mixed-reproductive strategies and copulating with males other than their social partner (reviewed in Westneat et al. 1990; Griffith et al. 2002). There is much debate over the relative importance of various ecological, life history, and behavioural factors that appear to influence interspecific variation in extra-pair paternity (Westneat & Stewart 2003). The majority of hypotheses proposed to explain this variation are based on genetic studies of north-temperate species; resident south-temperate and tropical species are grossly underrepresented in spite of the fact that the majority of bird species on Earth are concentrated in the tropics (Macedo et al. 2008, Stutchbury & Morton 2001; Tori et al. 2008). Without an understanding of extra-pair paternity in tropical species, we cannot draw broad conclusions about extra-pair paternity, or its role in sexual selection among socially monogamous birds (Macedo et al. 2008).

Though data are limited, genetic studies of 16 species to date suggest that extra-pair paternity rates vary markedly among tropical bird species, and may be comparable to rates seen in temperate birds (reviewed in Macedo et al. 2008; and see also Maguire & Mulder 2008; Krueger et al. 2008; Neto et al. 2010; Cramer et al. 2011). Although conclusions have limited scope with such an extreme minority of tropical bird species

sampled, the accumulated data suggest that an average of 18% of young result from extra-pair fertilizations, and 26% of broods contain extra-pair young. These rates are comparable to the levels of extra-pair paternity seen across all socially monogamous birds (extra-pair paternity accounts for 11.1% of young and 18.7% of broods; Griffith et al. 2002).

In this study, I describe the genetic mating strategy of the Rufous-and-white Wren (*Thryothorus rufalbus*). Rufous-and-white wrens are duetting neotropical songbirds that exhibit socially monogamous mating behaviour; one male and one female defend a territory together and build a globular nest (Mennill & Vehrencamp 2008). Breeding commences at the onset of the rainy season and continues asynchronously (due to high rates of predation) for several months (Topp & Mennill 2008). Males assist with nest building and nestling provisioning, but females alone are responsible for incubation. Over eight years of studying a colour-banded population in northwestern Costa Rica, observations have been collected from 176 breeding pairs and genetic samples have been collected from 51 broods, making this one of the more comprehensive long-term paternity studies in tropical birds to date.

Methods

Study population

Rufous-and-white Wrens are year-round residents of the mature humid forests and late-succession regrowth forests of the north Pacific slope of Costa Rica. I studied a population of Rufous-and-white Wrens in Sector Santa Rosa of the Guanacaste Conservation Area (10°40'N, 85°30'W). We observed birds daily throughout the breeding

seasons between approximately April and July of 2003 to 2010. The study population included 22.0 ± 1.5 pairs per year (average \pm SE; range: 17 to 31 pairs per year). I captured adult birds with mist nets, collected standard morphometric measurements, and assigned each individual a unique combination of three plastic colour bands and one numbered aluminum band. I obtained blood samples from adults through venipuncture of the brachial vein (35-75 μ l per bird). I determined the sex of each adult bird by observing singing and incubation behaviours (structural differences in songs distinguish between the sexes; incubation is limited to females; Mennill & Vehrencamp 2005; Topp & Mennill 2008).

For each pair, I defined the social father as the male who sang on the nesting territory, performed vocal duets with the social mother, and assisted with nest building and/or nestling provisioning. I defined the social mother as the female who sang on the nesting territory, provisioned nestlings, and incubated eggs. Among 176 breeding attempts monitored between 2003 and 2010, all birds bred as monogamous pairs, except one. In 2009 a case of polygamy occurred, where two nearby females paired with a single male and bred simultaneously in different nests at opposite sides of the male's territory. Each of these females had paired with the male as a monogamous partner in the two preceding years. I consider this behaviour atypical, and suggest that Rufous-and-white Wrens should be considered socially monogamous.

I monitored the reproductive activities of all pairs by watching the behaviour of the parents or by checking the nest for eggs. When nestlings were 3 to 6 days old, I used venipuncture to obtain a 10-25 μ L blood sample from the tibio-tarsal blood vessels (less than the recommendation for non-harmful sampling: Gaunt & Oring 1997). Blood was

preserved in ~1.5 mL of Queen's Lysis Buffer (Seutin et al. 1991). Predation and nest destruction was severe; most nests in the population were destroyed by white-faced capuchin monkeys (*Cebus capucinus*), White-throated Magpie-jays (*Calocitta formosa*), or snakes before they could be sampled.

Genetic analysis of paternity

I assessed the paternity of all sampled nestlings using four microsatellite loci (ThPI-14, ThPI-22, ThPI-26 and ThPI-30) that were originally isolated from the Banded Wren (*Thryothorus pleurostictus*; Brar et al. 2007). Rufous-and-white Wren DNA was isolated from field samples using a plate-based extraction method (I added ~10-15 μ l of whole blood sample to 160 μ l of digestion buffer and 5 μ l of 20 mM protease K). Plates were incubated at 37° C for a minimum of 6 hours and DNA was extracted following Elphinstone et al. (2003).

To assign paternity I ran polymerase chain reactions (PCR) following the cycling profile outlined in Brar et al. (2007): 1 cycle at 94° C for 2 min; followed by 30 cycles of 30 sec at 94° C, 30 sec at 58° C, and 30 sec at 72° C; followed by a final extension cycle of 1 min at 72° C. The annealing temperature of 58° C recommended by Brar et al. (2007) for ThPI-30 produced a high frequency of large-allele dropout; I eliminated the problem by decreasing the annealing temperature to 50° C for all ThPI-30 reactions. For all samples, I amplified microsatellite loci using 0.5 μ l of genomic DNA in 12.5 μ l reactions. PCRs contained 2.4 mM MgCl₂ (Applied Biosystems), 192.3 μ M of each dNTP (Promega), 0.17-0.29 μ M each of forward and reverse primers, 0.05 units of *Taq* polymerase (*Amplitaq*[®] DNA, Applied Biosystems), 1.25 μ l each of Bovine Serum Albumin (BSA) and buffer (Applied Biosystems), and 7.45 μ l of ddH₂O. Forward

primers were unlabeled and reverse primers were modified at the 5' end by a fluorescent label (IR Dye[®] 700 and IR Dye[®] 800; IDT[™] Primers). To visualize PCR fragments, I ran labeled PCR products on a LICOR 4300 DNA analyzer (v. 1.3.8-1, Biosciences) with positive and negative standards. Allele sizes were estimated using GENEIMAGR (version 4.05). In all cases, I ran nestlings on gels directly beside their putative parents to minimize inter-gel variability. Two independent observers scored allele sizes for all individuals to minimize observer-related bias.

Three of the four microsatellite loci were highly variable, with 9 to 14 alleles per locus and expected and observed heterozygosities of >60.7% based on samples of 56 (ThPI-22) and 57 (ThPI-14 and ThPI-30) adult birds (Table 1). The remaining locus, ThPI-26, was less variable, with only two alleles and expected and observed heterozygosities of >19.6% (Table 1). Alleles at all four loci appeared to abide by Mendelian inheritance, with parental alleles sorting to approximately 50% of offspring. I calculated allele frequencies and Hardy-Weinberg equilibrium probabilities using parental DNA with GenALEX version 6.4 (Peakall & Smouse 2006). Locus ThPI-26 was in Hardy-Weinberg Equilibrium, while the other three deviated from Hardy-Weinberg Equilibrium (ThPI-14, $p < 0.01$; ThPI-22 $p < 0.001$; ThPI-30 $p < 0.01$). The combined probability (using all four loci) of detecting an extra-pair nestling was 99.5% if the mother's genotype was known (45 broods), and 97.3% if the mother's genotype was unknown (6 broods).

I considered offspring to be extra-pair if they had allelic mismatches with their social father at two or more of the four loci. When possible, I assigned parentage to extra-pair young by running nestlings beside adult males that held territories proximate to the

nesting territory. I assessed genotyping error rates by amplifying 25 individuals from 2010 twice at two polymorphic loci (ThPI-14 and ThPI-30). The allele mismatch rate was zero, suggesting that the genotyping error rates are low.

All values are reported as mean \pm SE.

Results

I collected genetic data from 158 Rufous-and-white Wren nestlings in 51 broods. I sampled 7.3 ± 1.0 broods per year, except in 2004 when paternity wasn't sampled. Brood size ranged from 1 to 5 with an average brood size of 3.1 ± 0.1 nestlings.

Microsatellite paternity analysis revealed that Rufous-and-white Wrens follow a mixed reproductive strategy, where extra-pair young are found in a small minority of broods. Three of 158 nestlings (2%) were the result of extra-pair fertilizations. Three of the 51 broods sampled (6%) included one nestling with allelic mismatches to their social father at two of the four loci. I assigned paternity to the social father for the remaining 155 nestlings sampled; all nestlings matched their social father at all four loci. All offspring, including those sired by extra-pair males, matched the genotype of the social mother at all four loci.

I assigned paternity to two of the three extra-pair young. Both extra-pair nestlings were a genetic match at all four loci to an adult male in an adjacent territory. The nests of the extra-pair sires and the nests with the extra-pair young were separated by distances of 25 m and 150 m. I was unable to assign paternity for the third extra-pair nestling to any sampled male in the population at all four loci, including males in neighbouring territories. However, an unbanded bachelor male occupied a territory adjacent to the

nesting territory with the extra-pair young, and he is a likely candidate for the extra-pair sire.

Discussion

Rufous-and-white Wrens follow a mixed reproductive strategy, with extra-pair paternity found in a small minority of broods. Of all young sampled, 2% resulted from extra-pair fertilizations, and 6% of broods contained a single extra-pair offspring. All nestlings were the genetic offspring of their social mother, indicating that intraspecific brood parasitism is rare or absent in this species. For two of the three extra-pair young, I assigned paternity to a breeding male directly neighbouring the nesting territory. I could not assign paternity to the third extra-pair young, but suspect it was sired by an unsampled breeding male in a territory neighbouring the nesting territory.

The extra-pair paternity estimates I report here are lower than the average reported across north-temperate socially monogamous passerines (average extra-pair paternity rate across 130 species: 11.1% of offspring, 18.7% of broods; Griffith et al. 2002). However, extra-pair paternity in Rufous-and-white Wrens occurs at similar rates to two closely related neotropical wrens and in one temperate wren. In Buff-breasted Wrens (*Thryothorus leucotis*) extra-pair young accounted for 4% of nestlings and were found in 3% of broods ($n = 53$ young in 31 broods; Gill et al. 2005). In Banded Wrens (*Thryothorus pleurostictus*) extra-pair young accounted for 4% of nestlings and were found in 10% of broods ($n = 156$ young in 50 broods; Cramer et al. 2011). In Carolina Wrens (*Thryothorus ludovicianus*) no extra-pair young were found in 84 offspring from 16 broods (Haggerty et al. 2001). Together, these results suggest that low levels of extra-pair paternity may be common among *Thryothorus* wrens.

Studies of genetically monogamous bird species suggest that a number of ecological and behavioural traits are associated with low levels of extra-pair paternity. Ecological variables include low breeding density (e.g. Verboven & Mateman 1997), increased necessity for male parental care (e.g. Morton et al. 1998; Masello et al. 2002), low breeding synchrony (e.g. Stutchbury & Morton 1995; Morton et al. 1998), low adult mortality (e.g. Masello et al. 2002), long-term partnerships between males and females (Mauck et al. 1999; Wink & Dyrce 1999), and high costs to females seeking extra-pair copulations (e.g. Stanback et al. 2002). Low breeding density could influence extra-pair paternity rates by limiting opportunities to engage in extra-pair copulations; birds breeding at high densities may encounter opposite-sex individuals more often, presenting increased chances to encounter potential extra-pair partners (Griffith et al. 2002). Rufous-and-white Wrens defend very large multi-purpose territories arranged linearly or in clusters in mature tropical dry forests (mean territory size: 3.3 ± 0.3 acres or 13497 ± 1043 m²; Mennill & Vehrencamp 2008), and territorial neighbours are often separated by sizeable undefended spaces (Mennill & Vehrencamp 2008). These patterns may limit encounters with potential extra-pair mates. Nest location relative to a neighbour's nest may facilitate extra-pair copulations in this species; the nest of the neighboring extra-pair sire in 2008 was located only 25 m from the nest containing his genetic offspring, which is much less than the average distance between nests in the population (average unoccupied distance between two neighbouring territories is 103.0 ± 18.2 m; Mennill & Vehrencamp 2008).

In some birds, extra-pair paternity rates are related to the degree that male parental care is required to successfully rear young (Mulder et al. 1994; Birkhead & Moller 1996;

Gowaty 1996). In these species, females might seek extra-pair copulations when they can raise young with little investment from a male partner, and can therefore risk the cost of reduced male parental care (Griffith et al. 2002). In Rufous-and-white Wrens, both males and females are heavily involved in parental care activities. While both sexes invest heavily in nest building, females invest significantly more effort into nestling provisioning (Chapter 3). Behavioural observations of birds in my study population suggest that males are also involved in post-fledging care of young, at which point the female partner may initiate a second clutch. Since males play an integral role in raising young, it is possible that risks associated with extra-pair copulations (e.g. reduced male parental care) outweigh potential benefits, and thus promote low levels of extra-pair paternity in this species.

Behavioural characteristics may also correlate with low levels of extra-pair paternity in some birds (Farabaugh 1982; Langmore 1998). Rufous-and-white Wrens are duetting songbirds; both males and females possess a repertoire of song types which they may sing as solos, or combine into coordinated antiphonal and polyphonal duets (Mennill & Vehrencamp 2005). Extra-pair paternity has been investigated in only four duetting species to date (Dusky Antbirds, *Cercomacra tyrannia*: Fleischer et al. 1997; Australian Magpie-larks, *Grallina cyanoleuca*: Hall & Magrath 2000; Buff-breasted Wrens, *Thryothorus leucotis*: Gill et al. 2005; Purple-crowned Fairy Wrens, *Malurus coronatus*: Kingma et al. 2009), and rates appear unanimously low. The paternity guarding hypothesis for duet function suggests that males' response rates to their partner's songs should be highest during her fertile period in order to repel extra-pair males seeking copulations (Sonnenschein & Reyer 1983; Hall 2004). This pattern was seen in a

temporal study of Rufous-and-white Wren vocal behaviour during the breeding season (Topp & Mennill 2008). Because most social pairs in my study population are also genetically monogamous, perhaps duets function successfully as a paternity guard in this species. Alternatively, if paternity is maintained without the use of an acoustic guard, heightened responsiveness during the female's fertile period may be an attempt to guard the partnership, as divorce occurs at low rates in my study population, often at the onset of breeding (unpublished data). Experimental studies using acoustic playback techniques may be informative in distinguishing which is more likely in Rufous-and-white Wrens (Douglas & Mennill 2010); for the paternity guarding hypothesis to hold true, playback that simulates a male intruder should initiate a heightened response from the resident male when his partner is fertile.

In conclusion, this seven-year study reveals that extra-pair paternity occurs at low levels in Rufous-and-white Wrens. Further studies of the genetic mating strategies of temperate and tropical wrens would enable phylogenetic studies that may help to clarify ecological and behavioural factors influencing extra-pair paternity rates across closely related species. In general, more studies of the genetic mating systems of tropical species are needed in order to understand the role that extra-pair paternity plays in sexual selection in birds.

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Table 1. Characterization of four microsatellite loci used for paternity analysis of Rufous-and-white Wrens.

Locus	Annealing temp (°C)	Number of alleles*	Heterozygosity		Probability of exclusion†	
			expected	observed	P_{ex1}	P_{ex2}
ThPI-14	58	9	0.801	0.789	0.45	0.63
ThPI-22	58	11	0.715	0.607	0.32	0.51
ThPI-26	58	2	0.232	0.196	0.03	0.10
ThPI-30	50	14	0.856	0.737	0.57	0.72

*Data shown based on $n = 56$ (ThPI-22, ThPI-26) and $n = 57$ (ThPI-14, ThPI-30) adult birds sampled between 2003 and 2010.

† Probability of exclusion given when the genotype of one (P_{ex1}) or both (P_{ex2}) parents is known.

**Chapter 3: The relationship between singing behaviour and reproductive behaviour
in a neotropical duetting wren**

*This study is the product of joint research.

Chapter synopsis

In many tropical animals both sexes are elaborately ornamented, a pattern not usually seen in temperate ecosystems. In the north-temperate zone, elaborate vocalizations are produced mostly by males, but in tropical and south-temperate ecosystems it is not unusual for both sexes produce acoustic displays. When both sexes sing, animals may combine their songs in duets, acoustic displays where two animals vocalize in a coordinated fashion. Studies of some duetting birds show that duet rates peak prior to nest building and during renesting periods following predation events, suggesting that duets are associated with reproduction in tropical birds. In this study, I explore relationships between duetting behaviour and reproductive activities in a socially monogamous neotropical duetting songbird, the Rufous-and-white Wren (*Thryothorus rufalbus*). I test four established hypotheses for duet function that provide a direct link between vocal behaviour and reproduction. In general, the results of this study do not indicate a strong relationship between duetting and reproduction in Rufous-and-white Wrens. My findings provide no support for the reproductive synchrony, paternity guarding, or signalling quality hypotheses for duet function. My results provide mixed support for predictions of the signalling commitment hypothesis, where the duetting behaviour of females appears to signal willingness to invest in future reproductive activities to a partner. Previous studies of Rufous-and-white Wrens show that duets play an important role in acoustic contact, territory defence, and in mate guarding. Together with the present findings, cumulative investigations of this species reveal that duets play a more prominent role in other functions.

Introduction

The tropics hold the majority of Earth's biodiversity, and many tropical animals exhibit patterns of behaviour and natural history that are not well represented in north-temperate regions. Tropical species present an interesting opportunity to study the role of elaborate traits in mate choice because both sexes are often ornamented, a pattern not usually seen in temperate species (Langmore 1998). For example, in the neotropical Royal Flycatcher (*Onychorhynchus coronatus*), both males and females possess a brightly-coloured fan-shaped crest display (Lawrence 1862) that functions in courtship and intrasexual competition (Rievey 2010). In a West African cichlid (*Pelvicachromis taeniatus*), both sexes have exaggerated pectoral fins that they display toward their partner and quiver during courtship (Baldauf et al. 2010). In tropical Siamangs (*Hylobates syndactylus*), male and female breeding partners combine their voices in a spectacular display that functions in strengthening the pair-bond (Geissmann & Orgeldinger 2000). Thus, in tropical animals we might expect traits that play an important role in mate choice and reproduction to evolve in both sexes.

In many tropical species where both males and females sing, animals may combine their songs to form a duet. Duets are acoustic displays where two individuals, usually the male and female of a mated pair, vocalize together in a coordinated fashion (reviewed in Farabaugh 1982; Hall 2004, 2009). Duets occur in a wide variety of taxa including birds (Farabaugh 1982), insects (Bailey 2003), frogs (Emerson & Boyd 1999), primates (Geissmann 2002), and cetaceans (Lilly & Miller 1961). Numerous hypotheses have been proposed to explain duet function, and although the territory defence hypothesis has received the most empirical support (reviewed in Logue 2005, Douglas &

Mennill 2010), duets are increasingly understood to be multifunctional acoustic signals (e.g. Geissmann 2002; Grafe & Bitz 2004; Mennill & Vehrencamp 2008). In birds, studies of seasonal variation in duetting behaviour reveals that duet rates peak prior to nest building and again during renesting periods following predation events (Harcus 1977; Sonnenschein and Reyer 1983; Hall 2006; Topp & Mennill 2008). Thus, vocal duets appear to be associated with breeding in tropical birds with long-term socially monogamous partnerships (Wachtmeister 2001; Hall 2009). although the specific connection between duets and breeding behaviour is poorly understood.

Four hypotheses have been proposed to explain the link between duetting behaviour and reproductive behaviour (reviewed in Farabaugh 1982; Hall 2004, 2009). The *reproductive synchrony hypothesis* (Dilger 1953) suggests that duetting serves to synchronize reproductive activities between partners at the onset of breeding. Many duetting species live in tropical ecosystems which typically exhibit less pronounced changes in seasonality compared to temperate forests, and may provide fewer external cues to initiate breeding, such as changes in photoperiod (Kunkel 1974; Stutchbury & Morton 2001; Slater & Mann 2004). Studies of temperate female Ringneck Doves (*Streptopelia risoria*) demonstrate that male and female vocalizations can stimulate nest building and ovarian development in temperate birds (reviewed in Cheng 1992). The reproductive synchrony hypothesis suggests that duets may evoke a similar physiological response for the tropical birds that perform them, but this hypothesis has only been tested in a single species. When male White-browed Robin Chats (*Cossypha heuglini*) were experimentally prevented from duetting, pairs failed to construct a nest and breed, suggesting that duets facilitate reproduction in this species (Todt & Hultsch 1982),

although it is likely that experimental alterations influenced other aspects of behaviour related to reproduction. This hypothesis is also supported by indirect evidence in several duetting species where duets are most common before breeding and during reneating attempts (Harcus 1977; Sonnenschein & Reyer 1983; Topp & Mennill 2008), indicating that they may be important in coordinating reproductive activities.

The *paternity guarding hypothesis* (Sonnenschein & Reyer 1983) suggests that a duetting male should respond to his partner's songs at high rates to repel other males seeking extra-pair copulations. If duets function as a paternity guard, males with higher levels of duet responsiveness to their partner's songs during the female fertile period should benefit from diminished loss of paternity to extra-pair sires. There is little empirical evidence supporting the paternity guarding hypothesis, in large part because the genetic mating system of most duetting species is unknown (Hall 2009). A study in Buff-breasted Wrens (*Thryothorus leucotis*) showed that while extra-pair paternity rates are low, the proportion of female songs answered by males did not differ by breeding stage, suggesting that paternity is maintained without the use of an acoustic guard (Gill et al. 2005). A prediction of this hypothesis has also been tested using acoustic playback in the Plain Wren (*Thryothorus modestus*; Marshall-Ball et al. 2006). During a simulated intrusion, males did not show a greater tendency to answer their partner's songs during their mate's fertile period, as predicted under the paternity guarding hypothesis (Marshall-Ball et al. 2006).

The *signalling quality hypothesis* (Smith 1994) suggests that features of an individual's duetting behaviour (e.g. level of duet responsiveness, or timing of a bird's response to their partner) may communicate some aspect of individual quality to a

partner. This hypothesis predicts that birds that are adept at coordinating their songs with their partner's songs should exhibit high measures of reproductive success (e.g. larger clutches and broods) and survival, and low incidence of divorce or extra-pair mating (Hall 2004). Tests of this hypothesis require a long-term dataset on a population of colour-marked duetting birds, which are rare, and consequently this hypothesis has yet to be investigated.

Lastly, the *signalling commitment hypothesis* (Wickler 1980) suggests that effort allocated by an individual to performing duets with their partner's songs may provide information about their level of commitment to future reproductive activities (Hall 2004). An individual's duet responsiveness (the probability that an individual will respond to their partner's songs to create a duet) or an individual's level of duet output could potentially signal willingness to invest in the partnership (e.g. future parental care activities; Hall 2004). The relationship between duetting behaviour prior to breeding and parental effort during the breeding season, critical for testing the signalling commitment hypothesis, has not been tested to date. Indeed, the relative contribution of males and females during parental activities in socially monogamous neotropical birds has rarely been quantified in detail (Whittingham et al. 1996; Hall 1999).

In this study I explore relationships between singing and reproductive behaviours in a neotropical duetting songbird, the Rufous-and-white Wren (*Thryothorus rufalbus*). In this species adult birds form long-term socially monogamous partnerships and defend large, multi-purpose territories throughout the year. Partnerships are usually genetically monogamous, although low levels of extra-pair paternity occur (2% of young are extra-pair and 6% of broods contain extra-pair young; Chapter 2). Both male and female

Rufous-and-white Wrens have a repertoire of songs they can sing as solos or combine into duets (Mennill & Vehrencamp 2005). Both sexes create duets with their partner by responding to their partner's songs. As in most duetting species surveyed (Hall 2009), the majority of Rufous-and-white Wren duets are created by females responding to their partner's vocalizations (73%; Mennill & Vehrencamp 2005). Males and females have divergent seasonal singing patterns; female song peaks in the pre-breeding season and then declines, whereas male song peaks at the start of the breeding season when females are fertile (Topp & Mennill 2008). Duets are most frequent just prior to breeding, and duetting rates increase again during renesting attempts following frequent nest predation events (Topp & Mennill 2008).

In this study I have two main objectives. First, I describe Rufous-and-white Wren parental behaviours by comparing male and female investment in nest building and nestling provisioning activities. Very few detailed studies on colour-banded populations of neotropical birds exist, and knowledge of the relative contribution of males and females in parental care activities is limited. Second, I use eight years of field data to test predictions of four hypotheses to explain the function of duets in the context of reproductive behaviour. (1) Based on the reproductive synchrony hypothesis, I predict that pairs with high duetting rates will commence laying earlier than pairs with low duetting rates. (2) Based on the paternity guarding hypothesis, I predict that males who perform duets at a high rate and show high duet responsiveness to their partner's song will lose less paternity. (3) Based on the signalling quality hypothesis, I predict that birds with high duet responsiveness will have higher reproductive success (i.e. larger clutches and larger broods; Hall 2004). (4) Finally, based on the signalling commitment

hypothesis, I predict that individuals that duet more and show high duet responsiveness will invest more effort into parental activities. Testing the predictions of these hypotheses will allow us to comprehensively explore the role of duetting in reproduction in Rufous-and-white Wrens.

Methods

I studied a population of Rufous-and-white Wrens in Sector Santa Rosa of the Guanacaste Conservation Area, Costa Rica (10°40'N, 85°30'W) between 2003 and 2010. Rufous-and-white Wrens are year-round residents of the mature humid and late-successional regrowth forests in this park. Breeding in the study population commences at the start of the rainy season (Topp & Mennill 2008). The average date of the first sustained rainfall was May 13 (range: May 9 to May 19; 2003-2010). Birds were observed between approximately April and July each year, corresponding to the late pre-breeding season and the first months of the breeding season. The study population included 22.0 ± 1.5 pairs per year (average \pm SE; range: 17 to 31 pairs per year) distributed over approximately 7.5 km of neotropical forest.

General field methods

Adult birds were captured prior to breeding and each individual was given a unique combination of three plastic coloured bands and one numbered aluminum band. Standard morphometric measurements were taken, and blood was sampled via venipuncture of the brachial vein. Sex was determined from observations of singing and incubation behaviour (structural differences in male and female songs allow experienced observers to distinguish between the sexes: Mennill & Vehrencamp 2005; incubation is limited to females: Topp and Mennill 2008). For each territorial pair, I defined the social

father as the male who sang on the nesting territory, duetted with the social mother, and assisted with nest building and/or nestling provisioning. I defined the mother as the female who sang on the nesting territory, incubated eggs, and provisioned nestlings.

Measures of reproductive success

The reproductive behaviour of all pairs in the population was monitored throughout the end of the pre-breeding season and the first part of the breeding season. Nests were located by following birds with nesting material in their bills, and by searching areas where pairs sang during the dawn chorus. After nest construction was complete, the onset of egg laying was determined by watching the behaviour of the parents and by checking the nest for eggs. Whenever possible, the day on which the first egg was laid was recorded for all breeding pairs, as well as the total number of eggs laid. After clutches were complete, nests were monitored every 1-3 days until they were depredated or until eggs hatched, at which time the brood size was recorded. Clutch size often differed from brood size because unhatched eggs were common in my study population. Concurrent to this study, I assessed extra-pair paternity and assigned parentage using microsatellite paternity assignment techniques, as described in Douglas et al. (Chapter 2). Of the 51 pairs in that analysis, 44 pairs for which acoustic recordings were available were included in the current study.

Measures of parental behaviour

Both male and female Rufous-and-white Wrens are heavily involved in parental care activities such as nest building and nestling provisioning. I conducted detailed observations of nest building ($n = 20$ pairs) and nestling provisioning ($n = 23$ pairs) during the breeding seasons of 2009 and 2010. When possible, I observed each pair

across multiple sessions. I used the number of trips an individual made to the nest per hour of observation as a measure of parental investment. Observation periods were 1 h in length, and conducted between 0600 and 1000 h to minimize variation due to time of day. Observations commenced only when one of the focal birds returned to the nest to build or provision after the observer arrived. I collected all observations from a concealed position approximately 15 m from the focal nest. I do not believe the presence of the observer influenced the behaviour of focal birds because adults usually returned to the nest with nesting or provisioning material less than ten minutes after the researcher arrived.

To quantify nest building behaviour of males and females, I observed all pairs before females began lining the nest cup (males do not participate in lining the nest). I observed each pair for a minimum of one hour (mean \pm SE: 1.33 \pm 0.1 h per pair; range one to two 1-hour observation sessions per pair). Nest visits where an individual flew to the nest without nesting material, but rearranged existing nesting material while at the nest were counted as a building trip for that bird.

To quantify nestling provisioning behaviour of males and females, I observed nests when the chicks were in three age categories in an attempt to minimize the effect of nestling age on parental feeding rates (categories: 1-3 days after hatching, 4-7 days after hatching, and 8-11 days after hatching). I attempted to observe each nest three times, but predation and nest destruction by White-faced Capuchin monkeys (*Cebus capucinus*), White-throated Magpie Jays (*Calocitta formosa*), and snakes was so severe that most attempted nests were destroyed before I could collect data in all categories (mean \pm SE observations: 1.74 \pm 0.19 h per nest). Nest visits where the male did not enter the nest but passed material to the female inside the nest were considered a male provisioning trip.

On rare occasions, because of the high density vegetation at my study site, I could not identify the sex of the individual before they entered the nest. These observations accounted for 1.97% of the total observations for nest building (1.94% of 721 observations) and provisioning (2.05% of 292 observations), and I excluded them from the analyses. Because of the difficulty of collecting observations I was not able to include subtle details of investment (such as variation in quality or size of provisioned material) in the analyses.

Measures of vocal behaviour

Using recording data collected over eight years (2003-2010), I measured the singing behaviour of 176 territory-holding Rufous-and-white Wren pairs throughout the breeding season. For all analyses I selected recordings collected prior to each pair's first egg date (mean \pm SE: 8.15 \pm 1.29 hours recorded per pair). All pairs were monitored with both focal recordings (where birds were followed by a recordist for 1 to 4 hours per recording session) and automated recordings (where birds were recorded by unattended microphones for 4 to 24 hours per recording session). Focal recordings were collected between 0500 and 0900 h, and involved a recordist followed singing pairs around their territories with a directional microphone (Sennheiser ME66 or ME67 or MKH70) and a solid-state digital recorder (Marantz PMD-660 or PMD-670). Three types of automated recorders were used to collect longer recordings, where the recording devices were set up in a pair's territory for one to four days before being moved to a new territory. In 2003-2004, automated recorders consisted of an 8-microphone acoustic location system, which recorded pairs for the first four hours of each morning (full details in Mennill & Vehrencamp 2008). In 2005-2009, automated recorders consisted of a Sennheiser ME-62

omni-directional microphone and a K6 power module connected to a PMD-670 solid-state digital recorder powered by a sealed lead-acid battery (full details of this recording apparatus are given in Hill et al. 2006), which recorded pairs from a central location in their territory for 24-hour periods. In 2010 automated recorders consisted of Wildlife Acoustics SongMeters (Model: SM2), which recorded pairs from a central location in their territory for the first four hours of each morning. All recordings used in the analyses were of naturally occurring songs, outside of the context of any playback experiments or other manipulations that have been part of a long-term study of this population.

Analyses of recordings

I analyzed recordings using Syrinx-PC sound analysis software (John Burt, Seattle, Washington), and categorized vocalizations according to the individual singing, and whether the song was produced as a solo or duet. I classified a song as part of a duet when a social male and female's vocalizations overlapped or occurred ≤ 1.0 s of each other (following Mennill & Vehrencamp 2005). I further classified a duet as either a "male-created duet" (the male answered his partner's song within ≤ 1.0 s to create a duet) or "female-created duet" (the female answered her partner's song within ≤ 1.0 s to create a duet; Mennill & Vehrencamp 2005). I calculated duet responsiveness (the proportion of a partner's songs that a bird answers to create duets) for the male and female of each pair, following Hall (2009; female duet responsiveness = number of female-created duets divided by the sum of the number male solos and the number of female-created duets; male duet responsiveness = number of male-created duets divided by the sum of the number of female solos and the number of male-created duets). During song and duet rate calculations, I ignored between-song latencies of >60 min to minimize the influence of

long periods of silence in recordings. In my analyses of singing behaviour versus reproductive behaviour, I excluded pairs where recordings sampled fewer than 10 duets, to avoid rate calculations based on small numbers of sampled duets (average number of duets recorded per pair in the remaining dataset: 60.5 ± 14.3).

Statistical analyses

All analyses were conducted in JMP (v9.0). I transformed the data to meet the normality assumptions of parametric statistics. Where data could not be normalized, I used nonparametric statistics. For tests of relative male and female effort in nest building and nestling provisioning, I used female relative effort as my response variable (male relative effort and female relative effort are inversely related to each other, so that any variable that correlates positively with female relative effort correlates negatively with male relative effort, by definition). All values are presented as means \pm SE.

Results

Nest building and nestling provisioning behaviours

Male and female Rufous-and-white Wrens invested an equivalent amount of effort into the nest building process (Fig. 1a; paired t-test: $t = 1.1$, $p = 0.28$, $n = 20$ pairs). By contrast, females invested significantly more effort into nestling provisioning than males, typically investing three times the effort of their partners (Fig. 1b; Wilcoxon sign-rank test: $Z = 99.5$, $p < 0.001$, $n = 23$ pairs). Male age influenced relative nest building effort, where older males invested more than young males (Spearman's rank correlation: $\rho = 0.46$, $p = 0.04$, $n = 20$ males), but female nest building effort did not vary with age (Spearman's rank correlation: $\rho = -0.29$, $p = 0.22$, $n = 20$ females). There were no effects

of parent age on nestling provisioning effort for either sex (Spearman's rank correlations; males: $\rho = -0.02$, $p = 0.92$, $n = 23$ males; females: $\rho = 0.27$, $p = 0.21$, $n = 23$ females). Partnership length (i.e. number of seasons the focal pair had bred together as a pair) was not related to relative effort during nest building or nestling provisioning for either sex (Spearman's rank correlation: males: nest building: $\rho = 0.27$, $p = 0.25$, $n = 20$; nestling provisioning: $\rho = -0.15$, $p = 0.50$, $n = 23$; females: nest building: $\rho = -0.27$, $p = 0.25$, $n = 20$; nestling provisioning: $\rho = 0.15$, $p = 0.50$, $n = 23$). Predation was rampant in the population, so that few nests survived to hatching and fewer still to fledging. For two nests that fledged where I was able to observe the birds post-fledging, the fledglings spent the majority of the time in close proximity to the male while the female commenced a second clutch.

Reproductive synchrony hypothesis

My data reveal no relationship between all measures of duetting behaviour and first date of egg laying, and therefore do not provide support for the reproductive synchrony hypothesis. Specifically, I found no relationship between first egg date and the number of duets a pair produced per hour before laying (Pearson correlation: $r = 0.006$, $p = 0.96$, $n = 71$). I found no relationship between male duet responsiveness and laying date (Pearson correlation: $r = 0.07$, $p = 0.57$, $n = 71$); nor did I find a relationship between female duet responsiveness and laying date ($r = 0.13$, $p = 0.30$, $n = 71$).

Paternity guarding hypothesis

My data show no relationship between male or female duetting behaviour and reproductive strategy, and therefore do not provide support for the paternity guarding hypothesis. Males who lost paternity did not show a statistically different level of duet

responsiveness (50.5 ± 11.6 % of female songs answered to create duets; $n = 3$ broods with mixed paternity) compared to males that did not lose paternity (22.8 ± 3.1 %; $n = 41$ broods with all within-pair young; Mann-Whitney: $U = 1.4$, $p = 0.17$); indeed, this nonsignificant relationship went in the opposite direction of the prediction of this hypothesis. Males who lost paternity created duets at similar rates to males who did not lose paternity (broods with mixed paternity: 1.18 ± 0.86 male-created duets per hour, $n = 3$; broods with all within-pair young; 1.50 ± 0.23 male-created duets per hour, $n = 41$; $Z = 0$, $p = 1.0$), suggesting that a male's propensity to duet with his partner is not related to her subsequent reproductive strategy.

Similarly, my data reveal no relationship between female duetting behaviour and reproductive strategy. Females who engaged in a mixed-mating strategy did not show a statistically different level of duet responsiveness (3.0 ± 3.0 % of male songs answered to create duets, $n = 3$) compared to females that did not have extra-pair young (5.8 ± 6.0 %, $n = 41$; Mann-Whitney: $U = 0.84$, $p = 0.41$). Additionally, females who had extra-pair young created duets at a similar rate to females who did not have extra-pair young (broods with mixed paternity: 1.65 ± 3.20 female-created duets per hour, $n = 3$; broods with all within-pair young; 4.09 ± 0.86 female-created duets per hour, $n = 41$; $Z = 0.84$, $p = 0.40$), suggesting that a female's propensity to duet with her partner is also not related to her subsequent reproductive strategy.

Signalling quality hypothesis

My data reveal no relationship between duet responsiveness and measures of reproductive success, and therefore provide no support for the signalling quality hypothesis. For both sexes, I found no relationship between duet responsiveness and

clutch size (Kruskal-Wallis test: males: $H = 1.42$, $p = 0.49$, $n = 55$; females: $H = 6.47$, $p = 0.17$, $n = 55$). Similarly, for both sexes, I found no relationship between duet responsiveness and brood size (Kruskal-Wallis test: males: $H = 3.35$, $p = 0.50$, $n = 54$; females: $H = 3.85$, $p = 0.42$, $n = 54$).

Signalling commitment hypothesis

My data reveal mixed support for the signalling commitment hypothesis. Some measures of male duetting behaviour were related to the relative effort the parents devoted to nest building and nestling provisioning. I found a significant positive relationship between the number of male-created duets per hour and female relative effort in nest building (Fig. 2a; Spearman's rank correlation: $\rho = 0.84$, $p = 0.001$, $n = 12$). Male duet responsiveness showed a marginally significant relationship in the same direction, with a positive relationship between responsiveness and female relative effort in nest building (Fig. 2b; Spearman's rank correlation: $\rho = 0.56$, $p = 0.07$, $n = 12$). I found a marginally significant relationship between male the number of male-created duets per hour and female relative nestling provisioning effort (Fig. 3a; Spearman's rank correlation: $\rho = 0.53$, $p = 0.06$, $n = 12$). Male duet responsiveness was not related to female nestling provisioning effort (Fig. 3b; Spearman's rank correlation: $\rho = 0.17$, $p = 0.58$, $n = 12$).

Some measures of female duetting behaviour were also related to the relative effort the parents devoted to nest building and nestling provisioning. I found a marginally significant positive relationship between the number of female-created duets per hour and female relative effort in nest building (Fig. 2c; Spearman's rank correlation: $\rho = 0.57$, $p = 0.07$, $n = 12$). Female duet responsiveness showed no relationship with female relative

effort in nest building (Fig. 2d; Spearman's rank correlation: $\rho = 0.49$, $p = 0.13$, $n = 12$). The number of female-created duets per hour showed a significant positive relationship with relative contribution of the female to nestling provisioning (Fig. 3c; Spearman's rank correlation: $\rho = 0.63$, $p = 0.03$, $n = 12$). Female duet responsiveness showed a marginally significant relationship, where more responsive females provided a relatively greater effort with nestling provisioning (Fig. 3d; Spearman's rank correlation: $\rho = 0.50$, $p = 0.08$, $n = 12$).

Discussion

This long-term study of breeding activities and vocal behaviour fails to provide evidence of a strong relationship between duetting behaviour and reproductive activities in neotropical Rufous-and-white Wrens. The results do not support predictions of the reproductive synchrony, paternity guarding, and signalling quality hypotheses for duet function, and provide mixed support for predictions of the signalling commitment hypothesis. Specifically, female Rufous-and-white Wren duetting behaviour prior to egg laying appears to signal willingness to invest in future reproductive activities to a partner, whereas the reverse is true for males, where high duet rates are associated with minimal investment in parental care. Previous studies on Rufous-and-white Wrens show that duets play an important role in maintaining acoustic contact (Mennill & Vehrencamp 2008), in territory defence (Mennill 2006; Mennill & Vehrencamp 2008), and in mate guarding (Mennill & Vehrencamp 2008). Together with the findings of this study, the results of cumulative investigations indicate that duets do not appear to play a strong role in Rufous-and-white Wren reproductive behaviour, but instead play a greater role in other functions.

Nest building and nestling provisioning behaviours

Male and female Rufous-and-white Wrens invest a similar level of effort in the nest building process. Rufous-and-white Wren nests are complex globular structures with tunnel entrances woven from plant fibers (Brewer et al. 2001). Nest construction typically lasts for many days and may take a week or more to complete; having both partners involved in nest construction may reduce building time and enhance reproductive opportunities. Marked variation occurs in the number of building trips to the nest per hour, with some pairs making as few as seven trips to the nest per hour, and others making as many as 49 trips per hour. This variation may reflect the quality of the nesting territory, or a pair's motivation to breed.

In contrast to the sexual equality of nest building, female Rufous-and-white Wrens contribute significantly more effort to nestling provisioning than males, providing almost twice the effort of their partners. Parental investment theory predicts that individuals should balance the costs and benefits of parental behaviour to maximize their lifetime reproductive success (Winkler 1987). In species with bi-parental care where extra-pair paternity occurs, male paternal investment may not necessarily translate into fitness benefits (Chuang-Dobbs et al. 2001); thus, males may adjust their investment in provisioning according to confidence of paternity in their brood (Westneat & Sherman 1993; e.g. Davies et al. 1992; Chuang et al. 1999; Sheldon & Ellegren 1998, but see Dickinson 2003). Because extra-pair paternity occurs in 6% of Rufous-and-white Wren broods (Chapter 2), males may similarly alter their investment in rearing young, providing an explanation for intrasexual variation present in male provisioning behaviour.

Male and female territorial behaviour in Rufous-and-white Wrens may provide an alternative explanation for the female-biased effort seen during nestling provisioning. A study of seasonal variation in Rufous-and-white Wren vocal behaviour showed that male song rate was significantly higher during the nesting season than at the onset of breeding, whereas females showed the reverse pattern (Topp & Mennill 2008). At the time that females are incubating or provisioning nestlings, male Rufous-and-white Wrens may take on an expanded role in singing to defend territories. Playback studies have shown that although both sexes respond to simulated intruders, males respond more aggressively and more frequently than females, supporting the idea that males play a more active role in territory defence (Mennill 2006). Furthermore, male song has been shown to be inversely related to parental care in a number of species (e.g. Grieg-Smith 1982; Dittami et al. 1991; Hoi-Leitner et al. 1993), such that males with high singing rates do not invest as much in parental activities. Because male song output is elevated during the nesting season in Rufous-and-white Wrens, intersexual differences in territorial behaviour may account for divergent patterns of investment in provisioning between the sexes.

I found no relationship between partnership length and investment in nest building or nestling provisioning for either sex. Interestingly, the age of the male partner was related to effort invested in nest building, such that older males provided a significantly greater proportion of the relative effort during nest construction. Nest building has been shown to be a costly activity for female birds (e.g. Tomás et al. 2006). If this is true for Rufous-and-white Wrens, females may benefit from pairing with older males. Further study on the energetic demands of nest building and nestling provisioning activities in Rufous-and-white Wrens would be informative.

Reproductive synchrony hypothesis

The reproductive synchrony hypothesis (Dilger 1953) suggests that duets function to coordinate reproductive activities between pair members in environments with low seasonality (Sonnenschein & Reyer 1983). I predicted that if duets coordinate reproduction in Rufous-and-white Wrens, pairs that duct at high rates during the pre-breeding season would commence breeding earlier than pairs that duct at low rates. However, I found no relationship between any measure of duetting behaviour (total number of male- or female-created duets per hour, or male and female duet responsiveness) and first egg laying date, suggesting that duets do not facilitate earlier reproduction in Rufous-and-white Wrens. A previous study of birds in my study population showed high levels of duetting just prior to nesting and again following nest predation events (Topp & Mennill 2008). Rather than being associated with synchronizing reproductive activities, these peaks in duetting behaviour may be associated with increased importance of song in territory defence while pairs are choosing a new nest site, or an increased need for song in mate guarding prior to egg laying.

A future test of the reproductive synchrony hypothesis could evaluate whether pairs that start performing duets earlier in the pre-breeding season start to breed earlier. This would require thorough recordings for several months prior to breeding (recording efforts in this study were concentrated in the month preceding breeding and the first months of the breeding season, and do not extend far into the pre-breeding season). In addition, studies examining endocrine changes associated with duetting behaviour would help to elucidate a mechanism for this hypothesis. For this hypothesis to hold, the phenology of increases in duet frequency should immediately precede the phenology of

changes in reproductive hormone levels. The hormonal profile associated with duetting has only been examined in a single species, the Slate-coloured Boubou (*Laniarius funebris*). Female participation in duetting was correlated with plasma testosterone levels, but not with reproductive hormones (Schwabl & Sonnenschein 1992). Duets therefore do not appear to play an important role in achieving reproductive synchrony in the Slate-coloured Boubou, and the results of this study suggest a similar pattern in Rufous-and-white Wrens.

Paternity guarding hypothesis

Previous genetic analyses (Chapter 2) demonstrate that Rufous-and-white Wrens follow a mixed reproductive strategy, where a minority of nests (6%) include young sired by males in neighbouring territories to the cuckolded male. In this study, I found no relationship between male duet responsiveness prior to egg-laying and his partner's mixed reproductive strategy. Although my sample size is small (n=3 broods with mixed paternity), this result suggests that males do not duet with their partner in an attempt to guard paternity in their subsequent clutch. Similarly, I found no relationship between female duet responsiveness and reproductive strategy, suggesting that a female's propensity to duet is not indicative of her subsequent mate choice decisions. My findings contribute to a growing number of studies that do not support the paternity guarding hypothesis (reviewed in Hall 2009), and existing evidence in support of the hypothesis is indirect.

A critical prediction of the paternity guarding hypothesis is that male duet responsiveness rates should be highest during the female fertile period (Hall 2004). This prediction is based on the assumption that female song during the fertile period is

attractive to extra-pair males (Levin 1996). Male Rufous-and-white Wrens show their highest levels of duet responsiveness during the female fertile period, which has previously been suggested to indicate that duets may play a role in paternity guarding (Topp & Mennill 2008). However, female Rufous-and-white Wrens initiate relatively few songs during this period (Topp & Mennill 2008), which suggests they may not be attempting to attract extra-pair mates. Therefore, heightened male duet responsiveness during the fertile period may serve a more general mate guarding function, as opposed to a paternity guard. Findings from a playback study of Rufous-and-white Wrens support the idea that duets act as a mate guard; males responded most aggressively to simulated male intruders during the early breeding season (Mennill 2006). Although partnerships often last multiple breeding seasons, divorce is relatively common in my study population, and often occurs following nest failure at the onset of a new breeding attempt (unpublished data). Taken together with the results of the current study, I suggest that male Rufous-and-white Wrens are highly responsive to their partner's songs during her fertile period, not because of the threat of lost paternity, but perhaps because of the threat of partnership loss due to divorce in an environment with extensive nest predation.

Signalling quality hypothesis

Smith (1994) predicted that an individual's duetting behaviour might provide information to partners about their quality, such that attentive individuals (i.e. high-quality birds) would duet more and have higher responsiveness than less attentive individuals (i.e. poorer quality birds). Individual quality may be reflected in many different attributes including body condition, immunocompetence, or parasite load. Since individual quality should ultimately correspond to variation in reproductive success, I

predicted that if duetting behaviour signals individual quality in Rufous-and-white Wrens, birds with high duet responsiveness rates would have larger clutch and brood sizes. Contrary to my prediction, I found no relationship between male and female duet responsiveness and clutch or brood size, suggesting that Rufous-and-white Wrens do not use duets to signal information about individual quality. This is the first study to examine relationships between duet responsiveness and measures of reproductive success in a duetting species.

Signalling commitment hypothesis

Based on the signalling commitment hypothesis (Wickler 1980), I predicted that if duets signal commitment level to a breeding partner, birds that duet often and are highly responsive to their partner's songs should invest more effort into nest building and nestling provisioning. During nest building, the number of male-created duets per hour showed a significant positive relationship with relative effort invested by the male's partner, whereas male duet responsiveness showed a marginally significant relationship in the same direction. In other words, males that performed duets at a high rate prior to egg laying invested significantly less effort into the nest construction process, in direct contrast to my prediction. For nestling provisioning, I found no significant relationships between either measure of male duetting behaviour (male-created duets per hour, duet responsiveness) and relative effort invested by the male's partner, although male-created duets per hour showed a marginally significant relationship where males who duetted at high rates contributed less to nestlings. Together, these results strongly suggest that male Rufous-and-white Wren vocal behaviour does not reveal information about how much investment he will make to future reproductive activities; instead, vocal output appears to

reveal how little parental effort he will provide. Consequently, my results for male Rufous-and-white Wrens do not provide support for the signalling commitment hypothesis for duet function.

Female Rufous-and-white Wrens showed an opposite pattern to males. During nest construction, neither female duet responsiveness nor female-created duets per hour were related to female investment in nest building, although number of female-created duets per hour showed a marginally significant relationship where females who duetted at higher rates contributed more to nest building. For nestling provisioning, I found no relationship between female duet responsiveness and female relative effort invested. However, the number of female-created duets per hour showed a significant positive relationship with female provisioning effort, such that females who duetted more with their partner prior to breeding provisioned their nestlings at higher rates. Together, these results suggest that female duetting behaviour in Rufous-and-white Wrens reveals information about future parental investment to their partner, and lends support to the signalling commitment hypothesis for duet function. This finding is remarkable, considering that recordings included in the analyses were collected prior to the breeding season, often weeks before breeding commenced. Thus, females began advertising their parental ability well in advance of breeding. To our knowledge, this is the first study in duetting birds that has shown that participation in duets may signal female parental ability, much as male song has been shown to function in male temperate passerines (e.g. Greig-Smith 1982).

Summary

Analyses of data on duetting and reproductive activities collected over an eight-year period do not provide strong evidence linking duets and reproductive behaviour in Rufous-and-white Wrens. Previous research demonstrates that Rufous-and-white Wren duets play an important role in maintaining acoustic contact (Mennill & Vehrencamp 2008), in territory defence (Mennill 2006), and in mate guarding (Mennill 2006; Topp & Mennill 2008). This study shows that duets signal female willingness to invest in parental activities, but do not appear to be important in synchronizing reproduction, guarding paternity, or signalling quality to a mate. This study is one of the first to examine relationships between vocal duetting and reproduction using a comprehensive long-term dataset. More data on the social systems of duetting birds and parental care in duetting species are needed in order to better understand the function of duetting behaviour and shed light on the behavioural ecology of poorly studied tropical animals.

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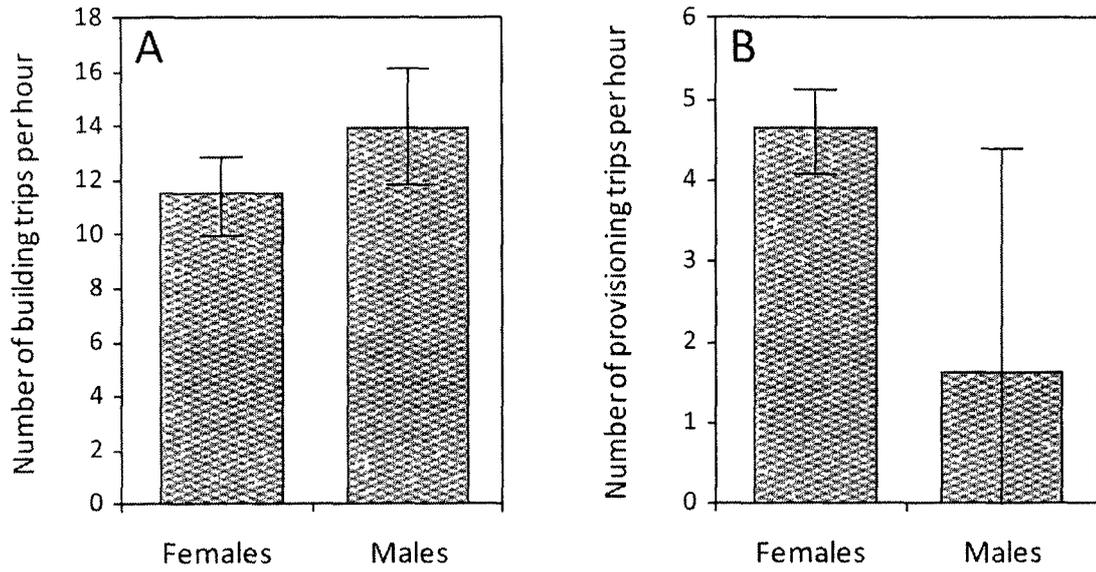


Figure 3.1. Trips to nest per hour (mean \pm SE) by male and female Rufous-and-white Wrens during (A) nest building (n = 20) and (B) nestling provisioning (n = 23).

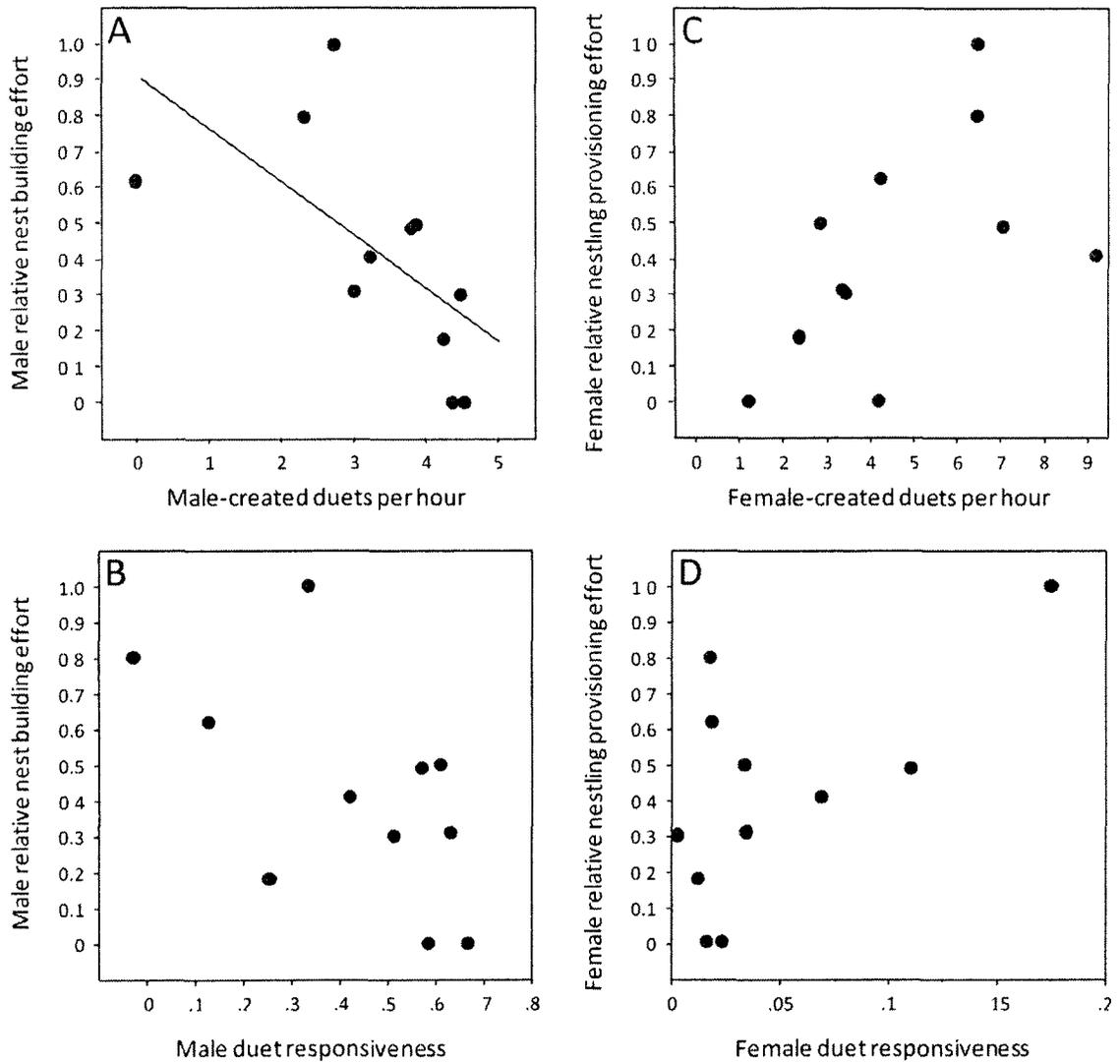


Figure 3.2. Relationships between male duetting behaviour (A, B) and female duetting behaviour (C, D) versus female relative nest building effort for Rufous-and-white Wrens. Regression lines are shown only for significant relationships.

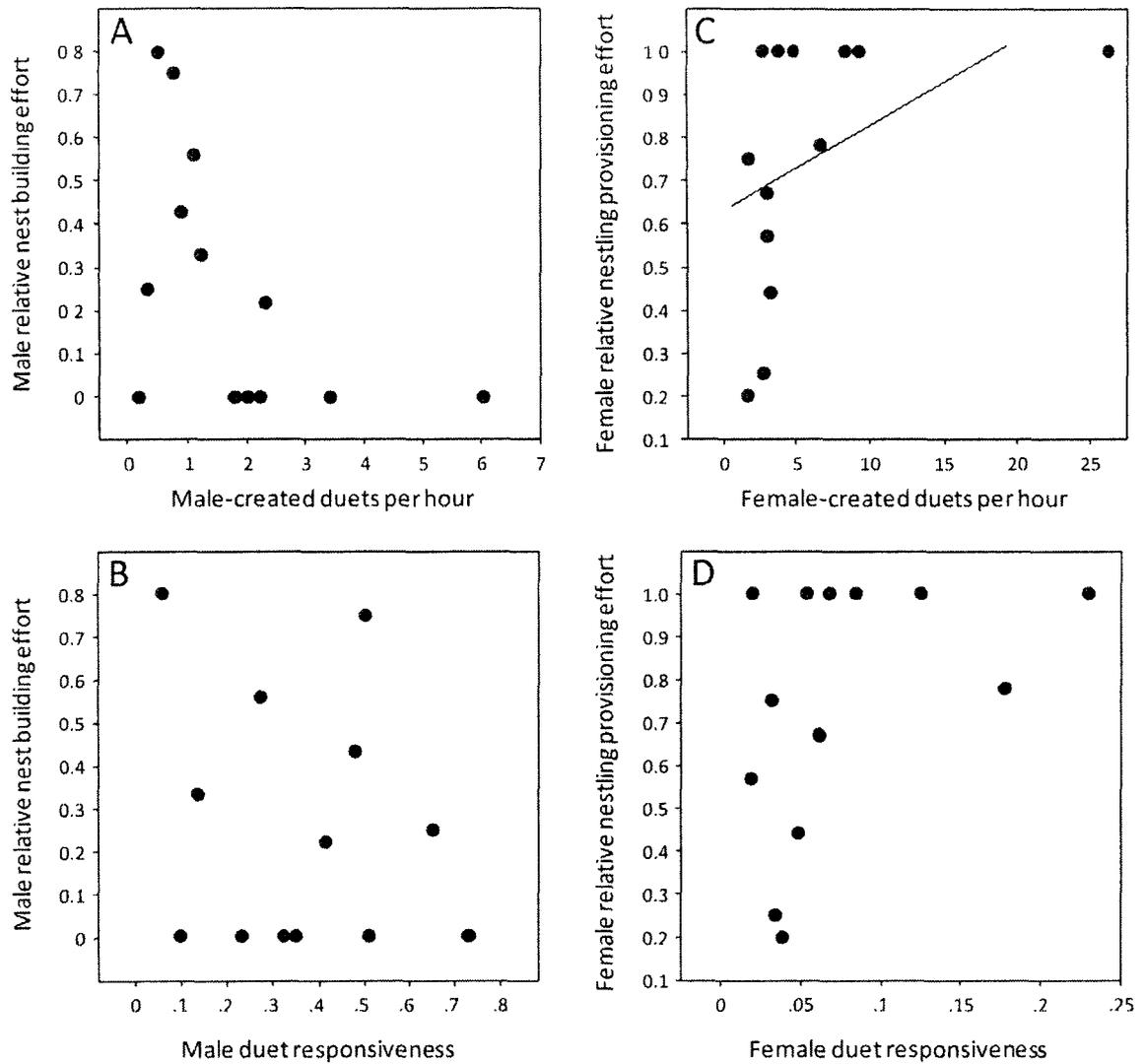


Figure 3.3. Relationships between male duetting behaviour (A, B) and female duetting behaviour (C, D) versus female relative nestling provisioning effort for Rufous-and-white Wrens. Regression lines are shown only for significant relationships.

Thesis summary

In many tropical animals both sexes are elaborately ornamented, a pattern not usually seen in temperate ecosystems. In the north-temperate zone, elaborate vocalizations are produced largely by males, but in tropical and south-temperate ecosystems both sexes produce complex acoustic displays. In species where both sexes sing, animals may combine their songs in duets, acoustic displays where two animals vocalize in a coordinated fashion. Duetting behaviour is poorly understood, in part because species that engage in these displays are concentrated in dense tropical habitats where field research is logistically challenging and where duetting animals are difficult to observe. In this thesis, I explored relationships between vocal duetting and reproductive behaviours in the Rufous-and-white Wren (*Thryothorus rufalbus*), a neotropical songbird where both sexes sing solo songs and where breeding partners coordinate their songs in vocal duets.

In Chapter 2 I described the previously unknown genetic mating strategy of the Rufous-and-white Wren (*Thryothorus rufalbus*). Based on eight years of data from a colour-banded population in northwestern Costa Rica, I analyzed observations of 176 breeding pairs and genetic samples from 51 broods, making this one of the most comprehensive long-term paternity studies in tropical birds to date. Microsatellite paternity analysis using four variable loci revealed that Rufous-and-white Wrens follow a mixed reproductive strategy, where extra-pair young are found in a small minority of broods. Three of 158 nestlings (2%) were the result of extra-pair fertilizations. Three of the 51 broods (6%) contained one nestling with allelic mismatches to their social father at two of the four loci. I assigned paternity to two of the three extra-pair young; both extra-

pair nestlings were a genetic match to an adult male in an adjacent territory. The nests of the two extra-pair sires and the nests containing the extra-pair young were separated by distances of 25 m and 150 m. The extra-pair paternity estimates found were lower than the average reported across north-temperate socially monogamous passerines (average rate: 11.1% of offspring, 18.7% of broods). However, extra-pair paternity in Rufous-and-white Wrens occurs at similar rates to two closely related Neotropical wrens, suggesting that low levels of extra-pair paternity may be common among neotropical *Thryothorus* wrens. In general, more studies of the genetic mating systems of tropical birds are needed in order to understand the role that extra-pair paternity plays in sexual selection in birds.

In Chapter 3 I explored relationships between singing and reproductive behaviours in Rufous-and-white Wrens. First, I described Rufous-and-white Wren parental behaviours by comparing male and female investment in nest building and nestling provisioning activities. Very few detailed studies on colour-banded populations of neotropical birds exist, and knowledge of the relative contribution of males and females in parental care activities is rudimentary. Second, I used eight years of field data to test predictions of four hypotheses that attempt to explain duets in the context of reproductive behaviour. The results of this study did not provide evidence of a strong relationship between duetting and reproduction in Rufous-and-white Wrens. My findings provide no support for predictions of the reproductive synchrony, paternity guarding, and signalling quality hypotheses for duet function, and provide mixed support for predictions of the signalling commitment hypothesis. In Rufous-and-white Wrens, female duetting behaviour appears to play a role in signalling willingness to invest in future reproductive activities to a partner. Previous studies on Rufous-and-white Wrens show that duets play

an important role in maintaining acoustic contact, in territory defence, and in mate guarding. Together with the findings of this study, the results of cumulative investigations on this species do not suggest that duets play a strong role in reproductive behaviour, but likely play a more prominent role in other functions.

More studies are needed on extra-pair paternity rates in duetting birds, as the genetic mating strategy of only four species (including this study) are currently known. These four studies suggest that extra-pair paternity rates are low among duetting species. If this holds true as the extra-pair paternity rate is determined in more duetting animals, researchers may need to revisit the hypotheses for duet function in light of this fact. Since extra-pair paternity is common in most bird species, low levels of extra-pair paternity in duetting species would provide insight into ecological and behavioural factors influencing interspecific variation in extra-pair paternity. More data on divorce rates and partner switching may provide additional insight into female mate choice and reproductive decisions in duetting birds. The *Thryothorus* wrens provide an interesting taxon for future phylogenetic analyses of the relationship between extra-pair paternity and vocal duetting, since this genus contains both duetting and non-duetting species. Genetic markers have been used to study four members of the genus, which should facilitate paternity analyses in the remaining species. Acoustic analyses have been conducted for most species in the genus, so acoustic data are readily available. This being said, future studies should also expand comparisons to other duetting groups, possibly including the African bush-shrikes or the babblers, to test whether the patterns seen within *Thryothorus* are more widely spread.

More information is needed on the relative investment of male and female partners during parental care in tropical birds in general, and in duetting species in particular. Although duets do not appear to be important in reproduction in Rufous-and-white Wrens, we will not fully understand the prevalence of duetting in socially monogamous species without at least a rudimentary understanding of the natural history and reproductive biology of these animals. This study revealed that effort in parental activities varied between the sexes, where males participated in both nest building and nestling provisioning, but investment in provisioning was highly variable. Future studies should explore male and female investment in parental care behaviours in other tropical duetting species to determine if this pattern is widespread.

The majority of research on acoustic communication has focused on temperate animals where typically only the male partner sings. In order to understand the prevalence of elaborate vocal displays in both sexes, more information is needed on the ecology and evolution of these ornaments in tropical animals. The results of this thesis suggest that vocal behaviour does not play a predominant role in the reproductive behaviours of Rufous-and-white Wrens, but that duets play more important roles in other functions such as territory defence, acoustic contact, and mate guarding. My research demonstrates that there is much to learn from studying both the vocal behaviour and reproductive activities of tropical animals, and that research on tropical animals can be a rich and rewarding area of investigation.

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