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Cooperative Functions of Duetting Behaviour in Tropical Wrens

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COOPERATIVE FUNCTIONS OF DUETTING BEHAVIOUR IN TROPICAL WRENS

By

KRISTIN ASHLEY KOVACH

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2013

Cooperative Functions of Duetting Behaviour in Tropical Wrens

by

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Declaration of Co-Authorship

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

I am the sole author of chapters 1 and 4, and the principal author of chapters 2 and 3. Both chapters 2 and 3 were conducted under the supervision of Dan Mennill (University of Windsor) who contributed input on the experimental design, analyses, and writing of the manuscripts, as well as logistical and financial support for field research; he shares authorship on both chapters 2 and 3. In addition, chapter 2 involved collaboration with Sandra Vehrencamp (Cornell University) and Michelle Hall (University of Melbourne), who provided input with playback stimulus preparation, experimental design, and editorial input; they share authorship on chapter 2. In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation were performed by myself, with input from my collaborators.

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-authors to include the above materials in my thesis.

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

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Abstract

Vocal duets occur when two breeding partners coordinate their songs into a joint display. Duetting serves functions both within and between pairs, and functionality is often context dependent. I explore the function of temporal coordination of male and female songs into duets, testing the hypothesis that coordinated duets are more threatening territorial signals than poorly coordinated duets or solos in three closely related species of wren. Results indicate that birds respond with similar levels of physical aggression to all three levels of coordination; however, they sing more duets in response to both categories of duets. I also explore duets and other vocalizations as they are used during breeding, testing the hypothesis that duets play a role in coordinating nest visitation. Contrary to my predictions, the birds sang the most duets during the incubation stage. My results suggest that duets are used for both territory defence and communication at the nest.

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Chapter 1

General Introduction

Animal Communication and Birdsong

Animals use a variety of signalling modalities to communicate information about themselves and their environment. The ability of animals to transmit signals between a sender and receiver, as well as to other animals in the vicinity, allows them to incorporate new information and to optimize decision making (Bradbury & Vehrencamp 2011). Animals can use information gained from a signaller to inform decisions about predation, foraging, and mating; as such, signalling is shaped by both natural and sexual selection. Signals may take many forms: they can be chemical, tactile, visual, electrical, or acoustic. Acoustic signalling is transitive, immediate, and long-ranging, and without the necessity of visual or tactile contact, acoustic signals are an ideal modality for communication over long distances or in dense environments. No other acoustic modality is better-studied than vocal behaviour (Catchpole & Slater 2008), which involves passing air over membranes in the throat. Birdsong has long been considered a model system for vocal communication (Bradbury & Vehrencamp 2011).

Birdsong is a multi-functional signal, used primarily for both mate attraction and territory defence (Catchpole & Slater 2008). Most birds hold territories during some or all of the year, and singing is an important way for birds to establish territory boundaries and defend valuable resources. In fact, song alone is enough to repel intruders from an unoccupied territory for a short amount of time (Krebs 1977). Additionally, female birds are attracted to male song (Eriksson & Wallin 1986) and can choose males based on the quality of their vocal signals (Vallet & Kreutzer 1995, Vallet *et al.* 1998). These functions are well-studied in north temperate birds, but much less is known about the functions of birdsong in the tropics (Slater & Mann 2004). Tropical birds typically hold territories throughout the year, rather than during a brief breeding season, and consequently experience different selective pressures on both their territorial and mating behaviour (Slater & Mann 2004). Perhaps correlated with the tendency for pairs to occupy territories year round, tropical birds are much more likely to exhibit female song in addition to male song, a characteristic that is quite rare in north temperate birds (Langmore 1998). The functions of female song are not well-understood; historical research has overlooked this largely tropical phenomenon, even suggesting that it may be a functionless behaviour in spite of very little research (Langmore 1998). More recent studies, however, consider female song to be an important behaviour in the ecology and evolution of birds, often in a similar manner as male song (e.g. Garamszegi *et al.* 2007). In this thesis, I explore the communicative functions of the songs of male and female tropical wrens (Family Troglodytidae), motivated by an interest in understanding the ecology and evolution of song in both sexes.

Vocal Duets

In a subset of species where both sexes sing, males and females coordinate their songs into vocal duets. Duetting is defined as the coordinated singing by two individuals of a mated pair so that their phrases alternate or overlap (Hall 2009). Duetting has evolved independently in many distinct phylogenetic groups, indicating that selection for the behaviour may be particularly strong (Hall 2004). Vocal duetting is relatively common in birds, with well over 200 species of birds producing some form of vocal duetting behaviour (Farabaugh 1982). The functions of duetting behaviour have become an important area of investigation, yet they remain poorly understood, often because it is difficult to tease apart predictions of various, non-mutually exclusive hypotheses (Hall 2009). Additionally, many studies report multiple functions of duets (e.g. Grafe & Bitz 2004, Benedict 2010) or reveal that some functions are context dependent (Mennill & Vehrencamp 2008). Hypotheses considering the functions of duets are often grouped into two categories: cooperative and competitive (Hall 2004). Cooperative functions of duets increase the fitness of both birds in the duetting pair. These functions include joint resource defence, pair bond maintenance, recognition and contact, and ensuring reproductive synchrony, among other cooperative functions that have received less empirical support. Competitive functions of duets increase the fitness of only one duetting pair member, often at the expense of the other, and include mate guarding and paternity guarding. Below are brief descriptions of each of these hypotheses for the function of duets.

Joint Resource Defence*.* This hypothesis suggests that pairs sing duets to defend resources and territories from conspecific rivals (Seibt & Wickler 1977). Resource defence is often considered a primary function of duetting, as duet songs mirror individual songs of north temperate birds in being loud, easy to locate, performed at territory boundaries, and used in interactions with neighbours (Hall 2009). Several studies reveal that pairs respond to playback by increasing duetting rates, indicating that duets are used in territory defence (reviewed in Douglas & Mennill 2010). Some evidence suggests that duets are more threatening than solo songs (e.g. Hall 2000, Molles & Waas 2006); however, many studies also find that birds exhibit similar levels of aggression to both duets and solo songs (e.g. Appleby *et al*. 1999, Grafe & Bitz 2004, Fedy & Stutchbury 2005, Mennill & Vehrencamp 2008, Bradley & Mennill 2009).

Pair Bond Maintenance*.* This hypothesis suggests that male and female birds strengthen and maintain their partnership by duetting with one another (Armstrong 1947). The strongest support for this hypothesis comes from research on primates, showing positive correlations between duetting behaviour and affiliative pair bond behaviours such as grooming in siamangs (*Hylobates syndactylus*; Geissman & Orgeldinger 2000). Additionally, duets are used as a mechanism to coordinate behaviours in sportive lemur pairs (*Lepilemur edwarsii)*; the pair is more likely to switch behaviours after performing a duet (Mendez-Cardenas & Zimmerman 2009).

Mate Recognition and Acoustic Contact*.* This hypothesis suggests that birds provide cues to their identity and location on the territory by performing duets (Cobb 1897). Birds are more likely to approach one another following duets (Logue 2007a, Mennill & Vehrencamp 2008), supporting the hypothesis that duets are used to maintain acoustic contact in the thick vegetation of the tropics. Logue suggests that mate recognition is important in monomorphic species in order to prevent accidental aggression toward the mate, particularly during interactions with territorial rivals (Logue 2007b).

Ensuring Reproductive Synchrony*.* This hypothesis suggests that birds duet to synchronize their reproductive physiology, ensuring that both birds are fertile during the same time period (Armstrong 1947). Song can stimulate reproductive physiology and nesting behaviour in birds (Brockway 1965), and tropical environments often have fewer seasonal cues by which birds can stimulate their reproductive systems (Slater & Mann 2004). Pairs of robin-chats (*Cossypha heuglini*) did not initiate nesting behaviour when experimentally prevented from duetting, providing further support for this hypothesis (Todt & Hultsch 1982).

Mate and Paternity Guarding*.* In contrast to the aforementioned cooperative functions of duetting, mate guarding and paternity guarding are considered competitive functions of duetting. The paternity guarding hypothesis suggests that males participate in duets in order to advertise the mated status of their partner, driving away other males and thus opportunities for their partner to participate in extrapair copulations (Sonnenschein & Reyer 1983). The mate guarding hypothesis suggests that birds of both sexes perform duets to advertise the status of their mate, but it suggests that the bird is guarding the mate from being usurped by other individuals, rather than merely guarding the paternity of the nest (Stokes & Williams 1968).

Duetting and Temporal Coordination

Temporal coordination is a defining feature of duets; without synchrony in timing, each bird's vocalization would be broadcast as a solo song (Hall 2004). In order to achieve temporal coordination, pair members must be attentive to one another and willing to respond (Hall 2009). Birds that are more attentive may be able to produce a faster response, and if this attentiveness is sustained over time, a more precisely coordinated duet, than birds that are not attentive to their partner. Evidence suggests that birds do base their timing on their partners. Black-bellied wrens (*Pheugopedius fasciatoventris*) base the timing of each phrase off of their partner's previous phrase rather than off of an internal tempo (Logue 2007b). Additionally, plain-tailed wrens (*Pheugopedius euophrys*) leave longer silent spaces between their notes if they are singing solo than they do if they are duetting, indicating that timing between notes is not based solely on a fixed action pattern (Fortune *et al*. 2012). Because partners do attend to each other in creating a duet, timing of duets could be a signal of attentiveness or commitment to the pair bond.

Precise coordination of vocal signals could also serve as a signal to neighbouring pairs, revealing information about how well the pair coordinates their behaviours. Male Australian magpie-larks respond to playback of precisely coordinated duets with more independent songs than they do to poorly coordinated duets (Hall & Magrath 2007), suggesting that highly-coordinated duets are a more intense signal between duetting territorial pairs. In addition, birds that had been together longer sang more highly coordinated duets than newly established pairs, indicating that it may take learning or practice to achieve precise coordination. These findings suggest that temporal coordination can act as an honest signal to other pairs of pair longevity, and in doing so could signal a pair's motivation or ability to act cooperatively.

Duetting behaviour is highly variable across animals in how often and how precisely the two animals coordinate their vocalizations (Hall 2009). The precision of temporal coordination in birds' songs is dependent on the species, the distance between partners, and the pair (Hall 2009). Duets that are composed of rapidly alternating songs, where the male and female alternate contributions without overlapping (or doing so excessively) are called "antiphonal duets". These duets are so well coordinated that they may be mistaken for the songs of one individual (Mann *et al.* 2009). Other birds loosely associate their songs with one another, where the songs may alternate or overlap, but they tend to be produced together. This type of duetting behaviour is called "polyphonal duetting." In most non-duetting species, the female simply does not sing; however, in a few species, both males and females sing, but they do not coordinate their songs into duets. Understanding why some species coordinate their songs into duets and some do not could provide insight into the functions and evolution of duetting behaviour.

Parental Care

Birds exhibit a wide range of parental care behaviour, ranging from those that leave their eggs and provide no care, to those with nestling periods of up to 303 days (Gill 2007). The amount of parental care necessary depends on the initial developmental state of the hatchling, which ranges from altricial nestlings (blind, naked, and helpless at hatching) to precocial (hatchlings that leave the nest immediately and follow their parents). All songbirds produce altricial young, which require substantial parental care. The nestlings rely on parents for food, thermoregulation, maintenance of the nest environment (e.g. clearing of waste and parasites), and protection. For adult birds, chicks are a liability, creating increased predation risk and increasing physiological stress on their parents (Trivers 1985). Caring for eggs and young requires a substantial commitment of time and energy, and birds must balance the costs of these behaviours with investment in their own survivorship (Gill 2007). Because raising young is one of the most energetically expensive times of a bird's annual cycle, pairs of some species have developed ways to share the costs of reproduction. Biparental care helps parents to accommodate demands of nestlings as well as maintain their own body condition, and seems to be a common solution to parental challenges, with 81% of species inferred to be using this strategy (Cockburn 2006). All songbirds exhibit biparental care, including all species of wrens.

Rufous-and-white wrens (*Thryophilus rufalbus*), a focal species in both data chapters of this thesis, share parental care duties between the male and female during most stages of the breeding cycle. Both the male and female help to construct a complex nest structure that is globular in shape and has a downward sloping entry tunnel. They create these nests by weaving together sticks and fungal rhizomes, and both males and females invest similar effort into nest building (Douglas 2011). Once the nest is complete and rainy season has begun, females begin to lay eggs. Only the female incubates the eggs, and while she does this, the male builds secondary nests that may be used for re-nesting later in the season. The incubation stage lasts for 16-18 days before the hatchlings emerge (Douglas 2011). The male and female share nestling provisioning duties; however, females invest much more in provisioning than do males, typically giving three times the effort (Douglas 2011). Observations in the field suggest that males may provide the care for fledgelings while females begin a second clutch (Douglas 2011).

Shared effort in parental care requires that birds assess the needs of their young as well as the behaviour of their partner in order to provide optimal care for the young while maintaining their own condition. Activity at the nest can draw attention to the nest, increasing the risk of predation; in fact, survivorship increases as parental activity at the nest is reduced (Martin *et al.*2000). This means that there is an optimal level of parental visitation to a nest in order to minimize the risk of predation and maximize the food intake of the offspring (Conway & Martin 2000). Predation risk is generally higher in tropical environments (Slater & Mann 2004), and is a significant factor in the survivorship of nestlings and eggs in my study population. For this reason, it would be useful for parents to communicate information about predation risk or the condition of the offspring before visiting the nest. Birds can communicate information about predation risk in other contexts (e.g. Templeton *et al*. 2005), and communication of this information between pair members near or at the nest may be particularly useful.

Vocal Behaviour during Parental Care

Rufous-and-white wrens build globular, opaque nests that are closed to the outside environment. This type of nest is advantageous because the nest architecture makes eggs invisible to predators (e.g. monkeys, snakes, other bird species), and may help protect against fluctuations in temperature (Gill 2007). However, this closed nest renders the parent and offspring blind to the outside environment and possible risks as they leave the nest. Vocal communication between a bird on the nest and a bird outside could provide information about environmental risks, allowing the bird on the nest to make a decision about exit that could affect its survivorship or that of the nest. Additionally, communication could provide information as to the state of the nestlings or the timing of previous partner visits, minimizing the risk of excess parental activity at the nest.

Few studies have directly investigated the role of vocal behaviour in coordinating parental activities around the nest (e.g. Halkin 1997, Ritchison 1983), but these studies indicate that vocalizations may be important. Northern cardinal (*Cardinalis cardinalis*) females use vocalizations on the nest to signal the male to either approach or avoid the nest (Halkin 1997). In this way, the female can protect the nest from becoming conspicuous by warning off the brightly coloured male if there are nearby predators. Some species with closed nests may be able to communicate in an opposite fashion, warning the bird inside of possible threats outside the nest. For example, zebra finches (*Taeniopygia guttata*) that nest in boxes produce quiet vocalizations at the nest, termed "private vocal duets," that may function in mate recognition, pair bond maintenance, and communication of threats in the external environment (Elie *et al.* 2010). Slate-coloured boubous (*Laniarius funebris*) use duets to coordinate incubation relief (Sonnenschein & Reyer 1983). Male black-headed grosbeaks (*Pheuticus melanocephalus*) also sing while incubating, both in response to neighbouring males and to signal to the mate that they are leaving the nest, whereas females rarely sing while incubating (Ritchison 1983). They also use songs in later stages of the breeding cycle in order to elicit begging calls from their young, allowing the parents to localize fledgelings and maintain the family group (Ritchison 1983). Female red-winged blackbirds (*Agelaius phoeniceus*), respond to male song from the nest with *chit* calls that encourage male parental care and nest defence (Yasukawa 1989). Though they are relatively uncommon (Leonard 2008), vocalizations from the nest exhibit a variety of ecological functions.

Study Species

Wrens (Family: Troglodytidae) are a group of monomorphic, plain-coloured birds well-known for their remarkable singing abilities (Brewer 2001). They range in colours of brown, rufous, gray, black, white, and beige, and are often barred, streaked, or spotted (Stiles & Skutch 1989). They are largely insectivorous and live in almost any habitat, most often remaining low in the vegetation or leaf litter. They are usually highly active but difficult to observe, with unexpectedly loud voices that seem to "dominate the soundscape" (Brewer 2001, Kroodsma & Brewer 2005). Tropical species often live in pairs year round. Most wren species build closed, globular nests with side entrance tunnels, and only the females incubate. Wrens use a large variety of calls, including *ticks, rattles, hoots,* and *whoops.* Within this group, there is a wide variety of female singing behaviour, ranging from a complete lack of female song, to female voices that are similar to males (Mann *et al*. 2009). My thesis focuses on three related species of wrens, chosen because they exhibit interesting differences in their singing and duetting behaviour (see figure 1.1).

Banded Wrens (Thryophilus pleurostictus)

Banded wrens have reddish brown backs and white on the cheeks and underparts. Their name comes from the heavy black barring on the flanks, and the tail also shows subtle dark barring. They are fairly common throughout their range from central Mexico to northwest Costa Rica on the Pacific slope (Brewer 2001). Banded wrens prefer dry forest habitats, and are often seen in open, thorny habitat foraging near the ground. They nest in bull's horn acacias (*Acacia cornigera*) and spiny palms (*Acrocomia sp.*), building a globular nest with a long entrance tunnel.

Males and females both sing, though their songs are only loosely associated, and they are not considered to be duetters (Mann *et al* 2009). Male song is very loud and frequency modulated, including a variety of different syllable types within each song. Female song is similar to that of males, but is often quieter and less stereotyped (Molles & Vehrencamp 1999). The male often sings from a high, conspicuous perch. Much research has been done on the characteristics and function of male song in the banded wren. Both banded wren songs and rufous-and-white wren songs generally begin with a few quiet introductory notes, often *clicks*, *whistles*, and *hoots,* then consist of a series of repeated notes in a trill or *rattle*, and are terminated by a single inflected note that is louder, higher pitched, and covers greater frequency bandwidth than the rest of the song (Mann *et al.* 2009). Males have large repertoires of about 20 songs, and they sing these in serial mode, switching song types nearly every song, though they switch less during aggressive encounters (Molles & Vehrencamp 1999, Molles 2006). Overlapping seems to be associated with earlier retreat, suggesting that it is a withdrawal signal, and song matching seems to be a signal of aggression (Vehrencamp *et al.* 2007). Male birds seem to distinguish between neighbours and strangers (Molles & Vehrencamp 2001), and they may also discriminate between young and old individuals based on their trill consistency, which increases with age (de Kort *et al.* 2009b). Different structures within the songs are used preferentially in different contexts; for example, when females are present, males sing songs with fewer *rattles* and *buzzes* and sing more compound songs (Trillo & Vehrencamp 2005). Banded wrens approach low and average performance songs more rapidly and remain in the area longer during playback than they do to high performance stimuli, and they seem to sing more in response to average songs than to low and high performance songs (de Kort *et al.* 2009a).

Rufous-and-white Wrens (Thryophilus rufalbus)

Rufous and white wrens are rufous above with white underparts. They are very similar in appearance to banded wrens, but have buffy flanks that lack barring and a more rufous tint to the plumage on their back. Rufous-and-white wrens inhabit mature, humid gallery forests, where they forage on the ground in the leaf litter. They are moderately abundant in these habitats from southern Mexico to Panama and in northern Colombia and Venezuela (Brewer 2001). Like banded wrens, they build globular nests with tunnel entrances in bull's horn acacias and spiny palms (Stiles & Skutch 1989). Where dry and humid forest habitats are adjacent, banded wren and rufous-and-white wren territories may abut, but they do not overlap.

Rufous-and-white wren song is tonal and flute-like, with a slower pace than songs of banded wrens. Their song is somewhat similar in structure to that of the banded wren, but the frequency is much lower, and there is less variety in syllables. Males and females both sing and coordinate their songs into polyphonal duets. Male song appears to be quieter in this species than in banded wrens. Male and female songs are similar, but female songs are often higher in frequency, quieter, and less consistent. Males sing far more often than females (Topp & Mennill 2008) and have a larger song repertoire, with an average of 10.8 songs for males and 8.5 for females (Mennill & Vehrencamp 2005). Males use repeat-mode singing, where they sing one song several times before switching, whereas females switch song types after fewer songs. Most duets are created by females responding to male song, and most female songs (about 79%) occur in duets. Pairs seem to have duet types, or duets that are consistently composed of the same male and female song types. Both male and female song output is seasonally variable, with females singing most often during pre-breeding season and less throughout the breeding season, and male song peaking slightly after females at the onset of rainy season (the fertile period). Duetting responsiveness peaks for males during the female fertile period, and female duet responsiveness is higher during pre-breeding and slightly higher after nest predation (Topp & Mennill 2008). Bachelor males sing more frequently than paired males, indicating that male song is attractive to females (Hennin *et al.* 2009). Rufous-and-white wrens usually sing at a height of about 5m, despite performing most activities, like foraging, on the ground (Barker & Mennill 2009).

In a natural context, pairs of rufous-and-white wrens sing duets farther away from territory boundaries and closer to the nest than expected by chance (Mennill & Vehrencamp 2008). They also tend to approach each other after singing a duet, with the initiator of the interaction moving further than the partner, suggesting that duets are a request for the location of the mate (Mennill & Vehrencamp 2008). Males and females respond to playback of duets by increasing their song rates and duetting rates (Mennill 2005). They respond similarly to solos and duets, and their duetting rate in response to territorial intrusion is more than five times their natural rate (Mennill & Vehrencamp 2008). The use of duets near the nest, tendency to approach each other after duetting, and increased duetting rates during territorial interactions suggest that duets are multi-functional signals for which function is dependent on the context.

Plain Wrens (Cantorchilus modestus)

Plain wrens are mostly grey on top, buffy coloured under the wing, and whitish grey underneath. Although they were once considered congeneric with banded wrens and rufous-and-white wrens in the genus *Thryothorus*, a recent reorganization of the genus has placed plain wrens in a separate genus (Mann *et al.* 2006). Plain wrens are quite common throughout their range from extreme southern Mexico to Panama on the Pacific slope (Brewer 2001). They tend to prefer mature, humid forest. They spend much of their time in the canopy, often in thick, tangled vines, but can also be seen foraging in vegetation near the ground (Stiles & Skutch 1989). Plain wrens hold smaller territories than rufous-and-white wrens, and one rufous-and-white wren territory may encompass a few plain wren territories. Plain wrens build globular nests with visor-shielded entrances, often in vine tangles (Stiles and Skutch 1989).

Plain wren songs are higher pitched, more rapid, and more frequency modulated than the other two species. They are an antiphonally duetting species, with males and females rapidly alternating phrases. Their songs usually begin with a male introductory phrase, followed by rapidly alternating female and male phrases (Cuthbert & Mennill 2007). Sometimes, however, males will join female songs to form a duet. Males sing introductory phrases in repeat mode, and most duets begin when females respond to this male phrase. Duets are sung with extreme precision so that gaps between male and female phrases are very short (on average 0.13 s), and overlapping of male and female phrases is very rare (occurring in 8/46 analyzed duets; Cuthbert & Mennill 2007). Data on duet function is lacking for plain wrens; however, previous studies of canebrake wrens (*Cantorchilus zeledoni;* the sister taxon to plain wrens) indicate that males and females sing duets for pair bond maintenance and territory defence. Like plain wrens, canebrake wrens sing with eventual variety, and song switching rates appear to have an anti-habituation function rather than signalling threat (Marshall-Ball & Slater 2004). Males and females of this species song match to playback of solos and duets, but they do not often duet match to playback (Marshall-Ball *et al.* 2006). They do seem to have sex-specific territory defence, although both birds will duet in response to intruders of either sex. While birds do not seem to improve the accuracy of their duets with increasing pair bond duration, they do become more consistent with their duet types, indicative of a possible function in pair bond maintenance (Marshall-Ball *et al.* 2006). Duet types are rarely shared with other pairs, though song sharing is common in both males and females (Marshall-Ball & Slater 2008), indicating that duet types are developed within the pair. As canebrake wrens and plain wrens are now considered separate species (Mann *et al.* 2006), more data are needed on plain wren song and function.

Thesis Goals

Vocal duets are a perplexing behavioural phenomenon, and understanding their functions and origins is the motivation of this thesis. In this thesis, I explore two hypotheses for the functions of duetting, both of which focus on the cooperative nature of vocal duets: joint territory defence and coordination of breeding behaviours. In the first data chapter of this thesis (chapter 2), I seek to determine if there is an advantage of coordinating vocalizations with a partner versus singing alone, using three species of tropical wrens that exhibit different types of duetting behaviour. In chapter 2, I examine the role that the temporal coordination of duets plays in territory defence by comparing the responses of territorial birds to highly coordinated and poorly coordinated duets, as well as alternating solo songs. A better understanding of coordination across related species could shed light on why precise coordination evolved in the first place. This chapter is written for submission to the journal Animal Behaviour. In the second data chapter (chapter 3), I explore duetting as a means of coordinating parental care behaviours by comparing duetting rates across breeding stages and nest development stages. I provide previously uncharacterized information about vocal behaviour and duetting at the nest. This chapter is written for submission to Journal of Ornithology. With these two chapters, I can examine duet functionality as a signal between pairs as well as within a pair, furthering our understanding of these multifunctional signals. Together, these two chapters investigate cooperative functions of duets in two different contexts: during territorial interactions (between pairs), and during breeding interactions (within pairs). By investigating two very different hypotheses on the function of duets, I hope to further our understanding of the context dependency and functionality of these vocal signals.

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Figures

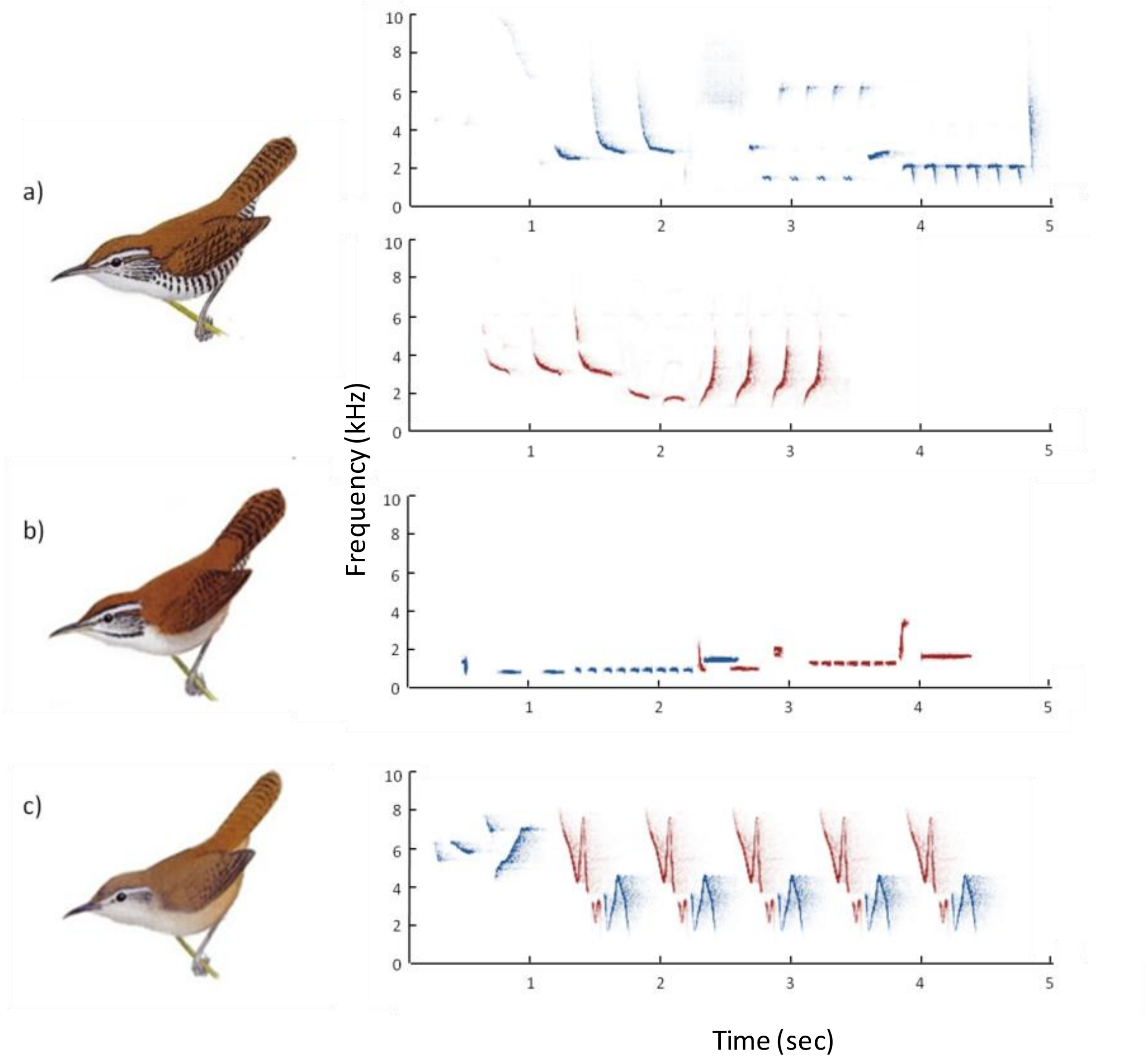

Figure 1.1. Illustrations and sound spectrograms of the three study species involved in this thesis. (a) Solo songs of banded wrens (*Thryophilus pleurostictus*; includes male song above and female below; this species does not produce coordinated duets); (b) a duet of rufous-and-white wrens (*Thryophilus rufalbus*); and (c) a duet of plain wrens (*Cantorchilus modestus*). Spectrograms have been altered to colour male contributions in blue and female contributions in red. Illustrations from Stiles and Skutch (1989).

Chapter 2

Timing isn't everything: Temporal coordination does not influence aggressive responses in three species of tropical wrens

*This chapter is the outcome of joint research

Chapter Summary

The coordinated vocal duets of tropical animals often play a role in territory defence. Because duet timing is dependent upon each animal’s attentiveness to its partner and ability or motivation to cooperate, precise coordination of duet elements could be an important signal to territorial rivals. I tested the hypothesis that highly coordinated duets are a signal of greater territorial threat than poorly coordinated duets or alternating solos in three species of territorial wrens. I chose three related species that show natural variation in the coordination of their duets: plain wrens (*Cantorchilus modestus*) are antiphonal duetters, rufous-and-white wrens (*Thryophilus rufalbus*) are polyphonal duetters, and banded wrens (*Thryophilus pleurostictus*) do not regularly perform duets. I created playback stimuli of highly coordinated duets, poorly coordinated duets, and alternating solos for each species, and played back these three conspecific treatments as well as a heterospecific control to territorial pairs of all three species. Wrens responded to all three conspecific treatments with higher levels of physical aggression than to controls; however, they did not respond differentially based on the level of duet coordination. They sang more duets in response to both duetting treatments than to alternating solos and controls, but they sang more solos in response to coordinated duets and alternating solos than they sang in response to un-coordinated duets. My results indicate that wrens respond with similar levels of physical aggression toward paired intruders regardless of their acoustic coordination; however, coordinated vocal responses are used more often in response to coordinated stimuli.

Introduction

Duets are the coordinated songs of two individuals, usually of a mated pair, such that the two animals' phrases alternate or overlap (Farabaugh 1982, Hall 2004, 2009). This phenomenon occurs in a variety of animals, including several clades of birds (Hall 2009), primates (Haimoff 1986), and insects (Bailey 2003). Because this behaviour has evolved independently in multiple phylogenetic groups (Farabaugh 1982), duetting appears to play an important role in the life histories of many species, and selection for duetting may be particularly strong (Hall 2004). In spite of a growing body of research, the ecology and evolution of duetting behaviour remains poorly understood (Hall 2009), possibly due to the multi-functional nature of duets both within and among species (Benedict 2010, Grafe *et al*. 2004, Mennill and Vehrencamp 2008). Our understanding of the functions and evolution of duets can be greatly enhanced by careful attention to the dynamics of coordination in male and female duet contributions.

Prior investigations provide support for several hypotheses for the functions of duets, and these hypotheses can largely be grouped into two categories: those in which pair members cooperate with one another for mutual benefit, and those in which pair members compete to work toward conflicting goals (Hall 2004). Cooperative functions include joint resource defence, maintaining the pair bond, and ensuring reproductive synchrony (Armstrong 1947). Competitive functions include mate guarding and paternity guarding (Levin 1996, Sonnenschein & Reyer 1983). Resource defence is a well-accepted function of male song in temperate songbirds (Catchpole and Slater 2008), where songs are predominantly performed by males alone. Duets mirror male song in that they are loud, easily localizable, performed at territory boundaries, and used in interactions between neighbouring birds (Hall 2009); for this reason, resource defence is often considered a primary function of duets (Hall 2009). Experimental research examining duetting as a mechanism for resource defence indicates that duets do function in this manner in many taxa (Douglas & Mennill 2010).

Vocal duets are relatively common among many bird species in the tropics (Farabaugh 1982), and tropical wrens, in particular, are well-known for their coordinated singing ability (Brewer 2001, Mann *et al*. 2009). Different clades of these territorial songbirds exhibit a wide variety of singing behaviour, ranging from species in which females produce no song at all, to species that produce such precisely-timed antiphonal duets that an observer may mistake the duets for songs of one individual (Mann *et al*. 2009). The “*Thryothorus* wrens” are a group of tropical birds in which duetting is common and present with highly variable degrees of temporal coordination (Mann *et al*. 2009). Formerly a single genus (*Thryothorus*), the group was recently split into four genera, each with variable degrees of duet coordination (Mann *et al*. 2006, 2009). For example, banded wrens (*Thryophilus pleurostictus*) exhibit male and female song but do not routinely sing coordinated duets (Molles & Vehrencamp 1999). Rufous-and-white wrens (*Thryophilus rufalbus*) exhibit coordinated, polyphonal duets, in which the male and female songs are loosely associated in time and often involve overlapping (Mennill & Vehrencamp 2005). Plain wrens (*Cantorchilus modestus*) sing highly coordinated antiphonal duets, where the male and female sing a series of rapidly alternating phrases with extremely small intervals of silence in between and very little overlap (Cuthbert & Mennill 2007). This remarkable variation in acoustic coordination provides an intriguing model system in which to study the ecology and evolution of temporal coordination.

Temporal coordination is a defining feature of duetting (Hall 2009), and thus, improving our understanding of the importance of temporal coordination in animal duets is likely to shed light on their function and evolution. Mechanistic studies of temporal coordination indicate that duetting birds base their timing on the immediately preceding notes of their mate (Logue *et al*. 2008, Fortune *et al*. 2012). Because temporal coordination requires a high level of attentiveness to the partner, birds that are more attentive may be able to more rapidly produce a response vocalization, and birds that sustain attentiveness may produce more precisely coordinated duets over time. Therefore, duet timing may serve as a signal of attentiveness, which may be important both to the partner and to nearby territorial rivals (Hall 2009). Male magpie-larks (*Grallina cyanoleuca*), for example, respond more aggressively to playback of highly coordinated duets than to playback of poorly coordinated duets (Hall & Magrath 2007), demonstrating that precise timing can be used as an indicator of high territorial threat. Additionally, magpie-lark pairs that have been together for longer periods sing duets that are more precisely coordinated, which indicates that precision of a duet could be dependent on learning (Hall & Magrath 2007). Together, these results suggest that duet precision may be an index of a pair's ability or motivation to act collectively, where pairs that produce highly coordinated duets may cooperate to perform other behaviours successfully as well. Precision in temporal coordination seems to be important; black-bellied wrens (*Pheugopedius fasciatoventris*) will stop their songs short when performing duets that have large gaps or overlapping phrases, indicating that birds are sensitive to their coordination (Logue *et al*. 2008). If other pairs use the duet coordination of rivals as a signal, they may be able to gain helpful information about the threat posed by an intruding pair.

In this study, I tested the hypothesis that highly coordinated duets communicate a more threatening territorial signal than poorly coordinated duets. I presented temporally altered duets, as well as alternating male and female solo songs, to pairs of three species of tropical wrens. I chose three sympatric species of wrens that vary in their natural degree of coordination: plain wrens produce highly coordinated antiphonal duets with alternating male and female contributions; rufous-and-white wrens produce less coordinated polyphonal duets with overlapping male and female contributions; and banded wrens do not routinely combine their male and female songs into duets. I predicted two possible outcomes: (1) wrens of all species would respond more aggressively to highly coordinated duets than to poorly coordinated duets and alternating solos; or, alternatively, (2) wrens would respond more aggressively to their species-typical form of song production (high coordination duets for plain wrens, low coordination duets for rufous-and-white wrens, and alternating solos for banded wrens). Because these three species of wrens have different forms of natural duets, if all three species show strong aggressive responses to coordinated stimuli, this could provide evidence of a pre-existing bias toward perceiving synchronous communication as a threat.

Methods

General Field Methods

I studied populations of three species of wrens in Sector Santa Rosa of the Guanacaste Conservation Area in northwestern Costa Rica (10^{o}, 40’N, 85^{o}, 30’W), an area that has been designated as a World Heritage Site by UNESCO (United Nations Organization for Education, Science and Culture). Playback studies were conducted from 24 April to 2 June, 2012, corresponding with the onset of the rainy season and the beginning of the breeding season (Topp & Mennill 2008). I conducted playbacks in the morning, between 06:00-11:00, when birds were most active. In total I studied 63 pairs of wrens (126 unique birds): 21 pairs of plain wrens, 22 pairs of rufous-and-white wrens, and 20 pairs of banded wrens. Rufous-and-white wrens are part of a long-term study population, and all males and most females were banded with unique colour combinations to facilitate distinction between pairs and between the sexes. Wren pairs tend to remain in stable territories throughout a season (plain wrens, approximately 400 m^2;

rufous-and-white wrens, approximately 10,000 m^2, banded wrens approximately 3,600 m^2; Cuthbert & Mennill 2007,Mennill & Vehrencamp 2005, Molles & Vehrencamp 2001). Although banded and plain wrens were not banded in this study, I ensured that I sampled unique pairs by moving at least 60 m between adjacent same-species playback territories for plain wrens and banded wrens.

Playback Treatments

To test the hypothesis that duet coordination plays a role in territorial signalling, I used a stereo playback design (Douglas & Mennill 2010) to simulate a rival pair singing inside the territory boundaries of my playback subjects. Subjects received four playback treatments: (1) highly coordinated conspecific duets, (2) poorly coordinated conspecific duets, (3) alternating male-and-female conspecific solo songs, and (4) a control stimulus of highly coordinated duets of a completely unfamiliar species (Australian eastern whipbirds, *Psophodes olivaceous*). Each subject pair received all four treatments during a one-hour playback session, which allowed us to rule out variation in responses that might arise by returning to the territory on different days (e.g. variation due to changes in breeding status, etc.). The order of the four playback treatments across sessions followed a factorial design.

Each playback stimulus was 3 min and was followed by 12 min of silence. The activity of the focal pair was observed throughout the 3 min of playback and for an additional 3 min after playback had ceased, so that I quantified responses during a 6 min window. This was followed by a 9 min recovery period, during which no data were recorded, before the next trial began. Preliminary trials confirmed that all three species left the area within a few minutes after the end of playback, convincing us that 9 min was an appropriate recovery period. I commenced the second treatment 15 min after the playback of the first stimulus began, the third treatment 15 min after that, and the fourth treatment 15 min after that. At the conclusion of the fourth treatment (i.e. 6 min from the start of the final treatment) the playback apparatus was collected and the observer left the territory. A trial was considered successful if at least one of the birds in the pair was seen or heard within 10 meters of the speakers for at least one of the four treatments (n=6 trials were excluded from analysis because no bird was seen or heard within 10 meters during any treatment). If other surrounding pairs were seen in the area, the trial was considered confounded and was not included in my analysis (n=7 trials were excluded for this reason).

Playback Stimuli

I created stereo playback stimuli from recordings of birds collected within my study site over the last ten years. By selecting historical recordings, and recordings collected from sites far away from the subjects' territories, I ensured that all playback stimuli were unfamiliar to the subjects. Rufous-and-white wren stimuli were selected from an archive of recordings of colour-banded birds; I chose birds that had been dead for more than two years at time of playback and were from areas at least 2 km from the site of playback. I used recordings of 20 different pairs to create the stimuli, using recordings of a single pair of birds in each stimulus set. Banded wren stimuli were selected from birds that were either dead or found at least 2 km from the site of playback. Stimuli for plain wrens were made from recordings collected in April 2012, at locations at least 2 km away from the site of playback. For banded and plain wrens, I created stimuli using recordings of 10 different pairs; each stimulus set was used during playback to two different pairs of subjects. Prior field observations verify that all three species remain in their territories over long periods, confirming that birds recorded far away from the site of playback would be unfamiliar to the subjects (Molles & Vehrencamp 2001, Mennill & Vehrencamp 2005, Cuthbert

& Mennill 2007). Eastern whipbird recordings (control stimuli) were collected in Atherton, Australia as part of another study (Mennill & Rogers 2006).

Stimuli were created in Audition (version 2.0; Adobe, San Jose, CA). I selected recordings with a high signal to noise ratio (assessed visually based on sound spectrograms) and where the male and female songs were not overlapping in either the time or frequency domain. To create stereo duet stimuli where the male duet contribution was broadcast from one loudspeaker and the female duet contribution from the other, I separated the male and female vocalizations into separate channels (see Mennill 2006, Douglas & Mennill 2010). Each individual bird's contribution was normalized to a peak amplitude of -1dB in Audition.

All conspecific treatments featured the same number of songs broadcast at the same amplitude. All three treatments for each pair consisted of the same song types recorded from the same male and female. Each stimulus was 3.0 min long, where duets were offered at a rate of one duet every 15 s, and alternating solos were given at a rate of one solo song every 7.5 s, thereby ensuring the same song output across all trials. The only difference between the three conspecific treatments (highly coordinated, poorly coordinated, and alternating solos) was the relative timing of male and female contributions. All duet stimuli were female-created duets, where the simulated male began singing and the simulated female joined the male song to create the duet. Previous research shows that most plain wren duets are female-created duets (Cuthbert and Mennill 2007) and the majority (75%) of rufous-and-white wren duets are female-created duets (Mennill & Vehrencamp 2005). Although both male and female banded wrens sing, they do not regularly perform duets (Mann *et al*. 2009). I modeled duet stimuli for this species on what is known for rufous-and-white wrens, as the two are sister species (Mann *et al*. 2006), and the songs of the two species are somewhat similar in the length and arrangement of syllables, although banded wren songs are higher in frequency and more frequency-modulated than rufous-and-white wren songs.

I defined a duet as alternating or overlapping male and female vocalizations where the male and female contributions were within 1 sec of each other (Mennill & Vehrencamp 2005). To synthesize coordinated duets, I created stimuli with a standard deviation in reaction time of 0 (i.e. no variation in delay between male and female components) and a mean reaction time that was typical based on published delays in male-to-female reaction times (Mennill & Vehrencamp 2005; Cuthbert & Mennill 2007). To synthesize uncoordinated duets, I created stimuli with a standard deviation in reaction time that was 1.5 times the published standard deviation in reaction time (Table 1). I created an Excel spreadsheet that used a random number generator, using the RandBetween command, to produce a list of variable reaction times that achieved the desired standard deviation between male and female components. To synthesize alternating solos, I created stimuli where male and female songs were presented in perfect alternation, with a silent interval of equal length between subsequent songs from the two channels.

Playback apparatus

The stereo playback apparatus consisted of two loudspeakers (Sony SRS A27) mounted one meter apart and one meter off of the ground on metal poles. Both the speakers and poles were painted with green, brown, and black spray paint to camouflage them. The speakers were mounted facing upward to avoid any effects of speaker directionality. A flag was hung one meter from the center of each speaker off to the side, so that the observer could estimate the distance between the birds and the speakers. The two speakers were connected by a 15m cable to a digital playback device (Apple iPod classic) operated by the observer. The observer sat in a position 15m from the loudspeakers, equidistant from the loudspeakers to avoid creating any asymmetric bias in the response of playback subjects.

I set up the apparatus near the center of the subjects' territories to minimize the possibility of inciting responses from neighbours (the territories of banded wrens and especially plain wrens are quite small, and the study populations are quite dense). I avoided areas within 25 meters of the subjects' nest for rufous-and-white and banded wrens, and 10m for plain wrens (due to smaller territory sizes). Once the apparatus was in place, I measured the output of each speaker using a sound level meter (Radio Shack 33-4050), broadcasting frequency-modulated synthetic tones that were normalized to the same level as playback stimuli. Years of field research on these three species revealed that the three species do not sing at the same amplitude; rufous-and-white wren songs are quieter than both banded wren and plain wren songs. For rufous-and-white wrens, the volume of the loudspeakers was adjusted to a level of 80dB relative to background noise at one meter distance from the speaker (as in Mennill & Vehrencamp 2005). For banded wrens and plain wrens, the volume was set at a maximum level of 90dB (as in Illes *et al.* 2006, for banded wrens). For all three species, these amplitudes produced sounds that approximated the natural level of live birds, based on my experience in the field. Eastern Whipbird duets were broadcast at the same volume as the stimuli for each trial (i.e. 80dB for playback to rufous-and-white wrens; 90dB for playback to the other two species).

I recorded vocal behaviour of playback subjects using a directional microphone (Sennheiser MKH 70) and a solid state digital recorder (Marantz PMD660). The microphone was mounted on a tripod next to the observer. The observer quietly dictated the physical behaviours of any responding birds into the microphone, following a technique that has been successful in previous playback studies (e.g. Bradley & Mennill 2009; Koloff & Mennill 2011).

Data Analysis

I visualized recordings of the playback trials using Syrinx-PC sound analysis software (J. Burt, Seattle, Washington). I identified songs, duets, and calls of the focal pair and scored the behaviours and distances dictated by the observer, using the time and frequency cursors in Syrinx-PC to highlight each vocalization and activity. This approach created a time-stamped series of the focal pair's songs and activities during playback. From these files, I extracted both vocal and behavioural response variables. I measured the following behavioural responses for both the male and female bird: (1) distance of closest approach to the loudspeakers, and (2) the number of times the bird passed across the loudspeakers (passes were counted if the bird passed under or over the apparatus within 5 horizontal meters). I also measured the following vocal response variables: (1) number of male solos, (2) number of female solos, (3) number of male-created duets (duets where the focal female sang first and the male joined to create the duet), and (4) number of female-created duets (duets where the focal male sang first and the female joined to create the duet). Because duetting was uncommon in my responses, I pooled the number of male and female created duets into one category classified simply as duets.

Statistical Analyses

I analyzed my data using generalized linear mixed models (GLMMs) in order to accommodate non-normal data and include a random effect of pair (Bolker et. al 2008). I included four main factors in my models: (1) playback treatment (four levels: coordinated duets, un-coordinated duets, alternating solos, and controls); (2) playback order (four levels: first, second, third, or fourth); (3) subject species (three levels: banded wrens, plain wrens, and rufous-and-white wrens); and (4) sex of the responding animal (two levels: male or female). I included all first-order interaction terms in my analyses, and I incorporated pair identity as a random effect to account for the fact that the same pair was sampled repeatedly across the four treatments. I ran the GLMM procedure once including all of the factors and all two-way interaction terms; I then excluded non-significant interaction terms and ran a second GLMM in order to achieve a better fit. Non-significant main effects were left in the model in order to report results of my hypothesis tests, and this did not affect the fit of my models. Post hoc pairwise contrasts for species and treatment were obtained through the GLMM interface, which increases p-values to correct for multiple comparisons (rather than decreasing the alpha value) using the sequential Bonferroni correction. All GLMMs were run in SPSS 21 (IBM, Chicago, IL).

Results

All three species of wren showed strong responses to conspecific playback compared to heterospecific controls. In most cases, both pair members approached the loudspeakers and produced vocal responses (79.4% of trials), while in some cases only the male approached and sang (20.6% of trials). I evaluated four variables summarizing the birds' responses to playback, two of which measured physical responses (distance of closest approach and number of passes over the loudspeaker) and two of which measured vocal responses (number of solo songs and number of duets).

Physical Response: Distance of Closest Approach

Wren pairs showed significant variation in their distance of closest approach across the four playback treatments (main effect of treatment: $F_{3,473} = 60.7$, $P < 0.001$; Table 2 provides a summary of the factors, interaction terms, and significance values for each GLMM). This effect was driven by close approaches to the three experimental treatments and distant approaches to the control treatment (post-hoc pairwise contrasts: all $P < 0.001$), with pairs responding similarly to all three conspecific experimental treatments (Fig. 2.1; all $P > 0.23$). Males and females showed the same pattern of response across the four treatments, although approach distance varied by sex, where males approached the speakers more closely than did females to all treatments (Fig. 1; main effect of sex: $F_{1,473} = 112.3$, $P < 0.001$). Distance of closest approach varied with the order of playback treatments ($F_{3,473} = 5.25$, $P = 0.001$). This effect was driven by birds coming closer to the second treatment than they did to the first ($P = 0.009$) or third ($P = 0.006$) treatment; the first, third, and last treatment were not significantly different from one another (all $P > 0.110$). All three wren species responded with similar approach distances (main effect of species: $F_{2,473} = 0.488$, $P = 0.61$).

I found three significant interaction terms for distance of closest approach (Table 2.2). Birds stayed farther away from the control treatment if it was given first in the series of treatments (order × treatment interaction: $F_{9,473} = 2.0$, $P = 0.04$). Banded wrens remained farther away from the loudspeakers than the other two species except during the two duet treatments, when they approached to a similar distance; and plain wrens came closest to all treatments except for un-coordinated duets (treatment x species interaction: $F_{6,473} = 2.5$, $P = 0.04$). Banded wrens came closer to the first treatment, while plain and rufous-and-white wrens came closer to the other three treatments (order × species interaction term: $F_{6,473} = 4.7$, $P < 0.001$).

Physical Response: Number of Passes

The number of times wrens passed over the speaker showed significant variation based on playback treatment (main effect of treatment: $F_{3,471}$= 25.5, P< 0.001), where the birds passed over the loudspeaker more in response to the three experimental treatments than to the control (Fig. 2.2; post-hoc pairwise comparisons: all P< 0.001). Females passed over the loudspeaker less often than males (main effect of sex: $F_{1,471}$ = 98.7, P< 0.001). The number of passes over the loudspeaker also varied by species (main effect of species: $F_{3,471}$ = 6.1, P = 0.002); this effect was again driven by plain wrens, which passed over the loudspeaker significantly more than banded wrens (P = 0.01), while rufous-and-white wrens passed over the loudspeaker an intermediate number of times compared to plain wrens (P = 0.14) and banded wrens (P = 0.14). Passing behaviour was also affected by the order of the treatments (main effect of order: $F_{3,471}$ = 4.7, P = 0.003); birds passed over the loudspeaker fewer times during the first compared to the last treatment (order 1 versus 4: P = 0.03) and more to the third than the fourth treatment (order 3 versus 4: P = 0.03).

Number of passes over the loudspeaker showed significant interaction terms in our model. Birds passed more often during the last treatment for all treatment types except for alternating solos, passing least often when alternating solos were the last treatment (treatment × order interaction: $F_{9,471}$ = 2.7, P = 0.005). Plain wrens passed over the loudspeaker more often across all treatments, followed by rufous-and-white wrens, and then banded wrens; however, for the un-coordinated duet treatment, banded wrens responded with slightly more passes over the loudspeaker than rufous-and-whites (species × treatment interaction: $F_{6,471}$ = 4.2, P < 0.001). Male and female banded wrens' passing behaviour was more similar to each other than to that of rufous-and-white and plain wrens, in which males respond more strongly than females (species × sex interaction: $F_{2,471} = 4.5$, $P = 0.011$). Again, banded wrens passed more often than other species for the first treatment and the least often of the three species for the second, third, and fourth treatments (species × order interaction: $F_{6,471} = 3.9$, $P = 0.001$).

Vocal Response: Number of Solo Songs

Vocal behaviour of wrens also showed significant variation based on playback treatment (Fig. 3; main effect of treatment: $F_{3,465} = 35.5$, $P < 0.001$). Birds sang significantly fewer solos in response to control playback compared to playback of the three experimental treatments (post-hoc pairwise comparisons: all $P < 0.001$). Additionally, birds sang more solos in response to alternating solos and coordinated duets than to uncoordinated duets (both $P < 0.001$), but un-coordinated duets were not significantly different from alternating solos ($P = 0.14$). Males showed significantly different responses based on species (main effect of species: $F_{2,465} = 7.2$, $P = 0.001$). This difference was driven by plain wrens, which sang significantly more solos than rufous-and-white wrens ($P = 0.002$) and banded wrens ($P = 0.019$); banded wrens and rufous-and-white wrens sang at very similar levels ($P = 0.38$). There was no evidence of a change in solo singing based on the order of the stimuli ($F_{3,465} =1.3$, $P = 0.269$). Males sang significantly more than females (main effect of sex: $F_{1,465} = 931.1$, $P< 0.001$).

Solo song responses showed significant interaction terms in my model. Birds sang more in response to alternating solos if they were presented first (order x treatment interaction: $F_{9,465} =6.3$, $P< 0.001$). Plain wrens respond more to coordinated duets than to uncoordinated duets, but similarly to coordinated duets and alternating solos, while the other two species respond similarly to all three experimental treatments (treatment x species interaction: $F_{6,465} = 12.4$, $P <$ 0.001). Plain wrens sang more during the fourth treatment than any others, while the other two species sang similarly to all orders of treatments (order x species interaction: $F_{6,465} = 12.7$, $P <$

0.001). In all species, females sang similarly few songs, whereas male plain wrens sang more than males of either of the other two species (species x sex interaction: $F_{2,465} = 29.9$, $P < 0.001$). Females responded with few solos to all treatments, whereas males responded differentially based on treatment as explained above (treatment x sex interaction: $F_{3,465} = 12.4$, $P < 0.001$).

Vocal Response: Number of Duets

Wrens showed significant variation in duetting behaviour based on both playback treatment (main effect of treatment: $F_{3,222} = 28.3$, $P < 0.001$) and species (main effect of species: $F_{2,222} = 5.9$, $P = 0.003$; Fig. 4). Similar to the results for male solo songs, pairs sang fewer duets in response to the control playback than to playback of coordinated duets ($P = 0.002$), un-coordinated duets ($P = 0.002$), and alternating solos ($P = 0.004$). They produced similar numbers of duets in response to playback of coordinated and un-coordinated duets ($P = 0.275$); and they sang more duets in response to both categories of duets than to alternating solos (coordinated duets: $P = 0.022$; un-coordinated duets: $P = 0.010$). The model indicated that pairs produced different numbers of duets depending on the order of the treatments (main effect of order, $F_{3,222} = 3.8$, $P = 0.012$); however, none of the pairwise comparisons showed any differences (all $P \geq 0.116$). Additionally, the whole model showed a significant effect of species; however, pairwise contrasts showed no significant differences between the three species (all $P \geq 0.142$).

Duet responses also showed significant interaction terms in my model. Pairs sang more duets in response to coordinated duet playback if this treatment was given first (order x treatment interaction: $F_{9,222} = 5.3$, $P < 0.001$). Plain wrens sang more duets to all experimental treatments (compared to the control), rufous-and-white wrens sang more duets to all experimental treatments, and banded wrens sang very few duets regardless of treatment (treatment x species interaction: $F_{6,222} = 5.3$, $P < 0.001$). Plain wrens sang fewer duets in response to the first treatment, while the other two species' duetting behaviour was not affected by order (order x treatment interaction: $F_{6,222} = 5.5$, $P < 0.001$).

Discussion

Three species of neotropical wrens responded strongly to playback of conspecific vocalizations compared to those of a heterospecific control, but none of the three species differentiated between playback of coordinated and uncoordinated duets in their physical response. Males and females showed the same pattern of response, although female responses were consistently lower in intensity. Interestingly, birds exhibited different patterns in their physical versus vocal responses. For vocal responses (number of duets and solos), birds showed different responses to the playback treatments, singing more duets in response to the two duet playback treatments. Birds responded with solos most often to alternating solos and coordinated duets, more so than to uncoordinated duets, which in turn elicited more solos than control treatments. Birds' solo song responses showed that birds differentiate between coordinated and uncoordinated songs, and duetting responses indicate that they differentiate between duets and alternating solos; however, their physical responses show that they did not treat coordinated duets as more aggressive signals than uncoordinated duets.

Only one other study has examined temporal coordination of duets as a territorial signal. Male Australian magpie-larks responded differentially to playback based on the degree of duet coordination, singing more solos in response to coordinated duets than uncoordinated duets (Hall & Magrath 2007). This result led us to hypothesize that duet coordination would function as an aggressive signal in wrens as well. The responses of wrens in the current experiment, however, provide mixed evidence that temporal coordination is a territorial signal in these three species. For physical responses (approach distance and passes) and duetting behaviour, I found that temporal coordination had no effect on the wrens' aggressive response. Wrens discriminated between high and low coordination in only one of four measures of aggressive response, singing more solos in response to high coordination and alternating solos. This result, using solos as a measure, is similar to the results seen in male magpie-larks (Hall & Magrath 2007), although my other variables show different patterns, and the alternating solo treatment was not applied in the magpie-lark study. I believe that a lack of physical differentiation between coordinated and uncoordinated duets suggests that they are perceived as similar threats by wrens, in contrast to the pattern observed for magpie-larks. Independent evolutions of duetting in wrens and magpie-larks—two distantly related taxa—may have given rise to different duet functions. Furthermore, magpie-larks always perform duets in close proximity to their partner, whereas wrens can perform duets with variable distances of separation (Mennill & Vehrencamp 2008). Given that temporal precision is easier to achieve from close distances (due of the slow speed of sound; Hall 2000), temporal coordination may be a better signal of pair quality in close-duetting magpie-larks than in birds that perform duets from further apart, in which case temporal precision may be a cue or signal of their spacing rather than their ability to coordinate.

Physical and Vocal Responses to Intruders

Duets could be threatening territorial signals in at least two ways: duets might pose a greater threat than solo songs because they are created by two territorial animals instead of one, or duets could pose a greater threat because those two animals have coordinated their vocalizations, indicative of cooperation. If territorial animals assess the degree of threat based solely on the number of birds singing, and not the degree of coordination in their songs, I would expect similar responses to all three of my experimental treatments, as seen in this study. A playback study of Kokako (*Callaeas cinerea*) found that territorial pairs respond more strongly to song playback of two intruders than one, indicating that the number of intruders changes the degree of threat (Molles & Waas 2006). If this is the case in these wren species, then responses to all three conditions would be similar, as all three would pose the same degree of threat (two birds intruding onto an established territory). However, prior research shows that rufous-and-white wrens respond with similar levels of aggression to both duets and solos of one sex (Mennill & Vehrencamp 2008), indicating that the number of intruders may not influence the aggressive responses of a territorial pair. Paired male canebrake wrens (*Cantorchilus zeledoni*) respond similarly to both solos and duets as well, although duets are more threatening than solos to unpaired males (Marshall-Ball *et al*. 2006). Taken together with the physical data presented here, these results indicate that paired wrens are using neither duet coordination nor number of intruders as a measure of territorial threat. The number of intruders may only be important to unpaired territory holders, and not to established pairs, as previously suggested in canebrake wrens (Marshall-Ball *et al.* 2006).

The number of duets and solo songs sung in response to playback revealed different patterns of responses than did physical measures. Birds sang more duets in response to playback of both coordinated and uncoordinated duets than to playback of solos, although they did not differentiate between coordinated and uncoordinated duets in their vocal responses. This indicates that the combination of a rival pair's songs into duets influences whether or not a pair will perform duets in response, but not whether the pair will respond physically to the intruders. Additionally, birds responded with more solo songs to both alternating solos and coordinated duets than they sang in response to uncoordinated duets. This may indicate that both solo singers and duetting pairs impose enough of a threat to warrant solo singing behaviour, and that the birds can discriminate between high and low coordination duets. The significant species by treatment interaction in solo singing behaviour indicates that this pattern was driven primarily by the responses of plain wrens, while the other two species respond similarly to the three experimental treatments. This is noteworthy because plain wrens naturally sing more highly coordinated duets, as do their congeners in the newly-defined *Cantorchilus* genus (Mann *et al*. 2009). Coordination may be a more powerful cue for plain wrens when determining the threat of a rival pair; when plain wren duets are poorly coordinated, it may be a more apparent signal than it would be for rufous-and-white wrens, whose duets are naturally loosely coordinated.

Birds did not discriminate between the three experimental treatments in their physical responses, but did so in their vocal responses. This difference could lie in the fact that physical responses may be intrinsically different than vocal ones. Searcy and Beecher (2009) suggest that only physical measures should be treated as aggressive responses, arguing that songs cannot be measured as an aggressive signal without first being correlated with known physically aggressive behaviours. A physical approach to a territorial rival (here, a loudspeaker) may be more of a deterrent than a vocal signal, implying that simply approaching the intruders could drive them away more effectively than singing. Because physical approach may also be more costly, this could be a last line of defence; birds might approach a territorial intruder only when singing fails to expel that intruder. This could explain my results for vocal behaviour; birds may sing at different rates based on the intruders' level of duet coordination, then approach if the intruder is not deterred. In my experiment, however, when birds either sang or approached the loudspeaker during playback, 20% of birds sang before approaching, and 80% approached before singing, regardless of sex. If birds approach an intruder before singing, it could indicate an advantage of quietly investigating a rival before a bird announces its presence. Differences in singing behaviour based on coordination of intruders in this case could arise if the duets are functioning within the pair rather than between pairs.

Species Effects and Phylogeny

The similarities and differences I found in physical and vocal behaviour seem to mirror the phylogenetic relationships between the three species, as well as the duetting styles delineated in a recent review of singing behaviour across the wrens formerly in the genus *Thryothorus* (Mann *et al*. 2009). Rufous-and-white wrens and banded wrens are sister taxa, in the newly distinguished genus *Thryophilus*, while plain wrens are in a different genus, *Cantorchilus* (Mann *et al*. 2006). *Thryophilus* wrens sing duets that are less frequent with more loosely associated polyphonal elements than those in the genus *Cantorchilus*, which sing complex and precisely-timed, antiphonal duets. Within the *Thryophilus* genus, banded wrens are not recognized as duetters (Mann *et al.* 2009), and although males and females produce songs, they rarely combine them into vocal duets, whereas rufous-and-white wrens associate their songs regularly into clear duets (Molles & Vehrencamp 1999, Mennill & Vehrencamp 2005, Mann *et al*. 2009). With these differences in mind, it is not surprising that I found a species effect for the duetting responses of my playback subjects, where banded wrens rarely sang duets, rufous-and-whites sang duets at an intermediate level, and plain wrens sang the most duets. Solo singing behaviour also mirrored the phylogenetic relationship, as banded wrens and rufous-and-white wrens showed more similar behaviour to each other than to plain wrens. Differences in vocal behaviour are highly related to phylogeny, but may also be related to ecological factors (Mann *et al*. 2009). Banded wrens and rufous-and-white wrens share many ecological similarities, foraging mainly on the ground and showing similar breeding and nesting ecology, whereas plain wrens spend most of their time foraging in the subcanopy or canopy, and exhibit different nesting behaviour (Ahumada 2001, Brewer 2001). These factors could also lead to divergence in duetting behaviour, and in functionality of duets.

Vocal variables were more likely to be influenced by species than were physical variables. Plain wrens passed over more often than the other two species, but showed the same pattern of response to the treatments. Plain wrens often approached high above the speakers, whereas the other two species often stayed only slightly above or below the apparatus. These differences in behaviour could present different risks in passing over the loudspeakers; it could be less risky for plain wrens to pass above the speakers than it is for the other two species to pass near the speakers. However, all three species came within similar approach distances. Similarity in physical responses, but not vocal, could indicate that physical behaviours such as approaching an intruder and increasing flights in the area are more conserved than the vocal behaviours in this group, which have diverged notably.

Duet Coordination as a Signal

If temporal coordination is not an aggressive territorial signal important in interactions between rivals, why then do wrens coordinate their songs into duets? It is possible that the coordination of duets is not used as a signal at all. Alternatively, coordination could be used as a signal within pairs. Many studies have supported within-pair functions of duets, such as maintaining acoustic contact (Mennill & Vehrencamp 2008), maintaining the pair bond (Hall 2000, Geissman *et al*. 2000), and recognizing a mate during aggressive interactions (Logue & Gammon 2004). Because responding quickly and consistently to a mate requires attentiveness (Hall 2009, Smith 1994), coordination could be a within-pair signal of attentiveness to the mate. Duets can serve a territorial function and within-pair functions simultaneously; happy wrens coordinated their song type with their mate rather than matching the song type of the intruder while duetting to defend their territory (Templeton *et al.* 2012). My results are consistent with the idea of duets having both a within-pair and between-pair function, based on higher levels of duetting in response to duets than to solo songs, but a lack of difference in physical responses to solos and duets.

Our data provide mixed support for joint resource defence hypotheses of duet function. Elevated physical and vocal responses to conspecific playback indicate that songs and duets are used for territorial defence. Yet wrens showed no evidence that coordinated songs sung in duets are more threatening than solo songs. While this supports the “joint resource defence” hypothesis of duet function, it may also provide evidence that duets have not evolved for this purpose, or that they have not evolved solely for this purpose. In order for this to be an ultimate function, birds need to gain an advantage by broadcasting duets rather than solos in a territorial context; however, in this experiment and others (e.g. Appleby *et al*. 1999, Bradley & Mennill 2009, Fedy & Stutchbury 2005, Grafe & Bitz 2004), both solo and duet songs receive similar aggressive responses, making an evolutionary advantage in territoriality unclear (but see Hall 2000, Molles & Waas 2006 for examples of duets eliciting a stronger response than solo songs). Levin (1996) found that bay wrens whose mates had been experimentally removed still retained their territories, so it is possible that in wren species, solo songs are just as effective at maintaining a territory as duets. If this is the case, these results indicate that evolution of precise coordination in duets evolved for purposes other than territory defence, possibly to serve within-pair functions. Rufous-and-white wrens use duetting within the pair to maintain contact with their mate, moving closer to each other after duets (Mennill & Vehrencamp 2008). Canebrake wrens, a sister species to plain wrens, seem to use their duets for territory defence and mate-guarding (Marshall-Ball & Slater 2006). It is possible that birds who are intruding on a territory, rather than established territory holders (tested in my experiment), would respond differently to duets or solos because it could give information about the probability of overtaking a territory (specifically, whether they will have to displace one bird or two). Removal experiments with replacement of speakers broadcasting solos or duets could further elucidate the utility of duet song versus solo song.

I tested the duet coordination hypothesis with three different species of wrens in hopes of elucidating evolutionary mechanisms of coordinated displays. I had expected either a species by treatment interaction in that birds would respond strongly to their species-specific form of duet, or a main effect in that all species would respond most strongly to highly coordinated duets. My results were not consistent with either prediction. Physically, all three species responded similarly, responding to all three experimental treatments more than the control (although plain wrens passed over the loudspeaker more often overall than the other species). Solo singing behaviour was greater in response to coordinated duets and alternating solos, which was driven mostly by plain wrens. This could mean that plain wrens are more threatened by duetting pairs that are well-coordinated and solo singers that may usurp a mate, than they are by poorly coordinated pairs. Apart from this, my species effects reflected differences in magnitude of responses rather than differences in overall patterns, where plain wrens simply sang more and passed over the loudspeaker more than the other species. I did find that banded wrens, while classified as non-duetting birds, produced more duets in response to manufactured duets (Fig. 3), despite this being an unnatural stimulus based on the current understanding that banded wrens do not routinely duet. This provides some evidence that the banded wrens perceive duets as signals, but more research needs to be done to determine the importance of this response. Overall, my species effects seem to mirror phylogenetic relationships, where my more closely related species behaved more similarly.

I conclude that these three tropical wren species do not respond with more physical aggression to precisely timed duets, indicating that they do not use duet coordination as a signal of territorial threat. However, the coordination of songs sung by intruders does influence the vocal behaviour of a territorial pair. While I do not show either predicted pattern of species differences, I do show influences of species on response variables that seem to be consistent with phylogenetic relationships.

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Tables and Figures

Table 2.1. Details of coordination of male and female playback stimuli for three species of tropical wrens, based on published delays in male and female duet contributions.

Species	Delay Type	Published delay*			Coordinated Stimuli			Uncoordinated Stimuli		
		Mean (sec)	SD (sec)	CV (%)	Mean (sec)	SD (sec)	CV (%)	Mean (sec)	SD (sec)	CV (%)
Rufous-and-white and banded wren	Male-to-Female delay†	2.156	0.911	42.2%	2.156	0	0	2.156	1.367	63.4%
Plain wren	I-to-F phrase delay‡	0.126	0.016	12.7%	0.126	0	0	0.126	0.024	19.0%
	F-to-M phrase delay‡	0.055	0.007	12.7%	0.055	0	0	0.055	0.011	19.1%
	M-to-F phrase delay‡	0.181	0.015	8.3%	0.181	0	0	0.181	0.023	12.4%

* Data for rufous-and-white and banded wrens from Mennill & Vehrencamp (2005); data for plain wrens from Cuthbert & Mennill (2007)

† All rufous-and-white and banded wren playback duets include just one male and one female song.

‡ Plain wren duets are comprised of male Introductory (I) phrases, female (F) phrases, and male (M) phrases (Cuthbert & Mennill, 2007).

Table 2.2. Factors and interaction terms used in the final models for each response variable. Non-significant interaction terms were removed from the original models where indicated. P-values less than 0.05 were considered significant.

	Closest Approach				**Number of Passes**				**Solo Songs**				**Duets**			
	df 1	***df 2***	***F***	***P***	***df 1***	***df 2***	***F***	***P***	***df 1***	***df 2***	***F***	***P***	***df 1***	***df 2***	***F***	***P***
Treatment	3	473	60.70	<0.001	3	471	25.52	<0.001	3	465	35.52	<0.001	3	222	28.30	<0.001
Order	3	473	5.25	0.001	3	471	4.69	0.003	3	465	1.31	0.269	3	222	3.76	0.012
Species	2	473	0.49	0.61	2	471	6.07	0.002	2	465	7.22	0.001	2	222	5.86	0.003
Sex	1	473	112.30	<0.001	1	471	98.65	<0.001	1	465	931.10	<0.001	*Not Applicable*			
Treatment × Order	9	473	2.02	0.04	9	471	2.69	0.005	9	465	6.29	<0.001	9	222	5.28	<0.001
Treatment × Species	6	473	2.48	0.04	6	471	4.18	<0.001	6	465	12.40	<0.001	6	222	5.27	<0.001
Treatment × Sex	*Removed*				*Removed*				3	465	12.40	<0.001	*Not Applicable*			
Order × Species	6	473	4.75	<0.001	6	471	3.91	0.001	6	465	12.69	<0.001	6	222	5.48	<0.001
Order × Sex	*Removed*				*Removed*				*Removed*				*Not Applicable*			
Species × Sex	*Removed*				2	471	4.54	0.011	2	465	29.86	<0.001	*Not Applicable*			

Figures

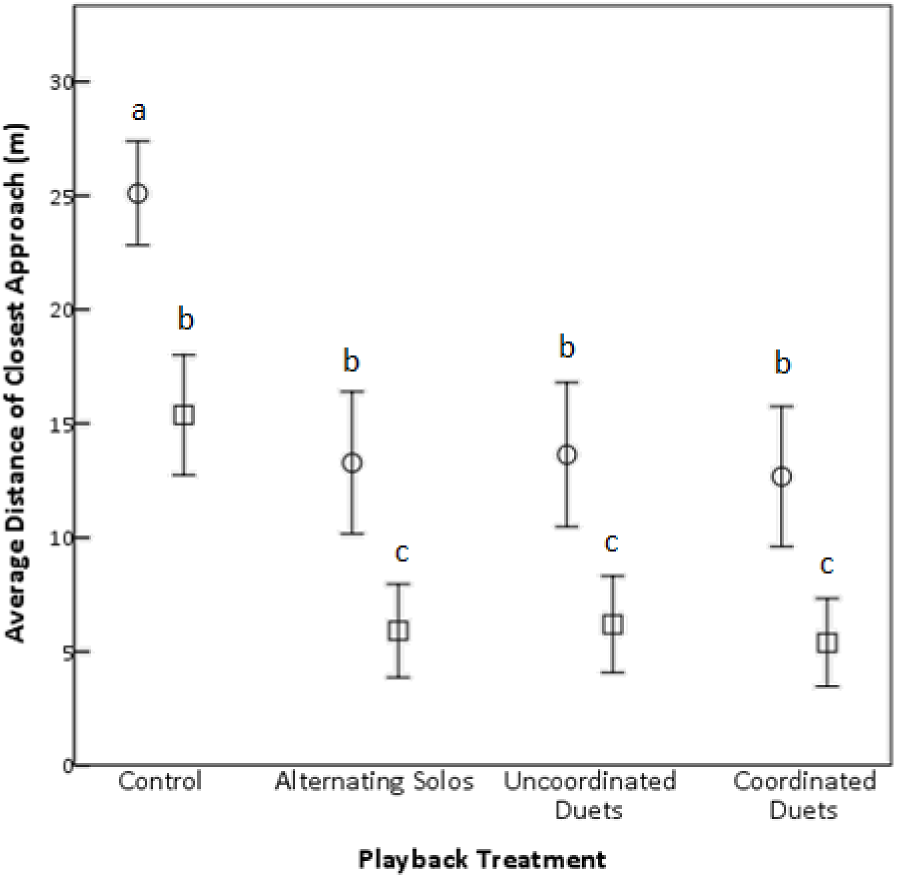

Figure 2.1. Average distances of birds to the speaker (in meters) during each playback treatment, separated by sex. Males (squares) always approached more closely than females (circles), but showed similar patterns of response by treatment type. All birds came closest to the three conspecific experimental treatments than they did to the heterospecific control treatment. Letters indicate significant differences between the sexes after post-hoc pairwise comparisons. Error bars indicate 95% confidence intervals.

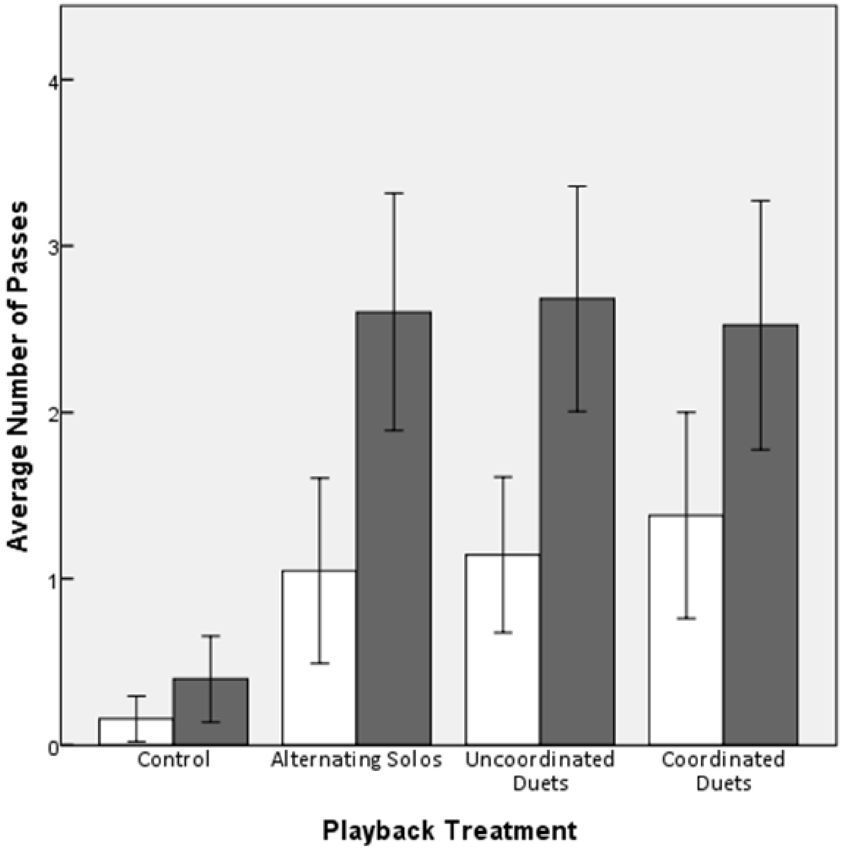

Figure 2.2. Average number of passes over the speakers during a six minute trial, split by sex. Males (grey bars) always passed over the speakers more often than females (white bars), and all birds passed more often during all three conspecific treatments than during the heterospecific control treatment. Error bars indicate 95% confidence intervals.

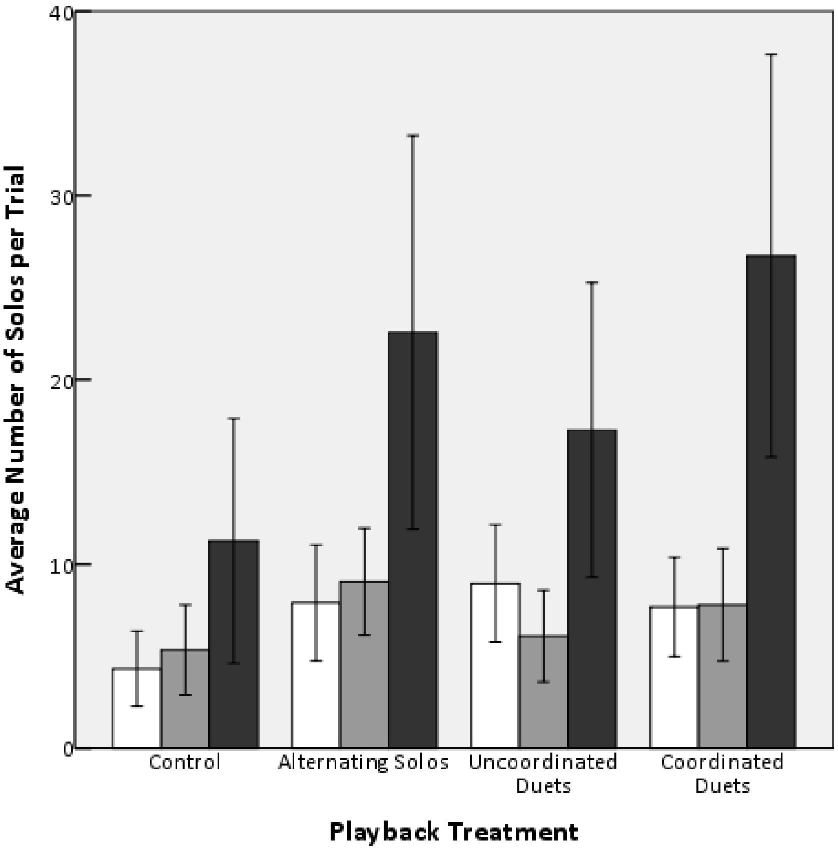

Figure 2.3. Average number of solo songs sung in response to playback, split by species. Plain wrens always sang more than banded and rufous-and-white wrens. White bars indicate banded wrens, grey bars indicate rufous-and-white wrens, and black bars show plain wren responses. Responses were higher to alternating solos and coordinated duets than to uncoordinated duets, which in turn was higher than the control. Error bars indicate 95% confidence intervals.

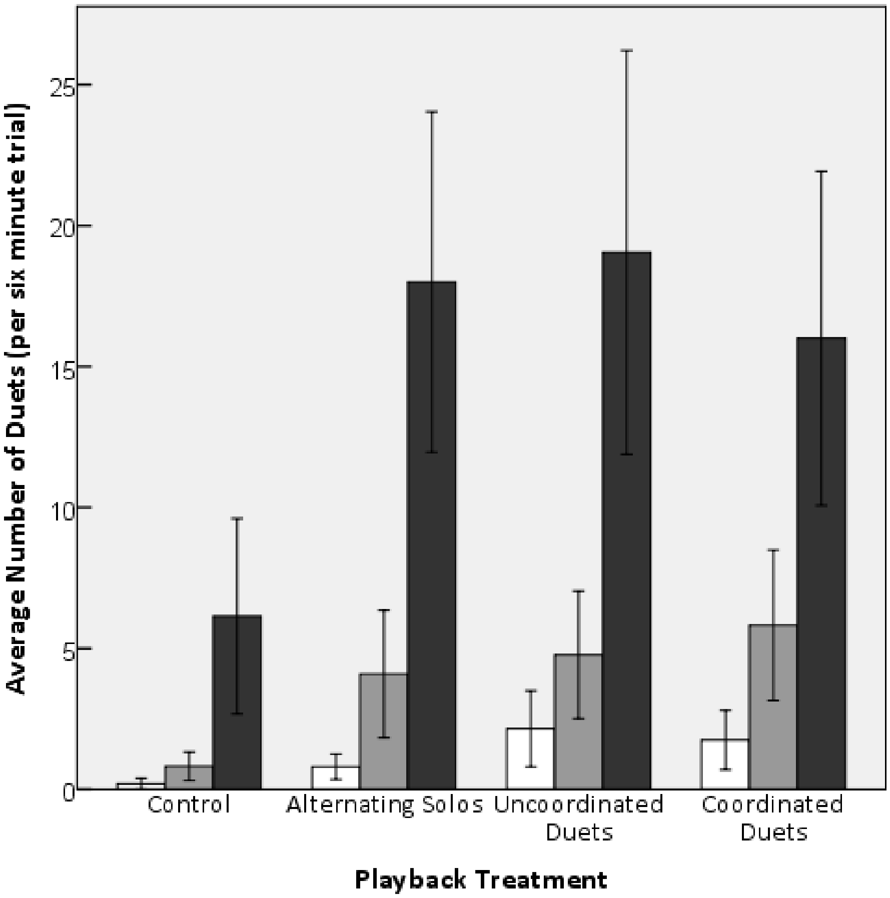

Figure 2.4. Average number of duets sung in response to playback, split by species. Plain wrens always sang more duets than rufous-and-white wrens, which sang more duets than banded wrens. White bars indicate banded wrens, grey bars indicate rufous-and-white wrens, and black bars show plain wren responses. Responses were higher to both duet treatments than to alternating solos, which was higher than the control. Error bars indicate 95% confidence intervals.

Chapter 3

Vocalizations and the coordination of nesting behaviours in duetting rufous-and-white wrens

Chapter Summary

The use of vocalizations, and particularly duets, as a means to coordinate parental activities has not often been considered. In bird species with biparental care, animals could benefit by using duets to communicate information about the needs of their offspring, minimizing the number of trips to the nest and thus reducing the risk of predation. Additionally, vocalizations could be used to communicate with a bird that is out of visual contact on a nest. In this study, I described rufous-and-white wren vocalizations used near and on the nest, and I characterize their association with visitations to the nest. I test four predictions for the functions of nest-associated vocalizations: (1) that duets and other vocalizations communicate information between the bird outside and inside the nest, (2) that duets are used to coordinate biparental visitation of the nest, (3) that duets allow pairs to minimize number of trips to the nest in high-predation tropical environments, and are therefore negatively associated with visits to the nest, and (4) that duets reveal a parent's investment, and are positively associated with visitation rates. My data show that birds vocalize before their partner leaves the nest more often when the nest is closed to the external environment than when the nest is open, indicating that vocalizations may act as an "all clear" signal to leave the nest. Duetting rates from near the nest were highest during nest building and incubation stages, but low during the hatchling stage; additionally, duets from on the nest were most common during incubation and low during the nest building and hatchling stages. Both of these results suggest that duetting is not used to coordinate visitation. I found no relationship between duetting rates and visitation rates. I conclude that birds may be using duets and other vocalizations near the nest to communicate with their partner during the incubation stage, but more research on communication near the nest is needed.

Introduction

Duetting behaviour occurs in many species of diverse animal taxa, including cetaceans (Schulz *et al.* 2008), primates (Haimoff 1986), insects (Bailey 2003), and birds (Hall 2009). Despite numerous published studies on duetting (reviewed in Hall 2009), the functions of coordinated male-female duets are poorly understood, and often multiple theories for different functions of duets are supported simultaneously (e.g. Grafe & Bitz 2004, Mennill & Vehrencamp 2008, Benedict 2010). While there are many hypotheses for the function of vocal duets (Hall 2004, 2009), only one hypothesis focuses on the possible reproductive functions of these coordinated pair vocalizations. The reproductive synchrony hypothesis suggests that duets may help to stimulate the pair's reproductive physiology to synchronize fertile periods (Todt & Hultsch 1982). Although many duetting birds cooperate to build nests and raise offspring, a function of duetting behaviour in coordinating parental activities is not often suggested (but see Langmore 1998, Sonnenschein & Reyer 1983).

Approximately 81% of bird species exhibit biparental care toward their offspring (Cockburn 2006), including many duetting species (Farabaugh 1982). When a pair cooperates to rear young, situations may arise where communication between the attending parents is beneficial, both to the fitness of the parents and their offspring. Parents could signal food necessity, alert the partner to potential danger, or coordinate visits to the nest using vocal communication. Because high levels of parental visitation to the nest can be correlated with negative nest outcomes by making the nest more conspicuous to predators (Martin 2000), animals would benefit by optimizing the level of activity at the nest to minimize nest conspicuousness and predation. Vocal behaviours could be useful for signalling this information between attending parents.

Given that vocalizations at the nest may also draw the attention of predators or parasites, it is essential that the benefits of these vocalizations outweigh the costs (Leonard 2008). The behaviour of singing from the nest is not well-described, despite being a relatively common trait in a few taxa. Outside of North America, this behaviour has not been systematically quantified. One review on the topic in North American birds suggests that singing on the nest may not be costly, although species that sing on the nest show greater variance in predation (Leonard 2008). Experimental red-winged blackbird (*Agelaius phoeniceus*) nests are predated more frequently when nest-associated *chit* calls are played from the nest, indicating a cost of vocalizing near the nest (Yasukawa 1989). However, females who answer their partner with *chits* from the nest are more successful, indicating a net benefit of vocalizations at the nest (Yasukawa 1989). In this case, calls seem to prompt nest defence by the male.

Vocalizations may also be used to coordinate visitation behaviours. Black-headed grosbeaks (*Pheuticus melanocephalus*) use vocalizations to coordinate incubation relief (Ritchison 1983). In northern cardinals (*Cardinalis cardinalis*), male nest visits are highly conspicuous, and females use acoustic signals to communicate whether males should approach or avoid the nest (Halkin 1997). The relationship between male cardinal visitation and the vocal interaction preceding it provides support for the hypothesis that the female's song provides information to her partner. Cardinals breed in open cup nests (Halkin & Linville 1999). Sitting on the cup nest, the incubating female can both incubate the eggs and assess potential threats near the nest simultaneously. In other species that breed in enclosed nests, however, the information available to the incubating parent may be minimal. In such species, it would be beneficial for incubating birds if the partner's vocalizations functioned as an "all clear" signal (Johnson & Kermott 1991) or allowed the incubating parent to distinguish the arrival of their partner from potential predators or rival conspecific animals (Neudorf *et al.* 2013). Great tits (*Parus major*) and blue tits (*Parus caeruleus*) are cavity-nesting species in which females produce quiet vocalizations from the nest during the dawn chorus (Gorrissen & Eens 2005). These quiet vocalizations at the nest could be used in close-range intrapair communication (or, perhaps, to communicate female ownership of the nest to other females; Gorrissen & Eens 2005). Similarly, box-nesting female zebra finches (*Taeniopygia guttata*) produce quiet calls at the nest, coordinating these with calls of the male outside the nest to create a type of "private vocal duet" (Elie *et al.* 2010). These duets may facilitate mate recognition and communicate threat information to the bird incubating in the enclosed nest box.

In tropical environments, prolonged breeding seasons and increased predation levels may lead to increased need for biparental care, and therefore coordination of behaviour between the sexes (Slater & Mann 2004). Duetting behaviour is often more common in the tropics than in temperate areas (Langmore 1998), possibly as a consequence of the longer breeding seasons and higher levels of predation. In light of these factors, having two birds involved in resource defence through song may be beneficial. Wrens (*Troglodytidae*) are a family of largely tropical birds that often exhibit female song and duetting behaviour (e.g. Mann *et al.* 2009), and in most cases, both parents provide parental care to the offspring (Brewer 2001). Consequently, wren pairs may benefit by communicating about the needs of their offspring and predation threats near the nest. Additionally, many wrens reproduce in closed, globular nests with narrow entrances (Brewer 2001). This type of nest may prevent the bird inside the nest from assessing the external environment, and prevent the parent outside the nest from visually assessing whether their partner is inside or not. As such, vocal communication with a partner outside the nest could improve the efficiency of parental behaviours, thus reducing stress on the parent and increasing parental fitness (Elie *et al.* 2010).

In this investigation, I focus on rufous-and-white wrens (*Thryophilus rufalbus*), a neotropical species in which both males and females sing songs as solos or in coordinated vocal duets. Breeding partners frequently combine their songs, overlapping male and female contributions, to perform duets (Mennill & Vehrencamp 2005). Duets are composed of the same songs that are sung as solos, but are coordinated in time with the songs of the partner. Rufous-and-white wrens also produce a variety of atonal calls, such as *ticks* and *rattles*, as well as more tonal calls such as *hoots* and *whoops* (personal observations). The functions of these vocalizations have received little or no attention. Prior studies reveal that duets are important for maintaining acoustic contact (Mennill & Vehrencamp 2008) and in joint resource defence (Mennill & Vehrencamp 2005, 2008; Mennill 2006), but it is apparent that they serve other functions as well. Over the last ten years of observing a colour-banded study population in Costa Rica, I have observed birds performing duets near the nest, sometimes with one parent producing their duet contribution from directly inside the nest. Spatial analysis of duetting behaviour confirms that rufous-and-white wrens sing duets nearer to the nest than expected by chance (Mennill & Vehrencamp 2008). The context and function of these duets near the nest is unknown, but appears to stand apart from resource defence.

In this study, I explored the role of vocalizations in and around the nest in duetting tropical rufous-and-white wrens. I conducted nest watches with simultaneous sound recordings on pairs of nesting rufous-and-white wrens during three stages of the breeding cycle: nest building, incubation, and hatchling provisioning. I first sought to describe the vocal behaviour of birds near the nest. My objective for this portion of the study was to describe vocalizations used near the nest, including songs, duets, and calls, Then, to evaluate the function of the vocalizations produced near the nest, as well test the functions of duets near the nest, I tested four predictions for relationships between vocal behaviour and nesting behaviour.

1) If wrens produce duets near the nest as a means of communicating about the current environment around the nest, , I predicted that duets would be equally common across the three main nesting stages (late nest building, incubation, and hatchling), given that the bird in the nest has equal need for information about the outside nest environment in all three stages. Further, I predicted that vocalizations would precede a bird's exit from the nest.
2) If wrens perform duets near the nest to coordinate nest visitation, I predicted that duets near the nest would be more common during the nest building stage and hatchling stage than during the incubation stage. In both the nest building and hatchling stages, both parents visit the nest, either to contribute to building the nest or to provision the young, providing a need to coordinate their visits. However, during incubation, only the female visits the nest. Because there is uniparental involvement at the nest during incubation and biparental involvement in the nest building and hatchling stages, similar rates of duetting during the latter two stages, and lower duetting rates during incubation could indicate that birds are coordinating behaviours only when they both have to visit.
3) If wrens perform duets near the nest to coordinate visits, with a goal of minimizing the number of trips to the nest (and consequently the conspicuousness of the nest), I predicted that high rates of duetting would be negatively correlated with visitation rates. Fewer parental visits are associated with lower predation rates (Martin *et al.* 2000), and birds reduce their provisioning rates in the presence of predators or when they have experienced high rates of predation (Martin & Ghalambor 1999, Ghalambor & Martin 2002).

Therefore, rufous-and-white wrens may increase fitness by minimizing trips to the nest.

4) If duets are used as a signal of parental investment, I predicted that high duetting rates would be positively related with high levels of visitation. The signalling quality hypothesis (Smith 1994) suggests that birds perform duets to communicate their quality to their mate, and therefore high quality pairs should both duet and provision more often. Additionally, the signalling commitment hypothesis (Wickler 1980) suggests that performing duets could communicate willingness to invest in reproductive activities with the mate, in which case, birds that duet more should provision more frequently. This relationship could vary with the nest stage, as vocalizations near the nest may become more risky as breeding season progresses.

Methods

I studied a population of rufous-and-white wrens in the Area de Conservacion Guanacaste, Sector Santa Rosa (10^{o}, 40'N, 85^{o}, 30'W), from April through July 2012 and April through June 2013. This time period encompassed the end of the non-breeding period, and the nest building, incubation, and hatchling stages. I carried out nest watches during each of these three stages at nests of 45 individually-marked pairs of wrens. Nests were usually 2-8 m high and in bull's horn acacia trees (*Acacia colinsii*). Due to intense predation by monkeys, snakes, and birds, I was unable to collect data for all three stages of the breeding cycle for many nests.

Nest watches were carried out both in person and using video cameras. All nest watches and videos commenced between 0620 h and 1000 h in the morning. In-person nest watches lasted 1-2 hours (1.27 hours ± 4.00 min average watch length). For in-person nest watches, the observer sat 6-10 m away from the nest under a camouflage screen. A shotgun microphone (model: Sennheiser MKH-70) was set up on a tripod next to the observer, directed toward the nest, to record all vocal behaviour of the birds, as well as descriptions of the parents' physical behaviour dictated by the observer. Recordings were collected with a digital recorder (model: Marantz PMD-660; 44.1 kHz sampling frequency with 16 bit accuracy). The observer dictated nest visits (entry and exit), the sex of the bird, a description of anything the bird brought to the nest (nest building material or food), the distance of the birds to the nest when singing, and other notable behaviours into the microphone.

High definition video cameras (Sony models: HDR-XR101, HDR-CX220, DCR-SR20) were set up to increase the sample size and to test whether the presence of the observer affected the behaviour of the nesting pairs. Cameras were set up 4 to 12 m from the nest and set to record in HD mode. The observer dictated the time, nesting pair, and stage of the nest before leaving the camera. Cameras recorded until the battery died or it was collected at the end of the morning. Consequently, video recording length varied (average ± SE length: 1.12 hours ± 2.28 min average length). To account for differences in nest watch length and recording length, all measures are presented controlled for length of observation period.

I analyzed the recordings of in-person watches using Syrinx-PC Sound Analysis Software (John Burt, Seattle, WA). I used the time and frequency cursors to annotate all nest visits and vocalizations of the birds. Calls, songs, and duets were annotated in this fashion. Video nest watches were analyzed using VLC media player; the observer noted the times of all vocalizations and nest visits of the focal pair.

During the nest building stage, some watches were conducted when the nest was in very early stages, and thus visibility was not limited for the bird inside the nest (figure 3.1 a,b), and some were conducted when the nest was further along and the parents could not be seen once they entered the nest (see figure 3.1 c). I noted these categories as either “open” or “closed”. This provided a natural experiment to test whether vocalizations are used to provide information to the bird inside the nest, as unfinished nests do not limit the visual perception of the bird inside the nest. Given that information would be most valuable to a bird before it leaves a closed nest, vocalizations prior to exit may be used to signal whether or not it is safe to exit. I compared the proportion of nest exits with vocalizations preceding them between pairs building open nests versus closed nests. For analyses comparing the three nest stages, only data for closed nests were used to ensure consistency across stages.

Statistical Methods

To determine whether the presence of an observer influenced the behaviour of the birds compared to the presence of a video camera, I compared the total number of entries and exits from the nest, and the total number of vocalizations, in the in-person watch data versus video data. The data were non-normal only in the incubation stage; therefore, I used t-tests to compare in-person versus video data in nest building and hatchling stages and Mann-Whitney U- tests for the incubation stage. I found that data collected by an observer did not differ from data collected by a camera in any breeding stage for total vocalizations per hour or for number of nest visits per hour (see table 3.1). I therefore pooled in-person and video data in the following analyses.

To test my first prediction, that vocalizations provide information to the bird on the nest, I calculated the proportion of nest exits preceded by a vocalization within 5 sec by either bird. I compared the proportions of visits between nests that were still open to the surrounding environment and nests that were completely opaque (closed) using Mann-Whitney U tests. For this analysis, in-person and video data could not be pooled because different stages of nest building (open versus closed) were recorded; therefore, I removed one of these sets of data (randomly via a coin flip) in order to maintain independence of samples.

To test my second prediction, that duetting behaviour is important in coordinating biparental nest visitation, I compared duetting rates both on and near the nest across the three breeding stages. I defined "near the nest" as within 15m; given the density of the tropical forest study site, this seemed to be a reasonable maximum distance from which a bird outside of the nest could assess the environment surrounding the nest. To make sure the status of the nest during nest building did not confound my comparison, I used only nest building watches in which the nest was classified as "closed." I used generalized linear mixed models with a Poisson distribution and a log link function to compare the three breeding stages, including pair as a random effect, in order to handle non-normal data and incorporate random effects (Bolker *et al.* 2008). For subsequent pairwise comparisons, I corrected for multiple comparisons using the built-in sequential Bonferroni feature of SPSS, which increases P-values rather than adjusting alpha values. Results were considered significant when $P < 0.05$.

To test my third and fourth prediction, that song rates predict nest visitation, I correlated both duetting rates and solo song rates with nest entries and exits per hour. As the relationships between song and visitation may change based on the status of the nest, I performed these correlations separately based on breeding stage. All duetting rate data were significantly non-normally distributed, so Spearman rank correlations were used for correlations using duetting rates, and Pearson correlations were performed for solo song rates.

Data were analyzed using IBM SPSS Statistics 20. Nest watches with no visitations were excluded from my dataset, unless it was clear that the bird was on the nest (i.e., I heard vocalizations from the nest). All tests are two-tailed with a significance threshold of 0.05. All values are presented as means ± SE.

Results

Description of calls near the nest

Rufous-and white wrens produced at least two unique vocalizations on and near the nest. One vocalization heard commonly on the nest, and less commonly near the nest, is a very high pitched, descending whistle which I called the "*squeal*" call (see figure 3.2). Both sexes produced the *squeal* call, which was often heard when one partner was on the nest and the other was nearby. Often when this call was given from the nest during the nest building stage, the partner would join the bird on the nest. Females also used these calls during duetting interactions with the male while incubating. During incubation, the female often gave *squeal* calls immediately preceding a song, so that her song bouts would consist of alternating *squeal* calls and solo songs. In these cases, the male did not go to the nest as he did during nest building. Male birds were heard making a *squeal* call before a song very infrequently. *Squeal* calls were more commonly given by females, although it was often hard to tell which bird produced this quiet call, and there were many occasions when the caller was unknown.

Rufous-and-white wrens also produced calls consisting of a series of high pitched, frequency modulated notes that often ended in a downslurred note; I referred to this call as the "*chitter*" call (see figure 3.2). These calls were heard in every stage of breeding but seemed most common during the hatchling stage. These vocalizations seemed to be given by the female, when the caller was known. Prior observations suggest that *chitter* calls are produced in excess immediately prior to copulation. Other vocalizations recorded near the nest were similar to vocalizations commonly used in other contexts by rufous-and-white wrens, including *whoops, hoots, rattles, ticks*, songs, and duets.

Description of songs near the nest

Birds sang from within the nest, although the behaviour was not common; 14.5% of songs in duets heard during all watches were from the bird within the nest. Singing near the nest was quite common (82% of songs in duets were near the nest; the remaining songs in duets were produced more than 15m from the nest). Females were most likely to sing and duet from the nest during incubation (see below). Overall, 22.7% of all nest entries and exits were associated with vocalizations (of 2,156 entries and exits, 490 were within 5 sec of vocalizations).When there were associated vocalizations, male birds often sang soon after departing the nest (15.9% of all visits had vocalizations after departure during nest building, and 17.0% of visits during hatchling provisioning). Females were not seen to perform this behaviour. Birds did not often enter the nest after vocalizations (115 of 1296 or 8.9% of entries were preceded by a vocalization). They also infrequently exited the nest after a vocalization (122 out of 1290 or 9.5% of all exits were preceded by a vocalization), although vocalizations often preceded departure from the nest during the incubation period (7 out of 15 or 46.7 % of all exits during incubation were preceded by a vocalization). Of the 7 visits that were preceded by a vocalization, in 2 cases the vocalizing bird was the female inside the nest and in 4 cases the vocalizing bird was the male outside the nest (in 1 case it was unknown who vocalized). In both cases where the female vocalized before exit, the vocalization was a *squeal* call; but all other vocalizations associated with exit were songs. After birds left the nest, they tended to fly toward the partner when the partner was nearby. During nest building, pair members would then forage near each other in the nest vicinity. Often, the pair members would join each other and fly out of view after one exited the nest.

Insights into the function of vocalizations near the nest

Vocalizations prior to exit from the nest varied with the stage of nest development (see figure 3.3). Vocalizations prior to nest exit were infrequent when the nest was in its early stages (i.e. an open nest) and visibility from inside the nest was good (6.0 ± 1.9 % of exits). Vocalizations were significantly more common when the nest was already opaque to the outside environment (18.1 ± 4.0 % of exits; Mann-Whitney: U=89.0, P=0.012).

The duetting rates of birds both on and near the nest were different based on breeding stage (see figure 3.4). The number of duets that birds sang near the nest varied significantly based on breeding stage (GLMM whole model: $F_{2,67} = 15.8$, $P < 0.001$). Birds sang more duets near the nest during the nest building and incubation stages than during the hatchling stage (nest building versus hatchling and incubation versus hatchling, both $P < 0.001$; nest building versus incubation $P = 0.15$). The number of duets that birds sang from directly on the nest also varied, though with a different pattern (GLMM whole model: $F_{2,67} = 17.7$, $P < 0.001$). Birds sang more duets on the nest during the incubation stage than during either hatchling or nest building (incubation versus hatchling and nest building versus hatchling, both P=0.02), with birds duetting at similar rates during hatchling and nest building stages ($P = 0.54$).

I found no relationship between the number of duets near the nest and the number of entries and exits per hour in any breeding stage (see figure 3.5; Spearman correlations: nest building: N =36 , $R_S = -0.167$, $P = 0.33$; incubation: N = 19, $R_S = -0.324$, $P = 0.17$; hatchling: N = 15, $R_S = 0.031$, $P = 0.91$). There were also no significant correlations between the number of solos near the nest and the number of entries and exits per hour in any stage (see figure 3.5; Pearson correlations: nest building: N = 36, R = -0.273, *P* = 0.11; incubation: N = 19, R = 0.060, *P* = 0.81; hatchling: N = 15, R = -0.237, *P* = 0.40).

Discussion

Rufous-and-white wrens vocalized prior to a bird leaving the nest more often when the nest was closed than when it was open, suggesting that birds were providing information to the bird on the nest before it exited into the outside environment. Birds sang more duets near the nest during incubation and nest building than during the hatchling stage. They also sang more duets while they were sitting in the nest during incubation than during the nest building and hatchling stages. The number of solos sung from near or on the nest did not differ between these three breeding stages. I found no evidence that birds who sang more duets or solos near the nest visited the nest more or less often than birds who sang fewer duets or solos near the nest. Taken together, these results indicate that vocalizations near the nest are important in communication between the bird within the nest and the bird outside.

I characterized, for the first time, the vocalizations that rufous-and-white wrens use in the vicinity of the nest. These include previously characterized vocalizations such as songs and duets, hoots, whoops, and atonal calls. However, I also described two new vocalizations, *squeals* and *chitters*, that often occur on the nest and have rarely been heard in other contexts. Although I did not quantify amplitude, *squeals* and *chitters* were noticeably quieter than either songs or other calls. These quiet vocalizations may function in close-range communication, as they were heard during the nest building stage when both pair members were on the nest, during duetting encounters when the female was incubating and the male was nearby, and during the hatchling stage, when the parent may have been communicating with the hatchlings. Zebra finches also make quiet calls while on the nest that function in mate recognition, as well as combining quiet calls to make private duets that maintain the pair bond (Elie *et al.* 2010). Similarly, black-capped chickadees (*Parus atricapillus*) produce quiet vocalizations near the nest during incubation. In these birds, the male sings quiet songs outside of the nest cavity, and the female emerges from the nest. The vocalizations seem to attract the mate when it is not visible, allowing birds to coordinate feeding activities at the nest hole (Ficken *et al.* 1978). Rufous-and-white wrens could be using quiet calls in a similar way: the *squeal* call seems to attract the mate to the area, and after a bird leaves the nest it most often moves toward its mate.

If duets near the nest played a role in coordinating the nest visitation of the parents, I predicted that duetting near the nest would be more common in the nest building and hatchling stages—the two stages when both parents frequently visit the nest to add nesting material or provision the young. In contrast to this prediction, my results reveal that duetting near the nest was more common during incubation and nest-building than during the hatchling stage. This result suggests that rufous-and-white wren duets do not serve to coordinate the nest visits of the two parents. Previous work on rufous-and-white wrens indicates that duet output peaks during the pre-breeding and nest building stages and decreases during the incubation and nestling stages of the breeding cycle (Topp & Mennill 2008). My results show an interesting deviation from this pattern. In the current study, I show high duetting rates near the nest during nest building, which corresponds with high duetting rates during the fertile period documented previously (Topp & Mennill 2008). However, prior analyses demonstrated that total duet output then drops off during the incubation and hatchling stages in previous research (Topp & Mennill 2008), whereas my data show that near-nest duetting rates increase slightly during incubation, dropping off later during the hatchling stage. This could indicate that while total output decreases, the proportion of duets sung near the nest during incubation is much greater than the proportion of duets near the nest during building. This change in locale of production could correspond with a change in the function of duets according to context.

Both the current data on near-nest duets and previous data on seasonal variation in vocal output (Topp & Mennill 2008) show low rates of duetting during the hatchling stage. If parents were using duets to coordinate visits, I had predicted that birds would duet more during the nest building and hatchling period, when both birds need to visit to build or provision young. High duetting rates near the nest during nest building, but not hatchling stages, indicate that there might be a cost to duetting too much near the nest. Because the parents' investment in nestlings is obviously higher than investment in an unfinished nest, duetting near the nest may be too risky at the hatchling stage. Parental activity at the nest increases predation risk (Martin *et al.* 2000), but the costs of vocalizations near and on the nest are poorly understood. Analysis of North American birds did not find a risk of higher predation or parasitism for birds that sing on the nest, although due to difficulties in collecting data from many sources, it is unclear whether the skew toward higher variance in predation could be interpreted as a cost associated with vocalizations on the nest (Leonard 2008). Red-winged blackbird nests with *chit* vocalizations being played experience higher predation when there are no attending parents; however, successful females answer their partner's songs with *chit* calls more often (Yasukawa 1989). This indicates that there is a net benefit to producing the *chit* call from the nest, despite the cost of making the nest more conspicuous. For rufous-and-white wrens, parents may benefit by visiting the nest quietly during the hatchling stage because predation risk is extremely high. If this is the case, they may use duets to coordinate during the nest building stage when the cost of discovery is low, but remain silent near the nest when there are hatchlings.

Interestingly, duetting from directly on the nest showed a different pattern, occurring most frequently during incubation and rarely during either nest building or hatchling stages. This result is more consistent with my hypothesis that duets might be used to provide information to a bird inside the nest than my hypothesis that birds use duets to coordinate parental visitation. If wrens use duets near the nest to provide information about the external environment, I might expect more duets during incubation, when the bird on the nest cannot assess visual cues about the environment for longer periods of time. In the incubation stage, the female bird is on the nest a large proportion of the time, whereas during the hatchling stage, the male and female visit more often but for shorter periods of time. This alone could account for more duets coming from directly on the nest; however, it does not account for duets near the nest being common during incubation, and duetting from on the nest is relatively rare even during incubation, despite the extensive time spent on the nest. Increased duetting behaviour from on the nest during the incubation stage may be an indication that birds are using these duets to communicate information to the bird sitting enclosed in the nest.

The use of vocalizations before nest exit when the nest is closed, but not open, provides additional support for the idea that rufous-and-white wrens use vocalizations to communicate information between visually separate birds. It could be that the outside partner is giving an "all clear" signal (Johnson & Kermott 1991), that the leaving partner is signalling their departure from the nest (Leonard 2008), or that the birds are locating each other using vocalizations, as has been shown for this species when performing duets outside the nest (Mennill & Vehrencamp 2008). In my data, most of the vocalizations preceding exit were given by the outside partner (during nest building, 65 of the exits with vocalizations preceding them were given by the outside partner, in 11 the vocalization was given by the exiting bird, and 15 were unknown). During incubation the pattern was similar, though there were fewer exits (in 6 exits the vocalization was given by the outside partner (male) and in 2 cases the vocalization was given by the departing female). This indicates that in some instances the bird in the nest may be announcing their departure, but the majority of the time, the partner's vocalization is preceding their mate's exit from the nest. This result is similar to the results of Halkin (1997) in which northern cardinals use vocal interactions while the female is on the nest to either attract or deter the male from visiting. In this case, however, the vocalizations of the bird off the nest are associated with the bird leaving the nest.

I had also predicted a relationship between duetting behaviour and nest visitation behaviour if birds were using duets to coordinate nest visitation. I found no evidence that duetting behaviour was correlated with visitation. If birds were using duets to coordinate trips to the nest and minimize visitation, I predicted that higher rates of duetting would be associated with fewer visits. Alternatively, if duetting behaviour is an indicator of parental investment (i.e. it signals the quality or commitment of the mate; Wickler & Seibt 1980, Smith 1994), I predicted higher duetting rates were associated with more nest visits. My data match neither prediction; I found no correlation between duetting rates and visitation. Based on my other data, birds may not be coordinating visitation with duets but may be communicating information to the bird inside the nest. In this case, duetting rates near and on the nest might be correlated instead with time spent on the nest. Additionally, duets are used for multiple functions in this species, including territory defence and maintaining contact (Mennill & Vehrencamp 2008), so duets near the nest may be functioning in this manner as well. If duets are used by this species to fulfill both of the functions that my opposing predictions are based upon, the relationships could cancel each other and give rise to no significant correlations.

The function of song on the nest has been given very little attention, possibly because it is not common; the only careful assessment to date suggests that this behaviour occurs in one tenth of breeding birds in North America (Leonard 2008). Within North American songbirds, the occurrence of song on the nest was dependent on family, sociality, and brood parasitism, and incubation sharing, feeding, and nest type were not significant factors (Leonard 2008). The occurrence of song on the nest has not been quantified for tropical species as far as I know. Several hypotheses have been proposed concerning the function of songs given on the nest: (1) to solicit feeding by the mate, (2) to signal departure from the nest, (3) to maintain contact with the partner, (4) to signal the mate to aid in nest defence, (5) to maintain territory boundaries, especially if territories are limited, (6) to invite extra-pair copulations, and (7) to assist in mate identification (Leonard 2008). Based on my data, acoustic contact and signalling departure seem most likely in rufous-and-white wrens. The only other species that is known to use duets to coordinate nest visitation is the slate-coloured boubou, which uses duets to coordinate incubation changeovers in the absence of visual cues (Sonnenschein & Reyer 1983). Rufous-and-white wrens do not use duets in this fashion, as only the female incubates; however, they do increase duetting rates during incubation. One other study quantified male song in relation to visitation in a related non-duetting species, the Carolina wren (*Thryothorus ludovicianus;* Neudorf *et al.* 2013). The authors suggest that for this species, the male sings to alert the female that he has provisioned the nestlings and to communicate his location. In my study, birds vocalized after about 17% of exits from the nest, which is substantially less than the 49% of visits in which male Carolina wrens sang after provisioning. However, my data are not separated by sex, and I rarely saw females sing after provisioning, so the numbers are not directly comparable and may in fact be more similar.

Our results indicate that duetting is not used to coordinate visitation to the nest between the male and female, at least during the hatchling stage. They do suggest, however, that duets and other vocalizations play a role in communication between parents at a nest, possibly conveying information to the partner with restricted information inside the enclosed nest. More research is needed to determine the functions of duetting on the nest. In particular, it would be helpful to determine whether duetting on the nest communicates information to the bird inside the nest or is simply a means to maintain acoustic contact with the bird within. A more comprehensive analysis of vocal interactions at the nest and subsequent behaviours of the birds would help to determine whether birds are duetting to determine if their partner is on the nest or are duetting to attract the mate off of the nest for other behaviours. Another area of interest would be to explore whether certain types of vocalizations are associated with a bird's exit from the nest versus birds vocalizing and remaining within the nest. Continued research on the vocalizations that birds produce near the nest will help to provide insight into the relationship between parental care and vocal behaviour.

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Tables and Figures

Table 3.1. Comparison of in-person and video nest watches. Data collected by an observer did not differ significantly from data collected by a camera in either total number of vocalizations per hour or number of nest visits per hour (independent samples t-tests; asterisks indicate values of U from Mann-Whitney tests on non-normal data).

	N		Mean Nest Visits per Hour				Mean Vocalizations per Hour			
	Observer	Video	Observer	Video	Test statistic	*P*	Observer	Video	Test statistic	*P*
Nest Building	20	16	46.6	56.4	t = 0.85	0.40	128.0	105.3	t = 0.94	0.35
Incubation	10	9	1.15	1.40	U =27.0	0.16	123.7	81.7	U = 49.0	0.78
Hatchling	4	11	16.0	20.3	t = 0.63	0.54	120.3	102.8	t = 0.53	0.60

Figure 3.1. Three photographs depicting different stages of nest building for rufous-and-white wrens. The top two photos are examples of “open” nests, where the birds could reliably see the outside nest environment from within the nest, and the bottom photo is an example of a “closed” nest. In photo a, the nest is in early stages and is a simple platform of sticks. Photo b shows a tunnel shaped nest taken head on so that the hole through the center is visible. While the nest is closer to completion, it is still open to the outside environment. Photo c shows a complete nest viewed from the side. The sides are opaque and the bird inside would be obstructed from seeing outside the nest in any direction.

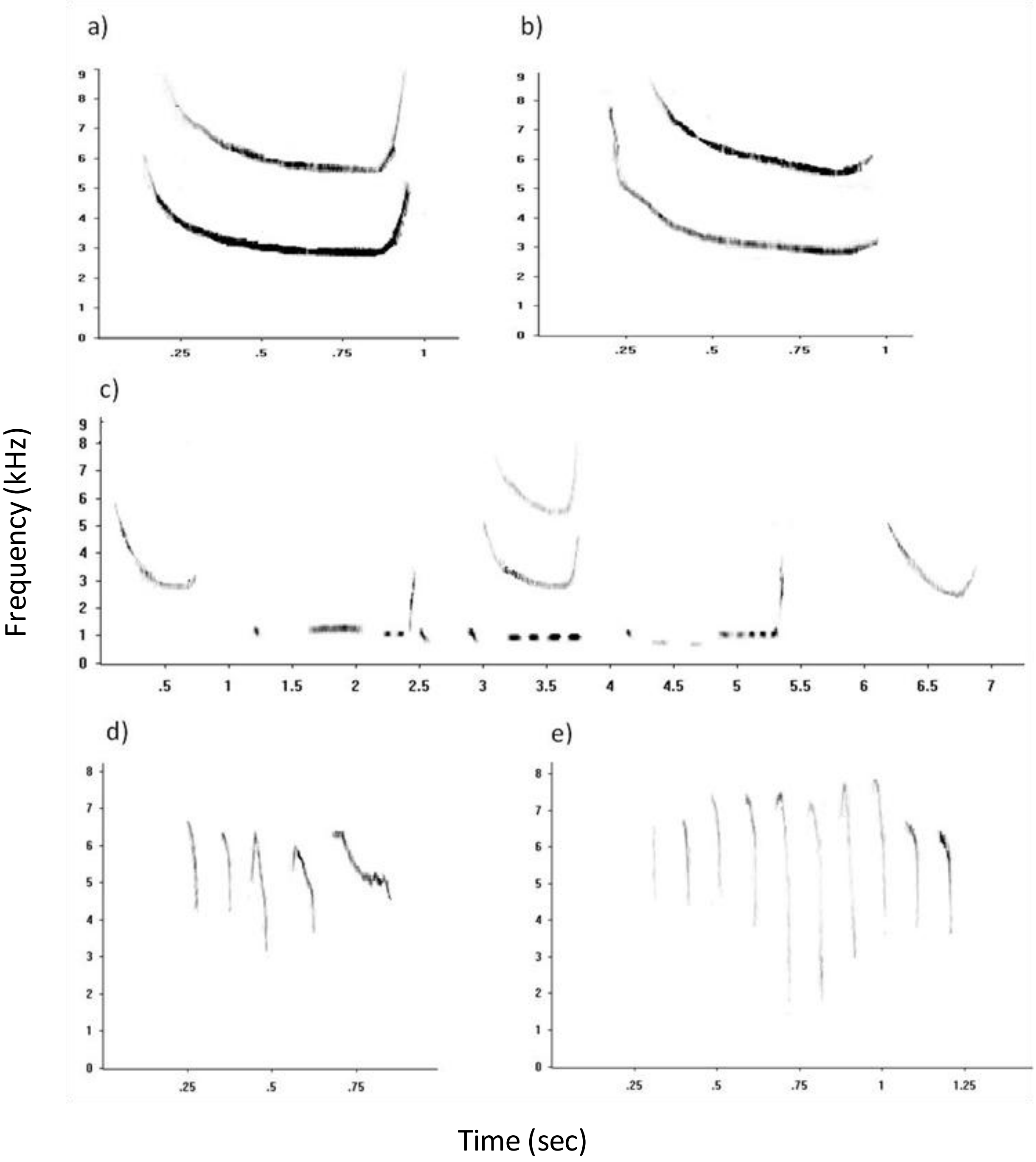

Figure 3.2. Spectrograms showing examples of calls used by rufous-and-white wrens on and around the nest: (a) a female squeal, (b) a male squeal, and (c) a female using squeal calls alternately with songs during a duet. Figures (d) and (e) show examples of two different *chitter* vocalizations recorded at the nest; the sex of the calling bird was unknown.

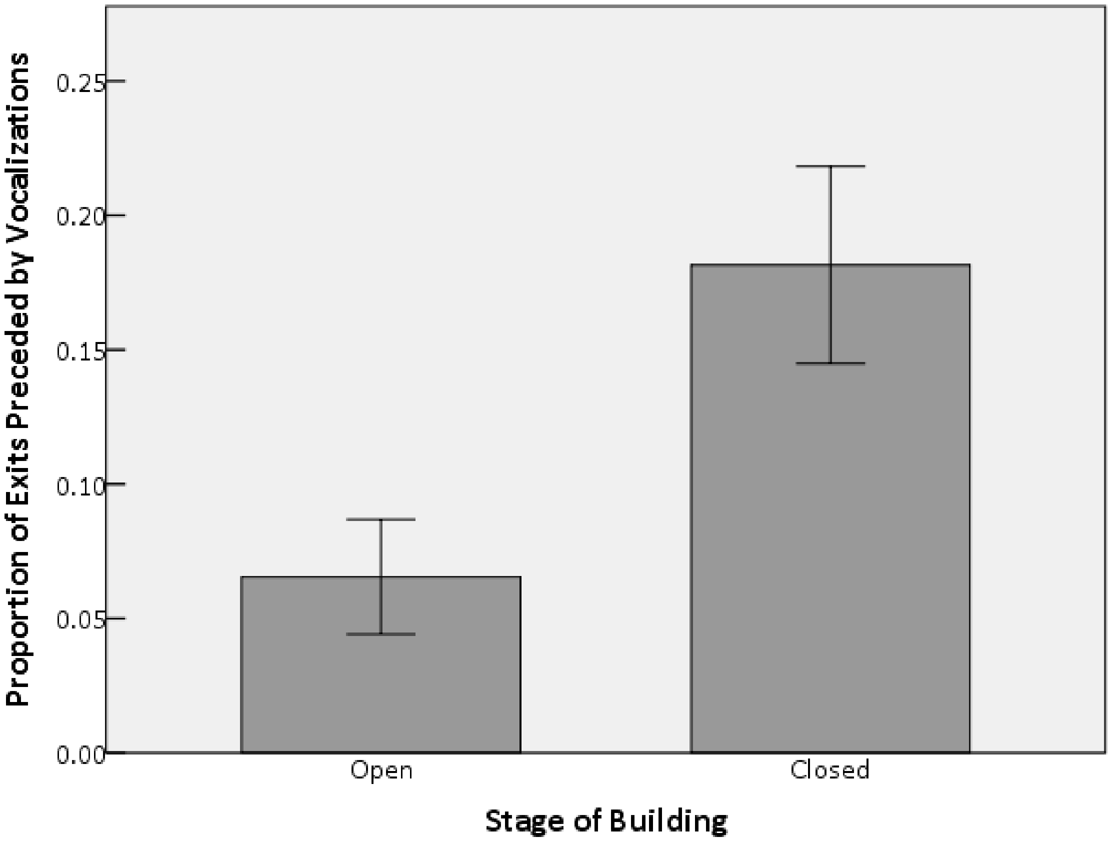

Figure 3.3. Rufous-and-white wrens were significantly more likely to vocalize before exiting once the nest was completed (completely opaque) than they were when the nest was still open to the outside environment. Error bars indicate ± SE.

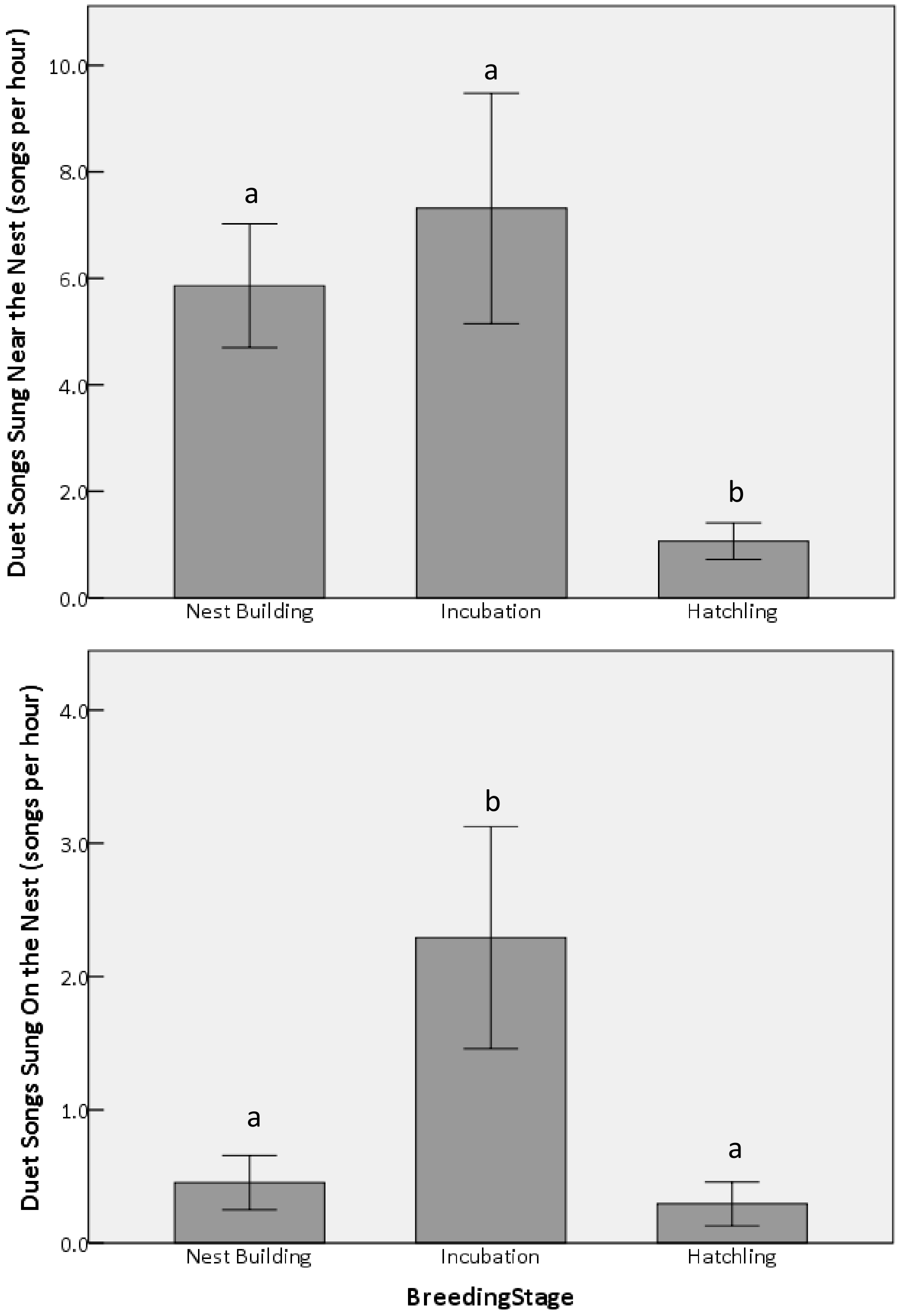

Figure 3.4. Rufous-and-white wren duets sung near and on the nest, per hour, by breeding stage. Duets near the nest show a different pattern based on breeding stage than duets sung on the nest. Error bars indicate mean ± SE. Significantly different breeding stages are indicated by different letters.

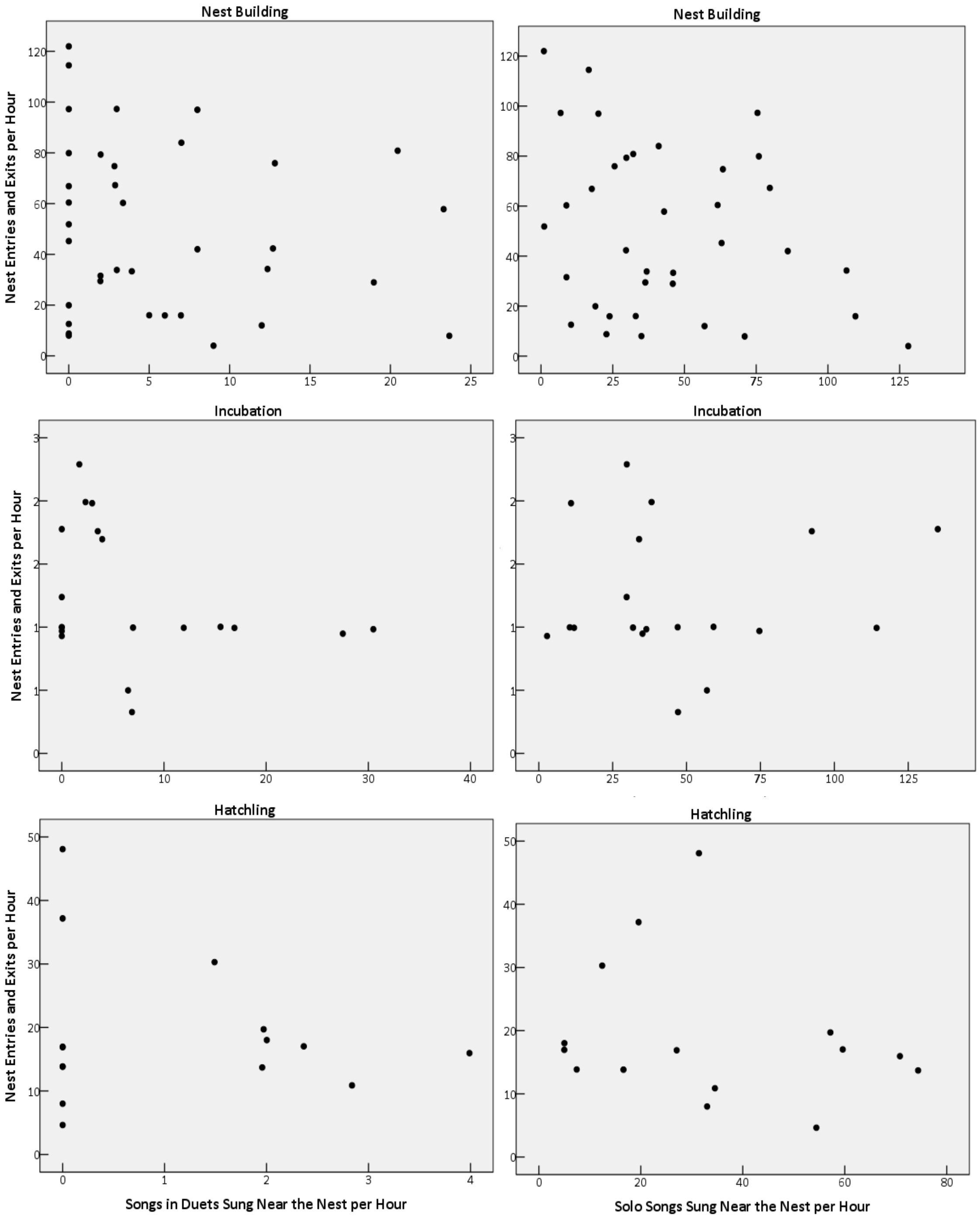

Figure 3.5. There was no correlation between duetting rates and visitation rates in any breeding stage for rufous-and-white wrens (left); nor was there any correlation between solo singing rates and visitation rates in any breeding stage (right); note different scales on both x and y axes.

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Chapter 4

General Discussion

Communication is an important behaviour in the lives of animals, allowing them to transmit information between individuals that may be important in determining future behaviours (Bradbury & Vehrencamp 2011). Acoustic communication is a widely-studied mode of communication, and because it is long-ranging, it is especially useful for animals living in heavily vegetated environments (Bradbury & Vehrencamp 2011). Birds use vocal signals for many different purposes, most importantly for mate attraction and territory defence (Catchpole & Slater 2008). Birdsong is a model system for communication and vocal behaviour; however, most of my knowledge is based on male song, and less is known about female singing behaviour (Catchpole & Slater 2008). Female song is uncommon in north temperate regions, but it is quite common in tropical regions (Langmore 1998). In many tropical bird species, females combine their songs with those of males to form duets, which are temporally coordinated vocalizations of a mated pair (Hall 2004). Duets have received significant attention in the literature, and yet their functions remain unclear; often studies conclude that duets are used for multiple functions that are context dependent (reviewed in Hall 2009). Currently, the primary function attributed to duets is joint resource defence (Hall 2004, 2009).

The first data chapter of my thesis (chapter 2) examined the function of duetting in territory defence. Temporal coordination is a defining feature of duets (Hall 2004), and I tested the hypothesis that highly coordinated duets are more threatening signals than poorly coordinated duets or alternating solos in the context of a territorial intrusion. I used three related species of neotropical wrens in order to test this hypothesis comparatively among species whose natural duetting styles corresponded with each of the treatment conditions: plain wrens (*Cantorchilus modestus*) duet antiphonally with a high degree of coordination, rufous-and-white wrens (*Thryophilus rufalbus*) sing loosely coordinated duets, and banded wrens (*Thryophilus pleurostictus*) are not known to duet (Mann *et al.* 2009). Physical responses of territorial pairs of all three species did not indicate that they differentiated between levels of coordination, but they did respond to conspecific playback more aggressively than they did to control playback. Vocal responses indicated that they performed more duets in response to playback of duets than playback of solos. Additionally, birds responded by singing more solos in response to playback of highly coordinated duets and alternating solos than they did to poorly coordinated duets, a pattern driven largely by plain wrens. This interaction of treatment and species could indicate that coordination is important only to species that show extremely high levels of coordination naturally. Species differences also indicated that the more closely related species behave more similarly to each other than to the non-congener. I concluded that coordination does not affect physical aggressive responses; however, vocal responses indicated that birds perceived differences in coordination.

In my second data chapter (chapter 3), I explored a within-pair function of duetting: whether duets are used as a means of coordinating breeding activities. Hypotheses for duet function that relate to breeding often deal with coordinating physiology (Todt & Hultsch 1982); however, using duets to coordinate parental nest visitation could also be important to optimize provisioning rates (Martin *et al.* 2000). For this chapter, I collected observational data through nest watches during each of three breeding stages: nest building, incubation, and hatchling provisioning. I tested the hypothesis that birds were using duets to coordinate nest visitation by comparing duetting rates over the three stages. My results revealed that birds do not seem to be using duets to coordinate visitation, as duetting rates from on the nest and near the nest are highest during incubation, when only one bird was visiting the nest. This, and a comparison of open versus closed nests, provides evidence that birds may be using duets and other vocalizations to transmit information between the bird inside the nest and the outside partner. I also tested whether duetting rates could predict visitation. I found no correlations between visitation rates and duetting rates during any of the three stages, providing further evidence that duets are not being used to coordinate nest visitation behaviour in rufous-and-white wrens.

These two approaches to the study of duetting provide further evidence that duets are multi-functional signals that can be used in a variety of contexts (i.e. Grafe & Bitz 2004, Mennill & Vehrencamp 2008, Benedict 2010). In chapter 2, I showed that duetting rates increased in response to duets, and less so in response to solos, indicating that duetting plays a role in defending a territory. The results of chapter 2 also indicate, however, that physical measures of aggression do not change based on the level of coordination or whether a song is broadcast as a solo or a duet. This provides some evidence that the coordination of songs into duets is not an important factor in whether a bird responds aggressively toward intruders. If coordination of songs into duets was not a threatening signal between pairs, perhaps it has a more important within pair function. Chapter 3 provided evidence that there is a within pair function of duetting during the breeding cycle. My results indicated that birds do not use duets to coordinate nest visitation, as visitation was lower during the hatchling stage, when birds have to cooperate to provide parental care, than during incubation, when only one bird is on the nest. Birds are, however, using duets near and on the nest, mostly during incubation. This could provide evidence that birds communicate information about the outside environment to the bird inside the nest. Taken together, these chapters support current literature indicating that duets serve multiple functions. The results presented in this thesis are consistent with the idea that duets are important in territorial defence, and provide new evidence that duetting may be a way to communicate with an incubating partner in the area around the nest.

An interesting avenue for future research, extending the results of chapter 2, would be to examine duet coordination in plain wren responses to the varying levels of coordination. I examined large scale behavioural and vocal responses; however, the fine details of vocal responses were not examined. Plain wrens commonly sang many duets in response to playback, making it possible to analyze the coordination of their responses. A closer look at the coordination coefficients of the responders may reveal interesting differences based on the coordination of the stimuli. Additionally, long-term studies could examine duet coordination over time, to see if pairs that have been together longer have more highly coordinated duets or respond more often to their partner. Comparative research is needed across many different duetting styles; further insight into the function of duets for a variety of species could reveal insightful patterns when mapped onto a phylogeny. It would be useful to compare functions and ecological correlates for species in related groups that show different types of duetting than the rest of their congeners (Hall 2009, Mann *et al.* 2009).

There is much to be learned about nesting behaviour and vocalizations, not only in these wren species, but in all tropical birds and even all birds generally. Nest associated vocalizations are often given little attention because they are rare (Leonard 2008); however, as they may risk reproductive success, they may be secretive behaviours, and their occurrence and importance may therefore be underestimated. A useful future avenue for research would be to determine the extent of duetting on the nest in other tropical duetting species, as well as categorization of subsequent behaviours in order to determine the function of duets on the nest. Additional research could further examine the functions of squeal or chitter calls using playback; although obtaining high quality recordings of these very quiet vocalizations for playback stimuli may prove difficult. Use of recording devices such as microphones and nest cameras in the nest may be a useful way to obtain further information about how vocalizations are related with visitation behaviour.

In summary, my research provides insight into the function of duets as multifunctional and context dependent signals. My research on coordination shows that the coordination of songs into duets does not seem to change the physical aggressive responses of the territorial pair, while still showing that duetting rates increase more in response to playback of duets compared to solos. My results indicate that duet coordination may not be used for signalling between pairs, and thus coordination may be more important within a pair. My research on nest-associated duets indicates that duets may be used to communicate between the bird within the nest and its partner outside the nest. I also show that vocalizations before exit may be used as an "all clear" signal to leave the nest (Johnson & Kermott 1991). Taken together, my two data chapters have provided further evidence that the function of duets is dependent on the context of the behaviour.

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