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ACOUSTIC AND VISUAL SIGNALS OF SPECIES DISCRIMINATION IN COMPETING NEOTROPICAL WRENS

By

KRISTINA GABRIELLA HICK

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Acoustic and Visual Signals of Species Discrimination in Competing Neotropical Wrens

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Declaration of Co-Authorship

I. Co-Authorship Declaration

I herby declare that this thesis incorporates material that is the results 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 Daniel Mennill and Stéphanie Doucet (University of Windsor) who contributed input on the experimental design, analyses, and writing, as well as providing funding for the field research. In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by myself, with input from my supervisors.

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.

This thesis includes one original paper that has been previously submitted for publication in a peer-reviewed journal, as follows:

Thesis Chapter	Publication title/full citation	Publication Status
Chapter 2	Hick, KG, Doucet, SM, Mennill, DJ. (Submitted). Interspecific vocal discrimination in neotropical wrens: responses to congeneric signals in sympatry and allopatry. Anim. Behav. ANBEH-A15-00321R.	Submitted

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I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has not been submitted for a higher degree to any other University or Institution.

Abstract

Animals must discriminate between individuals within their own species, and between individuals of their own species and individuals of competitor species, allowing animals to differentiate between threatening rivals, non-threatening individuals, and potential mates. Studying two competing neotropical wren species, I tested the influence of experience on species discrimination using acoustic playback. Contrary to my predictions, the playback experiment showed that species discrimination was not influenced by previous experience with a competitor species. I also studied the relative importance of acoustic and visual signals for intra- and interspecific discrimination using playback combined with presentation of visual models. The playback-and-model-presentation experiment showed that wrens in dense habitats use both acoustic and visual signals for species discrimination, but rely more on acoustic signals. My research provides insight into species discrimination and is the first study to investigate how male and female birds in the tropics use multimodal signalling for intra- and interspecific discrimination.

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CHAPTER 1

GENERAL INTRODUCTION

Introduction

Animals use diverse signals to communicate information about their species identity, sex, size, fighting ability, individual identity, and dominance (Bradbury & Vehrencamp, 2011). Animals must be able to identify individuals in a social context to distinguish between an individual that represents a threat versus an individual that may represent a potential mating opportunity (Bradbury & Vehrencamp, 2011). Animals communicate using a variety of signal modalities including chemical, tactile, acoustic, and/or visual signals. Animals use signals for mate attraction and intra- and inter-specific competition, and therefore animal signals are shaped by both natural and sexual selection (Bradbury & Vehrencamp, 2011). Birds are an ideal system for studying acoustic and visual communication because many species have complex songs and elaborate plumage which can be used for mate and species discrimination (Catchpole & Slater, 2008). My thesis investigates the influence of experience on species discrimination between two competing tropical birds, while also exploring their use of visual and acoustic signals for discrimination. In this General Introduction I provide an overview of topics relating to the function of bird song, intra- and interspecific discrimination, heterospecific aggression, and multimodal signalling, while also providing a description of my study species and study site. The information in this General Introduction provides the background for the two data chapters that follow.

The Functions of Bird Song

Avian vocalizations can be categorized as songs or calls. Songs are long, complex

vocalizations that are produced mainly in the breeding season to deter rival males and

attract females. Conversely, calls are shorter, simpler and are used by both sexes in

particular contexts such as signaling alarm or maintaining contact (Catchpole & Slater,

2008). Male birds tend to sing more than females, especially in north temperate species

where female song is rare or absent. Males use song to defend territories and resources

against intruders, and to attract and identify viable mates (Bradbury & Vehrencamp,

2011). A noteworthy experiment carried out by Krebs (1977) removed male great tits

(*Parus major*) from their territories and replaced half of the males with a speaker

broadcasting male great tit songs, and half were left empty and silent. Krebs (1977)

found that the silent territories were re-occupied significantly faster than the territories

with speakers, although eventually all territories were re-occupied. This study shows

that song is a successful deterrent for intruders for a limited time, but that the physical

presence of a bird is needed to defend the territory long-term (Krebs 1977). In addition

to the importance of male song in territory defense, females use song to assess the

quality of potential mates and they prefer males with high quality vocal signals (Marler

& Slabekoorn, 2004). An experiment by Eriksson and Wallin (1986) with pied flycatchers

(*Ficedula hypoleuca*), for example, showed that male song attracts females. They placed

male taxidermic mounts on nest boxes and found that females were more attracted to

nest boxes with the taxidermic mount and speakers broadcasting male pied flycatcher

song than nest boxes with only the taxidermic mount (Eriksson & Wallin, 1986). Taken

together, these and other studies reveal that bird song serves two critical within-species functions: resource defense and mate attraction (reviewed in Marler & Slabekoorn, 2004).

The majority of studies have focused on the functions of bird song and avian communication in temperate birds, but much less is known about avian communication signals in tropical bird species (Stutchbury & Morton, 2001). The tropics contain diverse bird species that experience significantly different ecological pressures compared to north temperate species. The majority of tropical birds hold and defend their territories throughout the year and experience prolonged breeding seasons (Slater & Mann, 2004; Stutchbury & Morton, 2001). Female song is much more common among tropical birds, perhaps due to the high selective pressures of defending territories year round; this trait is rare in north temperate birds (Slater & Mann, 2004; Stutchbury & Morton, 2001). A recent meta-analysis found that female song is widespread and is the ancestral state in birds (Odom et al. 2014). These characteristics of tropical birds make them important to study for understanding the evolution of communication signals in birds.

Species Discrimination

Animals have evolved complex species discrimination signals, which may include acoustic signals, visual signals, or signals in other modalities. We expect strong selection for signals to contain cues of species discrimination; misidentification may cause an animal to waste energy courting an individual who is not a viable mate, or an animal may lose resources if they fail to defend their territory against a legitimate threat

(Grether, 2011). Animals use species-specific templates to discriminate between

conspecific and heterospecific individuals on the basis of their signals. Some studies

suggest that this template is learned over time from experience with conspecific and

heterospecific animals (Catchpole, 1978; Grant & Grant, 1997; Irwin & Price, 1999; Lynch

& Baker, 1990; Matyjasiak, 2004), while others suggest that this template is innate and

modified through learning (Hauber et al., 2001; Sandoval et al., 2013).

Securing a mate is vital for animals to reproduce, yet discriminating between

competitors and non-competitors is important in social aspects not pertaining to mating

such as foraging, migration, and territory defense (Göth & Hauber, 2004; Grether, 2011).

Closely-related species have diverged in certain characteristics to allow for

discrimination to avoid unnecessary but costly fights or mating with the wrong species.

For example, a study of tropical seedeaters explored whether two recently-diverged

species, *Sporophila hypoxantha* and *S. palustris*, can discriminate between conspecific

songs and heterospecific songs. Males of both species responded most strongly to

conspecific songs, suggesting that song is maintaining reproductive isolation between

these species (Benites et al., 2014). Studies investigating species discrimination in

animals that live in zones of sympatry and allopatry can help us understand whether

experience affects discrimination. If closely-related species live in sympatry and compete

for resources, we expect them to discriminate and direct aggression towards

heterospecific animals (Grether et al., 2009). If species discrimination is learned and

influenced by experience, species living in isolation from each other in allopatry should

not be able to discriminate between one another. If, on the other hand, species

discrimination is innate, species discrimination should be present in both sympatric and allopatric populations.

Asymmetrical Interspecific Aggression

Birds have been shown to express more aggression towards a conspecific individual (i.e., a member of their own species) versus a heterospecific individual (i.e., a member of a different species; see Appendix at the end of the General Introduction for a list of some key terms). A conspecific intruder is more likely to usurp not only their territory and resources, but also their mate (Jankowski et al., 2010; Ord & Stamps, 2009), whereas heterospecific animals will only usurp resources and not mates. This means that animals should direct the highest aggression towards conspecific animals but direct some aggression towards heterospecific animals if they inhabit similar ecological niches and share common resources (Ord & Stamps, 2009).

In some cases, interspecific aggression is found to be asymmetrical, with one species being dominant and more aggressive than the other. The subordinate species is usually forced to inhabit suboptimal territories (Jermacz et al., 2015), which may ultimately result in niche partitioning between the species (Jakowski et al., 2010). This asymmetrical aggression and greater access to superior resources has been termed "behavioural dominance" or "social dominance" (Morse, 1974). Long-term dominance relationships formed due to asymmetrical aggression may result in directional selection, with the subordinate species having evolved larger niches to avoid being excluded from resources by the dominant species (Morse, 1974). The dominant species commonly has

characteristics that allow it to outcompete the subordinate species, such as larger body size, weaponry, and increased ability to dominate resources (Freshwater et al., 2014). It is important to understand how competitor species interact for a shared resource to gain insight into how changing distributions will affect the success of the subordinate species.

Multimodal Signalling

Animals use multiple signals for species discrimination to aid in more efficient species discrimination. There are many hypotheses explaining why multimodal signalling is beneficial (see Bro-Jørgensen, 2009), but there are two that are prevalent in the literature. The Multiple Messages Hypothesis states that each signal conveys unique information about the individual, allowing the receiver to acquire more information about the signaler than a unimodal signal (Hebets & Papaj, 2005). In contrast, the Redundant Signal Hypothesis states that the signals both convey the same information about the signaler allowing for more effective and error-free discrimination (Anderson et al., 2013; Partan & Marler, 2005). A variable environment is expected to promote the use of multimodal signalling so the receiver can acquire information from the sensory modalities that best transmits through the present conditions (Bro-Jørgensen, 2009). For example, if an auditory signal is not able to be heard due to high winds, a visual signal can still facilitate discrimination. This may be especially important in the noisy and densely vegetated environments of the tropics.

Birds can use both acoustic signals such as song and visual signals such as plumage for species discrimination. Birds are understood to have a poorly developed olfactory system, and they therefore rely more heavily on acoustic and visual signals for communication (Catchpole & Slater, 2008). Their use of these signals can differ depending on their environment. Bird song is able to travel farther and facilitates better communication in a densely vegetated habitat. However, sound transmits differently depending on habitat and environmental noise. The structural properties of the environment influence signal propagation, and thereby influence the evolution of animal acoustic signals (Morton, 1975; Wilkins et al., 2012). Birds living in urban areas have altered the frequency of their song so that they can be heard over anthropogenic noise (Brumm, 2006). For example, urban great tits (*Parus major*) sing at a higher frequency to allow their song to be heard over the low-frequency noise in their habitat (Slabbekoorn & Peet, 2003). Similarly, in the wild, birds adjust their songs to compensate for natural noise sources such as ocean surf (Gough et al., 2014) or loud streams (Brumm & Slabekoorn, 2005).

Visual signals can be used in open habitats, noisy environments, or at close distances in dense habitats (Grafe et al., 2012; Partan & Marler, 2005; Uy & Safran, 2013). Visual signals are unlikely be relied on as heavily in environments with dense vegetation or between animals with very similar or inconspicuous plumage. The amount of contrast with the background will affect how a visual signal is transmitted (Bradbury and Vehrencamp, 2011). Birds such as manakins and warblers species have evolved conspicuous plumage to better contrast against the background and enhance signal

transmission (Doucet et al., 2007; Marchetti, 1993). For example, in eight *Phylloscopus* warbler species, those living in darker habitats have more bright patches than birds living in habitats with high levels of light (Marchetti, 1993). Furthermore, when these bright patches were altered, it was found that increasing the conspicuousness of the birds increased their territory size, whereas decreasing their conspicuousness decreased their territory size, suggesting that brightness and conspicuousness in the environment plays a role in interspecific communication (Marchetti, 1993). Visual signals could serve an important role in discriminating between conspecific and heterospecific animals as well as between conspecific males and females.

Study Site and Species

My research investigates species discrimination between two closely related neotropical wrens (Mann et al., 2006): rufous-and-white wrens (*Thryophilus rufalbus*) and banded wrens (*T. pleurostictus*). Wrens (family: Troglodytidae) are a group of sexually monochromatic birds with relatively drab plumage that are widely recognized for their incredibly complex songs (Brewer, 2001). In most wren species, especially in the tropics, both males and females sing to attract mates and defend territories year round. Previous playback studies in these two species show that song plays a role in territory defense and mate guarding (Hall et al., 2015; Vehrencamp et al., 2014; Mennill & Vehrencamp, 2008; Topp & Mennill, 2008; Mennill, 2006; Molles, 2006; Molles & Vehrencamp, 2001), as well as interspecific discrimination (Molles & Vehrencamp, 2001).

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Both of my study species nest primarily in bull horn acacia trees (*Vachellia collinsii*; Joyce, 1993; Molles & Vehrencamp, 1999) and occupy similar foraging niches (Ahumada, 2001; Molles & Vehrencamp, 1999). Acacia trees contain spikes along with resident ants that act as a predator defense for the nesting wrens (Haemig, 2001). This species of tree has a symbiotic relationship with the ants, where it provides food and nest sites which the ants defend, thereby defending the tree against predators. These ants deliver a painful bite and sting and also produce a scent that many predators have learned to avoid; interestingly, these predator deterrents do not seem to deter wrens from nesting in acacia trees (Young et al., 1990; Goheen & Palmer, 2010). Acquiring a nest site is crucial for birds to successfully breed, and therefore suitable nest sites should be aggressively defended if they are in limited supply. Due to this overlap in resource use and the aggressive encounters between rufous-and-white wrens and banded wrens that we have observed in the field, we believe that these two species are ecological competitors.

My study site was in the Guanacaste Conservation Area in northwestern Costa Rica in two regions of this UNESCO World Heritage Site: Sector Santa Rosa (10°40'N, 85°30'W) and Sector Rincón de la Vieja (10°40'N, 85°, 30'W). Santa Rosa is a low-elevation, mature dry forest whereas Rincón de la Vieja is a mid-elevation rain forest. Both rufous-and-white wrens and banded wrens reside in Santa Rosa, inhabiting territories that do not overlap but may be abutting. Only rufous-and-white wrens are present in Rincón de la Vieja; acacia trees are not common at this site, where the wrens nest in other tree species with spines and with ant associations. Given that these two

wren species are closely-related, differ in their plumage and song, compete for resources, and live in zones of sympatry and allopatry, they are ideal for studying how experience influences species discrimination and the signalling modalities used for discrimination.

Rufous-and-white Wrens (Thryophilus rufalbus)

Rufous-and-white wrens have a rufous-coloured back with white underparts (Figure 1.1a). They are larger than banded wrens (14.5-16.5 cm in length; Brewer, 2001) and males are larger than females (male mass = 25.8 g, female mass = 23.7 g; Mennill & Vehrencamp, 2005). Males and females have similar plumage features but males are slightly larger (Mennill & Vehrencamp, 2005). Rufous-and-white Wrens' distribution ranges from Mexico to Columbia and Venezuela. They inhabit mature, dry deciduous forests and evergreen forests (Stotz et al., 1997), and build globular nests of grass and fibres with a tunnel entrance (Brewer, 2001). Both sexes contribute to parental care, and while males predominantly build nests, females perform all incubation, and both sexes take part in feeding the offspring. Rufous-and-white Wrens have large territories (1.35 ± 0.10 Ha, Mennill & Vehrencamp, 2008) that they defend from competitors year-round.

Rufous-and-white wrens sing slow, flute-like songs. Both males and females sing and are known for their duets, where males and females coordinate their songs so that their phrases alternate or overlap (Mennill & Vehrencamp, 2005). However, males have a significantly higher vocal output than females and sing more often during a song bout, repeating a song every 11.9 seconds whereas females repeat a song every 16.4 seconds

(Mennill & Vehrencamp, 2005). Males have repertoires that are on average 10.8 ± 0.7 song types and females average 8.5 ±0.7 song types (Mennill & Vehrencamp, 2005). Male songs are longer than female songs but females tend to sing slightly quieter, higher frequency songs (Mennill & Vehrencamp, 2005). Male and female song output varies throughout the breeding season (Topp & Mennill, 2008). Rufous-and-white wren songs sound substantially different from banded wren songs because they are much shorter and much lower in frequency (Figure 1.1).

Banded Wren (Thryophilus pleurostictus)

Banded wrens have reddish brown backs and white underparts with dark barring on their flanks (Figure 1.1b). Banded wrens are slightly smaller than rufous-and-white wrens (14-15 cm in length; Brewer, 2001) and females are slightly smaller than males (male mass = 20.3 g, female mass = 18.3 g; Hall et al., 2015). Their distribution ranges from central Mexico to the Pacific coast of northwestern Costa Rica (Brewer, 2001). Banded Wrens favour tropical dry scrub forest dominated by acacia trees (Brewer, 2001; Molles & Vehrencamp, 1999; Stotz et al., 1997) and are commonly found on the ground in more open areas or in vines. They also build globular nests with a tube entrance out of fine yellow grass and fibres (Brewer, 2001). Banded wrens have smaller territories (0.40 Ha; Trillo & Vehrencamp, 2005) than rufous-and-white wrens, and both sexes are known to defend territories year-round.

Banded wrens sing long, loud songs with a broad frequency range (Figure 1.1b). Banded wren songs contain a series of whistles and trills. Both sexes sing although

female songs are shorter and quieter than male songs (Hall et al., 2015). Banded wrens do not routinely duet, although males and females rarely overlap their songs in a way that is reminiscent of a rufous-and-white wren duet (Hall et al., 2015). Males have repertoires that are on average 19.7 song types (Molles & Vehrencamp, 1999) and females average 8.7 song types (Hall et al. 2015). Females have significantly lower song output compared to males and are not found to respond strongly to simulated territorial intrusions (Hall et al., 2015).

Thesis Goals

In this thesis, my goal is to investigate species discrimination signals in two competing tropical wrens. In Chapter 2, my goal is to evaluate how experience influences species discrimination by studying populations of rufous-and-white wrens that live in a zone of sympatry and a zone of allopatry with banded wrens. In Chapter 3, my goal is to investigate how birds use both acoustic and visual signals for inter- and intraspecific discrimination, as well as to understand the competitive relationship between these two wren species. Few studies have investigated how birds use both acoustic and visual signals and how a dense habitat affects the use of multimodal signals. Both chapters will enhance our understanding of how rufous-and-white wrens and banded wrens interact and compete for resources. Studying species discrimination in competitive species allows us to infer how species have diverged in their traits, allowing them to live in sympatry, and how they will alter niche partitioning in changing environments.

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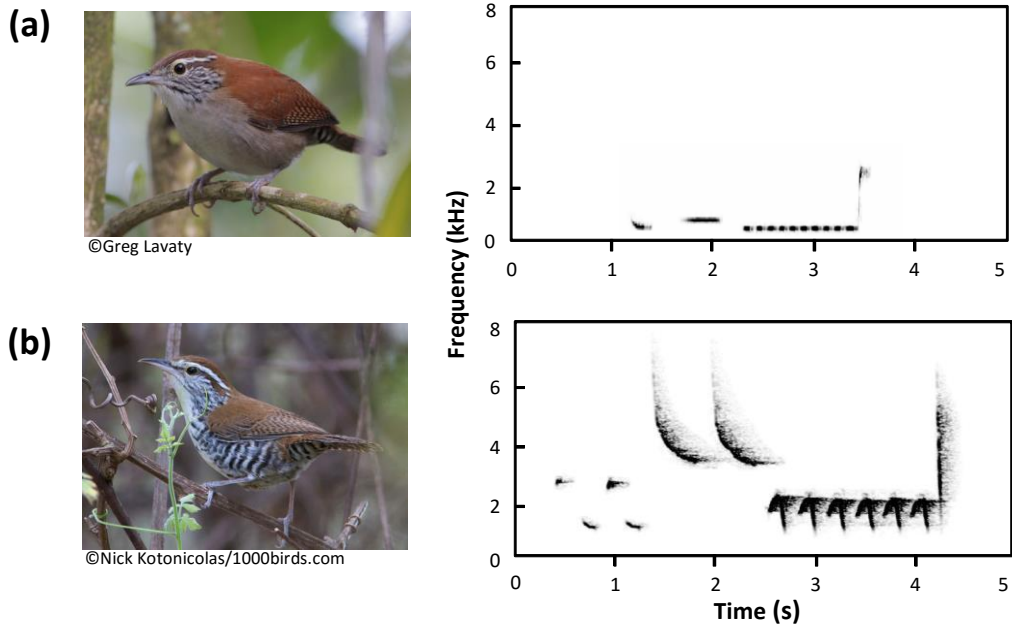
Figure 1.1. Pictures and sound spectrograms of the two study species in this study. (a) Male solo song of a rufous-and-white wren. (b) Male solo song of a banded wren.

Appendix A

List of Important Terms

Allopatry: Living in non-overlapping geographic areas

Sympatry: Living and interacting in over-lapping geographic areas

Conspecific animals: Animals belonging to the same species

Congeneric animals: Closely related animals of different species belonging to the same genus

Heterospecific animals: Animals belonging to different species

Intraspecific discrimination: Discrimination of animals within a species (i.e., males versus females; familiar neighbours versus strangers)

Interspecific discrimination: Discrimination of conspecific versus heterospecific animals

Signal: Structure that has evolved to convey information to a receiver

Multimodal signal: A signal containing properties of two or more signal modalities

CHAPTER 2

INTERSPECIFIC VOCAL DISCRIMINATION IN NEOTROPICAL WRENS: RESPONSES TO CONGENERIC SIGNALS IN SYMPATRY AND ALLOPATRY

Chapter Summary

When animals defend resources using territorial signals, they must distinguish between competitors and non-competitors. Conspecific animals routinely compete for resources and regularly engage in aggressive signaling exchanges. Heterospecific animals may also compete for resources, and therefore animals may direct their aggression towards heterospecific as well as conspecific rivals. In both cases, animals should benefit by discriminating between non-threatening individuals versus threatening conspecific and heterospecific competitors. Experience may play an important role in competitor discrimination; animals living in sympatry with heterospecific competitors may gain experience with heterospecific rivals, but animals living in allopatry will not. We investigated whether experience influences species discrimination between two congeneric neotropical wrens – rufous-and-white wrens (*Thryophilus rufalbus*) and banded wrens (*T. pleurostictus*) – that live in sympatry in parts of their range and allopatry in other parts of their range. We used playback to simulate the presence of male conspecific, congeneric, and control intruders in the territories of rufous-and-white wrens at sites where they are sympatric or allopatric with banded wrens. If species discrimination is influenced by experience, we predicted that wrens would always respond strongly to conspecific songs, but that in sympatry they would respond more strongly to the congeneric competitor than to the control songs. Conversely, we predicted that in allopatry wrens would exhibit similarly low responses to congener and control songs. In contrast to our predictions, we found that rufous-and-white wrens discriminate between conspecific and heterospecific animals, but that this response did

not differ in sympatry or allopatry, suggesting that experience with heterospecific

competitors does not influence interspecific discrimination in this species. By contrasting

the responses of sympatric and allopatric populations, we can better understand the

effect of experience on interspecific discrimination and gain insight into the evolution of

species discrimination signals.

Introduction

Species discrimination is the identification and differentiation of conspecific animals from heterospecific animals (Bradbury & Vehrencamp, 2011). Misidentifying the species of a potential rival or a potential mate can have significant fitness consequences (Grether, 2011), and given the high cost of territorial displays, selection should promote species discrimination (Grether et al., 2009). Species discrimination may be innate, it may be shaped by experience (i.e., previous interactions with heterospecific rivals), or it may be shaped by both genetics and experience. Experience with another species may allow animals to recognize competitors that they would not be able to identify in areas where heterospecific animals are absent. In spite of the large body of research on species discrimination (Grether, 2011), there is no consensus on the importance of experience for interspecific discrimination between closely related competitor species.

Animals are understood to construct species-specific templates, whether they are learned or innate, which they use to distinguish conspecific from heterospecific animals (Bradbury & Vehrencamp, 2011; Hauber & Sherman, 2001). Many studies suggest that animals have an innate species template, which they expand or modify through learning (Hauber et al., 2001; Sandoval et al., 2013). Other studies have suggested that there is a learned component to species discrimination, with animals learning the characteristics of conspecific animals through experience with parents or other individuals (Catchpole, 1978; Grant & Grant, 1997; Irwin & Price, 1999; Lynch & Baker, 1990; Matyjasiak, 2004). Species discrimination may involve phenotype matching, where an individual learns the phenotype of parents or kin and then uses this template

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to discriminate between conspecific and heterospecific animals (Bradbury &

Vehrencamp, 2011; Hauber & Sherman, 2001). This mechanism requires learning early in

life, but does not require prior experience with heterospecific animals since animals may

respond appropriately to any species whose phenotype is different from their own

(Kappeler, 2010). While phenotype matching is a potential mechanism for distinguishing

conspecific from heterospecific animals, it does not allow for the differentiation of

heterospecific competitors from heterospecific non-competitors.

Most animals respond more intensely to the signals of conspecific versus

heterospecific animals (e.g. frogs: Ryan & Rand, 1993; salamanders: Nishikawa, 1987;

insects: Anderson & Grether, 2010; fish: Johnson & Peeke, 1972; birds: Baker, 1991).

Conspecific animals are expected to pose a greater threat because they compete for

both resources and mates, whereas congeneric animals compete only for resources

(Jankowski et al., 2010; Ord & Stamps, 2009). Although interspecific discrimination plays

an important role in communication with conspecific animals, it can also facilitate

communication with heterospecific animals, particularly when two or more species

compete for access to similar resources such as foraging sites or nesting areas (Kodric-

Brown & Brown, 1978; Ord & Stamps, 2009). Species that compete for resources on a

regular basis should recognize each other as a potential threat. Red-cheeked

salamanders (*Plethodon jordani*), for example, exhibit similarly aggressive responses

towards both conspecific and congeneric rivals (northern slimy salamanders, *P.

glutinosus*) in areas of high interspecific competition, but more aggressive responses

towards conspecific than congeneric intruders in areas of low interspecific competition

(Nishikawa, 1987). Likewise, mountain chickadees (*Poecile gambeli*) respond strongly to the songs of both conspecific and heterospecific rivals (black-capped chickadees, *P. atricapillus*), suggesting that both species' songs are equally threatening signals, whereas the socially-dominant black-capped chickadees respond more strongly to conspecific songs (Grava, Grava, Didier, et al., 2012; Grava, Grava, & Otter, 2012). Species that never come into contact may not be able to discriminate between each other, as it may not be adaptive for species that have evolved in isolation to recognize one another (Grether et al., 2009).

Several studies have shown that birds have the ability to recognize closely related species as competitors, and this capacity appears to vary with experience. For example, blue-winged warblers (*Vermivora cyanoptera*) living in sympatry with golden-winged warblers (*V. chrysoptera*) respond aggressively to both conspecific and congeneric songs, showing more aggression towards conspecific songs (Gill & Murray, 1972). In allopatry, however, blue-winged warblers only respond aggressively to conspecific songs (Gill & Murray, 1972). This result is consistent with the idea that animals learn to distinguish threatening versus non-threatening rivals when they live in sympatry. Conversely, white-eared ground-sparrows (*Melozone leucotis*) show stronger aggressive responses to conspecific songs versus congeneric Prevost's ground-sparrow (*M. biarcuatum*) songs regardless of whether they live in sympatry or in allopatry (Sandoval et al., 2013). This latter result is more consistent with the idea that conspecific discrimination does not require learning. By contrasting the behaviour of more animals

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in sympatry versus allopatry, we can gain insight into the importance of experience in species discrimination.

In this study, we investigated species discrimination in neotropical wrens that live in zones of sympatry and allopatry in different parts of their ranges. Rufous-and-white wrens (*Thryophilus rufalbus*) and banded wrens (*T. pleurostictus*) are sister species (Mann *et al.,* 2006) that nest primarily in bullhorn acacias (*Vachellia collinsii*; Joyce, 1993; Molles & Vehrencamp, 1999) and occupy similar foraging niches (Ahumada, 2001; Molles & Vehrencamp, 1999). In zones of sympatry, banded and rufous-and-white wren territories do not overlap but may be abutting, with rufous-and-white wrens inhabiting mature evergreen forests and banded wrens favouring dry scrub forest. The two species are thought to engage in aggressive interactions where their territories meet (Battiston et al., 2015). We expect that rufous-and-white wrens living in sympatry with banded wrens have experience interacting with banded wrens, whereas the ones living in allopatry do not.

We tested the hypothesis that species discrimination in rufous-and-white wrens is influenced by experience by presenting conspecific and heterospecific songs to rufous-and-white wrens in an area of sympatry and allopatry with banded wrens. If species discrimination is influenced by experience, we predicted that rufous-and-white wrens living in allopatry with banded wrens would show a low response to both the congeneric and control songs, since neither represents a competitive threat, and that they would show a high response to conspecific songs. Conversely, we predicted that rufous-and-white wrens living in sympatry with banded wrens would show a stronger response to

the congeneric songs than to the control songs, because they do represent a competitive threat, and that they would show the highest response to conspecific songs. Alternatively, if species discrimination does not require experience to distinguish competitive versus non-competitive heterospecific individuals, we predicted that wrens' responses would not differ between sympatry and allopatry.

Methods

General Field Methods

We conducted a playback experiment at two sites within the Guanacaste Conservation Area in northwestern Costa Rica: Sector Santa Rosa (10°40'N, 85°30'W) and Sector Rincón de la Vieja (10°40'N, 85°, 30'W). Santa Rosa is a lowland dry-forest habitat where rufous-and-white wrens and banded wrens live in sympatry (hereafter, the "sympatric population"), with the former species occupying the mature evergreen habitats (Mennill & Vehrencamp, 2005), and the latter species occupying adjacent, less mature habitats (Molles & Vehrencamp, 1999). Rincón de la Vieja is a mid-elevation rainforest habitat where the two species live in allopatry (hereafter, the "allopatric population"). We have never encountered banded wrens at this second site. These two locations are approximately 45 km apart and separated by unsuitable habitat and we therefore do not expect dispersal to occur between them; analysis of thirteen years of banding returns from our laboratory suggests that rufous-and-white wrens disperse short distances from their natal territories. In the sympatric population, we studied only rufous-and-white wren pairs whose territory was within 200 m of a banded wren

territory to increase the chance that they would have had previous competitive interactions with the congeneric species. For all of these territories, we could hear banded wrens singing nearby, and we assume that the resident rufous-and-white wrens could hear the congeners as well.

We conducted playback experiments from early April to early June 2013, during the end of the dry season and beginning of the rainy season. This time of year coincides with the end of the non-breeding season, when birds defend territories, and the early part of the breeding season, when birds build nests and lay their first clutches of the year (Topp & Mennill, 2008). Birds in both the sympatric and allopatric population were in similar breeding stages of defending territories and building nests when the playback experiment was conducted. At this time of year, both rufous-and-white wrens and banded wrens are responsive to playback (e.g. Mennill, 2006; Molles & Vehrencamp, 2001). All playback experiments occurred between 0630 h and 1030 h, a time of day when countersinging interactions are common for rufous-and-white wrens (Mennill & Vehrencamp, 2005).

We captured birds in mist nets and uniquely colour-banded each captured animal to facilitate identification in the field (n = 63 of our 92 subjects were banded). For birds that we were not able to band (n = 29; 24 from Rincón de la Vieja and 5 from Santa Rosa), we distinguished between birds based on their ongoing occupation of the same area (as in Battiston et al., 2015; Kovach, Hall, Vehrencamp, & Mennill, 2014; Mennill, 2006), and we discriminated between males and females based on their vocalizations (Mennill & Vehrencamp, 2005). We conducted playback experiments to 46 rufous-and-

white wren pairs (92 birds): 24 pairs (48 birds) in the sympatric population and 22 pairs
(44 birds) in the allopatric population.

Natural competitive interactions

We scanned field notes from our research team that has been working at Sector
Santa Rosa for the past 13 years (2003-2015) to identify naturally-occurring competitive
interactions between rufous-and-white wrens and the two heterospecific animals.
Although we did not specifically target data collection on these interactions, an
anecdotal tally of these interactions provides context for interspecific aggression. Our
notes yielded reports of aggressive interactions between rufous-and-white wrens and
banded wrens on 11 occasions. Eight occasions were naturally-occurring aggressive
interactions when we observed rufous-and-white wrens and banded wrens producing
aggressive calls (including the harsh chattering calls produced by both species, as well as
the low-pitched *hoot* notes produced by rufous-and-white wrens; see Mennill &
Vehrencamp 2005), aggressive chases, and supplanting behaviour. Three occasions
occurred in the course of separate playback experiments and these instances included
aggressive calls, aggressive chases, and physical contact between rufous-and-white
wrens and banded wrens. We found zero observations of aggressive interaction
between rufous-and-white wrens and the control species in our playback experiment:
long-tailed manakins.

Playback Technique

We used playback experiments to simulate the presence of three species of birds intruding into the territories of rufous-and-white wrens: (1) a male rufous-and-white wren (conspecific treatment); (2) a male banded wren (congeneric treatment); and (3) an unrelated songbird (control treatment). We chose long-tailed manakins (*Chiroxiphia linearis*) as a control because they are sympatric with rufous-and-white wrens in both study locations (Garrigues & Dean, 2007), and because these frugivorous manakins are not ecological competitors with insectivorous wrens.

Each playback treatment consisted of 5 minutes of stimulus followed by a 5-minute silent period. We did not begin the playback until the subject pair was silent for at least 1 minute. A previous study of neighbour-stranger discrimination found that rufous-and-white wrens do not respond differently to conspecific versus heterospecific playback at the edge of their large territories (Battiston et al., 2015). Therefore we conducted all playback trials from a position near the centre of the subjects' territories. We observed the behaviour of the resident birds (both the male and the female) during the 5-minute stimulus period and the 5-minute silent period. To minimize carryover effects, each treatment was presented on separate, consecutive days, always from the same loudspeaker location and at the same time of day for each subject. To minimize order effects, we assigned the order of the three treatments according to a factorial design.

The playback apparatus was a camouflaged wireless speaker (Scorpion TX200, FOXPRO Inc.) hung in vegetation 1m above the ground. All treatments were played back

at 85dB SPL as measured with an analogue sound level meter (RadioShack 33-4050; C-weighting, fast response) positioned 1.0m in front of the speaker. Banded wrens produce louder songs than rufous-and-white wrens, and this amplitude reflects the average amplitude between the values that have been used in previous playbacks to the two species—80dB SPL has been used in playback studies of rufous-and-white wrens, and 90 B SPL in studies of banded wrens (Kovach et al., 2014; Mennill, 2006; Molles & Vehrencamp, 2001)—thereby ensuring that amplitude was not a confounding factor.

An observer (KGH) sat concealed in vegetation 15-20 m from the speaker and recorded all treatments using a shotgun microphone (Audiotechnica AT8015) and a solid state digital recorder (Marantz PMD660). Flagging tape was placed 2 m on either side of the playback speaker to aid in estimating the distance between the responding birds and the simulated intruder (i.e., the loudspeaker). The observer quietly dictated the identity and the behaviour of both the resident male and female, including their location in relation to the speaker. Trials where neighbouring pairs responded to the playback were aborted and repeated at least 1 week later (n=2 trials were repeated at a later date).

Playback Stimuli

We generated playback stimuli by isolating songs from recordings we collected in the Sector Santa Rosa study site over the preceding 11 years. We used recordings of male solo songs that we collected at locations ≥2 km away from the subjects' territories to ensure that all stimuli were unfamiliar to the subjects. Although rufous-and-white wrens are well-known for their male-female vocal duets, we chose to focus on male solo

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songs in this experiment because male rufous-and-white wrens have higher song output

than females (Mennill & Vehrencamp, 2005; Topp & Mennill, 2008) and respond more

intensely to playback (Mennill, 2006; Mennill & Vehrencamp, 2008). The stimuli were

prepared using Audition software (version 3.0; Adobe, San Jose, CA). We selected one

song from each source recording, choosing a song with a high signal-to-noise ratio

(assessed visually based on the spectrograms). We filtered out background noise with an

800-Hz high-pass filter (800-Hz is less than the minimum frequency of all songs used as

stimuli). We standardized song amplitude to -1 dB, so that all stimuli were broadcast at

the same amplitude. The prepared song was repeated at a rate of 1 song every 10

seconds for a total of 5 minutes. This song rate falls in the natural range of singing

behaviour for males of both study species (Mennill & Vehrencamp, 2005; Molles &

Vehrencamp, 1999). All birds in each population received different wren and control

playback stimuli to avoid pseudoreplication.

Measuring Subjects' Responses

In the laboratory, we used Syrinx-PC (J. Burt, Seattle, WA) to visualize the audio

recordings made during the playback trials, and we annotated all songs and duets of the

focal pair as well as their behaviours as dictated by the observer. This process created a

time-stamped record of all acoustic and behavioural measures. We then extracted the

following response measures for each male subject and each female subject: (1)

distance of closest approach, (2) latency to approach within 5 m of the speaker, (3)

number of songs initiated (the number of solo songs plus the number of duets where

the subject sang the first contribution), and (4) number of duets created (the number of

duets where the subject sang in response to its partner's song). Birds that did not

approach within 5 m of the speaker were given a latency score of 800 s (i.e., the length

of the trial plus 200 s). Birds that did not approach the playback area were given a

distance of closest approach score of 25 m since it was unlikely that the bird could have

been within that distance without the observer noticing. Excluding these trials from the

analysis did not change the significance of the results. These response variables are

commonly used to assess aggression and species discrimination in bird species (e.g. de

Kort et al., 2009; Grava, Grava, & Otter, 2012; Kovach et al., 2014; Sprau et al., 2013).

Statistical Analysis

We analyzed our data using generalized linear mixed models (GLMMs), which

allowed us to account for non-normal data and include a random effect (Bolker et al.,

2008). Our models included four main factors: (1) playback treatment (three levels:

conspecific, heterospecific, or control); (2) population (two levels: sympatric or

allopatric); (3) sex of the focal bird (two levels: male or female); and (4) order in which

the treatments were presented (three levels: first, second, or third). Pair identity was

included as a random factor since each pair received all three playback treatments. We

used a Poisson error distribution with a log link function for the acoustic variables (songs

initiated, duets created) and a Gamma distribution with a log link function for the

behavioural variables (latency to 5m, distance of closest approach). GLMMs with a

Poisson error distribution have been used in previous studies analyzing social aggression

with skewed count data, and Gamma distributions have been used for non-count data skewed to higher values (e.g. Hasegawa et al., 2014; Santos et al., 2009). We included all first-order interaction terms in our analyses. We ran post-hoc pairwise comparisons for all the main effects and first order interactions using a sequential Bonferroni correction which increases P values (rather than decreasing the alpha value) to adjust for multiple comparisons; we report corrected P values for post-hoc pairwise comparisons. The figures show post-hoc comparisons across the six groups shown, where groups that are not connected by the same letter are statistically different, whereas in the text we report post-hoc comparisons for the main effects. All analyses were conducted using SPSS software (version 21; IBM, Chicago, IL, U.S.A.).

Results

Overall, rufous-and-white wrens showed stronger responses to conspecific stimuli compared to congeneric stimuli and control stimuli, initiating more songs, performing more duets, and approaching the loudspeakers more closely. Responses did not differ, however, between the sympatric and allopatric populations. Males consistently showed significantly stronger responses to stimuli than females. Below, we present results for each of our four response measures, providing results for the effects of treatment, population, sex, and playback order in that sequence for all four response variables.

Distance of Closest Approach

Distance of closest approach differed significantly across experimental treatments (Table 1), with wrens approaching the speaker more closely for the conspecific treatment compared to the congeneric and control treatments (post-hoc pairwise comparisons: conspecific vs congeneric: $t_{256}=6.14$; conspecific vs control: $t_{256}=7.77$, $P<0.001$ for both), but approaching the congeneric playback more closely than the control playback ($t_{256}=2.15$, $P=0.032$; this post-hoc analysis does not include the effect of population and data from each population are pooled together). Importantly, the distance of closest approach for each treatment did not differ between the populations (Table 1, Figure 1a).

The distance of closest approach varied between the sexes (Table 1), with males approaching more closely than females ($t_{256}=8.54$, $P<0.001$). The distance of closest approach varied across the treatments for males versus females (Table 1), with males approaching more closely than females for all three treatments (conspecific male vs female: $t_{256}=6.31$; congeneric male vs female: $t_{256}=5.04$; control male vs female: $t_{256}=3.33$, $P<0.01$ for all). The sex × population interaction showed an overall effect (Table 1), with males in the allopatric population approaching more closely than males in the sympatric population ($t_{256}=2.21$, $P=0.03$). There was no difference between the females from each population ($t_{256}=0.92$, $P=0.36$).

Distance of closest approach did not vary with playback order (Table 1). The effect of playback order on distance of closest approach revealed an overall effect of population (Table 1); however, the post-hoc comparisons did not show any significant

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effects within the populations (overall tests: sympatric population: $F_{2, 256}$=1.98, P=0.14; allopatric population: $F_{2, 256}$=2.77, P=0.06).

Latency to approach within 5m

Latency to approach within 5m of the playback speaker differed significantly across treatments (Table 1), with wrens approaching within 5m sooner for the conspecific treatment versus the heterospecific treatments (post-hoc pairwise comparisons: conspecific vs congeneric: t_{256}=7.45; conspecific vs control: t_{256}=8.76, P<0.001 for both). There was no difference in the latency to approach within 5m for the congeneric versus control treatments (t_{256}=1.42, P=0.16). The latency to approach within 5m for each treatment differed between the populations (Table 1, Figure 1b), with wrens in the sympatric population approaching within 5m sooner than wrens in the allopatric population for the conspecific treatment (sympatric vs allopatric population: t_{256}=3.07, P=0.002). There was no difference between the populations in response to the congeneric and control treatments (congeneric sympatric vs allopatric: t_{256}=1.00, P=0.32; control sympatric vs allopatric: t_{256}=0.02, P=0.98).

Males showed shorter latencies to approach within 5m than did females (Table 1; t_{256}=4.82, P<0.001). There was also an effect of sex × treatment (Table 1); for the conspecific treatment males approached to within 5m sooner than females (t_{256}=6.16, P<0.001), but there was no difference between the sexes in response to the heterospecific treatments (congeneric male vs female: t_{256}=1.75, P=0.08; control male vs

female: $t_{256}=0.19$, P=0.85). The latency to approach within 5m did not differ between the populations for either sex (Table 1).

There was no overall order effect for latency to approach within 5m (Table 1), but there was a significant effect of population × playback order (Table 1). Wrens in the sympatric population showed the shortest latency to approach within 5m for the second playback versus the first and third (second vs first: $t_{256}=2.29$, P=0.04; second vs third: $t_{256}=2.64$, P=0.03). There was no significant difference for the latency to approach within 5m between the first and third playback trials ($t_{256}=0.36$, P=0.72).

Number of Songs Initiated

The number of songs initiated (solo songs plus the first song in a duet) differed significantly across treatments (Table 1), with more songs being initiated during the conspecific treatment versus both heterospecific treatments (post-hoc pairwise comparisons: conspecific vs congeneric: $t_{256}=10.81$, P<0.001; conspecific vs control: $t_{256}=10.8$, P<0.001) and no difference in the number of songs initiated during the congeneric versus control treatments ($t_{256}=0.23$, P=0.82). There was a significant population × treatment interaction (Table 1, Figure 2a), whereby wrens in the sympatric population initiated significantly more songs in response to the conspecific treatment than wrens in the allopatric population ($t_{256}=3.58$, P<0.001). There was no difference between the populations in response to the congeneric ($t_{256}=0.75$, P=0.45) and control treatments ($t_{256}=1.44$, P=0.15).

Males initiated significantly more songs than females (Table 1) in response to all three playback treatments (conspecific male vs female: t_{256}=14.16; congeneric male vs female: t_{256}=12.43; control male vs female: t_{256}=11.57, P<0.001 for all). There was a significant sex × population interaction, with females in the sympatric population initiating more songs than females in the allopatric population (t_{256}=3.55, P<0.001). Conversely, males in the allopatric population initiated more songs than males in the sympatric population (t_{256}=3.8, P=0.002).

The number of songs initiated differed with playback order (Table 1); birds initiated more songs for the first and third treatments compared to the second treatment (first vs second: t_{256}=4.01, P<0.001; third vs second: t_{256}=2.96, P=0.007). There was a significant playback order × treatment interaction (Table 1), with wrens initiating more songs if the congener treatment was presented first or third versus second (first vs second: t_{256}=5.31, P<0.001; third vs second: t_{256}=3.08, P=0.002).

Number of Duets Created

The number of duets created (the number of songs a bird sang in response to its partner's songs) differed significantly across treatments (Table 1), with birds creating significantly more duets during the conspecific treatment versus the heterospecific treatments (post-hoc pairwise comparisons: conspecific vs congeneric: t_{256}=3.83, P<0.001; conspecific vs control: t_{256}=3.7, P=0.001), but creating a similar number of duets in response to congeneric versus control treatments (t_{256}=1.69, P=0.09). These results showed a significant population × treatment interaction (Table 1), but this is

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likely driven by the strong treatment effect within a population because the post-hoc

comparison showed no significant patterns, with wrens in both populations creating a

similar number of duets in response to all treatments (Figure 2b).

The number of duets created by playback subjects varied between the sexes

(Table 1), with females creating significantly more duets than males (t_{256}=3.07, P=0.002).

In addition, females created significantly more duets than males for the conspecific

(t_{256}=2.68, P=0.008) and congeneric treatments (t_{256}=2.18, P=0.03). Males and females

created a similarly low number of duets for the control treatments (t_{256}=1.76, P=0.08).

The number of duets created varied with playback order (Table 1), with birds

creating more duets in response to the first playback compared to the second (t_{256}=2.51,

P=0.01), with the third playback eliciting an intermediate response. Additionally, the

results showed a significant population × order interaction (Table 1); in the allopatric

population birds created the greatest number of duets for the first playback versus the

second (t_{256}=2.56, P<0.03) with the third playback eliciting an intermediate response.

Discussion

We quantified the responses of rufous-and-white wrens to conspecific and

congeneric playback in two populations—one living in sympatry with banded wrens and

one living in allopatry—to study the influence of experience on species discrimination.

Our results demonstrate that rufous-and-white wrens discriminate between the songs of

conspecific and heterospecific rivals, but that this response does not differ between

areas of sympatry versus allopatry. Rufous-and-white wrens in both populations showed

intense responses towards conspecific playback and much weaker responses to both the congeneric and control playback. For the distance of closest approach, rufous-and-white wrens showed an intermediate response to the congeneric playback versus the conspecific and control, demonstrating that rufous-and-white wrens distinguish between congeneric rivals versus control stimuli. However, since this is the only instance of a significant difference in response to congeneric and control stimuli, it should be interpreted cautiously. Nevertheless, we found no differences in responses to congeneric rivals between the sympatric versus allopatric populations, and these results therefore provide no evidence that experience with sympatric congeners influences interspecific discrimination in rufous-and-white wrens.

Consistent with many previous studies, rufous-and-white wrens displayed more intense responses to conspecific songs than heterospecific songs (e.g. Baker, 1991; Catchpole, 1978; Grava, Grava, & Otter, 2012; Martin & Martin, 2001; Seddon & Tobias, 2010). This result held true for both sexes of wren, indicating that males and females both use song to distinguish conspecific animals. In contrast to our predictions, however, wrens displayed very little aggression in response to playback of a congeneric ecological competitor – banded wrens – in both the sympatric and allopatric populations. We expected that wrens in our allopatric population would exhibit weak responses to the congeneric treatment because they do not interact with banded wrens and thus should not perceive them as rivals for shared resources, whereas we predicted a stronger response to the congeneric treatment in the sympatric population where the two species interact and compete for nest sites. We did find that rufous-and-white wrens

approached the banded wren stimulus more closely than the control stimulus,

suggesting that they distinguished between congeners and non-competitors, but this

pattern did not differ between the sympatric and allopatric populations.

Studies investigating species discrimination in warblers (Brambilla et al., 2008)

and ground-sparrows (Sandoval et al., 2013) found that prior experience was not

necessary for animals to discriminate between competitors. Males of two different

subspecies of Moltoni's warblers (*Sylvia cantillans*), for example, showed a strong

response to playback of their own subspecies and a weak response to playbacks of the

other subspecies in both sympatry and allopatry (Brambilla et al., 2008). These studies

are consistent with our findings, whereby experience did not seem to influence species

discrimination in rufous-and-white wrens. By contrast, other studies have found

differing responses in areas of sympatry and allopatry. For example, indigo buntings

(*Passerina cyanea*) and lazuli buntings (*P. amoena*) responded similarly to conspecific

songs in sympatry and allopatry but more strongly to heterospecific songs in the

sympatric population than in the allopatric population (Baker, 1991).

Our results suggest that familiarity arising from previous experience with the

congener is not necessary for species discrimination. Rufous-and-white wrens are still

able to discriminate conspecifics from heterospecifics without previous experience with

the other species in our allopatric population. This finding suggests that the

discrimination ability is either innate or guided by phenotype matching. Phenotype

matching is a mechanism of species discrimination that does not require prior

experience with the heterospecific animals; instead, an individual uses the learned

template of their parents or kin to discriminate between conspecifics and heterospecifics (Hauber & Sherman, 2001; Irwin & Price, 1999). Our results are consistent with this idea. If rufous-and-white wrens learn to distinguish conspecific songs from all other songs, we would expect them to respond similarly to both congeners and other heterospecific songs, whether or not they live in sympatry or allopatry. Rufous-and-white wrens recognize conspecific animals as competitors and respond aggressively, but show little aggression in response to the songs of species that appear different from their own.

Another possible explanation for the low level of aggressive response by rufous-and-white wrens toward banded wren songs is that that they may not perceive the congeneric species as a threat. It is important to note, however, that response intensity might vary with each individual's experience with the congeneric species. A rufous-and-white wren that has had numerous competitive interactions with banded wrens may respond more aggressively to the congeneric playback than one that has rarely interacted aggressively with banded wrens. Although we have observed aggressive interactions between rufous-and-white and banded wrens, these may be rare occurrences, and perhaps these congeners are not threatening territorial rivals. Further, the two species may differ in their aggressiveness towards one another, causing their response to congeneric signals to vary. Asymmetry in aggressive responses to conspecifics and congenerics has been found in previous studies (see Jankowski et al., 2010; Martin & Martin, 2001; Robinson & Terborgh, 1995). For example, two species of chickadees show just such a relationship: black-capped chickadees (*Poecile atricapillus*)

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showed stronger responses to conspecific stimuli and little response to heterospecific

stimuli whereas mountain chickadees (*P. gambeli*) responded strongly to both stimulus

types (Grava, Grava, & Otter, 2012), suggesting that they are equally threatening to this

species. These two species use similar resources and the authors suggested that they are

competitors but that black-capped chickadees are the socially dominant species (Grava,

Grava, Didier, et al., 2012). Reciprocal playbacks to banded wrens would enhance our

understanding of the competitive relationship between the two wren species we studied

here. If these two species have a dominant/subordinate relationship with rufous-and-

white wrens being the dominant species (rufous-and-white wrens are larger than

banded wrens; Brewer, 2001), we would expect banded wrens to respond more similarly

to conspecific and congeneric stimuli. Investigating this type of relationship can provide

important information on how competitive species of birds may adapt if their

distributions are altered due to changing environments, which may force the birds

currently living in allopatry into sympatry (see Jankowski et al., 2010; Toms, 2013).

    Although we were unable to detect differences in how rufous-and-white wrens

responded to congeneric banded wrens in zones of sympatry and allopatry, we did find

differences in response to playback between the two populations. In particular, in

response to the conspecific treatment, wrens in the sympatric population initiated more

songs and approached within 5m of the speaker more quickly than wrens in the

allopatric population. Playback stimuli for both populations were composed of songs

from the sympatric population (i.e., Sector Santa Rosa). We chose to present only stimuli

made from recordings of the sympatric population because the congeneric banded wren

songs could only be recorded at the sympatric site, and thus we ensured that birds in

both populations received the same playback stimuli. These two populations are 45 km

apart, and therefore birds from these two locations may exhibit different dialects;

ongoing studies from our research group are quantifying these differences. The

existence of local dialects could be responsible for the stronger vocal and behavioural

responses to conspecific treatments in the sympatric population. In other songbirds,

territorial males respond more strongly to songs from a conspecific local dialect than to

a conspecific foreign dialect (e.g. Nicholls, 2007; Reichard, 2014; Searcy et al., 1997).

Interestingly, we found a sex difference in how birds responded to conspecific

stimuli in our two populations. Males in the allopatric population initiated more songs

and approached the speaker more closely than males in the sympatric population.

Females in the sympatric population, however, initiated more songs than females in the

allopatric population. The response of males is similar to a widespread pattern that

males respond more strongly to unfamiliar stimuli versus familiar stimuli, most

commonly found in neighbour-stranger discrimination studies (Temeles, 1994). Our

results do however contradict the studies previously mentioned where males respond

more strongly to local versus foreign dialects. The response of females is consistent with

the stronger response to local dialects seen in other male songbirds (Nicholls, 2007;

Reichard, 2014; Searcy et al., 1997). In a pattern that parallels our study, estradiol-

injected female song sparrows (*Melospiza melodia*; Searcy et al., 2002), great tits (*Parus

major*; Baker et al., 1987), and rufous-collared sparrows (*Zonotrichia capensis*; Danner et

al., 2011) showed more copulation solicitation displays to local male songs than to

foreign male songs. However, this is the first study to find female differences in response

to male local and foreign songs in the wild without injection of estradiol. It is interesting

to note, however, that the studies mentioned above were testing female preferences for

male song, whereas our study aimed to test how females respond to a male intruder.

Another possible explanation is that in order to defend their territory, females in the

sympatric population increased their song output to compensate for their mates' low

singing rate. Further work is required to understand this response, including reciprocal

playback of songs from our allopatric population to females in our sympatric population.

Rufous-and-white wrens and banded wrens use similar foraging sites and nest

sites in bullhorn acacia trees whose resident ants offer protection from predators

(Haemig, 2001). Nest sites are crucial for birds to successfully breed, and therefore they

should be aggressively defended. To minimize interference with nesting and

reproductive behaviour, we conducted playback experiments at least 15m away from a

tree containing a nest or that had been previously used as a nesting site. Conducting

playback near nesting trees might have elicited stronger responses to congeneric stimuli

in our experiment. The use of visual signals might also increase the strength of the

response by providing a close-range signal for the presence of a competitor in the

territory. Future studies should consider the addition of a visual model to elicit a

stronger response to playbacks while also testing the importance of visual and acoustic

signals for species discrimination (e.g. Uy et al. 2009; Uy & Safran 2013). Likewise,

reciprocal playback experiments directed at the competitor species, banded wrens,

would provide more insight into the relationship between these two species and how

they compete for resources. Investigating ecological interactions between competitor

species and how they coexist is important for understanding how they will react to

changing environmental conditions and how species discrimination signals have evolved.

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Tables

Table 2.1. Generalized linear mixed model results of the response of rufous-and-white wrens to playback simulating conspecific, congeneric, and control intruders. Significant values are in bold.

	Distance of Closest Approach			Latency to approach within 5m			Number of Songs Initiated			Number of Duets Created		
	F	df	P	F	df	P	F	df	P	F	df	P
Treatment	42.7	2, 256	**<0.001**	48.6	2, 256	**<0.001**	138.9	2, 256	**<0.001**	37.7	2, 256	**<0.001**
Population	0.7	1, 256	0.4	1.0	1, 256	0.31	0.8	1, 256	0.37	0.5	1, 256	0.5
Sex	93.3	1, 256	**<0.001**	23.8	1, 256	**<0.001**	543.4	1, 256	**<0.001**	18.1	1, 256	**<0.001**
Order	0.7	2, 256	0.49	0.3	2, 256	0.71	9.0	2, 256	**<0.001**	5.1	2, 256	**0.007**
Population × Treatment	2.8	2, 256	0.065	5.9	2, 256	**<0.001**	24.6	2, 256	**<0.001**	6.4	2, 256	**0.002**
Sex × Treatment	4.3	2, 256	**0.015**	608.1	2, 256	**0.003**	11.2	2, 256	**<0.001**	1.3	2, 256	0.27
Treatment × Order	1.5	4, 256	0.21	0.8	4, 256	0.54	13.5	4, 256	**<0.001**	1.5	4, 256	0.22
Sex × Population	6.4	1, 256	**0.012**	1.2	1, 256	0.27	55.8	1, 256	**<0.001**	0.9	1, 256	0.35
Population × Order	4.3	2, 256	**0.015**	11.0	2, 256	**<0.001**	0.8	2, 256	0.44	5.6	2, 256	**0.004**
Sex × Order	1.3	2, 256	0.27	0.5	2, 256	0.59	4.4	2, 256	**0.013**	1.5	2, 256	0.22
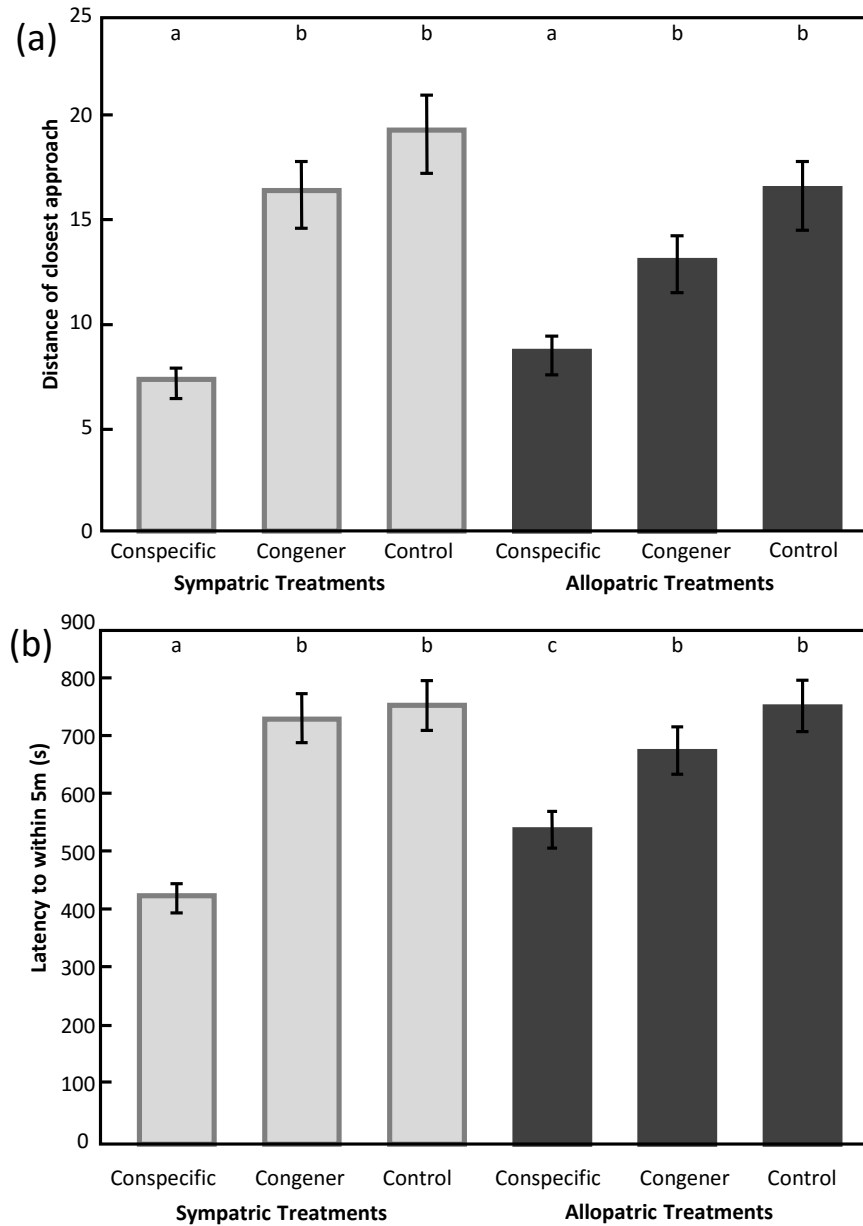
Figure 2.1. Behavioural response of rufous-and-white wrens to playback of male conspecific, congeneric and control songs in an area of sympatry and allopatry with banded wrens. (a) Distance of closest approach to the playback speaker. (b) Latency to within 5 m of the playback speaker. Different letters above bars indicate statistical significance. Graph shows mean values from treatment × population post-hoc pairwise comparison with standard error bars.
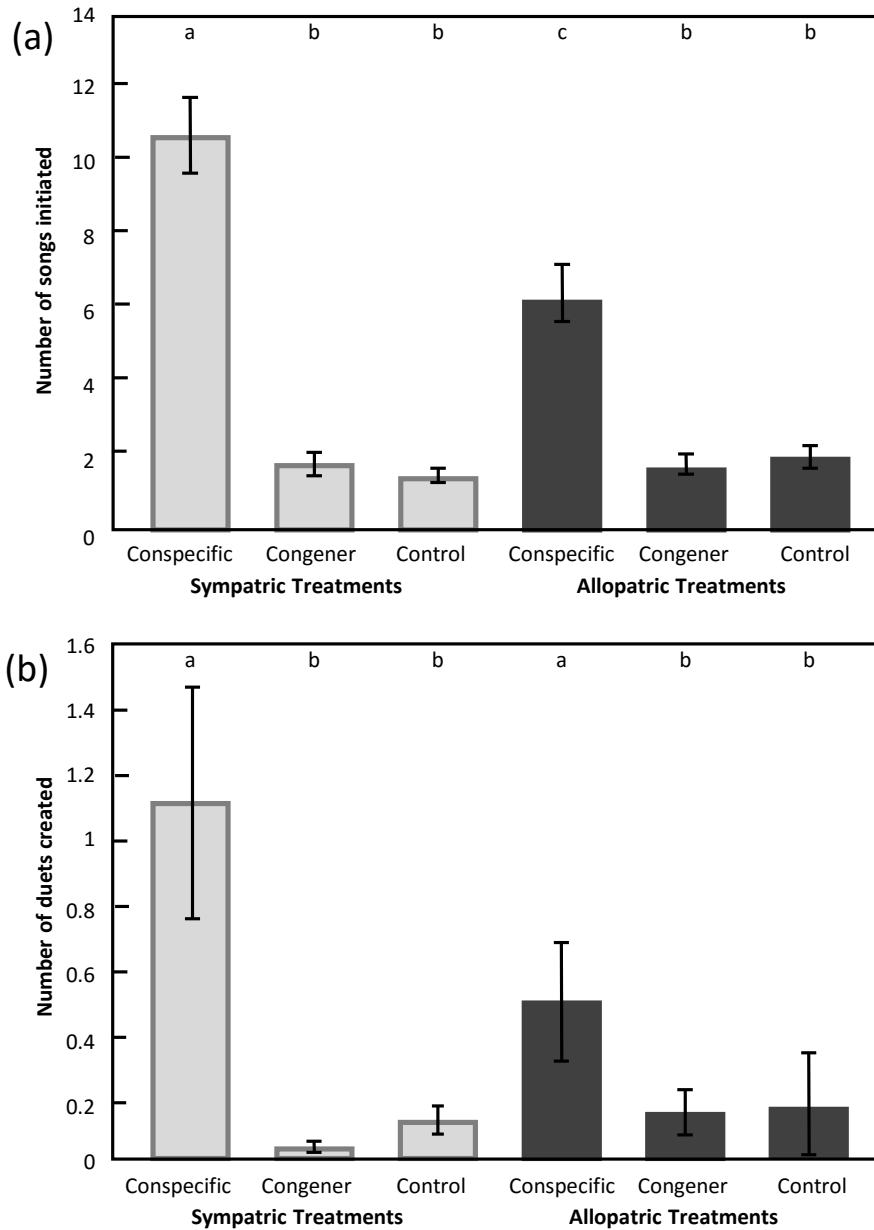
Figure 2.2. Acoustic response of rufous-and-white wrens to playback of male conspecific, congeneric and control songs in an area of sympatry and allopatry with banded wren. (a) Number of songs initiated (number of solos songs plus first song in a duet). (b) Number of duets created (song sung in response to a solo song). Different letters above bars indicate statistical significance. Graph shows mean values from treatment × population post-hoc pairwise comparison with standard error bars.

CHAPTER 3

DO NEOTROPICAL WRENS USE BOTH ACOUSTIC AND VISUAL SIGNALS FOR INTRA-AND INTERSPECIFIC DISCRIMINATION? A MODEL PRESENTATION AND PLAYBACK EXPERIMENT

Chapter Summary

Animals may use multiple signaling modalities to discriminate between conspecific and heterospecific animals, or between individuals that represent a threat or a potential mating opportunity. Multimodal signals used in intra- and interspecific discrimination can serve as redundant signals, or each modality may convey unique information. Different types of signals may differ in transmission efficiency through different habitats. In this study we investigated how two closely related wrens, rufous-and-white wrens (*Thryophilus rufalbus*) and banded wrens (*T. pleurostictus*), use acoustic and visual signals for species discrimination in tropical forest habitats. We coupled song playback experiments with visual models to assess the importance of these signals, both in combination and in isolation. We presented both rufous-and-white wrens and banded wrens with conspecific and congeneric song treatments, model treatments, and song accompanied by a model. We found that both species responded strongly to song playback and song playback accompanied by a model, but showed little or no response when the model was presented alone. These results suggest that wrens rely heavily on acoustic signals and very little on visual signals for discrimination. The species differed in their response to conspecific and congeneric trials, with rufous-and-white wrens showing little response to the congeneric trials but banded wrens responding strongly to both conspecific and congeneric trials. The asymmetrical response to the playback trials suggests that there may be a social dominance relationship between these two wren species, with rufous-and-white wrens being dominant over banded wrens. No previous studies have investigated the relative

importance of acoustic and visual signals in males and females for species discrimination

in tropical habitats. Our results suggest that acoustic signals are more important than

visual signals for inconspicuous animals living in dense environments.

Introduction

Social animals must discriminate between individuals that represent a threat versus individuals that do not represent a threat. For example, animals must distinguish between conspecific animals that are potential mating partners versus territorial intruders threatening their resources. Likewise, closely related species that live in sympatry and compete for resources should distinguish between individuals that are a competitor species and individuals that represent viable mates (Bradbury & Vehrencamp, 2011). Animals may use a diversity of signal modalities for intra- and interspecific discrimination – such as visual, acoustic, electrical, and chemical signals – and many animals appear to use multiple signals simultaneously (Bradbury & Vehrencamp, 2011; Grether, 2011). Several hypotheses have been proposed to explain the benefits of multimodal signals, with two hypotheses receiving the most attention. The first hypothesis proposes that multimodal signals serve as redundant signals and act as a backup for more accurate information transmission (Anderson et al., 2013; Partan & Marler, 2005). The second hypothesis proposes that each signal conveys unique information (Hebets & Papaj, 2005). Under either scenario, the use of multimodal signalling allows for more efficient and accurate intra- and interspecific discrimination.

The environment modifies how signals are transmitted, and we therefore expect habitat to have a strong influence on how animals use multimodal signals (Grether, 2011; Higham & Hebets, 2013; Morton, 1975; Wilkins et al., 2012). Acoustic signals are often used for long-range recognition because they can travel around obstructions, but they may not be easily accessible in noisy environments (Bradbury & Vehrencamp, 1998;

Wilkins et al., 2012). Visual signals can be easily obstructed by vegetation and therefore work best in open habitats or for close range recognition (Uy & Safran, 2013). A variable environment will promote the use of multimodal signals so that different signals can be perceived in the conditions through which they best transmit. Bornean rock frogs (*Staurois parvus*), for example, are thought to have evolved multimodal signals to overcome noise in their streamside habitat; males have modified the pitch, amplitude, and duration of advertisement calls to maximize signal transmission and also use numerous visual signals to communicate in a noisy environment (Grafe et al., 2012).

In dense habitats, acoustic signals are mainly used for long-range recognition whereas visual signals are mainly used for close-range recognition (Uy & Safran, 2013); therefore, it is beneficial for animals to use both signal modalities for efficient intra- and interspecific discrimination. In one study, male dart-poison frogs (*Epipedobates femoralis*) responded more strongly to a conspecific male model when its vocal sac was inflated and pulsating and accompanied by a male call, indicating that they used both visual and acoustic signals for species discrimination (Narins et al., 2003). Only three studies have investigated the simultaneous use of visual and acoustic signals for species discrimination in birds. In a study conducted on European warblers, playback accompanied by taxidermic models revealed that male blackcaps (*Sylvia atricapilla*) defending resources against garden warblers (*S. borin*) used both acoustic and visual signals for species discrimination (Matyjasiak, 2004). A study of chestnut-bellied flycatchers (*Monarcha castaneiventris*) found that birds use both song and plumage signals for species discrimination (Uy et al., 2009), but an additional study revealed that

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these signals were assessed sequentially in dense habitats, with song being used for long range recognition followed by plumage at close range, whereas song and plumage were used simultaneously in open habitats (Uy & Safran, 2013).

Multimodal signals may help animals discriminate between conspecific and heterospecific individuals, ensuring that aggression is properly directed towards the more threatening conspecific individuals that might usurp their mate and resources (Benites et al., 2014; Jankowski et al., 2010; Ord & Stamps, 2009). However, when conspecific and heterospecific animals use similar resources, it is beneficial to direct aggression towards conspecific as well as heterospecific rivals (Kodric-Brown & Brown, 1978; Ord & Stamps, 2009; Greenberg et al., 1994). This is especially true with closely-related species that compete for common resources and interact aggressively (Catchpole, 1978; Peiman & Robinson, 2010). Heterospecific aggression is widespread in animals (Peiman & Robinson, 2010), but is often asymmetrical, with one species being dominant over the other (Martin & Martin, 2001; Martin & Dobbs, 2015; Pearson & Rowher, 2000). Asymmetrical competition may be involved in niche partitioning in closely related species (Dingle et al., 2010), and can influence which species use particular resources (Carrete et al., 2010; Farwell & Marzluff, 2013; Peiman & Robinson, 2010). Commonly, the subordinate species is forced to inhabit less desirable territories (Jankowski et al., 2010; Morse, 1974; Pearson & Rowher, 2000). For example, Townsend's warblers (*Dendroica townsendi*) are more aggressive than hermit warblers (*D. occidentalis*); the former species has outcompeted and replaced the latter species, thereby shifting their hybrid zone (Pearson & Rowher, 2000). In some cases, the

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subordinate species will reduce singing and avoid the dominant species. For example, subordinate mountain wrens (*Troglodytes solstitialis*) sang fewer songs and stayed farther from the speaker when presented with dominant house wren (*T. aedon*) songs and control songs (Martin & Dobbs, 2015). However, this asymmetric relationship is not universal. For example, subordinate mountain chickadees (*Poecile gambeli*) responded aggressively to both conspecific and heterospecific songs of the dominant black-capped chickadees (*P.atricapillus;* Grava et al., 2012).

In this study, we combined song playback with presentation of visual models to investigate the importance of acoustic and visual signals for intra-and interspecific discrimination in two closely-related species. Our study species were rufous-and-white wrens (*Thryophilus rufalbus*) and banded wrens (*T. pleurostictus*). These two species both nest primarily in bullhorn acacia trees (*Vachellia collinsii*; Joyce, 1993; Molles & Vehrencamp, 1999) and occupy similar foraging niches (Ahumada, 2001; Molles & Vehrencamp, 1999). Rufous-and-white and banded wren territories do not overlap (rufous-and-white wrens occupy mature, evergreen forest and banded wrens inhabit drier, second-growth forest), but they often hold neighbouring territories, and we have observed aggressive competitive interactions between them in the field (Chapter 2). Our objective was to assess the importance of acoustic and visual signals for intra- and interspecific discrimination in two sympatric neotropical wrens. We delivered both conspecific and congeneric song playback, visual model presentation, and a combination of both song and model presentation to both males and females of the two wren species. Given that the mature forests preferred by rufous-and-white wrens appear to

be more densely vegetated, we quantified vegetation density in the two species'

territories. Given our anecdotal observation that there are fewer acacia nesting trees in

the evergreen forest habitat of rufous-and-white, we collected acacia tree abundance

data to better understand the distribution of one potentially limited resource for these

species. We were also interested in assessing the possible role of interspecific

dominance in mediating interactions between rufous-and-white wrens and banded

wrens because a previous playback study on rufous-and-white wrens showed that they

displayed little aggression towards control stimuli and banded wren song stimuli

(Chapter 2).

Methods

General Field Methods

We conducted this research in Sector Santa Rosa (10°40'N, 85°30'W), of the

Guanacaste Conservation Area in northwestern Costa Rica, a lowland dry-forest site. We

conducted playback experiments from early April to early June 2014, during the end of

the dry season and the beginning of the rainy season. At this time of year, wrens build

nests and defend territories (Topp & Mennill, 2008) and both study species are

responsive to playback (e.g. Mennill, 2006; Molles & Vehrencamp, 2001). We conducted

all playback and model-presentation experiments between 0630 and 1100 h, a time of

day when countersinging interactions are common for both species (Mennill &

Vehrencamp, 2005; Molles & Vehrencamp, 1999).

As part of our long-term study of this population of rufous-and-white wrens, we uniquely colour-banded as many animals as possible to facilitate identification in the field (n = 32 of 38 rufous-and-white wrens were uniquely colour banded). We were able to band only 2 of 34 banded wrens. For unbanded birds of both species, we distinguished between pairs based on their ongoing occupation of the same area, an approach that has proven effective in previous studies of these species (e.g. Mennill, 2006; Kovach et al., 2014, Battiston et al., 2015; Vehrencamp et al., 2014). We discriminated between males and females based on their sex-specific vocal traits (Mennill & Vehrencamp, 2005; Molles & Vehrencamp, 1999).

Playback Treatments

To study the role of acoustic and visual signals in species discrimination, we conducted experiments involving both rufous-and-white wrens and banded wrens. We used playback accompanied by a wooden model to simulate the presence of birds in wren territories. For each species, we delivered a song playback treatment (hereafter "Song Alone"), a model only treatment (hereafter "Model Alone"), and a treatment that featured both a model and song playback (hereafter "Model + Song"). In total, each pair received six treatments: (1) a conspecific Song Alone treatment, (2) a conspecific Model Alone treatment, (3) a conspecific Model + Song treatment, (4) a congeneric Song Alone treatment, (5) a congeneric Model Alone treatment, and (6) a congeneric Model + Song treatment.

Model Preparation

We chose to use wooden models to simulate both rufous-and-white and banded wrens; taxidermic mounts were not available for use as visual stimuli, and we did not wish to sacrifice live animals to create mounts. The wooden models were carved by skilled wood carvers from the Windsor Wood Carving Museum in Windsor, Ontario, Canada. We produced carved models of the two species that were the same size (130 mm from end of bill to end of tail; the mean size between the two wren species). When painting the wooden models, we selected paint colours that match reflectance spectra of museum specimens (see Figure 3.1 and Figure 3.2a,b). We measured spectral reflectance of both the models and of live birds using an Ocean Optics USB 2000 spectrometer and a PX-2 Flash lamp (Ocean Optics, Dunedin, FL). The reflectance probe was mounted in a black rubber holder to exclude all external light and keep the probe perpendicular to the feather surface at a fixed distance of 5mm. Working at the University of Michigan Museum Of Zoology in Ann Arbor, Michigan, U.S.A., we measured plumage reflectance of 11 rufous-and-white wren and 13 banded wren study skins collected from the Guanacaste Region in Costa Rica, as previous research has shown that museum specimens can accurately represent live birds (Doucet & Hill, 2009). Both species are sexually monochromatic (Brewer, 2001), and our measurement of 10 females and 14 males showed no noticeable differences between the sexes' reflectance curves. We collected five reflectance measurements for each of 10 body regions: belly, breast, crown, flank, mantle, black retrix, brown retrix, rump, undertail coverts and wing primaries. We measured reflectance spectra as the total reflectance across 300nm to

700nm, the bird-visible spectrum. We then tried to match the feather and paint colour

reflectance curves as closely as possible. We placed paint samples on plain white paper

and collected five reflectance measurements for the breast, black rectrices, and mantle

colours. We continued this process until the reflectance curves were as similar as

possible to the curves measured from the specimens. We used the same colours for

both species as the museum reflectance curves for rufous-and-white wren and banded

wren breast, black rectrices, and mantle did not differ.

Playback Stimuli

We generated playback stimuli by extracting songs from recordings collected at

our study site over the past 13 years. Given that the models represented strangers,

rather than familiar neighbours, we also chose to simulate the vocalizations of strangers

with playback. To ensure that the stimuli were unfamiliar to the subjects, we used

recordings that were collected ≥2km away from the subjects' territories. Playback

stimuli were composed of male solo songs repeated at a rate of one song every 10s for a

total of 5mins. This song rate falls in the natural range of singing behaviour for males of

both study species (Mennill & Vehrencamp, 2005; Molles & Vehrencamp, 1999). We

chose to focus on male solo songs in this experiment because although rufous-and-white

wrens are well-known for their vocal duets, female song is uncommon in banded wrens

(Hall et al., 2015; Molles & Vehrencamp, 1999; in response to our trials, 3% of banded

wren songs were duets, whereas 11% of rufous-and-white wren songs were duets).

Furthermore, in both rufous-and-white wrens and banded wrens, males have higher

song output than females (Mennill & Vehrencamp, 2005; Topp & Mennill, 2008; Molles & Vehrencamp, 1999) and males respond more intensely to playback than females (Hall et al., 2015; Mennill & Vehrencamp, 2008; Mennill, 2006; Hall et al., 2006).

We generated a playback lure to attract the birds to the playback area to ensure that all birds began the trials in a similar location and to ensure that they would be within sight of the model. The lure stimuli included both songs and calls. The lure began with a species-specific song repeated 5 times at a rate of one song every 10s, followed by 10s of species-specific calls (including whoops, rattles, and ticking) followed by 5s of silence repeated for a total of 5mins. Only one lure was used for each species to ensure that the aggressiveness of the calls presented did not influence the subjects' response.

We prepared all stimuli using Audition software (version 3.0; Adobe, San Jose, CA). We selected one song or set of calls from each source recording, choosing a song or call with a high signal-to-noise ratio (assessed visually based on the spectrograms). We filtered out background noise with an 800-Hz high-pass filter (800-Hz is less than the minimum frequency of all songs and calls in this dataset). We standardized amplitude to -1dB so that all stimuli would be broadcast at the same amplitude.

Playback Technique

We presented each song stimulus a maximum of 2 times (never twice to the same birds) and we used 10 different models (5 of each species), in alternation, to minimize pseudoreplication. The playback speaker and model were set up within 10m of a nesting tree near the middle of a pair's territory to simulate competition over their

shared resource. The lure speaker was set up 10m away from the playback speaker.

Once the focal pair was silent for at least 1min, the trial began with the lure phase

followed by the playback phase. The lure continued until a focal bird was within 5m of

the lure speaker, or for a maximum of 5mins (average length of lure: rufous-and-white

wrens: 161.0 ± 9.5s; banded wrens = 204.2 ± 13.4s). If the birds did not respond to the

first lure, we initiated a 2-min silent period followed by another 5-min lure period. If the

bird still did not come within 5m of the lure speaker after two lure phases, we

terminated the trial (n = 7 trials were terminated for this reason). During the lure phase

the model was covered with camouflage mesh fabric that the observer could slowly pull

aside using fishing line. Once the bird was within the experimental area, the 5-minute

trial began from the playback speaker with either acoustic stimuli, the presence of a

model, or both acoustic stimuli and model presentation followed by a 5-min silent

observation period. To minimize carryover effects, each treatment was presented on

separate, consecutive days, at the same time of day for each subject. To minimize order

effects, we used a factorial design to determine the order of the treatments.

The playback apparatus was a camouflaged, wireless speaker (Scorpion TX200,

FOXPRO Inc.) hung in vegetation 1m above the ground, with the model perched on a

branch within 0.5m above the speaker. All treatments were played back at 85dB SPL as

measured with a sound level meter (Casella, CEL-24X, Bedford, UK) positioned 1m in

front of the speaker. Banded wrens appear to produce louder songs than rufous-and-

white wrens, and this amplitude reflects the average amplitude between the values that

have been used in previous playback studies with the two species – 80dB SPL has been

used in playback studies of rufous-and-white wrens, and 90dB SPL in studies of banded

wrens (Mennill, 2006; Molles & Vehrencamp, 2001; Kovach et al., 2014) – thereby

ensuring that amplitude was not a confounding factor.

An observer (KGH) sat concealed in vegetation 15-20m from the speaker and

recorded all treatments using a shotgun microphone (Sennheiser MKH70) and a digital

recorder (Marantz PMD660). Flagging tape was placed 2m on either side of the playback

speaker or model to aid in estimating the distance between the responding birds and

the simulated intruder (i.e., the loudspeaker and/or model). Throughout the trial period

the observer quietly dictated the birds' identities as well as their behaviour and location

in relation to the speaker. Trials where neighbouring pairs responded to the playback

were aborted and repeated at least 1 week later. We conducted playback experiments

on 22 rufous-and-white wren territories and 21 banded wren territories. Of the 22

rufous-and-white wren pairs, one only had one unsuccessful treatment out of six

because they did not respond to the lure for one treatment (conspecific Model Alone).

Of the 21 banded wren pairs, four had one unsuccessful treatment (1 conspecific Song

Alone, 2 conspecific Model Alone, and 1 conspecific Model + Song) and one had two

unsuccessful treatments (conspecific model and congeneric song). This lack of response

to the lure could be due to habituation, as four out of the seven unsuccessful treatments

were given as the fifth or sixth treatments. However, due to time constraints, we did not

attempt to repeat these trials.

Response Measures

We visualized the audio recordings of the playback trials using Syrinx-PC sound analysis software (J. Burt, Seattle, WA). We annotated all songs and duets as well as the behaviour of the focal pair as dictated by the observer, creating a time-stamped record of all behavioural and acoustic measures. For each male and female subject, we extracted the following response variables: (1) distance of closest approach, (2) latency to approach within 5m of the playback apparatus, (3) number of songs initiated (the number of solo songs plus the first song in a duet), and (4) number of duets created (the number of songs where the subject sang in response to its partner thus creating a duet). However, we did not analyze the number of duets created when looking at banded wren responses since males and females do not routinely perform vocal duets (Hall et al., 2015; Molles & Vehrencamp, 1999).

Vegetation Measurements

We were interested in quantifying both visual obstruction due to vegetation density as well as nesting tree resource abundance to understand whether this might affect transmission of visual and acoustic signals for species discrimination. We collected vegetation data in late May and early June, 2014. We collected data in 21 rufous-and-white wren and 22 banded wren territories. We followed previously used methods (see Vermeire & Gillen, 2001; Roovers et al., 2005) to calculate visual obstruction by using a 1m high pole divided into alternating red and white 10-cm sections. The pole was placed perpendicular to the ground at the playback location where an observer counted the

number of 10-cm sections that were visible at distances of 5m, 10m and 15m. All three

distances were measured in all four cardinal directions relative to the playback location.

To better assess the competition between rufous-and-white wrens and banded

wrens over a shared resource, we investigated the abundance of one of the primary

resources for which they appear to compete: nesting trees. We counted the number of

acacia trees in 22 rufous-and-white wren and 21 banded wren territories to assess

resource availability.

Statistical Analysis

We analyzed our data using Generalized Linear Mixed Models (GLMMs). This

method allowed us to use a random effect while accounting for non-normal data, and

allowed us to include the six subjects that did not response to all of the treatments

(Bolker et al., 2008). We included pair identity as a random factor since most pairs

received all six treatments. When investigating variation in response between the two

focal species, our model included 5 main factors: (1) subject species (two levels: rufous-

and-white wren or banded wren); (2) treatment type (three levels: Model Alone, Model

+ Song, Song Alone); (3) stimulus species (two levels: conspecific or congeneric); (4) sex

(two levels: male or female); and (5) playback order (six levels: first – sixth). If playback

order was significant, we were only interested in a difference between the first and last

treatment, which would indicate a decreased response throughout the trials due to

habituation. When assessing differences between focal species, we included all first-

order interaction effects that contained subject species as one of the effects (e.g. subject

species × treatment type, subject species × stimulus species, subject species × sex, and subject species × playback order). When analyzing the data within each species, we included the 4 main factors previously mentioned excluding subject species. For these analyses, we included all first-order interaction effects except for the ones with playback order. We used a Poisson error distribution with a log link function for the acoustic variables (songs initiated and duets created) and a Gamma distribution with a log link function for the behavioural variables (distance of closest approach and latency to 5m). GLMMs with a Poisson error distribution have previously been used for social interaction variables with skewed count data, and Gamma distributions have been used for non-count data skewed to higher values (e.g. Hasegawa et al. 2014; Santos et al. 2009). We ran post-hoc pairwise comparisons for all main effects and first order interactions. To adjust for multiple comparisons, we used a sequential Bonferroni correction which increases P values (instead of decreasing the alpha value); we report corrected P values. When analyzing visual obstruction and vegetation data we used Mann-Whitney to compare non-normal data between the two species habitats. We conducted all analyses using SPSS software (version 21; IBM, Chicago, IL, U.S.A.).

Results

Rufous-and-white Wren Responses

Overall, rufous-and-white wrens responded most strongly to the Song Alone and Model + Song treatments and showed the weakest response to the Model Alone treatment. They also responded more strongly to the conspecific rather than the

congeneric trials for most of the acoustic and behavioural variables we measured. Finally, male rufous-and-white wrens consistently responded more strongly than females. There was one instance where a rufous-and-white wren male attacked a model, which occurred three times during the same congeneric Model + Song treatment. Tests of each of our response variables are provided below, first for behavioural responses followed by acoustic responses.

Behavioural Responses

In their distance of closest approach to the loudspeaker, rufous-and-white wrens showed a significant effect of treatment type (Table 3.1), approaching more closely to the Model + Song and Song Alone treatments versus the Model Alone treatments (post-hoc pairwise comparison: Model Alone vs Model + Song: $t_{229}=2.76$, P=0.01; Model Alone vs Song Alone: $t_{229}=3.91$, P<0.0001), but with no difference between the Model + Song and Song Alone treatments ($t_{229}=1.37$, P=0.17; Figure 3.3a). Rufous-and-white wrens also approached the speaker more closely for the conspecific treatments versus the congeneric treatments ($t_{229}=2.82$, P=0.005; Figure 3.3a). Within the conspecific trials, rufous-and-white wrens approached more closely for the Model + Song and Song Alone treatments versus the Model Alone treatments (Model Alone vs Model + Song: $t_{229}=2.93$, P=0.007; Model Alone vs Song Alone: $t_{229}=3.6$, P=0.001) but there was no difference between the Model + Song and the Song Alone treatments ($t_{229}=0.9$, P=0.37; Figure 3.3a). There was also a significant effect of sex (Table 3.1), with males

approaching significantly closer than females (t_{229}=7.57, P<0.0001). We found no significant effect of playback order (Table 3.1).

In their latency to approach within 5m of the playback apparatus and/or model, rufous-and-white wrens showed a significant effect of treatment type (Table 3.1), approaching more quickly in response to the Song Alone and Model + Song treatments versus the Model Alone treatments (post-hoc pairwise comparison: Model Alone vs Model + Song: t_{229}=2.94, P=0.007; Model Alone vs Song Alone: t_{229}=3.56, P=0.001), but no difference between the Song Alone and Model + Song treatments (t_{229}=0.68, P=0.5). Latency to approach was not influenced by stimulus species (Table 3.1). There was a significant effect of sex (Table 3.1) with males showing shorter latencies to approach within 5m (t_{229}=6.43, P<0.0001). Additionally, males and females differed in their response to the treatment types (Table 3.1); males approached within 5m more quickly for the Song Alone and Model + Song treatments versus the Model Alone treatment (Model vs Model + Song: t_{229}=3.88, P<0.0001; Model vs Song: t_{229}=3.94, P<0.0001), but did not differ in their latency to approach within 5m for the Song Alone and Model + Song treatments (t_{229}=0.18, P=0.86). Conversely, females did not differ in their response to treatment type (t_{229}<1.16, P=0.79). We found no significant effect of playback order (Table 3.1).

Acoustic Responses

The number of songs initiated by rufous-and-white wrens showed no significant effect of playback treatment (Table 3.1). There was, however, a significant effect of

stimulus species (Table 3.1), where rufous-and-white wrens initiated more songs in response to conspecific versus congeneric treatments (post-hoc pairwise comparison: t_{229}=5.69, P<0.0001; Figure 3.3b). There was a significant interaction effect for treatment type versus subject species (Table 3.1), showing that rufous-and-white wrens sang more songs in response to the conspecific stimuli versus the congeneric stimuli for all three treatments (Model Alone: t_{229}=3.18, P=0.002; Model + Song: t_{229}=4.66, P<0.0001; Song Alone: t_{229}=6.01, P<0.0001; Figure 3.3b). Furthermore, there was a significant effect of sex (Table 3.1), where males initiated significantly more songs than females (t_{229}=10.4, P>0.0001). Males initiated more songs than females in response to all three treatments (Model Alone: t_{229}=9.93, P<0.0001; Model + Song: t_{229}=9.87, P<0.0001; Song Alone: t_{229}=9.99, P<0.0001) and in response to all conspecific and congeneric treatments (conspecific: t_{229}=10.13, P<0.0001; congeneric: t_{229}=10.16, P<0.0001). There was a significant effect of playback order (Table 3.1); with the most songs sung in response to the fourth treatment, and the fewest for the fifth treatment, however, there was no significant difference between the number of songs initiated for trials presented first or last (t_{229}=2.28, P=0.21).

The number of duets created by rufous-and-white wrens showed a significant treatment effect (Table 3.1), with birds creating more duets in response to the Model + Song treatments versus the Model Alone treatments (post-hoc pairwise comparison: t_{229}=2.56, P=0.03; Figure 3.3c), but with no difference between the Model Alone and Song Alone treatments (t_{229}=1.61, P=0.22) or the Song Alone and Model + Song treatments (t_{229}=1.45, P=0.22; Figure 3.3c). There was no effect of stimulus species on

number of duets created (Table 3.1). There was a significant interaction effect of

treatment type by stimulus species (Table 3.1). Within the conspecific trials, rufous-and-

white wrens created more duets in response to the Song Alone and Model + Song

treatments versus the Model Alone treatments (Model Alone vs Model + Song:

t_{229}=3.14, P=0.006; Model Alone vs Song Alone: t_{229}=3.13, P=0.006; Figure 3.3c).

However, they did not differ in their response to conspecific Song Alone and conspecific

Model + Song treatments (t_{229}=0.24, P=0.81; Figure 3.3c). There was no significant

difference between responses to the treatment groups within the congeneric trials

(t_{229}<2.17, P>0.09; Figure 3.3c). Our results revealed a significant effect of sex (Table

3.1), with females creating more duets than males (t_{229}=3.03, P=0.003). The sexes also

differed in their response to the different stimulus species (Table 3.1). Males created

more duets in response to the conspecific trials versus the congeneric trials (t_{229}=2.44,

P=0.015); however, females did not differ in their response to conspecific versus

congeneric trials (t_{229}=1.4, P=0.16). There was a significant effect of playback order

(Table 3.1), with birds creating significantly more duets in response to the first trials

compared to all subsequent trials (t_{214}<3.55, P<0.03).

Banded Wren Responses

Overall, banded wrens responded more strongly to the Song Alone and Model +

Song treatments, and showed a weaker response to the Model Alone treatments, similar

to rufous-and-white wrens. Unlike rufous-and-white wrens, banded wrens showed

similar responses towards conspecific versus congeneric trials. Finally, male banded

wrens consistently responded more strongly than females for all variables. Tests of each

of our response variables are provided below, first for behavioural responses followed

by acoustic responses.

Behavioural Responses

The distance of closest approach for banded wrens showed a significant effect of

treatment type (Table 3.2), with banded wrens approaching the Song Alone and Model +

Song treatments more closely the Model Alone treatments (post-hoc pairwise

comparison: Model Alone vs Model + Song: $t_{174}=4.78$, P<0.0001; Model Alone vs Song

Alone: $t_{174}=4.45$, P<0.0001). However, there was no significant difference between the

distance of closest approach to the Song Alone and Model + Song treatments ($t_{159}=0.59$,

P=0.56; Figure 3.4a). There was no significant difference between the responses towards

conspecific versus congeneric stimuli (Table 3.2; Figure 3.4a). There was a significant

effect of sex (Table 3.3), with males approaching the speaker or model significantly more

closely than females ($t_{174}=6.52$, P<0.0001). Females and males differed in their response

to each treatment type (Table 3.2). Males approached more closely for the Song Alone

and Model + Song treatments versus the Model Alone treatments (Model Alone vs

Model + Song: $t_{174}=4.15$, P<0.0001; Model Alone vs Song Alone: $t_{174}=4.91$, P<0.0001), but

did not differ in their approach for the Song Alone versus Model + Song treatments

($t_{174}=1.49$, P=0.14). However, females approached the Model + Song treatment more

closely than the Model Alone treatments ($t_{174}=3.25$, P=0.004), but did not differ in their

approach for the Model Alone and Song Alone treatments ($t_{174}=1.87$, P=0.13) or the

Song Alone versus the Model + Song treatments (t_{174}=1.84, P=0.13). Lastly, there was a significant effect of playback order (Table 3.2), but the post-hoc comparison did not reveal any significant differences between different trial orders.

Banded wrens differed in their latency to approach within 5m of the playback apparatus for the three treatment types (Table 3.2). Consistent with rufous-and-white wrens, banded wrens approached within 5m more quickly for the Song Alone and Model + Song treatments versus the Model Alone treatments (post-hoc pairwise comparison: Model Alone vs Model + Song: t_{174}=4.37, P<0.0001; Model Alone vs Song Alone: t_{174}=4.83, P<0.0001), but they did not differ in their latency to approach within 5m of the Song Alone and Model + Song treatments (t_{174}=0.72, P=0.47). There was no significant effect of stimulus species (Table 3.3), or a significant interaction effect of treatment type by stimulus species (Table 3.2). There was, however, a significant effect of sex (Table 3.2,) with males approaching within 5m more quickly than females (t_{174}=4.67, P<0.0001). We also found a significant effect of playback order (Table 3.2), with the post-hoc comparison revealing that banded wrens approached within 5m more quickly for the third trial versus the sixth trial (t_{174}=3.0, P=0.047).

Acoustic Responses

The number of songs initiated by banded wrens showed a significant effect of treatment (Table 3.2), with birds singing more songs in response to the Song Alone and the Model + Song treatments compared to the Model Alone treatments (post-hoc pairwise comparison: Model Alone vs Model + Song: t_{174}=4.51, P<0.0001; Model Alone

vs Song Alone: $t_{174}=4.55$, P=0.001' Figure 3.4b), and they sang the same number of songs in response to the Song Alone and Model + Song treatments ($t_{174}=0.07$, P=0.94; Figure 3.4b). There was no significant effect of subject species, nor was there a significant interaction effect between treatment type and subject species (Table 3.2; Figure 3.4b). There was a significant effect of sex (Table 3.2), with males singing significantly more songs than females ($t_{174}=9.9$, P<0.0001). There was also a significant interaction effect of sex by treatment type (Table 3.2). Males sang the most songs in response to the Model + Song treatments compared to the other two treatments (Model Alone vs Model + Song: $t_{174}=6.12$, P<0.0001; Model + Song vs Song Alone: $t_{174}=1.98$, P<0.0001), and the fewest songs to the Model Alone treatment with an intermediate response to the Song Alone treatment (Model Alone versus Song Alone: $t_{174}=4.72$, P<0.0001). Additionally, female banded wrens initiated more songs in response to the Song Alone and Model + Song treatments compared to the Model Alone treatments (Model Alone vs Model + Song: $t_{174}=346$, P=0.001; Model Alone vs Song Alone: $t_{174}=3.78$, P=0.001), but they did not differ in their response to the Song Alone versus the Model + Song treatments ($t_{174}=0.39$, P=0.7). There was a significant effect of playback order (Table 3.2), with banded wrens singing significantly more songs during the first trial compared to all other trials ($t_{174}<5.73$, P<0.015).

Species differences in responses

Overall, in comparing the two subject species, rufous-and-white wrens and banded wrens did not differ in their behavioural responses to the six treatments, but

they did differ in their acoustic responses, with rufous-and-white wrens consistently

singing more than banded wrens. Tests of each of our response variables are provided

below, first for behavioural responses followed by acoustic responses.

Behavioural Responses

There was no effect of the subject species for distance of closest approach to the

playback (Table 3.3) or for the latency to approach within 5m of the playback (Table 3.3).

This held true for all of the subject species interaction effects for both behavioural

variables (Table 3.3).

Acoustic Responses

Rufous-and-white wrens initiated more songs than banded wrens in response to

playback (post-hoc pairwise comparisons: $t_{423}=2.75$, P=0.006; Figure 3.5a). Rufous-and-

white wrens sang more songs in response to the Model Alone treatments ($t_{423}=4.03$,

P<0.0001) and the Song Alone treatments ($t_{423}=2.27$, P=0.024) than banded wrens, but

both species responded similarly to the Model + Song treatments ($t_{423}=1.59$, P=0.11).

Rufous-and-white wrens initiated more songs than banded wrens to both the

conspecific and congeneric treatments (conspecific: $t_{423}=3.31$, P=0.001; congeneric:

$t_{423}=2.9$, P=0.038; Figure 3.5a). Additionally, males of both species initiated a similar

number of songs ($t_{423}=3.14$, P=0.28), whereas female rufous-and-white wrens sang more

songs than female banded wrens ($t_{423}=1.18$, P<0.0001).

Rufous-and-white wrens created significantly more duets than banded wrens

(post-hoc pairwise comparison: $t_{423}=2.7$, P=0.007; Figure 3.5b), with rufous-and-white

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wrens creating more duets in response to both the conspecific and congeneric

treatments (conspecific: t_{423}=2.92, P=0.004; congeneric: t_{423}=2.27, P=0.024; Figure 3.5b).

Vegetation Measurements

We measured vegetation characteristics in order to better understand visual

obstruction within the territories of our two study species, as well as the relative degree

of limitation of their nesting substrate: bull-horn acacia trees. We found no significant

difference in the percentage of visual obstruction caused by vegetation in the territories

of rufous-and-white wrens versus banded wrens' territories at each distance (Table 3.4).

Rufous-and-white wren territories had on average 34.8 ± 3.3 % visual obstruction caused

by the vegetation when values for all distances were combined, whereas banded wren

territories had on average 30.2 ±2.6 % visual obstruction (Table 3.4). In other words,

between 5-15m, approximately one third of the area 1m from the ground was obscured

by vegetation in the territories of both species.

Rufous-and-white wrens had significantly fewer acacia trees in their territories –

just a third the number of acacia trees – compared to banded wren territories (U=29,

P<0.0001) (Table 3.4).

Discussion

We investigated the use of acoustic and visual signals for species discrimination

in two congeneric neotropical wren species: rufous-and-white wrens and banded wrens.

Both species showed the strongest responses to the Song Alone and Model + Song

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treatments, and the weakest response to the Model Alone treatments, although there

was one instance where the Model + Song treatments received the strongest response

(i.e., the number of songs initiated by male banded wrens). Our findings suggest that

both species rely more on acoustic signals for interspecific discrimination, and rely less

on visual signals such as plumage colouration. We found that rufous-and-white wrens

approached the speaker more closely and created more duets for the conspecific Song

Alone and Model + Song treatments than for the Model Alone treatments. This pattern

suggests that rufous-and-white wrens may also rely more on acoustic signals for

intraspecific discrimination. Interestingly, the two species differed in their response to

conspecific and congeneric signals. Rufous-and-white wrens showed a stronger response

to the conspecific trials than to the congeneric trials, but banded wrens did not differ in

their response to conspecific versus congeneric trials. Our results show that birds living

in densely vegetated habitats use vocal and visual traits as intra- and interspecific

discrimination signals, but that they rely more on acoustic communication.

Rufous-and-white wrens are monochromatic, with birds of both sex exhibiting

similar plumage colouration that looks identical to the naked eye. However, the sexes

have obvious dimorphic song features where females sing quieter songs, shorter trill

components, and higher frequency elements (Mennill & Vehrencamp, 2005). Males and

females also sing separate repertoires of songs, which they can use to identify

individuals within their own species (Mennill & Vehrencamp, 2005). These pronounced

sex and individual differences in vocalizations may explain why rufous-and-white wrens

rely more on the acoustic signal for intraspecific discrimination. The songs of rufous-and-

white wrens and banded wrens differ substantially in their length and frequency (Figure 3.2) and consequently sound very distinct. Their plumage, however, is relatively similar with rufous brown colouration above and white below, with the primary difference being the dark barring on the flanks of banded wrens (Figure 3.2). Additionally, the plumage of both species is relatively drab and does not stand out against the brownish bark and leaf litter of the tropical dry forest (see Doucet et al., 2007). Consequently, it is not unexpected that these species would rely heavily on acoustic signals and less on visual signals for interspecific discrimination.

Only three previous studies have investigated the simultaneous use of both visual and acoustic signals for species discrimination in birds. One ground-breaking study compared the response of two sister taxa from the *Monarcha castaneiventris* complex of the Solomon Islands: the chestnut-bellied form (*M. c. castaneiventris)* and the white-capped form *(M. c. richardsii*; Uy et al., 2009)*. These species were presented with matching or mismatching taxidermic mounts and songs and were found to use both song and plumage for discrimination, but relied more heavily on plumage (Uy et al., 2009). Another study on *Monarcha* flycatchers found that subspecies in more densely vegetated forests use acoustic and visual signals sequentially, using song for long-range recognition and plumage for close-range discrimination (Uy & Safran, 2013). By contrast, subspecies in more open habitats use song and plumage simultaneously (Uy & Safran, 2013). These two studies show that birds are able to use both acoustic and visual signals for species discrimination but that they may differ in their use of these signals depending on their habitat. Further research is needed to demonstrate whether rufous-

and-white wrens and banded wrens use song and plumage sequentially in their tropical

forest habitat. Future studies comparing signal use in habitats with differing vegetation

density are required to demonstrate the influence of vegetation density on multimodal

signalling in birds. The vegetation density at our study site was moderately high, so we

would expect birds to rely more on the visual signal in a less densely vegetated habitat

or if they have plumage that contrasts against the background.

Rufous-and-white wrens were more responsive and they responded more

strongly to conspecific than to congeneric stimuli, whereas banded wrens responded

with similar intensity to both conspecific and congeneric stimuli. Such interspecific

variation in responses could be indicative of interspecific dominance between these two

species. Rufous-and-white wrens showed a lower response to the banded wren stimuli

and did not differ in their response to the different congeneric treatments, suggesting

that rufous-and-white wrens may not perceive banded wrens as a strong threat.

Conversely, banded wrens responded to conspecific and congeneric stimuli as though

they were equally threatening. One possible explanation is that banded wrens failed to

differentiate between conspecific and congeneric stimuli (Murray, 1981). However, the

substantial difference in the length, frequency, and amplitude of the songs of these two

species (Figure 3.2c) makes this explanation seem unlikely, especially with the additional

presentation of visual signals for many of the treatments. The presence of interspecific

dominance seems a more plausible explanation. Other species showing interspecific

dominance also demonstrate an asymmetry in their responses to congeneric and

conspecific signals (Martin & Martin, 2001; Martin & Dobbs, 2015; Pearson & Rowher,

2000). In chickadees, for example, dominant black-capped chickadees (*Poecile atricapillus*) were more vocal and responded more strongly to conspecific than heterospecific calls, whereas subordinate mountain chickadees (*P. gambeli*) responded similarly to both mountain and black-capped chickadee calls (Grava et al., 2012). Other indirect evidence supports the dominance of rufous-and-white wrens over banded wrens. For example, rufous-and-white wrens have larger territories than banded wrens (rufous-and-white wren territory size: 1.35 ± 0.10Ha, Mennill & Vehrencamp, 2008; banded wren territory size: 0.40Ha, Trillo & Vehrencamp, 2005). In addition, rufous-and-white wrens have larger body sizes than banded wrens (rufous-and-white wren body length = 14.5 – 16.5cm; banded wren body length = 14 – 15cm; Brewer, 2001) and are heavier than banded wrens (rufous-and-white wren male mass = 25.8g, female mass = 23.7g, Mennill & Vehrencamp, 2005; banded wren male mass = 20.3g, female mass = 18.3g, Hall et al. 2015); larger body size has previously been shown to relate to interspecific dominance (Funghi et al., 2014; Freshwater et al. 2014; Farwell and Marzluff, 2013). To conclude that these two species are ecological competitors, further research is required to assess whether banded wrens suffer fitness costs when living in sympatry with rufous-and-white wrens, and whether rufous-and-white wrens restrict the distribution of banded wrens.

Consistent with previous studies, we found that males were significantly more responsive to playback than females in both species (Fedy & Stutchbury, 2005; Mennill & Vehrencamp, 2008; Mennill, 2006; Hall et al., 2006). In our study, however, the two sexes differed in their response to the different treatment groups. In banded wrens,

males sang very few songs to the Model Alone treatment, the most songs to the Model + Song treatments and an intermediate number of songs to the Song Alone treatments. Females, by contrast, responded the least to the Model Alone treatment and with similar intensity to the Song Alone and Model + Song treatments. These results could be due to the lower response rate of females, which may not have allowed us to statistically differentiate between the Song Alone and Model + Song treatments. Nevertheless, our findings suggest that although male banded wrens relied on acoustic signals, the addition of the visual signal strengthened their response.

Rufous-and-white wrens and banded wrens live in a tropical dry forest characterized by dense vegetation. Our analyses suggest that, on average, vegetation obstructs 35% of the visual signals in rufous-and-white wren territories and 30% in banded wren territories (Table 3.4), even at distances as close as 10m, demonstrating that visual signals have limited transmission capabilities. Moreover, contrary to our predictions, the understory vegetation density in the habitats of each of our two focal species was not significantly different. These findings could have been affected by the fact that the measurements were taken during the beginning of the rainy season, when the tropical dry forest habitat of banded wrens starts to become more similar to the evergreen habitat of rufous-and-white wrens. Our vegetation measurements also revealed that banded wren territories contained significantly more acacia nesting trees than rufous-and-white wren territories. Rufous-and-white wrens may need to defend nesting trees more aggressively because the resource is more limited in their territories compared to banded wren territories.

One potential limitation of our study is that it relied on the use of wooden models to serve as a visual representation of conspecific and heterospecific animals. This is the first study to use both avian wooden models and song playback to investigate the importance of visual and acoustic signals for inter- and intraspecific discrimination. Our successful use of avian wooden models provides an alternative to sacrificing study animals to create taxidermic mounts. Although our wooden models were realistic (see Figure 3.1), and the colour matched the plumage reflectance of museum specimens, the lack of movement may have hindered the response of the birds. If the models produced movements such as wing flaps or tail cocking, they may have elicited stronger aggressive responses (Anderson et al., 2013). Experiments involving robotic birds (e.g. Patricelli et al., 2006; Balsby & Dabelsteen, 2002) show that movements can influence responses to model presentation experiments. Moreover, a previous study showed that birds use not only colour but also surface texture as a signal for species discrimination (Nemec et al., 2014). Red-backed shrikes (*Lanius collurio*) attacked a taxidermic model of a predator Eurasian jay (*Garrulus glandarius*) more often than a plush model, and attacked a silicone model the least (Nemec et al., 2014). Although our wooden models had feather-like texture carvings and looked more realistic than the plush model used in the aforementioned study (see Figure 3.1), the use of a taxidermic mount with feathers that rustled in the wind might have elicited a stronger response. Nevertheless, banded wren males sang the most songs to the Model + Song treatments, and in one instance a rufous-and-white wren attacked the model, which suggests that the birds were responding to the model.

Our study demonstrates that wrens living in tropical forests rely primarily on acoustic signals for inter- and intraspecific discrimination. We expect similar patterns among other species that have distinct songs but similar or cryptic plumage, especially in dense habitats with visual obstruction. The asymmetry in response to congeneric versus conspecific stimuli suggests the possibility of an interspecific dominance relationship between rufous-and-white wrens and banded wrens, which could have significant implications if shared resources become scarce and rufous-and-white wrens outcompete subordinate banded wrens. This is the first study to investigate multimodal signal use in both males and females in tropical habitats, and our experiment revealed that the use of different signal modalities, and the strength of responses, can vary both within and among species. More studies are needed to determine the breadth of these patterns, and how they vary across habitats and across species that differ in visual or acoustic conspicuousness.

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Tables

Table 3.1. Generalized linear mixed model results of the response of rufous-and-white wrens to playback and model presentation simulating conspecific and congeneric intruders. Significant values are in bold.

	Distance of Closest Approach			Latency to approach within 5m			Number of Songs Initiated			Number of Duets Created		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Treatment Type	**9.37**	**2,229**	**<0.0001**	**7.64**	**2,229**	**0.001**	1.19	2,229	0.31	**5.69**	**2,229**	**0.004**
Stimulus Species	**8.44**	**1,229**	**0.004**	1.06	1,229	0.3	**42.71**	**1,229**	**<0.0001**	2.33	1,229	0.13
Sex	**114.76**	**1,229**	**<0.0001**	**51.46**	**1,229**	**<0.0001**	**1193.6**	**1,229**	**<0.0001**	**25.37**	**1,229**	**<0.0001**
Playback Order	1.22	5,229	0.3	1.38	5,229	0.23	**7.33**	**5,229**	**<0.0001**	**26.92**	**5,229**	**<0.0001**
Treatment Type × Stimulus Species	1.73	2,229	0.18	1.14	2,229	0.32	**8.26**	**2,229**	**<0.0001**	**17.19**	**2,229**	**<0.0001**
Treatment Type × Sex	1.5	2,229	0.23	**3.68**	**2,229**	**0.027**	**3.29**	**2,229**	**0.04**	0.17	2,229	0.85
Sex × Stimulus Species	0.36	1,229	0.55	0.29	1,229	0.59	**18.74**	**1,229**	**<0.0001**	**12.69**	**1,229**	**<0.0001**

Table 3.2. Generalized linear mixed model results of the response of banded wrens to playback and model presentation simulating conspecific and congeneric intruders. Significant values are in bold**.**

	Distance of Closest Approach			Latency to approach within 5m			Number of Songs Initiated		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Treatment Type	**21.3**	**2,174**	**<0.0001**	**19.04**	**2,174**	**<0.0001**	**11.83**	**2,174**	**<0.0001**
Stimulus Species	0.48	1,174	0.49	0.002	1,174	0.96	2.67	1,174	0.1
Sex	**146.99**	**1,174**	**<0.0001**	**33.25**	**1,174**	**<0.0001**	**597.07**	**1,174**	**<0.0001**
Playback Order	**3.61**	**5,174**	**0.004**	**2.81**	**5,174**	**0.02**	**13.85**	**5,174**	**<0.0001**
Treatment Type × Stimulus Species	2.37	2,174	0.097	1.5	2,174	0.22	0.71	2,174	0.49
Treatment Type × Sex	**3.92**	**2,174**	**0.02**	0.83	2,174	0.44	**3.89**	**2,174**	**0.02**
Sex × Stimulus Species	3.45	1,174	0.07	1.91	1,174	0.17	2.3	1,174	0.13

Table 3.3. Generalized linear mixed model results of the response of rufous-and-white wrens and banded wrens to playback and model presentation simulating conspecific and congeneric intruders. Significant values are in bold.

|	Distance of Closest Approach			Latency to approach within 5m			Number of Songs Initiated			Number of Duets Created		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Subject species	1.26	1,423	0.26	0.67	1,423	0.41	**7.8**	**1,423**	**0.005**	**12.47**	**1,423**	**<0.0001**
Treatment Type	**29.87**	**2,423**	**<0.0001**	**26.63**	**2,423**	**<0.0001**	**22.69**	**2,423**	**<0.0001**	**6.25**	**2,423**	**0.002**
Stimulus Species	**12.55**	**1,423**	**<0.0001**	3.43	1,423	0.065	**11.57**	**1,423**	**0.001**	0.5	1,423	0.48
Sex	**236.21**	**1,423**	**<0.0001**	**75.94**	**1,423**	**<0.0001**	**1790.56**	**1,423**	**<0.0001**	**5.61**	**1,423**	**0.018**
Subject Species × Treatment Type	1.13	2,423	0.32	0.76	2,423	0.47	**20.79**	**2,423**	**<0.0001**	2.58	2,423	0.077
Subject Species × Stimulus Species	1.04	1,423	0.31	0.007	1,423	0.94	**14.55**	**1,423**	**<0.0001**	**9.79**	**1,423**	**0.002**
Subject Species × Sex	1.81	1,423	0.18	0.49	1,423	0.48	**1979**	**1,423**	**<0.0001**	3.02	1,423	0.083

Table 3.4. Vegetation visual obstruction and resource abundance data. We measured vegetation visual obstruction by counting the number of 10-cm bars that were concealed at distances of 5m, 10m and 15m at the playback location. Resource abundance data was collected by counting the number of nesting trees within a pair's territory. All data represent averages ± standard error. Results from Mann-Whitney test are shown. Significant values are in bold.

|	Rufous-and-white Wren	Banded Wren	*U*	*P*
Percent concealed at 5m	9.64 ± 2.13	11.36 ± 2.47	204	0.51
Percent concealed at 10m	35.95 ± 4.25	31.14 ± 3.3	204.5	0.52
Percent concealed at 15m	58.75 ± 4.26	48.01 ± 3.99	156	0.07
Number of Acacia Trees	7.38 ± 1.13	21.05 ± 1.23	**29**	**<0.0001**

Figures

Figure 3.1. Comparison picture of real rufous-and-white wren on left looking at model rufous-and-white wren on right.
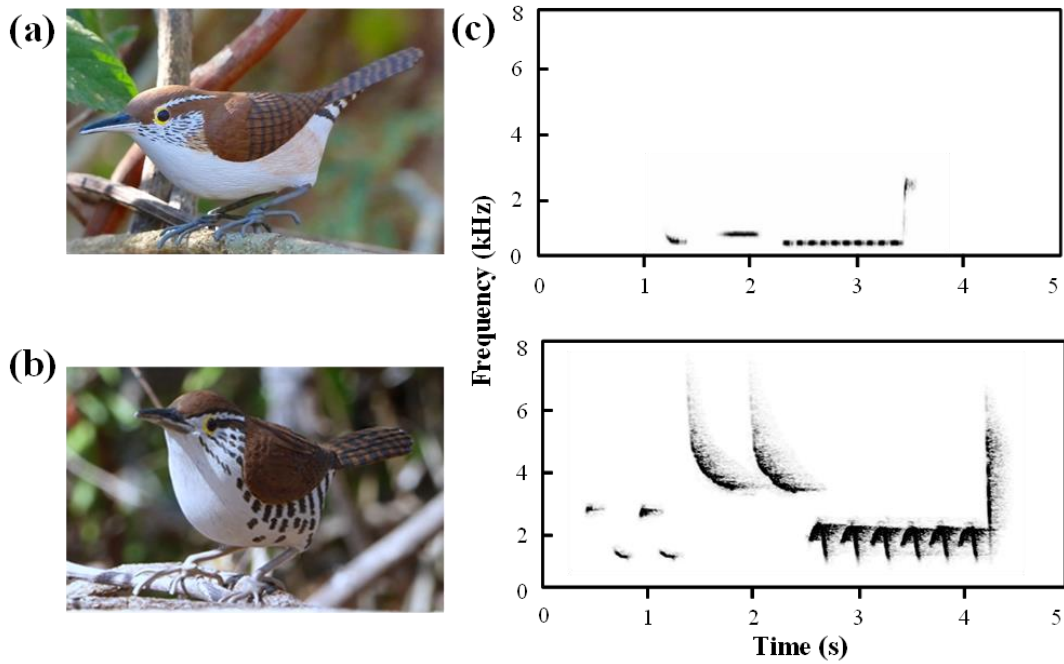
Figure 3.2. Comparison model pictures and spectrograms of both study species. (a) Picture of model rufous-and-white wren. (b) Picture of model banded wren. (c) Spectrograms of rufous-and-white wren song (top) and banded wren song (bottom).
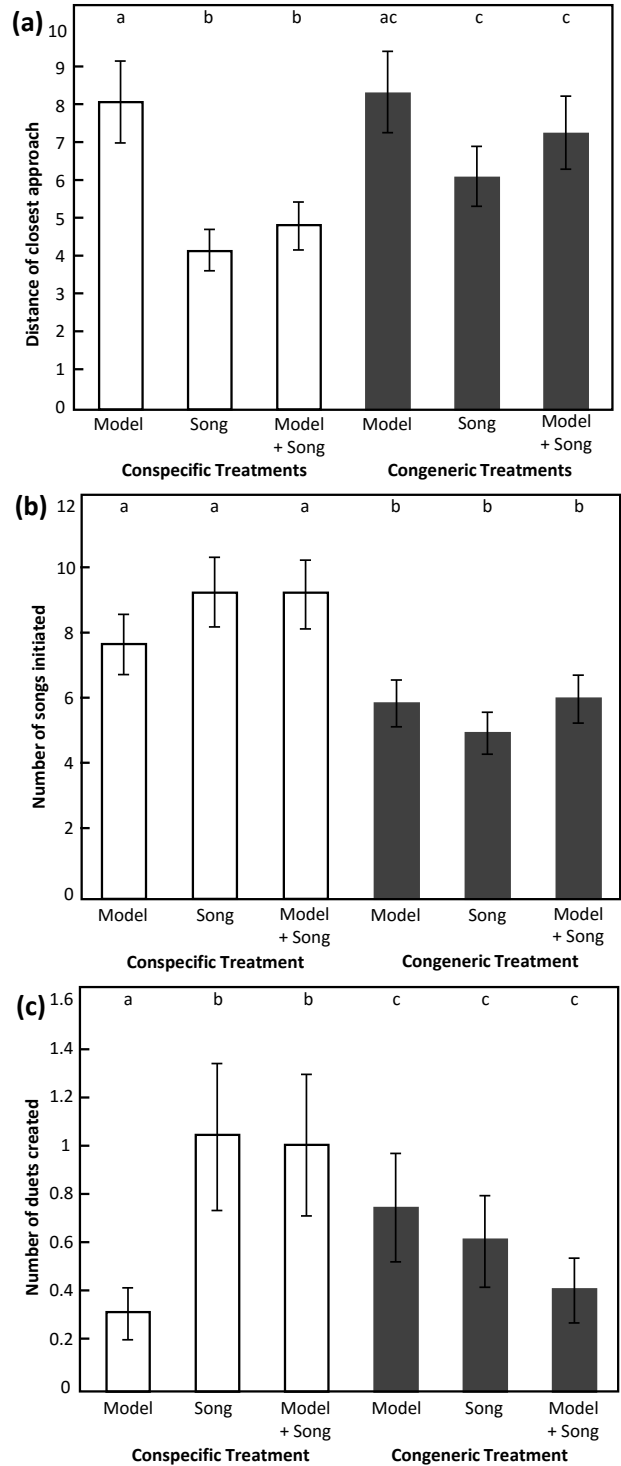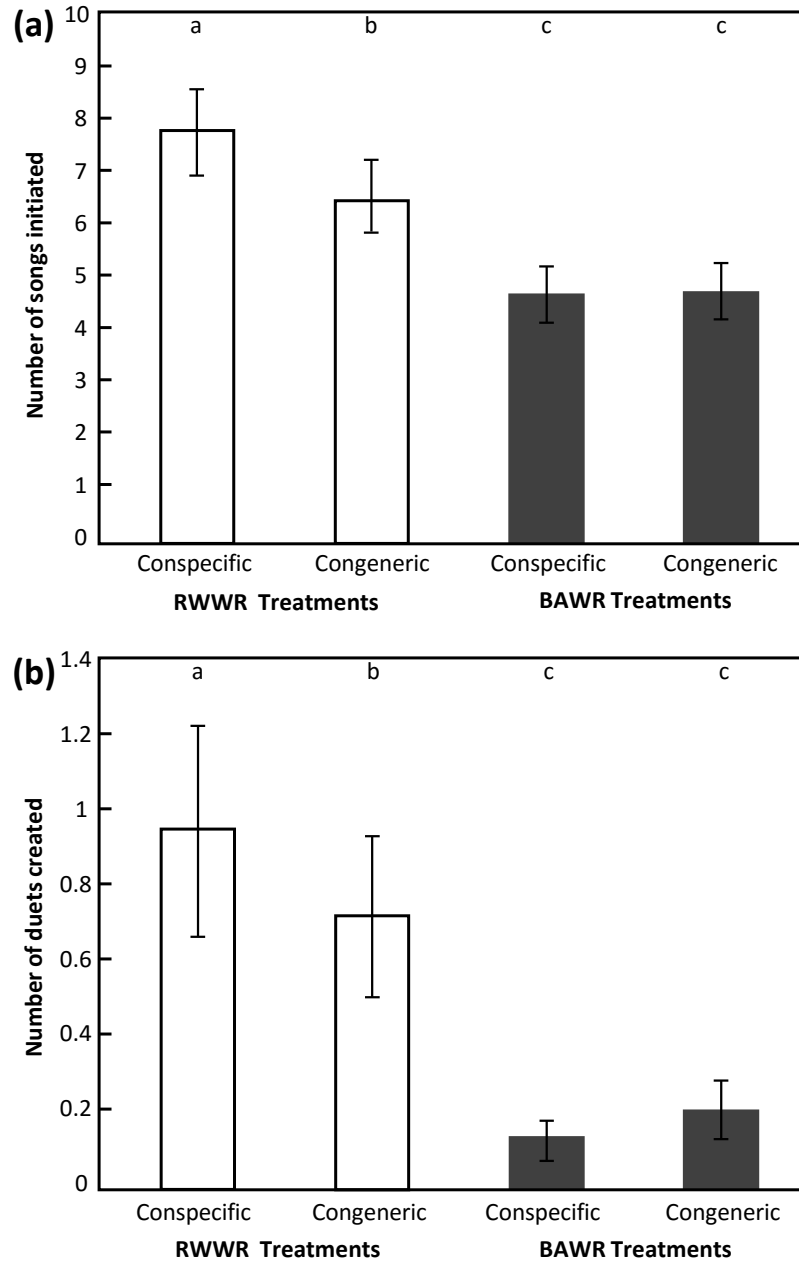
Figure 3.3. Response of rufous-and-white wrens to model presentation, song playback, and song playback and model presentation together of conspecific and congeneric stimuli. (a) Distance of closest approach to the playback speaker and/or model. (b) Number of songs initiated (number of solos plus first song of a duet). (c) Number of duets created (song sung in response to a solo song). Different letters above bars indicate statistical significance. Graph shows mean values from treatment × stimuli species post-hoc pairwise comparison with standard error bars.
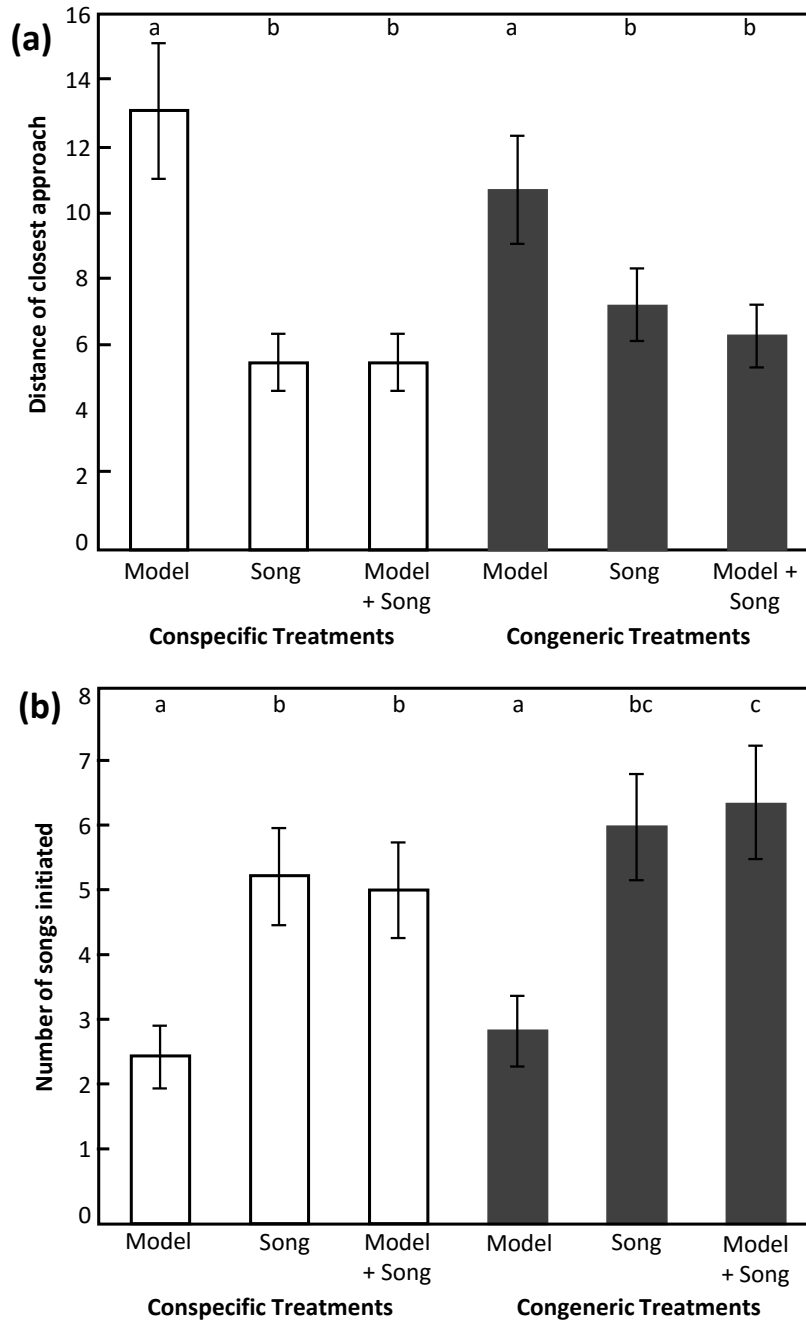
Figure 3.4. Response of banded wrens to model presentation, song playback, and song playback and model presentation together of conspecific and congeneric stimuli. (a) Distance of closest approach to the playback speaker and/or model. (b) Number of songs initiated (number of solos plus first song of a duet). Different letters above bars indicate statistical significance. Graph shows mean values from treatment × stimuli species post-hoc pairwise comparison with standard error bars.

Figure 3.5. Acoustic response of banded wrens and rufous-and-white wrens to conspecific and congeneric stimuli. (a) Number of songs initiated (number of solos plus first song of a duet). (b) Number of duets created (song sung in response to a solo song). Different letters above bars indicate statistical significance. Graph shows mean values from subject species × stimuli species post-hoc pairwise comparison with standard error bars.
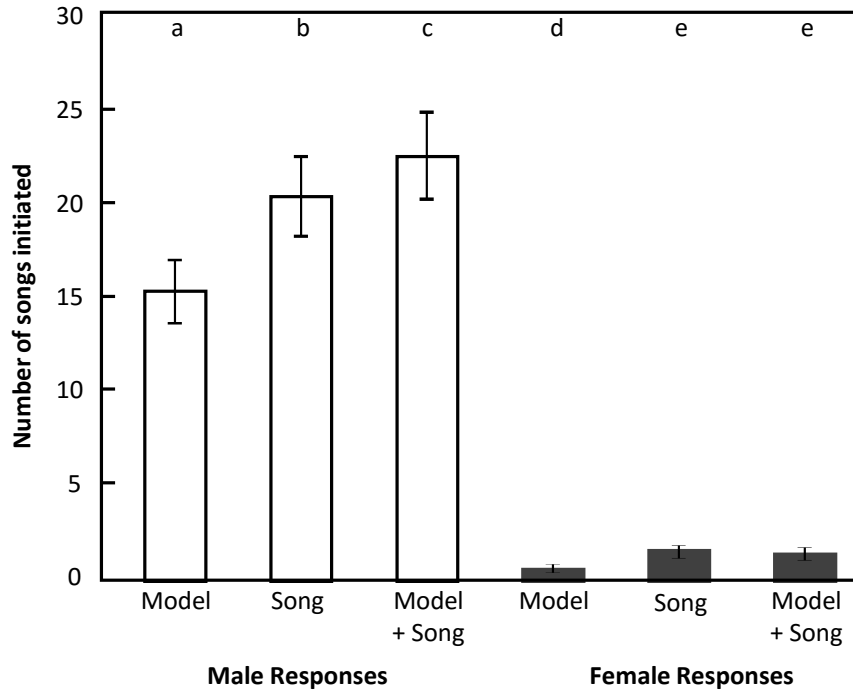
Figure 3.6. Number of songs initiated by male and female banded wrens to Model Alone, Song Alone, and Model + Song treatments. Different letters above bars indicate statistical significance. Graph shows mean values from sex × treatment post-hoc pairwise comparison with standard error bars.

CHAPTER 4

GENERAL DISCUSSION

Social animals should benefit from discriminating between individuals that represent a threat versus others who do not represent a threat and may represent a possible mating opportunity. It is especially important to discriminate between conspecific and heterospecific individuals for closely related species that compete for a shared resource so they can defend their territory and resources from the rival species. The ability to discriminate between conspecific and heterospecific animals may differ depending on the amount of contact an individual has had with a conspecific or heterospecific threat and therefore may differ in areas where two species live in sympatry versus allopatry. Due to the importance of properly identifying both conspecific and heterospecific individuals, animals may use multiple signal modalities for discrimination, such as acoustic and visual signals. A large body of research has focused on species discrimination, but there is no consensus on the importance of previous experience for species discrimination. Furthermore, little is known about the simultaneous use of both acoustic and visual signals for species discrimination. My thesis investigated the factors that affect intra- and interspecific discrimination signals in closely related neotropical wren species. My research advances this field of study by providing an example where experience does not influence species discrimination, and by showing that inconspicuous birds living in dense, tropical habitats rely very heavily on acoustic signals for intra- and interspecific discrimination.

My second chapter investigated how rufous-and-white wrens (*Thryophilus rufalbus*) differed in their response to playback of songs of conspecific rufous-and white wrens and congeneric banded wrens (*T. pleurostictus*) in an area where the two species

live in sympatry and an area where the two species live in allopatry. I tested the

hypothesis that rufous-and-white wrens would differ in their response to congeneric

stimuli in an area of sympatry and allopatry. I predicted that they would show a stronger

response to congeneric songs in the area of sympatry, where they interact and compete

with banded wrens, but show very little response to congeneric songs in allopatry,

where they have no experience with banded wrens and therefore may not perceive

their songs as a threat. I presented rufous-and-white wrens in the sympatric and

allopatric populations with conspecific rufous-and-white wren songs, congeneric banded

wren songs, and control long-tailed manakin songs. My results revealed that rufous-and-

white wrens always responded most strongly to the conspecific stimuli but showed very

little response to the congeneric stimuli and the control stimuli. Interestingly, these

responses did not differ in areas of sympatry versus allopatry. This pattern suggests that

in rufous-and-white wrens, species discrimination may be innate or that they always

show very little aggression towards any species that is different from their own. My

results were consistent with previous studies on white-eared ground-sparrows

(*Melozone leucotis;* Sandoval et al., 2013) and subspecies of Moltoni's warblers (*Sylvia*

cantillans; Brambilla et al., 2008). However, my findings were inconsistent with the

observation that the two species seem to compete for shared resources. Since rufous-

and-white wrens showed similarly low responses to both the congeneric and control

stimuli, I was interested in seeing if this may be due to a social dominance relationship

between rufous-and-white wrens and banded wrens by studying the reciprocal

playbacks to banded wrens, which was part of the motivation for chapter 3.

In chapter 3, I studied the importance of both acoustic and visual signals for intra- and interspecific discrimination between the two competing wren species previously mentioned. Only three previous studies have investigated how closely-related bird species use multiple signal modalities (Uy & Safran, 2013; Uy et al., 2009; Matyjasiak, 2004). I tested the hypothesis that these wren species use both acoustic and visual signals for species discrimination, but that they may rely more heavily on the acoustic signal. I also predicted that banded wrens, who live in more open habitats, would rely more on the visual signal than rufous-and-white wrens, who live in densely vegetated habitats. Additionally, I was interested in studying how these two species differ in their response to conspecific and congeneric stimuli to infer whether or not a social dominance relationship may exist between them. I presented both rufous-and-white wrens and banded wrens with conspecific and congeneric song playback alone, model presentation alone, and song playback and model presentation together to assess how they use acoustic and visual signals alone and together. I found that both species showed the lowest response to the model presented alone but showed equally high responses to the song playback alone and the song playback and model presented together. Additionally, rufous-and-white wrens were more responsive than banded wrens and responded the strongest to the conspecific stimuli and very little to the congeneric stimuli. Conversely, banded wrens responded with the same intensity to both the conspecific and congeneric stimuli. These results suggest that rufous-and-white wrens do not see banded wrens as a threat, but banded wrens perceive conspecific and congeneric stimuli as being equally threatening. This pattern suggests the possibility of

an inter-specific dominance relationship, where rufous-and-white wrens are the more dominant species and banded wrens are the more subordinate species. A similar pattern has been found in two subspecies of gray-breasted wood-wrens (Dingle et al., 2010) and between black-capped chickadees (*Poecile atricapillus*) and mountain chickadees (*P. gambeli*; Grava et al., 2012). My results demonstrate that these wren species rely more on acoustic signals than visual signals for intra- and interspecific discrimination and that they exhibit asymmetrical heterospecific aggression.

Taken together, these two experiments on species discrimination provide further evidence that species discrimination signals are complex and that animals have evolved multiple mechanisms for discriminating between competitor and non-competitor species. The results of chapter 2 indicate that the response to conspecific and congeneric stimuli did not differ in an area of sympatry or an area of allopatry. These findings suggest that birds differ in the mechanisms they use for species discrimination, and that species discrimination in rufous-and-white wrens may be innate (i.e., independent of experience with heterospecific animals) or they may always show very little response to any species that differs from their own. Chapter 3 demonstrates that wrens living in densely vegetated tropical habitats rely heavily on acoustic signals, such as song, and very little on visual signals such as plumage for species discrimination. This is not surprising, since both wren species are very secretive with cryptic plumage that does not stand out in the dense foliation. Both species also sing loud, distinctive songs that transmit further in the densely vegetated habitats. The reciprocal playbacks to banded wrens in chapter 3 may also help explain the low response to congeneric stimuli

by rufous-and-white wrens I found in chapter 2. My results indicate asymmetrical aggression between rufous-and-white wrens and banded wrens, which may suggest the presence of a social dominance relationship. Given that rufous-and-white wrens showed stronger responses, exhibit larger body sizes, and hold larger territories, I speculate that they are dominant over banded wrens. These findings demonstrate the importance of reciprocal playbacks to better understand competitive relationships between closely related species. This finding has important implications if environmental changes causes niche boundaries to change, which could lead to rufous-and-white wrens outcompeting banded wrens.

Future research could extend the findings in chapter 2 by conducting similar playbacks to other closely-related wren species that live in areas of sympatry and allopatry. For example, mountain wrens (*Troglodytes solstitialis*) and house wrens (*T. aedon*) show interspecific aggression (Martin & Dobbs, 2014), however it is not known how they interact in zones of sympatry and allopatry. One interesting finding from Chapter 2 was that females showed stronger responses to foreign versus local song dialects. This is the first study to find female differences in response to local and foreign song dialects without the injection of estradiol. Future research should present females with both male and female foreign and local songs in our sympatric population to understand whether this response is more widespread among rufous-and-white wrens.

Chapter 3 provides many interesting avenues for future research. For example, there is much to be learned about how animals use acoustic and visual signals in habitats with variable vegetation density. Similar playback experiments to other closely

related wrens and other species with differing degrees of conspicuousness, in terms of both plumage and song, could be conducted to compare with my results. I would expect more brightly coloured birds and birds with quieter songs or birds that sing infrequently to rely more on visual signals. Additionally, to conclusively demonstrate a social dominance relationship between rufous-and-white and banded wrens, it would be helpful to assess the quality of shared resources in each species' habitat to assess if rufous-and-white wrens are actively excluding banded wrens from higher quality habitats. A long-term removal experiment, where rufous-and-white wrens are removed from areas where they live in sympatry with banded wrens, could also be conducted to assess whether banded wren fitness increases in the absence of rufous-and-white wrens. These avenues for future research would allow us to obtain more information about the evolution of species discrimination mechanisms and signals while also providing insight into the competitive relationship between these two tropical wren species and how animals diverge in species discrimination signals.

In conclusion, my research provides insight into how previous experience affects species discrimination in tropical bird species and how inconspicuous wrens living in dense tropical forests use acoustic and visual signals. My research suggests that habitat vegetation, behaviour, and visual and acoustic conspicuousness have shaped how these species communicate and which signals they rely on based on which ones best transmit through their environment. My research also provides insight into how two species that use a similar resource to discriminate between one another to avoid costly fights. My research provides a basis for future research investigating the evolution of species

discrimination signals and understanding how closely related species compete and may

be affected by changes in their distributions due to changing environmental conditions.

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