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Complex singing behavior of the White-breasted Wood Wren (Henicorhina leucosticta)

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by

Zachary Thomas Harlow

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ABSTRACT OF THE DISSERTATION

Complex singing behavior of the White-breasted Wood Wren (*Henicorhina leucosticta*)

by

Zachary Thomas Harlow

Doctor of Philosophy in Biology

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Professor Charles E. Taylor, Chair

Neotropical bird species provide exceptional opportunities for advancing the understanding of avian communication systems. This dissertation focuses on the singing behavior of the White-breasted Wood Wren (*Henicorhina leucosticta*), which is not well understood even though it expresses behaviors such as female song and duetting that are at the forefront of research in avian communication. I present the background in this area of research and make the case that this species is of special interest to the study of avian communication because of its phylogenetic relationship to other duetting wrens and because its singing style is not easily categorized by singing behavior of other duetting species. In Chapter 2, I characterize the repertoire and singing behavior of this species and show that male repertoires are larger than those of females and can be classified into three groups based on spectral characteristics and song use. I describe unique high-frequency ‘introductory notes’ that directly precede songs within a bout but are facultatively included with the songs. In Chapter 3, I discuss playback experiments that suggest introductory notes act as a graded signal in certain song types during countersinging interactions. Differences in threat salience between four common song types was identified with one song type in particular that provoked lower song rates from subjects and was never song matched. These results suggest a separate function of this song type and together emphasize the potential role of structural cues in regulating singing interactions. In Chapter 4, I assess potential functions of duetting with two-speaker playback experiments and utilize a microphone sensor array developed at UCLA to localize the vocalizations of individuals. I show that in opposition to the mate-guarding hypothesis, the threat of unmated same sex intruders (solo playback) was not greater than the threat posed by paired intruders (duet playback). This supports territorial defense as the primary function of duets in this species. This research helps fill a taxonomic gap in our understanding of the singing behavior of tropical birds and suggests future directions of research for a better understanding of avian communication.

The dissertation of Zachary T. Harlow is approved.

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CHAPTER 1

Introduction

The White-breasted Wood Wren (*Henicorhina leucosticta*) provides exceptional opportunities for examining singing behavior and communication yet remains relatively unstudied. Here I review some of the characteristics that make this species a valuable study organism and introduce questions that I address in the following chapters. The White-breasted Wood Wren is a Neotropical passerine that maintains year-round territories and pair bonds in contrast to many temperate passerines. It is locally abundant with conspicuous vocalizations from both male and female birds. Both sexes sing solo songs and also combine their vocalizations to produce loosely coordinated duets. This species is phylogenetically basal to nearly all other duetting Wrens and is therefore integral to understanding song evolution in this group.

1.1 Differences between the avian biology of temperate and tropical systems

The field of avian behavioral ecology is dominated by studies of temperate bird systems that have produced a vast body of work and insights (Stutchbury and Morton 2008). However, tropical species provide strong counterpoints to many of the long-held paradigms of avian ecology and give context to the conditions underlying the evolution and diversity of many life history traits. For example, predation rates are typically higher in tropical regions and the environment is more climatically stable, leading to lower resource limitations and fewer constraints on the length of the breeding season (Cody 1966, Morton 1996, Russell 2000).

The basal metabolic rate of tropical birds is typically lower than that of temperate birds resulting in reduced overall energy expenditure (Wikelski et al. 2003). Tieleman et al. (2006) found that the field metabolic rates of House Wrens (*Troglodtes aedon*) were about 35% lower in the tropics than in temperate systems and showed comparatively little variation during the course of annual seasons. Life history characteristics associated with a low metabolic rate include smaller clutches, slower growth rates of hatchlings and longer-lived adults (Skutch 1949; Martin et al. 2001; Wikelski et al. 2003).

Smaller clutch sizes may also indicate that birds are sacrificing a higher annual fecundity for greater adult survival (Martin 1995; Bennett and Owens 2002). The neotropical Buff-breasted Wren (*Cantorchilus leucotis*) and north temperate Carolina Wrens (*Thryothorus ludovicianus*) have similar clutch sizes and incubation times but show the same tradeoff;

Buff-breasted Wrens invest more effort per nestling, which may be related to higher first-year survival, whereas Carolina Wrens raise multiple broods a year, which could account for the higher annual fecundity rates found in this species (Gill and Haggerty 2012).

Year round territories and pair bonds are common in the tropics, accounting for 60% of the passerines surveyed in Panama (Morton 1980). Many of these "stable" species have fewer extra-pair paternities and a greater overlap in sex roles (Morton 1996). Plumage is typically monomorphic between the sexes and the males of many species participate in nest building, the incubation of eggs, and the feeding of young in a similar capacity to female parental care (Skutch 1969).

The females of many species also sing in the tropics, a behavior that is the exception in temperate regions (Riebel 2003; Slater and Mann 2004). Female song in tropical species is a major distinction between temperate and tropical regions as also is monomorphic coloration and similar plumage patterning between sexes in many tropical birds (Morton 1996).

1.2 Vocal Behavior in Tropical Ecosystems

Animal communication mediates many important conspecific interactions such as mate choice and territorial defense. Avian vocal communication is highly developed and is strikingly different between temperate and tropical regions. While uncommon in temperate systems, female song is prevalent in the tropics and is especially common among species that maintain year round territories and pair bonds (Morton 1996; Slater and Mann 2004). The prevalence of female song in tropical systems is likely a product of the life history characteristics previously discussed that distinguish temperate and tropical regions that may have evolved through competition for mates or resources (Langmore 1998).

Many tropical species sing during both breeding and non-breeding periods in contrast to northern temperate zone species where singing is largely restricted to the breeding season, even in birds that are year-round residents (Morton 1996, Catchpole and Slater 1995). However, singing intensity between the sexes may fluctuate with respect to the breeding season in tropical birds. For example, female Rufous-and-White Wrens (*Thryophilus rufalbus*) sing and duet more often during the pre-breeding season while males sing and duet most often during the onset of female fertility (Todt and Naguib 2000; Topp & Mennill 2008).

Repertoire sizes differ dramatically within tropical Wren species from ~8 song types in the Rufous-and-White Wren (*Thryophilus rufalbus*, Mennill 2006) to 32 for the Carolina Wren (*Thyrothorus ludovicianus*, Morton 1987; see Mennill 2006 for list of studied species). The delivery of song types is also variable among the Wrens in that duetting birds are repeat-mode singers, delivering the same song type repeatedly with eventual variety, while nonduetting species are serial-mode singers, alternating song types with immediate variety (Mennill 2006).

An intuitively appealing explanation for the occurrence of large song repertoires in birds is that females prefer males with more song types. However, a repertoire-size effect on male reproductive success is not well supported in the literature and is inconsistent with the findings that there are also many tropical species with small repertories and with the common observation that many species lose song types when developing from juveniles to adults (Byers and Kroodsma 2009). The investment of time, effort, and neuronal resources required of a female to accurately assess differences in repertoire size may be too great to make the selection of males based on repertoire size feasible (Botero et al. 2008). Pattern- and time-specific responses may therefore have a greater influence on repertoire evolution than the number of song types alone. For a communication system in which multiple signals are used, the complex interplay between signalers and receivers could promote signal diversity without selecting for larger song repertoires (Byers and Kroodsma 2009).

Additional ways to generate song complexity without simply increasing the number of song types is through song type matching, repertoire sharing and duetting. Song type matching (replying to a rival with the same song type being sung) is a common component of communication within songbird species and an area in which selection for species-specific repertoire could act (Searcy and Beecher 2009). A number of studies have shown that birds that match another male's song type while countersinging produce a more threatening signal than when they sing a nonmatching song (reviewed by Todt and Naguib 2000).

Repertoire sharing (replying to a rival with a shared song other than the type being sung) is well characterized in four Wren species: Bay Wrens *Cantorchilus nigricapillus* (Levin 1996), Banded Wrens (*Cantorchilus pleurostictus*, Morton 1987), Carolina Wrens (*Thyrothorus ludovicianus*, Molles and Vehrencamp 1999), and Rufous-and-White Wrens (*Thryophilus rufalbus,* Mennill 2006). In three of these species in which it was characterized (all but the Bay Wrens), both sexes share more song types with nearby individuals than distant individuals.

Duetting behavior is the most complex form of vocal communication known in birds, consisting of coordinated song between both members of the pair. Defined broadly, duets may consist of overlapping bouts of vocalization between paired individuals singing in loose association with each other or displaying extended, tightly coordinated antiphonal bouts of song. Success of this complex vocal communication is evidenced by a minimum 220 bird species from at least 44 families known to duet (Farabaugh, 1982). The broad phylogenetic distribution of duetting suggests that there is strong selection pressure for this type of vocal communication independent of common ancestry (von Helversen 1980).

Which species duet and the function it serves between species likely depends on complex interactions between life history characteristics. Important factors may include the breeding system, dispersal distance, territoriality, breeding synchrony, mate selection, sex roles, and extra pair fertilization rate of a species (Hall 2004; Stutchbury and Morton 2001). However, additional insight comes from studying the interactions within a duetting pair. Behavioral experiments can show that duetting is a cooperative behavior or underlies a conflict between the sexes (Hall 2004).

Understanding the root functions of duetting in birds has value to diverse fields of science including behavioral neurobiology and endocrinology, interactive communication, cooperation and conflict between the sexes, as well as the neural basis of song production and perception (Margoliash 1997). Despite the impact duetting promises to understanding multiple aspects of animal communication, progress in this field has been slow in part due to the strong focus on northern temperate regions where female song and duetting are rare (Langmore 1998; Stutchbury and Morton 2001). Another obstacle has been that, historically duetting was regarded as a universally cooperative behavior without considering potential conflicts between the sexes (Stutchbury and Morton 2001).

In many duetting species, males and females combine specific song types from their repertoires to form non-random associations between song types called ‘duet types’ (see Logue 2006 for a list of species). In some species such as the Black-bellied wren (*Pheugopedius fasciatoventris*) non-random associations may result from a ‘duet code’ in which one sex responds to their partner’s songs based on a set of rules linking response songs to stimulus songs (Logue 2006). Non-random song type associations have also been identified in the Plain Wren (Mann et al. 2003), Rufous-and-White Wren (Mennill and Vehrencamp, 2005) and the Bay Wren (Levin 1996).

1.3 Study Species

1.3.1 The White-Breasted Wood Wren

The White-breasted Wood Wren (*Henicorhina leucosticta;* Cabanis, 1847) is a small non-migratory songbird in the family Troglodytidae, and is a common occupant of the lower understory of mature, lowland humid forest and edge habitat from Southern Mexico to Amazonia (Clements & Shany 2001). This species spends most of its time on or near the ground, where it feeds on insects and other arthropods in ground litter and fallen leaves trapped on understory vegetation. The sexes are monomorphic in plumage and both sexes contribute to nest building and feeding their young. They construct two types of nests: breeding nests and dormitory nests, which differ significantly in construction and location (Skutch 1960; Stiles and Skutch 1989). Females produce two eggs in a clutch and use breeding nests to incubate eggs and fledge young for a period of weeks between February and May. Breeding nests are located within 25 cm of the ground, sturdily constructed and highly cryptic (Skutch 1960). Dormitory nests are thin in comparison and located in lianas and thin saplings at a height of 0.3 – 3m and used year–round, with as many as four located within the same territory (Skutch 1960). The sexes rarely cohabit the same nest; however, fledglings sleep with the mother in dormitory nests while they are still dependent. Pairs occupy and defend territories year–round.

1.3.2 Taxonomic status of H. leucosticta

The *Henicorhina* Wood wrens stratify into four species by elevation. *H. leucosticta* is a lowland wood Wren. In areas of overlap *H. leucosticta* is at the lowest elevation and is replaced by the Grey-breasted Wood Wren (*H. leucophrys*) above 1300m and it is replaced by the Bar-winged Wood Wren (*H. leucoptera*) at ~1800 m (Dingle et al. 2006). Additionally, the Munchique Wood Wren (*Henicorhina negreti*) replaces *H. leucophrys* above ~2200 m across a restricted portion of the Cordillara Occidental in western Colombia (Salaman et al. 2003). *H. leucosticta* exhibits considerable vocal and behavioral variation across its range, over which historical taxonomies have split the taxon further into several species and even genera (Skutch 1960). More recent phylogenetic evaluation substantiates this distinction, revealing that *H. leucoptera* is paraphyletic with respect to *H. leucosticta* and contains considerable geographically structured genetic variation warranting full species status for at least the Central American, Amazonian, and Chocó populations within the species' range (Dingle et al. 2006).

At a broad phylogenetic scale, *Henicorhina* wood wrens are nested within the species group formerly recognized as the *Thryothorus* genus (Figure 1; Barker 2004; Mann et al. 2006). '*Thryothorus*' is the most thoroughly studied group of birds in terms of female song and duetting because most of the ~27 species exhibit one or both of these behaviors (Morton 1996; Mann et al. 2009). The new taxonomy distributes the species into four genera (*Thryothorus*, *Pheugopedius*, *Thryophilus* and *Cantorchilus*) that have been accepted by the South American Classification Committee of the American

Ornithologists' Union (Remsen et al. 2010). Duet complexity and precision is generally similar within each of the new genera but differs between groups, suggesting evolutionary transitions in singing style (Mann et al. 2009). How the singing behavior of the *Henicorhina* wood wrens fits into this picture is unknown. By defining the singing behavior of *Henicorhina leucosticta* I hope to contribute to the understanding of evolutionary transitions in song evolution in Neotropical wren species.

1.3.3 Vocal Behavior of the White-breasted Wood Wren

The song of the White-breasted Wood Wren is loud and clear with rich whistles, usually of three or four notes. The vocal behavior of *H. leucosticta* is unstudied relative to that of closely related Wren species. Like most Wrens, *H. leucosticta* is a repeat-mode singer where one song type is repeated many times and eventually changed to another song type. Bouts often begin with a series of four or five songs delivered in rapid succession and then continued at a slower rate for the remainder of the bout.

Like most other tropical Wrens, both males and females sing. They sing most often from a concealed position near the ground but will sing from perches up to 4 m when stimulated by playback of conspecific song. The form of the song is quite variable across the species' geographical range. The female song is generally higher in frequency than the male song, and contains 4 – 5 notes vs. 3 – 4 notes in males. An unusual feature of *H. leucosticta's* song (at least in some populations) is that nearly all song types are paired with one of several different high-frequency introductory note types (4 – 7 kHz) that may be rapidly repeated up to six times before a song begins. Introductory notes are most often used during territorial interactions and rarely continue through the terminus of a bout. In Chapter 2 (Repertoire structure and sex-specific singing behavior in the White-breasted Wood Wren (*Henicorhina leucosticta*)) I describe repertoire composition, song use and song sharing from a population in southern Chiapas, MX.

The White-breasted Wood Wren is highly territorial and commonly maintains territory boundaries through countersinging contests. In Chapter 3 (Song type salience and the use of introductory notes in the White-breasted Wood-Wren (*Henicorhina leucosticta*)), I assess whether the song type of an intruder affects the outcome of a countersinging interaction and compare responses to songs with and without the addition of introductory notes.

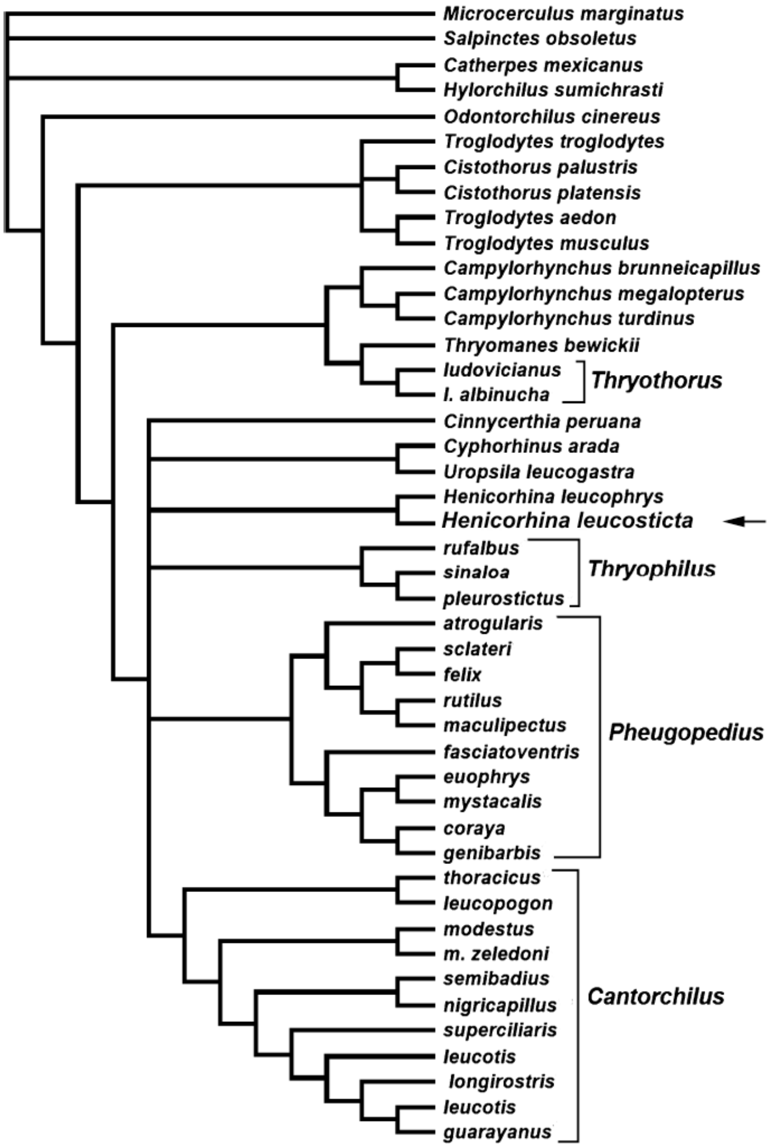
H. leucosticta is a duetting species, wherein both sexes sing in tandem to produce a coordinated singing display. The pairing of male and female song components is only loosely antiphonal with variable overlapping between male and female. In Chapter 4 (Functions of duetting in the White-breasted Wood Wren revealed by microphone-array recording and multispeaker playback), I assess the functional significance of duetting within a pair by utilizing a dual-speaker playback and a microphone sensor array to track the locations of responding pairs.

List of Figures

1. Phylogenetic framework (adapted from Mann et al. 2009) of the Wrens. Species groups highlighted in brackets were formerly of the *Thryothorus* genus and are labeled with the names of the new genera. The position of *Henicorhina leucosticta* is indicated with an arrow.

Figures

Figure 1



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CHAPTER 2

Repertoire structure and sex-specific singing behavior in the White-breasted Wood Wren (*Henicorhina leucosticta*)

The tropics harbor many avian species in which both males and females sing and the sexes combine their songs into vocal duets, unlike temperate regions, where duetting is rare. These species provide unique opportunities for examining intersexual communication yet they remain relatively understudied. Here I examine the singing behavior of the White-breasted Wood Wren (*Henicorhina leucosticta*) a Neotropical species that maintains year-round territories and pair bonds. Males and females sang similar songs but their repertoires did not overlap. Male songs were lower in frequency and contained an average of 3.0 notes versus 3.9 in females. Males had repertoires of about 20 song types which accounted for 96% of the total song output. Female repertoires were around 7 song types though this number may be conservative compared to a complete sampling of the repertoire. Within the study population, males and females both shared over 70% of their repertoire with neighbors and non-neighbors of the same sex. Females created 74.3% of duets by responding to male song and switching song types during a bout. This study highlights the sex-specific singing behavior of the White-breasted Wood Wren and establishes a baseline for further study to understand diverse singing styles of this group of tropical birds and the evolution of duetting.

2.1 Introduction

Vocal song systems are highly developed in birds and primarily serve to mitigate territorial interactions and communicate between the sexes. Most of what is known about birdsong comes from species in which only male birds sing, however female song is common, especially in tropical latitudes among non-migratory species that maintain year-round territories and long-term pair bonds. Mated pairs coordinate their singing efforts to produce duets in at least 220 species of birds (Farabaugh 1982). Intersexual differences in song use are not well studied but may hold important clues to evolution and function of bird communication. Here I describe the sex-specific singing behavior of the neotropical White-breasted Wood Wren (*Henicorhina leucosticta*) in order to better understand the evolution of female song and the complex behavior of duetting.

The Neotropical wrens represent an exceptional diversity of song systems, ranging from little or no female song in the Carolina wren (*Thryothorus ludovicianus; Brewer 2001)* to complex choruses in the Plain-tailed wrens (*Pheogopaedius euophrys*; Mann et al. 2006). The 27 species formerly composing the *Thryothorus* genus have received the greatest attention from researchers and have become a model system for studying the evolution of female song and duetting. However, recent taxonomic reshaping has placed these species into four separate genera and four additional related genera have been admitted into the clade (Chapter 1; Mann et al. 2003). One of these is the *Henicorhina* wood wrens. The White-breasted Wood Wren (*Henicorhina leucosticta*) is the most widespread and abundant of the wood wrens extending from southern Mexico through Columbia and Ecuador and eastward across much of Brazil. In spite of this, the singing behavior of the

White-breasted Wood Wren is not well understood (Morton 1996; Baker 1999; Mann et al. 2009). Historical disagreement about whether male and female singing should even be qualified as duetting in this species illustrates both the lack of information available for consensus as well as the relatively uncoordinated singing strategies of male and female White-breasted Wood Wrens. However, the loosely coordinated duets of this species offer a counterpoint to the highly syncopated antiphonal duets of species such as the Plain wren (*Cantorchilius modestus*) in which the precision of duets is a defining feature of their song.

There are three specific aims of this study: 1) describe and compare the sex-specific solo and duetting singing behavior of this species under natural field conditions; 2) quantify the song output, repertoire size and song sharing within a population of *H. leucosticta* as compared with other neotropical wren species; and 3) interpret these finding in context of the evolution of female song and the complex behavior of duetting.

2.2 Materials and Methods

2.2.1 Study Area

Fieldwork was conducted at the Estación Chajul, in the Montes Azules Biosphere Reserve, Chiapas, Mexico (16° 6′ °44"N and 90° 56′ 27"W) during the periods 7 – 28 May 2009, 16 January – 5 February 2010 and 12 May – 27 June 2010. The climate is hot and wet with temperatures ranging from 14°C to 38°C throughout the year and a mean annual temperature of 25°C (Garcia 1988). Rainfall ranges from 2500 to 3500 mm per year, with a summer maxima between June–September, preceded by a dry season starting in February (Mendoza & Dirzo 1999). The 331,000 ha Reserve, managed by Mexico City-based Natura Méxicana, accounts for most of the intact forest remaining within the 1.5 million hectares of the Selva Lacandona (Medellin 1994). The 50 ha study area reported on here is located on the northern bank of the Rio Lacantún. The primary vegetation cover is mature evergreen tropical forest, with patches of secondary forest resulting from tree falls and river flooding.

2.2.2 Territories

The spatial configuration of territories (Figure 1) was estimated by plotting sightings of both color-banded and unmarked birds onto a map of the study area. I assessed responses to playback of conspecific song along trails and recorded unprovoked singing opportunistically. A total of 40 individuals were captured in nylon mist-nets and each was uniquely banded with three divec color bands and one numbered aluminum band. Five individuals were banded prior to the May 2009 field season (June 2007 and December 2008), 19 were banded in May 2009, three in January 2010, and 13 in the May/June 2010 field season. Figure 1 represents the 30 banded individuals occupying territories during May and June 2010, from which most of the data for this chapter is derived. Adjacent territories were observed to share common borders; for example, a playback of male song from the point joining the territories of birds BRO, WWB, and KWO elicited close approaches and strong responses from all three males. Territory boundaries were thus drawn at the approximate midpoint between observed singing locations. Sightings were spatially calibrated to GPS referenced markers at 50 m intervals along trails (Trimble XH with external antennae, Trimble, Sunnyvale, CA) and referenced to a 0.8 m multispectral satellite image (IKONOS, April 2006, WGS84 UTM15N). The presence of large patches of unsuitable secondary habitat provided a “hard edge” for adjacent territories. Arroyo Jose provided another natural boundary to territory edges except for bird KWO, who was observed singing from the opposite edge of the stream on one occasion. Territory boundaries without a defined neighbor (adjacent white space on Figure 1) were based on a general estimate of the territory extent.

2.2.3 Field Recordings

I recorded songs from a total of 36 White-breasted Wood Wrens. Both males and females were color banded from 13 pairs with an additional 4 pairs where only the males were captured and banded. Vocal activities were recorded as WAV files at 48 kHz sample rate with a Marantz PMD661 digital recorder connected to a Sennheiser ME67/K6, with the time and identity of the bird noted in the recording. I define a bout in this species as a string of songs produced by the same individual that contains gaps between songs no longer than 20 seconds. An additional 48 hours of focal recording was gathered with 4-microphone autonomous recording units or “nodes” in 4 channel, 48 kHz sample rate, 16 bit .raw format (ENSBOX four channel recorders, Collier 2010). Node recordings were taken in 6-hour recording sessions; seven began at 0642 hr (SD 10.25 min). All eight node recordings were taken from separate locations over the course of five days between June 3, and June 22, 2010. On two days simultaneous recordings were made from two locations separated by approximately 50 meters to verify accuracy of the general range of sensitivity of the annotations and microphones respectively. On another day, two nodes were placed approximately 250m apart to compare song output in adjacent signaling neighborhoods.

2.2.4 Analysis of Solo Songs

For the purpose of this study, I define a song as a multiple–note vocalization delivered in a stereotypic manner and separated from individual specific vocalizations by greater than one second. Song types are structurally distinctive and can generally be easily discriminated by ear or comparing sound spectrograms (Figure 2). I annotated sound files with PRAAT acoustical software, by assigning an annotation tier to each singer on the recording and demarcating song intervals by start and stop times and labeling the song type identification. I then parsed textgrid annotation files with python scripts into excel spreadsheets to assess the structure of songs within bouts. I recorded the number of songs in a bout and the bout length. I also calculated the coefficient of variation in song duration and the start times of consecutive songs.

Additionally, I isolated one representative song type and spectrogram from each bout (node and hand-held recordings) to a separate folder organized by song types. For the four most common song types, I calculated maximum and minimum frequency used within a song and frequency bandwidth (F_{max}, F_{min}, and F_{band}) as well as the duration of songs (SongDur) and the number of notes (#Notes) within songs.

2.2.5 Song Output

I calculated song output as the number of songs from an individual per hour from 8 focal territories (Figure 1) recorded for 6 hours each. Birds recorded each day are as follows (male/female): UOU/RYGy (May 3; Red triangle), KWO/YYB and PKG/WYG (May 7; Orange triangles), KKG (May 10; Yellow circles), PKG/WYG and PPP/GyWY (May 20; Blue square), and BRO/GWK and WWB/RRG (May 22; Green diamonds). Song output was tabulated for each hour between 0700 h and 1200 h. To quantify song delivery rate, I tabulated the start times of all songs sung during the six-hour recording session for all focal birds present on the recording.

Songs were matched to individual singers by first assessing the general location of the singer relative to the territories of known birds and later annotated the node recordings in PRAAT by defining the beginning and end of the song and attributing the singer and song type to it. If the identity of the singer was ambiguous, the songs were compared to recorded examples from the potential singers. I then reviewed the annotations with associated files and evaluated any ambiguous calls. The sound and the fine structural characteristics of the spectrogram were sufficient in most cases for determining the singer. Cases in which territories were recorded from two locations simultaneously were also annotated separately and cross-referenced to roughly assess the detection sensitivity and accuracy of annotation labels. This method revealed a small number of conflicting identifications between annotations; however, nearly all of these vocalizations originated outside of the focal territory and did not substantially affect the data reported here.

2.2.6 Repertoire Size

I calculated repertoire size by summing the number of distinct song types represented from the recordings of each bird. For 5 males and 5 females, I tested the completeness of repertoire sampling by plotting the cumulative number of new song types against the cumulative number of bouts recorded to assess the completeness of repertoire sampling. The number of bouts recorded is a better metric for song type accumulation in this species than the number of songs recorded because consecutive songs within a bout are not independent in repeat-mode singers in which song types are often repeated many times before switching to another song type.

2.2.7 Song Sharing

Song sharing between neighbors is common in song repertoire species, and repertoire similarity typically decreases with the distance between territories (e.g. Brown et al. 1988; Hill et al. 1999; Beecher and Campbell 2005). I identified shared song types based on a visual assessment of similar frequency-temporal structural characteristics of the spectrogram.

Repertoire sharing was calculated between seven banded male White-breasted Wood Wrens each of which shared at least 13 songs with other individuals. Levels of song type sharing were calculated for each pairwise combination of males with the song type sharing coefficient (S = 2 * number of song types shared between two individuals / sum of the repertoire sizes of those individuals) to assess the amount of sharing within the population (McGregor and Krebs 1982; Molles and Vehrencamp 1999). To account for differences in repertoire sizes, I also calculated the corrected song type sharing coefficient as:

$$S_j\ (adjusted) = c\ /\ ((\ a + b + c\ ) - d)$$

where for two birds A and B, *c* is the number of syllables common to both birds' repertoires; *a* is the number of songs unique to A's repertoire; *b* is the number of songs unique to B's repertoire; and *d* is the difference in repertoire size between A and B. S_j was expected to be robust to inter-individual differences in repertoire size and sample size (Tracy and Baker 1999; MacDougall-Shackleton et al. 2009). For each male, I calculated the average S_j with all other subjects as an index of song sharing with the local population.

Song sharing was calculated at three levels: between neighbors, between non-neighbors, and between all individuals in the study population. Additionally, S_j was calculated for each individual relative to pooled sample of 16 song types recorded from individuals approximately 1 km outside the core study as a coarse representation of song repertoire sharing at this level of geographic separation.

2.2.8 Duetting

I define duets as temporally overlapping song bouts produced by a paired male and female in which the gap between songs from either sex does not exceed 10 seconds. Several aspects of duetting were considered. The duetting output (bouts of duetting per hour) and duetting proportion (number of duets to total song bouts) were calculated from the 8, 6-hr recordings. Female song was too rare on these recordings using the nodes to make broader assessments of the behavior, so additional recordings were included from handheld recordings made from the 12 May – 27 June 2010 field season as the periods 7 – 28 May 2009 and 16 January – 5 February 2010. 76 of the 120 additional duets were recorded within 10 minutes of a playback stimulus. The distribution of song types resulting from playback was not significantly different from duets recorded spontaneously so they were pooled for further analysis (Chi-squared = 21.3935, df = 20, p-value = 0.3743 males; 9.6452, df = 11, p-value = 0.5626 females). For each sex, I calculated the proportion of song output containing duets as the number of bouts containing a duet divided by the total number of bouts (solos + duets) from males and females. The creation of a duet was attributed to the sex that sang second, i.e. the bird that produced a duet by adding their contribution to a solo bout already in progress. I also assessed the amount of song switching in bouts relative to solo singing.

For each sex, I assessed the timing and coordination of solo songs and duets with the coefficient of variation (CV = SD/mean × 100) between the start times of consecutive songs. The CV was calculated within individuals and also between the male and female components of a bout. Thirty duet bouts and 53 solo bouts were analyzed for precision from four pairs.

To assess if males and females contribute song types to duets non-randomly, I compared the frequency distribution of song types between duet types and the total occurrences of song types from all of the recordings with a Chi-squared test using a simulated p-value based on 10,000 replicates. In addition, I plotted the observed and expected duet combinations from the population as a whole as a visual assessment of the presence of duet types. The null expectation of song type combinations was calculated as the cross product of male and female song type counts from the whole dataset scaled to the sample size of observed duets.

2.3 Results

I identified 535 bouts from node recordings for a total of 1278 bouts analyzed overall. The number of bouts recorded for each individual averaged 44.48 ± 53.9 bouts for males and 11.00 ± 9.93 bouts for females. Few general patterns fully characterized the singing behavior of the wood wren. Bout lengths varied from just a few songs to several hundred songs and may be delivered in precise measure or at nearly arbitrary intervals. The song rate within a bout also varied. Certain bouts commenced with a series of songs (mean ± SD: 5.25 ± 1.38 songs) that were delivered more quickly and consistently than the following songs in the bout (Figure 3; mean ± SD seconds; Fast: 1.21 ± 0.28; Normal: 4.37 ± 1.52). Females sang longer strings of quickly repeating songs than males and sometimes delivered songs in a “clumped” pattern, singing groups of two or three songs separated by gaps of several seconds.

2.3.1 Repertoire

Male and female White-breasted Wood Wrens sang non-overlapping repertoires of short repeated songs rarely exceeding 2 s. I identified 40 male song types and 10 female song types among the 37 individuals recorded (Figure 2). Along with a fixed series of notes comprising the song type, males also possessed a repertoire of up to six different introductory note types. When sung, they directly preceded the fixed portion of the song type and consisted of a single modulated high-frequency note or a trill of up to six notes in rapid succession. The spectral characteristics of introductory notes were specific to one or more different song types and were largely consistent between individuals. Thirty of the 40 male song types were associated with introductory notes, and 8 of the 10 remaining song types contained introductory-note-like elements apparently fixed within the song type. These song elements did not vary in number and were always sung with the song type. They were also lower in frequency and differed in other spectral characteristics compared to variable introductory notes.

Female songs were higher in frequency (max frequency: mean ± SD = female 4.00 ± 0.25 kHz; male 2.60 ± 0.10; *t*-test: $t_{5.5} = 5.20$, $P = < 0.003$) and contained more notes than male songs (mean ± SD = female 3.9 ± 0.74 notes; male 2.98 ± 0.28 notes; *t*-test: $t_{9.8} = 4.87$, $P = < 0.001$). Most male song types were composed of three notes between 1.5

and 3 kHz; however, several appeared to incorporate modified introductory notes into the set portion of the song type. The lengths of male song types were quite consistent, ranging from (mean ± SD) 1.109 ± 0.108 s to 1.307 ± 0.095 s. Song type st11 was unique in the repertoire in that it had a higher mean frequency (~3.8kHz), a larger frequency range (from 2.5-5kHz), and the shortest duration (~0.75 s). Small variations in the delivery of st11 between individuals suggest that it was composed of three continuously descending notes delivered without spaces between them. It was also the only common song type (rank order 6) that was never associated with introductory notes and was rarely, if ever, type matched. Potential differences in the function of st11 compared to other song types are discussed in Chapter 3.

The frequency distribution of song types was highly skewed by rank order in males with the three most common song types accounting for the majority of observations (Figure 4). Females have a less skewed rank order and three or more observations of every song type (Figure 4). Males have repertoires of about 21 songs (Figure 5). The addition of new songs to the repertoire diminished substantially after about 100 sampled bouts (~1,250 songs) suggesting that the repertoire was nearly complete for these four males.

A total of 10 female song types were identified within this population. The slower accumulation of song types in females suggests that female repertoires are substantially smaller than male repertoires; however, the low song output of females precluded the complete sampling of any female repertoires.

2.3.2 Singing activity

Song output was highly variable between individuals and sexes. Males sang dramatically more songs per hour than females: 96% of songs came from males. Males have a peak of singing early in the morning (Figure 6a). In contrast, female song was sporadic and three birds were not recorded at all during the morning (Figure 6b). Ninety one percent of female song was given from only two females (GyWY, WYG) involved in duetting with their partner and countersinging between each other. This was characteristic of female singing behavior recorded outside of focal node recordings. Most female song was associated with singing from other birds, either in response to their partner's song or to singing neighbors or playback. Males usually answered extended bouts of female singing to form duets, resulting in shorter female solo bouts than bouts that included duetting.

The six focal recording localities, each with two six-hour recordings, yielded a total of 375 bouts from among six focal males and 26 bouts from four of six focal females. Bout lengths ranged from 2 songs to 150 songs (mean ± SD = 27 ± 27.45 songs). The distribution of bout length was non-normal with a small number of bouts that contained many songs. In the two cases for which two microphones were used, microphone sensitivity was sufficient to detect the focal singers in both recordings. For the May 7th 2010 recording (Figure 1; orange triangles) some songs from bird KWO were faint in the south node recording and slightly underrepresented in the annotation while the opposite was true for bird PKG in the north node location. Songs from bird OWG and PPP were present on only the north and south speaker respectively. For the May 10th recording

(Figure 1; yellow circles) songs from male bird UNB5 were virtually undetectable from the west node recording and songs from bird BRO in the east node recording; however, all songs from bird KKG were detectable from both locations. The node recordings thus represent an approximate recording radius of 100 m.

The use of introductory notes differed by context. Introductory notes tended to be of higher frequency and thus attenuated faster in the rain forest environment. A number of songs recorded on the nodes were distant enough that the presence of introductory notes could not be verified and thus prevented an accurate assessment from node recordings. However, hand-held recordings from 2010 suggest some context to introductory note use. Of 242 recordings that were not in response to playback, 111 (46%) contained intro notes. Playback increased the use of introductory notes to 74% (n = 31 responses; two-proportion z-test: Z = -2.78, $P = 0.005$). Introductory notes were most common during the dawn chorus, occurring in 70% of bouts recorded before 0715 (n = 40). Introductory notes were usually limited to the first portion of the bout but were occasionally maintained throughout. Solo bouts containing introductory notes often appeared to be responses to songs from a mate or neighbor.

2.3.3 Song Sharing

All males shared at least 13 song types with other birds in the population (average 15.3 song types; $S_{adj} = 0.719 \pm 0.115$). Song sharing was not significantly different between neighbors and non-neighbors over the relatively short distances separating territories

(Wilcoxon signed-rank test; W = 51, P = 0.625) but sharing was greater within the study population relative to a sample of 16 song types recorded from approximately 1 km to the north (W = 147, P < 0.0001).

Females had higher levels of repertoire sharing than males (S_{adj} = 0.780 ± 0.125) but the trend only approached marginal significance (W = 271, P = 0.103). Neighbors and non-neighboring females were equally likely to share songs (W = 47.5, P = 0.4352); however, a low sample size of female recordings from outside the study area precluded an assessment of song sharing at larger spatial scales.

2.3.4 Duetting

White-breasted Wood Wrens sang loosely coordinate antiphonal duets that were created by either sex combining their song with that of a singing partner. Females created 74.3 percent of duets by responding to male song. Females sang more songs in association with duets than they did as solo songs; males sang more solo songs than songs associate with duets. As with the solo songs, the length of duet bouts was highly variable (mean ± SD = 49.1 ± 91.57 s).

The timing between male and female duet components varied from antiphonal duets that alternated with low variation (Figure 7a) to others that exhibited very little coordination (Figure 7b). Overall, the coefficient of variation in the timing between songs was high both between songs within a bout and between duetting partners (CV: male 40.16 ± 40.16; female 54.44 ± 27.73; between 68.02 ± 13.92); however, most duets contained at least some period of temporal coordination between singers. Completely antiphonal duets were rarely observed and only in shorter bouts.

Song switching, which involves a transition in song types within the same bout occurred in 26.9% of duet bouts and was equally likely to result from males switching songs as females (Males 13.1%; Females 13.8%). In cases in which both sexes switch songs, the song switches occur within several song exchanges of each other (mean ± SD = 14.70 ± 12.04 seconds). Females were the first to switch songs in 6 of 7 cases where both males and females switched songs, though this trend was not significant (binomial test: P = 0.125). The coordination of song switching was especially apparent in duets that included multiple song type switches from each sex.

Duets that succeeded a previous duet by less than 10 minutes were rarely the same duet type but often contained one of the song types present in the preceding duet and one or both birds usually changed song types between successive bouts. Females were more likely to initiate duets by combining their song to a males' solo song already in progress. When females were the first to respond, males often sang soon after them accounting for the rarity of female solos. Unmatched female solos were rare and usually shorter than the average duet.

Duet types were non-random with respect to song types present in the sample population as a whole (Figure 8a, b). A sufficient sample of duet types of most pairs was not achieved to accurately assess pair-specific duet types. Most duets were composed of common song types; however at the level of the study population, the composition of song types used in duets appeared to differ from singing as a whole (Chi-squared = 383.5625, df = 253, $P < 0.0001$).

2.4 Discussion

White-breasted wood wrens have relatively large repertoires that they use during solo singing or loosely coordinated duets. Pronounced differences exist between the singing behavior of males and females. Males have larger repertoires and sing more frequently than females. Songs in both sexes are short and stereotypic and delivered in repeat-mode with eventual variety.

Though not the highest observed, male repertoires of >20 song types in the White-breasted Wood Wren is relatively high compared to closely related wrens: >8 for Rufous-and-white Wrens (*Thryophilus rufalbus*; Mennill & Vehrencamp 2005), >15 for Bay Wrens (*Cantorchilus nigricapillus*; Levin 1996), >15 for Sinaloa Wrens (*Thryophilus sinaloa*; Brown and Lemon 1979), 20 for Banded Wrens (*Camplorhynchus zonatus*; Molles and Vehrencamp 1999), 29 for Happy Wrens (*Pheugopedius felix*; Brown and Lemon 1979; >25, Mann et al. 2003), and 32 for Carolina Wrens (*Thryothorus ludovicianus*; Morton 1987). Among the tropical wrens that have been studied, duetting species are all repeat-mode singers that sing with eventual variety (Happy Wrens, Brown and Lemon 1979; Buff-breasted Wrens, Gill et al. 2005; Rufous-and-white Wrens, Mennill & Vehrencamp 2005; Bay Wrens, Levin 1996; Plain Wrens, Mann et al. 2003), whereas both of the non-duetting tropical species are serial-mode singers that sing with immediate variety (Sinaloa Wren, Brown and Lemon 1979; Banded Wrens, Molles and Vehrencamp 1999). Repeat-mode singing may have been a pre-adaptation for the evolution of duetting in this group by allowing an individual to anticipate songs from its partner and precisely time its responses.

The coordination of song type delivery was highly variable in the White-Breasted Wood Wren, suggesting that precise timing is not a critical aspect of duetting, or that precision may be used as a graded threat against intruders. Precise duets were associated with high intensity responses to the songs of other birds or to playback. However, most female song occurs during higher intensity multi-way singing interactions, so further experimentation is required to establish a relationship.

The song output of females appears to be low in this species, and was generally limited to higher intensity interactions suggesting that they usually sing in response to a perceived threat and do not routinely signal their presence when unprovoked. Female Rufus-and-white Wrens sing about 10 songs an hour compared to less than one song per hour averaged across the continuous recordings in the current study on White-breasted Wood Wrens. Song output is similarly sex-biased in other wren species (Brown and Lemon, 1979; Mann et al. 2009). In Chapter 4, I discuss whether singing behavior in this species is driven primarily by cooperation or conflict between the sexes. I found that defending the mutual resources of the territory appears to be of greater importance to this species than defending mates from conspecifics.

Song type sharing among birds of the same sex was high in both males and females while no song types were shared between the sexes. Neighbors and non-neighbors shared similar portions of their repertoire suggesting that repertoire turnover occurs at slightly larger spatial scales than the area of this study site (~500 m^2). Only 3 of the 16 male song types recorded from birds 1km outside the core study area were not shared by birds in the core study area; the one female song recorded at this distance was not present in the study population. None of the song types most common at this study site were observed at a distance of 20km (n = 13), suggesting that the high repertoire overlap observed within the study population turns over at large spatial scales.

Song sharing in females may be higher than we observed in these data. Each of the 10 observed female song types was shared by at least three other females in the population, suggesting that incomplete sampling of female repertoires could be masking shared songs in the population. Unshared songs could also be masked; however, high female sharing is consistent with reports from Morton (Morton 1996; Stutchbury and Morton 2001) of unpublished data collected in Panama. There, females have small 'dialect' repertoires of 4 – 5 song types that are shared among all females in the population, while males have large repertoires of >30 song types very few of which were shared by adjacent males (Morton 1996). By this account, females in the Chiapas field site have larger repertoires (~10) and males have smaller repertoires (~20) with significantly more song sharing. Morton suggested that turnover in occupancy could be higher in males than in females, resulting in stable neighborhoods for females but not for males (Morton 1996). Such an asymmetry in territory tenure was not apparent in my population. Of the 13 birds banded in 2007 and 2008 only three males were still present May 2010, one who had three mates over that period. However, at least one female retained her territory and paired again following the loss of her mate. Three other pairs were completely replaced, and three males turned over territories, in pairs in which the females were unbanded. Thus, small differences may exist in territory turnover between sexes but it is not likely to be a strong driver of repertoire structure.

The duetting behavior of White-breasted Wood Wrens is unusual among tropical wrens studied for this behavior. Their singing style is not well categorized by the framework developed by Mann et al. (2009) to compare closely related species formerly assigned to the '*Thryothorus*' genus. Similar to *Thryophilus*, and *Pheugopedius*, females sing much less than males, duets contain variable overlaps, and duets are typically loosely coordinated. The song structure is quite different in *H. leucosticta*, with shorter songs composed of fewer notes and lacking the loud trill common among many members of the genus.

This study highlights the sex-specific singing behavior of the White-breasted Wood Wren to establish a baseline for the further study of this species and to facilitate the integration of *Henicorhina* song into the diverse singing styles present in closely related birds. Until recently, *H. leucosticta* was a missing member of the species group formerly recognized as the genus *Thryothorus* and the information presented here helps to fill a gap in this well studied lineage of tropical wrens, thereby opening the door to a greater understanding of the course that evolution has taken in forming duetting behaviors. I build on this foundation of singing behavior in the White-breasted Wood Wren to experimentally assess aspects of countersinging in chapters 3, as well as the function of duetting in this species in Chapter 4.

List of Figures

1. Estimated territories of banded and unbanded pairs (UNB1-UNB5) surrounding Estacion Chajul. Trails are marked at 50 m intervals with black circles. Territory holders are indicated by letter groups corresponding to the bird's color. Males are listed above females and unbanded females are indicated by the ♀ symbol. Territory boundaries are estimates; sides bordering other territories, secondary edges and Arroyo Jose are known with greater precision than borders next to undefined suitable habitat (white space; see text).

2. Spectrograms of the most commonly used male and female song types. The two top rows are male songs and the bottom row is female songs. The asterisk (*) denotes song type 11 discussed in the text.

3. Histogram of the intervals from the start of one song to the start of the next song. One hundred intervals are plotted from two individuals (KWO and PKG) from 10 bouts across 8 song types. The number of songs used from each bout included the total number of fast songs plus an equal number slow songs directly following the change in delivery rate.

4. The total number of times (bouts) in which a song type was recorded, ordered from most common to least common song types among all males (blue) and females (red) in the study.

5. Repertoire accumulation plots for four males (blues) and four females (reds) with the number of song types added to the repertoire on the y axis and number of bouts sampled on the x axis.

6. Variation in song output of White-breasted Wood Wrens in relation to time of day. (a) Males have a peak in song output at dawn, with consistent levels of singing between 9:00 and 12:00. (b) Female song is characterized by low song output and intermittent bouts of singing, most often in duets.

7. Two examples of duetting showing coordinated (a) and uncoordinated (b) duets. Note the male introductory note is emitted during the duet in b.

8. Some song type combinations (duet types) were more common than expected from song use overall (a) The number of duet types observed within the sample population out of all possible combinations of male and female song types. (b) The expected distribution of duet types if the probability of singing a song type in a duet was equal to the probability of singing a song type in any context (either during solo singing or duetting). Some frequently used song types were overrepresented in duets.

Figures

Figure 1

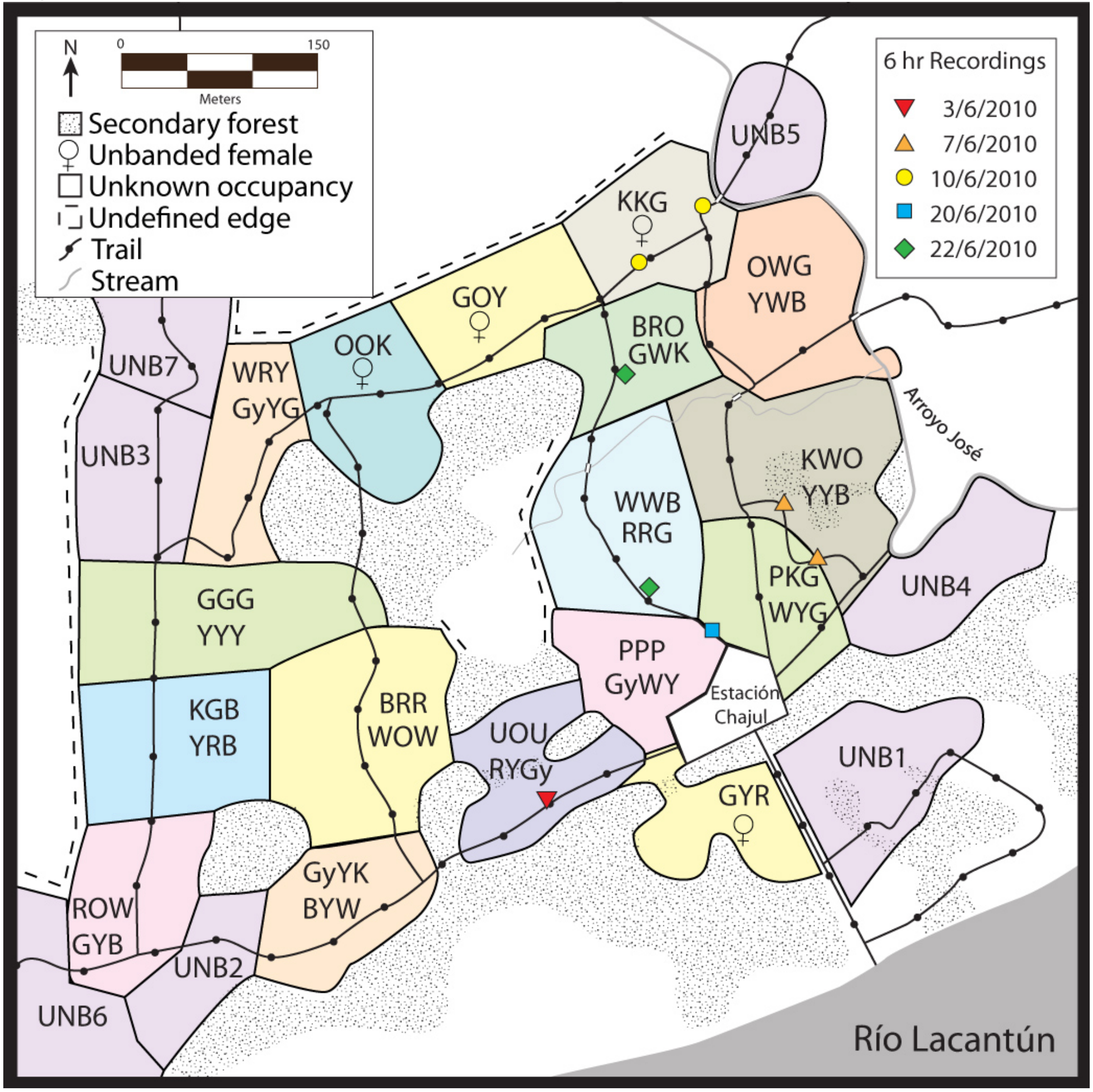


Figure 2

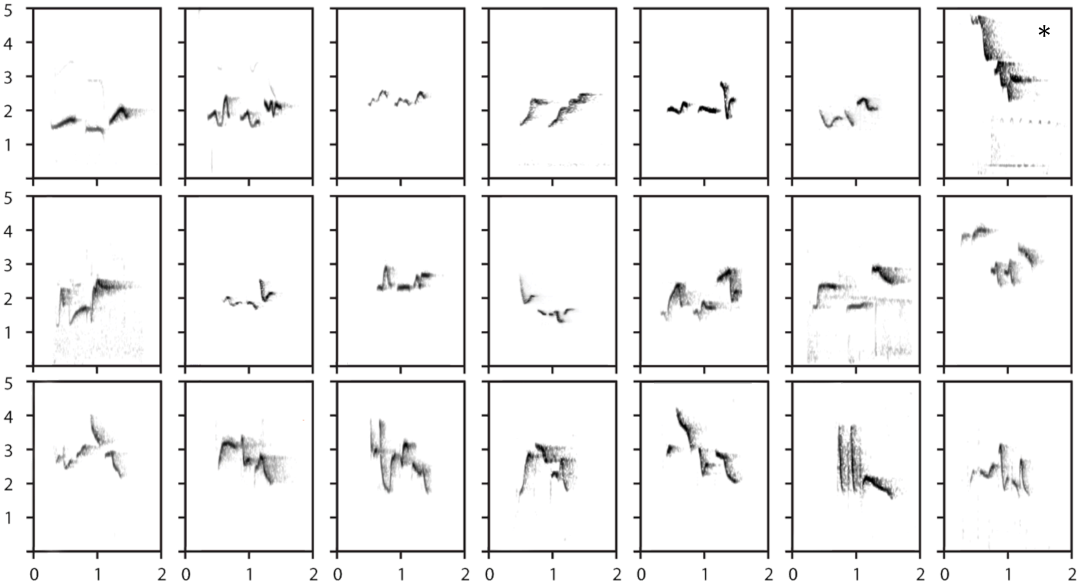


Figure 3

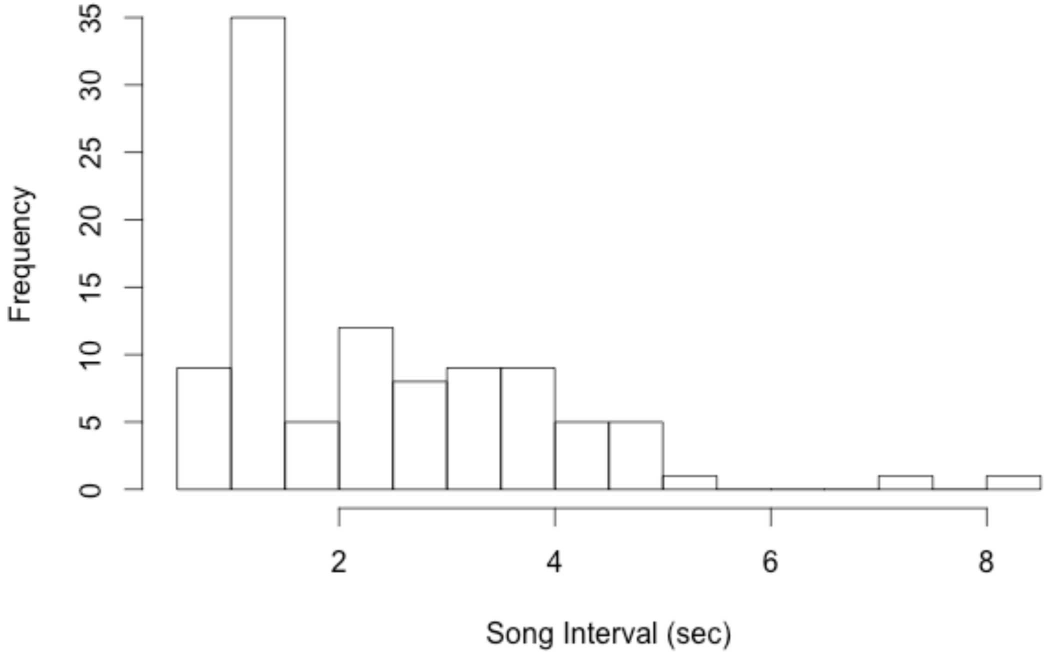


Figure 4

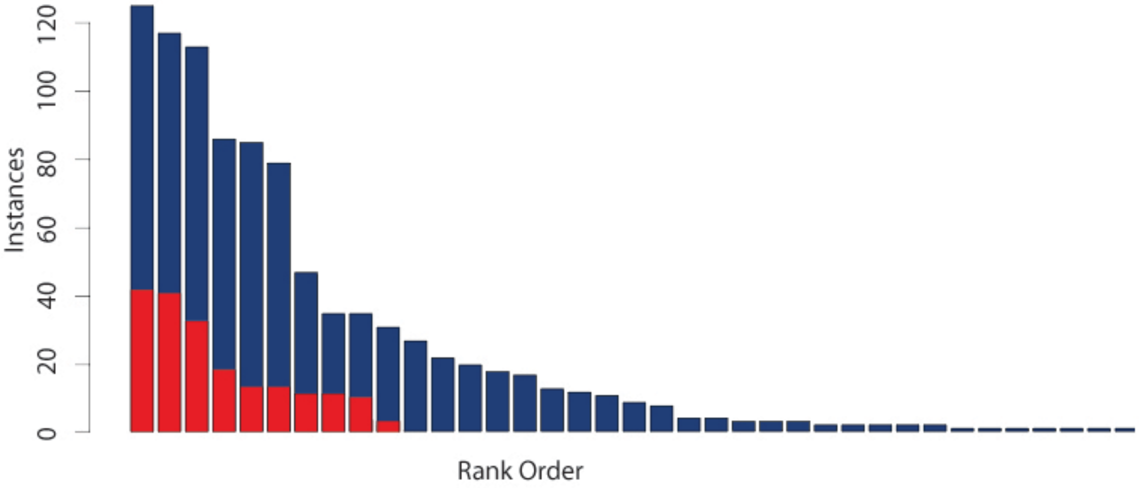


Figure 5

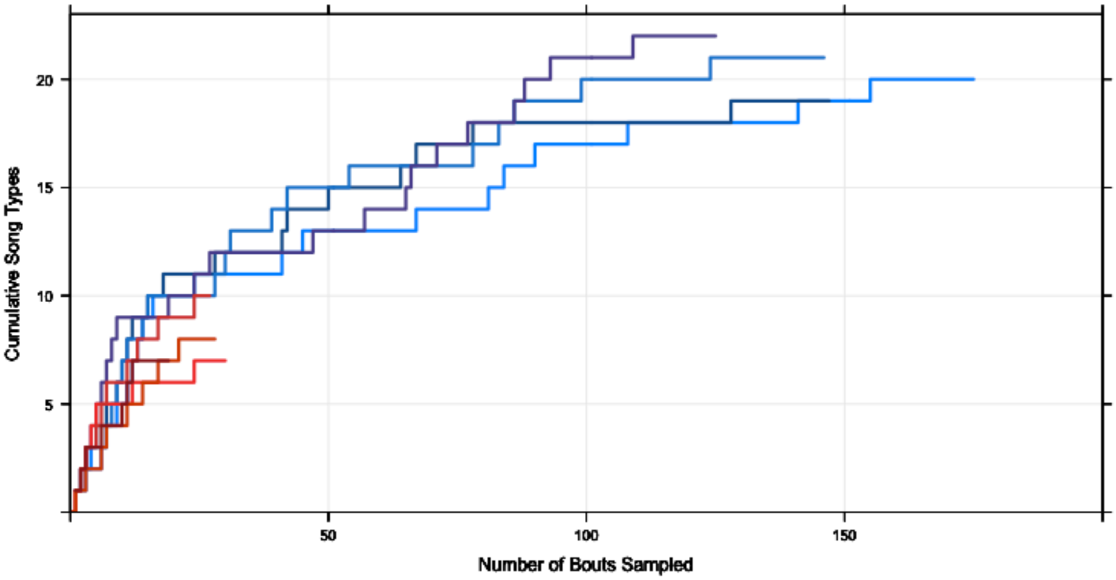


Figure 6

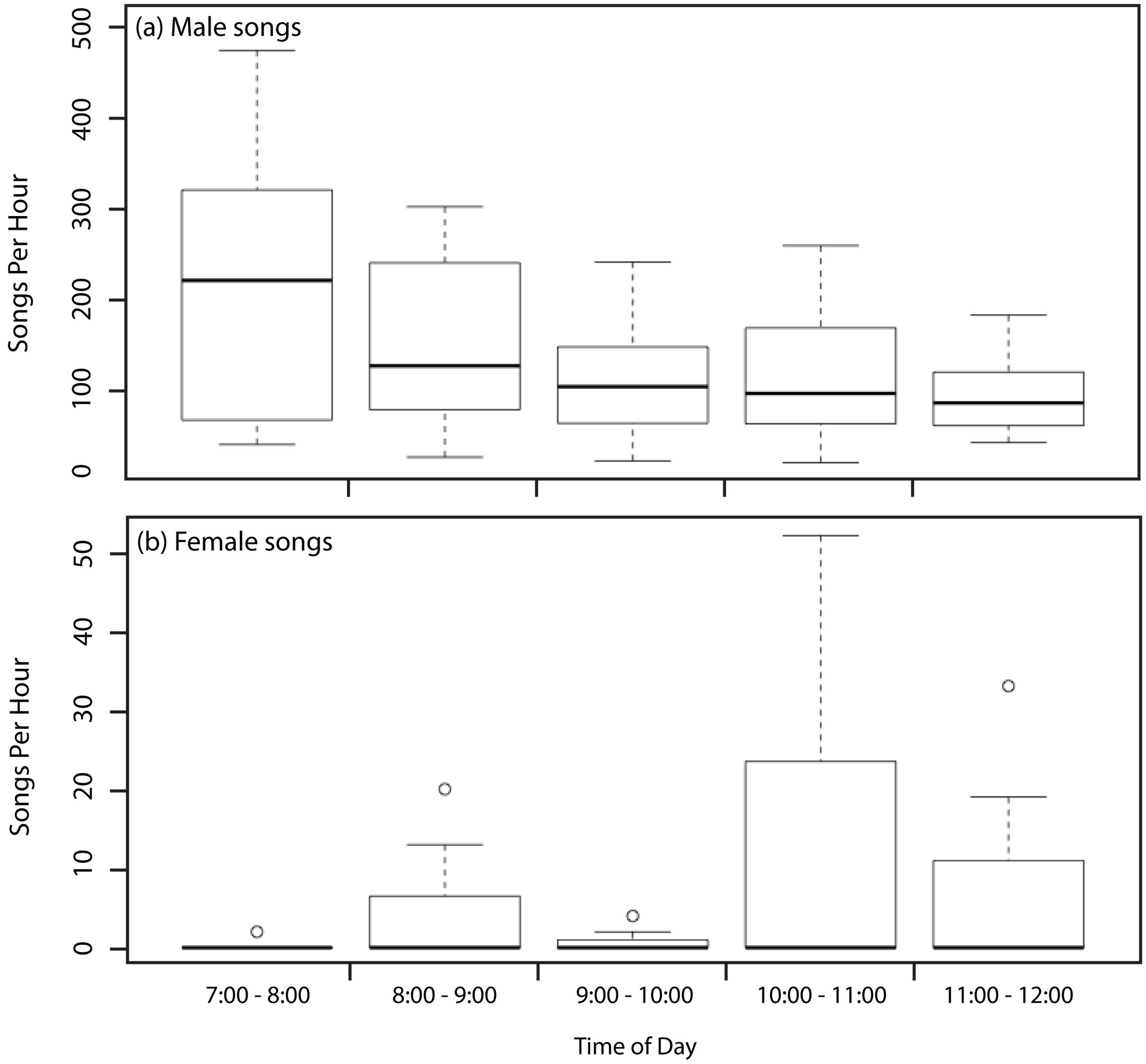

(a) Male songs
Songs Per Hour
0
100
200
300
400
500
(b) Female songs
Songs Per Hour
0
10
20
30
40
50
7:00 - 8:00
8:00 - 9:00
9:00 - 10:00
10:00 - 11:00
11:00 - 12:00
Time of Day

Figure 7

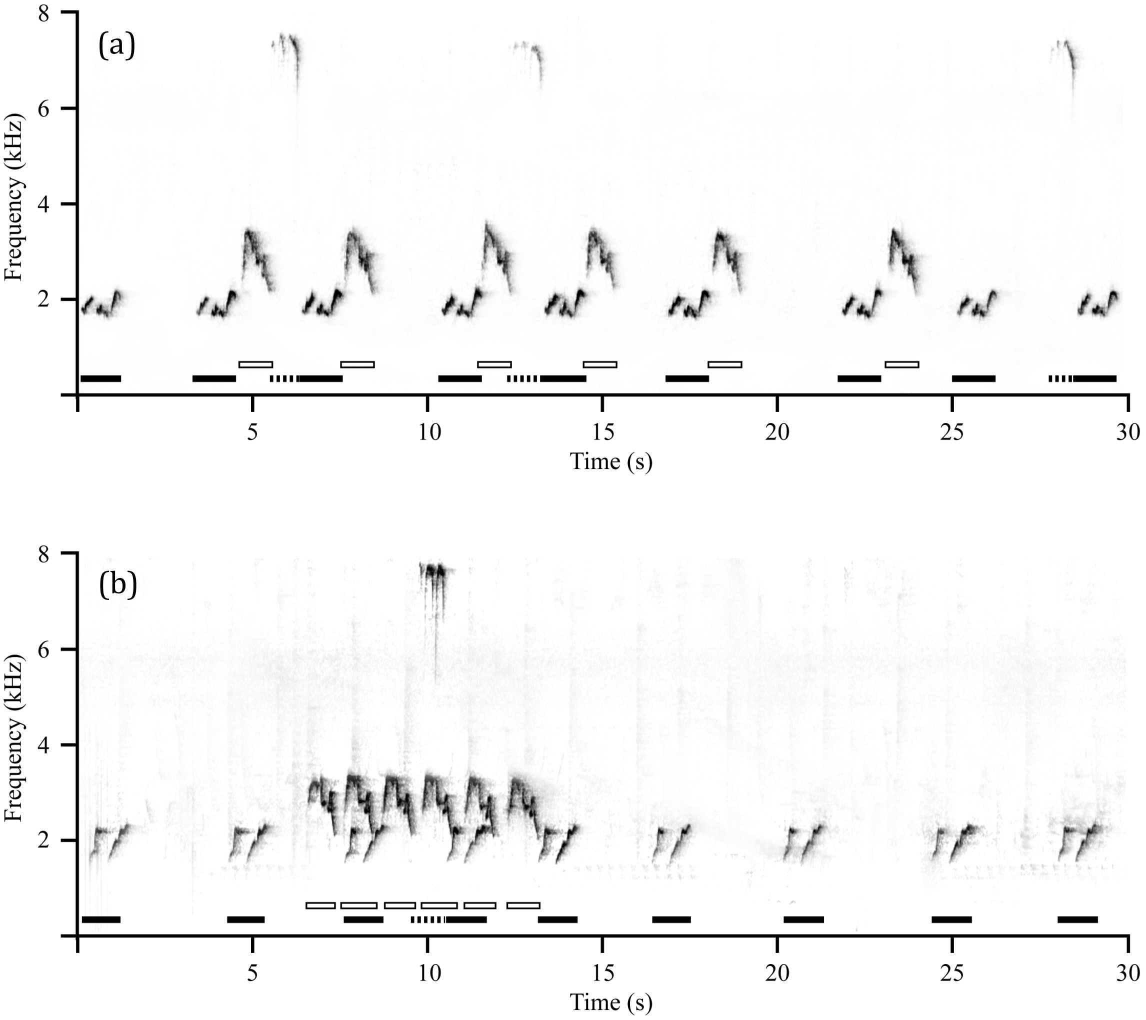
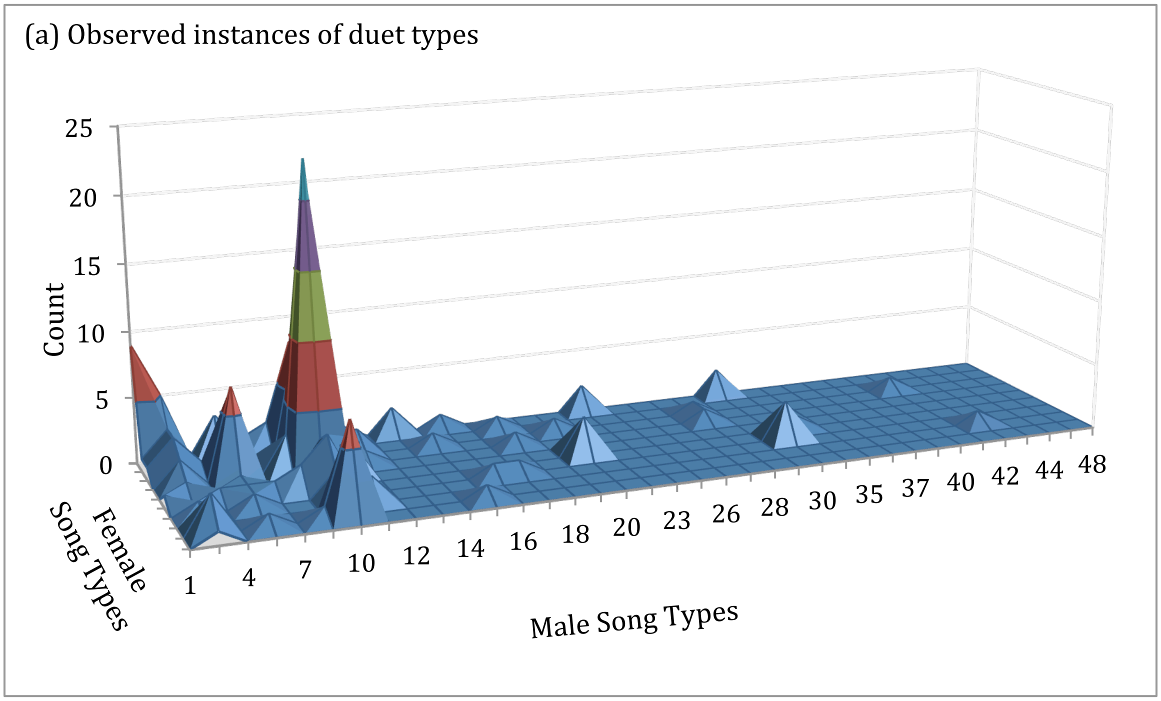
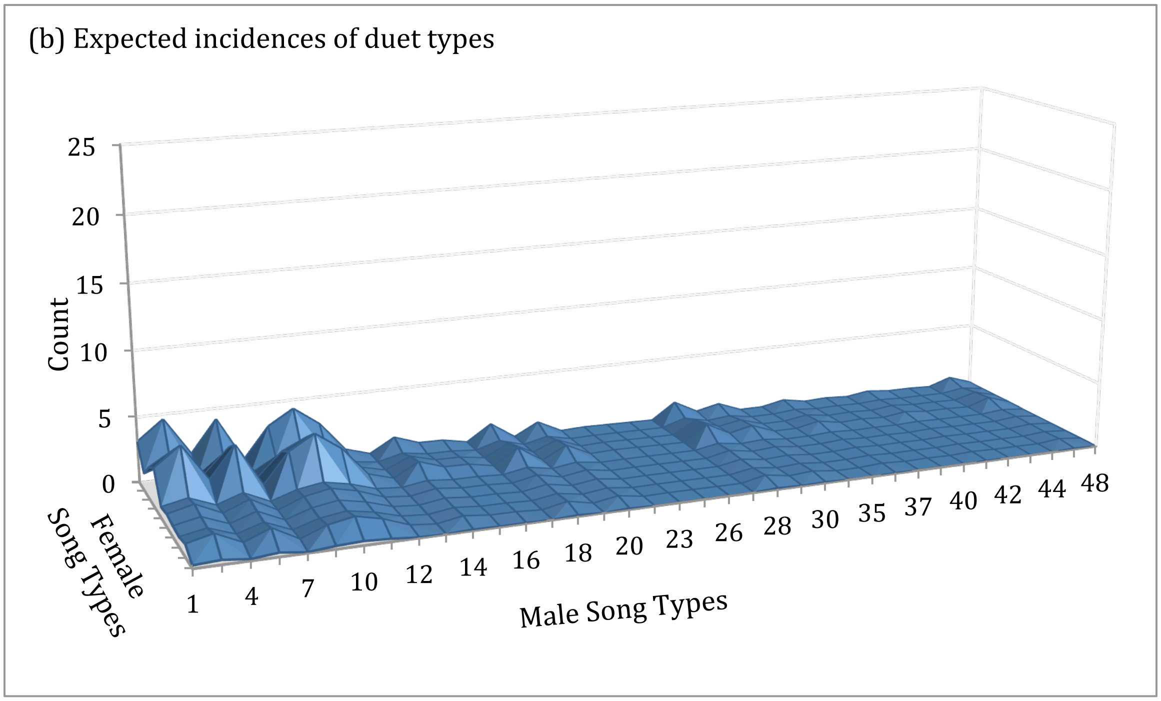

(a)
8
6
4
2
Frequency (kHz)
5
10
15
20
25
30
Time (s)
(b)
8
6
4
2
Frequency (kHz)
5
10
15
20
25
30
Time (s)

Figure 8


(a) Observed instances of duet types


(b) Expected incidences of duet types

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CHAPTER 3

Song type salience and the use of introductory notes in the White-breasted Wood-Wren (*Henicorhina leucosticta*)

Birds use song to attract prospective mates and to deter potential rivals from their territories. During male-male countersinging displays, males may manipulate the timing and song types they use depending on the behavior of their opponent. The signal value of behavioral responses is an intensively studied area, however in this chapter, I address two additional aspects that have received relatively little attention. One is the signal value of individual song types with respect to the salience of threat. Another, more uncommon feature, is the inclusion of ancillary 'accent' introductory notes on the signal value. Shared song types are presumed to have equivalent threat salience during countersinging contest, however focal males of my study animal, the White-breasted Wood Wren (*Henicorhina leucosticta*) varied their responses to playbacks of different shared song types. The time subjects took before responding to playback differed between song type treatments and one song type in particular provoked significantly lower song rates and was never song matched. These results and other behavioral observations suggest a categorical difference in signal function between song types though what this function might be remains uncertain. Introductory notes may have increased threat salience in one of the two song types tested; however, the trend is not strong enough to make definite conclusions. These data highlight the potential influence of small signal modifiers in influencing singing interactions and warrant the need for future studies.

3.1 Introduction

Multiple-song repertoires are common to over 75% of male oscine birds; these additional song types are often gained by innovation and by mimicry (MacDougall-Shackleton 1997). In many of these species, males use song during signaling contests to engage in territorial interactions and advertise for potential mates. Often, birds that sing with a repertoire of discrete song types will engage each other with countersinging in which two males sing overlapping bouts of song. Birds may communicate signals through these interactions by varying characteristics of the song (pattern-specific responses; Vehrencamp 2001) and the timing of songs (time-specific responses; Todt and Naguib 2000), and by approaching and sometimes physically attacking opponents (Krebs et al 1981). Responses that may increasing the threat salience of agonistic interactions include physical approach, matching a sender's signal, increased song rate, and overlapping an opponent's song (Searcy and Beecher 2009).

Many aggressive signaling systems are graded, meaning that the sender has a hierarchy of signals of increasing 'threat value'. Song-type matching, where an individual selectively chooses songs that match their opponent's song type, is thought to communicate aggression and a willingness to escalate the interaction (Krebs et al. 1981). The honesty of the signal is maintained by a receiver retaliation cost against bluffers rather than a production cost, vulnerability risk, or physical ability (Todt and Naguib 2000; Vehrencamp 2001; Mennill and Ratcliffe 2004). This kind of signal is known as a conventional signal because the form of the signal is arbitrary and is associated with a specific context by convention. Frequency matching, in which the spectral characteristics of a song are matched but the song type is unmatched, and repertoire matching, in which a different but shared song type is used, are two other examples thought to be conventional signals of aggression in some species (Burt et al. 2001; Beecher et al. 1996; Mennill and Otter 2007; Fitzsimmons et al. 2008; Naguib, 2005). Although not described in the avian literature, it is possible that song types within the shared repertoire of a communication network could acquire a graded signal value by convention, similar to that found in repertoire matching, where 'extra-threat' and 'reduced-threat' song types could influence the signaling interactions of countersinging contests.

Additionally, the signal value of song types may not be equivalent in all contexts. A number of species use songs selectively in specific contexts, effectively partitioning their repertoire into different functional groups (reviewed in Catchpole and Slater 1995). Male birds sing to resolve conflict with other males or to attract and retain mates. Song type groups are usually delineated similarly between intra- and intersexual communication. The chestnut-sided warbler (*Dendrioica pensylvanica*) for example has "accented-ending" and "unaccented-ending" song type categories. Accented-ending songs are commonly used by unmated males to attract mates and are highly stereotyped, including a characteristic pair of notes at the end of these songs. Unaccented-ending songs are more variable, compose most of the repertoire and are primarily used during male–male interactions (Byers 1995, 1996). Partitioned repertoires can add a higher degree of specificity to the signal thereby reducing the likelihood that a signal will be misinterpreted.

In addition to communication between sexes, vocal repertoires could be partitioned for communication within sexes although its occurrence is not established in birds. Variation in the spectral characteristics of song types may lead to the selection of certain song types for more specialized purposes, such as communicating with receivers at different distances. Such a signal may take on context-specific differences in the interpretation of the signal, increasing the specificity of the signal and reducing the likelihood of being misinterpreted, while also decreasing its signal value outside of that context.

Given this background, the male White-breasted Wood Wren, (*Henicorhina leucosticta*) sings song types that fall into three groups (reviewed in Chapter 1). Group 1 song types are typically three notes (mean ± SD = 3.00 ± 0.32) and are associated with an additional repertoire of introductory note trills (introductory notes hereafter). Introductory notes are not regularly included with the song but take the form of a single high frequency ascending or descending note or a trill of up to six or more notes preceding a song. Within Group 1 song types, introductory notes may function as a conventional signal of aggression because they may be either included or excluded from a songs within a bout, and could therefore be used to modify the threat salience of countersinging interactions independent of other timing- or pattern-specific signals. Group 1 song types include most song types in the repertoire and a great majority of the total song output of individuals. Group 2 song types are composed of three notes and lack introductory notes. The most common song type within this group is song type st11, which is unique because it spans the largest and highest frequency range of any male song type and is also the shortest in duration. Group 3 song types lack introductory notes and contain more notes per song (mean ± SD = 4.75 ± 0.707). Songs include relatively high frequency elements (max ± SD = 4.18 ± 0.475 kHz) as the first notes of the song type. Group 3 song types are not commonly sung nor widely shared between male repertoires. Male White-breasted Wood Wrens thus have two features of their repertoire that they may be able to manipulate when singing to other conspecifics in addition to pattern-specific and time-specific signals established in other species: signal differentiation through song type use, and signal modification through the inclusion or exclusion of introductory notes within Group 1 songs.

In this study, I explore the use of both of these potential mechanisms. I did so with a series of playback experiments simulating intruding males that differ in the song type they sung and the presence or absence of introductory notes. I compared agonistic responses of receiver birds to four different song types, three Group 1 song types and one Group 2 song type shared among males in the study population. Vocal and behavioral responses to playback that vary consistently between stimulus type would indicate that some song types themselves differ in signal value within the context of confrontational territory intrusions from male strangers. Responses that differ in magnitude across multiple agonistic behaviors would suggest song types are conventional signals in this context, employed to escalate or deescalate an agonistic interaction as a graded threat to receivers. Alternatively, asymmetric behavioral responses to differing song types, such that only some metrics of response differ between song types or responses differ in opposite directions, would indicate a mismatch between the function of a song type and its use as a conventional signal during countersinging displays.

I also explored the hypothesis that introductory notes function as a conventional signal of aggression during countersinging. If introductory notes increase the threat salience of a vocalization, songs that include introductory notes should provoke a greater response from territorial males than the same song type presented to males without introductory notes. I compared responses of free-living male White-breasted Wood Wrens to the playback of two Group 1 song types; one with and one without introductory notes preceding the playback songs.

3.2 Methods

3.2.1 Study population and study site

I presented a series of playback trials to 12 territorial male White-breasted Wood Wrens near Estación Chajul, in the Montes Azules Biosphere Reserve, Chiapas, Mexico (16º 6′ º44”N and 90º 56′ 27”W; Figure 1; See Chapter 1 for description). I conducted 65 experimental trials between 7 – 28 May 2009 and 4 additional trials between 23 – 27 June 2010. Three trials could not be completed because the birds YOGy, COO, and GyYY were no longer on their territories in 2010. There were a total of 69 trials during the two years.

3.2.2 Playback stimuli

To simulate intruders exhibiting different song type characteristics (either differing in song type or introductory note use) I presented each territorial male with six treatments: treatments 1 – 3 included Group 1 song types st1, st2, and st10 without the associated introductory note; treatment 4 included a commonly sung Group 3 song type (Group 3 songs never have introductory notes); and treatments 5 and 6 were again st1 and st2, but also included introductory notes (Figure 2). All song types were shared among the study individuals and were overall among the most commonly heard song types in the study population (rank order 5, 1, 2, and 6 with respect to treatment number). No Group 2 songs were common among all birds in the study and were therefore not evaluated.

During a three-week period prior to this reported experiment in 2009 and 2010, I created playback stimuli of six exemplar recordings representing each of the 6 treatments from all 12 subjects. All recordings used for playback were captured as 16-bit WAV files with a Marantz PMD 661 digital recorder with a Sennheiser ME67/K6 microphone. I generated stimuli by isolating songs with a high signal-to-noise ratio from field recordings and then normalized samples to 20 KU maximum amplitude with Raven 1.3 (Charif et al. 2006) and looped them every 4 seconds to produce a two minute stimulus similar in structure to that of naturally occurring songs. Thirty seconds of silence was also added to the beginning of the audio output to allow time to step away from the playback apparatus.

The six playback stimuli produced from each individual described above were presented to six other non-neighboring individuals at three different times of day: 0600 – 0830, 1100 – 1330, and 1600 – 1830. Each experimental trial lasted six minutes and consisted of a 2-min control period prior to the playback, a 2-min playback period and a 2-min post-playback period. Playback stimuli were presented as 16-bit WAV files at 44.1 kHz, from a Tivoli PAL portable speaker and an iPod hung approximately 1.5 m high on a small understory tree. Playback locations were consistent between trials and within 20 m of the territory edge directed to the territory center. Treatments were recorded and visually monitored from a partially concealed position, approximately 5 m from the playback experiment apparatus.

To quantify agonistic response to each playback, five measures of response were analyzed: (1) song matching, (2) closeness of approach, (3) the latency from the end of the first song to the beginning of the response, (4) the total number of songs during the treatment and post-playback periods and (5) song overlap.

3.2.3 Statistical analysis

I modeled vocal and physical responses to the six treatments with linear mixed models (LMEs) in R (R Core Development Team 2010) implementing the ‘‘nlme’’ package (Pinheiro et al. 2013). Parameters were fitted with restricted maximum likelihood procedures. Additionally, to assess the binomial distribution of the song matching response to treatments, I assessed generalized mixed models (GLMMs) with the “lme4” package (Bates et al. 2013). Parameters were estimated with the Laplace approximation. In both sets of models, subject identity was fitted as a random term to account for correlations of repeated measures within each subject (Cnaan et al. 1997). I first included interaction terms that have biological meanings in the model. Nonsignificant interactions were then dropped from the model.

Two sets of LMEs were performed. To assess the effect of song type on response variables, a LME was constructed between treatments 1 – 4 (see Figure 2) for three measures of response: closest approach, song latency and the total number of songs in the response. Song matching could not be assessed with parametric tests due to a zero sum value for treatment 3 (st11 was never matched) so Fisher’s exact test was performed for this measure of response. To test the statistical significance of observing a zero sum for this treatment, I used a permutation procedure with 1000 permutations per run. Song overlap was compared to null model of random overlap following Ficken et al. (1974).

A second set of models was developed to test for an effect of introductory notes by comparing treatments with matching song types in two treatments, both with and without introductory notes (Treatments 5 and 6 vs.1 and 2 respectively; see Figure 2).

3.3 Results

Male White-breasted Wood Wrens responded strongly to playback with song and by approaching the source of the playback in 57 of 69 experimental trials. Song rates during and after playback exceeded that of the pre-playback period and exceeded the average song output recorded for this species (see Chapter 1). Birds tended to avoid overlapping playback songs regardless of the treatment (Appendix A), while overlapping frequently occurred as a result of playback songs starting while a bird was already singing (Appendix B). One bird (YCC) did not respond to playback in five of six trials and therefore was excluded from analysis as a subject but was retained as a song donor to playbacks. Playbacks were presented near the borders of territories and it is likely that the remaining non-responding males were outside of hearing range or otherwise occupied from responding. This assessment was supported by the near random distribution of missing trials between bird, playback donor, time of day, or treatments (missing trials in 4 of 6 treatments; mean ± SD = 1.33±0.49 missed trials per treatment). These missed trials were therefore excluded from analysis.

3.3.1 Response to song type

Song latency was affected by song type (LME: $F_{24} = 3.506$, $P = 0.0307$; Table 1.1; Figure 3) with quicker responses to st2 than st11 or st10 ($T_{24} = 2.803$, P = 0.0099; $T_{24} = 2.710$, $P = 0.0122$) and intermediate responses to st1 (all $P > 0.14$; Table 1.2). The closest approach of subjects to the speaker was not significant overall between song types (LME: $F_{24} = 1.827$, $P = 0.1692$; Table 2.1; Figure 4) although there was tendency for males to approach st1 more closely than st10 and st11 (T_{24} = 1.939, P = 0.064; T_{24} = 1.608, P = 0.1209 respectively) and to approach st2 more closely than st10 (T_{24} = 1.663, P = 0.1092; Table 2.2). The affect of song type on song rate was marginally significant (F_{24} = 2.832, P = 0.0597; Table 3.1; Figure 5) but birds responded to st11 at lower rates than all other song types (all P < 0.038) and at otherwise very similar rates among treatments (all P > 0.75; Table 3.2). Additionally, birds never matched st11 (Permutation $\text{test}_{10,000}$ p < 0.001; mean ± SD = 0.55 ± 0.12) while song matching among other treatments levels was high (Figure 6; Table 4). The differences in song matching between other song types were not significant (all P > 0.14). This song type was only elicited twice as a response to playback; in one case representing only the first two songs in a bout of 54 songs. In the other instance, st11 was sung 15 times as the only response to a playback (bout length: mean ± SD = 34.51 ± 17.50).

3.3.2 Response to introductory notes

The affect of introductory notes on aggressive response was not distinct. Only song latency and song matching suggested that birds responded differently to songs presented with and without introductory notes and for both measures the effect was dominated by st1. Song latency was not directly affected by the presence of introductory notes (LME: $F_{1,23}$ = 1.795, P = 0.1934) or song type (LME: $F_{1,23}$ = 0.1780. P = 0.6768) and there was a significant interaction between the two (LME: $F_{1,23}$ = 5.817, P = 0.0243; Table 5.1; Figure 7). Subjects waited longer to respond to st1 when it had intros (T_{23} = -2.668, P = 0.0137); however, they responded at similar rates when st2 was presented with intros

(LME: T_{23} = 1.4111, P = 0.1716; Table 5.2). Song matching followed a similar pattern. Song matching was affected by introductory notes (GLMM: z = -2.178, P = 0.0294), but not song type (GLMM: z = -1.515, P = 0.1298) and there was a significant interaction between intro and song type (GMLL: z = 2.032, P = 0.0421; Table 6; Figure 8). Song type st1 was matched 78% of the time without introductory notes and only 43% of the time when introductory notes were included (t-test: p = 0.035).

The closest distance that males approached was similar with and without introductory notes (Figure 9). Similarly, the rate of singing in response to playback did not vary with the introductory notes in the playback (Figure 10). Closest approach was not affected by intros (LME: F_{23} = 0.312, P = 0.5820) or song type (LME: F_{23} = 0.014, P = 0.9062) and there was no interaction between intros and song type (LME: F_{23} = 0.296. P = 0.5918; Table 7.1; Figure 9). Contrasts between treatments were similarly unrelated (all $P > 0.4$; Table 7.2). Song rates were also unaffected by introductory notes (LME: F_{23} = 0.022, P = 0.8822) and song type (LME: F_{23} = 0.539, P = 0.4702) and there was no interaction between introductory notes and song type (F_{23} = 0.112, P = 0.7412; Table 8.1; Figure 10). Contrasts were also non-significant (all $P > 0.7$; Table 8.2).

3.4 Discussion

Male White-breasted Wood Wrens sing a variety of songs that fall into three broad groups based on the number of notes and the presence of intermittent introductory notes. Countersinging between males is mediated by pattern- and timing-specific responses similar to other species; however, data from this study suggest that song type and signal modifier cues may also provide information that birds use to assess an opponent or add specificity to a singing encounter. These may be conventional cues in the sense that their form is arbitrary and the meaning is defined by context rather than purely as an honest signal relating to physical fighting ability or dominance.

3.4.1 The signal value of song types

Males use song types from all three song groups during solo songs and within proximity to other singing males. Their signal value may not be equal within all contexts such as during confrontational countersinging with non-neighbors. All the song types examined here are shared between study individuals and among the most commonly recorded song types within natural singing contexts. The three Group 1 songs had similar spectral characteristics and were among the lower frequencies that the birds used. (see Chapter 1). In contrast, the Group 2 song type (st11) had the highest frequency recorded in the repertoire; it also had the widest bandwidth and is the shortest in duration. Treatments elicited high levels of song output and approach to the speakers, suggesting that stimuli represented a threat to the territory holder. The lower song rates and lack of song matching in response to st11 suggest that signal value of some song types may vary with respect to countersinging interactions. Song type st11 was commonly heard outside of territorial interactions yet was rarely sung in responses to playback of any treatment. These results suggest a potentially categorical difference in the signal value of st11 relative to other tested song types.

The underlying function of st11 was not evident from these experiments. Song type st11 was often heard outside of experimental treatments as a solo song or following the song of a distant neighbor or playback, but seldom in response to a near-by neighbor. Additionally, it was sometimes sung when neighboring males were already engaged in countersinging. These observations suggest that st11 could function as a general broadcast song to alert neighbors and other conspecifics of territory occupancy. Though uncommon, this song type was observed to occur in duets and during countersinging, suggesting that the function is not exclusive to communicate with distant neighbors.

3.4.2 The signal value of introductory notes

There was only weak support for an aggressive function of introductory notes. Most of the difference in response relating to introductory notes was concentrated in only one of the two song types presented with introductory notes (st1). That these two song types provoked responses that were more similar to each other than to the other two song types makes the reason for this disparity unclear. The faster response and increased song matching in response to st1 when introductory notes are included is consistent with an increase in the threat salience of this signal during countersinging interactions; however, the lack of agreement with st2 in this trend makes it difficult to draw any strong conclusions. The prevalence of introductory notes during countersinging as opposed to singing that doesn't involve other males suggests that other males are the intended receivers. An alternative explanation, not tested here, is that females are the intended recipients of this information. It is possible that introductory notes alert mates to a threat to territorial resources or invite the female to make her presence known, thereby reducing the chances that he is cuckolded. Both these alternatives seem unlikely given the relative unresponsiveness of females to male solo intruders compared to female or paired intruders (see chapter 4). Testing additional song types and considering the responses of alternative receivers will be necessary to establish a clear function of introductory notes.

The function of noncompulsory introductory notes similar to those found in the White-breasted Wood Wren remains unstudied in other species and this singing behavior appears to be very uncommon. Introductory notes are present in other wren species but differ in usage and spectral characteristics. For example, *Thryophilus sinaloa*, *T. pleurostictus*, and *T. rufalbus* begin songs with a series of introductory notes composed of clicks, whistles or hoots but these notes are always present in the song and are contained within a frequency bandwidth similar to the rest of the song (Mann et al. 2009). Tropical wren species within the *Cantorchilus* genus sing an introductory phrase that directly precedes duetting; however, these notes are not carried into the duet or added to individual songs within a bout (Mann et al. 2009). Such singing behaviors do not appear to have the equivalent potential of modulating the threat value of songs during the course of a countersing display.

The singing style of the Superciliated wren (*Cantorchilus superciliaris*) is more similar to *H. leucosticta* and is unique among the singing styles of other species in its genus. Mann et al. (2009) describe a separate repertoire of higher-frequency introductory 'calls' that is added to a portion of solo and duet songs in a similar fashion to introductory notes in *H. leucosticta*. During duets, introductory calls may be absent, combined with regular song types, or, differing from *H. leucosticta*, they may be sung without the regular song types at all (Mann et al. 2009). Additionally, both male and female *C. superciliaris* sing introductory calls and will sometimes engage in duets composed entirely of these calls (Mann et al. 2009). While introductory notes could also be defined as calls and appear to be analogous to introductory calls, I adopt the more general nomenclature of introductory notes because they always occur in combination with the song types in *H. leucosticta* and are rarely if ever sung without preceding a song. Also common to both species, duets are often imprecisely coordinated compared to the high precision duetting typical of the *Cantorchilus* genus. The broad overlap in singing styles between these species suggests that parallel selection may have shaped these singing behaviors. If this proves to be the case, the loosely coordinated duets of *H. leucosticta* may represent a derived singing style rather than an ancestral precursor to complex duets.

Introductory notes attenuated at shorter distances than the lower frequency song type notes suggesting that they may be most effective at communication to nearby individuals. Other species use low propagation signals in close–range agonistic and/or sexual contexts and loud well–propagating songs during long–range communication (Dabelsteen et al. 1998). Low-amplitude 'soft song' has been shown to predict aggression in some species (Searcy and Beecher 2009); however, these signals are not combined in a pairwise fashion with regular amplitude songs similar to introductory notes as recorded in this study.

3.4.3 Other signaling effects

It was important in this study that treatment song types were shared among study subjects, thereby constraining samples to a mostly continuous network of territories in which signaler familiarity could influence singing responses irrespective of treatment. Some species of birds are known to use structural cues in songs to assess the identity, quality or threat posed by birds within the signaling neighborhood (e.g. Byers 1996; Stoddard et al. 1990). These signaling effects were minimized in this study design by developing playbacks from only non-neighbors and administering playbacks of different birds to each study subjects. Additionally, all playbacks were used only once during experiments.

The propensity of study subjects to avoid overlapping playbacks is common in the literature (e.g.Wasserman 1977; Fitzsimmons 2008). These results are consistent with either the avoidance of overlap to prevent acoustic masking, or as a response to other information in the signal. The signal value of overlapping is a matter of debate (see Searcy and Beecher 2009; Naguib and Mennill 2010; Searcy and Beecher 2011). The main criticisms for drawing inferences from overlap are the lack of evidence that the signal increases in aggressive contexts, and little evidence that the signal predicts aggressive escalation. Most studies find that overlapping occurs less often than expected by chance (Searcy and Beecher 2009). However, a small number of studies suggest that some receivers do respond differentially to overlapping playback (e.g. Peake et al. 2001, 2002; Mennill and Ratcliffe 2004) suggesting that actively overlapping the songs of a signaler may still have a signal value, even if it occurs below levels expected if totally random.

My results suggest not only that song types may differ categorically in usage and threat salience in this species, but also that males could modify signals with high-frequency introductory notes to increase the threat salience. This type of signal modification has not been described previously and further research is necessary to define the extent of the functional differences between song type groups and introductory note modification in the *H. leucosticta*, as well as analogous processes occurring in other species. The Superciliated wren (*Cantorchilus superciliaris*) may utilize a similar behavior of signal modification that extends to both males and females and would therefore be a good study animal to augment the present work. This study reinforces the notion that signal modification may be more important for mediating agonistic interactions than is currently recognized.

List of Figures

1. Map of territorial males used in experiments near Estación Chajul, in the Montes Azules Biosphere Reserve, Chiapas, Mexico. Letters correspond to color band markings of the territory holder.

2. Spectrograms of song type treatments implemented in each trial with treatment names and corresponding song type names listed in the lower right. Numbers in the top right of each spectrogram are the treatment numbers referred to in the text. The dashed line encompasses treatments analyzed by song type whereas the solid line encompasses treatments that vary by the inclusion of introductory notes. Note that st1 and st2 are repeated in two treatments, 1 and 5, and 2 and 6 respectively.

3. Time delay or latency in seconds (mean ± SE) of subjects' singing response to each of the 4 song type treatment following the beginning of the playback.

4. Closest distance in meters (mean ± SE) of subjects that approached each of the 4 song type playback treatments.

5. Singing rates expressed in songs per minute (mean ± SE) by subjects responding to the 4 singing response to song type playback treatments.

6. Song matching in response to each of the 4 song type treatments plotted as a stacked bar chart with non-matching trials in red (below; "0") and matching trials in green (above; "1").

7. Time delay in seconds (mean ± SE) of subjects' singing response to song type st1 (left) and st2 (right), both without introductory notes (red; "no") and with introductory notes (green; "yes").

8. Song matching in response to song type st1 without introductory notes and with introductory notes (labeled st1 and st1 + intros), and st2 without introductory notes and with introductory notes (labled st2 and st2 + intros). Treatments are plotted as a stacked bar chart with non-matching trials in red (below; 0) and matching trials in green (above; "1").

9. Closest distance in meters (mean ± SE) that subjects approached song type st1 (left) and st2 (right), both without introductory notes (red; "no") and with introductory notes (green; "yes").

10. Singing rates expressed as songs per minute (mean ± SE) pooled across the 600 s focal period of subjects in response to song type type st1 (left) and st2 (right), both without introductory notes (no; red) and with introductory notes (yes; green).

List of Tables

8.2 Contrasts from the song rate model in Table 5.1 assessing the affect of introductory notes (intros) on song types st1 and st2; depicted in Figure 10.

Figures and Tables

Figure 1

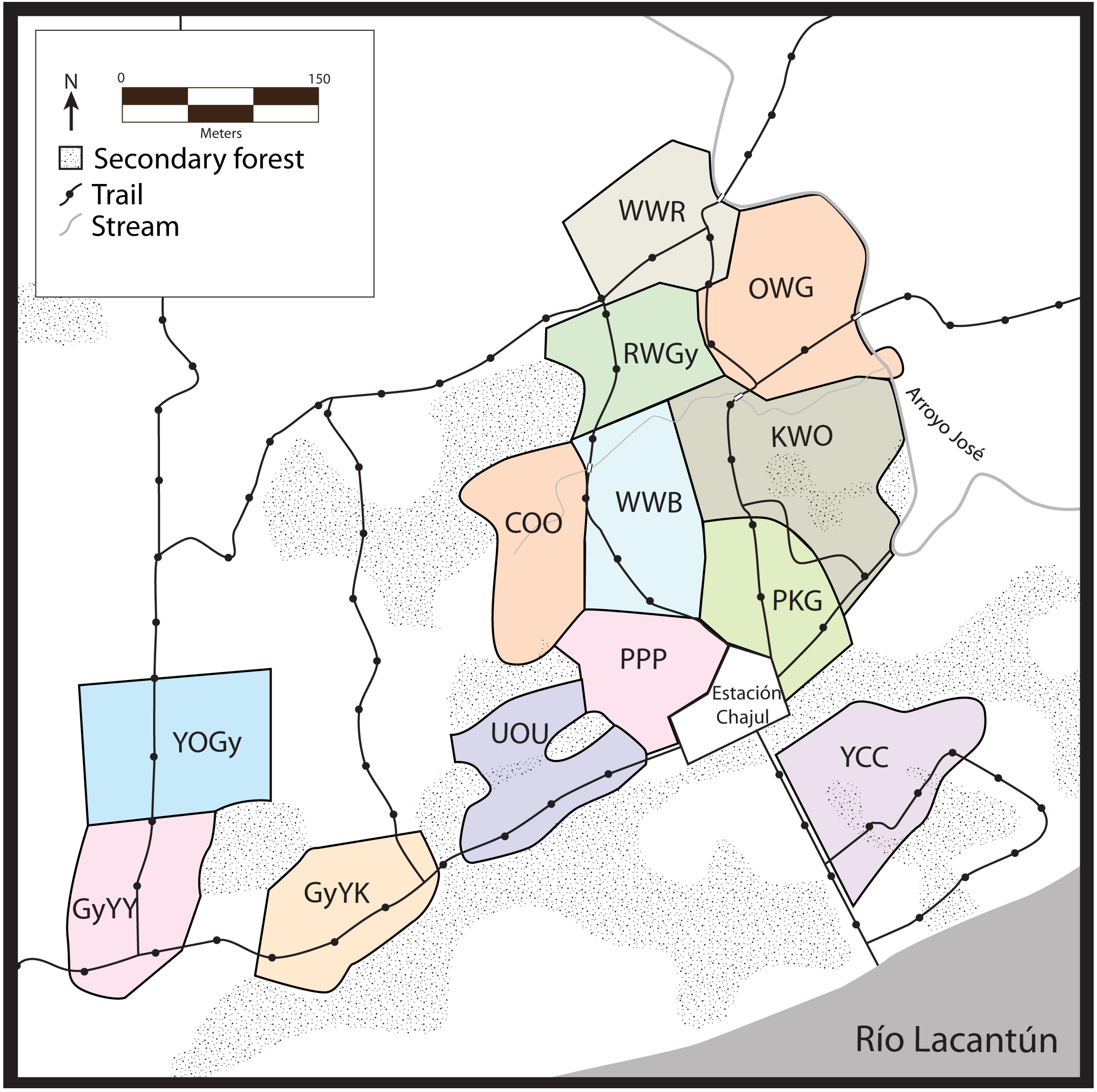
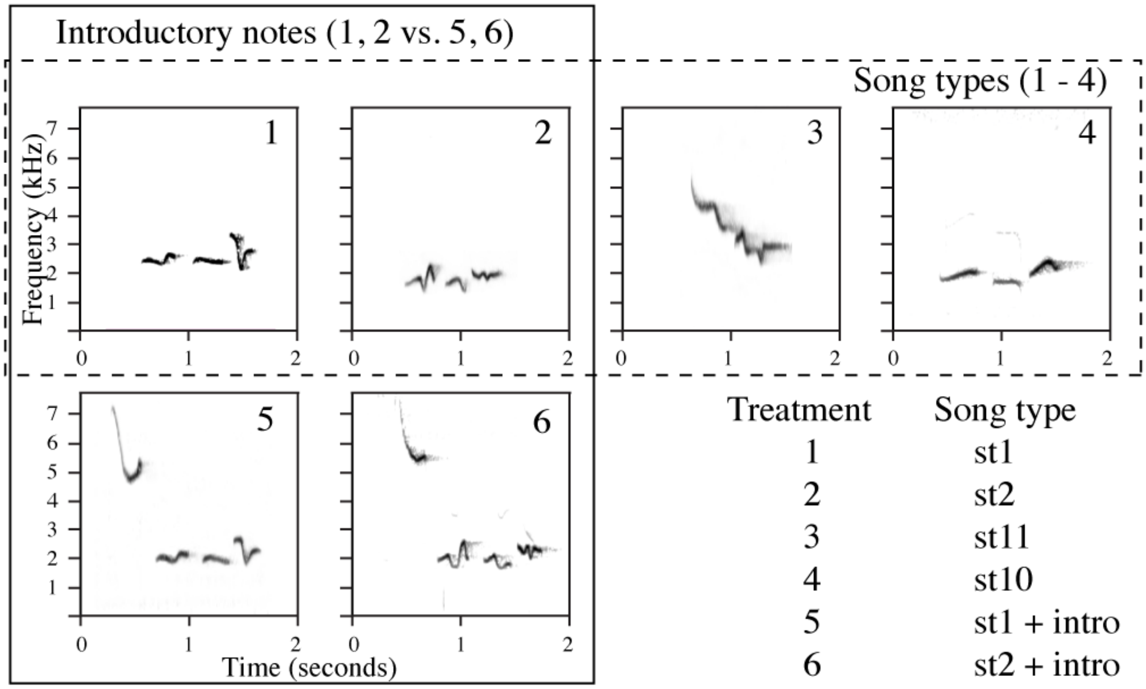


Figure 2



Treatment	Song type
1	st1
2	st2
3	st11
4	st10
5	st1 + intro
6	st2 + intro

Figure 3

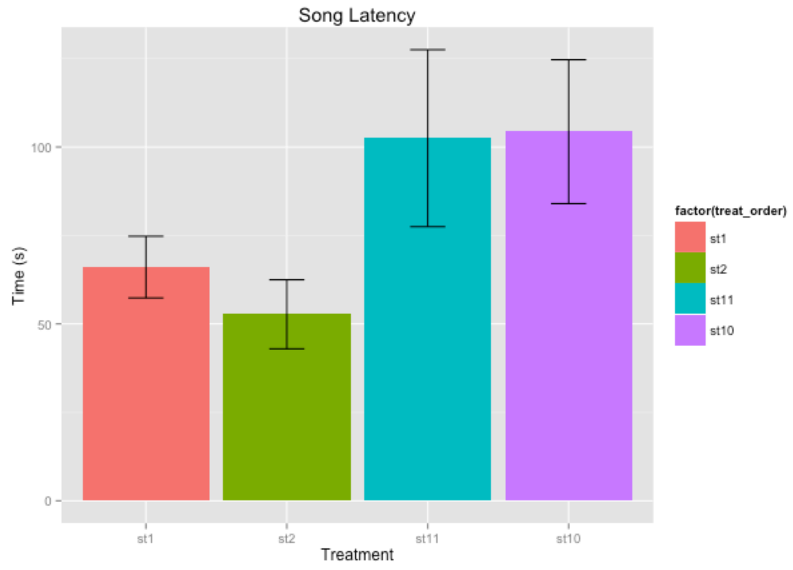


Table 1.1

Model: Song Latency	**Fixed effects**	**numDF**	***d.f***	***F***	***P<***
Intros × Song type	(Intercept)	1	24	187.322	<.0001
	Song type	3	24	3.506	0.0307

Table 1.2

Contrasts: Song Latency	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	8.084	0.915	24	8.839	<.0001
st1 × st2	-1.359	1.063	24	-1.279	0.2133
st1 × st10	1.620	1.066	24	1.520	0.1416
st1 × st11	1.341	1.016	24	1.320	0.1994
st2 × st10	2.979	1.063	24	2.803	0.0099
st2 × st11	2.700	0.996	24	2.710	0.0122
st10 × st11	-0.279	1.016	24	-0.275	0.7860

Figure 4

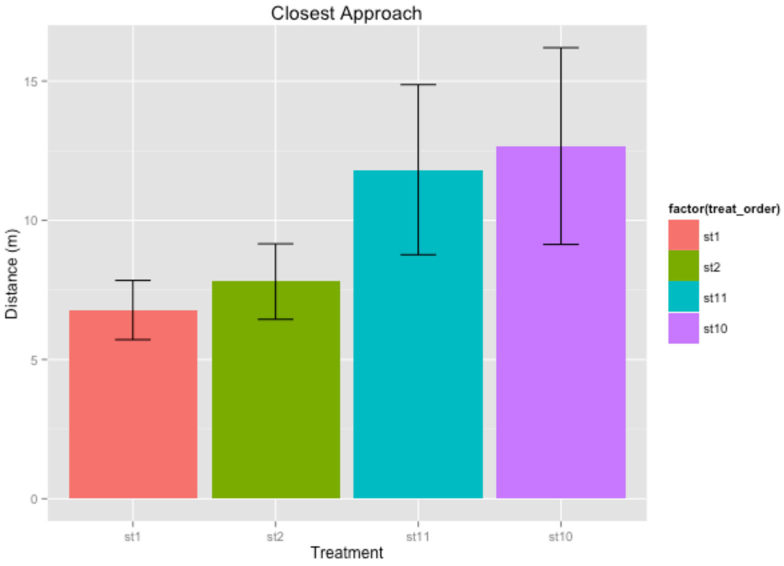


Table 2.1

Model: Closest Approach	**Fixed effects**	**numDF**	***d.f***	***F***	***P<***
Intros × Song type	(Intercept)	1	24	459.748	<.0001
	Song type	3	24	1.827	0.1692

Table 2.2

Contrasts: Closest Approach	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	1.802	0.202	24	8.941	<.0001
st1 × st2	0.091	0.278	24	0.326	0.7472
st1 × st10	0.553	0.285	24	1.939	0.0643
st1 × st11	0.437	0.272	24	1.608	0.1209
st2 × st10	0.462	0.278	24	1.663	0.1092
st2 × st11	0.346	0.264	24	1.311	0.2022
st10 × st11	-0.116	0.272	24	-0.426	0.6742

Figure 5

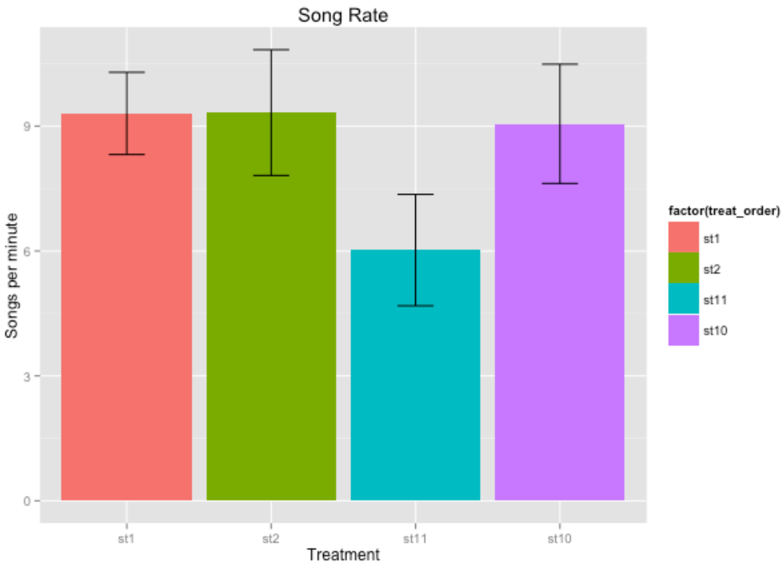


Table 3.1

Model: Song Rate	**Fixed effects**	**numDF**	***d.f***	***F***	***P<***
Intros × Song type	(Intercept)	1	24	275.933	<.0001
	Song type	3	24	2.832	0.0597

Table 3.2

Contrasts: Song Rate	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	6.041	0.529	24	11.430	<.0001
st1 × st2	-0.203	0.647	24	-0.314	0.7562
st1 × st10	-0.145	0.652	24	-0.222	0.8264
st1 × st11	-1.540	0.622	24	-2.476	0.0207
st2 × st10	0.059	0.647	24	0.091	0.9285
st2 × st11	-1.337	0.608	24	-2.198	0.0379
st10 × st11	-1.396	0.622	24	-2.244	0.0343

Figure 6

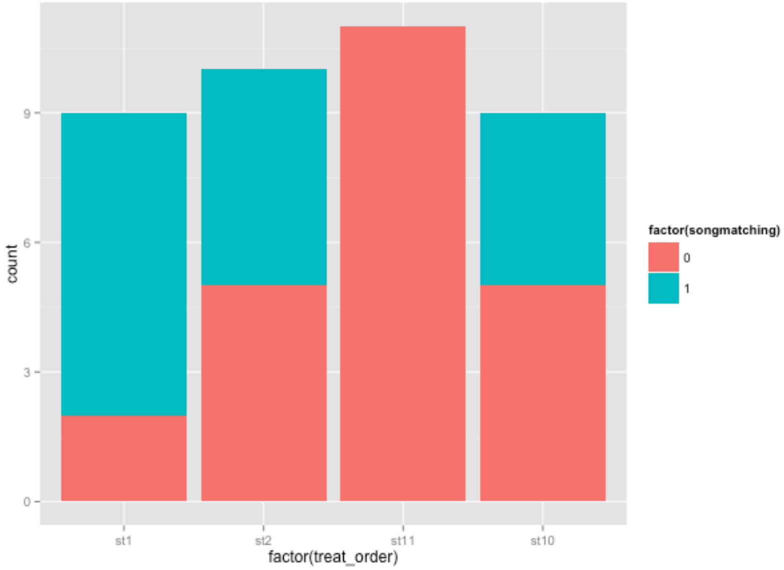


Table 4

Contrasts: Song Matching	**Estimate**	**Std.Error**	***z***	***P<***
Females:				
(Intercept)	1.283	0.816	1.573	0.116
st1 × st2	-1.285	1.029	-1.249	0.212
st1 × st10	-1.519	1.053	-1.442	0.149
st2 × st10	-0.234	0.926	-0.252	0.801

Figure 7

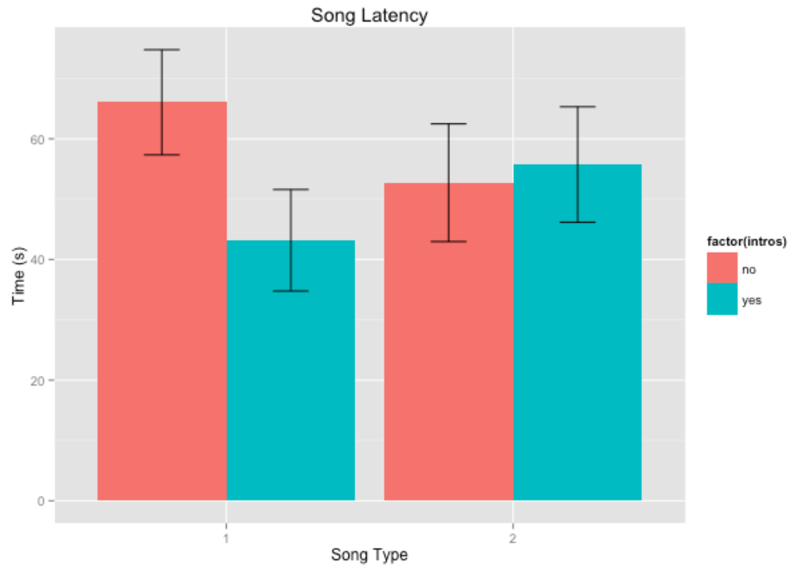


Table 5.1

Model: Song Latency	**Fixed effects**	**numDF**	***d.f***	***F***	***P<***
Intros × Song type	(Intercept)	1	23	259.170	<.0001
	Intros	1	23	1.795	0.1934
	Song type	1	23	0.178	0.6768
	Intros × Song type	1	23	5.817	0.0243

Table 5.2

Contrasts: Song Latency	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	8.130	0.611	23	13.311	<.0001
st1 × Intros	-1.877	0.703	23	-2.668	0.0137
st2 × Intros	-0.409	0.625	23	-0.654	0.5197
st1 × st2	-1.338	0.669	23	-2.000	0.0574
Song type × Intros	2.286	0.948	23	2.412	0.0243

Figure 8

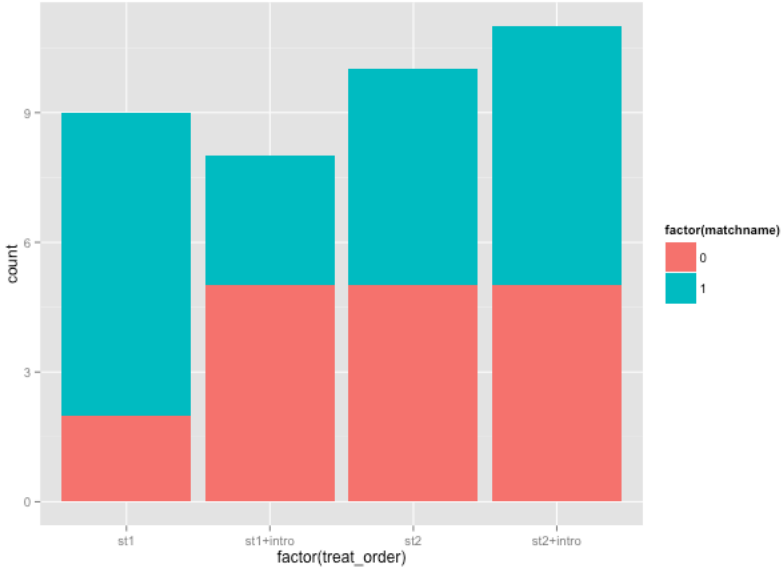


Table 6

Contrasts: Song Matching	**Estimate**	**Std.Error**	***z***	***P<***
Females:				
(Intercept)	1.861	1.022	1.82	0.0687
st1 × Intros	-2.863	1.314	-2.178	0.0294
st2 × Intros	0.547	1.020	0.536	0.5918
st1 × st2	-1.797	1.186	-1.515	0.1298
Song type × Intros	3.410	1.678	2.032	0.0421

Figure 9

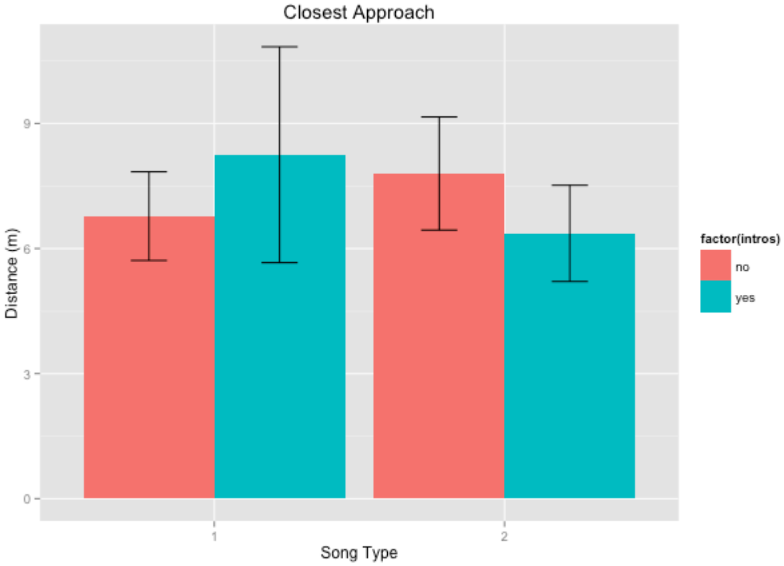


Table 7.1

Model: Closest Approach	**Fixed effects**	**numDF**	***d.f***	***F***	***P<***
Intros × Song type	(Intercept)	1	23	277.243	<.0001
	Intros	1	23	0.312	0.5820
	Song type	1	23	0.014	0.9062
	Intros × Song type	1	23	0.296	0.5918

Table 7.2

Contrasts: Closest Approach	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	1.802	0.221	23	8.159	<.0001
st1 × Intros	0.011	0.322	23	0.036	0.9718
st2 × Intros	0.224	0.290	23	0.774	0.4471
st1 × st2	0.091	0.304	23	0.298	0.7687
Song type × Intros	-0.235	0.433	23	-0.544	0.5918

Figure 10

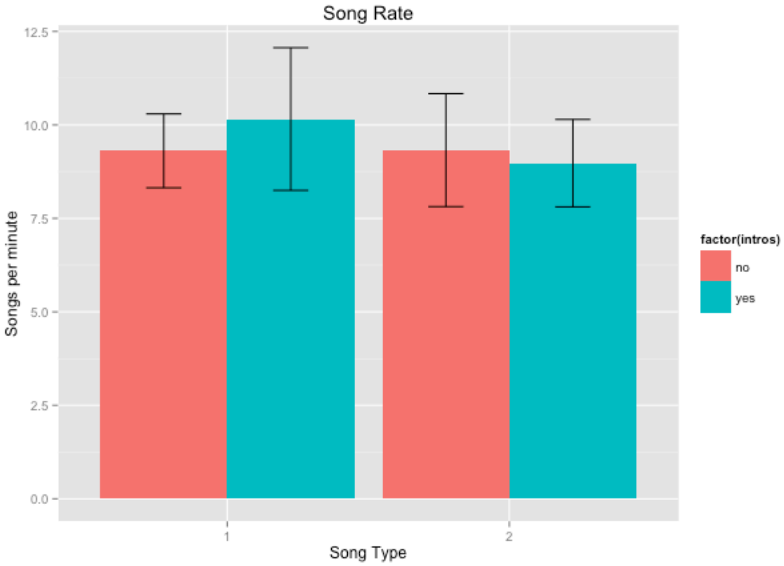


Table 8.1

Model: Song Rate	**Fixed effects**	**numDF**	***d.f***	***F***	***P<***
Intros × Song type	(Intercept)	1	23	278.046	<.0001
	Intros	1	23	0.022	0.8822
	Song type	1	23	0.539	0.4702
	Intros × Song type	1	23	0.112	0.7412

Table 8.2

Contrasts: Song Rate	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	6.033	0.468	23	12.896	<.0001
st1 × Intros	0.100	0.507	23	0.198	0.8450
st2 × Intros	0.128	0.451	23	0.284	0.7786
st1 × st2	-0.137	0.483	23	-0.284	0.7789
Song type × Intros	-0.228	0.683	23	-0.334	0.7412

Appendix A: Song Timing of Subject

							Subject's songs during PB			Subject's songs during silence				
Subject	Playback	Treatment	**Song** type	Intros	**Bout** overlap	**Total** songs(f)	Predicted (F_v)	Actual (v_f)	O - E	Predicted ($F_{v\ bar}$)	Actual ($f_{v\ bar}$)	O - E	X^2	p-value
BYW	**YOGy**	**1**	**st1**	**no**	**68.08**	**18**	**7.44**	**1**	**–**	**10.56**	**10**	**+**	**5.60**	**<0.05**
GyYY	**UOU**	**1**	**st1**	**no**	**73.55**	**20**	**8.81**	**3**	**–**	**11.19**	**8**	**+**	**4.74**	**<0.05**
KWO	GYK	1	st1	no	17.03	5	2.30	1	–	2.70	1	+	1.80	NS
PKG	WWB	1	st1	no	21.65	8	4.20	1	–	3.80	2	+	3.29	NS
PPP	**OWG**	**1**	**st1**	**no**	**59.49**	**29**	**12.66**	**5**	**–**	**16.34**	**18**	**+**	**4.81**	**<0.05**
UOU	**KYO**	**1**	**st1**	**no**	**87.09**	**25**	**12.06**	**1**	**–**	**12.94**	**14**	**+**	**10.24**	**<0.01**
YOGy	COO	1	st1	no	37.65	10	4.66	1	–	5.34	4	+	3.21	NS
COO	KWO	5	st1	yes	22.00	6	2.55	1	–	3.45	5	+	1.63	NS
KWO	**WWB**	**5**	**st1**	**yes**	**75.10**	**13**	**3.66**	**3**	**–**	**9.34**	**3**	**+**	**4.43**	**<0.05**
OWG	YCC	5	st1	yes	73.37	11	2.88	1	–	8.12	5	+	2.43	NS
PPP	**COO**	**5**	**st1**	**yes**	**46.62**	**10**	**3.06**	**2**	**–**	**6.94**	**2**	**+**	**3.89**	**<0.05**
UOU	**PKG**	**5**	**st1**	**yes**	**95.61**	**32**	**15.74**	**4**	**–**	**16.26**	**14**	**+**	**9.07**	**<0.01**
WWR	**PPP**	**5**	**st1**	**yes**	**63.15**	**16**	**7.12**	**1**	**–**	**8.88**	**2**	**+**	**10.59**	**<0.01**
BYW	**COO**	**2**	**st2**	**no**	**111.98**	**24**	**8.75**	**1**	**–**	**15.25**	**8**	**+**	**10.31**	**<0.01**
COO	**PKG**	**2**	**st2**	**no**	**123.23**	**29**	**3.48**	**4**	**+**	**25.52**	**14**	**+**	**5.28**	**<0.05**
KWO	**PPP**	**2**	**st2**	**no**	**79.11**	**17**	**6.17**	**2**	**–**	**10.83**	**6**	**+**	**4.97**	**<0.05**
OWG	GYK	2	st2	no	54.25	15	3.20	0	–	11.80	10	+	3.48	NS
UOU	**KYO**	**2**	**st2**	**no**	**81.51**	**35**	**22.81**	**2**	**–**	**12.19**	**21**	**+**	**25.36**	**<0.001**
WWR	KWO	2	st2	no	10.50	4	2.40	0	–	1.60	3	+	3.64	NS
YOGy	**UOU**	**2**	**st2**	**no**	**70.96**	**19**	**7.34**	**1**	**–**	**11.66**	**18**	**+**	**8.92**	**<0.01**
BYW	PPP	6	st2	yes	61.30	17	6.60	2	–	10.40	9	+	3.39	NS
COO	OWG	6	st2	yes	69.97	19	5.46	6	+	13.54	9	+	1.58	NS
GyYY	**WWR**	**6**	**st2**	**yes**	**53.45**	**16**	**6.01**	**0**	**–**	**9.99**	**16**	**+**	**9.64**	**<0.01**
KWO	**PPP**	**6**	**st2**	**yes**	**47.38**	**14**	**4.82**	**1**	**–**	**9.18**	**4**	**+**	**5.95**	**<0.05**
OWG	**PKG**	**6**	**st2**	**yes**	**85.40**	**17**	**2.60**	**3**	**+**	**14.40**	**7**	**+**	**3.87**	**<0.05**
PKG	GyYY	6	st2	yes	24.93	7	4.35	2	–	2.65	3	+	1.31	NS
PPP	YOGy	6	st2	yes	17.63	7	4.01	4	–	2.99	0	–	2.99	NS
UOU	**COO**	**6**	**st2**	**yes**	**68.70**	**19**	**7.35**	**1**	**–**	**11.65**	**12**	**+**	**5.49**	**<0.05**
WWB	UOU	6	st2	yes	100.29	30	13.57	15	+	16.43	9	–	3.51	NS
WWR	GYK	6	st2	yes	17.42	5	2.29	1	–	2.71	2	+	0.91	NS
YOGy	GYK	6	st2	yes	25.47	5	1.50	0	–	3.50	2	+	2.14	NS
BYW	PKG	4	st10	NA	54.25	11	3.29	1	–	7.71	7	+	1.66	NS
GyYY	**YCC**	**4**	**st10**	**NA**	**78.58**	**19**	**8.73**	**3**	**–**	**10.27**	**6**	**+**	**5.54**	**<0.05**
OWG	**YOGy**	**4**	**st10**	**NA**	**85.84**	**25**	**10.61**	**2**	**–**	**14.39**	**17**	**+**	**7.46**	**<0.01**
PPP	**KWO**	**4**	**st10**	**NA**	**41.80**	**10**	**4.19**	**1**	**–**	**5.81**	**2**	**+**	**4.93**	**<0.05**
UOU	OWG	4	st10	NA	77.39	29	5.35	8	+	23.65	17	+	3.19	NS
BYW	YOGy	3	st11	NA	11.54	4	2.67	0	–	1.33	2	+	3.01	NS
GyYY	PPP	3	st11	NA	39.99	14	5.73	2	–	8.27	8	+	2.44	NS
KWO	**UOU**	**3**	**st11**	**NA**	**76.86**	**18**	**6.40**	**2**	**–**	**11.60**	**5**	**+**	**6.78**	**<0.01**
OWG	**WWB**	**3**	**st11**	**NA**	**81.19**	**24**	**10.92**	**2**	**–**	**13.08**	**13**	**+**	**7.29**	**<0.01**
PPP	GYK	3	st11	NA	44.83	15	6.71	3	–	8.29	7	+	2.25	NS
YOGy	OWG	3	st11	NA	23.74	7	3.37	0	–	3.63	3	+	3.48	NS

Appendix B: Song Timing of Playback

							PB songs during subject's song			Playback songs during silence				
Subject	Playback	Treatment	Song type	Intros	Bout overlap	Total songs(f)	Predicted (F_v)	Actual (v_f)	O - E	Predicted (F_{v_bar})	Actual (f_{v_bar})	O - E	X^2	p-value
BYW	YOGy	1	st1	no	68.08	18	5.41	7	+	12.59	10	–	1.00	NS
GyYY	**UOU**	**1**	**st1**	**no**	**73.55**	**19**	**5.14**	**9**	**+**	**13.86**	**7**	**–**	**6.30**	**<0.05**
KWO	GYK	1	st1	no	17.03	5	1.73	3	+	3.27	1	–	2.51	NS
PKG	**WWB**	**1**	**st1**	**no**	**21.65**	**6**	**1.52**	**5**	**+**	**4.48**	**0**	**–**	**12.45**	**<0.001**
PPP	**OWG**	**1**	**st1**	**no**	**59.49**	**15**	**4.44**	**6**	**+**	**10.56**	**4**	**–**	**4.62**	**<0.05**
UOU	KYO	1	st1	no	87.09	22	6.58	10	+	15.42	11	–	3.05	NS
YOGy	COO	1	st1	no	37.65	10	3.19	5	+	6.81	4	–	2.18	NS
COO	KWO	5	st1	yes	22.00	6	1.37	0	–	4.63	5	+	1.40	NS
KWO	WWB	5	st1	yes	75.10	19	7.78	7	–	11.22	9	–	0.52	NS
OWG	YCC	5	st1	yes	73.37	20	7.71	5	–	12.29	14	+	1.19	NS
PPP	**COO**	**5**	**st1**	**yes**	**46.62**	**9**	**2.94**	**6**	**+**	**6.06**	**1**	**–**	**7.40**	**<0.01**
UOU	**PKG**	**5**	**st1**	**yes**	**95.61**	**25**	**9.25**	**14**	**+**	**15.75**	**7**	**–**	**7.31**	**<0.01**
WWR	**PPP**	**5**	**st1**	**yes**	**63.15**	**16**	**7.20**	**13**	**+**	**8.80**	**2**	**–**	**9.92**	**<0.01**
BYW	**COO**	**2**	**st2**	**no**	**111.98**	**28**	**7.75**	**15**	**+**	**20.25**	**12**	**–**	**10.13**	**<0.01**
COO	PKG	2	st2	no	123.23	31	7.91	11	+	23.09	16	–	3.38	NS
KWO	**PPP**	**2**	**st2**	**no**	**79.11**	**20**	**5.56**	**9**	**+**	**14.44**	**9**	**–**	**4.19**	**<0.05**
OWG	GYK	2	st2	no	54.25	14	3.38	5	+	10.62	9	–	1.03	NS
UOU	**KYO**	**2**	**st2**	**no**	**81.51**	**21**	**4.04**	**12**	**+**	**16.96**	**7**	**–**	**21.53**	**<0.001**
WWR	KWO	2	st2	no	10.50	3	0.62	1	+	2.38	2	–	0.30	NS
YOGy	UOU	2	st2	no	70.96	18	6.95	4	–	11.05	14	+	2.04	NS
BYW	PPP	6	st2	yes	61.30	16	5.51	6	+	10.49	8	–	0.63	NS
COO	OWG	6	st2	yes	69.97	18	7.98	4	–	10.02	8	–	2.39	NS
GyYY	**WWR**	**6**	**st2**	**yes**	**53.45**	**15**	**6.23**	**0**	**–**	**8.77**	**15**	**+**	**10.67**	**<0.01**
KWO	**PPP**	**6**	**st2**	**yes**	**47.38**	**12**	**4.84**	**9**	**+**	**7.16**	**2**	**–**	**7.29**	**<0.01**
OWG	PKG	6	st2	yes	85.40	22	8.64	7	–	13.36	12	–	0.45	NS
PKG	GyYY	6	st2	yes	24.93	7	1.94	2	+	5.06	3	–	0.84	NS
PPP	YOGy	6	st2	yes	17.63	7	4.65	3	–	2.35	0	–	2.94	NS
UOU	COO	6	st2	yes	68.70	18	6.38	6	+	11.62	11	–	0.06	NS
WWB	**UOU**	**6**	**st2**	**yes**	**100.29**	**26**	**9.37**	**6**	**–**	**16.63**	**5**	**–**	**9.35**	**<0.01**
WWR	GYK	6	st2	yes	17.42	5	2.01	2	–	2.99	2	–	0.33	NS
YOGy	**GYK**	**6**	**st2**	**yes**	**25.47**	**12**	**7.19**	**3**	**–**	**4.81**	**9**	**+**	**6.09**	**<0.05**
BYW	PKG	4	st10	NA	54.25	14	4.14	3	–	9.86	10	+	0.31	NS
GyYY	**YCC**	**4**	**st10**	**NA**	**78.58**	**20**	**5.73**	**10**	**+**	**14.27**	**7**	**–**	**6.89**	**<0.01**
OWG	YOGy	4	st10	NA	85.84	18	4.66	6	+	13.34	10	–	1.22	NS
PPP	**KWO**	**4**	**st10**	**NA**	**41.80**	**11**	**3.50**	**7**	**+**	**7.50**	**3**	**–**	**6.20**	**<0.05**
UOU	OWG	4	st10	NA	77.39	21	6.51	4	–	14.49	9	–	3.05	NS
BYW	YOGy	3	st11	NA	11.54	3	0.71	2	+	2.29	1	–	3.08	NS
GyYY	PPP	3	st11	NA	39.99	10	2.12	4	+	7.88	4	–	3.57	NS
KWO	**UOU**	**3**	**st11**	**NA**	**76.86**	**20**	**4.48**	**11**	**+**	**15.52**	**7**	**–**	**14.19**	**<0.001**
OWG	**WWB**	**3**	**st11**	**NA**	**81.19**	**21**	**4.58**	**9**	**+**	**16.42**	**10**	**–**	**6.77**	**<0.01**
PPP	**GYK**	**3**	**st11**	**NA**	**44.83**	**11**	**3.16**	**5**	**+**	**7.84**	**3**	**–**	**4.06**	**<0.05**
YOGy	**OWG**	**3**	**st11**	**NA**	**23.74**	**6**	**1.39**	**4**	**+**	**4.61**	**2**	**–**	**6.39**	**<0.05**

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CHAPTER 4

Functions of duetting in the White-breasted Wood-Wren revealed by microphone-array recordings and multispeaker playback

Birds sing to attract prospective mates and to deter potential rivals from their territories. Only males sing in most temperate passerine species; however, female song is common in the tropics, and in some species, pairs coordinate their songs into vocal duets. The role of duets in signaling cooperation and conflict within a pair reflects a species' life history and the evolutionary processes underling this complex form of communication. Here I examine the duetting behavior of the White-breasted Wood Wren (*Henicorhina leucosticta*) from a lowland rainforest in Southern Mexico. I utilized dual–speaker playback experiments that realistically simulated duetting intruders and a wireless sensor array developed at UCLA to localize the position of multiple interacting singers from recordings of their vocalizations. White-breasted Wood Wrens responded aggressively to playback by increasing their solo song and duetting output and by approaching the speakers. Opposite sex solos elicited responses from both sexes, and duets provoked greater or equal amounts of duetting from pairs compared to both same sex and opposite sex solos. My results support the view that the principle function of duetting in this species is cooperation for protecting mutual resources and argue against an important role for mate defense during this study period.

4.1 Introduction

Avian duets are joint vocal displays by mated individuals, in which singing is coordinated and usually alternates between partners. The degree of coordination varies from loosely overlapping bouts of song to precisely fitted antiphonal vocalizations (for reviews see Farabaugh 1982; Langmore 1998; Hall 2004; Mann et al. 2009). This complex behavior has been identified in 55 families of birds; however, the function of duetting appears to vary among species and there is no apparent explanation for that variation (Hall 2009). Nor is it known in most duetting species whether the fitness interests of each sex are aligned in producing this behavior or if they conflict. For example, species may sing duets cooperatively to defend mutual resources – especially if duets provide greater territorial defense and repel intruders more effectively than would singing alone (Langmore 1998; Hall 2000; Grafe and Bitz 2004). Alternatively, duetting may be directed toward establishing and maintaining pair bonds (Thorpe 1972; Todt and Hultsch 1982). If intruders represent a greater threat to their pair bond or mating status than to their territory, duets may advertise the partner's mated status to same-sex outsiders thereby preventing the partner from deserting (Hall 2000; Mulder et al. 2003), or from being usurped by same-sex rivals (Seddon and Tobias 2006; Grafe and Bitz 2004). Determining whether the dominant function of duetting in a particular species is cooperative or antagonistic, informs the observer how cooperation and conflict related to life history characteristics is achieved and complex communication systems evolved.

It should be possible to identify which explanation for duetting is more important by observing the birds' behavior toward simulated intrusions. Both the territory defense and mate defense hypotheses predict agonistic responses to simulated intruders in the form of elevated singing rates and approaches to playback speakers. However, predictions differ in the relative magnitude of responses to duets, same-sex solos, and opposite-sex solos. Duets that serve predominantly in mutual territory defense should: (1) be sung primarily in response to paired (duetting) intruders, who pose the greatest threat to the territory and little threat to the pair bond; (2) aggressive responses should be greater in magnitude toward duetting intruders than solo intruders; and (3) responses should not be biased towards same-sex intruders, unless a sex-specific division of labor exists in territorial defense. Alternatively, if duets function predominantly to defend mates, individuals should: (1) respond more strongly to same-sex solos than opposite-sex solos or to duets; and (2) join their partner to create duets more often and more promptly during same-sex solos. By creating duets a bird prevents its mate from solo-singing and thereby minimizes threats from same-sex rivals.

To help identify the role of duetting and distinguish between the territory and mate defense hypothesis, I employed two experimental techniques. First, I used a wireless eight-node (32 microphone) sensor array capable of accurately localizing vocalizations in playback conditions (Collier et al. 2010). Second, I implemented a multi-speaker playback design, a proven method for simulating two acoustic signals originating from separate sources (Logue and Gammon 2004; Mennill and Ratcliffe 2004; Rogers et al. 2004; Douglas and Mennill 2010). I observed and compared the response of territorial pairs to playbacks of simulated pairs of intruders or to simulated intrusions by a male or female alone.

4.2 Methods

4.2.1 Study population and experimental design

Field experiments were conducted between 17 January and 3 February 2010 near Estación Chajul, in the Montes Azules Biosphere Reserve, Chiapas, Mexico (16º 6′ º44"N and 90º 56′ 27"W; See Chapter 1 for a description of the field site). The experiments involved 14 individuals (7 pairs). Territories were determined during prior field seasons (see Chapter 1) by catching birds, attaching color bands to all males and 6 of 7 females, then visually tracking the color-marked individuals.

The microphone sensor array consisted of eight nodes, each a self-contained, four channel microphone array based on an embedded computer (VoxNet; Collier 2010). Nodes communicate to each other and to an on-site laptop via wireless networking, enabling remote monitoring and control of the array from outside of the focal recording area. Importantly, the VoxNet array can acoustically self-survey the relative locations of nodes without GPS coverage or manual surveying measurements, permitting the rapid deployment of the array. Localization methods utilized the relatively slow propagation of sound by cross-correlating time delays between recordings of an event (i.e. song) from each of the 32 microphones across a 0.1 m lattice of potential source locations. Source locations were estimated by summing the envelopes of the cross-correlation functions across the lattice then identifying the point that had the maximum correlation (see Collier 2010 for details of the procedure). In an earlier study, the array was capable of 0.445 m ± 0.500 m localization accuracy for free-ranging wild Mexican antthrush (*Formicarius* *moniliger*) songs from this field site using similar localization algorithms (Collier et al. 2010). That error in localization accuracy is likely to be a conservative estimate for White-breasted Wood Wren localizations given that Mexican antthrushes, unlike the wood wrens, sing mostly from the ground, where reflections and reverberations reduce the precision of localizations (Collier 2010). Multiple localizations of playbacks from the same location in the present study support this estimate.

I used a dual speaker playback design consisting of two loudspeakers (PAL, Tivoli Audio, Cambridge, MA, U.S.A.) mounted 1.5 m high on tripods or small trees and separated by 10 m. Speakers were placed parallel to territory boundaries at a distance of 20 m within the focal territory and facing the center of the territory. An iPod (Apple, Cupertino, CA, U.S.A.) connected both of the speakers through a stereo speaker cable fitted with 3.5 mm stereo plugs.

Playback treatments included: (1) male solos, (2) female solos, and (3) two-speaker (male + female) duets. To control for the possible effect of speaker position (i.e. responses influenced by the proximity of a bird or by the locations of it's preferred singing perches), I administered two rounds of duet playback as separate trials, with the male and female duet contributions reversed between speakers. Each pair was given one to two treatments (in a single day) separated by at least one hour.

Each experimental trial included 10 minutes of pre-playback recording, 2 minutes of playback, and eight minutes of post-playback recording. Male and female responses were localized with the program locGui (Travis Collier https://grassi2.ucdavis.edu/~travc/voxnet/Analysis/) and the distance between duetting partners calculated as well as the distance to each playback speaker in use. All treatments were given to territorial pairs between 0900 and 1200 on two consecutive days. To reduce any effect of presentation order on responses, I followed a counterbalanced design to determine the order of treatments. I included presentation order as a fixed effect during analysis to assess whether there was an interaction between order and treatment.

4.2.2 Playback Construction and Protocol

Playbacks were constructed from single channel digital recordings of non-neighboring birds from the local population (WAV files; 16-bit, 44 kHz). I selected two frequently used male song type (song type 1) and female song type (female song type 1) that were shared among all study subjects and that had been observed together as duets in the field (see Chapter 2). I viewed spectrograms of songs to selected exemplars with a high signal-to-noise ratio and little background noise within the frequency range of the White-breasted Wood Wren songs. I then normalized their amplitude and digitally filtered them below the minimum frequency and above 8 kHz. 120 second solo (single channel) stimulus files were constructed using AUDCITY 1.3.12 software (http://audacity.sourceforge.net). The playbacks consisted of the same sound repeated every four seconds, a rate within the normal range of song delivery in this population (see Chapter 2). To construct duets, male and female components were copied to separate channels of a blank 2-channel WAV file such that the female song component followed the male component by 0.1 seconds and retained the rate of one song (per sex) every 4 seconds. Thirty seconds of silence were added to the beginning of the playback to allow the observer time to step away from the playback apparatus.

4.2.3 Data Analysis

Statistical analysis was conducted through the use of linear mixed models (LME) in R 2.15.0 (R Core Development Team 2010) implementing the ‘‘nlme’’ package (Pinheiro et al. 2013). I used focal pair identity as a random effect and fixed-effect treatments were playback source (male solo, female solo, or duet), sex (male or female), and within pair trial number (first or second). I examined the interactions between treatment × sex and treatment × order. The interaction between treatment and trial number were never statistically significant so I dropped these terms from the final analysis and do not report them in the results.

My response measures included physical approach metrics and singing behavior metrics. I analyzed three physical approach metrics: closeness of approach, approach latency (the time from the first playback song to the first song sung within 20 m, and approach duration (the time spent within 20 m), in addition to three singing behavior metrics: song rate per minute, song latency, and song switching.

To assess the physical approach of the subjects, I analyzed the closest distance that each subject came to the playback speakers. If a subject did not sing in response to playbacks and was not observed to approach the playback source I used an arbitrary distance of 50 m, which is similar to the distances observed from other weak responses to playback (maximum distance 55 m). Birds were often visually occluded by vegetation, so the actual distances of non-singing birds sometimes had to be estimated without visual confirmation. I calculated the latency to approach as the amount of time lapsed from the end of the first song in the playback and the beginning of the first song in the response that occurred within 20 m of a playback speaker. If a bird did not approach within 20 meters during the 600 s focal period, an approach latency of 300 s was used that was comparable to other weak responses. Analyses also were run with a maximum approach latency of 600 s without changing the qualitative relationship between treatments; however, 300 s is a more conservative estimate and I will therefore discuss only these results. Finally, the approach duration was calculated as the number of seconds a singing bird spent within 20 m of a playback speaker during the 10-minute focal period.

To measure singing behavior I calculated singing rate of the response as the total number of songs per minute during the ten-minute assessment period including both playback and post-playback periods. In addition to overall song rate, I analyzed three categories of singing behavior individually: female solos, male solos, and duets. I compared the latency to response as the time lapsed from the end of the first song in the playback to the beginning of the first song of the response. If the subject did not sing until after the post playback period, the latency to sing was recorded as 300 s, commensurate with other weak responses.

Song-type switching was calculated as the number of song type changes in a bout. The amount of song switching did not necessarily equal the number of different song types sung in a trial because some birds switched back to a previous song type later in the trial.

I examined patterns of duet formation across treatments by noting which sex created and terminated each duet. The lack of precise alternation between male and female duet components precluded the assessment of duets on a per song basis (see Chapter 2). Rather, the bird that joined their partner's bout already in progress was counted as creating the duet. I considered a duet to be terminated by the sex that failed to respond to their partner's song. I assessed a bird's propensity to duet as the proportion of its mate's songs that were joined to form a duet divided by the total number of songs in the mate's response (solos and duets). For example, a male's duet propensity was the number of female-led duets / female-led duets + female solos. In other words it is a subjects' propensity to duet with a partner that is already singing.

4.3 Results

Male and female White-breasted Wood Wrens almost always responded strongly to playbacks that simulated territory intrusion by rival birds singing solos and duets. Their responses were graded by how closely they approached the intrusion source and by the heightened rate of their singing compared to the pre-playback period.

4.3.1 Approach

Closest approach distance was affected by treatment (LME: $F_{2,38} = 6.834$, $P = 0.0029$) with a non-significant trend for sex (LME: $F_{2,38} = 3.617$, $P = 0.0815$) with closer approaches by males than females (Figure 1). There was no interaction between treatment and sex (LME: $F_{2,36} = 0.289$, $P = 0.7510$; Table 1.1; Figure 1). Both sexes approached duet speakers closer than male treatment speakers (females; LME: $T_{19} = 2.096$, $P = 0.0497$; males; $T_{19} = 3.153$, $P = 0.0052$; Table 1.2). Approach latency was not significant for treatment (LME: $F_{2,38} = 2.801$, $P = 0.0733$) or sex (LME: $F_{2,38} = 3.874$, $P = 0.0729$), and there was no interaction between sex and treatment (LME: $F_{1,12} = 0.341$, $P = 0.713$, Table 2.1; Figure 2). There was a trend for males to approach duets sooner than female solos ($T_{19} = 2.016$, P = 0.0581) but all other contrasts were not significant (P > 0.19; Table 2.2). The time subjects spent within 20 m was influenced by treatment (LME: $F_{2,38} = 4.559$, $P = 0.0168$) and sex (LME: $F_{1,12} = 8.977$, $P = 0.0111$) with a non-significant interaction between treatment and sex (LME: $F_{2,38} = 0.350$, $P = 0.7067$; Table 3.1; Figure 3). Treatment was not a significant effect within sexes (Female LME: $F_{2,19} = 2.503$, $P = 0.1084$; Male LME: $\mathrm{F}_{2,19} = 2.556$, $P = 0.104$;) but there was a trend for both sexes (not significant in males) to stay longer within 20 m of duets than male solos (Females: $T_{1,19} = -2.235$, $P = 0.0376$; Males: $T_{1,19} = -1.955$, $P = 0.0655$; Table 3.2).

4.3.2 Singing Behavior

Song rate was influenced by treatment (LME: $F_{3,92} = 37.928$, $P = <0.0001$) and sex (LME: $F_{2,12} = 12.448$, $P = 0.0042$) and there was not a significant interaction between treatment and sex overall (LME: $F_{3,92} = 1.413$, $P = 0.244$; Table 4.1; Figure 4). Both sexes sang more in response to playback than during the pre-playback period (all $P \leq 0.03$; Table 4.2). Within sexes, only females had significant variation between other playback treatments, singing fewer songs in response to male solos than to duets or female solos ($T_{46} = 3.343$, $P = 0.0017$; $T_{46} = 4.462$, $P = 0.0001$ respectively).

Solo and duet rates followed different patterns (Figure 5). Duet rates were nearly equal between sexes (mean ± SD, females: 25.6 ± 15.8 songs/min, males: 24.5 ± 18.9 songs/min, paired t test: $t_{21} = 0.49$, $P = 0.628$) so sex was excluded from the final analysis of duet rate without changing any relationships. Duet rate was highly influenced by treatment (LME: $F_{3,95} = 46.370$, $P = <0.0001$; Table 5.1; Figure 5a) with a similar number of duets preceding playback and in response to male solos ($T_{95} = 1.267$, $P = 0.2084$) and more duets in response to duets and female solos (all contrasts $P < 0.0001$; Table 5.2). Subjects produced duets at similar rates in response to duets and to female solos ($T_{95} = -0.881$, $P = 0.3806$).

Solo song rate was influenced by playback treatment (LME: $F_{3,92} = 7.699$, $P = <0.0001$) and by sex (LME: $F_{3,12} = 12.047$, $P = 0.0005$) with higher song rates in males and no interaction between sex and treatment (LME: $F_{3,92} = 0.933$, $P = 0.4279$; Table 5.1; Figure

5b and 5c). Males sang significantly more solos in response to male solos than during pre-treatment intervals and marginally more solos in response to female solos than pre-treatment (T_{46} = 2.514 P = 0.0155; T_{46} = 1.941, P = 0.0583 respectively; Table 5.2). Females sang more solos in response to female solos than to any other treatment (P < 0.182) and more in response to duets than during the pre-treatment (T_{46} = 2.532, P = 0.0148) with an intermediate number of solos in response to male solos (pre × male solos: T_{46} = 1.701, P = 0.0956; duet × male solos: T_{46} = -0.237, P = 0.8135; Table 5.2).

Song latency was not affected by treatment ($F_{2,38}$ = 1.324, P = 0.2781) or sex ($F_{1,12}$ = 1.180, P = 0.2988) and there was not a significant interaction between treatment and sex ($F_{2,38}$ = 1.22, P = 0.3063; Table 6.1; Figure 6). There was little pattern in response between treatments within sexes (all P > 0.11; Table 6.2). Song latency models were sensitive to the inclusion of arbitrary values for the seven instances when a subject did not sing (5 female, 2 male); however, excluding these trials did not make differences between treatments significant (females: $F_{2,14}$ = 0.809, P = 0.4649; males: $F_{2,17}$ = 2.324, P = 0.1281).

The propensity to duet was affected by treatment ($F_{3,21}$ = 4.055, P = 0.0202) and sex ($F_{1,11}$ = 8.081, P = 0.016) and there was not a significant interaction between treatment and sex ($F_{2,21}$ = 1.353, P = 0.917; Table 7.1; Figure 7). Models were sensitive to the high heterogeneity of variance between treatments and sexes (group size ranged from 1 to 11 trials in which a partner's song could be answered to create a duets). Therefore, treatment level contrasts are presented for reference in Table 7.2 but are not discussed in detail within the text. Figure 7 is also consolidated by sex. Pooled across treatments, males joined more of their partner's songs to form duets (71 ± 10%) than females (23 ± 7%; t-test: $t_{26.5} = -3.752$, P = <0.001; Figure 7).

4.4 Discussion

Microphone sensor arrays enable the precise spatial visualization of multi-bird interactions important for delineating predictions for duetting. The VoxNet sensor array is portable and can be deployed within a few hours at a new location without the need of wires or GPS. Acoustic location systems enable the localization of all vocalizing birds within the recording range and thus avoid the need for capturing and fitting birds with radio transmitters and many of the challenges associated with radio tracking birds (Logue 2007). Multi-speaker playback experiments permit the discrimination of same-sex approach to the speakers and more accurately simulate intruders than single speaker playback methods (Rogers et al. 2004).

White-breasted Wood Wren pairs increased their song output and approached the speakers in response to playback. In particular, the aggressive behavior in response to simulated paired intruders suggests that duets, in this study, play an important role in territory defense as suggested by Thorpe (1972). Both sexes engaged in duets at similar levels between same-sex solos and duets, suggesting that birds don't increase responsiveness to their partners when facing unpaired same-sex intruders.

Viewed as a whole, White-breasted Wood Wrens exhibited aggressive behaviors consistent with a coordinated territorial defense strategy against conspecific rivals and showed little evidence of intra-pair conflict associated with mate defense. Although both territorial and mate defense hypotheses predict elevated responses toward intruders, they vary in their predictions between mated (i.e. duetting) and unmated (i.e. solo singing) rivals. Specifically, the mate defense hypothesis predicts that: (1) unpaired same-sex rivals pose the greatest threat to a birds partnership or mating status and should thus provoke the greatest response from individuals, (2) solos should rarely be produced in response to opposite-sex solo playback and (3) birds should primarily sing duets in response to same-sex solo playback. Male responses in this study do not support any of these predictions. Males responded with equal or greater intensity to duets than same-sex solos and showed no bias in approach to male speakers when faced with duets. In contrast females responded strongly to both treatments that contained female song (female solos and duets) and had a tendency to respond weakly to male solos at comparable rates to pre-treatment levels.

Males exhibited behaviors that were notably absent of any biases toward same-sex intruders and showed an equivalent or greater response to simulated duetting intruders than to male solos across response measures. Males approached speakers broadcasting duets more closely than same-sex solos and had a tendency to stay close to the playbacks for a longer period than during same sex solos. Males did not show a strong bias toward the male speaker during duets with close approaches often at intermediate distances to the two playback sources. None of these observations were consistent with behaviors expected of males defending partners from conspecific rivals and suggest that males regard intruders of both sexes as significant threats to their territory and of little threat to their pair bond.

Females also exhibited strong responses to both duets and female solos compared to male solos, producing more vocalizations and responding more quickly to duets and female solos. Similar responses to duets and female solos suggest that females pose a threat to other females regardless of the mating status of the intruder and are consistent with patterns of sex-specific territory defense found in other species.

Sex specific singing behavior is known in other duetting species, and may be especially prevalent in monochromatic species with year-round territories and pair bonds. For example, females respond more strongly to songs from their own sex, either solos or duets, and males respond to all conspecific playback in Happy wrens, *Pheugopedius felix* (Templeton et al. 2011), buff-breasted wrens, *Cantorchilus leucotis* (Gill et al. 2007), rufous-and-white wrens, *Thryophilus rufalbus* (Mennill 2006), tropical boubous, *Laniarius aethiopicus* (Grafe and Bitz 2004), and Steere’s liocichla, *Liocichla steerii* (Weng et al. 2012).

Sex-specific behaviors could result from intersexual conflict and mate defense, or be due to different territorial threats posed by intruders based on sexual size dimorphism or variation in sex ratio (Hall 2004). As pointed out by Winker et al. (1996), male White-breasted Wood Wrens are larger than females and are sexually monochromatic. My data show no evidence of biased sex ratios in the White-breasted Wood Wren (see Chapter 1). Sex-specific repertoires for this species may be better suited to same-sex territory defense and countersinging interactions than opposite-sex interactions.

My data showing low levels of intra-pair conflict in the White-breasted Wood Wren also supports the inference that pair bonds are relatively stable and that competition for resources associated with territories is stronger than competition for mates or mating opportunities. The rates of extra-pair paternity remain unknown for this species. However, territory turnover is likely relatively low in this population; many territories remained unchanged during three years of study at this site and some of the first banded pairs remained partnered at least five years (see Chapter 2).

The breeding season of the White-breasted Wood Wren is most predominant from April to September (Winker et al. 1996); my study showed little evidence of mate defense occurred during the fringe of the non-breeding season when females are not yet totally fertile. However, juveniles begin reaching sexual maturity by December (Winker et al. 1996) suggesting this could be a period of increased territorial intrusions and increased risk of mate loss or being usurped. The function of duets may change seasonally with respect to fertility in some species, as has been suggested in *T. rufalbus* (Topp and Mennill 2008). However, there is also evidence of intersexual conflict and mate defense during the nonbreeding and pre-breeding season, for example in the Australian magpie-larks (*Grallina cyanoleuca*; Hall 2000). Other species that respond aggressively to intruders with duets outside of the breeding season include the slate-coloured boubou (*Laniarius funebris*; Sonnenschein and Reer 1983), the bay wren, (*Cantorchilus nigricapillus*; Levin 1988), and the rufous-and-white wren (*Thryophilus rufalbus*; Topp and Mennill 2008).

No single explanation describes the function of duetting among all duetting species. Duetting is a phylogenetically diverse behavior that has arisen independently in multiple lineages and under potentially divergent selective constraints (Mann et al. 2009). Additionally, changing life-history characteristics within lineages may have led the exaptation of duets for different purposes between species (Mann et al. 2009; Bradley and Mennill, 2009). My study provides evidence that for the White-breasted Wood Wren, duetting does not enhance mate defense, but serves a beneficial function in defending a territory. More studies examining duetting both within and across lineages are required to establish connections between the evolutionary and life-history characteristics underlying the function of duetting in different species and enable inferences across organizational levels.

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Figures and Tables

Figure 1

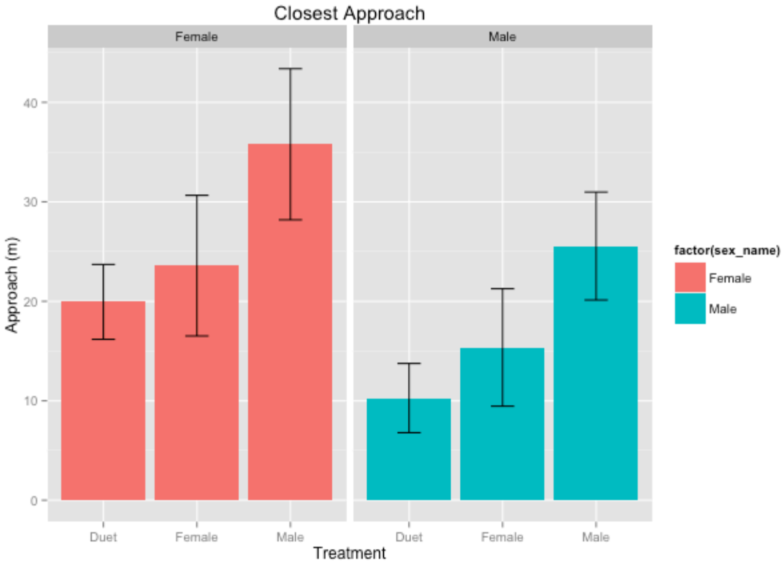

Table 1.1

Model: Closest Approach	**Fixed Effects**	**numDF**	***d.f***	***F***	***P<***
Treatment × Sex	(Intercept)	1	38	168.137	<.0001
	Treatment	2	38	6.834	0.0029
	Sex	1	12	3.617	0.0815
	Treatment × Sex	2	38	0.289	0.7510
Females Only	(Intercept)	1	19	112.344	<.0001
	Treatment	2	19	2.288	0.1288
Males Only	(Intercept)	1	19	60.248	<.0001
	Treatment	2	19	4.980	0.0182

Table 1.2

Contrasts: Closest Approach	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	4.239	0.525	19	8.074	<.0001
Duet × Female Solo	0.209	0.703	19	0.298	0.7691
Duet × Male Solo	1.474	0.703	19	2.096	0.0497
Female Solo × Male solo	1.264	0.812	19	1.558	0.1358
Males:					
(Intercept)	2.747	0.523	19	5.252	<.0001
Duet × Female Solo	0.781	0.666	19	1.173	0.2554
Duet × Male Solo	2.101	0.666	19	3.153	0.0052
Female Solo × Male solo	1.319	0.769	19	1.715	0.1026

Figure 2

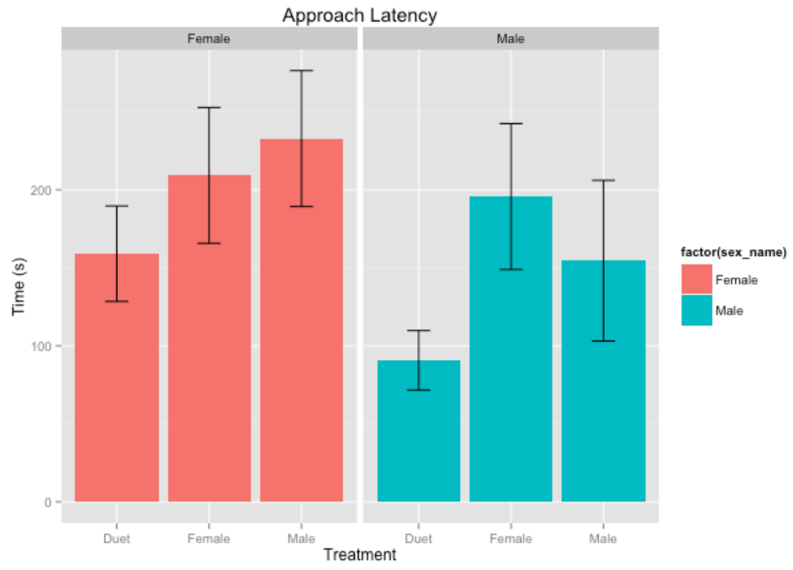

Table 2.1

Model: Approach Latency	**Fixed Effects**	**numDF**	***d.f***	***F***	***P<***
Treatment × Sex	(Intercept)	1	38	373.458	<.0001
	Treatment	2	38	2.801	0.0733
	Sex	1	12	3.864	0.0729
	Treatment × Sex	2	38	0.341	0.7130
Females Only	(Intercept)	1	19	215.653	<.0001
	Treatment	2	19	1.063	0.3650
Males Only	(Intercept)	1	19	158.769	<.0001
	Treatment	2	19	2.133	0.1459

Table 2.2

Contrasts: Approach Latency	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	11.734	1.251	19	9.377	<.0001
Duet × Female Solo	2.126	2.167	19	0.981	0.3389
Duet × Male Solo	2.914	2.167	19	1.344	0.1947
Female Solo × Male solo	0.787	2.503	19	0.315	0.7565
Males:					
(Intercept)	8.992	1.189	19	7.562	<.0001
Duet × Female Solo	4.152	2.060	19	2.016	0.0581
Duet × Male Solo	2.257	2.060	19	1.096	0.2869
Female Solo × Male solo	-1.896	2.378	19	-0.797	0.4352

Figure 3

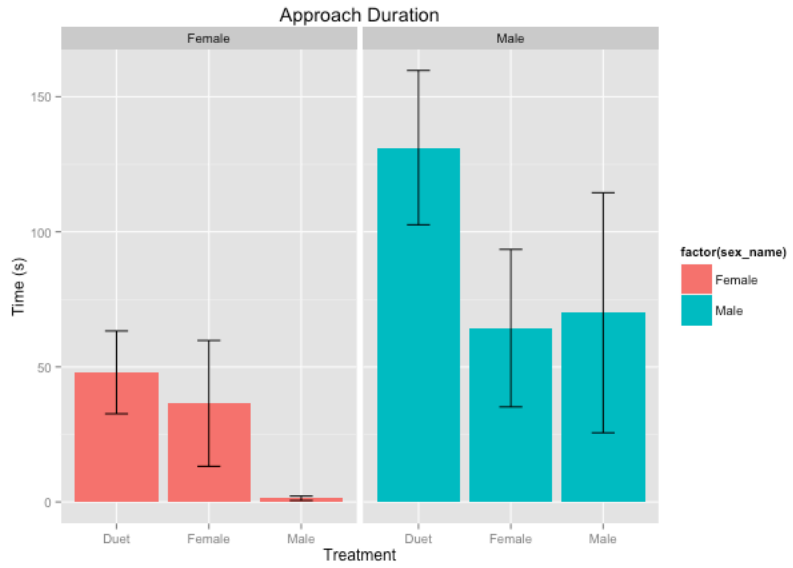

Table 3.1

Model: Approach Duration	**Fixed Effects**	**numDF**	***d.f***	***F***	***P<***
Treatment × Sex	(Intercept)	1	38	67.634	<.0001
	Treatment	2	38	4.559	0.0168
	Sex	1	12	8.977	0.0111
	Treatment × Sex	2	38	0.350	0.7067
Females Only	(Intercept)	1	19	21.388	0.0002
	Treatment	2	19	2.503	0.1084
Males Only	(Intercept)	1	19	40.933	<.0001
	Treatment	2	19	2.556	0.1040

Table 3.2

Contrasts: Approach Duration	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	5.248	1.141	19	4.600	0.0002
Duet × Female Solo	-1.653	1.976	19	-0.837	0.4133
Duet × Male Solo	-4.417	1.976	19	-2.235	0.0376
Female Solo × Male solo	-2.764	2.282	19	-1.211	0.2406
Males:					
(Intercept)	10.330	1.622	19	6.369	<.0001
Duet × Female Solo	-4.353	2.527	19	-1.723	0.1012
Duet × Male Solo	-4.940	2.527	19	-1.955	0.0655
Female Solo × Male solo	-0.587	2.918	19	-0.201	0.8426

Figure 4

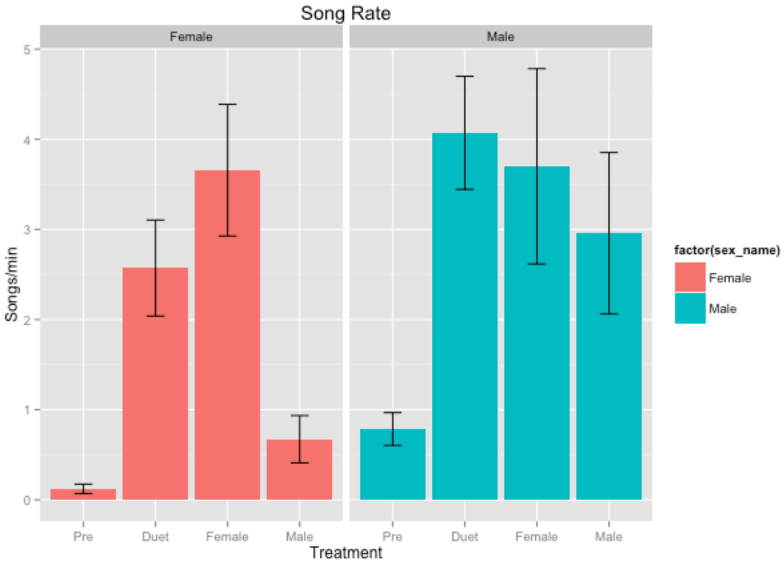

Table 4.1

Model: Song Rate	**Fixed effects**	**numDF**	***d.f***	***F***	***P<***
Treatment × Sex	(Intercept)	1	92	213.629	<.0001
	Treatment	3	92	37.928	<.0001
	Sex	1	12	12.448	0.0042
	Treatment × Sex	3	92	1.413	0.2440
Females Only	(Intercept)	1	46	80.048	<.0001
	Treatment	3	46	30.910	<.0001
Males Only	(Intercept)	1	46	133.601	<.0001
	Treatment	3	46	13.368	<.0001

Table 4.2

Contrasts: Song Rate	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	0.146	0.107	46	1.357	0.1812
Pre-treatment × Duet	1.268	0.170	46	7.467	<.0001
Pre-treatment × Female	1.702	0.219	46	7.766	<.0001
Pre-treatment × Male	0.465	0.219	46	2.122	0.0393
Duet × Female Solo	0.434	0.240	46	1.809	0.0769
Duet × Male Solo	-0.803	0.240	46	-3.343	0.0017
Female Solo × Male solo	-1.237	0.277	46	-4.462	0.0001
Males:					
(Intercept)	0.625	0.139	46	4.494	<.0001
Pre-treatment × Duet	1.272	0.227	46	5.609	<.0001
Pre-treatment × Female	1.174	0.293	46	4.012	0.0002
Pre-treatment × Male	0.885	0.293	46	3.022	0.0041
Duet × Female Solo	-0.097	0.321	46	-0.304	0.7625
Duet × Male Solo	-0.387	0.321	46	-1.207	0.2334
Female Solo × Male solo	-0.290	0.370	46	-0.782	0.4380

Figure 5

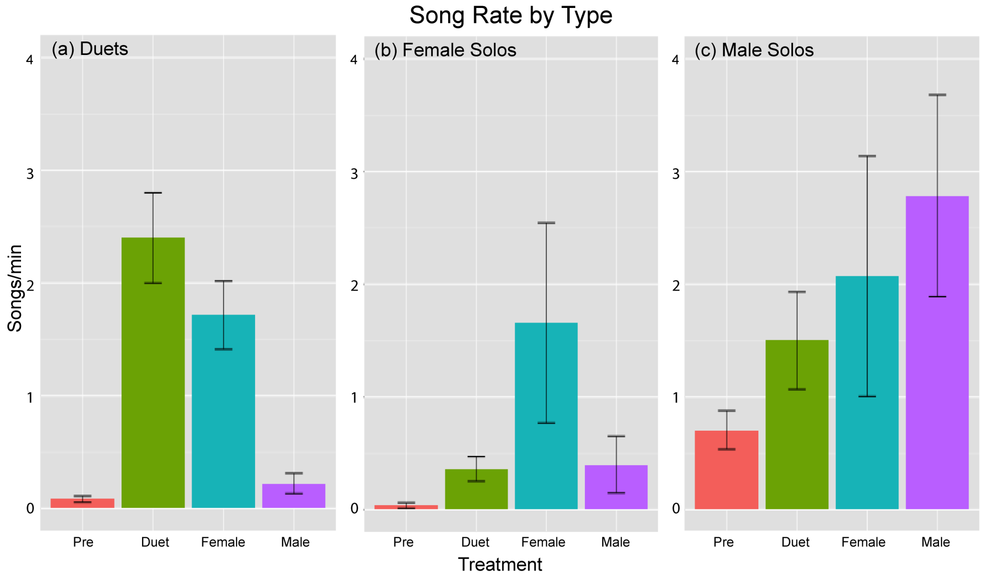

Table 5.1

Model	**Fixed effects**	**numDF**	***d.f***	***F***	***P<***
Duet Rate: Treatment	(Intercept)	1	95	82.451	<.0001
	treat_order	3	95	46.370	<.0001
Solo Rate: Treatment × Sex	(Intercept)	1	92	97.347	<.0001
	Treatment	3	92	7.699	0.0001
	Sex	1	12	22.047	0.0005
	Treatment × Sex	3	92	0.933	0.4279
Solo Rate: Females	(Intercept)	1	46	25.932	<.0001
	Treatment	3	46	8.356	0.0002
Solo Rate: Males	(Intercept)	1	46	71.244	<.0001
	Treatment	3	46	2.930	0.0434

Table 5.2

Contrasts:	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Duets:					
(Intercept)	0.108	0.078	95	1.380	0.1707
Pre-treatment × Duet	1.212	0.114	95	10.603	<.0001
Pre-treatment × Female	1.070	0.148	95	7.248	<.0001
Pre-treatment × Male	0.187	0.148	95	1.267	0.2084
Duet × Female Solo	-0.142	0.162	95	-0.881	0.3806
Duet × Male Solo	-1.025	0.162	95	-6.341	<.0001
Female Solo × Male solo	-0.883	0.187	95	-4.729	<.0001
Female Solos:					
(Intercept)	0.059	0.084	46	0.700	0.4872
Pre-treatment × Duet	0.366	0.144	46	2.532	0.0148
Pre-treatment × Female	0.895	0.186	46	4.801	<.0001
Pre-treatment × Male	0.317	0.186	46	1.701	0.0956
Duet × Female Solo	0.529	0.204	46	2.592	0.0127
Duet × Male Solo	-0.048	0.204	46	-0.237	0.8135
Female Solo × Male solo	-0.578	0.236	46	-2.451	0.0181

Table 5.2 (cont.)

Contrasts:	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Male Solos:					
(Intercept)	0.580	0.142	46	4.079	0.0002
Pre-treatment × Duet	0.366	0.246	46	1.486	0.1441
Pre-treatment × Female	0.618	0.318	46	1.941	0.0583
Pre-treatment × Male	0.800	0.318	46	2.514	0.0155
Duet × Female Solo	0.251	0.348	46	0.721	0.4743
Duet × Male Solo	0.434	0.348	46	1.244	0.2197
Female Solo × Male solo	0.182	0.402	46	0.453	0.6528

Figure 6

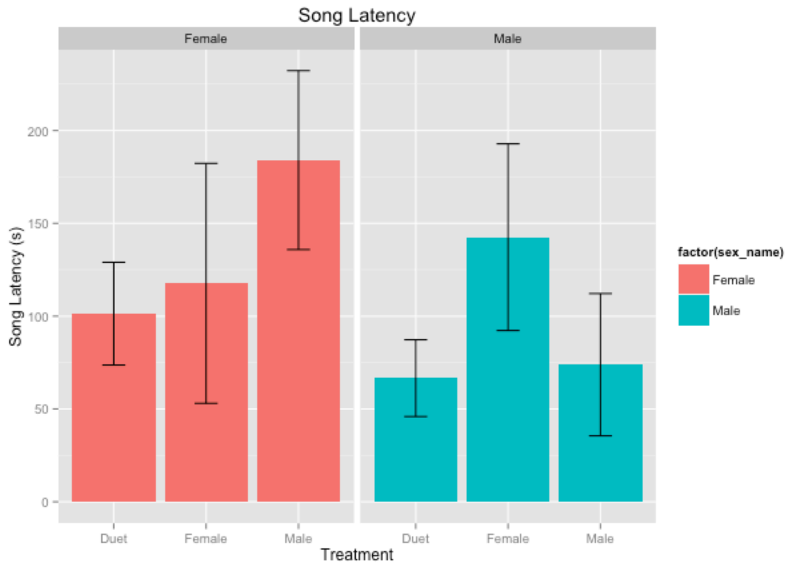

Table 6.1

Model: Song Latency	**Fixed effects**	**numDF**	***d.f***	***F***	***P<***
Treatment × Sex	(Intercept)	1	38	599.050	<.0001
	Treatment	2	38	1.324	0.2781
	Sex	1	12	1.180	0.2988
	Treatment × Sex	2	38	1.221	0.3063
Females Only	(Intercept)	1	19	288.664	<.0001
	Treatment	2	19	1.379	0.2758
Males Only	(Intercept)	1	19	296.966	<.0001
	Treatment	2	19	1.172	0.3311

Table 6.2

Contrasts: Song Latency	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	3.899	0.346	19	11.274	<.0001
Duet × Female Solo	0.306	0.589	19	0.519	0.6099
Duet × Male Solo	0.979	0.589	19	1.661	0.1132
Female Solo × Male solo	0.673	0.681	19	0.989	0.3352
Males:					
(Intercept)	3.646	0.317	19	11.506	<.0001
Duet × Female Solo	0.808	0.549	19	1.473	0.1572
Duet × Male Solo	0.053	0.549	19	0.097	0.9241
Female Solo × Male solo	-0.755	0.634	19	-1.192	0.2480

Figure 7

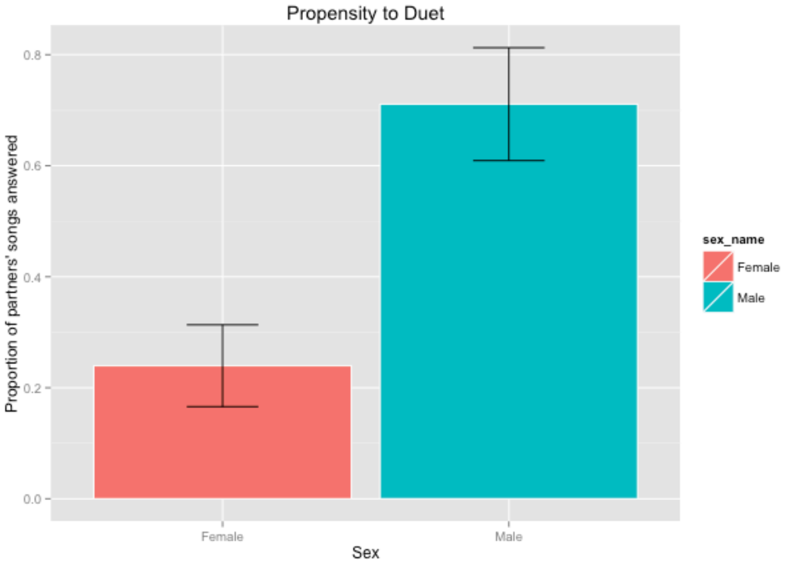

Table 7.1

Model: Propensity	**Fixed effects**	**numDF**	***d.f***	***F***	***P<***
Treatment × Sex	(Intercept)	1	21	49.590	<.0001
	Treatment	3	21	4.055	0.0202
	Sex	1	11	8.081	0.016
	Treatment × Sex	3	21	1.353	0.2845
Females Only	(Intercept)	1	16	11.463	0.0038
	Treatment	3	16	1.703	0.2066
Males Only	(Intercept)	1	5	52.074	0.0008
	Treatment	3	5	1.283	0.3756

Table 7.2

Contrasts: Propensity	**Value**	**Std.Error**	***d.f.***	***T***	***P<***
Females:					
(Intercept)	0.182	0.109	16	1.670	0.1143
Pre-treatment × Duet	0.130	0.195	16	0.667	0.5141
Pre-treatment × Female	0.329	0.195	16	1.692	0.1100
Pre-treatment × Male	-0.158	0.195	16	-0.814	0.4277
Duet × Female Solo	0.200	0.228	16	0.874	0.3950
Duet × Male Solo	-0.288	0.228	16	-1.263	0.2247
Female Solo × Male solo	-0.488	0.228	16	-2.137	0.0484
Males:					
(Intercept)	0.857	0.369	5	2.325	0.0676
Pre-treatment × Duet	-0.002	0.391	5	-0.006	0.9955
Pre-treatment × Female	-0.410	0.426	5	-0.963	0.3797
Pre-treatment × Male	-0.399	0.451	5	-0.883	0.4176
Duet × Female Solo	-0.408	0.250	5	-1.633	0.1633
Duet × Male Solo	-0.396	0.291	5	-1.360	0.2319
Female Solo × Male solo	0.011	0.336	5	0.033	0.9746

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