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UNIVERSITY OF MIAMI

THE ONTOGENY OF DUETS IN A NEOTROPICAL BIRD, THE CANEBRAKE
WREN

By

Karla D. Rivera-Cáceres

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

May 2017

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the requirements for the degree of
Doctor of Philosophy

THE ONTOGENY OF DUETS IN A NEOTROPICAL BIRD,
THE CANEBRAKE WREN

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(Ph.D., Biology)

The Ontogeny of Duets in a Neotropical Bird, the Canebrake Wren (May 2017)

Abstract of a dissertation at the University of Miami.

Dissertation supervised by Professor William A. Searcy.

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Interactions in which individuals exchange vocal signals are an important aspect of communication in many species. Human conversation provides one familiar example. Other examples drawn from songbirds are countersinging between territorial males and duetting between mated males and females. The development of vocal signals such as human speech and the song of songbirds has been amply studied, but, we know significantly less about the development of the rules that mediate vocal interactions in these groups. Because of the interactive nature of duets, duetting species provide an ideal model system to study the development of vocal interaction rules. Duets involve specific relationships among the vocalizations from different individuals. Two vocal interaction rules arise from these relationships: precise temporal coordination in the responses to a partner and non-random association of song types (i.e. duet codes). In this dissertation I study the proximate and developmental mechanisms by which a Neotropical bird, the canebrake wren (*Cantorchilus zeledoni*) acquire both vocal interaction rules. Regarding temporal coordination, results in this dissertation indicate a) that canebrake wrens achieve temporal coordination by dynamically modifying their singing tempo based on their own and their partner's

feedback, b) temporal coordination is acquired during early development very likely through learning and c) after re-pairing canebrake wrens perform duets with lower coordination but improve with time, indicating that they need a rehearsal period to achieve highly coordinated duets with a new partner. Regarding duet codes, my dissertation research indicates a) that juvenile canebrake wrens learn the duet codes of the adults they are reared by and b) adult canebrake wrens must learn a new duet codes when they acquire a new partner. Overall, results show that duet coordination and duet codes are honest indicators of pair-bond duration. In this dissertation I also provide evidence that vocal interaction rules in humans and songbirds are analogous and thus, the study of duetting rules in songbirds could help us understand the development, function, and mechanics of human conversation rules. These studies are the first to report the development of interaction rules in birds.

DEDICATION

I would like to dedicate this thesis to my daughter Priya. I hope to always be the example you deserve. All my love, always.

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Chapter 1

Introduction

Tinbergen (1963) suggested that how behaviors develop ought to be one of the four main questions addressed by the science of animal behavior. Since then, the arguments in favor of studying the ontogeny of behavior have grown. First, to be able to obtain an integrated view of any behavior, one has to understand not only the stage at which the behavior has an evident function but also the ontogenetic stages that lead to this behavior (Bateson & Laland, 2013). Thus, for example, studies of the ontogeny of dispersal, aggression, and dominance behaviors in canids and rodents have proven key to understanding the origin of different intra and interspecific adult behavioral phenotypes (Bekoff, 1977). Developmental studies of sexual behaviors in primates, including humans, have led to the discovery of the causes of early sexually dimorphic behaviors and their effect on adult sexual behavior (Bailey & Zucker, 1995; G. R. Brown & Dixson, 2000). Another example in rodents is that male rats that are reared by nurturing mothers (i.e. high levels of pup licking and grooming) exhibit phenotypes more resilient to anxiety as adults (Weaver et al., 2004).

Second, natural selection might act not only during the apparent functional stage of the behavior but also during its development, and thus the evolutionary trajectory of the behavior can be closely linked to its ontogeny (West-Eberhard, 2005). As one example, the learned aspects of song in many songbirds reflect the response of individuals to stress faced early in life and thus can provide an accurate indicator of individual (usually male) quality (Nowicki et al., 1998). Adult

swamp sparrows (*Melospiza georgiana*) that are deprived of food temporarily during early development show a lower repertoire size and lower quality of learned songs than swamp sparrows that developed normally (Nowicki et al., 2002a). In song sparrows, females respond more to songs that have been more accurately copied (Nowicki et al., 2002b). In European starlings (*Sturnus vulgaris*), nutritional history and social status during development affect the male's song repertoire size, a trait under sexual selection (Spencer et al., 2004). Finally, female zebra finches (*Taeniopygia guttata*) prefer songs of males that have not been stressed during early development and that consequently sing more syllable types per song (Spencer et al., 2005).

Song learning development

The song of songbirds has become the premier system used to address questions concerning the development of behavior (P. R. Marler & Slabbekoorn, 2004). Several decades of studies of song development have produced a comprehensive idea of how male songbirds acquire their individual song structures. Male songbirds consistently learn their songs during a critical period, usually at the juvenile stage. Juveniles develop their songs in two steps: first they listen to singing adults and memorize songs or parts of song (sensory phase); later, juveniles go through a period of rehearsal, when they improve the accuracy of their songs through practice (sensorimotor phase) (Nelson & Marler, 1994).

An array of laboratory and field experiments have shown that the details of song development vary across species (Catchpole & Slater, 2008). For example,

studies concerning the sensitive learning period show that some species are able to learn only during their first year of life, e.g. indigo buntings, *Passerina cyanea* (Lachlan et al., 2004), while others learn songs throughout their lives, e.g. canaries, *Serinus canaria* (Nottebohm et al., 1986). Most species need both exposure to tutor song and rehearsal to acquire normal repertoires while other species might only need the rehearsal period, e.g. grey catbirds, *Dumetella carolinensis* (Kroodsma et al., 1997). The degree to which birds are constrained on which song models they learn also differs between species: from a tight canalization to species-specific parameters, as in swamp sparrows (Ballentine et al., 2004), to being able to learn almost any vocalization, as in northern mockingbirds *Mimus polyglottos* (Derrickson, 1987).

Neural control of birdsong learning is performed by a discrete neural circuit of hormone-dependent nuclei in the anterior forebrain pathway (AFP) including the HVC, the robust nucleus of the arcopallium (RA), area X, the medial portion of the dorsolateral nucleus of the thalamus (DLM) and the lateral portion of the magnocellular nucleus of the anterior nidopallium (IMAN) (Brenowitz & Beecher, 2005). This architecture is similar to the architecture of many cerebral structures in mammals (Theunissen et al., 2008). HVC and RA are considered analogues of motor cortical areas specialized for vocal production, while area X is homologous to the basal ganglia and the auditory forebrain is analogous to auditory association cortex (Farries & Perkel, 2008).

The sensory phase of song learning occurs when the model songs are most likely stored in higher-order auditory forebrain areas (e.g. the caudal media

nidopallium) of young birds (Bolhuis et al., 2000). The sensorimotor phase occurs when the memorized songs are compared to auditory feedback from own song production which is processed by the brainstem and relayed to the forebrain and song production systems. The difference between stored and produced song is then used as a corrective signal which in turn modifies neural circuits involved in vocal production (Theunissen et al., 2008). Based on anatomical lesions and pharmacological studies it appears that LMAN region is involved in preventing the crystallization of songs during the learning period (Scharff & Nottebohm, 1991), while RA and HVC are involved in allowing the accurate production of memorized songs (Aronov et al., 2008; Simpson & Vicario, 1990).

Although, as reviewed above, much is known about the development of individual song repertoires and neural pathways controlling song production and learning, vocal signaling often involves structured interactions between multiple signalers. Songbirds therefore must develop not only the ability to produce their individual songs, but also the ability to use those songs in the most effective way in replying to other individuals. Important examples of vocal interactions in male songbirds are countersinging (Todt & Naguib, 2000), song type matching (Beecher et al., 2000) and frequency matching (Otter et al., 2002). One of the most complex type of vocal interaction, which is rather common in tropical regions, is duetting between mated pairs (Hall, 2009). My research has focused on the ontogeny of the rules that individuals use to vocally interact while duetting. In the next section, I cover the current knowledge of this behavior including what is known about its ontogeny.

Duetting

Duetting can be defined as the production of coordinated vocalizations by two individuals (Hall, 2009). The production of a duet is an obligatory interactive process that involves time and pattern specific relationships among the vocalizations from each individual. Two key aspects of duets arise from these relationships: precision in song answering – i.e. precise temporal coordination in the responses to a partner (Todt & Naguib, 2000) – and non-random association of song types – i.e. duet codes (Logue, 2007). Temporal association between songs from different individuals can also exist in species in which only the males sing, for example during countersinging (Geberzahn & Hultsch, 2004), but the coordination is nowhere as precise as in duetting species. Also, in non-duetting species, males can sing song types non-randomly with respect to the songs sung by other individuals, for example in song-matching (Beecher & Brenowitz, 2005), but these associations are often less strict than in duetting species (i.e. song 1 from male A does not have to be answered by song 2 of male B). Hence, in the strict sense temporal coordination and non-random association of vocalizations are absent in non-duetting species and thus are of special interest in duetting species (Hall, 2009).

Duetting is distributed across a variety of taxa including insects such as phaneropterid bushcrickets (Dobler et al., 1994), anurans such as the South African clawed frog, *Xenopus laevis* (M. L. Tobias et al., 1998), primates such as siamangs, *Hylobates syndactylus* (Maples et al., 1989) and many species of birds (Hall, 2009). Within birds, duetting has evolved multiple times and is

present in at least 18 passerine and 32 nonpasserine families (Benedict, 2008). Most duetting species are found in tropical and south-temperate regions; hence it has been hypothesized that ecological and life history differences between these regions and north-temperate areas might play a role in favoring duet evolution (Slater & Mann, 2004).

After a century of exhaustive research to elucidate the form of coordinated singing in birds, it is clear that there are multiple duetting patterns across species ranging from the alternation of simple songs by males and females, for example in eastern whipbirds, *Psophodes olivaceus* (Rogers, 2005), to unison singing of a single song type, for example in male-male pairs of lekking manakins, *Chiroxiphia spp* (Trainer et al., 2002), to alternation of multiple song types tied by a defined set of rules, as in black-bellied wrens, *Pheugopedius fasciatoventris* (Logue et al., 2008).

There has also been extensive research concerning the functions of duets. Studies show that duetting serves multiple purposes both within and between species. These functions include a) joint territorial defense for example in black-bellied wren, *Thryothorus fasciatoventris* (Logue & Gammon, 2004), Australian magpie-larks, *Grallina cyanoleuca*, (Rogers et al., 2004), and plain wrens, *Cantorchilus modestus zeledoni*, (now canebrake wren, *Cantorchilus zeledoni* (L. Marshall-Ball & Slater, 2008)), b) pair bonding for example in Australian magpie-larks (Hall & Magrath, 2007), and mate guarding, for example in tropical boubous, *Laniarius aethiopicus* (Grafe & Bitz, 2004).

Duet ontogeny

In contrast to the popularity of functional studies of duetting, the ontogeny of duets has largely been ignored. In part, it has been assumed that, at least in the passerine duetting species such as *Thryothorus* wrens (Mann et al., 2009), repertoire acquisition should not differ from the non-duetting species (Hall, 2009). There is some evidence that supports this view: slate-coloured boubous, *Laniarius funebris* (Wickler & Sonnenschein, 1989) and bay wrens, *Cantorchilus nigricapillus* (Levin et al., 1996) develop abnormal songs when raised in isolation, suggesting that song learning is important in duetting species. Some indirect evidence that supports the idea that individuals learn their song repertoires during early development (i.e. individual repertoire learning hypothesis) is that the repertoires of birds from numerous duetting species vary on a micro-geographic scale, for example in canebrake wrens (Marshall-Ball and Slater, 2008) and rufous-and-white wrens, *Thryothorus rufalbus* (Mennill & Vehrencamp, 2005).

However, it is unknown if the two key rules of duets (i.e. temporal precision and duet codes) are learned. Some indirect evidence supports the idea that performing temporally coordinated and coded duets requires learning. Birds from three species of *Thryothorus* wrens make phrase-by-phrase adjustments to their song tempo to be able to duet with precision: black-bellied wrens (Logue et al., 2008), plain-tailed wrens, *Pheugopedius euophrys* (Fortune et al., 2011), and canebrake wrens (Rivera-Cáceres, 2015). Such adjustments suggest that temporal precision is not the result of a genetically determined fixed action

pattern. Additionally, the non-random associations between the song types of partners (i.e. duet codes, Logue, 2006) of several species, including bay wrens (Levin, 1996) and canebrake wrens (Mann et al., 2003), are pair-specific (i.e. differ between pairs). Therefore, it is unlikely that these duet codes are innate, and thus they should require learning during early development, adulthood or both.

Study Species

The canebrake wren (*Cantorchilus zeledoni*) is a tropical songbird classified within the group of the former *Thryothorus* wrens (Mann et al., 2009). Until recently, the canebrake wren was considered a subspecies of plain wren (*Cantorchilus modestus zeledoni*). However, a study in 2015 revealed that enough genetic, morphological and behavioral differences existed to be considered a separate species (Saucier et al., 2015). Chapter 2 of this thesis was published before the official change was made. Thus, throughout Chapter 2 I continue to use the official name as it was correct at the time: plain wren. In chapters 3 and 4 I use the new name: canebrake wren.

Both male and female canebrake wrens sing often and are highly coordinated when duetting. Pairs associate their song types non-randomly – that is, they possess duet codes (Mann et al., 2003). Furthermore, male and female canebrake wrens possess sex specific repertoires (L. Marshall-Ball & Slater, 2008). Canebrake wrens inhabit second growth pasture (Mann et al., 2003), where they are relatively easy to observe compared to forest species. Finally, canebrake wren juveniles have been observed in adult territories, often duetting

with the adult pair (pers. obs.). Juveniles are highly responsive to playback and so are easy to capture. The occurrence of duetting in young juveniles provided the opportunity to study the early ontogeny of duetting in the field.

Canebrake wrens are distributed throughout the Caribbean Slope of southern Nicaragua, Costa Rica, and western Panama (Clements et al., 2016). Data for the second chapter and some of chapter 3 were collected at La Suerte Field Station (10°26'N, 83°47'W) in Costa Rica. The remaining data were collected at La Selva Biological Station (10°26'N, 83°59'W) in northeastern Costa Rica. Canebrake wren territories are distributed in overgrown pasture often including marshland (Mann et al., 2003). The area around both field stations includes a mixture of lowland moist forest, swamps, scrub, and cattle pasture, and thus plenty of canebrake wren territories are available (~20 at each station). The canebrake wren starts to breed late in the dry season (which lasts from January to May) and continues through the rainy season (L. Marshall-Ball & Slater, 2003). All studies for this dissertation were carried out during the breeding season (between April and August).

Canebrake wren song

In the canebrake wren, both male and female phrases are composed of stereotyped sequences of notes (i.e. between 1 and 5 continuous traces on a spectrogram). Phrases can be sung by one individual alone (solo song) or by a male and female together (duet song). Males possess two phrase categories: I (introductory) phrases and M phrases. I phrases are composed of 3-6 high frequency (4-9 kHz) notes that can be sung solo or as the beginning of a duet. M

phrases are composed of one or two lower frequency (1-4 kHz) notes that are usually (more than 90% of the time) sung during duets after a female answers an I phrase. Female canebrake wrens possess only one phrase category: F phrases. These are composed of a chevron-shaped wide-frequency (3.5-8.5 kHz) note plus one or two short low-frequency (1-4 kHz) notes. A typical duet is composed of the three phrase categories, starting with an I phrase followed by several cycles (on average 7) of alternating F and M phrases (Mann et al., 2003) (Figure 1.1). Each individual possesses on average more than 15 phrase types of each category. To perform a duet, members of a pair join their phrases in an alternating manner (i.e. avoiding overlap) and the phrase types that they use are linked in a non-random way (i.e. using duet codes, (Logue, 2007)) (Mann et al., 2003).

The purpose of this research was to determine for the canebrake wren the proximate mechanisms and ontogeny of two vocal interaction rules: duet temporal precision and duet codes. In the first observational study I recorded adult canebrake wrens to determine how duet coordination was achieved in this species. In this study, I addressed whether a) temporal coordination is the result of dynamic modification of the singing tempo of individuals and b) temporal precision is achieved by integrating an individual's own singing tempo and its partner's singing tempo. In the second study, I followed the development of juvenile canebrake wrens to determine if duet coordination and duet codes are acquired during early development. In this study, I addressed whether a) juvenile canebrake wrens show a lower level of duet coordination and adherence to duet

codes than adults, b) juveniles improve their temporal coordination and duet code adherence through time and c) juveniles use the same duet codes as their parents. The third study was composed of two field experiments to address the ontogeny of duetting rules during adulthood. First I temporarily removed one member of each pair of canebrake wrens that I sampled to determine whether adult duet codes are flexible and thus a) single adult canebrake wrens are able to immediately respond to unfamiliar phrases and b) whether their coordination is lower when answering these unfamiliar phrases. The second experiment involved the permanent removal of one member of each pair of canebrake wrens to induce new pair formations. In this second experiment, I determined whether adults need to learn new duet codes after re-pairing and thus a) individuals use the same or different duet codes with new partners compared to the duet codes they used with old partners, b) newly formed pairs of canebrake wrens perform duets with lower coordination and lower adherence to a consistent duet code and c) whether temporal coordination and duet code adherence improve with time.

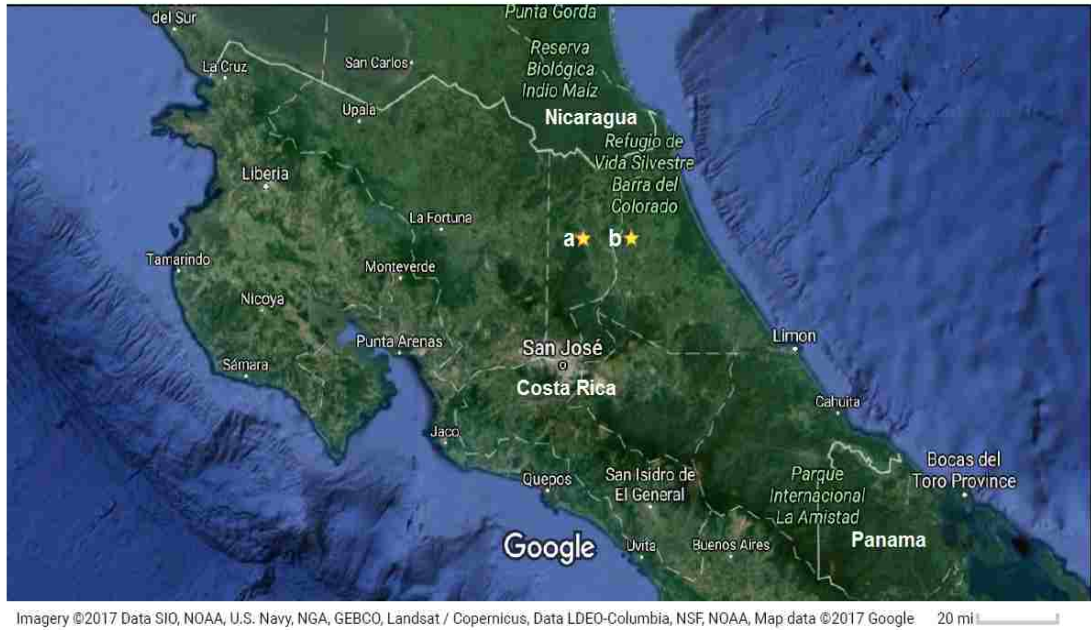


Figure 1.1 Map of study sites. a. La Selva, Biological Station. b. La Suerte, Field Station.

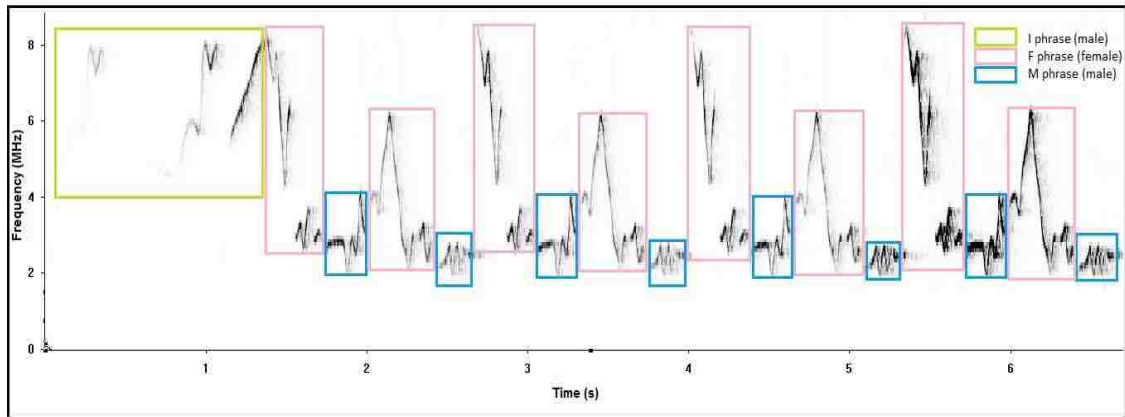


Figure 1.2 Example of a duet in canebrake wrens.

Chapter 2

Plain wrens (*Cantorchilus modestus zeledoni*) adjust their singing tempo based on self and partner's cues to perform precisely coordinated duets

Summary

Precise coordination appears to be an important signal in several duetting species. However, little attention has been directed to the proximate mechanisms that might drive this behavior. To perform highly coordinated duets, individuals can either have an intrinsic fixed singing tempo or modify their singing tempo based on cues in their own and their partner's songs. In this study, I determined whether autogenous and/or heterogeneous factors are associated with duet coordination in plain wrens, *Cantorchilus modestus zeledoni*, by analyzing recorded duets from 8 territorial pairs in the field. Previous research has determined that plain wrens perform highly coordinated antiphonal duets with almost no overlap. I found that to achieve such precise coordination individuals perform phrase-by-phrase modifications to the duration between two consecutive phrases (inter-phrase interval) based on a) whether their song is answered, b) the phrase type used in the duet and c) the position of the inter-phrase interval within the duet. Moreover, there are several sex differences in how individuals use these cues to modify their inter-phrase intervals. Females produce shorter inter-phrase intervals when their mates answer a phrase, whereas males produce longer inter-phrase intervals when their mates answer. Females modify their inter-phrase intervals based only on the phrase type their mates sing,

whereas males modify their inter-phrase intervals based on both the phrase that they sing and the phrase the females use to answer. Both males and females produce longer inter-phrase intervals for longer phrase types sung by their partners, but males do so with more precision than do females. Finally, both sexes increase their inter-phrase intervals as the duet progresses. That precise coordination is achieved by a complex and dynamic process supports the idea that this behavior could signal pair bond strength.

Background

Various animals perform cooperative display behaviors to defend shared resources (McComb, 1992). It has been hypothesized that through these cooperative displays, organisms may honestly advertise their competitive abilities (McComb et al., 1994; Radford, 2003). One feature of joint displays that might advertise competitive abilities is precise coordination (Hall, 2009). For duetting songbirds in particular it has been proposed that precise vocal coordination in duet performances, signals pair stability (Brumm & Slater, 2007), and thus has an intimidating effect on territorial rivals (Hall & Magrath, 2007).

Duet coordination varies greatly between species, from loose temporal association, e.g. banded wren, *Thryophilus pleurostictus* (Mann et al., 2009), to precisely-timed phrases either sung simultaneously, e.g. *Campylorhynchus* wrens (Selander, 1964) and lance-tailed manakin, *Chiroxiphia lanceolata* (DuVal, 2007) or in a non-overlapping manner (i.e. antiphonal; e.g. plain wren, *Cantorchilus modestus zeledoni* (Mann et al., 2009), orange-chinned parakeet,

Brotogeris jugularis (Power, 1966), Australian magpie larks (Hall, 2006) and yellow-naped amazon, *Amazona auropalliata* (Wright & Dahlin, 2007).

Presumably, it is among those species with precise timing that coordination is most difficult to achieve, and thus serves best as a signal of pair stability.

However, the mechanisms by which this complex behavior is achieved are poorly understood.

Three types of control are possible for individuals to be able to perform precisely-timed duets. First, individuals that engage in precisely timed duetting could sing temporally associated phrases as fixed action patterns initiated with a common cue (Payne & Skinner, 1970). Second, a singer could adjust its timing in response to what that singer itself has already sung (i.e. autogenous feedback) (Logue et al., 2008). Third, a singer could adjust its timing in response to what its partner has previously sung (i.e. heterogeneous feedback) (Fortune et al., 2011). A precisely-timed duet could then be the result of either a single type of control or a combination of different types of control.

To test these alternative mechanisms, Logue and collaborators (2008) analyzed naturally-sung duets from black-bellied wrens and measured the time at which each member of the pair started and ended a phrase. These authors also performed playback experiments in which a female or a male from a pair duetted artificially with the phrases coming from the speaker. Results indicate that a male modifies his singing timing firstly based on the beginning of the female's previous phrase and secondly on the beginning of his own previous phrase. A female modifies the timing of her song based on the ending of the male's previous

phrase and secondly on her own previous phrase. Fortune et al. (2011) analyzed duets and solo songs of plain-tailed wren and found that both males and females produce longer inter-phrase intervals (i.e. the interval from the ending of a bird's phrase to the beginning of its next phrase) when they sing alone. Fortune et al. (2011) also found that while duetting, males sometimes fail to sing their phrase and that in response females increase the inter-phrase interval duration. Finally, Templeton et al. (2013) performed a playback experiment with captive male happy wrens (*Pheugopedius felix*) and found that individuals are able to modify their singing tempo in response to that of the speaker. Thus, all three studies provide evidence that duet coordination is achieved through phrase-by-phrase adjustments based on autogenous and heterogeneous feedback.

Additional cues that have not previously been studied might also affect duet timing. In several duetting species, both males and females possess repertoires of song types and follow specific rules (duet codes) that determine which of their own song types they use to answer each of their partner's song types (Logue, 2006). If song types vary in duration, then which song type the singer produces and which song type the partner answers with could both affect duet coordination. If so, then one would predict that timing might be adjusted in response both to which song type the singer last sung and which song type the partner used to reply. Also, it is possible for singing rates to change as a duet progresses (Templeton et al., 2013). If so, then the timing with which answers are produced might vary systematically with position of a phrase (early versus late) in a duet.

In this study I use measurements of inter-phrase intervals of recorded duets in the field to test whether male and female plain wrens achieve precise duet coordination via the modification of their temporal singing pattern using phrase-by-phrase adjustments. I used General Linear Mixed Modeling to test whether the duration of inter-phrase intervals is associated with a) whether or not a phrase is answered, b) the previous phrase type sung by self, c) the phrase type that the duet partner uses to answer, and d) the position of the phrase within a duet.

Materials and methods

Ethics statement

Research in Costa Rica was performed under a scientific research permit (No. 05354) provided by the Ministry of Environment, Energy and Telecommunications (MINAET) of Costa Rica.

Plain wren duets

Plain wrens sing highly precise antiphonal duets which are composed of three sex-specific categories of phrases: two phrase categories sung by the male, an introductory phrase (I phrase) and an M phrase, and one phrase category sung by the female (F phrase). A typical duet is composed of all three categories of phrases, starting with an I phrase followed by several cycles (on average 7) of alternating F and M phrases. Each individual possesses a repertoire of each phrase category, composed of around 15-25 phrase types

(Mann et al., 2003). To sing a duet a pair follows a duet code (Logue, 2006) that for each member of a pair determines which phrase type to sing after a specific phrase type from its partner (Mann et al., 2003). This duet code is strictly followed; Mann et al. (2003) found that a switch in the males' phrases followed a switch in the females' phrases 88% of the time, while a switch in females' phrases followed a switch in males' phrases 86% of the time. Conversely, if females or males kept singing the same phrase type, then their partners remain using one phrase type 95% of the time.

Study site and subjects

Plain wrens were studied at La Suerte Field Station and its surrounding areas in northeastern Costa Rica (10°26'N, 83°47'W). The area around the field station includes a mixture of lowland moist forest, swamps, scrub, and cattle pasture, where plain wrens are common (Mann et al., 2003). I studied 8 pairs of territorial plain wrens during the summer of 2012 (June 18th- July 16th). All individuals were captured and provided with a unique combination of colored leg bands for further identification.

Data collection

To obtain duet and solo song recordings I used a Marantz PMD660 digital recorder and a unidirectional Sennheiser ME66 microphone. Songs were recorded under two conditions: natural singing (no artificial stimulus, from 6:00 am to 9:00 am) and in response to a simulated intrusion using playback (from 4:00 pm to 6:00 pm). At least 4 hours of recording per territory were made to get most or all of the birds' repertoire (Mann et al., 2003).

Data analysis

Song and duet repertoires

To create a library of each bird's repertoire, I created spectrograms of the recordings using SYRINX software (J. M. Burt www.syrinxpc.com) using a Hanning window and a 512pt FFT. Four songs of good quality were chosen as representatives of each phrase type for M and F phrase categories. The phrase types were determined based on visual inspection (Figure 2.1) (Nowicki & Nelson, 1990). To determine which male and female phrase types composed each duet I compared the classified phrase types to each duet spectrogram. A duet that comprised the same male and female phrases was designated as a duet type. Duets with the same male phrase and female phrase were considered the same duet type even if they contained different introductory phrases. Pairs sang on average 19.25 ± 3.25 duet types.

To assess whether plain wrens coordinate the timing of phrase alternation during duets using heterogeneous feedback, I measured the duration of the interval between two phrases from the same individual (hereinafter referred to as inter-phrase interval) using Raven Pro 1.4 (2011). The measurements were standardized using a Hanning window (window size 512 points) and a temporal resolution of 5.8ms. For every pair, three duets of each type were chosen randomly to measure inter-phrase interval duration for both male and female phrases. For each inter-phrase interval, I registered a) whether the phrase before the interval was answered by the mate, b) the position of the interval within the

duet (first interval, second interval, etc.), c) the subject's own phrase type before the interval, d) the phrase type that the mate used to answer, and e) the interaction between the subject's own phrase and the phrase its mate used to answer (Figure 2.2). As pairs use a duet code to sing a duet, the phrases that the male and the female use are correlated and thus c) and d) could have presented a problem of collinearity in the model (Dormann et al., 2013). However, as the duet codes are pair specific, the correlation coefficient was very low for the female model (0.118) and within the acceptable range for males (0.558) considering the large data set used (Booth et al., 1994). Thus these variables remained as independent explanatory factors in both models. I used source of recordings (natural vs. playback recordings) as a covariate. Source of recording had no significant effect on the duration of intervals for males (effect estimate=0.0000513, $T_{1860}=0.02055$, $p=0.98$) and thus was removed from the final model. The effect of source of recordings for females was significant (i.e. females produce shorter intervals when they answer to playback) and thus this covariate remained in the final model (effect estimate= -0.01, $T_{2440}=-5.74$, $p<0.0001$).

Statistical analysis

I used the function `lme` of the package `nlme` (Pinheiro et al., 2013) in R (version 2.15.1) to create two General Linear Mixed Models (GLMM, one for females and one for males) with individual and duet as nested random factors and variables a), b), c), d) and e) (above) as fixed factors. As the effect of phrase type was significant in predicting the duration of inter-phrase intervals, I created a

third GLMM to determine if there was a relationship between phrase type length and inter-phrase interval. A fourth GLMM was created to compare inter-phrase interval between males and females. For this model individual and duet were set as nested random factors while sex was set as the fixed factor. One way in which individuals could modify their inter-phrase interval duration according to the phrase type their mates use to answer is by increasing the intervals for longer phrase types. To determine if the females and males increased their inter-phrase intervals for longer phrase types I performed two GLMMs (one for females and one for males) with individual and duet as nested random factors and phrase type length as a fixed factor. R^2 marginal was calculated for both models using the `r.squaredGLMM` function (Johnson, 2014) in the MuMIn package (Bartoń, 2013).

The random structure of the models included Individual and Duet as nested random factors to account for the non-independent nature of the data. To select the optimal fixed effects structure for the models I followed the method suggested by Diggle et al. (2002). A full model (a+b+c+d, above) was compared to other models in which each explanatory variable was removed at a time using ML estimation and comparing the likelihood criteria. If the likelihood value was significantly lower for the model with the removed variable, then that variable remained in the final model. If there was no significant difference between the likelihood values of the model with and without the variable, then that variable was removed from the final model. I validated the final model by assessing violations to homogeneity and normality based on the graphic methods

suggested by Zuur et al. (2009). To determine violations of homogeneity, indicated by an increase in the spread for larger values, I plotted the residuals of each model against the fitted values and against each explanatory variable. To verify normality a histogram of the residuals for each model was built. When homogeneity was violated, I altered the variance structure of the fixed factor that seemed to have heterogeneous variances by using the function `varIdent` in the `lme` packet.

Results

In total 2578 inter-phrase intervals from 470 duets from 8 females (58.75 ± 8.20 duets per female) and 2254 intervals from 388 duets from 8 males (48.5 ± 7.15 duets per male) were analyzed. The number of inter-phrase intervals measured per pair was 323 ± 53.21 for females and 281 ± 40.30 for males. Inter-phrase interval duration was significantly longer in males than in females (male intervals = 0.499 ± 0.07 , female intervals = 0.33 ± 0.04 , effect estimate = -0.18 ± 0.01 , $t_{4230} = -17.51$, $p < 0.001$). Furthermore, female phrases were longer (0.447 ± 0.056 s) than male (M) phrases (0.29 ± 0.032 s, effect estimate = 0.137 , $t_{606} = 26.05$, $p < 0.001$).

Precise coordination in males and females

Both male and female plain wrens sang highly coordinated contributions to alternating duets. Females answered male phrases on average 0.064 ± 0.035 seconds after the end of each male phrase and overlapped only 2% of male phrases. Males answered female phrases on average 0.046 ± 0.034 seconds after the end of each female phrase and overlapped only 7% of female phrases.

Inter-phrase interval duration in females

A full model for females containing all the possible variables (see above) plus the interaction between female and male phrase was compared to models with fewer explanatory variables using the likelihood criteria. The interactions between male and female phrase and the female phrase variables were omitted from the final model as their contribution to explaining the duration of female inter-phrase intervals was non-significant. The remaining variables - male phrase, whether the phrase was answered or not and the position of the inter-phrase interval within the duet - significantly contributed to explaining the duration of female inter-phrase intervals (Table 2.1). The model selected to explain the duration of the inter-phrase intervals for females revealed that females produce significantly longer inter-phrase intervals when they were not answered by their partner (effect estimate= 0.036, $t_{2438}=8.81$, $p<0.001$, Figure 2.3a). There is a significant, but smaller effect of the phrase type that the male used to answer on the females' interval duration (effect estimate= -0.0006765, $t_{2438}= -7.03$, $p<0.001$), while there was no effect of the female's own prior phrase type (effect estimate= 0.0001, $t_{2438}= 0.97$, $p=0.34$). Lastly, females produced significantly longer inter-phrase intervals as the duet progressed (effect estimate= 0.0035, $t_{2438}=-19.95$, $p<0.001$, Figure 2.4a).

Inter-phrase interval duration in males

A full model for males containing all the possible variables (see above), plus the interaction between female and male phrase was compared to models with fewer explanatory variables using the likelihood criteria. The female phrase

was omitted from the final model as its contribution to explaining the duration of male inter-phrase intervals was non-significant. The remaining variables – the interaction between female and male phrase, male phrase, whether the phrase was answered or not and the position of the inter-phrase interval within the duet – significantly contributed to explaining the duration of male inter-phrase intervals (Table 2.2). The model selected to explain the duration of the inter-phrase intervals for males revealed that males produced significantly longer inter-phrase intervals when they were answered by their partner (effect size= -0.1, $t_{1861}=-11.75$, $p<0.001$, Figure 2.3b). Unlike females, males significantly modified their inter-phrase intervals based on the phrase they themselves used before the interval (effect estimate= 0.002, $t_{1861}= 6.35$, $p<0.001$) and on the interaction between that phrase and the phrase that the female used to answer him (effect estimate= -0.0004, $t_{1861}=-2.84$, $p=0.0044$). However, the effect of the interaction was small. Lastly, males produced significantly longer inter-phrase intervals as the duet progressed (effect estimate=0.006, $t_{1861}=-32.04$, $p<0.001$, Figure 2.4b).

Inter-phrase interval based on phrase type duration

I showed above that interval length depends on the phrase type of the partner, and now test whether birds modify their inter-phrase interval duration to accommodate variation in the length of their mates' phrase type. Both males and females significantly increased the duration of their intervals for longer phrase types from their mates. However, the effect was weak for females (effect estimate =0.35, $t_{324}=3.75$, $p<0.001$, R^2 marginal = 0.03, Figure 2.5a) and strong

for males (effect estimate =0.86, $t_{324}=24.18$, $p<0.001$, R^2 marginal= 0.60, Figure 2.5).

Discussion

Avoiding overlap: Answered vs unanswered phrases

Both female and male plain wrens perform phrase-by-phrase adjustments to their inter-phrase intervals using autogenous and heterogeneous feedback to achieve precise coordination. Similar adjustments are made by black-bellied wrens (Logue et al., 2008), plain-tailed wrens (Fortune et al., 2011) and happy wrens (Templeton et al., 2013). In plain wrens, however, there are sex differences in the way individuals adjust their inter-phrase intervals according to different cues. Males produce longer intervals than females regardless of other variables. The difference in duration of the intervals between males and females is consistent with the idea that individuals actively avoid overlapping their partners' phrases, as female phrases are longer than male (M) phrases. Avoiding overlap of partner's song has been regarded as an important consequence of precise coordination in other duetting species (Hall & Magrath, 2007; Templeton et al., 2013; J. A. Tobias & Seddon, 2009). Additionally, female plain wrens produce longer intervals when their partners do not answer while males produce shorter intervals when their partners do not answer. The duration of unanswered intervals for both males and females is very similar (Fig. 2.3) and thus could represent the intrinsic tempo (i.e. set internal rhythm with which individuals sing their songs) (Payne & Skinner, 1970), and each sex could then adjust its singing tempo based on both internal and external cues, e.g African barbets (Payne &

Skinner, 1970) and codas of sperm whales, *Physeter microcephalus* (Schulz et al., 2008). Males could then increase the duration of their intervals when they are answered to fit the longer duration of female phrases, while females decrease the duration of their intervals for the shorter male phrases. However, further research is needed to determine if an internal tempo exists in this species.

Adjusting interval duration based on phrase type and phrase type duration: evidence for coordination learning?

Plain wrens also show a sex difference in how they adjust their timing in response to phrase type. Females only modify their intervals based on the phrase type sung by their partner (heterogeneous feedback only), whereas males modify their intervals based on both their own phrase type and their partner's phrase type (autogenous and heterogeneous feedback).

Plain wrens adhere to a pair specific duet code, meaning that an individual chooses a specific song type based on the preceding song type sung by their mate (Marshall-Ball et al., 2006). Given that codes differ between pairs, it is likely that the rules to answer each phrase are learned. As partner phrase type affects the inter-phrase interval of both males and females it is possible that individuals are not just learning the phrase type they should use to answer but also the timing with which they should use that phrase. Templeton et al. (2013) found that male happy wrens are able to answer any of their mates' songs with the correct code (i.e. not using a random song) with a very short latency and without overlap (high precision), starting with their first response to the playback. Furthermore, Logue et al. (2008) found that female black-bellied wrens' response is influenced

by the starting time of their mates' songs. These results could suggest that happy wrens and black-bellied wrens are able to calculate the duration of the sung phrase ahead of time based on memory of their mates' repertoire and not just because of their fast auditory response time (Grimes, 1965; Power, 1966; Thorpe, 1963). One rule birds could learn to answer in a precise way is to wait more time for longer phrase types. Results indicate that male plain wrens strictly increase their inter-phrase intervals for longer female phrases. Females also produce longer inter-phrase intervals for longer male phrases but they do so with less consistency. It may be more important for males than for females to follow this rule, as female phrases are more variable in duration than are male phrases (female 0.186s- 0.629s C.V. = 15.38, male 0.131s-0.304s C.V. = 9.29).

Duet coordination and pair bond strength

That a trait is costly to produce is usually taken as evidence that the trait could be used as a signal to indicate quality (Grafen, 1990). In this study, I show that both male and female plain wrens produce longer inter-phrase intervals as the duet progresses. Two explanations seem possible for this pattern. First, a fixed internal tempo could indicate to slow down for later phrases. This internal tempo could be present in both sexes, or just in one sex and the other sex could adjust its singing timing based on the timing of its partner's song. The second possibility is that maintaining a high singing rate is costly and individuals find it difficult to maintain their initial tempo. Using a playback experiment, Templeton et al. (2013) showed that male happy wrens exposed to female playback with a constant tempo tended to slow their singing rate as the duet progressed, which

made later male phrases overlap with the female playback. Potentially, male happy wrens are able to dynamically alter their singing timing to avoid overlapping the female playback, which suggests that males are unable to avoid overlap for later phrases due to costs of maintaining high rates. In this study, I analyzed natural songs and thus it is not possible to discriminate between the two mechanisms that would cause plain wrens' tempo to increase as the duet progresses. However, results in black-bellied wrens (Logue et al., 2008) and magpie larks (Hall & Magrath, 2007) also suggest that performing long coordinated duets is costly and could indicate pair bond strength (but see (Benedict, 2010) for negative results).

If duet coordination is used as a signal either by the partner or neighboring pairs, individuals should be able to discriminate between coordinated and uncoordinated duets and give stronger responses towards coordinated duets. Kovach et al. (2014) suggested that coordination in three species of Neotropical wrens (including a different subspecies of plain wren, *C. modestus modestus*) is not a joint territorial signal because response to coordinated duets was not clearly stronger than response to solo songs. Nevertheless, Kovach et al. (2014) showed that plain wrens give a stronger response to coordinated than uncoordinated duets in terms of closest approach to the speaker and song production. Thus, it seems that even when birds respond equally strong, to duets and individual songs, they are able to discriminate between uncoordinated and coordinated duets, which accords with the idea that individuals are paying attention to duet coordination. The present study was carried out in the *C. M.*

zeledoni subspecies of plain wrens (sometimes called canebrake wrens) which differs in some aspects of its duet structure (Cuthbert & Mennill, 2007) from the *C. m. modestus* subspecies of plain wrens studied by Kovach et al. (2014). The duets of the two subspecies are sufficiently different that wrens of the *zeledoni* subspecies fail to respond to duets of the *modestus* subspecies (pers. obs.) Therefore, studies are needed that assess whether *C. modestus zeledoni* is able to discriminate between coordinated and uncoordinated duets.

Conclusions

Plain wrens achieve precise coordination by dynamically adjusting their singing tempo based on a) whether or not their phrase was answered, b) the phrase type that each individual sings and c) the position of the interval in the duet. The complexity of the adjustments made presumably requires sophisticated neural processing and may require learning. Accordingly, these results support the idea that coordination could be used as a signal of pair bond strength.

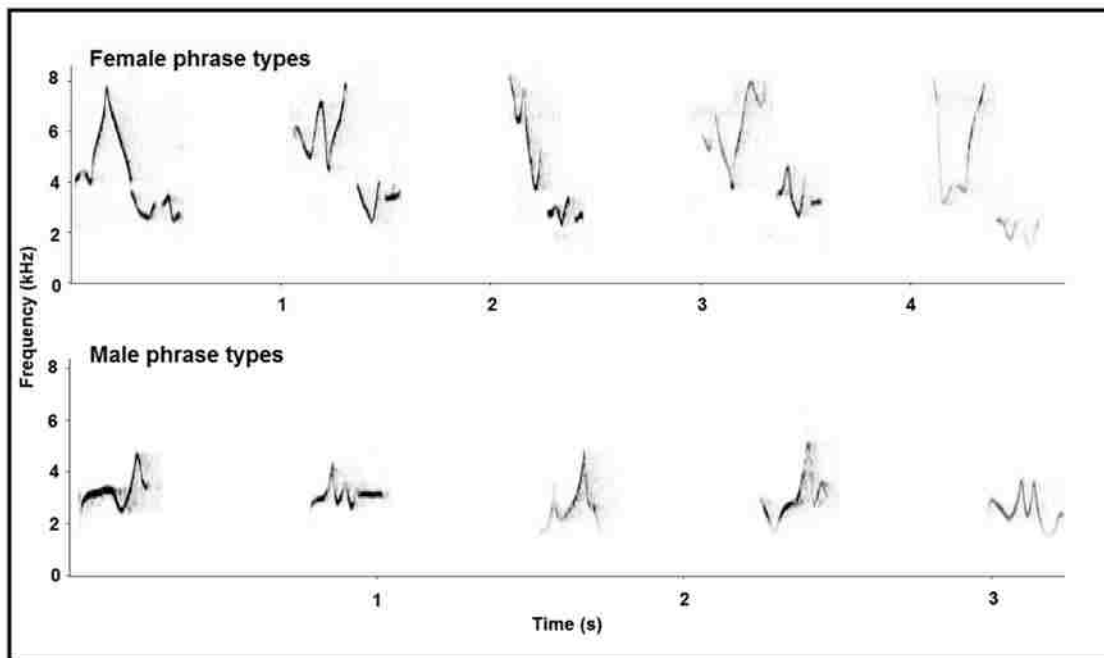


Figure 2.1 Example of five female phrase types sung by one female and five male phrase types sung by one male.

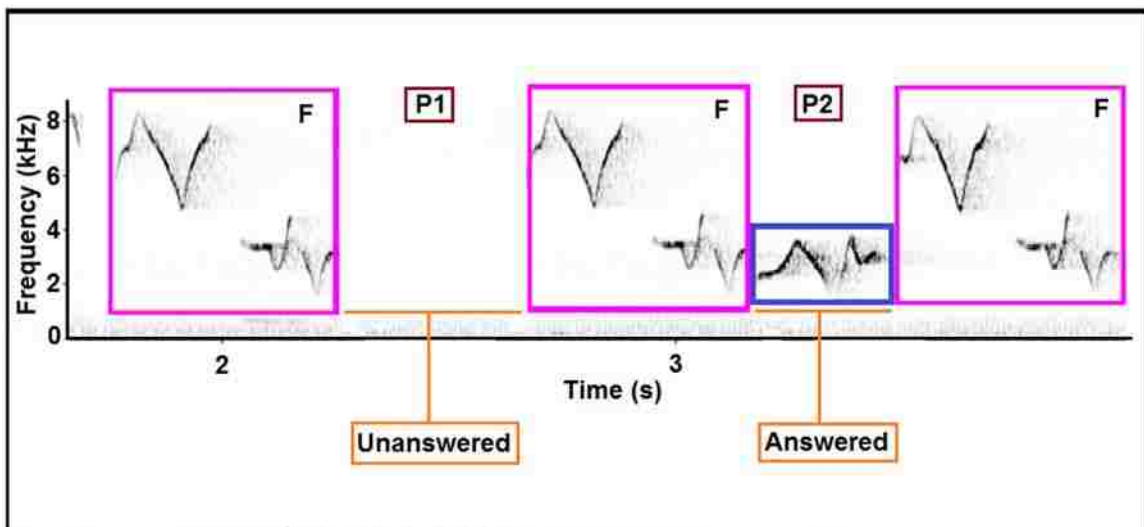


Figure 2.2 Example of measurements of inter-phrase interval durations performed in a duet. The following parameters were measured: whether or not an interval was answered (Answered, Unanswered), the position of the inter-phrase interval in the duet (P1, P2, etc.), the female phrase type (F) and the male phrase type (M).

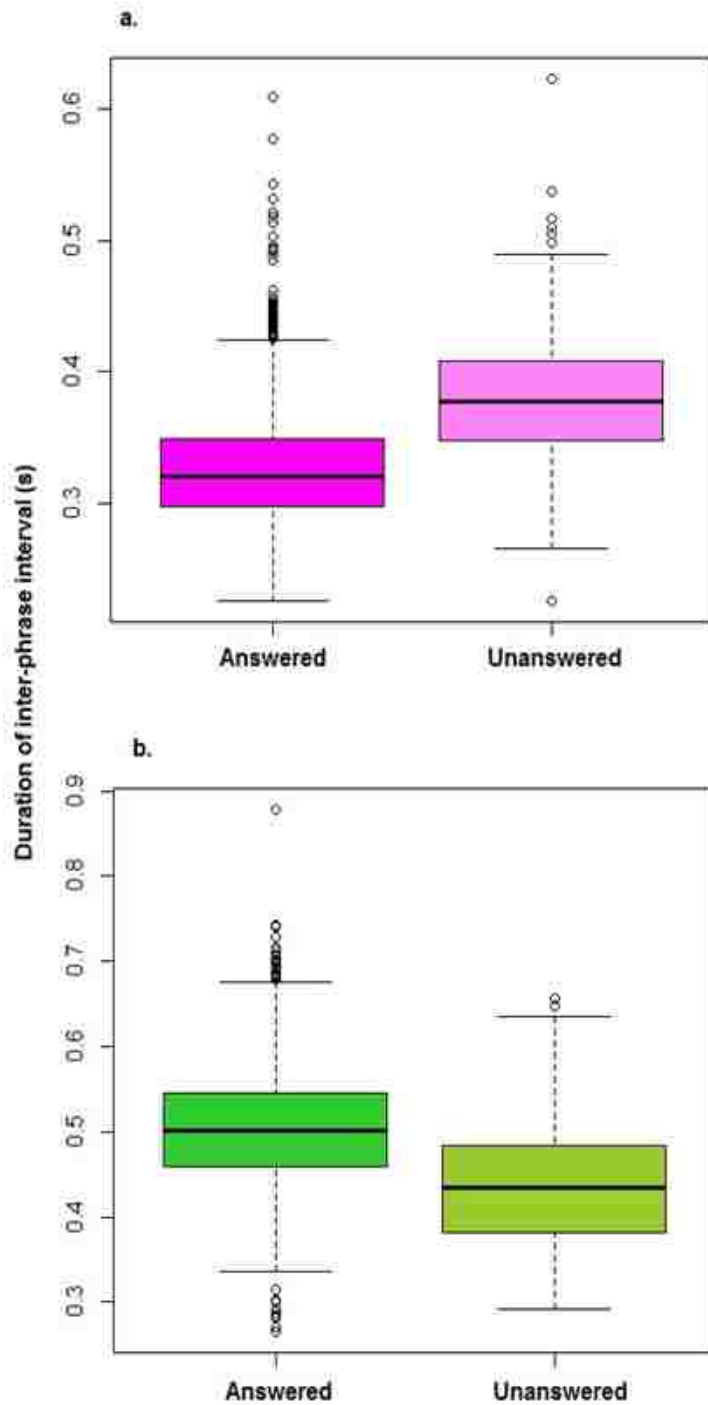


Figure 2.3 (a,b) Duration of inter-phrase interval depending on whether an individual was answered or not. a. Female inter-phrase intervals. b. Male inter-phrase intervals. Median (horizontal dark line in each box), quartiles (top and bottom of box), the 0.05 and 0.95 quantiles (tips of vertical whiskers) and extreme data points (open circles) are shown for each boxplot.

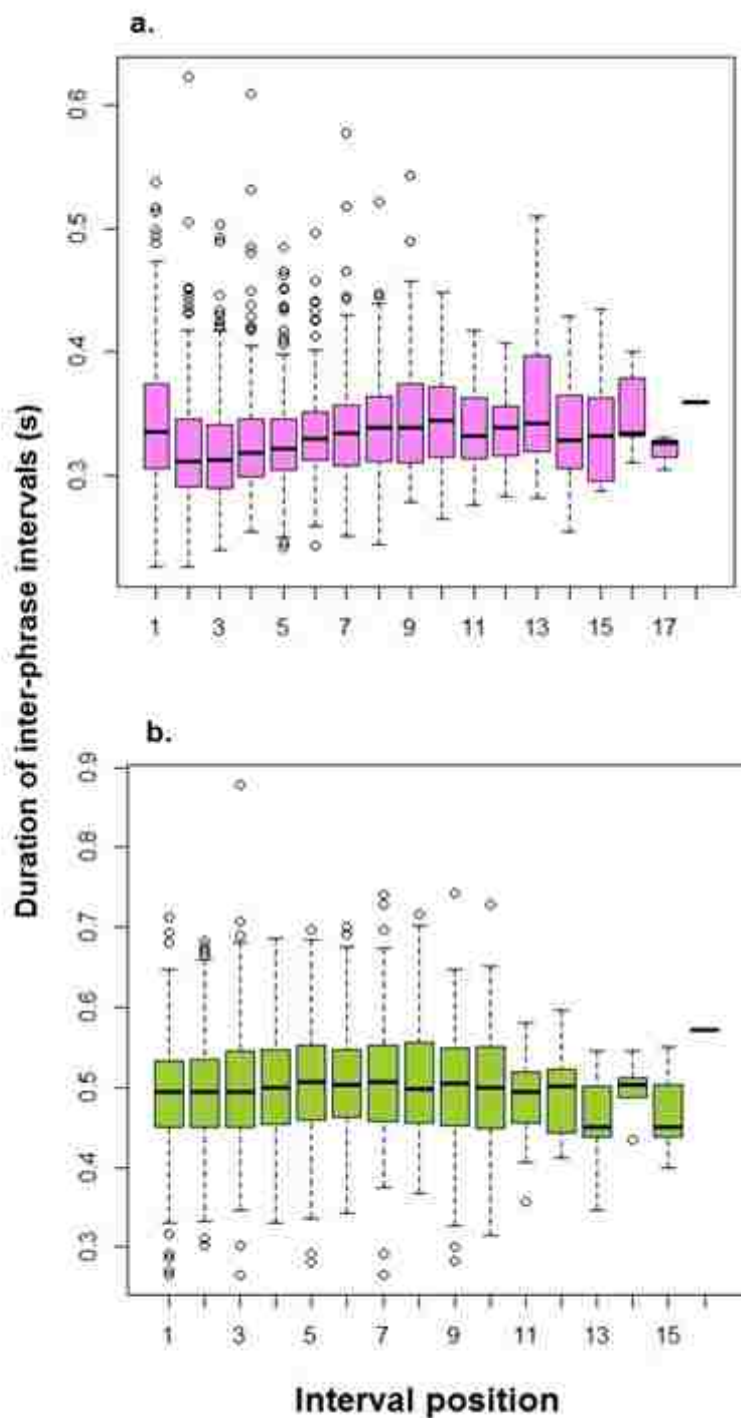


Figure 2.4 (a,b) Duration of inter-phrase interval depending on the position of the interval within a duet. a. Female inter-phrase intervals. b. Male inter-phrase intervals. Median (horizontal dark line in each box), quartiles (top and bottom of box), the 0.05 and 0.95 quantiles (tips of vertical whiskers) and extreme data points (open circles) are shown for each boxplot.

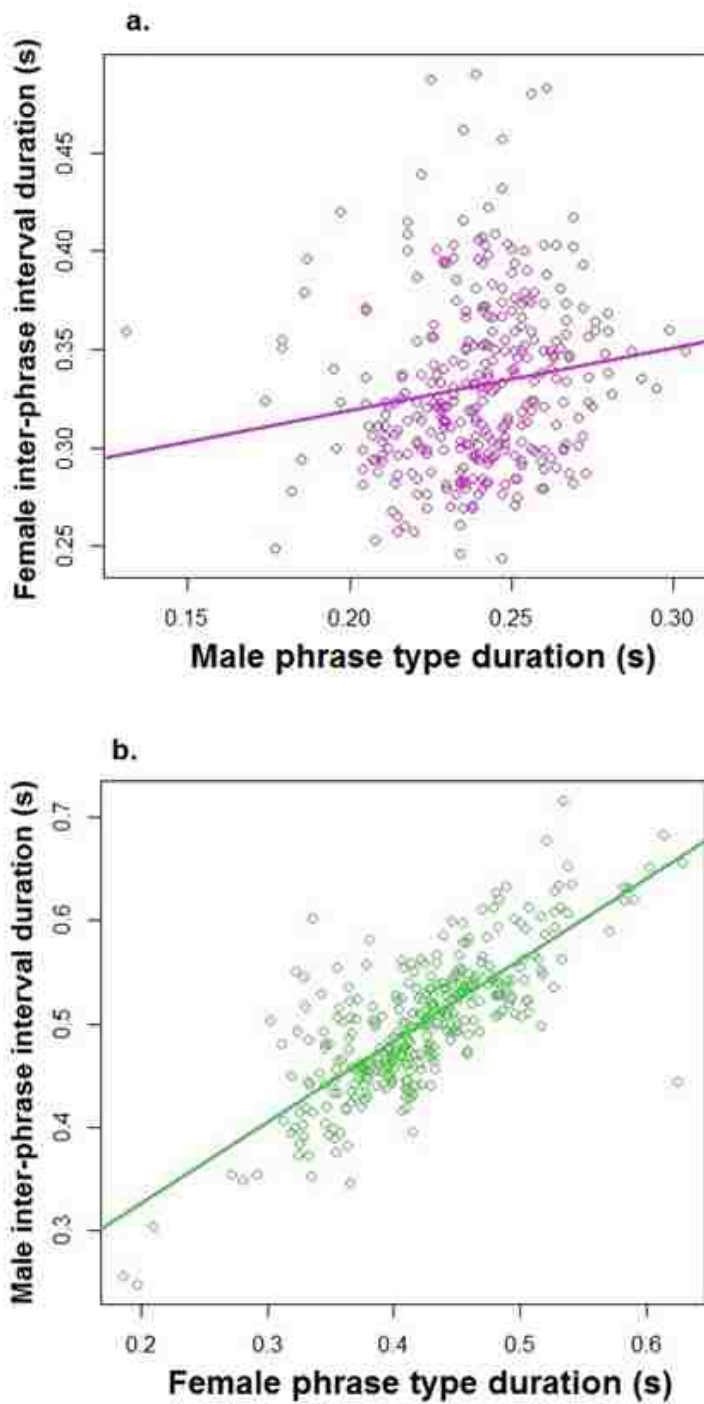


Figure 2.5 (a,b) Duration of inter-phrase interval depending on the duration of phrase type duration. a. Female inter-phrase intervals based on male phrase type length. b. Male inter-phrase intervals based on female phrase type length.

Table 2.1 Random and fixed structure selected for females' General Linear Mixed Model. I=individual, D=duet, A= whether the phrase was answered or not by mate, F= female phrase type, M= male phrase type, P= position of interval within a duet. For random effects, model with lowest AIC value was selected. For fixed effects models were compared through likelihood criteria. Each variable was removed sequentially. P values indicate the variables that significantly contribute to explaining duration of female's inter-phrase intervals. For the fixed structure model 3 was selected as removing more variables (models 4-6) significantly decreased the fit. For the random structure model 2 was selected as AIC value was lowest.

Model	Fixed effects	Log likelihood	Test	L. Ratio	P value
1	A+ F+ M+P + (F*M)	5717.629			
2	A+ F+ M+P	5714.756	1 vs 2	5.75	0.124
3	A+M+P	5717.628	2 vs 3	0.0007	0.862
4	A+ P	5714.75	3 vs 4	5.71	0.01
5	A+M	5537.981	3 vs 5	359.8	<0.0001
6	M+P	5672.546	3 vs 6	92.75	<0.0001

Table 2.2 Random and fixed structure selected for males' General Linear Mixed Model. I=individual, D=duet, A= whether the phrase was answered or not by mate, F= female phrase type, M= male phrase type, P= position of interval within a duet. For random effects, model with lowest AIC value was selected. For fixed effects models were compared through likelihood criteria. Each variable was removed sequentially. P values indicate the variables that significantly contribute to explaining duration of male's inter-phrase intervals. For the fixed structure model 3 was selected as removing more variables (models 4-6) significantly decreased the fit. For the random structure model 2 was selected as AIC value was lowest.

Model	Fixed effects	Log likelihood	Test	L. Ratio	P value
1	A+ F+M+ P+ (F*M)	4702.08			
2	A+ F+M+ P	4657.27	1 vs 2	89.48	<0.0001
3	A+M+ P+(F*M)	4702.02	1 vs 3	0.129	0.719
4	A +P+(F*M)	4669.19	3 vs 4	65.664	<0.0001
5	A +M+(F*M)	4301.65	3 vs 5	800.73	<0.0001
6	M +P+(F*M)	4645.33	3 vs 6	113.37	<0.0001

Chapter 3

Early development of vocal interaction rules in canebrake wrens

Summary

Although interactions in which individuals exchange vocal signals are an important aspect of communication in many animals the development of the rules governing such interactions has been little studied. Various species of tropical songbirds engage in vocal interactions in the form of duets between mated pairs. In some species duets show precise temporal coordination and follow rules (duet codes) governing which song type one bird uses to reply to each of the song types of its mate. In this study, I determined whether duet coordination and duet codes are learned during early development in one duetting species, the canebrake wren. Results show that juveniles acquire a duet code by singing with a mated pair of adults (presumably their parents) and that the juveniles gradually increase their fidelity to the code over time. Additionally, I found that juveniles initially exhibit poorer coordination than adults and improve their coordination as time progressed. I suggest that what is most important for juvenile wrens to learn are the general rules governing duet coordination and duet codes. This is the first study to report that the ontogeny of vocal interaction rules in songbirds is analogous to the ontogeny of some vocal interaction rules in humans.

Background

Signaling in general, and vocal signaling in particular often involves structured interactions between multiple signalers. Human conversation provides a familiar example (Jasnow & Feldstein, 1986; Logue & Stivers, 2012; Snow, 1977). Vocal interactions between other types of primates include call exchanges between group members in marmosets (Takahashi et al., 2013), and duetting between mated pairs of gibbons (Geissmann, 2002). Among non-human animals, the most complex vocal interactions studied to date occur in birds (Geberzahn & Hultsch, 2004). Male songbirds, for example, engage in vocal interactions during aggressive encounters such as countersinging (Todt & Naguib, 2000), song type matching (Beecher et al., 2000) and frequency matching (Otter et al., 2002). In all of these cases escalation in aggressiveness depends on the type of responses individuals give to each other (Akçay et al., 2013; Searcy & Beecher, 2009). Another important vocal interaction among birds is duetting between mated males and females, a behavior that is particularly common in tropical species (Hall, 2009).

To engage in any of these vocal interactions, individuals must develop not only the ability to produce their vocalizations, but also the ability to use those vocalizations effectively in replying to others. Previously, studies of the development of vocal signals have concentrated on the development of the structure of individual vocalizations (Beecher & Brenowitz, 2005; Doupe & Kuhl, 1999). These studies have shown strong analogies between the development of human speech and bird song (Doupe & Kuhl, 1999; P. Marler, 1970). For instance, vocal production learning (i.e. the ability to modify the structure of

vocalizations as a consequence of learning) occurs in birds and humans but not in non-human primates during early development (Egnor & Hauser, 2004), but see Takahashi et al. (2015) for an exception. It is unknown whether analogies also exist between the development of vocal interaction rules in humans and birds.

A few studies have addressed the development of vocal interaction rules in non-human primates, finding some analogies with humans. For instance, recent work suggests that turn-taking, the ability to exchange utterances rapidly but without overlap (Chow et al., 2015), is learned during early development in humans (Hilbrink et al., 2015). This ability seems to be learned also in marmosets (Takahashi et al., 2016). It has been also suggested for agile gibbons (*Hylobates agilis agilis*) that mother-daughter-interactions enhance vocal development and allow juveniles to learn temporal patterns needed to engage in duet singing (Koda et al., 2013). However, because most vocalizations in non-human primates are simple and innate (Egnor & Hauser, 2004), analogies between vocal exchanges in these species and human conversation are quite limited. Songbirds, with their complex, learned vocalizations, may provide a more appropriate animal model for studying analogs of human vocal interaction learning.

Two studies have indirectly addressed the early ontogeny of vocal interactions in temperate avian species. First, it has been shown that Common Nightingales (*Luscinia megarhynchos*) during early development learn not only individual song types, but also the order in which a group of songs is delivered

(Hultsch, 1989). Second, juvenile nightingales also distinguish the sequential association of different song groups (Hultsch, 1992). It has been argued that these two features suggest that juveniles learn contextual information of when and how the songs should be used during vocal interactions (Geberzahn & Hultsch, 2004). However, no direct test of vocal interaction learning has been performed in avian species.

Duetting requires engaging in vocal interactions that involve time and pattern specific relationships among the vocalizations from different individuals. In one duetting species, the canebrake wren (*Cantorchilus zeledoni*), individuals can acquire new interaction rules in adulthood when they obtain new mates, and the new rules develop gradually, which is suggestive of learning (Rivera-Cáceres et al., 2016). In black-bellied wrens, adults are able to answer to unfamiliar songs, suggesting that they can learn new rules to answer these songs (Logue, 2007). Furthermore, juveniles of some duetting species duet with adults during the sub-song stage (e.g. buff-breasted wrens (Farabaugh, 1982), black-bellied wrens, (Logue & Gammon, 2004) and canebrake wrens (pers. obs), in a way similar to how infant humans converse with their caregivers (Snow, 1977). It is then possible that the early ontogeny of these rules in some duetting species also involves learning. Duetting species thus provide an ideal model in which to search for vocal interaction learning.

Many duetting birds must abide by two interaction rules: precision in the timing of song answering (analogous to turn-taking in primates, Todt & Naguib, 2000), and non-random association of song types (i.e. duet codes) (Logue,

2007). The latter rule is absent in non-human primates. Humans, however, possess an analogous rule termed “adjacency pairs” in which the types of utterances used in exchanges are linked (e.g. question-answer exchanges) (Sacks, 2004). In this study, I address whether juvenile canebrake wrens first learn to duet and thus acquire both proper coordination and specific duet codes while they sing duets with adults during this stage. Regarding duet coordination, the learning hypothesis predicts that juveniles a) should perform duets with poorer coordination than adults and b) should improve their coordination with time. Regarding duet codes, this hypothesis predicts that juveniles a) should use the same code as the adults they sing with, b) should break the code more often than adults, and c) should break the code less often as time progresses. I tested these predictions by recording juvenile canebrake wrens for up to two months in the field and then determining how juvenile duets compared to adult duets and how juvenile duets changed over time. To my knowledge I am the first to provide direct evidence for the hypothesis that duetting birds learn to duet as juveniles.

Canebrake wrens (*Cantorchilus zeledoni*) are an ideal species to study the development of interaction rules because juveniles can be recorded singing with adults and because adult duet rules are so complex in this species. Adult pairs of canebrake wrens sing highly coordinated antiphonal (i.e. alternating) duets and associate their song types non-randomly (i.e. possess duet codes, Mann et al., 2003). Duets in this species are composed of three categories of phrases. Two of these categories are sung by males: introductory phrases (I phrases) which are used to begin songs and a separate set of male phrases (M phrases) which

are used later in the song. The remaining category is the set of phrases sung by the females (F phrases). Duets typically start with a single I phrase followed by an alternation of one F phrase and one M phrase (i.e. I(FM)_n): When a juvenile is present on a territory, it usually joins the duets of the adults by singing phrases specific to one sex.

Materials and Methods

Between the months of April and August of 2012 to 2016 I recorded duets performed by groups of territorial plain wrens composed of an adult female, an adult male and at least one juvenile. I started recording juveniles as soon as they were found, but I could not control the age of juveniles as this species has little reproductive synchronization (Marshall-Ball & Slater, 2003). Most individuals from the territories that I recorded were captured and provided with a unique combination of colored leg bands for further identification. In total I recorded eight groups (16 adults and 13 juveniles) around La Suerte Biological Station, Costa Rica (10°26'N, 83°47'W) and La Selva Biological Station, Costa Rica (10°26'N, 83°59'W). The study sites include a mixture of lowland moist forest, swamps, scrub, and cattle pasture, where plain wrens are common (Mann et al., 2003).

To confirm the genetic sex of the juveniles I obtained blood samples (~50µl) from the brachial vein and stored them in lysis buffer (Longmire et al., 1997) for nine out of the thirteen juveniles. I extracted the DNA using DNeasy Blood & Tissue Kits (Qiagen). DNA concentrations were measured using a Qubit 2.0 fluorometer (Life Technologies). I ran PCR amplification using the P0, P2 and P8 primers (Han et al., 2009). PCRs were successful for seven out of the nine

individuals as I was able to obtain clear bands on agarose gel identifying them as males or females. The two unsuccessful PCRs were run multiple times but failed to produce any visible bands in the agarose gel.

Data collection

Territorial birds were recorded between 6:00 am and 9:00 am. To obtain recordings of duets I used a Marantz PMD660 digital recorder and a unidirectional Sennheiser ME66 microphone. The birds on each territory were recorded at least once a week for one hour (average of days recorded per juvenile: 24.68 ± 17.2 s.d). The recording session started with a simulated intrusion using playback to increase the singing activity of the focal birds. The playback consisted of three bouts of canebrake wren duet songs. The playback was then repeated every 10 minutes until the recording session was over.

During each recording session two observers were present. One observer performed the recordings and the other observer followed the banded individuals with binoculars to determine which individuals sang each time. Only the duets in which both observers agreed on the individuals that had participated were analyzed.

Data analysis

To analyze all duets, I created spectrograms of each recording using SYRINX software (J. M. Burt www.syrinxpc.com) using a Hanning window and a 512 pt FFT and a temporal resolution of 5.8ms. All duets in which at least one adult and one juvenile sang were analyzed. For territories in which both female

and male juveniles were present I also included the duets performed solely by the juveniles.

To measure duet coordination, I determined whether each bird overlapped a song of a bird from the opposite sex by subtracting the end time of a phrase from the start time of the next immediate phrase from a bird of the opposite sex. I counted a phrase as overlapped if the result of the subtraction was negative. I then calculated the proportion of phrases overlapped per duet.

To classify the phrase types used by each bird in every duet I created a library of each bird's repertoire. I then compared the classified phrase types to each duet spectrogram. If an adult and a juvenile of the same sex both sang during a duet I determined whether the phrase types that they used were the same or not. The phrase types were determined based on visual inspection (Nowicki & Nelson, 1990).

Statistical analysis

To determine whether juveniles performed less coordinated duets than adults I used a generalized mixed model (GLMM, function lme of the package nlme, (Pinheiro et al., 2013) in R version 2.15.1). I used age of bird (adult or juvenile as a fixed factor) and sex and year as covariates. Because year and sex were non-significant ($p=0.35$ and $p=0.28$ respectively) they were removed from the model. I performed a second generalized mixed model to determine whether juveniles improved their coordination with time. I used day as a fixed factor and sex and year as covariates. Both covariates were dropped from the final model (sex $p=0.30$, year $p=0.31$).

To determine if juveniles broke the code more often than adults I used a third GLMM. I used age of birds (juvenile vs. adult) as a fixed factor. Sex and year were used as covariates, but were dropped from the final model (sex $p=0.40$, year= 0.28). A fourth GLMM was used to determine whether juveniles improved their duet code adherence with time. Day since first recording and type of duet (duet with both adults, duet with adult of the opposite sex, duet between juveniles) were used fixed factors. Sex and year were used as covariates. Sex was dropped from the model ($p=0.57$) but year was retained. Juveniles sang significantly more phrase types in 2013 than in 2014 (effect size= 1.13 , $t_6=4.41$, $p=0.004$) and 2016 (effect size= 0.85 , $t_6=3.43$, $p=0.01$).

For all GLMMs I used identity of the bird as a random factor as multiple duets from each individual were used in the analyses. All GLMMs were validated using the graphic methods suggested by Zuur et al. (2009).

To determine if juveniles used the same code as adults, I compared the song types used by juveniles and the adults of the same sex to answer each song type of the adult of the opposite sex. A heterogeneity G-test was applied to determine if the phrases that juveniles used to answer were chosen randomly or followed the same code as the adults. To calculate the expected values for the contingency table I used the inverse of the total repertoire size recorded from each juvenile.

Results

Sex-specific repertoires

Each of the 13 juveniles I recorded sang either only male phrases or only female phrases. I determined the genetic sex for seven juveniles: three females and four males. In all seven cases, the juveniles sang phrases that were appropriate for their genetic sex – that is males sang male phrases and females sang female phrases.

Duet coordination

Juvenile canebrake wrens overlapped significantly more songs of the opposite sex than did adults and thus exhibited poorer coordination (effect size= 0.425, $t_{24}=7.75$, $p<0.00001$, Fig. 1). Furthermore, juveniles significantly improved their coordination over time within the timeframe measured (average days recorded per juvenile 24.69 ± 17.2 s.d.). Although the effect size per day was small, overall juveniles decreased their overlapping proportion about 20% (effect size= -0.004, $t_{315}=-2.57$, $p=0.01$, Fig. 2). However, there is high variation in the rate at which juveniles decreased duet overlapping (Fig. 2).

Duet code adherence

All 13 juveniles matched the phrase type that the adult of the same sex used to answer phrases from the opposite sex with a probability far above chance ($G_{tot}=976.4$, d.f.=13, $p<0.0001$, Table 1, Fig. 3). However, juvenile canebrake wrens used more phrase types to answer the adult from the opposite sex than did adults (effect size= 0.283, $t_{24}=3.26$ $p=0.0032$, Fig. 4), which indicates that the duet codes of juveniles are less consistent than the duet codes

of adults. Furthermore, juveniles used more phrase types when they duetted only with the adult of the opposite sex than when they duetted with both adults (effect size=-0.54, $t_{317}=-4.4$, $p<0.00001$). These results indicate that juveniles copy the phrase type from the adult of the same sex to answer the adult of the opposite sex (i.e. follow the adult's duet code). Finally, juveniles used fewer phrase types to answer one phrase type of the opposite sex as time progressed (effect size=-0.015, $t_{318}=-3.83$, $p<0.0001$, Fig. 5) which indicates that their use of a defined duet code improved with time.

Discussion

Duet code development

This is the first study that provides direct evidence that a duetting bird learns a duet code during early development. First I showed that juvenile plain wrens use the same code as the adults with which they duet. Second, juveniles made more mistakes at following a duet code than adults, but improved with time.

A recent study showed that the duet codes of adult canebrake wrens are flexible and thus change when they acquire a new partner (Rivera-Cáceres et al., 2016). A second duetting species that is thought to have flexible duet codes is the black-bellied wren (Logue, 2006) as individuals are able to answer to unfamiliar phrases. The question then remains of why juveniles should learn a duet code from their parents if they have to invest in re-learning new rules when they mate. I hypothesize that what is important for juveniles to learn may be the general rules governing duet codes, rather than the specifics of the codes used

by their models. This proposed learning pattern is similar to human conversation learning. For instance, higher cognitive tasks needed to exchange ideas (such as the social manifestation of differences in perspective) can be learned throughout life (Doise et al., 1984). Still, exposure to speech interactions during early development is vital for the general ability of individuals to engage in conversations (Doise et al., 1975). Gibbons appear to also share this pattern as there is socially mediated vocal development in early stages with some flexibility in duetting performance in sub-adult and adult stages (Koda et al., 2013).

The study of the development of adjacency pairs (i.e. when turns between individuals that converse are functionally related to each other so that the first turn requires a certain type of answer, Sacks, 2004), the analogous rule to duet codes in birds, has been difficult. First, this rule can encompass many scenarios within conversations, as the general idea is that the rule is fulfilled if an individual makes a conversational contribution as is required, at the correct stage with the accepted purpose (Grice, 1975). Thus, protocols that can objectively target measurements of rule adherence can be difficult to generate. Still, by reviewing conversations of children around 2 years of age, researchers in conversations analysis have been able to determine that these young children can coherently relate to what was said by the previous speaker and frame their response accordingly. Thus, it seems that children develop the ability to engage in the collaborative activity that is required for adjacency pairs early in life (Wells, 1981). However, it is still unknown what the connectors between exchanges are and how explicit they have to be so that children understand their role in the

conversation at this age. Furthermore, it is unknown how and at what age this competence starts to develop in humans. In this study, I have found that duet codes develop during early development in a similar manner to adjacency pairs. Based on my results, I hypothesize that juvenile canebrake wrens require both adults to duet with them to start acquiring the connectors between I-F-M phrases, but as time progresses, juveniles memorize the connectors and can duet with the adult of the opposite sex without need of input from the same-sex parent. It is possible that children also need to listen to third party conversations to understand the general rule of adjacency pairs. Perhaps comparative analyses of children developing in different conditions (e.g. foster homes vs family homes) and their ability to find connectors in a cooperative conversation could help in understanding the mechanism by which this rule is acquired. Experimental studies with juvenile wrens could also help us understand whether duetting birds indeed need to hear duetting from other individuals to be able to develop their duet code rules.

Duet coordination development

That juvenile canebrake wrens fail to perform highly coordinated duets and that they tend to improve with time support the hypothesis that juveniles need a rehearsal period to be able to coordinate their duets. Here I thus provide evidence that an analogous rule to turn taking in humans (i.e. temporal coordination in duetting birds), also has an analogous development. In both species, this ability is acquired during early development. To my knowledge this is the first study to show that birds and humans not only share the ontogeny of

vocalization acquisition but also the ontogeny of the rules needed to use those vocalizations during vocal interactions.

A recent study of marmosets was the first to show that a non-human animal acquires the ability to exchange vocalizations without overlapping during early development (Chow et al., 2015; Takahashi et al., 2016). However, turn taking is somewhat different in marmosets than in humans because the timeframes of exchanges differ by several orders of magnitude: the silent gaps between two individuals' utterances are a few hundred milliseconds in humans (Levinson & Torreira, 2015) but up to 10 seconds in marmosets (Takahashi et al., 2013). By contrast, the silent gaps between the phrases of canebrake wrens are less than 100 milliseconds (Rivera-Cáceres, 2015). Furthermore, it has been shown that in order to keep silent gaps short, several species of duetting birds perform adjustments to their singing tempo based on the songs of their partners (Fortune et al., 2011; Logue et al., 2008; Rivera-Cáceres, 2015; Templeton et al., 2013). Furthermore, as in humans (Levinson & Torreira, 2015), birds in at least one duetting species are able to predict the end of their partners' vocalization (Logue et al., 2008). Thus, even though humans and marmosets are more closely related than humans and birds, it may still be more informative to draw comparisons between turn-taking in humans and duet precision in birds, especially as it has been suggested that turn-taking in marmosets and humans is analogous rather than homologous (Takahashi et al., 2016). The birdsong model provides us with the capacity to perform neurological and genetic studies in species that possess brain regions and genes dedicated to song learning and

production that are homologous or analogous to those that relate to speech learning and production in humans (Farries & Perkel, 2008).

My study has provided key evidence that vocal interaction rules are learned during early development, but further studies to examine this idea are still needed. For instance, in my study the rate of improvement in duet coordination was not consistent throughout all juveniles, leading to the large confidence interval I found around the mean rate of change (Fig. 2). The lack of a clear pattern of duet coordination improvement could be due to the need for a longer rehearsal period than the timeframe of my study. In the field, I only observed one pair of juvenile birds leaving their parental territory, more than two months after beginning to sing. Before they left, these two birds consistently performed highly coordinated duets. It could be that all juveniles achieve consistent high levels of coordination before leaving the natal territory. Other duetting species take from 5 months in bay wrens (Levin unpublished data) to 8 months in slate-coloured boubous, *Lanarius funebris* (Wickler & Sonnenschein, 1989) to develop a crystalized song repertoire, and thus it could take the same amount of time to develop the ability to perform highly coordinated duets. Testing this idea would require longer observation periods of juvenile duet development in the field.

Furthermore, no study has directly addressed the development of other types of vocal interactions in birds, but it will be very interesting to determine for instance if song type matching in songbirds (Todt & Naguib, 2000) develops in a similar fashion.

Conclusion

This study provides evidence that both duet coordination and duet codes are acquired through learning during early development in canebrake wrens: juvenile canebrake wrens improve both their temporal coordination and duet code adherence throughout time. Furthermore, the duet code that the juveniles acquire is the same code produced by their parents. The results point to important parallels between duet development in birds and the development of interaction rules in human speech.

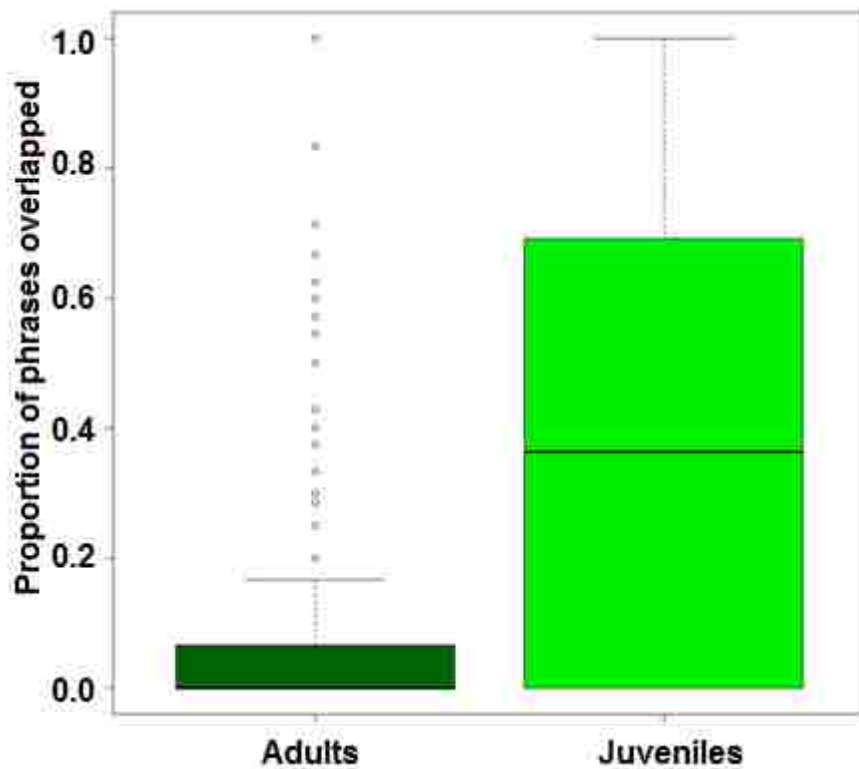


Figure 3.1 Proportion phrases of the opposite sex per duet that individuals overlapped with their own phrases. Median (horizontal dark line in each box), quartiles (top and bottom of box), the 0.05 and 0.95 quantiles (tips of vertical whiskers) and extreme data points (open circles) are shown for each boxplot.

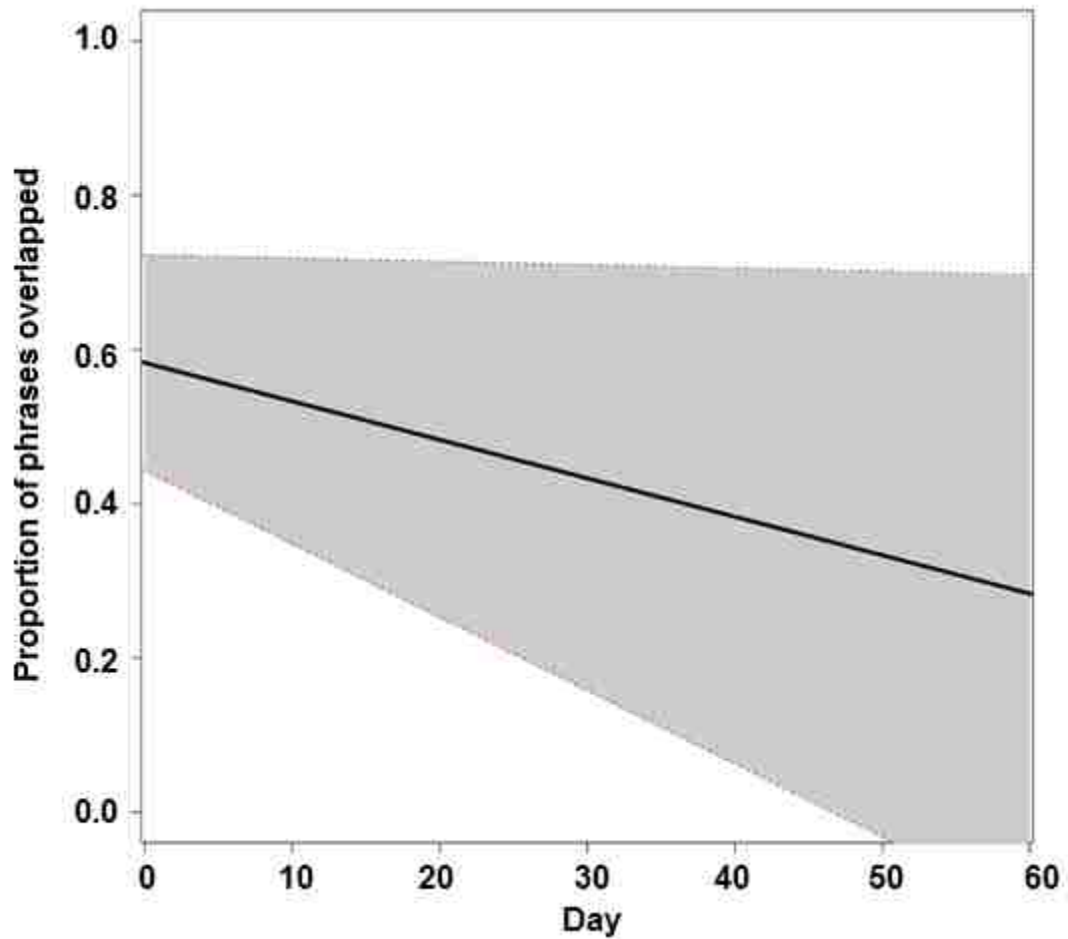


Figure 3.2 Proportion phrases of the opposite sex per duet that juveniles overlapped with their own phrases across time. The dashed black line represents the fixed effect of day over proportion of phrases overlapped. The shaded area between the red dashed lines represent the 95% confidence intervals of the GLMM.

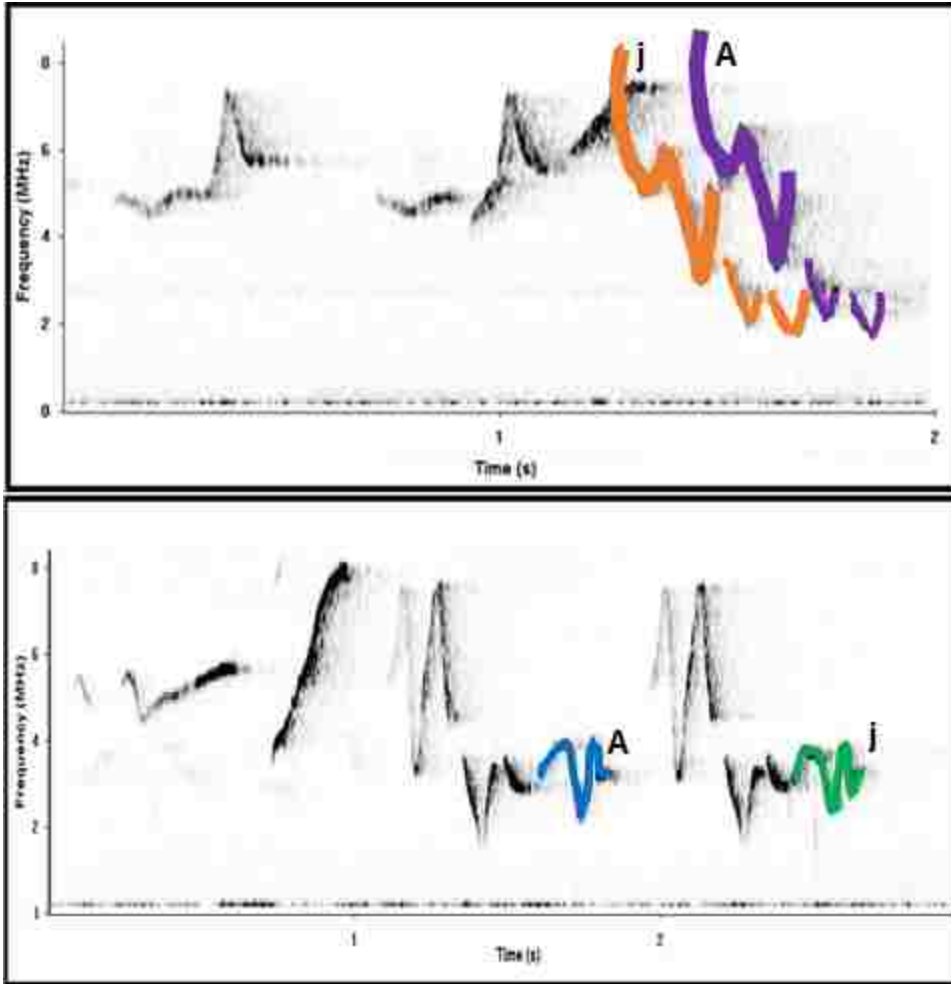


Figure 3.3 (a,b) Examples of duets where adults and juveniles participate. a. A phrase of a male is answered by the adult (A, violet) and juvenile (j, orange) females; both females used the same phrase type. A female phrase is answered by the adult (A, blue) and juvenile (j, green) male; both males used the same phrase type.

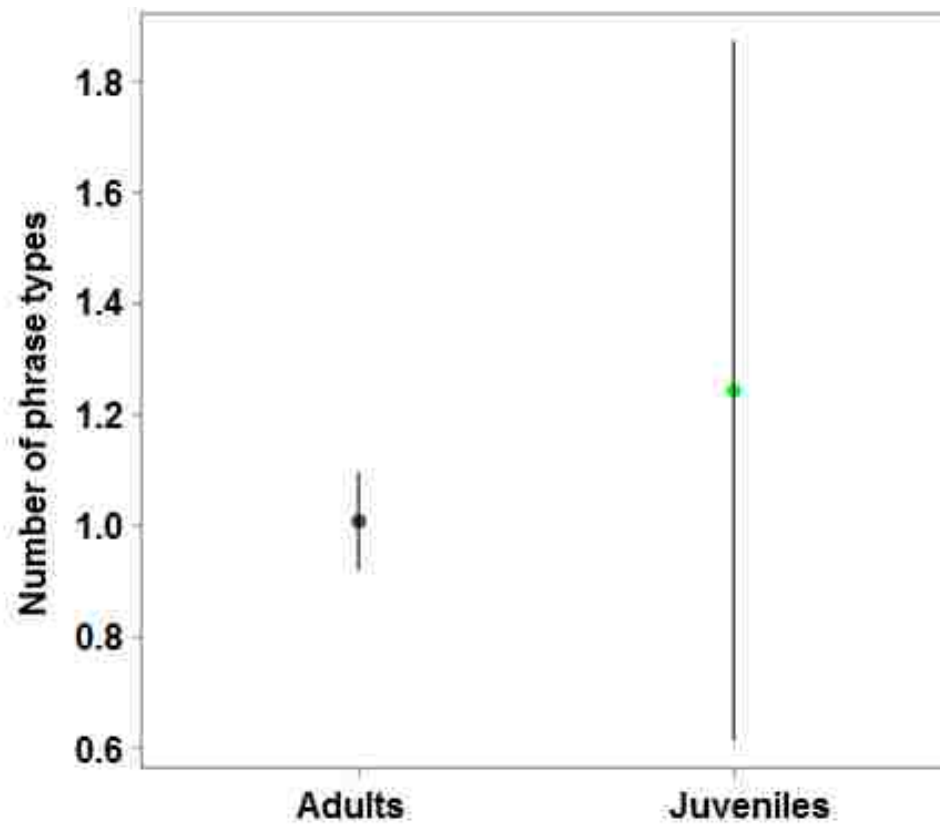


Figure 3.4 Number of different phrase types that individuals used to answer each of the phrase types of the adult of the opposite sex, average \pm standard deviation.

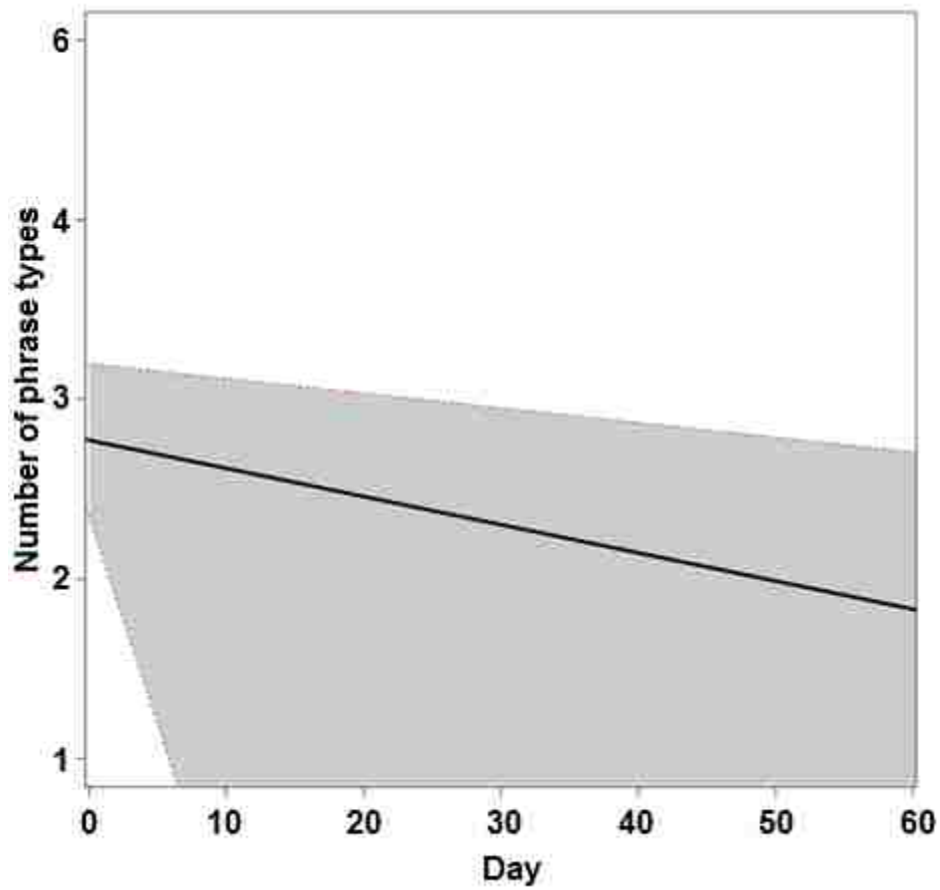


Figure 3.5 Number phrase types per duet that juveniles used to answer the phrases of the adult of the opposite sex across time. The gray area between the red dashed lines represent the 95% confidence intervals of the GLMM.

Table 3.1 G test for the phrase types that the juveniles used to answer different phrase types from the adults of the opposite sex. M=Phrases from juveniles that matched the adult phrase type, NM=Phrases from juveniles that did not match the adult phrase type, EM=Expected matches, ENM=Expected non-matches.

Individual	M	NM	Total	EM	ENM	G	df	p
G2F13	21	9	30	3.75	26.25	53.088	1	<0.0001
G2M13	18	17	35	4.375	30.625	30.908	1	<0.0001
H1M12	25	3	28	3.5	24.5	85.705	1	<0.0001
L2F13	52	1	53	6.625	46.375	206.61	1	<0.0001
PAEBF13	17	4	21	2.625	18.375	51.319	1	<0.0001
PAEBM13	24	2	26	3.25	22.75	86.245	1	<0.0001
G2F16	50	2	52	6.5	45.5	191.52	1	<0.0001
G2M16	95	3	98	12.25	85.75	369.07	1	<0.0001
G4F15	42	5	47	5.875	41.125	144.15	1	<0.0001
G4M15	24	3	27	3.375	23.625	81.777	1	<0.0001
BG1M15	41	7	48	6	42	132.5	1	<0.0001
LS2F16	18	6	24	3	21	49.47	1	<0.0001
LS2M16	4	3	7	0.875	6.125	7.876	1	<0.005
					Gtotal	1490.2	13	<0.0001
					Gpooled	1424.6	1	<0.0001
					Ghet	65.678	12	<0.0001

Chapter 4

Neotropical wrens learn new duet rules as adults

Summary

Although song development in songbirds has been much studied as an analog of language development in humans, the development of vocal interaction rules has been relatively neglected in both groups. Duetting avian species provide an ideal model to address the acquisition of interaction rules as duet structure involves time and pattern specific relationships among the vocalizations from different individuals. In this study I address the development of the most striking properties of duets: the specific answering rules that individuals use to link their own phrase types to those of their partners (“duet codes”) and precise temporal coordination. By performing two removal experiments in canebrake wrens (*Cantorchilus zeledoni*) I show that individuals use a fixed phrase repertoire to create new phrase-pairings when they acquire a new partner. Furthermore, immediately after pairing, individuals perform duets with poor coordination and poor duet code adherence, but both aspects improve with time. These results indicate that individuals need a learning period to be able to perform well coordinated duets that follow a consistent duet code. I conclude that both duet coordination and duet code adherence are honest indicators of pair-bond duration.

Background

Interactions in which individuals exchange vocal signals are an important aspect of communication in many animals (Searcy & Beecher, 2009). In temperate songbirds, males often engage in vocal interactions such as countersinging and song matching (Todt & Naguib, 2000), but the most complex vocal interactions occur in species that sing duets within pairs (Hall, 2009). Avian duets have features analogous to ones found in human conversation, for example rules on correct temporal coordination and on which elements can be used by one participant to answer another's vocal elements (Logue & Stivers, 2012). The development of vocal signals such as human speech and the song of songbirds has been amply studied (Doupe & Kuhl, 1999), but I know significantly less about the development of interaction rules in either group (Hall, 2009; Logue & Stivers, 2012).

Some species of duetting songbirds follow "duet codes" that specify which of a bird's own song phrases are used to answer each of its partner's phrases (e.g. own phrase A always answers partner's phrase X) (Hall, 2009; Logue, 2007). Juvenile birds of some species have been observed to duet with their parents (Hall, 2009), copying their own-sex parent's replies to the phrases of the opposite-sex parent (K.D.R-C. unpublished). These observations strongly suggest that some birds learn a duet code as juveniles. That code might then be retained throughout life, with phrase-pairing rules remaining constant regardless of partner identity (Levin, 1996); I term this idea the "inflexible code hypothesis." A difficulty with this hypothesis is that the phrase repertoires of same sex

individuals often show only limited overlap even within populations (E. D. Brown & Farabaugh, 1991; L. Marshall-Ball & Slater, 2008), so that a new mate will have many phrases that were not in the opposite-sex parent's repertoire and that were therefore not included in the parents' duet code. Furthermore, duet codes in many species are pair-specific (E. D. Brown & Farabaugh, 1991; Levin, 1996; Logue, 2006; L Marshall-Ball et al., 2006; Templeton et al., 2013; Voigt et al., 2006; Wickler, 1976), so that even when a new mate shares a phrase with an individual's opposite-sex parent, the rule that individual has learned on how the phrase should be answered may not match the rule that its new mate has learned. Finally, because precise temporal coordination in some species depends on the ability of the birds to anticipate the phrase type that will be sung by their mates, an inflexible code might produce poor temporal coordination. These difficulties suggest an alternative, "adult duet learning hypothesis," whereby individuals learn a new duet code and new timing patterns as adults each time they acquire a new mate.

Although this alternative hypothesis fits logically with the complexity of pair-specific duet codes, direct evidence on the learning of various aspects of duets is decidedly mixed. On one hand, established pairs in canebrake wrens adhere to a duet code more consistently than do newly-established pairs (L. Marshall-Ball & Slater, 2008), black-bellied wrens (*Pheugopedius fasciatoventris*) are able to answer to novel phrases (Logue, 2006) and in magpie larks (*Grallina cyanoleuca*) timing is more precise in established pairs than in new ones (Hall & Magrath, 2007). On the other hand, timing is not significantly better in established

pairs than in newer pairs in canebrake wrens (L. Marshall-Ball & Slater, 2008), canary-winged parakeets (*Brotogeris versicolorus* v.) (Arrowood, 1988), and California towhees (*Melospiza crissalis*) (Benedict, 2010). Particularly influential evidence against adult learning of duets came from a pioneering study of bay wrens (*Thryothorus nigricapillus*) by Levin (1996), in which birds were experimentally removed from established pairs in order to trigger new pair formation. From comparisons of the duets of new and old pairs, Levin concluded that learning was not required “for pair specificity and precision in the duets of new pairs of birds.” Levin, however, concentrated only on responses to phrases that were shared between the old and the new mates while ignoring responses to phrases exclusive to the new mate, which are the ones more likely to require learning. Furthermore, Levin (1996) focused on the stability of individual phrase repertoires rather than the consistency of phrase pairings. Finally, Levin’s (1996) conclusions could be based on Type II errors due to the small sample size (four individuals).

Mechanisms of development have implications for hypotheses on the function of duets. Wickler (1980) assumed that adult learning was needed to duet properly with a new partner, so that a pair-specific and precisely sung duet demonstrates that both participants have invested considerable time and energy in learning to duet with each other. Duet coordination and duet code adherence could then function as a signal of commitment by one of the partners to the other (Hall & Magrath, 2007) or as a signal by the pair to rivals in competition for territory that they are dealing with an established and committed pair (Hall,

2009). Neither of these signaling functions is possible if precise duets do not require adult learning.

To test the adult duet learning hypothesis, I performed two experiments in canebrake wrens. First I temporarily removed one member of a pair and tested the remaining bird's response to playback of its mate's phrases and of unfamiliar phrases. If learning is required to duet properly, duetting with the unfamiliar phrases should be less precise than with the familiar ones. Second I permanently removed one member of a pair to induce new pair formation, and compared duets recorded from old pairs and new pairs. The adult duet learning hypothesis predicts (1) that new pairs immediately after pair formation should show weaker adherence to a duet code and less precise temporal coordination than old pairs, and (2) that both adherence to a duet code and temporal coordination should improve in new pairs with time. My study is novel first in that I analyzed the responses of individuals towards phrases exclusively in the new mate's repertoire as well as to phrases shared by the old and new mates. Second, I treated the duet codes as an individual level behavior and thus analyzed the consistency of both female phrase pairings and male phrase pairings. Lastly, I measured duet code changes of new pairs with an unprecedented temporal resolution.

Materials and Methods

I performed the study at La Selva Biological Station and its surrounding areas in northeastern Costa Rica (10°26'N, 83°59'W). The area includes a mixture of lowland moist forest, swamps, and cattle pasture, where canebrake

wrens are common (Mann et al., 2003). Both experiments were conducted with approval of the University of Miami's Institutional Animal Care and Use Committee (protocols 12-115 and 15-064) and under a scientific research permit (No. 05354) provided by the Ministry of Environment, Energy and Telecommunications (MINAET) of Costa Rica. Experiment 1 was performed between May and August of 2013 and 2014 and experiment 2 between May and July of 2015. Recordings were made throughout using a Marantz PMD660 digital recorder and unidirectional Sennheiser ME66 microphones.

Canebrake wrens sing highly precise antiphonal duets that are composed of three sex-specific categories of phrases (Fig. 4.1): I and M phrases sung by the male, and F phrase sung by the female (L. Marshall-Ball & Slater, 2008) following an I(FM)_n sequence. Individuals possess a repertoire of 15-25 phrase types in each phrase category (Mann et al., 2003). To sing a duet, canebrake wrens strictly follow a pair-specific duet code (Mann et al., 2003). To perform coordinated duets, canebrake wrens modify their singing tempo based on the phrase types that their partners are singing (Rivera-Cáceres, 2015).

Experiment 1: Playback experiment

I performed playbacks to 17 individuals (9 females and 8 males) from 12 different territories. For the 5 pairs in which both individuals were tested, I performed the playback on different days with at least one week between trials. Prior to playback trials I recorded repertoires from each subject for at least six hours. To create a library of each individual's duet code, I created spectrograms of the recordings using SYRINX software (J. M. Burt www.syrinxpc.com) with a

Hanning window and a 512pt FFT and a temporal resolution of 5.8ms. The phrase types for both males and females in each duet were determined based on visual inspection (Nowicki & Nelson, 1990). The association between mates' phrase types (duet code) was determined based on at least three instances from different recordings where the phrase types were found together.

For removals, I lured canebrake wrens to mist nets using playback of recorded duets. If no bird was captured after 10 minutes of playback the experiment was stopped and another attempt was made at least 3 days later. If a member of the focal pair was captured, it was placed in a holding bag while the experiment was carried out and then released. The sex of the remaining bird was determined by its color band combination if the bird was banded ($n=13$) or by its sex-specific phrases if it was not banded ($n=4$). I waited two minutes after the partner was removed to start the experimental trials.

Trials were performed between 6:00 and 9:20 am (solar time), when canebrake wrens have high vocal activity (Mann et al., 2003). Each trial included three playback treatments: 1) Partner's Phrase – a phrase type from the subject's mate; 2) Unique Phrase – a phrase not present in the partner's repertoire but present in the repertoire of one bird from the same population; and 3) Other Population Phrase – a phrase from a different population and that was not known from the study population. Females were exposed to M phrases while males were exposed to F phrases. Each playback included five replicates of seven phrases, repeated at 10s intervals, followed by 90s of silence. The order of the

treatments was balanced across pairs. Information on treatment assemblage can be found in the supplementary methods.

Subjects were recorded throughout playback trials. From the recordings I determined: a) the proportion of phrases that were answered, b) the phrase type(s) that the birds used to answer, and c) the proportion of phrases that the birds overlapped with their own phrases.

For the Shared Phrase treatment, a G test was applied (Mann et al., 2003; Mennill & Vehrencamp, 2005) to determine whether the phrases that a focal bird used to answer were chosen randomly or followed the expected duet code. To calculate the expected values, I used the inverse of the average total repertoire size recorded from each individual and multiplied that value by the number of individuals that answered the playback. I compared these expected numbers to the observed number of individuals that answered with the predicted phrase type.

A generalized linear mixed model (GLMM) was used to determine whether there was an effect of treatment on the proportion of phrases that individuals answered. A second GLMM was used to determine whether there was an effect of treatment on the proportion of phrases from the playback that the focal individual overlapped with its own phrases. I use proportion of overlap as my measure of duet coordination because optimal coordination in this species involves little or no overlap (Rivera-Cáceres, 2015) and phrase overlapping should be easy for birds to detect (Dooling & Haskell, 1978). In both models I also included sex and the interaction between sex and treatment as fixed factors; order of treatment was used as a covariate and bout number and individual were

used as nested random factors. Fixed factors were left in the final models if $p \leq 0.05$, the interaction term was left in the final model if $p \leq 0.25$ (Sokal & Rohlf, 1995).

Experiment 2: Removal experiment

I first recorded the duets of 11 established pairs. At least 4 hours of recording were made per territory to get as much of the birds' repertoires as possible (Mann et al., 2003). Eight out of the 11 pairs had been recorded during the previous two years and these recordings were also used in reconstructing repertoires and duet codes. To create a library of each bird's duet code I used the same methods described in experiment 1 (see above). Each different IF phrase pairing was considered a rule within the duet code of females and each different FM phrase pairing was considered a rule within the duet code of males.

I performed a female or male removal from two territories each day, so that either the males or the females from two different territories could be exchanged (details in Appendix A). Each of the new pairs was composed of at least one member that belonged to the original 11 established pairs that were previously recorded. I monitored the birds that remained in their territories every day until they re-paired.

After a re-pairing event I recorded the new pair for one hour every day for an entire week and then one hour every week for up to one month. Five pairs were recorded for the entire month, four pairs were recorded for three weeks, two pairs were recorded for two weeks and two pairs were recorded for five days. I separately analyzed the contribution of each individual to the recorded duets.

In many cases, birds sang phrases with a new partner that I had not recorded from duets with the old partner. These phrases might represent new phrases added to the repertoire after re-pairing, or they might be phrases already present in the repertoire prior to re-pairing but missed by us due to incomplete sampling (Levin, 1996). To estimate repertoire sizes before re-pairing, I used the program EstimateS 9 (Colwell, 2013) to compute Coleman rarefaction curves (Coleman, 1981; Coleman et al., 1982) and 95% confidence intervals of unconditional variance for sample-based abundance data (Colwell et al., 2012). Details on calculation of curves can be found in Appendix A.

To determine if birds used the same answering code for phrase types that were shared between the old and the new mate (shared phrases) I used a Heterogeneity G-test. To calculate the expected values, I used the inverse of the repertoire size recorded from each individual. To determine if birds incorporated the phrases exclusively present in the new mates' repertoire (unshared phrases) in a new duet code I used a GLMM, to test whether canebrake wrens preferentially answered shared rather than unshared phrase types, and thus whether duets were formed by the shared phrases with a probability higher than expected by their frequency in the repertoires of individuals.

To determine whether the duet codes of individuals in new pairs are less consistent than those of individuals in more established pairs I calculated the Shannon index of diversity (H') of phrase pairings used by each individual (IF pairings for females and FM pairings by males). To test for differences in H' I used a GLMM with type of pair (established vs. new) as a fixed factor and

individual and pair as nested random factors. I also controlled for the effect of the number of phrase types used by the partner by including it as a covariate (effect magnitude=0.051, $t_7=4.11$, $p=0.0063$).

To determine whether individuals in new pairs improve their duet code consistency as time progresses I compared H' of the duet codes of individuals in new pairs using a GLMM with time (days 1-4, days 5-8 and weeks 2-4) as a fixed factor and individual and pair as nested random factors. The number of phrase types recorded from each bird's mate was used as a covariate (effect magnitude=0.039, $t_{25}=3.44$, $p=0.002$).

To measure duet coordination, I chose 5 duets with a clear delineation of the beginning and ending of phrases from each day and compared the observed number of phrase overlaps to the expected number found using a Monte Carlo randomization test (Appendix A) in the R package warbleR (Araya-Salas & Smith-Vidaurre, 2016). Individual tests were run for each singing bout (10,000 iterations). I then calculated the proportional difference between observed and expected overlaps as a measure of coordination performance (Overlap index). Positive overlap indices indicate that the observed number of overlaps exceeded those expected by chance (poor coordination), while negative overlap indices indicate less phrase overlap than expected by chance (good coordination). To determine whether coordination performance improves with time I used a GLMM with day after re-pairing as a fixed factor and individual and duet as nested random factors.

Sample sizes for all analyses in Experiment 2 are detailed in table 4.1.

All GLMMs were analyzed using the function `lme` of the package `nlme` (Pinheiro et al., 2013) in R (version 2.15.1). I validated all GLMMs (Experiments 1 and 2) by assessing violations to homogeneity and normality based on the graphic methods suggested by Zuur et al. (Zuur et al., 2009).

Results

Experiment 1: Playback experiment

Song answering

Individuals answered playback of all three treatments: phrases in their partners' repertoires, phrases that were not in their partner's repertoires but were present (though rare) in their population, and phrases from different populations that were unknown in their own population. However, birds answered significantly more often to their partners' phrases than to any of the phrases that were not in their partners' repertoires (Unique Phrases, effect magnitude=0.8 $t_{217}=3.2$, $p=0.0016$ or Other Population Phrases, effect magnitude = 0.88, $t_{217}=3.55$, $p=0.0005$; Fig. 4.2). In addition, males answered a higher proportion of phrases than did females (effect magnitude=1.81, $t_{15}=4.03$, $p=0.001$), but the magnitude of this effect was significantly higher only for unfamiliar phrases (Unique Phrases, effect magnitude= 1.25, $t_{217}=2.43$, $p=0.016$; Other Population Phrases, effect magnitude=1.04, $t_{217}=2.10$, $p=0.036$). The order of the treatments did not have a significant effect on the proportion of phrases produced by the individuals (effect magnitude=0.018, $t_{15}=0.81$, $p=0.42$).

Duet code consistency and temporal coordination

Males and females followed the corresponding duet code when answering their partners' phrases with a probability far above chance (8 out of 8 males sang the predicted phrase, $\chi^2=128.17$, $p<0.001$; 6 out of 9 females sang the predicted phrase, $\chi^2=75.77$, $p<0.001$). Individuals overlapped a significantly higher proportion of phrases that were not present in their partners' repertoires which indicates poorer coordination for any of the unfamiliar phrase types (Unique phrases, effect magnitude=0.32, $t_{12}=3.30$, $p=0.002$, Other population phrases, effect magnitude=0.25, $t_{12}=0.57$, $p=0.049$, Fig. 4.3). Sex and the interaction between sex and treatment and order of treatments had no significant effect on the proportion of overlapped phrases ($p=0.5$, $p=0.53$ and $p=0.24$ respectively) and thus were dropped from the final model.

Experiment 2: Removal experiment

Individual phrase repertoires

Individuals tended to use the same phrase repertoire to create duets with old and new mates. New phrase types found after re-pairing did not exceed the 95% upper confidence interval for the estimated repertoire size in three out of four males (I and M phrase types) and six out of eight females (F phrase types), indicating that these individuals did not change their repertoires after re-pairing. However, one female and one male sang one more phrase type than expected, and another female sang two more phrase types than expected, after re-pairing

(Table 4.2), and thus I cannot reject the possibility that a subset of individuals added a modest number of phrases after re-pairing.

Duet codes

Answers toward shared phrase types

Overall, wrens replied following their original duet codes more often than expected by chance when answering phrases from the new mates that were shared with their old mates ($G_{\text{total}}=155.3$, d.f.=13 $p<0.001$, Table 4.3). Six out of eight females used the same phrase type to answer a phrase type that was shared between the old and new mate with a frequency significantly higher than expected by chance, whereas the same was true for one of four males. The remaining three males and two females used a different duet code than they had with their old mate to answer the same phrases of the new mate (Table 4.3).

Answers toward unshared phrase types

Phrases only present in the new mate's repertoire were used in duets after re-pairing at a frequency (0.63 ± 0.15) equal to that expected from the frequency of these unshared phrases in the repertoire (0.64 ± 0.11) (effect magnitude=-0.01, $t_{13}=0.58$, $p=0.59$). Thus, unshared phrases were used as often as shared phrases in duets, demonstrating that birds adjusted their duet codes to include the novel phrases of their new mates.

Duet code consistency

The diversity indices (H') of duet codes of individuals after re-pairing were significantly higher than those of individuals in established pairs (effect magnitude=-0.54, $t_7=-13.95$, $p<0.0001$, Fig. 4.4). This result indicates that duet

code adherence in new pairs is less consistent than in established pairs. Furthermore, H' values for duet codes of new pairs during the first four days after re-pairing were significantly higher than H' on days four to eight (effect magnitude=-0.277, $t_6=-3.84$, $p<0.001$) and the latter were significantly higher than H' of duets recorded two to four weeks after re-pairing (effect magnitude=-0.45, $t_6=-5.27$, $p<0.0001$, Fig. 4.5). These results show that individuals require time to learn a consistent new duet code after acquiring a new mate.

Duet coordination

Coordination performance of new pairs immediately after pairing was lower than that of established pairs (effect magnitude=-0.22, $t_{19}=-2.85$, $p=0.01$) and subsequently improved significantly as time progressed (effect magnitude=-0.0097, $t_{288}=-2.66$, $p=0.0081$, Fig. 4.1,4.6).

Discussion

Duet code flexibility

The results of the two experiments together demonstrate that the duet codes of adult canebrake wrens are highly flexible after re-pairing events. In the playback experiment, adults of both sexes responded by duetting both to their mate's phrases and to phrases not present in their mates' repertoires. Although responses to unshared phrases were relatively infrequent, the occurrence of even a low level of response to novel phrases suggests how new code elements could be created. After re-pairing events in the second experiment, all individuals significantly modified their duet codes, in that all incorporated into their duets the phrase types of their new mates that were not shared with their old mates, which

required adopting new rules for phrase-pairings. In addition, 5 of 12 individuals showed no overall adherence to their old duet codes in answering phrases of their new mates that were shared with their old mates.

In general, individuals that switched mates used the same phrase repertoire to answer old and new mates. These results suggest that individual phrase repertoires might be acquired during a sensitive period in early development as in other wrens (Brenowitz et al., 1995; Kroodsma, 1974). However, because three individuals sang one to two phrase types above the 95% upper confidence interval I cannot reject the possibility that individuals possess the ability to learn new phrase types as adults (Brenowitz & Beecher, 2005) or that individuals memorize a larger repertoire than they sing with each partner and can decide which phrases to express depending on the identity of the partner (Vargas-Castro et al., 2015).

Levin (1996) found using similar methods that bay wrens do not significantly change their phrase repertoire to answer to new partners, and concluded from this that individuals do not change their duetting behavior after re-pairing. However, here I show that despite conserving the same phrase repertoire, canebrake wrens after re-pairing develop new rules on how their own phrase types link with their mates' phrase types. In the majority of instances, birds retained their old rule when replying to a phrase of their new mate that was shared with the old mate, but three out of four males and two out of eight females answered differently towards the same phrase types present in the repertoire of both old and new mates (Table S4.2). A potential explanation for why individuals

do not always use the same code as before is that even though codes are set at the individual level (Logue & Krupp, 2016) the development of a code requires both individuals to agree on which phrase pairings are allowed. In experiment 1, females answered M phrases correctly, demonstrating that they know the male's code (F-M pairings) as well as their own code (I-F pairings). Females may then use their memory of the F-M pairings developed with their former partner to influence the F-M pairings adopted by their new partner. Both females and males sometimes stop answering after their mates have answered for the first time in a given duet; for example, the female sings an F phrase, the male sings an M phrase, and the female fails to answer. After such aborted duets, birds often switch phrase types (KDRC pers. obs.), which is consistent with the hypothesis that feedback from partners is important in establishing a new duet code. It is important to note however, that individuals that did not follow the same code for phrases that I classified as shared could be interpreting these phrases as different types.

Learning is needed to develop a new code

Levin (1996) also proposed that learning did not play any role in the creation of new duet codes during adulthood in bay wrens. Since then it has been assumed that duet codes do not play a role in the process of pair formation and that they do not provide any information about the strength of a pair bond ((Hall, 2009) but see (L. Marshall-Ball & Slater, 2008)). However, the design of Levin's study rather addressed the question of whether individuals retain part of their codes when they switch mates and whether coordination remains high with

the retained phrase pairings. Levin's design thus focuses attention on those aspects of duetting with the new mate that ought not to require learning because they are retained from the old duet code. The present study demonstrates that canebrake wrens require learning to achieve both temporal precision and pattern-specificity of duets when answering to new phrase types as a) individuals are less coordinated when answering to unfamiliar phrases immediately after mate removal, but improve duet coordination with time and b) the duet codes of individuals in newly formed pairs are less consistent than those in more established pairs and consistency improves with time.

In a previous study of canebrake wrens, Marshall-Ball et al. (2008) found that that temporal coordination was no different for pairs that had been together for two or more years than for pairs that had formed within seven months. However, here I show that immediately after mate removal individuals overlapped significantly more than when they duetted with their established partners (experiment 2). After a low point immediately after re-pairing, coordination improved rather rapidly as time progressed (Fig 4.5), explaining why Marshall-Ball et al (2008) did not find poor coordination when averaging over pairs that had been together up to 7 months. Marshall-Ball et al. (2008) also tested the consistency of the "duet types" of pairs of canebrake wrens that had been together for more than two years versus pairs that had been together less than seven months. Their results suggested that codes become more stable with time, as the duet types of older pairs were more consistent than the ones of newer pairs. However, because the phrase pairings (i.e. duet types) were

regarded as a pair-level behavior (Brumm & Slater, 2007), only FM phrase pairings were taken in account and thus only male's adherence to codes was tested. These results complement my study because it appears that even after several months of being paired together, the duet codes of males are less consistent than the duet codes of males in more established pairs.

Implications for duet functions

This study establishes that both the consistency of duet codes and the precision of temporal coordination improve with time after re-pairing in canebrake wrens. These results are compatible with the hypothesis that duet coordination serves as a signal of pair quality and stability (Brumm & Slater, 2007). Thus, it is possible that in canebrake wrens, duet coordination could function as a pair-level signal to advertise pair quality to rivals in territory defense. This hypothesis is further supported in magpie larks by results showing stronger territorial responses to well-coordinated displays than to poorly-coordinated ones (Hall & Magrath, 2007). Although better coordination has been found in established pairs in some additional species, such as magpie-larks, there are some species for which this pattern does not hold, such as California towhees (Benedict, 2010). I suggest that the pattern of better coordination in established pairs is stronger and thus more easily demonstrated in species that have complex, highly coordinated duets than in species with simpler, looser duets.

As the development of a new pair-specific-duet code requires learning, duet code adherence could also function as an honest signal that advertises an individual's commitment to a mate (pair-bond maintenance hypothesis). In black-

bellied wrens, females adhere more strongly to their duet codes than do males (Logue, 2007). In this study, I found that male canebrake wrens tend to answer more to unfamiliar phrases than do females, and that males are more likely to change their phrase-pairing after re-pairing than females are. These results suggest that the selective pressure of following a duet code could be biased towards one sex or another, and might depend on the level of reproductive investment from each sex and the cost of losing a mate among other factors (Andersson, 1994).

Conclusions

This is the first longitudinal study that has addressed the ontogeny of pair-specific duet codes in new pairs. I found that the duet codes of adult females and males are flexible and change when re-pairing occurs. Furthermore, individuals need a learning period to be able to perform well coordinated duets that follow a consistent duet code. My results show that duet coordination and duet code adherence are honest indicators of pair-bond duration in canebrake wrens.

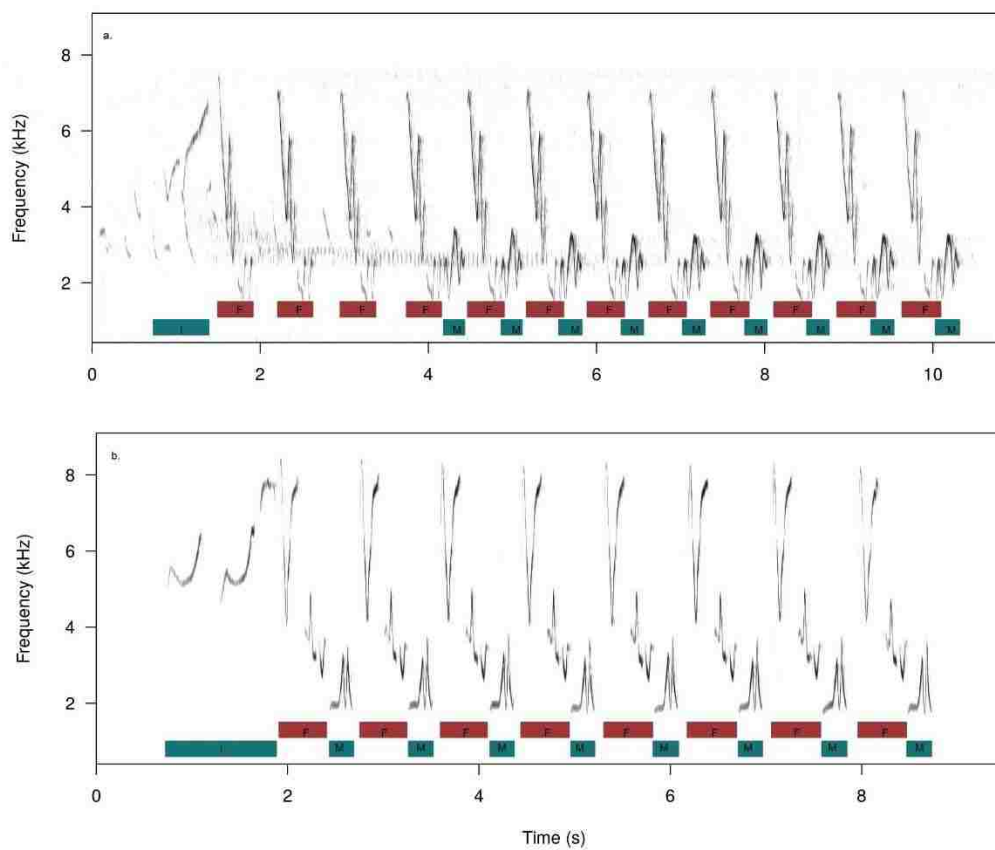


Figure 4.1a. Example of a low coordination performance duet performed by PAE3 four days after re-pairing. Eight phrases were overlapped by the male. b. Example of a high coordination performance duet performed by PAE3 twelve days after re-pairing. No phrases were overlapped. I: Introductory phrase sung by male, F: Female phrase, M: Male phrase.

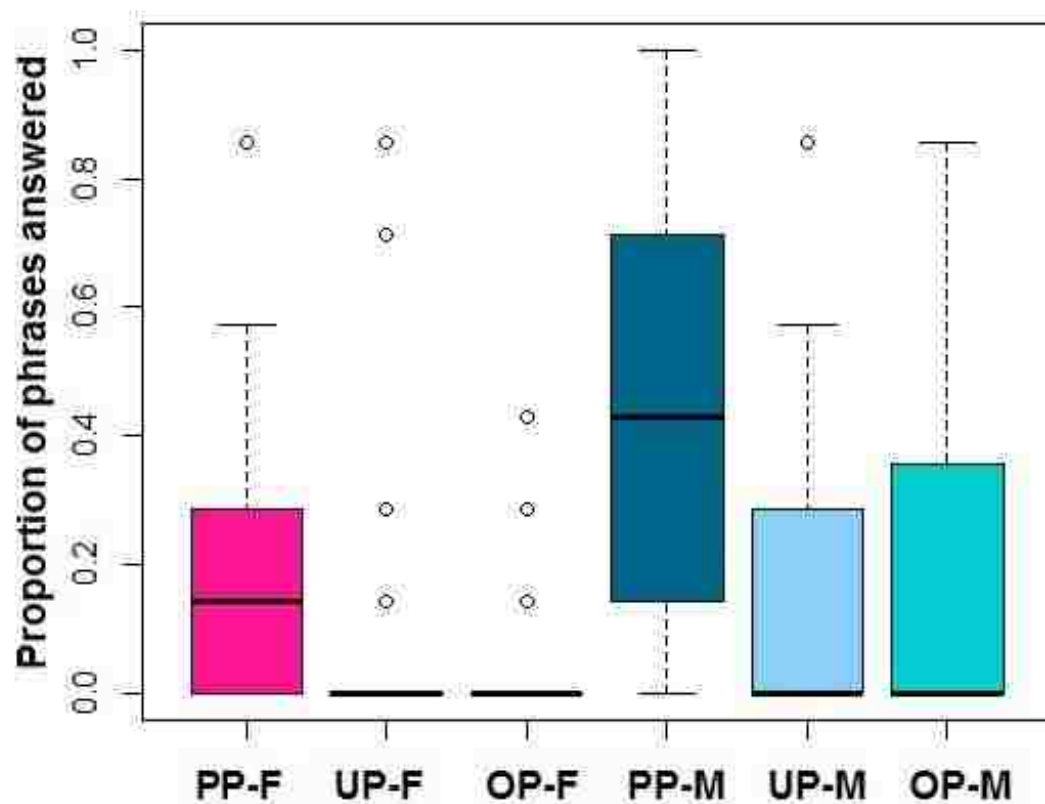


Figure 4.2 Proportion of phrases from playback that females (F) and males (M) answered with their own phrases for each treatment. SPP: Partner's phrase, UP: Unique phrase, OP: Other population phrase. Median (horizontal dark line in each box), quartiles (top and bottom of box), the 0.05 and 0.95 quantiles (tips of vertical whiskers) and extreme data points (open circles) are shown for each boxplot.

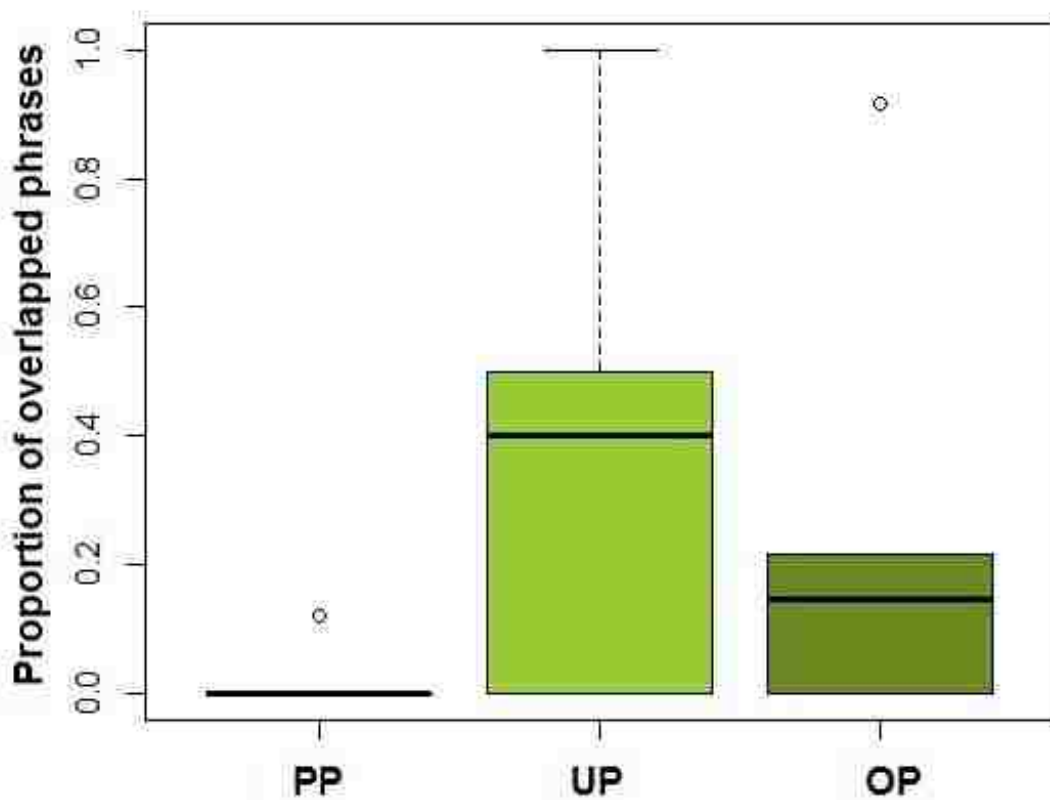


Figure 4.3 Proportion of phrases from playback that individuals overlapped with their own phrases. PP: Partner's phrase, UP: Unique phrase, OP: Other population phrase. Median (horizontal dark line in each box), quartiles (top and bottom of box), the 0.05 and 0.95 quantiles (tips of vertical whiskers) and extreme data points (open circles) are shown for each boxplot.

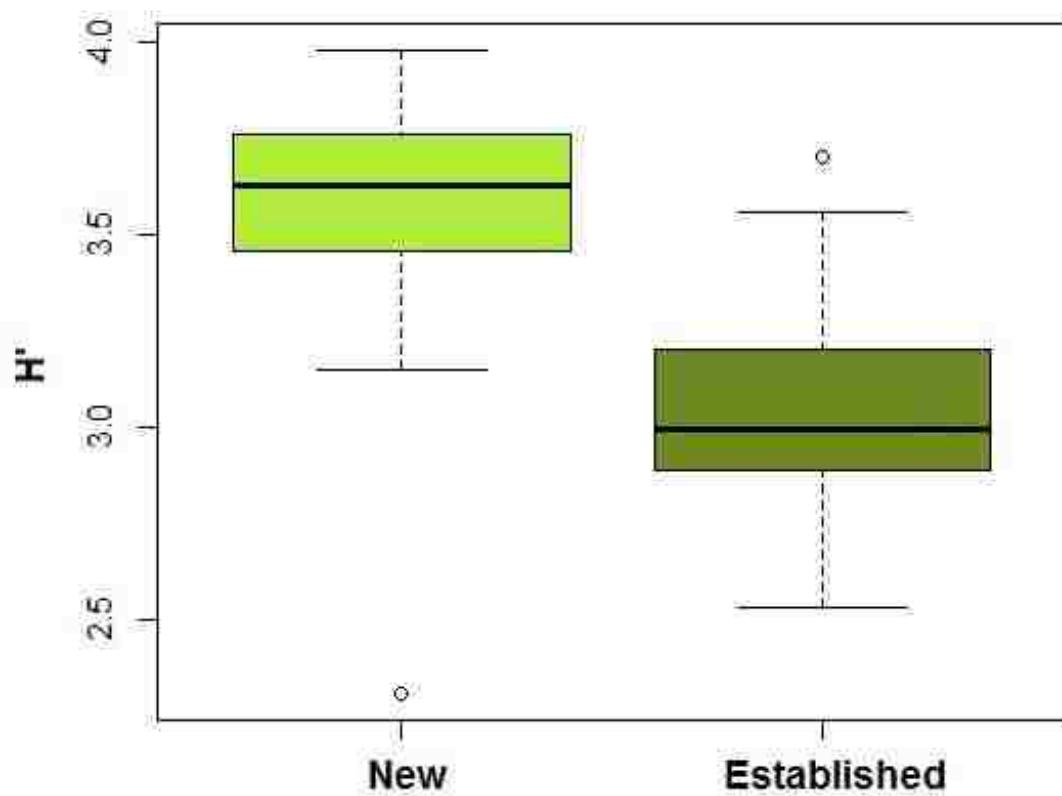


Figure 4.4 Diversity indices of male and female duet codes in established vs new pairs. Median (horizontal dark line in each box), quartiles (top and bottom of box), the 0.05 and 0.95 quantiles (tips of vertical whiskers) and extreme data points (open circles) are shown for each boxplot.

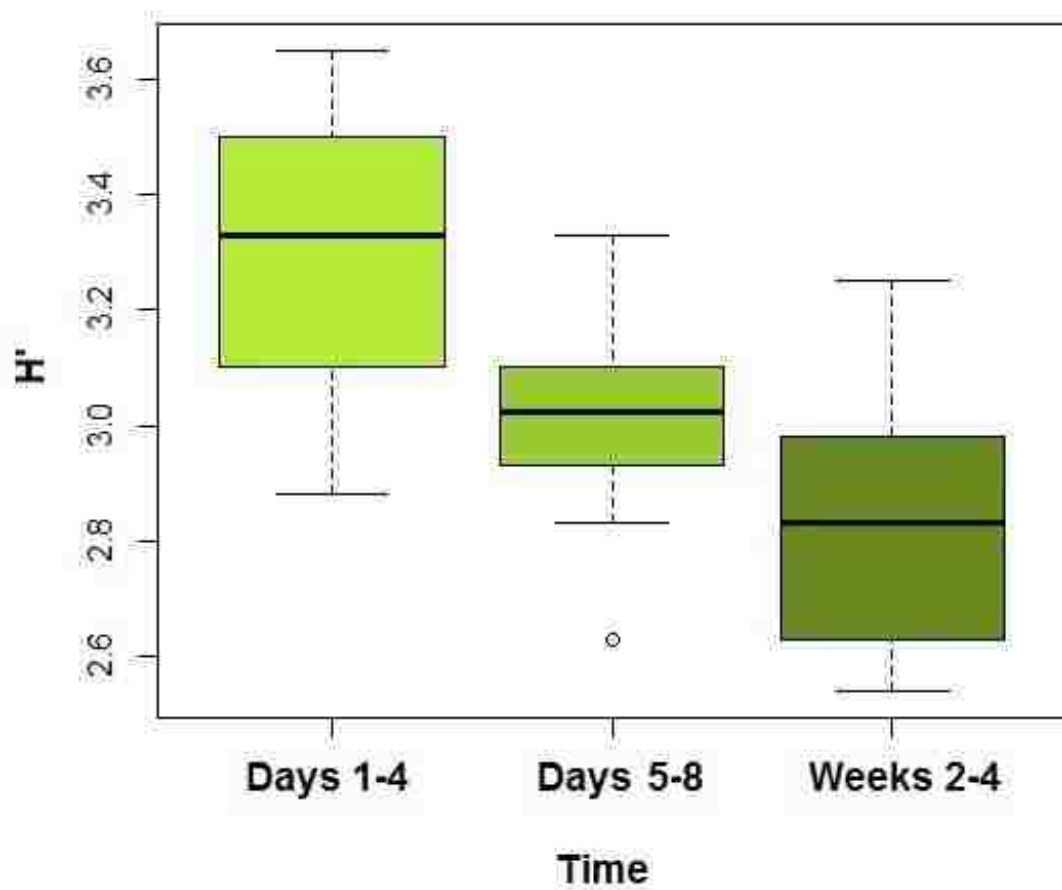


Figure 5 Diversity indices of individuals in new pairs across time. Median (horizontal dark line in each box), quartiles (top and bottom of box), the 0.05 and 0.95 quantiles (tips of vertical whiskers) and extreme data points (open circles) are shown for each boxplot.

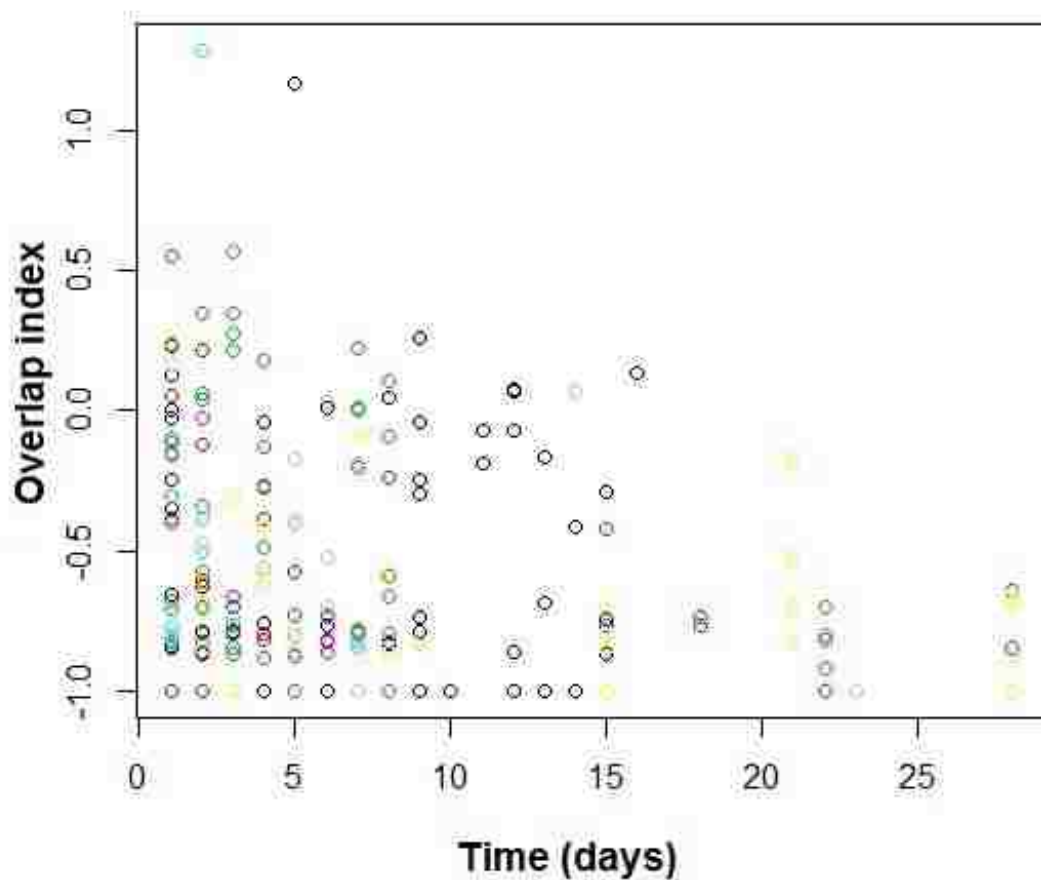


Figure 6 Coordination performances of new pairs across time. Different colors indicate different pairs. Overlap indices represent the proportional difference between observed and expected overlaps ($[\text{obs} - \text{exp}]/\text{exp}$).

Table 4.1 Birds included in each analysis performed in experiment 2, indicated with an X. Only the birds for which I had information from a previous and a new mate (n=12) were included in the Song Repertoire analysis (SR), the Shared Repertoire analysis (ShR) and the Unshared Repertoire analysis (UR). All birds (n=28) were included in the Duet Code Consistency analysis (DCC) and the Duet Coordination analysis (DCI). Only birds that were members of a newly formed pair (n=19) were included in the Duet Code Improvement analysis (DC) and the Coordination Improvement (CI) analysis.

Bird	Territory	Established pair ID	New pair ID	SR	ShR	UR	DCC	DCI	DC	CI
1	G2	G2 F	G2 F	x	x	x	x	x	x	x
2	G2	G2 M	None				x		x	
3	G2	Floater	G2 M				x	x	x	x
4	Casa 1	C1 F	C1 F	x	x	x	x	x	x	x
5	Casa 1	C1 M	None				x		x	
6	Casa 1	Floater	C1 M				x	x	x	x
7	G1	G1 F	LS1 F	x	x	x	x	x	x	x
8	G1	Floater	G1 M				x	x	x	x
9	G1	G1 M	L1 M	x	x	x	x	x	x	x
10	LS1	LS1 F	None				x		x	
11	LS1	LS1 M	LS1 M	x	x	x	x	x	x	x
12	L1	L1 F	L1 F	x	x	x	x	x	x	x
13	L1	L1 M	None				x		x	
14	BB2	BB2 F	BB2 F	x	x	x	x	x	x	x
15	BB2	BB2 M	PAE E M	x	x	x	x	x	x	x
16	BB2	Floater	BB2 M				x	x	x	x
17	PAE E	PAE E F	PAE E F				x	x	x	x
18	PAE E	PAE E M	None				x		x	
19	PAE2	PAE2 F	PAE2 F	x	x	x	x	x	x	x
20	PAE2	PAE2 M	None				x		x	
21	PAE2	Floater	PAE2 M				x	x	x	x
22	PAE3	PAE3 F	PAE3 F	x	x	x	x	x	x	x
23	PAE3	PAE3 M	None				x		x	
24	PAE3	Floater	PAE3 M				x	x	x	x
25	BB1	BB1 F	BB1 F	x	x	x	x	x	x	x
26	BB1	BB1 M	None				x		x	
27	BBarbol	BBarbol F	None				x		x	
28	BBarbol	BBarbol M	BB1 M	x	x	x	x	x	x	x
Totals				12	12	12	28	19	28	19

Table 4.2 Observed and estimated phrase repertoire sizes of individuals before and after acquiring a new mate. Estimated repertoire sizes were obtained by rarefaction curves. E: expected, CI: Confidence Interval, BR: before re-mating, AR: After re-mating.

Individual	Phrase category	Repertoire size E	U 95% CI	Repertoire size BR	New phrase types	Repertoire size AR
BB1F	F	35	54.56	17	10	27
BB2F	F	26.43	38.15	17	9	26
C1F	F	16.86	21.22	15	8	23
G1F	F	34.17	50.73	19	9	28
G2F	F	20.83	25.11	20	6	26
L1F	F	24.53	30.62	20	4	24
PAE2F	F	29.72	35.69	26	8	34
PAE3F	F	21.82	27.23	20	5	25
BBAM	I	31.43	36.08	26	3	29
G1M	I	36.44	62.25	13	13	26
LS1M	I	30.51	42.76	22	5	27
BB2M	I	36	53.71	17	6	23
BBAM	M	21.88	24.56	22	4	26
G1M	M	21.28	30.61	15	9	24
LS1M	M	23.33	28.26	15	5	20
BB2M	M	31.83	47.98	16	4	20

Table 3 G test comparing the responses of an individual (Ind) towards song types of the new mate that were shared with its old mate. M: matched old mate response, NM: no match. For expected values (EM: Expected match and ENM: Expected non-match) I used the inverse of the repertoire size of each individual. Some individuals follow the same rule and some create new rules. Terr: territory.

Ind	Terr	Sex	M	NM	Total	EM	ENM	G	df	p	
1	G2F	F	4	2	6	0.286	5.714	16.91	1	<0.001	
2	C1F	F	8	12	20	0.833	19.167	24.95	1	<0.001	
3	G1F	F	5	5	10	0.476	9.524	17.07	1	<0.001	
3	G1F2	F	7	19	26	0.963	25.037	17.29	1	<0.001	
4	L1F	F	4	15	19	0.826	18.174	6.861	1	<0.01	
5	LS1M	M	2	9	11	0.500	10.500	2.77	1	ns	
6	G1M	M	3	23	26	1.368	24.632	1.557	1	ns	
7	PAE2 F	F	1	17	18	0.643	17.357	0.177	1	ns	
8	PAE3 F	F	7	9	16	0.727	15.273	22.18	1	<0.001	
9	BB2F	F	1	15	16	0.727	15.273	0.096	1	ns	
10	BB2M	M	1	12	13	0.650	12.350	0.172	1	ns	
11	BB1F	F	3	5	8	0.320	7.680	9.136	1	<0.001	
12	BBAM	M	1	13	24	1.091	22.909	36.11	1	<0.001	
								Gtotal	155.3	13	<0.001
								G p	132.2	1	<0.001
								Gh	23.04	12	<0.025

Chapter 5

Conclusions

Vocal interactions in songbirds are common and mediate important interactions such as aggressive encounters and communication between mated pairs (Geberzahn & Hultsch, 2004; Hall, 2009). Studies of song development have concentrated on the development of individual vocalizations (Beecher & Brenowitz, 2005) while the development of vocal interaction rules has been rather neglected. The most complex vocal interactions occur in species that produce coordinated vocalizations (i.e. duets) within mated pairs. Duetting in many avian species involves rules governing temporal coordination and non-random pairing of song types (Hall, 2009). My research addressed the ontogeny and proximate mechanisms of two duetting rules in canebrake wrens.

Previous research has determined that canebrake wrens perform highly coordinated antiphonal duets with almost no overlap. By studying adult birds in the field, I found that canebrake wren pairs achieve precise coordination by performing phrase by phrase modifications to their individual singing tempo (Chapter 2). To alter their singing temporal patterns, individuals take into account acoustic feedback provided by themselves and their partners including whether their partners answer or not and also the phrase types that they and their partners have used. I also found that males modify their singing tempo with more precision than females. It has been shown that other duetting species such as black-bellied wrens (Logue et al., 2008), plain-tailed wrens (Fortune et al., 2011) and happy wrens (Templeton et al., 2013) also perform dynamic adjustments to their singing tempo to be able to duet with precision. These results suggest that

temporal coordination in several duetting species including the canebrake wren requires sophisticated neural processing.

Another unstudied question has been whether, in the same way that birds acquire their song repertoires during early development, juvenile birds in duetting species learn the rules they need to be able to duet during this stage. By following juvenile birds in the field (Chapter 3), I found that young canebrake wrens learn precise coordination and a duet code by singing with a mated pair of adults, presumably their parents. In such interactions, juveniles copy the answers of the same-sex adult to the song phrases of the opposite-sex adult.

Over the period in which I recorded juvenile song, the adherence of juveniles to a duet-code improves, in the sense that they are more and more likely to answer a specific phrase of the opposite-sex parent with a single phrase of their own. Over the same time period, juvenile canebrake wrens perform duets with significantly lower temporal coordination than do adults, but the frequency with which juveniles overlap the song phrases of the opposite-sex adults declines over time, indicating that they also acquire the ability to coordinate their duets at this stage. Because I have shown that duet codes and duet coordination are re-learned during adulthood (Chapter 4), I propose that what is most important for juvenile wrens to learn are the general rules governing duet coordination and duet codes, rather than the specifics of a particular code.

Finally, I have found that sex-specific song repertoires of canebrake wrens are set very early during development as young birds sing the song types that correspond to their genetic sex as soon as they begin to utter their first songs.

This result suggests that there is an innate sex template that specifies the types of songs each individual should sing.

It has been proposed that complex duetting features like precise coordination and duet code adherence are signals of pair-bonding strength (the pair-bond maintenance hypothesis (Wickler, 1980)). For these duet traits to signal commitment to a partner, the traits must be non-transferable between partners. Previous research has shown that duet codes in canebrake wrens are pair specific and thus fulfill that requirement (Mann et al., 2003). The fact that duet coordination in canebrake wrens is achieved by taking in to account the phrase types that individuals use to sing (i.e. their duet codes) suggests that precise coordination is also non-transferable. The second requirement of the pair-bond maintenance hypothesis is that the pair-bond traits should be costly to acquire. One way in which duetting rules could incur a cost is if developing these rules with a new partner requires an extended period of learning.

To determine if duet coordination and duet codes are learned during adulthood I performed a removal experiment and allowed new pairs to form (Chapter 4). By comparing the consistency of duet codes and temporal coordination in established and new pairs, I found that duet codes are less consistent and temporal coordination is less precise in new pairs than in established ones. I also determined that the consistency of duet codes and precision of temporal coordination in new pairs improved with time. Thus, I show that these rules are costly to acquire because they require a learning period after pair formation. I conclude that both duet coordination and duet code adherence

are honest indicators of pair-bond duration and are likely to signal pair stability either within the pair or to neighbors. This is the first longitudinal study that has addressed the ontogeny of pair-specific duet codes in new pairs. The result of this study overthrows the conclusion that learning is not required to duet with a new partner, which had been accepted for over 20 years.

This set of studies is the first to address the ontogeny of vocal interaction rules in birds. I have shown that the proximate mechanisms and ontogeny of duet codes and temporal precision can be as complex as the mechanisms and ontogeny of song repertoire acquisition. More studies that address the ontogeny of vocal interaction rules in duetting and non-duetting species are needed to be able to draw more general conclusions.

Human conversation possesses complex rules that are analogous to vocal interaction rules in birds. One set of human rules governs turn taking, in which two individuals exchange utterances separated by short silent gaps but with no overlap (Chow et al., 2015), a set of rules clearly analogous to those governing temporal precision in duetting birds. Additionally, humans possess a rule termed “adjacency pairs” in which utterance types are linked during exchanges (e.g. question-answer type of exchanges) (Sacks, 2004), which is analogous to duet codes. The birdsong model has been amply used as an analog for speech development in humans (Doupe & Kuhl, 1999). Many behavioral, neurological and genetic similarities have been discovered allowing us to understand the development of birdsong and human speech as I understand few other behaviors. Here I provide evidence that vocal interaction rules in humans and

songbirds are also analogous. Thus the study of duetting rules in songbirds can help us understand the development, function, and mechanics of human conversation rules.

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Appendix A

Supplementary methods for Chapter 4

Animal welfare

Throughout both experiments I ensured that direct manipulation of individuals was reduced to a minimum. All 17 birds in Experiment 1 were seen behaving normally immediately after the trials, normal behavior was maintained thereafter. In Experiment 2 I were able to monitor 18 out of 25 birds that remained territorial after the partner exchanges. All 18 birds were behaving normally immediately after the manipulations.

Experiment 1

Previous to the playback experiment, the pairs were recorded for at least six hours to obtain as much of their song repertoires as possible.

The phrases used for the luring playback were recorded in previous years from pairs that were not neighbors of the focal pair Playbacks for all treatments were assembled with Syrinx software using phrases selected for high signal to noise ratios. For male stimuli I only used M phrases because I phrases are commonly sung as solo songs and thus females often fail to respond to them. For female stimuli I used F phrases. Each phrase was repeated 7 times to form a bout, with 0.5 seconds between male phrases and 0.35 s between female phrases, matching the mean gap durations measured for the study population (KDRC unpublished). Each bout was repeated five times with 10 s between bouts. Treatments were separated by silent intervals of 90 seconds.

The population from which I recorded the phrases used in treatment 3 (Other Population Phrases) is located 50km away from my study site. While all of the territories included in this experiment were located less than 5km away from each other. In four seasons I never saw the phrase types used for treatment 3 in my study site.

Experiment 2: Partner exchanges

Partner exchanges were performed between 6:00 am and 9:20am (solar time). In total I performed 5 partner exchanges. For the 11th territory I performed a single female removal and placed her in one of the territories where a male had not re-mated. Some of the birds whose mates I removed re-mated with a floater rather than the exchanged bird (Table S1). I used playback of recorded duets to attract resident pairs to a mist net for capture. When a member of a focal pair was captured, I transported it in a holding bag to the territory where the second removal would take place. For the second removal I again used playback to attract the member of the resident pair of the same sex as the bird already captured. Once the second bird was captured, I released the first bird into its new territory. The second bird was placed in a holding bag, taken to the first territory and released there. The territories that were chosen for mate exchange were at a distance of at least 1km from each other. After a mate exchange was carried out I waited three days to perform the next mate exchange.

Experiment 2: Rarefaction curves

I used the repertoire recorded before mate removal as the reference sample with 1 hour of recording as the sample unit and extrapolated the rarefaction curves to include the sample units recorded after re-mating. At least four sample units per pair before mate exchange and five to twelve sample units per pair after mate exchange were included in the analysis. If the number of observed new phrase types after re-mating exceeded the upper 95% prediction interval I considered there to be a change of song repertoire. To calculate each curve and its corresponding confidence intervals I ran 100 randomizations without replacement and with the bias-corrected form of the Chao1 and Chao2 richness estimators (Chao, 2005). For this analysis I included 12 birds for which I had song repertoires before and after re-mating.

Experiment 2: Randomization test for duet coordination

The test in the R package warbleR (Araya-Salas & Smith-Vidaurre, 2016) shuffles sequences of signals and the silent intervals between signals many times to generate a null distribution of the number of overlaps expected if singing timing was independent from each other. Songs in randomized sequences are separated by silent intervals. The associated p-value is calculated as the number of random sequences with an equal or more extreme number of overlaps than the observed value. Significant song alternation was considered at p-value < 0.05. Individual tests were run for each singing bout (10 000 iterations).

Chao, A. 2005 Species richness estimation, Pages 7909-7916 in N. Balakrishnan, C. B. Read, and B. Vidakovic, eds. *Encyclopedia of Statistical Sciences*. New York, Wiley.

Araya-Salas, M. & Smith-Vidaurre, G. 2016 warbleR: an r package to streamline analysis of animal acoustic signals. *Methods in Ecology and Evolution*, n/a-n/a. (doi:10.1111/2041-210X.12624).