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THE SINGING BEHAVIOR OF SPLENDID FAIRY-WRENS (*MALURUS SPLENDENS*)

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CHAPTER 1: INTRODUCTION

Animals face a variety of challenges in their daily lives. They must find and secure resources, avoid becoming prey, and successfully negotiate interactions with mates and rivals to maximize reproductive success. In each of these contexts, individuals can exchange information through communication to influence the behavioral decisions made by their counterparts (Bradbury and Vehrencamp 2011). Communication is an important topic of study in the field of behavioral ecology, as communication behavior can have consequences for the reproductive success of individuals (e.g. Vasconcelos et al. 2011). On a larger scale, communication can play a role in processes such as speciation (Boughman 2002) and reinforcement (e.g. Höbel and Gerhardt 2003) and is therefore also integral to evolutionary biology in general.

During a signaling interaction, a sender can convey information about itself or the current environment using visual, acoustic, chemical, tactile, and electrical signals (Bradbury and Vehrencamp 2011). The specific form of a signal is shaped by the physical (Podos 2001), physiological (Ryan et al. 1992), and cognitive capabilities (Akre et al. 2011) of the sender and receiver, as these determine the range of possible signals that can be produced and perceived. Because the signaling environment can distort, degrade, and mask a signal as it propagates to the intended receiver (e.g. Wiley and Richards 1978, Forrest et al. 1993), the properties of the transmission medium, such as air temperature and humidity (Henwood and Fabrick 1979) or ambient light conditions (Gomez and Théry 2004), can further shape signal design. The interplay of these selective forces, together with social and sexual selection, has generated an incredible diversity of visual patterns, sounds, smells, and behaviors in the animal kingdom.

Many taxa, including insects, anurans, fish, reptiles, birds, and mammals, use acoustic signals to exchange information (e.g. Gerhardt and Huber 2002, Bass and McKibben 2003, Vergne et al. 2009, Catchpole and Slater 2008, Peters and Wozencraft 1989, Tyack and Clark 2000, respectively). Sound has several advantages that account for its ubiquitous use in communication; acoustic signals do not require ambient light or a direct line of sight between the sender and receiver, and they can quickly and reliably convey dynamic patterns over large distances (Bradbury and Vehrencamp 2011). Because sound production is energetically expensive and subject to constraints, acoustic signals can serve as honest indicators of characteristics such as body size (e.g. Davies and Halliday 1978), age (e.g. de Kort et al. 2009), stamina (e.g. Fischer et al. 2004), and condition (e.g. Mager et al. 2007).

One of the most striking displays of acoustic communication is the avian dawn chorus. There are a number of hypotheses for the social function of this burst of vocal activity; it can serve as an arena for mate attraction, function in mate guarding and stimulation, announce territory ownership, and enable individuals to assess and adjust their relationships with territorial neighbors (reviewed in Staicer et al. 1996). A male's performance during the dawn chorus can impact his reproductive success by influencing his mating opportunities (e.g. Poesel et al. 2006), as well as the reproductive decisions (Mennill et al. 2002) and investment of his social mate (Garcia-Fernandez et al. 2010). Consequently, singing behavior during the dawn chorus represents an important part of the picture for understanding how sexual selection shapes communication in songbirds.

In this dissertation, I address two key aspects of singing behavior, song type use and timing, and explore the extent to which these performance features can function as signals and signaling strategies in songbirds. Although individuals of some species sing only one type of song (e.g. field sparrows, *Spizella pusilla*, Zhang et al. 2016), in species such as nightingales (*Luscinia megarhynchos*), individuals can sing over 150 different song types (Kipper et al.

2004). Having a repertoire of multiple song types allows for increased versatility in singing behavior; song type use can follow a pattern of immediate or eventual variety (Hartshorne 1956), depend on social context (e.g. Trillo and Vehrencamp 2005), or vary interactively with respect to the songs of other individuals (e.g. Burt and Vehrencamp 2005). Songbirds with repertoires of any size can vary the timing of their songs, and doing so interactively with respect to the songs of other individuals can result in song overlapping and overlap avoidance. Patterns in song type use and timing can convey information to receivers (e.g. song type matching as an early threat signal in song sparrows, *Melospiza melodia*, Akçay et al. 2013), or function as signaling strategies that impact how the information in a performance is transmitted, received, or perceived.

In Chapter 2, I explore whether song type use during the dawn chorus could function as a signal and communication strategy in splendid fairy-wrens (*Malurus splendens*), a cooperatively breeding but reproductively promiscuous species. In several species in the genus *Malurus*, males produce trills in response to avian predator vocalizations (Greig and Webster 2014). Male splendid fairy-wrens have incorporated these trills into their dawn chorus performances, appending them as suffixes to their standard display songs (Greig and Pruett-Jones 2008). To gain insight into why a predator-elicited trill has been incorporated into the display repertoire of splendid fairy-wrens, I examined the potential costs and benefits associated with trill use during the dawn chorus. An analysis of the acoustic structure and propagation of splendid fairy-wren song types revealed that the addition of a trill suffix could represent a signaling strategy through which males are able to increase the detectability, localizability, and information content of their dawn chorus performances.

In Chapter 3, I discuss song overlapping, a common occurrence during the splendid fairy-wren dawn chorus and the subject of a recent controversy. Many studies have suggested that song overlapping functions as an aggressive signal in songbirds (reviewed in Naguib and Mennill 2010), but the possibility that overlapping is a chance occurrence has rarely been addressed (Searcy and Beecher 2009). It can be difficult to distinguish between intentional and accidental overlapping, in part, due to the lack of compelling null models for predicting chance levels of overlap. To address this issue, my coauthors and I developed the Song Overlap Null model Generator (SONG), a freely available software package for the statistical language R that uses resampling randomization to calculate the expected amount of overlap due to chance in a given interaction. We demonstrated the effectiveness of the SONG package by examining overlapping in three avian species: black-capped chickadees (*Poecile atricapillus*), rufous-and-white wrens (*Thryophilus rufalbus*), and long-tailed manakins (*Chiroxiphia linearis*). The potential applications for the SONG package extend well beyond song overlapping in birds; this tool can be used to study the coordination of any temporally patterned behavior, including visual displays, movements, and multimodal signaling interactions.

In Chapter 4, I apply the SONG package to investigate whether song overlapping could function as a signal or strategy during the dawn chorus of splendid fairy-wrens. There are many hypotheses for the function of song overlapping (reviewed in Helfer and Osiejuk 2015), and each one makes predictions about the contexts in which the behavior should occur as well as the relationships between the overlapper and overlapped individual. Splendid fairy-wrens are an excellent system in which to test the various predictions; as cooperative breeders with high rates of extra-pair paternity (Webster et al. 2004), the social system of splendid fairy-wrens is characterized by a complex blend of cooperation and competition among males. Consequently, singing interactions between splendid fairy-wrens during the dawn chorus can occur both within and across territory boundaries and between both related and unrelated sets of individuals, making it possible to tease apart whether overlapping arises through cooperative or competitive mechanisms. The latter appears to be the case in this species; overlap avoidance was observed in interactions between related individuals (i.e. fathers and sons), while significant overlapping was observed only in interactions between unrelated territorial rivals. The additional finding that males with the largest cloacal protuberances and tips (presumably the highest-quality males) were overlapped at the highest rates suggests that song overlapping might function as a masking strategy in this species.

In the chapters that follow, I use a combination of observational, computational, and experimental approaches to quantify singing behavior with the ultimate goal of shedding light on the functions of trill use, song overlapping, and the dawn chorus as a whole in splendid fairy-wrens. The research described in this dissertation contributes to our knowledge of how animals exchange information, and provides further evidence of the roles that natural, social, and sexual selection play in shaping signals and signaling behavior.

CHAPTER 2: THE POTENTIAL COSTS AND BENEFITS OF A TERMINAL TRILL IN SPLENDID FAIRY-WRENS

ABSTRACT

The form and function of acoustic signals are linked via selection for optimal transmission through the environment. In this chapter, we present a detailed analysis of the acoustic structure, use, and long-distance propagation of splendid fairy-wren song (*Malurus splendens*). Splendid fairy-wrens produce two song types, the Type 1 chatter and the Type 2 trill, and males often append the trill as a suffix to their Type 1 song during their dawn chorus performances. To gain insight into why this singing behavior evolved, we explored, from an acoustic perspective, the potential costs and benefits associated with the addition of the Type 2 suffix. Using an analytical model, we simulated the effects of atmospheric absorption and ground reflection to examine how splendid fairy-wren songs change in amplitude and frequency composition as they travel through the environment. We compared these patterns of attenuation to those in the natural environment as measured with a microphone array. Our analyses revealed that Type 1 and Type 2 song structures occupy distinct frequency ranges, attenuate at different rates, and vary in their susceptibility to distortion. Although the addition of the Type 2 suffix comes at the expense of Type 1 song duration and performance, combining the song types to form multi-component songs could increase the detectability, localizability, and information content of a male’s dawn chorus performance.

INTRODUCTION

Through natural and sexual selection, signals used in animal communication are shaped by many forces, including their functions and the environments through which they transmit. Acoustic signals that function in mate attraction and territoriality are generally most effective when detectable over a large area. This led Morton (1975) to hypothesize that the spectral and temporal features of a long-distance signal should function to maximize its broadcast area, reflecting the transmission properties of the habitat. The "acoustic adaptation hypothesis," as it has come to be known, has received some empirical support (Boncoraglio and Saino 2007, Ey and Fischer 2009) and has sparked many years of research on the relationship between the physical properties of the environment and the design of acoustic signals. Although Morton articulated the acoustic adaptation hypothesis specifically with broadcast area, and therefore attenuation, in mind, design features related to other acoustic properties, such as localizability, directionality, and susceptibility to distortion and degradation, can also be shaped by the environment (Wiley and Richards 1978).

The optimal propagation pattern of a signal depends on its social function. As a result, the distance to the intended receiver, and the cost of eavesdropping by unintended receivers, can shape a signal's broadcast area (e.g. Brenowitz 1982, Brown 1989, Piza and Sandoval 2016) and directionality (e.g. Patricelli et al. 2007, Yorzinski and Patricelli 2009). For signals with opposing messages, selection for and against acoustic properties such as localizability can result in diametrically opposed signal designs. For instance, alarm calls that signal for others to flee immediate danger are difficult to localize, generally taking the form of a high-frequency whistle with a gradual onset, while "assemble" alarm calls, which recruit individuals to a specific area, are highly localizable, often consisting of repeated, broadband notes with sharp onsets (Marler 1955, Klump and Shalter 1984, reviewed in Bradbury and Vehrencamp 1998).

Because of the strong connection between form and function in acoustic signals, it is possible to make inferences about the potential function of a signal through a detailed analysis of its acoustic structure and propagation. We used this approach to explore the possible function of the trill song of splendid fairy-wrens (*Malurus splendens*). Splendid fairy-wrens are a cooperatively breeding species, often forming social groups composed of a breeding pair and one or more helpers that aid in territory defense and offspring provisioning (Rowley and Russell 1997). Although socially monogamous, female splendid fairy-wrens seek extra-pair copulations, and more than half of nests contain young sired by males other than the social mate (Webster et al. 2004). Reproductive success through extra-pair paternity is not restricted to the dominant, breeding males; many “non-breeding” helper males are able to sire a small number of young within or outside of their social group (Webster et al. 2004). Consequently, the social system of this species is characterized by a mismatch of social and genetic relationships and a blend of cooperation and competition within territory boundaries. Splendid fairy-wrens mediate these social relationships through vocal communication, and they possess a signal repertoire containing two distinct song types: the Type 1 chatter song and the Type 2 trill song (Figure 2.1).

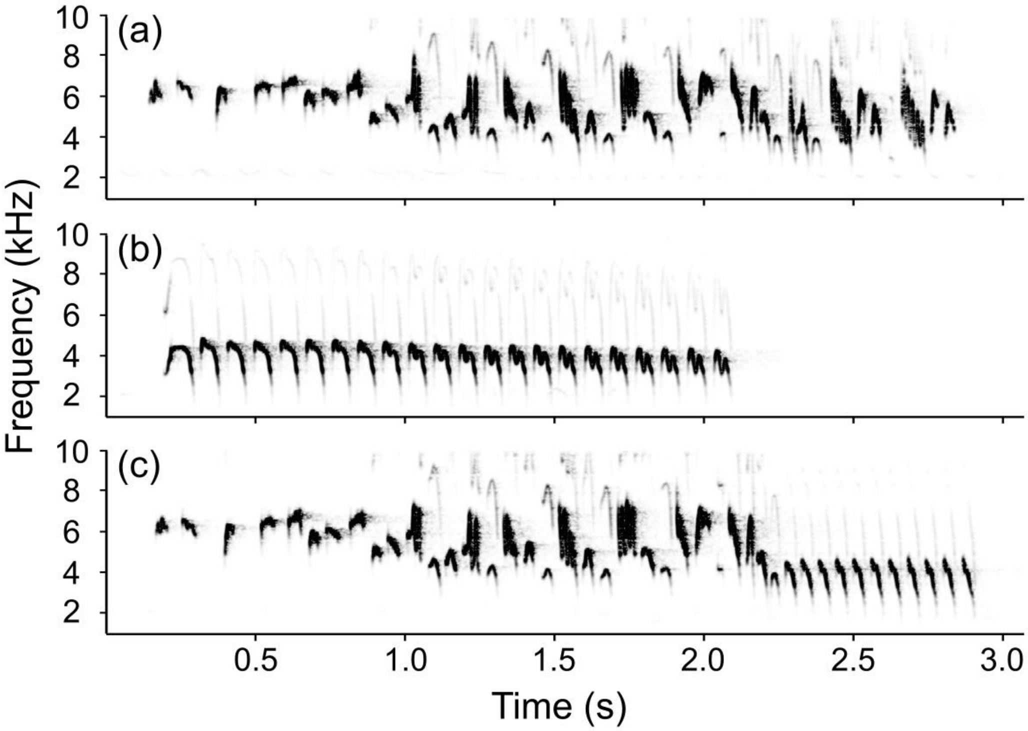

Figure 2.1. Spectrograms of the song types in the splendid fairy-wren repertoire: (a) the Type 1 song, (b) the Type 2 song, and (c) the Type 1 song with a Type 2 suffix (Type 1+2 song).

The Type 1 song consists of a variety of note shapes arranged in repeated syllables (Figure 2.1a). Male splendid fairy-wrens sing this song type at high rates early in the breeding season, but singing rates decrease dramatically after their chicks hatch, suggesting the Type 1 song might play a role in the attraction of social and extra-pair mates (Greig and Pruett-Jones 2008). Females also sing this song type, but at lower and more consistent rates throughout the breeding season, suggesting the Type 1 song likely functions in intra-group communication and territory defense as well (Greig and Pruett-Jones 2008). This song type appears to be individually distinctive in structure (pers. obs.), and splendid fairy-wrens are able to distinguish social group members from unfamiliar individuals on the basis of this vocalization (Payne et al. 1988, Johnson et al. in review).

The Type 2 song is a trill consisting of a series of frequency modulated notes (Figure 2.1b), and females rarely produce this vocalization (Zelano et al. 2001, Greig and Pruett-Jones 2008). Male splendid fairy-wrens often sing Type 2 songs in response to the vocalizations of avian predators such as grey butcherbirds (Zelano et al. 2001). Although predator-elicited, this song type does not appear to function as an alarm signal (Greig and Pruett-Jones 2009, Greig and Pruett-Jones 2010), but seems to function as a display that could be amplified when following an attention-grabbing predator vocalization (Greig and Pruett-Jones 2010). Male splendid fairy-wrens also incorporate this song type into their dawn chorus performances, either singing the Type 2 song alone or appending it to a Type 1 song to create a multi-component song with a trill suffix (Type 1+2 song, Figure 2.1c).

There are seven species in the *Malurus* genus that produce trills in response to predator vocalizations; like splendid fairy-wrens, superb (*M. cyaneus*), blue-breasted *(M. pulcherrimus*), and purple-crowned (*M. coronatus*) fairy-wrens have incorporated the trills into their dawn chorus performances, appending them to their standard display songs (Greig and Webster 2014). This pattern suggests that the trill vocalization originated in a predator context, and phylogenetic analysis indicates it was incorporated as a display three separate times in evolutionary history (Greig and Webster 2014). As habitat use, geographical distribution, and extra-pair paternity rates do not appear to be correlated with trill use in this family (Greig et al. 2013, Greig and Webster 2014), it remains unknown why this singing behavior evolved in these species.

Recombination of song components to form novel arrangements is common in songbirds (e.g. Slater and Ince 1979, Todt et al. 1979, Slabbekoorn et al. 2003), and these arrangements can become established in populations through cultural selection and drift (reviewed in Podos et al. 2004, Price 2008). In the subset of fairy-wren species that have incorporated trill vocalizations into their dawn chorus performances, songs including a trill component could have evolved through cultural mechanisms. Another possibility is that incorporating the trill vocalization allows a male fairy-wren to increase the effectiveness of his dawn chorus performance, perhaps by increasing its information content, broadening its intended audience, or improving its reception (Table 2.1).

Examining the contexts in which vocalizations are used and how receivers respond to them has revealed that songs, and components of songs, that vary in acoustic structure can contain unique information (e.g. Elfström 1990, Nelson and Poesel 2007) and can be used to communicate with different classes of receivers (e.g. Catchpole 1980, Nelson and Croner 1991, Fessl and Hoi 1996, Leedale et al. 2015). The distinct acoustic structures of the Type 1 and Type 2 songs of splendid fairy-wrens suggest that perhaps the song types differ in their information content or intended audiences. The Type 1 song appears to be used as a multi-purpose signal, functioning in mate attraction, intra-group communication, and territory defense (Greig and Pruett-Jones 2008). The Type 2 song also functions as a display (Greig and Pruett-Jones 2009), but whether it is intended for potential mates or territorial rivals is not clear (Greig and Pruett-Jones 2010), and the extent to which it contains information that is not available in the Type 1 song structure is unknown. If the Type 2 song conveys additional information about a male's quality or group membership, incorporating this song type into a dawn chorus performance could enable a male to broadcast this information to a large number of receivers during the time of day in which the environmental conditions are best for sound transmission (Henwood and Fabrick 1979).

Table 2.1. A summary of the hypothetical benefits and *a priori* predictions associated with the incorporation of the Type 2 trill structure into dawn chorus performances. The hypotheses marked with an asterisk (*) are specifically addressed in this study.

Description of benefit	Predictions for song type structure	Predictions for song type propagation	Predictions for song type use
Provide information about quality, status, or motivation (Greig and Webster 2014). *	If the trill is an honest signal, there should be evidence of performance costs or constraints that vary across individuals. A male's performance of the trill should correlate with other indicators of quality, status, or motivation.	No specific predictions	Males should incorporate the trill when it is beneficial to convey this information (e.g. territorial interactions, mate attraction attempts). A male's use of the trill should reveal evidence of performance costs or constraints that vary across individuals.
Provide information about social group membership or dialect identity.	Trill structure should be similar within social groups. If communicating dialect identity, trill structure should vary on a (micro-) geographic scale.	No specific predictions	Males should incorporate the trill during interactions with social group members and neighboring rivals.
Provide information about location. *	The trill should have features that increase the localizability of the performance or song (e.g. energy concentrated at low frequencies, frequency modulation, and note repetition).	The incorporation of the trill should provide a pattern of attenuation or degradation that varies predictably with distance to allow for ranging.	Males should incorporate the trill when it is beneficial to communicate location (e.g. when the intended receiver is distant, when ambient light levels are low).

Table 2.1. (continued)

Description of benefit	Predictions for song type structure	Predictions for song type propagation	Predictions for song type use
Communicate with multiple classes of receivers (e.g. social mate, group members, rivals, extra-pair mates).	There should be a relationship between the song structure and its intended receiver; short, simple, stereotyped songs typically function in male-male interactions (e.g. Catchpole 1980, Nelson and Croner 1991, but see Leedale et al. 2015).	There should be a relationship between the active space of the song type and the typical distance to the intended receiver.	Trill use should vary depending on the audience; songs containing both song types should be used when males are broadcasting to multiple receiver classes.
Attract the attention of conspecifics due to the association between the trill and danger (Greig and Webster 2014).	No specific predictions	No specific predictions	The trill should be used in display contexts while retaining its association with danger. If the trill functions as an alerting component (*sensu* Richards 1981), it should precede the Type 1 component in multi-component songs.
Increase the active space and detectability of his performance or song. *	The trill should have features that enhance its detection and propagation (e.g. energy concentrated at low frequencies, a narrow bandwidth).	The trill should have a larger active space than the Type 1 song.	Males should incorporate the trill when the intended receiver is distant.
Insure against distortion caused by absorption, reflection, and scattering. *	The trill should have features that resist distortion (e.g. a narrow bandwidth).	The frequency composition of the trill should change relatively little as it travels through the environment.	Trill use should be common, especially in environmental conditions in which distortion is likely.

As a consequence of their distinct acoustic structures, it is likely that the Type 1 and Type 2 songs of splendid fairy-wrens transmit differently through the environment and are perceived differently by receivers. As sound travels, high frequencies are absorbed and scattered to a greater extent than low frequencies (Wiley and Richards 1978); as a result, the Type 2 song structure, which has energy concentrated in a lower and narrower frequency range than the Type 1 song structure (Figure 2.1), may travel farther and with less distortion of its frequency composition. Lower frequency sounds tend to be more localizable as well, given that scattering can obscure the direction from which a sound originated (Wiley and Richards 1978). The Type 2 song might be more easily localized not only as a result of its frequency composition, but also due to its repetition of note structure, which facilitates binaural comparisons (Bradbury and Vehrencamp 1998). Thresholds for detection and discrimination on the part of the receiver also depend on acoustic structure; sounds with energy spread over a wide bandwidth have higher detection thresholds than sounds with energy concentrated in narrower frequency ranges (Lohr et al. 2003). Therefore, for a given signal-to-noise ratio, the Type 2 song structure may be more detectable than the Type 1 song structure. Considering these potential differences in the propagation and perception of splendid fairy-wren song types, incorporating the Type 2 song could enhance a male's dawn chorus performance by increasing its broadcast area, detectability, localizability, and resistance to distortion.

To explore the potential benefits associated with the use of the Type 2 trill during the dawn chorus, we performed a detailed analysis of the acoustic structure, long distance propagation, and use of splendid fairy-wren song. We used an array of microphones to record the dawn chorus performances of male splendid fairy-wrens at various distances, enabling us to quantify aspects of each male's performance as well as the overall effects of the natural environment on the propagation of Type 1 and Type 2 songs. Additionally, we devised acoustic filters based on a physical model of attenuation that simulate the effects of atmospheric absorption and ground reflection on the propagation of sound in the splendid fairy-wren's typical dawn chorus environment. Applying the filters to songs recorded at close range allowed us to examine how frequency-dependent attenuation is expected to alter the amplitude and frequency composition of the two song types as they travel through the environment.

METHODS

Study site and subjects

Splendid fairy-wrens are non-migratory, cooperatively breeding passerines endemic to Australia (Rowley and Russell 1997). They are often found in the semi-arid "mallee scrub" – a habitat characterized by mallee eucalypts and low shrubs such as *Senna* and saltbushes (Simpson and Day 2010). Male splendid fairy-wrens participate in the dawn chorus, singing as many as 9 songs per minute (Greig and Pruett-Jones 2008). Each male sings from a set of song posts at a height of approximately 1-2 m. Although song posts are often exposed, males sometimes also sing within shrubs. We conducted the fieldwork for this study at the Brookfield Conservation Park in South Australia during the breeding seasons (October – December) of 2014 and 2015. Each season, we monitored the behavior and reproductive success of 50-60 social groups (120-140 banded individuals; see Webster et al. 2004 for more details on the study site and field methodology). Processing the dawn chorus recordings for splendid fairy-wrens is time intensive due to their high singing rates and frequent song overlapping. As a result, only a small subset of the banded males (22 unique individuals) was included in this study. This fieldwork was conducted with approval from the University of South Australia Animal Ethics Committee

(Wildlife Ethics Committee approvals 17-2012, 18-2012, 21-2013), and the University of Chicago Institutional Animal Care and Use Committee (ACUP permit number 72322).

Acquisition and processing of close-range recordings

We recorded the dawn chorus performances of individual male splendid fairy-wrens using unidirectional shotgun microphones (frequency response: 40 Hz - 20 kHz ± 2.5 dB; model: ME 66 with K6 power module; Sennheiser Electronic GMBH & CO. KG, Wedemark, Germany) and digital recorders (model: PMD661 MKII; Marantz Corp., Kanagawa, Japan). Vocalizations were recorded in the uncompressed Waveform Audio File Format (.wav) at a sample rate of 44.1 kHz. To obtain recordings with high signal-to-noise ratio and little degradation, we collected recordings in as close proximity as possible without disturbing the singing individual; the majority of the recordings were collected 3-5 m from the focal male. Near the end of each male's performance, we confirmed the male's identity by sighting his color-band combination. We then extracted all clear songs from these recordings to build a song library for each male.

To examine the acoustic structure of splendid fairy-wren song types, we chose 10 examples each of Type 1, Type 2, and Type 1+2 songs for a total of 30 songs (40 song components) from 19 unique males. Examples were chosen based on recording quality such that the songs included had high signal-to-noise ratios and low degradation. These songs came from 19 different males (18 dominant males and one subordinate helper). Although some males appeared multiple times in the data-set, within each song component category (i.e. Type 1 alone, Type 2 alone, Type 1 initial component, Type 2 final component), each male was represented only once. All songs were bandpass filtered below 1 kHz to remove low-frequency noise. To account for varying recording distances and microphone sensitivities, we then adjusted the amplitude of each song to standardize the root-mean-square (RMS) amplitude across the data-set, setting the standardized RMS amplitude to the maximum possible value without clipping (rmsLeveler for MATLAB; D. McCloy, Seattle, WA, USA; MathWorks, Inc., Natick, MA, USA).

Analysis of acoustic structure

To quantify differences between Type 1 and Type 2 song structures at close-range, and to examine any changes when the song types were combined to form Type 1+2 songs, we measured nine aspects of acoustic structure (Table 2.2). Temporal features (i.e. the number of notes and duration) were measured through visual inspection of the spectrograms using Syrinx-PC (J. Burt, Seattle, WA, USA). Frequency measurements were calculated based on the power spectra of the songs (FFT size = 512, pwelch in MATLAB; MathWorks, Inc., Natick, MA, USA); for each song component, we calculated the area under the curve to find the frequency range that contained 75% of the total energy (hereafter called the “spectral focus”) and that which contained 99% of the total energy (i.e. the overall frequency range). Because animals perceive sound on a logarithmic rather than linear scale, we $\log_{10}$-transformed all frequency measurements for analysis and calculated bandwidth as a ratio of maximum to minimum frequency (Cardoso 2013).

Table 2.2. A description of the measurements of acoustic structure and their relationships with the first three principal components (PC 1-3) in the analysis. The proportion of the variance explained by each principal component is specified in parentheses in the corresponding column heading. The loadings of the acoustic measurements on each principal component are provided, and any instances in which the absolute values of the loadings were greater than 0.4 are in bold.

Measurement	Description	PC 1 (57.3%)	PC 2 (16.9%)	PC 3 (12.4%)
Number of notes	Number of continuous traces in a song	0.33	-0.25	**0.55**
Duration (s)	Time difference between the start of the first note and the end of the last note	0.32	-0.08	**0.64**
Note rate (notes s^{-1})	Number of notes divided by duration	-0.02	**-0.64**	-0.25
Frequency 12.5% (Hz)	Frequency below which 12.5% of the total energy occurs, $\log_{10}$-transformed	**0.40**	-0.07	-0.22
Frequency 87.5% (Hz)	Frequency below which 87.5% of the total energy occurs, $\log_{10}$-transformed	**0.40**	-0.19	-0.23
Bandwidth 75% (Hz)	Ratio of the frequency range containing 75% of the total energy [i.e. $\log_{10}$(frequency 87.5%) – $\log_{10}$(frequency 12.5%)]	0.30	**-0.42**	-0.17
Minimum frequency (Hz)	Frequency below which 0.5% of the total energy occurs, $\log_{10}$-transformed	0.39	0.26	-0.23
Maximum frequency (Hz)	Frequency below which 99.5% of the total energy occurs, $\log_{10}$-transformed	-0.28	-0.39	-0.04
Overall bandwidth (Hz)	Ratio of the frequency range containing 99% of the total energy [i.e. $\log_{10}$(maximum frequency) – $\log_{10}$(minimum frequency)]	-0.39	-0.30	0.19

Acquisition of neighborhood recordings

To collect recordings of splendid fairy-wren song at various distances, we used a 12-channel microphone array that recorded the dawn chorus performances of males as they moved around their territories (Figure 2.2). The microphone array consisted of six time-synchronized Wildlife Acoustics Song Meters (model: SM2+GPS; Wildlife Acoustics Inc., Concord, MA, USA) arranged in a pentagon with one recorder located in the center of the array. We fit each recorder with two omnidirectional microphones (frequency response: flat 20 Hz – 20 kHz; model: SMX-II; Wildlife Acoustics Inc., Concord, MA, USA), one attached directly to the recorder and the other attached by a 10 m cable, for a total of 12 microphones in the array. We mounted the microphones on trees at a height of approximately 1.75 m. The Song Meters were programmed to record from 4:00 to 7:00 a.m. as uncompressed stereo .wav files at a sample rate of 48 kHz.

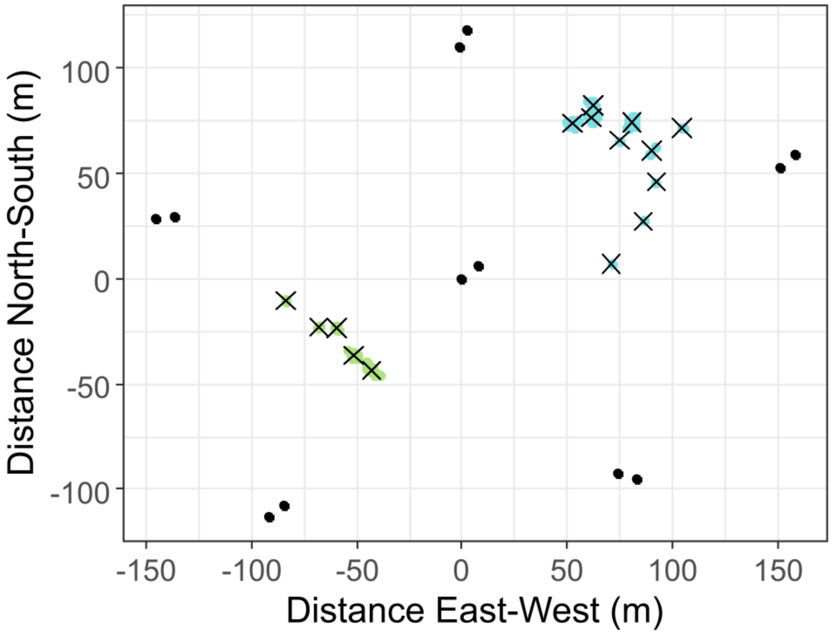

Figure 2.2. A map of the 12-channel microphone array and the song posts of two male splendid fairy-wrens during the dawn chorus. The black circles represent omnidirectional microphones, and the X symbols represent calculated song post locations. The estimated source locations for each male’s songs are indicated by the green or blue points.

Each placement of the microphone array recorded the dawn chorus performances of two to five males for a minimum of three days. To guide the positioning of the array microphones, we mapped the territories of the social groups of interest using handheld GPS units (model: eTrex 20; Garmin International Inc., Olathe, KS, USA). Territory mapping was conducted between the hours of 08:00 and 18:00 in the four weeks prior to the array placement, and it involved following focal individuals as they foraged and interacted with neighboring social groups, marking the coordinates each time the group ventured into an area in which they had not been seen previously. The locations at which we observed agonistic interactions between members of neighboring social groups were considered territory boundaries. Each mapping session lasted a minimum of 30 min, and each social group was revisited for additional mapping until the boundaries of their territory were well-defined. We also mapped the locations of the focal males' song posts during the dawn chorus, marking the coordinates of any location from which a male sang during at least one dawn chorus. We positioned the array microphones such that all of the focal males' song posts were included within the boundaries of the array.

Estimation of sound source locations

One advantage of using a time-synchronized microphone array is the ability to locate the source of a sound based on the time at which the sound arrives at each microphone (Blumstein et al. 2011, Mennill et al. 2012). The accuracy of a source location estimate depends on multiple factors, including the accuracy of the microphone location estimates, the number of channels in which the sound is detectable, the clarity of the recording (affected by the presence of masking noise and environmental features that contribute to sound degradation), and the acoustic structure of the sound in question (Mennill et al. 2012). To obtain the coordinates for each array microphone, we used a combination of static GPS sampling and trigonometry. Each recorder had two microphones, one attached directly to the recorder and the other tethered by a 10 m cable. For each microphone attached directly to the recorder, we used an approach tested by Mennill et al. (2012); we placed a handheld GPS unit (model: eTrex 20; Garmin International Inc., Olathe, KS, USA) immediately below the microphone, and used the "track" function to log the unit's location every 30 seconds for two to three hours. This process generated a cloud of over 200 points, the center of which represents the estimated "true" location of the microphone. To further improve the accuracy of this estimate, we removed the 10% of points that were farthest from the center, and calculated the average X and Y coordinates based on the remaining points. The locations of the tethered microphones were then calculated relative to the GPS-sampled microphones using a sighting compass and measuring tape.

We used Syrinx-PC (J. Burt, Seattle, WA, USA) to visualize and manually annotate the 12-channel neighborhood recordings, selecting the clearest portion of each song on the spectrogram of the recording by drawing a rectangle that specified the start time, end time, minimum frequency, and maximum frequency of the selection. Although each song appeared in multiple channels, songs were annotated only in the channel in which they were most clearly recorded. We used ArrayGUI for MATLAB (J. Burt, Seattle, WA, USA; MathWorks, Inc., Natick, MA, USA) to calculate the source locations of the annotated songs. This software uses spectrographic cross-correlation to determine the relative time-of-arrival of the annotated sound in each channel of the recording, then uses an optimization approach to determine the best location estimate given the locations of the microphones and the time-of-arrival differences (see Mennill et al. 2006). We considered location estimates reliable if (1) the quality index generated by ArrayGUI was 0.7 or higher, (2) the probability cloud generated by ArrayGUI had a small, circular distribution, (3) the location estimate fell in close proximity to the location estimates for songs occurring immediately before and/or after the song in question, and (4) the location estimate was concordant with field observations. Songs with unreliable source location estimates were excluded from the analyses of song amplitude.

Because the clarity of recorded songs varies depending on environmental conditions and slight changes in the position or orientation of singing males, errors arise in the sound localization analysis that make it appear as though males are moving between songs; in reality, males sing for stretches of time from specific song posts. To calculate the locations of these song posts, and to improve the accuracy of our distance estimates, we performed a cluster analysis for each male that grouped together any location estimates that occurred within 3 m of each other. To refine the clusters, we used the function "cluster_infomap" in the *igraph* R package (Csardi and Nepusz 2006). We then calculated the centroids of each cluster to obtain the coordinates of each male's song posts. The calculated song post locations for each male were consistent with our field observations. All calculations involving the distance from the source are based on song post location.

To assess the accuracy of the location estimates for splendid fairy-wrens in this 12-channel microphone array, we broadcast splendid fairy-wren song from a speaker (model: SME-AFS; Saul Mineroff Electronics Inc., Elmont, NY, USA) at a known location within the boundaries of each array site at a maximum sound pressure level of 75 dBC at 1 m from the speaker as measured using a sound pressure level meter (model: PSPL01; Pyle Audio, Brooklyn, NY, USA). The playback audio consisted of approximately 50 Type 2 songs interspersed with 10 second-long intervals of silence for a total duration of 5 min. During each playback, we sampled the location of the speaker using the static method described above and considered the resulting average coordinates to be the "true" location of the speaker. We then localized the source of each broadcasted song using ArrayGUI to calculate the predicted location of the speaker. Through an automated process, we randomly selected 10 of the approximately 50 location estimates to calculate the average predicted location of the speaker. We then calculated the distance between this predicted location and the speaker's "true" location to determine the error associated with the sound localization method. The speaker was stationary during the playback, and so any error associated with reorientation and the directionality of the sound was not measured. We repeated this procedure of random selection and calculation of error 1000 times to obtain an estimate of the average localization error for each array site. For the three neighborhood recordings included in our analysis of attenuation, the average localization error was between 0.6 and 1.76 m, suggesting that our estimates of source-to-microphone distance are reasonably accurate.

Processing of neighborhood recordings

After the source of each splendid fairy-wren song was localized, we used Syrinx-PC (J. Burt, Seattle, WA, USA) to create annotation selection boundaries that encompassed the entire duration of each song, from 1 to 24 kHz, in the channel in which it was most clearly recorded. We determined the identity of the singing male by comparing each song's location estimate to field observations of known song post locations. For cases in which the location estimate was insufficient for identifying the singer, we relied on the individual distinctiveness of splendid fairy-wren song (Greig and Pruett-Jones 2008) and determined singer identity by comparing the acoustic structure of the song to that of the songs in the library of focal recordings. In addition to the singer's identity, the song type (Type 1 or Type 2) was noted for each selection. For multi-component songs that contained both song type structures, each component was annotated separately, and the order in which they occurred (initial, middle, or final) was noted. Although males continue to sing at low rates after sunrise, they often sing the post-sunrise songs while foraging and from locations other than their typical song posts, so we excluded these songs from the analysis, defining a male's dawn chorus performance as all songs produced between 4:00 a.m. and sunrise, which ranged from approximately 5:50 a.m. to 6:15 a.m. in our data-set. We analyzed the dawn chorus performances of nine males recorded in five array sites, comprising a total of 1088 songs (1966 song components).

For a subset of the performances (five males recorded in three array sites, 707 songs, 1305 components), we sampled the songs at multiple distances by creating selections in all 12 channels of the microphone array recordings. After manually annotating each song in the channel in which it was most clearly recorded, we calculated the start and end times of each song in each of the remaining 11 channels based on source-to-microphone distances and a model of the speed of sound in humid air (Lord 2015). This automated method made it possible to create selections for songs recorded at far distances (i.e. when spectrograms are not reliable for manual annotation). To calculate the speed of sound appropriate for each neighborhood recording, we calculated the average air temperature associated with each recording and assumed an average relative humidity (65%) and standard atmospheric pressure (101.325 kPa; for more details about the weather parameters, see *Modeling the effects of frequency-dependent attenuation*). Each selection was then visually inspected for clarity and assigned a score on a scale of 0-3. A clarity score of 3 was assigned to selections that contained nothing other than splendid fairy-wren sound. Selections that received a clarity score of 2 contained small amounts of low-level noise. Clarity scores of 1 and 0 were assigned to selections that contained noise at or above the level of the signal of interest. Only the selections with clarity scores of 2 or higher (3146 of the 15660 multi-channel selections) were included in the analyses of song amplitude.

To quantify the amplitude of the ambient noise in the neighborhood recordings, we also created “empty” selections: 1.5 s long samples of the background noise level. For each channel, in each recording, we created two empty selections, one before the start of the dawn chorus and one after sunrise, for a total of 24 empty selections per recording.

Modeling the effects of frequency-dependent attenuation

To examine how atmospheric absorption and ground reflection affect the propagation of splendid fairy-wren song types, we devised an analytical model of sound attenuation that takes into account key features of the fairy-wren’s dawn chorus environment, including atmospheric conditions, the acoustic properties of the ground, and the typical song post height. For a sound composed of a single frequency, the sound pressure level at a given distance from the source can be expressed (in decibels) as

$$L_p(r) = L_w - A_{atm}(r) - A_{geom}(r) - A_{exc}(r). \tag{1}$$

In this expression, the sound pressure level L_p at a distance of r meters is dependent on the source radiation power level L_w, atmospheric absorption ($A_{atm}(r)$), spreading loss and ground reflection ($A_{geom}(r)$), and a number of additional attenuation mechanisms referred to as “excess attenuation” ($A_{exc}(r)$). Excess attenuation, which includes the effects of ground absorption, scattering, interference by obstacles, and refraction by wind shear and vertical temperature gradients, was not considered in our model.

Atmospheric attenuation is frequency-dependent and occurs as a result of viscous friction loss, loss by heat conduction, and the molecular relaxation of oxygen and nitrogen. For a sound wave of a single frequency, atmospheric attenuation can be expressed, in decibels, as

$$A_{atm}(r) = \alpha r, \tag{2}$$

where r is the distance from the source, and the absorption coefficient, α, can be expressed as

$$\alpha = \frac{20}{\ln 10}\, f^2 \left\{ 1.84 \cdot 10^{11} \frac{p_0}{p} \left(\frac{T}{T_0}\right)^{1/2} + \left[\frac{0.01275\, f_{rO}\, e^{\frac{-2239.1}{T}}}{f_{rO}^2 + f^2} + \frac{0.1068\, f_{rN}\, e^{\frac{-3352}{T}}}{f_{rN}^2 + f^2} \right] \left(\frac{T}{T_0}\right)^{-5/2} \right\}. \qquad (3)$$

In this expression, f is the frequency of the sound in Hz, T is the air temperature in Kelvin, T_0 is the reference temperature (293.15 K), p is the air pressure in atm, and p_0 is the reference pressure of 1 atm (International Organization for Standardization 1993, American National Standards Institute 1995, Bass et al. 1990, 1995, 1996). The parameters f_{rO} and f_{rN}, which represent the oxygen and nitrogen relaxation frequencies in Hz, respectively, are

$$f_{rO} = \frac{p}{p_0}\left(24 + 4.04 \cdot 10^4\, h\, \frac{0.02 + h}{0.391 + h}\right) \qquad (4)$$

and

$$f_{rN} = \frac{p}{p_0}\left(\frac{T}{T_0}\right)^{-1/2} \left(9 + 280\, h\, e^{-4.17\left[\left(\frac{T}{T_0}\right)^{-1/3} - 1\right]}\right), \qquad (5)$$

where h is the molar concentration of water vapor as a percentage (Bass et al. 1990, 1995, 1996).

Temperature and relative humidity have strong effects on the propagation of sound. To ensure that we parameterized our model with values that were representative of a splendid fairy-wren habitat before sunrise, we used of two sources of weather data. Each Song Meter is equipped with a built-in temperature sensor that logs the temperature of the air inside the unit every 5 minutes while recording. During the daytime, when direct sunlight warms the air inside the Song Meters, the sensor readings are likely to be overestimates of the ambient air temperature. In the hour before sunrise, however, the heat within the Song Meters has had time to dissipate overnight and the sensor readings are likely to be good estimates of the air temperature outside. For each neighborhood recording, we calculated the average Song Meter temperature for the one-hour period before sunrise to use as an estimate of the air temperature during the dawn chorus. To supplement these temperature measurements, and to obtain records of relative humidity, we explored data that were made available online from a personal weather station located approximately 17 km NE of our field site in Blanchetown, South Australia (Weather Underground Inc.).

According to these two data-sets, both temperature and relative humidity were highly variable day-to-day during the breeding seasons of 2014 and 2015, so we proceeded with four conditions corresponding to the approximate inter-quartile ranges of temperature (6 to 16 °C) and humidity (50 to 80%). The best propagation is achieved when both temperature and humidity are at their maxima, and the worst propagation occurs when both are at their minima. In the intermediate range, increasing temperature while decreasing humidity (or vice versa) has a balancing effect, resulting in an average level of attenuation between the two extremes. There are a number of temperature-humidity combinations that result in an average level of attenuation, but we chose to proceed with a temperature of 11 °C and a relative humidity of 65%.

Because splendid fairy-wrens sing from relatively low heights, interference by ground reflected sound waves is expected to have a measurable frequency-dependent effect on the propagation of their dawn chorus performances. Attenuation due to ground reflection and spreading loss can be expressed (in decibels) as

$$A_{geom}(r) = -20 \log_{10} \frac{p_{tot}(r)}{p_0}, \qquad (6)$$

where the total sound pressure at the receiver location p_{tot} is equal to the sum of the sound pressures of the direct and reflected waves. The reference value p_0 was set to 20 μPa for all decibel calculations. In an ideally still environment, the pattern of reflection is fully coherent (i.e. the phases of the direct and reflected waves differ by a constant), resulting in a consistent pattern of constructive and destructive interference. Fluctuations in the atmosphere, such as changing wind speed, temperature, and humidity, create turbulence that disrupts the coherence between the direct and reflected waves. As a result, ground reflection in a turbulent environment is characterized by a loss of coherence for sounds with wavelengths shorter than the scale of turbulence (Daigle 1979). Allowing for fluctuations in the amplitudes (A) and phases (δ) of the direct and reflected spherical waves, the total pressure at the receiver location can be expressed as

$$p_{tot} = \frac{A_d \exp(\frac{2\pi f}{c} \cdot r_d + \delta_d)}{r_d} + Q\,\frac{A_r \exp(\frac{2\pi f}{c} \cdot r_r + \delta_r)}{r_r}, \tag{7}$$

where f is the frequency of the sound in Hz, c is the speed of sound, r_d and r_r are the distances traveled by the direct and reflected waves respectively (Daigle 1979). The complex reflection coefficient Q can be expressed as

$$Q = R + (1 - R)F, \tag{8}$$

where R is the plane wave reflection coefficient, and the term $(1 - R)F$ accounts for the incident wave being spherical rather than planar. R can be expressed as

$$R = \frac{\sin\phi - \frac{\rho c}{Z_{ground}}}{\sin\phi + \frac{\rho c}{Z_{ground}}}. \tag{9}$$

For the simple case in which a sound wave is reflected by a planar surface (following Rudnick 1947),

$$F(w) = 1 + i\sqrt{\pi}w \exp(-w^2) \int_{-iw}^{\infty} \exp(-t^2)\, dt\,, \tag{10}$$

where ϕ is the angle of incidence of the sound onto the plane, ρ is the density of the air, and w is the numerical distance given by the expression

$$w = \sqrt{\frac{\pi f\, i\, r_r}{c}} \left(\sin\phi + \frac{\rho c}{Z_{ground}}\right). \tag{11}$$

We adopted an empirical single parameter model for the acoustic impedance of the ground:

$$Z_{ground} = \rho c \left[1 + 9.08 \left(\frac{f}{\sigma}\right)^{-0.75}\right] + j\rho c \left[11.9 \left(\frac{f}{\sigma}\right)^{-0.73}\right] \tag{12}$$

where σ is the flow resistance of the ground (Delany and Bazley 1970). At our field site, the ground is hard, characterized by compact soil and limestone, so we used a flow resistance value of 4000 kPa s m^{-2} that is appropriate for ground paved with small limestone chips. Assuming homogeneous isotropic turbulence on a length scale L, and a variable refractive index μ with a Gaussian-shaped correlation, the long-time average of the mean square sound pressure can be expressed as

$$\langle p_{tot}\rangle^2 = \frac{1}{r_d^2} + \frac{|Q|^2}{r_r} + \frac{2}{r_d\, r_r}|Q| \exp\left[-\sigma_d^2(1-\rho_\delta)\right] \cos\left(\frac{2\pi f}{c} \cdot (r_r - r_d) + \arg(Q)\right), \qquad (13)$$

where

$$\sigma_d = \sqrt{\pi} L \langle \mu^2 \rangle \left[\left(\frac{2\pi f}{c}\right)^2 r\right] \qquad (14)$$

and

$$\rho_\delta = \frac{\sqrt{\pi} L}{4}\left[\frac{1}{h_r}\operatorname{erf}\left(\frac{2h_f h_r}{L}\right) + \frac{1}{h_s}\operatorname{erf}\left(\frac{2(1-h_f)h_s}{L}\right)\right] \qquad (15)$$

In the above expressions, h_s and h_r represent the height above the ground of the sender and receiver, respectively, and

$$h_f = \frac{h_s}{h_r + h_s}. \qquad (16)$$

For sender height, we used a range of values that is typical for the song post heights of splendid fairy-wrens: 0.75 to 2.0 m (pers. obs.). To facilitate comparisons with the neighborhood recordings, we chose a receiver height of 1.75 m, the average height of the microphones in the arrays. With very large fluctuations of the refractive index μ, the pattern of reflection becomes fully incoherent, and the long-time average of the mean square sound pressure reduces to:

$$\langle p_{tot}^{(inc)}\rangle^2 = \frac{1}{r_d^2} + \frac{|Q|^2}{r_r^2}. \qquad (17)$$

Using the equations described above, we modeled the expected attenuation due to atmospheric absorption and ground reflection under two environmental conditions: a weakly turbulent atmosphere and a strongly turbulent atmosphere. For each meter of distance from 10 to 180 m, we calculated the attenuation for monochromatic sounds at frequencies of 1 kHz, 2 kHz, 3 kHz, and so on, up to a maximum frequency of 22 kHz. We then used these values to create equalization filters, which we applied to the 30 splendid fairy-wren songs recorded at close range to simulate the effects of attenuation. To facilitate comparisons with song amplitudes in the neighborhood recordings, we used Audacity (Audacity Team 2014) to mix the filtered songs with noise that was generated to have a sound level and frequency composition approximately equivalent to that of the background noise in the neighborhood recordings.

Analysis of song amplitude and frequency composition

To examine the relationships between splendid fairy-wren song amplitude, frequency, and distance, we calculated the amplitude and power spectrum (FFT size = 512) of each annotation made in Syrinx-PC using specSNR (J. Burt, Seattle, WA, USA) and the function “pwelch” in MATLAB (MathWorks, Inc., Natick, MA, USA). We applied this procedure to both the filtered close-range songs and the 12-channel neighborhood recordings to examine the predicted and observed attenuation of Type 1 and Type 2 songs.

We calculated the average median and average maximum amplitude of each selected song by dividing each selection into time bins of 0.005 seconds, calculating the median and maximum amplitudes within each time bin, and then averaging those values across the time bins for each selection. We calculated the amplitude measurements based on the spectrogram of the selection (FFT size = 512) in the 2400 to 10000 Hz range, a range that excludes high-frequency harmonics, but encompasses approximately 95% of the energy in splendid fairy-wren songs at close-range. We repeated this procedure with the “empty” selections to calculate the average median background noise level in the neighborhood recordings. We subtracted this value from each of the song amplitude measurements to obtain the average maximum signal amplitude, which is expressed in decibels as a ratio relative to a reference value of 20 μPa. It should be noted that because the array microphone sensitivities were not calibrated, the values reported here do not correspond to the actual sound pressure levels in the field. However, because sensitivity remained constant across microphones and recordings, and the nearest microphone for each male varied depending on his location within the array, the comparisons of relative amplitude and the relationship between amplitude and distance are assumed to be unaffected by the lack of calibration.

To examine how the frequency composition of splendid fairy-wren song changes as it travels through the environment, we compared the power spectra of the songs recorded at close range to those of the same set of songs that had been filtered to simulate attenuation. Specifically, we simulated the effects of atmospheric absorption and ground reflection without spreading loss in a weakly turbulent atmosphere. The amount of distortion is expected to be greater under these conditions than in a strongly turbulent environment due to the partial coherence of direct and reflected waves, which creates patterns of constructive and destructive interference that vary with frequency and distance from the source. For each song, at each distance over the range of 10 to 180 m, we calculated the ratio between the attenuated and original power for frequencies in the range of 2400 to 10000 Hz. We defined the amount of distortion (expressed in decibels) as the variance in this ratio across the frequency range.

Statistical analysis

We fit linear models ('lm' in the *stats* package, R Core Team 2015; 'lmer' in the *lmerTest* package with P-values based on Satterthwaite approximation, Kuznetsova et al. 2016) to investigate how acoustic structure and long-distance propagation varied with song type and usage (i.e. whether a song type was produced alone or as a component of a Type 1+2 song) and to examine the possible performance costs associated with each song type.

Because some measurements of acoustic structure were highly correlated, we performed a principal component analysis (PCA, 'prcomp' in the *stats* package; R Core Team 2015) to transform the nine acoustic measurements (Table 2.2). The first three principal components (PCs) had eigenvalues > 1 (5.16 for PC1, 1.52 for PC2, and 1.18 for PC3), and were used as response variables in linear mixed effect models to examine how acoustic structure varied with song type and usage. We also examined the relationships between (1) note rate and spectral characteristics such as minimum frequency ($\log_{10}$-transformed) and bandwidth, (2) song duration and time relative to sunrise, and (3) the initial and final component durations of Type 1+2 songs. For the analyses involving song amplitude and the rate of attenuation, rare song type combinations (Type 2+1 and Type 2+1+2; 10 of the 1088 songs) were removed from the analysis.

For the analyses of acoustic structure, allowing random intercepts for each individual male in the linear models did not significantly improve the fit with one exception; individual identity was included as a random effect for the lower bound of the spectral focus (frequency 12.5%). In the data-sets quantifying dawn chorus performances, there were repeated measures for males within each microphone array, multiple males recorded on each date, and for the analysis of amplitude, repeated measures of songs across multiple channels of the array (identified by event number). The maximal random effect structures were specified for the linear models of song and suffix duration; for individual identity nested within recording day, we allowed random intercepts as well as random slopes for each continuous variable and their interactions.

Due to the large amount of heterospecific noise during the dawn chorus, many observations of average maximum amplitude were excluded from the analysis. The lack of balance within groups in the resulting data-set likely accounted for the failure of convergence of models that included the maximal or close-to-maximal random effect structure (Bates et al. 2015). To ensure convergence, we excluded the event number, which, when included, resulted in a very large number of unbalanced groups, and the recording day, which accounted for only a small amount of variation that was not already captured by individual identity. For each individual and array channel, we allowed random intercepts as well as random slopes for the log-distance and time relative to sunrise, the latter accounting for the movement of males toward and away from the microphones as the dawn chorus progressed. Time relative to sunrise was scaled by a factor of 1000 before its inclusion in statistical models.

For each possible combination of fixed effects and interactions, we calculated the second-order Akaike's information criterion (AICc) and the Bayesian information criterion (BIC; 'dredge' in the *MuMIn* package for R, Barton 2016). We regarded the model with the lowest AICc score as the best model in all cases except for the analysis of average maximum amplitude. In this analysis, the model with the lowest AICc score was the complete model with more than 10 parameters. To avoid overfitting, we instead chose the model with the lowest BIC score, a criterion that penalizes more strongly for the number of parameters included in the model. Although the BIC does not perform as well as the AICc when selecting from a suite of models that does not include the “true” model (Aho et al. 2014), the model with the highest BIC score was more useful for this study, as it was more easily interpretable in a biological context.

After selecting the best model for each variable of interest, we inspected diagnostic plots of the residuals to ensure that no assumptions had been violated in fitting the models. This procedure revealed three outliers in the preliminary analyses of frequency and note rate, which were removed before selecting the models with the best fit.

RESULTS

To explore some of the potential benefits of incorporating the Type 2 song structure into dawn chorus performances, we quantified three aspects of splendid fairy-wren song: its acoustic structure, propagation, and use during the dawn chorus.

Acoustic structure of song types

A principal component analysis reduced the nine measures of acoustic structure to three variables that together explained 86.6% of the variation (Table 2.2, Figure 2.3). Using linear models, we analyzed the effects of song type and usage (i.e. whether a song type was produced alone or as a component of a Type 1+2 song) on each of the three principal components to examine the consequences of the addition of the Type 2 suffix. Song type, usage, and the interaction of these effects significantly predicted the first principal component ($R^2 = 0.93$, $F(3,36) = 184.6$, $P < 0.001$), which was related to the “spectral focus” of the song – the boundaries of the frequency range containing 75% of the total energy. Type 2 songs had lower spectral foci than Type 1 songs ($\beta = -3.79$, $SE = 0.26$, $t = -14.50$, $P < 0.001$). Both Type 1 and Type 2 song structures had lower spectral foci when combined as Type 1+2 songs than when produced alone (usage: $\beta = -0.67$, SE = 0.26, $t = -2.63$, $P = 0.013$, interaction of song type and usage: $\beta = -0.80$, SE = 0.37, $t = -2.17$, $P = 0.037$).

The second principal component, which was inversely related to the bandwidth of the spectral focus and note rate, was best predicted by song type, usage, and the interaction of these effects as well ($R^2 = 0.23$, $F(3,36) = 4.80$, $P = 0.006$). Type 2 songs had narrower spectral foci and slower note rates than Type 1 songs ($\beta = 1.69$, SE = 0.49, $t = 3.48$, $P = 0.001$), and Type 1 songs with suffixes had narrower spectral foci and slower note rates than Type 1 songs without suffixes ($\beta = 1.10$, SE = 0.49, $t = 2.26$, $P = 0.030$). Type 2 suffixes had broader spectral foci and faster note rates than Type 2 songs produced alone (interaction of song type and usage: $\beta = -2.41$, SE = 0.69, $t = -3.51$, $P = 0.001$). The third principal component was related to the number of notes and duration of the songs, and was best predicted by usage only ($R^2 = 0.60$, $F(1,38) = 58.65$, $P < 0.001$). The components of Type 1+2 songs contained fewer notes and had shorter durations than either of the song types produced alone ($\beta = -1.63$, SE = 0.21, $t = -7.66$, $P < 0.001$).

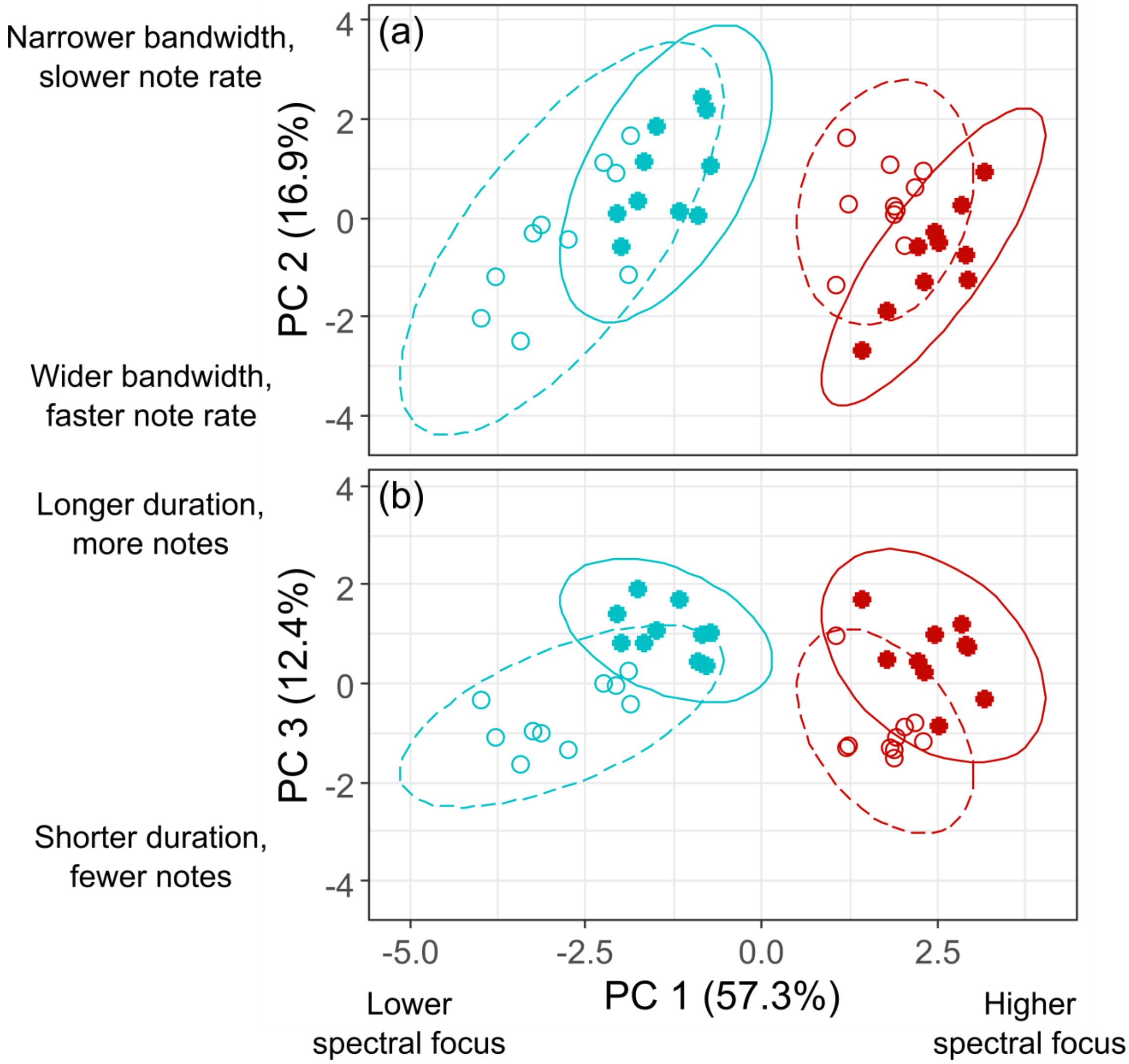

Figure 2.3. Plots of principal component scores depicting variation in acoustic structure. Type 1 song structures are indicated in red, and Type 2 song structures are indicated in blue. The point shape and line type indicate whether the song structures occurred alone (filled, solid) or as components of multi-component songs (open, dashed). The ellipses represent 95% confidence regions for each song type and order category, and each point represents one song or song component (N = 19 unique males, 30 songs, 40 song components in total). The proportion of the variance explained by each principal component is specified in the corresponding axis label.

We also used linear models to examine the relationships between the spectral and temporal features of the songs. Note rate and song type significantly predicted the minimum frequency of a song ($R^2 = 0.85$, $F(2,34) = 99.5$, $P < 0.001$, Figure 2.4b). Note rate and song type, with the addition of their interaction, predicted the lower bound of the spectral focus ($R^2 = 97.6$, Figure 2.4a) and the overall bandwidth ($R^2 = 0.82$, $F(2,34) = 84.8$, $P < 0.001$, Figure 2.4d). The correlation between minimum frequency and note rate was negative for both song types ($\beta = -0.018$, $SE = 0.004$, $t = -4.54$, $P < 0.001$). The correlation between the lower bound of the spectral focus and note rate was also negative, although the relationship was much stronger for Type 1 songs than for Type 2 songs (note rate: $\beta = -0.017$, $SE = 0.003$, $t = -5.25$, $P < 0.001$; interaction of song type and note rate: $\beta = 0.016$, $SE = 0.004$, $t = 4.35$, $P < 0.001$). The overall bandwidth of the song was positively correlated with note rate for both song types ($\beta = 0.024$, $SE = 0.005$, $t = 4.70$, $P < 0.001$).

Propagation of song types during the dawn chorus

Because attenuation due to atmospheric absorption, ground reflection, scattering, and interference are frequency-dependent, the spectral characteristics of the Type 1 and Type 2 song structures have consequences for their propagation. To examine how atmospheric absorption and ground reflection affect each song type, we devised acoustic filters based on the equations described in the methods, and applied the filters to songs recorded at close-range to simulate attenuation. The predicted patterns of attenuation for splendid fairy-wren song in strongly and weakly turbulent atmospheres are shown in Figure 2.5a. Because we standardized the root-mean-square amplitudes across the Type 1, Type 2 and Type 1+2 songs before simulating the effects of attenuation, the predicted average maximum amplitudes represent the case in which all songs were produced at the same average amplitude level.

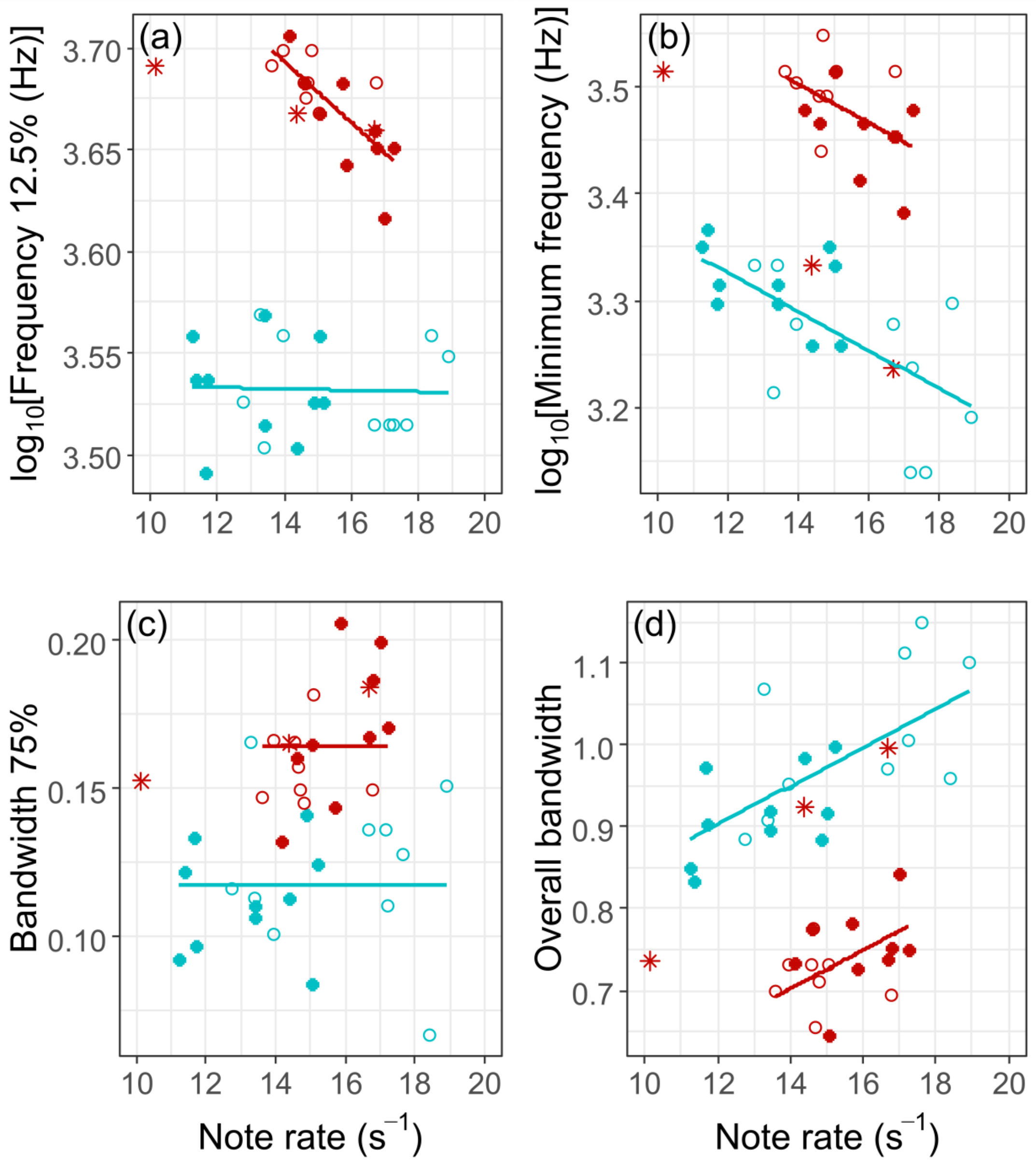

Figure 2.4. Frequency characteristics as a function of note rate for Type 1 and Type 2 song structures. Type 1 song structures are indicated in red, and Type 2 song structures are indicated in blue. The point shape indicates whether the song structures occurred alone (filled) or as components of multi-component songs (open). The lines were derived from the linear models with the lowest AICc scores. The points designated by asterisks (*) were removed from the data set as outliers before fitting the linear models. (N = 19 unique males, 30 songs, 40 song components in total).

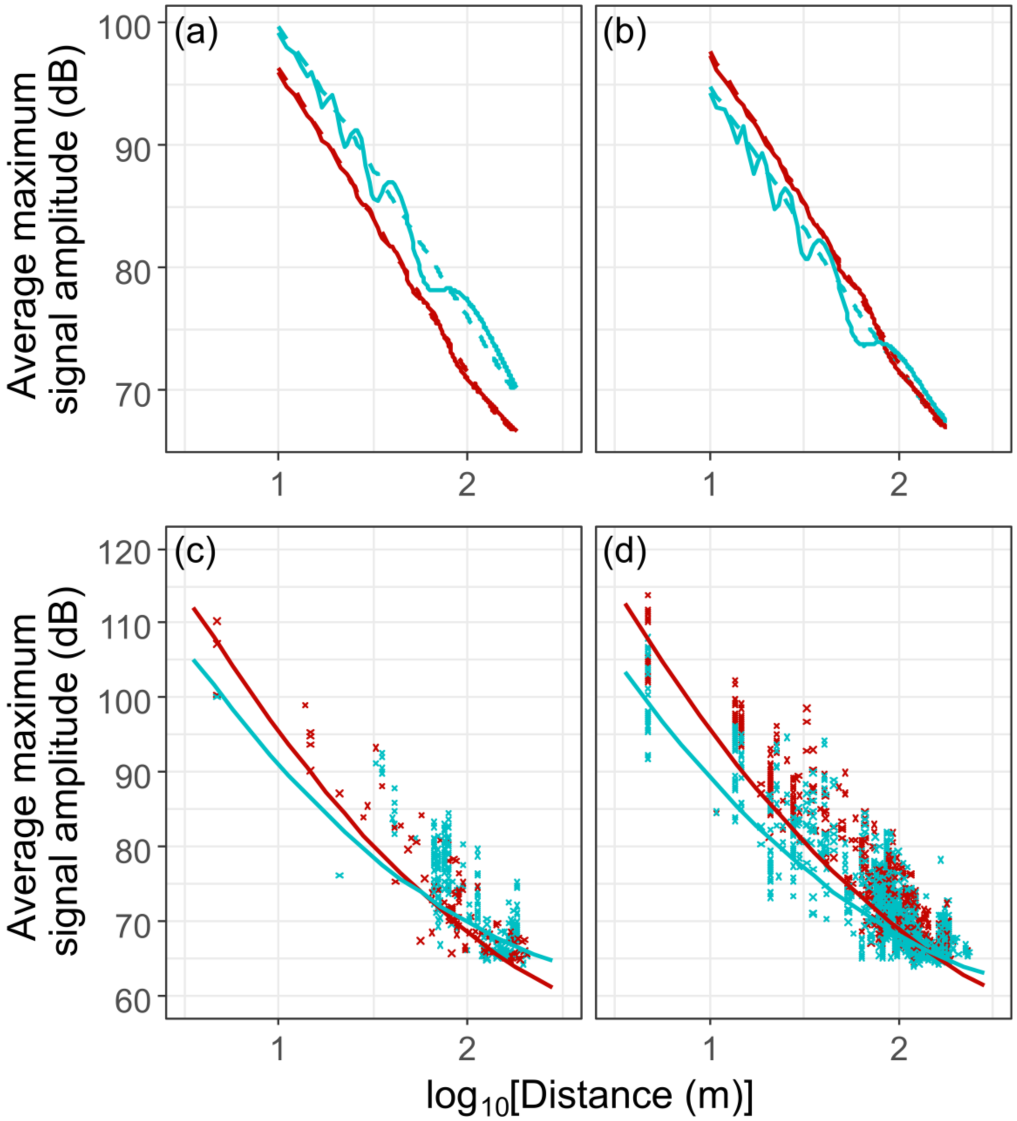

Figure 2.5. The attenuation of splendid fairy-wren song types. Type 1 song structures are indicated in red, and Type 2 song structures are indicated in blue. The left column of panels (a,c) shows the song structures when produced alone, the right column (b,d) shows the song structures when produced together as multi-component songs. The top row of panels (a,b) shows the predicted relationships between amplitude and distance calculated using our model of attenuation in strongly turbulent (dashed lines) and weakly turbulent (solid lines) atmospheres. The predicted relationships represent the case in which all songs are produced at the same average amplitude level. The bottom row (c,d) shows the observed relationships between amplitude and distance in the natural environment. Each point represents one song or song component, some recorded at multiple distances (N = 5 males, 3 recording sites, 3146 observations). Note: because the array microphone sensitivities were not calibrated, the amplitude values do not correspond to actual sound pressure levels in the field.

We directly studied the attenuation of splendid fairy-wren song in the natural environment by using microphone array recordings to examine the relationship between a song’s amplitude and the distance from its source (Figure 2.5b). The best model for the average maximum amplitude of a given song included the log-distance from the source (as a quadratic term), song type, its usage (i.e. whether produced alone or as part of a multi-component song), and the time relative to sunrise (as a quadratic term), with significant interactions between a subset of these effects (Table 2.3). According to this model, Type 2 songs were quieter than Type 1 songs at the source (i.e. the intercepts differed significantly, $P < 0.001$). Type 1 songs with suffixes were slightly, but not significantly, louder than Type 1 songs without suffixes ($P = 0.055$). The interaction of song type and usage was significant; Type 2 suffixes were produced at the lowest amplitude levels ($P < 0.001$). Over a range of distances from approximately 5 to 240 m, the Type 2 song structure attenuated less rapidly than the Type 1 song structure ($P < 0.001$). The average maximum amplitude and rate of attenuation varied significantly with the time relative to sunrise ($P < 0.001$); this relationship is likely due to the movement of males as they approached and retreated from the microphones over the course of the dawn chorus.

Table 2.3. The results of a linear mixed-effects model with song type, usage, and distance as predictors of the average maximum amplitude given the effects of overall attenuation in the natural environment. The fixed effect “usage” specifies whether a given song type structure was produced alone or as a component of a multi-component song.

Response variable: average maximum amplitude

Random effects:

Group	**Effect**	**Variance**	**Standard deviation**	**Correlation**		
Channel	Intercept	541.3887	23.2678			
	$\log_{10}$(Distance)	133.3867	11.5493	-0.99		
	Start time	0.4889	0.6992	-0.50	0.57	
ID	Start time	0.143	0.3781			
	Intercept	95.7172	9.7835			
	$\log_{10}$(distance)	18.8825	4.3454	-1		
Residual		6.2711	2.5042			

Fixed effects:

Coefficients	**Estimate**	**Standard error**	**Degrees of freedom**	**t**	**P**	
Intercept	136.487	13.744	81.4	9.931	< 0.001	*
Start time	65.418	13.260	2889	4.933	< 0.001	*
(Start time)^2	16.098	4.739	2732.1	3.397	< 0.001	*
Song type: Type 2	-10.695	1.474	3104.3	-7.258	< 0.001	*
Usage: component	0.467	0.244	3028.8	1.917	0.055	
$\log_{10}$(Distance)	-48.106	11.774	622.2	-4.086	< 0.001	*
[$\log_{10}$(Distance)]^2	7.062	2.844	3023.8	2.483	0.013	*
Start time x $\log_{10}$(Distance)	-70.762	13.456	2948	-5.259	< 0.001	*
(Start time)^2 x $\log_{10}$(Distance)	-16.987	4.737	2748.2	-3.586	< 0.001	*
Start time x [$\log_{10}$(Distance)]^2	18.692	3.415	2975.7	5.474	< 0.001	*
(Start time)^2 x [$\log_{10}$(Distance)]^2	4.409	1.180	2764.1	3.736	< 0.001	*
Song type: Type 2 x $\log_{10}$(Distance)	6.589	1.802	3105.2	3.657	< 0.001	*
Song type: Type 2 x [$\log_{10}$(Distance)]^2	-0.305	0.548	3105.5	-0.557	0.578	
Song type: Type 2 x Usage: component	-2.118	0.317	2949.1	-6.678	< 0.001	*

Because Type 1 and Type 2 song structures attenuate at different rates, the amplitude of a Type 2 suffix relative to its preceding Type 1 component is expected to increase with increasing distance from the source, but this relationship can be complicated by the effects of ground reflection in weak turbulence (Figure 2.6). Excess attenuation due to scattering, refraction, and interference by obstacles might complicate this relationship further, but the observed relative amplitude of Type 2 suffixes in the natural environment followed a similar pattern to those predicted by our models of attenuation.

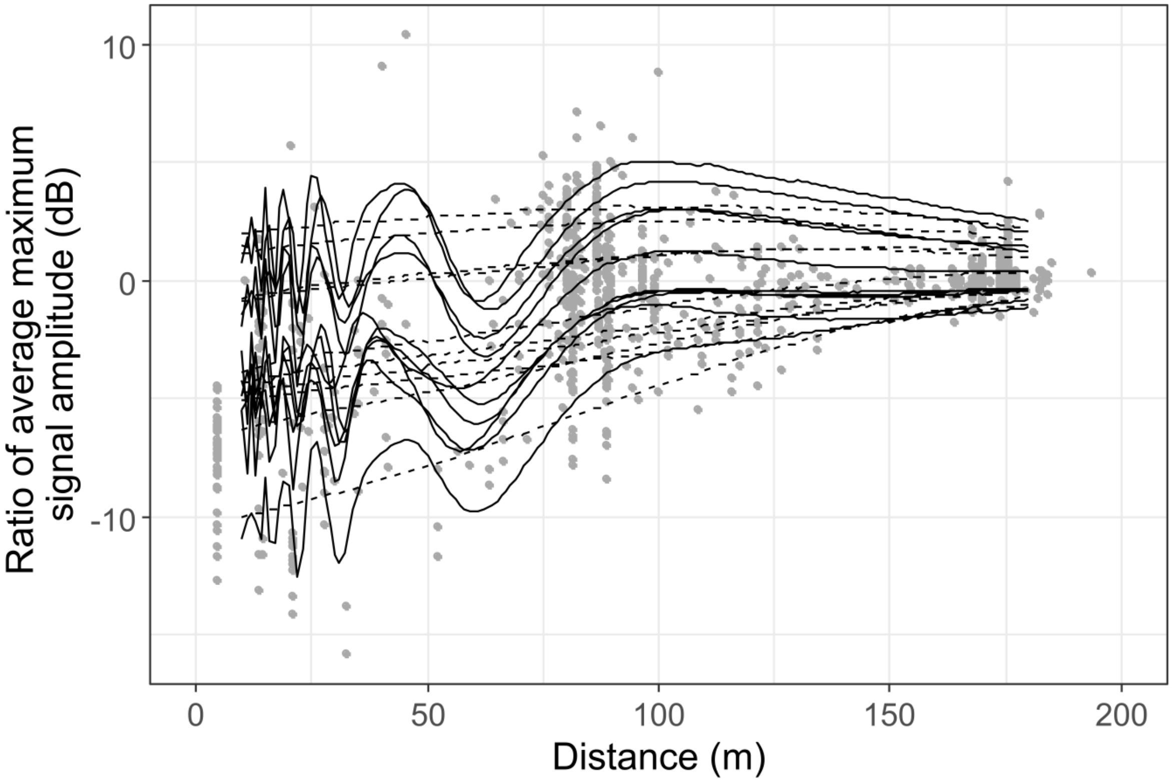

Figure 2.6. The relative amplitude of the Type 2 suffix as a function of the distance from the source. The relationships predicted by our model of attenuation in strongly and weakly turbulent environments are represented by dashed and solid lines, respectively, with one line representing each of the 10 close-range Type 1+2 songs included in the analysis. Each point represents one multi-component song recorded in the natural environment, some recorded at multiple distances. (N = 5 males, 3 recording sites, 800 observations).

We explored how the frequency composition of each song type is expected to change with distance by examining the power spectra of songs that had been filtered to simulate the effects of atmospheric absorption and ground reflection in a weakly turbulent atmosphere. According to the predictions from our model, the distortion of the frequency spectrum is expected to increase with increasing distance from the source, but the relationship is highly dependent on song type structure (Figure 2.7).

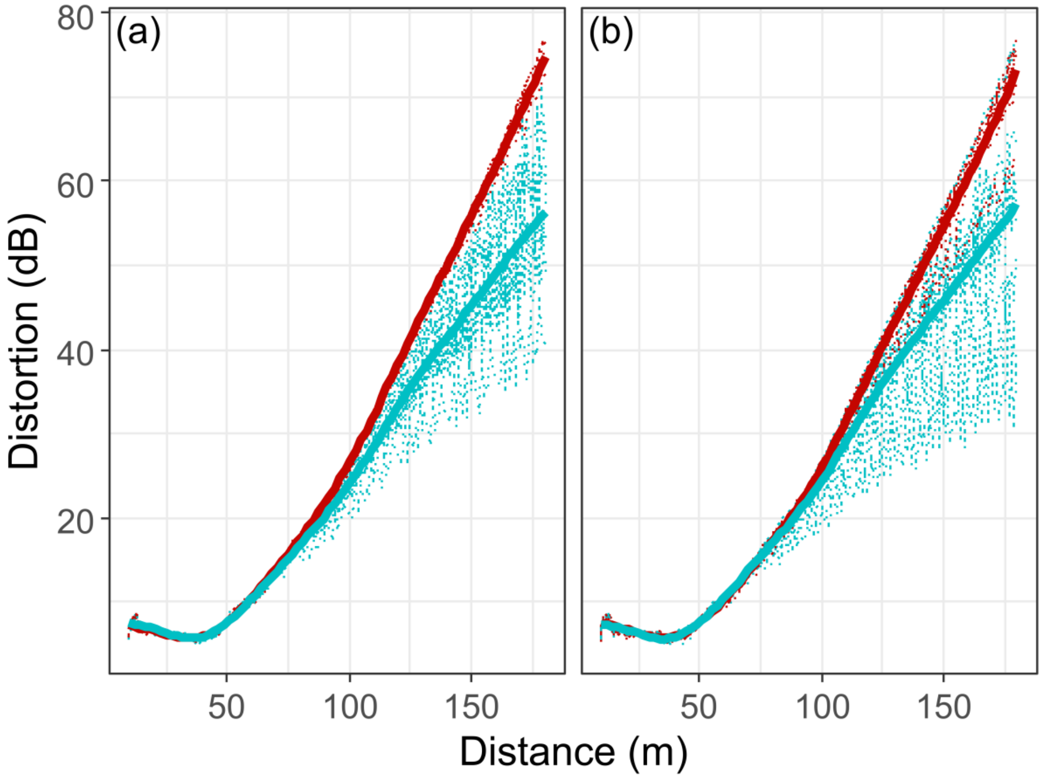

Figure 2.7. The predicted amount of spectral distortion in a weakly turbulent atmosphere as a function of the distance from the source. Type 1 song structures are indicated in red, and Type 2 song structures are indicated in blue. The left panel (a) shows the song structures when produced alone, the right panel (b) shows the song structures when produced together as multi-component songs. Each dotted line represents one song filtered to simulate attenuation across the range of distances (N = 19 unique males, 30 songs, 40 components), and the solid lines represent the average relationships for each song type.

Use of song types during the dawn chorus

We analyzed the performances of male splendid fairy-wrens to quantify the use of the Type 2 song structure during the dawn chorus, and we calculated song duration to explore whether there are costs associated with the performance of either of these song types. Male splendid fairy-wrens began singing 49.7 ± 13.6 min before sunrise (mean ± standard deviation; n = 9), with overall peak song rates occurring 25 to 30 min before sunrise (Figure 2.8a). Males sang both Type 1 and Type 2 songs during the dawn chorus, but songs that contained both song type structures (hereafter called "multi-component songs") were significantly more common (78.3 ± 16.0 % of a male's performance; mean ± standard deviation; n = 9; Friedman rank sum test $\chi^2 = 10.89$, df = 2, $P = 0.004$).

Song duration was significantly predicted by both the song type and the time relative to sunrise (Table 2.4). Multi-component songs were longer in duration than Type 1 and Type 2 songs (all $P < 0.001$), and the relationship between song duration and time relative to sunrise varied by song type; Type 2 song duration decreased monotonically as sunrise approached, but for Type 1 songs with and without suffixes, peak song duration occurred approximately 15 and 20 minutes before sunrise, respectively (Figure 2.8b). For Type 1+2 songs, the duration of the Type 2 suffix was predicted by the duration of the initial Type 1 component and the time relative to sunrise. The duration of the Type 2 component decreased with increasing Type 1 component duration ($\beta = -0.59$, SE = 0.064, $t = -9.18$, $P < 0.001$), and the slope of this relationship varied across individuals (Figure 2.9a). Like the overall duration of Type 1+2 songs, the duration of the Type 2 suffix changed nonlinearly with time relative to sunrise (time: $\beta = -0.83$, SE = 0.12, $t = -7.09$, $P < 0.001$; time^2: $\beta = -0.31$, SE = 0.03, $t = -9.20$, $P < 0.001$; Figure 2.9b).

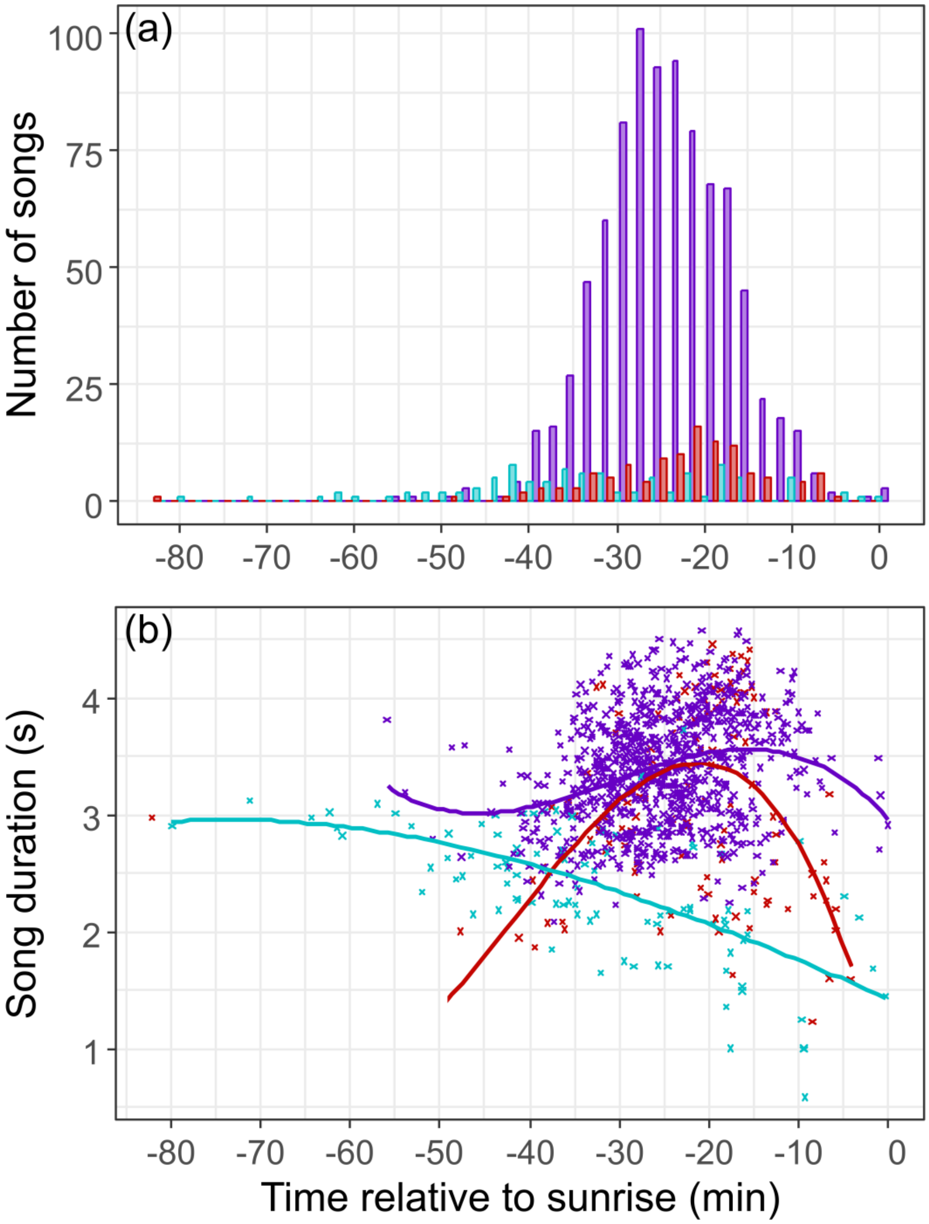

Figure 2.8. Song type use and duration over the course of the dawn chorus. (a) A histogram depicting song type use over the course of the dawn chorus. (b) Song duration as a function of time relative to sunrise. Type 1 songs are indicated in red, Type 2 songs are indicated in blue, and multi-component songs that contain both song types are indicated in purple. The lines were derived from the linear mixed effect model with the lowest AICc score. Each point represents one song (N = 9 males, 5 recording sites, 1088 songs).

Table 2.4. The results of a linear mixed-effects model with song type usage and start time relative to sunrise as predictors of song duration. The fixed effect “usage” specifies whether a song consisted of the Type 1 structure alone, the Type 2 structure alone, or both song type structures.

Response variable: song duration

Random effects:

Group	Effect	Variance	Standard deviation	
ID (nested within day)	Start time	0.052	0.2277	
	Intercept	0.219	0.4675	
Day	Start time	0	0	
	Intercept	0.071	0.2694	
Residual		0.112	0.335	

Fixed effects:

Coefficients	Estimate	Standard error	Degrees of freedom	t	P	
Intercept	2.485	0.249	10.5	9.998	< 0.001	*
Start time	-1.989	0.304	586.9	-6.532	< 0.001	*
(Start time)^2	-1.185	0.189	1072.2	-6.269	< 0.001	*
(Start time)^3	-0.198	0.038	1082.1	-5.183	< 0.001	*
Usage: Type 1 alone	-1.908	0.250	1084.3	-7.635	< 0.001	*
Usage: Type 2 alone	-1.262	0.228	1081.9	-5.538	< 0.001	*
Start time x usage: Type 1 alone	-2.618	0.478	1083.4	-5.476	< 0.001	*
(Start time)^2 x usage: Type 1 alone	-1.136	0.272	1083.2	-4.172	< 0.001	*
(Start time)^3 x usage: Type 1 alone	-0.105	0.046	1082.7	-2.266	0.024	*
Start time x usage: Type 2 alone	0.719	0.410	1081.2	1.754	0.080	
(Start time)^2 x usage: Type 2 alone	0.841	0.234	1081.2	3.592	< 0.001	*
(Start time)^3 x usage: Type 2 alone	0.164	0.041	1080.3	3.857	< 0.001	*

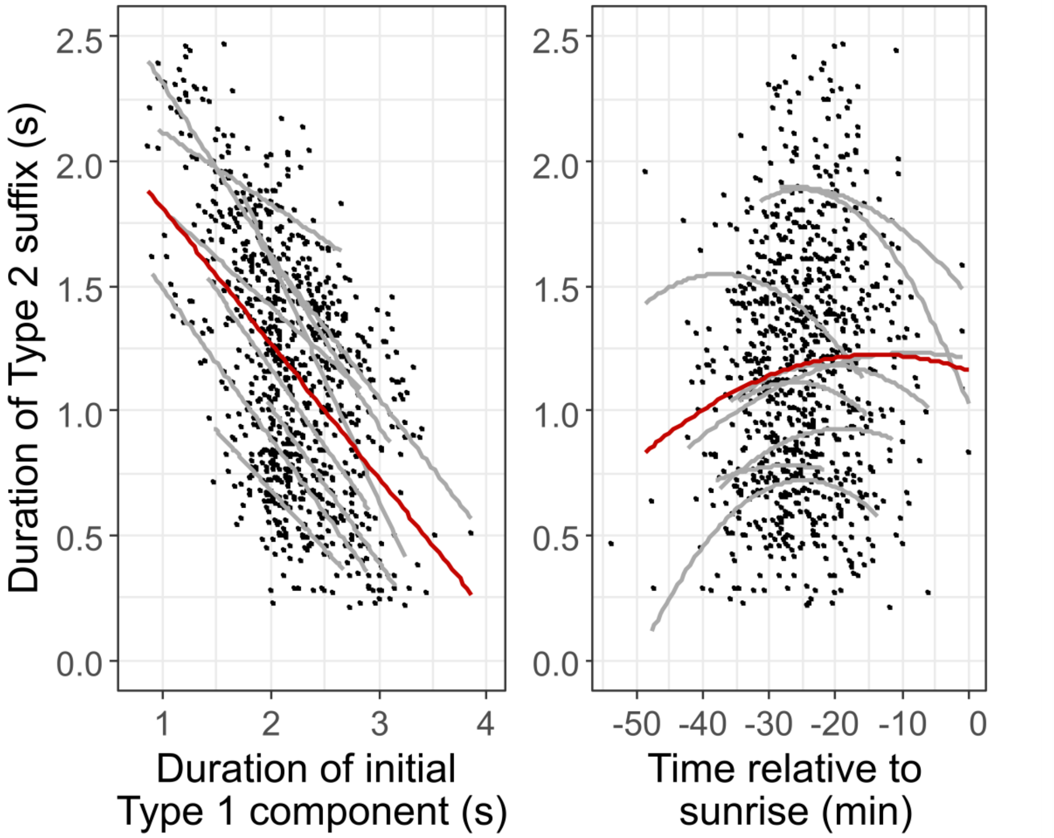

Figure 2.9. The duration of the Type 2 suffix as a function of the initial Type 1 duration and time relative to sunrise. The red lines represent the overall relationships, with individual regression lines for each male in gray. Each point represents one Type 1+2 song (N = 9 males, 5 recording sites, 861 songs).

DISCUSSION

Male splendid fairy-wrens possess two distinct song type structures, and although the Type 2 song is thought to have originated in a predator context (Greig and Webster 2014), males incorporate this song type into their dawn chorus performances, generally by appending the Type 2 trill as a suffix to their standard Type 1 display song. To gain insight into why this behavior evolved, we examined the acoustic structure, propagation, and use of splendid fairy-wren song types, exploring the extent to which the incorporation of the Type 2 song structure could enhance the information content and reception of a male's dawn chorus performance.

Splendid fairy-wren song type structures differ most significantly in the frequency ranges that they occupy; Type 2 songs have energy concentrated in lower and narrower frequency ranges than Type 1 songs. For songs containing both song type structures, the addition of the Type 2 suffix was correlated with changes in the preceding Type 1 structure; Type 1 songs with suffixes were shorter in duration and tended to have lower spectral foci, narrower bandwidths, and slower note rates than Type 1 songs without suffixes.

Our analysis of song structure also revealed that, for Type 2 songs, minimum frequency decreased, and bandwidth increased, with increasing trill rates across individuals. The relationship between trill rate and bandwidth is a well-known example of a performance constraint in songbirds; within and across many species, trill bandwidth has been found to *decrease* with increasing trill rate (Podos 1997, Podos 2017). The Type 1 song has a far more complex note structure and organization than the Type 2 trill, but we observed similar relationships between frequency composition and note rate. Because our analysis was based on a small number of observations (30 songs from 19 males), it is possible that we did not adequately capture the existing variation in bandwidth and note rate to detect this performance constraint. Sample sizes in published studies of vocal performance constraints range from 4 to 3298 vocalizations (median sample size = 42), but when sampling limitations are controlled, trade-offs have been detectable only in studies with sample sizes of at least 66 vocalizations (Wilson et al. 2014). Instead, our analysis may have captured the variation in performance quality among the males included in the sample. The finding that Type 1 songs with suffixes have slower note rates and narrower bandwidths than those without suffixes suggests that the addition of the Type 2 suffix is associated with a lower quality performance of the initial Type 1 component.

Because the splendid fairy-wren song types differ in their frequency compositions, we expected Type 1 and Type 2 songs to be transmitted differently by the environment. We compared the propagation patterns of the song type structures using a model of attenuation and by quantifying the amplitude of songs at various distances in the natural environment. Simulating the effects of atmospheric absorption and ground reflection on splendid fairy-wren song revealed that Type 2 song structures should attenuate less rapidly than Type 1 song structures. In weakly turbulent environments, partially coherent ground reflection is expected to have a greater effect on the amplitude of Type 2 song structures, resulting in a number of distances at which the trill is expected to be amplified as a result of constructive interference and attenuated as a result of destructive interference.

In the natural environment, we observed patterns of attenuation that were similar to those predicted by our physical models; Type 2 song structures attenuated less rapidly than Type 1 song structures. At distances of approximately 80 m, Type 2 song structures appeared to be amplified, which is consistent with our expectations of attenuation due to ground reflection in a an atmosphere with little turbulence. Based on the observed rates of attenuation, Type 2 song structures seem to be produced at lower amplitudes than Type 1 song structures.

As splendid fairy-wren song travels through the environment, its frequency composition becomes distorted by attenuation due to atmospheric absorption and ground reflection. Our simulation of these effects revealed that Type 2 songs should be less distorted than Type 1 songs at distances greater than 100 m from the source, although the distortion of the Type 2 structure is expected to be highly variable across distances and across songs (Figure 2.7). Preliminary comparisons of acoustic structure suggest that the expected amount of spectral distortion is best predicted by the shape of the notes used in the trill, which appears to be consistent within, but varies across, individuals. Whether higher quality males are able to produce trills that are more resistant to distortion is a worthwhile topic of future study. This variation in distortion might also have important implications for song learning, and therefore provide an opportunity for cultural selection (Hansen 1979), as males in this species learn their songs from their social fathers (Greig et al. 2012), but can adjust their trill structure to adopt note shapes used by neighboring males (pers. obs.).

The vast majority of work investigating the effect of the environment on acoustic signals has involved transmission experiments, which use speakers to simulate vocalizing animals. Passing sound through a speaker changes its frequency composition, and researchers typically compensate for this by recording a "model" sound at a short distance from the speaker that functions as a baseline for comparison (e.g. Dabelsteen et al. 1993). However, due to the frequency-dependence of attenuation and degradation (Wiley and Richards 1978), measuring the effect of the environment on artificially transformed sounds can bias comparisons of signals that differ in their frequency compositions, and this proved to be problematic when measuring the attenuation of splendid fairy-wren song as broadcasted by a speaker (unpublished data). The methods that we used in this study did not have these limitations; through the use of an analytical model of the transmission medium and microphone array recordings, we were able to examine the effect of the environment on splendid fairy-wren song in its natural form. By simulating the effects of atmospheric absorption and ground reflection, we were also able to measure the frequency composition of songs at specified distances without the complication of ambient noise, which can vary in amplitude level and frequency composition over time and space. A limitation of our technique was the inability to account for attenuation through other mechanisms such as scattering, interference by obstacles, and refraction, which would require complex calculations and a more detailed knowledge of the environmental conditions.

To quantify the use of the Type 2 song structure, and to explore whether there are performance costs that manifest in song timing or duration, we analyzed the dawn chorus performance characteristics of male splendid fairy-wrens. As sunrise approached, Type 2 songs became consistently shorter in duration. This pattern appears to be a result of shifting investment rather than fatigue; for Type 1 and multi-component songs, peak song duration occurred much later, coinciding with peak song rates such that singing effort reached its maximum approximately 25 before sunrise. The strong negative correlation between the initial and final component durations of Type 1+2 songs suggests that, as in superb fairy-wrens (Dalziell and Cockburn 2008) and chaffinches (*Fringilla coelebs*, Riebel and Slater 2003), the overall song duration of splendid fairy-wrens is constrained.

Although the addition of the Type 2 suffix occurs at the expense of Type 1 song duration and performance quality, approximately 80% of the songs produced during the dawn choruses in our sample contained both song type structures. Through our analyses of the acoustic structure, propagation, and use of the splendid fairy-wren song types, we have found evidence of several ways in which the incorporation of the Type 2 song structure could offset these costs by increasing the effectiveness of a male’s dawn chorus performance.

Increasing the information content of dawn chorus performances

If Type 2 song structure encodes information about a male’s quality, condition, status, or motivation, adding Type 2 suffixes to their standard display songs could allow males to communicate this information during the dawn chorus (Greig and Webster 2014). It is also possible that, with the addition of the suffix, a new source of information emerges related to performance constraints; trade-offs in song duration, note rate, and bandwidth suggest that the addition of the Type 2 suffix is costly, and the cost varies among males. If female splendid fairy-wrens are able to extract honest information about a male's value as a social or extra-pair mate from songs that contain both song type structures, a female preference for songs that include a Type 2 suffix could account for its frequent use during the dawn chorus.

Due to its spectral and temporal properties, the addition of a Type 2 suffix could also improve the location information in a male's song. Lower frequencies are less susceptible to scattering, which can obscure the direction from which sounds originate (Wiley and Richards 1978), and repetition of note structure provides the opportunity for binaural comparisons (Bradbury and Vehrencamp 1998). The addition of a component that enhances the localizability of an acoustic signal is a common strategy in animal communication; examples include the "chucks" of túngara frogs (*Physalaemus pustulosus*, Rand and Ryan 1981) and Richardson's ground squirrels (*Spermophilus richardsonii*, Sloan et al. 2005).

In addition to providing information about the direction of the source, the use of the Type 2 suffix could allow receivers to better estimate the distance to the singing male. Distance assessment, or "ranging," can be accomplished by attending to the degradation of sound, as degradation increases predictably with distance (reviewed in Naguib and Wiley 2001). Because attenuation is frequency-dependent, the relative attenuation of high frequencies could also function as a cue of distance for sounds that have both high and low frequency components (Wiley and Richards 1978). For splendid fairy-wren songs containing both song type structures, the relative amplitude of the Type 2 suffix is expected to increase predictably with distance from the source, although this relationship can be complicated by ground reflection in environments with low turbulence. If splendid fairy-wrens are familiar with the typical ratio of initial to final song amplitude at various distances, this feature could be used to approximate the distance to a singing male in the absence of visual contact.

By communicating both direction and distance, the Type 2 suffix could function as a location tag for the information contained in the preceding Type 1 structure. Producing an easily localizable song may be particularly important during the dawn chorus of splendid fairy-wrens. If females are seeking extra-pair copulations in the darkness before sunrise, males singing songs containing both song type structures might be more easily found at their song posts, resulting in higher success through extra-pair paternity. While very little is known about the pre-dawn behavior of female splendid fairy-wrens, there is evidence that female superb fairy-wrens leave their territories before sunrise to seek extra-pair copulations (Double and Cockburn 2000). The ability to estimate distance and direction based on song structure would be advantageous during interactions with neighboring males as well, allowing territory owners to monitor the locations of rivals without the energetic costs of patrolling the territory boundaries (Morton 1986).

Enhancing the reception of dawn chorus performances

The effectiveness of a male's dawn chorus performance depends not only on the information content of his song, but also its broadcast area, detectability, resistance to distortion, and ability to attract the attention of receivers. Greig and Webster (2014) suggested a potential benefit of the Type 2 suffix that exploits its typical association with predator vocalizations; the incorporation of the trill might attract the attention of conspecifics through its relationship with danger. In superb fairy-wrens, a species that often sings Type 2 trills followed by Type 1 "flourishes" (Langmore and Mulder 1992, Dalziell and Cockburn 2008), the trill could function as an alerting component to enhance the detectability of the flourish that follows (Richards

1981). In splendid fairy-wrens, however, the trill component almost always occurs after the Type 1 song; over 98% of the multi-component songs in our sample were arranged as Type 1 songs with Type 2 suffixes. Although a Type 2 suffix might attract the attention of receivers to the performance as a whole, it cannot function as an alerting component for the preceding Type 1 song structure in splendid fairy-wrens.

Because splendid fairy-wren song types propagate differently through the environment, incorporating the Type 2 song structure might increase the active space of a male's song, which depends on factors such as the intensity of the sound at its source, the detection and discrimination thresholds of the receiver, and the amount of masking noise (Marten and Marler 1977). Type 2 suffixes are produced at lower amplitudes, but attenuate less rapidly, than Type 1 songs. As a result, at distances greater than 160 m, the average maximum amplitude of the Type 2 suffix surpasses that of the initial Type 1 component. Because sounds with energy spread over a wide bandwidth have higher detection thresholds than sounds with energy concentrated in narrower frequency ranges (Lohr et al. 2003), it is likely that, for a given signal-to-noise ratio, a Type 2 song structure, which has a relatively narrow bandwidth, would be more detectable than the wider bandwidth Type 1 song structure. This bias in perception would further exaggerate the differences in the song type ranges, and so it is quite possible that the addition of the Type 2 suffix would increase the detectability of the song at long distances from the source.

When communicating over long distances, it is important that the receiver can not only detect the signal, but can also extract information from it. Encoding information in frequency modulations is expected to be the most reliable strategy for long-distance communication, as scattering and reverberation can more readily mask information encoded in modulations of amplitude (Wiley and Richards, 1978, Richards and Wiley 1980). Through atmospheric absorption, reflection, and scattering, frequency modulations are distorted by the environment as well, and as the bandwidth of a modulation increases, so does the opportunity for spectral distortion. As a result, the optimal acoustic structure for conveying information to distant receivers would be one that is highly modulated, but over a narrow range of frequencies. The Type 2 song fits this description well, and because it is more resistant to distortion than the Type 1 song, the addition of the Type 2 suffix could increase the distance at which receivers can extract information from a male’s song, especially if the two song types contain redundant information.

Since Morton (1975) proposed that the physical environment can act as a selective force on the acoustic structure of signals, much of the work inspired by the acoustic adaptation hypothesis has focused on comparisons across species and habitat types (reviewed in Ey and Fischer 2009); relatively few studies have examined how different vocalizations within a bird’s repertoire transmit through the environment. Those that have examined the propagation of various song or call types have found a relationship between the broadcast area and the typical distance to the intended receiver of the signal (Cosens and Falls 1984, Wallin 1987, Dabelsteen et al 1993, Balsby et al 2003, Barker et al. 2009, Rek 2013, Sandoval et al. 2015, Nelson et al. 2016, Piza and Sandoval 2016). In the case of splendid fairy-wrens, the Type 2 song structure appears to be better adapted for long distance communication, both in terms of its broadcast area and its resistance to distortion.

We have shown though our analysis of the acoustic structure, propagation, and use of splendid fairy-wren song types that, despite costs to Type 1 song duration and performance, there are several ways in which the addition of the Type 2 suffix could increase the effectiveness of a male’s dawn chorus performance. Along with splendid fairy-wrens, three species in the genus

Malurus have incorporated the predator-elicited trill structure into their displays: superb, blue-breasted, and purple-crowned fairy-wrens (Greig and Webster 2014). For superb and blue-breasted fairy-wrens, whose Type 1 and Type 2 song structures appear to occupy distinct frequency ranges, benefits similar to those described for splendid fairy-wrens might account for the incorporation of the trill into the standard display song. An interspecific study of the spectral characteristics of Type 1 and Type 2 songs in the genus *Malurus* could help determine whether advantages associated with trill structure and propagation are driving forces in the evolution of this singing behavior.

CHAPTER 3: THE SONG OVERLAP NULL MODEL GENERATOR (SONG): A NEW TOOL FOR DISTINGUISHING BETWEEN RANDOM AND NON-RANDOM SONG OVERLAP[1]

ABSTRACT

Song overlapping, a behavior in which an individual begins singing before its counterpart has completed its song, has been the subject of recent debate. Although many studies have suggested that song overlapping functions as a signal, the majority of these studies fail to address the possibility that overlapping is a chance occurrence. Part of the difficulty in determining whether overlap is intentional or accidental lies in the lack of compelling null models for estimating chance levels of song overlap. We have developed the Song Overlap Null model Generator (SONG), a software package for R. SONG uses resampling randomization to predict the expected amount of overlap due to chance, and is applicable to any system in which individuals engage in signaling interactions. To evaluate the effectiveness of SONG, we examined the overlapping behavior of three avian species: black-capped chickadees (*Poecile atricapillus*), rufous-and-white wrens (*Thryophilus rufalbus*), and long-tailed manakins (*Chiroxiphia linearis*). Our analyses revealed that black-capped chickadees avoided overlapping the songs of playback-simulated intruders, duetting wrens overlapped the songs of their mates, and manakins avoided overlapping the duets of their neighbors. We believe that SONG will prove to be a valuable tool for understanding signal timing in songbirds as well as other taxa.

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INTRODUCTION

Communication is essential for social behaviors such as mate attraction and resource defense. Many taxa use long-range acoustic signals to accomplish these goals, including mammals (e.g. Behr and von Helversen 2004, Charlton et al. 2007), birds (Catchpole and Slater 2008), amphibians (Gerhardt and Huber 2002), fish (Ladich 1997), and insects (Cade 1985). Through the use of these signals, individuals can communicate their quality (e.g. Forsman and Hagman 2006), condition (e.g. Wagner and Hoback 1999), and motivation (e.g. Searcy et al. 2006) to potential mates and territorial rivals.

Individuals often use signals interactively, adjusting the structure or timing of their signals in response to those of nearby conspecifics (Todt and Naguib 2000). During such interactions, individuals may produce signals synchronously, overlapping each other's signals. Overlapping is especially common in chorusing taxa (e.g. insects, anurans, and songbirds), in which individuals display simultaneously to attract mates (Greenfield 1994a, Todt and Naguib 2000). There are various processes by which overlap can occur. It can occur by chance when competition for acoustic space is high, or when individuals signal without reference to each other. In many species of insects and anurans, females have a strong preference for leading signals (i.e. signals occurring first in a sequence; e.g. Snedden and Greenfield 1998, Höbel and Gerhardt 2007), creating competition among males to begin signaling before nearby conspecifics. In insects, overlapping can emerge as a by-product of this competition (Greenfield and Roizen 1993).

Overlapping can also arise out of intentional processes. This is likely the case for duetting, which involves the coordination of two individuals' signals, usually the male and female of a mated pair. Overlapping is a defining feature of polyphonal duets, which have been observed in many taxa including primates (Haimoff 1986), anurans (Tobias et al. 1998), and birds (Hall 2009). During aggressive interactions between rivals, overlapping can function as a signal. Male grey tree frogs (*Hyla versicolor*) adjust their signal timing to overlap the calls of rival males during close-range interactions, signaling their intent to escalate the contest (Reichert and Gerhardt 2013).

Many studies have suggested that overlapping functions as an aggressive signal in songbirds (reviewed in Naguib and Mennill 2010). The majority of these studies, however, have neglected to address the possibility that song overlapping is a chance occurrence. In the studies that have explicitly tested this idea, overlapping occurred at or below chance levels (Wasserman 1977, Gochfeld 1978, Fitzsimmons et al. 2008, Foote et al. 2008, Maynard et al. 2012, Yang et al. 2014). As a result, whether song overlapping is truly a signal remains controversial (Searcy and Beecher 2009, Naguib and Mennill 2010, Searcy and Beecher 2011).

How do we calculate chance levels of overlap? One established technique is the duty cycle method, which states that during an interaction between individuals A and B, the probability that A’s song will overlap B’s song is equal to the proportion of time that B spends singing (Ficken et al. 1974). This method has a critical limitation: by collapsing singing behavior down into a simple probability, variation in song duration and timing become confounded. For example, as long as the ratio of song to silence remains the same, a rigidly periodic performance, a highly variable performance, and a single long song could all result in the same duty cycle. Therefore, according to this method, each of these performances would result in the same expected amount of chance overlap, even though the opportunities for overlap could be vastly different in each case.

Unlike the duty cycle method, randomization methods can take into account variation in song duration and timing. A randomization test estimates the expected value of a test statistic by rearranging the observed data many times, calculating the statistic for each rearrangement to generate a null distribution (Manly 2006). Randomization tests have proven useful for studying patterns in signaling behavior (e.g. call type matching: Janik 2000, Miller et al. 2004; mimicry: Payne et al. 2000), and recently, researchers have begun using resampling randomization to study song overlapping in birds (e.g. Maynard et al. 2012, Yang et al. 2014).

To make techniques like those described above more widely available for use, we have developed the Song Overlap Null model Generator (SONG). SONG is a freely available software package for the statistical language R (R Development Core Team 2013) that is freely available for download from the SONG GitHub repository (https://github.com/ChristinaMasco/song). Given an interaction, SONG uses resampling randomization to predict the amount of chance overlap, while giving users the option to preserve the natural variation song duration and timing. The input for the SONG package is a tab-delimited text file containing the start time, end time, and singer identity for each song during an interaction. These data can be acquired using sound annotation software packages such as Syrinx-PC (J. Burt, Seattle, WA, U.S.A.) or Raven (Cornell Laboratory of Ornithology, Ithaca, NY U.S.A.). SONG can accommodate files containing any number of individuals, and will calculate the amount of overlap for each possible pairwise interaction. Because the input is a simple list of start and end times, the SONG package can be used to investigate signal timing during any type of interaction.

Given an observed interaction between two individuals, A and B, the SONG package builds randomized performances for A and B independently, which are then assembled into randomized interactions (i.e. A vs. B-randomized and vice versa). The program then calculates the amount of overlap in each of these randomized interactions, generating a distribution that represents the amount of overlap expected due to chance. A comparison of the observed amount of overlap to this null distribution produces a P-value, calculated as the probability that a randomized interaction will result in an amount of overlap greater than observed. In other words, if individuals overlap each other's songs more often than expected by chance, the P-value will be close to zero; if individuals overlap each other's songs less often than expected by chance, the P-value will be close to one.

SONG contains two methods for measuring the amount of overlap: (1) the duration of overlap in seconds, and (2) the number of overlapping songs. A song is considered overlapping if it begins while another song is in progress. For calculating the expected amount of chance overlap, SONG contains three null models (SampleGaps, KeepGaps, and KeepSongOrder) that differ in the constraints they impose on the randomization procedure. Users can select whichever model creates randomized performances that most closely resemble the natural signaling behavior of their study system. (1) The SampleGaps randomization method preserves only the observed song durations, rearranging the order of the songs and placing them at random intervals to create randomized performances (Figure 3.1a). This method is most appropriate for systems in which signal timing is highly variable or unconstrained. (2) The KeepGaps randomization method preserves the observed song and inter-song interval durations, rearranging the order of the songs and intervals (Figure 3.1b). This procedure ensures that the observed variation in signal timing is preserved in each of the randomized performances. The KeepGaps method could be particularly useful for systems in which signal timing is periodic or highly constrained. (3) The KeepSongOrder randomization method preserves the observed song order in addition to the song and interval durations, rearranging only the interval order to create randomized performances

(Figure 3.1c). This method was designed specifically with dawn and dusk choruses in mind. During these prolonged performances, individuals of some species ‘drift’, shortening their songs and lengthening their pauses over time (e.g. Lambrechts and Dhondt 1988). In these cases, it may be important to preserve the relationship between song duration and time over the course of the interaction. Recognizing that users may wish to develop randomization methods that have not been included in the package, we built SONG to easily accommodate user-written methods as well.

In addition to the resampling randomization technique described above, we have included in the SONG package the traditional duty cycle method (Ficken et al. 1974). According to this method, the expected number of overlapping songs, E, during an interaction between two animals, A and B, is calculated as $E = N_A \cdot D_B + N_B \cdot D_A$. In this equation, N_A and N_B represent the number of songs produced by A and B respectively, while D_A and D_B represent each individual’s duty cycles, or the proportion of time spent signaling. We have also included a modified version of the duty cycle method (Maynard et al. 2012) that calculates the expected duration of overlap as $E = T(D_A \cdot D_B)$. In this equation, D_A and D_B represent each individual’s duty cycles, and T represents the total duration of the exchange.

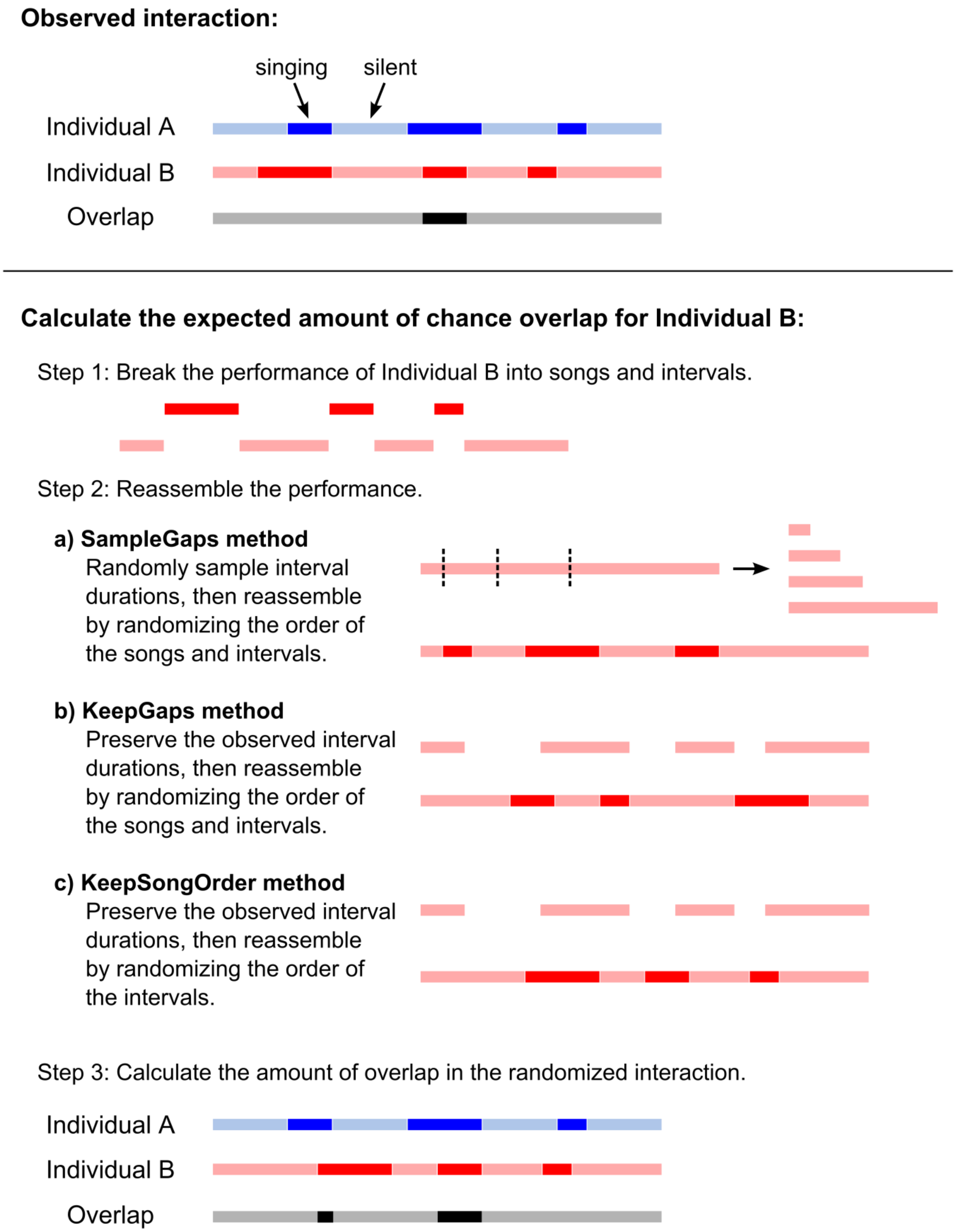

Figure 3.1. A schematic depicting the randomization process used by the SONG package. To calculate the expected amount of chance overlap for Individual B relative to Individual A, the

performance of Individual B is broken down into its components, reassembled in a random configuration, and compared to Individual A's observed performance. This process is repeated a user-defined number of times to generate a null distribution.

To evaluate the effectiveness of the SONG package, we examined overlapping in three avian species: black-capped chickadees (*Poecile atricapillus*), rufous-and-white wrens (*Thryophilus rufalbus*), and long-tailed manakins (*Chiroxiphia linearis*). Each of these species exhibits song overlapping in a different context, allowing us to investigate signal timing during different types of interactions. In this study, we asked whether overlapping occurred above chance levels during (1) responses by black-capped chickadees to playback-simulated territorial intruders; (2) duet interactions between male and female rufous-and-white wrens; and (3) interactions between long-tailed manakins and their neighbors on adjacent leks.

METHODS

For each of the three species, we assembled a data-set consisting of 10 pairwise interactions. Using the SampleGaps, KeepGaps, and KeepSongOrder methods, we calculated the expected duration of chance overlap for each of these interactions. Each analysis consisted of 1000 randomizations. We then compared the observed overlap to these expectations to obtain P-values for each interaction. Individuals were considered to be overlapping if $P \leq 0.025$, and avoiding overlap if $P \geq 0.975$. To provide a basis for comparison, we also analyzed these data-sets using the traditional duty cycle method. This method compares the observed and expected number of overlapping songs by means of a chi-squared test. Individuals were considered to be overlapping if they overlapped their counterparts more often than expected with $P \leq 0.05$.

Similarly, individuals were considered to be avoiding overlap if they overlapped their counterparts less often than expected with $P \leq 0.05$.

Data-set 1: Black-capped chickadees

Male black-capped chickadees engage in vocal interactions at territory boundaries during the breeding season (Mennill and Otter 2007). Song overlapping occurs during these interactions (Fitzsimmons et al. 2008, Figure 3.2a) and has been suggested to function as an aggressive signal (Mennill et al. 2002, Mennill and Ratcliffe 2004), although this idea has been controversial (Searcy and Beecher 2009, Naguib and Mennill, 2010). The data that we analyzed here came from a playback study by Baker, Wilson and Mennill (2012) designed to evaluate whether song overlap and other behaviors during countersinging interactions predict whether a chickadee will attack a rival. Baker et al. (2012) presented territorial male black-capped chickadees with a taxidermic mount of a male conspecific while playing chickadee songs on a fixed loop, recording the songs produced by the territorial male in response to the simulated intrusion. Baker et al. (2012) focused on a comparison of the vocal behavior of males that subsequently attacked the model, and those that did not. Here, we analyzed the overlapping behavior of the subjects with respect to the playback stimuli for both attackers and non-attackers. Our data-set consisted of interactions between 10 different males and the playback stimuli, five of which culminated in an attack of the model.

Data-set 2: Rufous-and-white wrens

Breeding pairs of rufous-and-white wrens combine their songs to produce vocal duets, resulting in variable degrees of overlap between male and female song (Mennill and Vehrencamp 2005, Figure 3.2b). The data that we analyzed here came from 8-channel microphone array recordings of rufous-and-white wrens, designed to passively sample the natural duetting behavior of breeding partners as they move around their large territories (Mennill et al. 2006, Mennill and Vehrencamp 2008). Our data-set consisted of interactions between 10 different females and their mates.

Data-set 3: Long-tailed manakins

Long-tailed manakins are a Neotropical species in which males perform highly synchronized male-male duets that are so well coordinated that the songs appear to originate from one individual (Trainer and MacDonald 1995). Maynard et al. (2012) noted that pairs of males appeared to vary the timing of their male-male duets with respect to the timing of the duets of the males in adjacent leks (Figure 3.2c). The data that we analyzed here came from stereo recordings obtained from two microphones positioned near adjacent leks (Maynard et al. 2012). Our data-set consisted of interactions between 10 male-male pairs and their neighboring male-male pairs.

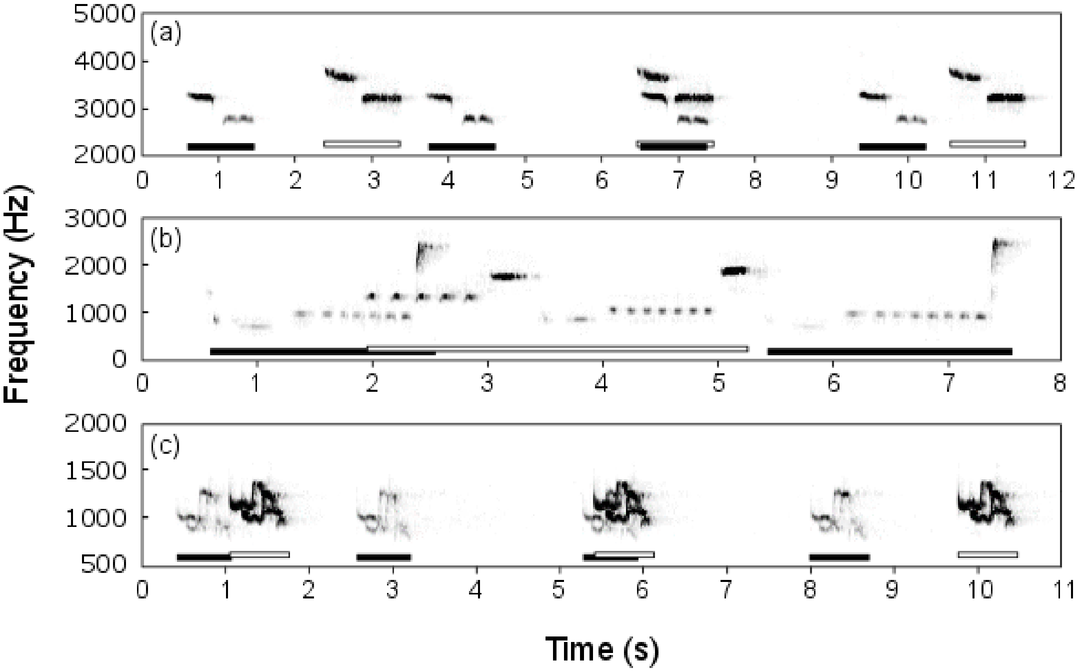

Figure 3.2. Sound spectrograms showing examples of overlapping in three species. (a) An interaction between two male black-capped chickadees, including one instance of overlap. The vocalizations of one male are underscored by black boxes, and the other male by white boxes. (b) A duet produced by a breeding pair of rufous-and-white wrens, in which the song from the female (underscored by a white box) overlaps one of the songs from the male (underscored by black boxes). (c) An interaction between two duetting male-male pairs of long-tailed manakins at adjacent lek sites, showing two instances of overlapping. The vocalizations of one pair of males are underscored by black boxes, and the other pair of males by white boxes.

RESULTS

More than half of the male black-capped chickadees included in this analysis overlapped the playback stimulus significantly less often than expected by chance (Table 3.1). When overlapping did occur, the playback stimulus began during the male’s song more often than vice versa (Wilcoxon signed rank test, $V = 48.5$, $P = 0.04$). Whether a male attacked the taxidermic mount was independent of the duration for which his songs were overlapped by the playback stimulus (Wilcoxon rank sum test, $W = 9.0$, $P = 0.55$). Overlap avoidance was also independent of attack (Fisher's exact test, $P > 0.44$ for all methods).

Table 3.1. The singing behavior of male black-capped chickadees in response to simulated territorial intrusions. For each interaction, the male's behavior is categorized as overlapping, avoiding overlap, or exhibiting no significant pattern (N.S.). The cases in which the various methods produced conflicting results are highlighted.

Male	SampleGaps		KeepGaps		KeepSongOrder		Duty Cycle	
	Pattern	P-value	Pattern	P-value	Pattern	P-value	Pattern	P-value
1	Avoid	> 0.999	Avoid	> 0.999	Avoid	0.999	Avoid	< 0.001
2	Avoid	0.993	Avoid	0.981	N.S.	0.973	Avoid	0.005
3	Avoid	> 0.999	Avoid	0.995	Avoid	0.993	Avoid	< 0.001
4	Avoid	> 0.999	Avoid	> 0.999	Avoid	0.998	Avoid	< 0.001
5	Avoid	0.988	N.S.	0.954	N.S.	0.964	Avoid	0.005
6	Avoid	0.984	N.S.	0.974	Avoid	0.977	N.S.	0.182
7	Avoid	0.999	Avoid	0.999	Avoid	0.999	Avoid	< 0.001
8	Avoid	> 0.999	Avoid	> 0.999	Avoid	0.999	Avoid	< 0.001
9	N.S.	0.967	N.S.	0.947	N.S.	0.930	Avoid	0.014
10	N.S.	0.506	N.S.	0.504	N.S.	0.521	N.S.	0.903

In about half of the rufous-and-white wren interactions, either one or both of the individuals in the breeding pair sang non-randomly with respect to their mate when randomization methods were used (Table 3.2). Two males and three females overlapped the songs of their mates more often than expected by chance (all $P < 0.025$). One breeding pair significantly avoided overlapping each other's songs ($P > 0.999$). According to the duty cycle method, half of the individuals (five males and six females) exhibited song overlapping (all $P < 0.05$), and no individuals avoided overlap.

Table 3.2. The singing behavior of rufous-and-white wrens during duet interactions. For each interaction, the behaviors of the male (a) and female (b) are categorized as overlapping, avoiding overlap, or exhibiting no significant pattern (N.S.) The cases in which the methods produced conflicting results are highlighted.

a) Male behavior with respect to their mates

Pair	SampleGaps		KeepGaps		KeepSongOrder		Duty Cycle	
	Pattern	P-value	Pattern	P-value	Pattern	P-value	Pattern	P-value
1	Overlap	< 0.001	Overlap	0.001	Overlap	< 0.001	Overlap	< 0.001
2	N.S.	0.768	N.S.	0.730	N.S.	0.745	N.S.	0.745
3	Overlap	0.011	Overlap	0.018	N.S.	0.026	Overlap	0.009
4	N.S.	0.251	N.S.	0.301	N.S.	0.310	Overlap	0.034
5	N.S.	0.293	N.S.	0.291	N.S.	0.295	N.S.	0.482
6	N.S.	0.409	N.S.	0.405	N.S.	0.385	N.S.	0.591
7	N.S.	0.174	N.S.	0.194	N.S.	0.177	N.S.	0.196
8	N.S.	0.392	N.S.	0.444	N.S.	0.402	Overlap	0.021
9	Avoid	> 0.999	Avoid	> 0.999	Avoid	> 0.999	N.S.	0.369
10	N.S.	0.045	N.S.	0.079	N.S.	0.073	Overlap	0.028

b) Female behavior with respect to their mates

Pair	SampleGaps		KeepGaps		KeepSongOrder		Duty Cycle	
	Pattern	P-value	Pattern	P-value	Pattern	P-value	Pattern	P-value
1	N.S	0.770	N.S	0.871	N.S	0.894	Overlap	0.024
2	N.S	0.026	N.S	0.052	N.S	0.049	Overlap	0.001
3	N.S	0.167	N.S	0.463	N.S	0.434	N.S.	0.088
4	Overlap	0.019	N.S	0.042	N.S	0.047	Overlap	< 0.001
5	Overlap	< 0.001	Overlap	0.003	Overlap	0.005	Overlap	< 0.001
6	N.S	0.489	N.S	0.765	N.S	0.811	N.S.	0.669
7	N.S	0.029	N.S	0.032	Overlap	0.011	Overlap	0.029
8	N.S	0.456	N.S	0.558	N.S	0.619	N.S.	0.639
9	Avoid	> 0.999	Avoid	> 0.999	Avoid	> 0.999	N.S.	0.281
10	Overlap	< 0.001	Overlap	< 0.001	Overlap	< 0.001	Overlap	< 0.001

In 7 of the 10 long-tailed manakin interactions, male-male pairs overlapped the duets of their neighbors significantly less often than expected by chance when randomization methods were used (Table 3.3). Overlap avoidance was reciprocal in two cases, for a total of 9 of the 20 male-male pairs avoiding overlap (all $P > 0.975$). According to the duty cycle method, 5 of the 20 male-male pairs exhibited overlap avoidance (all $P \leq 0.05$).

Table 3.3. The calling behavior of male-male pairs of long-tailed manakins during interactions with neighbors. For each interaction, the behavior of the male-male pair is categorized as overlapping, avoiding overlap, or exhibiting no significant pattern (N.S.). The cases in which the methods produced conflicting results are highlighted.

a) Male-male pair behavior with respect to their neighbors (e.g. Pair A versus Pair B)

Interact-ion	SampleGaps		KeepGaps		KeepSongOrder		Duty Cycle	
	Pattern	P-value	Pattern	P-value	Pattern	P-value	Pattern	P-value
1	N.S.	0.600	N.S.	0.656	N.S.	0.644	N.S.	0.492
2	N.S.	0.942	N.S.	0.903	N.S.	0.909	N.S.	0.389
3	Avoid	0.990	Avoid	0.983	Avoid	0.983	N.S.	0.444
4	N.S.	0.884	N.S.	0.878	N.S.	0.870	N.S.	0.225
5	Avoid	> 0.999	Avoid	> 0.999	Avoid	> 0.999	N.S.	0.068
6	N.S.	0.887	N.S.	0.902	N.S.	0.888	N.S.	0.303
7	Avoid	> 0.999	Avoid	> 0.999	Avoid	> 0.999	Avoid	0.042
8	N.S.	0.953	N.S.	0.947	N.S.	0.957	N.S.	0.206
9	Avoid	0.990	Avoid	0.979	Avoid	0.978	N.S.	0.106
10	N.S.	0.845	N.S.	0.806	N.S.	0.815	N.S.	0.674

Table 3.3 (continued)**.**

b) The reciprocal interactions (e.g. Pair B versus Pair A)

Interact-ion	SampleGaps		KeepGaps		KeepSongOrder		Duty Cycle	
	Pattern	P-value	Pattern	P-value	Pattern	P-value	Pattern	P-value
1	Avoid	> 0.999	Avoid	> 0.999	Avoid	> 0.999	N.S.	0.079
2	N.S.	0.488	N.S.	0.414	N.S.	0.418	N.S.	0.724
3	Avoid	0.997	Avoid	0.998	Avoid	0.993	Avoid	0.020
4	N.S.	0.627	N.S.	0.629	N.S.	0.605	N.S.	0.517
5	Avoid	> 0.999	Avoid	> 0.999	Avoid	> 0.999	Avoid	0.050
6	Avoid	> 0.999	Avoid	> 0.999	Avoid	> 0.999	Avoid	0.047
7	N.S.	0.877	N.S.	0.818	N.S.	0.829	N.S.	0.492
8	Avoid	> 0.999	Avoid	0.999	Avoid	0.999	Avoid	0.005
9	N.S.	0.963	N.S.	0.943	N.S.	0.949	N.S.	0.143
10	N.S.	0.666	N.S.	0.572	N.S.	0.57	N.S.	0.507

DISCUSSION

Overlapping is a taxonomically widespread feature of signaling interactions, thought to function as an aggressive signal in songbirds (Naguib and Mennill 2010). In many cases, however, it is unclear whether overlapping is the result of intention or chance, and the lack of a compelling null model has made distinguishing between these two possibilities difficult. To address this issue, we have developed SONG, a freely available software package for R designed to predict chance levels of song overlap.

To evaluate the effectiveness of this new tool, we used SONG to examine overlapping in three avian species: black-capped chickadees, rufous-and-white wrens, and long-tailed manakins. When confronted with a simulated intruder, more than half of the black-capped chickadees in this analysis avoided overlapping the playback stimulus. When overlap did occur, the playback stimulus overlapped the bird's song more often than vice versa, suggesting that instances of overlap were accidental rather than intentional. In our analysis, a male's overlapping behavior was independent of whether he eventually attacked the mount; a lack of relationship between overlapping and attack was found in the full data-set as well (Baker et al. 2012, n=38). Overlap avoidance has not been observed during natural countersinging interactions in this species (Fitzsimmons et al. 2008). Further investigation is necessary to determine whether overlapping functions as a signal in natural interactions as suggested by prior studies (Mennill et al. 2002, Mennill and Ratcliffe 2004).

For rufous-and-white wrens, our analyses revealed variation in duetting behavior. In some cases, the female overlapped the male's songs to form duets; in others, the male overlapped the female's songs. In half of the interactions analyzed, either one or both of the individuals in the breeding pair sang non-randomly with respect to their mate. Whether variation in duetting behavior is individually repeatable, or has any impact on reproductive success in this species is a matter of ongoing investigation. Analysis of longer recordings of these birds, along with parallel studies of their reproductive behavior, will enhance our understanding of vocal duets in rufous-and-white wrens.

For long tailed-manakins, half of the male-male pairs that we analyzed significantly avoided overlapping the duets of their neighbors. These results match the pattern found in the full data-set analyzed by Maynard et al. (2012). Because these duets primarily function in mate attraction, it is possible that overlap avoidance functions as a strategy to minimize signal masking. Investigating whether males that avoid overlapping their neighbors are more successful in attracting females would help shed light on the function of overlap avoidance in this species.

For each of the species analyzed, the duty cycle and randomization methods often produced conflicting results. Because the duty cycle method confounds variation in signal duration and timing, its estimations for chance overlap may be less accurate than the estimations generated by the randomization methods. This effect should be most pronounced in animals that show high variation in signal duration or signal timing. However, without knowing the true value of the amount of chance overlap in an interaction, we cannot be sure which method yields the most accurate prediction. Whether it is possible to rigorously test the accuracy of a song overlap null model is a worthwhile topic for future study.

It is important to note that, even though song overlapping may occur more often than expected during an interaction, this does not necessarily imply that overlapping functions as a signal. To demonstrate signal value, we recommend using SONG as a first step to inform subsequent experimentation. Searcy and Beecher (2009) have provided a useful set of criteria for assessing whether a behavior functions as an aggressive signal, emphasizing the importance of gathering evidence from both the sender's and receiver's perspectives. Helfer and Osiejuk (2015) have offered alternative explanations for the function of song overlapping, presenting a series of working hypotheses and specific predictions. Together, these works provide a valuable theoretical framework to guide future research on song overlapping.

Similarly, even though overlapping may occur less often than expected during an interaction, subsequent investigation is necessary to determine why individuals avoid overlap. Overlap avoidance may function as a strategy to minimize interference (Wasserman 1977, Egnor et al. 2007), facilitate mutual listening (Schwartz and Rand 1991), or in the case of mate attraction aggregations, maximize the overall duty cycle of the group (Greenfield 1994b). Beyond its utility in detecting overlap avoidance in response to conspecific and heterospecific sound, the SONG package may also prove useful in examining whether urban species exhibit overlap avoidance in response to intermittent anthropogenic noise.

Although the primary focus of this paper has been song overlapping in birds, the SONG package is not avian-specific. Signal timing in chorusing insects and anurans has also been an area of extensive study. In these taxa, the mechanisms involved in sound production constrain temporal features of the calls such as pulse rate and call duration, resulting in highly stereotyped performances (Gerhardt and Huber 2002). Methods included in the SONG package can preserve this stereotypy, potentially leading to more accurate estimations of chance overlap than the traditional duty cycle method.

The SONG package can also be used to study signaling interactions outside of the acoustic modality. Because the input is a simple list of start and end times, our package can be used to study the coordination of any temporally patterned behavior, including visual displays, movements, and multimodal signaling interactions.

CHAPTER 4: SONG OVERLAPPING IN SPLENDID FAIRY-WRENS

ABSTRACT

In songbirds, as in other chorusing taxa, overlapping is a common feature of signaling interactions. Although this behavior has been the subject of study for over 30 years, the function of song overlapping remains controversial. In this study, we quantified the singing behavior of splendid fairy-wrens (*Malurus splendens*), a cooperatively breeding but reproductively promiscuous species, to test the alternative hypotheses put forward for the function of song overlapping. Using a microphone array to simultaneously record the dawn chorus performances of multiple individuals, we discovered evidence of overlap avoidance among interacting kin and frequent overlapping among unrelated males that, in one of 14 cases, exceeded chance levels. The rate of overlap in an interaction (i.e. the observed amount of overlap in seconds divided by the total time spent singing by the individual being overlapped) was best predicted by the morphological characteristics of the overlapped male; the males with the largest cloacal protuberances and cloacal tips, traits that could increase the likelihood of successful fertilization, were overlapped by their counterparts at the highest rates. During simulated territorial intrusions, however, males overlapped the playback of conspecific song at or below chance levels. Overall, our findings best support the hypothesis that song overlapping functions as a masking strategy in splendid fairy-wrens.

INTRODUCTION

An animal can convey information to potential mates, rivals, and other receivers by encoding the information in the structure of a signal (e.g. fundamental frequency as an indicator of body size, Davies and Halliday 1978) as well as in the performance of a signal (e.g. song rate as an indicator of territory quality, Hoi-Leitner et al 1995). Additional sources of information can emerge when animals engage in interactive signaling behaviors, in which individuals dynamically adjust the structure or use of their signals in response to those other individuals (Todt and Naguib 2000). One example of an interactive singing behavior is song overlapping, which occurs when an individual begins singing before its counterpart has completed its song. There are a number of hypotheses for the function of this behavior (reviewed in Helfer and Osiejuk 2015), which fit into two broad categories: song overlapping could function as a signal that conveys information to the individual being overlapped or to third-party receivers, and song overlapping could function as a strategy to alter the receivers' detection or perception of the overlapping songs. In this chapter, we examined song overlapping in splendid fairy-wrens (*Malurus splendens*) to investigate whether this behavior could function as (1) an aggressive signal, (2) a signal of quality, (3) a signal of social group cohesion, (4) a masking strategy, (5) a modifier, or (6) a signal enhancement strategy in this species.

Song overlapping as an aggressive signal

Song overlapping has long been thought to function as an aggressive signal in songbirds (e.g. Brindley 1991; Dabelsteen et al. 1996, 1997). This hypothesis has received some empirical support (reviewed in Naguib and Mennill 2010); for example, overlapping behavior has been shown to vary with distance (van Dongen 2006, Araya-Salas et al. 2017) and threat level (Langemann et al. 2000) in aggressive contexts, and it can alter the behavior of the individuals whose songs are being overlapped (e.g. Mennill and Ratcliffe 2004, Hall et al. 2006). However, the possibility that song overlapping is a by-product of increased song rate during aggressive interactions has led Searcy and Beecher (2009) to conclude that overlapping "may not be a signal at all," and the signal value of song overlapping remains controversial (Naguib and Mennill 2010, Searcy and Beecher 2011). For song overlapping to function as a reliable signal of aggression, the occurrence of overlapping would need to be tightly linked to aggression on the part of the overlapper, and so it should occur more frequently than expected by chance in aggressive contexts. The individuals being overlapped should exhibit agonistic behaviors in response, and overlapping should predict increasingly aggressive behaviors on the part of the overlapper (Searcy and Beecher 2009).

Song overlapping as a signal of quality

In addition to signaling intent, song overlapping might communicate information about the quality or condition of the individuals involved in the interaction. Under this hypothesis, song overlapping could arise through competitive or cooperative means. Individuals might overlap rivals to signal their relative quality or condition to the individual who is overlapped as well as to any eavesdropping rivals and potential mates. Overlapping interactions have been shown to alter the behavior of eavesdropping females, revealing a preference by females for lagging songs (Amy et al. 2008) that can have consequences for the reproductive success of the males involved in these interactions (Mennill et al. 2002, Garcia-Fernandez et al. 2010). Alternatively, individuals could overlap in a cooperative fashion (i.e. duet) to jointly signal their quality or condition to potential mates, as is the case in long-tailed manakins (*Chiroxiphia linearis*, Trainer and McDonald 1993). By either mechanism, the honesty of song overlapping as a signal of quality is expected to be enforced by costs or constraints; therefore, under this hypothesis, higher-quality individuals should be able to engage in song overlapping at a higher intensity (Helfer and Osiejuk 2015). If song overlapping is a cooperative signal of quality, it should only occur among social group members, and the subordinate individuals in the interaction should derive benefits from participation, either directly or indirectly.

Song overlapping as a signal of social group cohesion

Song overlapping arising through cooperative means could also function as a signal of social group size or cohesion. Although two individuals singing in an alternating pattern could be perceived as one individual singing at a high rate, an overlapping exchange is a clear signal to third-party receivers that multiple individuals are engaged in a social interaction. For species that live and breed in social groups, numerical assessment based on vocalizations can play a role in aggressive interactions (e.g. McComb et al. 1994, Hale 2006), and the coordination of song could signal the cohesion of the group during interactions with territorial rivals as well as facilitate the coordination of activities within the social group such as cooperative defense, foraging, and care of offspring.

Song overlapping as a masking strategy

In addition to its potential to communicate information, song overlapping has the capacity to change how songs are detected, discriminated, and perceived by receivers. Song overlapping could function as strategy in which individuals mask the songs of their counterparts (Hultsch and Todt 1982), impeding the ability of receivers to discriminate one song from the other and rendering the information in the overlapped song unintelligible. Masking is expected to be a costly strategy, as it impacts the reception of the lagging songs as well as the leading songs. Consequently, masking is more likely to occur in species whose songs have information-rich suffixes; under these conditions, an individual could mask the information in its counterpart’s song while conveying its own information relatively unobscured. If song overlapping functions as a masking strategy, individuals should compete for the lagging position in an interaction, potentially by abbreviating their overlapped songs. In the context of mate attraction, lower-quality individuals should mask the songs of higher-quality individuals in an effort to disrupt the females' detection and discrimination of the more attractive songs.

Song overlapping as a modifier

Song overlapping can also function as a modifier, amplifying the information contained in the structure of the song itself (Helfer and Osiejuk 2015). When two songs overlap in time, third-party receivers are provided the opportunity to directly compare the features of the songs. For example, Montezuma oropendolas (*Psarocolius montezuma*) decrease the lowest peak frequency of their songs when overlapping the songs of their counterparts, a feature that is correlated with body size and more easily compared when songs overlap (Price et al. 2006). Overlapping may also provide receivers with direct comparisons of bandwidth and note rate, features that together can indicate the performance quality of the singing individual (Podos 1997, Podos 2017). If song overlapping functions as a modifier, third-party receivers would gain more reliable information from overlapping songs than solo songs, and song overlapping would be beneficial for the individuals involved regardless of whether their songs are in the leading or lagging position. The intensity of overlapping would be related to the relative quality of the interacting individuals; low-quality males should evade direct comparisons with high-quality males by avoiding overlap, and the highest rates of overlapping would be expected among the most evenly-matched individuals.

Song overlapping as a signal enhancement strategy

When overlapping in time is accompanied by overlapping in frequency, songs can constructively interfere to produce a combined signal of greater intensity (Rehberg-Besler et al. 2016). The "signal enhancement hypothesis" (Alexander 1975, Otte 1980) suggests that, in choruses characterized by high rates of signal overlap, the resulting increase in the overall active space of the chorus provides a *per capita* benefit to the participating individuals that exceeds the benefits associated with calling alone. Under this hypothesis, individuals should engage in song overlapping regardless of their social relationships, and all individuals should benefit from overlapping regardless of whether their songs are in the leading or lagging position.

Song overlapping as a chance phenomenon

Alternatively, song overlapping could have no function and arise due to chance (Searcy and Beecher 2009). In dense aggregations of singing individuals, such as those apparent during the dawn chorus, competition for acoustic space is high, and overlapping may be inevitable. When acoustic space is more available, overlapping can still arise due to the periodic nature of signaling; two individuals singing without reference to each other are expected to overlap by some amount that depends on their individual patterns of song duration and timing. Randomization methods can be used to calculate the expected amount of overlap due to chance in a given interaction, and they have proven useful for investigating the contexts in which song overlapping occurs (see Chapter 3; Masco et al. 2016, Araya-Salas et al. 2017). Overlapping that occurs at chance levels might not function as a reliable signal, as the link between the signal and the condition (e.g. the overlapper's intent to escalate a conflict) would be tenuous and provide little information for receivers. Song overlapping that occurs at chance levels may still have a strategic value, however, as even occasional overlap could mask, modify, or enhance the songs in an overlapping interaction.

Gaining insight into the potential function (or non-function) of song overlapping in a species requires teasing apart the various hypotheses described above. Investigating who overlaps whom and in what contexts can reveal, in part, what information this behavior could convey as well as what benefits it could confer on the interacting individuals. We used this approach to examine song overlapping in splendid fairy-wrens, a species of passerine that engages in singing interactions both within and across social groups. Splendid fairy-wrens exhibit facultative cooperative breeding, forming groups composed of a breeding pair and one or more helpers that aid in territory defense and offspring provisioning (Rowley and Russell 1997). Unlike many cooperatively breeding species, the rate of extra-pair paternity is high in splendid fairy-wrens; more than half of nests contain young sired by males other than the social mate (Webster et al. 2004). Consequently, there is a mismatch in the social and genetic relationships among group members, and some subordinate "non-breeding" males are able to achieve reproductive success through siring a small number of offspring within or outside of their social group (Webster et al. 2004). Because dominant males and their subordinate helpers jointly defend the territory and raise offspring, but are each seeking to attract extra-pair mates, the relationship between these individuals is characterized by both cooperation and conflict.

Although little is known about the copulation-seeking behavior of female splendid fairy-wrens, females of a closely-related species (superb fairy-wrens, *Malurus cyaneus*) seek extra-pair copulations during the dawn chorus (Double and Cockburn 2000). If splendid fairy-wrens engage in this behavior as well, the dawn chorus may play an important role as an arena for extra-pair mate attraction in this species. Male splendid fairy-wrens possess small song repertoires consisting of two song types: the Type 1 chatter song and the Type 2 trill song (Greig and Pruett-Jones 2008). During the dawn chorus, males sing at high rates and predominantly produce songs that contain both song types, singing the chatter song with a trill suffix (i.e. Type 1+2 song, see Chapter 2). Whether a male's dawn chorus singing behavior has an impact on his mating opportunities or his ability to secure paternity within his own nest is currently unknown.

Because the social system of splendid fairy-wrens is characterized by both cooperation and competition within and across territory boundaries, studying the singing behavior of this species provides an excellent opportunity to test the various hypotheses put forward for the function of song overlapping. To investigate the potential function of song overlapping in splendid fairy-wrens, we used a combination of observational and experimental approaches. Using a 12-channel microphone array, we quantified the occurrence of song overlapping during natural dawn chorus interactions and examined the relationships between this behavior and indicators of social dominance and male quality. We then conducted playback-simulated territorial intrusions during and after the dawn chorus to examine whether males adjust the timing of their songs during close-range, aggressive interactions with familiar and unfamiliar individuals. By revealing who engaged in song overlapping, whose songs were overlapped by others, and the contexts in which this behavior occurred, these approaches enabled us to explore whether song overlapping could function as a signal or strategy in splendid fairy-wrens.

METHODS

Study site and subjects

During the breeding seasons of 2013, 2014, and 2015 (October to December), we monitored the behavior and reproductive success of 50-60 social groups (120-140 banded individuals) at Brookfield Conservation Park in South Australia (see Webster et al. 2004 for more details on the study site and field methodology). This fieldwork was conducted with approval from the University of South Australia Animal Ethics Committee (Wildlife Ethics Committee approvals 17-2012, 18-2012, 21-2013), and the University of Chicago Institutional Animal Care and Use Committee (ACUP permit number 72322).

Adult splendid fairy-wrens were captured via mist net for color-banding, and while the males were in-hand, we measured various aspects of their morphology including weight, wing length, tarsus length, tail length, and the dimensions of the cloacal protuberance (Tuttle et al. 1996) and cloacal tip (Rowe et al. 2008). Although the ages of the males that were banded as nestlings were precisely known, the ages of the males captured for the first time as adults were unknown and were assumed to be a minimum of one year at the time of first capture. When morphological measurements were not collected during the same year as the dawn chorus recordings, measurements collected within 3 years of the recordings were used. Processing the dawn chorus recordings for splendid fairy-wrens is time intensive due to their high singing rates and frequent song overlapping. As a result, only a small subset of the banded males (10 unique individuals) was included in this study. One male was recorded in 2013 as a subordinate helper and again in 2014 as a dominant breeder on a new territory, yielding a total of 11 dawn chorus performances.

For each male included in our analysis, we determined his social rank (i.e. whether he was a dominant breeder or subordinate helper) based on behavioral observations, age, and plumage characteristics. For each pairwise singing interaction, we also determined whether the overlapper and the individual being overlapped shared a territory and whether they were socially related as father and son. Because the rate of extra-pair paternity is high in splendid fairy-wrens, it is currently unknown whether the three socially related father-son pairs included in this analysis were genetically related as well. Rates of nest depredation were high during 2013 and 2014, and none of the nests associated with the males included in this analysis fledged young. As result, we were unable to directly measure a male’s reproductive success.

Territory mapping

To guide the positioning of the array microphones and the placement of the speaker during playback-simulated intrusions, we mapped the territories of the social groups of interest using handheld GPS units (model: eTrex 20; Garmin International Inc., Olathe, KS, USA). ; During each mapping session, we followed focal individuals for 30 min to one hour as they foraged and interacted with neighboring social groups between the hours of 08:00 and 18:00, and we revisited each social group for additional mapping until the boundaries of the territory were well-defined. To obtain a map of the territory, we marked the coordinates each time the group, while exhibiting typical resident behavior (e.g. singing, foraging), ventured into an area in which they had not been seen previously. The locations at which we observed members of neighboring social groups engaging in agonistic interactions were considered territory boundaries. We also marked the coordinates of any location from which a male sang during at least one dawn chorus performance to ensure that all of the focal males' songs were recorded within the boundaries of the array.

Acquisition of focal recordings

We recorded the dawn chorus performances of individual male splendid fairy-wrens using unidirectional shotgun microphones (frequency response: 40 Hz - 20 kHz ± 2.5 dB; model: ME 66 with K6 power module; Sennheiser Electronic GMBH & CO. KG, Wedemark, Germany) and digital recorders (model: PMD661 MKII; Marantz Corp., Kanagawa, Japan). Vocalizations were recorded 3 to 5 m from the focal male as uncompressed Waveform Audio File Format (.wav) files at a sample rate of 44.1 kHz. We confirmed the identity of each singing male by sighting the color-band combination near the end of each recorded performance. We extracted all clear examples of song from these recordings to build a song library for each male. The song libraries were then used to identify males within the microphone array based on song structure and to create playback stimuli for simulated intrusions.

Acquisition and processing of neighborhood recordings

To investigate whether males engage in song overlapping during the dawn chorus, we used a 12-channel microphone array to record the performances of two to five males simultaneously. The microphone array consisted of six time-synchronized Wildlife Acoustics Song Meters (model: SM2+GPS; Wildlife Acoustics Inc., Concord, MA, USA), and at each recording site, we arranged them in a pentagon with one recorder located in the center of the array (Figure 2.2). Each recorder was fitted with two omnidirectional microphones (frequency response: flat 20 Hz – 20 kHz; model: SMX-II; Wildlife Acoustics Inc., Concord, MA, USA), one attached directly to the recorder and the other attached by a 10 m cable. We programmed the Song Meters to record from 4:00 to 7:00 a.m. as uncompressed stereo .wav files at a sample rate of 48 kHz.

With time-synchronized microphone arrays, researchers have the ability to locate the source of a sound based on the time at which the sound arrives at each microphone (Blumstein et al. 2011, Mennill et al. 2012). The accuracy of a source location estimate depends, in part, on the accuracy of the microphone location estimates, which we obtained using a combination of static GPS sampling and trigonometry. Each recorder had two microphones, one attached directly to the recorder and the other tethered by a 10 m cable. We used an approach tested by Mennill et al. (2012) to sample the location of each microphone attached directly to the recorder; we placed a handheld GPS unit (model: eTrex 20; Garmin International Inc., Olathe, KS, USA) immediately below the microphone, and used the "track" function to log the unit's location every 30 sec for two to three hours. This process generated a cloud of over 200 points, the center of which we assume provides the best estimate of the microphone’s location. To define the center, we removed the 10% of points that were farthest from the mean of all points, and then calculated the average X and Y coordinates based on the remaining points. We calculated the locations of the tethered microphones relative to the GPS-sampled microphones using a sighting compass and measuring tape.

We visualized and manually annotated the 12-channel neighborhood recordings using Syrinx-PC (J. Burt, Seattle, WA, USA), selecting the clearest portion of each song on the spectrogram of the recording by drawing a rectangle that specified the start time, end time, minimum frequency, and maximum frequency of the selection. Although each song appeared in multiple channels, songs were annotated only in the channel in which they were most clearly recorded. To calculate the source locations of the annotated songs, we used ArrayGUI for MATLAB (J. Burt, Seattle, WA, USA; MathWorks, Inc., Natick, MA, USA). This software determines the relative time-of-arrival of the annotated sound in each channel of the recording using spectrographic cross-correlation, then uses an optimization approach to determine the best location estimate given the locations of the microphones and the time-of-arrival differences (see Mennill et al. 2006). Location estimates were considered reliable if (1) the quality index generated by ArrayGUI was 0.7 or higher, (2) the probability cloud generated by ArrayGUI had a small, circular distribution, (3) the location estimate fell in close proximity to the location estimates for songs occurring immediately before and/or after the song in question, and (4) the location estimate was concordant with field observations, when available. Males sing for stretches of time from discrete locations within their territories (i.e. song posts), but because the clarity of recorded songs can vary depending on dynamic environmental conditions and slight changes in the position or orientation of singing males, errors arise in the sound localization analysis that make it appear as though males are moving very short distances between songs. To calculate the locations of the song posts, we performed a cluster analysis for each male that grouped together any location estimates that occurred within 3 m of each other. We used the function "cluster_infomap" in the *igraph* R package (Csardi and Nepusz 2006) to refine the clusters, and then calculated the centroids of each cluster to obtain the coordinates of each male's song posts. To assign song post locations to the songs without reliable estimates, we examined the song post assignments of the songs immediately before and after the song in question, and if a song post change was thought to have occurred, we visually inspected the spectrogram to compare the degradation of the song (an indicator of the distance from the microphone) to that of the songs immediately before and after. The song post locations that were calculated for each male were consistent with our field observations.

After we localized the source of each splendid fairy-wren song, we used Syrinx-PC (J. Burt, Seattle, WA, USA) to adjust each song's annotation selection boundaries such that the entire duration of the song was encompassed by the selection, made in the channel in which the start and end times of the song were clearest. We determined the identity of the male singing each song by comparing each song's location estimate to our field observations of known song post locations. When the location estimate was not sufficient for identifying the singer (i.e. when two males sang from a shared roost), we determined singer identity by comparing the acoustic structure of the song to that of the songs in the library of focal recordings, relying on the individual distinctiveness of splendid fairy-wren song (Greig and Pruett-Jones 2008). In addition to the singer's identity, we noted the composition of each song (i.e. the song type and order of its components). Although males continue to sing at low rates after sunrise, we defined a male's dawn chorus performance as all songs produced between 4:00 a.m. and sunrise, which ranged from approximately 5:50 a.m. to 6:15 a.m. in our data-set. In our analysis of song overlapping, we included the performances of 11 males recorded in 5 microphone arrays, for a total of 14 pairwise interactions and 1513 songs.

Playback-simulated intrusions

In 2015, we conducted a playback experiment to examine whether splendid fairy-wrens exhibit neighbor-stranger discrimination, and demonstrated that, based on the latency to first response and the occurrence of behaviors such as bill-wiping, mate-guarding, and singing, splendid fairy-wrens responded equally strongly to intrusions by familiar and unfamiliar individuals (Johnson et al. in review). As song overlapping was not examined in our previous study, we reanalyzed the vocal responses to the simulated intrusions to examine whether this behavior appears in aggressive contexts.

Each male was presented with the songs of (1) a dominant male splendid fairy-wren from a neighboring territory (the “neighbor” treatment), (2) a dominant male from five or more territories away (the “stranger” treatment), and (3) a red-capped robin (*Petroica goodenovii*, the control treatment). A total of 9 males received control, neighbor, and stranger treatments during the dawn chorus (starting approximately 40 min before sunrise), and 14 males received each of the treatments during the daytime (7:00-11:00 a.m.). For each trial, the speaker was placed on the ground, on the territory boundary that was shared with the individual whose songs were used for the neighbor treatment. Each focal individual received one treatment per day in a random order. For each trial, the stimulus was played in three sessions with 5 min of silence in between, yielding a trial duration of 18 min. For males that received treatments during both the dawn chorus and the daytime, two sets of neighbor stimuli and speaker locations were used to avoid repeating intrusions by the same individuals at the same locations. We used an amplified field speaker (model: SME-AFS; Saul Mineroff Electronics Inc., Elmont, NY, USA) and an iPod Classic (Apple Inc., Cupertino, CA, USA) to broadcast the playback stimuli at approximately 87.0 dB SPL 1 m from the speaker as measured using a sound pressure level meter (model: PSPL01; Pyle Audio, Brooklyn, NY, USA). The playback trials began 10-15 min after speaker placement to allow the focal individuals to resume normal behavior before the trial. If the focal individual did not resume normal behavior, the trial was canceled and attempted on a subsequent day.

We created the playback stimuli from our splendid fairy-wren song library, using only the songs that had been recorded during the same year as the experiment (2015). Each playback stimulus consisted of eight songs with 5 s of silence between each song (total duration ~ 1 min). For the neighbor and stranger treatments, we included both Type 1 and Type 1+2 songs. We standardized the maximum amplitude of each song before assembling the playback stimuli to ensure that the playback amplitude remained constant within and across trials. For each trial, we placed a Song Meter within the focal male's territory 50 to 150 m from the speaker to record the playback stimulus and any songs produced in response. We visualized and manually annotated the resulting recordings using Syrinx-PC to extract the start and end times of each song.

Analysis of song overlapping

During a typical dawn chorus performance by a male splendid fairy-wren, song rate and duration exhibit a quadratic relationship with respect to the time relative to sunrise; the performance begins with a "warm-up" period of increasing song rate and duration and ends with a "cool-down" period of decreasing song rate and duration, with a period of consistently high singing rates in between (see Chapter 2). Because song rate and duration are highly variable over the course of the dawn chorus performance, the expected amount of overlap due to chance should vary as well. To account for this, we included only the period with the highest singing rates in the overlap analysis, hereafter referred to as the “chorus phase.” For each array recording, the start and end of the chorus phase was determined by visually inspecting the relationship between the duration of the inter-song intervals and time relative to sunrise, defining the chorus phase as the time range in which the slope of the relationship was close to zero. Across the data set, the chorus phase consisted of 1034 songs in total, which represented approximately 68% of the 1513 songs produced across all three phases.

Because males sang from different locations within the microphone array and changed locations as they sang, the distances at which the songs were recorded were variable across and within performances. The speed of sound is slow enough that these differences in travel time could distort how we perceive song timing and the amount of overlap observed. To account for variable recording distances, we calculated the song-post-to-microphone distance for each song, as well as the speed of sound (Lord 2015) appropriate for each array recording based on the average air temperature associated with the recording, the average relative humidity during the dawn chorus (65%), and standard atmospheric pressure (101.325 kPa,). For each song, we then calculated the expected travel time and used this value to adjust the selection boundaries such that they represent the start and end times of the song at the source.

To find the average air temperature for each recording, we took advantage of the Song Meter’s built-in temperature sensor that logs the temperature of the air inside the unit every 5 min while recording. Although the inside of a Song Meter is expected to be warmer than the ambient air during the day due to the effects of sunlight, before sunrise, the sensor readings should be good estimates of the air temperature outside. For each recording, we calculated the average Song Meter temperature in the one-hour period before sunrise, and used this value as an estimate of the air temperature during the dawn chorus. To choose a value for the average relative humidity, we explored data that were made available online from a personal weather station located approximately 17 km NE of our field site in Blanchetown, South Australia (Weather Underground Inc.).

After extracting the chorus phase from each performance and correcting for source-to-microphone travel times, we used the R package, SONG (Masco et al. 2016), to calculate the observed and expected amounts of overlap for each pair-wise interaction of males within each microphone array. For all analyses, song overlapping was quantified as the total duration (in seconds) for which the lagging songs of one individual (i.e. the overlapper) overlapped the leading songs of another (i.e. the overlapped individual). Each estimate of the expected amount of overlap was based on 1000 randomizations. We used the KeepSongOrder method for randomization, which preserves the observed song order and song interval durations while randomizing the order of the song intervals. Using the KeepSongOrder method ensured that the interval durations characteristic of the chorus phase and the time at which songs are the longest in duration (approximately 25 to 30 mins before sunrise, see Chapter 2) would be preserved in the randomization process.

We also used the SONG package to examine song overlapping in response to simulated intrusions during the dawn chorus and daytime. We used the KeepGaps method for randomization in these analyses to preserve the fixed intervals of the playback stimulus. Each trial consisted of three consecutive playback sessions, and we calculated the observed and expected amounts of overlap for each of these sessions separately. The behavior of the focal male across the three sessions was fairly consistent, so in our analyses, we examined whether males overlapped the speaker non-randomly during at least one of the playback sessions.

Because the locations of the individuals responding to the playback stimuli were unknown, the start and end times of the songs could not be corrected for source-to-microphone distance before analysis.

Statistical analysis

Consistent with a two-tailed test for the comparisons of observed and expected overlap, individuals were considered to be overlapping if $P \leq 0.025$ and avoiding overlap if $P \geq 0.975$. We calculated the rate of overlap in a dawn chorus interaction as the observed amount of overlap in seconds divided by the total time spent singing by the overlapped individual, which represents the proportion of time a male was overlapped while singing. To investigate what male characteristics best predicted the rate of overlap in a dawn chorus interaction, we fit a linear model ('lm' in the *stats* package, R Core Team 2015). Morphological measurements of the males were highly intercorrelated, so we first performed a principal component analysis (PCA, 'prcomp' in the *stats* package; R Core Team 2015). The first principal component (PC1) had an eigenvalue of 2.51, explained 42% of the variance in morphology across males, and was related to the cloacal tip area (loading = 0.58), the cloacal protuberance volume (loading = 0.53), and tarsus length (loading = 0.52). Because cloacal protuberance size is known to correlate with the number of stored sperm (Tuttle et al. 1996) and whether a male sires offspring during a breeding season (Tarvin et al. 2005), we included PC1 for the overlapper, the individual being overlapped, and the difference in these scores in our linear model as proxies for male quality.

We also included as predictors the relative rank (above, below, or same), age (older, younger, or same), class (neighbor or groupmate), and social relationship (father, son, unrelated) of the overlapper relative to the individual being overlapped and their interaction terms. Although there were repeated measures within individuals and within each microphone array, the sample size was too small (n=14 interactions, 10 individuals) to include random effects in the model. For each possible combination of fixed effects and interactions, we calculated the second-order Akaike's Information Criterion (AICc) ('dredge' in the R package *MuMIn*, Barton 2016). We regarded the model with the lowest AICc score as the best model, and inspected diagnostic plots of the residuals to ensure that no assumptions had been violated in fitting the best model.

RESULTS

During the natural dawn chorus interactions (corrected for source-to-microphone distances), one of the 10 males overlapped the songs of its counterpart significantly more often than was expected by chance ($P \leq 0.025$), and two additional males showed a non-significant tendency to overlap their counterparts ($P = 0.027$ and 0.050). All three of these interactions occurred across territory boundaries, between dominant individuals who were unrelated. Two males significantly avoided overlapping the songs of their counterparts ($P \geq 0.99$); in one case, a dominant male avoided overlapping his son, who was his subordinate helper, and in the other case, a dominant male avoided overlapping his father on an adjacent territory. In each of these cases, the reciprocal interactions had nonsignificant patterns of overlap ($P = 0.07$ and 0.78, respectively). In the remaining father-son interaction, the dominant male and his subordinate helper overlapped each other at chance levels ($P = 0.63$ and 0.53, respectively).

In this data-set, correcting for the source-to-microphone distances changed the expected duration of overlap by -0.8 to +0.6 sec, and the observed duration of overlap by -2.4 to +2.5 sec; in some instances, the correction altered the interpretation of the interaction. Without the correction, the interactions with nearly significant overlapping in the corrected performances had *P*-values less than 0.025, and an additional male was found to be nearly significantly overlapping his unrelated neighbor (*P* = 0.036). The interaction with significant overlapping in the corrected performances had a pattern of overlapping that only approached significance without the correction (*P* = 0.037).

In the observed pairwise interactions, the average rate of overlap (i.e. the observed amount of overlap in seconds divided by the total time spent singing by the individual being overlapped) was approximately 16% of a male's total performance time. The model with the lowest AICc score for the observed rate of overlap included the PC1 score of the individual being overlapped and relative age as predictors ($R^2 = 0.80$, $F(3,10) = 13.4$, $P < 0.001$, Figure 4.1). Males with larger cloacal tips, protuberances, and tarsi were overlapped at higher rates (PC1: $\beta = 0.011$, SE = 0.004, t = 2.84, *P* = 0.018), and compared to interacting males of the same age, males overlapped their younger counterparts at lower rates ($\beta = -0.045$, SE = 0.017, t = -2.74, *P* = 0.021). The model with the next lowest AICc score (ΔAICc = + 0.6) included only the PC1 score of the individual being overlapped as a predictor ($R^2 = 0.57$, $F(1,12) = 18.0$, $P = 0.001$).

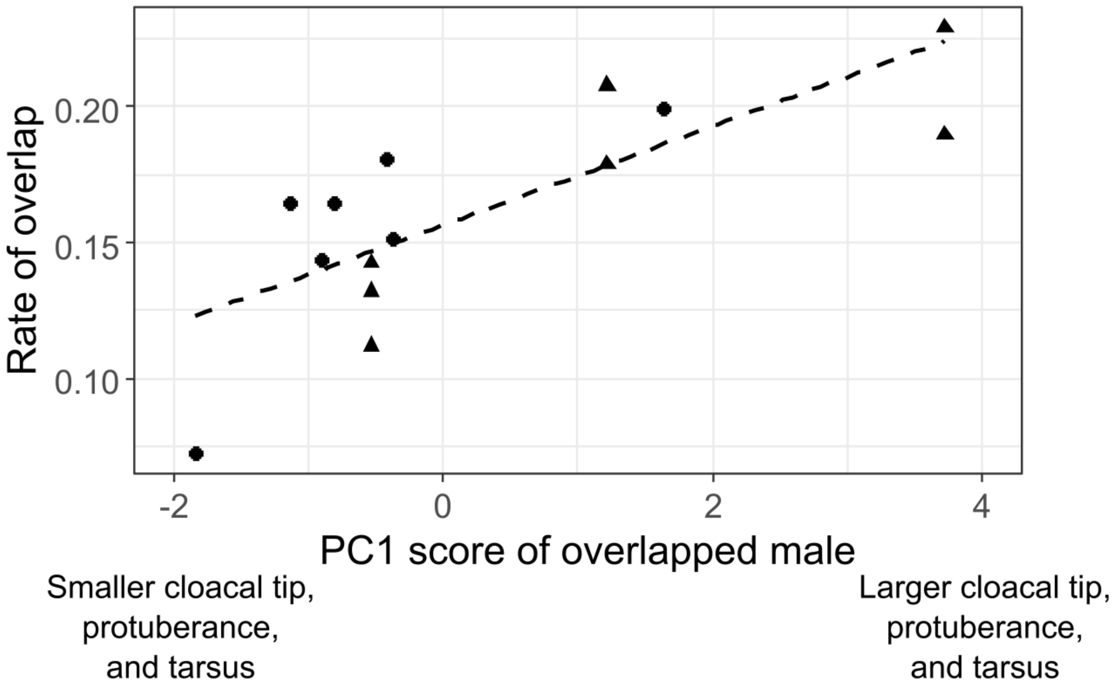

Figure 4.1. The relationship between the observed rate of song overlapping in an interaction and the morphological traits of the male being overlapped. The rate of overlap was calculated as the observed amount of overlap in seconds divided by the total time spent singing by the individual being overlapped. The first principal component of male morphology (PC1) explained 42% of the variance and was related to the cloacal tip area (loading = 0.58), the cloacal protuberance volume (loading = 0.53), and tarsus length (loading = 0.52). Each point represents one pair-wise interaction, and repeated measures for individuals are represented by triangles.

When we simulated intrusions via playback during the dawn chorus, males sang during all trials, either avoiding overlapping the playback of splendid fairy-wren song (neighbor treatment: 9 out of 9 males; stranger treatment: 5 out of 9 males), or overlapping the playback at chance levels (stranger treatment: 4 out of 9 males, Figure 4.2a). The control playback was significantly overlapped by 2 out of 9 males and avoided by 4 out of the 9 males. During the daytime, males sang during only 36 of the 42 trials, and the remaining trials were excluded from the analysis. Males generally avoided overlapping the playback of splendid fairy-wren song (neighbor treatment: 7 out of 13 males; stranger treatment: 10 out of 13 males), and no male overlapped the playback at higher than chance levels (Figure 4.2b). The control playback was significantly overlapped by 1 and avoided by 2 of the 10 males. There were no significant differences in overlapping behavior during the dawn chorus versus the daytime (McNemar's Chi-squared test, neighbor treatment: $\chi^2 = 2.25$, df = 1, $P = 0.13$; stranger treatment: $\chi^2 = 0.25$, df = 1, $P = 0.62$), and the response to neighbors and strangers did not significantly differ during the dawn chorus (McNemar's Chi-squared test, $\chi^2 = 2.25$, df = 1, $P = 0.13$) or daytime (McNemar's Chi-squared test, $\chi^2 = 0.57$, df = 1, $P = 0.45$).

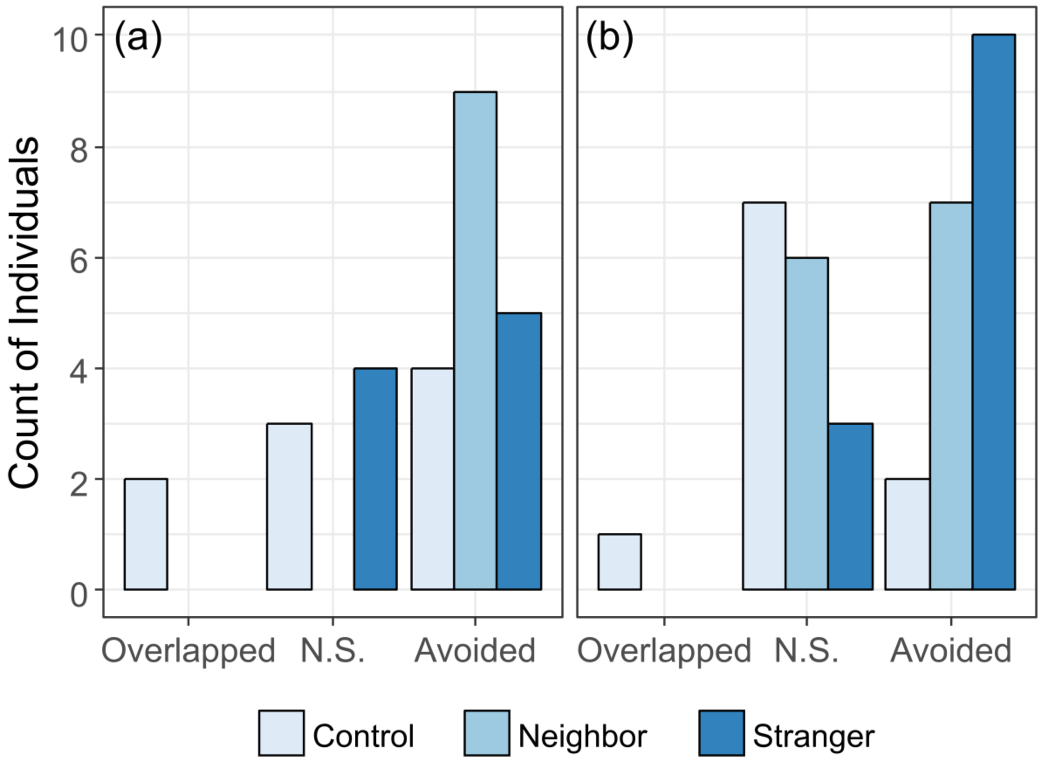

Figure 4.2. Male behavior in response to playback-simulated intrusions during the (a) dawn chorus and (b) daytime. Each trial consisted of three consecutive playback sessions, and the number of males that overlapped the playback non-randomly (i.e. “overlapped” or “avoided”) during at least one of the sessions is indicated for each stimulus type. Using a two-tailed test, individuals were considered to be overlapping the playback if $P \leq 0.025$ and avoiding overlap if $P \geq 0.975$.

DISCUSSION

Song overlapping commonly occurs during communication among songbirds, and there are a number of hypotheses for the function of this interactive signaling behavior (Helfer and Osiejuk 2015). To investigate whether song overlapping functions as a signal or a strategy during the dawn chorus interactions of splendid fairy-wrens, we used a combination of observational and experimental approaches that revealed the contexts in which overlapping occurs and the characteristics of the individuals involved.

During natural dawn chorus interactions, we found that male splendid fairy-wrens frequently overlapped their territorial rivals, and in one instance, the amount of overlap significantly exceeded the amount expected by chance. Conversely, males overlapped their groupmates and social kin at lower rates, in two instances, exhibiting overlap avoidance. The best predictor of the rate of overlap in a given interaction was the morphological characteristics of the male being overlapped; males with larger cloacal tips and protuberances were overlapped at higher rates. Cloacal protuberance size is correlated with the amount of stored sperm (Tuttle et al. 1996), and the cloacal tip is thought to facilitate sperm transfer by stimulating the female (Rowe et al 2008). Indicators of these male traits may play an important role in extra-pair mate choice, a context in which the qualities valued in a social mate (e.g. the ability to defend a high quality territory and raise offspring) would be less relevant. However, neither the morphology of the overlapper, nor the difference between the interacting males, predicted the rate of overlap. Age appears to play a role in male-male interactions during the dawn chorus; although males frequently overlapped their older and similarly-aged counterparts, males overlapped their younger counterparts at comparatively low rates. Overlapping behavior varied with context as well; during close-range aggressive interactions elicited by playback-simulated intrusions, males rarely overlapped the playback of splendid fairy-wren song. Overlap avoidance could be an important strategy during close-range aggressive interactions, allowing the individuals to perceive and respond to each other's signals without masking (e.g. Yang et al. 2014, Wilson et al. 2016).

Our use of a microphone array to quantify song overlapping enabled us to locate the source of each song during the dawn chorus and compensate for the variable travel times of the songs to the various microphones. The observed amount of overlap in the performances that were and were not corrected for source-to-microphone distance differed, and in three cases, this difference led to different conclusions about the observed amount of overlap relative to chance as calculated based on a null model.

Correcting for source-to-microphone distances effectively assumes that the observer of the overlapping interaction is either very close to or equidistant from the individuals engaged in the interaction. In a communication network, the distances between interacting individuals is likely to be unequal and can be variable if senders have multiple locations from which they signal. Consequently, when considering overlapping from the perspective of eavesdroppers or third-party receivers, it is important to remember that the amount of overlapping observed in a given interaction should be highly dependent on the relative position and movement of the observer. Likewise, the sender-to-receiver distances between interacting individuals can impact how overlapping is perceived by the individuals involved. Although we have accurate estimates for these distances in our data-set, male splendid fairy-wrens can sing from as many as a dozen song posts during a single performance, and analyzing the amount of overlap in each possible sender-receiver configuration is methodologically challenging. Computer simulations of individuals interacting at various distances may be the best option for investigating the effects of sender-receiver distance on the perception of song overlapping.

Our findings provide mixed support for the hypothesis that song overlapping functions as an aggressive signal in splendid fairy-wrens. As expected for a signal of aggressive intent, song overlapping, when observed at levels that exceeded chance, occurred in interactions between territorial rivals and did not occur in interactions between social group mates. During simulated intrusions, however, males approached the speaker but overlapped the playback of splendid fairy-wren song at low rates and in many instances, significantly avoided overlapping. The results of the playback experiment suggest that song overlapping does not function as a signal in close-range aggressive interactions, and that overlap avoidance might be an important strategy in this context. It remains possible that song overlapping is a low-level, long-distance signal of aggressive intent, a warding-off signal that gives way to more aggressive behaviors such as scolding and bill-wiping once territory boundaries are crossed. In this way, song overlapping would communicate that the sender is paying attention to a specific receiver, functioning as a pointing index signal as well (Bradbury and Vehrencamp 2011). For species with limited song repertoires, such as splendid fairy-wrens, directing songs at particular receivers through overlapping could be a simple, efficient mechanism for engaging the intended receiver (Helfer and Osiejuk 2015). Under this hypothesis, it is possible that higher quality individuals would be overlapped at higher rates; if males with larger cloacal tips and protuberances pose a greater threat, they may more often be the targets of attention and aggressive intent from a distance.

Song overlapping does not appear to function as a signal of quality in this species. There was no indication that song overlapping is an indicator of dominance status; both dominant and subordinate individuals overlapped their counterparts at high rates during the dawn chorus. The rate of overlap in a given interaction was best predicted by the morphological features of the male being overlapped and, contrary to the predictions of this hypothesis, independent of the features of the male performing the overlapping behavior.

Song overlapping during the dawn chorus does not seem to arise through a cooperative mechanism in splendid fairy-wrens; significant (and nearly significant) amounts of overlapping were only observed during interactions between territorial rivals, and overlap avoidance was observed in two out of the three father-son pairs included in this study. As a result, it is unlikely that song overlapping functions as a signal of group size or cohesion. Although song overlapping likely has an impact on the overall active space of the dawn chorus, it does not seem to function as a signal enhancement strategy in this species. Overlapping exceeded chance levels only once in our data-set, but the rate of overlap was generally high during the dawn chorus; in pairwise interactions, approximately 16% of a male’s total performance time was overlapped on average. As part of a communication network, males can have as many as six counterparts within range (pers. obs.), so the combined rates of overlap across the network of singing males could be much higher and result in the amplification of the overall chorus. The occurrence of overlap avoidance within father-son pairs, however, does not fit with the predictions of the signal enhancement hypothesis. Unlike chorusing frogs and insects that might require a “beacon effect” (Buck and Buck 1978) to attract females from far distances or to transient resources, female splendid fairy-wrens live in the midst of potential mates. Strategies that increase the effectiveness of the dawn chorus as a whole may not be required in splendid fairy-wrens, and any per capita benefits gained might not offset the costs of masking one’s own song while engaging in overlapping.

As a modifier, song overlapping could provide the opportunity for direct comparisons of song features such as minimum frequency, bandwidth and trill rate, but we found no support for this hypothesis in splendid fairy-wrens. Under this hypothesis, lower quality males should avoid overlapping to minimize the opportunity for direct comparisons with higher quality individuals. As song characteristics can vary with age (e.g. Gil et al. 2001), we would expect males of similar ages, and similar performance abilities, to engage in the highest rates of song overlapping. In contrast, our analyses revealed that young males overlapped older males and males of the same age at similar rates. Additionally, the males who were overlapped at the highest rates were those with the largest cloacal tips and protuberances. If there is a relationship between these morphological characteristics and song performance, this finding does not support the hypothesis that song overlapping functions as a modifier. The clearest test of this hypothesis would be to compare the acoustic features of leading, lagging, and solo songs to determine whether they differ in ways that are made more obvious by overlapping. Acoustic measurements are challenging to make based on microphone array recordings in which songs are distorted by both overlap and distance. Future work regarding this hypothesis should combine simultaneous close-range focal recordings with microphone arrays to obtain accurate measurements of song features in the context of song overlapping.

Overall, our findings best support the hypothesis that song overlapping functions as a masking strategy in splendid fairy-wrens. In line with this hypothesis, overlapping occurred between rivals, avoidance occurred between related individuals, and the males with the largest cloacal protuberances and tips were overlapped at the highest rates. As discussed previously, the cloacal protuberance and tip may play an especially important role in extra-pair mating, as these morphological traits are related to the success of fertilization. A male with a large cloacal tip and protuberance might be the most attractive candidate for females seeking extra-pair copulations during the dawn chorus, in which case, he would pose the greatest threat to the extra-pair mating success and within-pair paternity of his neighbors. Masking the songs of well-endowed males could disrupt female choice and result in a higher chance of siring offspring for the overlapper.

Considering the song structure of male splendid fairy-wrens, masking might represent a “spending a little to gain a lot” strategy (Helfer and Osiejuk 2015). During the dawn chorus, males often combine the two song types in their repertoires to produce a Type 1 “chatter” song with a Type 2 trill suffix. The trill portion of the song has the potential to increase the detectability, localizability, and information content of a male’s dawn chorus performance (see Chapter 2). Masking the trill of a rival through song overlapping could negate some of these benefits, while leaving the trill of the overlapper relatively unobscured. If the trill functions as a location tag that communicates both direction and distance for the information contained in the Type 1 chatter song (as discussed in Chapter 2), a properly timed song could mask a rival’s trill and effectively replace it with one’s own, linking a high quality song with the overlapper’s location. One caveat is that the two song types occupy distinct frequency ranges; when a Type 2 trill is overlapped by the beginnings of a Type 1 song, there is little to no frequency overlap. In these instances, overlapping may not mask the leading song per se, but could distract the attention of the receivers such that the perception of the overlapped song is hindered. To be sure that song overlapping function as a masking strategy in this species, future work should examine how males adjust their singing and movement behavior in response to being overlapped, and whether females exhibit a preference for unobscured song. An investigation of the reproductive consequences of song overlapping could shed additional light on the function of this behavior as well as the broader role that the dawn chorus plays in extra-pair mate attraction in splendid fairy-wrens.

CHAPTER 5: SUMMARY AND CONCLUSIONS

In this dissertation, I described several aspects of the singing behavior of splendid fairy-wrens, and explored the extent to which singing behaviors can function as signals and signaling strategies in songbirds. For splendid fairy-wrens, a cooperatively breeding species with high rates of extra-pair paternity, the use of trills (Chapter 2) and song overlapping (Chapter 4) appear to function as signaling strategies during the dawn chorus.

As discussed in Chapter 2, splendid fairy-wrens have two song types with distinct evolutionary histories: the Type 1 “chatter” song and the Type 2 trill. Although ancestral state reconstructions suggest the trill originated in a predator context (Greig and Webster 2014), males have incorporated this vocalization into their dawn chorus performances, appending trill suffixes to, on average, nearly 80% of their Type 1 songs. The addition of the trill suffix was associated with costs to Type 1 song duration and performance quality, and, because these costs vary among males, it is possible that this singing behavior reveals additional information about male quality or condition. As a consequence of its acoustic structure, the addition of the trill suffix may also enhance a male’s dawn chorus performance by increasing its broadcast area, detectability, localizability, and resistance to distortion. Overall, these findings suggest that, for male splendid fairy-wrens, the addition of the trill suffix can function as a signaling strategy that increases the effectiveness of their songs and dawn chorus performances.

Song overlapping can also impact the effectiveness of a dawn chorus performance, and in Chapter 4, I discussed the prevalence and potential function of this behavior in splendid fairy-wrens. Several pieces of evidence suggest that song overlapping functions as a masking strategy in this species. Overlap avoidance was observed in interactions between related individuals (i.e. fathers and sons), and significant overlapping was observed only in interactions between unrelated territorial rivals. Additionally, the males with the largest cloacal protuberances and cloacal tips (presumably the highest-quality males) were overlapped at the highest rates. Song overlapping may be a particularly effective strategy for splendid fairy-wrens as a consequence of their song type use. As discussed in Chapter 2, the trill that a male attaches to the end of his song could play an important role in ensuring that information about the male's quality or location reaches females seeking extra-pair copulations during the dawn chorus. By overlapping the latter portion of a rival's song, a male may be able to disrupt a female's ability to identify or locate her preferred extra-pair mate, while clearly broadcasting his own quality or location. Trill use and song overlapping may therefore represent a pair of signaling strategies that together can increase the effectiveness of a male's performance, while decreasing the effectiveness of rivals' performances during the dawn chorus.

Because song overlapping can arise as a result of chance, the signal value of song overlapping has recently been called into question (Searcy and Beecher 2009, Searcy and Beecher 2011). Establishing whether overlapping is a non-random occurrence can be difficult without a compelling null model for predicting the amount of chance overlap in an interaction (Naguib and Mennill 2010). The findings discussed in Chapters 3 and 4 illustrate the usefulness of the SONG package for uncovering patterns in signal timing and calculating chance levels of song overlap. Recent work has confirmed the robustness of resampling randomization methods for detecting non-random signal timing (specifically the "KeepGaps" method in the SONG package, Araya-Salas et al. 2017), and the use of the SONG package and similar methods is becoming a more widespread practice in the investigation of signal timing (e.g. Araya-Salas et al. 2017, Finton et al. 2017).

Although there is evidence that trill use and song overlapping represent important signaling strategies during the dawn chorus of splendid fairy-wrens, the part of the picture that remains unclear is the role that female preference has played in the evolution of these behaviors and the specific impact that singing behavior has on male reproductive success. These relationships can be hard to discern; playback-simulated intrusions by rivals elicit mate guarding behavior from male splendid fairy-wrens (Johnson et al. in review), which can make it difficult or impossible to assess, without interference, the responses of females to controlled playbacks of male singing behavior in the field. Regarding a male's reproductive success, the promiscuous behavior of splendid fairy-wrens makes it necessary to look both within the social pair and across the neighborhood to gain a complete picture (Webster et al. 2004). Unfortunately, mating success in each of these contexts is difficult to quantify; due to the very high rates of nest depredation (pers. obs.), the realized reproductive success of each male is an imperfect indicator of female choice. Although the data are limited in this regard, in future work, I aim to incorporate analyses of extra-pair paternity to more fully explore the relationship between singing behavior and reproductive success in splendid fairy-wrens, with the ultimate goal of gaining a better understanding of the function of the dawn chorus in this species.

The research presented in this dissertation contributes to our understanding of the signal value, and strategic value, of singing behavior. Examining the costs, benefits, and dynamics of singing behavior in splendid fairy-wrens has contributed to the body of evidence that song overlapping is a functional behavior in songbirds, and has provided additional insight into the strategies that animals use to encode and exchange information. The computational methods developed for this research (i.e. the analytical model of attenuation and the SONG package) have applications that extend well beyond the singing behavior of songbirds, and can facilitate future work on sound propagation and the temporal coordination of behavior.

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