Timing isn't everything: responses of tropical wrens to coordinated duets, uncoordinated duets and alternating solos

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The coordinated vocal duets of tropical animals often play a role in territorial signalling. Duet timing is dependent upon each animal's attentiveness to its partner and its ability or motivation to cooperate, and therefore precise coordination of duet elements could be an important signal to territorial rivals. We used a playback experiment to test the hypothesis that territorial animals distinguish between highly coordinated duets, poorly coordinated duets and alternating solos. We studied three related species of wrens that show natural variation in the coordination of their male and female songs: banded wrens, *Thryophilus pleurostictus*, occasionally perform loosely coordinated duets but more commonly sing solo songs; rufous-and-white wrens, *Thryophilus rufalbus*, regularly perform loosely coordinated duets; and plain wrens, *Cantorchilus modestus*, regularly perform highly coordinated duets. We used stereo duet playback to simulate highly coordinated duets, poorly coordinated duets, alternating solos and a heterospecific control. We played back these four treatments to 63 territorial pairs of wrens. Responses to experimental treatments were stronger than responses to control treatments. Response to the three experimental treatments varied among species. Banded wrens responded more strongly to both types of duets than to alternating solos, in terms of closest approach and number of passes over the speaker. Rufous-and-white wrens responded more intensely to alternating solos and coordinated duets than to uncoordinated duets, in terms of closest approach, number of passes over the speaker and number of songs initiated. Plain wrens responded most intensely to alternating solos in terms of closest approach, and no less intensely to alternating solos than to the other treatments in terms of other measures of response. Together, our results indicate that these three species of wrens distinguish between coordinated duets, uncoordinated duets and alternating solos. However, highly coordinated duets do not incite categorically stronger responses than uncoordinated duets or alternating solos in any of these species.

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Duets are the coordinated songs of two individuals, usually a mated pair, where the two animals' vocalizations alternate or overlap (Farabaugh, 1982; Hall, 2004, 2009). This phenomenon occurs in a variety of animals, including birds (Hall, 2009), primates (Haimoff, 1986), anurans (Tobias, Viswanathan, & Kelley, 1998) and insects (Bailey, 2003). Duetting behaviour shows a strong phylogenetic signal (Logue & Hall, 2014) and has evolved independently in multiple phylogenetic groups (Farabaugh, 1982). In spite of a growing body of research, the ecology and evolution of duetting behaviour remains poorly understood (Hall, 2009), possibly due to the multifunctional nature of duets both within and among species

(Benedict, 2010; Dahlin & Benedict, 2013; Grafe, Bitz, & Wink, 2004; Mennill & Vehrencamp, 2008). Our understanding of the functions of duets can be enhanced by careful attention to the dynamics of coordination in male and female duet contributions.

Hypotheses for the functions of duets can largely be grouped into two categories: those in which pair members cooperate with one another for mutual benefit, and those in which pair members compete to work towards conflicting goals (Hall, 2004). Cooperative functions include joint resource defence, maintaining the pair bond and ensuring reproductive synchrony (Armstrong, 1947), while competitive functions include mate guarding and paternity guarding (Levin, 1996; Sonnenschein & Reyer, 1983). Resource defence is a well-supported function of male song in north temperate songbirds (Catchpole & Slater, 2008), where songs are predominantly performed by males alone. Duets mirror male territorial song in that they are loud, easily localizable and used in interactions between neighbouring birds, and for this reason,

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resource defence is often considered a primary function of duets (Hall, 2009). Experimental research examining duetting as a mechanism for resource defence suggests that duets do function in this manner in many taxa (reviewed in: Dahlin & Benedict, 2013; Douglas & Mennill, 2010). However, previous studies have typically contrasted duet singing, male solo singing and female solo singing; this experimental design fails to control for the confounding difference in the number of intruders that are simulated. Differences in response to playback of duets and solos could be due to differences in the threat posed by a single intruder versus two intruders, rather than the coordination of songs into duets per se.

Temporal coordination is a defining feature of duetting behaviour, and as such, improving our understanding of the importance of coordination in animal duets is likely to shed light on their function and evolution. Duetting species can be grouped into two classes based on the form of their duets: those that answer their partner's song without fine-scale coordination of notes within the songs (e.g. polyphonal duetters) and those that coordinate the phrases within their synchronized songs (e.g. antiphonal duetters; Hall, 2009). Mechanistic studies of temporal coordination in antiphonal duetters indicate that duetting partners base their timing on the immediately preceding notes of their mate's song (Fortune, Rodriguez, Li, Ball, & Coleman, 2012; Logue, Chalmers, & Gowland, 2008). Coordinating songs requires attentiveness to the partner, and animals with higher levels of attentiveness may produce duets comprising a series of more precisely coordinated phrases. Therefore, duet timing could serve as a signal of attentiveness, which may be important both to the partner and to nearby territorial rivals (Hall, 2009). Male magpie-larks, *Grallina cyanoleuca*, for example, sing at a higher rate in response to playback of highly coordinated duets than to playback of poorly coordinated duets (Hall & Magrath, 2007), suggesting that precise timing can function as a more threatening territorial signal. In addition, magpie-lark pairs that have been together for longer periods sing more precisely coordinated duets, suggesting that duet coordination may indicate partnership length (Hall & Magrath, 2007). These results suggest that duet precision may be an index of a pair's ability or motivation to act collectively, where pairs that produce highly coordinated duets may cooperate to perform other behaviours successfully as well. Precision in temporal coordination seems to be important; black-bellied wrens, *Pheugopedius fasciatoventris*, will stop their songs short when performing duets that have large gaps or overlapping phrases, indicating that birds are sensitive to their own duet coordination (Logue et al., 2008). If other pairs use the duet coordination of rivals as a signal, they may be able to gain helpful information about the threat posed by a rival pair.

Vocal duets are relatively common among many birds in the tropics (Farabaugh, 1982). Tropical wrens, in particular, are well known for their ability to produce coordinated duets (Brewer, 2001; Mann, Dingess, Barker, Graves, & Slater, 2009). The 'Thryothorus wrens' are a group of tropical birds in which duetting is common, with highly variable degrees of temporal coordination (Mann et al., 2009). Formerly a single genus (i.e. *Thryothorus*), the group was recently split into four genera, and there is considerable variation in duetting behaviour and duet coordination between and within each of these new groups (Mann, Barker, Graves, Dingess-Mann, & Slater, 2006; Mann et al., 2009). Plain wrens, *Cantorchilus modestus*, sing highly coordinated antiphonal duets, where the male and female sing a series of rapidly alternating phrases with extremely small intervals of silence and very little overlap (Cuthbert & Mennill, 2007). Rufous-and-white wrens, *Thryophilus rufalbus*, show coordinated, polyphonal duets, in which the male and female songs are loosely associated in time and often involve overlapping (Mennill & Vehrencamp, 2005). Although male and female banded wrens, *Thryophilus pleurostictus*, both sing, they

historically have not been recognized as singing coordinated duets (Mann et al., 2009; Molles & Vehrencamp, 1999). However, recent detailed analysis demonstrates that males and females occasionally produce duets similar to the loosely coordinated duets of rufous-and-white wrens (Hall, Driscoll, & Vehrencamp, n.d.). This remarkable variation in acoustic coordination provides an intriguing model system in which to study the ecology and evolution of temporal coordination.

In this study, we tested the hypothesis that territorial animals respond differently to coordinated duets, poorly coordinated duets and alternating male and female solo songs. We presented temporally altered duets, as well as alternating male and female solo songs, to pairs of three species of tropical wrens that vary in their natural degree of coordination. We included three species of wrens in hopes of elucidating evolutionary mechanisms of coordinated displays. If birds distinguish between coordinated duets, uncoordinated duets and alternating solos, we predicted two possible outcomes: (1) wrens of all species would respond more intensely to highly coordinated duets than to poorly coordinated duets and alternating solos; or, alternatively, (2) wrens would respond more intensely to their species-typical form of song production (high-coordination duets for plain wrens, low-coordination duets for rufous-and-white wrens and banded wrens). Because these three species of wrens perform different types of duets, if all three species show strong responses to coordinated stimuli, this could provide evidence of a pre-existing bias towards perceiving highly coordinated duets as threatening signals.

METHODS

General Field Methods

We studied populations of three species of wrens in Sector Santa Rosa of the Guanacaste Conservation Area in northwestern Costa Rica (10°40′N, 85°30′W). Playback studies were conducted from 24 April to 2 June 2012, corresponding with the onset of the rainy season and the beginning of the breeding season (Topp & Mennill, 2008). We conducted playbacks in the morning, between 0600 and 1100 hours, when birds were most active. In total, we successfully completed trials with 63 pairs of wrens (126 unique birds): 21 pairs of plain wrens (42 unique birds), 22 pairs of rufous-and-white wrens (44 unique birds) and 20 pairs of banded wrens (40 unique birds), all of which were used in our analyses. The rufous-and-white wrens were part of an ongoing long-term study, and all males and most females were banded with unique combinations of coloured leg bands. Banded and plain wrens were not individually marked in this study, so we ensured that we sampled unique pairs by moving at least 60 m between adjacent same-species playback territories for plain wrens and banded wrens. For banded and plain wrens, we distinguished between sexes by careful attention to their songs, which are sexually distinct. Wren pairs tend to remain on stable territories throughout a season (plain wrens, approximately 400 m²; rufous-and-white wrens, approximately 10 000 m²; banded wrens approximately 3600 m²; Cuthbert & Mennill, 2007; Mennill & Vehrencamp, 2005; Molles & Vehrencamp, 2001).

Playback Treatments

We used a stereo playback design (Douglas & Mennill, 2010) to simulate a pair of wrens singing inside the territory boundaries of our playback subjects. Subjects received four playback treatments: (1) highly coordinated conspecific duets, (2) poorly coordinated conspecific duets, (3) alternating male-and-female conspecific solo songs and (4) a control stimulus of highly coordinated duets of a

completely unfamiliar species (eastern whipbirds, *Psophodes olivaceous*, from Australia). Each subject pair received all four treatments during a 1 h playback session, which allowed us to rule out variation in responses that might arise by returning to the territory on different days (e.g. variation due to changes in breeding status, etc.). The order of the four playback treatments across sessions followed a factorial design to minimize the bias created by order effects on treatment differences, such as carryover effects and priming.

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

Playback Stimuli

We created stereo playback stimuli from recordings of birds collected within our study site over the last 10 years. By selecting historical recordings and recordings collected from sites far away from the subjects' territories, we ensured that all playback stimuli were unfamiliar to the subjects. Rufous-and-white wren stimuli were selected from an archive of recordings of 20 different pairs of colour-banded birds; we chose birds that had been dead for more than 2 years at time of playback or whose territories were at least 2 km from the site of playback. Banded wren stimuli were selected from a colour-banded population, using recordings of birds that either had died or were found at least 2 km from the site of playback. Stimuli for plain wrens were made from recordings collected in April 2012, at locations at least 2 km away from the site of playback. For banded and plain wrens, we created stimuli using recordings of 10 different pairs; each stimulus set was used during playback to two different pairs of subjects. Prior field observations verified that all three species remain in their territories over long periods, confirming that birds recorded far away from the site of playback would be unfamiliar to the subjects (Cuthbert & Mennill, 2007; Mennill & Vehrencamp, 2005; Molles & Vehrencamp, 2001). Eastern whipbird recordings (control stimuli) were collected in Atherton, Australia as part of another study (Mennill & Rogers, 2006); four whipbird duet stimuli were used in alternation.

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

All conspecific treatments featured the same number of songs broadcast at the same amplitude. Each stimulus was 3 min long, where duets were presented at a rate of one duet every 15 s, and alternating solos were given at a rate of one solo song every 7.5 s, thereby ensuring the same song output across all trials. All three treatments for each pair were made from the same source recording. The sole difference between the three conspecific treatments (high-coordination duets, low-coordination duets and alternating solos) was the relative timing of male and female contributions. All duet stimuli were female-created duets, where the simulated male began singing and the simulated female joined the male song to create the duet. Previous research shows that most plain wren duets (Cuthbert & Mennill, 2007) and the majority (75%) of rufous-and-white wren duets are female-created duets (Mennill & Vehrencamp, 2005) and that female banded wrens are more likely to overlap or immediately follow their mate's songs than vice versa (Hall et al., n.d.). We modelled duet stimuli for banded wrens on what is known for rufous-and-white wrens, as the two species are closely related (Mann et al., 2006) and their songs are somewhat similar in the length and arrangement of syllables, although banded wren songs are much higher in frequency and more frequency-modulated than rufous-and-white wren songs.

We defined a duet as alternating or overlapping male and female vocalizations where the male and female contributions were within 1 s of each other (Mennill & Vehrencamp, 2005). To synthesize coordinated duets, we created stimuli with a standard deviation in reaction time of 0 (i.e. no variation in delay between male and female components) and a mean reaction time that was typical based on published delays in male-to-female reaction times (Cuthbert & Mennill, 2007; Mennill & Vehrencamp, 2005). Reaction times for banded wrens were based on the reaction times of their sister species, rufous-and-white wrens (Table 1). For plain wrens, the antiphonal duetting species, we varied the reaction time between the onset of male and female duet phrases, whereas for the other two species we varied reaction time between the onset of male and female song. To synthesize uncoordinated duets, we

Table 1
Details of the degree of coordination in male and female playback stimuli for three species of tropical wrens, based on published delays in male and female duet contributions

Species	Delay type	Published delay[a]	Coordinated stimuli	Uncoordinated stimuli
		Mean±SD (s)	Mean±SD (s)	Mean±SD (s)
Rufous-and-white and banded wren	Male-to-Female delay[b]	2.156±0.911	2.156±0	2.156±1.367
Plain wren	I-to-F phrase delay[c]	0.126±0.016	0.126±0	0.126±0.024
	F-to-M phrase delay	0.055±0.007	0.055±0	0.055±0.011
	M-to-F phrase delay	0.181±0.015	0.181±0	0.181±0.023

[a] Data for both rufous-and-white and banded wrens from Mennill and Vehrencamp (2005); data for plain wrens from Cuthbert and Mennill (2007).
[b] All rufous-and-white and banded wren playback duets include just one male and one female song.
[c] Plain wren duets include male introductory (I) phrases, female (F) phrases and male (M) phrases (Cuthbert & Mennill, 2007).

created stimuli with a standard deviation in reaction time that was 1.5 times the published standard deviation in reaction time (Table 1). We created an Excel spreadsheet that used a random number generator, using the RandBetween command, to produce a list of variable reaction times that achieved the desired standard deviation between male and female components. To synthesize alternating solos, we created stimuli where male and female songs were presented in perfect alternation, with a silent interval of equal length between subsequent songs from the two channels (approximately 6 s of silence, to achieve a delay of 7.5 s between the onset of the songs in the two channels).

Playback Apparatus

The stereo playback apparatus consisted of two loudspeakers (Sony SRS A27) mounted 1 m apart and 1 m off the ground on metal poles. Both the speakers and poles were painted in camouflage colours. The speakers were mounted facing upward to avoid any effects of speaker directionality. A flag was hung 1 m from each speaker off to the side, so that the observer could estimate the distance between the birds and the speakers. The two speakers were connected by a 15 m cable to a digital playback device (Apple iPod classic) operated by the observer. The observer sat in a position 15 m from the loudspeakers, equidistant from the loudspeakers.

We set up the apparatus near the centre of the subjects' territories to minimize the possibility of inciting responses from neighbours (the territories of banded wrens and especially plain wrens are quite small, and the study populations are quite dense). We avoided areas within 25 m of the subjects' nest for rufous-and-white and banded wrens, and 10 m for plain wrens (due to smaller territory sizes). Once the apparatus was in place, we measured the output of each speaker using a sound level meter (Radio Shack 33-4050), broadcasting frequency-modulated synthetic tones that were normalized to the same level as playback stimuli. Since rufous-and-white wren songs are quieter than both banded wren and plain wren songs, the volume of the loudspeakers was adjusted to a level of 80 dB relative to background noise at 1 m distance from the speaker for rufous-and-white wrens (as in Mennill & Vehrencamp, 2005) and 90 dB for banded wrens and plain wrens (as in Illes, Hall, & Vehrencamp, 2006, for banded wrens). For all three species, these amplitudes produced sounds that approximated the natural level of song from live birds, based on our experience in the field. Eastern whipbird duets were broadcast at the same volume as the stimuli for each trial (i.e. 80 dB for playback to rufous-and-white wrens; 90 dB for playback to the other two species).

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

Data Analysis

We visualized recordings of the playback trials using Syrinx-PC sound analysis software (J. Burt, Seattle, WA, U.S.A.). We identified songs and duets of the focal pair and scored the behaviours and distances dictated by the observer, using the time and frequency cursors in Syrinx-PC to highlight each vocalization and activity. This approach created a time-stamped annotation file of each focal pair's songs and activities during playback. From these files, we extracted two approach response variables and two vocal response variables: (1) distance of closest approach to the loudspeakers; (2) the number of times the bird passed under or over the loudspeakers; (3) the number of songs initiated (i.e. the number of solos plus the number of duets where the bird sang the first part of the duet); and (4) number of duets created (i.e. duets where the bird's partner sang first and the bird joined to create the duet).

Statistical Analyses

We analysed our data using generalized linear mixed models (GLMMs) to accommodate non-normal data and include a random effect of pair (Bolker et al., 2008). We included four main factors in our models: (1) playback treatment (three levels: coordinated duets, uncoordinated duets and alternating solos; a fourth level, the heterospecific control treatment, is included in models presented in the online Supplementary material); (2) playback order (four levels: first, second, third or fourth); (3) subject species (three levels: banded wren, plain wren and rufous-and-white wren); and (4) sex of the responding animal (two levels: male or female; we included sex as a factor because previous investigations have revealed that males and females respond differently to playback in our study species; Hall et al., 2006; Marshall-Ball, Mann, & Slater, 2006; Mennill, 2006; Mennill & Vehrencamp, 2008). For the target distribution in SPSS we used gamma regression for distance of closest approach and log linear for the remaining three variables. We included all first-order interaction terms in our analyses, and we incorporated pair identity as a random effect to account for the fact that the same pair was sampled repeatedly across the treatments. Post hoc pairwise comparisons were obtained through the GLMM interface, which increases P values to correct for multiple comparisons (rather than decreasing the alpha value) using the sequential Bonferroni correction; we report corrected P values for the post hoc pairwise comparisons. We conducted post hoc comparisons for all the main effects and first-order interactions in each GLMM, paying particular attention to the post hoc comparisons of the species $*$ treatment interactions (post hoc analyses for the interaction terms were pairwise comparisons of the treatments for each species). All GLMM analyses were run in SPSS 21 (IBM, Chicago, IL, U.S.A.).

RESULTS

Wrens showed strong responses to playback. For all four response variables, wrens showed significantly higher responses to conspecific stimuli than to control stimuli (see Supplementary material). Both pair members approached the loudspeakers and sang in 79.4% of conspecific playback trials, whereas only the male responded in the remaining 20.6%.

Closest Approach

The effect of experimental treatments on distance of closest approach differed among the three species of wrens (Fig. 1; treatment $*$ species interaction, Table 2): banded wrens approached coordinated and uncoordinated duets more closely than alternating solos (post hoc pairwise comparisons: $P \leq 0.05$) but they approached the two duet treatments similarly ($P = 0.80$); rufous-and-white wrens approached all three treatments similarly ($P \geq 0.45$); plain wrens approached alternating solos more closely than uncoordinated duets ($P = 0.01$), and they approached coordinated duets at a level intermediate between the other two treatments ($P \geq 0.08$). Across the three species combined, responses to the three treatments were similar (nonsignificant main effects of treatment and species, Table 2).
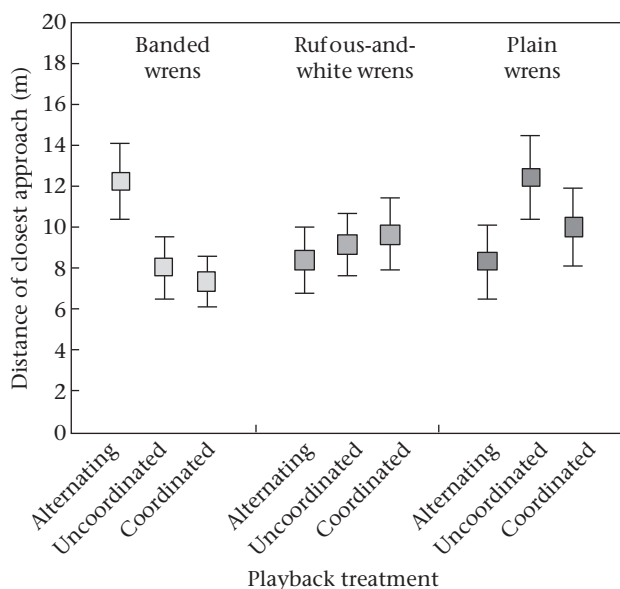
Figure 1. Average distance of closest approach to loudspeakers broadcasting simulated songs of a rival pair of conspecific animals, showing differences between three experimental treatments (alternating male and female solos, uncoordinated duets and coordinated duets) for three species of Neotropical wrens: banded wrens (light grey squares, at left), rufous-and-white wrens (medium grey squares, at centre) and plain wrens (dark grey squares, at right). Error bars show standard error around the mean.
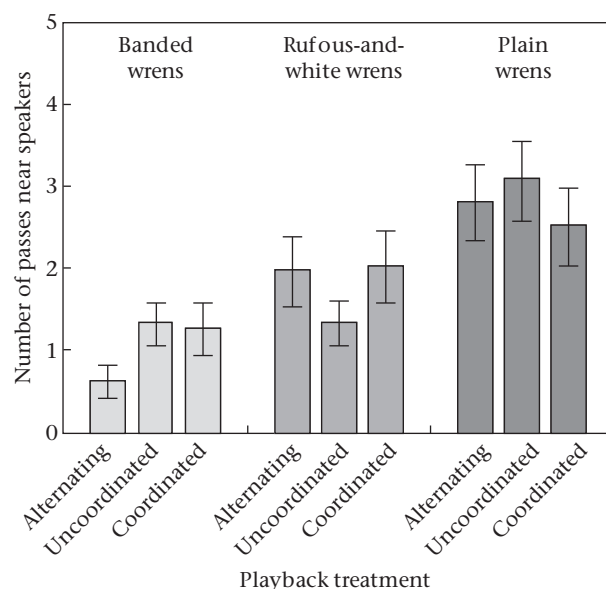

Figure 2. Average number of passes over a pair of loudspeakers broadcasting simulated songs of a rival pair of conspecific animals, showing differences between three experimental treatments (alternating solos, uncoordinated duets and coordinated duets) for three species of Neotropical wrens: banded wrens (light grey bars, at left), rufous-and-white wrens (medium grey bars, at centre) and plain wrens (dark grey bars, at right). Error bars show standard error around the mean.

Males approached loudspeakers more closely than did females in all treatments and in all species (Table 2, Supplementary material).

Closest approach varied with playback order (Table 2); birds approached the second stimulus more closely than the third stimulus (post hoc pairwise comparisons: $P = 0.002$) or the fourth stimulus ($P = 0.02$). Closest approach showed a significant species∗order interaction (Table 2); banded wrens approached the first two treatments more closely than the last two (post hoc pairwise comparisons: $P \leq 0.02$), rufous-and-white wrens approached the second treatment more closely than the other three treatment positions ($P \leq 0.02$), while plain wrens approached the fourth treatment more closely than the first treatment ($P = 0.02$).

Number of Passes

The number of times wrens passed near the speaker varied across the three experimental treatments for the three species (Fig. 2; treatment∗species interaction, Table 2): banded wrens made more passes in response to both duet treatments versus alternating solos (pairwise post hoc comparisons: $P \leq 0.005$) but made a similar number of passes during the two duet treatments ($P = 0.68$); rufous-and-white wrens made more passes in response to coordinated versus uncoordinated duet treatments ($P = 0.02$), but there was no statistically significant difference between alternating solos and both duet treatments ($P \geq 0.06$); and plain wrens showed no statistically significant differences across the three treatments ($P \geq 0.27$). Across the three species, birds made more passes in response to coordinated and uncoordinated duets versus alternating solos (post hoc pairwise comparisons: $P < 0.04$), but they made a similar number of passes in response to coordinated versus uncoordinated duets ($P = 0.49$; main effect of treatment, Table 2); this pattern appeared to be driven by banded wrens (Fig. 2). Across the three treatments, plain wrens made more passes than banded wrens (post hoc pairwise comparison: $P = 0.005$), and rufous-and-white wrens made an intermediate number of passes ($P \geq 0.08$; main effect of species, Table 2).

Table 2
Factors and interaction terms from the generalized linear mixed model used to analyse four aspects of the behavioural responses of three species of Neotropical wrens to playback simulating alternating solos, uncoordinated duets and coordinated duets

	Distance of closest approach			Number of passes over the speaker			Number of songs initiated			Number of duets created		
	F	df	P	F	df	P	F	df	P	F	df	P
Treatment	1.51	2, 346	0.223	4.16	2, 346	0.016	6.12	2, 346	<0.001	6.13	2, 346	0.002
Species	0.32	2, 346	0.729	5.76	2, 346	0.003	25.92	2, 346	0.002	4.31	2, 346	0.014
Sex	103.52	1, 346	<0.001	95.75	1, 346	<0.001	2029.61	1, 346	<0.001	98.39	1, 346	<0.001
Order	4.15	3, 346	0.007	1.36	3, 346	0.254	6.75	3, 346	<0.001	7.11	3, 346	<0.001
Treatment∗Species	3.55	4, 346	0.007	6.17	4, 346	<0.001	9.35	4, 346	<0.001	5.93	4, 346	<0.001
Treatment∗Sex	0.07	2, 346	0.936	2.08	2, 346	0.127	2.86	2, 346	0.059	3.51	2, 346	0.031
Treatment∗Order	0.64	6, 346	0.699	1.66	6, 346	0.129	6.47	6, 346	<0.001	2.29	6, 346	<0.001
Species∗Sex	0.2	2, 346	0.827	5.23	2, 346	0.006	40.59	2, 346	<0.001	37.04	2, 346	<0.001
Species∗Order	5.62	6, 346	<0.001	3.73	6, 346	0.001	7.14	6, 346	<0.001	5.58	6, 346	<0.001
Order∗Sex	0.15	3, 346	0.930	0.72	3, 346	0.540	5.10	6, 346	0.002	2.56	3, 346	0.055

Only the three experimental treatments are included here (see online supplement for analyses including the control treatment that show a significant response to conspecific versus heterospecific playback). P values less than 0.05 were considered significant.
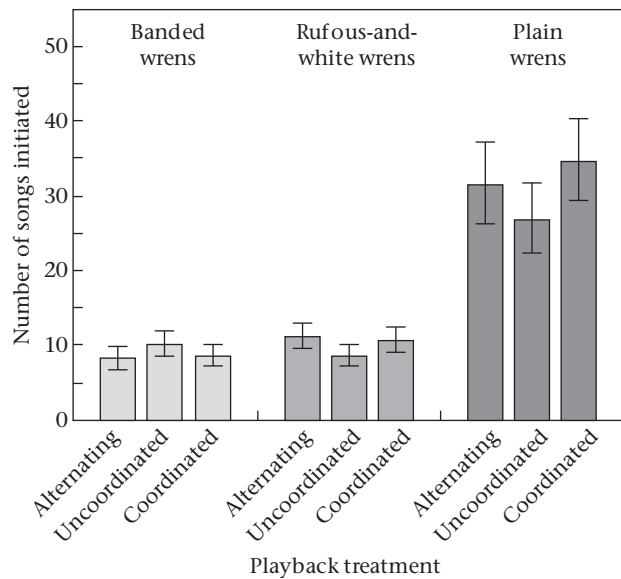
Figure 3. Average number of songs initiated (solo songs plus duets where the focal bird sang the first component of the duet) in response to playback simulating songs of a rival pair of conspecific animals, showing differences between three experimental treatments (alternating solos, uncoordinated duets and coordinated duets) for three species of Neotropical wren: banded wrens (light grey bars, at left), rufous-and-white wrens (medium grey bars, at centre) and plain wrens (dark grey bars, at right). Error bars show standard error around the mean.
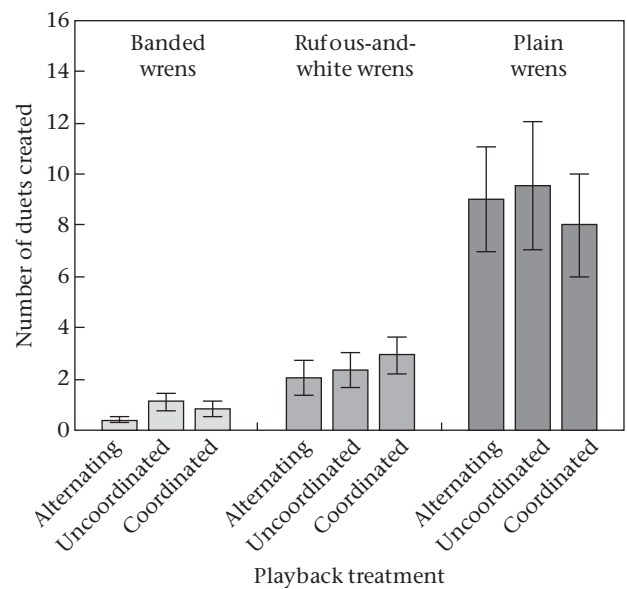

Figure 4. Average number of duets created (duets where the focal bird joined its partner's song to create a duet) in response to playback simulating songs by a rival pair of conspecific animals, in three experimental treatments (alternating solos, uncoordinated duets and coordinated duets) for three species of Neotropical wrens: banded wrens (light grey, bars at left), rufous-and-white wrens (medium grey bars, at centre) and plain wrens (dark grey bars, at right). Error bars show standard error around the mean.

Males passed over the loudspeaker more than females did (Table 2, Supplementary material). Number of passes showed a significant species * sex interaction (Table 2); both sexes of plain wren made more passes than banded wrens (post hoc pairwise comparisons: $P \leq 0.03$), whereas female plain wrens, but not males, made more passes than rufous-and-white wrens ($P = 0.04$).

Passing behaviour showed no effect of order (Table 2) but showed a significant order * species interaction (Table 2); rufous-and-white wrens passed over the fourth treatment more than the first (post hoc pairwise comparisons: $P = 0.02$), and banded wrens passed over the third treatment more than the first ($P = 0.007$).

Number of Songs Initiated

The effect of experimental treatment on the number of songs initiated (i.e. solos plus duets where the focal bird sang first) differed between the species (Fig. 3; significant treatment * species interaction, Table 2): banded wrens responded similarly to all experimental treatments (post hoc pairwise comparisons: $P \geq 0.13$); both rufous-and-white wrens and plain wrens initiated more songs to coordinated versus uncoordinated duets and alternating solos versus uncoordinated duets (all $P \leq 0.001$), but similar numbers of songs to alternating solos and coordinated duets ($P \geq 0.19$). Across species, wrens initiated more songs in response to both coordinated duets and alternating solos versus uncoordinated duets (post hoc pairwise comparisons; $P < 0.005$), but similar responses to coordinated duets and alternating solos ($P = 0.73$). Across treatments, the three species varied in their song initiation rate (Table 2); plain wrens initiated more songs than the other species (post hoc pairwise comparisons: both $P < 0.001$), and the other species initiated songs at similar levels ($P = 0.39$).

Males initiated more songs than females overall, but the effect of experimental treatment differed between the sexes (significant treatment * sex interaction, Table 2, Supplementary material); males initiated more songs for coordinated versus uncoordinated duets (post hoc pairwise comparison: $P < 0.006$) whereas females initiated more songs for both coordinated duets and alternating solos versus uncoordinated duets ($P \leq 0.04$). Song initiation also showed a significant species * sex interaction (Table 2); both sexes of plain wren initiated more songs than both sexes of banded and rufous-and-white wren, but the magnitude of the difference was greater for males (post hoc pairwise comparisons: $P \leq 0.004$).

Number of songs initiated varied with order (Table 2); birds initiated more songs in response to the second, third and fourth treatments compared to the first (post hoc pairwise comparisons: $P \leq 0.003$; all other comparisons $P \geq 0.40$). Song initiation showed a significant treatment * order interaction (Table 2); birds initiated more songs if alternating solos were presented in the second, third and fourth order versus the first position (post hoc pairwise comparisons: $P \leq 0.001$), or if coordinated or uncoordinated duets were presented in the fourth versus second, or second versus first position ($P \leq 0.003$). It also showed a significant species * order interaction (Table 2); rufous-and-white wrens only showed a higher initiation rate in response to the fourth versus first position ($P = 0.02$), whereas both plain and banded wrens showed higher initiation rates in response to the fourth versus first, second and third position ($P < 0.05$). It also showed a significant sex * order interaction (Table 2); males showed higher initiation rates in the third versus first, second and fourth order position ($P < 0.009$) whereas females showed higher initiation rates in the fourth versus third position and the second versus first position ($P < 0.008$).

Number of Duets Created

Number of duets created (i.e. duets where the focal bird responded to the song of its partner) showed a significant treatment * species interaction (Fig. 4, Table 2) but none of the post hoc comparisons showed significant differences (pairwise post hoc comparisons: all Bonferroni-corrected $P \geq 0.06$), probably owing to many trials in which the birds created no duets. Across species, the

number of duets created differed by treatment (Fig. 4, Table 2); wrens created similar numbers of duets during coordinated and uncoordinated treatments ($P = 0.62$), and significantly more duets in response to both categories of duets compared to alternating solos ($P < 0.02$). The model showed significant variation between species (Table 2), yet again none of the post hoc comparisons showed significant differences (all Bonferroni-corrected $P \geq 0.06$).

Overall, females created more duets than males, and the effect of treatment differed between the sexes (treatment∗sex interaction, Table 2, Supplementary material); females created more duets in response to both coordinated and uncoordinated duets versus alternating solos ($P \leq 0.02$) while males created more duets in response to coordinated duets versus alternating solos ($P = 0.04$). Number of duets created also showed a species∗sex interaction (Table 2); female plain wrens initiated significantly more duets than female banded wrens ($P = 0.04$) whereas none of the pairwise comparisons was significant for males ($P \geq 0.12$).

Number of duets created varied with playback order (Table 2); wrens created more duets in response to the first and second versus third or fourth position ($P \leq 0.02$). Number of duets created showed a significant order∗treatment interaction (Table 2); birds did not create more duets in response to coordinated duets if they were in the second position, but did so when alternating solos or uncoordinated duets were in the second position. Number of duets created also showed significant order∗sex and order∗species interactions (Table 2), although none of the post hoc comparisons showed significant differences.

DISCUSSION

Three species of Neotropical wrens showed strong responses to playback of coordinated duets, uncoordinated duets and alternating male and female solos, but weak responses to a heterospecific control treatment. We found no support for either of the two predicted outcomes: (1) that wrens of all species would respond more intensely to highly coordinated duets than to poorly coordinated duets and alternating solos, or (2) that wrens would respond most intensely to their species-specific singing style. For all measures of response, the effect of the experimental treatment differed between species and, if anything, birds tended to respond with the lowest intensity to their species-specific singing style. Banded wrens, the species that duets least frequently, responded more strongly to both types of duets than to alternating solos, in terms of their closest approach and number of passes near the speaker. Rufous-and-white wrens, the polyphonal duetter, responded more intensely to both alternating solos and coordinated duets than to uncoordinated duets, in terms of their number of passes near the speaker and number of songs initiated. Plain wrens, the antiphonal duetter, responded most intensely to alternating solos in terms of their closest approach, and no less intensely to alternating solos than to the other treatments, in terms of their other measures of response. Taken together, our results demonstrate that these Neotropical duetting songbirds distinguish between coordinated duets, uncoordinated duets and alternating solos, but fail to support the idea that coordinated duets incite categorically different responses than uncoordinated duets or alternating solos.

Playback studies to many duetting birds demonstrate that duets are common during territorial interactions, suggesting that they are important in interactions with rivals (reviewed in Hall, 2004, 2009; Douglas & Mennill, 2010). Duets could be important signals during territorial interactions in at least two ways: duets might communicate that two territorial animals are present instead of one, or duets might reveal that the two territorial animals are coordinating their behaviour, indicative of cooperation or readiness to produce a joint defence. In territorial kōkako, *Callaeas cinerea*, playback simulating a pair of duetting intruders incites a stronger response than playback simulating a single intruder producing both male and female duet contributions, indicating that the number of intruders changes the degree of threat (Molles & Waas, 2006). If the three species of duetting wrens in our study assess territorial rivals based solely on the number of individuals singing, and not the degree of coordination in their songs, then we would expect similar responses to all three of our experimental treatments, as we found for all species for one or more of our four measures of response. Prior research has shown that rufous-and-white wrens respond with similar levels of aggression to both duets and solos of one sex (Mennill & Vehrencamp, 2008), indicating that the number of intruders may not influence the approach responses of a territorial pair in one of the three species we studied here. Paired male canebrake wrens, *Cantorchilus zeledoni* (the sister species to plain wrens), also respond similarly to solos and duets, although unpaired males respond more strongly to duets than to solos (Marshall-Ball et al., 2006). Furthermore, similarly intense responses to solos and duets have been reported in many other duetting bird species (e.g. Bradley & Mennill, 2009; Dahlin & Wright, 2012; Fedy & Stutchbury, 2005; Grafe et al., 2004). Taken together with the data presented on similar approach responses across our experimental treatments, these results suggest that paired wrens regard all intruders as equally threatening, regardless of their number, or the degree of coordination in their songs. It is possible that the number of intruders may only be important to unpaired territory holders, and not to established pairs, as previously suggested in canebrake wrens (Marshall-Ball et al., 2006). Playback to unpaired animals would be required to test this idea (e.g. Seddon, Butchart, & Odling-Smee, 2002).

In our experimental design, the alternating solo treatment included solos by both the male and female to simulate intrusion by two birds, in contrast to previous studies that compared animals' responses to playback of duets versus solos from one bird at a time (simulating solitary intruders, e.g. Dahlin & Wright, 2012; Koloff & Mennill, 2011). In the latter design, different responses to duet versus solo playback could arise either because of coordinated singing or because two intruders are simulated rather than one. Our alternating solo treatment, involving the same number of male and female songs as the duet treatments, specifically tested the effect of two birds coordinating their songs to form duets, rather than singing independently. Our findings call into question the idea that duets create a stronger territorial signal than solo songs; alternating solos attracted a significantly reduced response only in approach responses of banded wrens (Figs. 2, 4). We encourage others to incorporate alternating solo treatments into future playback designs.

We presented the four playback treatments to each subject pair on the same day to minimize any variation that might occur due to changes in the subjects' breeding status, or other day-to-day changes. We found significant order effects in our analyses, although our factorial design minimized bias on treatment effects (each treatment was presented in each order an equal number of times). We encourage future investigators to attempt to space out treatments over longer periods, where possible, to decrease carryover effects. Nevertheless, order effects occurred in a previous study on banded wrens even when treatments were presented on different days, so factorial designs are preferable to randomized presentation order (Hall et al., 2006).

If duetting is not a strong territorial signal important in interactions between rivals, why then do wrens coordinate their songs into duets? One possibility is that coordinating songs to form duets functions as a signal within pairs. Many studies have supported the idea that duets serve within-pair functions, such as

maintaining acoustic contact (Mennill & Vehrencamp, 2008), maintaining the pair bond (Geissman & Orgeldinger, 2000; Hall, 2000) and recognizing a mate during aggressive interactions (Logue & Gammon, 2004). If a quick and consistent response to a mate requires attentiveness (Hall, 2009; Smith, 1994), duet coordination could be a within-pair signal of attentiveness. Duets can serve both between-pair and within-pair functions simultaneously; some duetting species coordinate their song type with their mate rather than matching the song type of a playback intruder while performing duets in response to rivals (Rogers, Mulder, & Langmore, 2006; Templeton, Ríos-Chelén, Quirós-Guerrero, Mann, & Slater, 2013).

Combining songs to form duets seems to influence whether or not a pair performs duets in response. Wrens created more duets in response to playback of both coordinated and uncoordinated duets than to playback of solos, although they did not differentiate between coordinated and uncoordinated duets in their duetting responses. Species variation in the frequency of duetting in response to playback (Fig. 4) was consistent with our designation of duetting as least frequent in banded wrens and most developed in plain wrens.

Our study is the second to contrast responses to coordinated and uncoordinated duets. In an antiphonal duetter, the Australian magpie-lark, males responded with higher song rates to coordinated duets than uncoordinated duets (Hall & Magrath, 2007). We found that banded wrens did not discriminate between these two treatments by any measure of response. However, rufous-and-white wrens passed near the speaker more often and initiated more songs in response to coordinated duets than to uncoordinated duets, while plain wrens also initiated more songs to coordinated duets than to uncoordinated duets, even though alternating solos and coordinated duets elicited responses of similar intensities in these two species. The magpie-lark study did not include an alternating solo treatment, so that component of the two investigations could not be compared directly. Taken together, these results indicate that species that duet frequently distinguish between duets based on the precision with which partners coordinate their phrases within duets, even though alternating solos elicit responses of similar intensity to coordinated duets. In contrast, the species that duets least frequently (banded wrens) did not distinguish between duets based on their precision, even though it responded more intensely to both types of duets than to alternating solos.

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Supplementary Material

Supplementary material for this article is available, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2014.06.012.

References

Armstrong, E. A. (1947). *A study of bird song*. London, U.K.: Oxford University Press.

Bailey, W. J. (2003). Insect duets: underlying mechanisms and their evolution. *Journal of Physiological Entomology, 28*, 157–174.

Benedict, L. (2010). California towhee *duets* are multi-functional signals for multiple receivers. *Behaviour, 147*, 953–978.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, J. R. P., Stevens, M. H. H., & White, J. S. S. (2008). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution, 24*, 127–135.

Bradley, D. W., & Mennill, D. J. (2009). Strong ungraded responses to playback of solos, duets, and choruses in a cooperatively breeding Neotropical songbird. *Animal Behaviour, 77*, 1321–1327.

Brewer, D. (2001). *Wrens, dippers, and thrashers*. New Haven, CT: Yale University Press.

Catchpole, C. K., & Slater, P. J. B. (2008). *Bird song: Biological themes and variations*. New York, NY: Cambridge University Press.

Cuthbert, J. L., & Mennill, D. J. (2007). The duetting behaviour of Pacific coast plain wrens (*Thryothorus modestus modestus*). *Condor, 109*, 686–692.

Dahlin, C. R., & Benedict, L. (2013). Angry birds need not apply: a perspective on the flexible form and multifunctionality of avian vocal duets. *Ethology, 120*, 1–10.

Dahlin, C. R., & Wright, T. F. (2012). Duet function in the yellow-naped amazon, *Amazona auropalliata*: evidence from playbacks of duets and solos. *Ethology, 118*, 95–105.

Douglas, S. B., & Mennill, D. J. (2010). A review of acoustic playback techniques for studying avian vocal duets. *Journal of Field Ornithology, 81*, 115–129.

Farabaugh, S. M. (1982). *The ecological and social significance of duetting*. In D. E. Kroodsma, & E. H. Miller (Eds.), *Acoustic communication in birds* (Vol. 2); (pp. 85–124). New York, NY: Academic Press.

Fedy, B. C., & Stutchbury, B. J. (2005). Territory defence in tropical birds: are females as aggressive as males? *Behavioral Ecology and Sociobiology, 58*, 414–422.

Fortune, E. S., Rodriguez, C., Li, D., Ball, G. F., & Coleman, M. J. (2012). Neural mechanisms for the coordination of duet singing in wrens. *Science, 334*, 666–670.

Geissman, T., & Orgeldinger, M. (2000). The relationship between duet songs and pair bonds in siamangs, *Hylobates syndactylus*. *Animal Behaviour, 60*, 805–809.

Grafe, T. U., Bitz, J. H., & Wink, M. (2004). Song repertoire and duetting behaviour of the tropical boubou, *Laniarius aethiopicus*. *Animal Behaviour, 68*, 181–191.

Haimoff, E. H. (1986). Convergence in the duetting of monogamous Old World primates. *Journal of Human Evolution, 15*, 51–59.

Hall, M. L. (2000). The function of duetting in magpie-larks: conflict, cooperation, or commitment? *Animal Behaviour, 60*, 667–677.

Hall, M. L. (2004). A Review of hypotheses for the functions of avian duetting. *Behavioral Ecology and Sociobiology, 55*, 415–430.

Hall, M. L. (2009). Vocal duetting in birds. *Advances in the Study of Behaviour, 40*, 67–121.

Hall, M.L., Driscoll, M.R.T., & Vehrencamp, S.L. (n.d.). Female song and vocal interactions with males in a Neotropical wren. Manuscript in preparation.

Hall, M. L., Illes, A., & Vehrencamp, S. L. (2006). Overlapping signals in banded wrens: long-term effects of prior experience on males and females. *Behavioral Ecology, 17*, 260–269.

Hall, M. L., & Magrath, R. D. (2007). Temporal coordination signals coalition quality. *Current Biology, 17*, 406–407.

Illes, A. E., Hall, M. L., & Vehrencamp, S. L. (2006). Vocal performance influences male receiver response in the banded wren. *Proceedings of the Royal Society B: Biological Sciences, 273*, 1907–1912.

Koloff, J., & Mennill, D. J. (2011). Aggressive responses to playback of solos and duets in a Neotropical antbird. *Animal Behaviour, 82*, 587–593.

Levin, R. N. (1996). Song behaviour and reproductive strategies in a duetting wren, *Thryothorus nigricapillus*: I. Removal experiments. *Animal Behaviour, 52*, 1093–1106.

Logue, D. M., Chalmers, C., & Gowland, A. H. (2008). The behavioural mechanisms underlying temporal coordination in black-bellied wren duets. *Animal Behaviour, 75*, 1803–1808.

Logue, D. M., & Gammon, D. E. (2004). Duet song and sex roles during territory defence in the black-bellied wren, *Thryothorus fasciatoventris*. *Animal Behaviour, 68*, 721–731.

Logue, D. M., & Hall, M. L. (2014). The evolution of vocal duetting in songbirds. *Proceedings of the Royal Society B: Biological Sciences, 281*, 20140103.

Mann, N. I., Barker, F. K., Graves, J. A., Dingess-Mann, K. A., & Slater, P. J. B. (2006). Molecular data delineate four genera of 'Thryothorus' wrens. *Molecular and Phylogenetic Evolution, 40*, 750–759.

Mann, N. I., Dingess, K. A., Barker, K. F., Graves, J. A., & Slater, P. J. B. (2009). A comparative study of song form and duetting in Neotropical *Thryothorus* wrens. *Behaviour, 146*, 1–43.

Marshall-Ball, L., Mann, N., & Slater, P. J. B. (2006). Multiple functions of duet singing: hidden conflicts and apparent cooperation. *Animal Behaviour, 71*, 823–831.

Mennill, D. J. (2006). Aggressive responses of male and female rufous-and-white wrens to stereo duet playback. *Animal Behaviour, 17*, 219–226.

Mennill, D. J., & Rogers, A. C. (2006). Whip it good! Geographic consistency in male songs and variability in female songs of the duetting eastern whipbird *Psophodes olivaceus*. *Journal of Avian Biology, 37*, 93–100.

Mennill, D. J., & Vehrencamp, S. L. (2005). Sex differences in the singing and duetting behaviour of Neotropical rufous-and-white wrens (*Thryothorus rufalbus*). *Auk, 122*, 175–186.

Mennill, D. J., & Vehrencamp, S. L. (2008). Context-dependent functions of avian duets revealed through microphone array recordings and multi-speaker playback. *Current Biology, 18*, 1314–1319.

Molles, L. E., & Vehrencamp, S. L. (1999). Repertoire size, repertoire overlap, and singing modes in the banded wren *(Thryothorus pleurostictus). Auk, 116*, 677–689.

Molles, L. E., & Vehrencamp, S. L. (2001). Neighbour recognition by resident males in the banded wren, *Thryothorus pleurostictus*, a tropical songbird with high song type sharing. *Animal Behaviour, 61*, 119–127.

Molles, L. E., & Waas, J. R. (2006). Are two heads better than one? Responses of the duetting kokako on one- and two-speaker playback. *Animal Behaviour, 72*, 131–138.

Rogers, A. C., Mulder, R. A., & Langmore, N. E. (2006). Duet duels: sex differences in song matching in duetting eastern whipbirds. *Animal Behaviour, 72*, 53–61.

Seddon, N., Butchart, S. J., & Odling-Smee, L. (2002). Duetting in the subdesert mesite *Monias benschi*: evidence for acoustic mate defence? *Behavioral Ecology and Sociobiology, 52*, 7–16.

Smith, W. J. (1994). Animal duets: forcing a mate to be attentive. *Journal of Theoretical Biology, 166*, 221–223.

Sonnenschein, E., & Reyer, H. U. (1983). Mate-guarding and other functions of antiphonal duets in the slate-coloured boubou (*Laniarus funebris*). *Zeitschrift für Tierpsychologie, 63*, 112–140.

Templeton, C. N., Ríos-Chelén, A. A., Quirós-Guerrero, E., Mann, N. I., & Slater, P. J. B. (2013). Female happy wrens select songs to cooperate with their mates rather than confront intruders. *Biology Letters, 9*, 20120863.

Tobias, M. L., Viswanathan, S. S., & Kelley, D. B. (1998). Rapping, a female receptive call, initiates male–female duets in the South African clawed frog. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 1870–1875.

Topp, S., & Mennill, D. J. (2008). Seasonal variation in the duetting behaviour of rufous-and-white wrens (*Thryothorus rufalbus*). *Behavioral Ecology and Sociobiology, 62*, 1107–1117.