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An experimental study of duet integration in the happy wren, *Pheugopedius felix*

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Pairs of duetting birds can sing coordinated duets with such precision that they are often mistaken for a single individual, yet little is known about how this impressive temporal synchronization is achieved. We experimentally examined duet coordination in male happy wrens, held briefly in captivity, by playing song phrases from their partner at different distances and tempos. Males were more likely to respond to songs played nearby, but did not vary their amplitude to compensate for their partner's simulated distance. Males modified their song rate to match the manipulated female playback tempo, indicating that they listen and respond to each female utterance. Each happy wren has a sex-specific repertoire of about 40 different song phrases and pairs combine particular phrases according to pair-specific duet 'codes', creating a further challenge for coordinating duets. We found that most males produced the appropriate phrase to reply to the female playback song in the absence of any other potential cues, sometimes delivering the correct song phrase type within 0.5 s of the start of the very first female playback heard. These experiments demonstrate rapid decision making and vocal production, indicative of sophisticated underlying cognitive processing, and provide a novel experimental technique to investigate the mechanisms controlling vocal duets.

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Auditory signals are widely used for communication and understanding the behavioural patterns that structure complex vocal interactions can provide important insights into the cognitive processes of animals. For example, rapid and precise acoustic interchanges between animals can indicate remarkable perception and information-processing abilities (Shettleworth 2010). Duetting is a particularly striking example of complex acoustic communication and duets are produced by a variety of animals, including insects (Bailey 2003), frogs (Tobias et al. 1998), primates (Geissmann 2002), and cetaceans (Schulz et al. 2008). Birdsongs are among the most elaborate of all acoustic duets (Catchpole & Slater 2008). Studies of complex avian duets may be of particular value for understanding cognition and there has been a recent surge in research into the form and function of these signals (Hall 2004, 2009).

In birds, duetting is primarily a tropical phenomenon, no doubt linked in part to female song being rare in the northern temperate

zone (Slater & Mann 2004). Why duetting has evolved, and is particularly common in the tropics, has been the subject of much study (Hall 2004, 2009), with considerable debate about whether it serves a cooperative function or represents a manifestation of sexual conflict within male–female pairs (e.g. Hall 2000; Marshall-Ball et al. 2006). Although detailed studies across many species have addressed the function of duetting, other, largely neglected, aspects of this behaviour, such as the remarkable degree of coordination between the birds involved, are equally compelling.

Duets vary between species, ranging from relatively simple, simultaneous singing (e.g. Radford 2003; Illes & Yunes-Jimenez 2009) to intricate, multipart performances (Mann et al. 2006), but some of the most impressive are those sung antiphonally. In these, the male and female sing alternating song phrases, frequently in rapid succession and with such precise timing that it often sounds as if only one bird is singing (Levin 1996; Mann et al. 2003). Singing perfectly timed, antiphonal duets poses a substantial challenge, especially given that different phrase types may vary in length and pairs can sing duets at varying distances. In fact, some researchers have anecdotally commented that the tempo of

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duetting becomes slower when birds are further apart (Farabaugh 1983). Furthermore, some species have large song repertoires, often with sex-specific phrase types and according to pair-specific combinations. With this system of 'duet codes' (Logue 2006), correctly replying involves appropriate phrase type selection in addition to timing. Thus, coordinating duets poses a complex cognitive problem for duetting species.

We quantified duet integration in pairs of happy wrens, small, resident songbirds from the dry forests of western Mexico. These birds sing sex-specific song phrases, and both the male and female have a repertoire of 30–40 phrase types, which can be repeated a variable number of times to form a song (Brown & Lemon 1979; Mann et al. 2009). Paired birds combine their song phrases to create duets, according to pair-specific duet codes (Templeton et al. 2013), which they use cooperatively in territorial defence (Templeton et al. 2011). In this experiment, we briefly held adult males in captivity and tested them with experimental playbacks to simulate their mate's singing. We varied the distance and timing of the female songs to examine how the males coordinate their songs to produce complex vocal duets. If males adjust their singing behaviour to maximize the communicative function of their duets, we predicted that they should select the appropriate song from their repertoire to pair with the playback and alter the tempo and amplitude of their singing in accordance with the varying female playback tempo and distance.

METHODS

Study Site and Subjects

The research was conducted from 26 May to 26 June 2011 at the Universidad Nacional Autónoma de México (UNAM) Chamela research station in Jalisco, Mexico. The station is surrounded by the tropical deciduous and semideciduous forests of the 131 km² Chamela-Cuixmala Biosphere Reserve. Happy wrens are common in the semideciduous forests and we have uniquely marked most birds in the population with coloured leg rings for individual identification. Subjects for this experiment came from 12 pairs for which we had previously mapped the territory boundaries and made high-quality duet recordings.

Each male was captured by mist net just before sunset (ca 1900 hours) on the day before the experimental playback. Subjects were transported back to the field station and placed in a small experimental cage (23 × 32 cm and 23 cm high) with several perches, ad libitum water and live mealworm, *Tenebrio molitor*, larvae. The cage was covered with thin cotton, which was opaque enough to minimize stress, while still letting through enough light to resemble the natural environment under the forest canopy. The cage was kept outside overnight, but within a wood and hardware cloth box (ca. 1 m³) inside a large insect-proof mesh enclosure, thus ensuring the bird remained undisturbed and away from any potential predators.

Playback Stimuli

Songs used for playback were recorded within the 3 weeks prior to the experiment from birds in the field using a Sennheiser ME67/K6 shotgun microphone and Marantz PMD 660 solid-state recorder, which was set to record uncompressed .wav files with a 41 kHz sampling rate. Songs were generally recorded at close range (<5 m). For playback, we randomly selected a single phrase type from all of the high-quality (high signal-to-noise ratio) recordings of that pair. We used the frequency cursor filter function of Syrinx (www.syrinxpc.com; J. Burt) to remove all male vocalizations and background noise from the recordings so that we had a high-quality

(high signal-to-noise ratio) sound file of a single female song phrase, from each focal male's partner. Phrase length varied somewhat across females, with a mean ± SE phrase length of 0.78 ± 0.04 s (range 0.55–1.18 s). Using Avisoft-SASLab Pro v5.1 (Avisoft Bioacoustics, Berlin, Germany), we duplicated these phrases and arranged them with appropriate lengths of silence (see below) to create the sequence of playback stimuli. All playback stimuli were normalized to –3 dB peak frequency and saved as uncompressed .wav files.

We created playback sequences of a female song phrase repeated six times with silent intervals varying across treatments to yield three different tempos (time between start of one phrase and start of next): 'fast' (mean ± SE = 1.44 ± 0.02 s), 'intermediate' (1.70 ± 0.04 s) and 'slow' (2.03 ± 0.03 s). The majority of original duet recordings used to create playback sequences were of intermediate timing, although happy wrens naturally vary the timing of their songs to cover this range.

Female happy wren song phrases consist of three to seven different and discrete elements and vary from about 0.6 to 1.3 s in duration. Male phrases are often rather longer (ca 0.9–1.5 s) and usually end with a trill in which the final element is repeated a variable number of times. In happy wrens, either sex can initiate or terminate a duet, although it is more common for males to initiate. When testing whether males follow a duet code, we assumed that the predicted answers should follow the same pattern as in our recordings of natural singing interactions. For some pairs we were able to obtain only a few recordings prior to the experiment and it is possible that these could have included atypical song combinations (e.g. song transitions or even 'disagreements'). It is therefore possible that in some cases the duet code we expected the male subject to follow was not the one conventionally adopted by that pair. However, any discrepancies should actually work against the hypothesis that males follow a duet code, thus adding a conservative element to our analysis.

Experimental Apparatus and Procedure

The playback experiments were conducted on a flat cinder-covered arena, 35 m long and next to where the captive bird was housed overnight. The forest adjacent to the test arena appeared to be without happy wrens and we never heard any wild happy wrens sing nearby. The subject's cage was raised on a platform 0.5 m above the ground at one end of the test arena. We used two recording systems, each composed of a Marantz PMD 670 recorder and Sennheiser ME66/K6 microphone. Microphone position and recorder volume were held constant throughout each set of playback trials. One microphone was placed beside the subject's cage and the other was placed 20 m away, with the microphone pointed directly at the subject's cage. Along the same line of sight, we placed three posts 0.8 m high at different distances (10 m, 20 m and 35 m) from the cage, on which the speaker was placed during the playback. A calibrated playback speaker (Pignose 7-100) connected to a PC laptop equipped with Syrinx was used for playback and moved between distances as appropriate to the sequence of trials. Each morning, the speaker was recalibrated in another location away from the test site (out of earshot) to play ca. 62 dB SPL at 9 m (range 59–65; a similar amplitude to natural happy wren songs) with a Sew 2310SL sound level meter set to maximum reading in fast mode (A-weighting). All dB values are with reference to 20 µPa. This calibration resulted in playback amplitude being standardized across treatments, distances and, as far as possible, subjects.

After the equipment was placed in the appropriate locations, we moved the subject's cage from the protective box to the test arena just before dawn (approximately 0700 hours). We then waited at least 15 min for the subject to recover from any potential stress

associated with the cage movement. No subject sang prior to our first experimental playback, which took place just after dawn (ca. 0715 hours), about the time when most wild birds began singing.

Only one subject was tested each day and two sequences of playbacks were carried out for each male. In the first ('distance experiment'), each male was tested with six repetitions of the same phrase recorded from his female played at the intermediate tempo. He received two such six-phrase playbacks 30 s apart from one distance, and this was then repeated at another distance 90 s later and so on until 12 playbacks had been carried out, four from each distance. Happy wrens sing duets at a variety of distances and each of the chosen distances (10 m, 20 m, 35 m) has been observed in wild pairs. The order of playbacks was varied between birds in a randomized blocked design. In the unusual situation where a bird failed to respond at least twice at each distance, further tests were conducted in an attempt to fill in missing data, although one bird had to be excluded from the analysis because of low rates of singing (he sang just two phrases during the experiments). The maximum SPL of the playback stimuli was measured after the experiment on three separate mornings and yielded a mean \pm SE of 45.9 ± 0.8 dB at 35 m, 55.5 ± 1.1 dB at 20 m and 64.6 ± 1.1 dB at 10 m.

Following a 10 min interval a second series of playbacks was conducted using the three different tempos played at 35 m ('tempo experiment'). For the first three birds, the tempo used was changed between successive groups of six female phrases. Thereafter we settled on a standard procedure where successive sequences of six phrases were played 60 s apart with three in a row at each tempo. These nine playbacks were then followed by a 10 min interval after which three more playbacks were conducted at each tempo, with the tempos tested in a different order for each subject. We saw no obvious differences in singing behaviour between the first three and the other subjects. Once again the order of testing was varied between birds. Playbacks were spread out to minimize habituation so that the whole procedure lasted ca. 60 min, after which the bird was released back on his territory. No ill-effects of the treatment were observed, and most males immediately began duetting with their partners upon release. Perhaps because of the short amount of time off territory or the fact that each male's songs were broadcast from his territory in his absence (details in Templeton et al. 2013), no male was usurped from his territory or mate.

Data Analysis

All recordings made were analysed with Avisoft SASlab Pro. Sonograms (256 FFT length; 44 kHz sampling rate) of all songs that each male sang during the playback period were classified by eye and a catalogue was prepared for each bird. We focused analyses on male phrases that were clearly stimulated by the playback, overlapping with or occurring just after the end of the female phrase, although in practice most males sang little outside these times. We made a number of measurements from the sonograms using the onscreen cursor function in Avisoft. (1) Latency of male response: we measured the time from the onset of the first female playback phrase to that of the first male response. (2) Length of male phrase: the duration of the phrase was also measured, using the cursor in Avisoft. (3) Male phrase completeness: the elements in each male phrase were counted and its completeness or incompleteness scored based on known song repertoires of each male. Songs were classified as 'complete' if the last element type was reached, regardless of whether it was repeated (males vary the number of repeats on almost all terminal trills), and 'incomplete' if they only consisted of one or more elements but did not reach the final one. (4) Tempo of male response: where a male responded to two female playback phrases in succession, the tempo (the time interval

between the onset of one phrase and that of the next) of his response was measured using the cursors. (5) Amplitude of male response: this was measured using recordings from the far microphone, located 20 m from the male, for comparison of male responses to playback at the three different distances. Only phrase types with which the male responded at all three distances of playback were analysed: this resulted in a sample of 10 birds, four with two phrase types, six with one. We selected a single element for amplitude analysis to minimize the number of songs that had to be excluded because of overlap with other sounds (playback or background noise). The selected element was typically in the middle of the song, because this was less likely to overlap with the playback and late elements are more often omitted if the song is cut short. Overlapping sounds were not a major problem. Songs of other species were seldom of high enough amplitude to influence the analysis, as confirmed by comparison of the same element with and without overlap. Overlap with the playback provided a potentially greater confound, as any influence of the playback stimulus would correlate with its distance from the microphone, so the few elements with overlap from the playback were excluded from the analyses. All recordings of each male's element to be compared were copied into a single file, with 1 s of silence inserted between each pair of elements. A root mean square plot of amplitude was then prepared for the complete file using 125 ms averaging time and normalized to the maximum amplitude (Zollinger et al. 2012). The amplitude cursor was then used to obtain the relative amplitude (in dB) of each element relative to the maximum. Thus we compared the amplitude of the same element produced by the male in response to a playback of female song at the three different distances.

Statistical analyses were conducted using SPSS v.19 (IBM, Armonk, NY, U.S.A.). We primarily used simple nonparametric comparisons and all significance tests were two tailed. To examine how response amplitude varied with playback distance and with position in a series of responses we used a general linear mixed model (GLMM) with subject as a random factor.

In addition to males, we tested three females with the equivalent playback of their mate's song, but failed to obtain useful data: one responded to only two stimuli and the others to none. Given that the male normally leads the duet, this is surprising but may suggest that females were more affected by being held in captivity. We intend to use refined versions of our procedure to test both sexes in the future.

This work was approved by the Mexican Secretary of the Environment and Natural Resources.

RESULTS

Males responded well to playback of their mate's song, and preferentially sang the song phrase that corresponded to the female playback phrase (Fig. 1). Eleven of 12 individuals responded frequently (the last did not sing and is not included in the analyses), with each using between three and nine phrase types during the course of the experiment (Table 1). All of these males sang the appropriate reply song at least once. Taking 30 phrase types as a conservative estimate of repertoire size (Brown & Lemon 1979; Mann et al. 2009), the probability that a randomly singing male would sing the correct phrase type would be between 3/30 and 9/30, depending on the number of different songs that the male sang during the trial. The observed frequency far exceeded that expected by chance (binomial test: $P < 0.0001$). Of the 11 males, eight used this song phrase most frequently (expected: 1/30; binomial test; $P < 0.0001$), and five sang the correct song as the very first phrase they produced. Males thus tended to follow a 'duet code' when replying to their mates.

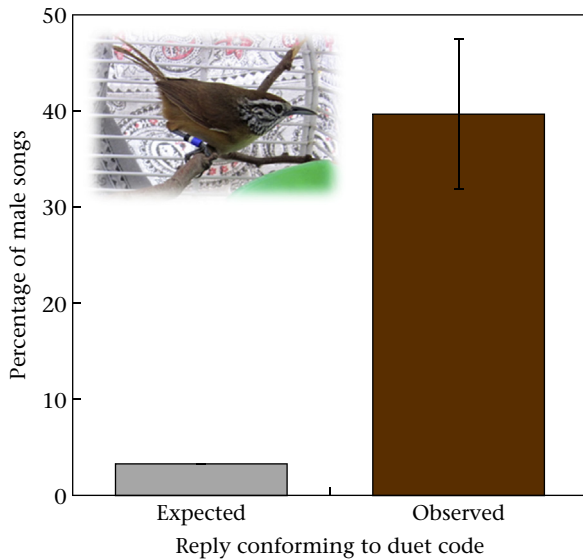


Figure 1. Mean \pm SE percentage of each male's songs sung, in answer to playback of his partner's song, with the same phrase type previously recorded in combination with it, and the percentage expected to be sung by chance.

The response of males to the playback was surprisingly rapid. Although none of the males sang before playback commenced, five of 11 males responded immediately to the very first female song phrase to create a duet. Even these initial duets were produced rapidly (mean \pm SE latency: 0.64 ± 0.04 s following the start of the female phrase) and timed to begin just as the female phrase ended (-0.07 ± 0.08 s after her phrase). Two different individuals not only responded immediately to the very first female song, but also used the appropriate phrase type, despite the short latency (0.53 s and 0.61 s from the start of the female phrase, respectively).

Male tempo was very well matched to that of the female playback (Fig. 2). This was true at all playback rates and even for the very first two female phrases played, indicating that males listen and respond to each female phrase. Male tempo tended to be greater than that of the female playback (binomial test: $P = 0.004$) and to get slower through the series of six female playbacks, the male song slipping in relation to the female, suggesting difficulty in maintaining a high rate. Thus, rather unexpectedly, latency of response to the first female song was shorter on average (mean of means = 0.67 s) than for any of the subsequent five (range 0.69 –

0.82 ; Friedman test: $\chi_{r5}^2 = 24.17$, $P < 0.001$). There was thus no evidence that the first female song alerted the male so that he was subsequently able to respond more rapidly.

Overall, male responsiveness varied with subject, distance and playback iteration. One male only sang two song phrases during the experiments, although on average subjects responded to more than half of the female playback songs (Table 1). The closer the playback, the more likely a male was to respond (Fig. 3a; Friedman test: $\chi_{r2}^2 = 10.23$, $P < 0.01$), suggesting that males preferentially respond when their females are nearby. Although males often responded to all six of the female songs played back in a sequence, there was an overall tendency for their response to decline through that sequence (Fig. 3b; Friedman test: $\chi_{r5}^2 = 26.10$, $P < 0.001$).

The proportion of songs that were incomplete varied substantially between birds and there was no tendency for later responses to be less complete (overall 20.6% to playback 6 as opposed to 20.4% to playback 1). Males were not more likely to abort song phrases during the fast playback, as might be predicted if birds found it difficult to keep up. If anything, incomplete songs were more frequent in response to slow female tempos (31.6%) than to fast ones (20.4%), suggesting that especially slow female tempos are perhaps even more unusual or difficult to follow, but numbers are low and there was much variation between birds.

We tested the hypothesis that males sing more loudly to females that are further away so as to maximize the chances of getting the signal across and to facilitate duet timing. Although there was a slight tendency for increased amplitude with increased distance, this was not significant when accounting for individual variation and position in the sequence of responses (GLMM: $F_{2,329} = 0.36$, $P = 0.6$; Fig. 3c). However, later responses in a series were sung at significantly lower amplitude than earlier ones ($F_{5,329} = 36.1$, $P < 0.0001$; Fig. 3d).

DISCUSSION

The males in these experiments were tested with only one of the 30–40 phrase types possessed by their mate. In response, they tended to use the phrase type out of their repertoire normally paired with that female phrase type during their duets, indicating that they follow a duet code when selecting phrase types. However, they did not follow this code exclusively and a likely explanation for this is that the male was attempting to lead the female to sing a different type. The male generally leads the duet in *Pheugopedius* wrens (Logue 2007b; Mann et al. 2009), meaning that when he switches to a new phrase type the female usually does so too. In this case, of course, being a recording, the female did not follow his switch. As we sometimes observe in wild pairs, several males switched back to the original phrase type after the simulated female failed to follow his change, implying that the male generally takes the lead but is also attentive to his mate's singing.

One of the most remarkable aspects of our findings was the speed of the males' response. Several males responded to the very first playback song just a fraction of a second after its start, suggesting that they were able to recognize it as a female happy wren song on the basis of its very first elements. Furthermore, in two cases the male responded to this first female phrase with the appropriate reply phrase, in keeping with their normal 'duet code'. For this to happen, the male must not only recognize that he has heard a female happy wren sing, but he must also correctly assess which song she has sung (of 30–40 phrase types), determine the appropriate reply from his repertoire (of 30–40 phrase types), and calculate the duration of the female's particular phrase type so he can accurately time his reply. That some males achieved this feat in the absence of any other cues indicates the sophisticated and rapid cognitive processing underlying this behaviour.

Table 1

Song phrase types produced by males in response to playback (PB) of a single phrase type from the repertoire of their own mate, together with the number of times each was used

Bird	Number of types used	Phrase type									Phrase from PB duet
		A	B	C	D	E	F	G	H	I	
Male 1	6	19	16	15	14	1	18				A
Male 2	6	6	2	4	15	6	2				D
Male 3	5	26	15	9	21	11					E
Male 4	3	51	35	24							A
Male 5	4	23	13	6	23						D
Male 6	8	1	5	2	5	2	10	21	5		B
Male 7	4	22	48	17	53						A
Male 8	4	38	7	2	3	1					A
Male 9	3	18	29	4							B
Male 10	7	13	46	8	22	25	8	22			B
Male 11	9	25	35	2	7	2	3	2	14	11	B

Data are combined from the distance and tempo trials. Figures in bold show those responses that were appropriate to the duet code of that particular pair, as shown in the right-hand column. The phrase types labelled with a particular letter in different birds are not necessarily the same phrase type.

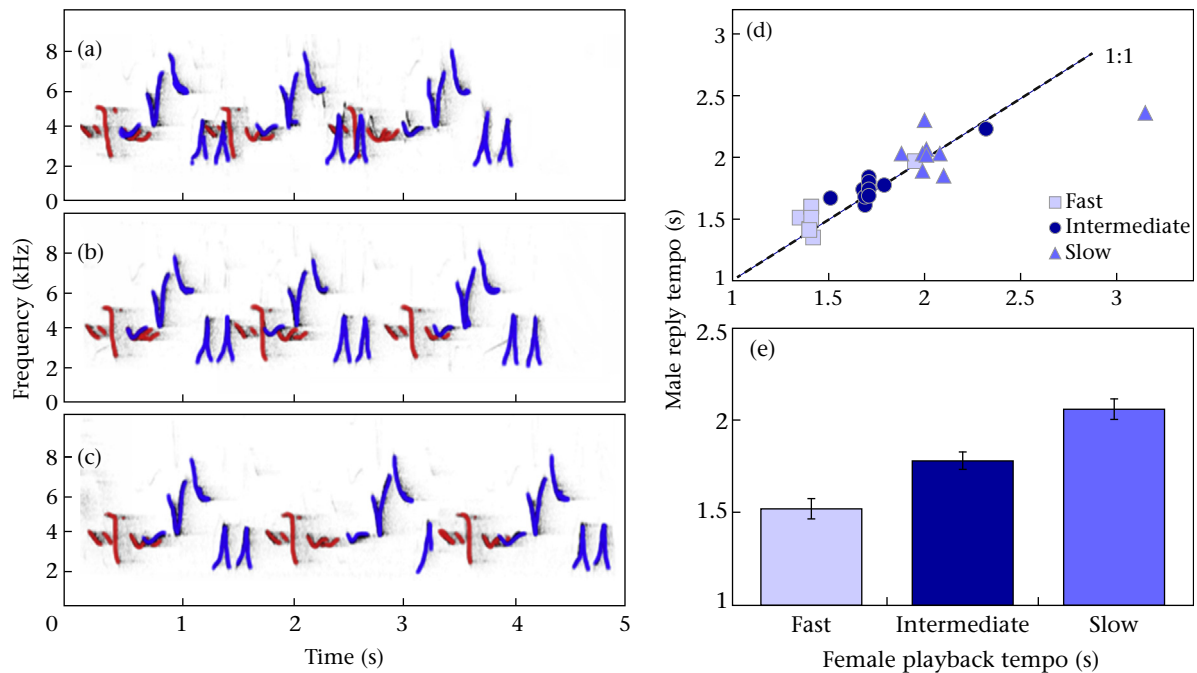


Figure 2. (a–c) Three responses from a single male (blue) to the same phrase type produced by his female mate (red) played at three different tempos: (a) fast (1.4 s), (b) intermediate (1.7 s) and (c) slow (2.0 s). (d) Tempo of male reply songs in relation to tempo of female playback songs. (e) Tempo of male reply songs across the three treatments.

Some earlier papers on the reaction times of duetting birds give figures even more rapid than those reported here: means of 144 ms in a pair of black-headed gonoleks, *Laniarius erythrogaster* (Thorpe 1963), 118 ms in Barbary shrikes, *Laniarius barbarus* (Grimes 1965)

and 164 ms in the orange-chinned parakeet, *Brotogeris jugularis* (Power 1966). However, the songs of each of these species are relatively short and simple, and it is not clear whether any of these responses involve selecting an appropriate phrase type in addition

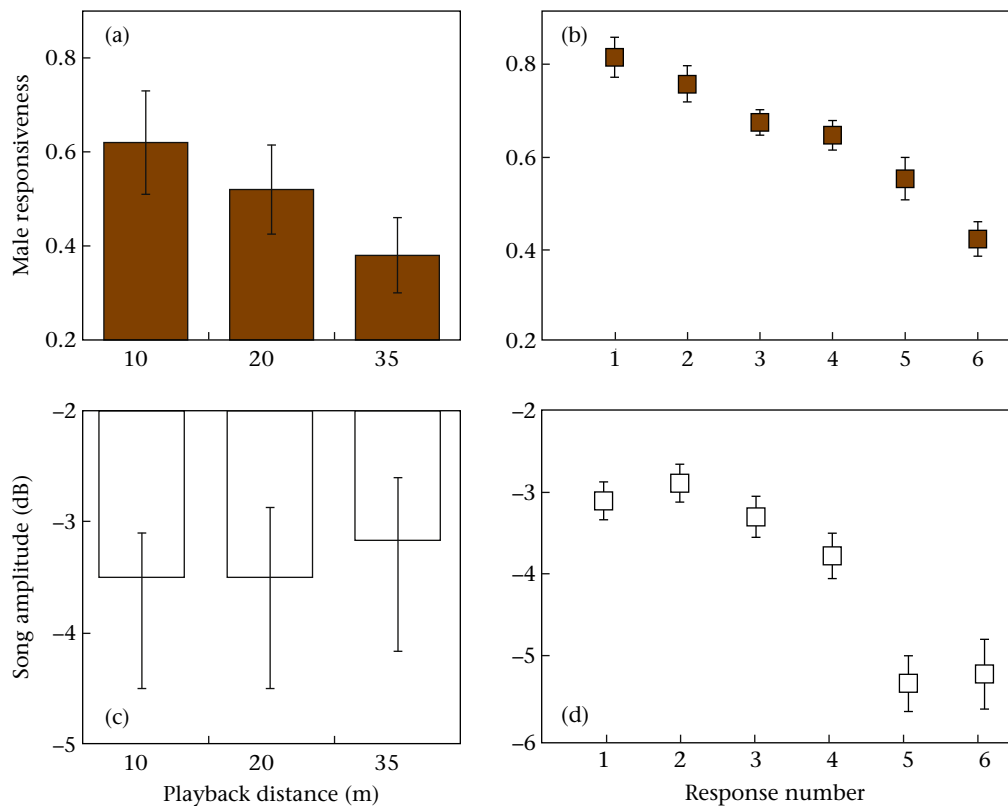


Figure 3. (a, b) The probability that a male would respond to playback of his female's songs in relation to (a) playback distance and (b) the position of the female playback phrase within the song series. (c, d) Maximum amplitude, dB(A), of a male's song in relation to (c) distance and (d) its position in the sequence of responses. Mean \pm SE proportions (a, b) or decibels (c, d) are shown.

to simply responding. These were also nonexperimental studies, with birds sometimes perched together (e.g. in the parakeets) so that the response may have been elicited by visual cues that preceded calling or through a rhythm that was established before the recordings were made. Thus it is possible that happy wrens can react even faster than we report here. Nevertheless, the results we present are the first we know of showing the speed of song type selection when replying in a duetting species.

Similar cognitive capacities may be found in the singing of nonduetting species. For example, in many territorial birds, neighbouring males countersing, alternating songs with each other. Often the specific song type selected or the exact timing of the reply is an important aspect of the signal (Catchpole & Slater 2008). Although most work on singing interactions has focused on signal function, a few studies have examined the latency of response to a rival's song. In countersinging meadowlarks, *Sturnella neglecta*, response latencies ranged from 3.8 to 8.9 s after the start of the previous bird's song (Falls 1985). Nightingales, *Luscinia megarhynchos*, often sing songs that overlap temporally with those of a neighbour, and response latencies for this species were considerably faster, at 0.3–1.6 s (Hultsch & Todt 1982; Kiefer et al. 2011; Geberzahn et al. 2013). In contrast to antiphonal duets, it is unclear how precisely timed an overlapping song must be for it to be an effective signal (and there is also debate about whether overlapping is in fact a signal; Searcy & Beecher 2009), but none the less, these latencies may represent the fastest response possible in these species. Both Falls (1985) and Geberzahn et al. (2013) reported that birds respond faster when hearing familiar songs and singing matching song types, suggesting the importance of pattern recognition and shared neural memory in determining reply latency. Although these species can time their songs to overlap or avoid overlapping with other individuals, duetting birds have the added challenge of being constrained in singing song phrases that both follow a duet code and are timed to follow an antiphonal duet structure.

In happy wrens, male and female phrases alternate in a cyclical manner, and we found that males adjust their song timing to correspond with that of the female. Even when the playback cycle was long, the male did not overtake her. An earlier observational study of barbets (Payne & Skinner 1970) concluded each sex has a rhythm of its own rather than influencing each other. However, in this case the song is not a tightly integrated duet like that of our wrens. Logue et al. (2007) studied similar issues in the closely-related black-bellied wren, *Pheugopedius fasciatoventris*, using natural recordings and playback to pairs in the field. Their results indicated that both males and females have internal tempos, but also adjust their timing in response to what their mate sings. The timing of the start of the female phrase influences the male but the timing of the end of the male phrase correlates more with when the female begins her phrase. As the phrases most often overlap, this implies that the female anticipates when his phrase will stop. Experimentally manipulating the tempo of the female phrases has allowed us to show that male happy wrens adjust their song rate to match their females. It is possible that females also adjust their tempo, accounting for the tight synchronization observed in our natural recordings. In another related species (*Pheugopedius euophrys*), Fortune et al. (2011) have recently shown that song nuclei (HVC) in the brains of both sexes respond to playback of a bird's mate's song, suggesting a neural mechanism that could help facilitate precisely coordinated duets in these wrens. There is clearly scope for more experimental work to tease apart the mechanics of the interaction between the sexes in duetting animals.

We also found that the closer the playback, the more likely the male was to respond. This may seem like a surprising finding given that happy wren songs are audible at much greater distances and

that birds respond to intruders much further away than this (Templeton et al. 2011). However, if duetting is primarily a joint signal directed to individuals outside the pair bond, males may be reluctant to sing when the female is further away. It may be more difficult to maintain duet precision at great distances, potentially producing a less potent signal. Also, it may not be in the male's best interests to advertise that he is far from his mate, because this might convey the impression of less active mate guarding or of a pair less likely to coordinate a united defence of their territory. As potential support for this idea, other species also seem more likely to respond to nearby mates (Hall & Magrath 2000). Mennill & Vehrencamp (2008) observed that free-ranging pairs of rufous-and-white wrens, *Thryophilus rufalbus*, produce duets at highly variable distances, some much greater than 100 m apart. So it is possible that our maximum distance of 35 m was not distant enough to necessitate an increase in amplitude. However, they also noted that the vast majority of duets were produced at close range (<10 m), similar to the results from this experiment. Using radio-telemetry, Logue (2007a) also noted higher response rates when pair members were closer together, and like Mennill & Vehrencamp (2008), showed that birds often approached their partner after duetting at a distance. It appears then that mate localization is at least sometimes an important function of duetting. Happy wren males were also more likely to respond to the earlier female phrases in a series of six and to do so at higher amplitude than their later responses. These changes may indicate that fatigue sets in when a male is stimulated to respond several times in quick succession, although longer series of songs are not uncommon in normal duetting. It is also possible that males lost motivation or interest in the playback towards the end of the series since the female recording failed to make subtle adjustments in timing and phrase selection to respond to his singing. However, we have observed a similar reduction in amplitude in later phrases of songs in wild birds under natural conditions (unpublished data), suggesting that the fatigue hypothesis may be valid.

We expected to find that males sang at higher amplitude to playback from further away to maximize the chances of getting the message across, just as captive zebra finches, *Taeniopygia guttata*, sing more loudly to females that are more distant (Brumm & Slater 2006). Whereas there may have been a slight tendency for this, it was not statistically significant. Consistent with this result is the hypothesis that duets are cooperative signals and are primarily directed to other birds outside the pair, rather than individual signals primarily directed towards a bird's mate (i.e. birds sing with, not to their mates). If so, each bird should attempt to maximize the transmission distance of its own song, so that it may not be surprising that the intrapair distance does not affect song amplitude. Perhaps happy wren pairs might instead modulate their singing by facultatively increasing song amplitude in response to a territorial challenge (Brumm & Todt 2004).

To conclude, these experiments have addressed how duetting birds integrate their duet contributions in the absence of other potential signals, and have revealed remarkably rapid cognitive processing in terms of reaction times, coordination and song type selection. In addition, we have introduced a new methodology that promises to be widely useful in future studies.

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References

- Bailey, W. J. 2003. Insect duets: underlying mechanisms and their evolution. *Physiological Entomology*, **28**, 157–174.
- Brown, R. N. & Lemon, R. E. 1979. Structure and evolution of song form in the wrens *Thryothorus sinloa* and *T. felix*. *Behavioral Ecology and Sociobiology*, **5**, 111–131.
- Brumm, H. & Slater, P. J. B. 2006. Animals can vary signal amplitude with receiver distance: evidence from zebra finch song. *Animal Behaviour*, **71**, 699–705.
- Brumm, H. & Todt, D. 2004. Male-male vocal interactions and the adjustment of song amplitude in a territorial bird. *Animal Behaviour*, **67**, 281–286.
- Catchpole, C. K. & Slater, P. J. B. 2008. *Bird Song. Biological Themes and Variations*. 2nd edn. Cambridge: Cambridge University Press.
- Falls, J. B. 1985. Song matching in western meadowlarks. *Canadian Journal of Zoology*, **63**, 2520–2524.
- Farabaugh, S. M. 1983. *A comparative study of duet song in tropical Thryothorus wrens*. Ph.D. thesis. University of Maryland.
- Fortune, E. S., Rodriguez, C., Li, D., Ball, G. F. & Coleman, M. J. 2011. Neural mechanisms for the coordination of duet singing in wrens. *Science*, **334**, 666–670.
- Geberzahn, N., Hultsch, H. & Todt, D. 2013. Memory-dependent adjustment of vocal response latencies in a territorial songbird. *Journal of Physiology*, **107**, 203–209.
- Geissmann, T. 2002. Duet-splitting and the evolution of gibbon songs. *Biological Reviews*, **77**, 57–76.
- Grimes, L. 1965. Antiphonal singing in *Laniarius barbarus* and the auditory reaction time. *Ibis*, **107**, 101–104.
- 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. A review of vocal duetting in birds. *Advances in the Study of Behavior*, **40**, 67–121.
- Hall, M. L. & Magrath, R. D. 2000. Duetting and mate-guarding in Australian magpie-larks (*Grallina cyanoleuca*). *Behavioral Ecology and Sociobiology*, **47**, 180–187.
- Hultsch, H. & Todt, D. 1982. Temporal performance roles during vocal interactions in nightingales (*Luscinia megarhynchos* B). *Behavioral Ecology and Sociobiology*, **11**, 253–260.
- Illes, A. E. & Yunes-Jimenez, L. 2009. A female songbird outsings male conspecifics during simulated territorial intrusions. *Proceedings of the Royal Society B*, **276**, 981–986.
- Kiefer, S., Scharff, C. & Kipper, S. 2011. Does age matter in song bird vocal interactions? Results from interactive playback experiments. *Frontiers in Zoology*, **29**, 1–8.
- 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. 2006. The duet code of the female back-bellied wren. *Condor*, **108**, 326–335.
- Logue, D. M. 2007a. Duetting in space: a radio-telemetry study of the black-bellied wren. *Proceedings of the Royal Society B*, **274**, 3005–3010.
- Logue, D. M. 2007b. How do they duet? Sexually dimorphic behavioural mechanisms structure duet songs in the black-bellied wren. *Animal Behaviour*, **73**, 105–113.
- Logue, D. M., Chalmers, C. & Gowland, A. H. 2007. The behavioural mechanisms underlying temporal coordination in black-bellied wren duets. *Animal Behaviour*, **75**, 1803–1808.
- Mann, N. I., Marshall-Ball, L. & Slater, P. J. B. 2003. The complex song duet of the plain wren. *Condor*, **105**, 672–682.
- Mann, N. I., Dingess, K. A. & Slater, P. J. B. 2006. Antiphonal four-part synchronized chorusing in a Neotropical wren. *Biology Letters*, **2**, 1–4.
- Mann, N. I., Dingess-Mann, K. A., Barker, F. K., 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. I. & Slater, P. J. B. 2006. Multiple functions to duet singing: hidden conflict and apparent cooperation. *Animal Behaviour*, **71**, 823–831.
- Mennill, D. J. & Vehrencamp, S. L. 2008. Context-dependent functions of avian duets revealed by microphone-array recordings and multispeaker playback. *Current Biology*, **18**, 1314–1319.
- Payne, R. B. & Skinner, N. J. 1970. Temporal patterns of duetting in African barbets. *Ibis*, **112**, 173–183.
- Power, D. M. 1966. Antiphonal duetting and evidence for the auditory reaction time in the orange-chinned parakeet. *Auk*, **83**, 314–319.
- Radford, A. N. 2003. Territorial vocal rallying in the green woodhoopoe: influence of rival group size and composition. *Animal Behaviour*, **66**, 1035–1044.
- Schulz, T. M., Whitehead, H., Gero, S. & Rendell, L. 2008. Overlapping and matching of codas in vocal interactions between sperm whales: insights into communication function. *Animal Behaviour*, **76**, 1977–1988.
- Searcy, W. A. & Beecher, M. D. 2009. Song as an aggressive signal in songbirds. *Animal Behaviour*, **78**, 1281–1292.
- Shettleworth, S. J. 2010. *Cognition, Evolution, and Behavior*. New York: Oxford University Press.
- Slater, P. J. B. & Mann, N. I. 2004. Why do the females of many bird species sing in the tropics? *Journal of Avian Biology*, **35**, 289–294.
- Templeton, C. N., Rivera-Cáceres, K. D., Mann, N. I. & Slater, P. J. B. 2011. Song duets function primarily as cooperative displays in pairs of happy wrens. *Animal Behaviour*, **82**, 1399–1407.
- Templeton, C. N., Rios-Chelen, A., Quiros-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.
- Thorpe, W. H. 1963. Antiphonal singing in birds as evidence for avian auditory reaction time. *Nature*, **197**, 774–776.
- 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, U.S.A.*, **95**, 1870–1875.
- Zollinger, S. A., Podos, J., Nemeth, E., Goller, F. & Brumm, H. 2012. On the relationship between, and measurement of, amplitude and frequency in birdsong. *Animal Behaviour*, **84**, e1–e9.