



Song duets function primarily as cooperative displays in pairs of happy wrens

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ARTICLE INFO

Article history:

Received 10 February 2011

Initial acceptance 21 March 2011

Final acceptance 9 September 2011

Available online 22 October 2011

MS. number: 11-00122R

Keywords:

cooperation
duetting
female song
happy wren
Pheugopedius felix
song
territory defence
Thryothorus felix
vocal communication

Vocal duetting occurs in diverse animal groups. Members of a mated pair may duet to communicate with each other or with other individuals. Many hypotheses have been proposed to explain the function of duets, and studies often provide support for the joint resource defence or mate-guarding hypotheses. We evaluated these hypotheses for the happy wren, *Pheugopedius felix*, using a two-speaker playback experiment. We observed the responses of happy wren pairs to playback of solo male, solo female and male/female duet songs, and compared these with heterospecific song control trials. Happy wrens responded aggressively to conspecific song playback by moving closer to their mate, approaching the playback speakers and increasing singing rates. Both sexes increased singing and especially duetting rates in response to conspecific playback. There were no differences in which sex initiated or terminated duets nor did birds vary the proportion of their partner's songs answered across conspecific treatments. Furthermore, neither sex treated unmated intruders (solo playback) as more threatening than mated intruders (duet playback). Together, these results argue against the mate-guarding hypothesis and instead indicate that duetting in happy wrens functions primarily in cooperative territory defence. Overall, males sang more than females, moved closer to the speakers and were more likely to answer their partner's songs, suggesting that males take a primary role in territorial defence. However, females also responded strongly, especially when female intruders were present (duet or female solo playback), which suggests a sex-specific division of labour in their territorial defence.

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Duets are complex acoustic displays involving the production of coordinated songs by two different animals (Hall 2009). Duetting has been reported in a number of animal groups, including insects (Bailey 2003), frogs (Emerson & Boyd 1999), mammals (Geissmann 1999; Muller & Anzenberger 2002; Schulz et al. 2008) and birds (Catchpole & Slater 2008; Hall 2009). In birds, vocal duets have been found in at least 420 species in 55 families (Farabaugh 1982; Benedict 2008; Hall 2009), with the majority of species being tropical (Langmore 1998; Stutchbury & Morton 2001; Slater & Mann 2004).

Many hypotheses have been proposed for the function of song duetting in birds (reviewed in Farabaugh 1982; Hall 2004, 2009; Douglas & Mennill 2010). Like solo song (Beecher & Brenowitz 2005; Catchpole & Slater 2008), duet songs seem to be

multipurpose signals that generally function to defend territories or communicate with mates. However, unlike solo songs, duets require that members of a pair are attentive to their mate's songs. Duet songs, and their relative degree of coordination, may therefore also function to communicate information about the quality or stability of a pair bond (Wickler 1980; Smith 1994; Hall & Magrath 2007). Duets may function more effectively than solo songs to help birds defend territories by providing a unified front against intruders (Logue & Gammon 2004; Rogers et al. 2004; Molles & Waas 2006) or allowing for a division of labour in territorial defence (Levin 1996; Marshall-Ball & Slater 2004). Another common hypothesis for duetting is that it functions in mutual mate or paternity guarding (Stokes & Williams 1968; Grafe & Bitz 2004; Mennill 2006; Rogers et al. 2007; Illes & Yunes-Jimenez 2009). Duetting has also been hypothesized to help maintain contact in dense habitats, synchronize reproductive efforts, and signal identity or commitment (reviewed in Farabaugh 1982; Hall 2004, 2009; Douglas & Mennill 2010). Numerous studies have been conducted to test each of these hypotheses, but because some studies support and others refute each hypothesis, experimental evidence is still somewhat conflicting as to the function of song duets.

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With such a wide variety of species singing duets, it is not surprising that experimental evidence suggests that duetting appears to serve a variety of different functions (Catchpole & Slater 2008; Hall 2009). Perhaps more surprising is that some studies have indicated that multiple functions can be served by duets even within single species, depending on the singing context (Sonnenschein & Reyer 1983; Grafe & Bitz 2004; Mennill & Vehrencamp 2008). Furthermore, the types of hypotheses that can be tested, and even the interpretation of the subjects' responses, may depend on the experimental design used (Douglas & Mennill 2010). For example, playing stereo duets using two speakers allows an experimenter to examine how birds respond to different parts broadcast from each speaker (e.g. male from one and female from the other), which is not possible with a single-speaker playback design (reviewed by Douglas & Mennill 2010). Some species even respond differently when the same stimuli are broadcast from two speakers instead of one (Molles & Waas 2006; Rogers et al. 2006). Stereo duet playback has now been used to examine duetting in a number of species, but results from these studies still suggest that duetting probably has different functions in different species (Hall 2004, 2009). Thus, previous studies have supported many different hypotheses for the function of duetting yet an overarching theory remains elusive. Examining duetting in more species of birds may help us to understand its function better.

We investigated song duetting in the happy wren, *Pheugopedius felix* (formerly *Thryothorus felix*). Happy wrens are small insectivorous birds which are endemic to the Pacific slope of Mexico (Brewer 2001). Male and female happy wrens sing sex-specific song types, and each bird has in the range of 30–40 different song types in its repertoire with no sharing of song types between the sexes (Brown & Lemon 1979; Mann et al. 2009). Both sexes can sing solo songs, although males generally sing many more solo songs than females. Mated pairs of males and females also sing duets which are generally sung in a 'reel' or 'train' format to make discrete duet songs of repeated male and female songs, with each sex's contribution alternating or slightly overlapping with the other's (Mann et al. 2009). Either sex can initiate or terminate a duet, although males are more likely to sing both the first and last songs in a duet (Brown & Lemon 1979; Mann et al. 2009).

We examined the function of solo and duet song in the happy wren by simulating territorial intrusions using a two-speaker playback experiment (Logue & Gammon 2004; Rogers et al. 2004; Douglas & Mennill 2010). We broadcast solo male, solo female, duet and heterospecific control songs to pairs of happy wrens and observed their behavioural and vocal responses to gain insights into the function of duetting and territorial defence in this species. Although both the mate guarding and joint territorial defence hypotheses predict increased duetting in response to conspecific playback, the critical distinction is in the relative threat posed by duets and same-sex solo intruders (Hall 2004). The mate-guarding hypothesis predicts that unmated solo intruders should elicit a stronger response from same-sex territory holders than should paired duetting intruders, whereas the territory defence hypothesis predicts that solo and paired intruders should elicit similar intensities of response. Thus, if duetting behaviour functions mainly in mutual mate guarding, we predict that subjects should rarely sing solos during opposite-sex playback and should move closer to their partner and create more duets during same-sex solo playback than during duet playback. If duetting instead functions primarily for joint territory defence, we expect no difference between the responses to these different sorts of playback or that duetting intruders will receive stronger responses than single same-sex intruders.

METHODS

Study Population

We studied happy wrens at the Estación de Biología Chamela, a field research station operated by the Universidad Nacional Autónoma de México (UNAM) and located in the state of Jalisco, Mexico (19° 30' N, 105° 03' W). The station is in the Chamela–Cuixmala Biosphere Reserve, an area largely covered in well-preserved tropical deciduous forest. In the dry season (generally November to June), most trees lose their leaves, although there are small numbers of evergreen trees located along the (dry) riverbeds.

Happy wrens are common throughout the reserve and seem especially abundant near the riverbeds in the dry season. This study was conducted on 17 pairs of happy wrens. Prior to this study, we marked at least one member of most pairs (six pairs with both, nine pairs males only, two pairs neither banded) with a unique combination of three plastic coloured bands and one numbered aluminium band, and mapped their territory boundaries. Territories vary in size, but are roughly 40 000 m² and are frequently centred near streambeds. We mapped territory sizes and boundaries by observing singing behaviour and territorial interactions with neighbours prior to the study. Our playback study was conducted from 16 to 23 July 2010, a period coinciding with the onset of breeding behaviour in most pairs (Brewer 2001; brood patches observed in some females in the population), but we do not have specific information on breeding stage for any of the pairs in this study.

Playback Stimuli

All of our playback stimuli were based on single-channel, duet recordings which were recorded from the same population in 2003 (Mann et al. 2009). Birds that were recorded in 2003 were also colour banded, but we did not find any of these birds in 2010. Thus our playback recordings should be true to any population-specific dialects that might exist, but should not be associated with any particular living individual (i.e. neighbours, etc.). We used a total of six duets originating from four different pairs and selected only recordings of especially high quality (i.e. strong signal to noise ratio, with no other species vocalizing in the background). These one-channel recordings were duplicated into two separate channels (left and right) using Audacity (www.audacity.sourceforge.net). We then used Syrinx (J. Burt, www.syrinxpc.com) to clean these files (details below) and create different playback stimuli, which were stored as uncompressed .wav files.

We first created stereo duet playback stimuli following Douglas & Mennill (2010). To create a two-channel recording that could be broadcast through two different speakers, we carefully removed all songs of one member of the pair from each channel using the frequency cursor filter function in Syrinx. Each stimulus was then normalized to peak at 0 dB. The end product was a two-channel, stereo sound file containing one channel of male songs and another channel of female songs, with the exact timing of the original duet retained (Fig. 1). Which channel (left or right) was used for the contribution of each sex (male or female) was determined randomly. Each duet lasted for approximately 10 s and contained about five songs from both the male and female (mean \pm SE: male songs: 5.5 \pm 0.4; female songs: 5.2 \pm 0.3). Each duet was repeated every 30 s to create a 150 s file which contained five total bouts of duet song (including approximately 25 male and 25 female songs in total), with the bouts separated by about 20 s of silence. This pattern of sound production is a realistic one for an actively singing pair.

We used these two-channel duet recordings to create playback stimuli for the solo male and solo female treatments. For both treatment stimuli, we retained one channel from the duet stimuli

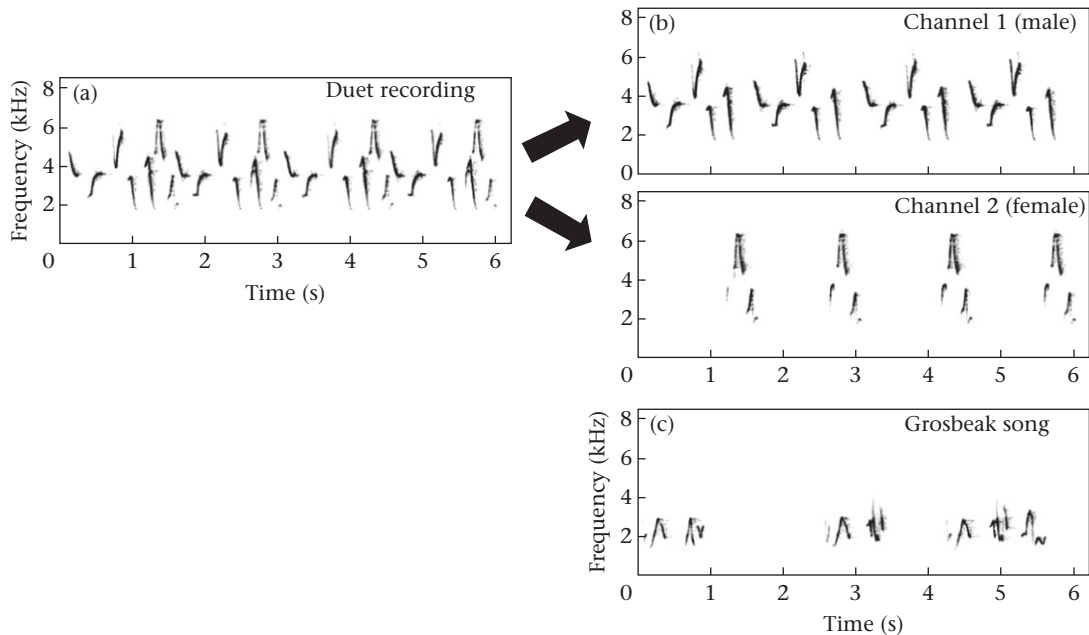


Figure 1. Playback stimuli were created from single-channel duet recordings (a). This particular duet song contains four songs each from the male and female and was created by the female (who sang second) and terminated by the male (who failed to answer the female's last song). From these duet recordings we isolated the contributions of the male and female into two separate channels to create a stereo recording (b). Single channels (male or female) from these recordings were broadcast as the male and female solo treatments (to different pairs of subjects). We used songs from yellow grosbeaks as control stimuli (c).

and inserted silence into the other channel. Thus, for each duet recording described above, we also made corresponding male solo and female solo playback stimuli. As control stimuli, we used songs from yellow grosbeaks, *Pheucticus chrysopheplus*, that had been recorded from three different individuals at the study site. The control stimuli were broadcast on one channel and had a similar pattern of songs and intervals between them to the treatment stimuli (Fig. 1), with approximately 10 s of songs followed by 20 s of silence, the entirety of which was repeated five times for a 150 s playback. Yellow grosbeaks are common at the study site and are conspicuous singers. Our playback stimuli appeared to mimic the singing of this species accurately since territorial male grosbeaks frequently countersang and closely approached the speaker during control playback trials.

Playback Procedure

Experiments were conducted between 0700 and 1030 hours (13 pairs) and between 1900 and 2030 hours (four pairs), the time periods that correspond with the highest rates of happy wren singing behaviour (C. N. Templeton, K. D. Rivera-Cáceres, N. I. Mann & P. J. B. Slater, unpublished data). We controlled for time of day, so that all treatments were presented to the same pair in the morning or evening, and we observed no obvious differences in response between morning and evening trials. For all treatments, we set up a pair of speakers (Pignose 7-100; frequency response 100 Hz–12 kHz) which were separated by 10 m. The speakers were connected to an Apple iPod, which we controlled from approximately 20 m away. Because the vegetation was thick and difficult to move through, we usually placed the speakers along a road or trail. Two observers monitored playback responses, with one on either side of the speakers. This set-up maximized our ability to locate birds and assess their distance from the speakers while minimizing our influence on their approach response. Each focal pair received each of the four treatments on consecutive days in a randomized order. Although we used female and male contributions of a duet playback as our female and male solo playback stimuli, we used

different playback exemplars for each pair (i.e. a given pair might hear duet A, male solo B and female solo C, whereas another pair might hear duet B, male solo C and female solo A).

During the 150 s playback and the following 10 min, we recorded all songs from the focal pair and estimated the distance of each bird from the playback speakers and from each other. Because of the difficulty in observing birds in the dense vegetation, we assessed distances by estimating the location of birds when they sang. When we did see the birds, we could confirm the accuracy of this estimation technique and in these cases we had correctly judged the location of the bird within about 1–3 m. For the solo and control treatments, we recorded distances relative to the active playback speaker. For the duet treatment, we recorded the distance of each subject to each speaker separately, and also noted which speaker (male or female) each bird approached more closely.

We recorded each song sung by the male and the female and noted whether these were solo songs or parts of a duet. We defined a duet as any occasion when at least one song each from the male and female was sung in succession, although a given duet song could include anywhere from two songs (one each) up to about 40 songs (20 each). Male and female songs are sung antiphonally or with slight overlap so duets are clearly defined units in this species (Mann et al. 2009). In practice there is no difficulty in distinguishing duets from occasions when male and female songs are sung in loose association because the timing of duets is fixed and precise. Male and female contributions to duets were not always equal and it was typical to have duets with unbalanced numbers of songs from the two members of the pair (e.g. two from the male, one from the female or vice versa). In addition to singing rate, we also noted how often each member of a pair switched song types during the trial, which sex initiated and terminated each duet, and the proportion of its partner's songs a given bird made into duets by responding with its own songs.

Data Analysis

Our basic approach to data analysis was to use general linear mixed models (GLMM) in JMP version 8.0 (SAS Institute Inc., Cary,

NC, U.S.A.) using the restricted maximum likelihood method. We used focal pair identity as a random effect and within-pair trial order (1–4), playback treatment (control, male solo, female solo, or duet), time of day (morning or evening) and, where appropriate, each focal bird's sex (male or female) as fixed effects. We also examined interactions between treatment and sex and between treatment and order. The time of day (morning/evening) factor or the treatment*order interactions were never significant (all $P > 0.2$), so we dropped these terms from the final analyses and do not report them in the results. If the treatment*sex interaction was not significant, we also reran the model without this term, although in no case did dropping this term substantially affect the results. We followed these analyses with post hoc Tukey tests (overall alpha = 0.05) to assess the statistical relationships between specific treatments, presentation orders and birds' sexes. For most statistical tests, our sample size was 17 pairs, although a few analyses necessitated using subgroups of the data (e.g. when examining the proportion of partner's songs a bird responded to, we could only examine trials where the partner sang at least once) and exact sample sizes for those comparisons not based on 17 pairs are indicated with the results. For all tests, we report two-tailed P values.

For distance measures, we analysed the closest distance that each subject came to the playback speaker(s). If a subject was not observed to approach the speaker during the trial we used an arbitrary distance of 80 m, which was similar to the distance (80–100 m) at which birds were observed in other weak responses and should generally be conservative because the territory is much larger than this. Given that our distance estimates were based primarily on estimating the location of singing birds rather than on visual observation, it is possible that silent birds were closer or further from the speakers in some trials. In addition to closest approach to the speakers, we measured the closest distance between members of a pair during each trial. If only one member of the pair was detected during a trial, we used 20 m as the distance between members. Again, this distance corresponded with larger distances observed during trials where both members were observed and should be conservative.

For song measures, we first calculated the overall singing rate per minute of each bird in a focal pair for each treatment as a general measure of each bird's territorial response level. We then analysed three different categories of singing behaviour: number of solo male, solo female and duet songs. We both compared the singing response across treatments within each of these categories and examined whether males and females sang at equal rates within treatment categories. Last, to examine whether individual birds or pairs responded with consistent singing behaviours to different types of intruders, we looked at the consistency in singing rate across treatments using Pearson correlations.

For song switching rate, we counted the total number of times a subject switched song types during the trial. Note that this is not necessarily the same as counting different song types used during the course of the trial, as some birds would sometimes switch back and forth between song types. In addition to the analyses described above, we examined whether members of a pair tended to switch song types at the same rate using linear regression.

To examine patterns of duetting across treatments, we analysed which sex created and terminated each duet song. The bird that sang second (thereby answering its partner's song) was said to have created the duet. Because duets are variable in length, the member not singing the last song (thereby failing to answer its partner's song) was said to have terminated the duet. We assessed the propensity to duet for each sex in each trial by calculating the proportion of the partner's songs that were joined to form duets (e.g. male propensity to duet = female-led duets)/(female-led

duets + female solo songs)). Last, we examined whether one sex was more likely to create or terminate duet songs and whether this varied across playback treatments.

The work was approved by the University of St Andrews Animal Welfare and Ethics Committee and conducted under Mexican Secretary of the Environment and Natural Resources Permit number 00504.

RESULTS

Approach

Closest approach distance was influenced by subject's sex (GLMM: $F_{3,109} = 11.48$, $P = 0.001$), treatment ($F_{3,109.1} = 11.68$, $P < 0.0001$) and trial order ($F_{3,110.1} = 3.38$, $P = 0.021$), and there was no interaction between treatment and sex ($F_{3,109} = 0.97$, $P = 0.41$). Playback trial order did not have a straightforward habituation effect on subject response, but instead responses decreased significantly during the second trial but then increased again so that the third and fourth trials were not distinguishable from either the first or second (Tukey test, overall alpha = 0.05). Males generally approached the speakers more closely than females (Fig. 2), but this difference was statistically significant only for the male solo playback (paired t test: $t_{16} = 4.38$, $P = 0.0005$; control: $P = 0.07$; duet: $P = 0.08$; female solo: $P = 0.16$). Male subjects approached the speakers more closely whenever happy wren song was broadcast compared with the control playback, but females approached closer to duet and solo female playback compared to the control treatment, with their response to solo male being intermediate and not statistically distinguishable from either group (Tukey tests; Fig. 2).

Within pairs, males moved significantly closer to the speaker than their mate during the male solo playbacks (equal 18%, male closer 82%, female closer 0%; sign test: $P = 0.0001$) and control playbacks (equal 17%, male closer 75%, female closer 8%; $P = 0.25$), but male and female partners did not differ in the distance they approached the speakers during female solo or duet playbacks and most commonly approached to equal distances (female solo: equal 64%, male closer 14%, female closer 21%; duet: equal 57%, male closer 36%, female closer 7%; sign tests: $P > 0.2$). Following

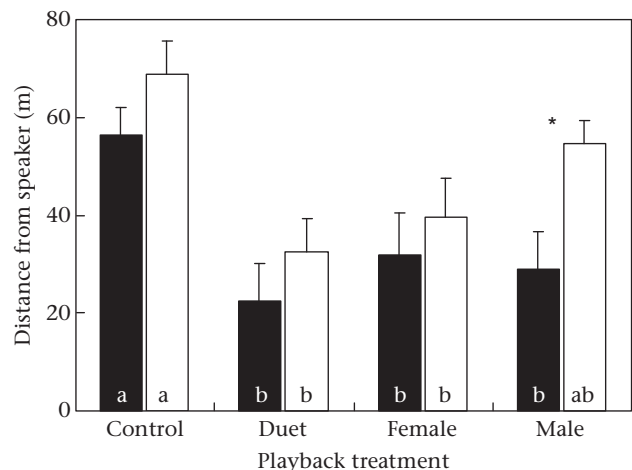


Figure 2. Closest approach to the speaker (mean + SE) for each sex in response to playback of grosbeak control, male–female duet, female solo and male solo stimuli. Male responses are shown in black and female responses in white. Within each sex, groups with the same letter are not statistically different from each other according to a Tukey test of honestly significant differences. Asterisk indicates a significantly different response between males and females.

playback, males were also more likely to stay near the speaker longer than their mates. This pattern was especially true for the male solo playback trials (equal 23% of trials, male longer 77%, female longer 0%; sign test: $P = 0.0001$). In duet playback trials, pairs were more likely to spend equal lengths of time near the speakers (equal 50%), although when one pair member responded for longer it was the male (male longer 50%, female longer 0%; sign test: $P = 0.015$). Female solo playback was the only stimulus that, for certain pairs, elicited longer responses by the female subjects than their mates (female longer 14%). However, the majority of pairs exhibited responses of similar duration (equal 57%) and overall one sex did not respond for longer than the other (male longer 28%; sign test: $P = 0.6$).

During stereo duet playback trials, each subject tended to approach the same-sex speaker. More males approached the male speaker than the female speaker (nine of the 12 males that approached moved closer to the male speaker, three moved equally close to both speakers, and none moved closer to the female speaker; sign test: $P = 0.039$), whereas approaching females tended to move closer to the female speaker than the male speaker (seven of the 10 females that approached the speakers moved closer to the female speaker, three approached the male and female speakers equally, and none approached the male speaker more closely; sign test: $P = 0.015$). When both the male and female approached the speakers, each sex tended to approach the same-sex speaker more closely than the opposite-sex speaker (7/10 pairs; 3/10 pairs approached both speakers equally, 0/10 approached the opposite-sex speaker more closely; sign test: $P = 0.015$).

Birds spent more time near their mate following intruder playback as compared with the control (GLMM: $F_{3,48} = 4.33$, $P = 0.009$; Tukey test; Fig. 3). Playback order affected response ($F_{3,45.8} = 4.25$, $P = 0.01$), again with distances significantly increasing in the second trial and then decreasing in subsequent trials to be indistinguishable from either the first or second trials (Tukey test). Playback treatment also affected response levels ($F_{3,45.1} = 5.39$, $P = 0.003$): males and females moved closer together following duet and solo female playback relative to the control, with the response to male solo playback statistically indistinguishable from these or the

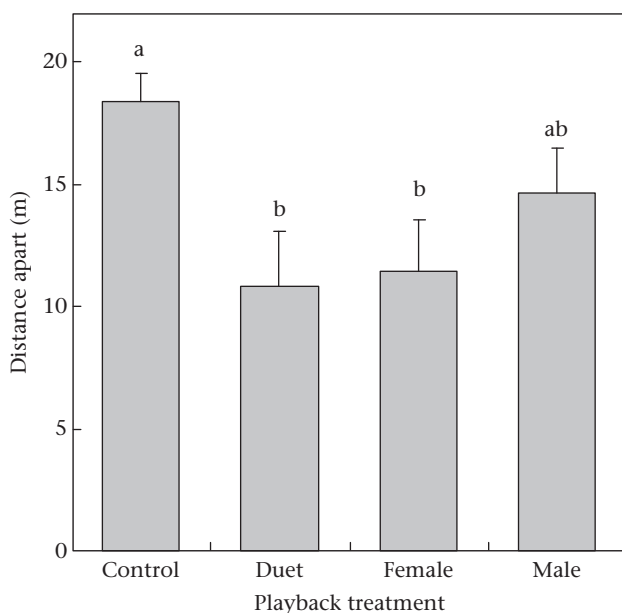


Figure 3. Closest distance (mean + SE) observed between the male and female of each mated pair during each playback treatment. Treatment groups with the same letter are not statistically different from each other.

control (Tukey test). The closest approach distance to the speaker(s) during a trial was also highly correlated between males and females within a pair (Pearson correlation: $r_{32} = 0.71$, $P < 0.0001$). There was a strong inverse relationship between the distance between birds in a pair and the number of duet songs they sang across all treatments, with birds producing more duets when in close proximity to each other (Pearson correlation: $r_{32} = -0.85$, $P < 0.0001$).

Singing Behaviour

Subjects' singing rates were affected by the playback treatment (GLMM: $F_{3,109.1} = 7.95$, $P < 0.0001$), their sex ($F_{3,109} = 20.90$, $P < 0.0001$) and the order of playback ($F_{3,109.7} = 2.84$, $P = 0.041$). However, we did not see any interactions between these factors in the model (treatment*sex: $P = 0.46$). Singing rates decreased slightly after the first trial but not between trials 2 and 4 (Tukey test). Males sang more songs in total than females across all treatments, with the greatest difference during the male solo playback treatment. Males had high song output in response to playback of any type of happy wren song (male solo, female solo or duet) relative to the control playback (Tukey test). Females exhibited a similar pattern, but showed a nonsignificant tendency to sing less to male solo playback (Tukey test).

Solo and duet song rates followed different patterns (Fig. 4). Solo song rates were primarily influenced by the bird's sex (GLMM: $F_{1,109} = 44.33$, $P < 0.0001$), with males singing more than females (Tukey test), but not by the treatment ($F_{3,109.3} = 1.93$, $P = 0.13$), order of trials ($F_{3,110.5} = 0.68$, $P = 0.57$) or treatment*sex interactions ($F_{3,109} = 2.10$, $P = 0.11$). In contrast, duetting rates were strongly affected by treatment ($F_{3,112.1} = 7.93$, $P < 0.0001$) and trial order ($F_{3,112.7} = 6.88$, $P = 0.0003$), but since both sexes must contribute to duets, they were not affected by the sex of a bird ($F_{1,112} = 0.29$, $P = 0.59$) or the interaction between sex and treatment ($F_{3,109} = 0.03$, $P = 0.99$). Order had a similar effect as described for other variables, with a pronounced decrease after the first trial and a rebound so that later trials were not significantly different from either the first or second trial (Tukey test). Subjects had higher duetting rates for female solo and duetting playbacks compared with the control, but somewhat lower responses to male solo playback, which was lower than female solo but not statistically different from the control or duet treatments (Tukey test).

Individual males showed fairly consistent singing levels among conspecific playback treatments (Pearson correlation: $r_{32} > 0.55$, $P < 0.020$ for all treatments; Fig. 5). However, females showed a more surprising pattern: most females that exhibited high singing rates during the duet playback had low or nonexistent singing rates for the female solo playback and vice versa (Fig. 5). We found a positive correlation between male and female singing rates across all treatments, with the highest correlations during the duet ($r_{32} = 0.74$, $P = 0.0008$) and solo female ($r_{32} = 0.68$, $P = 0.003$), and somewhat lower correlations during the solo male ($r_{32} = 0.62$, $P = 0.007$) and control ($r_{32} = 0.59$, $P = 0.012$) trials. There was a positive correlation between a female's response to duet playback and her response to solo male playback ($r_{32} = 0.61$, $P = 0.010$).

Males generally switched song types more frequently than females (paired t test: $t_{16} = 2.46$, $P = 0.016$), although, within a given treatment category, this difference was significant only for the duet playbacks (paired t test: $t_{16} = 2.20$, $P = 0.043$; all other treatments: $P > 0.3$). Despite males switching song types more than females, we observed a fairly strong correlation between male and female switching rates (Pearson correlation: $r_{32} = 0.70$, $P < 0.0001$). The correlation between male and female switching rates varied by treatment, with switching rates most highly coordinated during duet playback ($r_{32} = 0.80$, $P < 0.0001$), and somewhat less correlated during female solo playback ($r_{32} = 0.48$,

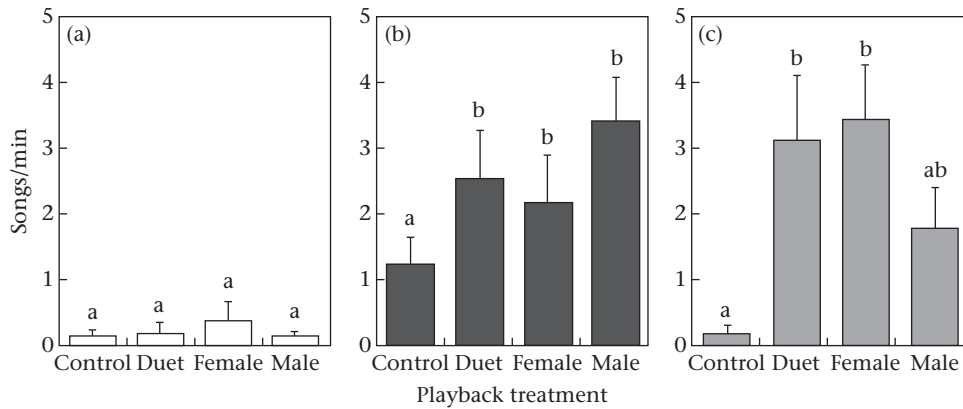


Figure 4. Singing rates in response to each playback treatment. Mean song rates per minute \pm SE are shown for (a) female solo songs, (b) male solo songs and (c) duet songs. Statistically indistinguishable groups within each singing style are shown with the same letter. In (c) the lower number of duets produced in response to the male solo playback is due primarily to reduced female singing during this treatment.

$P = 0.049$). Song switching rates were even less coordinated during the male playback trials ($r_{32} = 0.45$, $P = 0.073$). Switching rates were affected by the playback treatment ($F_{3,109.1} = 7.1$, $P = 0.0002$), but not order ($F_{3,109.7} = 2.1$, $P = 0.10$), sex ($F_{1,109} = 2.91$, $P = 0.09$) or

a treatment*sex interaction ($F_{3,109} = 0.38$, $P = 0.77$). Both sexes switched song types more during conspecific playbacks than the control (Tukey test).

Mean duet length was 4.61 ± 0.23 songs ($N = 13$ pairs, 32 total trials with at least one duet sung), and this did not vary across treatments (GLMM: $F_{3,17.1} = 2.24$, $P = 0.12$) or by trial order ($F_{3,17.5} = 1.48$, $P = 0.25$). When birds sang duets, females were more likely to create duets (by singing second) than males, with $78 \pm 4\%$ of duets across all trials being formed by females. Females were also slightly more likely to terminate duets (by failing to respond to their partner's song in a duet), with $63 \pm 5\%$ of duets terminated by the female across all trials. There were no effects of treatment ($P > 0.14$) or trial order ($P > 0.13$) on which sex initiated or terminated a duet. A bird's propensity to duet was affected by treatment (GLMM: $F_{3,62.6} = 6.88$, $P = 0.0006$), sex ($F_{1,64.9} = 12.79$, $P = 0.0007$) and trial order ($F_{3,65.8} = 3.84$, $P = 0.014$), with a nonsignificant interaction between treatment and sex ($F_{3,56.8} = 2.25$, $P = 0.09$; Fig. 6). Males were more likely to answer their partner's songs ($65 \pm 8\%$) than were females ($27 \pm 4\%$) across trials (paired t test: $t_{16} = 2.57$, $P = 0.015$). The likelihood of a male responding to its partner's songs was greater with conspecific playback but there were no differences between the three types of conspecific trials (Tukey test). In contrast, females increased the proportion of their partner's songs that they answered during female solo playback relative to the control. Female responses to solo male playback did not differ from the control treatment, and their response to duet playback was not statistically different from the female solo or control/solo male playback treatments (Tukey test). Responses also varied according to trial order in the same way as described previously.

DISCUSSION

Male and female happy wrens sang and approached the speakers during playback of intruder songs, indicating an aggressive response to territory intruders. In general, males had higher singing rates and approached the speakers closer than females. Most male subjects sang and approached the speaker whenever they heard an intruder, regardless of the intruder's sex or whether they heard duet or solo playback. In contrast, female subjects tended to sing and approach the speaker primarily when they heard playback of female songs, either in solo or duet form, with somewhat reduced responses to solo male intruders, although this difference was statistically significant with only some measures. Although females overall responded strongly to the duet and

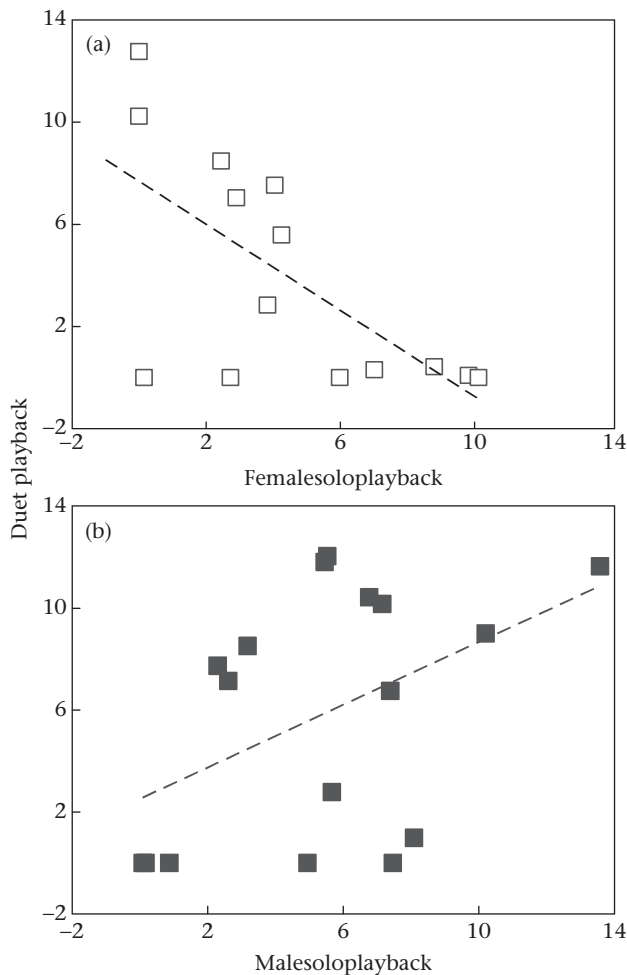


Figure 5. Patterns of response consistency across trials. (a) Female singing rates during female solo and duet playback (Pearson correlation: $r_{32} = -0.57$, $P = 0.01$). (b) Male singing rates during male solo and duet playback treatments (Pearson correlation: $r_{32} = 0.46$, $P = 0.05$).

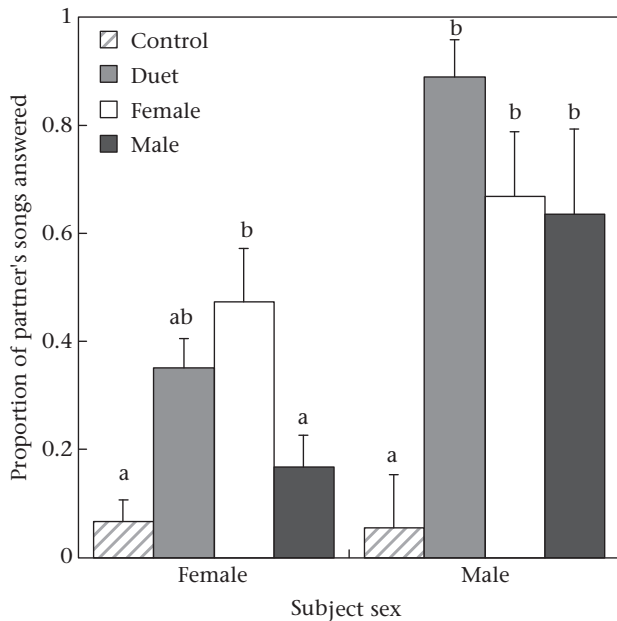


Figure 6. Male and female propensity to duet during each playback treatment. Within each sex, significantly distinct groups are distinguished by different letters.

female solo treatments, specific females tended to respond strongly only to one of these, with a significant negative correlation between their responses. When both sexes approached simulated duetting intruders, they exhibited sex-specific territorial defence, with about 75% of birds approaching the same-sex speaker more closely and none approaching the opposite speaker more closely. Duetting rates were higher in response to conspecific intruders, with the highest rates during solo female and duet playbacks. Females tended to create and terminate more duets than males, by singing second and failing to reply to their mate's final duet song, but we saw no effect of playback treatment on which sex initiated or terminated a duet. Last, we observed an unusual order effect, with subjects somewhat reducing their response to the second playback, but returning to the original response levels for subsequent trials.

Although increased singing and approach in response to playback is consistent with both mate guarding and joint territory defence, these hypotheses make different predictions as far as duetting behaviour is concerned (detailed in Hall 2004; Douglas & Mennill 2010). Specifically, in contrast to joint territorial defence, the mate-guarding hypothesis predicts that (1) solitary (i.e. solo singing), same-sex intruders should be perceived as a higher threat than paired, duetting intruders because solitary intruders represent a greater threat to a bird's mate or pairing status than paired intruders, but less of a threat to the territory. The mate-guarding hypothesis also predicts that birds (2) should rarely sing solos during opposite-sex playback and (3) should create duets primarily during same-sex solo playback. Our results do not support any of these predictions. In this study, solitary intruders were never treated as if they posed a higher degree of threat than paired intruders. We saw no effect of the mated status of an intruder on the solo singing rate of either males or females. Last, we saw no effect of treatment on which bird created duet songs. Thus, it appears that mate guarding is not the primary factor driving duetting behaviour in happy wrens. Instead, the results suggest that duetting has evolved mainly to facilitate joint territorial defence. However, we also observed several sex-specific differences in response to different types of territorial intruders suggesting that each sex has a slightly different strategy for responding to intruders.

Males tended to respond to all intruders equally strongly. This result could be explained in several ways. First, males may not be able to discriminate between male and female songs, although this seems unlikely given the clear acoustic differences between the sexes (Brown & Lemon 1979; Mann et al. 2009). Next, males may perceive male and female intruders as equally threatening, which is consistent with the territorial defence hypothesis (Wickler 1980). Last, males may discriminate between male and female intruders, but we were unable to distinguish between their responses to these stimuli with the measures we used (approach and song rate). It seems possible that males may be motivated both to drive away male intruders and to seek extrapair copulations with intruding females, but since both of these objectives are expressed as the male approaching the playback speakers and increasing song rates, it is difficult to distinguish between them. However, males were as likely to answer their partner's songs when single females intruded as they were when single males did so. Furthermore, during the duet treatment, most males selectively approached the male speaker, rather than the female speaker, and this suggests that they are more concerned with territorial or mate defence than attracting other females.

In contrast to male behaviour, females tended to respond more strongly when another female (either mated or unmated) intruded on their territories, although the statistical significance of these differences varied between measures. Females sang more and were more likely to answer their partner's songs when we simulated a female intruder than a solo male intruder. Furthermore, most female subjects preferentially approached the female speaker during duet playback. Because a female responds most strongly when it is another female intruding into her territory, regardless of whether this female is paired or not, it seems likely that females preferentially defend their territory, not their mate, from intruding females. This type of territorial defence may be particularly important if territories are limited, sex ratios are female biased, or high levels of divorce and re-pairing occur. It could be that in these situations, the act of a male joining his mate to produce duets, and potentially even the quality of the duets produced, might signal a male's commitment to his partner.

This general pattern of sex-specific territoriality is similar to that observed in several other species. In rufous-and-white wrens, *Thryophilus rufalbus* (Mennill 2006), buff-breasted wrens, *Cantorchilus leucotis* (Gill et al. 2007), tropical boubous, *Laniarius aethiopicus* (Grafe & Bitz 2004), and eastern whipbirds, *Psophodes olivaceus* (Rogers et al. 2007), females also generally respond only when they hear female song, either in solo or duet form, whereas males tend to respond to any conspecific playback. These studies have used a variety of methods, including single-speaker playback (Grafe & Bitz 2004), dual stereo speaker playback (Mennill 2006) and even live birds in cages (Gill et al. 2007), to simulate territorial intrusion. Thus, although many other response patterns have been described (reviewed in Hall 2009; Douglas & Mennill 2010), the pattern we observed seems common across a variety of species and playback methodologies. The order effect we observed, with decreased responses to only the second playback treatment, is more unusual. Clearly, it does not represent a straightforward habituation effect, but it may represent a trade-off between initial habituation after the first trial and heightened response from repeatedly facing territorial intruders, a situation that is unlikely to be common in nature.

We observed that birds were more likely to produce duets when they were close together, and on a number of occasions we saw pairs singing together when they were sitting beside each other. This pattern may be an artefact of the design of the study rather than a response to intrusion: since birds are more likely both to duet and to approach the speaker during playback, it is possible

that the close proximity of birds is driven principally by both birds approaching the speakers. However, similar results from natural singing interactions have been reported for magpie-larks, *Grallina cyanoleuca* (Hall & Magrath 2000), and Mennill & Vehrencamp (2008) observed that rufous-and-white wrens move closer to each other during bouts of duetting. These observations also argue against duetting being simply a means whereby birds locate or keep track of each other in dense vegetation (Logue & Gammon 2004).

The observation that birds switch song types more frequently during the duet playback is intriguing. It is possible that quickly cycling through a song repertoire is advantageous in territorial defence. Another possibility is that our subjects switched songs to try to match the songs of the intruder as an aggressive signal, as occurs in other species (Catchpole & Slater 2008). In aggressive situations, some duetting birds use song matching, with individual birds matching the same-sex intruder (Rogers et al. 2006) or pairs singing matching duet song types (Logue 2006). It is possible that our subjects did not possess appropriate songs to match the playback stimuli, although we do not yet have complete repertoire information for subjects. If different pairs of happy wrens use different duet 'codes' to combine male and female songs, as in some other species (Logue 2006), then it is also possible that subjects found it difficult to match both the playback songs and the songs of their partner (Marshall-Ball & Slater 2004; Rogers et al. 2006). For example, if a given female subject usually uses song type A to match a given song of her partner but the intruding female playback used song type B with the same male song, a female subject may be torn as to whether to match the playback female (type B) or sing the song that matches her mate (type A). This type of pattern may lead to the increased switching rates observed during the duet playback treatment, as shown by Marshall-Ball & Slater (2004). In fact we also observed several individuals (both males and females) switching back and forth between two song types during the experiment. More detailed studies are needed on the song repertoires of happy wrens and their use during territorial disputes.

Although females overall responded strongly to both female solo and duet playback, closely examining individual responses suggests that most females responded strongly to only one of these two treatments, that is, those females that responded most strongly to duet playback responded much less strongly or not at all to solo female playback and vice versa. Whether a female responds more strongly to duet or solo female playback may reflect other factors, such as her mate's response. We observed correlations between male and female singing rates, so it is possible that birds collect information about how their mate is responding to an intruder when making decisions about their own territorial response. It is also possible that variation in how different pairs respond to intruders reflects something about the social dynamics of that pair, relating, for example, to each individual's assessment of its current mate and/or territory quality. The specific stage of breeding may also influence a female's response to mated versus unmated intruders; it appears that female buff-breasted wrens may make such a distinction (Gill et al. 2007). In that species, females respond more to female intruders before they start breeding, but more to pairs of duetting intruders during breeding. Although we do not have any information on breeding stage for any of our subjects, the playback experiment was conducted during the breeding period, so it is possible that which stage each pair was at in the breeding cycle might influence their response to playback. Further study of territorial defence and duetting behaviour in relation to both breeding stage and within-pair social dynamics may help reveal more of the subtleties of duet function.

Acknowledgments

We thank Constantino Macias Garcia, Alejandro Rios Chelén, Edgar Avila Luna, Jorge Vega Rivera and the staff at the Chamela field station for logistical help. Patrice Adret and Ania Vargas helped record birds in the field, and three anonymous referees provided detailed and helpful comments. This research was funded by an NSF IRFP grant to C.N.T. and a Leverhulme Trust Emeritus Fellowship to P.J.B.S.

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