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8 **Riverside wren pairs jointly defend their territories against simulated**

9 **intruders**

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25

26 **Abstract**

27 Duets are a jointly-produced signal where two or more individuals coordinate their  
28 vocalizations by overlapping or alternating their songs. Duets are used in a wide array of  
29 contexts within partnerships, ranging from territory defence to pair bond maintenance. It has  
30 been proposed that pairs that coordinate their songs might also better coordinate other  
31 activities, including nest building, parental care, and defending shared resources. Here, we  
32 test in the riverside wren (*Cantorchilus semibadius*), a neotropical duetting species that  
33 produces highly coordinated duet songs, whether males and females show similar  
34 responses to playback. During territorial disputes in songbird species, individuals tend to  
35 direct their attention towards same-sex territorial intruders, but this bias might be less  
36 pronounced in duetting species. We performed a dual-speaker playback experiment to  
37 examine how mated individuals respond to speakers broadcasting female versus male duet  
38 contributions. We found that riverside wrens have high levels of converging behaviour by  
39 duetting and remaining in close proximity of one another when responding to simulated  
40 paired intruders. Males and females spent more than 80% of their time less than one meter  
41 apart while defending their territory. Both individuals in a pair aggressively engaged with  
42 both male and female simulated trespassers by approaching equally close and spending  
43 equal time near the two speakers. These results suggest that both sexes perceive a paired  
44 territorial intrusion as a similar threat and that both partners are highly invested in defending  
45 the shared resources. The current study is one of the few to demonstrate equal attention  
46 and aggression from mated pairs towards simulated same-sex and opposite-sex intruders.  
47 We suggest that pairs responding together, in close proximity of one another, might be  
48 favorable in duetting species when defending the territory because maintaining a close  
49 distance between partners facilitates the extreme coordination of their joint territorial  
50 signals.

51 *Keywords:* vocal duets, territorial defence, Riverside wren, *Cantorchilus semibadius*,

52 cooperation.

53

## 54 **Introduction**

55

56       The exchange of acoustic signals between individuals is a crucial component of  
57 many aspects of animal behaviour including mate attraction, territory defence, parent-  
58 offspring communication, and species recognition (Kroodsma & Miller, 1982; Searcy &  
59 Anderson, 1986; Catchpole & Slater, 2008). The information transmitted through these  
60 signals depends on the signaler's and receiver's social and spatial relations (Naguib, 2005).  
61 For instance, during territorial defence in songbird species, individuals must choose whether  
62 to avoid, tolerate, or fight intruders depending on the vocal interactions between all  
63 participants (Tanner & Adler, 2009). Territorial disputes become more complex in species  
64 where both sexes participate in defending the shared resources. For example, the majority  
65 of studies done so far have found that females and males mostly direct their attention and  
66 aggression towards same-sex intruders (Slagsvold, 1993; Levin, 1996b; Seddon, Butchart,  
67 & Odling-Smee, 2002; Grafe & Bitz, 2004; Mennill 2006; Rogers, Langmore, & Mulder, 2007,  
68 Cain, Ainsworth, & Ketterson, 2011). However, this sex-specific bias is not as consistent in  
69 species where both partners vocalize together (Hall & Peters, 2008; Mennill & Vehrencamp,  
70 2008; Illes & Yunes-Jimenez, 2009; Templeton, Rivera-Cáceres, Mann, & Slater, 2011),  
71 suggesting that the coordination required for the vocal behaviour might be facilitated by  
72 performing a joint defence and maintaining a close distance between partners. In a meta-  
73 analysis performed by Logue (2005) to test if duetting species showed a significant reduction  
74 in the sex-specific territorial defence behaviours, he found that a cooperative territorial  
75 defence was indeed more common in duetting than non-duetting birds.

76

77 Duets are mainly regarded as coordinated displays where individuals alternate or overlap

78 their vocalizations to achieve an outcome beneficial to both partners (Hall, 2009). Duets  
79 signal the stability of the partnership to territorial rivals (Hall, 2000; Mann, Marshall-Ball, &  
80 Slater, 2003; Hall & Magrath, 2007), enhance acoustic contact and pair bonding (Logue &  
81 Gammon, 2004; Mennill & Vehrencamp, 2008), and they might have a role in achieving  
82 reproductive synchrony (Hall, 2009). Duets are often performed in counter-singing  
83 interactions with neighbouring pairs and are frequently produced in response to territorial  
84 intrusions (Logue, 2005; Mennill & Vehrencamp, 2008; Hall, 2009). As avian duets are  
85 usually a multifunction joint signal, different species have been shown to use their  
86 coordinated vocalizations in different manners to solve territorial disputes (Hall, 2009;  
87 Douglas & Mennill, 2010; Dahlin & Benedict, 2014). There are two main ways in which duets  
88 seem to function cooperatively against intruders: through division of labour, where each  
89 member defends their territory and partner from the same-sex intruder (Levin, 1996b;  
90 Mennill, 2006; Mennill & Vehrencamp, 2008; Templeton et al. 2011), or by presenting a  
91 stronger unified front against trespassers (Hall, 2000; Hall & Peters, 2008; Dahlin & Wright,  
92 2012). In species that have sexes varying in weight, individuals might stay with the same-  
93 sex intruder to avoid the risks of interacting with bigger birds (Logue & Gammon, 2004;  
94 Marshall-Ball, Mann, & Slater, 2006). However, in species that perform duets with a fine-  
95 scale temporal coordination, it has been suggested that singing highly coordinated duets  
96 when defending a territory could signal a strong commitment within the pair and hence, a  
97 strong motivation to defend the territory (Hall, 2000; Marshall-Ball et al., 2006; Hall &  
98 Magrath, 2007; Logue, 2007). Because temporal coordination within duets is higher when  
99 pairs are closer together (Mennill & Vehrencamp, 2008; Templeton et al., 2013a), birds risk  
100 losing that precision if they split up and perform a same-sex defence strategy. If singing with  
101 temporal precision is an advantage when facing intruders then it seems likely that duetting  
102 pairs will try to maintain that precision by staying together.

103

104 In this study we investigated the degree of vocal duetting and the physical responses of  
105 riverside wrens, *Cantorchilus semibadius*, during territory defence. Riverside wrens sing  
106 some of the most complex and highly precise antiphonal duets (Mann, Dingess, Baker,  
107 Graves, & Slater, 2009). Despite the fact that partners reply immediately to one another (on  
108 average after 0.06 – 0.01 s), vocalizations rarely overlap (Mann et al., 2009). Riverside  
109 wrens are socially monogamous and pairs have year-round territories (Skutch, 2001). Both  
110 sexes perform solo songs and contribute to duets by selecting from a sex-specific repertoire,  
111 and it has been estimated that individuals of each sex possess as much as 40 phrase types  
112 in their repertoires (Walters, 2013). When performing duets, the pair follows a duet code  
113 (Logue, 2006), resulting in one or both individuals selecting a particular phrase type  
114 according to its partner's choice. We used a stereo-duet playback design to study the  
115 interactions within pairs and to disentangle the interactions between each bird and same-  
116 sex and opposite-sex intruders (speakers). Due to the highly precise acoustic coordination  
117 this species shows, we predicted that individuals would follow a joint defence strategy (Seibt  
118 & Wickler, 1977) rather than a division of labour strategy. We predicted males and females  
119 would respond together and stay in close proximity instead of splitting up spatially with males  
120 interacting mainly with the male intruder and females interacting mainly with the female  
121 intruder.

122

123

## 124 **Methods**

125

### 126 Field methods

127 We studied riverside wrens at Osa Conservation's Piro field station in Costa Rica. The  
128 station is in lowland and wet rainforest on the Osa Peninsula in southern Costa Rica  
129 (8°24'6.96" N, 83°20'10.74" W). Riverside wrens are common at the study site, especially

130 next to rivers and wetlands. Riverside wrens nest throughout the year and remain with their  
131 offspring for up to five months (Skutch, 2001). We have studied this population of riverside  
132 wrens since 2013, and we have colour banded and collected biometric data from more than  
133 100 individuals at the study site. Riverside wrens' territories have an average of  $0.61 \pm 0.04$   
134 ha (unpublished data). To estimate the size of the territories we followed the focal pairs  
135 during the recordings and we mapped their boundaries with a hand-held global positioning  
136 system (Garmin GPS-60SCx, Garmin, Olathe, KS, USA). Adult and juveniles were captured  
137 with mist nets and banded with a unique combination of leg bands, including three coloured  
138 plastic and one numbered metal band, for individual identification. We measured each bird's  
139 weight, wing length, and tail length upon capture. We distinguished juveniles from adults by  
140 the colour of the bill (yellow underside of bill in juveniles, dark bill in adults) and the colour  
141 of the eye (grey iris in juveniles, brown iris in adults). We distinguished females from males  
142 by the presence of a brood patch (if present), by their songs (see Fig. 1) and, if both adults  
143 were captured, also by the relative body measurements since sexes are moderately  
144 dimorphic. Males in the population ( $n = 51$ ) weigh on average 21.7g (SE = 0.21g) and have  
145 a wing length of 62.1cm (SE = 0.62cm); females ( $n = 41$ ) weigh on average 18.6g (SE =  
146 0.22g) and have a wing length of 59.3cm (SE = 0.35cm). For this experiment, we focused  
147 on 23 pairs of riverside wrens whose territories we had carefully mapped from April to June  
148 2015 (38 of these birds were previously captured and banded).

149

#### 150 Playback stimuli

151 For the playback stimuli, we used a total of 5 duets, each recorded from different pairs  
152 present in the study site. We chose local songs to ensure the stimulus was recognized and  
153 provoked a strong response, given that different populations might have different dialects.  
154 The stimuli songs were selected from the repertoire of a pair located at least 500 meters  
155 apart (more than 3 territories away) to reduce the chance that our focal birds would have

156 had any prior experience with the particular pair whose songs we broadcast. We recorded  
157 these songs using a Sennheiser ME66 directional microphone and a Marantz PMD670 solid-  
158 state digital recorder. We selected good quality songs (a high signal-to-noise ratio and no  
159 other vocalizations in the background) where the focal birds were singing side by side (less  
160 than a meter apart), to ensure that the degree of coordination was relatively consistent  
161 across stimuli (Mennill & Vehrencamp, 2008; Mann et al., 2009). To create stereo-duet  
162 playbacks (e.g. Mennill, 2006), we duplicated the one-channel recordings and then carefully  
163 removed all of the phrases from one sex in one file and all of the phrases from the other sex  
164 in a second file using the frequency cursor filter function in Syrinx (J. Burt, Seattle,  
165 Washington, USA). Afterwards, using Audacity (<http://www.audacityteam.org>), each file was  
166 normalized so that the peak amplitude was 0dB. We created a two-channel stereo sound  
167 file containing one channel with male songs and one channel with female songs, thus  
168 keeping the exact timing of the original duet. The contribution of each sex was randomly  
169 assigned to the left or right channel. The stimuli consisted of 10 bouts of duets, each with 7  
170 song phrases from each sex, separated by 10 seconds of silence, which is consistent with  
171 the mean phrases per duet and mean inter-phrase duration previously reported for this  
172 species (Mann et al., 2009). Each trial consisted of 5 min of pre-playback period, followed  
173 by 3 min of playback, and 5 min of post-playback period.

174

#### 175 Playback setup

176 We used two connected speakers (a Foxpro Fury and a FoxPro SP-55 External Speaker)  
177 to broadcast the male and female contributions as a stereo-duet playback (e.g. Mennill,  
178 2006). These two speakers produce standardized outputs (FoxPro Inc., PA, USA) and to  
179 our ears they sound equivalent in terms of quality and amplitude (e.g. Templeton et al., 2011,  
180 2013b). We randomized which speaker played the male/female contributions for each trial  
181 (with a coin flip), so even if there were differences between speakers they should not

182 produce any bias in the data. The speakers were set approximately 1-1.5m above the  
183 ground and 10m apart to facilitate accurate measures of which speaker each individual was  
184 more likely to approach. Riverside wrens commonly sing duets at this height and from this  
185 distance (EQG, personal observation). The speakers were placed within pair territories,  
186 preferentially along the river for better identification and tracking of individuals. The trials  
187 were performed at locations within the territory to avoid neighbour interference during the  
188 trials.

189

#### 190 Data collection

191 During the playback trials two observers monitored all playback responses. One observer  
192 stayed in the middle of the two speakers to accurately assess approaches to each speaker.  
193 The second observer was positioned 10m away to maximize the accuracy of distance  
194 measurements while minimizing our overall influence on the birds' approach response. Most  
195 of the time both of the focal birds were in sight and easy to track, but in some territories with  
196 especially dense vegetation the location of the birds was sometimes estimated from their  
197 songs. Because the speakers were 10m apart, whenever an individual was inside the 5m  
198 radius of either speaker it was considered to be closer to that speaker than to the other one.  
199 During the trials we recorded all vocalizations from the focal individuals and assessed the  
200 distance of each bird to both playback speakers and to each other as often as possible and  
201 every time any bird moved. Pair members were considered to be in close proximity (as  
202 opposed to apart) when they were one meter or less away from each other.

203

204 We carried out this experiment on 23 territories. In 22 territories at least one adult member  
205 was previously marked (39 colour-banded individuals in total). In the remaining territory  
206 where neither of the individuals were banded we distinguished each sex by the songs  
207 produced by each bird (Mann et al., 2009). The trials were conducted from the 11th to the



208 22<sup>nd</sup> of June 2015 at 0600-0900 h to minimize effects of date and time of day on the  
209 behavioural responses to playback. We chose this time period to conduct trials when birds  
210 are vocally active before temperature and humidity rise during the day and to avoid any  
211 potential confounding effect of the dawn chorus.

212

213 Before initiating the trial, we conducted a five-minute pre-playback period to ensure that  
214 birds were not provoked by other stimuli (e.g. other territorial intruders) and to obtain  
215 baseline data regarding the typical behaviour of pairs (vocal activity and distance between  
216 individuals) in the absence of territorial intruders. However, the number of trials in which  
217 birds were observed and/or sang during the pre-playback period was not large enough to  
218 create baseline values. Therefore, we used data collected during sound recordings from a  
219 random sample of 20 pairs made during 2015 and 2016 to determine the vocal activity and  
220 distance between pair members in natural contexts, unprovoked by playback.

221

## 222 Statistical Analyses

223 In 20 out of 23 territories both adult pair members approached the speakers during the  
224 playback. In the remaining three territories only males came within sight (we believe these  
225 females did not approach because they were incubating and reluctant to leave their nests).  
226 We excluded these three pairs from the analysis. Although juveniles were found in four  
227 territories, they never responded to playback—none of them sang nor approached the  
228 speakers—nor did their behaviour seem to affect the response of the adults, so we disregard  
229 their presence for statistical analyses. Thus, the final sample size for the analyses was 20  
230 pairs.

231

232 To determine the acoustic behaviour in response to a simulated intrusion we examined the  
233 following variables in each pair: 1) Number of duets sung, 2) number of duets where each

234 sex is the one to sing the first contribution, 3) number of duets where each sex is the one to  
235 stop singing (thus terminating the cycle of the duet), and 4) number of phrases sung by each  
236 sex while duetting.

237 To examine whether pairs sang more duets in response to playback than during the pre-  
238 playback period we used a Wilcoxon signed rank test for paired samples. To compare the  
239 number of duets where each sex sings the first contribution, the number of duets where each  
240 sex stops singing, and the number of phrases sung by each sex in natural and playback  
241 contexts we used Wilcoxon signed rank tests for paired samples. To analyse whether the  
242 proportion of duets where each sex sings first, the proportion of duets where each sex stops  
243 singing, and the proportion of phrases sung by each sex varied between natural and  
244 experimental contexts, we used Wilcoxon rank sum tests for unpaired samples.

245

246 To determine the physical behaviour during playback we examined the following variables  
247 in each pair:

248 1) Proportion of time pair members spent in close proximity ( $\leq 1\text{m}$ ), 2) time spent near each  
249 speaker (i.e.  $\leq 5\text{m}$ ) while pair members were in close proximity, 3) frequency of individuals  
250 approaching the same-sex or opposite-sex speaker while pair members were apart, and 4)  
251 closest approach distance of each individual to each speaker.

252 1) To test whether pairs spend more time in close proximity ( $\leq 1\text{m}$ ) than apart we used a one-  
253 sample Wilcoxon signed rank test. We compared the proportion of time in close proximity to  
254 the value of 0.5, since this is the proportion that corresponds to pairs spending the same  
255 time in close proximity and apart. 2) The time spent on the male versus female speaker  
256 while the pair members were in close proximity was analysed using a Wilcoxon signed rank  
257 test for paired samples. 3) The frequency of individuals from each sex approaching the  
258 same- or opposite-sex speaker when they were apart was analysed using a Fisher's exact

259 test. Only for this test we used a reduced sample of 14 males and 9 females. This was due  
260 to the fact that 10 birds (from five pairs) were never apart ( $>1\text{m}$ ) for the whole duration of  
261 the playback, and because we only considered individuals that were within the 5m radius of  
262 one or the other speaker and thus showed a clear preference. 4) To compare the closest  
263 approach between male and female individuals and between male and female simulated  
264 intruders we used generalized estimating equations (gee). This modelling approach was  
265 chosen because it accounts for the lack of independence among observations within  
266 territories (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). For this analysis, we used bird sex,  
267 speaker sex and an interaction term between them as covariates, and modelled closest  
268 approach with a gamma distribution that best fitted the error distribution.

269 We also compared the proportion of times seen in close proximity ( $\leq 1\text{m}$ ) in natural  
270 (unprovoked by playback) and experimental (playback trials) contexts with a Wilcoxon rank  
271 sum test for unpaired samples. A within-territory analysis was not possible because several  
272 pairs had few or no natural observations during pre-playback. Therefore, for the natural  
273 contexts we used recordings of a random sample of pairs recorded without the use of  
274 playback (unprovoked). We chose natural recordings that lasted at least 10 minutes to make  
275 sure we would sample a full range of behaviours and not just when birds were singing  
276 together. Because riverside wrens are very inconspicuous and extremely mobile, on several  
277 occasions during the natural recordings we were not able to assess where the individuals  
278 were. Therefore, instead of using the percentage of time pairs spent in close proximity or  
279 apart during the total time of recordings, every time it was possible to assess if pair mates  
280 were in close proximity or apart, it was done so. The proportion of far and close observations  
281 was then calculated from the total number for each separate observation within a recording  
282 for each distance class (i.e. each time the pair, or an individual became visible again during  
283 a recording so proximity could be assessed it was scored as near or far).

284

285 Statistical analyses were conducted using R 3.1.0 software (R Core Team 2014), using the  
286 packages *geepack* and *boot*.

287

#### 288 Ethical Note

289 The University of St. Andrews Animal Welfare and Ethics Committee approved this work.  
290 The handling and ringing of birds was done only by those with previous experience. During  
291 the captures we attempted to minimize the stress on birds and released them as soon as  
292 we had banded them. Birds were followed until we heard them singing again or until we saw  
293 them re-joining their partners. All birds were seen and recorded on the following days after  
294 capture.

295

296

#### 297 **Results**

298

299 Pairs responded to a simulated territorial intrusion by highly increasing their duetting  
300 output (duets per 3 min) from  $0.75 \pm 0.39$  during pre-playback to  $6.5 \pm 0.58$  during playback  
301 (Wilcoxon signed rank test,  $V = 190$ ,  $P < 0.001$ ). Duets comprised  $86 \pm 5.1\%$  of the total  
302 song output throughout playback. Territorial defence elicited riverside wren females to  
303 increase the proportion of duets in which they sang the first contribution from  $0.2 \pm 0.06$   
304 during natural context to  $0.44 \pm 0.5$  during playback (Wilcoxon rank sum test,  $W = 103.5$ ,  $P$   
305  $< 0.01$ ); and to decrease the proportion of duets in which they stopped singing from  $0.84 \pm$   
306  $0.4$  during natural context to  $0.62 \pm 0.7$  during playback (Wilcoxon rank sum test,  $W = 289.5$ ,  
307  $P = 0.01$ ). Throughout natural contexts, duets comprised  $91 \pm 3\%$  of the total song output,  
308 males sang more than females the first contribution in duets ( $V = 196.5$ ,  $P < 0.001$ ), males  
309 sang more phrases than females when duetting ( $V = 164.5$ ,  $P < 0.01$ ), and females stopped

310 singing in more duets than males ( $n = 20$ ,  $V = 2.5$ ,  $P < 0.001$ ). Throughout playback, males  
311 and females sang the first contribution in similar numbers of duets and sang similar number  
312 of phrases while duetting ( $V = 104.5$ ,  $P = 0.19$ ;  $V = 114$ ,  $P = 0.22$ ), but females stopped  
313 singing in more duets than males ( $V = 41$ ,  $P < 0.03$ ).

314

315 Pairs were significantly more likely to be in close proximity ( $\leq 1\text{m}$ ) during a simulated intrusion  
316 than during an unprovoked context (Fig. 2, Wilcoxon rank sum test,  $W = 53$ ,  $P < 0.001$ ).

317 While partners were seen within a meter of one another only 32% of the times during natural  
318 contexts, in response to playback, pairs spent on average 81% of the trial in close proximity  
319 (SE = 4.1%, median = 89.7%). During playback trials pair members spent significantly more  
320 time in close proximity ( $\leq 1\text{m}$ ) than apart (Wilcoxon signed rank test,  $n = 20$ ,  $W = 206$ ,  $P <$   
321  $0.001$ ), with five pairs never separating more than this distance during the entire playback  
322 period, moving together even when moving relatively large distances within their territory.  
323 In 14 out of the 20 territories, both individuals arrived simultaneously at the speakers. In the  
324 remaining territories, males arrived first but females joined them after less than 20 seconds.

325

326 When birds were in close proximity during playback, they spent equal amounts of time at  
327 both speakers (Fig. 3, Wilcoxon signed rank test,  $n = 20$ ,  $W = 103$ ,  $P = 0.95$ ). On average,  
328 pairs spent 53s (SE = 13.2s, median = 32.5s) close to the male speaker (i.e.  $\leq 5\text{m}$ ) and 51s  
329 (SE = 10.7s, median = 45.5s) close to the female speaker (i.e.  $\leq 5\text{m}$ ). During the relatively  
330 few time periods when birds were apart, individuals showed a same-sex bias in approach  
331 behaviour, with more males (11 out of 14) approaching closer to the male speaker and more  
332 females (7 out of 9) approaching closer to the female speaker (Fisher's Exact Test, two-  
333 sided,  $n = 23$   $P = 0.01$ ).

334

335 Overall, a male's closest approach distance to the male and female speakers was on  
336 average 3.4m (SE = 0.99m, median = 2m) and 5m (SE = 1.06m, median = 3m), respectively.  
337 For females, the closest approach distance to the male and female speakers was on  
338 average 4.9m (SE = 1.05m, median = 3m) and 4.8m (SE = 1.11m, median = 2.5m),  
339 respectively (Fig. 4). No significant statistical differences were found among sexes or among  
340 speakers. However, a trend ( $P = 0.076$ ) existed for the interaction term between sex and  
341 speaker suggesting males might approach closer to the male speaker but females did not  
342 discriminate.

343

344

## 345 **Discussion**

346

347 Riverside wrens primarily responded to simulated pairs of intruders by arriving  
348 together and staying in close proximity the majority of the time rather than responding at  
349 different times or approaching the speakers separately. Both pair members reacted with  
350 equal levels of aggression in their approaches to the two intruders: they were similarly close  
351 and spent comparable time next to the male and female speakers. The simulated intrusion  
352 elicited individuals to highly increase their duetting output and to show equal levels of vocal  
353 participation: pairs coordinated most of their songs to form duets and females sang the first  
354 contribution in as many duets and sang as many phrases as males during the territorial  
355 defence. Our findings suggest that riverside wrens not only display convergent behaviour  
356 during a paired intrusion but also that pair members are more invested in maintaining a  
357 cooperative territorial defence rather than performing same-sex specific responses.

358

359 The symmetry and intensity of the response in riverside wrens indicate that pair members  
360 are highly interested in defending the shared territory and that both individuals in a pair are

361 willing to actively engage with both intruders. Most previous studies with stereo duet  
362 playback have documented duetting pair members approaching male and female simulated  
363 individuals with different intensities. For example, eastern whipbirds (*Psophodes olivaceus*)  
364 show no coordination during defence and mostly same-sex aggression (Rogers et al., 2007);  
365 rufous-and-white wrens' (*Thryophilus rufalbus*) aggression is also biased towards same-sex  
366 intruders with females showing a weaker response overall (Mennill & Vehrencamp, 2008);  
367 happy wrens (*Pheugopedius felix*) approach closer to the same-sex speaker with none  
368 approaching closer to the opposite-sex speaker (Templeton et al., 2011); in black-bellied  
369 wrens (*Pheugopedius fasciatoventris*), although males respond strongly to both intruders,  
370 females approach closer to same-sex intruders (Logue & Gammon, 2004); and in barred  
371 antshrikes (*Thamnophilus doliatus*) males approached much faster and closer to the  
372 speakers compared to females, even if each sex showed similar responses to the two  
373 intruders (Koloff & Mennill, 2013). To our knowledge, in only three duetting species where  
374 distances between partners has been assessed, have equal levels of attention to the two  
375 simulated individuals been shown: magpie-larks (*Grallina cyanoleuca*) flew mostly as a  
376 'united pair' towards the speakers and made 93% of their flights together approaching the  
377 same speaker (Rogers et al., 2004); stripe-headed sparrows (*Peucaea ruficauda*) reacted  
378 with the same intensity in their physical response (Illes & Yunes-Jimenez, 2009); and in  
379 yellow napped amazon parrots (*Amazona auropalliata*), pair members showed an equally  
380 aggressive response, staying less than 10m apart during playback and approaching  
381 speakers mostly together (Dahlin & Wright, 2012).

382

383 In species that have size dimorphism, different levels of aggression might be predicted by  
384 territory holders because the bigger sex would experience lower costs when confronting any  
385 intruders (of either sex), therefore it should be this sex that would be more prone to  
386 intersexual territoriality (Logue & Gammon, 2004). However, it has been shown that

387 coordinated duets are an important signal during territorial encounters (Hall & Magrath,  
388 2007). Perhaps in riverside wrens the weight difference between sexes is not large enough  
389 to deter females from confronting intruding males. For them, the benefits of defending their  
390 territory and their mate are higher than the potential costs of interacting with larger  
391 individuals, especially if they engage in this competitive behaviour side by side with their  
392 partner (Hall, Rittenbach, & Vehrencamp, 2015). Considering that this species sings one of  
393 the most coordinated duets described so far (Mann et al., 2009) and that acoustic  
394 coordination improves when mates are closer (Mennill & Vehrencamp, 2008; Templeton et  
395 al., 2013a), it does seem likely that partners might jeopardize that synchrony if they were to  
396 confront their intruders separately. Therefore, remaining in close proximity ( $\leq 1\text{m}$ ) and  
397 displaying a joint behaviour during the defence of their territory could be highly important to  
398 both pair members in order to show commitment and stability to outsiders through song  
399 coordination. One alternative to the cooperative hypothesis is that the pairs remain in close  
400 proximity because individuals are preventing their mate from engaging in extra pair  
401 copulations (i.e. mate-guarding, Stokes & Williams, 1968). We did not test for the responses  
402 to simulated solo intruders, so we cannot reject the possibility that individuals might perform  
403 a close joint defence as an attempt to guard the pair bond. In duetting species that have  
404 year-round territories and long-term partnerships, the defence of the shared resources and  
405 the partnership are tightly connected because acquiring a new mate or territory can both be  
406 challenging (Rogers et al., 2004, Hall & Peters, 2008, Logue & Hall, 2014). Riverside wrens  
407 share several activities including nest building and parental care (Skutch 2001 and EQG,  
408 personal observation), which suggests males and females benefit from maintaining and  
409 protecting the pair bond as well as the territory (Hall, 2004, Rogers et al., 2004, Logue &  
410 Gammon, 2005).

411



412 While pairs spent the vast majority of the trial in close proximity, when they did separate,  
413 each individual primarily approached the same-sex speaker. This observation could support  
414 the same-sex defence (Logue & Gammon, 2004; Mennill & Vehrencamp, 2008) or the mate-  
415 guarding hypothesis. Under same-sex defence, the risks of interacting with a bigger  
416 individual are greater for females if their mates do not join them. Hence, when they are apart  
417 there is less threat if they follow a division of labour where females confront females while  
418 males confront males. Under the mate-guarding hypothesis, individuals seek to advertise  
419 their partner's mated status by singing and showing themselves to the same-sex intruder.  
420 We found that riverside wrens approach the speakers within a distance close enough to  
421 engage in direct contact, suggesting that birds are prepared to physically challenge  
422 intruders. Additionally, we did find a trend (albeit not significant) that male riverside wrens  
423 approached closer to the male speaker than to the female one, also showing that males are  
424 perhaps more invested in confronting other males. The turnover rate in riverside wren  
425 territories is actually high (around 50% of the birds either leave the territory or die every  
426 season, E. Quirós-Guerrero own data), which shows that mate change is likely so birds must  
427 treat same-sex individuals as a strong threat. Divorce entails a cost because it could lead to  
428 a loss of the territory or other resources in it or because experience improves the breeding  
429 success between mates (Benedict, 2008). Thus, it is not surprising that when riverside wrens  
430 are apart, each mate is more likely to engage with an individual endangering their territory  
431 ownership as well as their mated status (Logue, 2005; Pärn, Lindström, Sandell, &  
432 Amundsen, 2008). Further work investigating the responses of males and females towards  
433 single intruders might help elucidate if there are any sexual conflicts within the partnership  
434 in this species. Additionally, it would be very interesting to address in the future the effect of  
435 varying distances between simulated intruders seeing how close riverside wrens remained  
436 in response to this study.

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438

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440

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451 **References**

452

453 Benedict, L. (2008). Occurrence and life history correlates of vocal duetting in North  
454 American passerines. *Journal of Avian Biology*, 39(1), 57-65.

455 Cain, K. E., Rich, M. S., Ainsworth, K., & Ketterson, E. D. (2011). Two sides of the same  
456 coin? Consistency in aggression to conspecifics and predators in a female songbird.  
457 *Ethology*, 117(9), 786-795.

458 Catchpole, C. K., & Slater, P. J. B. (2008). *Bird Song: Biological Themes and Variations* (2nd  
459 edn). Cambridge, United Kingdom: Cambridge University Press.

460 Dahlin, C. R., & Benedict, L. (2014). Angry birds need not apply: a perspective on the flexible  
461 form and multifunctionality of avian vocal duets. *Ethology*, 120(1), 1-10.

462 Dahlin, C. R., & Wright, T. F. (2012). Duet Function in the Yellow- Naped Amazon, Amazona

463           aeropallata: Evidence From Playbacks of Duets and Solos. *Ethology*, 118(1), 95-105.

464 Douglas, S. B., & Mennill, D. J. (2010). A review of acoustic playback techniques for studying  
465           avian vocal duets. *Journal of Field Ornithology*, 81(2), 115-129.

466 Fedy, B. C., & Stutchbury, B. J. (2005). Territory defence in tropical birds: are females as  
467           aggressive as males?. *Behavioral Ecology and Sociobiology*, 58(4), 414-422.

468 Grafe, T. U., & Bitz, J. H. (2004). Functions of duetting in the tropical boubou, *Laniarius*  
469           *aethiopicus*: territorial defence and mutual mate guarding. *Animal Behaviour*, 68(1),  
470           193-201.

471 Hall, M. L. (2000). The function of duetting in magpie-larks: conflict, cooperation, or  
472           commitment?. *Animal Behaviour*, 60(5), 667-677.

473 Hall, M. L. (2004). A review of hypotheses for the functions of avian duetting. *Behavioral*  
474           *Ecology and Sociobiology*, 55(5), 415-430.

475 Hall, M. L. (2009). A review of vocal duetting in birds. *Advances in the Study of Behavior*,  
476           40, 67-121.

477 Hall, M. L., & Magrath, R. D. (2007). Temporal coordination signals coalition quality. *Current*  
478           *Biology*, 17(11), R406-407.

479 Hall, M. L., & Peters, A. (2008). Coordination between the sexes for territorial defence in a  
480           duetting fairy-wren. *Animal Behaviour*, 76(1), 65-73.

481 Hall, M. L., Rittenbach, M. R., & Vehrencamp, S. L. (2015). Female song and vocal  
482           interactions with males in a neotropical wren. *Frontiers in Ecology and Evolution*, 3, 12.

483 Illes, A. E., & Yunes-Jimenez, L. (2009). A female songbird out-sings male conspecifics  
484           during simulated territorial intrusions. *Proceedings of the Royal Society of London B:*  
485           *Biological Sciences*, 276(1658), 981-986.

486 Koloff, J., & Mennill, D. J. (2013). The Responses of Duetting Antbirds to Stereo Duet  
487           Playback Provide Support for the Joint Territory Defence Hypothesis. *Ethology*, 119(6),  
488           462-471.

489 Kroodsma, D. E., Miller, E. H., & Ouellet, H. (Eds.). (1982). *Acoustic communication in birds*  
490 (Vol. 2). New York, United States of America: Academic Press.

491 Levin, R. N. (1996). Song behaviour and reproductive strategies in a duetting wren,  
492 *Thryothorus nigricapillus*: II. Playback experiments. *Animal Behaviour*, 52(6), 1107-  
493 1117.

494 Logue, D. M. (2005). Cooperative defence in duet singing birds. *Cognition, Brain, Behavior*,  
495 9, 497-510.

496 Logue, D. M. (2006). The duet code of the female black-bellied wren. *The Condor*, 108(2),  
497 326-335.

498 Logue, D. M. (2007). How do they duet? Sexually dimorphic behavioural mechanisms  
499 structure duet songs in the black-bellied wren. *Animal Behaviour*, 73(1), 105-113.

500 Logue, D. M., & Gammon, D. E. (2004). Duet song and sex roles during territory defence in  
501 a tropical bird, the black-bellied wren, *Thryothorus fasciatoventris*. *Animal Behaviour*,  
502 68(4), 721-731.

503 Logue, D. M., & Hall, M. L. (2014). Migration and the evolution of duetting in songbirds.  
504 *Proceedings of the Royal Society of London B: Biological Sciences*, 281(1782),  
505 20140103.

506 Mann, N. I., Marshall-Ball, L., & Slater, P. J. (2003). The complex song duet of the plain  
507 wren. *The Condor*, 105(4), 672-682.

508 Mann, N. I., Dingess, K. A., Barker, K. F., Graves, J. A., & Slater, P. J. (2009). A comparative  
509 study of song form and duetting in neotropical *Thryothorus* wrens. *Behaviour*, 146(1),  
510 1-43.

511 Marshall-Ball, L., Mann, N., & Slater, P. J. B. (2006). Multiple functions to duet singing:  
512 hidden conflicts and apparent cooperation. *Animal Behaviour*, 71(4), 823-831.

513 Mennill, D. J. (2006). Aggressive responses of male and female rufous-and-white wrens to  
514 stereo duet playback. *Animal Behaviour*, 71(1), 219-226.

515 Mennill, D. J., & Vehrencamp, S. L. (2008). Context-dependent functions of avian duets  
516 revealed by microphone-array recordings and multispeaker playback. *Current Biology*,  
517 18(17), 1314-1319.

518 Naguib, M. (2005). Singing interactions in songbirds: implications for social relations and  
519 territorial settlement. In McGregor, P.K. (Ed.), *Animal communication networks* (pp.  
520 300-319). Cambridge, United Kingdom: Cambridge University Press.

521 Pärn, H., Lindström, K. M., Sandell, M., & Amundsen, T. (2008). Female aggressive  
522 response and hormonal correlates—an intrusion experiment in a free-living passerine.  
523 *Behavioral ecology and sociobiology*, 62(10), 1665-1677.

524 Rogers, A. C., Ferguson, J. E., Harrington, H. M., Mcdowell, S., Miller, A., & Panagos, J.  
525 (2004). Use of stereo duet playback to investigate traditional duet playback methods  
526 and mechanisms of cooperative territorial defence in magpie-larks. *Behaviour*, 141(6),  
527 741-753.

528 Rogers, A. C., Langmore, N. E., & Mulder, R. A. (2007). Function of pair duets in the eastern  
529 whipbird: cooperative defense or sexual conflict?. *Behavioral Ecology*, 18(1), 182-188.

530 Searcy, W. A., & Andersson, M. (1986). Sexual selection and the evolution of song. *Annual*  
531 *Review of Ecology and Systematics*, 17(1), 507-533.

532 Seddon, N., Butchart, S. H., & Odling-Smee, L. (2002). Duetting in the subdesert mesite  
533 *Monias benschi*: evidence for acoustic mate defence?. *Behavioral Ecology and*  
534 *Sociobiology*, 52(1), 7-16.

535 Seibt, U., & Wickler, W. (1977). Duettieren als Revier- Anzeige bei Vögeln. *Ethology*, 43(2),  
536 180-187.

537 Skutch, A. F. (2001). Life history of the Riverside Wren. *Journal of Field Ornithology*, 72(1),  
538 1-11.

539 Slagsvold, T. (1993). Female-female aggression and monogamy in great tits *Parus major*.  
540 *Ornis Scandinavica*, 155-158.

541 Stokes, A. W., & Williams, H. W. (1968). Antiphonal calling in quail. *The Auk*, 83-89.

542 Tanner, C. J., & Adler, F. R. (2009). To fight or not to fight: context-dependent interspecific  
543 aggression in competing ants. *Animal Behaviour*, 77(2), 297-305.

544 Templeton, C. N., Rivera-Cáceres, K. D., Mann, N. I., & Slater, P. J. (2011). Song duets  
545 function primarily as cooperative displays in pairs of happy wrens. *Animal behaviour*,  
546 82(6), 1399-1407.

547 Templeton, C. N., Mann, N. I., Ríos-Chelén, A. A., Quiros-Guerrero, E., Garcia, C. M., &  
548 Slater, P. J. (2013). An experimental study of duet integration in the happy wren,  
549 *Pheugopedius felix*. *Animal Behaviour*, 86(4), 821-827.

550 Templeton, C. N., Ríos-Chelén, A. A., Quiros-Guerrero, E., Mann, N. I., & Slater, P. J.  
551 (2013). Female happy wrens select songs to cooperate with their mates rather than  
552 confront intruders. *Biology letters*, 9(1), 20120863.

553 Walters, H. (2013). *Song sharing in the riverside wren (Cantorchilus semibadius) a neo-*  
554 *tropical duetting passerine* (M.Sc. Thesis). Frontier Costa Rica Forest Research  
555 Programme. University of Cumbria, Carlisle, United Kingdom.

556 Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). Zero-truncated  
557 and zero-inflated models for count data. In *Mixed effects models and extensions in*  
558 *ecology with R* (pp. 261-293). Springer New York.

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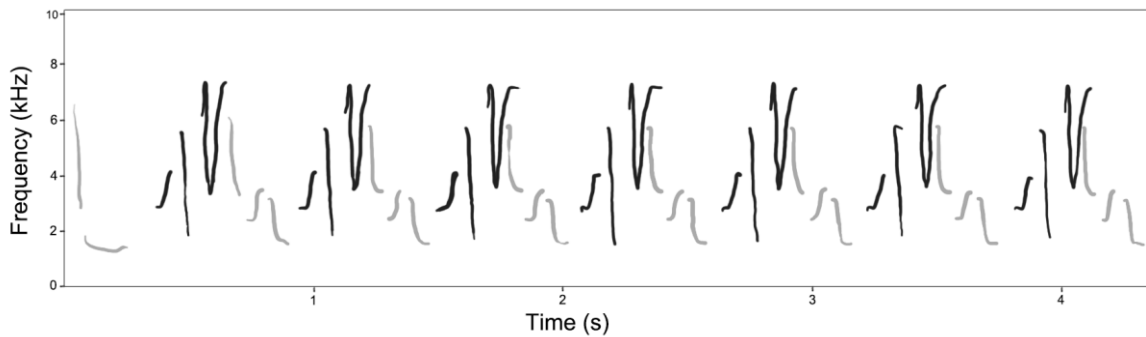
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580 **Figures**

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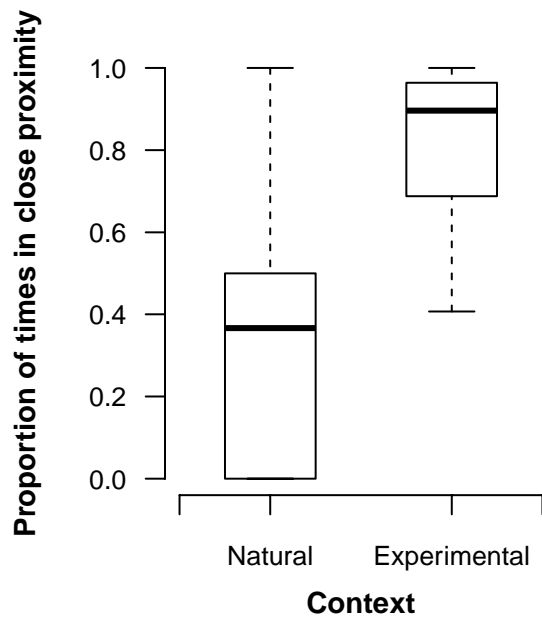
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584 Figure 1. Tracing of a spectrogram illustrating an example of the high coordination in a single  
585 riverside wren duet song type. The male contribution is depicted in grey and includes an  
586 introductory phrase (I phrase) and the male sex specific phrase (M phrase). The female  
587 contribution is depicted in black and includes a female sex specific phrase (F phrase). Pairs  
588 have repertoires of approximately 40 of these song types.

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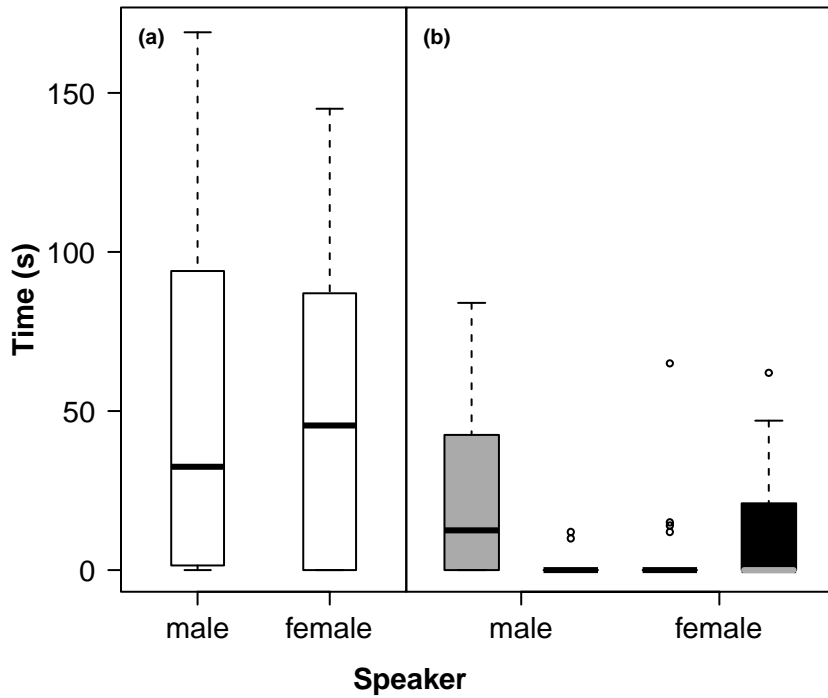




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592 Figure 2. Boxplot of proportion of times pairs were seen in close proximity ( $\leq 1\text{m}$ ) in the  
593 natural and experimental contexts across territories.

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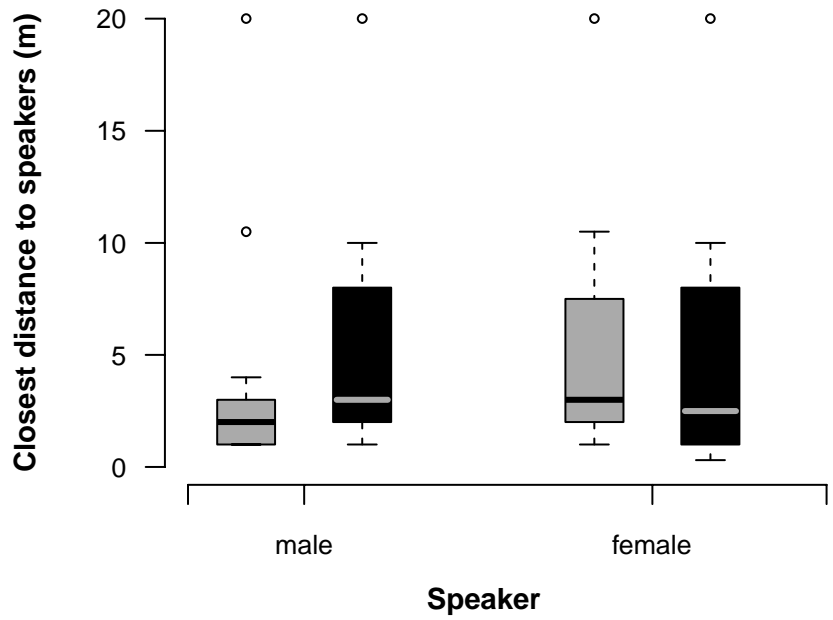
596 Figure 3. Boxplots of time during trial spent on the male and on the female speaker. The trial  
 597 lasted approx 180s. a) When in close proximity ( $\leq 1$ m apart), pairs approached both speakers  
 598 equally. b) When individuals were not in close proximity ( $< 20\%$  of the time), birds were more  
 599 likely to approach the same sex speakers; male behaviour is represented with the grey  
 600 boxplots and female behaviour is represented with the black boxplots.

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606 Figure 4. Boxplot of closest approach distance of males and females to the male and female  
 607 speaker. Male individuals are represented with the grey boxplots; female individuals are  
 608 represented with the black boxplots.