



Behavioral Ecology (2015), 00(00), 1–9. doi:10.1093/beheco/arv144

## Original Article

# An experimental test of duet function in a fairy-wren (*Malurus*) with moderate cuckoldry rates

Jenelle Dowling and Michael S. Webster

Cornell Lab of Ornithology, Department of Neurobiology and Behaviour, Cornell University, 159 Sapsucker Woods Road, Ithaca, NY 14850, USA

Received 28 May 2015; revised 31 August 2015; accepted 2 September 2015.

Individuals within social groups commonly combine vocal signals, forming duets. Although these displays have been described across taxa, their function is not fully understood. In birds, territory defense is a well-supported function of duetting, but additional functions are likely. Extrapair paternity (EPP) is common in birds, and males at risk of cuckoldry are expected to guard paternity in many ways, possibly including acoustic guarding with duets. Theoretical models predict species with moderate-to-high EPP rates will invest most in paternity-guarding, yet few studies have investigated duet function in such species. We conducted a dual-speaker playback experiment to test duet function in the red-backed fairy-wren, *Malurus melanocephalus*, which has moderately high EPP rates. Breeding groups were presented with 3 playback treatments: male solo, female solo, and duet. The territory defense hypothesis predicts pairs will respond aggressively and sing duets during duet playback in early breeding stages but predicts no same-sex bias in response to male/female songs. Acoustic paternity-guarding predicts males will duet with mates during male playback and in their mate's receptive stage, and they will bias aggression toward the speaker playing male song. Focal pairs duetted more during playback than controls and most during pre-breeding. Males and females responded more strongly to duet than solo playback, especially during pre-breeding, but neither showed same-sex response bias. Results most strongly supported the territory defense hypothesis, providing evidence that, despite cuckoldry risk, vocal duets were not used primarily for paternity-guarding. This helps us toward understanding the function of vocal displays across different mating systems.

**Key words:** acoustic mate-guarding, dual-speaker playback experiment, duet function, red-backed fairy-wren, territory defense, vocal communication.

Vocal duets and choruses have been described for mammals, frogs, insects, and birds (Thorpe et al. 1972). In birds, the function of duets appears to vary across species, and several non-mutually exclusive functions have been proposed (Hall 2004; Dahlin and Benedict 2013). Most studies indicate that duets have multiple functions that allow them to communicate with mates and individuals outside of the pair bond, with 1 common function being territory defense (Mulder et al. 2003; Fedy and Stutchbury 2005; Hall 2009; Benedict 2010; Dahlin and Wright 2012; Dahlin and Benedict 2013).

An additional hypothesis—acoustic mate-guarding—suggests that an individual joins its mate's song in duet to signal their mate's paired status to rivals and protect paternity or their position in the partnership (Stokes 1968; Sonnenschein and Reyer 1983). Studies of several species have found support for the acoustic

mate-guarding hypothesis for males (Levin 1996; Mennill 2006), for females (Seddon and Tobias 2006; Rogers et al. 2007; van den Heuvel et al. 2013), or for both members of the pair bond (Sonnenschein and Reyer 1983; Marshall-Ball et al. 2006). A subset of the acoustic mate-guarding hypothesis, specific to male-guarding of females, is the paternity-guarding hypothesis. Though this hypothesis has gained support in several previous studies, most support is indirect and/or observational (Sonnenschein and Reyer 1983; Levin 1996; Marshall-Ball et al. 2006). Two studies (Mennill 2006; Rogers et al. 2007) provide direct experimental support, but the results of these studies also support a function in territory defense. Many other studies do not support acoustic paternity-guarding (e.g., Hall and Magrath 2000; Gill et al. 2005; Hall and Peters 2008a), and overall, this hypothesis has found less direct, experimental support than the territory defense hypothesis (Hall 2009).

However, the function of duetting likely varies across species with different mating systems and levels of cuckoldry. For example,

Address correspondence to J. Dowling. E-mail: jld276@cornell.edu.

theoretical models (Kokko and Morrell 2005; see also Hall and Peters 2008b) predict that males should guard little in populations where cuckoldry rates (i.e., extrapair paternity [EPP]) are very low or very high. Accordingly, we can predict that males will be most likely to exhibit paternity-guarding behaviors, possibly including acoustic paternity-guarding, in species with moderately high EPP rates (Kokko and Morrell 2005). Because most previous studies of duet function have been conducted in species with either very low (e.g., Hall and Magrath 2000; Gill et al. 2005; Hall and Peters 2008b; Douglas et al. 2012; Hall et al. 2015; but see Benedict 2010) or unknown (e.g., Logue and Gammon 2004; Bradley and Mennill 2009) rates of EPP, it is perhaps unsurprising that few previous studies find direct support for the acoustic paternity-guarding hypothesis.

We conducted an experimental test of several hypotheses for the function of duetting in the red-backed fairy-wren, *Malurus melanocephalus*, a cooperatively breeding Australian bird species with moderately high EPP rates (54% of young result from extrapair copulations [EPCs], Webster et al. 2008). In this species, all members of the cooperatively breeding group (male, female, and auxiliary helpers) sing, and songs are often joined together in polyphonic duets (Thorpe et al. 1972; Hall and Peters 2008b; Dowling and Webster 2013). Like in other species of *Malurus*, extrapair matings are rarely observed in red-backed fairy-wrens (Webster M, personal communication; Rowley and Russell 1990; Karubian 2002). Males of this species leave their breeding territories, intrude on territories of neighboring males, and display to females with puff-back displays, food and petal carries, and sexual chases (Karubian 2002). EPCs are rarely observed during or after courtship displays from extrapair males (Dowling J, unpublished data; Karubian 2002). This suggests that EPCs occur at other times, and female red-backed fairy-wrens may make predawn forays to the territories of the extrapair mate she chooses based on diurnal displays, as in other species of *Malurus* (Double and Cockburn 2000). Red-backed fairy-wrens males physically guard their mates by remaining close during their fertile stage (Dowling J, in preparation; Karubian 2002). This and other mate-guarding behaviors, possibly including duetting, may allow a male to prevent rival males from intruding and displaying to his mate, thereby interfering with the mate's ability to assess and select an extrapair mate, which may in turn prevent females from later engaging in extraterritorial forays and EPCs.

Duets in this species are composed of nonidentical songs sung with overlap (Thorpe et al. 1972; Hall and Peters 2008b; Dowling and Webster 2013), and thus fit a broad definition of duetting (i.e., coordinated singing by 2 individuals so that their phrases alternate or overlap, Hall 2009). Overlapping songs in red-backed fairy-wrens do not, however, meet all characteristics of more specific definitions of duetting (i.e., a constant time lag between the contributions of the 2 individuals, and a stereotyped structure that occurs repeatedly and predictably in time, Langmore 2002) because the time lag between song contributions is moderately variable, notes within songs are not consistently antiphonally coordinated, and songs sung in duets show no apparent differences from solo songs (Dowling and Webster 2013). Nonetheless, in this species, song overlap occurs much more often than expected if individuals sing randomly with regard to each other (Dowling and Webster 2013). Our understanding of these types of duets is limited, and functional studies are needed for insights into the diversity and evolution of different forms of coordinated vocal display.

Red-backed fairy-wrens are an ideal system for studying the function of vocal duets, particularly the paternity-guarding hypothesis, because relatively high cuckoldry rates and pressure from intruding rival males predict males will use a suite of guarding behaviors to maintain paternity, possibly including acoustic guarding. Accordingly, we designed our experiment and analyses to specifically test the paternity-guarding hypothesis, rather than the more general acoustic mate-guarding hypothesis. As a result, we did not specifically test for female acoustic guarding of the male in this study, though we address this hypothesis to the extent possible with our data.

In this study, we used a dual-speaker playback experiment (Logue and Gammon 2004; Rogers et al. 2004; Mennill 2006) to test several hypotheses for the function of duetting in the red-backed fairy-wren (Table 1). Specifically, we were able to directly determine whether duets in red-backed fairy-wrens function primarily for territory defense, for acoustic paternity-guarding, or for some other purpose. The territory defense and acoustic paternity-guarding hypotheses both assume that the intended receivers of duets are individuals outside of the pair bond (Table 1, i.e., duets function for communication with conspecifics on other territories, Mennill 2006). The territory defense hypothesis (Seibt and Wickler 1977) predicts that because duets are territorial “keep-out” signals, pair members will respond to a simulated territorial intrusion

**Table 1**  
**Hypotheses for duet function tested and predictions for dual-speaker playback experiment**

Hypothesis	Focal pair movement response	Duet response
Territory defense Duet to create stronger “keep-out” signal to same-sex and opposite-sex rivals	Male and female response: Stronger response to duet than solo Especially in pre-breeding stage Response to male speaker = female speaker	Duet rate: Higher in playback period than pre- or post-playback Especially in pre-breeding stage Higher in duet treatment than either solo Higher in pre-breed than other stages
Paternity-guarding Males duet with their mates to signal their mated status and repel rivals who threaten paternity, especially when mates fertilizable	Male response: Stronger response to male than female speaker Stronger response to male solo than duet	Duet rate: Higher in playback period than pre- or post-playback Especially in female receptive stage Higher when female receptive than other stages Male answer female songs: Higher rate in male solo than other treatments Higher rate when female receptive than other stages

(playback) by singing duets, will do so most often in the early breeding stages when territories are first being established, and will not discriminate among male and female intruders. Pairs also are expected to respond with higher aggression and duet rate to duet than to solo treatments, especially during the pre-breeding stage, as intruders singing duets rather than solos represent a greater threat, possibly leading to a territorial claim. However, we point out that in some previous studies, authors suggest that equivalent responses to all treatments may also support the territory defense hypothesis (Benedict 2010; Dahlin and Wright 2012). Pairs are expected to respond equally to both the male and female contributions to the duet, as both male and female intruders threaten territory ownership in species where both sexes are territorial.

The acoustic paternity-guarding hypothesis (Stokes 1968; Sonnenschein and Reyer 1983), on the other hand, posits that males duet with their mates as an agonistic signal, intended for same-sex rivals, which communicates that the female is paired. Thus, the acoustic paternity-guarding hypothesis first predicts that a male will respond more strongly to the male than to the female speaker during stereo duet playback, because that male's duet contribution is a signal intended for him, a same-sex rival (Mennill 2006). Second, this hypothesis predicts that unmated solo territorial intruders should elicit a stronger response from same-sex individuals than should paired duetting intruders (Templeton et al. 2011), because the relative paternity threat is higher for solo than for paired intruders (Hall 2004). This hypothesis lastly predicts that a male will sing in response to his mate's songs to form duets more often when a male intrusion is simulated (Levin 1996) and during her receptive stage, when the threat of cuckoldry is highest.

Our previous work using correlative approaches (Dowling and Webster 2013) suggested that duetting functions in territorial defense and not in mate-guarding in this species, but these 2 hypotheses are notoriously difficult to separate using observational data alone (Hall 2009; Douglas and Mennill 2010). This study is the first to use a dual-speaker playback design to investigate the function of duetting in a species with moderately high levels of EPP and so provides a critical piece of the puzzle for understanding how coordinated displays function across different mating systems.

## METHODS

### General field methods

We conducted a dual-speaker playback experiment in the 2011/2012 breeding season (October–January). Male and female duet contributions were broadcast through different speakers (see Logue 2005; Mennill 2006). This setup allowed assessment of each focal pair member's response to a male and a female song simultaneously and allowed us to test a critical prediction that separates the territory defense and acoustic paternity-guarding hypotheses (Mennill 2006).

Red-backed fairy-wrens are a territorial species and defend relatively large areas for breeding and foraging; territories are on average  $103.4 \pm 35$  m across ( $N = 14$  territories, Dowling J, unpublished data). Males exhibit 2 distinct breeding plumage types: red–black plumage (greater than 66% of plumage is red and black, scored on 6 body regions) or brown plumage (less than 33% of plumage is red and black) (Karubian et al. 2008). Although some males show intermediate plumage scores, plumage type generally shows a bimodal distribution in this species (Webster et al. 2008). Males in 9 focal

groups were breeding in red–black plumage (greater than 85% of each focal male's plumage was red and black), whereas males in 7 breeding groups had brown plumage. Subjects in this study were 16 breeding groups, 5 of which had an auxiliary helper (son from previous year in all cases). In red-backed fairy-wrens, pair bonds between close relatives occasionally form and are associated with changes in EPP allocation (Varian-Ramos and Webster 2012). To avoid potential biases, we ensured that in all 16 breeding groups, the social pairing was between unrelated birds.

Focal birds were captured in mist nets and marked with a unique color band combination, allowing individual identification. Breeding stages were defined as follows: 1) the pre-breeding stage, before the female began nest building ( $N = 36$  experiments conducted an average of  $26 \pm 6.5$  days before first egg was laid); 2) the fertile stage, between the first observed solicitation or copulation and when the penultimate egg was laid ( $N = 54$  experiments conducted an average of  $4 \pm 5.5$  days before first egg was laid); and 3) the incubation stage, the period when the female was incubating her clutch of eggs ( $N = 45$  experiments conducted an average of  $11 \pm 2.4$  days after first egg was laid).

### Playback stimuli

We created stimulus songs for 3 different treatment categories: male solo, female solo, and simultaneous duet. Sets of stimulus songs played to each focal group were unique (16 different male solos and 16 different female solos) and were from high-quality recordings made during the dawn chorus, following playback protocols as in Greig and Webster (2013). We used Syrinx PC (J. Burt, Seattle, WA) to create all stimuli. Amplitudes of songs were normalized to 3500u (the highest amplitude possible without waveform clipping), and songs were bandwidth filtered so that the entire file ranged from 2400 to 17800 Hz, which left spectral space occupied by song elements intact (Greig et al. 2013). We created duet stimuli by combining the male and female solos used for solo stimuli (matched for length) into stereo files using Audacity 1.2.5 (D.M. Mazzoni, Canada, <http://audacity.sourceforge.net/>, last accessed 11 September 2015). Male and female duet components were combined so that they began at the exact same time for all duet treatments. For each treatment type, we combined 14 copies of the same stimulus onto 1 track so that 1 stimulus was played every 20 s and the total playback length was 5 min. Tracks started with 20 s of silence and ended after the 14th stimulus. This song rate was chosen because red-backed fairy wrens sing approximately 1 song for every 20 s during aggressive interactions and during the dawn chorus (Dowling and Webster 2013). Simultaneous duets were used because the majority of red-backed fairy-wren duets are sung with complete or near complete overlap; 79% of duets measured have  $< 1$  s between the start of the first bird's song and the start of the responding bird's song (Dowling and Webster 2013).

### Playback procedure

Each focal group was played with all 3 playback treatments (male solo, female solo, and duet) once in each of 3 breeding stages (pre-breeding, fertile, and incubation). We conducted 135 total playbacks on 16 groups. All but 3 of the groups received playbacks during all 3 breeding stages; the 3 groups that did not were each missing 1 playback. We conducted 44 duet treatment, 45 male solo treatment and 45 female solo treatment playbacks. Stimuli played to each group were recorded from non-neighboring birds from the same population that were at least 5 territories away. Playback of each

treatment was separated by approximately 60 min ( $91 \pm 31$  min, ranging from 40- to 200-min separation). For the duet treatment, we balanced the number of times the female solo was played on the left channel and the male solo on the right ( $N = 22$ ) and vice versa ( $N = 22$ ). The duet presented to each pair was composed of the same male solo and female solo that was presented to them in the solo treatments. A unique set of playback stimulus songs was presented to each focal group, and the same set was presented to that group in a different order in each of 3 breeding stages (once each with male solo first, female solo first, and duet played first, with the treatment for the first experiment for each group determined randomly).

Experiments were conducted between 05:30 and 12:30, which is after the red-backed fairy-wren dawn chorus ends and is an active time of day for singing and other behaviors (Dowling J, unpublished data). At the start of each experiment, 2 speakers (Pignose Legendary 7–100, Pignose-Gorilla, Las Vegas, NV) were set up 10 m apart, within the focal territory and greater than 10 m from a territory boundary. Songs were played from an iPod nano (Apple Inc., Cupertino, CA) connected to each speaker via a 30-m cable. The first observer stood about 20 m from the speakers, with both speakers in clear view, and operated the iPod, whereas the second observer kept track of focal birds. When all members of the focal group were located within 25 m of speakers, the playback began. For solo treatments, the male solo and female solo were played from the same speaker, and for duet treatments, the speaker that played the male and female contribution to the duet was determined beforehand and balanced. Songs were broadcast at 90 dB at 1 m. Each of 2 observers was equipped with a Marantz PMD661 solid-state digital recorder (Marantz America, Itasca, IL) and Sennheiser ME67 highly directional long-gun microphone (Sennheiser Corp., Old Lyme, CT) with a Rycote softie windshield and mount. Observers dictated the bird's behaviors and details about vocalizations into the recorder during the experiment.

We observed birds for a 5-min pre-playback period of silence, 5-min playback period, and 5-min post-playback period of silence. During these observation periods, both observers immediately began to dictate the following behaviors as they occurred: counts of male, female and helper solos, duets and who initiated each, distance of each group member from each speaker throughout the entire period, closest approach of each group member to each speaker, number of flights over each speaker, all group member flights and flight following, distance between group members, and latency to approach within 5 m of each speaker. Distance was estimated using the distance between the 2 playback speakers (10 m) as a standard. When birds did not approach within 5 m of speakers during the entire period, their latency to approach was coded as the total time in the period (the maximum possible latency given the period length).

## Statistical methods

To test predictions that involved focal pair movements, we determined how focal birds' movement response varied during the playback period across treatments and breeding stages. We combined several movement responses into one principal component response score for the breeding male and another for the breeding female of the group (Table 2). A higher response score indicates a stronger response to playback (less time to approach closely, closer approach, more time spent close, and more flights over speakers). We ran a linear mixed-effect model for each response variable (male response PC1 and female response PC1) using the lme4 package in R v.3.1.2

**Table 2**  
Correlations of 4 behavioral response variables with the first principle component in a Principle component analysis

	Male PC1	Female PC1
Eigenvalue	2.74	2.57
Percent variation	68.5	64.2
Amount of time spent within 5 m	-0.84	-0.86
Closest approach	0.80	0.79
Latency to approach within 5 m	0.93	0.86
Number of flights over	-0.72	-0.69

Response variables describe focal male and female's strongest response to either speaker in a trial.

(R Development Core Team 2014) with breeding group number as a random effect, and playback treatment, breeding stage, breeding male plumage type, and all interactions as fixed effects.

To determine how pair members responded to the male speaker versus the female speaker when both were presented simultaneously, we calculated the difference in response to one speaker versus the other (e.g., number of male flights over the male speaker minus the number of male flights over the female speaker), just within the dual-speaker duet treatment. We used 1-sample *t*-tests in JMP version 10 (SAS Institute Inc., Cary, NC) to determine if that difference was significantly different from 0. We did this for each of the 4 movement responses. For this analysis, responses were averaged across breeding stages to avoid pseudoreplication, because multiple experiments on the same group were conducted during each of 3 breeding stages. Results changed only minimally when tests were run on each breeding stage separately, and all differences are reported in results.

To test predictions involving vocal responses, we first determined how focal birds' duet response varied across trial types (pre-playback, playback, and post-playback), playback treatments, and breeding stages. We ran a generalized linear mixed model (GLMM) with a Poisson distribution with number of duets as the response variable and amount of time as an offset argument. The model included breeding group number as a random effect, and trial type, playback treatment, and breeding stage as fixed effects. Next, we determined how the male's song answering behavior (the proportion of female songs the male answered to form a duet) varied across playback treatments and breeding stages. We used a GLMM with binomial counts as outcomes (number of times the male joined the female's songs to form a duet) and a weight argument specified (number of female songs) to analyze the proportion of female songs the male answered. The model included breeding group number as a random effect, and playback treatment and breeding stage as fixed effects.

To test for physical mate-guarding during experiment trials, we tested the effect of playback treatment and breeding stage on movements that the male made toward the female (proportion of female flights male followed and male–female distance). We ran a GLMM either with binomial counts as outcomes (proportion of female flights the male followed) and a weight argument specified or a Poisson distribution with distance between male and female and amount of observation time as an offset argument.

All models were first run including all interactions, and nonsignificant interactions were then removed in future model iterations. All models were also run with the presence/absence of an auxiliary helper, breeding male plumage, and treatment presentation order as a fixed effects, which were removed from final models when

nonsignificant (unless specified in results). Periods during the experiment, where either the male or female was absent (off territory or at the nest), were excluded from all analyses.

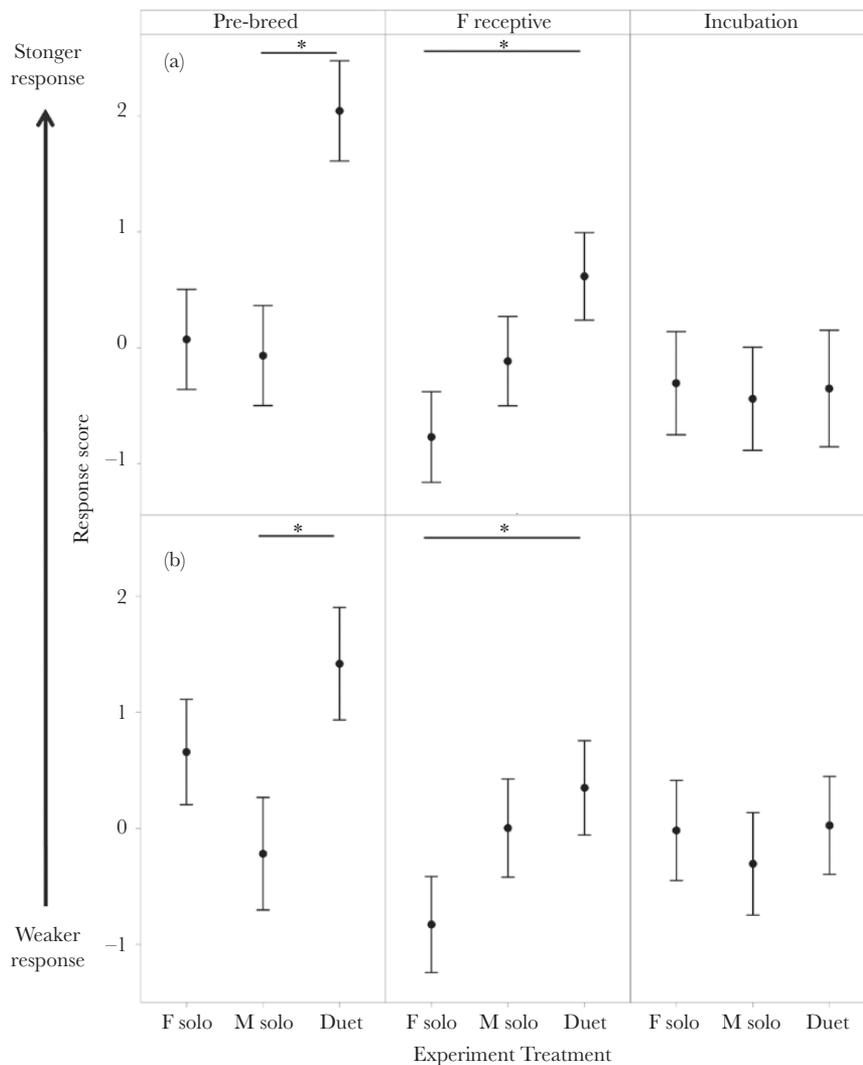
## RESULTS

### Movement responses

Females showed a stronger movement response to the duet treatment than to the male or female solo treatment in the pre-breed and fertile stages of their nesting cycle (LMM: playback treatment  $\times$  breeding stage:  $\chi^2 = 12.0$ , degrees of freedom [df] = 4,  $P = 0.018$ ,  $N = 117$  observations of 16 groups, Figure 1a, Supplementary Table S1). Males showed a similar pattern, with response to duet treatment higher than male or female solo treatments in pre-breeding and female receptive stages, although the overall pattern was not significant (LMM: playback treatment  $\times$  breeding stage:  $\chi^2 = 9.2$ , df = 4,  $P = 0.056$ ,  $N = 122$  observations of 16 groups, Figure 1b, Supplementary Table S1). Overall, within each experimental period, the male's response did not differ from that of the female (2-tailed paired  $t$ -test:  $t_{16} = -0.4$ ,  $P = 0.7$ ). These results provide partial support for the first 2 predictions of the territory

defense hypothesis, albeit with stronger support for females than for males. We found a significant interaction between breeding stage and male plumage type, such that brown males responded more aggressively than red-black males to simulated intrusion in their mate's fertile stage (plumage type  $\times$  breeding stage:  $\chi^2 = 9.2$ , df = 2,  $P = 0.010$ ).

We found no difference in the response of each pair member to the male speaker versus the female speaker (Figure 2). Responses to the speakers did not differ with respect to the proportion of time spent within 5 m (1-sample  $t$ -test, males:  $t_{16} = 0.12$ ,  $P = 0.91$ ; females:  $t_{16} = 0.28$ ,  $P = 0.79$ ), closest approach (males:  $t_{16} = -1.19$ ,  $P = 0.3$ , females:  $t_{16} = -2.13$ ,  $P = 0.051$ ), latency to approach within 5 m (males:  $t_{16} = 0.71$ ,  $P = 0.49$ ; females:  $t_{16} = -0.42$ ,  $P = 0.68$ ), or number of flights over the speaker (males:  $t_{16} = 1.07$ ,  $P = 0.3$ ; females:  $t_{16} = 0.18$ ,  $P = 0.86$ ). These results do not support the critical prediction of the acoustic paternity-guarding hypothesis (that males will show more aggression to the male speaker) and instead support the territory defense hypothesis. Females showed a trend of approaching the male speaker more closely than the female speaker, but this result was not significant. When we looked at responses within each breeding stage, we saw no difference in results, except



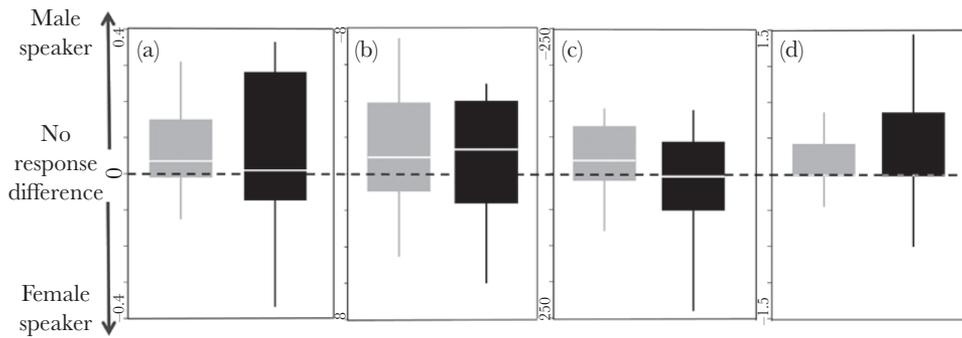
**Figure 1** Female (a) and male (b) mean behavioral response ( $\pm$  SE) to different experimental treatments: female solo, male solo, and duet. \* $P < 0.05$ .

that during the pre-breeding stage females approached the male speaker more closely ( $t_{16} = -3.4, P = 0.007$ ) and approached the male speaker more quickly than the female speaker ( $t_{16} = -2.8, P = 0.017$ ). This result does not provide clear support for either the territory defense or the acoustic paternity-guarding hypothesis and suggests that to females, males may pose a greater threat than other females, possibly due to their larger size (Schwabl et al. 2015), but the nature of the threat is unclear.

**Vocal responses**

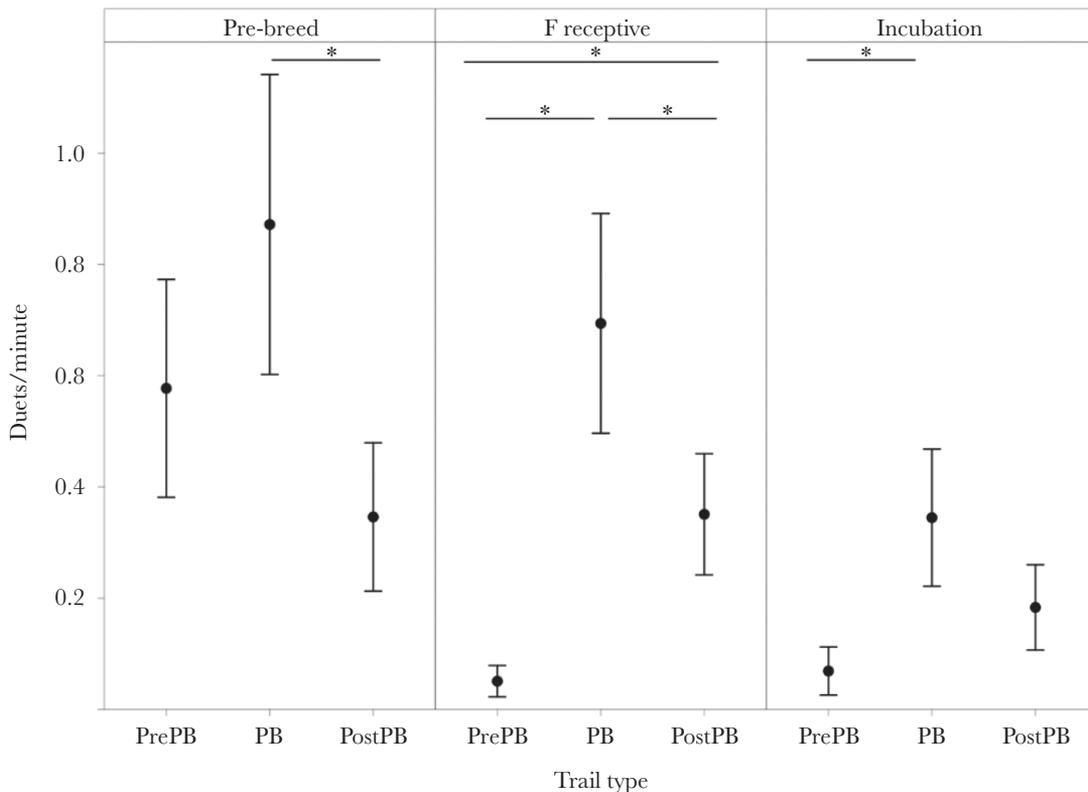
Focal pairs sang more duets during the playback period, regardless of treatment played, than in the pre- or post-playback

control period (GLMM: trial type  $\times$  breeding stage:  $\chi^2 = 17.34, df = 4, P = 0.002$ , Figure 3, Supplementary Table S2). This pattern was similar across all 3 breeding stages, but strongest in the female’s fertile stage (Figure 3). Duet rate differed across breeding stages ( $\chi^2 = 16.05, df = 2, P = 0.0003, N = 114$  observations of 16 groups), but not across playback treatments ( $\chi^2 = 0.89, df = 2, P = 0.64$ ), and there was no significant interaction between breeding stage and treatment ( $\chi^2 = 4.44, df = 4, P = 0.35$ ). Duet rates were highest in the pre-breeding stage (post hoc  $z$ -test of GLMM least-squares means: pre-breed vs. fertile:  $z = 2.5$ , standard error [SE] = 0.3,  $df = 2, P = 0.0335$ ; pre-breed vs. incubation:  $z = 3.89$ , SE = 0.4,  $df = 2, P = 0.0003$ ), intermediate in the fertile stage



**Figure 2**

Box and whisker plots showing difference in response to speaker playing a male song versus speaker playing a female song for the focal female (gray bars) and focal male (black bars). (a) Proportion of time spent within 5 m of each speaker; (b) closest approach to each speaker (meters), (c) latency to approach within 5 m of each speaker (seconds), and (d) number of flights over each speaker. Horizontal lines represent 25th quartile, median, and 75th quartile. Whiskers represent maximum and minimum values for data. Data are averaged across breeding stages. There were no significant differences in responses to the male versus female speaker for either sex.



**Figure 3**

Variation in mean duet rate ( $\pm$  SE) across 3 playback periods (pre-playback, playback, and post-playback) and across 3 breeding stages.  $*P < 0.05$ .

(fertile vs. incubation:  $z = 2.5$ ,  $SE = 0.3$ ,  $df = 2$ ,  $P = 0.0328$ ), and lowest in the incubation stage. These results support one prediction of the territory defense hypothesis and may provide partial support for one prediction of the acoustic paternity-guarding hypothesis. We also found that in groups with auxiliary helpers, pair members sang more duets during experiments than pairs without helpers ( $z = -2.14$ ,  $SE = 0.63$ ,  $df = 1$ ,  $P = 0.0325$ ).

We found no difference in the proportion of female songs the male answered across playback treatments (GLMM:  $\chi^2 = 1.08$ ,  $df = 2$ ,  $P = 0.58$ ,  $N = 64$  observations of 16 groups) or breeding stages ( $\chi^2 = 0.24$ ,  $df = 2$ ,  $P = 0.89$ ), which does not support the acoustic paternity-guarding hypothesis. We saw different results for female duet rates with males. During the female fertile stage, when compared with the female solo treatment (experiment treatment  $\times$  breeding stage:  $\chi^2 = 10.2$ ,  $df = 4$ ,  $P = 0.037$ ,  $N = 70$  observations of 16 groups), females answered a higher proportion of their mate's songs in the duet treatment (post hoc  $z$ -test of GLMM least-squares means:  $z = -2.69$ ,  $SE = 0.6$ ,  $df = 2$ ,  $P = 0.02$ ) and (marginally) in the male solo treatment ( $z = -2.3$ ,  $SE = 0.7$ ,  $df = 2$ ,  $P = 0.056$ ).

### Physical mate-guarding

We found no difference in the proportion of female flights that the male followed across playback treatments (GLMM:  $\chi^2 = 1.61$ ,  $df = 2$ ,  $P = 0.45$ ,  $N = 79$  observations of 16 groups) or breeding stages ( $\chi^2 = 3.97$ ,  $df = 2$ ,  $P = 0.14$ ) and no significant interaction between the 2 ( $\chi^2 = 3.69$ ,  $df = 4$ ,  $P = 0.45$ ). Likewise, we found no difference in the distance between the male and female across playback treatments ( $\chi^2 = 2.48$ ,  $df = 2$ ,  $P = 0.29$ ,  $N = 98$  observations of 16 groups), though the pair stayed closer during the female's fertile stage than during incubation (post hoc  $z$ -test of GLMM least-squares means:  $z = -3.11$ ,  $SE = 0.14$ ,  $df = 2$ ,  $P = 0.0054$ ). In groups with helpers, males followed their mate less than males without helpers in the incubation stage (helper presence  $\times$  breeding stage:  $\chi^2 = 8.8$ ,  $df = 2$ ,  $P = 0.012$ ). In addition, brown males followed their mates more than red-black males, across all stages and treatments ( $\chi^2 = 6.8$ ,  $df = 1$ ,  $P = 0.0097$ ).

## DISCUSSION

We used a dual-speaker playback design to distinguish between the territory defense and acoustic paternity-guarding hypotheses, which are difficult to separate using behavioral observations or a traditional single-speaker playback experiment (Hall 2009; Douglas and Mennill 2010). Our results suggest that the primary function of duets in the red-backed fairy-wren is territory defense (see also Dowling and Webster 2013).

Our results support 4 out of 5 predictions of the territory defense hypothesis. One prediction, that duet rate will be higher in the duet treatment, was not supported, but it is important to note that we may see no difference in duet rate across playback treatments because pairs respond to duet playback with physical aggression and that may prevent or distract them from duetting simultaneously. Our results do not support the one prediction of the acoustic paternity-guarding hypothesis that differs critically from predictions of the territory defense hypothesis (same-sex bias in response to playback speakers, Table 1), meaning that the pair's behavior was not in line with the predictions of the paternity-guarding hypothesis.

We found that pairs responded to a simulated territorial intrusion (playback, all treatments) by singing duets, particularly during the female's fertile stage. This may suggest that males use duets to

repel rivals, particularly when the risk of paternity loss is at its highest. On the other hand, total duets included both those initiated by males and those by females, and if paternity-guarding is a function of duetting, we would also expect that males would answer their mate's songs more in the fertile stage, a pattern we did not see. We also find no support for female acoustic guarding of males because females did not show more aggression to the female speaker or female solo treatment and did not answer more of their mate's songs in duet when an intrusion from a solo female was simulated. Taken together, these results suggest that duets in this species function primarily as a keep-out signal meant for individuals outside of the group.

Our results complement those from other studies: across bird species, territory defense seems to be a main function of duetting, but duetting also seems to have evolved for multiple functions (Hall 2009; Dahlin and Benedict 2013). Duets create a strong signal that is loud and easy to locate and may serve as a stronger signal of territory ownership than does solo song (Hall 2009). Indeed, several studies show that duets are more threatening territory displays than are solo songs (Hall 2009). In addition to being louder and easier to locate, duets also provide more information to the receiver about the senders than do solo songs: a duet signals when both members of the pair are present and ready to attend to a threat and provides information about the quality and condition of each duet contributor as well as information about the quality of their pair bond (Hall 2009).

We chose to investigate the acoustic paternity-guarding hypothesis because duetting has been primarily studied in species where EPP rates are low (e.g., Hall and Magrath 2000; Gill et al. 2005; Hall and Peters 2008b; but see Benedict 2010) and/or details of cuckoldry and EPP rate are unknown (e.g., Logue and Gammon 2004; Bradley and Mennill 2009). Investigating vocal strategies across a wide range of mating systems is crucial to understanding how they evolve. Theoretical models predict little mate-guarding when pair members are very faithful or very unfaithful (Kokko and Morrell 2005). Because red-backed fairy-wrens show an intermediate level of cuckoldry (54% of young result from EPCs, Webster et al. 2008), we predicted that red-backed fairy-wren males would guard mates heavily and possibly use duetting as part of a larger suite of paternity-guarding behaviors. Despite the fact that mate-guarding should be important to species with moderately high rates of EPP, our results do not support the predictions of the acoustic paternity-guarding hypothesis, including the critical prediction of the hypothesis (same-sex bias in response to playback of male and female duet contributions).

If mate-guarding strategies should be well developed in species with moderate levels of cuckoldry, why did we find little support for the acoustic paternity-guarding hypothesis? There are several potential explanations. First, it is possible that red-backed fairy-wren males rely mainly on nonvocal forms of mate-guarding, such as mate following or physical aggression. Physical mate-guarding has been studied in 2 other fairy-wren species, one where pair members are extremely faithful (purple-crowned fairy-wrens) and one where pair members are extremely unfaithful (superb fairy-wrens), and neither guard mates heavily (Mulder 1997; Hall and Peters 2008a), as predicted by theoretical models. In this study, we found that male red-backed fairy-wrens showed some physical guarding behaviors during simulated intrusions, but males did not target physical aggression on intruding males, as one would expect if they were guarding their mates from rivals. Male red-backed fairy-wrens do show physical guarding of fertile mates (Dowling

J, in preparation; Karubian 2002), but the behavior was not pronounced during experimental trials. Another explanation is that acoustic guarding may not be favored in this species because it has little impact on EPP rates, which may be the case if females engage in EPCs regardless of male-guarding efforts. We expect male-guarding may prevent neighboring males from intruding and displaying to their mates, which is expected to reduce cuckoldry. If this is the case, guarding does not necessarily eliminate a female's opportunities to obtain EPCs, because in the closely related superb fairy-wrens, females sneak out for EPCs under cover of darkness (Double and Cockburn 2000) and it is possible that female red-backed fairy-wrens may do the same.

Another possible reason we see little acoustic and behavioral mate-guarding in our study is that EPP rates may be too high to favor acoustic mate-guarding in this species, and they may exhibit the behavior predicted by Kokko and Morrell's (2005) model for species with high cuckoldry rates (i.e., low levels of mate-guarding). This also seems unlikely because red-backed fairy-wrens are faithful to one another almost as often as they are unfaithful (given that EPP rates are 54% of young), which predicts they should show maximal levels of mate-guarding. One last possibility is that we may have missed a finer-scale pattern, as some males within our study population may have reduced need to guard their mates. Specifically, theoretical models (Kokko and Morrell 2005) predict that unattractive males should guard their mates at high levels, because they are vulnerable to cuckoldry and have a low probability of siring extrapair young of their own, whereas attractive males should guard their mates little, because they will receive a higher payoff from seeking EPCs (i.e., their attractiveness both protects them from cuckoldry and increases success with additional females). In our study species, red-black males appear to be relatively attractive, compared with males that breed in brown plumage (Karubian 2002; Webster et al. 2008). We would expect more mate-guarding behavior (including acoustic guarding) from brown than red-black males. Indeed, we found that during experimental trials, brown males showed more aggression to intruders than red-black males during their mate's fertile stage, and they followed their mates more across all treatments and stages. Future studies will be tailored to address the possibility that duet function differs between attractive and unattractive red-backed fairy-wren males.

## CONCLUSION

Our study joins the majority of studies to date on the function of avian duets in supporting the territory defense hypothesis (Hall 2009; Dahlin and Benedict 2013). Given the substantial empirical support for this hypothesis, duetting and chorusing may have evolved multiple times in various groups primarily to serve a territorial defense function. Duets, whether antiphonal or overlapping, are often louder, longer, and easier to locate than solo songs, and also signal when more than 1 individual is present and attending to the threat (Hall 2009), which may make them ideal keep-out signals. This study is the first to investigate the function of duetting in a species known to have moderate levels of cuckoldry and thus helps to fill an important gap in our knowledge on the role of the mating system in the evolution of coordinated vocal displays. This and other empirical research into the function of duetting in taxonomically diverse systems help to further our understanding of the evolutionary underpinnings of complex vocal signals when the pressures of defending a territory, coordinating breeding, and successfully guarding a mate vary across species with different mating systems.

## SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

## FUNDING

Research was supported by a National Science Foundation (USA) grant to M.S.W. (grant number 0818962). The National Science Foundation Graduate Research Fellowship Program and the Cornell University Student Research Grant in Animal Behavior provided additional grant support.

We thank E. Greig and S. Kaiser for helpful comments on previous versions of this manuscript and E. Greig for input on research design and data analysis. T. Daniel and B. Congdon provided logistic support in the field, and a large number of excellent field assistants were instrumental in data collection. L. Nietmann and A. Potticary in particular helped with playback experiments and sound recording in the field, and A. Buermeyer, A. Hasler, and M. Ocana assisted with data transcription.

**Handling editor:** Naomi Langmore

## REFERENCES

- Benedict L. 2010. California towhee vocal duets are multi-functional signals for multiple receivers. *Behaviour*. 147:953–978.
- Bradley DW, Mennill DJ. 2009. Solos, duets and choruses: vocal behaviour of the rufous-naped wren (*Campylorhynchus rufinucha*), a cooperatively breeding neotropical songbird. *J Ornithol*. 150:743–753.
- Dahlin CR, Benedict L. 2013. Angry birds need not apply: a perspective on the flexible form and multifunctionality of avian vocal duets. *Ethology*. 120:1–10.
- Dahlin CR, Wright TF. 2012. Duet function in the yellow-naped amazon, *Amazona auropalliata*: evidence from playbacks of duets and solos. *Ethology*. 118:95–105.
- Double M, Cockburn A. 2000. Pre-dawn infidelity: females control extra-pair mating in superb fairy-wrens. *Proc Biol Sci*. 267:465–470.
- Douglas SB, Heath DD, Mennill DJ. 2012. Low levels of extra-pair paternity in a neotropical duetting songbird, the rufous-and-white wren (*Thryothorus rufalbus*). *Condor*. 114:393–400.
- Douglas S, Mennill D. 2010. A review of acoustic playback techniques for studying avian vocal duets. *J Field Ornithol*. 81:115–129.
- Dowling JL, Webster MS. 2013. The form and function of duets and choruses in red-backed fairy-wrens. *Emu*. 113:282.
- Fedy BC, Stutchbury B. 2005. Territory defence in tropical birds: are females as aggressive as males? *Behav Ecol Sociobiol*. 58:414–422.
- Gill SA, Vonhof MJ, Stutchbury BJM, Morton ES, Quinn JS. 2005. No evidence for acoustic mate-guarding in duetting buff-breasted wrens (*Thryothorus leucotis*). *Behav Ecol Sociobiol*. 57:557–565.
- Greig EI, Price JJ, Pruett-Jones S. 2013. Song evolution in Maluridae: influences of natural and sexual selection on acoustic structure. *Emu*. 113:270–281.
- Greig EI, Webster MS. 2013. Spatial decoupling of song and plumage generates novel phenotypes between 2 avian subspecies. *Behav Ecol*. 24:1004–1013.
- Hall ML. 2004. A review of hypotheses for the functions of avian duetting. *Behav Ecol Sociobiol*. 55:415–430.
- Hall ML. 2009. A review of vocal duetting in birds. *Adv Stud Behav*. 40:67–121.
- Hall ML, Magrath RD. 2000. Duetting and mate-guarding in Australian magpie-larks (*Grallina cyanoleuca*). *Behav Ecol Sociobiol*. 47:180–187.
- Hall ML, Peters A. 2008a. Do male paternity guards ensure female fidelity in a duetting fairy-wren? *Behav Ecol*. 20:222–228.
- Hall ML, Peters A. 2008b. Coordination between the sexes for territorial defence in a duetting fairy-wren. *Anim Behav*. 76:65–73.
- Hall ML, Rittenbach MRD, Vehrencamp SL. 2015. Female song and vocal interactions with males in a neotropical wren. *Front Ecol Evol*. 3:1–13.
- van den Heuvel IM, Cherry MI, Klump GM. 2013. Land or lover? Territorial defence and mutual mate guarding in the crimson-breasted shrike. *Behav Ecol Sociobiol*. 68:373–381.
- Karubian J. 2002. Costs and benefits of variable breeding plumage in the red-backed fairy-wren. *Evolution*. 56:1673–1682.

- Karubian J, Sillett TS, Webster MS. 2008. The effects of delayed plumage maturation on aggression and survival in male red-backed fairy-wrens. *Behav Ecol.* 19:508–516.
- Kokko H, Morrell L. 2005. Mate guarding, male attractiveness, and paternity under social monogamy. *Behav Ecol.* 16:724–731.
- Langmore NE. 2002. Vocal duetting: definitions, discoveries and directions. *Trends Ecol Evol.* 17:451–452.
- Levin R. 1996. Song behaviour and reproductive strategies in a duetting wren, *Thryothorus nigricapillus*. 2. Playback experiments. *Anim Behav.* 52:1107–1117.
- Logue DM. 2005. Cooperative defence in duet singing birds. *Cogn Brain Behav.* IX:497–510.
- Logue DM, Gammon DE. 2004. Duet song and sex roles during territory defence in a tropical bird, the black-bellied wren, *Thryothorus fasciatoventris*. *Anim Behav.* 68:721–731.
- Marshall-Ball L, Mann N, Slater P. 2006. Multiple functions to duet singing: hidden conflicts and apparent cooperation. *Anim Behav.* 71:823–831.
- Mennill D. 2006. Aggressive responses of male and female rufous-and-white wrens to stereo duet playback. *Anim Behav.* 71:219–226.
- Mulder RA. 1997. Extra-group courtship displays and other reproductive tactics of superb fairy-wrens. *Aust J Zool.* 45:131–143.
- Mulder RA, Bishop H, Cooper M, Dennis S, Koetsveld M, Marshall J, Saunders BL, Langmore NE. 2003. Alternate functions for duet and solo songs in magpie-larks, *Grallina cyanoleuca*. *Aust J Zool.* 51:25–30.
- R Development Core Team. 2014. R: a language and environment for statistical computing (reference index, version 3.1.2). Vienna (Austria): R Foundation for Statistical Computing.
- Rogers A, Ferguson J, Harrington H, McDowell S, Miller A, Panagos J. 2004. Use of stereo duet playback to investigate traditional duet playback methods and mechanisms of cooperative territorial defence in magpie-larks. *Behaviour.* 141:741–753.
- Rogers AC, Langmore NE, Mulder RA. 2007. Function of pair duets in the eastern whipbird: cooperative defense or sexual conflict? *Behav Ecol.* 18:182–188.
- Rowley I, Russell E. 1990. “Philandering”—a mixed mating strategy in the splendid fairy-wren *Malurus splendens*. *Behav Ecol Sociobiol.* 27:431–437.
- Schwabl H, Dowling J, Baldassarre DT, Gahr M, Lindsay WR, Webster MS. 2015. Variation in song system anatomy and androgen levels does not correspond to song characteristics in a tropical songbird. *Anim Behav.* 104:39–50.
- Seddon N, Tobias J. 2006. Duets defend mates in a suboscine passerine, the warbling antbird (*Hypocnemis cantator*). *Behav Ecol.* 17:73.
- Seibt U, Wickler W. 1977. Duettieren als Revier-Anzeige bei Vögeln. *Z Tierpsychol.* 43:180–187.
- Sonnenschein E, Reyer HU. 1983. Mate-guarding and other functions of antiphonal duets in the slate-coloured boubou (*Laniarius funebris*). *Ethology.* 63:112–140.
- Stokes AW. 1968. Antiphonal calling in quail. *Auk.* 85:83–89.
- Templeton CN, Rivera-Cáceres KD, Mann NI, Slater PJB. 2011. Song duets function primarily as cooperative displays in pairs of happy wrens. *Anim Behav.* 82:1399–1407.
- Thorpe WH, Hall-Craggs J, Hooker B, Hooker T, Hutchison R. 1972. Duetting and antiphonal song in birds: its extent and significance. *Behaviour.* (Suppl 18):1–197.
- Varian-Ramos CW, Webster MS. 2012. Extrapair copulations reduce inbreeding for female red-backed fairy-wrens, *Malurus melanocephalus*. *Anim Behav.* 83:857–864.
- Webster M, Varian C, Karubian J. 2008. Plumage color and reproduction in the red-backed fairy-wren: why be a dull breeder? *Behav Ecol.* 19:517.