

VOCAL DUETTING AND MALE MATING STRATEGY IN A PROMISCUOUS BIRD

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The question of how animals adapt their signals and behaviors to variable physical and social environments is central to the field of behavioral ecology. Throughout an animal's lifetime, both the social context they experience and their own characteristics can change. Much is known about the mechanism, function and evolutionary origin of reproductive strategies across taxa, especially tactics that are fixed over time. Much less is known about plasticity in mating and signaling strategies within an individual's lifetime. Here, we investigate how male mating and signaling strategies vary with attractiveness in a promiscuous, duetting bird species, the Red-backed Fairy-wren. We conducted behavioral observations and experimental tests to determine: 1. the function of vocal duets, 2. how signal function and general mating strategy vary with male attractiveness and 3. whether these mating and signaling strategies are effective and yield fitness returns. We found that male birds use duets to mediate both territorial and breeding interactions. The degree to which males use duets for acoustic mate guarding (i.e. duetting to repel rivals and protect paternity), and whether they employ other physical mate guarding tactics, depends on the social environment each male experiences. Unattractive males (those who are not preferred by females and have little success obtaining extra-pair matings) used duets for acoustic mate guarding and invested in intense physical guarding of mates. Attractive males used duets primarily for territory defense and invested most in obtaining additional matings. Both physical and acoustic guarding were effective, since they prevented intrusions from rival males, but acoustic guarding repelled rivals to a lesser degree. Each male's strategy appeared to provide him optimal reproductive success. Specifically, unattractive males had higher within-pair reproductive success, attractive males higher extra-pair reproductive success, resulting in equal total reproductive success between

male types. Our research fills an important gap in our understanding of how flexible mating strategies can evolve, since there appears to be selection for males to adjust the mating strategy they use continually, depending on the mating opportunities available to them. Our studies also show that duet function can vary, depending on the signaler and his social priorities.

BIOGRAPHICAL SKETCH

Jenelle Dowling's interest in the natural world has followed her closely throughout her life. A strong childhood interest has manifested itself in several forms, first as childhood exploration, later as a desire to introduce others to the natural world, and now for her profession. Upon entering college at University of Maryland, Baltimore County (UMBC), she conversed with several professors about her interests and was eventually lead to Dr. Kevin Omland, who studies avian biology and evolution. She described to him a broad interest in zoology, animal behavior and evolution. He asked that she participate in his field research, despite her less-than-focused interests and mispronunciation of zoology. Joining the research team as often as possible, she slogged through streams to set up mist-nets and ran through wheat fields with fellow students while hollering to flush birds into the nets.

This first experience with formal field biology felt much more like a homecoming than an introduction. She soon began an independent research project with Dr. Omland that allowed her to supervise students in the field and provided the opportunity to interact with graduate students and understand the intricacies of graduate level scientific investigation. After graduation, she accepted a position with Dr. Pete Marra with the Smithsonian Migratory Bird Center. She conducted fieldwork with birds for two years, all over the world, and worked as an educator for the Smithsonian Neighborhood Nestwatch program in the summers. In this citizen science project, she conducted hands-on research on birds with hundreds of homeowners, teachers and students.

After she acquired experience with science education, evolutionary biology, behavioral ecology, and community outreach, she pursued a doctoral program that would integrate all of these elements while allowing her to make novel inquiries and enhance our general understanding of behavioral ecology. At this point, she began working with Michael Webster as a graduate student in both the Neurobiology and Behavior department and the Cornell Lab of Ornithology's Macaulay Library at Cornell University.

For my Grandmother, Dorothy and my Mother, Jo Ann, who showed me how to be strong

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PREFACE

Humans have long appreciated the myriad sounds, colorful displays, elaborate movements and complex scents created by plants and other animals, though they differ greatly from our own communications. These signals mediate social interactions that determine an individual's survival and reproduction, and this selection in turn determines how signal traits evolve. I am intrigued by the question of how animal communication evolves in an environment with variable and shifting social pressures, ecological conditions and increasingly, human impact. To investigate this, I conducted an in-depth study of how vocal signals and mating strategies are shaped by an individual's social environment. My goal is to further our understanding of the micro-evolutionary processes that shape communication and behavior.

The work outlined here explores the evolution of behavioral strategies in variable social environments. Determining how animals adjust their mating behaviors to achieve optimal reproductive success is a central question in organismal biology. Much is known about mating strategies in species where males have discrete phenotypes that are fixed throughout their lives (due to genetic polymorphism or some other fixed physiological or morphological characteristic) but less is known about how plasticity in mating strategy within a male's lifetime evolves. Evolution can drive flexibility in male mating strategy over a short time scale if choosing the correct strategy has a sufficient influence on a male's reproductive success.

During my dissertation research, we conducted an in-depth study of the behavior of the Red-backed Fairy-wren, a promiscuous Australian passerine that exhibits unique vocal behaviors. We discovered that this species sings an unusual, overlapping duet (described in detail in chapter 1) that they use to mediate interactions with neighbors and lay claim to breeding territories (chapter 2).

In addition to using duets for territory defense, male birds also used duets and other behaviors to mediate breeding interactions, in a way that depended on the social environment

each individual male experienced, and these strategies affected their reproductive success (detailed in chapter 3). Duetting with a fertile mate is called acoustic mate guarding, and it has long been assumed that it is used to signal the mated status of the female and repel rival males, thus protecting the signaler's paternity. Male Red-backed Fairy-wrens that do not have many mating opportunities outside of their pair bond (i.e. unattractive males) sang more duets with their fertile mates than attractive males (those who have success obtaining extra-pair matings). This suggests that unattractive, but not attractive males use duets to guard their mates.

Unattractive male Red-backed Fairy-wrens also physically guarded their mates (remained present constantly, followed closely and aggressively defended against rivals) in addition to acoustic mate guarding. We experimentally tested the effectiveness of acoustic mate guarding by removing males and using playbacks to simulate either acoustically guarding his mate, or not. We found that both types of guarding, mate following and duetting, prevented rival males from intruding onto a focal male's territory. Acoustic guarding decreased intrusion rate to a lesser degree than physical guarding, and did not prevent males that had already intruded from courting females (chapter 4). These results suggest that duetting may serve as a long-range signal, acting as the first tier of defense against intruders, whereas physical guarding may more directly prevent paternity loss once an intruder has arrived on the territory.

Attractive males did very little acoustic or physical mate guarding and instead invested in leaving their territory to seek additional matings. During behavioral observations, attractive males left their territories and displayed to neighboring females more than unattractive males (chapter 3). In addition, when a male was experimentally removed from the territory, attractive males intruded on that territory more often and unattractive males less often than expected, given their representation in the population (chapter 4).

The strategies that each type of male adopted allowed each to achieve reproductive

success that was optimal, given their mating opportunities. Specifically, unattractive males prevented their mates from cuckolding them through physical and acoustic mate guarding, which resulted in higher within-pair reproductive success than we observed for attractive males. Attractive males gained additional matings by leaving the territory and displaying to neighboring females, which resulted in higher extra-pair reproductive success than unattractive males. The total reproductive success of both male types was equal, suggesting their chosen strategy was adaptive and provided them optimal reproductive success, given the mating opportunities available to them.

These results indicate an adaptive signaling strategy may have evolved in response to each male's specific social environment. This provides one of the first examples of a bird species where males optimize their reproductive success by adopting a conditional mating strategy based on their attractiveness. Our research fills an important gap in our understanding of how flexible mating strategies can evolve, since there appears to be selection for males to adjust the mating strategy they use continually, according to their attractiveness. Alternative male reproductive tactics are well studied, and we know how and why fixed tactics evolve, but we know less about how and why strategies change within a male's lifetime. Few studies have described the specific micro-evolutionary processes that take place and lead to flexibility in a mating strategy. Here, we provide evidence that males that change their strategy depending on the physical attributes they currently possess can optimize their reproductive success, which may drive the evolution of plasticity in mating strategy.

Our studies also showed that the function of vocal duets can change, depending on the signaler and the context. Males that were at high risk of cuckoldry used duets to guard mates. This supports previous findings that vocal duets are multifunctional signals. Our results additionally suggest that the same vocal signals can be used for different functions, depending on the social priorities of the signaler.

CHAPTER 1

THE FORM AND FUNCTION OF DUETS AND CHORUSES IN RED-BACKED FAIRY-WRENS

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Abstract

In many avian species, both members of a pair or all members of a breeding group sing, often in duets or choruses. Previous studies indicate that duets often function in territorial defence. However, other potential functions, such as acoustic mate-guarding, need further study, because most species that have been studied have low or unknown rates of extra-pair paternity (EPP) and theoretical models predict species with moderate rates of EPP will guard more than those with very low or high rates. We conducted behavioural observations to describe the form of duets and test hypotheses for the function of duets in breeding groups of Red-backed Fairy-wrens (*Malurus melanocephalus*), a species with moderately high rates of EPP. Within duets, notes overlapped in frequency and time, rather than being antiphonally coordinated. Duets and choruses occurred more in pre-breeding than other stages of breeding, and more in inter-group than intra-group contexts, supporting a territorial defence function. Contrary to predictions of an acoustic mate-guarding function, males did not duet with mates more when they were fertile. Likewise, functions of breeding coordination and group establishment were not supported. Thus, Red-backed Fairy-wrens may use duets for territorial defence rather than

mate-guarding, despite their moderately high rates of EPP. Investigating how vocal behaviour varies across different mating systems allows us to test theoretical predictions and determine how mating system affects the evolution of signalling strategies in birds and other taxa.

Introduction

In many species of group-living animals, members of the same group perform coordinated displays. Such displays include vocal duetting and chorusing, in which two (duet) or more (chorus) members of a group sing together such that their respective song phrases alternate or overlap (Hall 2004). Duets and choruses have been reported in mammals, frogs, insects and birds (e.g. Lilly and Miller 1961; Mitani 1985; Tobias et al. 1998; Bailey 2003).

In many avian families, females sing (Langmore 1998; Garamszegi et al. 2007), especially in the tropics (Slater and Mann 2004), and in many of these species, females join other group members to sing in duets or choruses (Thorpe et al. 1972). Songs that are coordinated between two individuals are defined as duets, whereas those between more than two individuals are labelled choruses (Brown and Farabaugh 1991). Across species, duet structure varies widely, with many species performing precisely coordinated antiphonal (or alternating) duets (e.g. Hall 2006; Mann et al. 2009) and other species performing duets in which the individual notes of song overlap in frequency and time but are not precisely coordinated. This latter category includes cases where identical songs (unison songs; Thorpe et al. 1972; Wickler and Seibt 1980a; Trainer et al. 2002) or different songs (polyphonic songs; Thorpe et al. 1972; Hall and Peters 2008a) are sung simultaneously by different individuals. Choruses are necessarily more complex than duets but are likely to have similar functions (Bradley and Mennill 2009).

In birds, the function of duets and choruses appears to vary between species, and several non-mutually exclusive functions have been proposed (Table 1). Research has yielded considerable empirical support for the territorial defence hypothesis, which appears to be the

most broadly applicable function of duetting (Hall 2009). However, there is also some evidence that duets and choruses can function in establishing or maintaining bonds, and coordinating behaviours within a breeding group (e.g. Benedict 2010). In cooperatively breeding species, helpers may sing to form choruses to increase the threat of the territorial display (Radford 2003; Seddon and Tobias 2003; but see Baker 2009; Bradley and Mennill 2009) and duets also may enhance within-group cohesion (Baker 2009).

Table 1. Definitions and predictions of hypotheses tested for the function of duetting and chorusing in Red-Backed Fairy-wrens

Hypothesis	Definition	Who expected to duet?	Nest stage duetting expected	Interaction context duetting expected	Additional predictions
Territory defense (Seibt and Wickler 1977)	Duet to create stronger “keep out” signal to rivals	All group members	Early breeding stages	Inter-group interaction contexts only	
Group establishment and cohesion (Wickler and Seibt 1980a)	Duet to signal attentiveness to one another and establish group bond	All group members	Early breeding stages	Intra- and inter-group contexts	Groups with newly formed pairs duet more than those who bred together previously
Breeding coordination (Sonnenschein and Reyer 1983)	Female joins duet to exchange information about breeding status and coordinate behaviors	Female joins male and helper songs	When female initiates nesting (before laying)	More in intra-group contexts	
Mate-guarding (Stokes and Williams 1968, Sonnenschein and Reyer 1983)	Males initiate songs and join female songs in her fertile stage to signal her mated status and repel rivals	Male joins female songs	When female fertile	More in inter-group interaction contexts (potential rivals present)	Males initiate more songs in female’s fertile stage and aggressive contexts

Some studies have provided support for the mate-guarding hypothesis (Seddon and Tobias 2006; Topp and Mennill 2008) but others do not (Hall and Magrath 2000; Gill et al. 2005; Hall and Peters 2008b), and the mate-guarding hypothesis is generally less well-supported than the territorial defence hypothesis. Theoretical models (Kokko and Morrell 2005) predict that males should guard little in species with low rates of EPP, in which females are generally faithful to their mates, and also in species with extremely high rates of EPP, as high levels of infidelity both decrease the efficiency of mate-guarding and provide a greater incentive to seek extra-pair matings. It follows that species with moderately high, but not extremely high, rates of EPP will be most likely to exhibit mate-guarding behaviours, including acoustic mate-guarding. Because most previous studies of the function of duets have been conducted on species with either very low rates of EPP (Hall 2009; but see Benedict 2010) or in duetting species for which rates of EPP are not known (Bradley and Mennill 2009), it may be unsurprising that few previous studies have supported the acoustic mate-guarding hypothesis.

Fairy-wrens in the genus *Malurus* are small, insectivorous passerine birds. Both males and females sing, often with overlap in some species (Rowley and Russell 1997). However, vocal behaviour has been studied in only 4 of 11 species of *Malurus*: the Red-winged Fairy-wren (*M. elegans*; Payne et al. 1991), Superb Fairy-wren (*M. cyaneus*; Cooney and Cockburn 1995), Splendid Fairy-wren (*M. splendens*; Zelano et al. 2001; Greig and Pruett-Jones 2008) and Purple-crowned Fairy-wren (*M. coronatus*; Hall and Peters 2008a). Further, much of this work has focused on solo songs rather than duets. Previous studies of fairy-wren song suggest that solo songs function mainly for territorial defence (Payne et al. 1991). Solo songs might also function in signaling social relationships or group membership, because young birds learn songs from their social parents (Greig et al. 2012) and individuals can distinguish non-group members from group members based on song (Payne et al. 1988, 1991). Duetting has been studied in only one species of fairy-wren, the Purple-crowned Fairy-wren, which duets

to defend territories (Hall and Peters 2008a). However, the mating system of the Purple-crowned Fairy-wren differs markedly from that of other species of *Malurus*, in that they have very low rates of EPP, suggesting high female fidelity (Kingma et al. 2009). Thus, as predicted by theoretical models (Kokko and Morrell 2005), males expend little effort in mate-guarding and do not use duetting to guard their mates acoustically (Hall and Peters 2008b).

We investigated the structure and function of duets in the Red-backed Fairy-wren, a cooperatively breeding, promiscuous species of *Malurus* in which all members of the cooperatively breeding group sing (male, female and auxiliaries). Relative to avian species in other genera, this species has high rates of EPP. However, within *Malurus*, rates of EPP in Red-backed Fairy-wrens are at intermediate levels (54% of young result from extra-pair copulations; Webster et al. 2008), when considered along the spectrum from Purple-crowned Fairy-wrens (4.4% of young; Kingma et al. 2009) to Superb Fairy-wrens (76% of young; Mulder et al. 1994). Thus, theoretical models predict that mate-guarding should be strongly favoured in Red-backed Fairy-wrens, which makes this an ideal species to test hypotheses for the function of duetting. We recorded songs and used intensive behavioural observations to: (1) confirm that Red-backed Fairy-wrens are indeed singing duets and choruses (i.e. multiple individuals overlap their songs in a non-random fashion), (2) describe the structure of duets and choruses and (3) distinguish among several hypotheses for the function of duets and choruses (Table 1). This study provides the first description of singing behaviour and the form of duets in the Red-backed Fairy-wren, and is one of the first studies to investigate duetting behaviour in a species with moderately high rates of EPP.

We chose to investigate the hypotheses for duet function in Table 1 based on their general empirical support across avian species and also their relevance to our study species. We investigated the territorial defence hypothesis (Seibt and Wickler 1977) because it is generally well supported in many species, including the closely related Purple-crowned Fairy-wren (Hall and Peters 2008b). We investigated the group-establishment hypothesis (Wickler

and Seibt 1980a) because, although Red-backed Fairy-wrens tend to maintain pair-bonds from year to year (M. S. Webster, unpubl. data), they experience some instability in group composition, including disappearance of mates and subsequent re-pairing, as well as the occasional addition or loss of helpers (Varian-Ramos et al. 2010). This may select for signalling strategies that establish group membership. We tested the breeding coordination hypothesis (Sonnenschein and Reyer 1983) because Red-backed Fairy-wrens breed cooperatively in groups and also nest asynchronously across the population, which suggests that a female may need to signal her initiation of nesting and coordinate breeding behaviours between all members of the group (in an ask and answer style; Sonnenschein and Reyer 1983), because there is no population-wide cue to signal the start of synchronous breeding (Hall 2009; Benedict 2010). We investigated the mate-guarding hypothesis (Stokes and Williams 1968; Sonnenschein and Reyer 1983) because of its particular relevance to duetting species with moderate rates of EPP (detailed above).

Materials and methods

Field methods

We conducted behavioural observations in the breeding season (October–January) of 2010–11 and 2011–12 near Herberton, northeastern Queensland, Australia (145°25'E, 17°23'S). Basic breeding data have been collected on this study population during each breeding season since 2003 (see Webster et al. 2010). In this study, all birds in focal groups were marked with a unique combination of colour-bands and basic breeding data were collected as per previous studies (Webster et al. 2008; Varian-Ramos and Webster 2012). First observations of the day (2010–11: observation = 68 h, 2011–12: observation = 27 h) were started within 30 min of sunrise. Second observations of the day were started immediately after the first and all began within 4 hours of sunrise (before 1030 hours). In 2010–11, 50 hours of observation occurred later in the morning, in 2011–12, 69 hours of observation occurred later (a separate

experiment was being conducted immediately after sunrise in 2011–12, so behavioural observations were often started later). The period between sunrise and late morning is a peak time for singing by Red-backed Fairy-wrens (J. L. Dowling, pers. obs.). Observation sessions lasted either 1 hour (in 2011–12) or 2 hours (2010–11); we conducted a total of 214 hours of observation. At the start of an observation, all members of one focal group were located and one observer continually tracked and tallied the behaviour of each member on a data sheet for 1 or 2 h. We were able to track the location of each member of a group and determine the identity of each bird that sang on most occasions for the whole observation session; on average, over all observations, the singers of only two of 19 songs were not identified.

We conducted observations on 57 focal breeding groups, each of which was observed for an average of 3.74 hours (s.d. ± 3.46) over the study. Within the 1- or 2- hour observation sessions (161 sessions), periods within sessions were categorised as intergroup, when members of a breeding group interacted with conspecifics outside of their group, or intragroup, when members interacted only with their breeding group. Of the 236 total periods identified within the 214 hours of observation, 81 were intergroup periods (8 hours of observation) and 155 were intragroup periods (206 hours of observation). Intergroup periods were recorded in 52% of observation sessions and when they occurred, intergroup periods lasted a mean of 6.04 min (s.d. ± 6.67).

In Red-backed Fairy-wrens, young (typically males) often remain on their natal territory and assist their parents at the nest, either in the same season they hatch or in the next breeding season (Varian-Ramos et al. 2010). Of the 57 focal groups observed, 39 were pairs with no helper, 15 had one helper, two had two helpers and one group had three helpers. The total song output for a group did not increase with an increase in the number of helpers (one-way analysis of variance (ANOVA): $F_{2, 154} = 0.4$, $P = 0.668$), but the one group with three helpers did have a higher song output than all other groups. To be conservative we excluded this unusually large group from all analyses. During observations, tallies of the number of

songs sung by helpers is the sum of song contributions from all helpers, but in the two groups with two helpers, one of the helpers tended to sing and the other was mostly silent (J. L. Dowling, pers. obs.), which appears to explain why total song output did not increase in these groups. All helpers in 2010–11 were confirmed as males; those in 2011–12 were assumed to be male (helpers in this species are almost always male, Varian-Ramos and Webster 2012). Where helpers were present ($n = 17$ groups), most were the social offspring of the breeding male and female from the previous season (88% of groups), some were the social offspring of only one of the breeding adults (24% of groups; this occurred when one member of the breeding pair died or disappeared) and some were not the social offspring of either breeding adult (12% of groups; this occurred when an unrelated helper joined a group at the beginning of the breeding season, presumably after dispersing from their natal group; J. Dowling, pers. obs.). Genetic analyses were not conducted to determine parentage of these offspring for this study.

For each focal group, observations were conducted during three breeding stages: (1) the pre-breeding stage, defined as the period before the female began nest building ($n = 64$ hours of observation); (2) the fertile stage, when the first solicitation or copulation was seen until the penultimate egg was laid ($n = 54$ hours of observation); solicitations or copulations were seen an average of 6 days (s.d. ± 3.5) before first egg is laid, and no observations were conducted in the fertile stage after the day the first egg was laid; and (3) the post-fertile stage, which was the time between completion of the clutch and fledging of young ($n = 96$ hours of observation). One pre-breeding observation session was conducted on a day that the female was later seen building but we included this in analyses because we confirmed she was very likely not yet fertile at that time (her first egg was laid 23 days later).

We collected data on the number of solo, duet and chorus songs that were sung and the amount of time each group member spent within their territory. We also collected data on physical spacing and interactions between group members, which are not presented here. We

kept a tally of solo songs and, for duets and choruses we used a stopwatch to measure the time between the start of the first song in a duet or chorus and the start of the responder's song (reaction time). We defined solo songs as songs where one individual sang alone and no other individual in the group sang within 1 second of the end of the song. In the field, each duet or chorus was categorised based on the reaction time of the responder. We defined categories of duets based on analyses of spectrograms of duets audio-recorded during a subset of initial observations (see below).

Because the habitat of this species at our study site is open and individuals often perch off of the ground when singing duets, we were able to determine visually which group members were participating in a duet or chorus (although we were not able to determine which notes within the duet they were contributing). This allowed us to collect data on which group members contributed to each duet or chorus and also determine whose song initiated the duet and whose song followed in cases where songs did not overlap completely.

Analysis of recordings of songs and behavioural observations

To determine if songs of Red-backed Fairy-wrens overlapped in a non-random fashion (i.e. a true duet or chorus), we used Chi-square tests to determine if the song of one individual overlaps another's more often than expected if they time their songs randomly with regard to one another. We calculated Chi-square values by determining the number of times an individual sings while another is singing and the number of times they sing while the other individual is silent, and compared these to the number of times they are expected to sing if the birds sing independently of one another. Expected values were calculated following established methods for determining temporal patterning of singing (Ficken et al. 1974). Observations were excluded if the male, female or helper (if applicable) were not present or did not sing during the observation. To analyse the probability that a helper and a breeding male or female will duet or chorus, duets or choruses initiated by the breeder were lumped

with duets or choruses initiated by the helper because helpers very infrequently initiated duets or choruses ($n = 5$ helper-initiated duets or choruses in 214 hours of observation).

To describe the acoustic structure of duets and choruses and define categories based on degree of overlap, we analysed a sample of recordings of 38 duets and choruses from 15 groups, recorded in 2009–10 and 2010–11. Songs were recorded with a Marantz PMD661 solid-state digital recorder (Marantz America, Itasca, IL, USA) and Sennheiser ME67 highly directional long-gun microphone with a Rycote softie windshield and mount (Sennheiser Corp., Old Lyme, CT, USA). Spectrograms were created in Raven 1.4 (Cornell Laboratory of Ornithology, Ithaca, NY, USA) (16-bit sample format; discrete Fourier transform (DFT) = 512 samples; frequency resolution = 124 Hz; time resolution = 11.6 ms; frame overlap = 50%). We measured reaction time (the amount of time between the start of the first song in a duet or chorus and the start of the responder's song), percentage of the notes that overlapped in the duet or chorus, bandwidth (the frequency range that includes 90% of the energy in the song selection and is bounded by the upper and lower quartile frequencies) and length of song (s). To investigate how Red-backed Fairy-wren duets or choruses differ from solo songs, we compared songs in the duet or chorus to solo songs sung by the same individual and analysed these differences quantitatively and qualitatively. Observations of duetting behaviour and initial analyses suggested that duets and choruses separate naturally into categories based on reaction times of the duetting or chorusing partners. We used K-means clustering (Hartigan and Wong 1979), based on measurements of reaction time and percentage of note overlap, to define quantitatively the discrete categories we observed.

Duets and choruses from this sample of recordings grouped into three clusters: (1) duets and choruses with reaction times of <1 second (i.e. complete or near-complete overlap of songs; $n = 12$ duets or choruses); (2) reaction times ± 1 second (incomplete overlap; $n = 22$ duets or choruses) and (3) no overlap but <1 second passed between the end of the first song and start of the second song (non-overlapping duets or choruses; $n = 4$ duets or choruses).

The acoustic structure of solo songs does not differ between males, females and helpers (J. L. Dowling, unpubl. data). To determine differences in song structure between solos, duets and choruses, we used paired t-tests to compare the bandwidth and length of an individual's solo song to the bandwidth and length of a duet or chorus that they contributed to. Because we were not able to separate the two (or more) songs that make up a duet (or chorus) from one another, we were not able to compare a solo song of an individual to just their song within a duet, but rather compared it to the entire duet. We also visually inspected spectrograms of songs sung in a duet to determine if they appeared subjectively different from that individual's solo song (in terms of note spacing, number of notes and shape of notes).

Because we were not able to separate songs of each contributor to a duet or chorus we were unable to analyse quantitatively the note structure of each contributor within the duet or chorus because many of their notes overlap. Red-backed Fairy-wrens songs have a high rate of notes per second (average 17 ± 3 notes per second) (J. L. Dowling, unpubl. data), which prevents us from using the traditional method of observing and denoting who contributed which duet note in the field (as in Mann et al. 2006). In this species, duetting and chorusing individuals also typically sing in close physical proximity (typically within 1 m of one another), which prevented us from investigating fine-scale note structure by recording duet contributors separately (as in Voigt et al. 2006).

We calculated the proportion of total songs that were duets or choruses by summing the number of songs that included two or more individuals singing together (either songs overlapping or birds singing within 1 second of one another) and dividing by the total number of all singing instances from all group members (solos and all types of overlapping and non-overlapping duets and choruses). We calculated the female's response rate to the song of the male by summing (1) the number of songs the male and female sang with complete overlap and (2) the number of over-lapping and non-overlapping duets or choruses in which the female answered a song initiated by the male, then dividing that sum by the total number of

all female singing instances. The same calculations were done to determine the female's response rate to the song of a helper.

We calculated the male's response rate to song of the female by summing (1) the number of songs the male and female sang with complete overlap and (2) the number of overlapping and non-overlapping duets where the male answered a song initiated by the female then dividing that sum by the total number of all female singing instances. We did not calculate the male's response rate to the helper song because none of the hypotheses tested made predictions about when the male should duet with the helper. We calculated rates of song initiation by males to test a prediction of the mate-guarding hypothesis (see Table 1 and description of this prediction below). Initiation rates for males were calculated by summing the solos, duets and choruses initiated by the male and dividing by the time (min) that he was present during each observation session.

For all calculations, we recorded whether songs occurred when each of the other group members was present or absent. For calculations of duets and choruses, we considered whether all relevant group members were present to be sure this did not affect tallies of duets and choruses (e.g. a male can only duet with a female if he is present so only songs sung when both were present were used to calculate such proportions).

Statistical analyses

Data were mainly analysed using R version 2.15.3 (R Core Development Team 2013). All proportional data were analysed using generalised linear mixed models (GLMM) with binomial counts as outcomes and a weight argument specified. Rate data (i.e. rates of song initiation – see Results) were analysed using a GLMM with a Poisson distribution and an offset argument. All GLMM were run using the `glmer` function in the `lme4` package within R. Chi-square tests and percentage calculations were done in Microsoft Excel 2011 (Microsoft Corporation, Redmond, WA, USA). Some statistical tests (ANOVA, paired t-tests and K-

means clustering) were performed in JMP version 10 (SAS Institute Inc., Cary, NC).

Tests of hypotheses

To investigate hypotheses for the function of duetting in Red-backed Fairy-wrens (Table 1), we used GLMM with the identity of the breeding group as a random effect (to control for multiple observations conducted for each breeding group in a single year). Fixed effects in models were: behavioural interaction context (categorical: inter-group and intra-group), breeding stage (categorical: pre-breeding, fertile and post-fertile) and age of pair-bond (categorical: 1 = pairs and groups in which the male and female are breeding together for the first time; 2 = pairs and groups in which the male and female have bred together previously). For age of pair-bond, the category 1 includes all pairs that are newly formed, including those with helpers; for groups with helpers, they are the offspring of only one of the breeding adults or are not related to either breeding adult. Category 2 includes all pairs that had bred together previously, including pairs with helpers; in this category, helpers are the offspring of both breeding adults and were retained as helpers from the previous breeding season. We did not consider the addition of a related helper to the group a change in composition of the group because such helpers always hatched in the group and did not join through the formation of a new group at the beginning of the breeding season the way that a new breeding partner or unrelated helper does.

To test the territorial defence and group-establishment hypotheses, we determined how the above predictors affected the proportion of total songs that were duets. The territorial defence hypothesis predicts that a higher proportion of songs will be duets during early stages of breeding and, if territorial defence is the only function of duetting, duets will only occur during inter-group contexts. The group-establishment hypothesis predicts that groups that have not spent time together as a group will duet to establish or signal their pair-bond or commitment to one another more often than groups that have bred together previously. It also

predicts that duets will occur during both intra-group and inter-group contexts (because a response that forms a duet can signal a bird's attentiveness to another in either context).

To test the breeding coordination hypothesis, we determined whether the female's response rate to the male and helper depended on stage of breeding and interaction context. This hypothesis predicts that females will respond to a higher proportion of male and helper songs in her fertile stage than in other stages of breeding, and in intra-group more than inter-group contexts. The female is expected to respond to songs of other group members (forming a duet) to provide information on her readiness to initiate breeding, so that the group may coordinate their breeding behaviours.

To test the mate-guarding hypothesis, we determined whether the response rate of the male to the female and the rate of song initiation by the male depended on breeding stage and interaction context. This hypothesis predicts that a male will respond to a higher proportion of a female's songs in her fertile stage than in other stages, and more in inter-group contexts (when potential rivals are present) than intra-group contexts. It also predicts that males will initiate more songs during the female's fertile stage than in other stages, because song initiation elicits a response from the female; essentially, the male guards the female (by confirming that she is present) through her acoustic guarding of him (Sonnenschein and Reyer 1983). We analysed duets that occurred between the breeding male and female because acoustic and behavioural mate-guarding are expected to occur mainly between members of a breeding pair. Auxiliary helpers who are related to the breeding male also might be expected to guard the breeding female (Welbergen and Quader 2006). We generally expect a son to protect his father's paternity to increase his indirect genetic representation, but when such mother guarding should occur depends also on offspring viability (Welbergen and Quader 2006). Because this information is not available for Red-backed Fairy-wrens, we made no predictions for when helpers should duet with the breeding female.

We did not test the identity hypothesis, which suggests that individuals use duetting to

identify and localise the members of their breeding group in both agonistic and non-agonistic contexts (Logue and Gammon 2004). We do not have sufficient data on approach behaviour after duets (which would allow us to test the partner locating function of duetting). In addition, we feel that this duet function is unlikely for our study species because they occupy an open habitat and appear to remain in contact using a simple contact call (Logue and Gammon 2004; S. Shah, E. I. Greig and M. S. Webster, unpubl. data) that varies between individuals (J. L. Dowling, unpubl. data), which is likely to make these calls sufficient to identify and locate fellow group members.

Results

Non-random overlap of songs

Males sang 34% of their songs ($n = 1147$ total male songs in 211 hours of observation) in duets with females and females spent only 0.51% of their time singing. Females sang 44% of their songs ($n = 1099$ total female songs in 190 hours of observation) in duets with males and males spent only 0.46% of their time singing. Helpers sang 22% of their songs ($n = 345$ total helper songs in 73 hours of observation) in duets with males and 21% of their songs in duets with females, and helpers spent only 0.33% of their time singing. Overall, our analyses indicate that Red-backed Fairy-wrens sing true duets or choruses, as the overlap in song of different individuals that we observed occurred more often than expected if individuals sing randomly with regard to each other. In 75% of the observation sessions when the female sang ($n = 102$ observation sessions), she sang more often when the male was singing and less often when he was silent than expected if she sings randomly (of those significant, 97% were significant at $P < 0.001$, one was significant at $P < 0.01$ and one at $P < 0.05$). Similarly, in 76% of observation sessions where the male sang ($n = 97$ observation sessions), he sang more often when the female was singing and less often when she is silent than expected if he sings randomly (of those significant, all but one were significant at $P < 0.005$). Finally, helpers sang

more often than expected if singing randomly when the male was singing (78% of observation sessions, $n = 27$ observation sessions, all $P < 0.001$) and also when the female was singing (71% of observation sessions, $n = 28$ observation sessions, all $P < 0.001$).

Form of duets

Members of Red-backed Fairy-wren groups join their songs together in duets and choruses with varying degrees of overlap (see Methods). Field observations and inspection of a sample of spectrograms of recorded duets or choruses suggest that duetting or chorusing birds did not coordinate notes to avoid overlap and did not sing antiphonally. Of the sample of 38 recordings of duets or choruses we analysed, 34 (90%) included notes that overlapped in frequency and time. The four duets and choruses with no overlap did not have precisely coordinated notes within the duet, but songs were separated in time, with the second song initiated <1 second after the end of the first. Within the overlapping duets or choruses, an average of 71% (s.d. $\pm 15\%$) of the notes within the song overlapped in frequency and time (see Fig. 1). The duets and choruses we analysed had an average bandwidth of 3064.76 Hz (s.d. ± 766.1 , $n = 38$) and an average length of 4.24 seconds (s.d. ± 0.8 , $n = 38$).

Pair-wise comparisons of the broad-scale structure of this sample of songs revealed that duets and choruses were longer than the solo songs of their contributors (two-tailed paired t-test: $t_{30} = -2.37$, $P = 0.024$), but these solo songs and duets did not differ in bandwidth (two-tailed paired t-test: $t_{30} = -1.75$, $P = 0.09$). Subjectively, when we visually compared spectrograms of duets and choruses to solo songs of the same individuals that contributed to the duet ($n = 30$ comparisons), we also saw little difference in fine-scale song structure (Fig. 1). The general shapes of the songs were similar and there were no noticeable

differences in spacing of notes, number of notes and shape of notes between songs sung in solos and in duets and choruses.

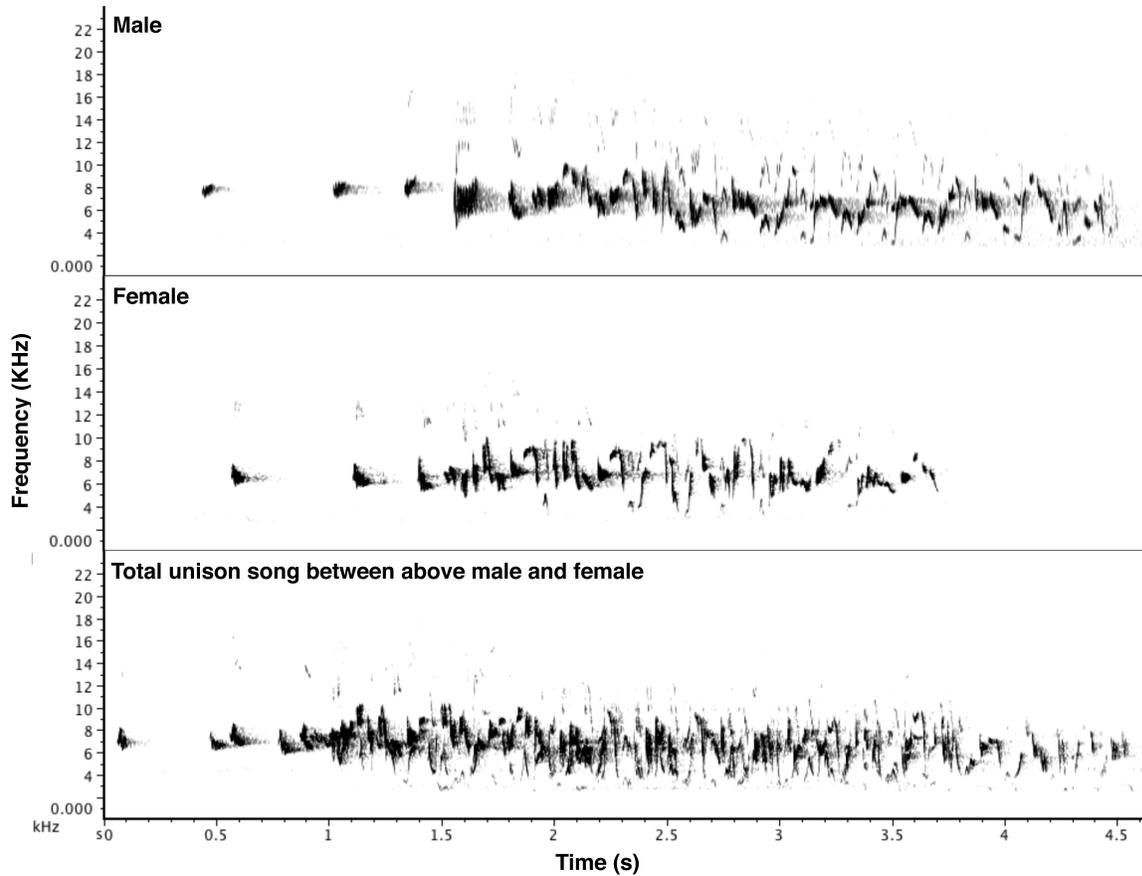


Figure 1. Spectrograms of a song of a breeding male (top), his social mate (middle) and a duet with near complete overlap recorded from this same pair (bottom).

Additionally, the two (or more) songs that made up the duet or chorus appeared non-identical (i.e. they were not unison songs). However, it was often difficult to assess note characteristics and more quantitative analyses were not possible owing to overlap of notes. These results provide preliminary impressions of the fine-scale structure of Red-backed Fairy-wren duets and choruses but further quantitative analysis is needed.

Solo, duet and chorus singing behaviour

During our systematic behavioural observations, males, females and helpers initiated a similar number of songs (solo songs and initiating songs in duets or choruses). Rates of song initiation by males and females were similar in pairs without helpers (male average 6.2 songs h⁻¹ of observation (s.d. ±9.8); female: 6.3 songs per hour of observation (s.d. ±11.6); two-tailed paired t-test, $t_{106} = -0.09$, $P = 0.93$, $n = 107$) and in groups with one or more helpers (male average, 2.9 songs per hour of observation (s.d. ±5.1); female average, 2.2 songs per hour of observation (s.d. ±3.5); two-tailed paired t-test, $t_{43} = 1.18$, $P = 0.25$, $n = 44$). Helpers initiated a similar number of songs (average 3.2 ± 6 songs per hour of observation) as adult males (two-tailed paired t-test, $t_{43} = -0.78$, $P = 0.44$, $n = 44$) and adult females (two-tailed paired t-test, $t_{43} = -1.62$, $P = 0.11$, $n = 44$).

For duets and choruses during behavioural observations, reaction times of duet partners varied greatly. When duets and choruses across all groups were analysed, most had near complete overlap (78.8% had complete or near-complete overlap, 13.2% had incomplete overlap and 8% had no overlap; total $n = 684$ duets or choruses). Within male and female duets in which initiator and answerer could be determined (i.e. duets that did not overlap completely), duets were initiated by the male and answered by the female in more than half of non-overlapping duets (59%, $n = 88$ duets) and most duets with incomplete overlap (68%, $n = 50$ duets), but many duets were initiated by females and answered by males as well. For groups with helpers, most songs sung with complete overlap (78.3%, $n = 65$ of 83 songs) were choruses involving all members of the group (male, female and helper or helpers), few involved only the male and female (9.6%, $n = 9$) and even fewer were between helpers and a single breeder (8.4% for male or helper, $n = 7$; 3.6% female or helper, $n = 3$).

Function of duets

In the model used to test the territorial defence and group-establishment hypotheses, breeding

stage (GLMM, $\chi^2 = 61.51$, d.f. = 2, $P < 0.0001$) and interaction context ($\chi^2 = 193.12$, d.f. = 1, $P < 0.0001$) accounted for most of the variation in the proportion of total songs that were duets or choruses, whereas length of pair-bond was not a significant predictor ($\chi^2 = 2.65$, d.f. = 1, $P = 0.10$). Interpretation of the fixed-effects tests (GLMM 1, Table 2) shows that when compared to the fertile stage, a higher proportion of songs were duets or choruses in the pre-breeding and a lower proportion were duets or choruses in the post-fertile stage (Fig. 2), and a higher proportion of songs were duets or choruses in inter-group contexts than in intra-group contexts (Fig. 3). These results support both predictions of the territorial defence hypothesis but only one prediction of the group-establishment hypothesis. It is important to note that although inter-group interactions occurred in more than half of the observation sessions they generally did not last long and so represent only a small proportion of observation time.

Table 2. Results of generalised linear mixed models (GLMM) testing hypotheses outlined in Table 1

GLMM 1 tests the territorial defence and group-establishment hypotheses, using proportion of songs that are duets as the response variable ($n = 193$ observation periods for 54 groups). GLMM 2 tests the breeding coordination hypothesis, using female's response rate to male as the response variable ($n = 148$ observation periods for 47 groups). GLMM 3 tests the breeding coordination hypothesis, using female's response rate to helper as the response variable ($n = 83$ observation periods for 23 groups). GLMM 4 and 5 are used to test the acoustic mate-guarding hypothesis: GLMM 4 uses male's response rate to female as the response variable ($n = 148$ observation period for 47 groups), whereas GLMM5 uses the male's song-initiation rate as the response variable ($n = 228$ observation periods for 56 groups)

	Fixed effect	Estimate	Std. Error	Z value	P
GLMM 1	(Intercept)	1.29	0.21	5.95	<0.0001
	Pre-breed stage	0.44	0.12	3.62	<0.001
	Post-fertile stage	-0.85	0.19	-4.56	<0.0001
	Pair bond age	-0.48	0.30	-1.63	0.10
	Interaction context	-1.61	0.12	-13.90	<0.0001
GLMM 2	(Intercept)	1.21	0.38	3.192	<0.01
	Pre-breed stage	0.44	0.24	1.86	0.06
	Post-fertile stage	-0.87	0.36	-2.40	0.02
	Interaction context	-1.62	0.22	-7.30	<0.0001
GLMM 3	(Intercept)	-8.38	4.34	-1.93	0.05
	Pre-breed stage	0.23	0.77	0.30	0.77
	Post-fertile stage	-0.80	1.41	-0.57	0.57
	Interaction context	-4.44	0.78	-5.71	<0.0001
GLMM 4	(Intercept)	0.86	0.37	2.36	0.02
	Pre-breed stage	0.73	0.24	3.09	<0.01
	Post-fertile stage	-0.31	0.35	-0.88	0.38
	Interaction context	-1.68	0.22	-7.70	<0.0001
GLMM 5	(Intercept)	-0.43	0.13	-3.38	<0.001
	Pre-breed stage	0.12	0.08	1.46	0.14
	Post-fertile stage	-0.80	0.11	-7.58	<0.0001
	Interaction context	-2.57	0.07	-35.53	<0.0001

In the models used to test the breeding coordination hypothesis, interaction context accounted for most of the variation in both the female's response rate to the male (GLMM, $\chi^2 = 53.29$, d.f. = 1, $P < 0.0001$) and her response rate to the helper ($\chi^2 = 32.55$, d.f. = 1, $P < 0.0001$). Breeding stage was also a significant predictor of the female's response rate to the male ($\chi^2 = 16.87$, d.f. = 2, $P < 0.001$), but not for her response rate to the helper ($\chi^2 = 0.76$, d.f. = 2, $P = 0.69$). Interpretation of the fixed-effects tests (GLMM 2, Table 2) shows that there was no difference in the female's response rate to the male between fertile and pre-breeding stages but that the female responded to a higher proportion of male songs in the fertile stage

than in the post-fertile stage. Females responded to a higher proportion of male and helper songs in inter-group contexts than in intra-group contexts but breeding stage was not a significant predictor in the model for the female's response rate to the helper (GLMM 3, Table 2). These results do not support either prediction of the breeding coordination hypothesis.

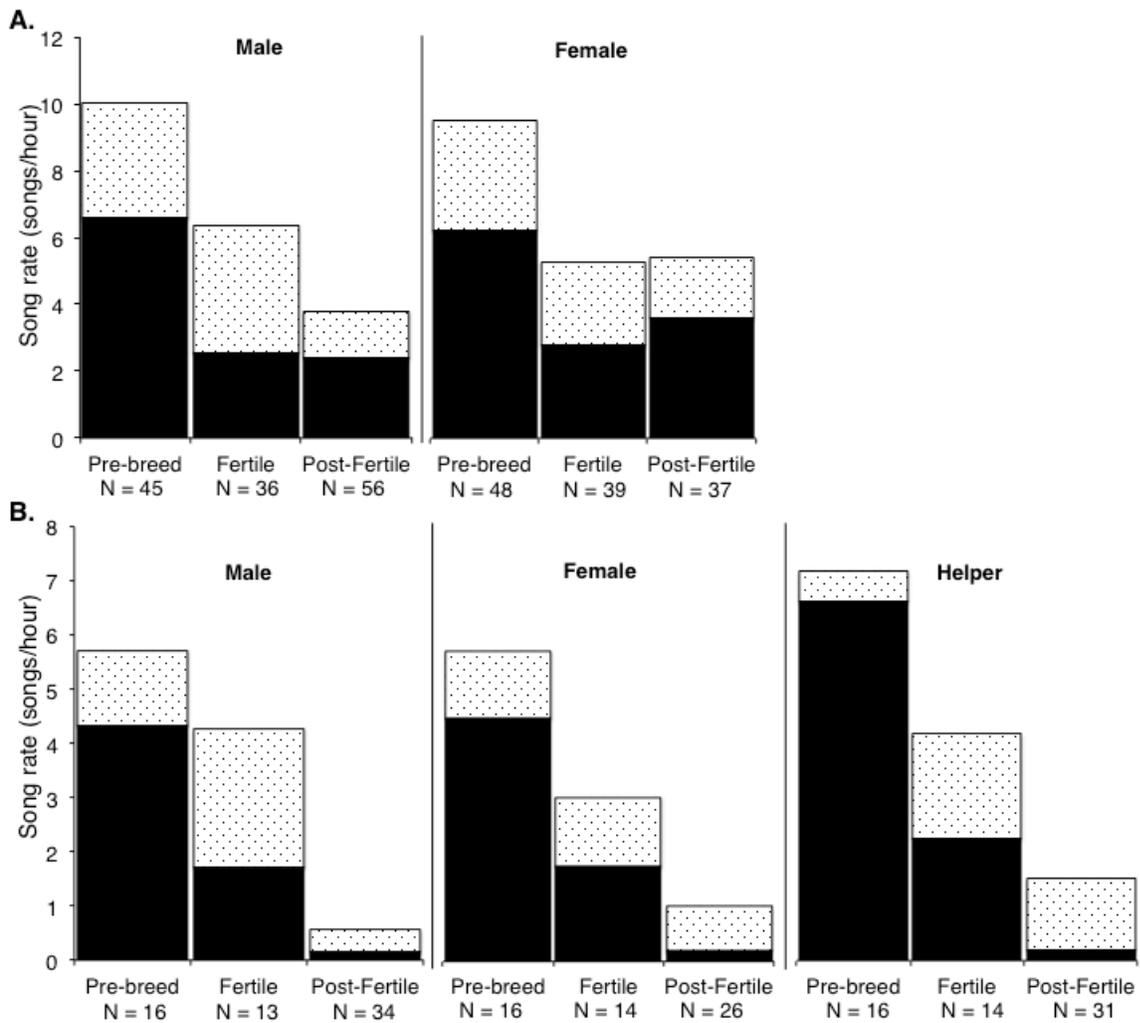


Figure 2. Number of songs sung by each group member in each stage of breeding, including those that were sung with others (duet or choruses, dark bars) and solo songs (light stippled bars): (a) pairs without helpers ($n = 147$ hours of observation of 34 pairs); (b) groups with helpers ($n = 67$ hours of observation of 21 groups). n is the hours of observation for each category, listed below the x -axis. Each chart shows counts (songs per hour) of songs sung solo and songs sung with other individuals, so each duet or chorus is counted for every contributor.

In the model used to test the mate-guarding hypothesis, breeding stage was a significant predictor in the model for the male's response rate to the female (GLMM, $\chi^2 = 17.05$, d.f. = 2, $P < 0.001$) and male song-initiation rates ($\chi^2 = 98.61$, d.f. = 2, $P < 0.0001$), but interaction context accounted for most of the variation in the model in both the male's response rate to the female ($\chi^2 = 59.26$, d.f. = 1, $P < 0.0001$) and male song-initiation rates ($\chi^2 = 1262.51$, d.f. = 1, $P < 0.0001$). Interpretation of the fixed-effects tests (GLMM 4, Table 2) shows that, when compared to the fertile stage, males responded to a higher proportion of female songs in the pre-breeding stage and a lower proportion in the post-fertile stage. There was no difference in male initiation rates between fertile and pre-breeding stages and males initiated more songs in the fertile stage than in the post-fertile stage (GLMM 5, Table 2). Males responded to a higher proportion of female songs (GLMM 5, Table 2) and initiated more songs (GLMM 5, Table 2) in inter-group contexts than in intra-group contexts. These results support only two of four predictions of the mate-guarding hypothesis.

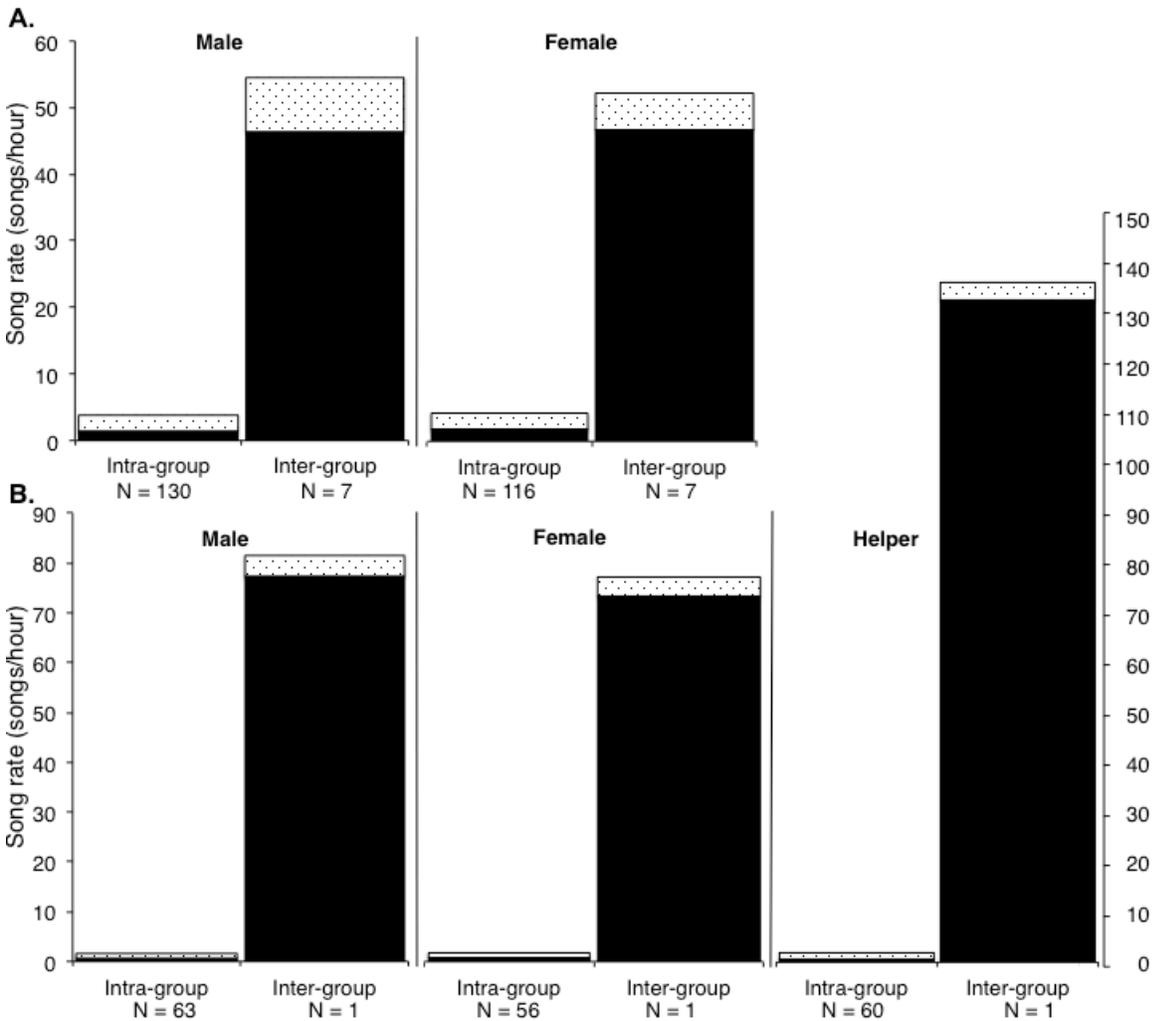


Figure 3. Number of songs sung by each group member in each interaction context (inter-group *v.* intragroup), including those that were duets or choruses (dark bars) and solo songs (light stippled bars): (a) pairs without helpers ($n = 147$ hours of observation of 39 pairs); (b) groups with helpers ($n = 79$ hours of observation of 17 groups). n is the hours of observation for each category, listed below the x -axis. Each chart shows counts (songs per hour) of songs sung solo and songs sung with other individuals, so each duet or chorus is counted for every contributor.

Discussion

In this study, we describe the vocal behaviour of the Red-backed Fairy-wren, with specific attention to the form and function of duets and choruses. This species differs from most other duetting species that have been studied to date in that they have moderately high rates of EPP. We would thus predict high levels of mate-guarding, making it an ideal species for testing the

acoustic mate-guarding hypothesis as well as other hypotheses for duetting.

Do Red-backed Fairy-wrens duet?

Our analysis shows that group members were significantly more likely to sing when another group member was singing (which forms a duet or chorus), and less likely to sing when others were silent. Those cases where the difference was not significant were likely to be a result of small sample sizes, because there was generally little singing activity during these observations (an average of 1.9 songs per hour of observation by the focal individual, compared to an average of 6.7 songs per hour of observation in cases where the singing overlap was significant). These results indicate that Red-backed Fairy-wrens coordinate the timing of their songs so that they overlap and thus fit the definitions for duets and choruses (Hall 2009). Further analyses that investigate reaction times of duetting or chorusing partners, using time as a continuous variable rather than a categorical variable as done here, would lend further support and would also test the precision of song overlap. This type of analysis is difficult for this species because individuals often duet while perched close to one another (within 1 meter), which prevented us from easily recording each individual separately to determine which partner contributed each note of a duet.

Form of duets and choruses

Red-backed Fairy-wrens sing duets and choruses in which non-identical songs overlap in frequency and time rather than precisely coordinated duets with antiphonal notes or phrases (Fig. 1). Duets with this type of overlapping, polyphonic structure have been described in a few other species (Reyer and Schimdl 1988; Brown and Farabaugh 1991; Hall and Peters

2008b), and other types of overlapping duets (unison songs) have also been described (Thorpe et al. 1972; Wickler and Seibt 1980a; Trainer et al. 2002). Interestingly, similar overlapping, polyphonic songs have been described for the Purple-crowned Fairy-wren, which sings a duet style almost identical to that of Red-backed Fairy-wrens (Hall and Peters 2008b), suggesting that this may be the common form of duetting in *Malurus*.

On a proximate level, fairy-wren duets or choruses may not include precise antiphonal alternation of notes because fast rates of notes (average 17 notes per second s.d. ± 3 , $n = 44$ songs for Red-backed Fairy-wrens, J. L. Dowling, unpubl. data) and J. L. Dowling, unpubl. data) make antiphonal coordination difficult (Goller and Suthers 1996). Additionally, on a functional level, Red-backed Fairy-wren duets or choruses may not be precisely coordinated because overlap of notes may be beneficial. It has been proposed that overlap of notes makes a duet or chorus more threatening (Naguib and Mennill 2010) and also that overlap may be a type of coordination that signals the quality of the duetting individuals and the pair-bond (Hall and Magrath 2007). Because Red-backed Fairy-wrens (this study) and Purple-crowned Fairy-wrens (Hall and Peters 2008a) seem to use duets and choruses as coordinated displays meant to deter territorial rivals, we might expect overlapping duets and choruses to be favoured in these species, if they are more threatening, but this remains to be tested with playback experiments. Another possible explanation for this duet form is that it may represent an early transitional stage in the evolution of a coordinated, antiphonal duet (Mann et al. 2009). This hypothesis has also never been tested and comparative phylogenetic studies of duet form, possibly in *Malurus*, are necessary.

The function of duets

Overall, the results of this study support the territorial defence hypothesis. Although some predictions are supported for other hypotheses (group-establishment and mate-guarding), none of the critical predictions of these hypotheses (those that are not shared to some extent between hypotheses) is supported. Thus, our study joins the majority of studies of the function of avian duets to date in supporting the territorial defence hypothesis (Hall 2009). Given the substantial empirical support for this hypothesis, duetting and chorusing may have evolved multiple times in various groups at least partly to serve a territorial defence function. Duets, whether antiphonal or overlapping, are often louder, longer and easier to locate than solo songs, and also signal that more than one individual is present and attending to the threat (Hall 2009), which may make them ideal as keep-out signals.

Support for the territorial defence hypothesis is based partly on observations during inter-group interactions. These interactions are frequent, but brief, and are characterised by extremely high overall rates of song (Fig. 3). Despite few hours of observation in inter-group contexts, we found that most songs in these contexts were sung as duets or choruses (as compared to intra-group contexts). The low overall rates of singing in intra-group contexts, however, may prevent detection of similar patterns in these contexts.

The group-establishment hypothesis was not directly supported by our data. This hypothesis predicts that group members will invest in duetting or chorusing during formation of groups and signal commitment (Wickler and Seibt 1980b) by learning to coordinate a duet or chorus with the group (Arrowood 1988; Smith 1994). We found no support for the critical prediction that groups who have never bred together should duet more than those that have bred together. Although we found that Red-backed Fairy-wrens duet more in the pre-breeding

stage, duets occurred more often in inter-group than intra-group contexts instead of equally in both (as predicted by this hypothesis). Likewise, previous investigations of this hypothesis have found little support for it (Arrowood 1988; Levin 1996; but see Wickler and Seibt 1980b). Although the result that duets and choruses occur more in inter-group than intra-group contexts suggests that duets and choruses are not used for group cohesion, the fact that some duets or choruses do still occur in intra-group contexts may mean that they play a role in group cohesion or some other intra-group communication, which we have not ruled out in this study.

Neither prediction of the breeding coordination hypothesis was supported, as duets occurred primarily during inter-group interactions and females did not duet more with other group members when initiating nests (a critical prediction of the hypothesis). Females could potentially use signals other than song to signal breeding status and coordinate with group members. Indeed, fertile females solicit copulations with wing-flutter displays 6 days (s.d. ± 3.5 , maximum 18 days, $n = 11$ copulations) before her first egg is laid and often conspicuously perch with nesting material in view of other group members during nest-construction (J. L. Dowling, unpubl. data), both of which may signal breeding status to other group members. Previous studies supporting this hypothesis based that support on predictions shared between breeding coordination and territorial defence hypotheses (Hall 2006; Topp and Mennill 2008; see Table 1), whereas other studies (including this study) examined whether females duet more specifically within their fertile stage. Future studies should use this more restricted prediction to distinguish between breeding coordination and territorial defence.

Despite theoretical predictions that species with moderately high rates of EPP should

guard mates heavily, acoustic mate-guarding does not seem to be a primary function of duetting and chorusing in Red-backed Fairy-wrens. This is somewhat surprising because the overlapping form of Red-backed Fairy-wren duets and choruses could allow the joining bird to mask the signal of the initiator of the duet or chorus, which is the presumed goal if the joining bird is acoustically guarding his partner (Tobias and Seddon 2009). Although the mating system and the duet form of Red-backed Fairy-wrens suggest that a mate-guarding function of duetting and chorusing is likely, the critical prediction of this hypothesis (that males will duet more with their mate when she is fertile, Table 1) was not supported by our results.

As an alternative to our interpretation, Red-backed Fairy-wrens may use duetting and chorusing for mate-guarding, but in a way that escaped detection in our study. For example, if extra-pair mating occurs pre-dawn in Red-backed Fairy-wrens, then acoustic mate-guarding is likely to occur at that time as well, instead of during the day, a possibility not investigated in this study. Pre-dawn extra-pair copulations have been suggested in one species of *Malurus* (Double and Cockburn 2000), and may also occur in others because daytime extra-pair copulations are almost never observed in species of *Malurus* (Rowley and Russell 1990). In addition, our current study focussed on duetting and chorusing behaviour of whole breeding groups, but future research in this system could investigate differences in duetting and chorusing behaviour (including acoustic mate-guarding) between males that vary in attractiveness, because male mating strategies might depend on attractiveness (Karubian 2002). Another alternative is that Red-backed Fairy-wren males may guard their mates but use behaviours other than duetting and chorusing to do so, such as close following (e.g. see Hall and Peters 2008b).

Conclusions

Our study is the first to describe the vocal behaviour of the Red-backed Fairy-wren, a cooperatively breeding and promiscuous species of *Malurus*. Red-backed Fairy-wrens sing polyphonic duets and choruses that are unique when compared to other duetting species but similar to other chorusing species (Reyer and Schimdl 1988; Brown and Farabaugh 1991) and also to duets in the congeneric Purple-crowned Fairy-wren (Hall and Peters 2008a). Our results support a territorial defence function for duets and choruses and, taken together with many previous studies (Hall 2009), this seems to be one major function of vocal duets across avian species. However, almost all previous studies that support territorial defence as the main function of duets and choruses have been of species with low or unknown rates of EPP (but see Benedict 2010), and theory predicts mate-guarding (including acoustic) should be most strongly favoured in species with moderately high rates of EPP. Investigating the function of duets or choruses across taxa that vary broadly in their mating systems and reproductive strategies will help fill this important gap in our understanding of duetting behaviour.

The variability seen within the genus *Malurus* provides an excellent model for examining the evolution of songs and other signals. Within the genus, some species coordinate songs into duets (this study; Hall and Peters 2008a) whereas it seems that others do not (Cooney and Cockburn 1995; Greig and Pruett-Jones 2008). Within *Malurus*, species also differ with regard to the number of song-types (E. I. Greig and M. S. Webster, unpubl. data), the use of predator-elicited song (Greig and Pruett-Jones 2008; E. I. Greig and M. S. Webster, unpubl. data), and alarm call and response behaviour (Magrath et al. 2009). The genus also shows variation in mating systems, for example with regard to extra-pair copulation seeking behaviour of males and rates of EPP (Mulder et al. 1994; Webster et al. 2008; Kingma et al.

2009), and within some species individual males appear to adopt different mating strategies (Karubian 2002; Cockburn et al. 2009). This variation provides fertile ground for examination of the general rules that govern the evolution of broader reproductive strategies, including signalling strategies, such as duets and choruses, and can contribute to a general framework for understanding signalling behaviours exhibited by animals in diverse taxa.

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CHAPTER 2

AN EXPERIMENTAL TEST OF DUET FUNCTION IN THE MODERATELY PROMISCUOUS RED-BACKED FAIRY-WREN

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Abstract

Individuals within social groups commonly combine vocal signals, forming duets and choruses. Although these displays have been described across groups, their function is not fully understood. In birds, territory defence is a well-supported function of duetting, but the behavior frequently has multiple functions. Although extra-pair paternity is common in birds, few studies have addressed the function of duetting in mate-guarding. Theoretical models predict that mate-guarding behaviors, possibly including duetting, will be common in species with intermediate promiscuity. We conducted a dual-speaker playback experiment to test duet function in a moderately promiscuous bird, the Red-backed Fairy-wren, *Malurus melanocephalus*. Breeding groups were presented with a male solo, female solo and a duet played from two speakers. 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 playback speakers. Acoustic mate-guarding predicts male aggressive response and duets during male playback in his mate's receptive stage, and biased aggression to the male speaker. Focal pairs duetted more during playback than controls and

most in pre-breeding. Males and females responded more strongly to duet than solos, especially during pre-breeding, but had no same-sex bias in response to playback speakers. Together, these results provide partial support for both hypotheses, but most for territory defence. This provides experimental evidence that, despite moderately high promiscuity rates, vocal duets are not used primarily to guard mates, which helps us toward understanding the function of coordinated vocal displays across different mating systems.

Introduction

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). Most studies indicate that duets have multiple functions that allow them to communicate with mates and individuals outside of the pair-bond, with one common function being territory defence (Hall 2009; Benedict 2010; Dahlin and Benedict 2013).

An additional hypothesis – acoustic paternity-guarding – suggests that a male joins his mate’s song in duet to signal her mated status to rival males and protect his own paternity (Stokes 1968; Sonnenschein and Reyer 1983). This paternity-guarding hypothesis has gained support in two studies (Mennill 2006; Rogers, Langmore, and Mulder 2007), though Rogers’ 2007 study supports female acoustic guarding of the male/pairbond). Mennill’s (2006) study is the only to our knowledge to find support for male acoustic guarding of paternity, but the results of this study also support a function in territory defence. Most other studies do not support acoustic mate guarding (Hall and Magrath 2000; Gill et al. 2005; Hall and Peters 2008a), and overall, this hypothesis is generally less well-supported than the territory defence hypothesis (Hall, 2009).

The function of duetting likely varies across species with different mating systems and levels of promiscuity. For example, theoretical models (Kokko and Morrell, 2005; see also Hall & Peters, 2008b) predict that males should guard little in populations where promiscuity (i.e., extrapair paternity, EPP) is low, because there is little need to guard mates in this case, and also in species where EPP rates are extremely high, because a high level of promiscuity both decreases the efficacy of mate-guarding and increases the incentive to seek extra-pair

matings. Accordingly, we can predict that males will be most likely to exhibit mate-guarding behaviours, possibly including acoustic paternity-guarding, in species with moderately high EPP rates. 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, Heath, and Mennill 2012; Hall, Rittenbach, and Vehrencamp 2015) or unknown (e.g. (Logue and Gammon 2004; Bradley and Mennill 2009) rates of EPP it is perhaps unsurprising that few previous studies support 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 and promiscuous tropical Australian bird. 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, which are non-identical songs sung with overlap (Thorpe et al. 1972; Hall and Peters 2008b; Dowling and M.S. Webster 2013). Songs sung in duets show no apparent differences from solo songs (they are qualitatively and quantitatively similar (Dowling and M.S. Webster 2013). Moreover, Red-backed Fairy-wrens are an excellent species for examining the acoustic paternity-guarding hypothesis, because they have moderately high rates of EPP (54% of young result from extra-pair copulations, Webster, Varian and Karubian, 2008), so we expect high levels of mate-guarding, potentially including acoustic paternity-guarding. Our previous work using correlative approaches (Dowling & Webster, 2013) suggested that duetting functions in territorial defence and not in mate-guarding in this species, but these hypotheses are notoriously difficult to separate using observational data alone (Hall 2009; Douglas and Mennill 2010).

In this study we used a dual speaker playback experiment to test several hypotheses

for the function of duetting in the Red-backed Fairy-wren (Table 1). Specifically, we aimed to determine whether duets in Red-backed Fairy-wrens function primarily for territory defence, for acoustic paternity-guarding, or for some other purpose. 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, so provides a critical piece of the puzzle for understanding how coordinated displays function across different mating systems.

Methods

Tests of hypotheses

We conducted a dual speaker playback experiment in the 2011/12 breeding season (October-January). Male and female duet contributions were broadcast through different speakers (see Logue 2005; Mennill 2006). This setup allows assessment of each focal pair member's response to a male and a female song simultaneously, and allows us to test a critical prediction that separates the territory defence and acoustic paternity-guarding hypotheses (Mennill 2006).

The territory defence 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). The territory defence hypothesis (Seibt and Wickler, 1977) predicts that because duets are territorial "keep-out" signals, pair members will respond to a simulated territorial intrusion (playback) by singing duets, will not discriminate among male and female intruders, and will do so most often in the early breeding stages (when territories are first being established). Pairs also are expected to respond more strongly to duet than to solo treatments, especially during the pre-breeding

stage, when territories are still being established, since intruders singing duets rather than solos represent a greater threat, possibly leading to a territorial claim. Pairs may respond equally to both the male and female contributions to the duet, since both male and female intruders are threatening invaders. The acoustic paternity-guarding hypothesis (Sonnenschein and Reyer, 1983, Stokes and Williams, 1968), on the other hand, posits that males duet with their mates as an agonistic signal, intended for same-sex rivals, which signals that the female is paired. Thus, this hypothesis predicts that males will respond more strongly to the male than to the female speaker during duet playback, since that male's duet contribution is a signal meant to elicit an agonistic interaction with the focal male. In addition, the acoustic paternity-guarding hypothesis predicts that the male will respond to his mate's songs to form duets more often during her receptive stage (when the threat of cuckoldry is highest).

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.	<u>Male and female response:</u> - Stronger response to duet than solo • Especially in pre-breeding stage - Response to male speaker = female speaker	<u>Duet rate:</u> - 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.	<u>Male response:</u> - Stronger response to male than female speaker	<u>Duet rate:</u> - Higher in playback period than pre or post-playback • Especially in female receptive stage - Higher when female receptive than other stages <u>Male answer female songs:</u> - Higher rate in male solo than other treatments - Higher rate when female receptive than other stages

General field methods

Red-backed Fairy-wrens are a territorial species and defend relatively large areas for breeding and foraging; territories are on average 103.4 ± 35 meters across (J. Dowling, unpublished data). Males exhibit two distinct breeding plumage types: red-black plumage (greater than 66 percent of plumage is red and black, scored on 6 body regions, Karubian et al, 2008) or brown plumage (less than 33 percent of plumage is red-black). While 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 percent of each focal male's plumage was red-black), while 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 all 16 breeding groups, the social pairing was between unrelated birds, such that mate guarding would be relevant.

Focal birds were captured in mist-nets and marked with a unique color band combination, allowing individual identification. Breeding stages were defined as: (1) the pre-breeding stage, the stage before the female began nest building (N =36 experiments conducted an average of 26 ± 6.5 days before 1st egg was laid); (2) the fertile stage, when the first solicitation or copulation was observed in the group until the penultimate egg was laid (N =54 experiments conducted an average of 4 ± 5.5 days before 1st 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 ± 2.4 days after 1st egg was laid).

Playback stimuli

We created stimulus songs for three 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, U.S.A.) 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 2400-17800 Hz, which left spectral space occupied by song elements intact (Greig, Price, and Pruett-Jones 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/>). For each treatment type, we combined 14 copies of the same song onto one track so that one song was played every 20 seconds and the total playback length was 5 minutes. Tracks started with 20 seconds of silence and ended after the 14th song. This song rate was chosen because Red-backed Fairy wrens sing approximately one song every 20 seconds during aggressive interactions and during the dawn chorus (J. L. Dowling and M.S. Webster, 2014). 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 second between the start of the first bird's song and the start of the responding bird's song (Dowling and M.S. Webster 2013).

Playback procedure

Each focal group was played all three playback treatments (male solo, female solo and duet) once in each of three breeding stages (pre-breeding, fertile, incubation). We conducted 135 total playbacks on 16 groups conducted during each of 3 breeding stages. All but three of the

groups got playbacks from all three breeding stages; the three for which we did not were missing one playback. Playbacks included 44 in the duet treatment, 45 with the male solo and 45 with the female solo. Stimuli played to each group were recorded from non-neighbouring birds from the same population that were at least 5 territories away. Playback of each treatment was separated by approximately 60 min (91 ± 31 min, ranging from 40 – 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 comprised of the same male solo and female solo that were 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 three 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 (J. L. Dowling, personal observation). At the start of each experiment, two speakers (Pignose Legendary 7-100, Pignose-Gorilla, Las Vegas, NV, U.S.A.) were set up 10 meters apart, within the focal territory and greater than 10m from a territory boundary. Songs were played from an iPod nano (Apple Inc., Cupertino, CA) connected to each speaker via a 30 meter cable. The first observer stood about 20 meters from the speakers, with both speakers in clear view, and operated the iPod, while the second observer kept track of focal birds. When all members of the focal group were located and within 25m 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 volume of 90 dB at 1 meter. Each of two observers was equipped with a Marantz PMD661 solid-state digital recorder (Marantz America, Itasca, IL, USA) and Sennheiser ME67 highly directional long-gun microphone (Sennheiser Corp., Old Lyme, CT, USA) with a Rycote softie windshield and mount. Observers dictated the bird's behaviors on vocalizations into the recorder during the experiment.

We observed birds for a 5-minute pre-playback period of silence, 5-minute playback period, and 5-minute 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 meters of each speaker. Distance was estimated using the distance between the two playback speakers (10 meters) as a standard.

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 (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.

Table 2. Correlations of four 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 5m	-0.84	-0.86
Closest approach	0.80	0.79
Latency to approach within 5m	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.

To determine how pair members respond to the male speaker versus the female speaker when both are 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 one-sample *t*-tests in JMP version 10 (SAS Institute Inc., Cary, NC) to determine if that difference was significantly different from zero. We did this for each of the four movement responses. For this analysis, responses were averaged across breeding stages to

avoid pseudo-replication, since multiple experiments on the same group were conducted during each of three 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 generalised 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 towards the female (proportion of female flights male followed and male-female distance). We ran a generalised linear mixed model (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, which were then removed when not significant. All models were also run with presence/absence of an auxiliary helper,

breeding male plumage and treatment presentation order as a fixed effects, which were removed from final models when non-significant (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*breeding stage: $\chi^2=12.0$, $df=4$, $P=0.018$, $N = 117$ observations of 16 groups, Figure 1a, Table 3). Males showed a similar pattern, with response to duet higher than male or female solo treatments in pre-breeding and female receptive stages, although the overall pattern was not significant (LMM: playback treatment*breeding stage: $\chi^2=9.2$, $df=4$, $P=0.056$, $N = 122$ observations of 16 groups, Figure 1b, Table 3). Overall, within each experimental period, the male's response did not differ from that of the female (two-tailed paired t -test: $t_{16} = -0.4$, $P = 0.7$). These results provide partial support for the first two predictions of the territory defence 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*breeding stage: $\chi^2=9.2$, $df=2$, $P=0.010$).

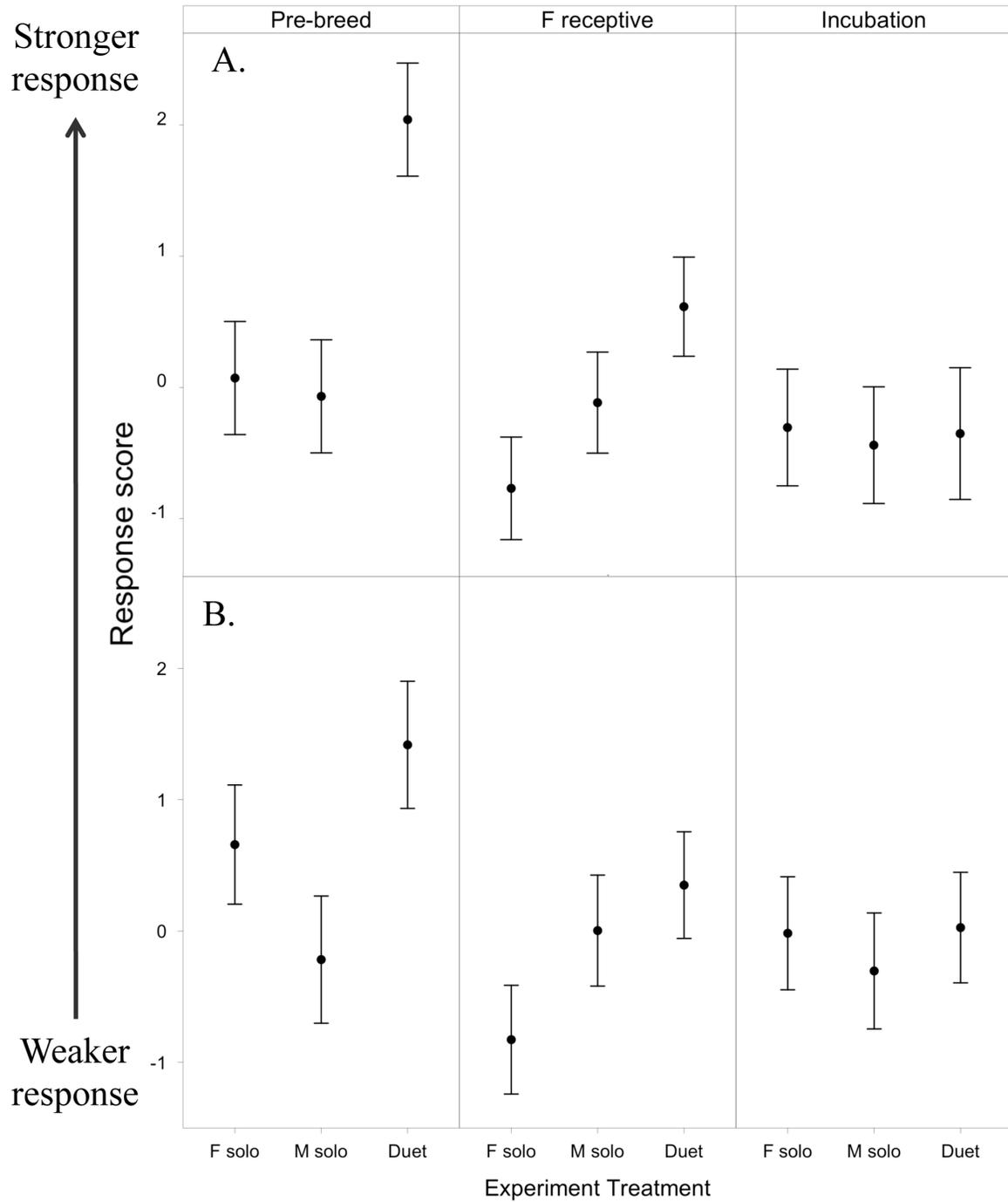


Figure 1. Female (A) and male (B) mean behavioral response (\pm SE) to different experimental treatments: female solo, male solo and duet.

Table 3. Results of a linear mixed-effects model (LMM) testing differences in female (A) and male (B) movement responses between experiment treatments and breeding stages.

A. Response: Female movement response principal component one

Full LMM		Chi sq	Df	p value
Experimental treatment		23.5	2	<0.0001
Breeding stage		11.3	2	0.0035
Experimental:Breeding stage		11.9	4	0.0177
Post-hoc contrasts	Estimate	SE	z ratio	p value
Breeding stage = Pre-breed				
F solo - M solo	-0.14	0.48	-0.29	0.9552
F solo - Duet	1.97	0.48	4.07	0.0003
M solo - Duet	2.11	0.48	4.36	0.0001
Breeding stage = Female receptive				
F solo - M solo	0.65	0.41	1.58	0.2616
F solo - Duet	1.38	0.41	3.39	0.0029
M solo - Duet	0.73	0.4	1.82	0.1683
Breeding stage = Incubation				
F solo - M solo	-0.13	0.51	-0.26	0.9624
F solo - Duet	-0.05	0.56	-0.08	0.9962
M solo - Duet	0.09	0.56	0.16	0.9862

B. Response: Male movement response principal component one

Full LMM		Chi sq	Df	p value
Experimental treatment		7.85	2	0.01977
Breeding stage		8.44	2	0.01468
Experimental treatment:Breeding stage		9.23	4	0.05567
Post-hoc contrasts	Estimate	SE	z ratio	p value
Breeding stage = Pre-breed				
F solo - M solo	0.88	0.53	1.65	0.2293
F solo - Duet	-0.76	0.53	-1.43	0.3282
M solo - Duet	-1.64	0.56	-2.93	0.0116
Breeding stage = Female receptive				
F solo - M solo	-0.83	0.45	-1.85	0.1587
F solo - Duet	-1.18	0.43	-2.74	0.0199
M solo - Duet	-0.35	0.44	-0.79	0.7129
Breeding stage = Incubation				
F solo - M solo	0.29	0.48	0.6	0.8199
F solo - Duet	-0.04	0.46	-0.09	0.9951
M solo - Duet	-0.33	0.47	-0.7	0.7614

We found no difference in the response of each pair member to the male speaker versus the female speaker (Fig. 2). Responses to the speakers did not differ with respect to the proportion of time spent within 5 meters (one-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 meters (males: $t_{16}=0.71$, $P=0.49$; females: $t_{16}=-0.42$, $P=0.68$), or number of overflights (males: $t_{16}=1.07$, $P=0.3$; females: $t_{16}=0.18$, $P=0.86$). 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 that during the pre-breeding stage females approach the male speaker more closely ($t_{16}=-3.4$, $P=0.007$) and approach the male speaker more quickly than the female speaker ($t_{16}=-2.8$, $P=0.017$). These results do not support the critical prediction of the acoustic mate-guarding hypothesis (that males will show more aggression to the male speaker) and instead support the territory defence hypothesis.

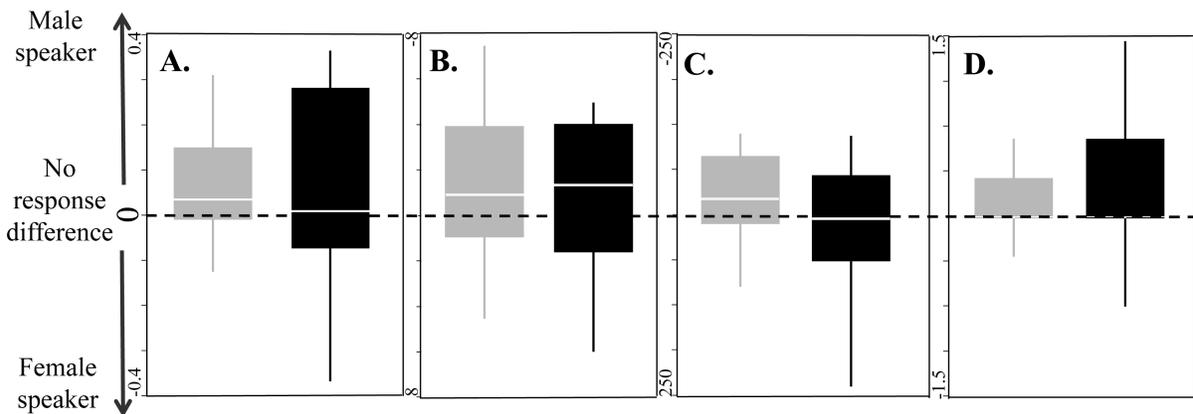


Figure 2. Box and whisker plots showing difference in response to speaker playing a male song versus speaker playing a female song for the focal female (gray bars) and focal male (black bars). A) proportion of time spend within 5 meters of each speaker, B) closest approach to each speaker (meters), C) latency to approach within 5 meters 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.

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*Breeding stage: $\chi^2=17.34$, $df=4$, $P=0.002$, Fig. 3, Table 4). This pattern was similar across all three 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: $z = 2.5$, $SE = 0.3$, $df=2$, $P = 0.0335$, pre-breed vs. incubation: $z = 3.89$, $SE = 0.4$, $df=2$, $P = 0.0003$), intermediate in the fertile stage (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 defence hypothesis and one prediction of the acoustic mate-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 mate-guarding hypothesis. We saw different results for female duet rates with males. During the female fertile stage, when compared to the female solo treatment (experiment treatment*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 condition (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 condition ($z = -$

2.3, $SE = 0.7$, $df = 2$, $P = 0.056$).

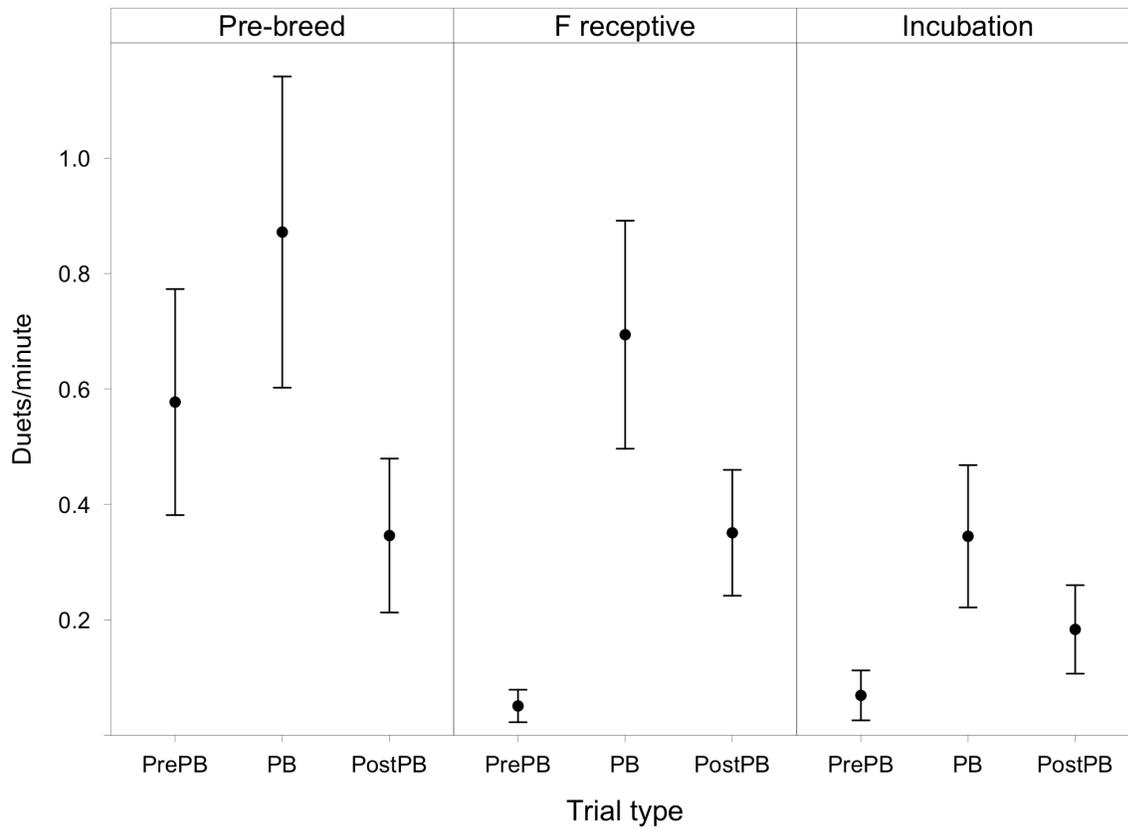


Figure 3. Variation in mean duet rate ($\pm SE$) across three playback periods (pre, playback and post) and across three breeding stages.

Table 4. Results of generalised linear mixed models (GLMM) testing differences in vocal responses between playback periods, treatments and breeding stages.

Response: Duet rate (duets/minute)

Full LMM		Chi sq	Df	p value
Trial type (pre, playback, post)		30.05	2	<0.0001
Breeding stage		18.17	2	0.0001
Trial type:Breeding stage		17.34	4	0.0017
Post-hoc contrasts	Estimate	SE	z ratio	p value
Breeding stage = Pre-breed				
PrePB - PB	-0.41	0.3	-1.39	0.3454
PrePB - PostPB	0.51	0.37	1.37	0.3594
PB - PostPB	0.92	0.35	2.65	0.022
Breeding stage = Female receptive				
PrePB - PB	-2.61	0.51	-5.14	<0.0001
PrePB - PostPB	-1.93	0.52	-3.69	0.0006
PB - PostPB	0.68	0.21	3.18	0.0043
Breeding stage = Incubation				
PrePB - PB	-1.61	0.62	-2.59	0.0261
PrePB - PostPB	-0.98	0.66	-1.48	0.2988
PB - PostPB	0.63	0.41	1.53	0.2757

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 two ($\chi^2=3.69$, $df=4$, $P=0.45$). 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*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 that helped to distinguish between the territory defence and acoustic mate-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 defence, with secondary support for an acoustic paternity-guarding function.

Our results support four out of five predictions of the territory defence 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. One of the predictions supporting the territory defense hypothesis differed critically from predictions of the acoustic mate-guarding hypothesis (same-sex bias in response to playback speakers, Table 1). Although, it is important to note that overall, duetting species show less same-sex bias in their response to stereo duet playback than non-duetting species (Logue 2005). This might mean that in duetting species, cooperative defence is paramount, and pairs may respond to speakers jointly, even if male song is used to guard paternity. This suggests that response to dual speaker playback may not conclusively separate the two functions.

We found some partial support for the acoustic mate-guarding hypothesis, since pairs responded to a simulated territorial intrusion (playback, all treatments) by singing duets, particularly in the female's fertile stage. This suggests that males use duets to repel rivals, but only 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. Taken together, these results suggest that duets in this species function primarily as a keep-out signal meant for individuals outside of the group, but may also secondarily function to acoustically guard paternity.

Our results complement those from other studies: across bird species, territory defence 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 that 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 mate-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) and/or details of promiscuity 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). Since Red-Backed Fairy-wrens are moderately promiscuous (54% of young result from extrapair copulations; Webster et al, 2008), we predicted that Red-

backed Fairy-wren males would guard mates intensely, and possibly use acoustic mate-guarding as part of a larger suite of mate-guarding behaviors. Despite the fact that mate-guarding should be important to species with moderately high rates of EPP, our results support few predictions of the acoustic mate-guarding hypothesis, and do not support 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 promiscuity, why did we find little support for the acoustic paternity-guarding hypothesis in this study? There are several potential explanations. First, it is possible that Red-backed Fairy-wren males use non-vocal forms of mate-guarding, such as mate following or physical aggression. Physical mate-guarding behaviors have been studied in two closely related species, one extremely faithful (Purple-crowned Fairy-wrens) the other extremely promiscuous (Superb Fairy-wrens), and neither guard mates intensely (Mulder 1997; Hall and Peters 2008a), as predicted by theoretical models. In this study, we found that male Red-backed Fairy-wrens show some physical guarding behaviors during simulated intrusions (closer male-female distance in female fertile stage, but no difference in distance or mate-following behavior between playback and control periods or across playback treatments) and did not target aggression on intruding males (no more aggression to the male than female speaker in any treatment). We find that focal males showed some physical and possibly some acoustic guarding of fertile mates, but neither behavior was pronounced.

Second, another possible explanation for why we see little acoustic and behavioral mate-guarding in our study is that EPP rates may be too high to favor mate guarding in this species, and they may exhibit the behavior predicted by Kokko and Morrell's (2005) model for highly

promiscuous species (i.e. low levels of mate guarding). This also seems unlikely, since Red-backed Fairy-wren pairs are faithful to one another about half of the time, which suggests they should show maximal levels of mate-guarding. To rule out this possibility, future studies could investigate duet function in species with EPP rates lower than those of Red-backed Fairy-wrens, but higher than the very faithful species previously studied.

Third, 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) also predict that unattractive males should guard their mates at high levels, since they are highly vulnerable to cuckoldry and have a low probability of siring extra-pair young of their own, whereas attractive males should guard their mates little, since 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 to males that breed in brown plumage: red-black males sire more extra-pair young than do brown males (Webster et al. 2008), and females prefer to associate with red-black over brown males in choice trials (Karubian 2002). We would predict that brown males would show more mate-guarding behavior (including acoustic guarding) than do Red-black males, and we do indeed find that during experimental trials, brown males showed more aggression to intruders during their mate's fertile stage and followed their mates more across all treatments and stages. Although we find no firm evidence for acoustic guarding by brown males in this study, 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 territorial defence hypothesis (Hall 2009). Given the substantial empirical support for this hypothesis, duetting and chorusing may have evolved multiple times in various groups at least partly to serve a territorial defence function. Duets, whether antiphonal or overlapping, are often louder, longer and easier to locate than solo songs, and also signal that more than one 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 promiscuity, and thus helps to fill an important gap in our knowledge of the role of mating system in the evolution of coordinated vocal displays. This study helps to further our understanding of the potential evolutionary underpinnings of complex vocal signals like duetting when the pressures of defending a territory, coordinating breeding and successfully guarding a mate vary widely across species with different mating systems.

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CHAPTER 3

MALE ATTRACTIVENESS PREDICTS MATE-GUARDING STRATEGY AND PATERNITY IN A PROMISCUOUS SONGBIRD

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Abstract

Animals face conflicting demands on the time and energy available for mating effort. How this conflict resolves may depend on individual quality, including attractiveness. In species with extra-pair copulations (EPCs), males trade off mate guarding with seeking EPCs, and the strategy that affords a male the highest reproductive success may depend on his attractiveness to females. We hypothesized that males that differ in attractiveness would have different optimal mating strategies, predicting that attractive males would invest more in seeking EPCs, resulting in higher extra-pair success, whereas relatively unattractive males would guard their mates more, leading to higher within-pair success. We tested these predictions in the Red-backed Fairy-wren (*Malurus melanocephalus*). In this species, males can breed either in red/black or brown female-like plumage, and red/black males, regardless of age, are preferred in female choice experiments over brown males. Our hypothesis predicted that brown males would mate guard more than red/black males and, because RBFW sing duets, we also expected that brown males would duet with mates as an acoustic form of mate guarding. We

found that during observations and simulated intrusions, red/black males, especially older males, invested more in seeking EPCs, whereas brown males invested in physical and acoustic mate guarding. Consequently, brown males had higher within-pair success and red/black males had higher extra-pair success, resulting in similar total reproductive success between male types. Thus, males appear to optimize their reproductive success by balancing investment in mate guarding and EPC seeking in a way that is appropriate to their level of attractiveness. This result indicates that in addition to adjusting behavioral tactics with their physiological state and environmental/social conditions, males adjust mating strategies depending on their quality, and proper adjustment yields fitness benefits. This extends our knowledge of the factors that influence how males choose strategies and broadens our understanding of how behavioral plasticity in general can evolve.

Introduction

The balance of an animal's investment in parental effort, mating effort and self-maintenance can decide whether they survive and reproduce (Westneat and Sherman 1993). Thus animals are selected to invest most in behaviors that profitably yield fitness returns (Magrath and Komdeur 2003). In species that form social pair bonds, mating effort can take the form of investment in reproduction with the social mate or investment in seeking additional mating opportunities, such as extra-pair copulations (EPC). The net benefit to the male of seeking EPC will depend on the fitness gains of the EPC, relative to the potential gains from conflicting behaviors- mate guarding, territory defense and parental care (Westneat *et al.* 1990). Since EPC seeking and mate guarding can both potentially increase an individual's reproductive success, a trade-off exists (Westneat *et al.* 1990; Harts and Kokko 2013). EPC seeking can enhance extra-pair reproductive success, but may cost within-pair success, whereas mate guarding may prevent females from engaging in EPCs, but also may cost energy and EPC opportunity (Komdeur 2001). Males appear to adjust investment according to the risk of losing paternity and also to the opportunity for gaining additional matings, as assessed from their social environment (Wilson and Swaddle 2013).

Whether a male benefits from investment in seeking EPCs over investment in mate-guarding may also depend on his attributes (Trivers 1972). If females prefer a certain male phenotype, then males with that phenotype will receive more of a fitness benefit in return for their investment in seeking additional mating opportunities, compared to males with the less preferred phenotype, and may also pay little cost to within-pair paternity because they likely are also preferred by their own social mates (Burley *et al.* 1994; Kokko and Morrell 2005). Non-preferred males, on the other hand, will not receive this fitness payoff for their EPC

investment, and are instead predicted to invest in mate guarding to maintain within-pair paternity by preventing their mates from copulating with more attractive males (Kempnaers *et al.* 1995; Kokko and Morrell 2005).

Male mating tactics have been well studied, especially those produced by genetic polymorphisms (Gadgil 1972; Oliveira *et al.* 2008). Several studies have focused on strategies that are flexible within a male's lifetime (Gross 1996; Greene *et al.* 2000). Conditional strategies that exhibit plasticity with a male's physiological condition (Humfeld 2013), competitive ability (Taborsky 1998; Painting and Holwell 2014) and social and environmental conditions (Oliveira *et al.* 2008), appear to be widespread. However, despite its intuitive appeal, the hypothesis that males differing in attractiveness will have different optimal mating strategies has received little empirical study (but see Johnsen and Lifjeld 1995). This hypothesis predicts that attractive males, as preferred extra-pair partners, will invest more time/effort in seeking EPCs and receive an extra-pair benefit from doing so, whereas unattractive males instead will invest in mate guarding and receive a within-pair reproductive benefit. This hypothesis also predicts that, as a result of their increased impetus to guard, unattractive males may use a suite of mate-guarding behaviors. This suite may include acoustic mate guarding, wherein a male joins his mate's song in duet to signal her mated status to rival males (Sonnenschein and Reyer 1983). We tested these predictions by conducting behavioral observations and playback experiments with a promiscuous bird species that sings vocal duets.

The Red-backed Fairy-wren (*Malurus melanocephalus*) is a cooperatively breeding and promiscuous tropical Australian bird that has moderately high levels of extra-pair paternity (EPP, 54% of young result from extra-pair copulations, 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 (i.e., non-identical songs sung with overlap, Thorpe *et al.* 1972; Hall and Peters 2008; Dowling and Webster 2013), especially between the breeding pair. Similarly-aged Red-backed Fairy-wren males can breed in one of two plumage types (Karubian 2002): red/black males have elaborate, colorful plumage (>66% of plumage is red and black), whereas brown males have mostly dull, female-like plumage (less than 33% of plumage is red and black). Plumage color is bimodal (Webster *et al.* 2008) and few males breed in intermediate plumage (33-66% red/black plumage). Plumage color appears to affect attractiveness of a male, as females prefer red/black males, regardless of age, over brown in choice trials (Karubian 2002), and red/black males have been found to sire more extra-pair young (Webster *et al.* 2008).

The hypothesis that males differing in attractiveness have different optimal mating strategies led us to make several specific predictions for our study species. First, we predicted that red/black males would seek EPCs more, resulting in higher extra-pair success, whereas unattractive brown males would guard mates more, resulting in higher within-pair success. Second, we predicted that mate guarding by brown males would be especially strong when their mate was fertilizable and receptive to matings. Finally, because this species sings duets (Dowling and Webster 2013), and brown males were expected to show a high level of guarding, we also predicted that brown males would use vocal duetting as part of a suite of mate guarding behaviors that also included physical guarding behaviors and aggression towards intruding male rivals.

Methods

General field methods

We conducted fieldwork each year from 2009/10 through 2013/14 during the breeding seasons (October-January) near Herberton, Queensland, Australia (145°25'E, 17°23'S). The study site is located in open sclerophyll forest with tall eucalypt over-story and grass under-story with sporadic shrub layer dominated by *Lantana* spp. Basic breeding biology data have been collected continually on this study population during each breeding season since 2003 (see Webster *et al.* 2010). All birds in focal groups were marked with a unique combination of colour bands and basic breeding biology data were collected as per our previous studies (see Webster *et al.* 2008; Varian-Ramos *et al.* 2010).

Experiments and observations were started at first light, with some starting later if two were conducted consecutively. The period beginning at sunrise and extending to late morning represents a peak time for singing for Red-backed Fairy-wrens (J. Dowling, personal observation). Sexes of adults were identified using behavioural and morphological traits. All successful and most unsuccessful nesting attempts were monitored and individuals were re-sighted at least once per week. Active nests were visited at least once every other day to determine nest stage, lay date, clutch size, hatch date, and to band and collect a small blood sample from nestlings on feather pin break (ca. day 6 after hatching). In this species, young (typically males) often remain on their natal territory and assist their parents at the nest as auxiliary helpers in the next breeding season (Varian-Ramos *et al.* 2010). All members of focal breeding groups were captured and a small (80 microliter) blood sample was taken from the jugular vein using a 29-gauge insulin syringe.

Focal Behavioral observations

Observations lasted either one (2010/11-2013/14) or two hours (2009/10). Observations conducted in 2012/13 were excluded, as bird behavior that season was affected by a severe brush fire that modified the habitat and breeding of the birds. For each focal group, one observer located and continually tracked and tallied the behaviour of each member on a data sheet. The open habitat allowed us to keep track of each group member's movements and determine the identity of each bird that sang.

We conducted observations of 73 focal breeding groups during each of three breeding stages, for a total of 244 observation hours. Breeding stages were categorized as: (1) the pre-breeding stage, the period before the female began nest building (N = 69 observation hours); (2) the female receptive stage, when the first solicitation or copulation was seen until the penultimate egg was laid (N = 70 observation hours, solicitations/copulations seen an average of 6 ± 3.5 days before first egg is laid); and (3) the post-receptive stage, which was the time period between clutch completion and fledging (N = 105 observation hours).

Breeding males had red/black plumage in 53 groups and brown plumage in 20 groups. We also categorized males based on age, with males 2 years or younger considered "young" (n = 31 groups) and males > 2 years old considered "old" (n = 42 groups). We chose this age classification because it divided our focal males approximately in half and because previous research shows marked differences in mate guarding behaviors in male Red-backed Fairy-wrens after the age of two (Potticary et al, in review). Across groups observed, 51 were pairs with no auxiliary helper, 18 groups had one helper, two had two helpers, and one group had three helpers. Effect of auxiliary helper presence on behavior was determined statistically.

We collected data on the number of solo and duet songs sung and determined who initiated and who answered each duet. We defined ‘solos’ as songs where one individual sang alone, while in ‘duets’, another individual in the group sang within one second of the first song’s end. We also recorded all bird movements and locations, including flights, during each observation.

We used the following response variables in analyses: (1) *proportion of female songs the male joined in duet*, calculated by summing the number of duets the male sang with the female and dividing by the total number of songs (duets and solos) the female sang while the male was present; (2) *proportion of female flights the male followed*, calculated by summing the number of female flights greater than 5 meters that the male followed (i.e. made flight in same direction less than 5 seconds after) and dividing by the total number of flights the female made while the male was present; (3) *proportion of time the male spent close to the female*, calculated by dividing the amount of time the pair was less than 10 meters apart by the total time the group was observed; and (4) *proportion of time the male was present*, calculated by dividing the amount of time the male spent on the territory by the total time the group was observed. For reproductive success analyses, we had data on total reproductive success for each season for 67 males and data on proportion of within versus extra-pair young in the nest for 43 nests.

Playback experiment

We conducted a dual-speaker playback experiment in 2011/12 (Dowling and Webster, in prep) to test for differences in breeding group responses to simulated intrusions of a male, a female, and a breeding pair, a common method used to test responses to different types of

intruders (Douglas and Mennill 2010). Subjects were 16 breeding groups, five of which had one auxiliary helper. Seven groups contained brown breeding males, and nine contained red/black males. Each focal group was played three playback treatments – a male solo song played from one speaker, a female solo song from one speaker, and a duet played from two speakers. One experiment, including all three treatments, was conducted on each group in each of three breeding stages. Breeding stages were defined as above, except experiments in the post-receptive stage were always conducted during the incubation stage, the period when the female was incubating her clutch of eggs. We conducted 135 playbacks in total (16 groups, 3 playback treatments, conducted once per each of three breeding stages, with three groups each missing one playback during one breeding stage).

Stimuli played to each group were made from high quality recordings of non-neighbouring birds (at least 5 territories away) from the same population (for detailed experimental methods, see Dowling and Webster, in prep). Playback of each treatment was separated by at least 60 min. For the duet treatment, the male and female duet contribution were played from two speakers (male contribution from one speaker and female contribution from the other), 10 meters apart to simulate a duet realistic for this species (Dowling and Webster 2013). The duet presented to each pair was comprised of the same male solo and female solo that were presented to them in the solo treatments, as previous results indicate that the duet songs do not differ from solo songs (Dowling and Webster 2013). A unique set of playback stimulus songs was presented to each focal group (16 total playback sets), and the same set was presented to that group in a different order in each of the three 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.

Songs were broadcast at volume of 90 dB at 1 meter at the start of playback. Each of two observers recorded all bird vocalizations, along with a dictation of the bird's behaviors, using a Marantz PMD661 solid-state digital recorder (Marantz America, Itasca, IL, USA) and Sennheiser ME67 highly directional long-gun microphone (Sennheiser Corp., Old Lyme, CT, USA) with a Rycote softie windshield and mount.

We determined each group member's approach behavior for each treatment by recording distance from each speaker, closest approach, number of over-flights and latency to approach within 5 meters. Response score for the male and female were calculated by combining all approach behaviors in a principal component analysis, which was used as the response variable in statistical tests. For each experiment, we observed birds for a 5-minute pre-playback control period of silence, 5-minute playback period, and 5-minute post-playback control period of silence. After determining that behavior in the playback period differed from the control periods (i.e. the playback elicited a response), only playback periods were used in analyses. Our hypothesis predicts that when a rival male intrudes on the focal male's territory, unattractive males (who are invested in maintaining within-pair paternity) will show aggression towards the intruder, especially when his mate is fertile, while attractive males (who are invested in increasing extra-pair paternity) will do so to a lesser degree.

Paternity assignment

To assign parentage and determine male reproductive success, DNA was extracted from blood samples collected from captured adults and nestlings, and individuals were genotyped using a panel of highly polymorphic microsatellite loci as described in Baldassarre and Webster (2013). In brief, we PCR-amplified seven microsatellite loci from the DNA extracted from

each individual sample, and size-separated the amplified fragments (alleles) on an ABI Prism 3730® automated sequencer. Allele sizes were scored for each individual at each locus using the program GeneMapper (Applied Biosystems), and these genotypes were compared across individuals to determine parentage of nestlings using CERVUS 3.0 (Kalinowski et al. 2007).

Statistical analyses

Data were analysed using R version 3.1.2 (R Development Core Team 2015). We used generalized linear mixed models (GLMM) from the `glmer` or `lmer` functions in R package `lme4` for models that did not conform to linear model assumptions. Models with a proportion as the response were modeled with binomial counts as outcomes and a weight argument specified. Rate data were modeled with a Poisson distribution and an offset argument. We used linear mixed models (LMM, `lmer` function in R package `lme4`) for data that did conform to linear model assumptions and generalized linear models (GLM) for data that did not require a random effect.

To test for general male mating strategies, we constructed a model for each of the four male behaviors we measured during behavioral observations: proportion of female songs the male joined in duet, proportion of female flights the male followed, proportion of time the male spent close to the female, and amount of time the male was present. Male age and male plumage type were entered as fixed effects. To test for mate guarding strategies across breeding stages, we used the same four response variables and male plumage/age type lumped into three categories: young red/black males, old red/black males and brown males. Old brown males (N = 5) were lumped with young brown males (N = 15) for this more complex model, since samples size for each alone was too small for appropriate analysis. Male

age/plumage type and breeding stage were entered as fixed effects in these models. All mixed models included year and group number as random effects, since observations were repeated during each of three breeding stages and observations were conducted during three years.

To test for difference in male aggression towards different intruder types, we determined how focal birds' movement response varied across treatments within the female's fertile stage. We combined several movement responses into one principal component response score for the breeding male. 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) with breeding group number as a random effect and playback treatment, breeding stage, breeding male plumage type and all interactions as fixed effects.

To test for differences in reproductive success between male plumage/age types, we constructed one model with the number of young in the nest that were within pair young as the response variable, one with number of extra-pair young as the response variable and one model with total reproductive success (number of within-pair young sired plus number of extra-pair young sired within a given year) as the response variable. We also looked at cuckoldry rates by using the proportion of young in the nest that were within-pair young as the response variable. Each model had male plumage/age type as the predictor variable.

For all models, we tested for effects of weather (heat index, humidity and average wind speed) and auxiliary presence (present/absent) by adding these as covariates in each model, where appropriate. The covariates were removed from models when non-significant. We conducted post-hoc tests using the lsmeans package in R. Least-squares means were

computed for specified factors or factor combinations from each model, and we made comparisons among them using the Tukey method to adjust P values when multiple comparisons were made. Least-squares means were back-transformed to show response in the original scale in figures and tables. Principal components analyses for male response during playback experiment were done using the R function `prcomp`.

Results

Behavioral observations

Brown males of all ages had a higher duet rate with females than did old red/black males, but young red/black and young brown males had similar rates (Table 1, Figure 1a). Young brown males followed mates more than did old red/black males, and old brown males showed a similar trend that was marginally non-significant after correction for multiple comparisons (Table 1, Figure 1b). Brown males spent more of their time close to their mates than red/black males, regardless of age (Figure 1c). Finally, brown males and young red/black males spent more time on the territory than did old red/black males (Table 1, Figure 1d), although all males spent the majority of time on the territory.

Table 1. Results of generalized linear mixed models, describing the effect of age and male plumage on four male mate guarding behaviors: the male's duet rate with his mate, male mate following, male time spent close (<10m) to his mate and time male spent on territory.

Proportion of female songs the male joins in duet		Chi sq	Df	p value	
Age		1.21	1	0.27	
Plumage type		4.63	1	0.03	
Age:Plumage type		4.19	1	0.04	
Post-hoc contrasts		Estimate	SE	z ratio	p value
Brown,Young - R/B,Young		0.45	0.43	1.04	0.73
Brown,Young - Brown,Old		-1.05	0.95	-1.11	0.69
Brown,Young - R/B,Old		1.33	0.50	2.68	0.04
R/B,Young - Brown,Old		-1.50	0.98	-1.52	0.42
R/B,Young - R/B,Old		0.88	0.54	1.62	0.37
Brown,Old - R/B,Old		2.38	0.85	2.80	0.03
Proportion of female flights the male follows		Chi sq	Df	p value	
Age		1.89	1	0.17	
Plumage type		8.24	1	0.004	
Age:Plumage type		0.54	1	0.46	
Post-hoc contrasts		Estimate	SE	z ratio	p value
Brown,Young - R/B,Young		0.73	0.40	1.82	0.26
Brown,Young - Brown,Old		0.12	0.61	0.19	1.00
Brown,Young - R/B,Old		1.36	0.36	3.77	0.001
R/B,Young - Brown,Old		-0.62	0.62	-1.00	0.75
R/B,Young - R/B,Old		0.62	0.40	1.56	0.40
Brown,Old - R/B,Old		1.24	0.55	2.28	0.10
Proportion of male time close to female		Chi sq	Df	p value	
Age		0.12	1	0.73	
Plumage type		84.66	1	<.0001	
Age:Plumage type		0.27	1	0.60	
Post-hoc contrasts		Estimate	SE	z ratio	p value
Brown,Young - R/B,Young		1.75	0.23	7.54	<.0001
Brown,Young - Brown,Old		-0.09	0.48	-0.18	1.00
Brown,Young - R/B,Old		1.89	0.34	5.54	<.0001
R/B,Young - Brown,Old		-1.84	0.47	-3.92	0.001
R/B,Young - R/B,Old		0.14	0.32	0.45	0.97
Brown,Old - R/B,Old		1.98	0.37	5.30	<.0001
Proportion of male time on territory		Chi sq	Df	p value	
Plumage type		4.00	1	0.05	
Age		12.98	1	0.0003	
Plumage type:Age		0.78	1	0.38	
Post-hoc contrasts		Estimate	SE	z ratio	p value
Brown,Young - R/B,Young		0.50	0.31	1.63	0.36
Brown,Young - Brown,Old		0.80	0.99	0.80	0.85
Brown,Young - R/B,Old		2.16	0.48	4.52	<.0001
R/B,Young - Brown,Old		0.30	0.98	0.31	0.99
R/B,Young - R/B,Old		1.66	0.45	3.71	0.001
Brown,Old - R/B,Old		1.36	0.93	1.46	0.46

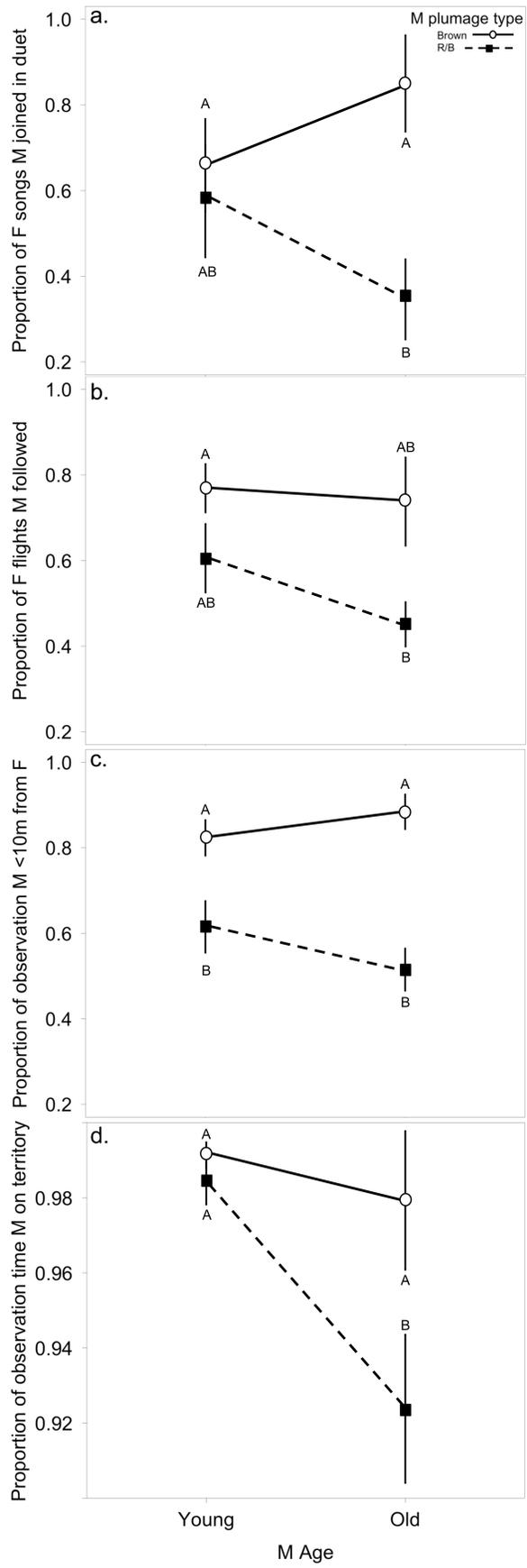


Figure 1. Effect of age and male plumage type on four mate guarding behaviors: male duet rate with mate (a), male mate following (b), male time close to mate (c) and time male on territory (d). Top lines show data for brown males (open circles), lines on bottom show data for red/black males (closed squares). Points show mean \pm one standard error. Levels with different letters are significantly different from one another. Data were scaled differently for the proportion of male time on territory because males of all types spent the majority of their time on their territory.

Mate-guarding strategies across breeding stages

Both brown and young red/black males joined a higher proportion of their mate's songs in duet than did old red/black males in the female's receptive stage, but we did not see this pattern in the pre-breed or post-receptive stages (Table 2, Figure 2a). Males in groups with auxiliary helpers joined their mate's songs in duet less than did males without helpers in all breeding stages (Post-hoc z-test of GLMM least-squares means: $z = -5.43$, $SE = 0.54$, $df = 1$, $P \leq 0.0001$). Brown males (young and old combined) followed a higher proportion of their mate's flights than did old red/black males in the pre-breed stage, and showed the same trend in the receptive stage, but this trend was not significant (Table 2, Figure 2b). Brown males spent more time close to their mate than did old red/black males in the pre-breed stage, and both brown and young red/black males spent more time close to their mate than old red/black males in the post-receptive stage, but we saw no differences between male types in the receptive stage (Table 2, Figure 2c).

Old red/black males in groups with helpers spent less time close to their mate than those without helpers (Post-hoc t-test of LMM least-squares means: $t = -3.86$, $SE = 0.09$, $df = 1$,

$P = 0.0001$). Brown and young red/black males spent more of their time on their territory than old red/black males in all three breeding stages (Table 2, Figure 2d). Males (all types) with helpers spent more time on their territory when their mate was receptive than males without helpers (Post-hoc z-test of GLMM least-squares means: $z = 2.39$, $SE = 0.6$, $df = 1$, $P = 0.017$).

Table 2. Results of mixed models, describing the effect of male age/plumage, breeding stage and auxiliary presence on four male mate guarding behaviors: the male's duet rate with his mate, male mate following, male time close to mate, and time male spent on territory.

Proportion of female songs the male joins in duet	Chi sq	Df	p value	
Breeding Stage	13.31	2	0.001	
Male age/plumage type	5.34	2	0.07	
Aux	29.46	1	<.0001	
Breeding Stage:Male age/plumage type	17.37	4	0.002	
Post-hoc contrasts				
Pre-breed Stage	Estimate	SE	z ratio	p value
Brown - YoungRB	-0.12	0.64	-0.19	0.98
Brown - OldRB	0.53	0.48	1.09	0.52
YoungRB - OldRB	0.65	0.67	0.97	0.59
Female receptive Stage				
Brown - YoungRB	0.75	0.61	1.22	0.44
Brown - OldRB	2.35	0.54	4.32	<.0001
YoungRB - OldRB	1.61	0.66	2.43	0.04
Post-receptive Stage				
Brown - YoungRB	1.12	0.94	1.19	0.46
Brown - OldRB	0.85	0.84	1.01	0.57
YoungRB - OldRB	-0.27	0.67	-0.40	0.91
Proportion of female flights the male follows	Chi sq	Df	p value	
Male age/plumage type	19.48	2	<.0001	
Breeding Stage	0.16	2	0.92	
Male age/plumage type:Breeding Stage	4.01	4	0.41	
Post-hoc contrasts				
Pre-breed Stage	Estimate	SE	t ratio	p value
Brown - YoungRB	-0.03	0.22	-0.13	0.99
Brown - OldRB	0.44	0.11	4.05	0.0002
YoungRB - OldRB	0.47	0.22	2.13	0.08
Female receptive Stage				
Brown - YoungRB	0.12	0.20	0.60	0.82
Brown - OldRB	0.26	0.11	2.29	0.06
YoungRB - OldRB	0.14	0.19	0.70	0.76
Post-receptive Stage				
Brown - YoungRB	-0.03	0.18	-0.14	0.99
Brown - OldRB	0.13	0.17	0.74	0.74
YoungRB - OldRB	0.15	0.11	1.34	0.37

Proportion of male time close to female	Chi sq	Df	p value	
Breeding Stage	55.68	2	<.0001	
Male age/plumage type	3.43	2	0.18	
Aux	3.09	1	0.08	
Breeding Stage:Male age/plumage type	10.13	4	0.04	
Male age/plumage type:Aux	12.16	2	0.002	
Post-hoc contrasts				
Pre-breed Stage	Estimate	SE	t ratio	p value
Brown - YoungRB	0.18	0.09	1.95	0.13
Brown - OldRB	0.32	0.13	2.50	0.03
YoungRB - OldRB	0.14	0.11	1.22	0.44
Female receptive Stage				
Brown - YoungRB	0.15	0.11	1.33	0.38
Brown - OldRB	0.13	0.12	1.17	0.47
YoungRB - OldRB	0.01	0.06	-0.23	0.97
Post-receptive Stage				
Brown - YoungRB	0.02	0.10	0.20	0.98
Brown - OldRB	0.46	0.16	2.95	0.01
YoungRB - OldRB	0.44	0.15	2.98	0.01
Proportion of male time on territory	Chi sq	Df	p value	
Breeding Stage	22.705	2	<.0001	
Male age/plumage type	27.08	2	<.0001	
Aux	0.08	1	0.78	
Breeding Stage:Aux	60.14	2	<.0001	
Breeding Stage:Male age/plumage type	16.28	4	0.003	
Post-hoc contrasts				
Pre-breed Stage	Estimate	SE	z ratio	p value
Brown - YoungRB	0.52	0.64	0.81	0.70
Brown - OldRB	2.36	0.47	5.01	<.0001
YoungRB - OldRB	1.84	0.68	2.70	0.02
Female receptive Stage				
Brown - YoungRB	-0.46	0.58	-0.78	0.72
Brown - OldRB	2.31	0.51	4.49	<.0001
YoungRB - OldRB	2.77	0.59	4.73	<.0001
Post-receptive Stage				
Brown - YoungRB	0.89	0.59	1.50	0.29
Brown - OldRB	1.91	0.64	3.01	0.01
YoungRB - OldRB	1.02	0.44	2.34	0.049

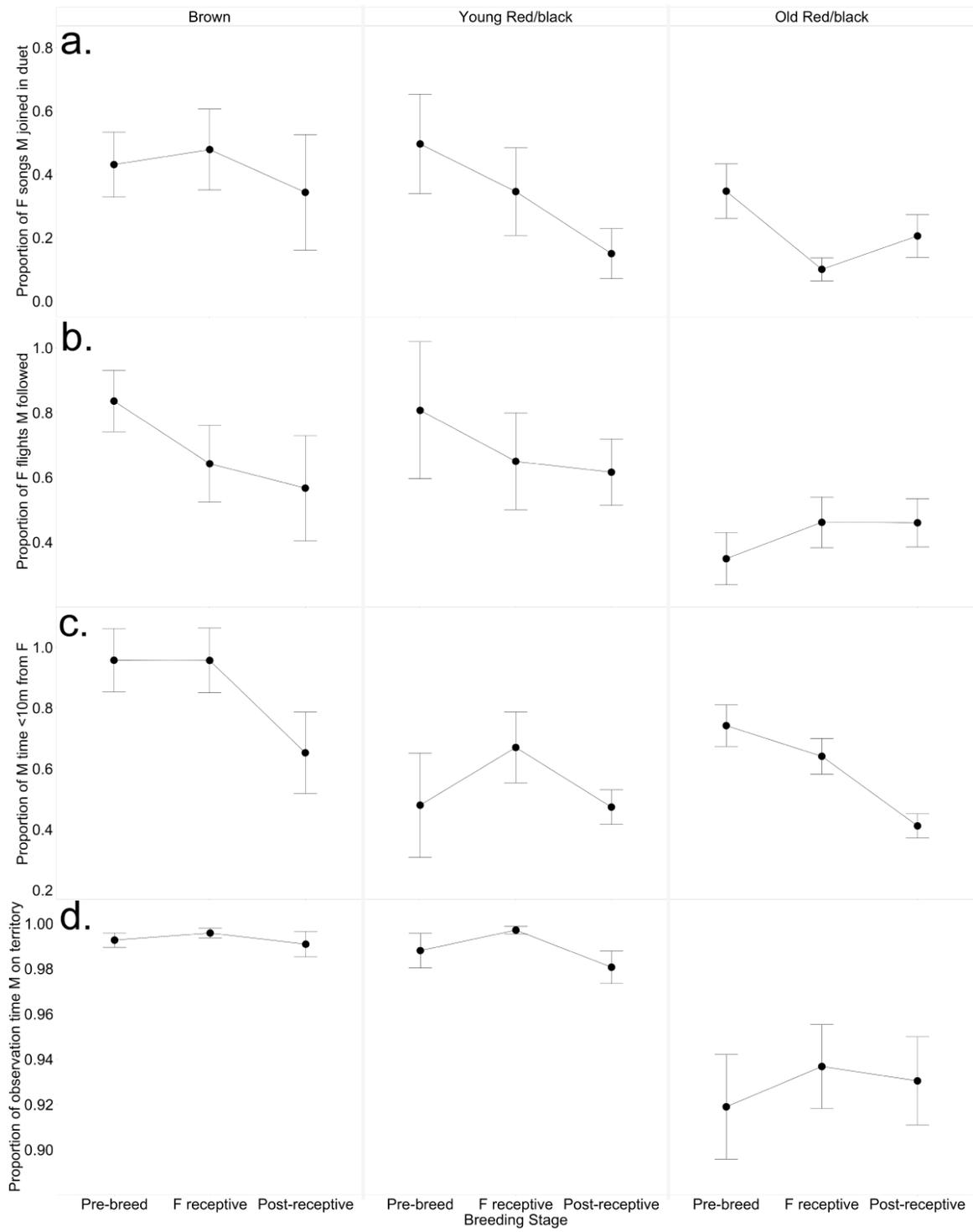


Figure 2. Effect of male age/plumage type on four male mate guarding behaviors: the male's duet rate with his mate (a), male mate following (b), male time spent <10 meters from his mate (c) and time male spent on territory across three breeding stages (d) across three breeding stages, pre-breed, female receptive and post-receptive stages. Young males are ≤ 2 years-old, old males are 3 or more years old. Points show mean \pm one standard error.

Playback experiment

We found that within the female's receptive stage, brown males showed a stronger aggressive response than did red/black males to a simulated male intruder, but not to a female or an intruding breeding pair (Table 3, Figure 3).

Table 3. Results of linear mixed model used to determine how male aggressive response varied with his plumage type (red/black vs. brown), the type of intrusion simulated (solo male, solo female or mated pair) and interactions.

Response: Male response to simulated intrusion

Full LMM		Chi sq	Df	p value
Plumage type		7.47	1	0.01
PB.treatment		8.11	2	0.02
Plumage type:PB.treatment		0.93	2	0.63

Post-hoc contrasts

	Estimate	SE	t ratio	p value
M solo treatment				
Brown - RB	-1.86	0.76	-2.45	0.02
F solo treatment				
Brown - RB	-0.96	0.73	-1.31	0.20
Duet				
Brown - RB	-1.38	0.72	-1.93	0.06

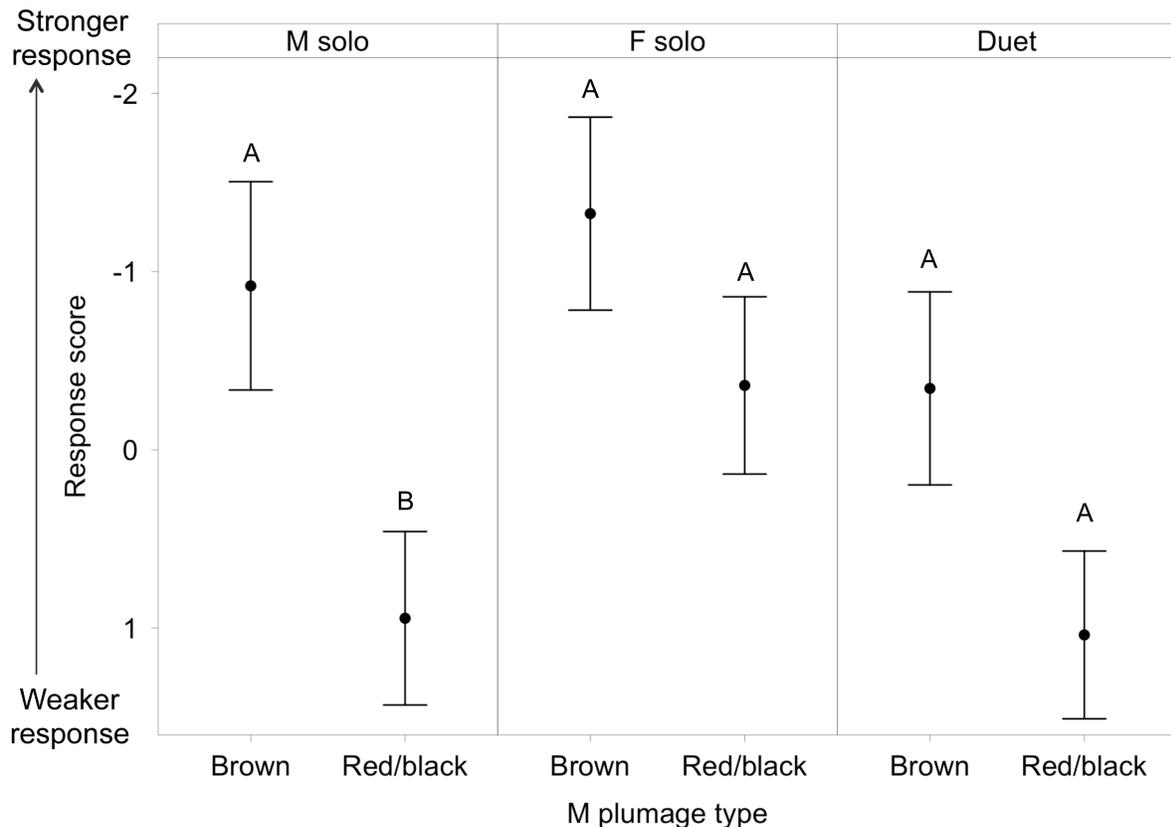


Figure 3. Variation in male aggressive response across types of simulated intrusions (solo male, solo female or mated pair) and between male plumage types (red/black vs. brown). Levels with different letters are significantly different from one another. All simulated intrusions were done during the female's receptive stage.

Variation in reproductive success across male types

Brown males sired a higher number of within-pair young than did old red/black males (Figure 4a, Post-hoc z-test of GLM: $z = -2.18$, $SE = 0.34$, $df = 2$, $P = 0.029$), whereas brown and young red/black males sired a similar number ($z = -1.29$, $SE = 0.34$, $df = 2$, $P = 0.198$). Brown males sired fewer extra-pair young than did both young red/black males (Figure 4b, $z = 2.11$, $SE = 1.03$, $df = 2$, $P = 0.034$) and old red/black males ($z = 2.27$, $SE = 1.02$, $df = 2$, $P = 0.023$).

Concordantly, all three male types had similar total reproductive success (GLM, $\chi^2 = 0.25$, d.f. = 2, $P = 0.88$). Proportion of EPY in a male's nest was equal across male types (GLM, $\chi^2 = 5.52$, d.f. = 2, $P = 0.063$), but we see a trend of old red/black males being cuckolded more than brown males ($z = 2.28$, $SE = 0.51$, $df = 2$, $P = 0.0586$).

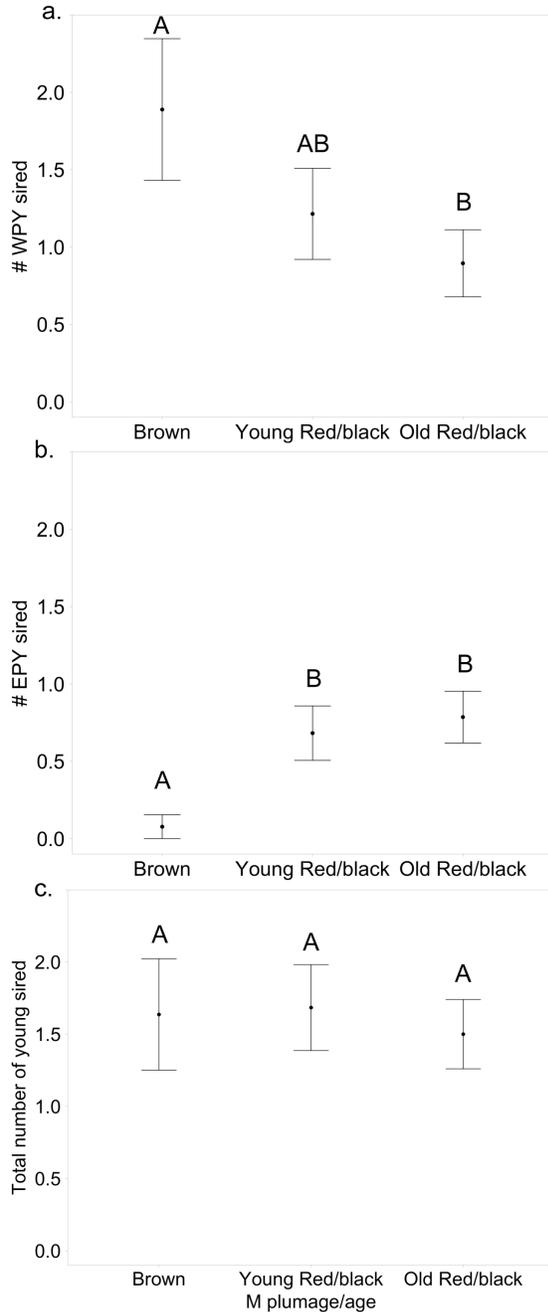


Figure 4. Number of within-pair young (a), number of extra-pair young (b) and total number of young (c) sired by brown, young red/black and old red/black males.

Discussion

Male mating strategies

Mating strategies adopted by males in our study depended on both age and plumage type in some cases, and only on plumage type in others (Figure 1). Brown males spent almost all of their time on their territory regardless of age, whereas red/black males were much more likely to leave the territory on a foray when they were older, although all male types spent the majority of their time on their territory. For all four mate guarding behaviors, both ages of brown males showed differences from red/black males, especially during the female's receptive stage (Figure 2). In all cases, young brown males guarded more and stayed on the territory longer than did old red/black males, whereas old brown and young red/black males tended to show intermediate behaviors (Figures 1 and 2).

These results have several implications. Although female Red-backed Fairy-wrens prefer to associate with red/black males, regardless of age, in choice experiments (Karubian 2002), females may find older red/black males more attractive than younger males, a pattern that may have been obscured in choice experiments by the female's inability to assess the unfamiliar males presented. In a previous study of Red-backed Fairy-wrens we found a trend of older red/black males siring more extra-pair young than young red/black males, suggesting they may find them more attractive (Webster *et al.* 2008).

Indeed, across studies of many different animal species, the most consistent factor that correlates with EP success is male age (Cleasby and Nakagawa 2012). This may explain our finding that younger red/black males adopt intermediate mate guarding and EPC seeking

strategies, since they may be less attractive than old red/black males. In general, females may prefer older males as mates because they provide direct and indirect benefits, including those indirect benefits associated with genetic quality, which longevity may indicate (Brooks and Kemp 2001). Our results add credence to this hypothesis.

On the other hand, our finding that both young and old red/black males sire more extra-pair young than brown males may indicate that red/black males are more attractive than brown males as extra-pair partners regardless of age (see also Webster et al. 2008), which instead supports the hypothesis that male plumage signals may be more important in mate choice than male age. Across species, females appear to choose mates based on multiple criteria, though some traits are more important than others in mate choice (Burley 1981; Andersson and Simmons 2006). Plumage type and age are likely both important mate choice criteria in our study species and further manipulative study is needed to determine the relative importance of each.

Another non-mutually exclusive interpretation for these results is that young red/black males have inaccurately or incompletely assessed their own attractiveness (Johnsen and Lifjeld 1995). For instance, if a male assesses his attractiveness through interactions with females (e.g. rejected copulations and avoidance flights) and other males (e.g. intrusions onto territory), then males that recently transitioned from unattractive to attractive status may not have enough information to make an accurate assessment. This is also likely in our study population, since 9 of the 15 young red/black males in our study were brown at the beginning of the season, when their pair bond formed, meaning that the majority of them were unattractive in their recent history. This may partly explain why young red/black males show intermediate mate guarding and EPC seeking strategies.

Male response to simulated intrusion

Brown males responded with more aggression than did red/black males to a simulated intrusion by a male during their mate's receptive stage, supporting the prediction that unattractive males will guard their fertile mates more than attractive males. The fact that we saw this pattern only when an intrusion by a male, but not female or pair, was simulated, supports our hypothesis, since it suggests that unattractive, but not attractive, males will put effort into repelling rival male territory intruders. Nonetheless, there may be alternative explanations for this result. We make the assumption here that close approach and flights over a speaker represent an aggressive attempt by the male to remove the intruder from the territory and prevent him from interacting with his mate, but since approach behavior doesn't necessarily indicate aggression, we cannot draw strong conclusions from this. Animal responses to playback experiments can be difficult to interpret (McGregor 1992).

Variation in mating strategy with auxiliary presence

We found that in all breeding stages, males in groups with auxiliary helpers sang duets less with their mates than did males without helpers, and old red/black males with helpers spent less time close to their mate than did males without helpers. Both results suggest that breeding males may be relieved of some mate guarding duties when helpers are present, as described in Red-backed Fairy-wrens by Potticary et al. (in review). Auxiliary helpers that are related to the breeding male might be expected to guard the breeding female to protect their relatedness to offspring (Welbergen and Quader 2006). We generally expect a son to protect his father's paternity to increase his potential for indirect fitness benefits, but when such mother guarding should occur depends also on offspring viability (Welbergen and Quader 2006). Because this

information is not available for Red-backed Fairy-wrens, we made no predictions for when helpers should duet with the breeding female. We also found that males (all plumage/age types) with helpers spent more time on their territory when their mate was receptive than did males without helpers. This may suggest that males with helpers are relieved of close mate guarding duties, but remain on the territory to “keep an eye on” helpers, which are reproductively mature and occasionally father young (Webster *et al.* 2008).

Another possible explanation is that males with helpers remain on the territory more than males without helpers because they do not need to foray as much, if the helper’s mate guarding efforts increase the breeding male’s total reproductive success enough so that extra-pair mating and its associated costs are less worth his effort. These results are consistent with results of Potticary *et al.* (in review), who found that Red-backed Fairy-wren males appear to use extra time afforded by the presence of an auxiliary helper to invest in foraging and self-maintenance rather than extra-pair mating. Also consistent with Potticary *et al.* (in review), we found no effect of auxiliary presence on male reproductive success, suggesting that helpers might have little actual influence on male reproductive success, but they may instead increase breeding male survival by allowing a male to increase his somatic investment (Magrath and Yezerinac 1997; Russell *et al.* 2007; Potticary *et al.*, in review).

Reproductive success across male types

We found that male plumage/age types that differ in mating strategies also show different patterns of reproductive success. Unattractive brown males, which invest strongly in mate guarding, had higher within-pair paternity than did old red/black males, whereas attractive red/black males, which invest in seeking extra-pair matings, had higher extra-pair paternity

than did brown males. Each of these reproductive success components sum to similar total reproductive success between plumage/age types, suggesting that the strategy each male type adopts provides him with an optimal success benefit for his level of attractiveness.

Our results differ from those of Webster et al (2008), who found that number of within-pair young was equal across male age/plumage types, whereas we found that brown males sired more within-pair young than old red/black males in our study. In Webster et al's (2008) study, red/black males (young and old) sired more extra-pair young than brown males and consequently had higher total reproductive success than brown males, instead of equal reproductive success across male types, as we found. Likewise, cuckoldry rates in a study conducted from 1997-2000 differed (brown and young red/black males were cuckolded more than old/red black males, Karubian 2002) from both our study and Webster et al's (2008) study (cuckoldry rates were equal across male types for both).

The Red-backed Fairy-wrens in our current study may have experienced a social environment different from birds in the previous studies. For example, density has been shown to affect both risk of cuckoldry and intensity of mate guarding behaviors in birds (Moller and Birkhead 1993; Westneat and Sherman 1997). Mate guarding behavior was not quantified in these previous studies, but some change between study-years in the characteristics of the social environment may have caused brown males to guard mates more strongly, which may explain the higher within-pair success we see for brown males (compared to old red/black males). Another possible explanation is that differences in paternity allocation across study-years may result from differences in female preferences across years. Changes across years in types of male signal traits females prefer have been demonstrated (Chaine and Lyon 2008) and flexibility in preference for males with certain

traits or behaviors could benefit females when social and physical environment vary (Qvarnstrom 2001; Alonzo and Sinervo 2001). This may be relevant to Red-backed Fairy-wrens, since they live in variable tropical habitat (Webster *et al.* 2010) and the benefits each male type provides (e.g. offspring care and mate-guarding, Karubian 2002) may be more important to females in some years than in others.

This possibility points out the importance of considering the female's role in the mating decision, since mating requires a compromise between two individuals, each employing strategies to increase their own fitness. Females are usually not coerced into mating, but instead, they play an active role in mate choice and decision making (Cordero and Eberhard 2003; Brennan *et al.* 2007). Indeed, we find that an additional female behavior, the proportion of the male's songs the female joins in duet, strongly predicts paternity; females that duet with their mates more cuckold them less (Dowling *et al.*, in preparation). Together with possible variation in female mate preference over time, these results suggest that in our study system, males exhibit strategies that we expect will prevent female infidelity, but whether a female mates within or outside of her pair bond appears to be a consequence of both sex's decisions (Westneat and Stewart 2003).

Conclusion

Determining how animals adapt their mating behaviors to achieve optimal reproductive success is a central question in organismal biology. Much is known about mating strategies in species where males have discrete phenotypes that are fixed throughout their lives (due to genetic polymorphism or some other fixed physiological or morphological characteristic, Oliveira *et al.* 2008) but less is known about plasticity in mating strategy within a male's

lifetime. Evolution can also drive flexibility in male mating strategy over a short time scale if choosing the correct strategy has a strong influence on a male's reproductive success (Painting and Holwell 2014).

Here, we show that male Red-backed Fairy-wrens adjust the degree to which they invest in mate guarding over EPC seeking depending on whether their age and plumage type is preferred by females, and proper adjustment yields reproductive success returns. This provides one of the first examples of a bird species where males optimize their reproductive success by adopting a conditional mating strategy based on their attractiveness. This fills an important gap in our understanding of how flexible mating strategies can evolve, since there appears to be selection for males to adjust the mating strategy they use continually, according to their attractiveness. We also show that the function of vocal duets can depend on the communication needs of the signaler, since only males that were at high risk of cuckoldry by rivals (unattractive males) used duets to guard mates. This supports previous findings that vocal duets are multifunctional signals (Dahlin and Benedict 2013) and additionally suggests that how duets are used depends on the social priorities of the signaler.

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CHAPTER 4

AN EXPERIMENTAL TEST OF THE EFFECT OF PHYSICAL AND ACOUSTIC MATE GUARDING ON INTRUDER BEHAVIOR IN A PROMISCUOUS BIRD

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Abstract

Animals face conflicting demands on their time and energy. In promiscuous species, males trade off investing in mate guarding with seeking extra-pair copulations (EPC). Mate guarding likely evolved to prevent paternity loss, and may take the form of physical or acoustic guarding, especially in duetting birds. Mating strategy theory hypothesizes that males of differing quality have different optimal mate guarding strategies and predicts that attractive males (those preferred by females) will invest more in EPC seeking, whereas unattractive males will invest more in mate guarding. However, this assumes that physical and acoustic mate guarding effectively deter rivals. We tested these assumptions in a promiscuous bird with discrete variation in male attractiveness, the Red-Backed Fairy-wren (*Malurus melanocephalus*). All males physically guard mates, and unattractive males also appear to do so with vocal duets. We used a removal experiment to determine the effectiveness of physical and acoustic mate guarding and also which males (attractive vs. unattractive) invest in EPC seeking. We temporarily removed males in two conditions: no mate guarding (removed completely) and acoustic mate guarding (female guarded with mate's songs from speaker).

We compared intrusion rate, intruder display rate and intruder identity to controls. Intrusion rates were higher than controls in the no mate guard condition, and intermediate in the acoustic guarding condition. This suggests that both deter intruders, but with acoustic guarding somewhat less so. Display rate during removals was higher than for controls, regardless of acoustic guarding, indicating that physical, but not acoustic, guarding deters intruder displays. Intruders were disproportionately attractive, red-black males, suggesting they invest in EPC seeking, whereas unattractive brown males do not. Results support the hypothesis that mating strategy varies with attractiveness and provide the first experimental evidence that acoustic guarding effectively deters rivals. This deepens our understanding of mating strategy evolution, by showing that strategies vary flexibly with male quality. This also confirms that mate guarding is an important mating strategy that is likely to yield fitness returns.

Introduction

In species that form social pair bonds, male mating effort can take the form of investment in reproduction with a social mate, or, in species that also participate in extra-pair copulations (EPCs), males can invest in seeking additional mating opportunities. Males of differing quality are expected to have different optimal mating strategies and this hypothesis predicts that attractive males (those preferred by females) will invest more in EPC seeking, whereas unattractive males will invest more in mate guarding (Trivers 1972; Kempenaers *et al.* 1995; Kokko and Morrell 2005, Dowling and Webster, in prep). This hypothesis hinges on the assumption that mate guarding, be it by close following, frequent copulation, or vocal duetting (i.e. acoustic mate guarding, wherein a male duets with his mate to signal her mated status to rivals, (Stokes 1968; Sonnenschein and Reyer 1983; Moller and Birkhead 1991) effectively ensures paternity (Moller & Birkhead, 1991). Physical mate guarding (close following) and frequent copulation are behaviors commonly seen in species across taxa (Brylawski and Whittingham 2004) while acoustic mate guarding appears to be a more specialized behavior that occurs in certain species of duetting birds (Hall 2009).

Recently, the question of whether physical mate guarding is an effective mechanism of assuring a male's paternity has received both observational and experimental attention (Brylawski and Whittingham 2004; Marthinsen *et al.* 2005). Results of these studies suggest that physical guarding decreases intrusion rates of rival males (Dickinson 1997) and decreases the prevalence of extra-pair young in the nest, both during short-term (Chuang-Dobbs *et al.* 2001; Brylawski and Whittingham 2004) and long term male detention experiments (MacDougall-Shackleton *et al.* 1996; Currie *et al.* 1999). However, in all previous studies,

males were removed completely, preventing researchers from measuring the effectiveness of the male's physical and acoustic presence in a graded fashion (but see Dickinson 1997).

Moreover, the effectiveness of acoustic mate guarding has not previously been studied. In general, mate guarding is not considered a primary function of duet (Hall 2009) and whether vocal duets can effectively deter intrusions has never been experimentally tested. We do not yet know if both the physical and acoustic presence of a male is necessary to deter rivals (Sonnenschein and Reyer 1983). Determining whether acoustic mate guarding is effective is crucial to our understanding of how paternity assurance strategies evolve across species that may use different types of behaviors, including vocal signals, to assure paternity.

We investigated the relative effectiveness of physical and acoustic mate guarding by separating the two mate guarding behaviors into separate experimental conditions, a novel experimental approach that allows us to control the male's mate guarding effort and determine if song alone effectively deters intruders. We used the promiscuous Red-backed Fairy-wren (*Malurus melanocephalus*) as a study system. Unattractive males in this species sing vocal duets that function as part of a suite of mate guarding behaviors (Dowling and Webster, in prep). Male Red-backed Fairy-wrens guard mates physically by staying in close proximity (<10 meters) and following female flights (Dowling and Webster, in prep). Unattractive males guard mates acoustically by either singing over or immediately following their songs, particularly during their fertile stage (Dowling and Webster 2013, Dowling and Webster, in prep). To determine how the physical and acoustic presence of the male affects intrusion rate, we conducted short-term (1 hour) detentions of males in three experimental conditions: (1) No mate guarding (male removed completely); (2) Acoustic mate guarding only (male removed

but female “guarded” with her mate’s songs played from a speaker); and (3) Control (male captured and released, so permitted to guard mate physically and acoustically). During experiment periods, we observed the female/pair and quantified the number and type of intrusions and characteristics and displays of intruders.

We predicted that if physical and acoustic mate guarding are equally effective (i.e. vocal duets alone deter rivals), then intrusion and intruder display rates will be equally low when both types of guarding occur and when just acoustic guarding occurs (i.e. the no mate guard condition will have the highest intrusion rate, the acoustic mate guard condition and control will be equally low). If physical mate guarding is more effective than acoustic guarding, then we expect intrusion and display rates will be low in the condition where physical mate guarding does occur and high in the two conditions where it does not (i.e. control will be lower than no mate guard and acoustic mate guard conditions).

If acoustic mate guarding is effective, but not as effective as physical mate guarding, then intrusion and display rates will be lowest in the condition where physical mate guarding occurs, intermediate when just acoustic guarding occurs, and highest in the condition where both are prevented (i.e. control lowest, acoustic mate guarding intermediate, no mate guard condition highest). If acoustic mate guarding is not effective, then we expect intrusion and display rates will be as high when acoustic guarding occurs as when no guarding occurs (i.e. no mate guard and acoustic mate guard conditions will be equally high and both higher than control). If neither physical nor acoustic guarding is effective at deterring intruders, then intrusion and display rates will be equal across conditions.

To investigate how male mating strategy varies with attractiveness, we focused on

how neighboring males responded when the focal male was removed and determined which males (attractive vs. unattractive) intruded to seek matings with neighbors over guarding their own mates. Similarly aged Red-backed Fairy-wren males with red/black plumage are preferred in female choice experiments (Karubian 2002) over brown males, who have mostly dull, female like plumage. Despite these differences in extra-pair reproductive success and female association preference, the two male types seem to both have success at siring young (Webster *et al.* 2008, Dowling and Webster, in preparation). We hypothesize that males that differ in attractiveness have different optimal mating strategies and predict that older red-black males will intrude on unguarded females (in the no mate guard and acoustic mate guard conditions) at rates higher than those predicted by their representation in the population, while brown and young red/black males will remain on their territories to guard their mates, a pattern we see in observational studies of this species (Dowling and Webster, in prep).

Currently, much is known about alternative male reproductive tactics across taxa (Oliveira *et al.* 2008), especially tactics that are fixed across a male's lifetime (Gadgil 1972), while less is known about flexible male strategies (Taborsky 1998; Humfeld 2013), and in particular, we lack a complete understanding of fundamental questions about male mating strategies, like the factors that influence how males choose strategies. For instance, the hypothesis that males that differ in attractiveness have different optimal mating strategies for mate guarding and EPC seeking has received little empirical study (but see Johnsen and Lifjeld 1995). In addition, we have a limited understanding of whether (and what components of) mate guarding behaviors are effective. These two questions are intimately tied, and understanding how male mating strategies evolve requires that we describe how male mate

guarding and EPC seeking efforts vary with male quality, and that we investigate the fitness benefits of mate guarding. We addressed both questions in this study, using a novel experimental approach that allows us to control the male's physical and acoustic mate guarding effort.

Methods

General field methods

We conducted fieldwork in the beginning of the breeding season (September-November) in 2013 on Lake Samsonvale (27°16' S, 152°41' E), 30 km northwest of Brisbane, Queensland, Australia. Study subjects were 25 breeding Red-backed Fairy-wren pairs in an early breeding stage (pre-breeding or fertile stage, before incubation onset). Breeding males had red/black plumage (greater than 66 percent of plumage is red and black, scored on 6 body regions, Karubian et al, 2008) in 17 groups and brown plumage (less than 33 percent of plumage is red-black) in 8 groups. We also categorized males based on age, with males two years or younger considered "young" and males > two years old considered "old". All brown males were one year old (young), while eight red/black males were one or two years old (young) and nine were three or more years old (old). We chose this age classification because it divided our focal males approximately in half and because previous research shows marked differences in mate guarding behaviors in male Red-backed Fairy-wrens after the age of two (Potticary et al, in review). Basic breeding biology data have been collected continually on this study population during each breeding season since 2011. All birds in focal groups were marked with a unique combination of color bands and basic breeding biology data were collected as per our previous studies (see (Webster *et al.* 2008; Varian-Ramos *et al.* 2010).

Experiments were started at first light, after the Red-backed Fairy-wren pre-dawn chorus had ended, and were completed before 10:00.

Experimental setup

We manipulated the breeding male's acoustic and behavioral mate guarding in one of three conditions: (1) No mate guard, male captured and removed from the territory where he was unable to sing or follow the female (prevents both acoustic and physical mate guarding); (2) Acoustic mate guard, male captured and removed, but recordings of his songs were played by observers to imitate a duet with the female (female acoustically but not physically guarded); and (3) Control, male captured and then immediately released (acoustic and physical mate guarding allowed). Males (and females) were allowed a 10-15 minute re-acclimation period and we waited until the female/pair resumed normal foraging before starting observations. We observed each female/pair for one hour after the male was captured and quantified female/pair behavior and intrusion behavior of neighboring males. In all cases where the male was detained, he was kept in the shade in a standard-size birdcage covered with a breathable dark cloth, provided with food and water, several territories away. Male detentions did not exceed two hours.

Observation procedure

In all conditions, the female/pair was followed within 15-20 meters throughout the 60-minute observation by two observers. Observers continually dictated female/pair distance from where the male was captured, distance between male and female, general behavior (movements,

foraging, flights and who follows), any vocalizations, and any intruding conspecifics (description and identity, plumage percentage, distance from female/pair, all behaviors and displays and female/male reaction). Males who were considered ‘intruders’ on focal territories exhibited specific behaviors that indicated they were on an extra-territory courtship foray. Red-backed Fairy-wrens, like many other fairy-wren species, engage in forays and courtship displays that are directed primarily at extra-pair females (Mulder *et al.* 1994; Green *et al.* 1995; Karubian 2002). Males on foray exhibit a long, undulating flight (similar to the ‘sea-horse’ flight described for the Splendid and Superb fairy-wren; Rowley and Russell 1990; Mulder 1997). Displays performed by intruding males included puff-back displays, female feeding, courtship chases and petal displays (Karubian and Alvarado 2003). At the end of the observation, we noted the length of the observation and the male was retrieved and released (if applicable). On capture, we estimated the focal male’s plumage percent and categorized him as either red/black or brown (no males in experiment had intermediate plumage type).

Acoustic mate guarding procedure

For playback in the acoustic mate guarding condition, we recorded and used the focal male’s own songs, which were recorded during the dawn chorus, one to three days before the experiment, with a Marantz PMD661 solid-state digital recorder (Marantz America, Itasca, IL, USA) and Sennheiser ME67 highly directional long-gun microphone with a Rycote softie windshield and mount (Sennheiser Corp., Old Lyme, CT, USA). We created spectrograms in Raven 1.4 (Cornell Laboratory of Ornithology, Ithaca, NY, USA, 16-bit sample format; discrete Fourier transform (DFT) = 512 samples; frequency resolution = 124 Hz; time

resolution = 11.6 ms; frame overlap = 50%). We clipped 20 high quality songs from spectrograms for each male and saved them as 16-bit wave files. Amplitude 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 2400-17800 Hz, which left spectral space occupied by song elements intact (Greig *et al.* 2012; Greig *et al.* 2013).

Song playback

We used program SYRINX-PC (J. Burt, Seattle, WA, U.S.A.) on a Panasonic Toughbook laptop computer (Model CF-45; Pentium MMX, 266 MHz, 32 KB RAM, 16 bit sound card) for song playback in the field. The laptop was connected to an amplifier (Pignose Legendary 7-100, Pignose-Gorilla, Las Vegas, NV, U.S.A.) with a 30-meter cable. One observer carried the speaker and the second observer controlled playback using the laptop. The first observer remained within 15-20m of the female, so that playback was audible to her, but our presence did not affect her behavior in any obvious way. When the female sang a song or song introduction notes, the second observer played one of 20 recordings of her mate's songs, cycling through all songs at random throughout the observation. Once the observation began, if the female had not yet sung or if her location was unknown, we played a male's song to solicit singing (lure song). We played approximately the same number of recorded male songs during all acoustic mate guarding trials (29 ± 9.7 playbacks).

Statistical analyses

Intrusion and display rate data were analysed using R version 3.1.2 (R Development Core

Team 2015). We used generalized linear mixed models (GLM) from the glm functions in R package lme4 for all models, since the response variables we measured did not conform to linear model assumptions. Count data were modeled with a Poisson distribution, with number of intrusions and number of intruder displays considered as counts and total observation time as an offset argument. We tested for effects of weather (heat index, humidity and average wind speed) and focal male plumage type by adding these as covariates in each model. The covariates were removed from models when non-significant and patterns described in results otherwise.

We conducted post-hoc tests using the lsmeans package in R. Least-squares means were computed for specified factors or factor combinations from each model, and we made comparisons among them using the Tukey method to adjust P values when multiple comparisons were made. Least-squares means were back-transformed to show response in the original scale in figures. Chi-square tests and percentage calculations were used to determine which male types intrude on unguarded females and were done in Microsoft Excel 2011 (Microsoft Corporation, Redmond, WA, USA). We calculated the expected number of intruders of each type for the population by multiplying the total number of intrusions by each male type's prevalence in the population (proportion of total males that were old red/black, young red/black and brown).

Results

Effectiveness of mate guarding

When males were prevented from both physically and acoustically guarding (no mate-guard

condition), mean intrusion rate was higher than the control condition (Figure 1, post-hoc z-test of GLM least-squares means: $z = 2.36$, $SE = 0.76$, $df = 2$, $P = 0.0484$). When we acoustically guarded the female with playback of the male's song (acoustic mate guard condition), intrusion rate was intermediate between no mate guard and control conditions (i.e. rate did not differ from the control condition, $z = 1.74$, $SE = 0.77$, $df = 2$, $P = 0.19$ or the no mate-guard condition, $z = 1.1$, $SE = 0.40$, $df = 2$, $P = 0.51$). We found no effect of focal male plumage/age on intrusion rate (GLM, $\chi^2 = 0.34$, d.f. = 2, $P = 0.84$).

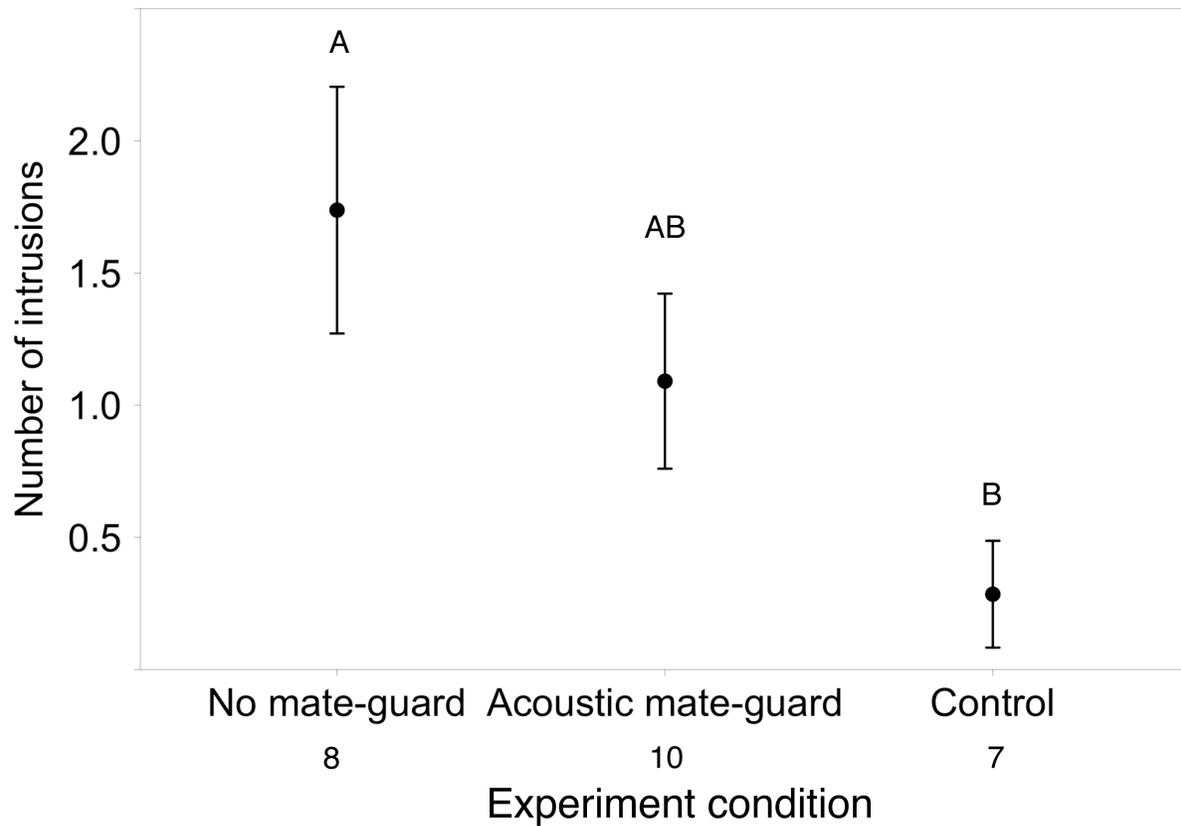


Figure 1. Rate of intrusions (mean \pm SE) from foraging males across three experimental conditions. Levels with different letters are statistically different from each other. Sample

size (number of focal breeding pairs) listed below each condition.

When the focal male was not physically present, display rate by intruding males was higher than control conditions, both in the no mate-guard (Figure 2, $z=3.15$, $SE = 1.02$, $df = 2$, $P = 0.005$) and acoustic mate-guard conditions ($z=2.99$, $SE = 1.02$, $df = 2$, $P = 0.008$). The number of displays did not differ between the no mate-guard and acoustic mate guard conditions ($z=0.64$, $SE = 0.26$, $df = 2$, $P = 0.79$). Observations where there were no intrusions (and hence no displays) were included in the calculation so that we could determine the overall display rate across conditions, since only one intrusion occurred during a control condition observation. If we look only within observations where intrusions occurred, display rate does not differ between acoustic mate guarding and no mate guarding conditions ($z = 0.44$, $SE = 0.26$, $df = 1$, $P = 0.6635$), suggesting that the acoustic presence of a male does not prevent an intruder from displaying, once he's already arrived on the territory. The rate of display by rival males who had already intruded (observations with no intrusions were excluded) was lower when the resident male was an old red/black male than when he was a young red/black ($z = 1.95$, $SE = 0.39$, $df = 2$, $P = 0.076$) or brown male ($z = 1.78$, $SE = 0.39$, $df = 2$, $P = 0.051$), though these differences were marginally non-significant.

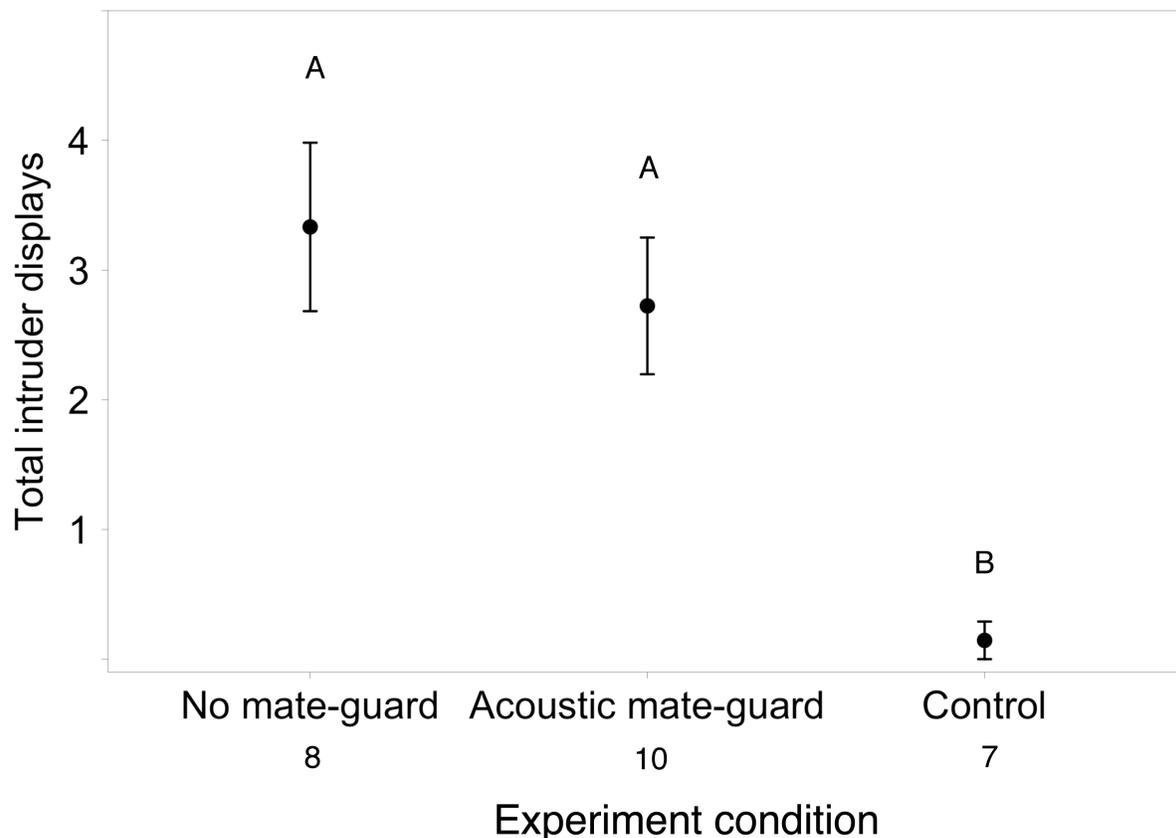


Figure 2. Total number of displays (mean \pm SE) from foraging males across three experimental conditions. Levels with different letters are statistically different from each other. Sample size (number of focal breeding pairs) listed below each condition.
Which males intrude on unguarded females?

In the year of this study, our study population was composed of 40% old red/black males (> two years old), 30% young red/black males and 30% brown males. When intrusions occurred during focal male detention (no mate-guard and acoustic mate-guard conditions, N = 11 observations), the males that intruded were old and red/black significantly more often than expected and brown or young red/black significantly less often than expected if male types intrude according to their representation in the population (Table 1). In other words, although old red/black males make up only 40% of the populations, they were responsible for 78% of

intrusions.

Table 1. Results of a Chi Square test used to determine if males of each age/plumage type intrude more often than predicted by their representation in the total population.

Intruder age/plumage type	Intrusions observed	Intrusions expected from population frequency	Chi Square	P value
Old red/black	18	9.28	12.363	<0.001
Young red/black	2	6.86		
Brown	3	8.32		
Total	23	23		

Discussion

Effectiveness of mate guarding

When the focal male was detained in the no mate guarding condition, territorial intrusions from neighboring males were much higher than they were for controls (Figure 1). This result suggests that a male can effectively deter rivals by either physically or acoustically guarding his mate, but we cannot determine which had more of an effect, since we were not able to prevent the male from singing. Intrusion rates in the acoustic mate guarding condition were intermediate between the no mate-guard condition and control, suggesting that duetting with the female effectively decreases intrusion rates, but not to the same degree as when both physical and acoustic mate guarding occur. The fact that acoustically guarding the female maintained intrusions at rates similar to when the male is present (control condition) suggests that vocal duets alone may effectively prevent males from intruding.

There are several possible alternative explanations for why intrusion rates in the acoustic mate guarding condition are intermediate to the other conditions. First, acoustic mate guarding may or may not be as effective as physical mate guarding, but we were not able to detect that difference due to low sample size. If in a larger study, we found that the

difference between acoustic mate guarding and no mate guarding conditions was significant, this would suggest that acoustic guarding decreases intrusion rates substantially, compared to when the female is not guarded at all. Alternatively, if we observed a significant difference between acoustic mate guarding and control conditions, this would suggest that intrusion rates are much lower when males physically guard in addition to acoustically guarding. Second, intrusion rates may have been higher than controls during acoustic mate guarding due to imperfect acoustic mate guarding playback or presence of the experimenters. Although we modeled the duetting rules used in the experiment from our observations of natural duets (Dowling and Webster 2013), duet timing and structure are often more subtle than can be appreciated by a human observer. Actual duets may more effectively deter rival males than our simulated duets, meaning we cannot say conclusively that acoustic mate guarding is less effective than physical mate guarding.

Rival males were more likely to intrude and display in the no mate-guard and acoustic mate guard conditions than they were in controls (Figure 2) and when a male does intrude (i.e. when observations with no intrusions are excluded from analyses), he is just as likely to display to a female when she is acoustically guarded as when she is not guarded at all. This suggests that the physical presence of a male is necessary to prevent an intruder from displaying to a female. Taken together with intrusion rates, this may suggest that acoustic mate guarding is an effective long-range signal that prevents males from entering the territory, but physical mate guarding by an actual male is necessary for preventing males from displaying to the female once they've already entered the territory.

These results have important implications for understanding the function of duets.

Vocal duets often have multiple functions, and although both within and extra-pair communication functions have been tested in many avian species, most species seem to use duets at least partly to communicate with individuals outside of their pair-bond (especially in territorial contexts (Dahlin and Benedict 2013)). Our novel experimental method allowed us to determine how individuals outside of the pair bond respond to vocal duets that occur when the male is not physically present. We showed that these duets affect the behavior of extra-pair receivers, such that intrusion is suppressed to the same degree as when the male is physically present. The fact that duet did not prevent males from displaying once they had intruded may demonstrate that duets are limited in their effect on intruders and may serve as a first tier defense.

This provides support specifically for the hypothesis that males can use vocal duets to help prevent rival males from usurping their paternity or position in the pair bond (i.e. acoustic mate guarding, Hall 2004), since the intruders who were repelled were those on courtship forays, though other types of non-courtship intrusions would need to be quantified to rule out territory defense functions of duet. The acoustic mate guarding hypothesis has found sparse support across duetting species (Hall 2009). Experimentally testing the effect of vocal duets on receivers outside of the pair bond by removing the male and duetting with the female may prove more fruitful for testing whether a species uses vocal duets to guard mates. This experimental approach more directly addresses the question of whether duets repel rivals than traditional methods like dual speaker playback experiments. These experiments yield results that are difficult to interpret, since predictions for how subjects will respond to playback overlap across several hypotheses (Douglas and Mennill 2010).

In our study species, unattractive males invest in physical and acoustic mate guarding (Dowling and Webster, in prep), which suggests that rival males may threaten their paternity/pair bond position more often than attractive males (who guard less). This predicts that in our experiment, unattractive males' territories will be intruded upon more often than those of attractive males. We found no effect of male plumage/age type on intrusion rate, suggesting that intruders were equally likely to visit neighboring territories regardless of the attractiveness of the resident male.

When we excluded observations with no intrusions, intruding males were more likely to display to a female if the resident male was brown or young red/black than if he was old red/black, but these differences are marginally non-significant. There are several potential explanations for this trend: old red/black males could be dominant to other male types or more aggressive, which suppresses displaying behavior of intruders, or females mated to brown and young red/black males may be more likely to engage in extra-pair copulations (as previous studies on this species suggest, Karubian 2002), which means displaying to their mates has a higher potential fitness payoff for intruders.

Which males intrude on unguarded females?

When the focal male was detained (in the no mate-guard and acoustic mate guard conditions, Table 1), males who intruded were much more likely to be old red/black males than brown or young red/black males, given the proportion of old red/black males in the population.

Removing the focal male can be thought of as an experimental presentation of an unguarded female, and all male types should have equal opportunity to intrude, since removing the male

removes effects of suppression from dominance and risk of aggression. This result suggests that intruding on neighboring territories and courting females is a mating strategy commonly used by old red/black males, but not brown or young red/black males (Dowling and Webster, in prep). This supports the hypothesis that male strategy varies with attractiveness, suggesting that attractive old, red/black males adopt an EPC seeking strategy, and unattractive brown and young red/black males may adopt a heavy mate guarding strategy. Although the fact that unattractive males were not foraging does not necessarily mean they were mate guarding.

Conclusions

The evolution of male mating strategies is a well-studied research topic in behavioral ecology. As a result, much is known about the mechanism, function and evolutionary origin of alternative male reproductive tactics across taxa (Oliveira *et al.* 2008), especially tactics that are genetically, physiologically or morphologically fixed (Gadgil 1972). Less is known about strategies that are flexible within a male's lifetime (Taborsky 1998; Humfeld 2013), and in particular, we know little about factors that influence how males choose strategies. In addition, although a large body of research on mate guarding effectiveness has confirmed that such guarding can prevent intrusions and protect paternity (Brylawski and Whittingham 2004; Marthinsen *et al.* 2005), we still lack an understanding of the components of mate guarding that make it adaptive. Specifically, we do not yet know whether the vocal presence of a male effectively deters rivals, which is crucial to our understanding of how paternity assurance strategies evolve across species that may use vocal signals to assure paternity.

Here, we sought to further our knowledge of mating strategy evolution be

investigating how male quality affects the strategies they choose (Kokko and Morrell 2005) and the effectiveness of acoustic and behavioral mate guarding. We investigated these questions using a novel experimental approach that allows us to control the male's physical and acoustic mate guarding effort and this approach can serve as a useful tool for research into both mate guarding effectiveness and vocal duet function, since it allows for a more direct test of the acoustic mate guarding hypothesis than traditional methods (Douglas and Mennill 2010).

We found that physical mate guarding prevents males from intruding and displaying, while acoustic guarding prevents intrusions (albeit possibly to a lesser degree) but not displays. This suggests that acoustic mate guarding effectively deters rival at long range, but male presence is required to prevent courtship by rivals, which confirms that the vocal presence of a male is an important component of the mate guarding strategy and vocal duets in this species may have evolved at least partially for this function. We also find that attractive males are the ones doing the intruding, which supports the hypothesis that whether a male invests in mate guarding or EPC seeking depends at least in part on his attractiveness and confirms that quality is an important factor that determines which strategy a male chooses. Together, these results inform our understanding of male strategies by filling in crucial gaps in what we know about the effectiveness of physical and acoustic mate guarding and flexibility in mating strategies across males of different quality. This furthers our understanding of how animals adapt their mating behaviors to help them achieve optimal mating success.

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