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No evidence for acoustic mate-guarding in duetting buff-breasted wrens (*Thryothorus leucotis*)

Abstract There are few empirical tests of the acoustic mate-guarding hypothesis for the function of duetting in birds. This hypothesis states that when females are fertile, males initiate many songs or answer most of their mates' solo songs to form duets and repel rival males seeking extra-pair copulations. We tested the hypothesis by comparing song initiation and answer rates of males and females in socially monogamous buff-breasted wrens (*Throthorus leucotis*) during pre-fertile and fertile periods. During pre-fertile periods, males often sang for short periods before being answered by their mates, yet first duets were formed earlier relative to dawn and more duets were given during the dawn chorus on pre-fertile than fertile mornings. Males initiated more songs during pre-fertile than fertile periods, whereas there was no difference between stages in female song initiation rates. The proportion of songs answered by individuals of both sexes did not differ between breeding stages. Other mate-guarding behaviours, such as frequent copulation and maintaining close proximity to mates when fertile, did not appear to be important in this species, as no copulations were observed and there was no difference in the time pairs spent in close proximity when females were fertile or not. Parentage analysis revealed that only 3% of 31 broods had young that were likely the result of extra-pair paternity. These findings do not support the acoustic mate-guarding hypothesis, and suggest that the low rate of extra-pair paternity in buff-breasted wrens was maintained without the use of acoustic or traditional paternity guards.

Keywords Acoustic mate-guarding · Breeding synchrony · Duet · Monogamy · Parentage

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Introduction

Duetting, precisely timed alternating or overlapping singing by pairs or groups, occurs in over 200 avian species, most of which reside in tropical regions (Farabaugh 1982). Given the high degree of complexity in song structure and in the coordination of the behaviour of two or more individuals (e.g. Mann et al. 2003), duetting has been viewed traditionally as a cooperative pair display involved in the establishment and maintenance of partnerships and mutually defended territories, or the stimulation and coordination of reproduction in species with long-term partnerships (reviewed in Hall 2004). Recent playback studies support this cooperative view of duetting, finding that pairs duet more frequently in response to simulated intrusions of paired than single birds (Hall 2000; Seddon et al. 2002; Grafe and Bitz 2004), and that individuals were equally likely to answer their mates' songs and produce duets regardless of the sex of the simulated intruder (Mulder et al. 2003; but see Levin 1996a; Logue and Gammon 2004).

Instead of viewing duetting as primarily a cooperative endeavour, Sonnenschein and Reyer (1983) suggested

that males participate in duets to acoustically guard their mates from rivals seeking extra-pair copulations (see also Brown and Lemon 1979; Farabaugh 1982 who propose a similar hypothesis for guarding the pair bond). Thus, the "acoustic mate-guarding hypothesis" suggests that duetting arose from a conflict of interest between the sexes: female song attracts extra-pair males, whereas males answer their partners' songs to form duets and deter rivals. Alternatively, males may guard their mates by initiating many songs, thereby forcing their mates to duet at high rates or else risk their partners attracting rival females (Sonnenschein and Reyer 1983; Hall and Magrath 2000). The critical prediction of this hypothesis is that both song initiation rates and song answer rates will be highest during the females' fertile period (Hall and Magrath 2000), when extra-pair copulations are mostly likely to result in the production of extra-pair young (Birkhead and Moller 1992). Some studies support the acoustic mate-guarding hypothesis, although fertile periods were inferred rather than known directly (Sonnenschein and Reyer 1983). By contrast, Hall and Magrath (2000) monitored individual reproductive attempts and analysed singing behaviour in relation to known fertile periods, but found no evidence for acoustic mate-guarding in Australian magpie-larks (*Grallina cyanoleuca*) (see also Whittingham et al. 1997; Logue and Gammon 2004).

We tested the acoustic mate-guarding hypothesis that male buff-breasted wrens (*Thryothorus leucotis*) prevent their mates from engaging in extra-pair behaviour by answering all songs initiated by females or by initiating many songs themselves. Unlike congeneric bay wrens (*T. nigricapillus*) in which females almost always initiate duets (Levin 1996a) or plain wrens (*T. modestus*) in which males initiate most duets (Mann et al. 2003), buff-breasted wrens of both sexes may initiate duets and both sexes sing solo songs (Farabaugh 1983; S.A. Gill, unpublished data). Thus, we investigated whether males or females, or both, guard their mates by initiating more songs, or by answering more frequently songs initiated by their mates during fertile than pre-fertile periods (Hall and Magrath 2000). We observed duetting during the dawn chorus, the period of intense singing that occurs around sunrise in many species (Staicer et al. 1996), including buff-breasted wrens (S.A. Gill, unpublished data). If female fertility influences duetting behaviour in buff-breasted wrens, we expected that such an effect would be most pronounced during this diel peak at dawn, which also corresponds to the time at which female buff-breasted wrens lay their eggs and hence may be most fertile as they ovulate their subsequent eggs (Gill 2003a). We tested the following predictions: (1) pairs start duetting earlier at dawn and duet at higher rates when females are fertile than pre-fertile; (2) individuals of both sexes initiate more songs during fertile periods; and (3) males and females answer more of their mates vocalisations when females are fertile than non-fertile.

In addition to acoustic mate-guarding, we also examined whether buff-breasted wrens use physical mate-guarding and high copulation frequency (reviewed in Birkhead 1998) as alternative ways to ensure paternity in their mates' clutches. In duetting magpie-larks, males protected their paternity by staying close to their mates when fertile, rather than guarding their mates acoustically or by copulating frequently (Hall and Magrath 2000). Whether this may be a general pattern for duetting species is unknown, because the use of alternative paternity guards has not been investigated in other duetting species. Males guard their mates in some non-duetting tropical species (Komduer et al. 1999), but not in others (Moore et al. 1999; Robertson et al. 2001; Stutchbury and Morton 2001). Yet extra-pair paternity appears to be uniformly low in the tropical species studied to date (reviewed in Stutchbury and Morton 2001), including two duetting species (Fleischer et al. 1997; Hall and Magrath 2000). Low breeding synchrony, common in low latitude areas, may decrease the benefits to individuals of both sexes from seeking extra-pair copulations (Stutchbury and Morton 2001). Thus, we also examined the degree of breeding synchrony within this population. Finally, we determined the frequency of extra-pair paternity in our population of buff-breasted wrens to consider the extent to which males were vulnerable to losses in paternity.

Methods

Study species and population

Buff-breasted wrens are small (16–23 g) insectivorous passerines, which inhabit secondary forests from central Panama to southeastern Brazil (Brewer and MacKay 2001). They are sexually monochromatic, but slightly sexually dimorphic; within pairs males are larger than their mates (Gill et al., unpublished data). Buff-breasted wrens are paired and territorial year-round, and are often found in family groups, as independent offspring may remain in natal territories for several months to over a year after becoming independent. Breeding occurs during the wet season (April–October), with clutch initiation closely tied to the onset of rains that mark the transition between dry and wet seasons (Ahumada 2001; S.A. Gill, unpublished data). Paired birds duet throughout the year, with males and females producing sex-specific components (Farabaugh 1983; S.A. Gill, unpublished data), which are easily distinguishable in the field. Males and females may give their respective sex-specific songs without being accompanied by their partners, thereby forming sex-specific solo songs.

We studied buff-breasted wrens from February to May 1997, February to July 1998, and February to June 1999, in a 22-ha secondary forest located at the convergence of the Chagres River and Panama Canal in central Panama (9°7′N, 79°42′ W; see Gill and Stutchbury 2005, for details of the study area). The study population consisted of 18–24 territories, on which we colour-banded paired adults and any retained offspring. Each individual was given a unique combination of a single aluminium and one to three colored-celluloid bands to facilitate individual recognition. All individuals were caught in non-breeding condition and so could not be sexed by the presence of brood patches or cloacal protuberances. Sex was provisionally assigned based on body-size measurements taken at the time of banding (length of unflattened wing chord, tail, bill, and tarsus, and mass), and confirmed by observing sex-specific songs (Farabaugh 1983), egg-laying bouts (Gill 2003a), and female-only incubation (Brewer and MacKay 2001). None of the provisional sex assignments were subsequently found to be in error. Adults were distinguished from juveniles by morphology where possible (ossified skull and rust-colored iris versus unossified skull and grey or brown iris) and by following

behavioural observations of banded groups. Members of a pair typically foraged in close proximity, and roosted and duetted together, while offspring often foraged and sang alone, and roosted separately from adults.

We located and monitored buff-breasted wrens dormitory and breeding nests through pre-breeding and breeding periods to determine the breeding stage at which observations were made. We conservatively defined the fertile period as five days before laying until the day on which the penultimate egg was laid (modal clutch size = 3 eggs, range = 2–3, n=42), as copulations likely have the highest probability of fertilisation during this period (Birkhead 1998). Observations in the pre-fertile period occurred between 9–38 days prior to clutch initiation, a period which likely encompassed gonadal recrudescence in buff-breasted wrens (see Wikelski et al. 2003).

We calculated a breeding synchrony index for each breeding female as the number of fertile females (excluding the focal female) on each day that a focal female was fertile divided by the product of the number of those days and the number of other females in the population (Kempenaers 1993). The overall synchrony index for the population is the mean of the breeding females. We calculated synchrony indices for each of the 2 years (1998, 1999) during which we observed duetting. The values of these indices probably overestimated actual breeding synchrony, because some buff-breasted wrens continued to initiate clutches after we left our field site.

Duetting observations

We selected pairs for observations using a stratified random sampling design such that all pairs were observed once in a randomly determined order before the series of watches was re-initiated. Each day before dawn, an observer arrived on a focal territory 5–15 min before the wrens left their dormitory nests. When the location of dormitory nests was known, an observer positioned him- or herself within 10 m of it and noted the time the wrens emerged from nests. Observers recorded the time when the first duet was given on focal territories, and counted all vocalisations produced beginning with the first duet for 30 min. These counts included male-initiated and female-initiated duets, as well as those vocalisations produced only by males (male solo songs, trills, trill wops, and wops) or only by females (female solo songs and weeohs; Farabaugh 1983). We recorded and reviewed daily dawn chorus vocalisations during the first month of observation to ensure we could reliably distinguish male- and female-initiated duets in the field. We identified the sex of the individual that initiated the duet for 92±15.4% (mean±SD; n=695) of duets. A 30-min observation period was selected based on our impression (S.A.G. and M.J.V., personal observation) that song rates decreased substantially even before 30 min had passed. This was later confirmed by comparison with song rates at other times of the day (S.A. Gill, unpublished data). Solo songs given before the first duet were also quantified but analysed separately from songs given after the first duet, since pre-duet solo songs were not given on all territories (see Results). Buff-breasted wren duets are intense and can be heard up to two territories away. Thus, when weather conditions permitted (low wind and no rain), up to three neighbouring territories were monitored concurrently. We present the time of the first duet as minutes relative to sunrise, which are represented as SR- or SR+ depending on whether the duet occurred before or after sunrise, respectively. Sunrise times for Gamboa were obtained from the United States Naval Observatory Astronomical Applications Department website at http://aa.usno.navy.mil/AA/.

From the dawn chorus observations, we calculated song initiation rates and answer rates for males and females during pre-fertile and fertile periods. Song initiation rates included all duets initiated by the male or female plus their sex-specific solo songs given within the 30-min observation period. Answer rates were calculated as the number of songs initiated by the partner that were answered to form a duet divided by the total number of songs initiated by the partner (Levin 1996b). If an individual did not initiate any songs

during the observation period, then the answer rate of the partner was scored as a missing value.

Alternative paternity guards

To examine whether buff-breasted wrens used mate guarding or frequent copulation as paternity guards (see Birkhead 1998), we performed focal observations between 0600 and 1300 hours during both pre-fertile and fertile periods in 1998 and 1999. Observations commenced when we spotted one individual within the pair and ended when we lost sight of both individuals for 2 min. During focal observations, we quantified: (1) the time individuals spent at <5 or >5 m from their partners, from which we calculated the proportion of time partners spent within 5 m of each other; (2) the number of movements by males and females towards their partners; (3) the number of times males and females followed their partners when they had moved >5 m from them; (4) the number of copulations or copulation attempts; and (5) the number of border disputes or intrusions onto territories. Buff-breasted wrens made few large movements over 5 m; instead they typically moved fairly rapidly using small perch changes or flights (<0.5 m) during which it was difficult to score one individual as being responsible for moving closer to the other. As a result, there were many zeros in the data set. Since the behaviours represented by variables (2) and (3) result in the maintenance of a particular distance between individuals within a pair, we grouped them for analysis. The mean±SD duration of our focal observations was 19.9±9.7 min (n=10 pairs). The length of time we watched pairs differed between pre-fertile and fertile stages. However, the results of analyses that compared mate-guarding between stages did not differ when we used the number of movements per observation or the number of movements per min.

Parentage analysis

At the time of banding, we collected 20–50 μl blood samples from territorial males and females and their offspring via puncture of the brachial vein for parentage analysis. We bled offspring as nestlings (12–14 days old if nest accessible; modal brood size=2 young, range=1–3), recently after fledgling, or while they were still on their natal territories as independent juveniles. We sampled a total of 27 adult males, 25 adult females, and 53 offspring from 31 broods (produced by 22 pairs), for a total of 105 individuals over three field seasons. We sampled all young present in 17 broods and partially sampled young from the remaining 14 broods.

DNA was extracted from blood in lysis buffer (0.01 M Tris HCl, 0.01 M EDTA, 0.01 M NaCl, 1% n-lauryl-sarcosine) with 7.5 M ammonium acetate and precipitated in isopropanol. We used the allelic variation present at 5 microsatellite loci to determine the paternity of each nestling. Two of these loci, TA-A5–15 and TA-C3(B)-2 were isolated from the house wren (*Troglodytes aedon*; Cabe and Marshall 2001), Cp 51 was isolated from the song wren (*Cyphorhinus phaeocephalus*; Hughes and Robinson 2001), McyU4 was isolated from the superb fairy wren (*Malurus cyaneus*; Double et al. 1997) and FhU2 was isolated from the pied flycatcher (*Ficedula hypoleuca*; Primmer et al. 1996).

We used an Applied Biosystems 9700 thermocycler to amplified genomic DNA from each individual sample in a 10 μl PCR reaction with 50 ng of template DNA, 0.3 pmol end-labelled (^{33}P ATP; Dupont) forward primer, 0.3 pmol unlabeled forward primer, 0.6 pmol reverse primer, 200 μM dNTPs, PCR reaction buffer (Quiagen), 0.25 U *Taq* polymerase (Quiagen), and 2.5 mM MgCl$_2$. Amplifications consisted of an initial 2 min 94°C denaturing step, followed by 35 cycles of: 15 s at 96°C, 15 s annealing step at about 50°C, 30 s extension at 72°C, and finished with a single extension step of 72°C for 5 min. To visualise, products were run on a 6% denaturing polyacrylamide gel. Several positive (samples of known size) and one negative controls were included on each gel. Following electrophoresis, gels were dried and exposed to autoradio-

graph film for 24–48 h. We scored the size of each PCR fragment relative to the reference samples on each gel.

Departures from Hardy-Weinburg equilibrium and linkage equilibrium were examined using FSTAT 2.9.3 (Goudet 1995). In addition, the probability of parental exclusion when neither (P_{EX1}), or one (P_{EX2}), parent is known (equations in Marshall et al. 1998), and estimated null allele frequencies at each locus (following Summers and Amos 1997), were calculated using CERVUS 2.0 (Marshall et al. 1998). Parentage was assessed with a likelihood-based approach using CERVUS 2.0. The parameters entered into the simulation were as follows: 27 male candidate parents [considered only in the year(s) they were present], proportion of loci typed equal to 0.95, proportion of candidates sampled set at 0.90, and typing error rate of 0.01. We considered parent-offspring pairs only if they matched at least one allele at each locus, regardless of the confidence level. When multiple male candidates matched all offspring in a brood at all loci, for the sake of parsimony we assumed that the social father was the genetic parent. When the social father did not match all offspring at all loci, we designated the candidate male with the highest likelihood as the genetic parent.

Statistical analyses

We tested the residuals of untransformed and transformed data for departures from normality and the data for equality of variance using Shapiro-Wilks test and Levene test, respectively (SPSS 1999). The residuals were not normally distributed and could not be normalised by transforming them. Accordingly, we used non-parametric Wilcoxon matched-pairs tests to examine differences in singing behaviour and proximity during fertile and pre-fertile periods, and to examine sex differences in song initiation rates and responsiveness within breeding stages.

Results

Duetting during pre-fertile and fertile periods

The first duet of the morning was often preceded by a short period (typically <5 min long) in which male, but never female, buff-breasted wrens gave solo songs. The number of males engaged in solo singing prior to duetting with their mates differed depending on whether their mates were fertile, as fewer males gave solo songs during fertile (5/21) than pre-fertile periods (13/20; χ^2=7.057, P=0.008). Moreover, males produced fewer solo songs during fertile than pre-fertile periods (mean±SD= 0.4±0.98 male solo songs in the fertile period; 7.7±17.54 in pre-fertile period; Wilcoxon matched-pairs test, Z=2.943, n=20, P=0.003).

Possibly as a result of their earlier onset of song, males initiated most of the first duets of the morning, regardless of breeding stage (males initiated 20/22 first duets in the pre-fertile period; 14/18 duets in the fertile period (sample sizes are not equal because we did not determine which individual initiated the first duet in four cases); χ^2=1.339, P=0.25). The time at which the first duet of the morning was produced differed between breeding stages. Pairs gave their first duet approximately 8 min later on days when females were fertile (mean±SD time of first duet relative to sunrise=SR+2.4±13.41 min) than on pre-fertile days (SR-5.7±5.72 min; Z=3.045, n=25, P=0.002).

During the dawn chorus, singing by pre-fertile and fertile pairs differed, as the number of duets produced
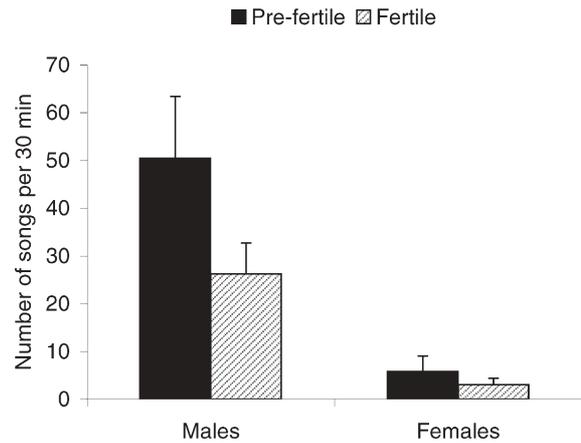

Fig. 1 The number of male- and female-initiated songs given by buff-breasted wrens (*Thryothorus leucotis*) during the dawn chorus in pre-fertile and fertile periods (n=21 pairs). Values are mean±SE

during the fertile period (mean±SD=8.4±1.04 duets per 30 min) was less than half that given during the pre-fertile period (18.6±3.59 duets; Z=3.267, n=23, P=0.001). The percentage of duets initiated by males was variable among pairs, ranging from 33.3 to 100%. Overall, however, males initiated most duets regardless of breeding stage (pre-fertile: mean±SD=82.2±20.1% of duets initiated by males; fertile: 79.6±20.7%; Z=0, n=21). Overall, males initiated more songs (all male-initiated duets plus male solo songs) during pre-fertile than fertile periods (Fig. 1; Z=2.033, n=21, P=0.04), whereas female song initiation rates did not vary with fertility stage (Fig. 1; Z=1.691, n=21, P=0.1). However, the degree to which song-initiation rates decreased between stages did not differ between the sexes (males: median decline in song-initiation rates = −45.2%; females: −0.50%; Z=0, n=21, P>0.9). In both pre-fertile and fertile stages, males initiated approximately 10 times as many songs as their partners (pre-fertile: Z=4.017, n=22, P=0.0001; fertile: Z=3.458, n=21, P=0.0005).

Answer rate was not influenced by female fertility for either males (Fig. 2; Z=0.889, n=16, P>0.3) or females (Z=1.694, n=21, P>0.1). During both periods, males were more likely to sing in response to their mates' vocalizations than were females (pre-fertile: Z=2.179, n=19, P=0.03; fertile: Z=2.618, n=16, P=0.03).

Alternative paternity guards

Instead of guarding females by forming duets, male buff-breasted wrens may use other behaviours to prevent their mates from engaging in extra-pair matings. Although pair members spent more time in close proximity when females were fertile (mean±SD=71.9±29.5% of observation period within 5 m) than not fertile (55.5±39.3%), the difference was not significant (Z=0.918, n=10, P>0.3). The close association of pair members when females were fertile appeared to be driven by female rather than male behaviour. Females made more movements toward their
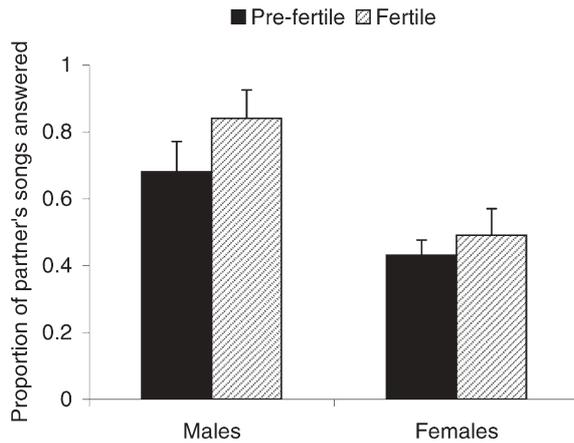
Fig. 2 The proportion of songs initiated by their partners that males (n=16) and females (n=21) answered to form duets during pre-fertile and fertile periods. Sample sizes differ between the sexes because some females did not initiate songs during observations. Values are mean±SE

Table 1 Movements of male and female buff-breasted wrens (*Thryothorus leucotis*) towards their mates during pre-fertile and fertile periods (n=10 pairs). Counts include the number of movements of greater than 5 m that were followed by the partner, as well as the number of movements toward the partner. Test statistics refer to the results of Wilcoxon matched-pairs tests

	Pre-fertile	Fertile	Z	P
Males	0.5±0.3	0.9±0.5	0.405	0.6
Females	0.6±0.3	2.2±0.8	1.944	0.047

mates than males when they were fertile (Table 1; Z=2.20, n=10, P=0.028), but not during pre-fertile periods (Z=0.535, n=10, P>0.5). Moreover, the females' stage of breeding affected their own behaviour, as females made more movements to keep close to their mates during their fertile than non-fertile periods (Table 1). Males did not differ between periods in the amount they followed their mates (Table 1).

Male buff-breasted wrens did not appear to frequently copulate with their mates as a strategy to guard paternity, as we did not observe copulations during focal observations on pre-fertile or fertile pairs (n=6.6 h). In species that copulate frequently to guard paternity, pairs may copulate up to 6 times per hour (e.g. Beasley 1996), and, thus, even a short duration of observations should reveal a similar strategy, were it to exist, in buff-breasted wrens. Nevertheless, because of the limited duration of our observations on fertile pairs, we examined a second set of observations on fertile females made when pairs were constructing their breeding nests (n=10 h observation made 2–5 days before clutch initiation). We observed no within-pair or extra-pair copulations during these additional observations. Our only observation of copulation in buff-breasted wrens came during a spot sample of a banded pair, 1 month before the pair initiated their first clutch.

Table 2 Observed number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosity, probability of exclusion when neither parent is known (P_{EX1}) and when one parent is known (P_{EX2}), and estimated null allele frequency by locus calculated from genotypes of 105 buff-breasted wrens. H_E was calculated using the formula $(1-\Sigma P_i^2)/(n-1)$. Overall values are 5-locus means for number of alleles and heterozygosity. The overall values for the probability of exclusion are the products of individual values, and assume linkage equilibrium among loci

Locus	N_A	H_O	H_E	P_{EX1}	P_{EX2}	Null freq.
TA-A5–2	2	0.46	0.49	0.12	0.18	0.029
TA-C3(B)-2	15	0.89	0.89	0.61	0.76	−0.006
Cp51	9	0.78	0.78	0.40	0.59	0.003
McyU4	3	0.41	0.39	0.08	0.16	−0.021
FhU2	2	0.55	0.48	0.12	0.18	−0.062
Overall	6.2	0.62	0.61	0.83	0.94	

Territorial intrusions

Territorial intrusions when females are fertile may provide evidence that male and female buff-breasted wrens engage in extra-pair behaviour. Although buff-breasted wren pairs spent more than 50% of focal observations within 5 m of each other, such close proximity seemed unrelated to the degree of territorial intrusions experienced. We observed no intrusions or border disputes during observations on pre-fertile and fertile pairs (6.6 h). From a larger sample of focal observations (n=323.1 h) conducted during non-breeding and breeding periods, we observed only one border dispute and three intrusions by single non-territorial birds or retained offspring for a rate of 0.01 territorial intrusion per h. These intrusions were detected in April (n=1), May (n=2), and June (n=1), periods that correspond with potential fertile periods of females. However, in no case did intrusions occur on territories with fertile females.

Breeding synchrony

Breeding was asynchronous in both years of the study. The mean±SD synchrony index was 9.7±5.2% (n=36 nests) and 10.5±5.0% (n=37 nests) in 1998 and 1999, respectively.

Parentage in buff-breasted wrens

Two of the five microsatellite loci used in this study were highly variable, with 9–15 alleles per locus, and observed and expected heterozygosities of >78%, based on genotyping 105 individuals (Table 2). The remaining three loci were not so variable, with 2–3 alleles per locus, and relatively low heterozygosities of 39–54%. All loci conformed to the expectation of HWE and were unlinked, and null allele frequencies were uniformly low (Table 2).

All 53 offspring had one allele at each of five loci in common with their social mother, indicating that no offspring were the result of intraspecific brood parasitism and that maternity may be assigned based on behavioural

observation. For 51 of 53 offspring, paternity was assigned to the social father, as each had at least one allele at each locus in common. For 38 of these offspring, the social father had the highest likelihood of paternity, whereas for the remaining 13 offspring, between 1–3 candidate males per offspring had a higher probability of paternity than the social father. Two offspring (4% of 53) in 1 of the 31 (3%) broods had an allele at one locus that could not be matched with the social father. The most likely father of the extra-pair offspring was a male who defended a territory approximately 200 m away from the focal territory (one territory intervened between the territories of the extra-pair male and focal female).

Discussion

We found no evidence in support of the acoustic mate-guarding hypothesis in buff-breasted wrens. Contrary to predictions of this hypothesis, pairs began duetting earlier relative to sunrise, gave more duets during the dawn chorus (see also Gill 2003a), and males initiated more vocalisations during pre-fertile than fertile periods. Neither males nor females varied their answer rates by breeding stage, nor did they initiate more songs during the female's fertile period. Moreover, males did not protect their paternity by spending more time in close proximity to their mates when they were fertile, or by frequently copulating with their mates. Despite the apparent lack of paternity guards, however, only 2 young (4% of 53 young) from the brood of a single female were confirmed to be the result of an extra-pair mating. Thus, our findings suggest that the very low rate of extra-pair paternity in buff-breasted wrens was maintained in the absence of the paternity guards we examined.

Male buff-breasted wrens initiated more songs in the pre-fertile period than in the fertile period of their partners. Similar results have been found in a number of temperate-zone species in which only males sing (e.g. Sheldon 1994; Titus et al. 1997; Gil et al. 1999; but see Mace 1987; Welling et al. 1995; see also Hall and Magrath 2000). There may be several explanations for our results. First, many buff-breasted wren offspring that have stayed on their natal territories disperse in the late non-breeding or pre-breeding seasons. Thus, this period could be marked by high intrusion rates and high risk of mate loss. Accordingly, individuals may participate in duets to prevent their mates from pairing with prospecting birds or from being usurped by them. The risk of mate take-over and usurpation seems low in buff-breasted wrens, as they rarely occur (Gill and Stutchbury, unpublished data), and intrusions onto territories were relatively rare (this study). Second, in some temperate species, males sing at lower rates when their mates are fertile, possibly because they cannot simultaneously stay close to their fertile partners and sing from exposed perches (e.g. Hanski and Laurila 1993; Sheldon 1994). By contrast, buff-breasted wrens likely do not experience a similar trade-off since males do not leave their partners to sing; rather, song answering

and mate-guarding conceivably would occur concurrently.

Two alternative hypotheses seem more likely to explain the seasonal change in singing behaviour in buff-breasted wrens. Males may sing more during pre-fertile periods to guard their partnership rather than their paternity (reviewed in Hall 2004). Buff-breasted wrens have long-term partnerships, which may persist for the entirety of an individual's lifetime (Gill and Stutchbury, unpublished data). However, of the 11 divorces we observed, 8 were initiated by females, each of which occurred in the period preceding reproduction. Thus, males may guard their partnerships by initiating more songs thereby preventing their mates from prospecting for vacancies when divorces are more likely to occur. Second, elevated rates of song initiation and duetting in general occur concurrently with the period during which gonads are likely to be recrudescing and sex hormones are seasonally elevated (e.g. Wikelski et al. 2003). If true, then initiating and answering songs to form duets with partners may stimulate or coordinate reproductive behaviour (Armstrong 1947; Kroodsma 1976). Further studies are needed to distinguish between these two alternatives. In buff-breasted wrens, divorces occur only within pairs that have not bred together (Gill and Stutchbury, unpublished data), thus, the "mate-defence" hypothesis predicts that inexperienced pairs should duet more during the pre-fertile period than experienced pairs, but little or no difference in duetting between experienced and inexperienced pairs should be evident during the breeding stage. The "reproductive-synchrony" hypothesis predicts that all pairs should exhibit a peak in duetting during the pre-fertile period and that seasonal changes in singing frequency should be related to seasonal increases in gonads and circulating hormone concentrations.

Females might sing when they are fertile to attract males for extra-pair copulations (Mace 1987), a hypothesis that predicts that females should initiate more songs when they are fertile than pre-fertile. On average, song initiation rates of female buff-breasted wrens did not differ with fertility, as females initiated fewer than six songs over 30 min during the pre-fertile period and even fewer songs during their own fertile period (see also Levin 1988; Hall and Magrath 2000). Thus, females in duetting species do not seem to be singing to attract mates for extra-pair copulations. Hall and Magrath (2000) suggested two alternative explanations for the decreased song initiation rates of fertile females. Lower song initiation rates might make it difficult for males to localise their fertile mates, allowing females to seek extra-pair copulations. During our observations, paired birds spent considerable periods of time close together, and females did not appear to be evading their mates as they, more often than males, maintained their close proximity. Alternatively, fertile females may be constrained in singing because of the concurrent cost of egg production. Although song production is assumed to be costly, in general singing does not appear to be so for most birds (e.g. Ward et al. 2003), and, thus, females may not trade-off song and egg production.

Most buff-breasted wren pairs were faithful, resulting in a very low rate of confirmed extra-pair paternity (3% of broods). Although all social fathers shared at least one allele at each locus with their offspring, other candidate males had a higher probability of siring young than social fathers in 13 cases. Paternity studies of temperate passerines have found that extra-pair fathers typically are immediate neighbours (e.g. Stutchbury 1998; Stutchbury et al. 1997; Webster et al. 2001). Since most candidate males (9 of 13) in this study did not reside on neighbouring territories, territorial intrusions were rare, and social father-offspring genotypes were compatible, we assigned paternity to social fathers. Male buff-breasted wrens did not employ any obvious paternity guards, as we found no evidence for either mate-guarding or frequent copulation. Similarly, extra-pair paternity was not detected in either purple sandpipers (*Calidris maritima*; Pierce and Lifjeld 1998) or Capricorn silvereyes (*Zosterops lateralis*; Robertson et al. 2001), despite the absence of mate-guarding and infrequent within-pair copulations. Genetic monogamy may be maintained without paternity guards in these species because low genetic variation among males means that females may have little incentive to seek extra-pair fertilisations (Pierce and Lifjeld 1998; Robertson et al. 2001). By contrast, in duetting magpie-larks genetic monogamy apparently is enforced by males that stay close to their fertile mates (Hall and Magrath 2000).

With or without paternity guards, genetic monogamy has been found in a number of non-passerine and passerine species (reviewed in Griffiths et al. 2002), although it is still unclear why it is favoured in some species, but not others (reviewed in Neudorf 2004). Studies of genetically monogamous species have proposed a variety of non-mutually exclusive reasons for the lack of extra-pair behaviour, including breeding asynchrony (e.g. Stutchbury and Morton 1995; Morton et al. 1998), low breeding density (e.g. Verboven and Mateman 1997), low benefits or high costs to females (e.g. Quinn et al. 1999; Stanback et al. 2002), high degree of male parental care, including incubation (e.g. Morton et al. 1998; Masello et al. 2002), high longevity of breeders (e.g. Masello et al. 2002), and long-term partnerships (e.g. Bolen 1999 in Chu et al. 2002). Buff-breasted wrens exhibit several of these traits, including long-term partnerships (Gill 2003b), long lifespans (S.A. Gill, unpublished data), high paternal care (Gill and Stutchbury, 2005), and low breeding synchronisation (this study), which may act alone or synergistically to favour genetic monogamy within pairs.

Mate-guarding has been traditionally used to imply the protection of paternity by males and is widely used in this context. In the duetting literature, mate-guarding may entail this widely understood guarding of paternity within the restricted period of the female's fertile period (e.g. Hall and Magrath 2000; this study), but the term has been recently co-opted to connote the more general guarding of the mate against the *pairing* attempts of rival individuals, which may occur throughout the year (see, e.g., Levin

1996a; Grafe and Bitz 2004). In her recent review, Hall (2004) noted that this idea is also known as "mate defence", and we strongly recommend future authors use this term rather than the historically loaded term of mate-guarding, which should be reserved solely for studies addressing male behaviour in the context of paternity guarding. By doing so, findings from studies considering whether males and females participate in duets to defend their partnerships (the mate defence hypothesis) versus whether males sing to guard their paternity (the acoustic mate-guarding hypothesis) would be more accurately conveyed to a broader audience.

In conclusion, we have shown that buff-breasted wrens exhibited very low rates of extra-pair paternity in the absence of paternity guards, acoustic or otherwise. With no support for the acoustic mate-guarding hypothesis and little evidence for extra-pair behaviour in duetting species to date (Hall and Magrath 2000; this study), we question whether males of duetting species need to guard their mates against challenges to their paternity. We strongly encourage further tests of the acoustic mate-guarding hypothesis, as well as investigations of alternative hypotheses that may better explain seasonal changes in singing in duetting species.

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