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Sexual selection and reproductive strategies in songbirds

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**Singing and the risk of paternity loss
in winter wrens: territory defence
or quality advertisement?**

Mathew L. Berg & Jan Komdeur

Animal Behaviour, provisionally accepted

ABSTRACT

Males may increase their fitness through extra-pair copulations (EPCs) that result in extra-pair fertilisations, but they also risk lost paternity when their own mate engages in EPCs. Like many birds, winter wrens (*Troglodytes troglodytes*) commonly engage in EPCs so the fitness costs of cuckoldry to males are considerable. Males may minimise their loss of paternity by defending the mate or breeding territory against intruding males or advertising their own quality when their mate is fertile (e.g. through singing performance). In this paper, we provide evidence that investment in singing during song contests is adjusted to the likelihood of cuckoldry. When faced with simulated territory intrusions by unfamiliar males (male models and song playback), male wrens sang significantly more during the fertile breeding stage than before or after this period. However, males did not adjust their song rate according to breeding stage when intruders were not present. Other measures of territorial response were not consistent with the idea that males use territory or mate defence as paternity assurance, instead indicating that territory defence either remains stable or declines as the breeding cycle progresses. Our data suggest that song rate and approach behaviour may be distinct functional components of resource defence in wrens, and we discuss how such behaviour may function as paternity assurance.

INTRODUCTION

Extra-pair copulations (EPCs) resulting in extra-pair fertilisations (EPFs) are widespread in birds (e.g. Birkhead *et al.* 1990; Gibbs *et al.* 1990; Birkhead & Møller 1992a; Kempenaers *et al.* 1992; Dixon *et al.* 1994; Wetton *et al.* 1995; Griffith *et al.* 2002). In order to increase reproductive success, males should not only seek EPCs for themselves, but also prevent their mates from copulating with extra-pair males. Males may employ a number of anti-cuckoldry tactics to achieve this (Birkhead *et al.* 1987; Birkhead & Møller 1992a; Birkhead 1998). These may involve following the female or staying near her for long periods (mate guarding), frequent copulations, territorial behaviour to deter competing males, or singing to advertise quality (Birkhead 1998). Because these behaviours are all likely to entail substantial costs in terms of energy, temporal constraints on other activities, or risk of injury or predation (e.g. Stamps 1994; Catchpole & Slater 1995; Birkhead 1998; Komdeur 2001), males are expected to adjust their investment in such anti-cuckoldry behaviour to the risk of paternity loss (e.g. their mate's fertile period or the presence of male intruders; Møller 1987a; Askenmo *et al.* 1992; Hanski 1994). Evidence from several studies in birds has indicated that mate guarding (e.g. Alatalo *et al.* 1987; Møller 1987b; Hanski 1994; Gray 1996; Komdeur *et al.* 1999; Komdeur 2001) and copulation frequency (e.g. Sheldon 1994; Mougeot *et al.* 2001) are important paternity guards that are adjusted to the risk of paternity loss. However, despite much recent interest, corresponding evidence relating to territory defence and singing is limited (see Møller 1990; Birkhead 1998; Rodrigues, 1998).

Two main mechanisms have been proposed to explain the role of singing and territory defence in paternity assurance. First, territorial behaviour may reduce cuckoldry by keeping competing males away from the main breeding area and thereby limiting their access to the pair female ('mate defence' hypothesis, e.g. Hinde 1956; Birkhead 1979; Møller 1987a; Stamps 1994; Maher & Lott 1995; Langmore 1996; Tobias & Seddon 2000). This hypothesis predicts that males should invest more in territorial activity during the pair female's fertile period, such as territory advertisement, patrolling and aggressively responding to intruders.

Second, the 'fertility announcement' hypothesis (Møller 1991a) postulates that males advertise the fertility of their mate by singing in order to signal their quality. By doing so, they may reduce cuckoldry by deterring competing males and encouraging mate fidelity. This may also increase a male's own EPC success if females prefer to mate with males singing at a high rate (Møller 1991a). This hypothesis specifically predicts that males will engage more in advertisement singing during the fertile period (e.g. Møller 1991a; Gil *et al.* 1999). Following Møller's (1991a) paper, a number of studies have tested this hypothesis by examining the natural singing behaviour of males during the breeding season, with conflicting results. While several studies have found no support for this prediction (11 out of 13 studies reviewed in Gil *et al.* 1999; Amrhein *et al.* 2004; Turner & Barber 2004), a number of other studies have found patterns of male singing corresponding to female fertility (e.g. Greig-Smith 1982; Mace 1987; Møller 1988; Pinxten & Eens 1998; Forstmeier and Balsby 2002). In light of these contradictory results, the generality of the fertility announce-

ment hypothesis is clearly in doubt and further studies are needed to elucidate the circumstances and mechanisms through which song production can be adjusted to female fertility.

Two recent studies on eavesdropping and extra-pair mating (Otter *et al.* 1999; Mennill *et al.* 2002) suggest an intriguing mechanism through which this process may operate. In these studies, female black-capped chickadees (*Poecile atricapilla*) and great tits (*Parus major*) were shown to eavesdrop on male song contests, and use the information gathered on the relative quality of males in their subsequent extra-pair mating decisions. In this way, male performance during song contests may represent a link between singing performance and paternity assurance. Males would then be expected to increase their investment specifically in song contests when paternity is at risk (i.e. the pair female's fertile period). In this way, singing during song contests, advertisement singing and other forms of territorial activity (e.g. patrolling and approaching intruders) could be considered functionally distinct behaviour.

Few studies examining the relationship between male song production and female fertility have simultaneously gathered data on male territory defence and singing in the context of direct singing contests. As such, there is little empirical evidence to date from species that exhibit a high rate of singing after pair formation to compare the relative roles of mate defence and quality advertisement in paternity assurance. Here, we examine these explanations by simultaneously studying advertisement singing, territory patrolling and territorial response to intruders (counter-singing and approach behaviour) throughout the breeding season in the winter wren (*Troglodytes troglodytes*). An increase in advertisement singing during the fertile period would offer support for the fertility announcement hypothesis, while a corresponding increase in territory patrolling and response against intruders during the fertile period would support the mate defence hypothesis. However, if only singing effort against intruders increases during the fertile period, this would be consistent with the idea that male wrens invest more in singing contests to advertise their quality to eavesdropping females as a way to enhance their paternity.

In the wren, sexes are morphologically similar and highly cryptic, but males vigorously defend exclusive breeding territories and sing in bouts regularly throughout the breeding season, even after pairing (e.g. Armstrong 1955; Cramp 1988). Territory intrusions by males are common and result in vigorous bouts of counter-singing and posturing, occasionally culminating in chases and fights (Armstrong 1955; pers. obs.). In contrast, female wrens rarely leave their breeding territories (Armstrong 1955; pers. obs.). Therefore, most EPCs probably occur on the female's territory and will be at least in part a reflection of male-male competition. The level of EPFs is substantial, accounting for 18.7% offspring ($n = 134$) in 37.9% of broods in our population ($n = 29$; chapter 7; see also Burn 1996). Nevertheless, males commonly provide care to nestlings (Armstrong 1955; Burn 1996; pers. obs.). Given the opportunities for EPCs and the high fitness costs of cuckoldry, male wrens should be under strong selection to develop effective paternity guards. However, in contrast to many birds (Birkhead *et al.* 1987; Birkhead & Møller 1992a), male wrens typically do not guard their fertile mates and copulations are relatively infrequent (Burn 1996; this study; pers. obs.). Thus, singing to advertise quality and/or deter competing males may be a particularly important means of paternity assurance for male wrens.

MATERIAL AND METHODS

Study population

This study was conducted in Vosbergen forest reserve, Paterswolde, The Netherlands (ca. 50 ha, 53°08' N, 6°35' E). Data were gathered during the main breeding season, after territory establishment, of the winter wren, in 2001-2002 (intrusion experiments) and 2002 (natural behavioural observations). Adult wrens were caught in mist-nests and colour-banded for individual identification. Sexes were distinguished based on the presence of a brood patch and behavioural cues such as singing and intra-sexual aggression (see Armstrong 1955). During catching, males were frequently caught intruding onto neighbouring territories.

Territory borders were estimated by plotting the location of singing males and noting territorial interactions weekly. Nests were located by searching through the vegetation on every territory weekly, and breeding activities were monitored by checking all known nests twice weekly. During each check the status of each nest was classified as follows: (i) incomplete/in construction, (ii) finished but unlined (not occupied by a female), (iii) feather lined (occupied by a female), (iv) laying (eggs cold, clutch incomplete), (v) incubated eggs, (vi) nestlings, (vii) previously occupied, and (viii) predated. Nests were never used for more than one breeding attempt. Males build and maintain several nests continuously throughout a season, to which they attempt to attract females. When a female selects a male, she lines the nest with feathers (Armstrong 1955). The precise period of fertility of wrens is not known, but in most birds the fertile period begins about 10 days before the first egg is laid and ends when the final egg is fertilized (Birkhead and Møller 1992b). Therefore, males were considered paired to a fertile female from ten days before a new egg appeared in one of his nests until the penultimate egg of the clutch was laid. Although some wrens are polygynous, we did not use males paired to both a fertile and a non-fertile female at the time of the experiment. All males tested were mated during the breeding season.

Natural territorial behaviour observations

To examine natural levels of singing and investment in territorial defence, we performed focal watches of 17 males from 7 May through 18 June, between 0700 and 0900 h WEST, because territorial intrusions and EPCs in most bird species occur more frequently in the morning (e.g. Møller 1987a; Birkhead and Møller 1992a; but see Birkhead *et al.* 1996). One to five focal watches (1.9 ± 1.2 SD per male) were performed on each male, on different days, during one or more of the following breeding periods: paired with fertile mate, incubation (after clutch completion), nestlings and the post-nesting non-fertile period (following nest predation and/or fledging, until 10 days before the next egg appears on the territory). No observations were made before pairing as males may use song more during this period to advertise for a mate. It was easy to locate and follow male wrens due to their conspicuous behaviour and frequent vocalisations, and the relatively open habitat of the study site. During focal watches a single male was followed for a continuous period of 20 mins during which we recorded the following male behaviour: (i) the

number of separate songs (hereafter referred to as ‘spontaneous songs’, assumed to function in territory advertisement or mate attraction (Armstrong 1944, 1955; Catchpole & Slater 1995)), (ii) the number of alarm calls, and (iii) the number of single movements > 10 m (this is assumed to indicate the rate of territory patrolling or vigilance). During 11 h of focal watches involving 17 males with a fertile female, mate guarding (female initiated movements > 10 m when followed by the male) or copulations were never observed. As song length does not vary much the number of songs over such a period is an accurate indication of overall song output in wrens (i.e. the product of song rate and song length; Clark 1949). We considered movement rate an indication of investment in territory patrolling. Males were followed from a distance of approximately 20 m and care was taken to avoid disturbance; as the study site is a popular public recreational reserve the birds were accustomed to regular close human presence. Focal watches were not performed during rain because the birds are relatively inactive and rarely sing when it is raining.

Simulated territory intrusion experiments

In order to quantify intra-sexual territorial aggressiveness of male wrens, intrusions were simulated using a mount and song playback of a male wren ($n = 38$, including three males with unknown breeding stage). Trials were conducted from 15 May through 20 June (mean trial date 27 May \pm 12 days SD) during the post dawn morning period 06.00 and 12.00 h (mean trial time 09.08 \pm 1.47 h SD). Mounts consisted of freeze-dried wrens collected in The Netherlands but away from the study site, and mounted on sticks *ca.* 1 m high in a neutral posture. To reduce effects arising from particular mounts or songs (Wiley 2003), mounts of three wrens and song playbacks from three territory-holding individuals (separated by > 3 territories) were selected randomly for each trial (giving a total of nine different combinations of experimental stimuli). Playbacks comprised consecutive songs recorded from a spontaneously singing unfamiliar male (> 3 territories away from focal birds) on the study site. Songs were recorded onto Sony Metal-XR cassettes (to maintain fidelity at high frequencies) using a Sony WM-D6C cassette recorder and a Telinga directional microphone with a fibreglass parabolic reflector. Songs were transferred to CD with an inter-song interval of 10 s and played back using a Sony CFD-V3 portable CD player (Sony Corporation, Tokyo, Japan) adjusted to 67 dB SPL at 10 m in front of the speaker. These values approximately correspond to the volume (Holland *et al.*, 2000) and song rate during natural antiphonal (competitive) song bouts between male wrens (Armstrong, 1944, 1955; Clark 1949; Brémond & Aubin 1992; Holland *et al.* 2000). Playbacks lasted for 20 min, which corresponds with the duration of bouts of high frequency singing among male wrens during the breeding season in natural situations (Clark 1949; Armstrong 1955; pers. obs.) or during interactive playback experiments (Brémond & Aubin 1992). The length of the playbacks gave us a good ability to distinguish differences in song rate between males and detect males that were unwilling to maintain high song rates for extended periods. Wrens typically responded actively for the entire duration of playbacks (see Results).

As a control, the experiments were replicated on a random sub-sample of focal males ($n = 21$, including three males with unknown breeding stage) using a mount and song

playback of a blue tit (*Parus caeruleus*) during 2001, a presumably neutral avian intruder (non predator, non-wren) that occurs naturally on the study site. Wren and blue tit trials were performed on consecutive days and the order was randomised. Unlike the natural singing observations, the playback experiments included the pre-fertile, fertile or post-nesting non-fertile periods. We included the pre-fertile period as we were directly testing territorial behaviour, and we did not perform experiments during the incubation and nestling periods so that male parental effort would not influence the experimental results. Each focal male was used only once, during a randomly assigned breeding period, to avoid habituation effects. The mount and song playback was placed 30 m from the focal male on his territory and at least 20 m inside the territory borders, in order to eliminate variation associated with detection time or male presence or absence on the territory, or interference from neighbours. From a distance of 20 m from the male and mount, we scored the following response variables for a period of 20 mins from the start of the trial (at 30 s intervals): the number of songs sung by the focal male (hereafter referred to as 'challenge songs', i.e. singing when directly challenged by an intruder) within 10m (measured horizontally) of the model/playback, the latency to approach within 10 m of the model/playback (males that did not approach within 10 m during the trial were given a latency of 20 mins), the time spent within 10 m of the model/playback, the closest approach (scored in four distance classes horizontally from the model/playback: 0.0-1.0 m, 1.1-5.0 m, 5.1-10.0 m and >10.0 m) and the number of 30 s periods during which the focal male made physical attacks on the model (the actual number of individual attacks happen too quickly to be quantified under field conditions). Attacks consisted of landing on the model with bouts of rapid pecks on the head and body of the model for a period of time from 1 s up to approximately 1 min. When calculating the song rate, we excluded the time period before the male began responding (latency), and any 30 s intervals when the male was observed to physically attack the model since the birds can not sing during this activity and the actual duration of attacks (i.e. the proportion of the 30 s period actually spent attacking) varied greatly.

In all cases, the observers were randomly assigned to observations and were blind to the breeding period. Observers sat quietly behind vegetation and took great care to avoid disturbing the wrens excessively during trials.

Data analyses

Statistical analyses followed Quinn and Keough (2002) and were performed in SPSS 12 for Windows (SPSS Inc., USA). We compared the behaviour of males in relation to time of day, date, year and breeding stage. Latency to respond during experimental trials displayed a highly skewed distribution (skewness 2.453 ± 0.383 SE) and was therefore rank-transformed prior to analysis. Unless otherwise stated, analyses were performed using general linear models (MIXED procedure) with fixed effects, using restricted maximum likelihood (REML) estimation to account for missing data (breeding stage unknown for three males). Because focal watches of spontaneous singing patterns were performed on some males several times across different days and at different breeding periods, these analyses were performed with male identity as a 'subject' (random effect). One male did

not respond during the trial period and was excluded from the subsequent analyses. To aid the interpretation of non-significant comparisons, we report the estimates and 95% confidence intervals (CI) supported by the data (Colegrave & Ruxton, 2003). To account for multiple comparisons between groups, we report significance and adjusted α values based on the Bonferroni method (Holm 1979). Means are given \pm SEM, P -values are two-tailed and the null hypothesis was rejected when $P < 0.05$.

RESULTS

Natural territorial behaviour

We observed a mean male song rate (songs per minute) during focal watches ('spontaneous' song rate) of 2.61 ± 0.18 SE (range 0.40-4.35). We found no relationships between date (independent variable) and either spontaneous song rate ($F_{1,29.923} = 0.139$, $P = 0.712$, estimate = 0.005 ± 0.013 songs per min, 95% CI -0.022 to 0.032; Fig. 4.1a) or movement rate ($F_{1,29.740} = 0.047$, $P = 0.829$, estimate $< 0.001 \pm 0.002$ moves per min, 95% CI -0.003 to 0.004). Similarly, we found no relationships between time of day (independent variable, h) and either spontaneous song rate ($F_{1,28.294} = 0.374$, $P = 0.546$, estimate = -0.238 ± 0.389 songs per min, 95% CI -1.034 to 0.559; Fig. 4.1b) or movement rate ($F_{1,28.509} = 0.323$, $P = 0.574$, estimate = -0.026 ± 0.046 moves per min, 95% CI -0.120 to 0.068).

Comparing breeding periods (fertile, incubation, nestlings and post-nesting non-fertile), we detected no significant difference in either spontaneous song rate ($F_{3,27.852} = 0.157$, $P = 0.924$, 95% CI of differences ≥ -1.6 to ≤ 1.1 songs per min; Fig. 4.2) or movement rate ($F_{3,27.572} = 0.046$, $P = 0.987$, 95% CI of differences ≥ -0.16 to ≤ 0.15 moves per min). Alarm calling was only observed on one occasion (five calls) during focal watches and was not incorporated into the analyses.

Experimental responses to intruders

To ensure that male wrens were responding to the simulated intrusions directly and not some other aspect of the experimental treatment, we compared responses during the wren trials (male wren mount and playback) with the responses of the same males during control blue tit trials (blue tit mount and playback, $n = 21$). Male wrens displayed significantly stronger territorial responses during wren trials than during control trials according to all response variables (Table 4.1).

In order to test whether male wrens differed in their responses to either experimental or control trials between breeding periods, we compared male behaviour during the pre-fertile, fertile (up to 10 days prior to egg laying) and post-fertile (following nest depredation or fledging but prior to the subsequent fertile period) breeding stages. During control trials, we found no effects of breeding stage on any response variable (song rate: $F_{2,15} = 0.220$, $P = 0.805$, 95% CI of differences ≥ -1.944 to ≤ 3.580 songs per min; latency: $F_{2,15} = 0.554$, $P = 0.586$, 95% CI of differences ≥ -12.319 to ≤ 9.521 latency ranks; time spent < 10 m: $F_{2,15} = 2.667$, $P = 0.102$, 95% CI of differences ≥ -3.199 to ≤ 7.848 min; closest

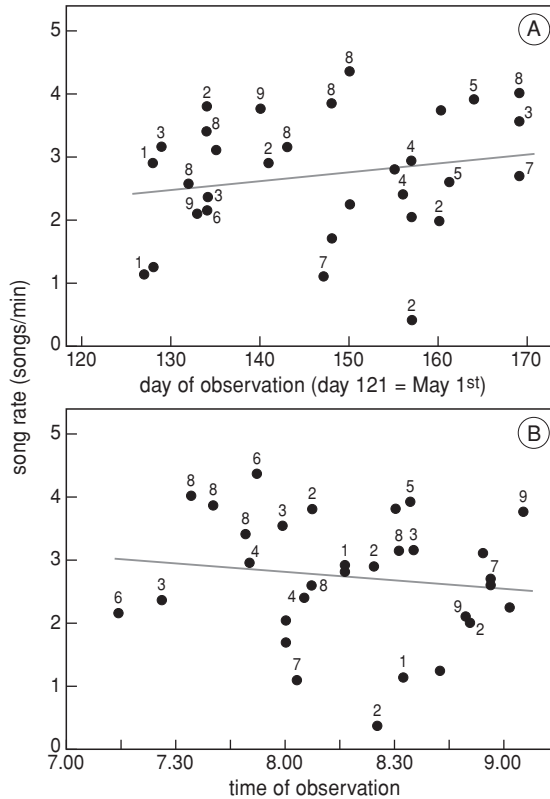


Figure 4.1. (A) The relationship between date and spontaneous song rate produced by male wrens during focal watches throughout the main breeding season. Dashed line indicates non-significant fit line ($P = 0.712$). Note that nine males are represented on two or more different days (1.9 ± 0.3 SE observations per male) in this figure, and these males are labelled (one to nine). Statistical analyses use a repeated measures design (see text). $n = 17$ males. (B) The relationship between time of day and spontaneous song rate produced by male wrens during focal watches during the post-dawn morning period. Day 121 is May 1st. Dashed line indicates non-significant fit line ($P = 0.546$). Note that nine males are represented on two or more different days (1.9 ± 0.3 SE observations per male) in this figure, and these males are labelled (one to nine). Statistical analyses use a repeated measures design (see text). $n = 17$ males.

approach: logistic regression, $\chi^2_4 = 3.033$, $P = 0.552$, Nagelkerke pseudo $r^2 = 0.178$). These conclusions were robust to controlling for date, time and year. Wrens other than the territory-holding male never responded to control trials, and no physical attacks on the blue tit model were observed.

We observed a mean challenge song rate (songs per minute during experimental intrusions) of 4.5 ± 0.4 (range 2.6-7.0; $n = 11$) during pre-fertile trials, 5.6 ± 0.9 (0.74-12.0; $n = 13$) during fertile trials, and 2.7 ± 0.5 (0.4-4.8; $n = 11$) during post-fertile trials. We detected no significant relationships between challenge song rate (dependent variable) and date ($F_{1,31} = 0.360$, $P = 0.553$, estimate -0.025 ± 0.042 SE songs per min, 95% CI of

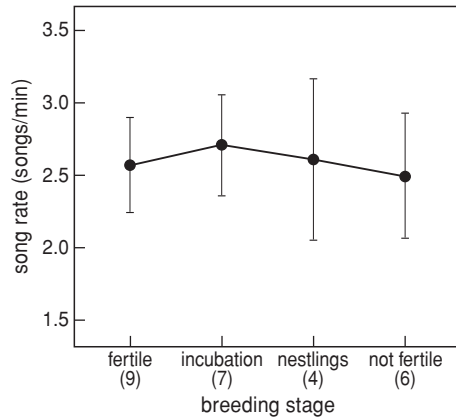


Figure 4.2. Mean spontaneous song rate \pm SE produced by male wrens during focal watches across different breeding periods. When males were observed more than once in a single breeding period, their average score for each breeding period is shown. No significant differences were found between groups (see text). Sample sizes for each breeding period (number of individuals) are given on the figure.

Table 4.1. The territorial behaviour of male wrens when compared between male wren and blue tit simulated territory intrusions (model and song playback). Wren and blue tit trials were performed on each male on consecutive days, during the same breeding stage and in random order. Closest approach is expressed in four distance classes (1 = 0.0-1.0 m, 2 = 1.1-5.0 m, 3 = 5.1-10.0 m, 4 = >10.0 m). Values show mean \pm SEM (range); for latency we present the geometric mean (see Methods). Paired *t*-tests, $n = 21$ males.

Behaviour	Wren intrusion	Blue tit intrusion	95% confidence interval of difference	<i>t</i>	<i>P</i>
Song rate (songs min ⁻¹)	4.06 \pm 0.40 (0.40-7.03)	0.65 \pm 0.34 (0.0-7.00)	2.27-4.55	6.238	<0.001
Latency (min)	2.6 \pm 0.6 (0.0-19.0)	11.8 \pm 1.7 (0.0-20.0)	5.8-13.0	5.059	<0.001
Time spent < 10 m (min)	13.0 \pm 1.5 (1.0-20.0)	1.6 \pm 0.6 (0.0-10.0)	8.2-14.5	7.444	<0.001
Closest approach					
0-1m	42.9%	0.0%	0.9-1.9	5.451	<0.001
1.1-5m	33.3%	19.0%			
5.1-10m	23.8%	42.9%			
>10m	0.0%	38.1%			

difference -0.111 to 0.0605), time of day ($F_{1,31} = 0.139$, $P = 0.712$, estimate -0.103 ± 0.276 SE songs per min, 95% CI of difference -0.664 to 0.458) or year ($F_{1,31} = 0.730$, $P = 0.399$, estimate -0.880 ± 1.030 SE songs per min, 95% CI of difference -2.980 to 1.221). Therefore, these variables were not included in further analyses. In addition, all conclusions were robust to controlling for these variables.

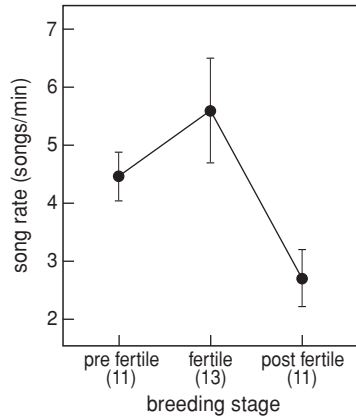


Figure 4.3. Mean challenge song rates \pm SEM produced by male wrens in response to simulated male intrusions (male wren model and song playback) during the pre-fertile, fertile (up to 10 days prior to egg laying) and post-fertile (following nest depredation or fledging but prior to the subsequent fertile period) breeding stages. Sample sizes (number of individuals) are given on the figure.

Challenge song rate differed significantly among the breeding stages ($F_{2,32} = 4.610$, $P = 0.017$, partial $\eta^2 = 0.224$; Fig. 4.3). To examine hypothesised differences between breeding periods, we used planned comparisons to test for a difference in song rate during the fertile period compared to the pre-fertile and post-nesting periods combined, and for a difference in song rate between the pre-fertile and post-fertile periods. These comparisons revealed that challenge song rates were significantly higher during the fertile breeding period compared to the pre-fertile and post-nesting periods ($t_{32} = 2.470$, $P = 0.019$, contrast estimate = 2.009 ± 0.813 SE; Fig. 4.3), and this conclusion is robust to Bonferroni correction for multiple comparisons (adjusted $\alpha = 0.025$). In addition, we found a non-significant tendency for challenge song rates to be higher during the pre-fertile period compared to the post-fertile period (contrast estimate = 1.752 ± 0.992 SE, $t_{32} = 1.767$, $P = 0.087$).

We detected no significant effects of date, time of day or year on latency (all main effects: $P > 0.070$), time spent $<10m$ ($P > 0.410$) or closest approach ($P > 0.713$). We found no significant differences between breeding periods any of time spent $<10m$ or closest approach, but there was a significant effect of breeding stage on latency (Table 4.2). Post-hoc analyses revealed that latencies were significantly shorter in the pre-fertile breeding compared to the fertile (mean difference -9.601 ± 4.038 SE latency ranks, $P = 0.024$, 95% CI of difference -17.826 to -1.376) and post-fertile breeding stages (mean difference -10.097 ± 4.203 SE latency ranks, $P = 0.022$, 95% CI of difference -18.652 to -1.530), while latency did not differ between the fertile and post-fertile breeding stage (mean difference -0.490 ± 4.038 SE latency ranks, $P = 0.904$, 95% CI of difference -8.715 to 7.736).

During experimental trials (wren model with playback), physical attacks on the model were observed from only three males: one during the pre-fertile stage (occurring during

Table 4.2. The territorial behaviour of male wrens during simulated territory intrusions (male wren model and song playback) during the pre-fertile, fertile (up to 10 days prior to egg laying) and post-fertile (following nest depredation or fledging but prior to the subsequent fertile period) breeding stages. Closest approach is expressed as frequencies (% cases) in four distance classes. Latency rank-transformed prior to analysis. Model fit is an indication of the proportion of variance in the behaviour explained by the breeding period; for multinomial logistic regression analysis of closest approach, we have presented the Nagelkerke pseudo r^2 . See the text for post-hoc comparisons between breeding periods. Values show untransformed mean \pm SEM (range). $n = 11$ (pre-fertile), 13 (fertile) and 11 (post-fertile).

Behaviour	Pre-fertile	Fertile	Post-fertile	Statistic	d.f.	<i>P</i>	Model fit
Latency (mins)	1.6 \pm 1.0 (0.0-19.0)	3.2 \pm 1.4 (0.0-9.5)	3.1 \pm 0.9 (0.0-9.5)	3.755*	2, 32	0.034	0.190‡
Time spent <10 m (mins)	14.1 \pm 1.6 (5.5-20.0)	14.7 \pm 1.9 (2.0-20.0)	10.2 \pm 2.1 (1.0-20.0)	1.604*	2, 32	0.217	0.091‡
Closest approach							
0-1m	54.5%	46.2%	27.3%	4.765†	4	0.312	0.144**
1.1-5m	36.4%	38.5%	27.3%				
5.1-10m	9.1%	15.4%	45.5%				
>10m	0%	0%	0%				

* ANOVA (F)

† multinomial logistic regression (χ^2)

‡ Partial Eta²

** Nagelkerke statistic (pseudo r^2)

one 30 s observation interval), and two during the fertile period (comprising seventeen 30 s observation intervals). Alarm calls (two) were only observed during one blue tit trial. We never observed copulations, courtship displays or mate guarding between the focal male and his partner during trials. Females were never observed responding to playbacks of responding males, although their cryptic habits made systematic observations of female activities during trials impossible.

DISCUSSION

Fertility announcement

Our results indicated that spontaneous singing or territory patrolling (movement rate) were not adjusted to the breeding period, and in particular the fertile period of the pair female. We also found no relationship between these behaviours and either time of day or date, although the latter pattern was not unexpected even if wrens did adjust singing to fertility as a high rate of nest predation (*ca.* 85%) and frequent reneating resulted in low breeding synchrony in the local wren population in this study. Although the relatively small sample sizes of these observations dictate that such negative relationships should be interpreted with some caution, in most cases the estimates of effect sizes derived from these analyses indicate that the effect sizes supported by these data are likely to be fairly small and not very biologically significant. Furthermore, we found no relationship

between breeding stage and either song rate or other measures of territorial response during control intrusion trials, which provides additional support for the idea that male wrens do not adjust territorial patrolling to female fertility.

The fertility announcement hypothesis (Møller 1991a) predicts that males will engage more in spontaneous (advertisement) singing during their mate's seasonal and diurnal fertile periods. Thus, our observations of singing in wrens are not consistent with this hypothesis. In line with our results, the majority of studies testing this hypothesis to date have found no support for this prediction (11 out of 13 studies reviewed in Gil *et al.* 1999; Amrhein *et al.* 2004; Turner & Barber 2004; but see Greig-Smith 1982; Mace 1987; Møller 1988; Pinxten & Eens 1998; Forstmeier and Balsby 2002). In species where spontaneous singing does seem to increase with fertility, song may instead function to stimulate the female reproductive cycle, to promote copulation solicitations from the pair female or to gain copulations with extra-pair females (Hinde & Steel 1978; Greig-smith 1982; Morton *et al.* 1985; Pinxten & Eens 1998; Mota 1999; Amrhein *et al.* 2004). Alternatively, some studies intending to report only spontaneous song rate may report a greater proportion of singing during natural contests between males during the fertile period, as intrusions by extra-pair males may be higher during the female's fertile period (e.g. Currie *et al.* 1998). More detailed studies in species where fertility announcement through song has been reported would be required to investigate this possibility.

Mate defence and paternity assurance

In general, our results do not support the mate defence hypothesis (e.g. Hinde 1956; Birkhead 1979; Møller 1987a; Stamps 1994; Maher & Lott 1995; Langmore 1996; Tobias & Seddon 2000). In addition to the lack of any significant associations involving advertisement singing or territory patrolling (discussed above), our experiments revealed that latency to respond, time spent in close proximity of the intruder and distance of closest approach did not indicate a stronger response during the pair female's fertile period compared to the pre-fertile and post-nesting post-fertile periods. Instead, latency to approach the intruder was significantly shorter in the pre-fertile period (presumably indicating a stronger territorial response). This pattern was apparently associated with the breeding cycle itself rather than other seasonal variation because there were no significant effects of date or time of day on any of the territorial response variables that we recorded. These three variables are measures of territorial approach behaviour, which presumably indicate vigilance, aggression and/or territorial motivation (see also chapter 3).

Singing as paternity assurance

Challenge song rate (i.e. song rate in the presence of a male conspecific intruder) showed a markedly different pattern in relation to breeding stage compared to the measures of territorial approach behaviour discussed above. Challenge song rate was significantly higher during the fertile period compared to the pre-fertile and post-fertile periods combined, but tended to decline between the pre-fertile and post-fertile periods. Such fine-tuning of male behavioural mechanisms with the female fertility period may suggest that they are adaptations to sperm competition (Birkhead 1998).

The mate defence hypothesis provides one explanation for why males should increase investment in singing when faced with a male conspecific intruder and when the pair female is fertile (Langmore 1996). However, as discussed above this pattern was not reflected in any of our other measures of territorial response, such as latency to respond to an intruder. This suggests that performance (song rate) during singing contests between males has a function during the fertile period distinct from territorial competition and the expulsion of intruding males, and may represent a different kind of information. In light of recent findings about the importance of eavesdropping by females on male singing contests for female (extra-pair) mate choice, it is possible that these results may be related to advertising male quality to females that are eavesdropping on male singing contests. Males could encourage mate fidelity and increase their own mating opportunities if females prefer to copulate with males that perform well in singing contests. A recent experiment on black-capped chickadees (*Poecile atricapillus*) has shown that better performance of males during song contests decreased the rate of cuckoldry in the subsequent nests of those males (Mennill *et al.* 2002). Male great tits (*Parus major*) that performed better during experimental song contests decreased the subsequent frequency of excursions onto neighbouring territories by their mates (Otter *et al.* 1999). Apparently, females in these species use information gathered by eavesdropping on male singing contests to guide their future mating decisions, and this may lead to more escalated singing contests between males when fertile females are eavesdropping (Johnstone 2001). In this way, the pair female rather than the intruding male may be the primary receiver of male challenge songs during the fertile period, and the primary purpose of singing during contests may be to advertise quality rather than direct defence of the breeding territory. It should be possible to further address this issue by considering how males behave during sing contests in relation to the proximity or within-pair and extra-pair females. Future work is also required to investigate how females respond to male singing contests in this species. Unfortunately, we have no information on the role of singing performance on (extra-pair) mate choice in wrens, and no data regarding the behaviour of female wrens during male singing contests.

Our results are in contrast to the outcomes of playback experiments in yellowhammers (Hiatt & Catchpole 1982) and stonechats (Greig-smith 1982), which showed that the peak of singing is also when males are most aggressive. Langmore (1996) has offered experimental evidence in polygynandrous dunnocks (*Prunella modularis*) that both song and territory defence function as mate defence. Dunnock males produced more spontaneous songs, and were more likely to respond and sang more in response to song playbacks when they were part of female biased breeding groups. Furthermore, when females were experimentally removed from breeding groups males dramatically decreased their song output and eventually abandoned their territories. Tobias and Seddon (2000) have shown that male robins exhibit more territorial aggression (significantly closer approach and more physical attacks, tendency for shorter latency) to experimental intrusions (male conspecific mounts with no song playback) during the pair female's fertile period compared to the pre-fertile and post-fertile periods, but did not find a difference in challenge song rate between breeding periods. To our knowledge, no previous study has demon-

strated an increase in singing in response to intruders during the fertile period of the pair female.

One shortcoming of our experimental design is that we used only three model wren and three playback stimuli, leading to some degree of pseudoreplication (Kroodsma 1989; Wiley 2003). Both models and playbacks were presented at random with respect to the focal male and the breeding stage (observers were blind with respect to the breeding stage), but our conclusions are based on the assumption that these models and playback songs represent a single class of stimulus (Wiley 2003). Irrespective of this concern, we believe that the differences found between territorial approach (e.g. latency) and challenge song rate are worthy of consideration.

Conclusions

Investment in challenge song could be a particularly effective form of paternity assurance for wrens if female wrens do make mate choice decisions based on information gathered by eavesdropping on male singing contests. Strong performance during sing contests may not only reduce the likelihood of cuckoldry and mate-switching (which is common in wrens; Armstrong 1955; Garson 1980), but also enhance a male's territory persistence (Naguib *et al.* 1999; Peake *et al.* 2001; Peake *et al.* 2002) and his success in obtaining extra-pair copulations with neighbouring females. Conversely, mate guarding may be a relatively poor paternity assurance strategy in wrens because of their cryptic habits and dense habitat, and because it is often mutually exclusive with territory defence or engaging in song contests with competitors (Slagsvold *et al.* 1994). We never observed mate guarding during this study.

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