

Costs of sleeping in: circadian rhythms influence cuckoldry risk in a songbird

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Summary

1. Circadian (i.e. daily) regulation of behaviours is thought to provide fitness benefits to organisms by enabling them to anticipate diel changes in the environment, such as sunrise.
2. A common behaviour among socially monogamous songbirds that usually takes place in the early mornings is extra-pair mating, that is copulating with partners outside of the social pair bond.
3. Thus, variation in when individuals begin their daily activity may influence their reproductive success; early risers may be better able to gain copulations and guard their partners, thus minimizing their risk of being cuckolded compared with late risers. Sexual selection may thus play an important role in shaping circadian behaviours, but this assumption has yet to be tested in free-living animals.
4. Here, we experimentally weakened endogenous circadian rhythmicity, and thus, anticipation of dawn in male great tits (*Parus major*) in the wild through the subcutaneous administration of implants filled with melatonin shortly before egg-laying began in this population. Melatonin is a hormone released during the dark phase at night and is one important cue animals use to entrain their circadian clock.
5. Experimental individuals delayed the onset of daily activity compared with controls and were more likely to be cuckolded compared with control males. Manipulation did not alter other behavioural traits observed; no difference between treatments was observed in activity levels during the day or in the end time of daily activity.
6. These results strongly support the assumption that selection, particularly sexual selection, shapes the circadian phenotypes of wild vertebrates which enable anticipation of important and predictive diel changes in an individual's biotic and abiotic environment.

Key-words: biological rhythms, daily rhythms, melatonin, passerine

Introduction

Nearly all organisms studied to date possess daily (i.e. circadian) rhythms (Dunlap 1999; Bell Pedersen *et al.* 2005). It is hypothesized that these rhythms provide a fitness advan

tage by enabling organisms to alter physiology and behaviour in anticipation of diel changes in the environment (Daan 1981; Pittendrigh 1993) and thereby maximizing reproduction and survival. Indeed, traits that characterize circadian phenotypes, such as the period length of an endogenous rhythm as well as its phase relationship relative

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to environmental stimuli, vary between individuals and have been found to be heritable, suggesting that such traits are capable of responding to selection (Daan & Beersma 2002; Michael *et al.* 2003; Helm & Visser 2010). Whether and how such individual variation could be maintained by selection in wild animals is poorly understood.

The hormone melatonin is a significant component of the circadian system in all vertebrates. Melatonin is primarily released by the pineal gland during the dark phase of the night. The day night pattern of melatonin release, in conjunction with other relevant physiological signals of the environment, is an important cue used by vertebrates to entrain and reset their endogenous circadian clock to external stimuli (Gwinner & Brandstätter 2001). In many species of birds, melatonin is therefore an essential part of circadian function (Gwinner & Hau 2000; Gwinner & Brandstätter 2001). For example, removal of the pineal gland abolishes daily behavioural rhythms in European starlings (*Sturnus vulgaris*) held under constant conditions in the laboratory, while daily timed administration of melatonin restores these rhythms (Gaston & Menaker 1968; Gwinner & Benzinger 1978; Heigl & Gwinner 1994). Similarly, long release melatonin implants administered to house sparrows (*Passer domesticus*) and starlings, releasing constantly high night time like levels of melatonin, decrease behavioural rhythmicity under constant conditions (Turek, McMillan & Menaker 1976; Beldhuis, Dittami & Gwinner 1988).

Laboratory studies of bacteria, plants and animals support the hypothesis of a fitness advantage for a circadian machinery that provides appropriate matching of the period length of an organismal rhythm with that of the environmental light:dark cycle (Ouyang *et al.* 1998; Beaver *et al.* 2002; Green *et al.* 2002; Dunlap, Loros & DeCoursey 2004; Johnson 2005). However, caution needs to be used when extrapolating laboratory based observations to free living animals, as behaviours and mechanisms often vary between these two environments (Calisi & Bentley 2009). For example, the golden hamster (*Mesocricetus auratus*), a model system of circadian rhythms, displays different patterns of daily activity in the wild compared with activity patterns observed in the laboratory (Gattermann *et al.* 2008).

To understand the selective forces acting in nature and how they shape phenotypes, free living animals must be experimentally studied in their natural habitat. At present, however, only a few studies have attempted to determine the relationship between circadian phenotypes and fitness in a natural setting. A unique set of experiments using surgical ablations of the suprachiasmatic nucleus (SCN), the main circadian pacemaker in the brain, showed significant survival detriments in rodents (DeCoursey, Walker & Smith 2000), while a more recent experiment in which laboratory mice carrying a mutation for a circadian gene were released into outdoor enclosures yielded equivocal results regarding fitness effects (Daan *et al.* 2011). These few examples exploring the fitness function of circadian

rhythms have focused primarily on the survival value of these rhythms (DeCoursey *et al.* 1997; Alpatov *et al.* 1999; DeCoursey, Walker & Smith 2000). The relationship between these rhythms and reproductive success remains unexplored, particularly in the wild.

In competitive environments, it is predicted that individuals that are better able to exploit temporally limited resources, would have increased fitness (Pittendrigh 1993). In the early breeding season, many species of birds advance the timing of their activity onset relative to dawn, and dawn is the time of day when most extra pair copulations are expected to occur (Double & Cockburn 2000; Dolan *et al.* 2007). Other behaviours, such as participation in the dawn chorus, occur during this time period, which may enhance a male's ability to protect paternity in their mate's nest while also increasing extra pair mating success (Mace 1987; Poesel *et al.* 2006). In free living blue tits (*Cyanistes caeruleus*), the onset of a male's morning song is correlated with extra pair mating success (Poesel *et al.* 2006; Kempnaers *et al.* 2010). Similar to most songbirds, male great tits (*Parus major*) are observed singing before dawn near their mate's roosting cavity during the period leading up to and during the female's fertile period (Hinde 1952). Moreover, recent findings indicate that male great tits that were sired by an extra pair male have a shorter circadian rhythm compared with their siblings sired by the social father (Helm & Visser 2010). As extra pair paternity is common in this species as well (e.g. Van Oers *et al.* 2008), anticipation of dawn and a concomitant early activity onset may be a beneficial trait, increasing both extra pair fertilizations opportunities and mate guarding. Experimental manipulation of great tit circadian phenotypes during this period presents an ideal opportunity to test whether circadian based behavioural rhythms influence aspects of reproductive success in nature.

Here, we experimentally manipulated melatonin, a main hormonal component of the circadian system in birds (Gwinner & Hau 2000; Gwinner & Brandstätter 2001), in the early breeding season in two successive years and recorded the impact of this manipulation on daily activity patterns of male great tits. This manipulation provides continuous night time like circulating melatonin levels and delays the onset of circadian activity rhythms in captive house sparrows relative to lights on (i.e. no anticipation of the daily dark to light transition) (Hau & Gwinner 1994, 1995). We predicted that melatonin administration to wild male great tits in the early breeding season would similarly delay their daily activity onset, which in turn would decrease their reproductive success.

Materials and methods

VALIDATION OF HORMONE MANIPULATION

To test the effectiveness of melatonin implants to alter melatonin levels in male great tits, thirteen captive males were housed in outdoor aviaries at the Max Planck Institute for Ornithology,

Radolfzell, Germany, just prior to the first field season, in February 2010. All birds received a small subcutaneous implant either filled with crystalline melatonin (Sigma; $n = 6$) or left empty ($n = 7$) on 1 February 2010. Implants were 10 mm long silastic tubes (1.47 mm i.d., 1.96 mm o.d.) (Dow Corning), closed on both ends with silastic glue and implanted under the skin on the flank. To determine the effect of implant treatment on circulating levels of melatonin, each bird was bled twice, once during the dark of night at 04:00 on February 9th and then again during the daylight at 14:00 on February 10th. Blood samples were collected by puncturing the wing vein, and $c. 150 \mu\text{L}$ of blood was collected in heparinized capillary tubes and kept cool on ice. Samples were then centrifuged, and plasma was collected and stored frozen at

-80°C until assayed for melatonin. Quantification of plasma melatonin was performed via RIA as previously described (Goymann, Trappschuh & Fusani 2008). The detection limit of this assay is 0.66 pg mL^{-1} with a mean recovery of 82%. The intra assay coefficient of variation using standard melatonin was 3.4%, and the intra extraction coefficient of variation using a chicken pool was 7.9%.

FIELD METHODS AND MELATONIN MANIPULATION

Free living male great tits were captured during mid to late March in 2010 and 2011 ($c. 2-3$ weeks prior to onset of egg laying) in an established nest box population near Radolfzell, Germany ($47^\circ44'24''\text{N}$, $8^\circ58'48''\text{E}$). Individuals were randomly assigned to either receive a melatonin implant ($n = 5$ in 2010, $n = 12$ in 2011) or a control implant ($n = 5$ in 2010, $n = 11$ in 2011, for implant details see above). To enable recording of activity patterns, all males also received a small ($<0.5 \text{ g}$) radiotransmitter (BD 2N; Holohil Systems, Ltd., Carp, ON, Canada) secured to the back of the bird using eyelash glue and tissue adhesive (for attachment methods see Cochran & Wikelski 2005; Adelman *et al.* 2010). Following initial capture and implantation, daily activity patterns (i.e. beginning and end of daily activity) were recorded in the field. All nests of implanted individuals found nesting on our study site, regardless of whether or not activity data could be ascertained, were followed from egg laying until fledging; the fate of nest success or failure was recorded.

All experimental procedures follow NIH guidelines for the Care and Use of Experimental Animals and were approved by the appropriate authority, the animal welfare and nature conservation departments of the regional council (Regierungspräsidium Freiburg).

CALCULATING DAILY ACTIVITY ONSET RELATIVE TO SUNRISE AND THE END OF DAILY ACTIVITY RELATIVE TO SUNSET AND DAYTIME ACTIVITY LEVELS

Activity patterns were assessed from implanted birds during 2011 field season using stationary automated recording units (ARUs) connected to an H antenna. In total, data could be recorded from 10 control implanted and eight melatonin implanted birds, each for between 2 and 19 days. Birds with fewer than 2 days of quality recording data were excluded from analysis. The ARUs were placed within the study site and programmed to search for and record the signal strength of a given frequency assigned to implanted birds once a minute; background noise was also recorded at each recording time. The method for calculating daily activity patterns was performed in R and has been described elsewhere (Dominoni *et al.* 2014) (for R script see Appendix S1, Supporting Information). Briefly, first, the signal to noise ratio was computed at each recording time point for a specific transmitter frequency. The signal strength of an inactive bird is relatively constant from 1 min to the next, resulting in relatively low variance in

signal, whereas an active bird moving relative to the stationary antenna produces a large amount of variance from 1 min to the next in the strength of the transmitter signal (Kjos & Cochran 1970; Adelman *et al.* 2010; Kays *et al.* 2011). This pattern in variance structure lends itself well to a slightly modified behavioural change point analysis (BCPA) (Gurarie, Andrews & Laidre 2009). This method calculated the time of onset and offset of daily activity relative to sunrise and sunset, respectively. Sunrise and sunset times were derived from the United States Naval Observatory data base (www.usno.navy.mil/USNO); treatment (melatonin or control) was spread across the study site to minimize localized environmental factors influencing our results. Briefly, daily activity onset is determined to be the point at which the variance structure changes from relatively low variance to relatively high variance, that is a behavioural change. Similarly, daily activity offset was calculated as the point where variance structure changes from relatively high variance to relatively low variance in the signal strength within a window from 2 h before sunset to 2 h after sunset.

To assess the potential differences in activity during daylight hours between the treatments, a 30 min window of recordings of transmitter signal strength recorded by the stationary ARU was analysed for activity rate during the morning (08:00 to 08:29). Periods of activity were determined using methods described previously. Briefly, a change in transmitter signal strength of $\geq 4 \text{ dB}$ from one recording to the next indicates that the bird was active (Kjos & Cochran 1970; Bisson *et al.* 2009; Kays *et al.* 2011). A change in signal strength of $<4 \text{ dB}$ was scored as inactivity. An individual's activity score was calculated by dividing the number of active periods over 30 possible recording periods (i.e. one period scored every minute beginning with 8:00).

NEST FATE AND PATERNITY ANALYSES

In both the 2010 and 2011 field season, all nest boxes were checked regularly for active nests, and active nests were followed throughout incubation and nestling rearing. Specifically, all boxes in the population were checked for nest building and the presence of eggs every 1-3 days. Once clutches were complete, nests were checked at least twice during the incubation period. Once the nestlings hatched, the nests were checked every 3 days until day 15. At 15 days post hatching, all nestlings were weighed to the nearest 0.1 g using a Pesola spring balance and tarsus was measured to the nearest 0.1 mm using a digital calliper, and each nestling received an individually numbered metal ring. After this, nests were checked regularly to confirm fledging and to determine which individuals did not survive until fledging.

In this population, we found active nests of 10 control males ($n = 3$ in 2010; $n = 7$ in 2011) and nine melatonin treated males ($n = 3$ in 2010; $n = 6$ in 2011). During nestling feeding, a small blood sample was taken in all nestlings and their parents in our population for DNA for subsequent paternity analysis. To collect blood samples, the wing vein was punctured with a sterile 26G needle, and whole blood was collected in Queen's Lysis buffer.

Paternity analysis was performed for all nestlings that successfully fledged. Genomic DNA from both the nestlings and the parents were isolated using the PureGene DNA Isolation Kit (Qiagen, Hilden, Germany), and PCR was carried out using the Multiplex PCR kit (Qiagen). Five microsatellite regions (PmaCAn1, PmaGAn27, PmaTGAn42, PmaGAn30 and PmaD22; see Saladin *et al.* 2003), known to enable successful parentage assignment in this species, were amplified (Helm & Visser 2010). PCR products were run on an ABI PRISM[®] 3130 Genetic Analyser (Applied Biosystems, Foster City, CA, USA) with a molecular size standard (GeneScan[™] 500 LIZ[®]; Applied Biosystems). Sizes of the amplification products were determined using commercial

software (GENEMAPPER 4.0; Applied Biosystems), and parentage was assigned using CERVUS 3.0 (Marshall *et al.* 1998; Kalinowski, Taper & Marshall 2007). Critical values were calculated using the following parameters in CERVUS: 10 000 cycles, 98% of loci typed, error rate 0.001% and 32 candidate parents. In this way, the tolerance of mismatches was set to accept mismatches until a logarithm of odds score of 2.31 (95%) and 0.31 (85%). An individual was categorized as extra pair young if there were one or more mismatches and CERVUS based analyses did not recognize the social father as the most likely father.

DATA ANALYSIS

The effect of treatment on the onset and offset of daily activity and amount of activity after sunrise were analysed using linear mixed effects models, controlling for the individual ID (random intercept). The effect of treatment and year, and their interaction on the number of fledglings (within pair + extra pair) (family Poisson), and the proportion of fledglings in a nest sired by the social (focal) implanted male (family binomial) was analysed using generalized linear models. The effect of treatment and year on date of first egg in the nest, combined mass of all nestlings in a nest, as well as the average weight and tarsus length of nestlings 15 days post hatch, were analysed with generalized linear models (family Gaussian). As part of a separate study (Greives *et al.* 2012), some females mated with experimental males had also been implanted with melatonin (one of six females in 2010 and 5 of the 13 females in 2011); including implant presence and type for females into our models did not affect results and are therefore not reported. All analyses were performed using R v. 3.0 using the LME4 package (Team 2011). All data are reported as mean \pm 1 SEM.

Results

MELATONIN TREATMENT VALIDATION

Overall, a main effect of treatment was found with birds that received melatonin implants having significantly elevated plasma melatonin levels compared with control implanted individuals ($F_{1,11} = 267.03$, $P < 0.001$). Both day and night time melatonin levels were elevated in melatonin implanted individuals compared with control birds. Within subjects, there was also a significant effect of sampling time (day vs. night: $F_{1,11} = 80.94$, $P < 0.001$), with melatonin levels being elevated during the night. Importantly, the daytime melatonin levels in melatonin treated male birds were similar to the night time levels found in the control birds (mean \pm SEM, Control daytime: 81.37 ± 19.79 pg mL⁻¹; Control Night: 797.45 ± 94.29 pg mL⁻¹; Melatonin day: 869.53 ± 82.08 pg mL⁻¹; melatonin night: 2127.00 pg mL⁻¹), indicating that melatonin implanted birds received consistent around the clock night time like melatonin levels.

EFFECT OF MELATONIN MANIPULATION ON DAILY ACTIVITY

Male great tits carrying melatonin implants ($n = 8$) delayed their daily activity by *c.* 10 min, indicating that experimental individuals were less able to anticipate sunrise

compared with control males ($n = 10$; $t_{1,16} = 2.36$, $P = 0.015$, Fig. 1a). Treatment specifically affected activity onset; no effect of treatment was observed on the time that the birds ended daily activity ($t_{1,16} = 0.71$, $P > 0.1$, Fig. 1b), nor the amount of activity they displayed during daylight hours ($t_{1,14} = 1.32$, $P > 0.1$) (Fig. 1c).

EFFECT OF MELATONIN MANIPULATION ON REPRODUCTIVE SUCCESS

The observed genetic reproductive success (within pair + extra pair fledglings) of melatonin implanted males tended to be lower than that of control males, although this effect did not reach significance ($Z_{1,15} = 1.74$, $P = 0.08$). The nest of one melatonin implanted male

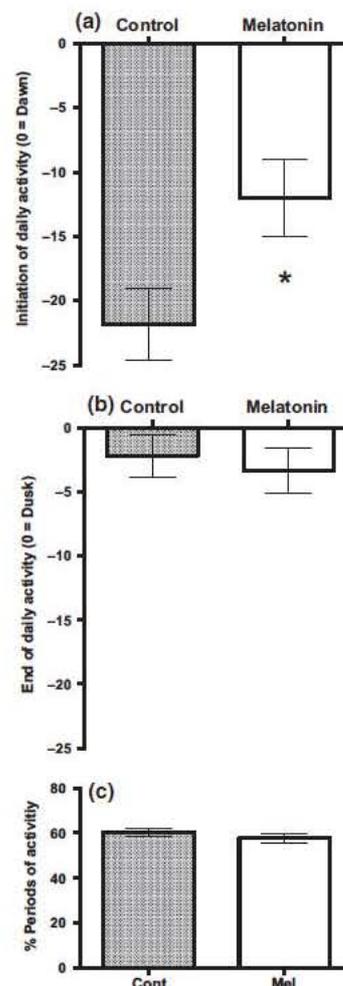


Fig. 1. Melatonin delays onset but not end of daily activity. (a) Male great tits carrying melatonin implants ($n = 8$) initiated daily activity *c.* 12 min prior to dawn, while control implanted males ($n = 10$) initiated daily activity *c.* 22 min prior to dawn. No effect of melatonin treatment was observed on either (b) the time individuals ended daily activity in the evening or (c) on their activity levels during daylight hours. All values are means \pm SEM. * denotes $P < 0.05$.

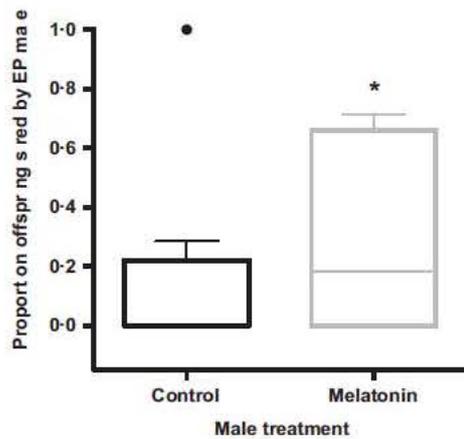


Fig. 2. Experimental males suffered increased cuckoldry. Male great tits that received melatonin implants ($n = 8$) were more likely to raise a greater proportion of offspring that sired by an extra pair father compared with control implanted ($n = 10$) males, depicted here with the box plot. On average, control males raised $0.7 (\pm 0.4)$ extra pair young in their social nest, whereas $1.9 (\pm 0.8)$ of the offspring in the nest of melatonin implanted males were sired by extra pair males. Note the outlier on top left of figure: this male had a control implant, and all but one nestling failed to fledge, with the one fledgling being sired by an extra pair male. Comparison finds a significant effect of treatment with or without this data point included. * denotes $P < 0.05$.

failed prior to us obtaining DNA samples from the nestlings. This individual was removed from further analysis on cuckoldry rate and subsequent measures of rearing (nestling mass and tarsus length). Melatonin implanted males ($n = 8$) fledged a significantly greater proportion of extra pair young compared with control males ($n = 10$; $Z_{1,14} = 2.05$, $P = 0.041$, Fig. 2); However, neither year ($Z_{1,14} = 0.54$, $P = 0.6$) nor an interaction of year and treatment ($Z_{1,14} = 1.145$, $P = 0.25$) had an influence on the proportion of young sired by extra pair males.

Melatonin treatment did not affect the males' ability to raise offspring; there were no differences in the date the nest was started (lay date: $t_{2,16} = 1.01$, $P = 0.33$), in the number of offspring fledged from the males' own nests ($t_{2,16} = 0.77$, $P = 0.45$), nor was the body mass or size of their nestlings different from that of nestlings raised by control males (body mass: $t_{2,15} = 1.18$, $P = 0.26$; tarsus length $t_{2,15} = 1.02$, $P = 0.32$) (Table 1). Furthermore, no significant effect of year or the interaction of year and treatment were observed for these variables (all $P > 0.1$).

Discussion

We observed that birds provided with continuous night time levels of melatonin delay their onset of daily activity compared with control individuals. Further, nestlings of melatonin treated males were more likely to be sired by an extra pair male compared with those of control individu-

als. Combined, these observations suggest that the melatonin rhythm influences onset of daily activities and that manipulation of the melatonin rhythms decreases one major aspect of reproductive success, namely being cuckolded.

Males great tits were provided with constant release melatonin implants in the current study. This manipulation provided unnatural continuous night like melatonin titres during both day and night, and has previously been used successfully in laboratory studies to weaken circadian function (Turek, McMillan & Menaker 1976; Beldhuis, Dittami & Gwinner 1988; Hau & Gwinner 1994, 1995). In the current study, melatonin implanted males became active later during the time of the dawn chorus and were observed to be more likely to be cuckolded; no difference in activity following sunrise was observed between treatment groups. This observation fits with other reports suggesting that extra pair copulations occur early in the morning (Double & Cockburn 2000; Poesel *et al.* 2006; Dolan *et al.* 2007; Kempnaers *et al.* 2010) and that birds joining the dawn chorus earliest during the day are the most successful at gaining extra pair paternity (Poesel *et al.* 2006; Kempnaers *et al.* 2010). Further, our findings that melatonin implanted males, which become active later in the morning, are more likely to suffer paternity loss is also in line with the observation that offspring sired by extra pair fathers possess shorter endogenous periods (τ) than their nest mates sired by the social father (Helm & Visser 2010). The study by Helm & Visser (2010) also detected for the first time in a non laboratory animal that variation of circadian rhythms is heritable. These observations, combined with a recent report of differences in circadian phenotypes between recently diverged forest dwelling and city dwelling European blackbirds, suggest that circadian phenotypes are likely targets of selection (Dominoni *et al.* 2014) and that sexual selection is one selective force that may be capable of shaping circadian phenotypes.

While our results indicate an increased loss of a male's within pair paternity as a result of receiving the melatonin manipulation, a significant effect on total reproductive success was not observed, although the results show a trend in the expected direction. The lack of a significant difference in the total number of offspring fathered by melatonin implanted vs. control individuals may be a result of the relatively small sample size we were able to obtain due to logistical limitations of this type of research in a free living system. Indeed, the final sample size, and thus our statistical power, in the current investigation is relatively low compared with other investigations which have sought to link physiological manipulations with patterns of paternity (e.g. Raouf *et al.* 1997; Foerster & Kempnaers 2004); thus, caution must be taken in interpreting these data. Alternatively, the lack of a significant difference observed between our melatonin treated and control individuals could be due to a higher number of extra pair offspring sired by melatonin treated individuals. This is unlikely, as melatonin treated individuals delayed their onset of

Table 1. Melatonin treatment did not alter the date of first egg laid by the paired female, total mass of all nestlings within the nest, or the average nestling mass or tarsus length of nestlings 15 days post hatch compared with control treatment. All data are mean \pm SEM

Treatment	Date of first egg	Total brood mass day 15	Nestling mass day 15	Nestling tarsus day 15
Control	April 18.0 \pm 2.3 day	102.1 \pm 8.9 g	15.7 \pm 0.7 g	19.2 \pm 0.3 mm
Melatonin	April 15.8 \pm 1.9 day	108.7 \pm 7.9 g	16.8 \pm 0.4 g	19.6 \pm 0.1 mm

activity, probably limiting the opportunity to obtain extra pair matings.

In seasonally breeding mammals, melatonin manipulation can directly alter reproductive function (Goldman 2001). While in birds, melatonin has been found to alter specific components that modulate the reproductive endocrine axis such as gonadotropin inhibitory hormone (GnIH) (Ubuka *et al.* 2005; Chowdhury *et al.* 2010), specific effects of melatonin on fertility have not been found in birds (Dawson *et al.* 2001). Indeed, melatonin implants in the current study did not inhibit the males' ability to fertilize eggs or raise offspring as all nests sampled had at least one nestling sired by the social male, and the condition of nestlings raised by implanted males did not differ from those raised by control males. Additionally, in a related study, the fecundity of female great tits (clutch size, number of nestlings) from the same study population was not altered by melatonin implants (Greives *et al.* 2012), supporting the interpretation that the effects of the melatonin manipulation on reproductive success were mediated through the circadian phenotype.

The current data, in demonstrating a significant delay in daily activity onset and an increased likelihood of being cuckolded, suggest a role for properly functioning circadian rhythms on reproductive success, an important component of fitness. Pioneering studies performed by DeCoursey and colleagues demonstrated a strong role for properly functioning circadian rhythms in the survival of small mammals (DeCoursey *et al.* 1997; DeCoursey, Walker & Smith 2000). These studies disrupted circadian rhythms through surgical lesions of a portion of the brain, the SCN of the hypothalamus, known as the master circadian oscillator (Stephan & Zucker 1972; Zucker 2001). More recently, instead of using surgical manipulation to disrupt circadian rhythms in a naturalistic setting, researchers followed strains of inbred laboratory mice mutant for the circadian gene *Per2* or wild type at this allele in an outdoor enclosure and monitored survival and reproduction. This study found no difference in the reproductive success between the mutant and wild type mice based on the genotype distribution of subsequent cohorts, but found variation in survival among the genotypes: at first, *Per2* homozygous mutant mice had a survival disadvantage, which was then followed by an unexpected survival advantage in these mutant mice compared with the wild type genotype (Daan *et al.* 2011). It has often been observed that animals display strong differences when observed in the wild compared with laboratory based observations (Calisi & Bentley 2009),

suggesting a need to study wild animals in the field. Indeed, the authors investigating laboratory mice in a more naturalistic environment noted that these strains of mice, while nocturnal in wheel running activity in the laboratory, demonstrated strong diurnal patterns of feeding activity in these enclosures (Daan *et al.* 2011). The current experiment builds upon these previous studies of circadian rhythms in a naturalistic setting and demonstrates a role for these rhythms in influencing reproductive success in addition to their previously demonstrated role in enhancing survival.

Our results demonstrate that experimentally altered onset of circadian activity reduces a male's fitness via an increased risk of being cuckolded, implicating a potential role for sexual selection, in addition to natural selection, to shape circadian traits in a wild vertebrate. Further experiments will address how females assess male wake up times. Likely candidates are an early song onset, but it is also conceivable that other traits are influenced by early wake up times that signal male quality to female great tits. The method of subcutaneous delivery of melatonin represents a relatively easy and minimally invasive way to experimentally alter circadian behavioural rhythms in wild vertebrates, creating further experimental opportunities to examine the evolutionary forces acting on these rhythms in nature.

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Data accessibility

Data deposited in the Dryad Digital Repository: <http://doi:10.5061/dryad.cb91k> (Greives *et al.* 2015). The R-script for extracting beginning and end of daily activity from the automated telemetry data has been uploaded as Supporting Information.

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