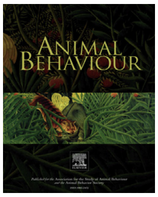
Animal Behaviour 205 (2023) 65–78

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav



Reproductive fitness is associated with female chronotype in a songbird

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ARTICLE INFO

Article history:
Received 29 November 2022
Initial acceptance 9 February 2023
Final acceptance 17 July 2023

MS number 22-00560R

Keywords:
activity
chronobiology
great tit
incubation
reproduction
rhythm
timing
wild clock

Research on biological rhythms has revealed widespread variation in diel timing within populations. Repeatable individual chronotypes have been linked to performance in humans but, in free-living species, benefits of chronotype are poorly understood. To address this gap, we investigated fitness correlates of incubation patterns in female songbirds (great tit, *Parus major*) at urban and forest sites. We confirm repeatable chronotypes ($r \geq 0.31$) and show novel links between chronotype and reproductive success. In both habitats, females that started activity earlier in the day raised more fledglings. We also observed that forest females started their day at a similar time throughout the breeding season, whereas urban females tied their onset of activity closely to sunrise. Our study points to possible mechanisms that underlie chronotype variation and provides sought-after evidence for its relevance to fitness.

Owing to the rotation of the Earth around its axis, no environments are completely constant across the 24 h day. Hence, for organisms, appropriate diel timing of activities and physiology relative to environmental cycles is thought to be important for fitness (Kronfeld-Schor & Dayan, 2003). Still, interindividual differences in diel activities can be large, whereby individuals display highly consistent temporal phenotypes called 'chronotypes' (Alós et al., 2017; Helm et al., 2017; Roenneberg et al., 2003). Chronotype, first defined for laboratory rodents (Ehret, 1974), has gained major research importance in human studies, where millions of subjects have been scored (Roenneberg et al., 2019). Human chronotype has been associated with genetic variants (e.g. in clock genes), performance, and physical and mental health (Jones et al., 2019; Roenneberg et al., 2003). For example, in athletes, performance depends on chronotype and can be enhanced by modifying wake-up time (Facer-Childs & Brandstaetter, 2015).

Interest in chronotype is rapidly increasing in ecology and evolution (Alós et al., 2017; Helm et al., 2017; Maury et al., 2020) fuelled by remote and automated tracking technology (e.g. transmitters or on-site loggers; Dominoni et al., 2013; Graham et al., 2017; Maury et al., 2020). Simultaneous data collection from many individuals is paving the way for studying fitness implications of particular chronotypes, the mechanisms that generate them and the maintenance of interindividual variation (Hau et al., 2017; Helm et al., 2017; Martorell-Barceló et al., 2018). While ecological data are becoming increasingly available for chronotype studies, our understanding of the causes and consequences of its variation has been hindered by conceptual challenges, and by bias in the sex and traits investigated.

Conceptually, disentangling factors that contribute to variation in chronotype requires engaging with the complexity of diel timing. Timing is based on circadian rhythms which closely interact with ambient light (de Coursey, 2004). This ancient clock system integrates genetically controlled molecular clocks with various sensory

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https://doi.org/10.1016/j.anbehav.2023.08.018

pathways, primarily those that perceive and transduce light (Cassone et al., 2017; Helm, 2020; Stevenson & Kumar, 2017). Through further physiological pathways, additional environmental features (e.g. ambient temperature, predation risk) and state (e.g. nutrition, reproductive stage) modify timing (Helm et al., 2017). Chronotype is a phenotype defined by consistent timing of an individual's rhythmic features (e.g. activity onset), relative to an external temporal reference and to conspecifics measured under similar conditions (Helm et al., 2017). The external reference is a fixed environmental phase in the diel cycle at the location of an animal. Choosing an external reference is, however, challenging. Chronotype in human studies usually refers to time after midnight (hereafter called ‘clock’ chronotype; Jones et al., 2019; Roenneberg et al., 2003). This also works well for some other species that repeat diel routines at a relatively fixed time of day, for example, seabirds that under continuous polar light return to breeding colonies at roughly constant clock time (de Coursey, 2004; Huffeldt & Merkel, 2016). Yet, many species time their activity by tracking changing features of the natural light environment, such as sunrise and sunset (Bennie et al., 2014). Therefore, most ecological studies use annually and spatially variable aspects of the solar day (e.g. sunrise) as external references to calculate chronotype (hereafter called ‘relative’ chronotype; Graham et al., 2017; Maury et al., 2020). Species and even local populations may differ in the extent to which they time their activities based on fixed (i.e. ‘clock’ chronotypes) or temporally changing (i.e. ‘relative’ chronotype) features of the environment (Da Silva et al., 2014; da Silva & Kempenaers, 2017; Dominoni et al., 2013; Helm et al., 2017). Furthermore, animals typically adjust their behavioural responsiveness to light conditions over time, space and life cycle stage, so that use of relative chronotype can obscure consistent variation in timing (Daan & Aschoff, 1975). Thus, neither reference fully captures the animals' chronotypes (da Silva & Kempenaers, 2017; Shaw & Cresswell, 2014) and variation in both clock and relative chronotypes should be investigated in parallel to understand variation in chronotype in wild animals. Such integrative research is currently missing.

Investigation of chronotype–fitness associations must also be broadened in scope. Fitness studies on wild chronotypes have until recently mostly focused on males (but see Maury et al., 2020), partly due to extrapair mating and to conspicuous features such as courtship, song and ornaments (Hau et al., 2017; Pagani-Núñez & Senar, 2016). For example, avian observational and experimental studies suggest that early active males may sire more extrapair young than late active males (Greives et al., 2015; Kempenaers et al., 2010), and that such differences could be based on endogenous circadian clocks (Helm & Visser, 2010). However, females are disproportionately more involved in reproductive activities (Mace, 1985), and mating represents a small fraction of factors that shape the fitness landscape of chronotypes. For example, offspring must develop to sexual maturity, potentially requiring extensive parental care, and adults must survive, forage and maintain sufficient body condition to generate offspring. Thus, data are needed on the ecological and evolutionary implications of variation in female chronotype across diverse life cycle stages.

To investigate the fitness implications of chronotype, birds offer excellent study opportunities because their conspicuous behaviours and nest-bound reproductive outcomes are often easily trackable. In this study, we leveraged data from wild birds to (1) disentangle factors that contribute to explaining variation in clock and relative chronotypes, and (2) document links between chronotype and reproductive success. We examined a well-studied songbird whose chronotype has been shown to be repeatable, the great tit, *Parus major* (Graham et al., 2017; Meijdam et al., 2022; Stuber et al., 2015). We inferred the female's chronotype by measuring behaviour during incubation, a critically important postzygotic stage of avian reproduction, while monitoring reproductive success (Capilla-Lasheras, 2018; Graham et al., 2017; Gwinner et al., 2018; Maury et al., 2020). Because features of the environment can influence chronotype (e.g. McGlade et al., 2023), we included data from two habitat types, urban and forest, which often affect the diel rhythm of animals (diurnal animals in urban habitats often have earlier chronotypes; e.g. Dominoni et al., 2013; Miller, 2006). Our analyses also controlled for additional sources of environmental variation (e.g. temperature; Dominoni et al., 2014; Lehmann et al., 2012) and breeding conditions that are known to influence variation in chronotypes (Cooper & Voss, 2013; Gwinner et al., 2018).

The great tit is a small passerine species, widely distributed across Europe and Asia. Great tits reproduce every year, lay one clutch per year in our study area and are female-only, intermittent incubators. They spend nights on their nests, but in the daytime alternate between nest attendance (i.e. on-bouts) and foraging outings (i.e. off-bouts; Diez-Méndez et al., 2021). From small temperature loggers inserted into nests of urban- and forest-breeding great tits, we first inferred both clock and relative chronotype of incubating females and assessed the consistency of chronotype across the breeding season. As our measure of chronotype, we focused on activity onset (beginning of the first incubation off-bout of the day), which in birds is particularly robust and sometimes associated with male fitness (Dominoni et al., 2013; Graham et al., 2017; Hau et al., 2017; Kempenaers et al., 2010; Pagani-Núñez & Senar, 2016), but we also report end of activity (beginning of the last incubation on-bout of the day) and duration of activity (difference in time between activity onset and activity end). Then, we linked incubation chronotype to reproductive success from these same nests to test associations between female chronotype and fitness. Our research spanned 3 years and multiple breeding locations in Scotland, ranging from oak forests to urban settings.

METHODS

Study Populations and Field Protocols

We studied five nestbox-breeding populations of great tits (nestbox details: Woodcrete material, 260 × 170 mm × 180 mm deep, hole diameter 32 mm, Schwegler, Schorndorf, Germany) during the breeding seasons of 2016, 2017 and 2018 (April to June). Three study populations were located in ancient deciduous forests, dominated by oak species (*Quercus* sp.): Scottish Centre for Ecology and the Natural Environment (SCENE; $N = 28$ nestboxes included in the study; 56°7′N, 4°36′W), Sallochy Forest ($N = 8$ nestboxes included in the study; 56°7′N, 4°36′W) and Cashel Forest ($N = 31$ nestboxes included in the study; 56°6′N, 4°34′W). The remaining two populations were situated in an urban park (Kelvingrove Park; $N = 14$ nestboxes included in the study; 55°52′N, 4°16′W) and a suburban park (Garscube estate; $N = 9$ nestboxes included in the study; 55°54′N, 4°19′W) within the city of Glasgow (U.K.). Both urban sites contained a mixture of open land, small shrubs and sparse woodland with introduced and native tree species. For further details on the study sites, see Branston et al. (2021) and Jarrett et al. (2020).

All nestboxes were checked weekly from 1 April for signs of nest-building activity and egg laying. Once a new completed clutch was detected, we calculated the date of clutch completion (from the number of eggs present between two consecutive nestbox visits, assuming that females laid one egg per day). After the estimated earliest possible date of hatching (assuming a minimum incubation length of 14 days from the date of clutch completion; Gosler, 1993), nestboxes were checked every other day, allowing determination of the exact date of hatching based on nestling presence and appearance. Thirteen days after hatching, all nestlings within a brood were weighed (± 0.01 g) and ringed for individual identification ($N = 57$ broods of 13-day-old nestlings). Nestboxes were checked again > 21 days after hatching to determine the number and identity of any dead nestlings remaining in the nest. As our sample size varied slightly for each trait under investigation (see below), we provide a breakdown of sample sizes per habitat, year and trait in Table A1. Sunrise and sunset times at SCENE (56°7′46″N, 4°36′46″W) and Glasgow (55°52′11″N, 4°16′56″W) were obtained from www.timeanddate.com. Our data are collected from individual nestboxes, rather than from identified females. Thus, some individuals might have been recorded in multiple years. Given that our study was spread across five sites over 3 years, the potential bias introduced by this methodological limitation is expected to be minimal (Table A1).

Incubation Temperature Data

To quantify incubation behaviour in female great tits, we deployed small temperature loggers (iButtons DS1922L-F5, Thermochron, www.thermochron.com) inside their nests (Fig. A1). We programmed iButtons to record temperature (± 0.0625 °C) every 3 min (Capilla-Lasheras, 2018). The iButtons were placed carefully next to the eggs (after the third egg of the clutch had been laid), covered with a small piece of white cloth, and attached to the base of the nest by a green wire anchored by a small fishing weight (Fig. A1).

Environmental Temperature Data

To control for variation in environmental temperature when quantifying incubation behaviour (Capilla-Lasheras, 2018), daily mean temperatures for the breeding seasons of 2016, 2017 and 2018 were obtained from the U.K. Met Office for an area close to our forest sites (Tyndrum; 56°25′N, 4°42′W) and city sites (Bishopton; 55°54′N, 4°30′W). We also incorporated daily mean temperatures in our statistical models explaining variation in incubation behaviour (details below).

Quantification of Incubation Behaviour

Some individuals removed iButtons from the nest cup and pushed them to the side of the nestbox, so that these iButtons did not record incubation temperature accurately. These cases of failed incubation temperature data collection were identified by visual inspection of the incubation temperature time series blind to factors in the analysis and were removed from the data set. When this occurred, we discarded the affected days of observation until the following iButton exchange. Our incubation analyses only included days of incubation after the clutch was completed and started no earlier than 15 days before the hatch date. From a total of 1283 days of observations, the largest data set included in the analysis contained 729 days of incubation temperature recordings from 102 clutches (sample sizes vary slightly across statistical models; details are given in the Results and Table A1).

Incubation behaviour (e.g. on- and off-bout timing) was determined using the R package incR (v1.1.0; Capilla-Lasheras, 2018; Gwinner et al., 2018), choosing parameter values for incRscan validated for great tit incubation (Capilla-Lasheras, 2018; lower.time = 22, upper.time = 3, sensitivity = 0.15, temp.diff = 8, maxNightVar_accepted = 2). In short, to determine incubation on- and off-bouts, incRscan used variation in incubation temperatures during a time window (2200–0300 hours, lower.time and upper.time parameters) in which females were assumed to incubate constantly, unless incubation temperatures dropped more than two degrees (maxNightVar_accepted parameter; see more details in Capilla-Lasheras, 2018). For each incubating female, we determined: first morning off-bout, last evening on-bout and duration of active day (i.e. time difference between the first morning off-bout and the last evening on-bout).

DATA ANALYSIS

General Modelling Procedures

All analyses and visualizations were performed in R (version 4.2.1; R Core Team, 2022). Generalized linear mixed models (GLMM) were employed to explain variation in several incubation and reproductive traits (see below). For each of these traits, we built a full model that contained all explanatory variables and interactions of interest for each trait (see below). Then, we used likelihood ratio tests (LRTs) to assess the statistical importance of each model predictor. We removed nonsignificant interactions from the initial full models to ease biological interpretation of single effect predictors. However, we did not apply model simplification beyond nonsignificant interactions and present the resulting full model outputs. Linear and quadratic terms were retained in all models and fitted using orthogonal polynomials to improve model convergence and assess their statistical importance independently. Random effects were present in every model as specified for the analysis of each response variable (details below). We formally tested for nonzero model slopes of interactive terms using Wald chi-square tests implemented in the R package car (v3.1.0; Fox & Weisberg, 2019) via its linearHypothesis function. All statistical models were performed using the R package lme4 (v1.1.29; Bates et al., 2015). Gaussian model residuals were visually inspected to check the assumption of normality using the R package performance (v0.10.1; Lüdecke et al., 2021). The R package DHARMa (v0.4.5; Hartig, 2018) was employed to check the normality of residuals in non-Gaussian models.

Statistical models

Incubation behaviour. We analysed clock (i.e. time after midnight) and relative (i.e. time relative to sunrise or sunset time) onset and end of diel activity. To account for differences in photoperiod throughout the breeding season, we calculated relative onset as the time of the first incubation off-bout minus sunrise time for each day (i.e. positive values represent onset of activity after sunrise, whereas negative vales indicate an onset of activity earlier than sunrise). Similarly, relative end of activity was defined per day as the time of the last on-bout minus sunset time (i.e. positive values represent end of activity after sunset, whereas negative values indicate an end of activity earlier than sunset). Full models for onset and end of activity (both clock and relative metrics) included as explanatory variables habitat (urban versus forest), clutch size (as a continuous predictor), mean daily temperature (as a continuous predictor) and days before hatching (as a continuous predictor whose minimum value was 1, i.e. 1 day before hatching, included as a quadratic and a linear term; these terms effectively modelled within-female variation in onset and end of activity). Additionally, we controlled for between-nest differences in timing of reproduction by including the date of incubation start (i.e. clutch completion date) as a fixed-effect predictor (in number of days after 1 April; included as a quadratic and a linear term; these terms effectively modelled among-female, i.e. cross-sectional, variation in onset and end of activity). Temporal predictors of incubation behaviour were included in the analysis as linear and quadratic terms given the evidence for negative quadratic temporal effects on incubation reported before (Cooper & Voss, 2013; Gwinner et al., 2018). We also included the interactions between habitat and days before hatching (both quadratic and linear terms), and between habitat and incubation start date (both quadratic and linear terms). Breeding attempt identity (included as a 90-level factor for nestbox identity, 79 of the 90 nestboxes included in the analysis, i.e. 87%, were used in a single year only), site (five-level factor) and year (three-level factor) were included as random-effect intercepts. Using the same model structure, we analysed the duration of the active day of incubating females, defined as the time interval between the first incubation off-bout and the last on-bout per day.

We used the amount of variation explained by breeding attempt identity to calculate within-breeding-attempt consistency (i.e. repeatability) in female chronotype, but we do acknowledge that this calculation could be improved by tracking individual females across multiple breeding years (see Discussion). Specifically, consistency in female onset, end and duration of activity was calculated as the proportion of variation explained by the breeding attempt identity random effect in the linear mixed models presented above (i.e. including year and site as random effects), as implemented in the R package rptR (Stoffel et al., 2017). Female chronotype for subsequent analyses (see below) was defined as the average within-nest onset of activity, but we also report consistency (i.e. repeatability) for end and duration of activity. We additionally analysed incubation start dates using a Gaussian GLMM with clutch size and habitat as fixed effects, and breeding attempt identity, site and year as random effects.

Survival of nestlings to fledging and nestling weight. A Poisson GLMM was used to explain variation in the number of nestlings that survived to fledging. The probability of total brood failure (i.e. the probability that no nestling survived to fledging) was modelled using a binomial GLMM. Given the lack of zero values (which Poisson distributions do have), an LMM was employed to analyse the number of nestlings that survived to fledging excluding broods in which no nestlings survived. Variation in the average 13-day-old nestling's weight per brood was analysed using an LMM. These models included habitat (urban versus forest), female chronotype (see definition above), hatching date (as a continuous variable in days after 1 January; included in the model as a linear and a quadratic term) and clutch size as fixed-effect predictors. The interactions between hatching date and habitat, and between female chronotype and habitat, were also added. Breeding attempt identity (90-level factor for survival analysis and 53-level factor in nestling weight analysis), site (five-level factor) and year (three-level factor) were included as random-effect intercept.

Ethical Note

Nestlings were captured and minimally disturbed (for weighing) in their nestboxes under ringing licences granted to the authors by the British Trust of Ornithology. We adhered to the ASAB/ABS Guidelines for the use of animals in research. This project did not involve harmful manipulations of the study individuals or their environmental conditions and no institutional approval was required.

RESULTS

Correlates and Consistency of Incubating Female Chronotype

We recorded nest temperatures in 2016, 2017 and 2018, and analysed a maximum of 729 days of great tit incubation in 102 clutches (median = 7 days of incubation per clutch; range 1–15 days; see details of sample sizes in Table A1). Urban great tit females laid their eggs and started incubation earlier in the year than forest females, and thus experienced shorter days with later sunrise and earlier sunset (start of incubation date: $\text{mean}_{\text{urban}} \pm \text{SE} = 30$ April ± 1.09 days, $\text{SD}_{\text{urban}} = 5.68$ days, $N_{\text{urban}} = 27$ clutches; $\text{mean}_{\text{forest}} \pm \text{SE} = 8$ May ± 0.61 days, $\text{SD}_{\text{forest}} = 5.30$ days, $N_{\text{forest}} = 75$ clutches; $\chi^2_1 = 10.59$, $P = 0.001$). Therefore, we detail results separately for the two habitats, but all data were analysed together in overarching models.

We found that, at the population level, clock time of activity onset was affected by habitat and by the date when incubation started (interaction 'Incubation start date*habitat'; Fig. 1a, b, Table 1). Urban females closely tracked the seasonally advancing sunrise time (Fig. 1a), but forest females largely ignored this advance and started their activity at a similar time throughout the season (Fig. 1b; the slope in Fig. 1b was not significantly different from zero: $\chi^2_1 = 1.01$, $P = 0.315$). Whereas early breeding urban females started activity later than forest females, for late breeding birds the pattern reversed, so that urban females started their day earlier than forest females (Fig. 1a, b). In contrast, at the population level, end of activity was similar in both habitats and became progressively earlier with later incubation start date (Fig. A2, Table A2). Overall, the active day lengthened over the breeding season for urban but tended to shorten for forest females (Fig. A3, Table A3). These patterns at the population level for clock time of onset and end of activity were broadly matched by temporal variation within clutches (i.e. variation between the first and last day of incubation of a clutch; Table 1, Table A2, Figs A4, A5). Ambient temperature and clutch size did not affect clock time of onset and end of activity (Table 1, Table A2).

Relative onset of activity also depended on habitat and on the date when incubation started (Fig. 1c, d, Table 2). Females that initiated incubation later in the year began their day relative to sunrise progressively earlier in the city, but progressively later in the forest (interaction 'Incubation start date*habitat'; Fig. 1c, d, Table 2). The end of activity relative to sunset advanced consistently over the season in both habitats (Fig. A2c, d, Table A4). Ambient temperature and clutch size did not affect relative time of onset and end of activity (Table 2, Table A4). These patterns at the population level for relative time of onset and end of activity were broadly matched by temporal variation within clutches (i.e. variation between the first and last day of incubation of a clutch; Table 2, Table A4, Figs A4, A5).

We identified consistent individual differences in the time of onset of activity (i.e. female chronotype). Between-female differences explained 31% of the variation in clock onset time (LRT on the breeding attempt ID random effect: $\chi^2_1 = 133.18$, $P < 0.001$; repeatability [95% confidence interval, CI] = 0.31 [0.21, 0.41]). Analyses of relative onset time (i.e. correcting for changes in sunrise time) yielded similar results, with consistent between-female differences in onset of activity (repeatability [95% CI] = 0.32 [0.22, 0.41]). We also found consistent between-female differences in the end of activity, both in clock (repeatability [95% CI] = 0.26 [0.17, 0.35]) and relative end of activity (repeatability [95% CI] = 0.27 [0.17, 0.35]) and consistent between-female differences in the duration of the active day (repeatability [95% CI] = 0.20 [0.13, 0.28]).

Fledging Success and Prefledging Weight

We detected substantial variation between broods in the number of nestlings that survived to fledging. Relative female chronotype predicted the number of surviving nestlings: the earlier the female chronotype, the more nestlings fledged (Fig. 2a, Table 3). This effect was consistent across habitats (i.e. nonsignificant interaction between female chronotype and habitat; Table 3) and was robust to controlling for clutch size (Table 3). The number of surviving chicks was also affected by habitat (Table 3): urban females fledged 1.48 nestlings less than forest females (i.e. a decrease

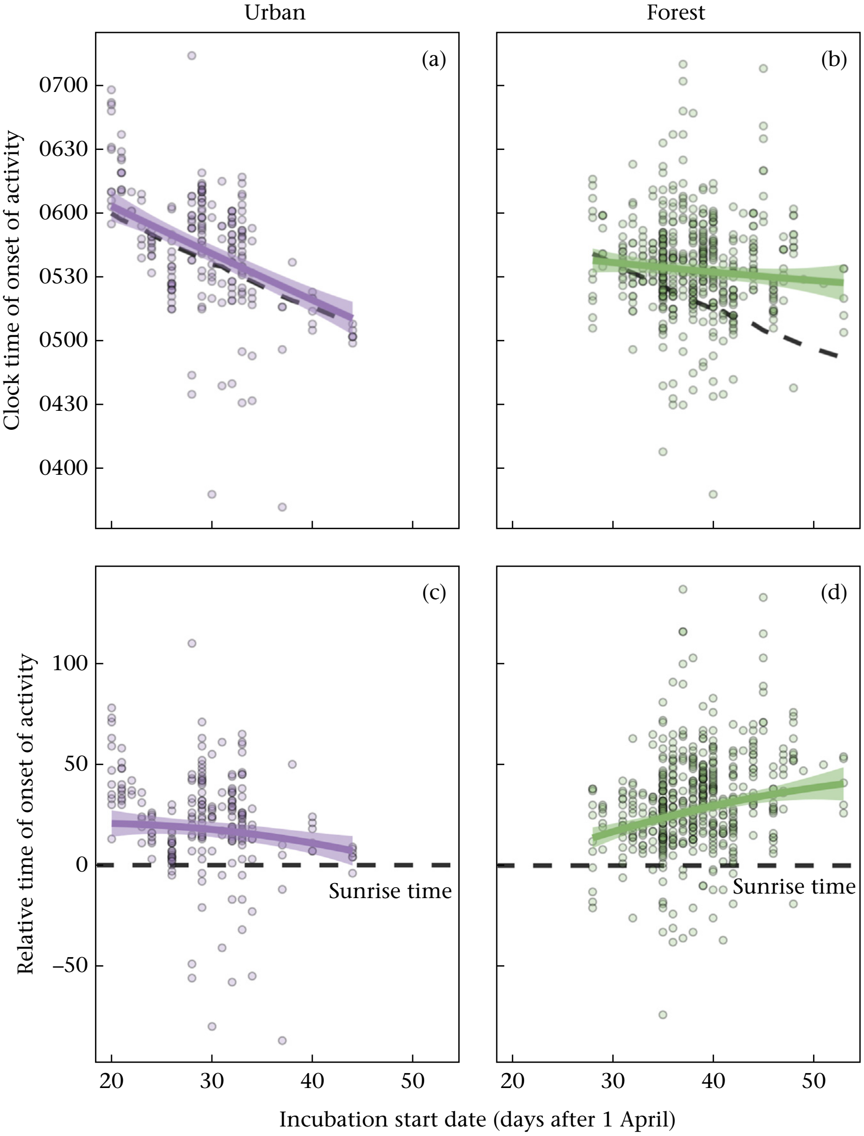

Figure 1. (a, b) Clock time (time of activity onset) and (c, d) relative time of activity onset (minutes after sunrise) in (a, c) urban and (b, d) forest great tit females. Points represent data while thick solid lines and shaded areas provide mean model predictions ± 1 SE (see model coefficients in Tables 1 and 2). Dashed lines indicate sunrise time.

in surviving nestlings of 46%; Table 3). Conversely, clock chronotype of females did not predict the number of surviving nestlings (Table A5).

To investigate the mechanism that could have generated the decreasing reproductive success with later relative female chronotype, we performed two additional analyses. First, we assessed whether relative female chronotype was associated with total brood failure and found no support (i.e. no significant effect of relative female chronotype on the probability that no nestling survives to fledging, Table A6). Second, we tested whether relative female chronotype predicted the number of nestlings surviving to fledging in successful broods (i.e. those that fledged at least one offspring) and confirmed that earlier relative chronotypes fledged more offspring than later chronotypes (Table A7).

Mean body mass of 13-day-old nestlings was affected by habitat, hatching date and clutch size. Forest nestlings were on average 2.69 g (95% CI = [1.67, 3.70]; Table 4) heavier than urban nestlings of the same age. In both habitats, prefledging weight was higher for broods that hatched in the middle of the season (Fig. 2b), and nestlings from larger clutches were on average lighter (Table 4). Neither relative nor clock female chronotype affected prefledging weight in either habitat (relative chronotype: $\chi^2_1 = 0.20$, $P = 0.656$; clock chronotype: $\chi^2_1 = 0.06$, $P = 0.801$; interaction terms between chronotype and habitat were nonsignificant, for relative chronotype: $\chi^2_1 = 0.48$, $P = 0.489$; for clock chronotype: $\chi^2_1 = 0.36$, $P = 0.551$).

DISCUSSION

Recent research has identified surprisingly high variation in chronotype of free-living animals, but determinants and effects of this variation are still largely unclear. Our study is among the few that have identified fitness correlates of (relative) chronotype in female animals. We first showed high repeatability of timing, and thus corroborated evidence of chronotype as a consistent individual trait in birds, including in our study species (Graham et al., 2017; Meijdam et al., 2022; Stuber et al., 2015). We then showed that the relative chronotype of female great tits, measured during the incubation period, predicted reproductive success, such that early rising females raised more offspring to fledging than late (relative)

Table 1
Likelihood ratio test results and model coefficients for predictors explaining variation in clock time of female onset of activity (i.e. time of first incubation off-bout)

Fixed effect	Estimate	SE	95% CI	χ^2	*df*	*P*
Intercept	324.12	7.91	308.62, 339.63			
Incubation start date^2	3.64	54.35	−102.88, 110.17	0.00	1	0.948
Incubation start date^1	−365.30	72.17	−506.74, −223.85			
Days before hatching^2	−44.97	19.83	−83.82, −6.11	5.11	1	**0.024**
Days before hatching^1	−44.63	40.15	−123.33, 34.07			
Mean daily temperatures	0.48	0.35	−0.20, 1.17	1.87	1	0.172
Clutch size	−0.13	0.89	−1.89, 1.62	0.02	1	0.883
Habitat						
Urban	–	–	–			
Forest	7.54	4.61	−1.50, 16.58			
Incubation start date^1*Habitat				6.02	1	**0.014**
Incubation start date^1*Forest	290.09	113.67	67.30, 512.87			
Days before hatching^1*Habitat				8.71	1	**0.003**
Days before hatching^1*Forest	143.60	48.31	48.90, 238.30			

Model coefficients (‘Estimate’) for clock time onset of activity (given in min after 0000 hours) are shown along with standard errors and 95% confidence intervals (95% CI). Superscripts ‘1’ and ‘2’ refer to linear and quadratic terms, respectively. $N = 729$ days of incubation. Significant results are highlighted in bold. LRT results for ‘Habitat’, ‘Incubation start Date^1’ and ‘Days before hatching^1’ are not provided as these terms were part of a significant interaction present in the final model. The interactions ‘Incubation start date^2*Habitat’ ($\chi^2_1 = 0.98$, $P = 0.323$) and ‘Days before hatching^2*Habitat’ ($\chi^2_1 = 1.01$, $P = 0.316$) were not significant and were dropped from the model.

Table 2
Likelihood ratio test results and model coefficients for predictors explaining variation in relative time of female onset of activity (i.e. time of first incubation off-bout minus sunrise time)

Fixed effect	Estimate	SE	95% CI	χ^2	*df*	*P*
Intercept	12.22	7.56	−2.60, 27.04			
Incubation start date^2	−28.70	51.99	−130.61, 73.21	0.27	1	0.604
Incubation start date^1	−113.30	67.99	−246.56, 19.96			
Days before hatching^2	−49.87	19.27	−87.64, −12.11	6.64	1	**0.010**
Days before hatching^1	162.30	38.98	85.90, 238.70			
Habitat						
City	–	–	–			
Forest	11.46	4.27	3.09, 19.83			
Mean daily temperatures	0.42	0.34	−0.25, 1.08	1.46	1	0.227
Clutch size	−0.09	0.87	−1.80, 1.61	0.01	1	0.916
Incubation start date^1*Habitat				8.30	1	**0.004**
Incubation start date^1*Forest	327.37	108.76	114.21, 540.53			
Days before hatching^1*Habitat				4.76	1	**0.029**
Days before hatching^1*Forest	103.20	46.91	11.26, 195.14			

Model coefficients (‘Estimate’) for relative onset of activity (given in min after sunrise) are shown along with standard errors and 95% confidence intervals (95% CI). Superscripts ‘1’ and ‘2’ refer to linear and quadratic terms, respectively. $N = 729$ days of incubation. Significant results are highlighted in bold. LRT results for ‘Habitat’, ‘Incubation start Date^1’ and ‘Days before hatching^1’ are not provided as these terms were part of a significant interaction present in the final model. The interactions ‘Incubation start date^2*Habitat’ ($\chi^2_1 = 1.84$, $P = 0.175$) and ‘Days before hatching^2*Habitat’ ($\chi^2_1 = 0.68$, $P = 0.409$) were not significant and were dropped from the model.

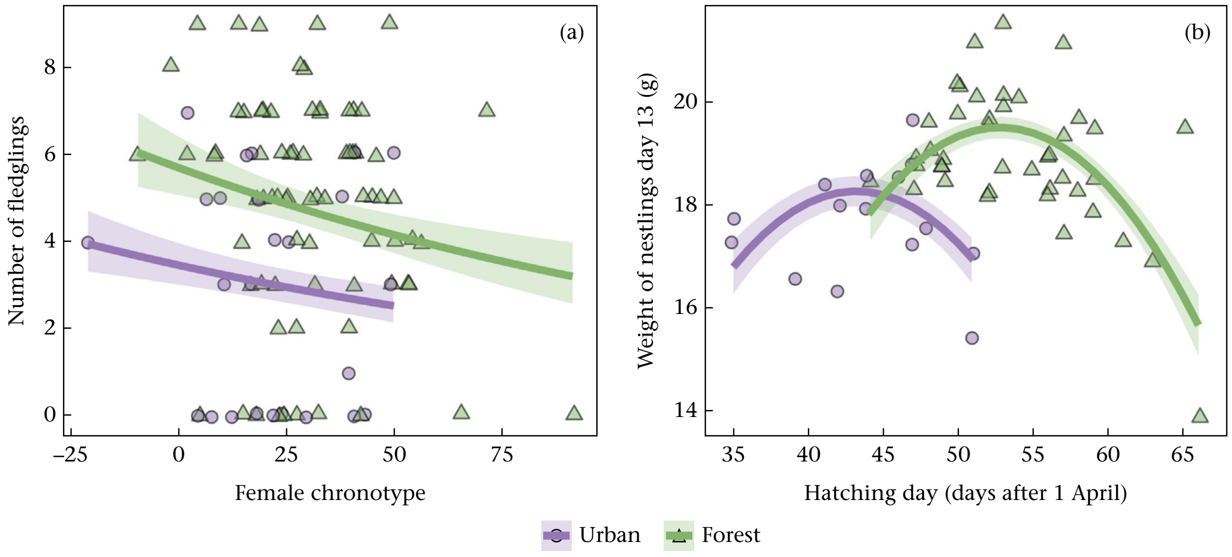

Figure 2. (a) Effect of relative female chronotype (relative time of activity onset, given as minutes after sunrise) on survival ($N = 101$ broods; Table 3) and (b) effect of seasonal time on weight ($N = 57$ broods; Table 4) of 13-day-old nestlings. Points represent data, while thick lines and shaded areas provide mean model predictions ± 1 SE (Table 3 and 4).

Table 3
Likelihood ratio test results and model coefficients for relative female chronotype and other predictors hypothesized to explain variation in nestling survival to fledging

Fixed effect	Estimate	SE	95% CI	χ^2	*df*	*P*
Intercept	0.14	0.27	−0.38, 0.66			
Hatching date^1	−0.08	0.82	−1.69, 1.53	0.01	1	0.919
Hatching date^2	−0.37	0.59	−1.53, 0.79	0.40	1	0.527
Female chronotype	−0.11	0.05	−0.22, 0.00	4.02	1	**0.045**
Habitat				5.12	1	**0.024**
City	–	–	–			
Forest	0.38	0.17	0.05, 0.71			
Clutch size	0.14	0.03	0.08, 0.21	18.66	1	**<0.001**

Model coefficients ('Estimate') are shown in their link scale (logit) along with standard errors and 95% confidence intervals (95% CI). Superscripts '^{1}' and '^{2}' refer to linear and quadratic terms, respectively. $N = 101$ broods. Significant results are highlighted in bold. The interactions 'Days before hatching^1*Habitat' ($\chi^2_1 = 0.05$, $P = 0.831$), 'Days before hatching^2*Habitat' ($\chi^2_1 = 0.01$, $P = 0.927$) and 'Female chronotype*Habitat' ($\chi^2_1 = 0.48$, $P = 0.489$) were not significant and were dropped from the model.

chronotypes. As predicted based on previous studies (Capilla-Lasheras et al., 2022; Dominoni et al., 2013), we also found that urban great tits breed earlier in the season than nonurban great tits.

Early rising may be beneficial for replenishing energy stores after the night, and the ability of small passerine birds to successfully forage peaks in the early morning, once light conditions are suitable (Kacelnik, 1979; Pagani-Núñez & Senar, 2016). However, foraging in the early morning can be costly because of low ambient temperatures and increased predation risk (McNamara et al., 1994). It has been speculated that in addition to the endogenous circadian clock, the condition or reserves of an individual relative to environmental demands influences activity onset. However, the direction of such an influence is still unclear: good condition might either advance or delay the time of activity onset. For example, early song production has been interpreted as an honest signal of male quality, suggesting that superior condition is required for an early start of the day (Murphy et al., 2008). Conversely, birds in superior condition might also be able to afford a longer rest, circumventing a need to forage early in the morning. Thus, incubating females of several species responded to eased environmental conditions by delayed activity onset, for example when their energy reserves were boosted through warming of the nest (Arct et al., 2022; Bryan & Bryant, 1999; Gwinner et al., 2018). Likely, links between a bird's condition and rising time are sensitive to ecological and life history contexts.

Balancing costs and benefits of early rising might be intricate during incubation. For uniparental incubators, self-maintenance is weighed up against maximal offspring development (Nord & Cooper, 2020). This trade-off is heightened during early morning hours when incubators must replenish energy stores. Yet, because the typically low morning temperatures risk cooling of the eggs, an incubating female should delay leaving the nest until she can forage efficiently. Early rising may thus indicate superior foraging skills of incubating females, as proposed for courtship song and provisioning by males (McNamara et al., 1987; Murphy et al., 2008; Pagani-Núñez & Senar, 2016). If so, the higher reproductive success we found for early rising females might be an indirect result of the females' superior condition, as previously proposed for early breeding females (Verhulst and Nilsson 2008). It is also possible that early rising might be indicative of females in poor condition that cannot tolerate further depletion of energy and, hence, need to leave the nest early when eggs are at high risk of cooling (Nord & Cooper, 2020). However, our results do not support this alternative interpretation.

The ability to perform efficiently early in the day likely also depends on circadian mechanisms that facilitate an early start, as demonstrated in human athletes (Vitale & Weydahl, 2017). Reproductive advantages due to circadian-based early rising have been proposed for male great tits whose circadian rhythm affects extrapair paternity (EPP) and have been supported by experiments on the same study species (Greives et al., 2015; Hau et al., 2017; Helm & Visser, 2010). Great tit chicks with fast circadian clocks were significantly more likely to be sired through EPP, and males whose circadian system was pharmacologically delayed lost paternity (Greives et al., 2015; Helm & Visser, 2010). As in these other studies, our work found benefits for the early bird, without indicating what benefits or costs, in turn, might arise for late chronotypes.

A putative circadian basis to an early chronotype could involve several mechanistic features. These include a fast clock (i.e. short free-running period; Helm & Visser, 2010), but also individual variation in sensitivity to light (Brown et al., 2008; Helm et al., 2017; Jones et al., 2019; Tudorache et al., 2018). A contribution of light response pathways to the chronotype–fitness link is suggested by our findings for clock and relative timing. Fitness effects were evident only for chronotype relative to sunrise, whereas the clock time of activity onset showed no association.

We detected unexpected differences in response to sunrise, but not sunset, between females at urban and forest sites. Forest females started activity at almost constant times of day, despite the rapid advance of sunrise time over the breeding season. Conversely, urban females were far more responsive to light and largely tracked the rapid advance of sunrise. This finding was counter to the expectation that in urban habitats, where artificial light at night is prevalent, the birds' responsiveness to natural light changes would be reduced (Dominoni et al., 2013; Roenneberg et al., 2007), or that, like some species under continuous light, birds in light-polluted areas might not use light conditions to time their activities (Huffeldt & Merkel, 2016). It is possible that habitat differences other than light levels contributed to the differences in behaviour. For related blue tits, *Cyanistes caeruleus*, the same study habitats differed in quality, with poorer adult state and reproductive success in the city (Capilla-Lasheras et al., 2017; Pollock et al., 2017). Thus, some urban great tit females may have needed to forage at the earliest opportunity to replenish their resources, without an apparent impact on reproductive success. Disentangling effects of the internal circadian clock on chronotype from those of the birds' body condition would require experimental examination (Dominoni et al., 2013; Greives et al., 2015).

The only other study we are aware of that has examined reproductive success relative to incubation chronotype did not find such an association (Maury et al., 2020). This investigation differed in several aspects, including use of the European starling, *Sturnus vulgaris*, as a study species. While we cannot explain the different findings, we speculate that colonial breeding of the studied starlings may have affected synchronicity, and thereby altered or obscured effects of chronotype (Gwinner, 1966; Menaker & Eskin, 1966). In other contexts, fitness implications of chronotype are also beginning to arise. For example, a recent study on fish showed that under fishery pressure, chronotype was associated with differential survival (Martorell-Barceló et al., 2018). Still, we are far from understanding how variation in chronotype is maintained.

Our study results come with some caveats. Because we report correlative data from wild birds, we cannot assess whether chronotype was affected by the local microenvironment, either directly or via differences in individual quality (Diez-Méndez, Cooper, et al., 2021; Maury et al., 2020). We recorded female chronotype only during one life cycle stage, incubation, similar to earlier studies on males that considered only courtship (Murphy et al., 2008). Thus, the consistency of chronotype across life stages remains to be

Table 4
Likelihood ratio test (LRT) results and model coefficients for relative female chronotype and other predictors hypothesized to explain variation in prefledging weight of nestlings on day 13

Fixed effect	Estimate	SE	95% CI	χ^2	*df*	*P*
Intercept	16.95	0.70	15.58, 18.32			
Hatching date^1	−16.18	3.56	−23.16, −9.20			
Hatching date^2	−10.29	1.91	−14.03, −6.55	19.88	1	**<0.001**
Female chronotype	0.06	0.14	−0.21, 0.34	0.20	1	0.656
Habitat						
City	–	–	–			
Forest	2.69	0.52	1.67, 3.70			
Clutch size	−0.16	0.08	−0.31, −0.01	4.05	1	**0.044**
Hatching date^1*Habitat				10.87	1	**0.001**
Hatching date^1*Forest	20.86	5.23	10.61, 31.11			

Model coefficients (‘Estimate’) are shown along with standard errors and 95% confidence intervals (95% CI). Superscripts ‘^{1}’ and ‘^{2}’ refer to linear and quadratic terms, respectively. $N = 57$ broods. Significant results are highlighted in bold. The interactions ‘Days before hatching^2*Habitat’ ($\chi^2_1 = 0.04$, $P = 0.847$) and ‘Female chronotype*Habitat’ ($\chi^2_1 = 0.02$, $P = 0.892$) were not significant and were dropped from the model.

tested. Similarly, we inferred chronotype from onset of activity across multiple days of the same breeding event, and we could not assess consistency of chronotype for the same female across multiple breeding seasons. Correlated environmental conditions or female body condition within breeding attempts could have potentially increased the estimate of chronotype consistency. Comparing our estimates of chronotype with quantifications from studies that track individuals across multiple breeding seasons will greatly expand the significance of our results and shed new light on the environmental contributions to chronotype.

Nevertheless, our study strengthens the evidence for variation in chronotype in free-living animals and provides a sought-after link to reproductive success. We have extended the circadian focus of chronotype studies by indicative findings on light pathways and confirmed the importance of looking at both relative and clock time, as previously suggested for avian incubation (Shaw & Cresswell, 2014). Future challenges, likely requiring experimental approaches, are a disentangling of effects of the endogenous clock from body condition, and determination of counterbalancing benefits that maintain variation in chronotype.

Author Contributions

R.J.W. and B.H. conceived the study. R.J.W., C.L.O.M., D.M.D. and B.H. collected the data. P.C.-L. performed all statistical analyses, with input from R.J.W., C.L.O.M., D.M.D. and B.H. P.C.-L. and B.H. wrote the first draft of the manuscript, with major contributions by R.J.W. All authors read and revised the manuscript.

Data Availability

All R scripts and data sets needed to reproduce the analyses presented in this paper are available at https://doi.org/10.5281/zenodo.7967106.

Declaration of Interest

The authors declare no conflict of interest.

Acknowledgments

We thank Ally Phillimore and an anonymous referee for their comments on the manuscript. R.J.W. received funding from a BBSRC-DTP studentship. D.M.D. and P.C-L. are funded by a NERC grant (grant no. NE/S005773/1).

References

Alós, J., Martorell-Barceló, M., & Campos-Candel, A. (2017). Repeatability of circadian behavioural variation revealed in free-ranging marine fish. *Royal Society Open Science, 4*, Article 160791. https://doi.org/10.1098/rsos.160791

Arct, A., Martyka, R., Drobniak, S. M., Oleś, W., Dubiec, A., & Gustafsson, L. (2022). Effects of elevated nest box temperature on incubation behaviour and offspring fitness-related traits in the Collared Flycatcher *Ficedula albicollis*. *Journal of Ornithology, 163*(1), 263–272. https://doi.org/10.1007/s10336-021-01944-3

Bates, D., Machler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, 67*, 1–48.

Bennie, J. J., Duffy, J. P., Inger, R., & Gaston, K. J. (2014). Biogeography of time partitioning in mammals. *Proceedings of the National Academy of Sciences of the United States of America, 111*(38), 13727–13732. https://doi.org/10.1073/pnas.1216063110

Branston, C. J., Capilla-Lasheras, P., Christopher, L., Griffiths, K., White, S., & Dominoni, D. M. (2021). Urbanisation weakens selection on the timing of breeding and clutch size in blue tits but not in great tits. *Behavioral Ecology and Sociobiology, 75*, 155. https://doi.org/10.1007/s00265-021-03096-z

Brown, S. A., Kunz, D., Dumas, A., Westermark, P. O., Vanselow, K., Tilmann-Wahnschaffe, A., Herzel, H., & Kramer, A. (2008). Molecular insights into human daily behavior. *Proceedings of the National Academy of Sciences of the United States of America, 105*(5), 1602–1607. https://doi.org/10.1073/pnas.0707772105

Bryan, S. M., & Bryant, D. M. (1999). Heating nest-boxes reveals an energetic constraint on incubation behaviour in great tits, *Parus major*. *Proceedings of the Royal Society B: Biological Sciences, 266*, 157–162.

Capilla-Lasheras, P. (2018). incR: a new R package to analyse incubation behaviour. *Journal of Avian Biology.* , Article e01710. https://doi.org/10.1111/jav.01710

Capilla-Lasheras, P., Dominoni, D. M., Babayan, S. A., O'Shaughnessy, P. J., Mladenova, M., Woodford, L., Pollock, C. J., Barr, T., Baldini, F., & Helm, B. (2017). Elevated immune gene expression is associated with poor reproductive success of urban blue tits. *Frontiers in Ecology and Evolution, 5*, 64. https://doi.org/10.3389/fevo.2017.00064

Capilla-Lasheras, P., Thompson, M. J., Sánchez-Tójar, A., Haddou, Y., Branston, C. J., Réale, D., Charmantier, A., & Dominoni, D. M. (2022). A global meta-analysis reveals higher variation in breeding phenology in urban birds than in their non-urban neighbours. *Ecology Letters, 25*, 2552–2570. https://doi.org/10.1111/ele.14099

Cassone, V. M., Paulose, J. K., Harpole, C. E., Li, Y., & Whitfield-Rucker, M. (2017). Avian circadian organization. In V. Kumar (Ed.), *Biological timekeeping: Clocks, rhythms and behaviour* (pp. 241–256). Springer India.

Cooper, C. B., & Voss, M. A. (2013). Avian incubation patterns reflect temporal changes in developing clutches. *PLoS One, 8*(6), Article e65521. https://doi.org/10.1371/journal.pone.0065521

Daan, S., & Aschoff, J. (1975). Circadian rhythms of locomotor activity in captive birds and mammals: Their variations with season and latitude. *Oecologia, 18*, 269–316. https://doi.org/10.1007/BF00345851

da Silva, A., & Kempenaers, B. (2017). Singing from North to South: Latitudinal variation in timing of dawn singing under natural and artificial light conditions. *Journal of Animal Ecology, 86*, 1286–1297. https://doi.org/10.1111/1365-2656.12739

da Silva, A., Samplonius, J. M., Schlicht, E., Valcu, M., & Kempenaers, B. (2014). Artificial night lighting rather than traffic noise affects the daily timing of dawn and dusk singing in common European songbirds. *Behavioral Ecology, 25*, 1037–1047. https://doi.org/10.1093/beheco/aru103

de Coursey, P. (2004). The behavioral ecology and evolution of biological timing systems. In J. C. Dunlap, J. J. Loros, & P. de Coursey (Eds.), *Chronobiology: Biological timekeeping* (pp. 27–65). Sinauer.

Diez-Méndez, D., Cooper, C. B., Sanz, J. J., Verdejo, J., & Barba, E. (2021). Deconstructing incubation behaviour in response to ambient temperature over different timescales. *Journal of Avian Biology.* , Article e02781. https://doi.org/10.1111/jav.02781

Diez-Méndez, D., Sanz, J. J., & Barba, E. (2021). Impacts of ambient temperature and clutch size on incubation behaviour onset in a female-only incubator songbird. *Ibis, 163*, 1056–1071. https://doi.org/10.1111/ibi.12937

Dominoni, D. M., Carmona-Wagner, E. O., Hofmann, M., Kranstauber, B., & Partecke, J. (2014). Individual-based measurements of light intensity provide new insights into the effects of artificial light at night on daily rhythms of urban-dwelling songbirds. *Journal of Animal Ecology, 83*(3), 681–692. https://doi.org/10.1111/1365-2656.12150

Dominoni, D. M., Helm, B., Lehmann, M., Dowse, H. B., & Partecke, J. (2013). Clocks for the city: Circadian differences between forest and city songbirds. *Proceedings of the Royal Society B: Biological Sciences, 280*(1763), Article 20130593. https://doi.org/10.1098/rspb.2013.0593

Ehret, C. F. (1974). The sense of time: Evidence for its molecular basis in the eukaryotic gene-action system. *Advances in Biological and Medical Physics, 15*, 47–77. https://doi.org/10.1016/b978-0-12-005215-8.50009-7

Facer-Childs, E., & Brandstaetter, R. (2015). The impact of circadian phenotype and time since awakening on diurnal performance in athletes. *Current Biology, 25*, 518–522. https://doi.org/10.1016/j.cub.2014.12.036

Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (3rd ed.). Sage.

Gosler, A. (1993). *The great tit*. Hamlyn.

Graham, J. L., Cook, N. J., Needham, K. B., Hau, M., & Greives, T. J. (2017). Early to rise, early to breed: A role for daily rhythms in seasonal reproduction. *Behavioral Ecology, 28*(5), 1266–1271. https://doi.org/10.1093/beheco/arx088

Greives, T. J., Kingma, S. A., Kranstauber, B., Mortega, K., Wikelski, M., van Oers, K., Mateman, A. C., Ferguson, G. A., Beltrami, G., & Hau, M. (2015). Costs of sleeping in: Circadian rhythms influence cuckoldry risk in a songbird. *Functional Ecology, 29*, 1300–1307. https://doi.org/10.1111/1365-2435.12440

Gwinner, E. (1966). Entrainment of a circadian rhythm in birds by species-specific song cycles (Aves, Fringillidae: *Carduelis spinus, Serinus serinus*). *Experientia, 22*, 765.

Gwinner, H., Capilla-Lasheras, P., Cooper, C., & Helm, B. (2018). 'Green incubation': Avian offspring benefit from aromatic nest herbs through improved parental incubation behaviour. *Proceedings of the Royal Society B: Biological Sciences, 285*, Article 20180376.

Hartig, F. (2018). *DHARMa: Residual diagnostics for hierarchical (multi-Level / mixed) regression models*. R package version 0.2.4 https://cran.r-project.org/package=DHARMa.

Hau, M., Dominoni, D., Casagrande, S., Buck, C. L., Wagner, G., Hazlerigg, D., Greives, T., Hut, R. A., & Hau, M. (2017). Timing as a sexually selected trait: The right mate at the right moment. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372*, Article 20160249.

Helm, B. (2020). Clocks and calendars in birds. In F. J. P. Ebling, & D. Piggins (Eds.), *Neuroendocrine clocks and calendars* (pp. 119–142). Springer.

Helm, B., & Visser, M. E. (2010). Heritable circadian period length in a wild bird population. *Proceedings of the Royal Society B: Biological Sciences, 277*, 3335–3342. https://doi.org/10.1098/rspb.2010.0871

Helm, B., Visser, M. E., Schwartz, W., Kronfeld-Schor, N., Gerkema, M., Piersma, T., & Bloch, G. (2017). Two sides of a coin: Ecological and chronobiological perspectives of timing in the wild. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372*, Article 20160246. https://doi.org/10.1098/rstb.2016.0246

Huffeldt, N. P., & Merkel, F. R. (2016). Sex-specific, inverted rhythms of breeding-site attendance in an Arctic seabird. *Biology Letters, 12*, Article 20160289. https://doi.org/10.1098/rsbl.2016.0289

Jarrett, C., Powell, L. L., McDevitt, H., Helm, B., & Welch, A. J. (2020). Bitter fruits of hard labour: Diet metabarcoding and telemetry reveal that urban songbirds travel further for lower-quality food. *Oecologia, 193*(2), 377–388. https://doi.org/10.1007/s00442-020-04678-w

Jones, S. E., Lane, J. M., Wood, A. R., van Hees, V. T., Tyrrell, J., Beaumont, R. N., Jeffries, A. R., Dashti, H. S., Hillsdon, M., Ruth, K. S., Tuke, M. A., Yaghootkar, H., Sharp, S. A., Jie, Y., Thompson, W. D., Harrison, J. W., Dawes, A., Byrne, E. M., Tiemeier, H., & Weedon, M. N. (2019). Genome-wide association analyses of chronotype in 697,828 individuals provides insights into circadian rhythms. *Nature Communications, 10*, 343. https://doi.org/10.1038/s41467-018-08259-7

Kacelnik, A. (1979). The foraging efficiency of great tits (*Parus major* L.) in relation to light intensity. *Animal Behaviour, 27*, 237–241. https://doi.org/10.1016/0003-3472(79)90143-X

Kempenaers, B., Borgström, P., Loës, P., Schlicht, E., & Valcu, M. (2010). Artificial night lighting affects dawn song, extra-pair siring success, and lay date in songbirds. *Current Biology, 20*, 1735–1739. https://doi.org/10.1016/j.cub.2010.08.028

Kronfeld-Schor, N., & Dayan, T. (2003). Partitioning of time as an ecological resource. *Annual Review of Ecology, Evolution, and Systematics, 34*, 153–181. https://doi.org/10.1146/annurev.ecolsys.34.011802.132435

Lehmann, M., Spoelstra, K., Visser, M. E., & Helm, B. (2012). Effects of temperature on circadian clock and chronotype: An experimental study on a passerine bird. *Chronobiology International, 29*(8), 1062–1071. https://doi.org/10.3109/07420528.2012.707159

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software, 6*(60), 3139.

Mace, R. (1985). Importance of female behaviour in the dawn chorus. *Animal Behaviour, 34*(2), 621–622.

Martorell-Barceló, M., Campos-Candela, A., & Alós, J. (2018). Fitness consequences of fish circadian behavioural variation in exploited marine environments. *PeerJ, 6*, Article e4814. https://doi.org/10.7717/peerj.4814

Maury, C., Serota, M. W., & Williams, T. D. (2020). Plasticity in diurnal activity and temporal phenotype during parental care in European starlings, *Sturnus vulgaris*. *Animal Behaviour, 159*, 37–45. https://doi.org/10.1016/j.anbehav.2019.11.004

McGlade, C. L. O., Capilla-Lasheras, P., Womack, R. J., Helm, B., & Dominoni, D. (2023). Experimental light at night explains differences in activity onset between urban and forest Great tits. *Biology Letters*. https://doi.org/10.1098/rsbl.2023.0194

McNamara, J. M., Houston, A. I., & Lima, S. L. (1994). Foraging routines of small birds in winter: A theoretical investigation. *Journal of Avian Biology, 25*(4), 287–302. https://www.jstor.org/stable/3677276.

McNamara, J. M., Mace, R. H., & Houston, A. I. (1987). Optimal daily routines of singing and foraging in a bird singing to attract a mate. *Behavioral Ecology and Sociobiology, 20*, 399–405. https://doi.org/10.1007/BF00302982

Meijdam, M., Müller, W., Thys, B., & Eens, M. (2022). No relationship between chronotype and timing of breeding when variation in daily activity patterns across the breeding season is taken into account. *Ecology and Evolution, 12*, Article e9353. https://doi.org/10.1002/ece3.9353

Menaker, M., & Eskin, A. (1966). Entrainment of circadian rhythms by sound in *Passer domesticus*. *Science, 154*, 1579–1581.

Miller, M. W. (2006). Apparent effects of light pollution on singing behaviour of American robins. *Condor, 108*, 130–139. https://academic.oup.com/condor/article/108/1/130/5563689.

Murphy, M. T., Sexton, K., Dolan, A. C., & Redmond, L. J. (2008). Dawn song of the eastern kingbird: An honest signal of male quality? *Animal Behaviour, 75*, 1075–1084. https://doi.org/10.1016/j.anbehav.2007.08.020

Nord, A., & Cooper, C. B. (2020). Night conditions affect morning incubation behaviour differently across a latitudinal gradient. *Ibis, 162*, 827–835. https://doi.org/10.1111/ibi.12804

Pagani-Núñez, E., & Senar, J. C. (2016). More ornamented great tit *Parus major* fathers start feeding their offspring earlier. *Ardea, 104*, 167–175. https://doi.org/10.5253/arde.v104i2.a1

Pollock, C. J., Capilla-Lasheras, P., McGill, R. A. R., Helm, B., & Dominoni, D. M. (2017). Integrated behavioural and stable isotope data reveal altered diet linked to low breeding success in urban-dwelling blue tits (*Cyanistes caeruleus*). *Scientific Reports, 7*, 5014. https://doi.org/10.1038/s41598-017-04575-y

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org/.

Roenneberg, T., Kumar, C. J., & Merrow, M. (2007). The human circadian clock entrains to sun time. *Current Biology, 17*(2), 44–45. https://doi.org/10.1016/j.cub.2006.12.011

Roenneberg, T., Pilz, L. K., Zerbini, G., & Winnebeck, E. C. (2019). Chronotype and social jetlag: A (self-) critical review. *Biology, 8*, 54. https://doi.org/10.3390/biology8030054

Roenneberg, T., Wirz-Justice, A., & Merrow, M. (2003). Life between clocks: Daily temporal patterns of human chronotypes. *Journal of Biological Rhythms, 18*, 80–90. https://doi.org/10.1177/0748730402239679

Shaw, P., & Cresswell, W. (2014). Latitudinal variation in day length and working day length has a confounding effect when comparing nest attentiveness in tropical and temperate species. *Journal of Ornithology, 155*, 481–489. https://doi.org/10.1007/s10336-013-1029-1

Stevenson, T. J., & Kumar, V. (2017). Neural control of daily and seasonal timing of songbird migration. *Journal of Comparative Physiology, 203*, 399–409. https://doi.org/10.1007/s00359-017-1193-5

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution, 8*, 1639–1644. https://doi.org/10.1111/2041-210X.12797

Stuber, E. F., Dingemanse, N. J., Kempenaers, B., & Mueller, J. C. (2015). Sources of intraspecific variation in sleep behaviour of wild great tits. *Animal Behaviour, 106*, 201–221. https://doi.org/10.1016/j.anbehav.2015.05.025

Tudorache, C., Slabbekoorn, H., Robbers, Y., Hin, E., Meijer, J. H., Spaink, H. P., & Schaaf, M. J. M. (2018). Biological clock function is linked to proactive and reactive personality types. *BMC Biology, 16*, 148. https://doi.org/10.1186/s12915-018-0618-0

Vitale, J. A., & Weydahl, A. (2017). Chronotype, physical activity, and sport performance: A systematic review. *Sports Medicine, 47*, 1859–1868. https://doi.org/10.1007/s40279-017-0741-z

Appendix

Table A1
Sample sizes in urban and forest habitats across the 3 years of study for each trait investigated

Trait	Year	Urban	Forest	Units
Onset of activity	2016	14 (4)	216 (30)	Days of observation (no. of clutches)
	2017	103 (11)	203 (33)	Days of observation (no. of clutches)
	2018	89 (12)	104 (12)	Days of observation (no. of clutches)
End of activity	2016	14 (4)	209 (30)	Days of observation (no. of clutches)
	2017	102 (11)	201 (33)	Days of observation (no. of clutches)
	2018	84 (12)	97 (12)	Days of observation (no. of clutches)
Duration of activity	2016	14 (4)	209 (30)	Days of observation (no. of clutches)
	2017	102 (11)	201 (33)	Days of observation (no. of clutches)
	2018	84 (12)	97 (12)	Days of observation (no. of clutches)
Nestling survival	2016	4	30	No. of clutches
	2017	11	33	No. of clutches
	2018	11	12	No. of clutches
Fledgling weight	2016	4	24	No. of broods
	2017	6	10	No. of broods
	2018	5	8	No. of broods

Table A2

Likelihood ratio test (LRT) results and model coefficients for predictors explaining variation in female clock time end of activity (i.e. time of last daily incubation on-bout)

Fixed effect	Estimate	SE	95% CI	χ^2	*df*	*P*
Intercept	1,155.28	12.71	1,130.38, 1,180.18			
Incubation start date^2	−32.84	62.95	−156.21, 90.54	0.27	1	0.603
Incubation start date^1	−270.15	69.80	−406.95, −133.34	9.08	1	**0.003**
Days before hatching^2	19.59	34.98	−48.97, 88.15	0.31	1	0.576
Days before hatching^1	105.38	70.80	−33.39, 244.15			
Habitat						
Urban	–	–	–			
Forest	20.90	6.79	7.60, 34.20			
Mean daily temperatures	−0.07	0.62	−1.28, 1.14	0.01	1	0.904
Clutch size	−2.59	1.43	−5.40, 0.23	3.23	1	0.072
Days before hatching^1*Habitat				15.93	1	**<0.001**
Days before hatching^1*Forest	−342.66	85.19	−509.63, −175.68			

Model coefficients (‘Estimate’) for clock time end of activity (model coefficients are given in min after 0000 hours) are shown along with standard errors and 95% confidence intervals (95% CI). Superscripts ‘1’ and ‘2’ refer to linear and quadratic terms, respectively. $N = 707$ days of incubation. Significant results are highlighted in bold. LRT results for ‘Habitat’ and ‘Days before hatching^1’ are not provided as these terms were part of a significant interaction present in the model. The interactions ‘Incubation start date^2 × Habitat’ ($\chi^2_1 = 0.60$, $P = 0.440$), ‘Incubation start date^1*Habitat’ ($\chi^2_1 = 0.13$, $P = 0.719$) and ‘Days before hatching^2 × Habitat’ ($\chi^2_1 = 1.84$, $P = 0.175$) were not significant and were dropped from the model.

Table A3

Likelihood ratio test (LRT) results and model coefficients for predictors explaining variation in the duration of the active day of incubating great tit females, calculated from clock time (i.e. time of last daily incubation on-bout minus time of first daily incubation off-bout

Fixed effect	Estimate	SE	95% CI	χ^2	*df*	*P*
Intercept	828.65	13.79	801.62, 855.68			
Incubation start date^2	−144.54	66.08	−274.05, −15.02	4.71	1	**0.030**
Incubation start date^1	−32.34	75.67	−180.66, 115.97	0.18	1	0.670
Days before hatching^2	67.10	40.34	−11.97, 146.17	2.76	1	0.097
Days before hatching^1	153.84	81.12	−5.15, 312.84			
Habitat						
Urban	–	–	–			
Forest	14.30	7.11	0.36, 28.24			
Mean daily temperatures	−0.60	0.71	−1.99, 0.79	0.72	1	0.396
Clutch size	−2.59	1.52	−5.56, 0.39	2.90	1	0.088
Days before hatching^1*Habitat				23.22	1	**<0.001**
Days before hatching^1*Forest	−473.36	97.43	−664.32, −282.41			

Model coefficients (‘Estimate’) are shown along with standard errors and 95% confidence intervals (95% CI). Superscripts ‘1’ and ‘2’ refer to linear and quadratic terms, respectively. $N = 707$ days of incubation. Significant results are highlighted in bold. LRT results for ‘Habitat’, ‘Incubation start date^1’ and ‘Days before hatching^1’ are not provided as these terms were part of a significant interaction present in the model. The interactions ‘Incubation start date^2*Habitat’ ($\chi^2_1 = 0.25$, $P = 0.616$), ‘Incubation start date^1*Habitat’ ($\chi^2_1 = 1.979$, $P = 0.160$) and ‘Days before hatching^2*Habitat’ ($\chi^2_1 = 0.756$, $P = 0.385$) were not significant and were dropped from the model.

Table A4

Likelihood ratio test (LRT) results and model coefficients for predictors explaining variation in female relative end of activity (i.e. time of last incubation on-bout minus sunset time)

Fixed effect	Estimate	SE	95% CI	χ^2	*df*	*P*
Intercept	−118.84	12.90	−144.12, −93.55			
Incubation start date^2	−29.95	64.22	−155.82, 95.93	0.20	1	0.658
Incubation start date^1	−520.10	73.64	−664.44, −375.76	30.44	1	**<0.001**
Days before hatching^2	21.25	35.15	−47.64, 90.15	0.36	1	0.548
Days before hatching^1	−80.29	71.23	−219.90, 59.32			
Habitat						
Urban	–	–	–			
Forest	13.64	7.05	−0.18, 27.46			
Mean daily temperatures	−0.10	0.62	−1.32, 1.12	0.02	1	0.875
Clutch size	−2.65	1.45	−5.49, 0.18	3.33	1	0.068
Days before hatching^1*Habitat				14.36	1	**<0.001**
Days before hatching^1*Forest	−328.15	85.67	−496.07, −160.24			

Model coefficients (‘Estimate’) for relative time of end of activity (model coefficients are given in min after sunset) are shown along with standard errors and 95% confidence intervals (95% CI). Superscripts ‘1’ and ‘2’ refer to linear and quadratic terms, respectively. $N = 707$ days of incubation. Significant results are highlighted in bold. LRT results for ‘Habitat’ and ‘Days before hatching^1’ are not provided as these terms were part of a significant interaction present in the model. The interactions ‘Incubation start date^2*Habitat’ ($\chi^2_1 = 0.35$, $P = 0.557$), ‘Incubation start date^1*Habitat’ ($\chi^2_1 = 0.35$, $P = 0.553$) and ‘Days before hatching^2*Habitat’ ($\chi^2_1 = 1.96$, $P = 0.162$) were not significant and were dropped from the model.

Table A5

Likelihood ratio test (LRT) results and model coefficients for clock time female chronotype and other predictors hypothesized to explain variation in nestling survival to fledging

Fixed effect	Estimate	SE	95% CI	χ^2	*df*	*P*
Intercept	0.18	0.27	−0.35, 0.70			
Hatching date^1	−0.49	0.86	−2.17, 1.20	0.32	1	0.572
Hatching date^2	−0.60	0.59	−1.74, 0.55	1.07	1	0.300
Clock female chronotype	−0.01	0.06	−0.13, 0.10	0.06	1	0.801
Habitat				4.32	1	**0.038**
Urban	–	–	–			
Forest	0.36	0.17	0.02, 0.69			
Clutch size	0.14	0.03	0.08, 0.21	17.04	1	**<0.001**

Model coefficients (‘Estimate’) are shown in their link scale (logit) along with standard errors and 95% confidence intervals (95% CI). Superscripts ‘1’ and ‘2’ refer to linear and quadratic terms, respectively. $N = 101$ broods. Significant results are highlighted in bold.

Table A6

Likelihood ratio test (LRT) results and model coefficients for predictors explaining variation in total brood failure (i.e. probability that no nestling survives to fledging)

Fixed effect	Estimate	SE	95% CI	χ^2	*df*	*P*
Intercept	−0.72	1.37	−3.41, 1.97			0.598
Hatching date^1	−3.31	4.38	−11.89, 5.26	0.58	1	0.447
Hatching date^2	−1.78	3.35	−8.33, 4.78	0.30	1	0.587
Female chronotype	0.29	0.30	−0.30, 0.88	0.94	1	0.333
Habitat				1.18	1	0.277
Urban	–	–	–			
Forest	−0.86	0.78	−2.39, 0.67			
Clutch size	−0.05	0.19	−0.41, 0.31	0.07	1	0.796

Model coefficients (‘Estimate’) are shown along with standard errors and 95% confidence intervals (95% CI). Superscripts ‘1’ and ‘2’ refer to linear and quadratic terms, respectively. $N = 101$ broods.

Table A7

Likelihood ratio test (LRT) results and model coefficients for predictors explaining variation in the number of nestlings that survived to fledging, after excluding broods in which no nestling fledged (i.e. excluding total failure broods)

Fixed effect	Estimate	SE	95% CI	χ^2	*df*	*P*
Intercept	−0.41	0.73	−1.85, 1.02			
Hatching date^1	−3.07	1.67	−6.34, 0.20	3.32	1	0.068
Hatching date^2	−1.08	1.41	−3.85, 1.68	0.59	1	0.443
Female chronotype	−0.34	0.16	−0.65, −0.03	4.45	1	**0.035**
Habitat				3.67	1	0.055
Urban	–	–	–			
Forest	0.89	0.46	−0.01, 1.78			
Clutch size	0.73	0.09	0.55, 0.92	45.81	1	**<0.001**

Model coefficients (‘Estimate’) are shown along with standard errors and 95% confidence intervals (95% CI). Superscripts ‘1’ and ‘2’ refer to linear and quadratic terms, respectively. $N = 82$ broods. Significant results are highlighted in bold.

Figure A1. iButton set-up for incubation data collection. (a) Preprogrammed iButton wrapped in fabric and attached to wire (weight 28.35 g) and iButton field label. (b) Detail of a nest cup with the iButton device positioned among great tit eggs.

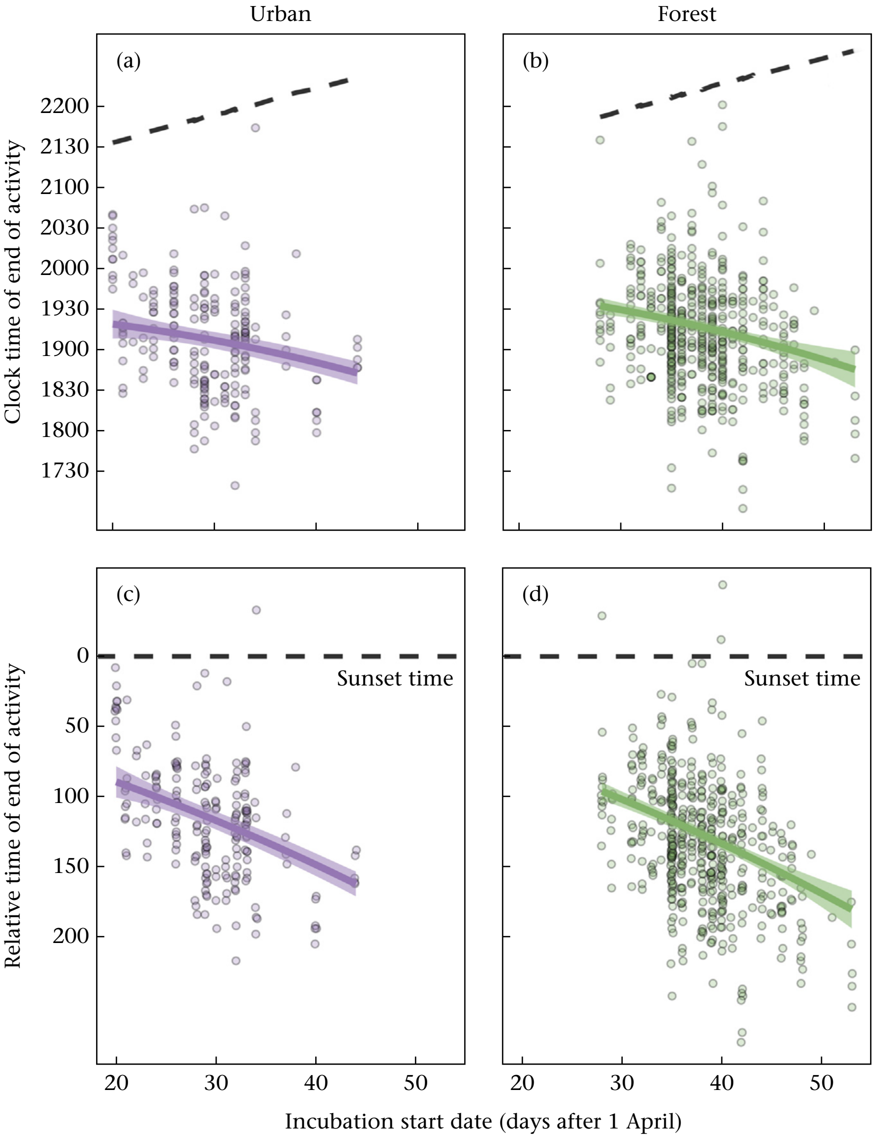

Figure A2. (a, b) Clock and (c, d) relative end of activity (i.e. time of the last incubation on-bout minus sunset time; positive values indicate minutes after sunset, whereas negative values indicate minutes before sunset) for incubating (a, c) urban and (b, d) forest female great tits throughout the breeding season. Points represent data, while thick solid lines and shaded areas provide mean model predictions ± 1 SE. Dashed lines indicate sunset time.

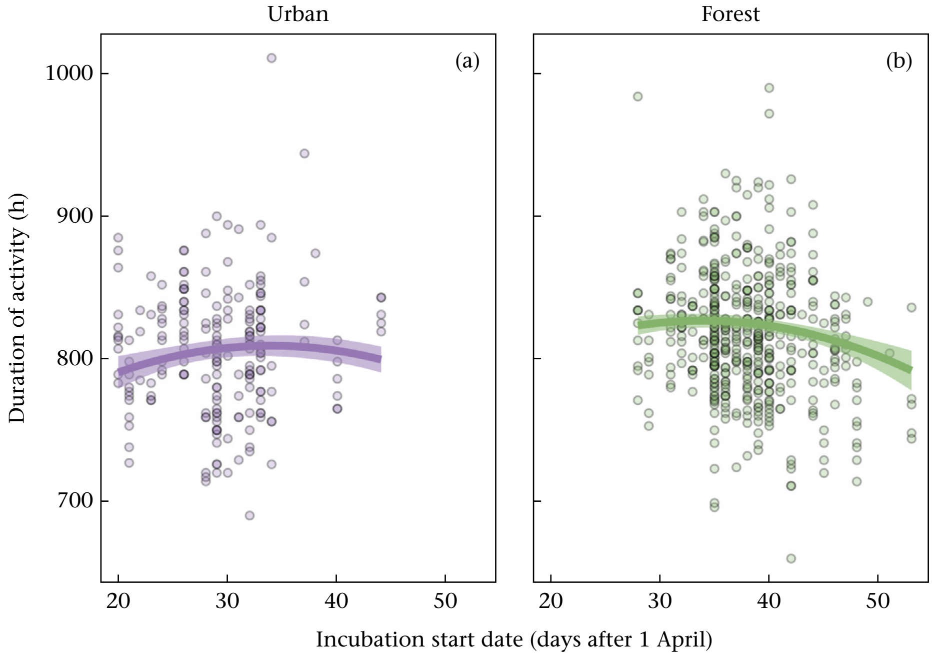

Figure A3. Duration of the active day (i.e. time difference between the first incubation off-bout and the last incubation on-bout per day) in incubating (a) urban and (b) forest female great tits throughout the breeding season. Points represent data, while thick solid lines and shaded areas provide mean model predictions ± 1 SE (see model coefficients in Table A4).

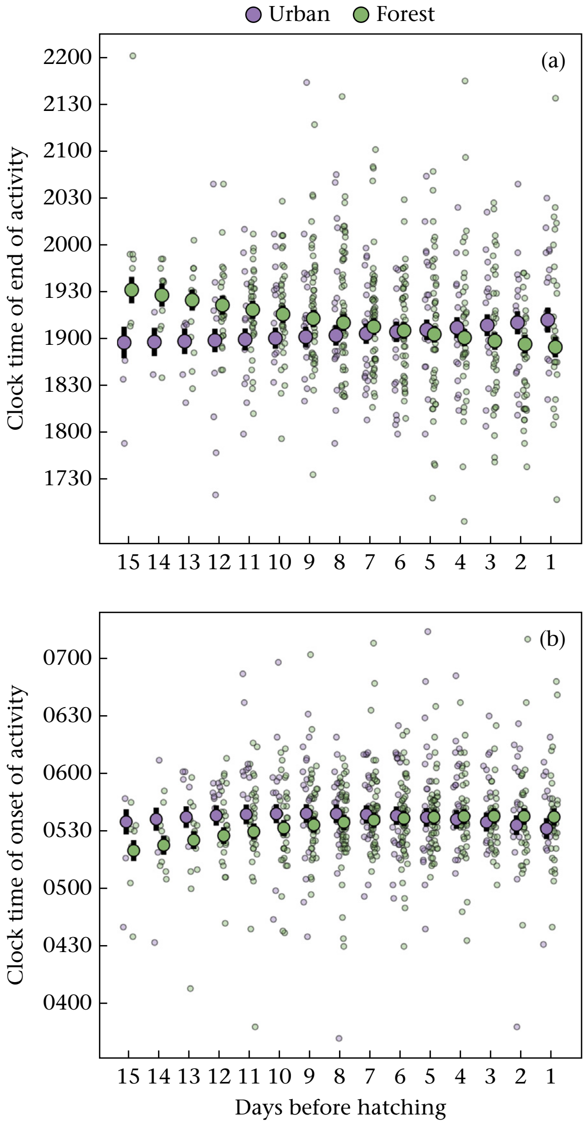

Figure A4. (a) Clock end of activity and (b) clock onset of activity (i.e. time of the last incubation on-bout and time of the first incubation off-bout) per day of incubation (from 15 to 1 days before hatching; '0' would indicate day of hatching) for urban and forest female great tits. Small transparent points represent data, while large solid points and bars provide mean model predictions ± 1 SE for each day of incubation (see model coefficients in Tables 1 and A2).

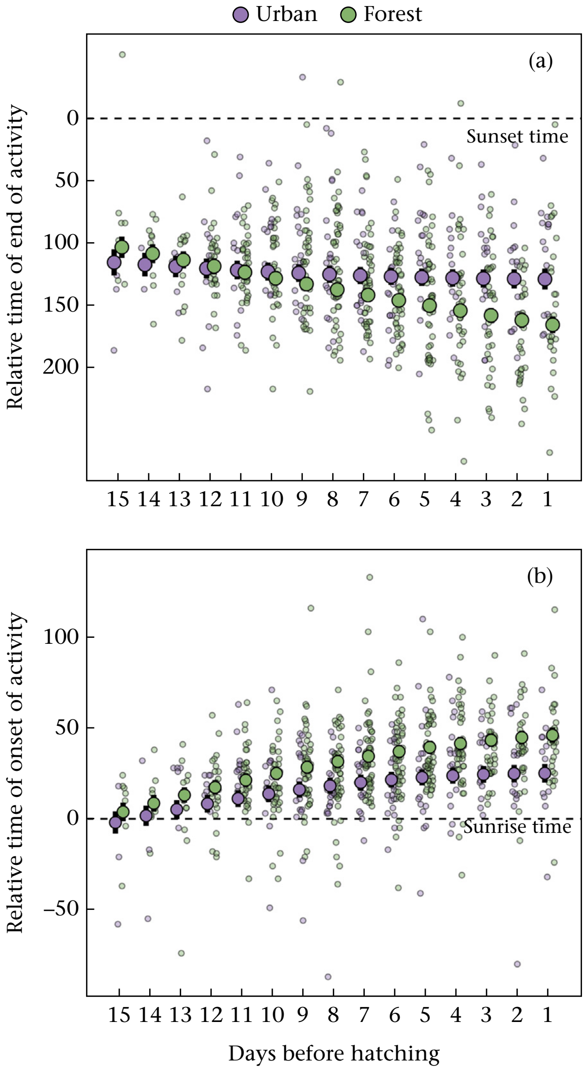

Figure A5. (a) Relative end and (b) relative onset of activity (i.e. time of the last incubation on-bout minus sunset time, and time of the first incubation off-bout minus sunrise time; positive values indicate minutes after sunset/sunrise, whereas negative values indicate minutes before sunset/sunrise) per day of incubation (from 15 to 1 days before hatching; '0' would indicate day of hatching) for urban and forest female great tits. Small transparent points represent data, while large solid points and bars provide mean model predictions ± 1 SE for each day of incubation (see model coefficients in Tables 1 and A3). Dashed lines indicate sunset time.