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Reproductive fitness is associated with female chronotype in a songbird

Authors:

Robyn J. Womack[1#], Pablo Capilla-Lasheras[1#@], Ciara L. O. McGlade[2], Davide M. Dominoni[1], Barbara Helm[1, 3]

[#]: joint first authors

[@]: corresponding author - pacapilla@gmail.com

Affiliations:

[1] Institute of Biodiversity, Animal Health & Comparative Medicine, Graham Kerr Building, University of Glasgow, G12 8QQ Glasgow, UK

[2] School of Biological Sciences, Queen's University Belfast, BT9 5DL Belfast, UK

[3] Swiss Ornithological Institute, Seerose 1, 6204 Sempach, Switzerland

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Abstract

Research on biological rhythms has revealed widespread variation in 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 fitness. In both habitats, females that started activity earlier in the day raised more fledglings. We also observed that forest females started their day at similar times 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.

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Introduction

Due to the rotation of the Earth around its axis, no environments are completely constant across the 24 hour day. Hence, for organisms, appropriate diel timing of activities and physiology relative to environmental cycles is thought to be important for fitness [1]. Still, inter-individual differences in diel activities can be large, whereby individuals display highly consistent temporal phenotypes called "chronotype" [2–4]. Chronotype, first defined for laboratory rodents [5], has gained major research importance in human studies, where millions of subjects have been scored [6]. Human chronotype has been associated with genetic variants (e.g., in clock genes), performance and, physical and mental health [2,7]. For example, in athletes, performance depends on chronotype and can be enhanced by modified wake-up time [8].

Interest in chronotype is also rapidly increasing in ecology and evolution [3,4,9], fuelled by remote and automated tracking technology (e.g., transmitters or on-site loggers [9–11]). 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 inter-individual variation [4,12,13]. Still, our understanding of causes and consequences of chronotype in free-living animals has been hindered by shortcomings when assessing chronotype, and by bias in the sex and traits investigated.

First, disentangling contributing factors to variation in chronotype requires engaging with the complexity of diel timing, which is based on circadian rhythms in interaction with light, further environmental features and state-dependent modifiers [4]. Chronotype is broadly defined as consistent timing of an individual's rhythmic features (e.g., activity onset), relative to an external reference and to conspecifics measured under similar conditions [4]. Choice of temporal reference is challenging. Human studies usually refer to a fixed time of day (hereafter called 'absolute time') [2,7]. In contrast, because

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animals often track natural light environments [14], most ecological studies refer to annually and spatially variable aspects of the solar day (e.g., sunrise; hereafter called 'relative time') [9,11]. However, animals typically change their relationship to these solar features over time and space [15] so that, for example, latitude, daylength and life-cycle stage can bias or alter findings [16,17]. Therefore, variation in both absolute and relative timing of activities should be investigated in parallel to understand variation in chronotype in the wild.

Secondly, investigation of fitness effects must be broadened in scope. Fitness studies on wild chronotypes have mostly focused on males (but see [9]), partly due to extra-pair mating and to conspicuous features such as courtship, song and ornaments [12,18]. For example, avian observational and experimental studies suggest that early-active males may sire more extra-pair young than late-active males [19,20], and that such differences could be based on endogenous circadian clocks [21]. However, females are disproportionately more involved in reproductive activities [22], and mating represents but a fraction of factors that shape the fitness landscape of chronotype. 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 for implications of female chronotype, and for diverse life-cycle stages.

To investigate fitness implications of chronotype, birds offer excellent study opportunities because their behaviours and reproductive outcomes are unusually tractable. Therefore, in this study, we leverage data from wild birds to i) disentangle particular factors that contribute to explain variation in chronotype, and ii) newly document links between chronotype and reproductive fitness. In a well-studied songbird, the great tit (*Parus major*), we inferred female chronotype through measuring behaviour during incubation, a critically important post-zygotic stage of avian reproduction, while

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monitoring reproductive success [9,11,23,24]. Because features of the environment can influence chronotype, we also included data from two habitat types, urban and forest, which often affect the diel rhythm of animals [10,25].

Great tits are female-only intermittent incubators, who spend nights on their nest, but at daytime alternate between nest attendance (i.e., on-bouts) and foraging outings (i.e., off-bouts) [26]. From small temperature loggers (i.e., iButtons) inserted into nests of urban and forest-breeding great tits, we first infer incubation chronotype of females for both absolute and relative time, and assess consistency of chronotype across the breeding season. As our measure of chronotype, we focus on activity onset (inferred from the first incubation off-bout of the day), which in birds is particularly robust and sometimes associated with male fitness [10–12,18,20], but we also report end and duration of activity. Secondly, we link incubation chronotype to reproductive outcomes from these same nests to test associations between fitness and female chronotype. Our research spans three years and multiple breeding locations in Scotland, ranging from oak forests to urban settings.

Results

Correlates and consistency of incubating female chronotype

We recorded nest temperatures in 2016, 2017 and 2018, and analysed 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 S11). 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: $mean_{urban}$ ± SE = 30th April ± 1.09 days; N_{urban} = 27 clutches; $mean_{forest}$ ± SE = 8th May ± 0.61 days; N_{forest} = 75

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clutches). Therefore, we detail results separately for the two habitats, although all data were analysed in overarching models.

We found that, on population level, absolute time of activity onset was affected by habitat and by the date when incubation started (interaction 'Incubation start date × habitat': $\chi^2_{df=1} = 11.41$, $p = 0.001$; Table 1; Figure 1a & 1b). Urban females closely tracked the seasonally advancing sunrise time (Figure 1a), but forest females largely ignored this advance and rose at similar absolute time throughout the season (Figure 1b). Whereas early-breeding urban females started activity at a later absolute time than forest females, for late-breeding birds the pattern reversed, so that urban females started their day earlier than forest females (Figure 1a & 1b). In contrast, the mean end of activity was similar in both habitats and became progressively earlier with later incubation start date (Figure S1; Table S1 -S4). Overall, the active day lengthened over the breeding season for urban but shortened for forest females (Figure S2 & Table S5 & S6). Time left until hatching also affected timing, whereas ambient temperature and clutch size did not (Tables 1, S1 -S6).

Relative to sunrise, females started their day on average 28.5 min after sunrise (SE = 0.91 min; range = [-87 min, 137 min]), but onset of activity depended on habitat and on the date when incubation started (Figure 1c-1d; 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': $\chi^2_{df=1} = 11.44$, $p = 0.001$; Figure 1c-1d). The end of activity relative to sunset advanced consistently over the season in both habitats ('Incubation start date': $\chi^2_{df=1} = 36.78$, $p = 0.001$; Figure S1c-S1d; Table S3 & S4). Effects of the time left until hatching, ambient temperature and clutch size were similar to those for absolute time (Tables 1, S1 - S6).

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The population pattern described above was broadly matched by temporal variation within clutches (i.e., variation between the first and last day of incubation of a clutch; Tables 1-2; Figures S3-S4). We identified consistent individual differences in the time of onset of activity (i.e., female chronotype). Among-female differences explained 31 % of the variation in absolute onset time (LRT on the nest-box ID random effect: $\chi^2_{df = 1}$ = 134.30, $p < 0.0001$; among-female repeatability [95%CI] = 0.31 [0.21, 0.40]). Analyses of relative onset time (i.e., correcting for changes in sunrise time) yielded similar results, with consistent among-female differences in onset of activity (repeatability [95%CI] = 0.32 [0.22, 0.41]). We also found consistent among-female differences in the end of activity, both in absolute (repeatability [95%CI] = 0.25 [0.16, 0.34]) and relative end of activity (repeatability [95%CI] = 0.25 [0.17, 0.34]); and consistent among-female differences in the duration of the active day (repeatability [95%CI] = 0.20 [0.11, 0.28]).

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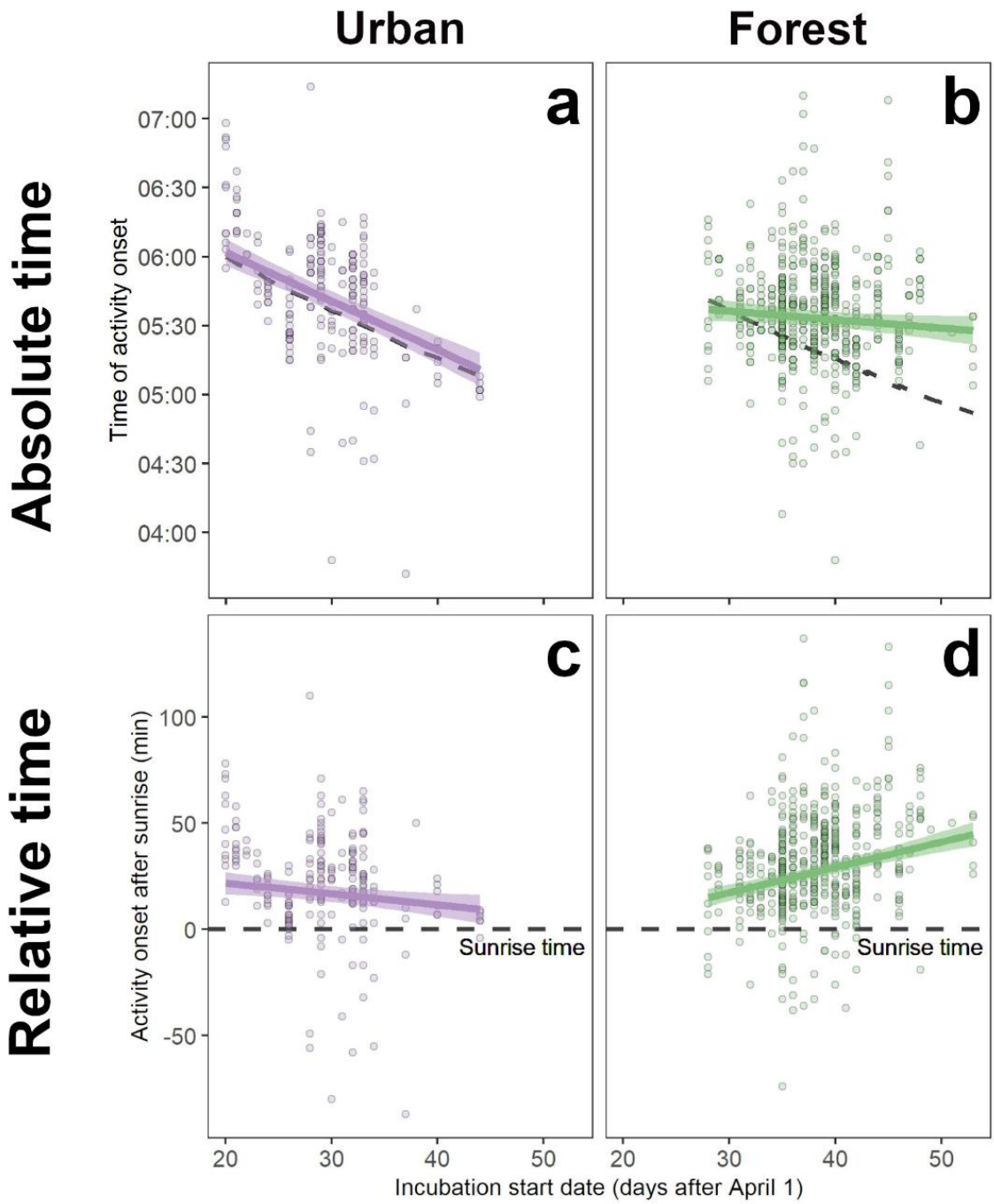

Figure 1. Mean absolute and relative time of activity onset in urban and forest great tit females. (a) On a population level, urban females advanced their absolute time of activity onset throughout the breeding season, seemingly tracking the seasonal advance of sunrise time (dashed line); whereas (**b**) the mean onset of activity of forest females remained relatively constant throughout the breeding season. Consequently, (**c**) urban females modified their onset of activity relative to sunrise over the breeding season only slightly, while forest females started their activity progressively later relative to sunrise. Points represent raw data, where each point shows the mean timing of one female, while thick solid lines and shaded areas provide mean model predictions ± 1 SE (see model coefficients in Tables 1 and 2).

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Table 1. (a) Likelihood-ratio test (LRT) results for predictors explaining variation in absolute time of female onset of activity (i.e., time of first incubation off-bout). Significant terms are highlighted in bold and italic type. 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 ('Incubation start Date[1] × Habitat' and 'Days before hatching[1] × Habitat'). The significance of these three terms is given by the LRT results of the interactions. **(b)** Minimal adequate model coefficients (i.e., after model simplification using likelihood-ratio tests) for absolute onset of activity (model coefficients are given in min after 00:00 h). Model coefficients ('Estimate') are shown along with standard errors ('SE') and 95% confidence intervals ('95% CI' obtained using 500 parametric bootstrap simulations of the model). 'df' = degrees of freedom for LRTs. Superscripts '[1]' and '[2]' refer to linear and quadratic terms, respectively; reference level for Habitat is 'urban'.

Absolute onset of activity (N = 729 days of incubation)

(a) Likelihood-ratio test results

	χ^2	Df	P
Incubation start date[1] × Habitat	***11.41***	***1***	***0.001***
Incubation start date[2] × Habitat	0.82	1	0.364
Days before hatching[1] × Habitat	***7.52***	***1***	***0.006***
Days before hatching[2] × Habitat	1.55	1	0.213
Habitat	-	-	-
Incubation start date [1]	-	-	-
Incubation start date[2]	0.01	1	0.938
Days before hatching[1]	-	-	-
Days before hatching[2]	***4.78***	***1***	***0.029***
Clutch size	0.01	1	0.935
Mean daily temperatures	1.87	1	0.171

(b) Model coefficients

Fixed effects	**Estimate**	**SE**	**95% CI**	
Intercept	329.07	4.243	320.60	337.47
Habitat (forest)	7.367	4.535	-2.01	16.74
Incubation start date [1]	-360.4	66.238	-495.40	-231.50
Days before hatching[2]	-43.42	19.817	-81.98	-7.48
Days before hatching[1]	-33.25	39.318	-112.30	46.60
Incubation start date[1] × Habitat (forest)	293.83	83.825	135.80	459.87
Days before hatching[1] × Habitat (forest)	130.97	47.35	35.57	219.11

Table 2. (**a**) Likelihood-ratio test (LRT) results for predictors explaining variation in relative time of female onset of activity (i.e., time of first incubation off-bout minus

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sunrise time). Significant terms are highlighted in bold and italic type. (**b**) Minimal adequate model coefficients (i.e., after model simplification using likelihood-ratio tests) for relative onset of activity. Model coefficients ('Estimate') are shown along with standard errors ('SE') and 95% confidence intervals ('95% CI' obtained using 500 parametric bootstrap simulations of the model). 'df' = degrees of freedom for LRTs. Superscripts '1' and '2' refer to linear and quadratic terms, respectively; reference level for Habitat is 'urban'.

Relative onset of activity (N = 729 days of incubation)

(a) Likelihood-ratio test results

	χ^2	Df	P
Incubation start Date^1 × Habitat	***11.44***	***1***	***0.001***
Incubation start Date^2 × Habitat	1.67	1	0.197
Days before hatching^1 × Habitat	***3.93***	***1***	***0.048***
Days before hatching^2 × Habitat	1.09	1	0.297
Habitat	-	-	-
Incubation start Date^1	-	-	-
Incubation start Date^2	0.3	1	0.584
Days before hatching^1	-	-	-
Days before hatching^2	***6.31***	***1***	***0.012***
Clutch size	< 0.01	1	0.98
Mean daily temperatures	1.49	1	0.222

(b) Model coefficients

Fixed effects	**Estimate**	**SE**	**95% CI**	
Intercept	42.44	11.75	17.95	66.04
Habitat (forest)	-42.79	17.00	-76.02	-9.37
Incubation start date^1	-0.514	0.38	-1.28	0.26
Days before hatching^2	-0.131	0.05	-0.23	-0.03
Days before hatching^1	-0.068	0.84	-1.68	1.50
Incubation start date^1 × Habitat (forest)	1.71	0.49	0.76	2.68
Days before hatching^1 × Habitat (forest)	0.991	0.48	0.07	1.96

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Effects of female chronotype on fledging success and pre-fledging weight

We detected substantial variation between broods in the number of nestlings that survived to fledging. Female chronotype, derived from activity onset relative to sunrise, predicted the number of surviving nestlings: the earlier the female chronotype, the more nestlings fledged ($\chi^2_{df=1} = 5.49$, $p = 0.019$; Figure 2a; Table 3a). This effect was consistent across habitats (interaction between female chronotype and habitat, $\chi^2_{df=1} = 0.43$, $p = 0.513$; Table 3a) and was robust to controlling for clutch size ($\chi^2_{df=1} = 19.31$, $p < 0.0001$; Table 3a). The number of surviving chicks was also strongly affected by habitat ($\chi^2_{df=1} = 6.82$, $p = 0.009$; Table 3a; urban females fledged 0.66 nestlings less than forest females (i.e., a decrease in surviving nestlings of 48%; Table 4a). Conversely, absolute chronotype of females did not predict the number of surviving nestlings (Table S7).

To investigate the breeding parameters that could have generated the decreasing reproductive success with later female chronotype relative to sunrise, we performed two additional analyses. Firstly, we assessed whether female chronotype was associated with total brood failure, and found no support (effect of female chronotype on the probability that no nestling survives to fledging: $\chi^2_{df=1} = 0.51$, $p = 0.475$; Table S8). Secondly, we tested whether 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 chronotypes fledged more offspring than later chronotypes ($\chi^2_{df=1} = 7.03$, $p = 0.008$; Table S9 & S10).

Mean body mass of 13-day old nestlings was affected by habitat, hatching date and clutch size. Forest nestlings were on average 2.73g (95%CI = [1.66, 3.86]; Table 3b; Table 4b) heavier than urban nestlings of the same age. In both habitats, pre-fledging weight was higher for broods that hatched in the middle of the season (Figure 2b), and nestlings from larger clutches were on average lighter ($\chi^2_{df=1} = 4.10$, $p = 0.043$; Table 3b; Table 4b).

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Neither relative nor absolute female chronotype affected pre-fledging weight in either habitat (relative chronotype: $\chi^2_{df = 1} = 0.20$, $p = 0.656$; absolute chronotype: $\chi^2_{df = 1} = 0.31$, $p = 0.580$; interaction terms between chronotype and habitat were non-significant, for relative chronotype: $\chi^2_{df = 1} < 0.01$, $p = 0.939$; for absolute chronotype: $\chi^2_{df = 1} < 0.01$, $p = 0.938$).

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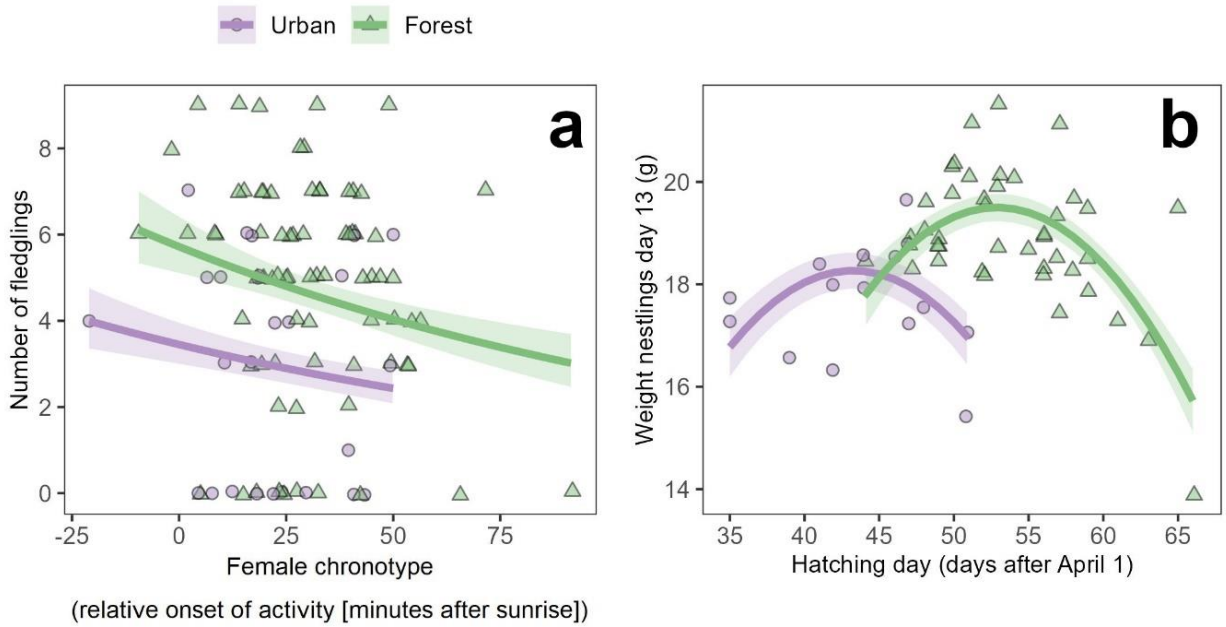

Figure 2. Effects of relative female chronotype and seasonal time on pre-fledging survival and weight. (**a**) Both in urban and forest nests, progressively later chronotype of the breeding female was negatively associated with the number of nestlings that survived to fledgling (N = 101 broods; Table 3a and 4a). **(b)** Urban nestlings on day 13 of their life were lighter than forest nestlings (N = 57 broods; Table 3b and 4b). Variation in the weight of 13-day old nestlings showed a similar seasonal pattern in forest and city habitats. Points represent raw data, while thick lines and shaded areas provide mean model predictions ± 1 SE (Table 4).

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Table 3. Likelihood-ratio test (LRT) results for relative female chronotype and further predictors explaining variation in (**a**) nestling survival to fledging and (**b**) weight of 13-day old nestlings. Significant terms are highlighted in bold and italic type. 'df' = degrees of freedom for LRTs. Superscripts '1' and '2' refer to linear and quadratic terms, respectively.

a Number of nestlings that survived to fledging (N = 101 broods)

	χ^2	df	P
Hatching date^1 × Habitat	0.02	1	0.885
Hatching date^2 × Habitat	0.03	1	0.865
Habitat	***6.82***	***1***	***0.009***
Relative female chronotype × Habitat	0.43	1	0.513
Relative female chronotype	***5.49***	***1***	***0.019***
Hatching date^1	0.08	1	0.778
Hatching date^2	0.40	1	0.527
Clutch size	***19.31***	***1***	***<0.001***

b Pre-fledging weight of nestlings (N = 57 broods)

	χ^2	df	P
Hatching date^1 × Habitat	***11.80***	***1***	***0.001***
Hatching date^2 × Habitat	0.09	1	0.766
Habitat	-	-	-
Relative female chronotype × Habitat	0.01	1	0.939
Relative female chronotype	0.20	1	0.656
Hatching date^1	-	-	-
Hatching date^2	***19.69***	***1***	***<0.001***
Clutch size	***4.10***	***1***	***0.043***

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Table 4. Minimal adequate model coefficients (i.e., after model simplification using likelihood-ratio tests) for relative female chronotype and further predictors explaining variation in (**a**) number of nestlings surviving to fledging and (**b**) pre-fledging weight of nestlings on day 13. Model coefficients ('Estimate') are shown along with standard errors ('SE') and 95% confidence intervals ('95% CI' obtained using 500 parametric bootstrap simulations of the model). Superscripts '1' and '2' refer to linear and quadratic terms, respectively; reference level for Habitat is 'urban'.

a Number of nestlings that survived to fledging (N = 101 broods)

Fixed effects	Estimate	SE	95% CI	
Intercept	0.327	0.245	-0.111	0.810
Habitat (forest)	0.394	0.135	0.141	0.651
Relative female's chronotype	-0.007	0.003	-0.013	-0.001
Clutch size	0.145	0.031	0.084	0.203

b Pre-fledging weight of nestlings (N = 57 broods)

Fixed effects	Estimate	SE	95% CI	
Intercept	16.920	0.697	15.333	18.463
Hatching date^1 × Habitat (forest)	21.053	5.224	9.255	33.048
Habitat (forest)	2.725	0.516	1.657	3.857
Hatching date^1	-16.152	3.604	-24.565	-7.949
Hatching date^2	-10.234	1.916	-14.896	-5.539
Clutch size	-0.164	0.076	-0.336	0.010

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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 chronotype, in particular in female animals. We firstly show high repeatability of timing, and thus corroborate the interpretation of chronotype as a consistent individual trait. We then show that the 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 chronotypes.

Early rising may be beneficial as energy stores are depleted, and the ability of small passerine birds to successfully forage peaks in the early morning, once light conditions are suitable [18,27]. However, foraging in the early morning can be costly because of low ambient temperatures, and may be constrained by condition of an individual and its endogenous circadian clock.

Balancing costs and benefits of early rising might be intricate during incubation. For uniparental incubators, self-maintenance is weighed up against maximal offspring development [28]. This trade-off is heightened during early morning hours, when incubators must replenish energy stores while the typically low morning temperatures risk cooling of the eggs. Hence, an incubating female should delay leaving the nest until she can forage efficiently, and promptly return to the nest. Early rising may thus indicate superior condition of incubating females, as proposed for courtship song and provisioning of males [18,29,30].

Alternatively, the ability to perform efficiently early in the day could be based on circadian mechanisms that facilitate an early start, as demonstrated in human athletes [31]. Reproductive advantages due to circadian-based early-rising have been proposed for

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male great tits whose circadian rhythm affects extra-pair paternity (EPP), and have been supported by experiments on the same study species [12,19,21]. 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 [19,21]. 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 early chronotype could involve several mechanistic features. These include a fast clock (i.e., short free-running period [21]), but also individual variation in sensitivity to light [4,7,32,33]. A contribution of light pathways to the chronotype - fitness link is indicated by our comparison of absolute and relative timing. Fitness effects were evident only for chronotype relative to sunrise, but not for absolute timing. This suggests that for incubating females, shifting their response in relation to light cues is more important for fitness than maintaining absolute timing.

We also 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 would be reduced [10,34]. 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 [35,36]. Thus, some urban great tit females may have foraged at the earliest opportunity, to replenish their resources. Disentangling effects of the circadian clock from those of the birds' body condition would require experimental examination [10,19].

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The only other study we are aware of that examined reproductive success relative to incubation chronotype did not find such an association [9]. This investigation differed in several aspects, including use of the European starling (*Sturnus vulgaris*) as 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 [37,38]. 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 [13]. Still, we are far from understanding how variation in chronotype is maintained.

Our study results come with some slight caveats. Firstly, because we report correlative data from wild birds, we cannot assess whether chronotype was affected by the local micro-environment, either directly or via differences in individual quality [9]. Secondly, we have recorded female chronotype only during one life-cycle stage, incubation, similar to earlier studies on males that considered only courtship [30]. Thus, the consistency of chronotype across seasons and life stages remains to be tested.

Nonetheless, our study strengthens the evidence for variation in chronotype in free-living animals and provides a sought-after link to reproductive success. We extend the circadian focus of chronotype studies by indicative findings on light pathways, and confirm the importance of looking at both, relative and absolute time, as previously suggested for avian incubation [17]. Future challenges, likely requiring experimental approaches, are a disentangling of effects of endogenous clock from body condition, and determination of counter-balancing benefits that maintain variation in chronotype.

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Methods

Study populations and field protocols

We studied five nest-box breeding populations of great tits (*Parus major*; nest-box details: woodcrete material, 260H x 170W x 180D, hole diameter 32 cm, Schwegler, 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 nest-boxes included in the study; 56° 7' N, 4° 36' W], Sallochy Forest [N = 8 nest-boxes included in the study; 56° 7' N, 4° 36' W] and Cashel Forest [N = 31 nest-boxes included in the study; 56° 6' N, 4° 34' W]). The remaining two populations were situated in an urban park (Kelvingrove Park [N = 14 nest-boxes included in the study; 55° 52' N, 4° 16' W]) and a suburban park (Garscube estate [N = 9 nest-boxes included in the study; 55°54' N, 4°19' W]) within the city of Glasgow (UK). 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 [35,36,39]

All nest-boxes were checked weekly from April 1 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 nest-box 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 [40]), nest-boxes were checked every other day, allowing determination of exact date of hatching based on nestling presence. 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). Nest-boxes were checked again > 21 days after hatching to determine the number and identity of any dead nestlings remaining in the

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nest. As our sample size varied slightly per each trait under investigation (see below), we provide a breakdown of sample size per habitat and year in Table S11. 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 nest-boxes, 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 three years, the potential bias introduced by this methodological limitation is expected to be minimal (Table S11).

Incubation temperature data

To quantify incubation behaviour in female great tits, we deployed small temperature loggers (iButtons DS1922L-F5, Thermochron) inside their nests (Figure S5). We programmed iButtons to record temperature (± 0.0625 °C) every 3 min [23]. 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 (Figure S5).

Environmental temperature data

To control for variation in environmental temperature when quantifying incubation behaviour [23], daily mean temperatures for the breeding seasons of 2016, 2017 and 2018 were obtained from the UK 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).

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Ethical statement

All field procedures were carried out under the appropriate Home Office project licence and BTO ringing licence held by Barbara Helm.

Quantification of incubation behaviour

Some individuals removed iButtons from the nest cup and pushed them to the side of the nest-box, 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 dataset. Our incubation analyses only included days of incubation after the clutch was completed and started no earlier than 15 days before the hatch date. In total, 729 days of iButton incubation temperature recordings from 102 clutches were included in the analysis (sample sizes vary slightly across statistical models; details are given in the result section and Table S11)

Incubation behaviour (e.g., on- and off-bout timing and length) was determined using the R package incR [23,24], choosing parameter values for *incRscan* validated for great tit incubation ([23]; *lower.time*=22, *upper.time*=3, *sensitivity*=0.15, *temp.diff*=8, *maxNightVar_accepted*=2). For each incubating female, we determined: first morning off-bout, last evening on-bout, and duration of active day (e.g., time difference between the first morning off-bout and the last evening on-bout) [23].

Data analysis

General modelling procedures

All analyses and visualisations were performed in R (version 4.1.1 - [41]). Generalised linear mixed models were employed to explain variation in several incubation and reproductive

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traits (see below). For each of these traits, we first built a global model that contained all explanatory variables and interactions of interest for each trait (see below). Then, we used likelihood-ratio tests (LRTs) to investigate the importance of each model predictor. Starting from each global model, nested models were sequentially built and compared via LRTs until a minimal adequate model was reached (i.e., a model containing only significant terms). The statistical significance of terms present in the minimal adequate model was assessed dropping such terms (one at a time) from the model and comparing this new simpler model against the minimal adequate model via LRT. The statistical importance of predictors not included in the minimal adequate model was calculated in a similar way, using LRTs which compared the minimal adequate with and without the focal predictor. Quadratic terms were only included in models that also contained linear effects. When two-way interaction terms were present in a model, the single effect predictors that formed the interaction were always included. Random effects were present in every model as specified for the analysis of each response variable (details below). All statistical models were performed using the R package *lme4* [42]. Linear model residuals were visually inspected to check the assumption of normality. The R package *DHARMa* [43] was employed to check the normality of residuals in generalised linear models.

Global models

Incubation behaviour: We analysed absolute and relative onsets and ends of 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

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defined daily 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).

Global models for onset and end of activity (both absolute 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 zero [i.e. hatching day], 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 absolute among-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 April 1; included as a quadratic and a linear term - these terms effectively modelled among-female [e.g., cross-sectional] variation in onset and end of activity). 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). Nest-box identity (90-level factor), site (5-level factor) and year (3-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 interval (in absolute time) between the first incubation off-bout and the last on-bout per day. Consistency (e.g., repeatability) in female onset, end and duration of activity, was calculated as the proportion of variation explained by between-nest variation in the linear mixed models presented above, as implemented in the R package *rptR* [44]. Female chronotype was defined as the average within-nest onset of activity, but we also report end and duration of activity.

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Survival of nestlings to fledging and nestling weight

A Poisson generalised linear mixed model was used to explain variation in the number of nestlings that survived to fledgling. The probability of total brood failure (i.e., the probability that no nestling survived to fledging) was modelled using a binomial GLMM. A LMM was employed to analyse the number of nestlings that survived to fledgling excluding broods were no nestlings survived. Variation in the average 13-day old nestling weight per brood was analysed using a LMM. These models included habitat (urban *versus* forest), female chronotype (see definition above), hatching date (as a continuous variable in days after January 1; 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. Nest-box identity, site (5-level factor) and year (3-level factor) were included as random effect intercept.

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