

Effects of Temperature on Circadian Clock and Chronotype: An Experimental Study on a Passerine Bird

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Daily schedules of many organisms, including birds, are thought to affect fitness. Timing in birds is based on circadian clocks that have a heritable period length, but fitness consequences for individuals in natural environments depend on the scheduling of entrained clocks. This chronotype, i.e., timing of an individual relative to a zeitgeber, results from interactions between the endogenous circadian clock and environmental factors, including light conditions and ambient temperature. To understand contributions of these factors to timing, we studied daily activity patterns of a captive songbird, the great tit (*Parus major*), under different temperature and light conditions. Birds were kept in a light (L)-dark (D) cycle (12.5 L:11.5 D) at either 8°C or 18°C with ad libitum access to food and water. We assessed chronotype and subsequently tested birds at the same temperature under constant dim light (LL_{dim}) to determine period length of their circadian clock. Thermal conditions were then reversed so that period length was measured under both temperatures. We found that under constant dim light conditions individuals lengthened their free-running period at higher temperatures by 5.7 ± 2.1 min ($p = .002$). Under LD, birds kept at 18°C started activity later and terminated it much earlier in the day than those kept under 8°C. Overall, chronotype was slightly earlier under higher temperature, and duration of activity was shorter. Furthermore, individuals timed their activities consistently on different days under LD and over the two test series under LL_{dim} (repeatability from .38 to .60). Surprisingly, period length and chronotype did not show the correlation that had been previously found in other avian species. Our study shows that body clocks of birds are precise and repeatable, but are, nonetheless, affected by ambient temperature. (Author correspondence: marina.lehmann@uni-konstanz.de)

Keywords: Activity patterns, *Parus major*, Phase, Period length, Q₁₀, Repeatability, Temperature compensation, Timing

INTRODUCTION

As environments fluctuate substantially over the 24-h day, the scheduling of daily activities is thought to be closely linked to fitness. Being in time can give an advantage, e.g., in foraging for food, finding a mating partner, or escaping predators (Kronfeld-Schor & Dayan, 2003; Moore & Doherty, 2009; Sakai & Ishida, 2001). Within a population, however, not every individual is on the same schedule, so that there is variation in timing traits such as phase angle difference relative to zeitgebers, such as sunrise or sunset. In humans, individual time patterns are relatively consistent and have a heritable component (Merrow et al., 2005; Roenneberg & Merrow, 2007). Individuals can be classified into “chronotypes” (e.g., morning or evening types), although additional factors, such as age and social context, time of year, and ontogeny, also affect timing (Brown et al., 2008; Natale & Di Milia, 2011; Roenneberg et al., 2007; Strang-Karlsson

et al., 2010). Much less is known about consistency of chronotypes and other aspects of scheduling, e.g., onset and end of activity, in wild-derived animals. If timing was similarly consistent, as has been suggested in some studies (Bertin et al., 2007; Murphy et al., 2008), schedules should be under selection depending on relative costs and benefits. Specifically, selection should act on the mechanisms that regulate the expression of a given chronotype in a given environmental context.

One of the key factors that influences chronotype is the free-running period length (τ) of the underlying circadian clock, which is also known to differ within populations and between species (Daan & Beersma, 2002; Helm & Visser, 2010; Wyse et al., 2010). In many species, including humans (Allebrandt & Roenneberg, 2008; Brown et al., 2008), mice (Dallmann et al., 2011), birds (Aschoff, 1965; Aschoff & Wever, 1962, 1966), and insects (Fleury et al., 2000), chronotype correlates with

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τ (Aschoff & Pohl, 1978), such that an individual with a fast clock ($\tau < 24$ h) would be an early chronotype. However, there are also examples for an absence of such a relationship, possibly due to additional factors that could modify entrainment and weaken the correlation (Brown et al., 2008; Fleissner & Fleissner, 2002; Helm & Visser, 2010; Shimizu & Masaki, 1997). Besides light, one aspect of the natural environment that shapes schedules is ambient temperature (Aschoff, 1979; DeCoursey, 1960; Redlin, 2001; Rensing & Ruoff, 2002; Sweeney & Hastings, 1960). Temperature can affect timing directly, as seen by modified activity patterns and behavior in response to changing thermal conditions (DeCoursey, 1960; Vivanco et al., 2010). In addition, temperature could affect timing by modifying the underlying circadian period length. A rise in temperature increases the reaction rate of most biochemical processes (Ruoff & Rensing, 2004), so that the ratio of two reactions measured at temperatures that differ by 10°C (i.e., the so-called " Q_{10} ") is usually ~ 2 –3. In contrast, however, the circadian clock is largely temperature-compensated (Morrow et al., 2005), i.e., its period length stays roughly the same over a range of ambient temperatures. Correspondingly, a Q_{10} of ~ 1 is characteristic of circadian processes (Rensing & Ruoff, 2002). However, temperature compensation is not perfect. A number of studies on endotherms and ectotherms report change of period length dependent on ambient temperature (Aschoff, 1979; Pohl, 1968; Sweeney & Hastings, 1960) (Supplementary Table 1). Q_{10} values are within a range of .85–1.4 which implies up to 40% differences in cycle length for a difference of 10°C. According to some studies, e.g., on chaffinches, differences in period length of that magnitude can strongly influence activity timing under light-dark cycles (Aschoff & Wever, 1966). If similar temperature-dependent changes of the clock occurred in the wild, this could thus be highly relevant for fitness. Therefore, a better understanding of temperature effects on timing under constant and synchronizing conditions could be an important step towards clarifying the role of circadian clocks for timing in the "real world" (Menaker, 2006).

Herein, we investigate the effects of normally experienced temperatures (Gosler, 1993) on τ and chronotype in a species with a relatively well-known ecology. Great tits (*Parus major*) are widespread songbirds whose behavior has also been studied with regard to temporal patterns (Bednekoff & Houston, 1994; Charmantier et al., 2008; Hinde, 1952; Kacelnik & Krebs, 1982; Nussey et al., 2005; Ydenberg, 1984). As in other birds, male great tits are more successful at defending their territory and attracting females by singing early in the day, and thereby produce more offspring (Daan & Aschoff, 1982; Dolan et al., 2007; Murphy et al., 2008; Poesel et al., 2007). In related blue tits (*Cyanistes caeruleus*), early-singing males gained fitness via extra-pair offspring (Kempnaers et al., 2010; Poesel et al., 2007). Thus, an early chronotype, and possibly a correspondingly short

τ , may be advantageous. A previous study examined a large number of individuals of a great tit population and found an average τ of 23.8 h (Helm & Visser, 2010). Females seemed to prefer males with a fast clock for extra-pair matings, all the more if their social mate had a slower clock, which would be consistent with links between fast clocks and early chronotypes. The present follow-up study aims at examining the still poorly understood links between circadian clock, chronotype, and ambient temperature. To investigate these links, we subjected birds from a wild population to two temperature treatments (8°C and 18°C). We also tested whether chronotype was consistent within individuals (i.e., repeatable), and whether it correlated with circadian period length by keeping the same birds under a light (L)-dark (D) cycle and constant dim light (LL_{dim}). We found an effect of temperature on τ as well as on activity schedules, but no correlation between τ and chronotype. High repeatability confirmed chronotype is a consistent trait in these birds, at least under captive conditions.

METHODS

Birds used for the experiments were 24 male great tits that were obtained from a long-term study population at Hoge Veluwe (The Netherlands) (Helm & Visser, 2010). The birds were taken in and hand-raised during the breeding season of 2009. Family size varied from single birds to up to four brothers. Because of earlier suggestions that paternity could be related to clock properties, we scrutinized relatedness by molecular genetic analysis (Helm & Visser, 2010). We found no indication of extra-pair offspring (i.e., there were no half-siblings in the set of birds). During the experiment, birds were kept in 24 separate indoor aviaries ($2 \times 2 \times 2.25$ m). For the entire duration of the experiment, each bird was thereby individually recorded in its own aviary. It had previously been shown by spatial autocorrelation that social effects on timing between neighboring birds were absent (Helm & Visser, 2010). Birds received fresh water and were fed ad libitum with a constant daily amount of food, consisting of a mixture of minced beef, proteins, and vitamins, complemented by sunflower seeds, fat balls, a mix of dried insect, and calcium and mineral supplements.

The studied birds had previously been used in an experiment on the effect of temperature on the timing of reproduction from December 2009 to September 2010 (see Schaper et al., 2012) and were then kept at 8°C except for a 5-d warmer period (18°C) for one group. Our experiment started on 13 November 2010 and ended on 28 December 2010. We divided the birds into two groups experiencing ambient temperatures in the aviaries of either 8°C or 18°C, such that each new group consisted of a similar number of birds from the earlier experimental groups. Temperature treatments were alternated between neighboring aviaries (Figure 1).

At the start of the experiment, all birds were kept under a daily light:dark regime of 12.5 L:11.5 D for 17 d

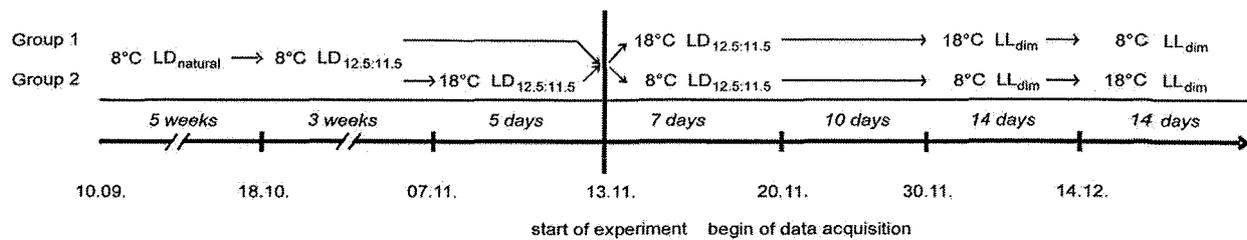


FIGURE 1. Experimental set up of light and temperature conditions. Birds were kept for 10 d under a daily 12.5 L:11.5 D regimen at either 8°C or 18°C. Afterwards, the light was set to constant dim light (LL_{dim}), and temperatures stayed the same for 14 d. For another 14 d under LL_{dim} , temperature settings were crossed over between groups.

(Figure 1). The main light sources were three high-frequency fluorescent tubes. At night, a small green lamp provided dim light of .5 lux at perch level. After 1 wk of habituation, we recorded activity for 10 d to assess onset and offset of activity and thereby chronotype (LD experiment). The light condition was then changed to constant dim light (LL_{dim}), whereby, until the end of the study, the night lamp was used as sole illumination (Figure 1). Thereby, we measured the free-running period under the temperatures to which birds had been pre-exposed. Two weeks after the shift to LL_{dim} conditions, temperatures were changed in a crossover design from 8°C to 18°C, or from 18°C to 8°C, respectively, for a second 2-wk series of measurements under LL_{dim} (Figure 1). Thus, the free-running period of each bird was measured under both temperature conditions, but in a different sequence. Light and temperature conditions were set and monitored for each individual aviary. Based on sensor recordings, temperatures fluctuated by ± 1 –2°C around the set point. Intensity of the constant dim light was measured once in each aviary for each temperature condition. Although light intensity varied from .4 to .8 lux between aviaries, it was unrelated to ambient temperature. During the LL_{dim} experiment, we avoided food entrainment by feeding birds at randomized times. Exact feeding times were noted for subsequent data correction.

For activity monitoring, we used radar detectors (Conrad Electronics, Hirschau, Germany). One detector per aviary recorded movements of the single bird for subsequent computer storage (Helm & Visser, 2010). These radar detectors use the Doppler principle to record even small movements by sending out a signal in the microwave range that is reflected by the bird. Superimposing the reflected over the original signal then reveals information about the speed and proximity of the bird relative to the sensor. Any number of bird movements within a 2-s interval was counted as activity, and the total number of 2-s intervals containing activity was stored every 2 min (i.e., a number between 0 and 60). In parallel to movements, temperature and light information (on/off) was recorded as well.

All activity caused by feeding the birds or measuring light intensity was removed before data analysis by marking it as missing values. Birds responded to

changes in light condition from LD to LL_{dim} initially by irregular activity patterns. Therefore, the first 5 d of activity were not used for determination of period length (τ). For the same reason, the first 2 d of data after switching of temperatures were excluded from analysis. Two additional days of data were excluded for one bird at the start of the first temperature treatment (LL_{dim1}) and of another one at the end of the second temperature treatment (LL_{dim2}). Both birds showed an abrupt change in behavior, probably due to external disturbance. Because of sensor malfunction during LD, one bird had to be removed from the chronotype analysis; for another there was no activity information available.

To calculate τ , we used Lomb-Scargle periodogram analysis (Ruf, 1999), implemented in the software program ChronoShop (Spoelstra, 2010). τ was estimated from 9 d of data for the first temperature treatment and from 13 d of data for the second temperature treatment. These time series are long enough to allow stable estimates of τ (Helm & Visser, 2010). For estimation of timing patterns under LD, we used measurements derived from 10 d of data.

Depending on the individual bird, the activity-onset was either a sudden event or a drawn-out increase in activity. The same was true for activity-offset with even greater variation. We used an edge detector (Helm & Gwinner, 2005) to determine the time of greatest increase in activity in the morning (within 1 h before and after lights-on, respectively) and of greatest decrease in activity in the evening (within 2 h before and after lights-on, respectively) for each bird. This method allows separating the main activity interval from recurring lesser increases and decreases in activity, yielding a relatively late calculated activity-onset and early activity-offset. Duration of activity was the interval between activity-onset and activity-offset. Chronotype was calculated as a bird's mid-activity (midpoint between activity-onset and activity-offset) relative to mid-light-time (mid-time between lights-on/lights-off). An early chronotype is thereby characterized by a positive phase angle difference (i.e. phase advance) between midpoint of activity and mid-light-time, whereas a negative phase angle difference indicates a late (i.e. phase-delayed) chronotype.

The derived timing parameters were analyzed by linear mixed models using restricted maximum-likelihood estimation methods (Genstat, 1993). This approach implements a repeated-measures design in all cases to take account of the individuals that were measured multiple times. Effects of temperature treatment and τ were modeled as fixed factors and covariates for chronotype as response variable. For τ as response variable, temperature treatment and trial (first vs. second treatment) were included as fixed factors. The interaction between these factors was included to test for carry-over effects, i.e., to investigate whether τ was influenced by sequence of treatments. Effects of family and individual were usually included in the model as random effects, except for analyses in which we modeled them as fixed factors for significance testing (Genstat, 1993). Significance levels were derived from Wald statistics that asymptotically follow a χ^2 distribution. As significance levels can depend on the order of factors within the model, we tested each factor by removing as well as adding it to the model. Usually sequence of entrance did not affect results; but, if it did, we always used the most conservative outcome. We progressively reduced models until only factors were retained that had significant effects on timing; in all cases, the reported statistics for each factor are derived from the most parsimonious model. Estimates of repeatability followed the analysis of variance (ANOVA)-based procedure suggested by Lessells and Boag (1987), using the ANOVA and LM (linear models) function from the *stats* package implemented in the software package R (R Development Core Team, 2012). Data were tested for deviation from normality, and specifically for heteroscedasticity between temperature groups using Bartlett's test. In some cases, data were not normally distributed and had heterogeneous variances (Genstat, 1993). If heteroscedasticity was detected, we added corresponding analyses after rank transformation (Conover & Iman, 1981). Data were also characterized by descriptive methods (mean \pm SD) and by nonparametric tests.

All experiments were conducted under license of the Animal Experimental Committee of the KNAW (DEC

protocol no. NIOO 10.12) and in accordance with international ethical standards (Portaluppi et al., 2010).

RESULTS

Birds Under LD Cycles (LD)

On average, the chronotype, as defined by the midpoint of activity, preceded mid-light-time by 13.5 ± 1.35 min (mean \pm SEM; $n = 217$ observations from 22 birds) (Figures 2 and 3). Onset of activity, as estimated by an edge detector as the time of greatest increase in activity, occurred 5.9 ± 1.26 min after lights-on, and completion preceded lights-off by 33.0 ± 2.50 min (Figures 2 and 3).

Linear mixed-model analysis revealed significant effects of temperature on chronotype (Wald₁ = 5.12, $p = .024$; Figure 2, Table 1), with a tendency for birds to show a slightly earlier chronotype if kept under higher temperatures. However, because there was an indication of heteroscedasticity (Bartlett's test: $\chi^2 = 4.31$, $df = 1$, $p = .038$), we also used a more conservative approach over ranks, for which significance levels depended on the sequence of factors in the analysis (most conservative Wald₁ = 2.98, $p = .084$; Table 1). In the evening, activity ended much earlier under warmer than under colder temperatures (Wald₁ = 16.30, $p < .001$; Table 1). Conversely, in the morning, birds under warmer temperatures were active a bit later (Wald₁ = 4.41, $p = .065$ [most conservative statistic]; Table 1), so that, overall, the activity period was shortened under warm temperatures (Wald₁ = 5.97, $p = .015$; Figure 2, Table 1). The effect of temperature on chronotype derived from a greater advancing effect of temperature on activity-offset compared to that on activity-onset. All activity patterns showed clear consistency between the repeated recordings of individuals, as indicated by significant effects of individual on all parameters of timing ($p < .001$; Figure 3, Table 1). Overall, differences between individuals were associated with a much larger proportion of variance than differences between temperature treatments (activity-onset: individual 36.3%, treatment 4.9%, residual 58.8%; activity-offset: individual 32.4%, treatment 10.3%, residual 57.3%; activity duration: individual

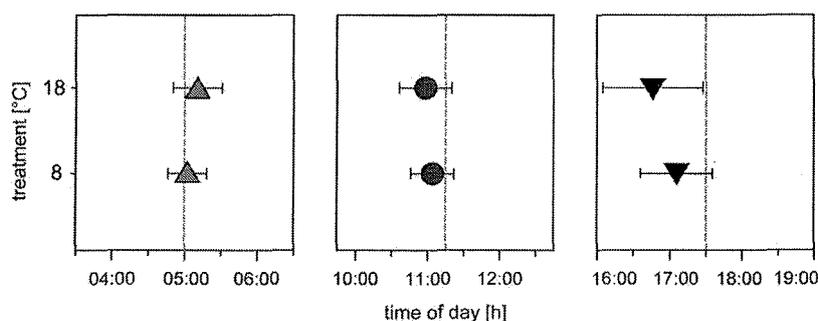


FIGURE 2. Activity timing of great tits under two temperature conditions. Data points show onset (triangles), midpoint (circles), and offset (inverted triangles) of activity in relation to lights-on, mid-day, and lights-off (dashed lines), respectively. Error bars indicate standard deviation; n is the total number of measurements collected from the 22 birds ($n_{\text{onset}} = 217$, $n_{\text{midpoint}} = 217$, $n_{\text{offset}} = 217$).

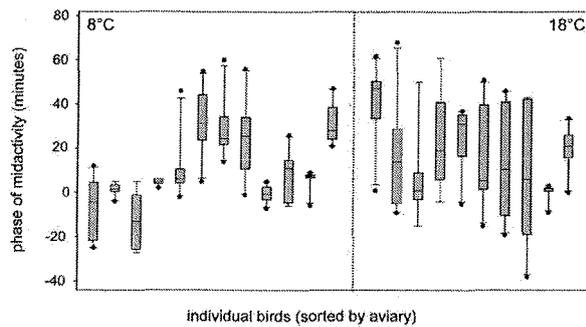


FIGURE 3. Variation in chronotype under LD (defined as difference of midpoint of activity from mid-day in minutes). Boxplots summarize all recorded activities of each individual. Error bars above and below the box indicate the 90th and 10th percentiles; dots indicate outliers. Positive values represent birds whose activity preceded mid-light-time. Boxplots are sorted by temperature treatment and aviary number; n is the total number of measurements collected from the 22 birds ($n = 217$).

27.9%, treatment 16.6%, residual 55.5%; chronotype: individual 39.7%, treatment .3%, residual 60.0%.

Repeatability was calculated by ANOVA for a total sample size of 22 birds with 10 d of data in each case and ranged between .38 and .45, depending on timing parameter and method of calculation (i.e., raw data vs. ranks; Table 2).

Birds Under Constant Dim Light (LL_{dim})

Circadian period length τ for all 24 birds was on average $23.77 \pm .18$ h (range: 23.33–24.07 h; skewness: $-.66 \pm .34$; kurtosis: $-.79 \pm .67$; $n = 48$, for individual actograms, see Supplementary Figure 1) and differed significantly from 24 h (Wilcoxon: $W_{48} = 5$, $p < .001$). Figure 4 shows the period lengths of all birds for the two temperature treatments. Ambient temperature had a clear lengthening effect on τ (Wald₁ = 10.01, $p = .002$, $n = 48$). There was no difference between the two trials (Wald₁ = 2.07, $p = .150$, $n = 48$), and nor were there effects of sequence of treatment (8°C to 18°C or vice versa; Wald₁ = 2.33, $p = .127$, $n = 48$). Period length was longer at 18°C ($23.81 \pm .17$ h,

range: 23.47–24.07 h; $n = 24$) compared to at 8°C ($23.72 \pm .2$ h, range: 23.33–23.93 h; $n = 24$). Nevertheless, the Q_{10} value was extremely close to 1 ($1.004 \pm .0074$ [mean \pm SD]; $n = 24$). We found no evidence for an influence of period length on chronotype (Wald₁ = .01, $p = .918$, $n = 20$). Period length was significantly different between individuals (Wald₂₃ = 76.18, $p < .001$, $n = 48$; Figure 4) and highly repeatable within an individual over both treatments (between .53 and .60; Table 2).

DISCUSSION

We show that change in ambient temperature had an effect on both τ and on entrained schedules of great tits. Birds had clocks, on average, that were faster than 24 h, and chronotypes that were slightly early in comparison to the mid-light-time. Repeatability of individual patterns indicated consistent daily timing, but we did not detect a correlation between τ and chronotype. τ was on average <24 h and was close to the previously determined estimate from the same population (Helm & Visser, 2010). In the previous study, birds were kept at $\sim 20^\circ\text{C}$ and had a mean τ of 23.83 h. This value is almost identical to the mean τ reported here for birds kept under 18°C (23.81 h).

Higher ambient temperatures had a significant lengthening effect on τ (Figure 4). Usually, higher temperature increases the speed of biochemical reactions, whereas circadian clocks are largely temperature-compensated. In our study, the circadian clock was mostly temperature-compensated, but the longer τ suggested that biochemical processes underlying the clock were in fact slightly slowed. This slowing effect is, however, barely reflected in the Q_{10} , as it is the ratio of the two period lengths taken at the different temperatures. Q_{10} values are known to vary between species. For example, in lizards, bats, or mice, temperature effects seemed to be greater than in birds (Sweeney & Hastings, 1960). In comparison to some other species, the Q_{10} of 1.004 reported here shows almost perfect temperature compensation (Supplementary Table 1). The Q_{10} for

TABLE 1. Effect of temperature and individual on activity timing (Wald test)

Variable	n	Temperature				Individual			
		Wald ₁	p	Wald ₁ on ranks	p on ranks	Wald ₂₀	p	Wald ₂₀ on ranks	p on ranks
Onset	217	10.07	.002	3.41	.065	92.70	<.001	114.05	<.001
Offset	217	19.51	<.001	16.30	<.001	86.88	<.001	86.75	<.001
Duration	217	5.97	.015	—	—	78.49	<.001	—	—
Chronotype	217	5.12	.024	2.98	.084	98.88	<.001	116.67	<.001
		Wald ₁	p	Wald ₁ on ranks	p on ranks	Wald ₂₃	p	Wald ₂₃ on ranks	p on ranks
Period length (τ)	48	6.97	.008	10.01	.002	60.87	<.001	76.18	<.001

Temperature had an effect on time of onset, offset, and duration of activity, as well as on chronotype (midpoint of activity) under LD and an effect on period length τ under LL conditions. All values were highly consistent within individuals. Parameters that were not normally distributed were also analyzed over rank-transformed data. Degrees of freedom are indicated by subscripts; n indicates the total number of measurements collected from the 22 birds.

TABLE 2. Repeatability of activity timing, chronotype (midpoint of activity), and period length \pm SEM

Variable	n	Repeatability	F, df_{num} , df_{den}	Repeatability ranks	F, df_{num} , df_{den}
Onset	217	.395 \pm .09***	7.50, 21, 195	.452 \pm .09***	9.20, 21, 195
Offset	217	.397 \pm .09***	7.54, 21, 195	.384 \pm .09***	7.17, 21, 195
Duration	217	.399 \pm .09***	7.55, 21, 195	—	—
Chronotype	217	.397 \pm .09***	7.51, 21, 195	.436 \pm .09***	8.63, 21, 195
Period length (τ , LL _{dim})	48	.528 \pm .15*	3.23, 24, 23	.604 \pm .13**	4.05, 24, 23

Data for onset and end of activity and for period length were not normally distributed, and repeatability was calculated over ranks as well. For period length, temperature was included in the ANOVA model as a covariate. Asterisks denote significance levels for repeatability in the respective ANOVA models (* $p < .05$, ** $p < .01$, *** $p < .001$); n indicates the total number of measurements collected from the 22 birds; df_{num} = numerator degrees of freedom; df_{den} = denominator degrees of freedom.

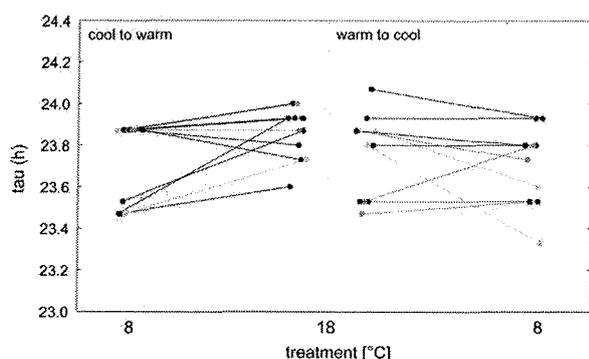


FIGURE 4. Change of period length τ in individual birds due to temperature treatments. Overall, period length increased when temperatures rose and decreased when temperatures dropped. Lines connect the values of a given bird ($n_{cool\ to\ warm} = 13$, $n_{warm\ to\ cool} = 11$).

other songbird species (finches) was also close to 1 ($\sim .99$), and thereby almost completely temperature-compensated (Enright, 1966; Pohl, 1968, 1974). However, the effects of temperature on even a very well compensated clock could be larger under natural conditions where individuals experience a much broader range of temperatures than the tested 10°C difference.

The advancing effect of warmer temperature on chronotype was relatively small (.3% of variance) (Figure 2). This could be partly due to the fact that each bird under LD experienced only one temperature treatment, which reduced sample size and allowed comparison of chronotype only between groups but not within individuals. Another possible reason for the overall small effect is the method of calculating chronotype. We defined chronotype as the midpoint between onset and termination of activity in relation to the mid-light-time, so that changes in morning and evening behavior partly offset each other. However, a closer look at the activity patterns reveals that activity-offset depended more strongly on ambient temperature than activity-onset. There is also a difference in variability of the two values, apart from temperature effects, which is much higher for the completion than for the onset of activity (Daan & Aschoff, 1975) (Figure 3). A similar pattern has been noted in free-living great tits, for which seasonal variation was low for awakening times but substantial

for roosting times (Hinde, 1952). The greater sensitivity to temperature of activity-offset in comparison to activity-onset adds to the idea of more rigidly controlled timing in the morning. For a bird like the great tit, which prefers to sleep in cavities or sheltered places with low light levels, it is particularly important to anticipate the right time for rising. Most important activities happen in the morning when territories are defended, females are attracted, and mating takes place (Hinde, 1952; Kacelnik & Krebs, 1982; Poessel et al., 2007). In contrast, in the evening the birds can perceive light change outside their cavity, and precise scheduling may be less consequential.

Although advantages of an early chronotype for great tits are thought to be numerous and activity-onset is accurately timed, these advantages must be counterbalanced by factors that keep their chronotype from extreme earliness. Benefits of an early rise may be limited by food that may not yet be available (Bednekoff & Krebs, 1995; Sibly, 1975), by females not responding to courtship (Mace, 1986), or by light conditions (Kacelnik, 1979; Thomas et al., 2002). Furthermore, cold night and morning temperatures may add an energetic cost of leaving the roosting site too early in the day (Bednekoff et al., 1994; Krams et al., 2010). To the extent that these factors exert selection pressures, any evolutionary responsiveness of birds requires that timing be consistent within an individual but variable within a population (Lynch & Walsh, 1998). This was the case in our study population for annual breeding schedules, and selection on timing traits and their heritable components has been shown (Husby et al., 2010; Nussey et al., 2005). In our study on daily timing, we found significant differences between individuals and substantial repeatability of individual schedules, indicating birds show consistent behavior, at least, under laboratory conditions (Figure 3). Repeatability was of comparable magnitude across timing parameters (Table 2), partly because duration of activity and chronotype are related by their derivation to activity-onset and activity-offset. To our knowledge, highly repeatable time patterns of the free-running and entrained clock are a novel finding for animals taken from the wild and all the more important because they indicate that timing might be similarly consistent in the real world where selection takes place.

Taken together, the observed differences between morning and evening in temperature sensitivity and variability suggest that not chronotype as a whole is subject to selection but rather its temporal components, e.g., onset and end of activity. These components could be differently modified in organisms depending on the general lifestyle of a species. Our inability to detect correlation between chronotype and τ , despite effects of temperature on both parameters, could be related to complex interactions of the circadian clock and chronotype. This interaction can also be influenced by many additional factors, such as light conditions, ambient temperature, food availability, or social context (Beersma et al., 2008; Fleissner & Fleissner, 2002; Helm & Visser, 2010). A huge effort is required to examine this in captivity, and it may yield results that are not directly applicable to the situation in the wild. Therefore, data from birds in their natural environment are needed to shed more light on the relationship of τ and chronotype and their impact on timing, behavior, and survival.

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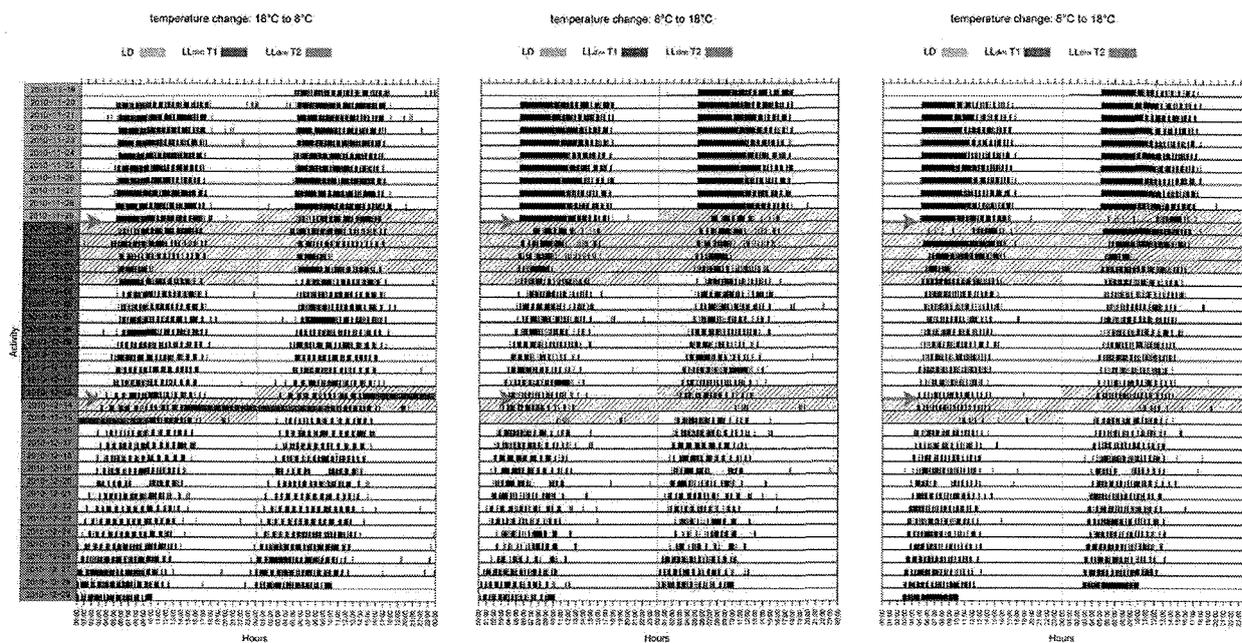
SUPPLEMENTARY TABLE 1. Response of circadian period length to a 10°C increase of ambient temperature in various species (adapted from Aschoff, 1979; Sweeney & Hastings, 1960)[†]

Species	Active period	Thermoregulation	Q ₁₀	Source
<i>Carduelis flammea</i>	Day	Endotherm	.99	Pohl (1974)
<i>Carpodacus mexicanus</i>	Day	Endotherm	.99	Enright (1966)
<i>Fringilla coelebs</i>	Day	Endotherm	.99	Pohl (1968)
<i>Macaca nemestrina</i>	Day	Endotherm	1.01	Tokura & Aschoff (1983)
<i>Drosophila pseudoobscura</i>	Day	Ectotherm	1.02	Pittendrigh (1954)
<i>Lacerta sicula</i>	Day	Ectotherm	1.02	Hoffmann (1957)
<i>Uca pugnax</i>	Day	Ectotherm	1.0	Brown & Webb (1948)
<i>Euglena gracilis</i>	Day	Ectotherm	1.01-1.1	Bruce & Pittendrigh (1956)
<i>Glis glis</i>	Night	Endotherm	1.03	Pohl (1968)
<i>Perognathus longimembris</i>	Night	Endotherm	1.01	Lindberg et al. (1971)
<i>Peromyscus leucopus</i>	Night	Endotherm	1.1-1.4	Rawson (1959)
<i>Myotis lucifugus</i>	Night	Endotherm	1.4	Rawson (1959)
<i>Thamnophis radix</i>	Night	Ectotherm	1.04	Heckrotte (1975)
<i>Admetus pumilio</i>	Night	Ectotherm	1.02	Beck (1972)
<i>Periplaneta americana</i>	Night	Ectotherm	1.06	Bünning (1958)
<i>Neurospora</i>	Night	Ectotherm	1.03	Pittendrigh et al. (1959)
<i>Gonyaulax polyedra</i>	Night	Ectotherm	.85	Hastings & Sweeney (1957)

[†]The majority of references cited can be found in these publications and are listed below.

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SUPPLEMENTARY FIGURE 1. Actograms (double-plot) of three individuals throughout the experiment. Hatched areas indicate days excluded from analysis. Arrows indicate treatment changes, i.e., from LD to LL_{dim} (upper arrow) and from 8°C to 18°C or 18°C to 8°C, respectively (lower arrow). Left actogram: 18°C to 8°C, T accelerates. Middle actogram: 8°C to 18°C, T decelerates. Right actogram: 18°C to 8°C, T unchanged.