Heritable circadian period length in a wild bird population

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Timing is essential, but circadian clocks, which play a crucial role in timekeeping, are almost unaddressed in evolutionary ecology. A key property of circadian clocks is their free-running period length (τ), i.e. the time taken for a full cycle under constant conditions. Under laboratory conditions, concordance of τ with the ambient light–dark cycle confers major fitness benefits, but little is known about period length and its implications in natural populations. We therefore studied natural variation of circadian traits in a songbird, the great tit (*Parus major*), by recording locomotor activity of 98 hand-raised, wild-derived individuals. We found, unexpectedly, that the free-running period of this diurnal species was significantly shorter than 24 h in constant dim light. We furthermore demonstrate, to our knowledge for the first time in a wild vertebrate, ample genetic variation and high heritability ($h^2 = 0.86 \pm 0.24$), implying that period length is potentially malleable by micro-evolutionary change. The observed, short period length may be a consequence of sexual selection, as offspring from extra-pair matings had significantly shorter free-running periods than their half-siblings from within-pair matings. These findings position circadian clocks in the 'real world' and underscore the value of using chronobiological approaches in evolutionary ecology. Evolutionary ecologists study variation and its fitness consequences, but often have difficulties relating behavioural variation to physiological mechanisms. The findings presented here open the possibility that properties of internal, circadian clocks affect performance in traits that are relevant to fitness and sexual selection.

Keywords: clock; circadian; heritability; sexual selection; extra-pair; *Parus major*

1. INTRODUCTION

The importance of accurate timing for living organisms is widely acknowledged (e.g. Cuthill & Houston 1997; Purves *et al.* 2001), but biological clocks are poorly understood in terms of evolutionary ecology (Kyriacou *et al.* 2008). Daily timing processes in many organisms, from bacterial photosynthesis to wake-up time in humans, are based on underlying, endogenous rhythms (Dunlap *et al.* 2004; Foster & Kreitzman 2005; Koukkari & Sothern 2006). This becomes evident if all temporal information is removed and organisms are exposed to constant light and temperature. Under such conditions, clocks continue ticking but drift progressively from the solar day, assuming an internal rhythm with a period length that differs slightly from 24 h (hence, circadian, from *circa* = about, and *dies* = day). Circadian rhythms help organisms anticipate upcoming environmental conditions and are essential for the integration of internal fluctuations in physiology (Daan & Aschoff 1982; Decoursey 2004; Koukkari & Sothern 2006; Brown *et al.* 2008; Martino *et al.* 2008; Turek 2008). In the remarkably successful study of circadian rhythms, free-running period length τ has been a focal trait (Kenagy 1980; Koukkari & Sothern 2006; Brown *et al.* 2008; Takahashi *et al.* 2008; von Schantz 2008). Period length varies within groups of study organisms, but also between species (Aschoff 1979; Daan & Beersma 2002).

For example, τ tends to be longer than 24 h in diurnal animals and shorter than 24 h in nocturnal animals at low light levels (Aschoff 1979).

(a) Circadian period length and daily timing

Under normal daylight conditions, circadian rhythms synchronize to the daily change in light conditions. However, individuals differ markedly in how their circadian rhythm aligns itself with the light–dark (LD) cycles (the so-called 'phase of entrainment'). Individuals can hence be classified into 'chronotypes', depending on whether they are active relatively early or late in the day (Duffy *et al.* 2001; Koukkari & Sothern 2006; Allebrandt & Roenneberg 2008). In many species, these individual differences in chronotype are related to free-running period length τ (Duffy *et al.* 2001; Allebrandt & Roenneberg 2008; Brown *et al.* 2008; von Schantz 2008). Under LD cycles, individuals with shorter τ tend to be active earlier in the day and to display more or longer lasting activity than those with longer τ (Aschoff & Wever 1966; Fleury *et al.* 2000; Brown *et al.* 2008). In humans, individuals with shorter τ tend to define themselves as earlier chronotypes than those with longer τ (Duffy *et al.* 2001; Brown *et al.* 2008). Corresponding information from wild organisms under natural conditions exists for species or populations, but not for individuals (Fleury *et al.* 2000; Kronfeld-Schor & Dayan 2003; Kyriacou *et al.* 2008). Thus, variation in τ is expected to have consequences for timing in free-living animals, but robust information is sorely missing.

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(b) *Fitness consequences of τ*

Fitness implications of τ under natural conditions are not well known. Slight deviations of τ from 24 h could be adaptive because they may affect phase of entrainment and promote adjustment to seasonally changing daylength (Daan & Aschoff 1982; Fleury *et al.* 2000; Daan & Beersma 2002; Johnson *et al.* 2003). By contrast, large deviations of τ from the 24 h day appear to be harmful (Daan & Beersma 2002). If τ is genetically altered, or if organisms are exposed to artificial LD cycles that differ notably from 24 h, fitness and health decrease (Ouyang *et al.* 1998; Koukkari & Sothern 2006; Emerson *et al.* 2008; Martino *et al.* 2008). We therefore expect that in natural populations, τ will have a fitness peak near environmental rhyhmicity of 24 h (Daan & Beersma 2002). Slight deviations from 24 h could be favoured by selection for particular clock properties, e.g. for advantages of early chronotype or of easy adjustment to changing conditions (Johnson *et al.* 2003). If differences in τ are associated with a steep fitness peak (e.g. under stabilizing selection), variation in τ could be low. Heritable variation could then be depleted and evolutionary change constrained (i.e. genostasis; Bradshaw 1991; Price & Boag 1993). These ideas can be evaluated by studying τ in natural populations. Information on distribution of τ may provide insight into adaptiveness, while information on genetic contributions to variation, and ideally on fitness implications of differences in τ, is crucial for understanding micro-evolutionary processes (Price & Boag 1993; Roff 1997; Daan & Beersma 2002).

(c) *Approach*

We examined circadian traits in a songbird species, the great tit (*Parus major*), which is widely studied by evolutionary ecologists (Nussey *et al.* 2005; Charmantier *et al.* 2008; Van Oers *et al.* 2008; Visser 2008). We recorded free-running period length τ of birds that hatched in the wild from free-living parents and were raised in captivity to minimize environmental effects. Birds were studied under constant dim light (LL, *ca* 0.5 lux) to assess circadian period length in the absence of environmental rhythmicity (Dunlap *et al.* 2004; Foster & Kreitzman 2005; Koukkari & Sothern 2006). For exploratory analyses of possible implications of τ, we collected additional data on three aspects of the birds' biology that are associated with variation in clocks.

(i) We addressed the relationship between τ and chronotype by brief sampling of activity patterns under LD cycles. Great tits and birds of several other species are thought to be under pressure during the breeding season to commence daily activities early in the morning when territorial and reproductive behaviours peak (Daan & Aschoff 1982; Dolan *et al.* 2007; Poesel *et al.* 2007; Murphy *et al.* 2008). Consequently, a short τ, which is associated with early chronotype, could be advantageous.

(ii) We also assessed paternity because extra-pair (EP) young are common in our study population (Van Oers *et al.* 2008). Information on paternity is important for calculation of heritability, and EP paternity itself may be related to timing. In several avian species, including blue tits (*Cyanistes caeruleus*), EP success was highest for males that

initiated morning song the earliest (Daan & Aschoff 1982; Dolan *et al.* 2007; Poesel *et al.* 2007; Murphy *et al.* 2008).

(iii) Last, we related τ to reproductive timing by recording lay date in captivity. Circadian clocks contribute to seasonal timing by providing reference time for photoperiodic time measurement and possibly also by links to circannual rhythms (i.e. rhythms that underlie annual processes; Gwinner 1986; Sharp 2005; Bradshaw & Holzapfel 2007; Foster & Kreitzman 2009; Liedvogel *et al.* 2009). Our study population has been extensively monitored for seasonal timing under climate change. Despite high selection pressure for early breeding, the birds' reproductive schedules do not keep pace with an advancing food peak (Visser *et al.* 1998; Visser 2008). Limited flexibility of breeding schedules could arise from mechanistic constraints, including circadian contributions, which we hereby examine (Silverin *et al.* 1993; Majoy & Heideman 2000; Bradshaw & Holzapfel 2007; Liedvogel *et al.* 2009; Visser *et al.* in press).

(d) *Objectives*

Our primary goal was investigation of individual variation in period length. Based on data of 98 great tits from 20 broods, we first derived information on the distribution of τ within the population. We then used these phenotypic data and information on relatedness to address genotypic contributions to variation in τ. This is based on the rationale that resemblance between family members (e.g. parents and offspring, siblings) allows an estimate of the relative importance of genetic variation. Using data from genetically confirmed full siblings, we calculated broad-sense heritability (h^2) to estimate the extent to which phenotypic variation is determined by genotypic variation (Conner & Hartl 2004). However, broad-sense h^2 can be inflated by environmental variation (Roff 1997). We therefore used the differences in paternity to explore common-environment effects on τ. EP young share maternal origin and a common nesting environment until hand-raising with their within-pair (WP) half-siblings. If differences in τ between broods were largely owing to a common environment, there should be little difference between EP and WP young. Conversely, strong effects of paternity on τ would support high additive genetic variation and emphasize genotypic contributions of WP and EP fathers. A secondary goal of our study was an exploratory analysis of implications of period length. We therefore related, on an individual basis, findings on τ to those on chronotype and lay date in captivity.

2. MATERIAL AND METHODS

(a) *Birds and housing conditions prior to circadian experiments*

Great tits were collected in spring of 2005 and 2006 in the Hoge Veluwe study population (52°05′ N, 05°50′ E) of the Netherlands Institute of Ecology (NIOO-KNAW) as entire broods at day 10 after hatching. Parents were caught prior to removing the brood and blood-sampled to obtain a DNA sample. The nestlings were transported to the NIOO-KNAW and hand-raised and housed as described elsewhere (Drent *et al.* 2003). Young birds were paired in

December and were allowed to breed and moult in $2 \times 2 \times 2$ m aviaries (see Visser *et al.* 2009 for a description). All seasonal activities were closely monitored, and for the 86 birds that initiated broods, exact lay dates are known. Temperatures were controlled and recorded every 10 min. Aviaries were illuminated by artificial light. From December until the beginning of circadian experiments, photoperiod was increased twice a week following the natural, local increase in day length (i.e. exposing birds to daily LD cycles from a minimum of 7.45 L : 16.15 D to a maximum of 16.30 L : 7.30 D at the winter and summer solstice, respectively). The main sources of light were two 'True Light' high-frequency fluorescent tubes, and additional natural light was provided using a tubular daylighting device (SolaTube) that was opened and closed when the True Light tubes were turned on and off, respectively. In combination, this provided about 700 lux at perch level. For half an hour before the True Light tubes were switched on, and half an hour after they were turned off, an 8 W light bulb mimicked dusk and dawn.

(b) *Circadian experiments*
Birds were examined for temporal behaviour in the breeding aviaries in their second autumn at the end of moult. In order to collect data on individual circadian clocks, birds had to be tested in isolation from all external time cues including those from conspecifics (Dunlap *et al.* 2004; Foster & Kreitzman 2005; Koukkari & Sothern 2006). Hence, birds were tested in batches during times when aviary space was available. In 2006, female activity was examined from 15 September, and males were subsequently examined from 13 October. In 2007, females were monitored from 28 August (see below for details). All birds were first briefly recorded under LD cycles. Light conditions were kept constant based on the amount of natural daylight on the day the experiment began. Accordingly, for the groups tested in 2006, day length was fixed at 12.7 h and for the group tested in 2007, at 14.4 h.

Subsequently, LD cycles were replaced by constant conditions to measure circadian rhythms. Lights were dimmed because constant bright light disrupts clocks (Dunlap *et al.* 2004; Foster & Kreitzman 2005; Koukkari & Sothern 2006). During exposure to constant LL, light was provided using a green light night lamp of 0.5 lux at perch level. Light intensity was measured at perch level using a lux light meter (Nieaf Instruments NI-L204) at least once per experiment. Food was constantly available and replenished at random times between 8.00 and 15.00 to avoid entrainment to the feeding regime. To avoid possible communication between family members, birds were placed randomly into neighbouring aviaries. Furthermore, testing by spatial autocorrelation methods indicated that social effects between neighbouring birds were absent. Movements were measured using radar-based detectors (Conrad Electronics) connected to a computer. If the bird moved within a 2 s interval, a movement was detected, and every 2 min the total number of 2 s intervals in which a movement was detected was stored (i.e. a number between 0 and 60). Activity counts in 2 min bins were smoothed by a 5 bin running average. Overall, data were available for 98 individuals tested in three sets. In 2006, female activity was recorded for 4 days under LD cycles and subsequently for 20.5 days under LL. Males were recorded for 3 days under LD cycles but owing to technical problems, for only 12.3

days under LL. To attain a longer series of measurements, we recorded females in 2007 for 32.4 days under LL following 4 days under LD cycles. In some cases, equipment failure created missing data. Estimates of τ were unaffected by missing values, but for phase estimates, short gaps (up to 8 bins) were filled by means of preceding and subsequent values. For a single male, phase could not be estimated owing to a larger gap in the data.

(c) *Derivation of timing traits and statistical analyses*
We derived τ by the Lomb–Scargle periodogram analysis (Ruf 1999), implemented in the software program CHRONOSHOP (courtesy of Kamiel Spoelstra). Several individuals showed some degree of internal desynchronization under LL, but in all cases, a dominant τ exceeded the significance threshold ($p = 0.05$). We used the long recording of females in 2007 to examine whether the differences in series length affected estimates of τ. We investigated at which time after onset of LL period length stabilized, and whether the estimated τ depended on length of recording (courtesy of Julia Diegmann). Even when estimated from the first 3 days after exposure to LL, period length was significantly correlated with estimates that were based on longer time series. From day 6, estimates of τ were stable and accurate. Thus, τ could be estimated with confidence from short series. Estimates based on 7 versus 27 days, respectively, differed maximally by ± 0.17 min. Therefore, we removed the first 5 days after exposure to LL and calculated τ over 15.0 days in females and over 7.3 days in males.

We also estimated chronotype under LD cycles as phase of entrainment, given by centre of gravity (COG; Kenagy 1980), which is a particularly robust measure. However, data on chronotype are preliminary because the interval from which we estimated chronotype was short, and because sensors also recorded minor movements. We used an edge detector (Helm & Gwinner 2005) to determine, individually for each bird, the time of greatest increase in activity in the morning (within 2 h before and after lights-on) and of greatest decrease in activity in the evening (within 4 h because of greater variation). This method identifies the time of core activity, yielding a relatively late calculated onset and early calculated termination of activity. Duration of activity was the interval between onset and termination. Phase was calculated as a bird's activity timing relative to the time of lights-on, mid-light time and lights-off, respectively. Thus, a positive estimate of phase under LD cycles indicates early (i.e. phase-advanced) chronotypes, and a negative estimate indicates late (i.e. phase-delayed) chronotypes. The overall level of activity of a bird was the mean number of hops per 2 min bin. Activity levels were calculated separately under LL and LD cycles, but the two measures were closely correlated ($r = 0.64$; $p < 0.001$; $n = 97$).

Timing traits were analysed by linear mixed models implementing restricted maximum-likelihood estimation methods. These have high flexibility and are suitable for unbalanced designs, as was the case for unequal clutch sizes in our study (GENSTAT 6.0. VSN International, Genstat 1993). We modelled effects of sex, brood, EP paternity, year of study and lay date as fixed factors and covariates to test for statistical significance. Significance was derived from Wald statistics which asymptotically follow a χ^2-distribution. Since significance levels can depend on the order of entry of factors, we tested each factor by adding as well as removing it from the model, but found no discrepancies. Experiments
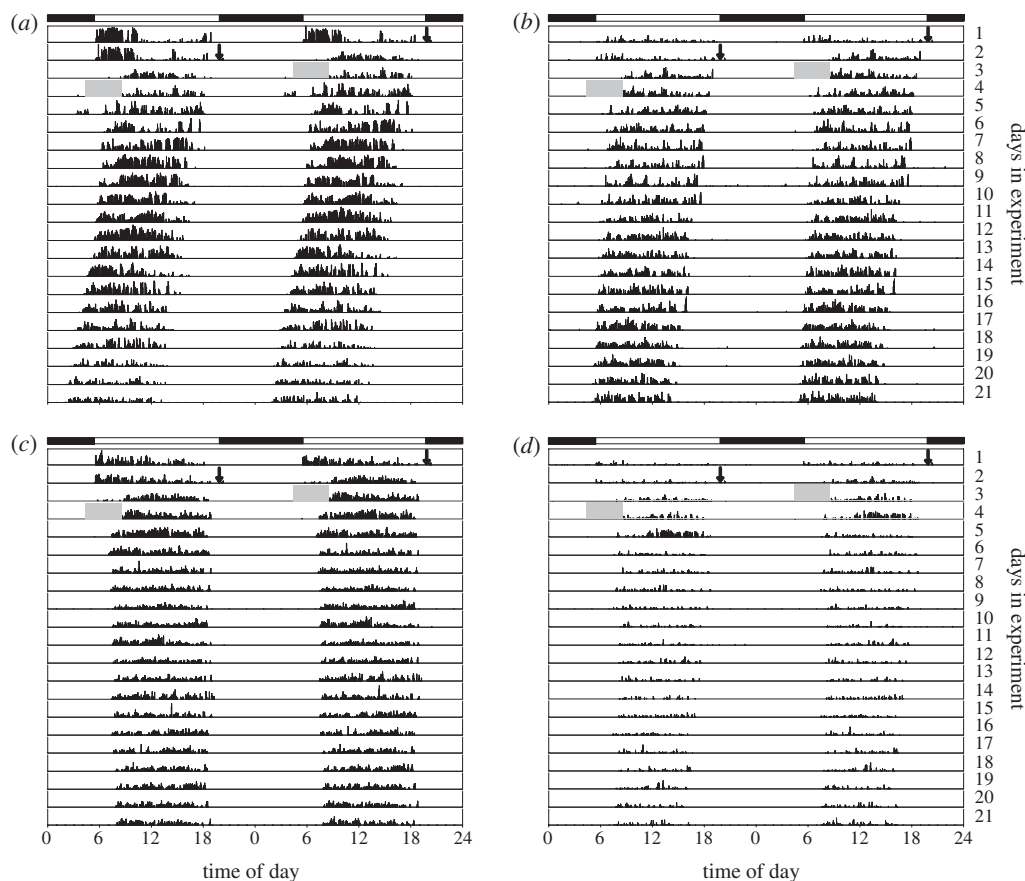
Figure 1. Activity timing of great tits. Actograms illustrate the distribution of movements (as black marks) over time for four females (*a–d*) collected from a single brood in 2007. The graphs show the number of recorded movements during subsequent 2 min bins across the time of day (records of each day are duplicated for greater clarity). Each line represents a day in the experiment (column to the right: day number). Bar on top indicates initial LD cycles (white = light; black = darkness); black arrows indicate onset of LL; grey blocks show brief failure of sensor. (*a,b*) Extra-pair daughters; (*c,d*) within-pair daughters.

in 2006 and 2007 differed in initial LD conditions, but because we found no effects of study year on τ we pooled the data. In broods with both EP and WP young, we tested for effects of EP paternity, brood and their interaction. Variance components and estimates of heritability (h^2) were derived from a random model. We used data from all WP young to estimate broad-sense h^2, reducing the model to the single remaining significant factor, family. We derived h^2 from the variance component associated with family divided by the total phenotypic variation (Falconer & Mackay 1996) as twice the intra-class correlation coefficient, treating the 20 families as class (Sokal & Rohlf 1995), and computed standard errors by an approximate formula for unequal family sizes (Roff 1997). We then used data from broods with both WP and EP young to attain relative proportions of variance associated with a common environment (shared nest and same mother), and with paternal genotype. In addition, we conducted similar, exploratory analyses of chronotype. Data were characterized by descriptive methods (mean ± s.d.) and correlational techniques.

(d) *Paternity analyses*

Genomic DNA from 5 µl blood from both parents and the chicks was isolated using the PureGene DNA Isolation Kit (Gentra Systems, USA). Polymerase chain reactions (PCR) were carried out in a 10 µl volume using the Multiplex PCR kit (Qiagen, The Netherlands). We used a microsatellite protocol with an annealing temperature at 60°C.

Micro-satellite markers (for the 2006 birds: Pma CAn1, Pma GAn27, Pma GAn42, Pma GAn30 and Pma D22, and for the 2007 birds: Pma CAn1, Pma TAGAn86, Pma GAn27, Pma C25 and Pma D22) were selected as described by Saladin *et al.* (2003). Fluorescent PCR fragments were visualized by capillary electrophoresis on an ABI3130 Genetic Analyzer (Applied Biosystems) and the sizes of the PCR products were determined using GENEMAPPER 4.0 software (Applied Biosystems). Individuals were categorized as WP young if all loci or all but one locus matched those of the social father (none of the young had a single mismatch).

3. RESULTS

Circadian period length τ differed considerably between the 98 recorded great tits from 20 broods (figures 1 and 2). Overall, τ was significantly shorter than 24 h and its distribution was slightly left-skewed (figure 2; mean ± s.d.: 23.83 ± 0.21; range: 23.4–24.4 h; $t_{97} = -8.33$; $p < 0.001$; skewness: 0.42 ± 0.25; kurtosis: 0.07 ± 0.49). Birds with shorter τ had higher activity levels (under LL: $r = -0.23$; $p = 0.025$; under LD cycles: $r = -0.17$; $p = 0.090$). As detailed in table 1, τ did not differ between the sexes and was unrelated to the lay date of a bird, regardless of whether females only or all birds were analysed. The only factors that had significant effects on τ were brood of origin and paternity. Paternity analyses revealed that four of the 20 broods contained both
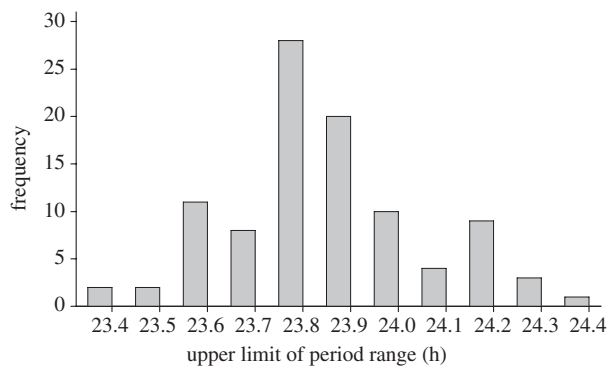
Figure 2. Distribution of free-running period τ in 98 great tits. Bars give frequency of τ for intervals of 0.1 h.

Table 1. Factors influencing period length τ. (Results from linear mixed-model analysis of τ of locomotor activity in wild-derived great tits ($n = 98$), all factors and covariates modelled as fixed effects; results on lay date are based on the subset of 86 laying birds.)

variable	Wald statistic	p-value
brood	$\text{Wald}_{19} = 77.1$	<0.001
extra-pair paternity	$\text{Wald}_1 = 11.6$	<0.001
chronotype (COG)	$\text{Wald}_1 = 2.0$	0.159
sex	$\text{Wald}_1 = 1.7$	0.197
lay date	$\text{Wald}_1 = 0.1$	0.772

EP young (EP; $n = 11$) and WP young (WP; $n = 10$). EP paternity occurred preferably in broods with slow clocks, as evident from longer τ of WP young with EP siblings compared with those without EP siblings (figure 3; EP broods: 24.12 ± 0.16; WP broods: 23.79 ± 0.19; $t_{85} = 5.21$; $p < 0.001$). EP young differed from their half-siblings by considerably shorter τ (figure 3; EP: 23.79 ± 0.14; WP: 24.12 ± 0.16; $t_{21} = -5.21$; $p < 0.001$), but were similar to WP young on a population level (EP: 23.79 ± 0.14; WP: 23.83 ± 0.21; $t_{96} = -0.65$; $p = 0.517$). Based on data from full genetic siblings (i.e. WP young only), τ had a high h^2 of 0.86 ± 0.24. In clutches with both EP and WP young, brood, which combines maternal contribution and common environment, was associated with 23 per cent of variation, while EP paternity accounted for 59 per cent of overall phenotypic variation, although standard errors for these estimates were high (table 2).

From the data collected during brief exposure to simulated LD cycles, we derived preliminary estimates of chronotype. Activity was timed relatively early (figure 1). The COG, used as a proxy for chronotype, preceded midday by 52.5 ± 22.3 min. The morning increase in activity occurred on average 16.7 ± 11.2 min after lights-on, and the evening decrease 51.1 ± 22.3 min before lights-off. Early chronotypes had higher activity levels ($r = -0.27$; $p = 0.007$) and were active longer than late chronotypes ($r = -0.38$; $p < 0.001$). However, we found no relationship between chronotype and τ ($r = -0.04$; $p = 0.701$; table 1). When we subjected chronotype to full analysis of factors related to timing, we found no effects of brood ($\text{Wald}_{18} = 18.2$, $p = 0.44$), EP paternity ($\text{Wald}_1 = 0.02$, $p = 0.88$) and
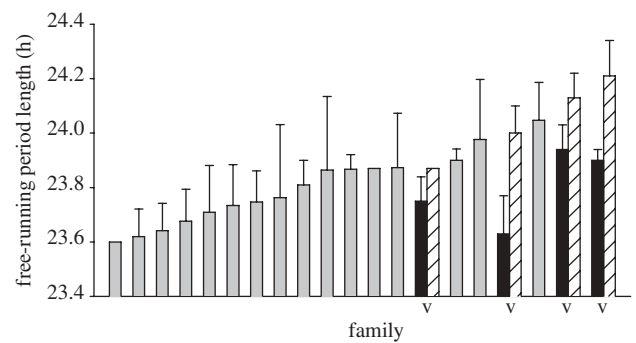
Figure 3. Free-running period of great tit broods. Bars show family means with s.e.m. ordered by τ of within-pair (WP) young. Broods with exclusively WP young are shown by grey bars. Broods with extra-pair (EP) young have two bars joined by a V: black bars, EP young; hatched bars, WP young.

Table 2. Estimated variance components ($\pm$s.e.m.) from linear mixed-model analysis of τ in the four broods of great tits ($n = 21$) that contained both extra-pair and within-pair young.

random term	variance component	proportion of total variance (%)
brood	0.01394 ± 0.0134	22.9
extra-pair paternity	0.03571 ± 0.0523	58.7
residual (individual)	0.01120 ± 0.0040	18.4
total phenotypic variance	0.06085	100

lay date ($\text{Wald}_1 = 3.1$, $p = 0.08$). We detected effects of two factors that were associated with differences in LD conditions, study year ($\text{Wald}_1 = 9.6$, $p = 0.002$; earlier timing in 2007 than in 2006) and sex ($\text{Wald}_1 = 11.6$, $p < 0.001$; earlier timing of male than female activity).

4. DISCUSSION

Our data demonstrate that τ, a fundamental property of the circadian system, is variable and highly heritable in a natural bird population. High h^2 of period length suggests that fast micro-evolutionary adjustments are conceivable (Bradshaw 1991; Price & Boag 1993). To our knowledge, evidence for genetic variation in τ is a novel finding for wild-derived vertebrates. In invertebrates, heritability was calculated from full-sibling analyses of τ in one population of nemobiine crickets, and from selective breeding in a second. Estimated h^2 differed between 0.17 and 0.78, respectively, and τ and phase under LD cycles were uncorrelated (Shimizu & Masaki 1997). The findings of selectable variation in a clock trait converge with circumstantial evidence for adaptive adjustment of circadian systems, inferred from latitudinal clines in clock properties and gene variants (Daan & Aschoff 1982; Pittendrigh & Takamura 1989; Price & Boag 1993; Decoursey 2004; Johnsen *et al.* 2007; Allebrandt & Roenneberg 2008; Kyriacou *et al.* 2008). Such micro-evolutionary adjustments could counteract possible constraints on flexibility that are thought to be imposed by circadian clocks (Kronfeld-Schor & Dayan 2003).

In our study, the factors that mediate selection on τ are still unclear. With respect to seasonal timing, we found no relationship between τ and lay date in captivity, which in our great tit population is a good proxy for laying date of these females in the wild (Visser *et al.* 2009). Similarly, in studies of selection lines of rodents (Majoy & Heideman 2000), circadian differences were unrelated to individual differences in reproductive response to photoperiod. It has been argued that evolutionary adjustments of seasonal timing may occur downstream from the clock because circadian modifications are expected to have pleiotropic effects (Majoy & Heideman 2000; Bradshaw & Holzapfel 2007). By contrast, recent findings from a closely related songbird, the blue tit, do point to a possible link between clock gene variants and seasonal timing (Liedvogel *et al.* 2009).

With respect to daily behaviour, our study confirmed that birds with shorter τ had higher activity levels, but we did not detect the links between τ and chronotype shown in some other studies (Aschoff & Wever 1966; Fleury *et al.* 2000; Johnson *et al.* 2003; Brown *et al.* 2008). Lack of an association is probably a consequence of short sampling under LD cycles (figure 1), but could also be related to complex entrainment of multiple oscillators (Daan & Beersma 2002; Decoursey 2004; Koukkari & Sothern 2006; Beersma *et al.* 2008; Brown *et al.* 2008) and to the particular light simulation (Fleissner & Fleissner 2002). Our preliminary study of chronotype indicated differences between study years and sexes. Both factors were confounded with expected effects of the different initial LD cycles (Daan & Aschoff 1975). An alternative interpretation, possible sex differences in phase control, would merit closer investigation.

Our findings offer a surprising clue to possible selection on τ. Data from EP young suggest that differences in τ may be associated with traits that in free-living great tits are implicated in sexual selection. EP young were found in broods with slow clocks and differed from their WP half-siblings by a significantly shorter τ (figures 1 and 3). Assuming that period lengths of offspring partly reflect those of their fathers, the data suggest that females choose males with fast clocks for EP matings, in particular if their social mate has a slow clock. Sexual selection for heritable, fast clocks could thereby contribute to adaptive adjustments of circadian rhythms. Studies of several avian species have demonstrated territorial and reproductive benefits of early activity, including high EP success (Daan & Aschoff 1982; Dolan *et al.* 2007; Murphy *et al.* 2008). EP matings are thought to be constrained by spatial limitations, but temporal niches, like early-morning hours, may provide opportunities to increase reproductive success for socially monogamous birds such as great tits (Dolan *et al.* 2007; Murphy *et al.* 2008). Our data further suggest that EP success could be related to more or longer lasting activity, rather than only to early rising because fast clocks were associated with high activity. EP paternity and sexual selection could potentially explain the finding that the τ of our wild bird population is significantly shorter than 24 h (Dolan *et al.* 2007). Directional selection for short τ is also suggested by the near-significant left-skew in its distribution (Price & Boag 1993).

We conclude that circadian systems in the wild may be highly variable and malleable in response to both natural and sexual selection. In great tits, slight deviations of τ from 24 h could be rewarded by reproductive benefits, but benefits of clocks with longer period lengths are still unclear. Reproductive benefits of particular chronotypes could be widespread. Many organisms show pronounced circadian rhythms in sexual traits, for example, flowering in plants and sperm production and sex steroid levels in mammals, including humans (Smale *et al.* 2005; Koukkari & Sothern 2006). Timing of these traits relative to mating opportunities and environmental conditions could confer major fitness benefits to individuals with particular circadian traits. Our findings are an important step forward in relating circadian biology to evolutionary ecology, and thereby contribute to the timely quest for the role of clocks in the real world (Menaker 2006).

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