Temperature has a causal effect on avian timing of reproduction

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Many bird species reproduce earlier in years with high spring temperatures, but little is known about the causal effect of temperature. Temperature may have a direct effect on timing of reproduction but the correlation may also be indirect, for instance via food phenology. As climate change has led to substantial shifts in timing, it is essential to understand this causal relationship to predict future impacts of climate change. We tested the direct effect of temperature on laying dates in great tits (*Parus major*) using climatized aviaries in a 6-year experiment. We mimicked the temperature patterns from two specific years in which our wild population laid either early ('warm' treatment) or late ('cold' treatment). Laying dates were affected by temperature directly. As the relevant temperature period started three weeks prior to the mean laying date, with a range of just 4°C between the warm and the cold treatments, and as the birds were fed ad libitum, it is likely that temperature acted as a cue rather than lifting an energetic constraint on the onset of egg production. We furthermore show a high correlation between the laying dates of individuals reproducing both in aviaries and in the wild, validating investigations of reproduction of wild birds in captivity. Our results demonstrate that temperature has a direct effect on timing of breeding, an important step towards assessing the implication of climate change on seasonal timing.

Keywords: timing of reproduction; birds; great tit; temperature; climate change

1. INTRODUCTION

Timing of reproduction and growth are life-history decisions with major fitness consequences. For many species, there is a relatively brief period in the year where conditions are favourable and it is at this time that the most demanding stages of the life cycle have to take place (Lack 1968). This period of favourable conditions varies from year to year and, as a consequence, the annual timing of many species varies annually. While photoperiod plays an important role in timing (Murton & Westwood 1977; Farner 1985; Dawson *et al.* 2001), it cannot account for this annual variation. The environmental variable that most clearly affects this variation in timing is temperature: many species breed, flower, end hibernation, etc. earlier in warm years (Parmesan 2007) as is often shown using correlations between temperature and mean population timing (Dunn 2004).

The population effects of temperature on avian timing of reproduction are mainly due to individuals reproducing earlier in warmer springs (Nussey *et al.* 2005; Charmantier *et al.* 2008). Thus, timing of reproduction is a so-called phenotypically plastic trait: the same genotype expresses different phenotypes under different environmental conditions (Pigliucci 2001). However, very little is known about whether there is a causal effect of temperature on timing of avian reproduction, or whether the correlations come about via a third derived variable (for instance, food abundance). It is essential that we understand this causal relationship between temperature and timing. Global climate change has already led to substantial shifts in timing (Parmesan 2007) and as temperatures are predicted to rise even further (IPCC 2007), we are faced

with the challenge to predict responses to these future climate scenarios. It makes an important difference whether temperatures affect timing directly. If that is the case, we can use reaction norms of timing versus temperature in a model to forecast future timing. But if temperature only impacts timing via food abundance, we also need to study the relationships between temperature and food abundance phenology, and between food abundance phenology and avian timing to be able to predict future impacts of climate change on seasonal timing.

The oldest experiments investigating the effect of temperature on timing of reproduction date back to 1937 (Suomalainen 1937) and since then many more papers have addressed this question, mostly using either temporal patterns in circulating hormones related to reproduction (El Halawani *et al.* 1984; Wada *et al.* 1990; Silverin & Viebke 1994; Maney *et al.* 1999; Wingfield *et al.* 2003; Perfito *et al.* 2005; Silverin *et al.* 2008) or gonadal development (Wendell Burger 1948; Engels & Jenner 1956; Lewis & Farner 1973; Storey & Nicholls 1982; Jones 1986; Silverin & Viebke 1994; Wingfield *et al.* 2003; Dawson 2005; Perfito *et al.* 2005; Silverin *et al.* 2008) as proxies for timing of reproduction. No clear pattern emerges from these studies: there is variation both among species and within populations of the same species on how temperatures affect the timing of hormonal or gonadal reproductive patterns. There are moreover potential problems with such proxies, as their relationships with timing of reproduction (i.e. laying dates) are not always straightforward.

Only very few studies have looked at the effect of temperature on avian timing of reproduction directly by obtaining laying dates under controlled conditions. Meijer *et al.* (1999) kept three groups of starlings

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(*Sturnus vulgaris*) in indoor chambers and increased ambient temperature by 5°C at a different period for each of these groups. They demonstrated that, on average, the first egg was laid 7–8 days after the increase in temperature, regardless of the date when the temperature was increased. A similar advance of egg laying was obtained a few days after discontinuing the cooling of nest-boxes in outdoor aviaries (Meijer *et al.* 1999). More recently, Salvante *et al.* (2007) have showed that zebra finches (*Taeniopygia guttata*) kept at 7°C significantly increased their energy intake, delayed their egg-laying initiation and laid fewer eggs at a slower rate, compared with birds kept at 21°C. Although manipulating temperature experienced by free-living birds is much more challenging, a few field studies have heated and/or cooled nest-boxes in the field. The results obtained in these experiments are, to some extent, in contradiction with those described in aviaries. Neither Nager & Van Noordwijk (1992) nor Yom-Tov & Wright (1993) found that manipulating overnight temperatures in nest-boxes altered laying dates in blue and great tits, respectively.

Temperature may affect laying dates in different ways, as it is experienced at different times during the development of the reproductive system. Temperature can function as a cue that predicts the forthcoming of favourable conditions (Wingfield *et al.* 1992), such as the period of maximal food abundance. But temperature may also play a direct role at the time of egg formation: there are costs associated both with the development of the reproductive organs (Williams & Ames 2004) and with producing eggs (Stevenson & Bryant 2000; Visser & Lessells 2001; Vezina & Williams 2002). At low temperature, these costs are higher (Stevenson & Bryant 2000) and thus may constrain laying date. Both in the starling (Meijer *et al.* 1999) and the zebra finch studies (Salvante *et al.* 2007), as well as in field studies with great and blue tits (Nager & Van Noordwijk 1992; Yom-Tov & Wright 1993), the effects of temperature were studied just prior to egg laying, and thus the effects of temperature could have been that it lifted the constraints on egg laying. No study using egg laying date directly to measure timing has looked at how temperature may play a role as a predictor for favourable periods.

Many field studies have demonstrated a correlation with temperatures experienced weeks before laying (Van Balen 1973; Perrins & McCleery 1989; Visser *et al.* 2006), and thus these temperatures earlier in the season may be of importance. During spring, temperatures will increase. Common practice is however, to use as a treatment, two fixed temperatures, unrelated to natural variation in temperature. A further problem is that these temperatures are often chosen to be extremely different. For instance, in the zebra finch study (Salvante *et al.* 2007), the two temperature treatments were kept constant at 7 and 21°C. To put this in perspective, the average difference in temperatures between a warm and a cold spring in the period relevant for great tits in The Netherlands is just 4°C. Thus, when studying the effects of temperature it would be better to use realistic temperature patterns (with small variations between treatments, but high temporal variations within treatments), as we do not know where the information that wild

birds use to time their reproduction is ‘coded’ in these temperature patterns.

The aim of this paper is to assess whether temperature has a direct effect on laying dates in great tits (*Parus major*) by determining laying dates under two temperature regimes, using temperature-controlled aviaries (one pair per aviary). We mimicked the temperatures from two specific years in which our long-term study population of great tits laid either very early or very late. We ran experiments for 6 years, allowing us to look at year-to-year variations even though the temperature patterns are aimed to be the same in all years.

2. MATERIAL AND METHODS

Pairs of great tits were kept in indoor aviaries (2 × 2 × 2.25 m) under artificial light. Photoperiod was increased twice a week following the natural increase in day length (i.e. from 7.45 L : 16.15 D to 16.30 L : 7.30 D at the winter and summer solstice, respectively). The main source of light were two high-frequency fluorescent tubes, but half an hour before these were switched on, and half an hour after they were turned off, an 8 W light bulb mimicked dusk and dawn. Temperatures were controlled ± 0.5°C by either heating or cooling the air that was circulated in the aviary. The realized temperatures were measured in each of the aviaries and recorded every 10 min. From these, average hourly temperatures were calculated.

Temperature patterns that birds were exposed to during the experiment mimicked two different years. As an early year (the warm treatment), we used data from 1998 with a mean laying date 20.8 April for our long-term great tit population at the Hoge Veluwe (The Netherlands). As a late year (the cold treatment) we used 1986, with a mean laying date 2.8 May, a difference of 10 days. For these years, we obtained the daily minimum and maximum temperatures as measured by the Royal Netherlands Meteorological Institute (KNMI) in De Bilt. Using these minimum and maximum values, hourly temperatures were calculated using a sine wave model (Baskerville & Emin 1969), with the lowest temperature at 03.00. Thus, the target temperatures were set hourly from the end of January to June, resulting in both a daily and seasonal pattern of temperatures. We alternated the temperature patterns over the aviaries over the years (i.e. the odd-numbered aviaries would be in the warm treatment in one year and in the cold treatment the next year).

The same sets of target temperatures were used every year. However, owing to various technical problems, the realized temperatures varied over the years. In addition to small-scale divergences that are inevitable when running experiments for several months, the system was, in general, unable to produce temperatures below 0°C, and on hot and sunny days temperatures were sometimes higher than the target values. As we were logging the actual temperatures, this enabled us to carry out some of the analysis using the realized temperatures to determine in which period temperatures played a role, rather than using temperature treatment (a class variable) as an explanatory variable.

Birds were caught from the wild from two Dutch forests (approximately 10 km apart): Warnsborn (WB; 1999, partly 2000 and partly 2004) and the Buunderkamp (BU; partly 2000, 2001, 2002, 2003 and partly 2004). The birds were introduced into the aviaries between mid-January and mid-February (photoperiod of less than 9 hours), although

some birds in 2004, when an additional 20 aviaries were included, were captured and introduced into the aviaries later (up to 10 March). The majority of the birds were thus introduced well before the critical photoperiod inducing the start of the rapid gonadal growth (between 11 and 12 hours; Silverin *et al.* 1993) and approximately three months before the start of the breeding period in these areas. From all birds, tarsus length and weight at catching were measured. Also, their age was determined (first-year breeder or older bird) from the contrast between the primary and greater coverts (Jenni & Winkler 1994).

The birds were given a constant daily amount of food throughout the experiment (consisting of fly pupae, sunflower seeds, fat, a mix of dried insects and Cédé mix: a mixture of proteins, vitamins, minerals and trace elements) as well as water, nesting material and a surplus of calcium.

Two nest-boxes were provided per aviary, which were checked daily for eggs (checking was carried out without disturbing the birds, as the bottom part of the nest-box containing the nest cup could be pulled out into the corridor for inspection). The date that the first egg was found is the laying date. Eggs were weighed and measured on the day they were laid, stored at -80°C and replaced by dummy eggs.

After the birds had incubated the clutch of dummy eggs for 5 days, the experiment was terminated. All birds were used only in one year and released at the end of the experiment in the forest where they were originally captured. Some of these birds were found breeding in nest-boxes in the wild in later years (and in a very few cases before they were put in the aviaries), and we thus have laying dates of these birds both in captivity and in the wild.

We ran the experiments for 6 years. In the first 5 years, we had 16 climatized aviaries and, in the last year, we had 36. In total, we obtained 73 laying dates. In the other 43 cases, either one of the birds of a pair died ($n=8$) or no eggs were found ($n=35$), although sometimes a nest was built. We analysed (using Proc GLM in SAS 8.02 with binominal errors) whether the probability to lay ($n=108$) was related to the temperature treatment, which it was not ($\chi^2_1 = 1.77$, $p = 0.096$). There were, however, year differences ($\chi^2_5 = 15.97$, $p = 0.007$), with some years where all birds laid (2000) and other years where just half the pairs laid (2002).

The data were analysed using generalized linear models (the Proc GLM of SAS 8.02) with laying date as a response variable. The main explanatory variables were either treatment (as a class variable: warm or cold) or the actual measured temperatures (as a continuous variable). For the latter analysis, the mean daily temperature for each of the aviaries in each of the years was calculated for eight 10-day periods (51–60 January, 61–70 January up to 121–130 January; note that the temperature periods are given as Julian dates). To avoid mixing non-experimental (from the period prior to when the birds were introduced in the aviaries) and experimental temperatures, we start at 50 January for the temperature period, the date that in all years all birds were in the aviary (except in 2004 when 12 birds were introduced later, for these birds we included the outside temperatures for all days up to the day a pair was put into its climatized aviary). We tested for an effect of the date the pair was introduced in the aviary by including this date in the statistical model. The end date for the temperature periods was set to 130 January at which date 9 out of the 73 laying pairs had already laid their first egg.

Additional explanatory variables were female age (first year versus older, class variable), male age (class variable) and female area of origin (the forest where the female was caught, class variable) as well as all two-way interactions. We applied a step-down approach, so that all variables and interactions with a p -value < 0.05 were taken out of the model.

Clutch size was analysed for the 38 out of 73 pairs that started laying. In the remaining 35 pairs, we did not consider the clutches as complete either because females did not start incubating or because eggs were removed and/or destroyed by one of the birds. The variation in clutch size (overall mean 8.0, range 4–13, the mean clutch size in the wild for this population is 9) was neither explained by treatment nor by realized temperatures, nor by laying date, year, female age or female area, nor by any of the two-way interactions.

3. RESULTS

(a) *The effect of temperature on laying dates*

Laying dates were significantly affected by temperature, with birds in the warm treatment generally laying earlier, but the effects of treatment on laying date varied from year to year (a significant year $\times$ treatment interaction; table 1; figure 1): while in 1999, the pairs in the warm treatment laid 7 days earlier than those in the cold treatment; in 2001, 2002, 2003 and 2004, the differences were 2, 28, 5 and 4 days, respectively. In 2000, however, the pairs in the warm treatment laid 8 days *later* than the cold treatment. The temperature effects were therefore in the predicted direction (earlier laying in warmer years) in 5 out of 6 years.

Owing to the differences in the target temperatures and those actually realized between years, birds were exposed to different temperature patterns in different years. We tested whether these realized temperature patterns could explain the year $\times$ treatment interactions, as well as determining in which period temperatures are affecting laying date. Determining in which period temperature plays a role may be relevant for the question of whether temperature is used as a cue by the birds. In the final model, two temperature periods remained (table 2), the periods 111–120 January and 121–130 January (both with a negative estimate; table 2; figure 2). When added to the model of table 2, neither treatment ($F_{1,68} = 1.10$, $p = 0.30$) nor treatment $\times$ year ($F_{5,63} = 1.97$, $p = 0.10$) was significant, indicating that variation in these two temperature periods account for the year-to-year variation in treatment effect.

(b) *Age effects*

In our experimental set-up, we used both birds that were born in the previous breeding season (young birds) and birds that were at least in their second year (old birds). In the aviaries, the old birds laid a week *later* than the young birds (tables 1 and 2), which is not what is generally found in the wild (Curio 1983; Robertson & Rendell 2001; Gienapp *et al.* 2006). Constraining the analysis to only those years for which we have laying dates of at least two old and two young females (1999, 2000 and 2004; $n=44$) does not alter this result: the old females lay 9 days later than the young females.

Table 1. Analysis of laying date (in April dates, i.e. 31 = 1 May) of great tits that were kept in climatized aviaries either under the temperatures mimicking a cold or a warm spring (treatment). (There are in total 73 laying dates over 6 years (1999–2004, factor with six levels). Females were either born in the previous breeding season (young) or earlier (old) and were caught in two areas, Warnsborn (WB) or Buunderkamp (BU).)

variable	<i>F</i> -statistics	<i>p</i> -value	level	estimate (s.e.)
treatment	$F_{1,59}=5.01$	0.029		
year	$F_{5,59}=3.34$	0.010		
female area	$F_{1,59}=4.06$	0.048	WB	55.08 (5.29)
			BU	45.24 (3.96)
female age	$F_{1,59}=5.18$	0.026	young	−7.78 (3.42)
			old	0
year × treatment	$F_{5,59}=2.55$	0.037	1999 cold	−6.44 (7.07)
			1999 warm	−13.36 (7.00)
			2000 cold	−8.93 (5.63)
			2000 warm	−1.21 (5.63)
			2001 cold	−5.47 (5.90)
			2001 warm	−7.30 (6.15)
			2002 cold	23.87 (7.56)
			2002 warm	−4.07 (6.48)
			2003 cold	7.27 (7.45)
			2003 warm	2.53 (6.48)
			2004 cold	4.06 (5.02)
			2004 warm	0

Figure 1. Laying date of first eggs (with $\pm$ s.e., in April dates, i.e. 31 = 1 May) of great tits that were kept in climatized aviaries under the temperatures mimicking either a cold (*c*) or a warm (*w*) spring. (There are in total 73 laying dates over 6 years ((a) 1999 *w*=6 and *c*=4, (b) 2000 *w*=7 and *c*=7, (c) 2001 *w*=6 and *c*=7, (d) 2002 *w*=5 and *c*=3, (e) 2003 *w*=5 and *c*=3 and (f) 2004 *w*=10 and *c*=10). Females originated from two areas, WB (filled circles) or BU (filled triangles)).

(c) Spatial variation

In the analysis with treatment (but not with the actual temperatures), there was also a significant effect on the area where the female was caught (table 1): females from Warnsborn (WB) laid 10 days later than those caught at the Buunderkamp (BU). In the wild, BU birds also lay slightly earlier than WB birds, on average 1.6 days (19 years of laying dates from the wild for both populations: effect of site $F_{1,18}=4.42$, $p=0.0498$; year effect (as a class variable) $F_{18,18}=142.5$, $p<0.0001$).

(d) Timing of reproduction under controlled versus wild conditions

For a subset of the birds (21 females), we have laying dates both from the wild (the BU area) and from the aviaries. Most of these were birds that were released after being kept in the aviaries and were found breeding in nest-boxes in the wild in later years ($n=16$), but in some cases birds had already bred in the wild before being taken into captivity ($n=5$). Overall, those females laid later in captivity than in the wild (repeated-measures ANOVA

Table 2. Analysis of laying dates (in April dates, i.e. 31 = 1 May) against two mean daily temperatures that were significant in a model with initially eight mean temperature 10-day periods (51–60 January, 61–70 January up to 121–130 January). (Also in the model was female age (young versus old). Both year (1999–2004, factor with six levels) and the area where the female was caught were not significant.)

variable	<i>F</i> -statistics	<i>p</i> -value	level	estimate (s.e.)
female age	$F_{1,69}=9.83$	0.0025	young old	201.00 (49.14) 210.48 (49.00)
temperature 111–120 Jan	$F_{1,69}=8.91$	0.0039		–4.21 (1.41)
temperature 121–130 Jan	$F_{1,69}=8.87$	0.0040		–8.59 (2.88)

Figure 2. The laying dates (as the residual from a model with female age (categorical) in April dates) against two mean temperatures (table 2): the periods 111–120 and 121–130 January (note that 1 April = 90 January). (Filled symbols are the warm treatment and open symbols are the cold treatment; different symbols represent different years (1999–2004)).

Figure 3. Laying dates of female great tits for which a laying date was obtained both in the wild (BU) and in the aviaries (laying dates are relative to the mean laying date of that year for either the BU population or the aviary birds). (Both the regression line (solid) and the $x=y$ line (dashed) are given.)

on laying dates: $F_{1,20}=58.00$, $p<0.0001$; birds lay on average 21.7 days earlier in the wild). For a more accurate comparison between breeding in captivity and breeding in the wild, we controlled for the year-to-year variations in laying dates. Laying dates were therefore standardized by subtracting the mean laying date for that year in the aviaries (aviary laying dates) or in the BU population (wild laying dates). The laying dates in the aviaries were clearly correlated with the laying date in the wild of these same females ($F_{1,19}=6.28$, $p=0.021$; figure 3) and even more striking is that the slope of the regression is not significantly different from 1 (0.99 ± 0.39) and the intercept is not significantly different from 0 (1.76 ± 1.75 ; $p=0.33$). Thus, among-individual variations in laying dates in the aviaries clearly reflect among-individual variations in laying dates in the wild (figure 3).

We have similar data for 11 males as breeding partners both in the wild and in captivity. Again, we find a relationship but now the earlier the pair in the aviary laid, the later the male's mate in the wild laid ($F_{1,10}=6.50$, $p=0.029$, slope -1.36 (s.e. 0.53)). It is not clear why this relationship is opposite in the males, but we do want to point out that laying dates are under female rather than male control, and that male breeding periods are less repeatable across years than the female ones (Van Noordwijk *et al.* 1981; Caro *et al.* 2009).

4. DISCUSSION

Avian timing of reproduction in the wild is clearly correlated with temperature (Dunn 2004): in warm years, the mean population laying date is earlier than in cold years. Very few studies have looked at the causal relationship between timing and temperature using laying dates under controlled conditions. The two papers that did this (Meijer *et al.* 1999; Salvante *et al.* 2007) focused on the effect of temperature just prior and during egg production, i.e. whether temperature can lift constraints on egg production. In this study, we look at the causal effects of temperature when temperature provides information on the optimal time of reproduction, i.e. with temperature as a cue.

We find that temperature does affect timing directly, demonstrating that temperature has a direct effect on timing rather than via a third variable such as a food abundance. The strength of the effect, however, varied from year to year, even under controlled conditions (table 1; figure 1). When the realized temperature is used rather than treatment, the year-specific temperature effects and the year differences themselves are no longer significant (table 2), demonstrating that the year-to-year variations were probably due to variation in the realized temperature patterns. The temperature periods that affect laying date are 111–120 January and 121–130

January (21 April–10 May). This is a later period than the period over which the temperatures correlated best with laying dates of Dutch great tits in the wild, which is 16 March to 20 April (Visser *et al.* 2006). Even when we correct for the difference in laying date in aviaries and the wild (21.7 days; see §3d and below), the aviary birds use a temperature period with a starting date of two weeks later, but with a similar end date. Thus, while in the wild, temperatures five weeks prior to the mean laying date seem to play a role, in the aviaries this is the three weeks prior to the mean laying date. The degree to which temperatures affect laying dates is striking: more than $4 \text{ d } ^\circ\text{C}^{-1}$ for the 111–120 January temperatures, and even double that for the 121–130 January period (table 2). The range of temperatures in the experiment was small, approximately 4°C for both periods, but realistic as this is also the range of spring temperatures (for the relevant period) in The Netherlands. This small range is, however, in sharp contrast to the temperature range used in other studies on the role of temperature on seasonal timing (Dawson 2005; Salvante *et al.* 2007; Silverin *et al.* 2008).

The experiment was designed to study the effect of temperature as a cue for laying dates. The period over which temperatures played a role was the period of three weeks prior to the mean laying date. This makes it likely that temperature played a role as a cue rather than as lifting a constraint. Great tits are the so-called income breeders (Meijer & Drent 1999), i.e. they produce their eggs from the nutrients and energy intake on the days the egg is formed. As egg formation takes 4 days (Kluyver 1951), it is unlikely that temperature three weeks before egg laying would affect the energetics of egg production. Furthermore, the birds had *ad libitum* food and the temperatures in the relevant periods varied at most 4°C . The results do, however, indicate that the period in which temperatures play a role as a cue is late in the cascade of physiological events leading up to laying the first egg. It may well be possible that the onset and first stages of gonadal growth are primarily driven by photoperiod, and that the fine-tuning effect of temperatures is only at the end of gonadal growth, or even after full gonadal development, but before initiation of vitellogenesis and rapid follicle development (Wingfield & Kenagy 1991). Experiments as ours, but which also include physiological measurements, are needed to shed light on this.

Laying dates in the aviaries differ from laying dates in the wild in at least three important ways. First, on average, captive birds breed later than wild birds. Late laying dates in captivity have already been reported in several tit populations (Lambrechts & Dias 1993; Lambrechts *et al.* 1999). The exact reason for this delay still unknown but probably refers to the lack of some specific cues or resources (Lambrechts *et al.* 1999) used by the birds to time their breeding, or general stress caused by artificial environments (Caro *et al.* 2007). For example, in a Corsican population of blue tits that usually breeds two months later in captivity than in the field, increasing the size of outdoor aviaries, which creates more natural breeding conditions in captivity, causes a significant advancement in laying dates (Caro *et al.* 2007), similar to those observed in the field.

Second, while in the aviaries, the period in which temperatures play a role as a cue is late in the cascade of physiological events leading up to laying the first egg,

in the wild, temperature seems to play a role earlier. For our long-term Hoge Veluwe population, the best correlating temperature period is 16 March–20 April (Visser *et al.* 2006), with the temperatures just prior to egg laying playing a less important role in determining laying date than the long-term temperature (Gienapp *et al.* 2005). But these conclusions come from correlations between temperature and laying dates, and thus these differences may be due to indirect effects of temperatures in the wild, for instance via an effect of temperature on food abundance, which would then affect laying date.

Third, in the aviary experiment, first-year great tit breeders laid earlier than older females (tables 1 and 2), which is in contrast to what is often found in the wild, both for laying dates (Curio 1983; Robertson & Rendell 2001; Gienapp *et al.* 2006) and for the seasonal maturation of the reproductive system (Silverin *et al.* 1997; Deviche *et al.* 2000; Caro *et al.* 2006). It is likely that these older females had already reproduced in the wild before they were brought into the aviaries (for five cases, we actually have records of these females breeding in the wild), and there is accumulating evidence that great and blue tits make use of earlier breeding experience in their subsequent timing (Grieco *et al.* 2002; Gienapp & Visser 2006). One way this former experience may play a role is via the physiological transduction of the spring increase in photoperiod. Female starlings that have already been exposed to a photoperiodic cycle developed their reproductive axis more quickly than females of the same age class, which had no previous experience with photoperiodic cycles (Sockman *et al.* 2004). We might expect photoperiodic experience to also be involved in the present age-class difference observed in captivity, but in a slightly different manner. Female great tits that had already reproduced in the wild may have imprinted on some particular aspects of the photoperiodic cycle (i.e. light quality and intensity) and/or its interaction with other environmental cues (temperatures). These aspects were probably not similarly reproduced in captivity (i.e. artificial lightning) and may have delayed their onset of laying. Contrarily, first-year breeders had no experience with any previous seasonal increase in photoperiod (especially natural photoperiods in the wild), and their laying dates were therefore not affected by the novelty of artificial photostimulation. This explanation and the unknown mechanisms involved are quite similar to what has been hypothesized for the lack of laying behaviour in captive wild-caught females compared with hand-reared females (Baptista & Petrinovich 1986): females gain experience with environmental cues during their first breeding season and rely on these sets of cues to time their subsequent breeding events.

In one of the analysis (using treatment as the main explanatory variable), we found a significant influence of the female's native forest (table 1), but not in the analysis with actual temperature (table 2). It is not clear whether the possible difference between areas is due to genetic differences between these populations. There is a small but significant difference in wild laying dates between these populations in the predicted direction ($F_{1,18} = 4.42$, $p = 0.05$, 1986–2005; mean laying date: WB = 24.7 and BU = 23.1 April). However, the two forests are connected by other suitable habitat and hence it seems unlikely that there is no exchange of individuals between these populations. The distance between them is just 10 km.

We cannot rule out that the effects of the native forest come about through small differences in the way the birds were caught. In WB, we caught the birds on the edge of the forest, in an adjacent garden, while in BU, the birds were caught in the middle of the nest-box area.

Studies on the effects of temperature on timing have mostly been carried out by measuring temporal patterns either in circulating hormone levels or in gonadal sizes. The correlations between these measures and timing of reproduction (i.e. laying dates) are not always clear. But do laying dates obtained in controlled temperature aviaries tell us something about laying dates in the wild? The conditions in the aviaries are obviously very different and potentially important cues are missing (see above). The birds in the aviaries lay late relative to laying dates in the wild but despite this, we found a very good correlation between laying dates of the same female obtained in the aviaries and in the wild (figure 3). We thus clearly show that the variation among females in the wild is reflected in the variation among females in the aviaries. As females in the aviaries have *ad libitum* food, this would also indicate that differences among birds in the wild is not due to differences in the degree to which they are constrained (i.e. their ability to produce eggs under early spring conditions), but that it is more likely that these differences are due to the way birds perceive and process cues similar to photoperiod and temperature.

How temperature affects laying date in a mechanistic sense is largely unknown. It has been suggested that the effects of temperature on gonadal growth could be mediated through a neuroendocrine mechanism controlling luteinizing hormone release (Silverin *et al.* 2008; but see Wingfield *et al.* 2003), while the effects of temperature on the termination of breeding and gonadal regression could be mediated through the control of prolactin and/or thyroid hormone levels (Dawson & Sharp 1998; Maney *et al.* 1999; Wingfield *et al.* 2003). Because studies manipulating temperature and photoperiod in captivity have generally produced contrasting results, much remains to be done in order to understand how temperature acts on the hypothalamo-pituitary-gonadal (HPG) axis. It seems, however, that female gonads may be more sensitive to temperature than to male testes (Wingfield *et al.* 1997), and that temperature sensitivity may be more pronounced in populations breeding at lower latitudes (Wingfield *et al.* 2003; Silverin *et al.* 2008). As a consequence, because the effects of temperature on the HPG axis seem rather subtle and hard to detect, future investigations should first focus on low latitudinal populations of females and use realistic patterns of photoperiod and temperature. By doing so, we would maximize our chances of understanding these fine-tuning effects of temperature on bird reproductive development. Finally, the recent discoveries of gonadotropin-releasing hormone and gonadotropin-inhibitory hormone receptors at the level of the gonads (Bentley *et al.* 2008), combined with a possible control of reproduction lying at the level of the female ovary (Caro *et al.* 2009), have opened the way to future exciting investigations.

The temperatures in the climatized aviaries affected the laying dates of the great tits as the different treatments had different temperature patterns. Any information coded in these patterns can be used by the birds, which would not be the case if we used constant temperatures, as

is often done. Also, it ensures that we use realistic temperature values rather than the extreme differences in temperature often used in experiments. This is especially relevant when studying the informative value of temperature (as a cue), as temperatures outside the natural range should be meaningless to the birds. However, in the temperature patterns used here, there is (as intended) temporal autocorrelation in the temperatures, which makes it difficult to assess which period matters. Now we have shown that temperature plays a direct role in timing of reproduction, we can start using artificial temperature patterns to address that question.

The experiments presented here are a step towards a better understanding of the mechanisms underlying phenotypic plasticity of ecological relevant traits. This integration should lead in the long run to an understanding of selection not on the phenotypes but on the underlying physiological response mechanisms. Especially for phenotypic plastic traits such as laying date, it is these 'rules' on which selection will operate (Visser 2008). The only route towards such an understanding is to integrate the knowledge on the ecology and evolution of phenotypic variation with the detailed knowledge on the physiology underlying the perception and integration of environmental variables (Dawson 2008; Wingfield *et al.* 2008). This integration is especially pressing as organisms now need to survive and reproduce in a warming world in which the rules they are using may no longer be adaptive (Visser 2008).

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