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RESEARCH ARTICLE

Temperature has a causal and plastic effect on timing of breeding in a small songbird

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ABSTRACT

Phenotypic plasticity is an important mechanism by which an individual can adapt its seasonal timing to predictable, short-term environmental changes by using predictive cues. Identification of these cues is crucial to forecast the response of species to long-term environmental change and to study their potential to adapt. Individual great tits (*Parus major*) start reproduction early under warmer conditions in the wild, but whether this effect is causal is not well known. We housed 36 pairs of great tits in climate-controlled aviaries and 40 pairs in outdoor aviaries, where they bred under artificial contrasting temperature treatments or in semi-natural conditions, respectively, for two consecutive years, using birds from lines selected for early and late egg laying. We thus obtained laying dates in two different thermal environments for each female. Females bred earlier under warmer conditions in climate-controlled aviaries, but not in outdoor aviaries. The latter was inconsistent with laying dates from our wild population. Further, early selection line females initiated egg laying consistently ~9 days earlier than late selection line females in outdoor aviaries, but we found no difference in the degree of plasticity (i.e. the sensitivity to temperature) in laying date between selection lines. Because we found that temperature causally affects laying date, climate change will lead to earlier laying. This advancement is, however, unlikely to be sufficient, thereby leading to selection for earlier laying. Our results suggest that natural selection may lead to a change in mean phenotype, but not to a change in the sensitivity of laying dates to temperature.

KEY WORDS: Correlated response, Selection, Great tit, Phenotypic plasticity, Timing of breeding

INTRODUCTION

The effects of global climate change are omnipresent and can severely modify environmental conditions for wild populations (Parmesan and Yohe, 2003; Walther, 2010). Phenological traits in particular are highly sensitive to these environmental modifications. This has led to phenological changes with subsequent mismatches between trophic levels, in numerous taxa, covering all trophic levels (Cohen et al., 2018; Parmesan, 2006; Root et al., 2003; Visser and Gienapp, 2019), because trophic levels do not necessarily shift their timing at the same rate in response to increasing temperature (Thackeray et al., 2010; Visser and Holleman, 2001; Visser et al., 1998; Voigt et al., 2003). It remains largely unknown, however, to which processes these phenotypic changes are attributed. As such, distinguishing between genetic changes and phenotypic plasticity (Gienapp et al., 2008; Merilä and Hendry, 2014), i.e. the environmentally induced production of different phenotypes by a single genotype (Pigliucci, 2001), hampers predictions of species adaptations to ongoing climate change.

In temperate zone birds, the breeding period is short and varies yearly as a result of annual variation in environmental conditions. Consequently, females need to track this inter-annual variation to optimally time their breeding (i.e. egg laying) to when food resources are most abundant in order to support successful rearing of offspring (Charmantier et al., 2008; Perrins, 1965; Sheldon et al., 2003; van Noordwijk et al., 1995). Females thus need to be able to ‘predict’ when to initiate breeding, for which they use environmental cues. Photoperiod is an important cue, as it gives initial important information to track the time of the year and activates the reproductive system (Dawson et al., 2001; Farner, 1985). However, its invariability from year to year does not allow females to track year-to-year variation in local conditions (Bradshaw and Holzapfel, 2007; Visser et al., 2004) and, as such, photoperiod alone cannot explain individual year-to-year differences in the timing of breeding. Temperature is highly variable between years and provides information about local conditions, such as insect emergence, which allows females to fine-tune the timing of breeding (Dawson, 2008; Wingfield and Kenagy, 1991; Wingfield et al., 1992) and subsequently match offspring needs to food abundance. Temperature is the major driving force of biological seasonality in temperate zones (Parmesan, 2007) and the most influential environmental cue in fine-tuning the timing of avian seasonal breeding (Caro et al., 2013; Lambrechts and Visser, 1999; Visser et al., 2009).

Spring temperatures could affect the timing of breeding directly or indirectly, by altering other environmental conditions, which subsequently causally affect the timing of breeding. Previous work has suggested that the former is the case, i.e. that temperature has a direct effect on the timing of egg laying directly, rather than acting via, for example, food phenology (Schaper et al., 2011), as shown in a 6 year study in great tits (*Parus major*) (Visser et al., 2009). In that study, breeding pairs were housed in climate-controlled aviaries and went through a breeding season in either a warm or a cold treatment, mimicking a warm and cold spring, respectively. Birds initiated egg laying significantly earlier in the former. It is important to point out here, that in the study by Visser et al. (2009), the laying date comparison was performed between individuals, thus showing between-individual variation. In addition, the effect of temperature on egg laying varied strongly between the 6 years of the study, despite controlled conditions.

Here, we firstly set out to study whether plasticity in laying date is due to the direct or indirect (e.g. via food phenology) effect of temperature on the timing of egg laying by subjecting birds to

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Received 15 November 2019; Accepted 17 March 2020

contrasting temperatures in climate-controlled aviaries for 2 years and gathering within-individual data on egg laying. As such, the real effect of temperature is less likely to remain undetected or clouded by differences between individuals (e.g. genetic and physiological factors, physical condition, etc.). In addition, we housed pairs in semi-natural conditions (i.e. outdoor aviaries) for two consecutive years that differed in environmental conditions, including temperature. Based on previous studies both in the wild (e.g. Both and Visser, 2001; McCleery and Perrins, 1998) and in captivity (Schaper, 2012; Visser et al., 2009), we expected females to lay earlier in the warm environment than in the cold. If this was indeed the case, we expected that the earliest egg-laying female would have experienced a steeper increase in average daily temperature in the days prior to initiating egg laying compared with the earliest female in the cold environment. This, because an increase in temperature shortly prior to egg laying has been shown to advance the timing of egg laying in great tit females (Schaper et al., 2012).

Secondly, we had the unique opportunity to test whether these birds, which originated from selection lines for early and late timing of breeding through bi-directional genomic selection (Gienapp et al., 2019; Verhagen et al., 2019b), showed a difference in the average laying date (i.e. the elevation of the reaction norm) between selection lines, independent of environment. As shown previously, early selection line females laid on average about 6 days earlier than late selection line females (Verhagen et al., 2019b). This difference between selection lines is, under the influence of environmental cues, ultimately rooted in the cascade of (epi)genetic and physiological processes underlying egg laying. How these mechanisms are affected by temperature remains obscure and potential pathways are discussed elsewhere (Caro et al., 2013). However, we expected that early selection line females would lay earlier across environments compared with late selection line females.

Lastly, we tested whether selection for laying date had a correlated response to selection on the sensitivity to temperature (i.e. the slope of the reaction norm). Previously, Ramakers et al., (2019) studied whether reaction norms would evolve under current climate change in our long-term study population of great tits in the Hoge Veluwe from which these selection line birds originated (see Verhagen et al., 2019b). By quantifying selection on, and predicting the evolution of, the timing of breeding reaction norm in response to temperature over three time periods, they showed that laying dates did advance over the time periods examined, but the sensitivity of laying dates to temperature (i.e. laying date plasticity) did not (Ramakers et al., 2019). As such, we did not expect a difference in laying date plasticity between the selection lines.

Currently, knowledge on how cues are perceived (Caro et al., 2013; Dawson, 2008) and on how individuals vary in their perception (individual-by-environment interaction or I×E) is still scarce (Lyon et al., 2008; Visser, 2008; Visser et al., 2010). Identification of these cues and understanding of the responses of breeding plasticity to selection, therefore, are crucial to forecast species' responses to long-term environmental change and to study the potential for adaptation to such change.

MATERIALS AND METHODS

Selection lines in timing of breeding

Selection lines were created for early and late timing of breeding in great tits (*Parus major* Linnaeus 1758) using genomic selection, which was moderately strong and in both directions (Gienapp et al., 2019; Verhagen et al., 2019b). Briefly, nestlings (F_1 generation) were taken from wild broods of our long-term study population in the Hoge Veluwe National Park, The Netherlands (52°02′07″N, 5°51′32″E) in which the mother had initiated egg laying either extremely early (early selection line) or extremely late (late selection line) in the wild. These chicks were genotyped using a 650 SNP chip (Kim et al., 2018) in order to predict their 'genomic breeding values' (GEBVs, i.e. the value estimating the relationship between genotype and phenotype based on genetic markers). The F_1 generation individuals with the most extreme GEBVs were selected for early and late selection line breeding pairs to produce the F_2 generation in captivity. Eggs were transferred to wild foster nests, where they were incubated and hatched. Subsequently, 10 days post-hatching, F_2 generation chicks were brought into the aviary facilities at the NIOO-KNAW (Wageningen, The Netherlands) for further hand-raising. In turn, the F_2 offspring were genotyped and, based on the most extreme GEBVs, selected to produce the F_3 generation, which was then genotyped and selected. This study was performed under approval by the Animal Experimentation Committee (DEC), Amsterdam, The Netherlands, protocol NIOO 14.10.

The results of the selection line study are described elsewhere (Verhagen et al., 2019b). In short, early selection line birds laid on average earlier than late selection line birds, and this difference in laying date increased (from about 2 to 10 days) over the generations (F_1 to F_3). Line effects for the F_1 and F_2 generation were non-significant, but line differences were highly significant for the F_3 generation. On average early selection line birds laid about 6 days earlier than late selection line birds (Verhagen et al., 2019b). Note that these results were found in the birds housed in outdoor aviaries (see below). No differences were found between selection lines and treatments in birds that were housed in climate-controlled aviaries (see below; Verhagen et al., 2019a).

Outdoor aviaries

The outdoor aviaries are described in detail in Verhagen et al. (2019b). In short, from January 2017 onwards, F_3 generation pairs (n=40) were housed in 40 outdoor aviaries (4.2×1.9×2.1 m) where the birds were subjected to natural photoperiod and temperatures. These pairs had the most extreme GEBVs (see above) within the F_3 generation and functioned as breeding pairs to produce eggs (F_4 generation) to be put in the wild as part of another study. Temperatures were recorded in 20 out of the 40 aviaries every 10–30 min using loggers (Thermochron iButton) throughout the breeding season.

Climate-controlled aviaries

The climate-controlled aviaries have been described in detail elsewhere (Verhagen et al., 2019a) but, briefly, 36 pairs of the F_3 generation of the selection lines (see 'Selection lines in timing of breeding', above) were housed in 36 climate-controlled aviaries in January 2017. These birds had less extreme GEBVs (see above) compared with the birds housed in the outdoor aviaries. In the climate-controlled aviaries, birds received an artificial photoperiod that mimicked the change in natural photoperiod. In addition, two contrasting temperature treatments (Fig. 1A) were provided, mimicking an extreme cold (2013) or extreme warm (2014) spring in The Netherlands (for details, see Verhagen et al., 2019a). This was reflected in (average) egg-laying dates between these years in the Hoge Veluwe population, where females (n=47 for which we obtained laying dates for both 2013 and 2014) laid ~24 days earlier in 2014 than in 2013 (Fig. 2A). Every hour the temperature changed to follow as closely as possible the observed hourly temperatures in these years (note that the minimum temperature in the aviaries was 2°C so any temperature below 2°C in the temperature time series from outside was set to 2°C).

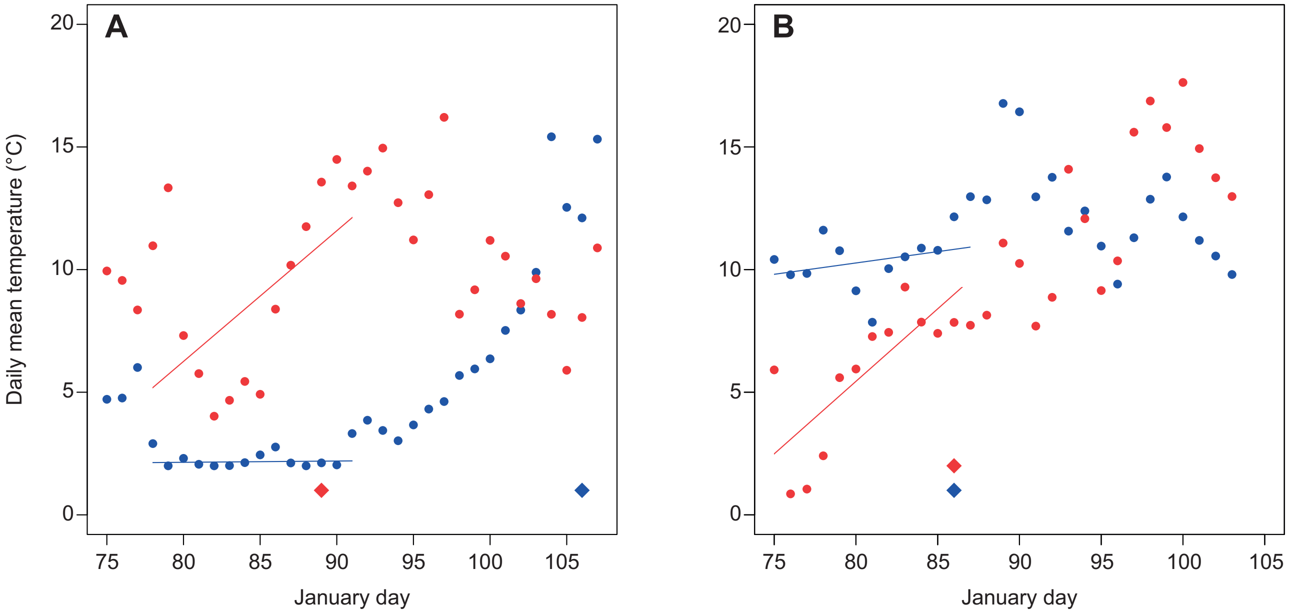

Fig. 1. Mean daily temperature profiles during the great tit breeding season (16 March to 20 April) in the climate-controlled and outdoor aviaries. (A) Temperature profiles of the climate-controlled aviaries (*n*=36) for the cold (blue) and warm (red) treatment. (B) Temperature profiles of the outdoor aviaries (*n*=40) from 2017 (blue) and 2018 (red). Note that for the outdoor aviaries, temperature is shown until 15 April. The filled diamonds indicate the first egg laid in the warm (red) and cold (blue) temperature treatment or in 2017 (blue) and 2018 (red), for the climate-controlled and outdoor aviaries, respectively. The regression lines indicate the relationship between date and mean daily temperature for the 11 day period prior to breeding initiation. Dates are in January days (January day 90=30 March).

Mimicking these natural temperature patterns is important to be able to infer realistic conclusions. It is still a challenge to define what information in an experienced temperature profile is used to time breeding. However, previous research indicated that the seasonal increase in temperature, rather than the average temperature explains the fine-tuning in the timing of avian breeding (Schaper et al., 2012). The combination of selection line and temperature treatment resulted in four groups (*n*=9 pairs per group) in the climate-controlled aviaries: 'early-warm', 'early-cold', 'late-warm' and 'late-cold'.

Birds in both aviary types were fed *ad libitum* with several food sources and had water available for drinking and bathing (for details, see Visser et al., 2011).

Breeding seasons and laying dates

All birds went through their breeding season in 2017 and were housed in single-sex groups afterwards. In January 2018, the same pairs were housed in the same outdoor or climate-controlled aviary that they were in for the 2017 breeding season to go through their second breeding season. In the climate-controlled aviaries, when

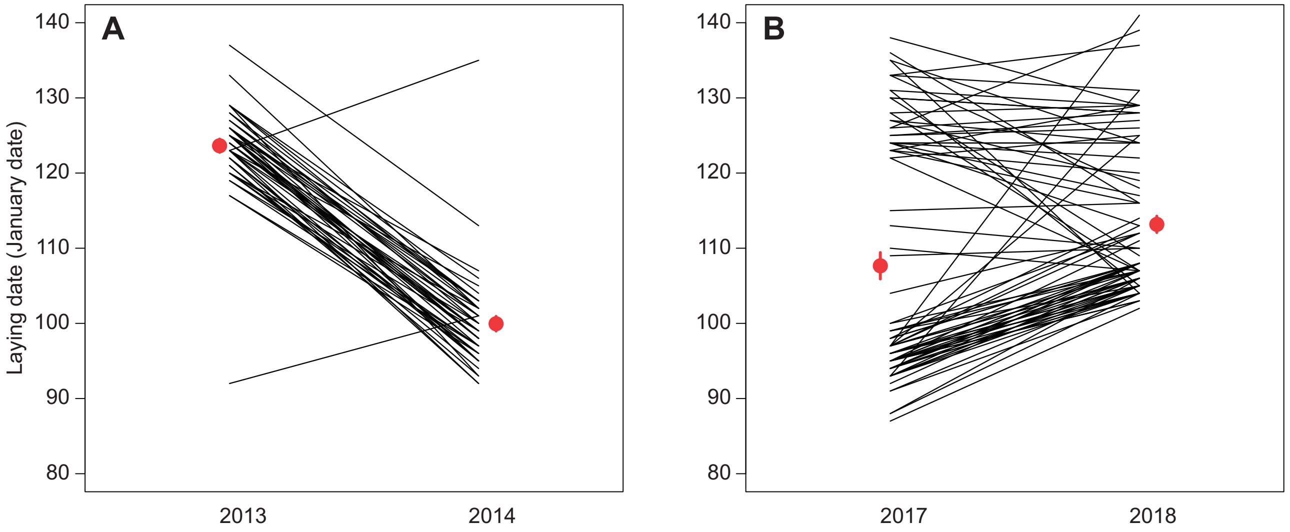

Fig. 2. Individual reaction norms and mean laying dates for female great tits in Hoge Veluwe National Park. (A) Data for 2013–2014, the years for which the temperatures were used for the experiments in the climate-controlled aviaries. (B) Data for 2017–2018, the years in which the open aviary experiment was performed. Laying dates are presented as January dates (e.g. January day 90=30 March), with mean (±s.e.m.) values indicated by the red circles. Females (*n*=47) laid on average ∼24 days earlier in 2014 than in 2013, and females (*n*=85) laid on average ∼5.5 days earlier in 2017 than in 2018.

pairs were subjected to the warm temperature treatment in 2017, they received the cold treatment in 2018 and vice versa. During these breeding seasons, nest boxes in all the aviaries were checked twice a week for nest building, and daily, when a female had completed her nest, for eggs. Laying dates (i.e. the first day an egg was laid by a female) were recorded as January dates (i.e. 1 January=1, 1 April=91, etc.). Some females did not initiate egg laying in one or both environments. As such, we were able to obtain two laying dates recorded in two different environments for 34 out of 40 outdoor aviary females and 32 out of 36 climate-controlled aviary females.

Statistical analysis

While for the climate-controlled aviaries we knew which temperature profile was associated with early laying in the wild (Fig. 2A), as we mimicked temperature from an early and a warm year (Fig. 2A), this was not the case for the outdoor aviary years. To determine the difference between temperatures in 2017 and 2018 in the outdoor aviaries, we used a *t*-test to compare mean daily temperature from 16 March to 15 April in the two years. This is the period in which the temperatures correlated best with mean annual laying dates in our long-term wild population in the Hoge Veluwe National Park (Visser et al., 2006).

To analyse the reaction norms, we used a mixed model analysis of variance (procedure lmer, package lme4, R 3.5.1, R Development Core Team 2018; http://www.R-project.org/). For the outdoor aviary females, we estimated the effects of the fixed effects year, selection line and their interaction together with the random effects female identity and female identity nested in female family, with the following model:

$$\text{Laying date} = \text{year} \times \text{selection line} + (1|\,\text{female family} : \text{female identity}). \tag{1}$$

For the climate-controlled aviaries, we estimated the effect of the fixed effects treatment, selection line and their interaction, age and order of treatment together with the random effects female identity and female identity nested in female family, with the following model:

$$\text{Laying date} = \text{year} \times \text{selection line} + \text{order} + \text{age} + (1|\,\text{female family} : \text{female identity}). \tag{2}$$

We could not test for female age in the outdoor aviaries, because for these aviaries, age is completely confounded by year. A significant selection line term indicates that selection lines differ in their average laying date in the average environments (i.e. the elevation of the reaction norm). The interaction between year or treatment, depending on the aviary type, and selection line was tested. A significant interaction term indicates that selection lines differ in their degree of plasticity, or sensitivity, in laying date in response to temperature (i.e. the slope of the reaction norm). Non-significant effects were eliminated in a stepwise model reduction procedure (procedure KRmodcomp, package pbkrtest).

In addition, as a preliminary indication, we tested whether the average daily increase in temperature differed between temperature environments in both outdoor and climate-controlled aviaries, as a previous study showed that great tits used the increase in temperature rather than the mean warm temperature to time their breeding (Schaper et al., 2012). For this, we used the 11 days prior to the earliest egg-laying date in the environment where egg laying was initiated first, starting within the period that correlates best with the timing of breeding in the wild (see above; Visser et al., 2006). We used year and treatment as a fixed effect for the outdoor and climate-controlled aviaries, respectively.

RESULTS

Timing of breeding is directly affected by temperature

In the outdoor aviaries, females laid earlier in 2018 than in 2017 (year=−6.32±1.57, $F_{1,33}$=16.24, *P*<0.001; Fig. 3A). Average daily temperatures were significantly lower in 2018 than in 2017 (*t*=2.27, d.f.=38.78, *P*=0.029; Fig. 1B), meaning that, unexpectedly, the outdoor aviary females laid earlier in the colder environment (but see ‘Average daily increase in temperature prior to egg laying’, below). Compared with the wild females in Hoge Veluwe National

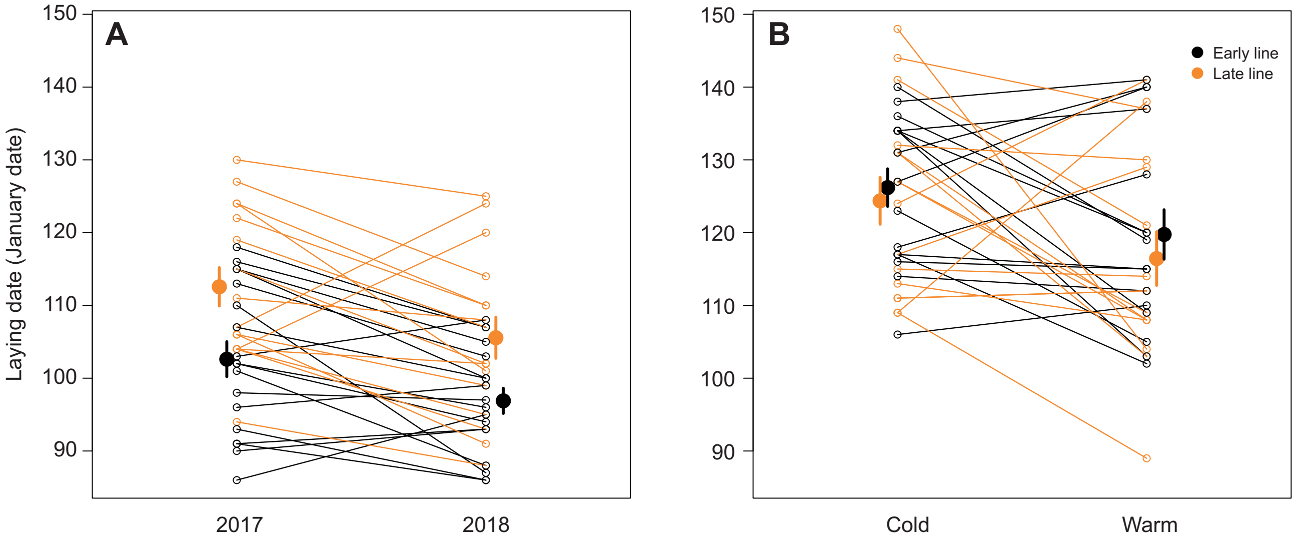

Fig. 3. Individual reaction norms and mean laying dates for female great tits in outdoor aviaries and in the temperature-controlled aviaries in 2017 and 2018. (A) Data for females in the outdoor aviaries. (B) Data for females in the two temperature treatments in the climate-controlled aviaries. Laying dates are presented as January dates (e.g. January day 90=30 March), with filled circles indicating means (±s.e.m.). In the outdoor aviaries, late selection line females (orange lines) showed a higher elevation in mean laying date compared with early selection line females (black lines). In the climate-controlled aviaries, there was no significant difference in elevation between selection lines.

Park in the same years, the average response in plasticity in laying date was in the opposite direction (year×location=11.84±2.31, $F_{1,116}$=25.88, P<0.0001; Fig. 2B; see Discussion).

In the climate-controlled aviaries, laying dates were significantly affected by temperature (controlled aviaries: $\Delta_{\mathrm{cold-warm}}$=7.2 days), with birds laying earlier in the warm treatment, indicating a direct effect of temperature on the timing of egg laying (treatment=−7.19±2.69, $F_{1,31}$=7.17, P=0.012; Fig. 3B). This average response in plasticity in laying date was in the same direction, though the slope was less steep, as that of the wild females in Hoge Veluwe National Park in the same years (Hoge Veluwe: $\Delta_{2013-2014}$=23.7 days, n=47 females; Fig. 2A; see Discussion).

Order of treatments and age of birds

In the climate-controlled aviaries, we found no effect of the order of the treatments to which the birds were subjected (treatment order=−0.34±3.52, $F_{1,28.7}$=0.002, P=0.96), meaning that plasticity in laying date was not influenced by first experiencing a cold spring, followed by a warm spring or vice versa. Further, we found no effect of age on the timing of breeding in the climate-controlled aviaries (age=0.86±2.64, $F_{1,30}$=0.09, P=0.762).

Selection on timing of breeding results in a change in reaction norm elevation between selection lines

In the outdoor aviaries there was no difference in plasticity in laying date (i.e. the reaction norm slope) between the early and late selection line (selection line×year=−1.28±3.09, $F_{1,32}$=0.16, P=0.69; Fig. 3A). However, the late selection line females showed a significantly higher elevation in the reaction norm for the timing of egg laying (i.e. they laid later independent of the temperature) of about 9 days (selection line=9.31±3.01, $F_{1,32}$=8.73, P=0.004).

Between the early and late selection line females in the climate-controlled aviaries, there was no difference in plasticity in laying date (selection line×treatment=−1.50±5.29, $F_{1,30}$=0.08, P=0.784) or elevation (selection line=−1.35±4.10, $F_{1,30}$=0.11, P=0.747; Fig. 3B).

Average daily increase in temperature prior to egg laying

Outdoor aviaries

Both in 2017 and in 2018, the earliest female started egg laying on 27 March, or January day 86 (Fig. 1A). The profiles of increasing temperature of the 11 days (75–86 January) before the first female initiated breeding differed significantly for 2017 and 2018 (year=−4.58±0.87, $F_{1,22}$=27.5, P<0.0001; Fig. 1A). Temperatures in this 11 day period increased on average by 0.09±0.09°C day^{-1} in 2017, but by 0.59±0.16°C day^{-1} in 2018, and this increase was significantly different between years (year=−4.75±0.87, $F_{1,22}$=27.5, P<0.0001).

Climate-controlled aviaries

The earliest females initiated breeding on 30 March (i.e. January day 89) and 16 April (i.e. January day 106) in the warm and cold treatment, respectively (Fig. 1B). We tested the mean increase in temperature in the same period (January day 79–90) for both treatments (Fig. 1B). The main daily temperature over this 11 day period increased 0.53±0.29°C day^{-1} in the warm treatment and 0.005±0.02°C day^{-1} in the cold treatment. This increase was significantly different between treatments (treatment=6.05±1.13, $F_{1,20}$=28.6, P<0.0001).

DISCUSSION

We studied whether temperature directly affects the timing of egg laying and whether selection on timing of breeding results in a correlated response to selection in the plasticity in laying date, using females from lines artificially selected for early and late egg laying. We found that females in climate-controlled aviaries on average initiated egg laying earlier under warmer conditions, showing that there is within-individual plasticity in laying date in response to temperature. However, this response was not observed in the outdoor aviaries. Further, we found no difference in the degree of laying date plasticity between selection lines for both aviary types. However, in outdoor aviaries, early selection line females initiated egg laying ∼9 days earlier compared with late selection line females. Selection on timing of breeding, therefore, results in a change in phenotype in the average environment, but not in a correlated response to selection on the degree of plasticity in laying date.

Currently, it is poorly understood what components of the temperature profiles – mean, minimum, maximum, change, etc. – are used by birds to predict their breeding and how this information is perceived, transduced and ultimately translated into egg laying. Interestingly, the temperature profiles provided in this study show periods of increasing mean daily temperature before females started initiating egg laying (Fig. 1). An increase in temperature for a period of a week has previously been shown to advance the timing of egg laying in great tit females (Schaper et al., 2012). In the climate-controlled aviaries, there was no clear temperature increase over the 11 day period in the cold treatment, compared with the steep increase in temperature in the same 11 day period for the warm treatment (Fig. 1A). This could explain why females in the cold treatment started egg laying later. Interestingly, despite 2018 showing lower mean daily temperatures compared with 2017 (Fig. 1B), females laid on average earlier in the outdoor aviaries in 2018. Also, in these aviaries, the increase in temperature 11 days before the earliest female initiated egg laying was steeper in 2018 than in 2017 (Fig. 1B). These preliminary results in both the climate-controlled and outdoor aviaries concur with those of a previous study in which it was shown that great tits used the increase in temperature rather than the mean warm temperature to time their breeding (Schaper et al., 2012). As we show that temperature directly affects egg laying, future studies could try to pin-point which components of the temperature profiles birds use.

The results from the outdoor aviaries were, however, not consistent with the average breeding plasticity in the Hoge Veluwe population in those 2 years. There, the wild birds laid ∼5.5 days earlier in 2017 than in 2018. We cannot explain this difference but we can speculate as to the reason. A key difference is that the birds in our aviaries were not constrained by food availability in the period prior to and during egg laying. As such, one potential reason why birds would lay earlier in colder years with abundant food resources is that it enables them to produce a second brood. In colder years, these second broods are more profitable, because colder years lead to a later and perhaps a wider food peak. However, in the wild, birds do not lay early in those potentially ‘second brood years’ because of a constraint in food resources during egg production. In the outdoor aviaries, this constraint is lifted and therefore birds potentially lay early in cold years (Fig. 3A). In accordance with this speculation, all early-laying females in the wild laid later in cold years (Fig. 2B), while some of the late females laid earlier in cold years. This results in ample individual variation in laying date plasticity. We do, however, want to point out that in the field some clutches were abandoned before we could identify the female. This could have led us to mistakenly identify a replacement clutch as the first clutch of that female in that year. In addition, there is the possibility of an age effect on laying date as shown previously in wild populations of great tits and pied flycatchers (Jarvinen, 1991; Nager and van Noordwijk, 1995). However, we could not test this as age is completely confounded by year.

In the climate-controlled aviaries, the breeding time reaction norms were in the same direction as in the wild (Figs 2A and 3B). However, they showed great individual variability in slope compared with those of Hoge Veluwe females in 2013 and 2014 (Fig. 3B), which could have been caused by genetic differences between individuals, lack of specific cues (Lambrechts and Visser, 1999) or a disrupted correlation between cues as a result of the (semi) artificial environments (Bentley et al., 1998). In addition, eggs or first clutches could have been missed, but this is less likely given the daily checks of the nest boxes in the aviaries when laying was initiated. The great variability in individual slopes could have led to decreased plasticity in average laying date of the selection line females in the climate-controlled aviaries. This indicates that temperature is unlikely to be the only environmental driver affecting laying date plasticity and that other environmental factors are also involved, whether in interaction with temperature or not. A recent study in wild tree swallows (*Tachycineta bicolor*), for example, found that timing of breeding was mainly influenced by latitude and temperature, the latter in interaction with breeder density (Bourret et al., 2015).

Here, we found a difference in the timing of breeding in the average environment between the early and late selection line birds in the outdoor aviaries, but not in the climate-controlled aviaries. It is likely that the environments perceived in the outdoor aviaries, i.e. semi-natural conditions, give better or more complete information [e.g. proper (correlations between) environmental cues] for timing of breeding. Further, genomic selection on the timing of breeding resulted in a selection response in the outdoor aviaries (Verhagen et al., 2019b), but not in the climate-controlled aviaries (Verhagen et al., 2019a). Possible reasons could be that females lack specific cues (Lambrechts et al., 1999) or experience a disrupted correlation between predictive cues (Bentley et al., 1998) in artificial conditions, and that this, in combination with a different genetic make-up (outdoor aviaries are more extreme), did not result in a difference in reaction norm elevation in climate-controlled aviaries.

While genomic selection on timing resulted in a change in phenotype in the average environment, at least in the outdoor aviaries, we found no correlated response to selection on plasticity in laying date, independent of aviary type. This is in agreement with a recently performed study in the long-term study population at the Hoge Veluwe from which these aviary birds originate (Ramakers et al., 2019). This study found a directional selection on the elevation, but not the slope of the laying date reaction norm to temperature. However, we must interpret the results from the aviaries with some reservation, because, unlike Ramakers et al. (2019), we studied a limited number of females. With the strength of genomic selection on egg laying being moderate (Verhagen et al., 2019b), we may not have been able to detect changes in reaction norm slopes. In addition, because of this low sample size, we were unable to test the individual variation in plasticity (I×E) and whether it has a genetic basis (i.e. genotype-by-environment interaction or G×E). Further, both aviary types experienced two environments unlike other long-term studies performed in wild populations. Still, these results are promising for future studies (see below), especially given the fact that they focus on patterns within individuals.

Global climate change will continue to disrupt the synchrony between interacting trophic levels, and therefore responding through phenotypic plasticity will probably not be sufficient in the long run (Thackeray et al., 2016; Visser, 2008; Visser and Gienapp, 2019; Visser et al., 2004). Genetic shifts in reaction norms are thus necessary for species to resolve the asynchrony in phenology between consumer and prey, but these shifts remain scarce (Merilä and Hendry, 2014). In order for such a shift to occur, genetic variation in the mechanisms underlying phenological traits is necessary and we need to find where in these mechanisms this variation resides for selection to act upon. Experiments on temperature effects on the timing of breeding contribute to our understanding of how birds respond to environmental cues. Here, by using a within-individual experimental approach, we show that temperature directly affects the timing of egg laying in a song bird. Thus, natural selection may lead to a change in phenotype in the average environment, but will probably not result in a correlated response to selection on the degree of plasticity in laying date. Finding a direct effect of temperature on the timing of egg laying is exciting, as it advances our understanding of the mechanisms underlying breeding decisions under climate change. Data and results from this study will be important in future studies that, for example, investigate within-individual DNA methylation patterns in contrasting treatments analysing plasticity in laying date.

Acknowledgements

We thank Marylou Aaldering, Coretta Jongeling, Franca Kropman and Ruben de Wit for taking care of the birds, and Jeroen Laurens and Gilles Wijlhuizen for technical assistance prior to and during the experiments. We are grateful to Jip Ramakers for help with I×E and G×E analyses and Samuel Caro for constructive comments on the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.E.V.; Methodology: P.G., M.E.V.; Software: P.G.; Formal analysis: I.V.; Investigation: I.V., B.M.T.; Resources: M.E.V.; Writing - original draft: I.V.; Writing - review & editing: I.V., B.M.T., P.G., M.E.V.; Visualization: I.V.; Supervision: M.E.V.; Funding acquisition: M.E.V.

Funding

This study was supported by a European Research Council Advanced Grant (339092 – E-Response to M.E.V.).

Data availability

Raw data supporting this manuscript are available from DataverseNL: https://hdl.handle.net/10411/NNHAJF

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