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

Irene Verhagen<sup>1,2</sup>, Barbara M Tomotani<sup>1</sup>, Phillip Gienapp<sup>1,3</sup> & Marcel E Visser<sup>1</sup>

<sup>1</sup> Department of Animal Ecology, Netherland Institute of Ecology (NIOO-KNAW), The Netherlands

<sup>2</sup> Present address: Wageningen University & Research, Wageningen, The Netherlands

<sup>3</sup> Present address: Michael-Otto-Institut im NABU, Bergenhusen, Germany

**Corresponding author:**

Irene Verhagen  
P.O. Box 50, 6700 AB Wageningen, the Netherlands  
i.verhagen@nioo.knaw.nl

**Running title:** Direct effect temperature on avian egg-laying

**Key words:** correlated response to selection, great tit, phenotypic plasticity, timing of breeding

**Summary statement:** Temperature directly affects avian breeding time. However, sensitivity to temperature did not differ between two lines genetically selected for breeding time, but early selection line birds did lay earlier independent of environment.

## 26    **Abstract**

27    Phenotypic plasticity is an important mechanism by which an individual can adapt its seasonal  
28    timing to predictable, short-term environmental changes by using predictive cues. Identification  
29    of these cues is crucial to forecast species' response to long-term environmental change and to  
30    study their potential to adapt. Individual great tits (*Parus major*) start reproduction early under  
31    warmer conditions in the wild, but whether this effect is causal is not well known. We housed  
32    36 pairs in climate-controlled aviaries and 40 pairs in outdoor aviaries, where they bred under  
33    artificial contrasting temperature treatments or in semi-natural conditions, respectively, for two  
34    consecutive years, using great tits from lines selected for early and late egg laying. We thus  
35    obtained laying dates in two different thermal environments for each female. Females bred  
36    earlier under warmer conditions in climate-controlled aviaries, but not in outdoor aviaries. The  
37    latter was inconsistent with laying dates from our wild population. Further, early selection line  
38    females initiated egg laying consistently ~9 days earlier compared to late selection line females  
39    in outdoor aviaries, but we found no difference in the degree of plasticity (i.e. the sensitivity to  
40    temperature) in laying date between selection lines. Because we find that temperature causally  
41    affects laying date, climate change will lead to earlier laying. This advancement is however  
42    unlikely to be sufficient, thereby leading to selection for earlier laying. Our results suggest that  
43    natural selection may lead to a change in mean phenotype, but not to a change in the sensitivity  
44    of laying dates to temperature.

## Introduction

Effects of global climate change are omnipresent and can severely modify the 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 numeral 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 to the 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 attribute these phenotypic changes. 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 due to yearly varying environmental conditions. Consequently, females need to track this inter-annual variation to optimally time their breeding (i.e. egg-laying) to the time 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 starts up the reproductive system (Dawson et al., 2001; Farner, 1985). However, its year-to-year invariability 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 variation in timing of breeding. Temperature is highly variable between years and provides information about local conditions, such as insect emergence, which allows female to fine-tune their timing of breeding (Dawson, 2008; Wingfield and Kenagy, 1991; Wingfield et al., 1992) and subsequently match offspring needs to food abundance. So far, temperature is the major driving force of biological seasonality in temperate zones (Parmesan, 2007) and the most influential environmental cue in fine-tuning timing of avian seasonal breeding (Caro et al., 2013; Lambrechts and Visser, 1999; Visser et al., 2009).

Spring temperatures could directly affect timing of breeding or its effect can be indirect, where temperature affects other environmental conditions, which subsequently causally affect timing of breeding. Previous work has suggested that the former is the case, i.e. that temperature affects

78 timing of egg-laying directly, and not acting via e.g. food phenology (Schaper et al., 2011), as  
79 shown in a previous six-year study in great tits (*Parus major*) (Visser et al., 2009). In that study,  
80 breeding pairs were housed in climate-controlled aviaries and went through a breeding season  
81 in either a warm or a cold treatment, mimicking a warm and cold spring, respectively. Birds  
82 initiated egg-laying significantly earlier in the former. It is important to point out here, that in  
83 the study by Visser et al. (2009) the laying date comparison was performed *between* individuals,  
84 thus showing between-individual variation. In addition, the effect of temperature on egg-laying  
85 varied strongly between the six years of the study, despite controlled conditions.

86 Here, we first set out to study whether plasticity in laying date is due to the direct or indirect  
87 (e.g. via food phenology) effect of temperature on timing of egg-laying by subjecting birds to  
88 contrasting temperatures in climate controlled aviaries for two years and gathering *within*  
89 individual data on egg-laying. As such, the real effect of temperature is less likely to stay  
90 undetected or clouded by differences between individuals (e.g. genetic and physiological  
91 factors, physical condition, etc.). In addition, we housed pairs in semi-natural conditions (i.e.  
92 outdoor aviaries) for two consecutive years that differed in environmental conditions, including  
93 temperatures. Based on the previous studies in both the wild (e.g. Both and Visser, 2001;  
94 McCleery and Perrins, 1998) and captivity (Schaper, 2012; Visser et al., 2009), we expected  
95 females to lay earlier in the warm environment compared to the cold. If indeed females would  
96 lay earlier in the warm environment, we expected that the earliest egg-laying female would  
97 have experienced a steeper increase in average daily temperatures in the days prior to initiating  
98 egg-laying compared to the earliest female in the cold environment. This, because an increase  
99 in temperatures shortly prior to egg-laying has been shown to advance the timing of egg-laying  
100 in great tit females (Schaper et al., 2012).

101 Secondly, we have the unique opportunity to test whether these birds, which originated from  
102 selection lines for early and late timing of breeding through bi-directional genomic selection  
103 (Gienapp et al., 2019; Verhagen et al., 2019b), would show a difference in the average laying  
104 date (i.e. the elevation of the reaction norm) between selection lines, independent of  
105 environment. As shown previously, early selection line females laid on average about six days  
106 earlier compared to late selection line females (Verhagen et al., 2019b). This difference between  
107 selection lines is, under the influence of environmental cues, ultimately rooted in the cascade  
108 of (epi)genetic and physiological processes underlying egg-laying. How these mechanisms are  
109 affected by temperature remain 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 to 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*) using genomic selection, which was moderately strong and in both directions (Gienapp et al., 2019; Verhagen et al., 2019b). Briefly, nestlings (F<sub>1</sub> generation) were taken in 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) of 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<sub>1</sub> generation individuals with the most extreme GEBVs were selected for *early* and *late* selection line breeding pairs to produce the F<sub>2</sub> generation in captivity. Eggs were transferred to wild 'foster-nests', where they were incubated and hatched. Subsequently, 10 days post-hatching F<sub>2</sub> generation chicks were brought into the aviary facilities at the NIOO-KNAW (Wageningen, the Netherlands) for further hand raising. In their turn, the F<sub>2</sub> offspring were genotyped and, based on the most extreme GEBVs, selected to produce the F<sub>3</sub> generation, which was then genotyped and selected. This study was performed under the 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., 2019a). 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<sub>1</sub> to F<sub>3</sub>). Line effects for the F<sub>1</sub> and F<sub>2</sub> were non-significant, but line differences were highly significant for the F<sub>3</sub> generation. On average *early* selection line birds laid about six days earlier compared to *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*

For a detailed description, see Verhagen et al. (2019b). In short, from January 2017 onwards, F<sub>3</sub> generation pairs (n = 40) were housed in 40 outdoor aviaries (4.2 x 1.9 x 2.1m) where the birds were subjected to natural photoperiod and temperatures. These pairs had the most extreme GEBVs (see above) within the F<sub>3</sub> generation and functioned as breeding pairs to produce eggs (F<sub>4</sub> 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 minutes 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<sub>3</sub> 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) as compared to 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 compared to 2013 (Fig. 2A). Every hour temperatures 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). 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 fine-tuning avian timing of 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 they were in in the breeding season of 2017 to go through their second breeding season. In the climate-controlled aviaries, when 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 know which temperature profile is associated with early laying in the wild (Fig. 2A), as we mimic temperature from an early and a warm year (Fig. 2A), this is not the case for the outdoor aviary years. To determine a difference between temperatures in 2017 and 2018 in the outdoor aviaries, we tested with a t-test the differences in mean daily temperatures between 2017 and 2018 from 16 March – 15 April. This is the period in which the temperatures correlate the 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). 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:

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

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:

laying date = year  $\times$  selection line + order + age + (1|female family:female identity)

We could not test for female age in the outdoor aviaries, because for these aviaries, age is completely confounded with 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 temperatures 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 temperatures 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 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 compared to 2017 (year =  $-6.32 \pm 1.57$ ,  $F_{1,33} = 16.24$ ,  $p < 0.001$ , Fig. 3A). Average daily temperatures are significantly lower in 2018 compared to 2017 ( $t = 2.27$ ,  $df = 38.78$ ,  $p = 0.029$ , Fig. 1B), meaning that, unexpectedly the outdoor aviary females laid earlier in the colder environment (but see “Average daily increase in temperature prior to breeding” below). Compared to the wild females in Hoge Veluwe National Park in the same years, the average response in plasticity in laying date is in the opposite direction (year  $\times$  location =  $11.84 \pm 2.31$ ,  $F_{1,116} = 25.88$ ,  $p < 0.0001$ , Fig. 2B, see “Discussion”).

In the climate-controlled aviaries laying dates were significantly affected by temperature (controlled-aviaries:  $\Delta_{\text{cold-warm}} = 7.2$  days), with birds laying earlier in the warm treatment, indicating a direct effect of temperature on timing of egg-laying (treatment =  $-7.19 \pm 2.69$ ,  $F_{1,31} = 7.17$ ,  $p = 0.012$ , Fig. 3B). This average response in plasticity in laying date is in the same direction, though less steep in slope, compared to 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 the birds*

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

#### *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  $\times$  year =  $-1.28 \pm 3.09$ ,  $F_{1,32} = 0.16$ ,  $p = 0.69$ , Fig. 3A). However, the *late* selection line females showed a significantly higher elevation in the reaction norm for timing of egg-laying (i.e. they lay later independent of the temperature) of about nine days (selection line =  $9.31 \pm 3.01$ ,  $F_{1,32} = 8.73$ ,  $p = 0.004$ ).

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

#### *Average daily increase in temperature prior to egg-laying*

Outdoor aviaries: Both in 2017 and 2018, the earliest female started on 27 March, or 86 January (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 =  $-4.58 \pm 0.87$ ,  $F_{1,22} =$

27.5,  $p < 0.0001$ , Fig. 1A). Temperatures in this 11-day period increased on average with  $0.09 \pm 0.09$  °C/day in 2017, whereas in 2018 this increase was  $0.59 \pm 0.16$  °C/day and this increase was significantly different between years (year =  $-4.75 \pm 0.87$ ,  $F_{1,22} = 27.5$ ,  $p < 0.0001$ ).

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

## Discussion

We studied whether temperature directly affects timing of egg-laying and whether selection on timing of breeding results in a correlated response to selection in 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 to 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 temperatures 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 is no clear temperature increase over the 11-day period in the cold treatment, when comparing the steep increase in temperatures in 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 that 2018 shows

lower mean daily temperatures compared to 2017 (Fig. 1B), females laid on average earlier in the outdoor aviaries in 2018. Also, in these aviaries the increase in temperatures 11 days before the earliest female initiated egg-laying is steeper in 2018 compared to 2017 (Fig. 1B). These preliminary results in both the climate-controlled and outdoor aviaries, are in concurrence with a previous study in which was shown that great tits used the increase in temperature rather than the mean warm temperatures to time their breeding (Schaper et al., 2012). Since we show that temperature directly affects egg-laying, future studies can try to pin-point which components of the temperature profiles birds use.

The results from the outdoor aviaries are, however, not consistent with the average breeding plasticity in the Hoge Veluwe population in those two years. There, the wild birds laid ~5.5 days earlier in 2017 compared to 2018. We cannot explain this difference but we can speculate. A key difference is that the birds in our aviaries are 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’ due to 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 lay later in cold years (Fig. 2B), while some of the late females lay 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 get abandoned before we identify the female. This could have led us to mistakenly identifying 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 with year.

In the climate-controlled aviaries the breeding time reaction norms are in the same direction as in the wild (Figs. 2A, 3B). However, they show great individual variability in slope compared to 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 due to (semi) artificial environments (Bentley et al., 1998). In addition, eggs or first clutches could have been missed, but this is less likely due to 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 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, as opposed to 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, due to this low sample size, we were unable to test the individual variation in plasticity ( $I \times E$ ) and whether it has a genetic basis (i.e. genotype-by-environment interaction or  $G \times E$ ). Further, both aviary types experienced two environments compared to other long-term studies performed in wild populations. Still, these results are promising for future studies (see below), especially due to 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 likely 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 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 timing of egg-laying in a song bird. Thus, natural selection may lead to a change in phenotype in the average environment, but will likely not result in a correlated response to selection on the degree of plasticity in laying date. Finding a direct effect of temperature on 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.

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## **Author contributions**

MEV and PG performed and coordinated the genomic selection. IV and BMT performed the experiments and collected the data. IV analysed the data and wrote the manuscript. All co-authors contributed critically to the drafts and gave final approval for publication.

## **Competing interests**

414 All authors declare to have no competing interests that might have influenced this manuscript.

415

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418

#### 419 **Data availability**

420 Raw data supporting this manuscript will be made available at <https://dataverse.nl> after  
421 acceptance.



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Figures

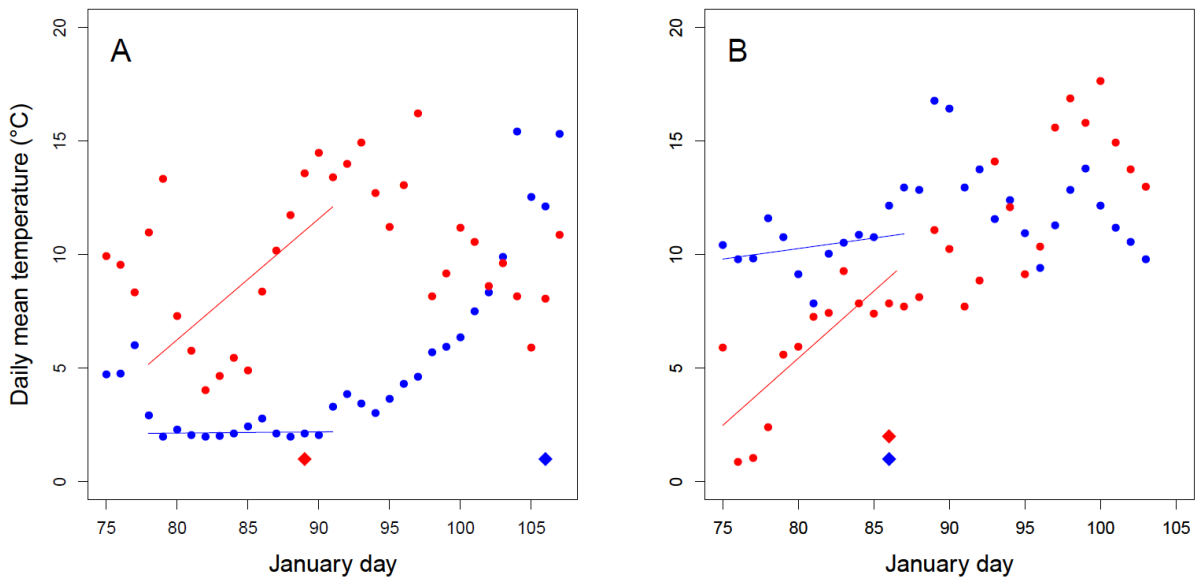


Figure 1. **Mean daily temperature profiles during the breeding season (16 March – 20 April) in the climate controlled aviaries (A, n=36) and outdoor aviaries (B, n=40).** For the climate controlled aviaries (A) the temperature profiles for the cold (blue) and warm treatment are shown (red), where for the outdoor aviaries (B) the temperature profiles of 2017 (blue) and 2018 (red) are shown. Note that for the outdoor aviaries temperatures are 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 90 = March 30).

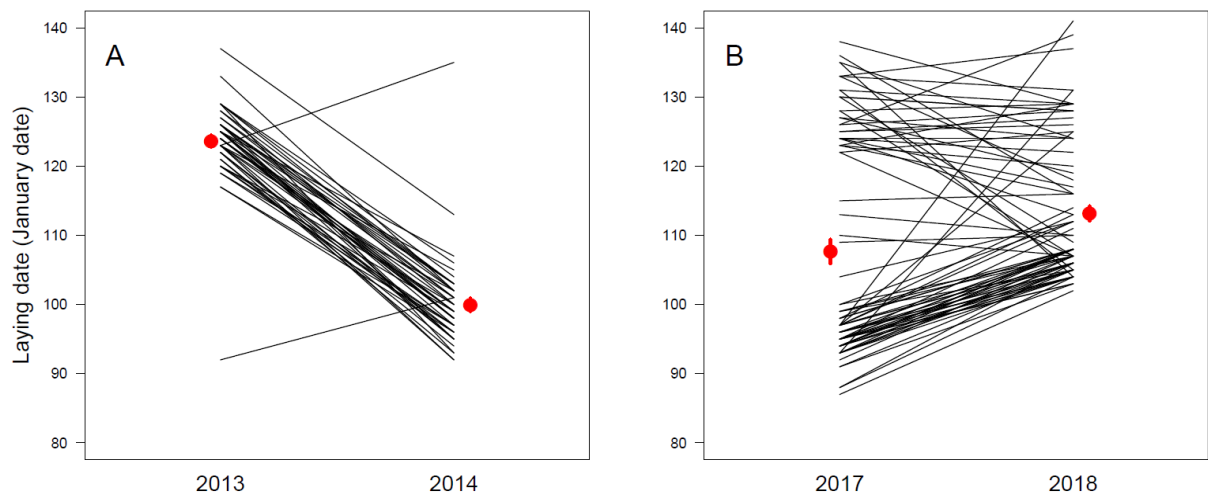


Figure 2. **Individual reaction norms, presented together with mean laying dates ( $\pm$  s.e.m; the red dots) for females in Hoge Veluwe National Park for 2013-2014 (the years for which the temperatures used in the climate controlled aviaries) (A) and 2017-2018 the years in which the open aviaries experiment was performed) (B).** Laying dates are presented as January dates (e.g. 90 January = 30 March). Females ( $n=47$ ) laid on average  $\sim 24$  days earlier in 2014 compared to 2013 and females ( $n=85$ ) laid on average  $\sim 5.5$  days earlier in 2017 compared to 2018.

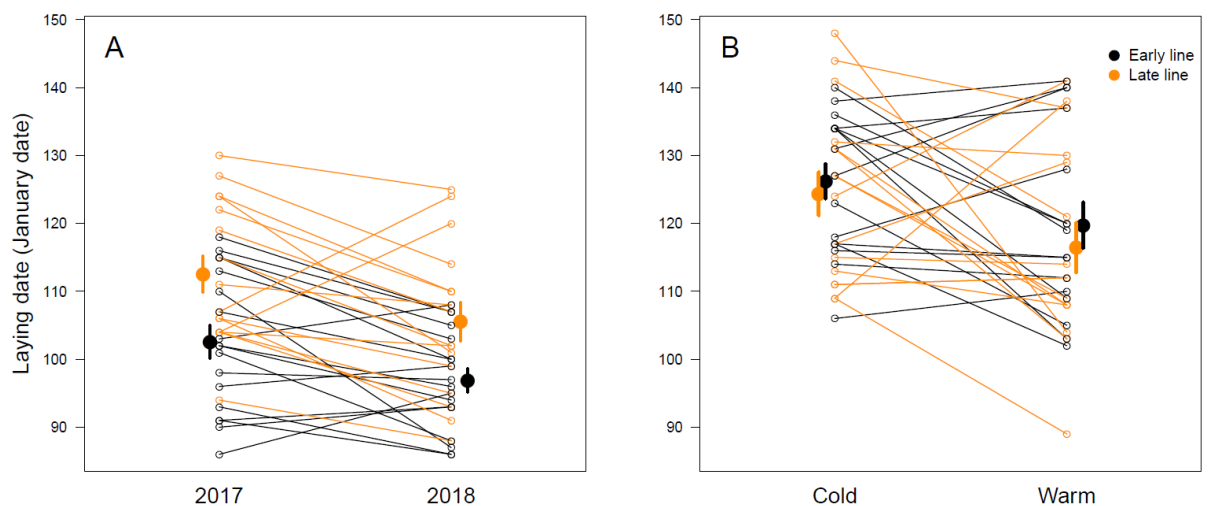


Figure 3. **Individual reaction norms, presented together with mean laying dates ( $\pm$  s.e.m; the black and orange dots) in two years (2017 and 2018) for the outdoor aviary females (A) and in two temperature treatments in the climate-controlled aviary females (B).** Laying dates are presented as January dates (e.g. 90 January = 30 March). In the outdoor aviaries, late selection line females (orange lines) show a higher elevation in mean laying date compared to the early selection line females (black lines). In the climate-controlled aviaries, there is no significant difference in elevation between selection lines.