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Geographic differences in the phenology of gonadal development and moult, but not of egg laying, are genetically based in a small songbird

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To forecast how fast populations can adapt to climate change, it is essential to determine the evolutionary potential of different life-cycle stages under selection. In birds, timing of gonadal development and moult are primarily regulated by photoperiod, while laying date is highly phenotypically plastic to temperature. We tested whether geographic variation in phenology of these life-cycle events between populations of great tits (*Parus major*) has a genetic basis, indicating that contemporary genetic adaptation is possible. We carried out a common garden experiment in which we bred first- and second-generation pairs in captivity originating from eggs from Gotland (Sweden) and Hoge Veluwe (The Netherlands), two populations that showed different temperature sensitivity of laying date in a recent meta-analysis. We recorded the phenology of egg-laying, moult and gonadal size in early spring. We found no significant differences in laying date between the populations, but they did differ in moult timing and testis size. This implies that under climate change the timing of gonadal development and moult, which are mainly regulated by photoperiod, will not respond to increased temperature but can respond by genetic adaptation in response to selection, while the opposite holds for laying date, perhaps indicating that plasticity is constraining genetic adaptation.

1. Introduction

To forecast how fast populations can adapt to climate change, it is essential to estimate the genetic variation in life-history traits that are under selection. One avenue to estimate such genetic variation is to use quantitative genetics to estimate additive genetic variation in traits [1,2]. However, even for cases where there is genetic variation and the trait is under selection, often no genetic adaptation is observed [3,4]. Many reasons for this so-called evolutionary stasis have been put forward, such as correlations with other traits under selection but that are not necessarily measured, the strength of selection that varies in time and space and overestimation of the degree of heritability of the trait [3,4]. A complementary approach is to explore geographical differences in these traits and whether any differences across populations are of genetic origin [5]. Showing such genetic differences demonstrates that a trait shows local adaptation and, hence, that evolution has occurred. This then provides insights into the potential for genetic adaptation in current populations under selection.

A phenotype that is currently under selection due to climate change is avian timing of reproduction (or phenology [6]), a life-history trait with major fitness consequences in wild birds [7,8]. Birds need to time their annual life cycle events such that they are happening at a time when the environment is most suitable. This is especially true for the period of chick rearing but is also important at other times, such as egg laying and moult, which can be energetically costly [9,10]. Also, it is key that these life-history events do not overlap [11]. One seasonal event in temperate zone birds is that they regress their gonads in autumn, in response to shortening of the photoperiod, and need to regrow them every spring, in response to lengthening of the photoperiod. For instance, the testes can increase in size several 100-fold at the start of the breeding season [12]. This gonadal development needs to be completed before eggs or sperm can be produced. After reproduction, birds moult their flight feathers, which is also an energetically demanding period that should not substantially overlap with rearing the offspring. The seasonal timing of all these life cycle events is regulated by several interrelated physiological mechanisms [13].

(a) Laying date

Avian laying date is highly plastic to temperature and thus there is ample year-to-year variation in the timing of reproduction within individuals [14–16]. This is ultimately because the optimal time for reproduction also varies between years as, for example, the phenology of food needed to raise the offspring is temperature sensitive and thus the peak in resource availability occurs at different times in different years. Birds therefore use cues to time their reproduction, with photoperiod and ambient temperature as the two key environmental variables [17–19]. Longer photoperiods and higher temperatures (within a 'photoperiod sensitivity window') lead to earlier egg laying dates within individuals [14]. In many species, climate change has led to an unequal shift in the phenology of birds compared with their prey [15,20–22]. The resulting phenological mismatch [23] then leads to directional selection on seasonal timing [8] (i.e. to selection on sensitivity to photoperiod and/or temperature). But whether such selection will also lead to genetic adaptation depends on the amount of within-population genetic variation.

There have been ample studies estimating the within population genetic variation in avian laying date (i.e. the date the first egg of the first clutch of the season is laid). This includes quantitative genetics [16,24,25], molecular variation in genes associated with timing [26] and selection lines on seasonal timing [27]. The emerging pattern of these studies is that while there is genetic variation, partly due to immigration, on which selection can act, this variation is moderate, making any response to selection slow. In line with this, no genetic adaptation of laying date has ever been detected in long-term studies of wild birds [3,28].

(b) Gonadal development

In birds from the temperate zone, gonadal development is not phenotypically plastic to temperature, and photoperiod is the main cue. When starlings (*Sturnus vulgaris*) are kept under conditions where the annual photoperiod regime is condensed to six months, the gonadal regression and growth also occur twice in 12 months [29]. This is also shown in experiments where perceived photoperiod was manipulated, which showed that gonadal development is effected by photoperiod (while laying date was not) [30–32] (but see [33]). Most notably, the initial gonadal growth phase is highly heritable in female, but not male, great tits [34], indicating that, at least for females, there is ample genetic variation in sensitivity to photoperiod. In contrast, the timing of gonadal development is not effected by ambient temperature, as shown in starlings [35], white-crowned sparrows (*Zonotrichia leurophrys*) [36], house finches (*Haemorhous mexicanus*) [37] or great tits (*Parus major*) [38–40].

(c) Moult

Seasonal timing of moult in songbirds is also under the control of photoperiod [32,41]. However, an effect of temperature on moult timing has been reported in some studies. For example, while gonadal growth was not affected by temperatures in starlings (as described above [35]), moult started earlier at higher temperatures [35]. In some cases, an effect of temperature was only found at extreme temperatures (e.g. tree sparrows at 30°C [42]; white crowned sparrows at 30°C [43]). In a number of these studies, birds were not allowed to breed, either because only males were used or because birds were kept in individual cages. When breeding birds are studied, results are less clear, some showing birds moulting earlier in warmer temperatures [39], while in others there was no difference [38]. Breeding is known to have an effect on moult timing, this is supported by experiments where implants of testosterone delay moult onset [44]. In the wild, overlap between moult and breeding occurs but usually in late breeders, and delaying the termination of breeding (chick rearing) does not necessarily affect moult onset [45,46]. In correlative studies with wild songbirds, moult differs from year to year at the population [46,47] (but see [48]) and individual levels [45], but is not necessarily correlated to changes in ambient temperatures [46]. Overall the literature suggests that while photoperiod has a strong effect on moult timing and duration [49], temperature could play a role on timing of moult; however, it is unclear whether its effect is direct or indirect via termination of breeding or other preceding stages of the annual cycle [35,37–39].

(d) Geographic variation in phenology

Relatively few studies have used a between-population approach to test for variation in laying dates, although studies of other seasonal traits exist [32,50,51]. One of the few examples is an analysis of laying dates of blue tits (*Cyanistes caeruleus*) in two evergreen woodland and two deciduous woodland habitats [52]. Two of these habitats (Muro and Pirio, Corsica, France) are just

25 km apart but differ in their laying date by a month [53,54]. Porlier *et al*. [52] showed that these populations were sensitive to temperature in very different photoperiodic time windows in spring. Similarly, Bailey *et al*. [55] made use of the geographic variation in environmental conditions across the great tit's breeding range. They used long-term data from 34 great tit and 33 blue tit populations from across Europe and determined the photoperiodic window for which the population was sensitive to temperature and how sensitive (in days per °C) the population was. This large-scale comparative analysis confirmed that great tit populations differ in their temperature sensitivity of laying date, depending on the forest habitat they breed in.

To study to what extent this geographic variation in laying date has a genetic basis, we carried out a two-generation common garden experiment in which we bred great tits that originated from eggs retrieved from two of the populations shown by Bailey *et al*. [55] to differ in temperature sensitivity. Such a common garden approach can show whether there are genetic differences underlying this difference in cue sensitivity, as the confounding effects of different environments that shape the phenotype, as it occurs in the wild, are lifted. The two-generation set-up of the common garden experiment is essential as using either adult birds caught at different locations, or birds raised from eggs laid at different locations may lead to phenotypic differences resulting from carry-over effects of the location in which the adults were raised or the eggs produced. In a two-generation set-up, eggs are also laid in a common garden, excluding such carry-over effects, and thus any phenotypic differences between birds originating from different populations must be genetic.

If the common garden experiment shows that there are genetic differences between the populations (i.e. that there is local adaptation), this would demonstrate that evolution has occurred over time, providing insights into the potential for genetic adaptation in current populations under selection. We measured three life cycle events: laying date of the breeding pairs (primarily sensitive to temperature) as well as seasonal timing of moult and gonadal development (primarily sensitive to photoperiod). We also compared the difference in laying date in this common garden experiment with the predictions of the Bailey *et al*. [55] statistical model.

2. Material and methods

(a) Setting up common garden experiment

(i) Birds

In the spring of 2021, freshly laid, unincubated eggs were transported from eight populations to the Netherlands Institute of Ecology (NIOO-KNAW): La Rouvière (France), Boshoek (Belgium), Wytham Woods (England), Gotland (Sweden) and four Dutch populations (Hoge Veluwe, Vlieland, Oosterhout and Liesbos). The eggs were then taken to a Dutch population (Bennekomse Bos, lat: 52 003; long: 5708) where they were placed in foster nests for incubating and early parental care. At 10 days post-hatching, these chicks were transported to the NIOO and hand raised, following procedures as outlined in [56]. They were blood sampled and based on their genotypes (using five microsatellite regions Pma-TGAn33, PmaC25, PmaTAGAn71, PmaGAn27 and PmaD10 [57]) and that of the potential parents, they were assigned to a family following a standard protocol [58].

For a number of these populations, there were severe problems leading to very low numbers of chicks (see electronic supplementary material for more info on all eight populations). Therefore, we only included offspring from Gotland (Sweden, lat: 57.063° N, long: 18.278° E) and Hoge Veluwe (The Netherlands, lat: 52.041° N, long: 5.856° E) in our analysis. Note that due to the different timing of egg laying between the two populations, the Gotland eggs were placed 7 days later at the foster parent nests than the Hoge Veluwe eggs (7 May versus 30 April).

The following year (2022) we formed first-generation (F1) breeding pairs (within populations) from these birds and kept them from January onwards in pairs in open aviaries at the NIOO with ad libitum food (constant daily amount of food consisting of a mixture of minced beef, proteins and vitamins, sunflower seeds, fat, a mix of dried insects, a mixture of proteins, vitamins, minerals and trace elements (Ce'De'-mix), a surplus of calcium, water for drinking and bathing, nesting material and four nestboxes) as nesting opportunities. The eggs produced where collected every morning and put in an egg turner (i.e. a device that gently rocks eggs during storage—a CocinaCo 154 Eggs Quail Turner Tray Container).

Within 5 days of laying, eggs were taken to the Bennekomse Bos to foster parents. We placed Gotland and Hoge Veluwe eggs together in foster broods (with a total clutch between 5 and 11 eggs, 9 on average) to ensure common conditions during incubation and early chick rearing. Note that as there was no difference in laying date between the populations for the F1 pairs, eggs were taken to their foster nests during the same period. At day 10 post-hatching the chicks were taken to the NIOO, hand raised, blood sampled and assigned to a family.

In the following year (2023), 20 Gotland and 20 Hoge Veluwe second generation (F2) breeding pairs were set-up. As these F2 birds originate from eggs produced in a common garden setting, and thus any carry-over effects of the location the eggs were produced are excluded, any differences between them will be genetic.

(ii) Aviaries

Breeding pairs were set up in 40 outdoor aviaries of 4 m × 2 m × 2 m (l × w × h) with mesh on one side, allowing natural light and ambient temperatures. Despite being exposed to natural light, all aviaries are still darker than natural conditions. This causes the birds in aviaries to consistently lay later than wild birds if left without additional light. Thus, a fluorescent light tube provided additional light in the morning for all breeding pairs. In January and February, lights were on from sunrise until midday (i.e. same as normal housing conditions) and from March onwards lights were on 2 h and 15 min before sunrise until midday. This additional light was crucial for eggs to be laid, while foster nests were still available to produce the F2 birds. The aviary building consisted of two rows of aviaries (20 west-facing and 20 east-facing) and to minimize the impact of any systematic variation in conditions between aviaries, we kept the Gotland and Hoge Veluwe pairs in alternating aviaries.

(b) Phenotyping

(i) Laying date

Laying dates were recorded for both the Hoge Veluwe and Gotland F1 and F2 generations (see electronic supplementary material, appendix A for laying dates of F1 birds from other populations). Nest boxes were checked daily for nest-building progress and new eggs, and the laying date was the day the first egg was laid. Eggs were replaced by plastic dummy eggs, and upon clutch completion females were allowed to incubate for four complete days after which nests and dummy eggs were removed on the fifth day. Frequently, pairs would initiate replacement clutches. Here, we only analyse the laying date of the first broods, i.e. the first clutch of the season.

(ii) Moult

Moult was scored once in both years at the end of the breeding season (F1 = 17 June 2022 and F2 = 16 June 2023). We inspected the right wing of each bird and gave 10 scores per bird, one for each primary feather (P1–P10), from 0 to 5 (0 old feather, 5 fully grown), reflecting how much it had grown [59]. Then, we obtained a moult score per individual by converting each of the 0–5 moult scores into an approximate proportion of feather grown (following [60]), multiplied by the respective mass of that particular feather, and finally summed the values of all feathers. This resulted in a single value ranging between 0 and 1. The mass-corrected moult score serves as a proxy for moult timing because all birds were scored on the same day, and feather mass increases linearly throughout the season [60–62]. Thus, the larger the score, the further the bird is in its moult progress and consequently the earlier it started moulting.

(iii) Gonadal size

We measured the gonadal sizes of F2 birds on 22, 23, 26 and 27 February 2024. Birds, 19 Hoge Veluwe and 19 Gotland pairs, were kept in the same outdoor aviaries as in the F2 breeding season of 2023. Alternating between pairs from Gotland and Hoge Veluwe, birds were put under isoflurane and then decapitated. We measured the length (mm), width (mm) and fresh mass (mg) of their gonads and calculated testis and ovary volume as $V = 4/3\pi a^2 b$, where a is width (mm)/2 and b is length (mm)/2.

(c) Statistics

In total, 7 Hoge Veluwe and 8 Gotland F1 pairs and 20 Hoge Veluwe and 20 Gotland F2 pairs were used (number of pairs that produced a clutch was 7, 7, 20 and 17, respectively). We used data from both the F1 and the F2 generation, but note that the F1 birds originate from eggs laid in the two populations and if there are carry-over effects these may, to some extent, explain any phenotypic differences we find between the populations.

Laying date, moult score and gonad volume were analysed using linear mixed models using the lmer function of lme4 [63] in R v. 4.3.3 [64], with population (Gotland or Hoge Veluwe), generation (F1 or F2), (for moult and gonads) sex (male or female), as well as their interactions (all two-way and (for moult score) a three-way interaction) as explanatory variables, and family as random effect (to account for the fact that some birds were siblings). We used a likelihood ratio test to generate p-values. For testis, the right testis was analysed (as one left testis was missing, reducing the sample size and as left and right testis volume were highly correlated (Pearson correlation: 0.96)). Following Schaper *et al*. [34], we use the ^{10}log gonadal volume as the distribution of the logged values better followed a normal distribution.

(d) Calculation of the predicted laying dates

We used the statistical model from Bailey *et al.* [55] to predict the difference in laying date between the Gotland and the Hoge Veluwe F1 and F2 pairs. In this paper, for 34 populations of great tits, including Hoge Veluwe and Gotland, an absolute sliding time window approach (as implemented in the R package ClimWin [65,66]) was used to calculate for each population a relationship between temperature and laying date. First, the sensitivity window (date open and date close) for which mean temperature correlated best with the annual laying date of that population was calculated, and next the temperature sensitivity of laying date to temperature was determined (day per °C; see Fig. 1 in [55]). The Hoge Veluwe had a window that opens 12 March and closes 19 April, for Gotland this was 15 March and 25 May, respectively. The sensitivity for the Hoge Veluwe population was 3.25 day/°C and for Gotland 5.73 day/°C.

To use the Bailey *et al.* model to predict laying dates under Dutch conditions in 2022 and 2023, we first calculated the day length at which the sensitivity window opens and closes (which in the Bailey model is expressed in dates rather than day length) at the location of origin. Next, we calculated the mean temperature for the Netherlands (as recorded at the

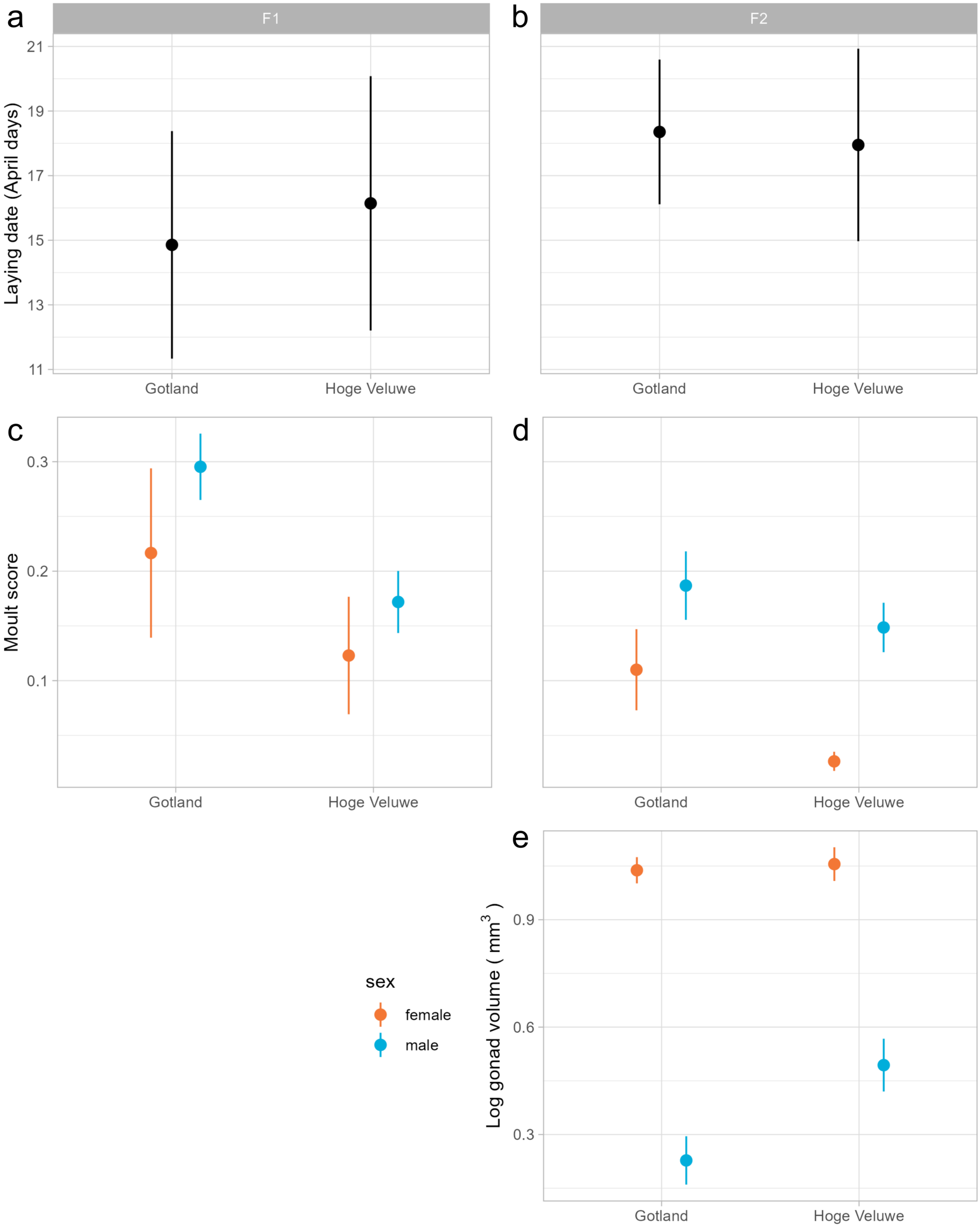

Figure 1. Seasonal phenotypes of great tits originating from Gotland or Hoge Veluwe. Mean laying date (in April date, i.e. 31 March = day 0; with s.e.) for (a) F1 and (b) F2 generation. Moult score (with s.e.) taken mid-June for (c) F1 and (d) F2 generation, and (e) ^{10}Log ovary volume (with s.e., mm^3) for F2 females and ^{10}Log testis volume (with s.e., mm^3) of F2 males as measured at the end of February.

meteorological station closest to de Hoge Veluwe (<1 km; Deelen) over these ‘photoperiod sensitivity windows’ and then used the temperature sensitivities from the Bailey *et al*. model to calculate the population-specific laying date for the 2 years. The model predicted a difference of 18 days in the laying date of the Gotland birds compared with the Hoge Veluwe birds (see Results). But note that these calculations could only be done after the sensitivity window closed so this was not a prediction made before the breeding season started.

3. Results

We found no significant differences in laying date (in April date, i.e. 31 March = day 0) between the two population for F1 and F2 birds nor between generations (population × generation $\chi^2(1) = 0.012$, $p = 0.91$, population $\chi^2(1) = 0.71$, $p = 0.40$, generation $\chi^2(1) = 0.048$, $p = 0.83$; figure 1*a* and *b*). Pairs from Gotland and the Hoge Veluwe differ in their moult scores in mid-June (no significant three-way and two-way interactions (all $p > 0.20$), population $\chi^2(1) = 4.80$, $p = 0.028$ (GO: 0.19, s.e. 0.04; HV: 0.12, s.e. 0.04), Sex $\chi^2(1) = 15.87$, $p < 0.001$ (female: 0; male: 0.087, s.e. 0.021), generation $\chi^2(1) = 4.0159$, $p = 0.045$ (F1: 0; F2: −0.073, s.e. 0.037); figure 1*c* and *d*) and in the volume of male testis as measured at the end of February (population $\chi^2(1) = 6.10$, $p = 0.014$ (GO: 0.20, s.e. 0.09; HV: 0.51, s.e. 0.08); figure 1*e*) but not in female ovary volume (population $\chi^2(1) = 0.088$, $p = 0.77$; figure 1*e*). Therefore, there were genetic differences between the populations in seasonal timing of male and female moult and male gonadal development but not in female ovary volume or laying date.

The lack of difference in laying date contrasts to what the statistical model of Bailey *et al.* [55] predicted based on Dutch temperatures in 2022 (F1) and 2023 (F2). For the F1 generation, the model predicted laying dates of 31 March for birds originating from Gotland and 18 April for birds originating from Hoge Veluwe. For the F2 generation, the predicted laying dates were Gotland 30 March and 17 April for Hoge Veluwe. Thus, in both years a difference of 18 days was predicted.

4. Discussion

To test for genetically based geographic variation in seasonal timing, we carried out a common garden experiment in which we bred great tits from two populations shown to have different temperature sensitivity of laying date (i.e. results from a recent meta-analysis across European populations [55]). We bred F1 and F2 pairs in open aviaries, originating from eggs collected in Gotland (Sweden) and Hoge Veluwe (Netherlands). We found no significant differences in laying date between the birds of different origins, but they did differ in moult timing and male testis size in spring.

A statistical model [55] predicted a difference in laying date of 18 days between the Gotland and Hoge Veluwe populations, so it is interesting that no difference was found (figure 1*a*,*b*). Our results thus show that caution is needed when interpreting population differences identified by large-scale comparative analysis as being genetic differences between populations as these differences are not necessarily signs of local adaptation.

The laying dates observed in the aviaries might be different phenotypes when compared with laying dates in the wild. For instance, in aviaries the birds get ad lib food and thus the environment under which we obtained seasonal timing phenotypes of the Hoge Veluwe and Gotland birds differs from the natural environment. Evidence for this comes from a lack of a correlation between the mean annual laying date in the aviaries (the ones we used in the common garden experiment here) and the mean annual laying date of the wild Hoge Veluwe population over 8 years for which we have data for both sets (M.E. Visser, 2025, unpublished.). However, if birds from Gotland and the Hoge Veluwe have different reaction norms of laying date versus temperatures (in slope or elevation) then even if the common garden environment is not the same as the natural environment, we would still expect the laying dates to differ in these 2 years. Moreover, great tit pairs from selection lines for early and late laying did reproduce at different times in the same aviaries as we used here [14], showing that meaningful differences in laying date can be obtained using this set-up.

Male testis size in early spring differed between birds originating from Gotland and Hoge Veluwe, whereas female ovary size did not (figure 1*e*). This finding in our between-population study is in contrast to the within-population variation observed in captive birds originating from the Hoge Veluwe population, where there was ample genetic variation in female ovary size but not in male testis size [34]. For females, the heritability was the largest (0.78) in early spring (February [34]) and for this reason, we also measured gonadal size in early spring (end of February). The difference in testis size between the Gotland and Hoge Veluwe populations indicates that over time there has been a genetic change in cue sensitivity of gonadal development.

Laying date is strongly temperature sensitive and much less photoperiod sensitive, while the opposite holds for gonadal development. For moult, there is clear evidence for sensitivity to photoperiod, but less so for temperature [49]. In our experiments, there was a difference in moult timing between the two populations (figure 1*c*,*d*), which is arguably more likely to be due to genetic variation in photoperiodic sensitivity rather than temperature sensitivity.

We studied the seasonal timing of three events: laying date, gonadal development and moult. The timing of these three events needs to be coordinated as, for instance, females cannot produce eggs and males cannot produce sperm without fully developed gonads, and an overlap between moult and chick feeding comes with an energetic cost [67,68]. Furthermore, gonadal development and, to a lesser extent, moult are primarily sensitive to photoperiod, while timing of egg laying is highly sensitive to temperature. This implies that under climate change the timing of gonadal development and moult will not respond to increased temperatures, but that laying date will advance. Climate change may thus lead to a desynchronization of events within the annual cycle of an individual [23,46].

Interestingly, the two life cycle events of which the seasonal timing is regulated mainly by photoperiod showed significant genetic differences between the Gotland and the Hoge Veluwe populations, while laying date, which is regulated by temperature, did not. This is possibly because the two populations have genetically differentiated over time in their sensitivity to photoperiod, and also potentially because under the current selection pressure the scope for genetic change in photoperiodic sensitivity is larger than that for temperature sensitivity. This is in line with the well-known genetic differences in laying date in two Corsican blue tit populations: Pirio and Muro. The populations differ in their laying date by a month and indeed are sensitive to temperature in very different periods in spring (Muro: day 42-73, Pirio day 86-105 (in January date, i.e. 31 December = day 0 [52]). Common garden experiments show that these differences between populations are due to the genetic differences in their sensitivity to photoperiod rather than temperature [33]. This is in line with another study that carried out a common garden experiment with two races of grouse (*Lagopus lagopus*) from different latitudes: the willow ptarmigan (*Lagopus lagopus lagopus*) from northern Norwegian and the red grouse (*Lagopus lagopus scoticus*) from Scotland. The red grouse laid their first eggs after the photoperiod was increased to 15 h, while the willow ptarmigan laid at a photoperiod of 19 h [69]. These results are further supported by studies under common environment conditions using wild caught dark-eyed juncos (*Junco hyemalis carolinensis* and *Junco hyemalis hyemalis*) [50,51]. Specifically, these populations breed in distinct latitudes and when maintained in a common environment under simulated photoperiods differ in traits related to timing of migration and breeding [32,70]. Overall, these studies show that even for a temperature sensitive life cycle event like laying date, genetic differentiation between populations operates via photoperiodic sensitivity.

In conclusion, our results appear to indicate that seasonal timing of life cycle events that are mainly affected by photoperiod will not shift due to increasing temperatures under climate change but that they are more likely leading to genetic change in response to selection. For life cycle events that are temperature sensitive the opposite holds; a phenotypic plastic response rather than genetic change is likely to be observed. This is an intriguing pattern, though we acknowledge that more empirical testing is needed. Our results may also imply that plasticity is constraining genetic adaptation [71,72] as laying date is very plastic to temperature, but there is no difference in laying date between populations in a common garden situation. This is also in line with the finding that in great tits, selection on the reaction norm for laying date versus temperature is stronger for elevation (that might run via photoperiodic sensitivity) rather than slope (which is affected by temperature sensitivity [28]).

Ethics. All experimental procedures in the lab and in the field were carried out under licenses of the Central Authority for Scientific Procedures on Animals (Project AVD 80100 2019 9005) and the Animal Welfare Body (IvD) of the Royal Netherlands Academy of Sciences (KNAW; protocol NIOO21.04).

Data accessibility. Data and statistical code are available [73].

Supplementary material is available online [74].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. M.E.V.: conceptualization, data curation, formal analysis, methodology, writing—original draft, writing—review and editing; K.v.O.: methodology, writing—review and editing; A.C.: resources, writing—review and editing; S.M.D.: resources, writing—review and editing; C.V.J.: resources, writing—review and editing; E.M.: resources, writing—review and editing; M.R.: investigation, methodology, writing—review and editing; B.M.T.: conceptualization, formal analysis, investigation, methodology, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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