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

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

3

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

19

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

22

23 **Summary statement:** Temperature directly affects avian breeding time. However, sensitivity
24 to temperature did not differ between two lines genetically selected for breeding time, but early
25 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.

45 **Introduction**

46 Effects of global climate change are omnipresent and can severely modify the environmental
47 conditions for wild populations (Parmesan and Yohe, 2003; Walther, 2010). Phenological traits
48 in particular are highly sensitive to these environmental modifications. This has led to
49 phenological changes with subsequent mismatches between trophic levels, in numeral taxa,
50 covering all trophic levels (Cohen et al., 2018; Parmesan, 2006; Root et al., 2003; Visser and
51 Gienapp, 2019), because trophic levels do not necessarily shift their timing at the same rate to
52 the increasing temperature (Thackeray et al., 2010; Visser and Holleman, 2001; Visser et al.,
53 1998; Voigt et al., 2003). It remains largely unknown, however, to which processes attribute
54 these phenotypic changes. As such, distinguishing between genetic changes and phenotypic
55 plasticity (Gienapp et al., 2008; Merilä and Hendry, 2014), i.e. the environmentally induced
56 production of different phenotypes by a single genotype (Pigliucci, 2001), hampers predictions
57 of species adaptations to ongoing climate change.

58 In temperate zone birds, the breeding period is short and varies yearly due to yearly varying
59 environmental conditions. Consequently, females need to track this inter-annual variation to
60 optimally time their breeding (i.e. egg-laying) to the time when food resources are most
61 abundant in order to support successful rearing of offspring (Charmantier et al., 2008; Perrins,
62 1965; Sheldon et al., 2003; van Noordwijk et al., 1995). Females thus need to be able to ‘predict’
63 when to initiate breeding, for which they use environmental cues. Photoperiod is an important
64 cue, as it gives initial important information to track the time of the year and starts up the
65 reproductive system (Dawson et al., 2001; Farner, 1985). However, its year-to-year
66 invariability does not allow females to track year-to-year variation in local conditions
67 (Bradshaw and Holzapfel, 2007; Visser et al., 2004) and as such, photoperiod alone cannot
68 explain individual year to year variation in timing of breeding. Temperature is highly variable
69 between years and provides information about local conditions, such as insect emergence,
70 which allows female to fine-tune their timing of breeding (Dawson, 2008; Wingfield and
71 Kenagy, 1991; Wingfield et al., 1992) and subsequently match offspring needs to food
72 abundance. So far, temperature is the major driving force of biological seasonality in temperate
73 zones (Parmesan, 2007) and the most influential environmental cue in fine-tuning timing of
74 avian seasonal breeding (Caro et al., 2013; Lambrechts and Visser, 1999; Visser et al., 2009).

75 Spring temperatures could directly affect timing of breeding or its effect can be indirect, where
76 temperature affects other environmental conditions, which subsequently causally affect timing
77 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

110 et al., 2013). However, we expected that early selection line females would lay earlier across
111 environments compared to late selection line females.

112 Lastly, we tested whether selection for laying date had a correlated response to selection on the
113 sensitivity to temperature (i.e. the slope of the reaction norm). Previously, Ramakers et al.,
114 (2019) studied whether reaction norms would evolve under current climate change in our long-
115 term study population of great tits in the Hoge Veluwe from which these selection line birds
116 originated (see Verhagen et al., 2019b). By quantifying selection on, and predicting the
117 evolution of, the timing of breeding reaction norm in response to temperature over three time
118 periods, they showed that laying dates did advance over the time periods examined, but the
119 sensitivity of laying dates to temperature (i.e. laying date plasticity) did not (Ramakers et al.,
120 2019). As such, we did not expect a difference in laying date plasticity between the selection
121 lines.

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

128 **Materials and methods**

129

130 *Selection lines in timing of breeding*

131 Selection lines were created for early and late timing of breeding in great tits (*Parus major*)
132 using genomic selection, which was moderately strong and in both directions (Gienapp et al.,
133 2019; Verhagen et al., 2019b). Briefly, nestlings (F₁ generation) were taken in from wild broods
134 of our long-term study population in the Hoge Veluwe National Park, The Netherlands
135 (52°02'07" N, 5°51'32" E) of which the mother had initiated egg-laying either extremely early
136 (*early* selection line) or extremely late (*late* selection line) in the wild. These chicks were
137 genotyped using a 650 SNP chip (Kim et al., 2018) in order to predict their 'genomic breeding
138 values' (GEBVs, i.e. the value estimating the relationship between genotype and phenotype
139 based on genetic markers). The F₁ generation individuals with the most extreme GEBVs were
140 selected for *early* and *late* selection line breeding pairs to produce the F₂ generation in captivity.
141 Eggs were transferred to wild 'foster-nests', where they were incubated and hatched.
142 Subsequently, 10 days post-hatching F₂ generation chicks were brought into the aviary facilities
143 at the NIOO-KNAW (Wageningen, the Netherlands) for further hand raising. In their turn, the
144 F₂ offspring were genotyped and, based on the most extreme GEBVs, selected to produce the
145 F₃ generation, which was then genotyped and selected. This study was performed under the
146 approval by the Animal Experimentation Committee (DEC), Amsterdam, The Netherlands,
147 protocol NIOO 14.10.

148

149 The results of the selection line study are described elsewhere (Verhagen et al., 2019a). In short,
150 *early* selection line birds laid on average earlier than *late* selection line birds, and this difference
151 in laying date increased (from about 2 to 10 days) over the generations (F₁ to F₃). Line effects
152 for the F₁ and F₂ were non-significant, but line differences were highly significant for the F₃
153 generation. On average *early* selection line birds laid about six days earlier compared to *late*
154 selection line birds (Verhagen et al., 2019b). Note that these results were found in the birds
155 housed in outdoor aviaries (see below). No differences were found between selection lines and
156 treatments in birds that were housed in climate-controlled aviaries (see below, Verhagen et al.,
157 2019a).

158

159 *Outdoor aviaries*

160 For a detailed description, see Verhagen et al. (2019b). In short, from January 2017 onwards,
161 F₃ generation pairs (n = 40) were housed in 40 outdoor aviaries (4.2 x 1.9 x 2.1m) where the
162 birds were subjected to natural photoperiod and temperatures. These pairs had the most extreme
163 GEBVs (see above) within the F₃ generation and functioned as breeding pairs to produce eggs
164 (F₄ generation) to be put in the wild as part of another study. Temperatures were recorded in 20
165 out of the 40 aviaries every 10-30 minutes using loggers (Thermochron iButton) throughout the
166 breeding season.

167

168 *Climate controlled aviaries*

169 The climate-controlled aviaries have been described in detail elsewhere (Verhagen et al.,
170 2019a), but briefly, 36 pairs of the F₃ generation of the selection lines (see ‘Selection lines in
171 timing of breeding’ above) were housed in 36 climate-controlled aviaries in January 2017.
172 These birds had less extreme GEBVs (see above) as compared to the birds housed in the outdoor
173 aviaries. In the climate-controlled aviaries, birds received an artificial photoperiod that
174 mimicked the change in natural photoperiod. In addition, two contrasting temperature
175 treatments (Fig. 1A) were provided mimicking an extreme cold (2013) or extreme warm (2014)
176 spring in the Netherlands (for details see Verhagen et al., 2019a). This was reflected in (average)
177 egg-laying dates between these years in the Hoge Veluwe population, where females (n=47 for
178 which we obtained laying dates for both 2013 and 2014) laid ~24 days earlier in 2014 compared
179 to 2013 (Fig. 2A). Every hour temperatures changed to follow as closely as possible the
180 observed hourly temperatures in these years (note that the minimum temperature in the aviaries
181 was 2°C so any temperature below 2°C in the temperature time series from outside was set to
182 2°C). Mimicking these natural temperature patterns is important to be able to infer realistic
183 conclusions. It is still a challenge to define what information in an experienced temperature
184 profile is used to time breeding. However, previous research indicated that the seasonal increase
185 in temperature, rather than the average temperature explains fine-tuning avian timing of
186 breeding (Schaper et al., 2012). The combination of selection line and temperature treatment
187 resulted in four groups (n = 9 pairs per group) in the climate-controlled aviaries: ‘early-warm’,
188 ‘early-cold’, ‘late-warm’ and ‘late-cold’.

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

191

192 *Breeding seasons and laying dates*

193 All birds went through their breeding season in 2017 and were housed in single-sex groups
194 afterwards. In January 2018, the same pairs were housed in the same outdoor or climate-
195 controlled aviary they were in in the breeding season of 2017 to go through their second
196 breeding season. In the climate-controlled aviaries, when pairs were subjected to the warm
197 temperature treatment in 2017, they received the cold treatment in 2018 and vice versa. During
198 these breeding seasons, nest boxes in all the aviaries were checked twice a week for nest
199 building and daily, when a female had completed her nest, for eggs. Laying dates (i.e. the first
200 day an egg was laid by a female) were recorded as January dates (i.e. 1 January = 1, 1 April =
201 91, etc.). Some females did not initiate egg-laying in one or both environments. As such, we
202 were able to obtain two laying dates recorded in two different environments for 34 out of 40
203 outdoor aviary females and 32 out of 36 climate-controlled aviary females.

204

205 *Statistical analysis*

206 While for the climate-controlled aviaries we know which temperature profile is associated with
207 early laying in the wild (Fig. 2A), as we mimic temperature from an early and a warm year (Fig.
208 2A), this is not the case for the outdoor aviary years. To determine a difference between
209 temperatures in 2017 and 2018 in the outdoor aviaries, we tested with a t-test the differences in
210 mean daily temperatures between 2017 and 2018 from 16 March – 15 April. This is the period
211 in which the temperatures correlate the best with mean annual laying dates in our long-term
212 wild population in the Hoge Veluwe National Park (Visser et al., 2006).

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

218

219 $\text{laying date} = \text{year} \times \text{selection line} + (1|\text{female family}:\text{female identity})$

220

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

224

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

226

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

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

243

244 **Results**

245

246 *Timing of breeding is directly affected by temperature*

247 In the outdoor aviaries, females laid earlier in 2018 compared to 2017 (year = -6.32 ± 1.57 , $F_{1,33}$
248 = 16.24, $p < 0.001$, Fig. 3A). Average daily temperatures are significantly lower in 2018
249 compared to 2017 ($t = 2.27$, $df = 38.78$, $p = 0.029$, Fig. 1B), meaning that, unexpectedly the
250 outdoor aviary females laid earlier in the colder environment (but see “Average daily increase
251 in temperature prior to breeding” below). Compared to the wild females in Hoge Veluwe
252 National Park in the same years, the average response in plasticity in laying date is in the
253 opposite direction (year \times location = 11.84 ± 2.31 , $F_{1,116} = 25.88$, $p < 0.0001$, Fig. 2B, see
254 “Discussion”).

255 In the climate-controlled aviaries laying dates were significantly affected by temperature
256 (controlled-aviaries: $\Delta_{\text{cold-warm}} = 7.2$ days), with birds laying earlier in the warm treatment,
257 indicating a direct effect of temperature on timing of egg-laying (treatment = -7.19 ± 2.69 , $F_{1,31}$
258 = 7.17 , $p = 0.012$, Fig. 3B). This average response in plasticity in laying date is in the same
259 direction, though less steep in slope, compared to the wild females in Hoge Veluwe National
260 Park in the same years (Hoge Veluwe: $\Delta_{2013-2014} = 23.7$ days, $n=47$ females, Fig. 2A, see
261 “Discussion”).

262

263 *Order of treatments and age of the birds*

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

269

270 *Selection on timing of breeding results in a change in reaction norm elevation between selection* 271 *lines*

272 In the outdoor aviaries there was no difference in plasticity in laying date (i.e. the reaction norm
273 slope) between the *early* and *late* selection line (selection line \times year = -1.28 ± 3.09 , $F_{1,32} =$
274 0.16 , $p = 0.69$, Fig. 3A). However, the *late* selection line females showed a significantly higher
275 elevation in the reaction norm for timing of egg-laying (i.e. they lay later independent of the
276 temperature) of about nine days (selection line = 9.31 ± 3.01 , $F_{1,32} = 8.73$, $p = 0.004$).

277 Between the *early* and *late* selection line females in the climate-controlled aviaries, there was
278 no difference in plasticity in laying date (selection line \times treatment = -1.50 ± 5.29 , $F_{1,30} = 0.08$,
279 $p = 0.784$) or elevation (selection line = -1.35 ± 4.10 , $F_{1,30} = 0.11$, $p = 0.747$, Fig. 3B).

280

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

282 Outdoor aviaries: Both in 2017 and 2018, the earliest female started on 27 March, or 86 January
283 (Fig. 1A). The profiles of increasing temperature of the 11 days (75-86 January) before the first
284 female initiated breeding differed significantly for 2017 and 2018 (year = -4.58 ± 0.87 , $F_{1,22} =$

285 27.5, $p < 0.0001$, Fig. 1A). Temperatures in this 11-day period increased on average with 0.09
286 ± 0.09 °C/day in 2017, whereas in 2018 this increase was 0.59 ± 0.16 °C/day and this increase
287 was significantly different between years (year = -4.75 ± 0.87 , $F_{1,22} = 27.5$, $p < 0.0001$).

288 Climate-controlled aviaries: The earliest females initiated breeding on 30 March (i.e. 89
289 January) and 16 April (i.e. 106 January) in the warm and cold treatment, respectively (Fig. 1B).
290 We tested the mean increase in temperatures in the same period (79-90 January) for both
291 treatments (Fig. 1B). The main daily temperature over this 11-day period, increased 0.53 ± 0.29
292 °C/day in the warm treatment and 0.005 ± 0.02 °C/day in the cold treatment. This increase was
293 significantly different between treatments (treatment = 6.05 ± 1.13 , $F_{1,20} = 28.6$, $p < 0.0001$).

294

295 **Discussion**

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

307 Currently, it is poorly understood what components of the temperature profiles, mean,
308 minimum, maximum, change, etc., are used by birds to predict their breeding and how this
309 information is perceived, transduced and ultimately translated into egg-laying. Interestingly,
310 the temperature profiles provided in this study show periods of increasing mean daily
311 temperature *before* females started initiating egg-laying (Fig. 1). An increase in temperatures
312 for a period of a week has previously been shown to advance the timing of egg-laying in great
313 tit females (Schaper et al., 2012). In the climate-controlled aviaries, there is no clear temperature
314 increase over the 11-day period in the cold treatment, when comparing the steep increase in
315 temperatures in same 11-day period for the warm treatment (Fig. 1A). This could explain why
316 females in the cold treatment started egg-laying later. Interestingly, despite that 2018 shows

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

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

343 In the climate-controlled aviaries the breeding time reaction norms are in the same direction as
344 in the wild (Figs. 2A, 3B). However, they show great individual variability in slope compared
345 to Hoge Veluwe females in 2013 and 2014 (Fig. 3B), which could have been caused by genetic
346 differences between individuals, lack of specific cues (Lambrechts and Visser, 1999) or a
347 disrupted correlation between cues due to (semi) artificial environments (Bentley et al., 1998).
348 In addition, eggs or first clutches could have been missed, but this is less likely due to the daily
349 checks of the nest boxes in the aviaries when laying was initiated. The great variability in

350 individual slopes could have led to decreased plasticity in average laying date of the selection
351 line females in the climate-controlled aviaries. This indicates that temperature is unlikely to be
352 the only environmental driver affecting laying date plasticity and that other environmental
353 factors are also involved, whether in interaction with temperature or not. A recent study in wild
354 tree swallows (*Tachycineta bicolor*), for example, found that timing of breeding was mainly
355 influenced by latitude and temperature, the latter in interaction with breeder density (Bourret et
356 al., 2015).

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

368 While genomic selection on timing resulted in a change in phenotype in the average
369 environment, at least in the outdoor aviaries, we found no correlated response to selection on
370 plasticity in laying date, independent of aviary type. This is in agreement with a recently
371 performed study in the long-term study population at the Hoge Veluwe from which these aviary
372 birds originate (Ramakers et al., 2019). This study found a directional selection on the elevation,
373 but not the slope of the laying date reaction norm to temperature. However, we must interpret
374 the results from the aviaries with some reservation, because, as opposed to Ramakers et al.
375 (2019), we studied a limited number of females. With the strength of genomic selection on egg-
376 laying being moderate (Verhagen et al., 2019b), we may not have been able to detect changes
377 in reaction norm slopes. In addition, due to this low sample size, we were unable to test the
378 individual variation in plasticity (I×E) and whether it has a genetic basis (i.e. genotype-by-
379 environment interaction or G×E). Further, both aviary types experienced two environments
380 compared to other long-term studies performed in wild populations. Still, these results are
381 promising for future studies (see below), especially due to the fact that they focus on patterns
382 *within* individuals.

383 Global climate change will continue to disrupt the synchrony between interacting trophic levels,
384 and therefore responding through phenotypic plasticity will likely not be sufficient in the long
385 run (Thackeray et al., 2016; Visser, 2008; Visser and Gienapp, 2019; Visser et al., 2004).
386 Genetic shifts in reaction norms are thus necessary for species to resolve the asynchrony in
387 phenology between consumer and prey, but these shifts remain scarce (Merilä and Hendry,
388 2014). In order for such a shift to occur, genetic variation in the mechanisms underlying
389 phenological traits is necessary and we need to find where in these mechanisms this variation
390 resides for selection to act upon. Experiments on temperature effects on timing of breeding
391 contribute to our understanding of how birds respond to environmental cues. Here, by using a
392 *within*-individual experimental approach, we show that temperature directly affects timing of
393 egg-laying in a song bird. Thus, natural selection may lead to a change in phenotype in the
394 average environment, but will likely not result in a correlated response to selection on the degree
395 of plasticity in laying date. Finding a direct effect of temperature on timing of egg-laying is
396 exciting, as it advances our understanding of the mechanisms underlying breeding decisions
397 under climate change. Data and results from this study will be important in future studies that,
398 for example, investigate within-individual DNA methylation patterns in contrasting treatments
399 analysing plasticity in laying date.

400

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407

408 **Author contributions**

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

412

413 **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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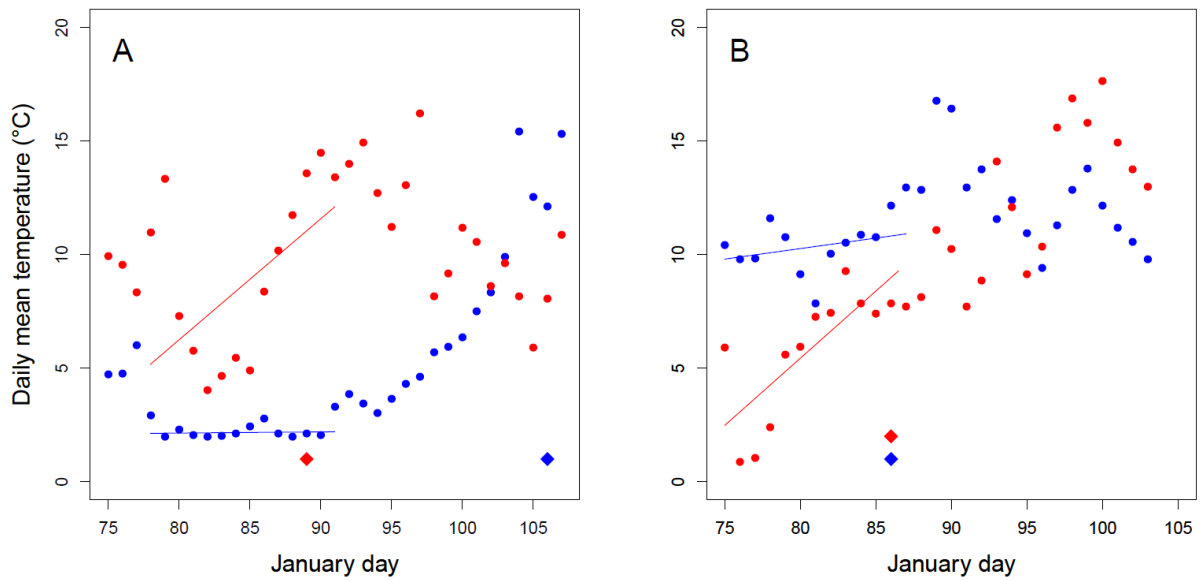
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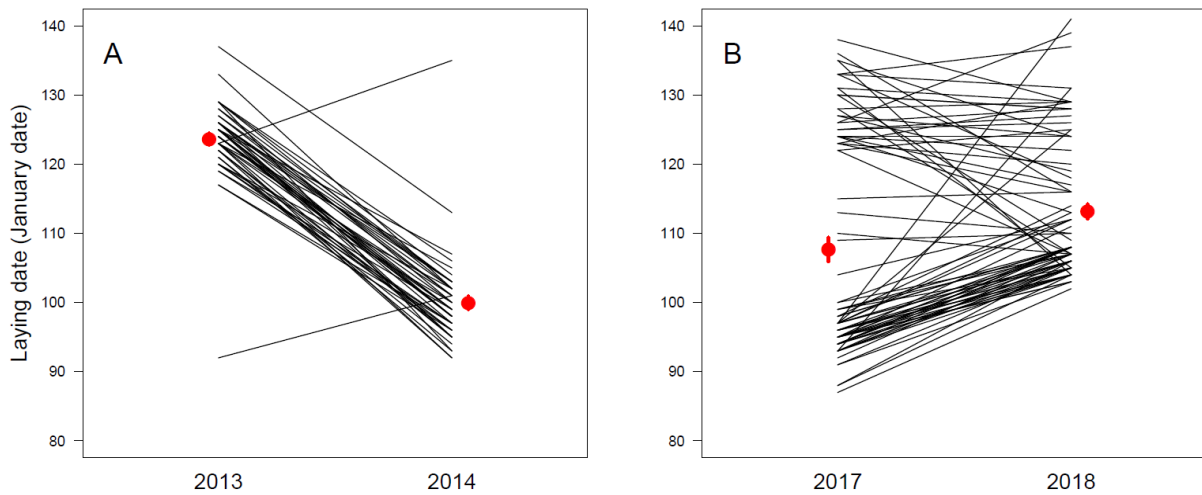
552 **Figures**



553

554 **Figure 1. Mean daily temperature profiles during the breeding season (16 March – 20 April) in the**
555 **climate controlled aviaries (A, n=36) and outdoor aviaries (B, n=40).** For the climate controlled
556 aviaries (A) the temperature profiles for the cold (blue) and warm treatment are shown (red), where for
557 the outdoor aviaries (B) the temperature profiles of 2017 (blue) and 2018 (red) are shown. Note that for
558 the outdoor aviaries temperatures are shown until 15 April. The filled diamonds indicate the first egg
559 laid in the warm (red) and cold (blue) temperature treatment or in 2017 (blue) and 2018 (red), for the
560 climate controlled and outdoor aviaries, respectively. The regression lines indicate the relationship
561 between date and mean daily temperature for the 11-day period prior to breeding initiation. Dates are in
562 January days (January 90 = March 30).

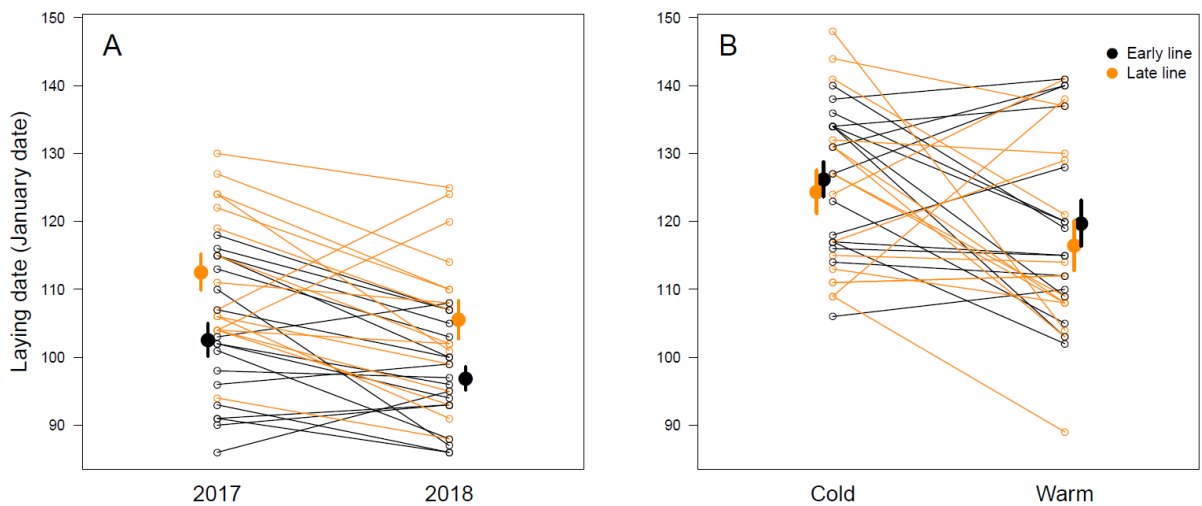
563



564

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

571



572

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