

Kimmitt Abigail A (Orcid ID: 0000-0003-0044-8297)  
Becker Daniel (Orcid ID: 0000-0003-4315-8628)  
Rosvall Kimberly (Orcid ID: 0000-0003-3766-9624)  
Ketterson Ellen D (Orcid ID: 0000-0002-7375-6605)

## Plasticity in female timing may explain earlier breeding in a North American songbird

Abigail A. Kimmitt<sup>1,2\*</sup>, Daniel J. Becker<sup>3</sup>, Sara N. Diller<sup>1</sup>, Nicole M. Gerlach<sup>4</sup>, Kimberly A. Rosvall<sup>1</sup>, Ellen D. Ketterson<sup>1,5</sup>

<sup>1</sup> Department of Biology, Indiana University, 1001 E. Third St., Bloomington, Indiana 47405

<sup>2</sup> Department of Ecology and Evolutionary Biology, University of Michigan, 1105 North University Ave, Ann Arbor, MI 48109

<sup>3</sup> Department of Biology, University of Oklahoma, 730 Van Vleet Oval, Norman, OK 73019

<sup>4</sup> Department of Biology, University of Florida, P.O. Box 118525, Gainesville, FL 32611

<sup>5</sup> Environmental Resilience Institute, Indiana University, 717 E. Eighth St., Bloomington, Indiana 47408

\* Corresponding author: [akimmitt@umich.edu](mailto:akimmitt@umich.edu)

**Keywords:** climate change; timing of breeding; selection; phenotypic plasticity; phenological shifts; bird

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/1365-2656.13772](https://doi.org/10.1111/1365-2656.13772)

This article is protected by copyright. All rights reserved.

## Abstract

1. Many species have shifted their breeding phenology in response to climate change. Identifying the magnitude of phenological shifts and whether climate-mediated selection drives these shifts is key for determining species' resilience to climate change. Birds are a strong model for studying phenological shifts due to numerous long-term research studies; however, generalities pertaining to drivers of phenological shifts will emerge only as we add study species that differ in life history and geography.

2. We investigated 32 years of reproductive timing in a non-migratory population of dark-eyed juncos (*Junco hyemalis*). We predicted that plasticity in reproductive timing would allow females to breed earlier in warmer springs. We also predicted that selection would favour earlier breeding and asked whether the temperatures throughout the breeding season would predict the strength of selection.

3. To test these predictions, we examined temporal changes in the annual median date for reproductive onset (i.e., first egg date) and we used a sliding window analysis to identify spring temperatures driving these patterns. Next, we explored plasticity in reproductive timing and asked whether selection favoured earlier breeding. Lastly, we used a sliding window analysis to identify the time during the breeding season that temperature was most associated with selection favouring earlier breeding.

4. First egg dates occurred earlier over time and strongly covaried with April temperatures. Further, individual females that bred in more than one year, typically bred earlier in warmer Aprils, exhibiting plastic responses to April temperature. We also found significant overall selection favouring earlier breeding (i.e., higher relative fitness with earlier first egg dates)

and variation in selection for earlier breeding over time. However, temperature across diverse climatic windows did not predict the strength of selection.

**5.** Our findings provide further evidence for the role of phenotypic plasticity in shifting phenology in response to earlier springs. We also provide evidence for the role of selection favouring earlier breeding, regardless of temperature, thus setting the stage for adaptive changes in female breeding phenology. We suggest for multi-brooded birds that advancing first egg dates likely increases the length of the breeding season, and therefore, reproductive success.

## 1. Introduction

Phenological shifts are one of the most documented responses to climate change (Parmesan and Yohe 2003; Scheffers et al. 2016; Piao et al. 2019), suggesting that many species can adapt to warming temperatures (Charmantier and Gienapp 2014; Saalfeld and Lanctot 2017). If phenological shifts are directly linked to demographic traits (e.g., reproductive success, annual survival), understanding the underlying processes of these shifts will have relevance for predicting population dynamics and resulting species persistence (Miller-Rushing et al. 2010; McLean et al. 2016; Iler et al. 2021). And yet, the relative role of microevolutionary change in response to selection and phenotypic plasticity in driving phenological shifts remains under debate (Charmantier and Gienapp 2014).

Plastic responses allow individuals to respond more quickly to a changing climate compared to microevolutionary change in response to selection (Sih et al. 2010; Charmantier and Gienapp 2014; Beaver et al. 2017), such that evidence for plasticity has been widely reported to account for current phenological shifts observed in animals (Réale et al. 2003; Boutin and Lane 2014; Charmantier and Gienapp 2014; Crozier and Hutchings 2014). However, as plasticity might not always be adaptive under rapidly changing environmental conditions (Ghalambor et al. 2007), microevolutionary change in combination with plasticity will likely be necessary for long-term response to climate change (Gienapp et al. 2013). Birds have served as a strong model for studying the role of plasticity versus microevolutionary change in driving phenological shifts due to long-term research studies and citizen science projects that monitor avian breeding populations (Charmantier and Gienapp 2014; McLean et al. 2016). In this study, we add to this growing body of literature, by investigating how changes in the environment over time shape these drivers of phenological shifts in a North American songbird.

As seasonal environments are variable, the environment that shapes plastic versus selective responses likely differ (Gavrilets and Scheiner 1993). Phenotypic plasticity allows individuals that breed in more than one season to track or partially track optimal breeding phenology over time (de Villemereuil et al. 2020). Plasticity in avian breeding phenology is therefore likely shaped by the environment in early spring prior to egg laying, as environmental variables, such as temperature, can act as cues for females to time their final stages of reproductive development leading up to the first egg (Williams 2012). Breeding phenology, however, can also be modified by plastic behaviours, such as incubation initiation or length, driven by environmental conditions after the first egg is laid (Cresswell and McCleery 2003). Environmental variables that are varying as a result of climate change (e.g., temperature) may also drive directional selection favouring earlier breeding in birds by affecting offspring survival either directly (e.g., effects on thermoregulation) or indirectly (e.g., effects on prey or predators) (Charmantier and Gienapp 2014). Evolutionary adaptation can then occur if directional selection favouring earlier breeding acts on heritable traits with genetic variation (Hoffmann and Sgrò 2011). In multi-brooded birds, the breeding season spans over a few months and variable climatic conditions, such that the environment shaping selection might differ from the environment driving plasticity in breeding phenology (Gavrilets and Scheiner 1993; Bonamour et al. 2019).

Here, we used long-term data collected from Dark-eyed Juncos (*Junco hyemalis*), a north-temperate sparrow found in Canada and the United States, to investigate changes in breeding phenology and the underlying drivers of these changes. As a geographically widespread species, the junco includes populations that vary greatly in migratory strategy, phenology, and morphology (Nolan et al. 2002; Milá et al. 2007). Juncos have also served as a model songbird species for studies of ecology and evolution over the past few decades (Ketterson and Atwell 2016).

Specifically, we focused on a breeding population of Carolina Dark-eyed Juncos (*J. h. carolinensis*) that resides in the Appalachian Mountains year-round, with some individuals migrating short distances (e.g., altitudinal migrants). We first asked whether median monthly air temperature in early spring changed over a 32-year study period, predicting that spring temperatures would increase over time. We next compared annual average first egg dates (i.e., initiation of breeding, or the date of first egg laid in the year) to spring temperatures over time and predicted that first egg dates would be earlier in response to a warming climate. We then used a random regression model approach to evaluate the degree of female plasticity in response to spring temperatures. We predicted that females can plastically adjust their phenology to lay earlier in warmer years. If among-individual variation in plasticity is detected, then selection might be able to act on phenology, and the trait could evolve given a genetic basis of phenology (Nussey et al. 2007). Next, we asked whether selection favoured earlier breeding by assessing the relationship between female annual relative fitness and first egg date. Finally, we used a sliding window approach to identify climatic drivers of the strength of selection across our study period. We predicted that selection would favour earlier breeding and that strength of selection would vary over time in response to temperature, such that selection would be stronger in warmer springs. We also expected that selection would be predicted by temperatures after females invested in egg-laying because the thermal environment during this time might directly affect chick survival and recruitment (Visser et al. 2006; Bonamour et al. 2019; Sauve et al. 2021).

## **2. Materials and Methods**

### **a) Study system and breeding data**

Since 1983, a breeding population of Dark-eyed Juncos has been monitored at Mountain Lake Biological Station (MLBS) and the surrounding Jefferson National Forest (37°22'N, 80°32'W) (Chandler et al. 1994). All monitoring protocols were approved by Indiana University's Institutional Animal Care and Use Protocol (#12-050). At the beginning of each breeding season (April-May), birds on the study site were caught using mist nets or Potter traps and banded with a unique USFWS metal band and distinctive combinations of colour bands. Researchers searched for nests every year, identifying parents and tracking the progress of the nest. First egg date, expressed as ordinal date, was observed directly, or for nests found after the start of egg-laying, was calculated based on the day nestlings hatched or left the nest (Nolan et al. 2002). Breeding data from 1983–2015 were used for this study except for 2013 due to limited research effort. Records where female ID or first egg date were unknown were removed. Female subjects that were implanted with exogenous testosterone during a separate five-year study in the population were also removed (Clotfelter et al. 2004; Ketterson et al. 2005).

To calculate true first egg dates, we excluded any known re-nests. Also, knowing that the first nest found for a female might not be her true first nest, we eliminated nests whose first egg dates came later than each year's median first egg date from known re-nests. Our data filtering resulted in 1,244 first nests of 935 female juncos between 1983 and 2015; females had one to five years of data ( $\bar{x} = 1$ ). Annual differences in research effort (number of nests found) did not explain variation in first egg dates (see Supplementary Materials; Fig. S1). Because the distributions of first egg dates were not normal in some years, we calculated median annual first egg dates from first nests. Using both first nests and re-nests for each year, we calculated the annual total number of eggs and total number of fledglings produced by each female. Females were grouped into two age classes based on plumage (Pyle 1997) or records from previous

breeding seasons: second years (SY; first breeding season) and after second years (ASY; second or later breeding season).

## **b) Temperature data**

Between November 16, 1971 and January 31, 1998, temperature data (daily minimum;  $T_{\min}$  and maximum temperature;  $T_{\max}$ ) were collected from MLBS via a National Oceanic and Atmospheric Administration (NOAA) weather station (Network ID GHCND: USC00445828, hereafter, “Logger A”). On June 24, 1994, a second data logger (Campbell CR10) was established at MLBS that records temperature every half hour. To permit comparing data across devices, we calculated daily  $T_{\max}$  and  $T_{\min}$  from this MLBS data logger (hereafter, “Logger B”). From  $T_{\min}$  and  $T_{\max}$ , we calculated a daily midpoint (median) temperature ( $T_{\text{mid}}$ ) for both loggers. Since the two weather stations overlapped from 1994-1997, we confirmed that Datasets A and B were strongly correlated and then combined the datasets (*see Supplementary Materials*).

Monthly average  $T_{\min}$ ,  $T_{\max}$ , and  $T_{\text{mid}}$  were calculated for March–August for each year. Data were available for all years (1983-2015), except for missing March and April data for 1991 and 2002 and missing March data for 2004.

## **c) Temporal patterns**

All statistical analyses were conducted in R (version 4.0.0). We fit a linear model (LM) with year as the predictor variable to first investigate change over time in median first egg date. Most female juncos lay their first egg in late April–early May, and the final stages of reproductive development can take anywhere from days to weeks leading up to the first egg (Williams 2012). Temperatures in early spring prior to egg laying likely have the greatest influence on female

reproductive timing ('environment of [reproductive] development'), whereas temperatures later in the season could affect offspring survival and female reproductive fitness ('environment of selection') (Gavrilets and Scheiner 1993). Therefore, to determine how temporal variation related to median annual first egg date, we used a sliding window approach to identify the time period over which temperatures during the breeding season predict population median first egg date (van de Pol et al. 2016). We used a range of start dates for our climatic window (i.e., ordinal date 60–120) alongside varying window lengths (i.e., 10–50 days), corresponding to approximately the month before the earliest first egg date (day 95) through mid-June when the latest first egg date was recorded (Nolan et al. 2002); temperatures during these dates could reasonably affect females' initiation of laying. For each climatic window, we derived the mean temperature per year using our daily data for  $T_{\min}$ ,  $T_{\max}$ , and  $T_{\text{mid}}$ . Owing to missing March and April data for 1991 and 2002, missing April data for 2003, and missing March data for 2004, we excluded data from these years to facilitate model comparison. We then fit our LM for median egg one date with year and this new temperature variable as predictor variables, using Akaike's information criterion adjusted for small sample sizes (AICc) and Akaike weights ( $w_i$ ) to compare across 35 climate windows for each temperature variable ( $T_{\min}$ ,  $T_{\max}$ , and  $T_{\text{mid}}$ ). We considered models within two  $\Delta\text{AICc}$  of the top model to be competitive (Burham and Anderson 2002). We then used the most competitive climate window for each temperature measure in our plasticity analyses.

#### **d) Phenotypic plasticity**

To assess the degree of individual female plasticity in first egg date in relation to temperature, we used a random regression model (RRM) approach (Nussey et al. 2007). RRM's are a particular case of generalised linear mixed models (GLMMs) where individuals vary in the

elevation (i.e., intercept) and slope of their reaction norms. For females that bred in at least three years of our study ( $n=62$  individuals representing 190 first egg date observations, hereafter “returning females”), we fit a RRM with a fixed effect of temperature (see above), a random intercept of female ID, and their interaction (i.e., a random slope) for  $T_{\min}$ ,  $T_{\text{mid}}$ , and  $T_{\max}$  with the *lme4* package (Pinheiro and Bates 2006). We included age as a covariate and a random intercept for year. We fit each RRM with REML to derive marginal and conditional  $R^2$  as measures of fit ( $R^2_m$  and  $R^2_c$ ; (Nakagawa et al. 2017) and to estimate variance for random effects. We used sequential likelihood ratio tests (LRTs) to assess if random intercepts and slopes were significantly different from zero (i.e., denoting significant inter-individual variation in reaction norms) (Nussey et al. 2005).

#### **e) Selection analyses**

We estimated selection acting on the start of breeding as the slope of a regression of relative fitness (i.e., total number of fledglings per year per female divided by annual population mean total fledglings) on first egg date (Lande and Arnold 1983). As relative fitness was zero inflated, we used a compound Poisson GLMM with the *cplm* package (Zhang 2013). Within this GLMM, we adjusted for age and annual total number of eggs per female by including these as fixed effects (Marrot et al. 2017). Annual total number of eggs per female was included as a covariate in this model because variation in this trait likely predicts relative fitness regardless of lay date. We also standardised first egg dates annually (zero mean and unit variance) to control for environmental covariance between fitness and this trait across years (Kingsolver et al. 2001; Marrot et al. 2018). Owing to missing values, this analysis included 1,182 first nests from 898 females. We included year and female ID as random intercepts to control for multiple observations per year and females

that bred more than one year; we also included a random slope of first egg dates by year to account for temporal variation in selection gradients. We also derived  $R^2_m$  and  $R^2_c$  as measures of model fit (Nakagawa et al. 2017). To then assess temporal variation in selection acting on the start of breeding, we fit a secondary GLMM with an equivalent model structure and an interaction between standardised first egg date and year.

Lastly, we tested for relationships between temperature and the strength of selection on first egg date. We again used a sliding window approach to identify the time period over which temperatures during the breeding season predict selection on first egg date (van de Pol et al. 2016). We used start dates ranging from ordinal date 60–160 alongside varying window lengths (i.e., 10–80 days), corresponding to approximately the month before the earliest first egg date (day 95) to the end of August, when juncos are undergoing autumn moult and chicks are no longer in the nest (Nolan et al. 2002). We then fit our selection GLMM with an interaction between first egg date and this new temperature variable, using AICc and Akaike weights to compare among climatic windows. Owing to missing temperature data, we used a reduced dataset of 1,052 first nests from 817 females to compare GLMMs (88 climatic windows per temperature measure).

### 3. Results

#### a) Temporal patterns

April average  $T_{mid}$ , April–August  $T_{min}$ , and June–August  $T_{max}$  significantly changed over time (1983–2015) at MLBS. No other temperature variables exhibited significant change over time (Table S1; Fig. 1).

Median first egg date decreased significantly over time  $\beta = -0.23$ ,  $p = 0.044$ ,  $R^2 = 0.10$ ) as females laid their first egg earlier in more recent years (Fig. 2A). This univariate LM predicted the

Author Manuscript

median first egg dates at the start (1983) and most recent year (2015) to be 132 and 125 respectively, resulting in a predicted net advancement of seven days. When using a sliding window approach to identify the temperature most associated with first egg dates (after accounting for year effects), we identified 11 competitive windows for  $T_{\min}$ , with the top window spanning from ordinal date 100 to 119 (April 10–April 29 in a non-leap year,  $w_i = 0.07$ , Table S2). In contrast, we identified the same and sole competitive window for  $T_{\text{mid}}$  and  $T_{\text{max}}$  as spanning from ordinal date 90 to 119 (March 31–April 29 in a non-leap year;  $w_i = 0.55$  and  $0.83$ , respectively, Tables S3 and S4). For the top  $T_{\min}$  model ( $R^2 = 0.26$ ), first egg date decreased weakly by mean spring temperature ( $\beta = -0.87$ ,  $t = -1.89$ ,  $p = 0.07$ ) and year ( $\beta = -0.18$ ,  $t = -1.77$ ,  $p = 0.09$ ; Fig. 2B). For the top  $T_{\text{mid}}$  model ( $R^2 = 0.50$ ), first egg date decreased significantly by mean spring temperature ( $\beta = -1.90$ ,  $t = -4.22$ ,  $p < 0.001$ ) but not year ( $\beta = -0.13$ ,  $t = -1.66$ ,  $p = 0.11$ ; Fig. 2C). Lastly, for the top and equivalent  $T_{\text{max}}$  model ( $R^2 = 0.59$ ), first egg date decreased significantly by mean spring temperature ( $\beta = -1.67$ ,  $t = -5.33$ ,  $p < 0.001$ ) and year ( $\beta = -0.22$ ,  $t = -3.35$ ,  $p < 0.01$ ; Fig. 2D).

## b) Phenotypic plasticity

We used RRM models to assess phenotypic plasticity in the relationship between timing of breeding and each of our most competitive climate windows per each temperature variable. For 62 females studied across at least three years, individuals generally started breeding significantly earlier with warmer spring temperature after adjusting for female age (Table 1). The population-level temperature effect on first egg date was significantly negative for both  $T_{\min}$  and  $T_{\text{mid}}$  ( $T_{\min}$ :  $\beta = -0.96$ ,  $t = -2.06$ ,  $p = 0.05$ ;  $T_{\text{mid}}$ :  $\beta = -1.35$ ,  $t = -2.29$ ,  $p = 0.03$ ), whereas that for  $T_{\text{max}}$  was weaker but consistent in directionality ( $\beta = -0.72$ ,  $t = -1.45$ ,  $p = 0.17$ ). For each temperature window,

random effects explained substantially more variation in first egg date than fixed effects alone (i.e.,  $R^2_c > R^2_m$ ; Table 1). LRTs revealed that females showed moderate variation in elevation (i.e., estimated first egg date at the average temperature), primarily for  $T_{mid}$  and  $T_{max}$ , but never in slope (i.e., individual response to inter-year variation in temperature) of the breeding phenology reaction norm for average spring temperatures (Fig.3; Table 1). We also did not observe significant inter-annual variation in first egg dates for this subset of females for any temperature measure (Table 1). Therefore, females displayed significant population-level phenotypic plasticity to temperature. Females also differ in when they initiate breeding based on an average spring temperature (elevation), but we did not find evidence of inter-individual variation in the degree of plasticity (slope).

### c) Selection analyses

We estimated overall strong negative selection on first egg date ( $\beta = -0.16, t = -4.42, p < 0.001$ ; Fig. 4A), in which females that initiated breeding earlier had higher reproductive fitness than females that initiated breeding later in the breeding season. Annual total number of eggs per female was under positive selection, in which individuals that produced more eggs also had more successful fledglings ( $\beta = 0.05, t = 5.28, p < 0.001$ ). Older females had marginally higher relative fitness ( $\beta = 0.12, t = 1.87, p = 0.06$ ). The model explained 6.8–13% of the variance in relative fitness ( $R^2_m$  and  $R^2_c$ , respectively). When considering an additional GLMM to test for year-dependent selection gradients, we found no support for an interaction between first egg date and time ( $\beta = -0.002, t = -0.30, p = 0.76$ ). However, this model did identify strong inter-year variation in random slopes by year ( $\sigma^2 = 0.08, LRT = 19.06, p < 0.001$ ). Therefore, selection favouring earlier breeding displays strong inter-annual variation.

We then used another sliding window approach to flexibly identify the temperature period most strongly associated with selection on first egg date. Across 88 climatic windows per temperature measure, we found no support for temperature dependence in selection gradients. For average  $T_{\min}$  and  $T_{\text{mid}}$ , all candidate GLMMs were within  $2 \Delta\text{AICc}$  of the top model, and 56% of candidate GLMMs for  $T_{\max}$  were also within  $2 \Delta\text{AICc}$  of the top model. Additionally, interaction terms between the mean temperature within each climate window and first egg date were consistently non-significant (i.e., 95% confidence intervals always crossed zero) across all considered windows, although effect sizes were moderately stronger (though still non-significant) for longer climate windows (i.e., 80 days) that corresponded to mid-May–August through mid-June–September (Fig. 5). Thus, we did not find support for warmer temperatures in any climatic window to be associated with selection favouring earlier breeding.

#### 4. Discussion

We investigated shifts in breeding phenology of a North American songbird, the dark-eyed junco, over a 32-year period and in relation to spring temperatures and found a net change of seven days in first egg date. Temperatures in the springs and summers have grown warmer, and females are initiating reproduction earlier than in the past. April temperatures were the best predictors of female first egg date. We also found evidence for plasticity in driving these phenological changes. For a subset of returning females with sufficient multi-year data to evaluate the reaction norm between breeding phenology and each temperature variable, females typically bred earlier in warmer years. When we evaluated these reaction norms, however, we found no significant inter-individual differences in slope with only moderate inter-individual differences in elevation for maximum and midpoint temperatures. Therefore, females displayed significant population-level

phenotypic plasticity to warming spring temperatures. Females differed in when they initiated breeding based on an average spring temperatures but did not show strong inter-individual variation in the degree of their plastic response to different temperatures. Considering that such individual differences are a prerequisite for selection to act, selection might be able to act on the average phenotype but might not be able to act on the plasticity in breeding phenology itself, which has been observed in other wild bird populations (Nussey et al. 2005; Brommer et al. 2008; but see Husby et al. 2010). In other words, we do not currently observe steeper plastic responses in females that could be favoured over more subtle plastic responses as temperatures become more extreme in the future.

We also found evidence that selection favouring earlier breeding varied over time. Changes in breeding phenology is likely adaptive as the directional change in the trait over time mirrors the overall negative selection on phenology (Radchuk et al. 2019). This result confirms a previous finding from a 17-year subset of our study population data, in which males and females from 1990–2007 produced significantly more offspring when females initiated egg laying earlier (Gerlach 2010). However, unlike studies of other avian species (Marrot et al. 2018; Helm et al. 2019), temperatures spanning multiple climatic windows did not predict strength of selection on egg one dates, as might be expected if selection were responding to a warming climate. Our results do not suggest that temperature is acting as a driving environmental variable of selection favouring earlier breeding. Instead, other factors may be affect selection on female phenology, such as other environmental variables (e.g., precipitation; Dunn and Winkler 2010) or selection on male reproductive timing, which in turn could stimulate earlier egg-laying in females (Watts et al. 2016). While selection and plasticity both likely played a role in driving earlier breeding, we find greater support for plastic responses of breeding phenology to warming temperatures.

### **a) Plastic versus evolutionary responses to climate change**

Microevolutionary responses to climate change are predicted to result from directional selection favouring earlier breeding to alleviate the negative effects of phenological mismatches (Charmantier and Gienapp 2014) or to benefit from a longer breeding season (Møller et al. 2010; Dunn and Møller 2014). Without knowledge of the heritability of timing of reproduction, however, evidence of selection favouring earlier breeding is insufficient to conclude that microevolutionary change is occurring, as genetic and environmental effects can be difficult to disentangle (Merilä 2012; Helm et al. 2019). There is some evidence of microevolutionary changes in phenology across taxa that are likely adaptive shifts in response to climate change (Van Asch et al. 2013; Manhard et al. 2017; Helm et al. 2019). In the case of the junco, while we found strong overall selection favouring earlier breeding, and that this selection varied over time, the strength of selection was not associated with a diverse set of climatic windows during the breeding season. In other words, females that bred earlier in years when temperatures were warmer during periods of reproductive development or chick development did not experience higher relative fitness. While females that breed earlier overall have higher reproductive fitness, this adaptive change does not appear in response to warming temperatures. As we did not measure genetic variation or heritability of reproductive timing, we cannot conclude whether microevolutionary change might account for the observed shifts in timing. Future work should integrate genomic quantitative genetic approaches with breeding data to determine the role of microevolution in phenological shifts, which could in turn have important implications for conservation genomics (Gienapp et al. 2017).

Author Manuscript

Additionally, phenotypic plasticity, which can allow for more rapid changes in phenotype than microevolutionary changes, may account for why earlier breeding was related to increases in fitness over time (Van Buskirk et al. 2012; Charmantier and Gienapp 2014; Beever et al. 2017). Numerous studies support plasticity as a mechanism for coping with climate change (Charmantier et al. 2008; Phillimore et al. 2016; Verhagen et al. 2020), despite its limitations in promoting population persistence in the face of climate change (Ghalambor et al. 2007; Gienapp et al. 2013; Duputié et al. 2015). We found that returning females typically initiated egg laying earlier in warmer springs. Females exhibited moderate variation in the intercept of the relationship between lay date and temperature, suggesting that individuals differ from one another in their phenology based on the average environment. The reaction norms, however, did not show significant among-individual differences in slope, meaning that females do not substantially vary from one another in how much they adjust their phenology to different temperatures they experience. One important caveat is that our sample size of returning females for the plasticity analysis ( $n = 62$ ) was relatively small due to low survivorship (i.e., annual survival probability calculated for 1994–2000 was  $0.49 \text{ years} \pm 0.03 \text{ SE}$ , Reed et al., unpublished data). Therefore, we might have been unable to fully capture variation in plastic responses to the environment within the population. We cannot exclude the possibility that selection might act on this inter-individual variation and serve as a possible explanation for the observed phenological shift. Selection acting on plasticity could play an important role in allowing breeding phenology shifts to keep up with the rate of environmental change (Gienapp et al. 2013).

**b) *Winners vs. losers in relation to climate change***

Global change biologists often discuss ‘winners’ and ‘losers’ in relation to climate change, typically in the context of range shifts (Crick 2004; Bateman et al. 2016; Tayleur et al. 2016). Here, we extend these concepts of winning and losing to variation in brood number per season. In our system, juncos are multi-brooded, as females can re-nest as many as five times and can fledge up to three successful nests (Nolan et al. 2002). Reproductive success of multi-brooded birds is likely dependent on the duration of the breeding season (Dunn and Møller 2014), an effect echoed in our finding that juncos that bred earlier tended to fledge more offspring that year, presumably resulting from having more time for breeding attempts. Previous evidence suggests that population dynamics differ between species that vary in breeding duration: multi-brooded species are often found to exhibit stable or growing populations compared to declining populations of single-brooded species (Dunn and Møller 2014). Multi-brooded species might thus be “winners” relative to current-day climate change, as they have higher reproductive success because of extended breeding seasons. Therefore, warmer springs may allow females to breed earlier and extend their breeding season, despite the lack of evidence that temperatures predicted strength of selection favouring earlier breeding. We cannot directly test this hypothesis because junco nests become more challenging to find later in the breeding season, resulting in an inaccurate count of the number of nests per female each season. Future studies in multi-brooded species should examine whether females had higher number of nesting attempts or successful broods in warmer years if possible. However, while breeding phenology predicts reproductive success, and likely in turn population dynamics, thermal tolerance and habitat adaptation are also essential for population persistence. Because our study population occurs at a high elevation, persistent increases in temperature could eventually result in population decline and range shifts, as the population cannot move to higher elevations.

### **c) Future directions**

Accurate predictions of future responses to climate change will require further consideration of mechanisms of female reproductive timing (Williams 2012; Chmura et al. 2020; Kimmitt 2020). Past and ongoing work in the junco are elucidating the physiological mechanisms driving reproductive timing in females based on life history, including endocrine systems and costs of early breeding (Greives et al. 2016; Graham et al. 2019; Kimmitt et al. 2019; Kimmitt et al. 2020). However, more research is necessary to understand how females integrate supplementary cues, such as temperature, to regulate the final stages of their reproductive development and ovulation (Wingfield et al. 2016; Chmura et al. 2020). Through our analysis of this 32-year dataset, we found that plasticity in female timing is likely relevant for population persistence. Further work on the proximate mechanisms of female timing will improve forecasts of the effects of climate change on birds.

### **Data Availability Statement**

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.4j0zpc8fd> (Kimmitt et al., 2022).

### **Acknowledgments**

We acknowledge the significant role of the late Val Nolan Jr., who established this field site for long-term study as a co-PI with Ellen Ketterson in the early 1980s. We also thank Ketterson lab members and colleagues who have contributed to this long-term dataset, with a special thanks to long-term lab managers and field assistants, Eric Snajdr, Charles Ziegenfus, and Sarah

Author Manuscript

Wanamaker. We also acknowledge support from MLBS and its directors, Henry Wilbur and Edmund “Butch” Brodie III, as well as MLBS staff. We thank associate editor, Ally Philimore, for his feedback and assistance with the sliding window analysis. Finally, we thank Allie Byrd, Alex Jahn, Katie Talbott, and Sarah Wanamaker for discussion and feedback on the manuscript.

Long-term data collection was supported by NSF (#8718358, 9408061, 9728384, 0216091, 0519211, 0820055, 1257474). AAK was supported by the NSF Graduate Research Fellowship. SND received funding from the IU School of Public and Environmental Affairs.

### **Conflict of Interest**

The authors declare no conflicts of interest.

### **Authors’ Contributions**

Abigail A. Kimmitt conceived and designed the study, curated data, conducted statistical analysis and was the primary author of the manuscript; Daniel J. Becker conducted statistical analysis, drafted sections of the manuscript, and revised the manuscript; Sara N. Diller participated in data curation and analysis and revised the manuscript. Nicole M. Gerlach conceived and designed the study, curated data, and revised the manuscript. Kimberly A. Rosvall conceived and designed the study and revised the manuscript. Ellen D. Ketterson conceived and designed the study, secured funding for data collection, and revised the manuscript. All authors gave final approval of this publication.

## References

- Bateman, B. L., A. M. Pidgeon, V. C. Radeloff, J. VanDerWal, W. E. Thogmartin, S. J. Vavrus, and P. J. Heglund. 2016. The pace of past climate change vs. potential bird distributions and land use in the United States. *Global change biology* 22:1130-1144.
- Beever, E. A., L. E. Hall, J. Varner, A. E. Loosen, J. B. Dunham, M. K. Gahl, F. A. Smith, and J. J. Lawler. 2017. Behavioral flexibility as a mechanism for coping with climate change. *Frontiers in Ecology and the Environment* 15:299-308.
- Bonamour, S., L.-M. Chevin, A. Charmantier, and C. Teplitsky. 2019. Phenotypic plasticity in response to climate change: the importance of cue variation. *Philosophical Transactions of the Royal Society B* 374:20180178.
- Boutin, S. and J. E. Lane. 2014. Climate change and mammals: evolutionary versus plastic responses. *Evolutionary Applications* 7:29-41.
- Brommer, J. E., K. Rattiste, and A. J. Wilson. 2008. Exploring plasticity in the wild: laying date-temperature reaction norms in the common gull *Larus canus*. *Proceedings of the Royal Society B: Biological Sciences* 275:687-693.
- Burham, K. and D. Anderson. 2002. Model selection and multimodel inference: A practical information-theoretic approach. Springer. New York.
- Chandler, C. R., E. D. Ketterson, V. Nolan Jr, and C. Ziegenfus. 1994. Effects of testosterone on spatial activity in free-ranging male dark-eyed juncos, *Junco hyemalis*. *Animal Behaviour* 47:1445-1455.
- Charmantier, A. and P. Gienapp. 2014. Climate change and timing of avian breeding and migration: evolutionary versus plastic changes. *Evolutionary Applications* 7:15-28.
- Charmantier, A., R. H. McCleery, L. R. Cole, C. Perrins, L. E. Kruuk, and B. C. Sheldon. 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. *science* 320:800-803.
- Chmura, H. E., J. C. Wingfield, and T. P. Hahn. 2020. Non-photoc environmental cues and avian reproduction in an era of global change. *Journal of Avian Biology* 51.
- Clotfelter, E. D., D. M. O'Neal, J. M. Gaudioso, J. M. Casto, I. M. Parker-Renga, E. A. Snajdr, D. L. Duffy, V. Nolan Jr, and E. D. Ketterson. 2004. Consequences of elevating plasma testosterone in females of a socially monogamous songbird: evidence of constraints on male evolution? *Hormones and behavior* 46:171-178.
- Cresswell, W. and R. McCleery. 2003. How great tits maintain synchronization of their hatch date with food supply in response to long-term variability in temperature. *Journal of Animal Ecology* 72:356-366.
- Crick, H. Q. 2004. The impact of climate change on birds. *Ibis* 146:48-56.
- Crozier, L. G. and J. A. Hutchings. 2014. Plastic and evolutionary responses to climate change in fish. *Evolutionary Applications* 7:68-87.
- de Villemereuil, P., A. Charmantier, D. Arlt, P. Bize, P. Brekke, L. Brouwer, A. Cockburn, S. D. Côté, F. S. Dobson, and S. R. Evans. 2020. Fluctuating optimum and temporally variable selection on breeding date in birds and mammals. *Proceedings of the National Academy of Sciences* 117:31969-31978.
- Dunn, P. O. and A. P. Møller. 2014. Changes in breeding phenology and population size of birds. *Journal of Animal Ecology*:729-739.
- Dunn, P. O. and D. W. Winkler. 2010. Effects of climate change on timing of breeding and reproductive success in birds. *Effects of climate change on birds*:113-128.

- Duputié, A., A. Rutschmann, O. Ronce, and I. Chuine. 2015. Phenological plasticity will not help all species adapt to climate change. *Global change biology* 21:3062-3073.
- Gavrilets, S. and S. M. Scheiner. 1993. The genetics of phenotypic plasticity. V. Evolution of reaction norm shape. *Journal of evolutionary biology* 6:31-48.
- Gerlach, N. M. 2010. Causes and consequences of extra-pair mating in the Dark-eyed Junco: Implications for female, male, and offspring fitness, sexual selection, and the sources of individual variation. Indiana University.
- Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional ecology* 21:394-407.
- Gienapp, P., S. Fior, F. Guillaume, J. R. Lasky, V. L. Sork, and K. Csilléry. 2017. Genomic quantitative genetics to study evolution in the wild. *Trends in Ecology & Evolution* 32:897-908.
- Gienapp, P., M. Lof, T. E. Reed, J. McNamara, S. Verhulst, and M. E. Visser. 2013. Predicting demographically sustainable rates of adaptation: can great tit breeding time keep pace with climate change? *Philosophical Transactions of the Royal Society B: Biological Sciences* 368:20120289.
- Graham, J. L., C. M. Bauer, B. J. Heidinger, E. D. Ketterson, and T. J. Greives. 2019. Early-breeding females experience greater telomere loss. *Molecular ecology* 28:114-126.
- Greives, T. J., A. M. Fudickar, J. W. Atwell, S. L. Meddle, and E. D. Ketterson. 2016. Early spring sex differences in luteinizing hormone response to gonadotropin releasing hormone in co-occurring resident and migrant dark-eyed juncos (*Junco hyemalis*). *General and comparative endocrinology* 236:17-23.
- Helm, B., B. M. Van Doren, D. Hoffmann, and U. Hoffmann. 2019. Evolutionary response to climate change in migratory pied flycatchers. *Current Biology* 29:3714-3719. e3714.
- Hoffmann, A. A. and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. *Nature* 470:479-485.
- Husby, A., D. H. Nussey, M. E. Visser, A. J. Wilson, B. C. Sheldon, and L. E. Kruuk. 2010. Contrasting patterns of phenotypic plasticity in reproductive traits in two great tit (*Parus major*) populations. *Evolution: International Journal of Organic Evolution* 64:2221-2237.
- Iler, A. M., P. J. CaraDonna, J. R. Forrest, and E. Post. 2021. Demographic consequences of phenological shifts in response to climate change. *Annual Review of Ecology, Evolution, and Systematics* 52:221-245.
- Ketterson, E., V. Nolan, and M. Sandell. 2005. Testosterone in females: mediator of adaptive traits, constraint on sexual dimorphism, or both? *the american naturalist* 166:S85-S98.
- Ketterson, E. D. and J. W. Atwell. 2016. *Snowbird: integrative biology and evolutionary diversity in the Junco*. University of Chicago Press.
- Kimmitt, A. A. 2020. Females as the gatekeepers to seasonal breeding: what we can learn by studying reproductive mechanisms in both sexes. *Integrative and Comparative Biology* 60:703-711.
- Kimmitt, A. A., J. W. Hardman, C. A. Stricker, and E. D. Ketterson. 2019. Migratory strategy explains differences in timing of female reproductive development in seasonally sympatric songbirds. *Functional Ecology* 33:1651-1662.
- Kimmitt, A. A., D. M. Sinkiewicz, and E. D. Ketterson. 2020. Seasonally sympatric songbirds that differ in migratory strategy also differ in neuroendocrine measures. *General and comparative endocrinology* 285:113250.

- Kimmit, Abigail et al. (2022), Plasticity in female timing may explain earlier breeding in a North American songbird, Dryad, Dataset, <https://doi.org/10.5061/dryad.4j0zpc8fd>
- Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. *The American Naturalist* 157:245-261.
- Lande, R. and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution*:1210-1226.
- Manhard, C. V., J. E. Joyce, and A. J. Gharrett. 2017. Evolution of phenology in a salmonid population: a potential adaptive response to climate change. *Canadian Journal of Fisheries and Aquatic Sciences* 74:1519-1527.
- Marrot, P., A. Charmantier, J. Blondel, and D. Garant. 2018. Current spring warming as a driver of selection on reproductive timing in a wild passerine. *Journal of Animal Ecology* 87:754-764.
- Marrot, P., D. Garant, and A. Charmantier. 2017. Multiple extreme climatic events strengthen selection for earlier breeding in a wild passerine. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372:20160372.
- McLean, N., C. R. Lawson, D. I. Leech, and M. van de Pol. 2016. Predicting when climate-driven phenotypic change affects population dynamics. *Ecology letters* 19:595-608.
- Merilä, J. 2012. Evolution in response to climate change: in pursuit of the missing evidence. *BioEssays* 34:811-818.
- Milá, B., J. E. McCormack, G. Castañeda, R. K. Wayne, and T. B. Smith. 2007. Recent postglacial range expansion drives the rapid diversification of a songbird lineage in the genus Junco. *Proceedings of the Royal Society B: Biological Sciences* 274:2653-2660.
- Miller-Rushing, A. J., T. T. Høye, D. W. Inouye, and E. Post. 2010. The effects of phenological mismatches on demography. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365:3177-3186.
- Møller, A., E. Flensted-Jensen, K. Klarborg, W. Mardal, and J. Nielsen. 2010. Climate change affects the duration of the reproductive season in birds. *Journal of Animal Ecology* 79:777-784.
- Nakagawa, S., P. C. Johnson, and H. Schielzeth. 2017. The coefficient of determination  $R^2$  and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of the Royal Society Interface* 14:20170213.
- Nolan, V., E. Ketterson, D. Cristol, C. Rogers, E. Clotfelter, R. Titus, S. Schoech, and E. Snajdr. 2002. Dark-eyed Junco (*Junco hyemalis*). In *The Birds of North America* 716 (A. Poole and F. Gill, Editors). Birds of North America, Philadelphia, PA, USA.
- Nussey, D., A. Wilson, and J. Brommer. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of evolutionary biology* 20:831-844.
- Nussey, D. H., E. Postma, P. Gienapp, and M. E. Visser. 2005. Selection on heritable phenotypic plasticity in a wild bird population. *Science* 310:304-306.
- Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421:37-42.
- Phillimore, A. B., D. I. Leech, J. W. Pearce-Higgins, and J. D. Hadfield. 2016. Passerines may be sufficiently plastic to track temperature-mediated shifts in optimum lay date. *Global change biology* 22:3259-3272.

- Piao, S., Q. Liu, A. Chen, I. A. Janssens, Y. Fu, J. Dai, L. Liu, X. Lian, M. Shen, and X. Zhu. 2019. Plant phenology and global climate change: Current progresses and challenges. *Global change biology* 25:1922-1940.
- Pinheiro, J. and D. Bates. 2006. *Mixed-effects models in S and S-PLUS*. Springer Science & Business Media.
- Pyle, P. 1997. *Identification guide to North American birds: a compendium of information on identifying, ageing, and sexing "near-passerines" and passerines in the hand*. Slate Creek Press.
- Radchuk, V., T. Reed, C. Teplitsky, M. Van De Pol, A. Charmantier, C. Hassall, P. Adamík, F. Adriaensen, M. P. Ahola, and P. Arcese. 2019. Adaptive responses of animals to climate change are most likely insufficient. *Nature communications* 10:1-14.
- Réale, D., A. G. McAdam, S. Boutin, and D. Berteaux. 2003. Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270:591-596.
- Saalfeld, S. T. and R. B. Lanctot. 2017. Multispecies comparisons of adaptability to climate change: A role for life-history characteristics? *Ecology and Evolution* 7:10492-10502.
- Sauve, D., V. L. Friesen, and A. Charmantier. 2021. The effects of weather on avian growth and implications for adaptation to climate change. *Frontiers in Ecology and Evolution* 9:5.
- Scheffers, B. R., L. De Meester, T. C. Bridge, A. A. Hoffmann, J. M. Pandolfi, R. T. Corlett, S. H. Butchart, P. Pearce-Kelly, K. M. Kovacs, and D. Dudgeon. 2016. The broad footprint of climate change from genes to biomes to people. *Science* 354.
- Sih, A., J. Stamps, L. H. Yang, R. McElreath, and M. Ramenofsky. 2010. Behavior as a key component of integrative biology in a human-altered world. *Integrative and comparative biology* 50:934-944.
- Taylor, C. M., V. Devictor, P. Gaiüzère, N. Jonzén, H. G. Smith, and Å. Lindström. 2016. Regional variation in climate change winners and losers highlights the rapid loss of cold-dwelling species. *Diversity and Distributions* 22:468-480.
- Van Asch, M., L. Salis, L. J. Holleman, B. Van Lith, and M. E. Visser. 2013. Evolutionary response of the egg hatching date of a herbivorous insect under climate change. *Nature Climate Change* 3:244-248.
- Van Buskirk, J., U. Candolin, and B. Wong. 2012. Behavioural plasticity and environmental change.
- van de Pol, M., L. D. Bailey, N. McLean, L. Rijdsdijk, C. R. Lawson, and L. Brouwer. 2016. Identifying the best climatic predictors in ecology and evolution. *Methods in Ecology and Evolution* 7:1246-1257.
- Verhagen, I., B. M. Tomotani, P. Gienapp, and M. E. Visser. 2020. Temperature has a causal and plastic effect on timing of breeding in a small songbird. *Journal of Experimental Biology* 223.
- Visser, M. E., L. J. Holleman, and P. Gienapp. 2006. Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird. *Oecologia* 147:164-172.
- Watts, H. E., B. Edley, and T. P. Hahn. 2016. A potential mate influences reproductive development in female, but not male, pine siskins. *Hormones and behavior* 80:39-46.
- Williams, T. D. 2012. *Physiological adaptations for breeding in birds*. Princeton University Press.

Wingfield, J. C., N. Perfito, R. Calisi, G. Bentley, T. Ubuka, M. Mukai, S. O'Brien, and K. Tsutsui. 2016. Putting the brakes on reproduction: implications for conservation, global climate change and biomedicine. *General and comparative endocrinology* 227:16-26.

## Figures and Tables

Table 1. Random effect estimates from RRM testing for temperature-driven plasticity in first egg date for repeatedly sampled females ( $n = 62$  individuals). Each RRM was fitted with REML and used the top temperature window from the climate window analyses of first egg date (see Figure 1 and Tables S2–4). Test statistics and p values were derived from sequential LRTs.

Term	$T_{\min}$			$T_{\text{mid}}$			$T_{\max}$		
	$\sigma^2$	LRT	p	$\sigma^2$	LRT	p	$\sigma^2$	LRT	p
Female ID	0	2.19	0.14	5.52	2.91	0.09	22.9	4.09	0.04
Temperature female ID	1.88	3.80	0.15	0.59	0.97	0.62	0.001	0.02	0.99
Year	2.46	0.31	0.58	3.39	0.10	0.75	5.54	0.19	0.66
Residual	121.1			127.4			128.4		
R2m	0.04			0.04			0.02		
R2c	0.22			0.18			0.17		

Figure 1. Independent relationships between year and monthly average minimum ( $T_{\min}$ ), midpoint ( $T_{\text{mid}}$ ), and maximum ( $T_{\max}$ ) temperature in March-August from 1983 to 2015. All prediction lines and confidence bands from the GAMs are created as a function of year as a smooth term and overlaid with original data. Solid lines indicate significant change over time, whereas dashed lines indicate no significant change over time.

Figure 2. (A) Average first egg date of females is shown as a function of year only (B-D) Average first egg date is shown as a function of each average temperature identified from the sliding

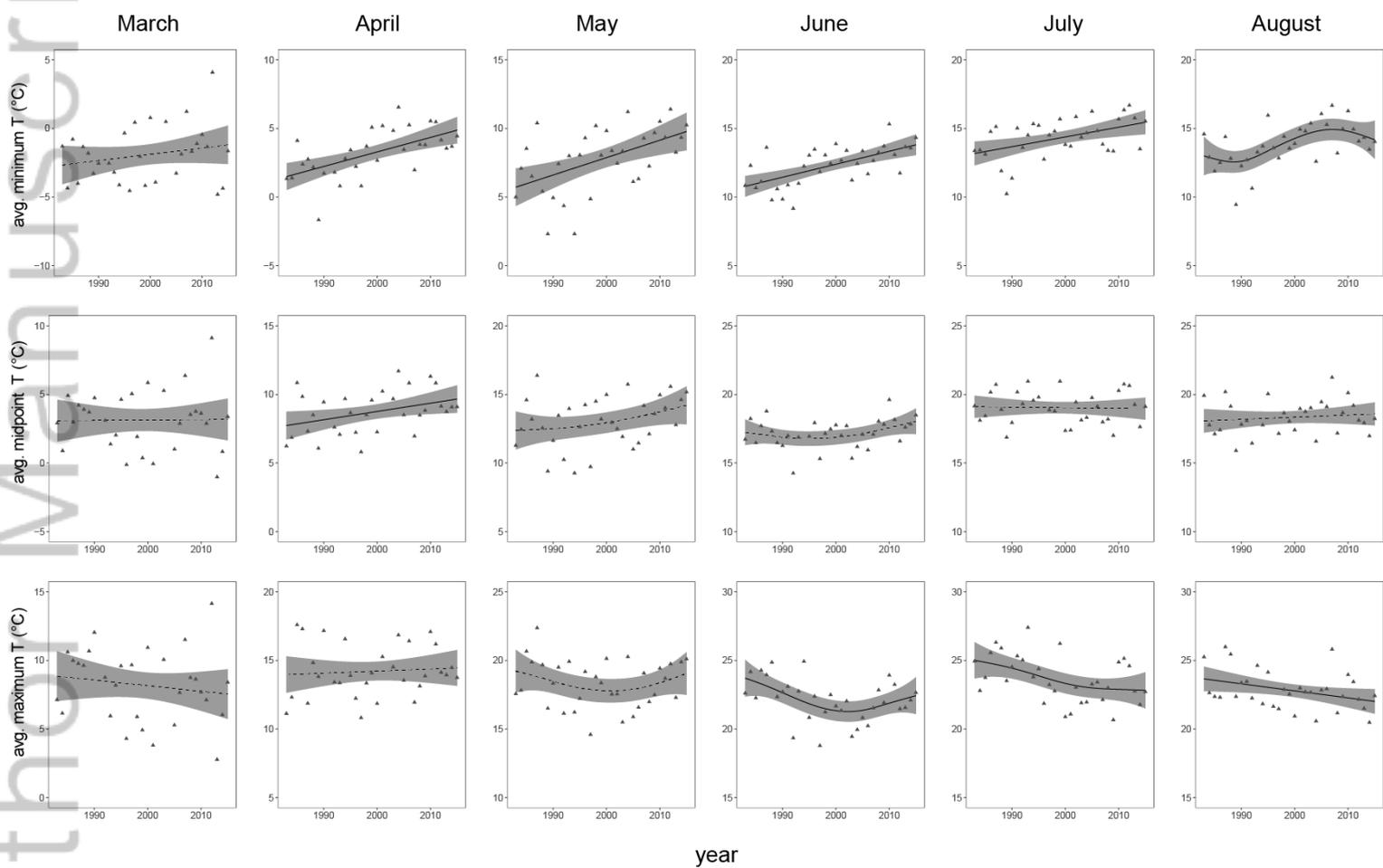
window analyses ( $T_{\min}$ ,  $T_{\text{mid}}$ ,  $T_{\max}$ ) using LMs that account for year. Fitted values and 95% confidence bands from the LMs are overlaid with original data.

Figure 3. Fitted values of RRM testing for temperature-driven plasticity in first egg date. The thick line and grey band show the overall reaction norm for each temperature measure after controlling for female age and associated 95% confidence interval, respectively. Thin lines show fitted reaction norms for each individual (holding each female at the ASY class).

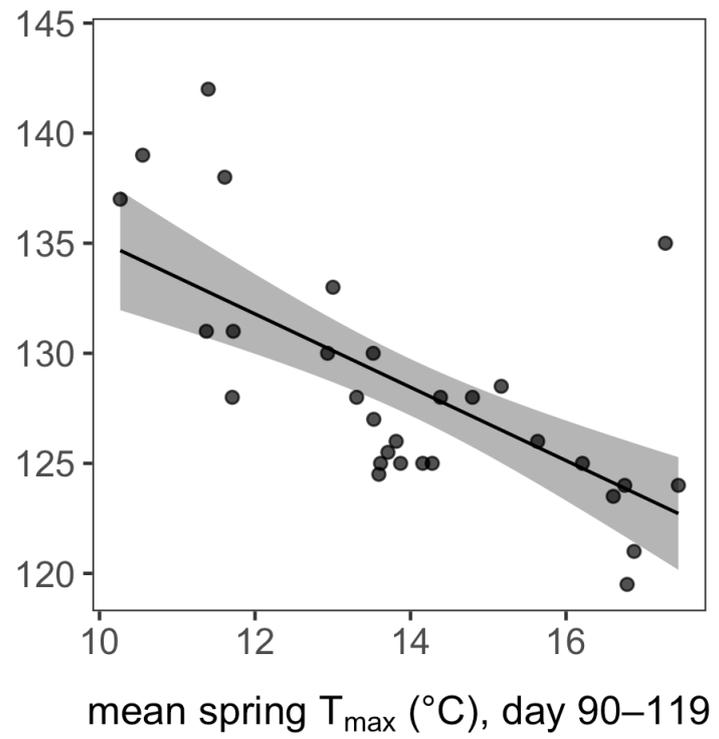
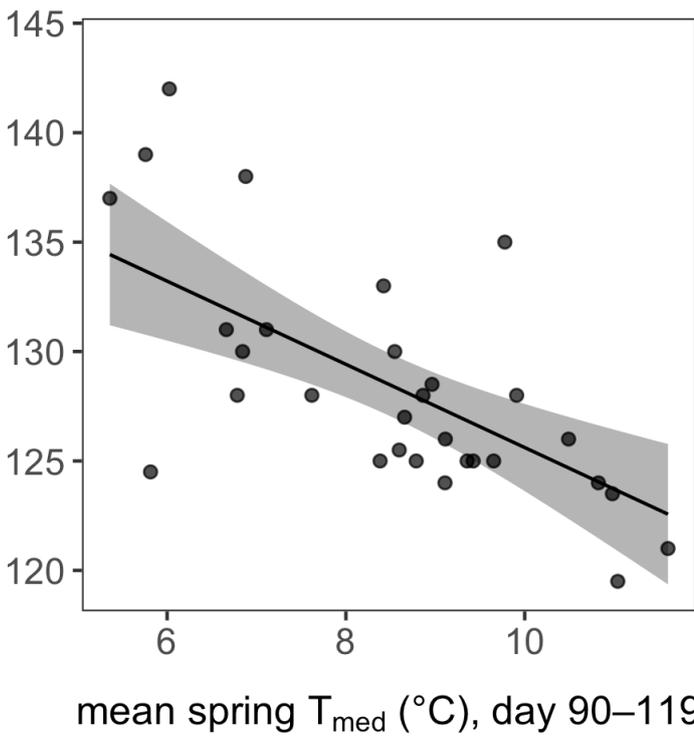
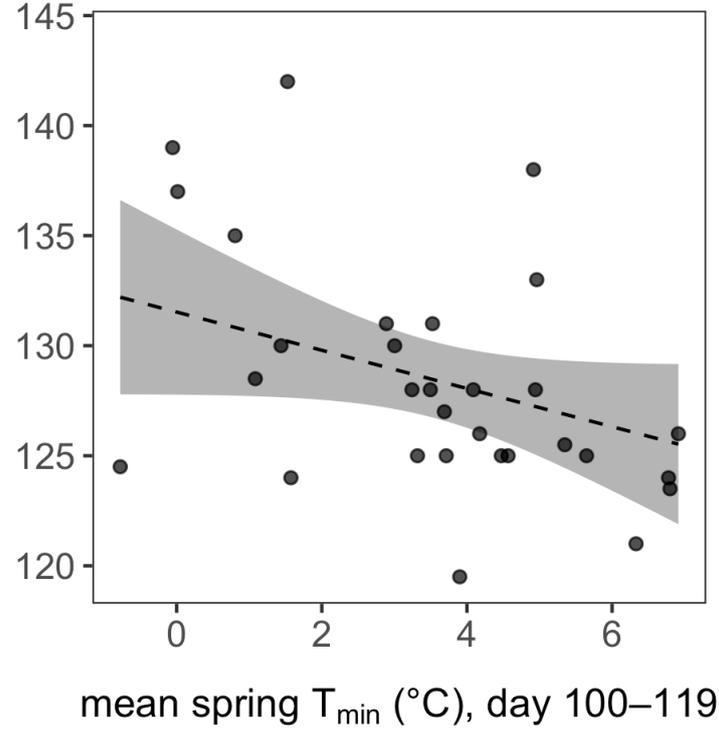
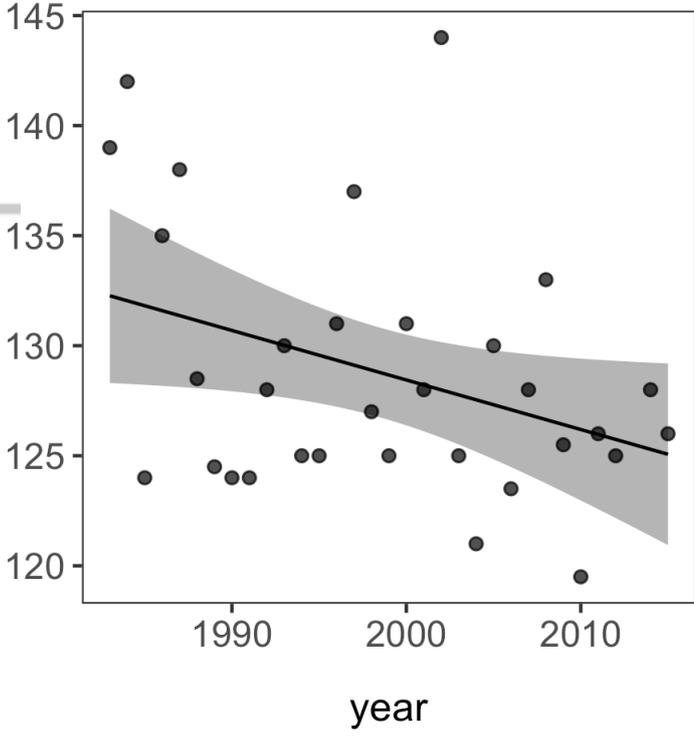
Figure 4. (A) Results of the GLMM predicting relative fitness as a function of first egg date after adjusting for annual total number of eggs per female and female age. Points display individual nest data. The solid line and grey band show fitted values and 95% confidence intervals (derived with a year-stratified bootstrap procedure using 100 replicates). Relative fitness is shown with a modulus transformation given the right-skew. (B) Fitted values for an equivalent GLMM with an interaction between first egg date and time, with annual slopes shown coloured by year.

Figure 5. Estimated coefficient ( $\beta$ ) and 95% confidence interval for the interaction between first egg date and mean temperature from GLMMs across all considered climate window start dates (ordinal date) and durations. Results are coloured by  $\Delta\text{AICc}$  from candidate GLMMs, and the dashed line shows  $\beta=0$ , representing neutral temperature-dependent selection on breeding phenology. Sliding window analyses were performed for daily  $T_{\min}$ ,  $T_{\text{mid}}$ , and  $T_{\max}$ .

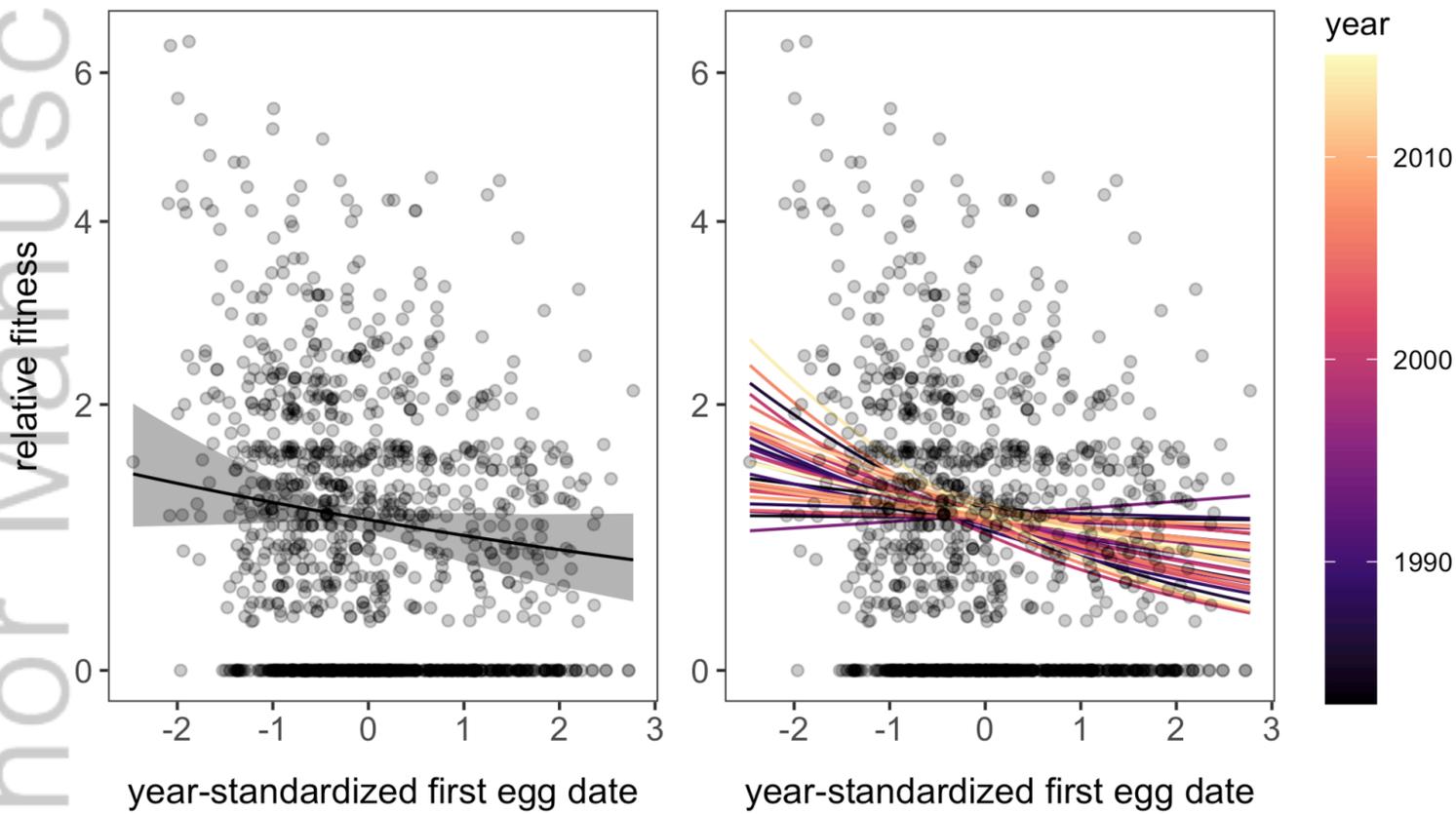
Manuscript  
Author



JANE\_13772\_Fig1\_new.png

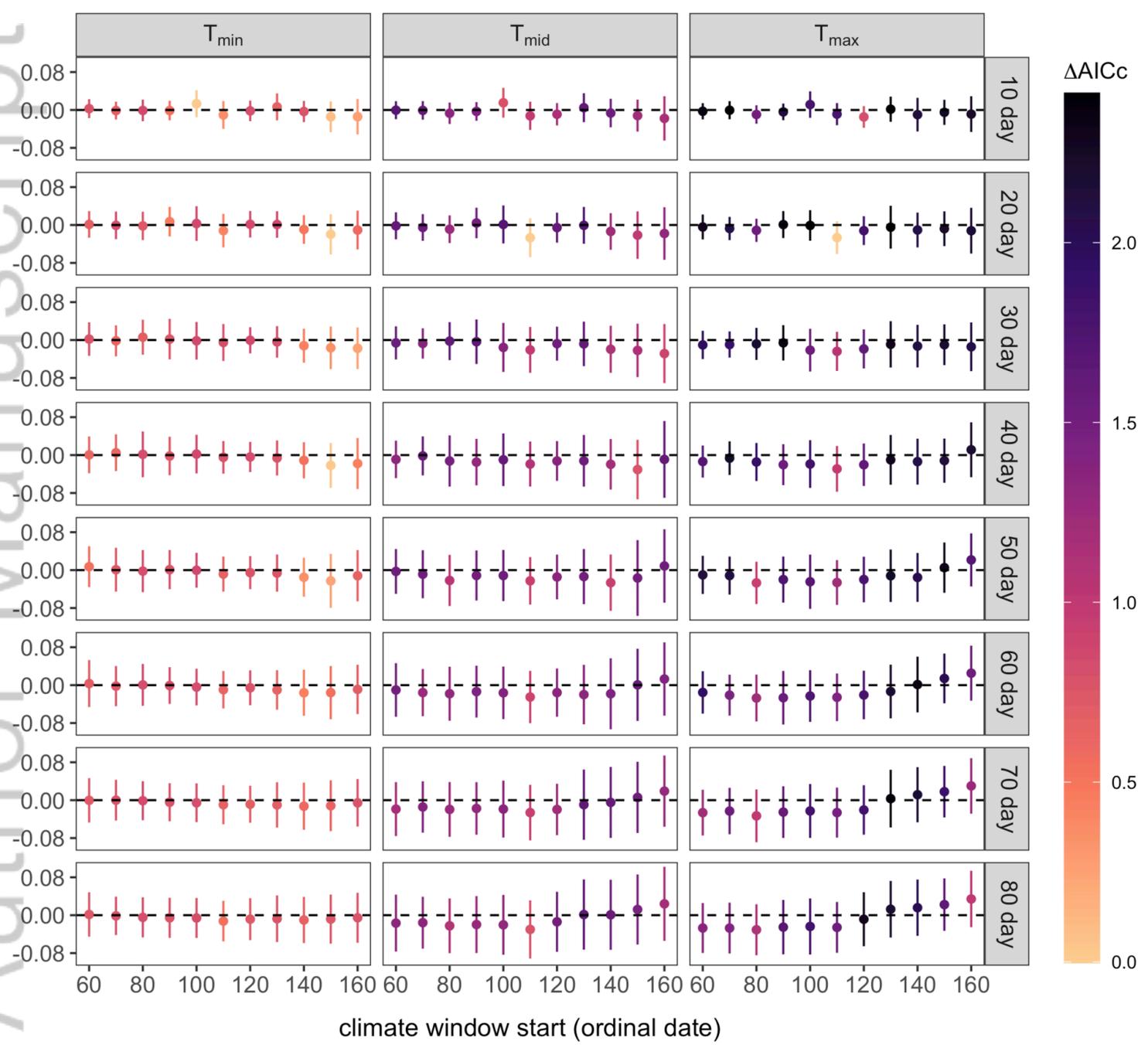


JANE\_13772\_Figure 2.png

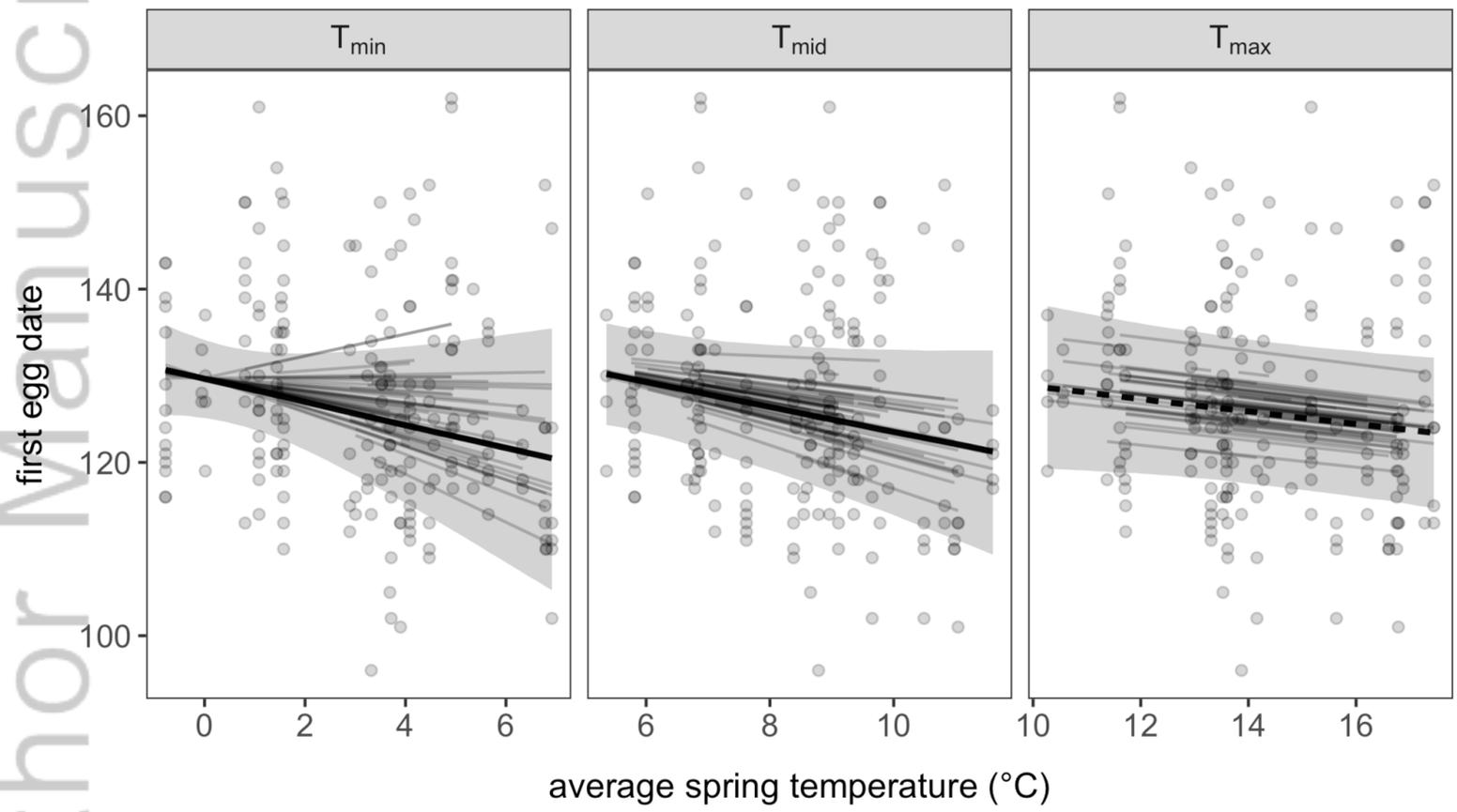


JANE\_13772\_Figure 4.png

$\beta$  and 95% CI for first egg date \* mean temperature



JANE\_13772\_Figure 5.png



JANE\_13772\_Figure\_3.png

1 **Plasticity in female timing may explain current shifts in breeding phenology of a North**  
2 **American songbird**

3

4

5 **Abigail A. Kimmitt<sup>1,2\*</sup>, Daniel J. Becker<sup>3,4</sup>, Sara N. Diller<sup>1,5</sup>, Nicole M. Gerlach<sup>6</sup>, Kimberly**  
6 **A. Rosvall<sup>1</sup>, Ellen D. Ketterson<sup>1,3</sup>**

7

8 <sup>1</sup> Department of Biology, Indiana University, 1001 E. Third St., Bloomington, Indiana 47405

9 <sup>2</sup> Department of Ecology and Evolutionary Biology, University of Michigan, 1105 North  
10 University Ave, Ann Arbor, MI 48109

11 <sup>3</sup> Environmental Resilience Institute, Indiana University, 717 E. Eighth St., Bloomington, Indiana  
12 47408

13 <sup>4</sup> Department of Biology, University of Oklahoma, 730 Van Vleet Oval, Norman, OK 73019

14 <sup>5</sup> Department of Biological Sciences, Western Michigan University, Kalamazoo, MI 49008

15 <sup>6</sup> Department of Biology, University of Florida, P.O. Box 118525, Gainesville, FL 32611

16 \* Corresponding author: [akimmitt@umich.edu](mailto:akimmitt@umich.edu)

17

18

19 **Keywords:** climate change; timing of breeding; selection; phenotypic plasticity; phenological  
20 shifts; bird

Author Manuscript

21 **Abstract**

- 22 1. Climate change has driven changes in breeding phenology. Identifying the magnitude of  
23 phenological shifts and whether selection in response to climate change drives these shifts  
24 is key for determining species' reproductive success and persistence in a changing world.
- 25 2. We investigated reproductive timing in a primarily sedentary population of the dark-eyed  
26 junco (*Junco hyemalis*) over 32 years. We predicted that juncos would breed earlier in  
27 warmer springs in response to selection favouring earlier breeding.
- 28 3. To test this prediction, we compared the annual median date for reproductive onset (i.e.,  
29 egg one date) to monthly spring temperatures and examined evidence for selection  
30 favouring earlier breeding and for plasticity in timing.
- 31 4. Egg one dates occurred earlier over time, with the timing of breeding advancing up to 24  
32 days over the 32-year period. Breeding timing also strongly covaried with maximum April  
33 temperature. We found significant overall selection favouring earlier breeding (i.e., higher  
34 relative fitness with earlier egg one dates) that became stronger over time, but strength of  
35 selection was not predicted by temperature. Lastly, individual females exhibited plastic  
36 responses to temperature across years.
- 37 5. Our findings provide further evidence that phenotypic plasticity plays a crucial role in  
38 driving phenological shifts in response to climate change. For multi-brooded bird  
39 populations, a warming climate might extend the breeding season and provide more  
40 opportunities to re-nest rather than drive earlier breeding in response to potential  
41 phenological mismatches. However, as plasticity will likely be insufficient for long-term  
42 survival in the face of climate change, further research in understanding the mechanisms

43 of female reproductive timing will be essential for forecasting the effects of climate change  
44 on population persistence.

## 45 **1. Introduction**

46 Climate change is greatly affecting plant and animal life (Root et al. 2003; Scheffers et al.  
47 2016; Staudinger et al. 2013). Phenological shifts are common (Piao et al. 2019; Scheffers et al.  
48 2016), suggesting that many species can adjust to climate change (Charmantier and Gienapp  
49 2014; Saalfeld and Lanctot 2017). Identifying the magnitude of phenological shifts and their  
50 selective drivers in response to climate change are key for conservation efforts (Charmantier &  
51 Gienapp 2014). Numerous studies have investigated phenological shifts in passerine birds in the  
52 last two decades; however, the number of long-term datasets for unique species that can account  
53 for breeding timing as well as reproductive success is limited (i.e., <10 passerine species).  
54 Additionally, these species greatly vary in life history (e.g., migratory strategy [migrant vs.  
55 resident], breeding duration [single- vs. multi-brooded], nesting strategy [cavity vs. open cup  
56 nesting], diet, and habitat) and geography, all of which could directly affect selection pressures  
57 on breeding phenology (Dunn & Møller 2014). Investigating the potential drivers of phenological  
58 shifts in additional species with distinctive life histories will allow for more accurate predictions  
59 of which populations will be able to adapt to the changing climate. To date, many studies  
60 investigating phenological shifts in birds have been focused on European species, with some work  
61 in North America species that are predominantly migratory, but see (Wilson *et al.* 2007; Watts *et*  
62 *al.* 2019). In this study, we contribute to this growing body of literature by analysing a long-term  
63 data set of the breeding efforts of a North American, resident songbird population.

64 The relative role of microevolutionary change versus behavioural plasticity in driving  
65 phenological shifts remains under debate (Charmantier & Gienapp 2014), as it likely varies across  
66 species in relation to their life history and their ability to adapt to climate change. Climate change  
67 could drive directional selection favouring earlier breeding in birds by influencing phenology in

68 related trophic levels (e.g., prey, competitors), thus affecting offspring survival (Charmantier &  
69 Gienapp 2014). Evolutionary adaptation can then occur if directional selection favouring earlier  
70 breeding acts on heritable traits with genetic variation (Hoffmann & Sgrò 2011). Phenological  
71 changes may also reflect behavioural plasticity, or the ability of an individual to modify its  
72 behaviour based on the environment (Sih *et al.* 2010; Van Buskirk, Mulvihill & Leberman 2012;  
73 Beever *et al.* 2017). Behavioural plasticity and its underlying mechanisms can allow individuals  
74 to respond more quickly to a changing climate, as compared to microevolutionary change in  
75 response to selection (Sih *et al.* 2010; Charmantier & Gienapp 2014; Beever *et al.* 2017).  
76 However, plasticity is not always adaptive (Duputié *et al.* 2015) and is unlikely to be sufficient  
77 to allow populations to respond long-term to climate change (Ghalambor *et al.* 2007; Gienapp *et*  
78 *al.* 2013).

79 Here, we used long-term data collected from Dark-eyed Juncos (*Junco hyemalis*), a north-  
80 temperate sparrow found in Canada and the United States, to investigate changes in their breeding  
81 phenology. Juncos serve as a model songbird species for studies of ecology and evolution  
82 (Ketterson & Atwell 2016). Specifically, we focused on a breeding population of Carolina Dark-  
83 eyed Juncos (*J. h. carolinensis*) that resides in the Appalachian Mountains year-round, with some  
84 individuals migrating short distances (e.g., altitudinal migrants). We first asked whether median  
85 monthly air temperature in early spring changed over the 32-year study, predicting that spring  
86 temperatures would increase over time. We next compared annual average egg one dates (i.e.,  
87 initiation of breeding, or the date of first egg laid in the year) to spring temperatures over time and  
88 predicted that egg one dates would be earlier over time in response to a warming climate. We also  
89 asked whether selection acted on earlier breeding by assessing the relationship between female  
90 annual relative fitness and egg one date. We then used a model comparison framework to identify

91 climatic drivers of the strength of selection across our study period. We predicted that selection  
92 would favour earlier breeding, especially in warmer springs. Lastly, we used a random regression  
93 model approach to evaluate the degree of female plasticity in response to spring temperatures. We  
94 predicted that individuals that bred in multiple years would vary their initiation of breeding in  
95 response to changing spring temperatures proportional to annual differences in temperature.

96

## 97 **2. Methods**

### 98 **a) Study system and breeding data**

99 Since 1983, a breeding population of Dark-eyed Juncos has been monitored at Mountain  
100 Lake Biological Station (MLBS) and the surrounding Jefferson National Forest (37°22'N,  
101 80°32'W) (Chandler *et al.* 1994). At the beginning of each breeding season (April-May), birds  
102 on the study site were caught using mist nets and Potter traps and banded with a unique USFWS  
103 metal band and distinctive combinations of colour bands. Researchers searched for nests every  
104 year, identifying parents and tracking the progress of the nest. Egg one date, expressed as Julian  
105 date, was observed directly, or for nests found after the start of egg-laying, was calculated based  
106 on the day nestlings hatched or left the nest (Nolan *et al.* 2002). Breeding data from 1983–2015  
107 were used for this study except for 2013 due to limited research effort. Records where female ID  
108 or egg one date were unknown were removed. Female subjects that were implanted with  
109 exogenous testosterone during a five-year study were (Clotfelter *et al.* 2004; Ketterson, Nolan  
110 & Sandell 2005) were also removed.

111 To calculate true egg one dates, we excluded any known re-nests. Also, knowing that the  
112 first nest found for a female might not be her true first nest, we eliminated nests whose egg one  
113 dates came later than each year's median egg one date from known re-nests. Our data filtering

114 resulted in 1,244 first nests of 936 female juncos between 1983 and 2015. Annual differences  
115 in research effort (number of nests found) did not explain variation in egg one dates (see  
116 Supplementary Materials; Fig. S1).

117 Because the distributions of egg one dates were not normal in some years, we calculated  
118 median annual egg one dates from first nests. Using both first nests and renests for each year,  
119 we calculated the annual total number of eggs and total number of fledglings produced by each  
120 female. Females were grouped into two age classes based on plumage (Pyle 1997) or records  
121 from previous breeding seasons: second years (SY; first breeding season) and after second years  
122 (ASY; second or later breeding season). Finally, since most open-cup nests fail due to predation  
123 (Ricklefs 1969), we estimated annual predation rates of nests by calculating the annual  
124 percentage of nests that failed at the egg or nestling stage before fledging.

125

## 126 **b) Temperature data**

127 Between November 16, 1971 and January 31, 1998, temperature data (daily minimum;  $T_{\min}$   
128 and maximum temperature;  $T_{\max}$ ) were collected from MLBS via a National Oceanic and  
129 Atmospheric Administration (NOAA) weather station (Network ID GHCND: USC00445828,  
130 hereafter, “Logger A”). On June 24, 1994, a second data logger (Campbell CR10) was established  
131 at MLBS that records temperature every half hour. To permit comparing data across devices, we  
132 calculated daily  $T_{\max}$  and  $T_{\min}$  from this MLBS data logger (hereafter, “Logger B”). From  $T_{\min}$   
133 and  $T_{\max}$ , we calculated daily median temperature ( $T_{\text{med}}$ ) for both loggers. Since the two weather  
134 stations overlapped from 1994-1997, we confirmed that Datasets A and B were strongly  
135 correlated and then combined the datasets (*see Supplementary Materials*).

136 Monthly average  $T_{\min}$ ,  $T_{\max}$ , and  $T_{\text{med}}$  were calculated for March–May for each year. Data  
137 were available for all years (1983–2015), except for missing March and April data for 1991 and  
138 2002 and missing March data for 2004.

139

### 140 **c) Temporal patterns**

141 All statistical analyses were conducted in R (version 4.0.0). Temperature is not expected  
142 to exclusively change linearly over time, so we first fit generalized additive models (GAMs) with  
143 a smooth term for year to flexibly determine temporal trends in average  $T_{\min}$ ,  $T_{\max}$ , and  $T_{\text{med}}$  during  
144 spring (March–May) when birds were initiating breeding.

145 We fit a GAM with a smooth term for year to first investigate change over time in median  
146 egg one date. Most female juncos lay their first egg in late April–early May, and the final stages of  
147 reproductive development can take anywhere from days to weeks (Williams 2012), such that  
148 temperatures prior to laying likely have the greatest influence on female reproductive timing.  
149 Therefore, to determine how annual spring temperatures and temporal variation related to median  
150 annual egg one date, we fit independent GAMs with smooth terms for both year and each of 9  
151 temperature variables (average  $T_{\text{med}}$ ,  $T_{\min}$ , and  $T_{\max}$  for March, April, and May). We then compared  
152 model fit using Akaike information criterion corrected for small sample size (AICc) and Akaike  
153 weight ( $w_i$ ; Burham & Anderson 2002). We considered models within two  $\Delta\text{AICc}$  of the top model  
154 to be competitive. All GAMs were fit with the *mgcv* package using a Gaussian distribution and  
155 thin plate splines (Wood 2017). We used maximum likelihood (ML) for model selection but refit  
156 all final GAMs using restricted ML (REML). We used the most competitive temperature  
157 covariates in our subsequent selection and plasticity analyses.

158

159 **d) Selection analyses**

160 Selection acting on start of breeding was defined as the slope of a regression of relative  
161 fitness (i.e., total number of fledglings per year per female divided by annual population mean  
162 total fledglings) on egg one date (Lande & Arnold 1983). We adjusted for age, annual total eggs  
163 per female, and predation rate when estimating selection acting on egg one date by including these  
164 as fixed effects (Marrot, Garant & Charmantier 2017). As relative fitness was zero inflated, we  
165 used compound Poisson generalized linear models (GLMs) or generalized linear mixed models  
166 (GLMMs) with the *cplm* package (Zhang 2013); however, we also estimated the linear selection  
167 gradient using a conventional LMM (Lande & Arnold 1983). We ran analyses on egg one dates  
168 standardized annually (zero mean and unit variance) to control for environmental covariance  
169 between fitness and this trait across years (Kingsolver *et al.* 2001; Marrot *et al.* 2018). Owing to  
170 missing values, these fitness analyses included 1,182 first nests from 898 females. We included  
171 year and female ID as random intercepts to control for multiple observations per year and females  
172 that bred more than one year.

173 We ran selection analyses and model comparisons using two approaches. First, we derived  
174 selection gradients on a per-year basis using GLMs. We then compared univariate linear  
175 regressions with each of our primary temperature variables identified from our GAM analyses  
176 using AICc and Akaike weights (Burham & Anderson 2002). To account for uncertainty in  
177 estimates of selection gradients, these models included weighting by the annual sample size, ( $\bar{x}=37$   
178 first nests  $\pm 2$  SE). Next, we tested for relationships between temperature and the strength of  
179 selection on egg one date at the individual level using GLMMs fit to our full dataset. We compared  
180 variants of our base selection GLMM with an interaction between egg one date and each  
181 temperature variable, again using AICc and Akaike weights. We derived a marginal and

182 conditional  $R^2$  as measures of fit ( $R^2_m$  and  $R^2_c$ ; (Nakagawa, Johnson & Schielzeth 2017). Owing to  
183 missing temperature data, we used a reduced dataset of 1,123 first nets from 871 females to  
184 compare GLMMs.

185

### 186 e) Behavioural plasticity

187 To assess the degree of individual female plasticity in egg one date in relation to spring  
188 temperature, we used a random regression model (RRM) approach (Nussey, Wilson & Brommer  
189 2007). RRM are a particular case of GLMMs where individuals vary in the elevation (i.e.,  
190 intercept) and slope of their reaction norms. For females that bred in at least three years of our  
191 study ( $n=62$  individuals representing 206 egg one date observations, hereafter “returning  
192 females”), we fit a RRM with a fixed effect of temperature, a random intercept of female ID, and  
193 their interaction (i.e., a random slope) using ML with the *lme4* package (Pinheiro & Bates 2006).  
194 We again included age, annual total eggs per female, and predation rate as covariates and included  
195 an additional random intercept for year. We again compared among temperature predictors using  
196 AICc and Akaike weights and refit RMMs with REML to derive  $R^2_m$  and  $R^2_c$  and estimate variance  
197 for random effects. We then used sequential likelihood ratio tests to assess if random intercepts  
198 and slopes were significantly different from zero (i.e., denoting significant inter-individual  
199 variation in reaction norms) (Nussey *et al.* 2005). To visualize individual female slopes, we held  
200 each female at the ASY age class at its mean annual clutch size and annual predation rate.

201

## 202 3. Results

### 203 a) Spring temperatures

204 March average  $T_{\text{med}}$ ,  $T_{\text{min}}$ , and  $T_{\text{max}}$  did not significantly change between 1983 and 2015 at  
205 MLBS. April average  $T_{\text{med}}$  and  $T_{\text{min}}$ , but not  $T_{\text{max}}$ , significantly increased over time. Finally,  
206 average May  $T_{\text{min}}$  significantly increased over time, but  $T_{\text{med}}$  and  $T_{\text{max}}$  did not (Table S1; Fig. 1).

207

## 208 **b) Timing of reproductive onset**

209 Median egg one date varied significantly over time ( $F_{1,1} = 4.43$ ,  $p = 0.044$ ,  $R^2 = 0.10$ ), with  
210 a 13-day difference from the first year (May 19, 1983) to the final year (May 6, 2015; Fig. 2A).  
211 Considering the largest difference in breeding phenology over the 32 years, females advanced egg  
212 one dates up to 24 days over the study (range=April 30-May 24). When testing effects of monthly  
213 temperatures after accounting for nonlinear effects of year, the best GAM included April average  
214  $T_{\text{max}}$  ( $w_i = 0.97$ ; Table S2). In this model ( $R^2 = 0.65$ ), egg one date was predicted by the average  
215 April  $T_{\text{max}}$  ( $F_{1.7, 2.1} = 14.84$ ,  $p < 0.0001$ ) and year ( $F_{2.4, 3.0} = 5.28$ ,  $p = 0.007$ ) (Fig. 2B). Since an  
216 April temperature was the best predictor of lay date, we proceeded using only the three April  
217 temperature variables for selection and plasticity analyses.

218

## 219 **c) Selection analyses**

220 We observed strong selection on egg one date, and the gradient from our full dataset  
221 GLMM was significantly negative (i.e., selection favouring earlier breeding;  $\beta = -0.16$ ,  $t = -4.42$ ,  
222  $p < 0.001$ ). This estimate was identical to the selection gradient from an analogous LMM ( $\beta = -0.16$ ,  
223  $t = -4.91$ ,  $p < 0.001$ ). Annual total eggs per female was under positive selection, in which individuals  
224 that produced more eggs also had more successful fledglings ( $\beta = 0.05$ ,  $t = 5.28$ ,  $p < 0.001$ ). Older  
225 females had marginally higher relative fitness ( $\beta = 0.12$ ,  $t = 1.89$ ,  $p = 0.06$ ). Annual nest predation  
226 rates did not predict relative fitness ( $\beta = -0.09$ ,  $t = -0.38$ ,  $p = 0.71$ ). The overall (GLMM) selection

227 gradient was similar to the mean of the per-year estimates ( $\bar{x}=-0.18 \pm 0.03$  SE). Annual selection  
228 gradients showed strong inter-year variation ( $\sigma^2=0.04$ ) and became significantly more negative  
229 (i.e., more strongly favouring earlier breeding) with time (Fig. 3;  $\beta=-0.01$ ,  $p=0.04$ ,  $R^2=0.11$ ).

230 When comparing temperature-dependent models of selection on egg one date, all three  
231 April temperature measures received equivalent support (Table S3). When analysing selection  
232 gradients directly, models including average April  $T_{\max}$  and  $T_{\min}$  received the most support from  
233 AICc ( $w_i=0.36$  and  $0.35$ ), but warmer temperatures were nevertheless not associated with selection  
234 gradients ( $T_{\max}$ :  $\beta=0.01$ ,  $p=0.55$ ,  $R^2=0$ ; Fig. 4A;  $T_{\min}$ :  $\beta=-0.01$ ,  $p=0.59$ ,  $R^2=0$ ). Individual-level  
235 GLMMs with interactions between egg one date and temperature did not differentiate between  
236 April temperature predictors ( $w_i=0.32-0.35$ ; Table S3). We similarly found no significant  
237 interaction between egg one date and April temperature (e.g.,  $T_{\max}$ :  $\beta=-0.0004$ ,  $t=-0.02$ ,  $p=0.98$ ;  
238 Fig. 4B). Thus, in both analyses, warmer April temperatures were not associated with selection  
239 favouring earlier breeding.

240

#### 241 **d) Behavioural plasticity**

242 We used RMMs to assess phenotypic plasticity in the relationship between timing of  
243 breeding and temperature. For 62 females studied across at least three years, RMMs found most  
244 support for an association between egg one date and April average  $T_{\min}$  ( $w_i=0.60$ ) and  $T_{\text{med}}$   
245 ( $w_i=0.36$ ,  $\Delta\text{AICc}=1.04$ ), but not with  $T_{\max}$  ( $w_i=0.04$ ,  $\Delta\text{AICc}=5.33$ ; Table S4). Individuals started  
246 breeding significantly earlier with warmer April average  $T_{\min}$  ( $\beta=-1.33$ ,  $t=-2.50$ ,  $p=0.02$ ) and  $T_{\text{med}}$   
247 ( $\beta=-1.67$ ,  $t=-2.70$ ,  $p=0.01$ ). Importantly, individuals did not vary in their elevation (i.e., estimated  
248 egg one date at the average temperature) nor their slope (i.e., individual response to inter-year  
249 variation in temperature) for either competitive temperature measure (Fig. 5; Table S5). Therefore,

250 females displayed significant population-level phenotypic plasticity, but not inter-individual  
251 variation in plasticity.

252

#### 253 **4. Discussion**

254 We investigated shifts in phenology over a 32-year period and in relation to spring  
255 temperatures and found a net change of 12 days in egg one date and a maximum between-year  
256 advance of 24 days. Springs have grown warmer, and females are initiating reproduction earlier  
257 than in the past. We also found evidence of selection favouring earlier breeding that has also  
258 become stronger over time. However, unlike studies of other avian species [25, 30], spring  
259 temperatures did not predict strength of selection on egg one dates, suggesting that other factors  
260 may be driving shifts in phenology. Among the many possibilities are some combination of abiotic  
261 factors (Dunn & Winkler 2010) or even advances in male reproductive stimulating earlier egg-  
262 laying in females (Watts, Edley & Hahn 2016). It is also possible that females respond to a  
263 temperature threshold in the spring to initiate laying, explaining why our temperature measures  
264 were not related on selection on timing of breeding.

265 We found strong evidence for plasticity in driving these phenological changes. For a subset  
266 of returning females with sufficient multi-year data, females bred earlier in the warmer of the three  
267 or more springs in which they bred. However, females exhibited very little inter-individual  
268 variation in the degree of plasticity, leaving little variation on which selection might act. While  
269 selection and plasticity both likely played a role in earlier breeding associated with warmer springs,  
270 the pattern may be more a result of plasticity than selection.

271

272 **a) Plastic versus evolutionary responses to climate change**

273           Microevolutionary responses to climate change are predicted to result from directional  
274 selection favouring earlier breeding to alleviate the negative effects of phenological mismatches  
275 (Charmantier & Gienapp 2014). Without knowledge of the heritability of timing of reproduction,  
276 however, evidence of selection favouring earlier breeding is insufficient to conclude that  
277 microevolutionary change is occurring, as genetic and environmental effects can be difficult to  
278 disentangle (Merilä 2012; Helm *et al.* 2019). There is some evidence of microevolutionary changes  
279 in phenology across taxa that are likely adaptive shifts in response to climate change (Van Asch *et*  
280 *al.* 2013; Manhard, Joyce & Gharrett 2017). In the case of the junco, while we found strong overall  
281 selection favouring earlier breeding, and this selection has become stronger over time, the strength  
282 of selection was not associated with the observed changes in spring temperature. We note that we  
283 did not measure genetic variation or heritability of reproductive timing. Therefore, we cannot  
284 conclude whether microevolutionary change might account for the observed shifts in timing.  
285 Future work should integrate genomic quantitative genetics approaches with this breeding data to  
286 determine the role of microevolution in phenological shifts, which could in turn have important  
287 implications for conservation genomics (Gienapp *et al.* 2017).

288           Additionally, behavioural plasticity, which can allow for more rapid changes in phenotype  
289 than microevolutionary changes, may account for why earlier breeding was related to increases in  
290 fitness over time (Van Buskirk, Candolin & Wong 2012; Charmantier & Gienapp 2014; Beever *et*  
291 *al.* 2017). Numerous studies support behavioural plasticity as a mechanism for coping with climate  
292 change (Charmantier *et al.* 2008; Phillimore *et al.* 2016; Verhagen *et al.* 2020), despite its  
293 limitations in promoting population persistence in the face of climate change (Ghalambor *et al.*  
294 2007; Gienapp *et al.* 2013; Duputié *et al.* 2015). We found that returning females initiated egg  
295 laying earlier in warmer springs. However, there was very little among-individual variation in the

296 degree of plasticity upon which selection might act, suggesting that microevolutionary change in  
297 plasticity itself is not a likely explanation for the observed change.

298

299 **b) Winners versus losers in relation to climate change**

300 Global change biologists often discuss ‘winners’ and ‘losers’ in relation to climate change,  
301 typically in the context of range shifts (Crick 2004; Bateman *et al.* 2016; Tayleur *et al.* 2016).  
302 Here, we extend these concepts of winning and losing to migratory strategy and breeding season  
303 length. Short-distance migrants and residents often experience longer breeding seasons than long-  
304 distance migratory species. This is true in part because they typically breed at lower latitudes  
305 where spring comes earlier and also because they do not lose time to the migratory journey leaving  
306 time for multiple broods (Newton 2010). Juncos in our study population have a longer breeding  
307 season than closely related long-distance migrant populations (Nolan *et al.* 2002). Females can re-  
308 nest as many as five times and can fledge up to three successful nests.

309 The advancement in breeding phenology reported here is supported by a previous finding  
310 that multi-brooded species tend to exhibit larger advances in breeding phenology than single-  
311 brooded species, likely because multi-brooded species are experiencing longer breeding seasons  
312 with warmer springs (Dunn & Møller 2014). Thus multi-brooded populations are expected to have  
313 higher reproductive output than migratory populations that are typically single- or double-brooded  
314 (Halupka & Halupka 2017), an effect echoed in our finding that juncos that bred earlier tended to  
315 fledge more offspring that year, presumably an effect of having more time for breeding attempts  
316 (Dunn & Møller 2014). Warmer springs may benefit this population by allowing females to breed  
317 earlier and extend their breeding season, despite the lack of evidence that warmer spring  
318 temperatures predicted stronger selection favouring earlier breeding.

319 Overall, females can respond flexibly to changes in temperature, but individuals do not  
320 strongly vary in their plastic response to temperatures. However, plasticity alone will likely be  
321 insufficient for populations to survive in the long-term when facing climate change (Gienapp *et*  
322 *al.* 2013). Since our study population occurs at high elevation, persistent increases in temperature  
323 could eventually result in population decline, as the population cannot shift any further up the  
324 mountains.

325

### 326 **c) Future directions**

327 Accurate predictions of future responses to climate change will require further  
328 consideration of mechanisms of female reproductive timing (Williams 2012; Chmura, Wingfield  
329 & Hahn 2020; Kimmitt 2020). Past and ongoing work in the junco is elucidating the physiological  
330 mechanisms driving reproductive timing in females based on life history, including endocrine  
331 systems and costs of early breeding (Greives *et al.* 2016; Graham *et al.* 2019; Kimmitt *et al.* 2019;  
332 Kimmitt, Sinkiewicz & Ketterson 2020). However, more research is necessary to understand how  
333 females integrate supplementary cues, such as temperature, to regulate the final stages of their  
334 reproductive development and ovulation (Wingfield *et al.* 2016; Chmura, Wingfield & Hahn  
335 2020). Via our analysis of this 32-year dataset, we found that flexibility in female timing is likely  
336 relevant for population persistence, and further work on the proximate mechanisms of female  
337 timing will improve forecasts on the effects of climate change on birds.

338

### 339 **Acknowledgments**

340 We acknowledge the significant role of the late Val Nolan Jr., who established this field site for  
341 long-term study as a co-PI with Ellen Ketterson in the early 1980s. We also thank Ketterson lab

342 members and colleagues who have contributed to this long-term dataset, with a special thanks to  
343 long-term lab managers and field assistants, Eric Snajdr, Charles Ziegenfus, and Sarah  
344 Wanamaker. We also acknowledge support from MLBS and its directors, Henry Wilbur and  
345 Edmund “Butch” Brodie III, as well as MLBS staff. Finally, we thank Allie Byrd, Alex Jahn,  
346 Katie Talbott, and Sarah Wanamaker for discussion and feedback on the manuscript.

347

### 348 **Funding**

349 Long-term data collection was supported by NSF (#8718358, 9408061, 9728384, 0216091,  
350 0519211, 0820055, 1257474). AAK was supported by the NSF Graduate Research Fellowship.  
351 SND received funding from the IU School of Public and Environmental Affairs.

352

### 353 **Authors’ Contributions**

354 AAK conceived and designed the study, curated data, conducted statistical analysis and was the  
355 primary author of the manuscript; DJB conducted statistical analysis, drafted sections of the  
356 manuscript, and revised the manuscript; SND participated in data curation and analysis and  
357 revised the manuscript. NMG conceived and designed the study, curated data, and revised the  
358 manuscript. KAR conceived and designed the study and revised the manuscript. EDK conceived  
359 and designed the study, secured funding for data collection, and revised the manuscript. All  
360 authors gave final approval of this publication.

361

### 362 **Competing Interests**

363 The authors declare no competing interests.

364

365 **Data Accessibility**

366 Data will be made available on Dryad pending manuscript acceptance.

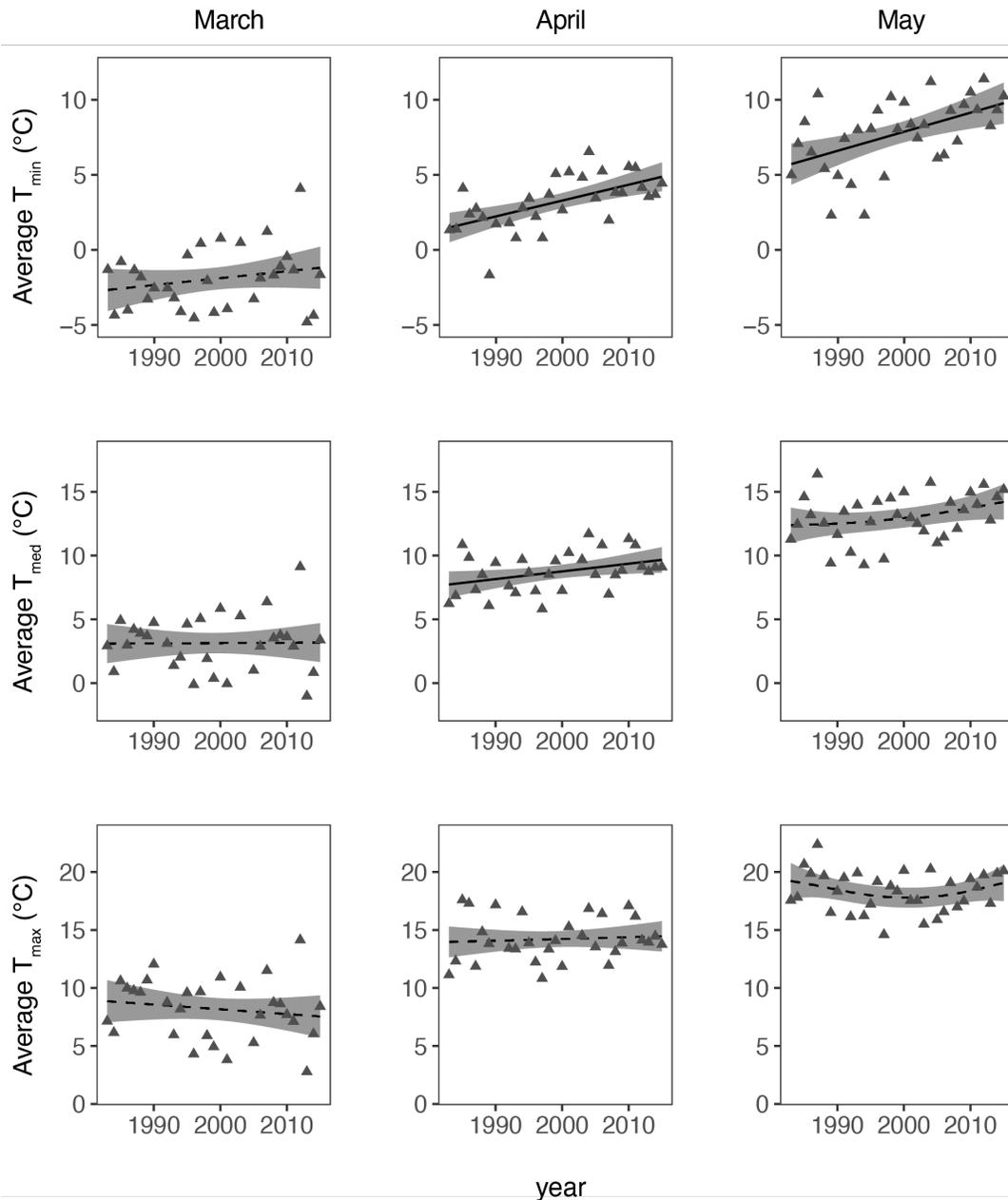
367 **Figures**

368

369 Figure 1. Independent relationships between year and median, minimum, and maximum  
370 temperatures in March-May from 1983 to 2015. All prediction lines and confidence bands from  
371 the GAMs are created as a function of year as a smooth term and overlaid with original data.

372 Average April  $T_{med}$ , April  $T_{min}$ , and May  $T_{min}$  change significantly over time (**April  $T_{med}$** :  $F_{1,1}=$   
373  $4.79$ ,  $p=0.037$ ,  $R^2=0.11$ ; **April  $T_{min}$** :  $F_{1,1}=15.86$ ,  $p<0.001$ ,  $R^2=0.33$ ; **May  $T_{min}$** :  $F_{1,1}=11.41$ ,  $p=$   
374  $0.002$ ,  $R^2=0.25$ )

375



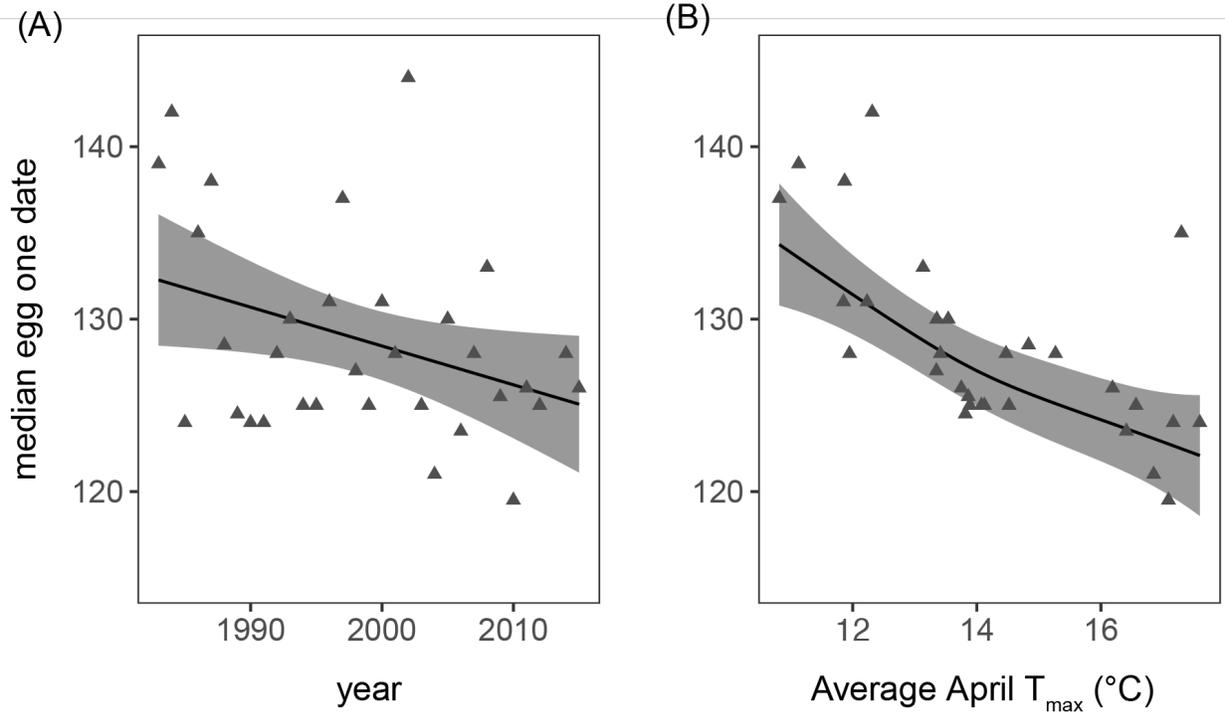
376

377

378 Figure 2. (A) Average egg one date of females is shown as a function of year only ( $R^2=0.10$ ). (B)

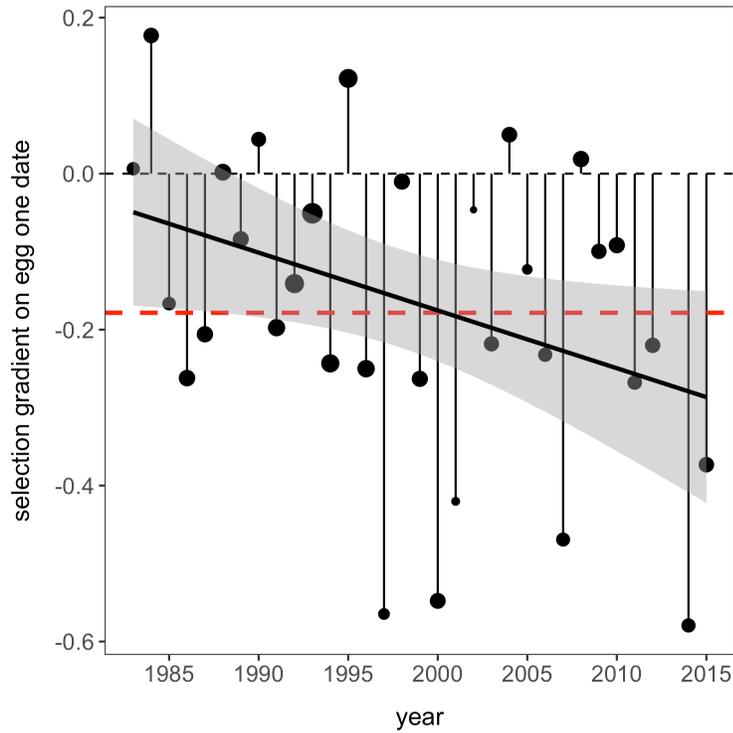
379 Average egg one date is shown as a function of April average maximum temperatures when the

380 model also accounts for nonlinear effects of year ( $R^2=0.65$ ). Fitted values and 95% confidence  
381 bands from the GAMs are overlaid with original data.  
382

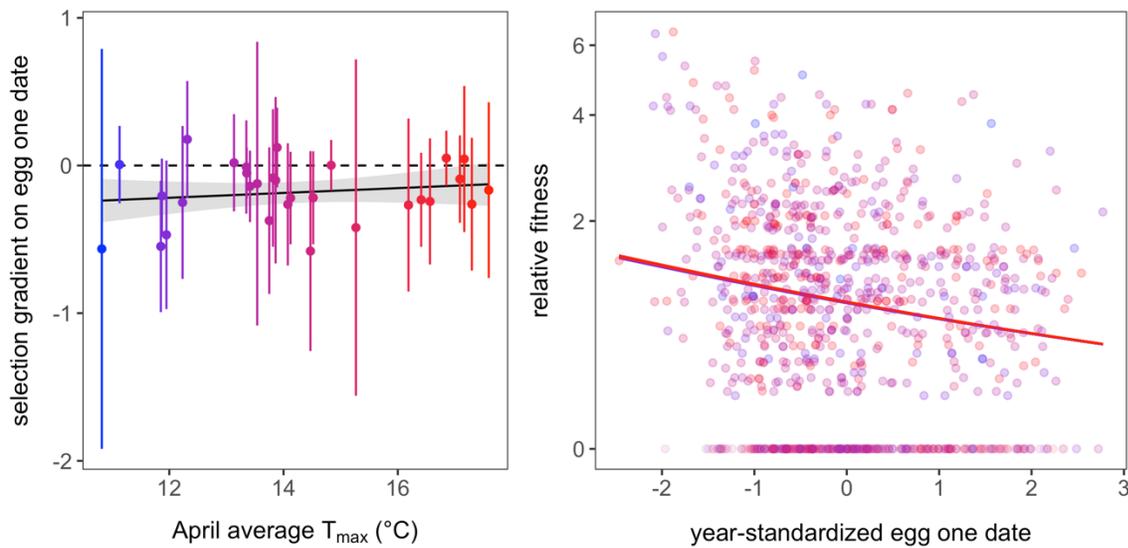


383  
384

385 Figure 3. Per-year selection gradients on egg one date estimated with compound Poisson GLMs  
386 after adjusting for annual total eggs per female and female age. The dashed line shows  $\beta=0$ ,  
387 whereas the red line displays the mean selection gradient across the 32 years. The solid line and  
388 grey band show fitted values and 95% confidence intervals from a linear model that included  
389 weighting by annual sample size (points are scaled by sample size).  
390

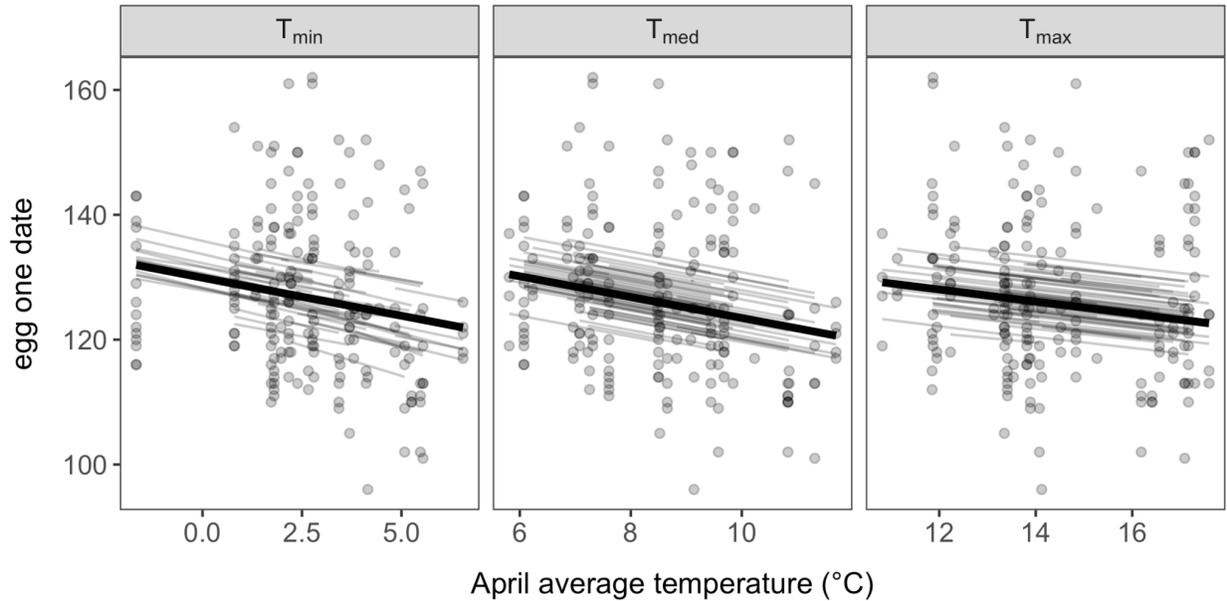


392 Figure 4. April temperature does not predict variation in selection on egg one date. (A) Results of  
393 a linear model using the per-year selection gradients as the response variable (after adjusting for  
394 female age and annual total eggs per female), with the solid line and grey band showing fitted  
395 values and 95% confidence intervals. The linear model included weighting by annual sample size,  
396 which is illustrated through point size. (B) Results of a GLMM predicting relative fitness as a  
397 function of the interaction between maximum April temperature and egg one date (after adjusting  
398 for female age, annual total eggs per female, and annual predation rate). Points display individual  
399 nest data and lines show fitted values, with colours indicating temperature as in A. Relative fitness  
400 is shown with a modulus transformation given the right-skew in this variable.  
401



402  
403

404 Figure 5. Fitted values of RRM testing for temperature-driven plasticity in egg one date. Thick  
405 lines show the overall reaction norm for each April temperature measure (displayed in order of  
406 Akaike weights) after controlling for female age, annual total eggs per female, and annual  
407 predation risk, whereas thin lines show reaction norms for each individual female. Random effects  
408 were visualized by holding each female (assumed to be ASY) at its mean annual total eggs per  
409 female and annual predation rate.  
410



411  
412

413 **References**

414

415 Bateman, B.L., Pidgeon, A.M., Radeloff, V.C., VanDerWal, J., Thogmartin, W.E., Vavrus, S.J.

416 & Heglund, P.J. (2016) The pace of past climate change vs. potential bird distributions and land

417 use in the United States. *Global change biology*, **22**, 1130-1144.

418 Beever, E.A., Hall, L.E., Varner, J., Loosen, A.E., Dunham, J.B., Gahl, M.K., Smith, F.A. &

419 Lawler, J.J. (2017) Behavioral flexibility as a mechanism for coping with climate change.

420 *Frontiers in Ecology and the Environment*, **15**, 299-308.

421 Burham, K. & Anderson, D. (2002) Model selection and multimodel inference: A practical

422 information-theoretic approach. Springer. *New York*.

423 Chandler, C.R., Ketterson, E.D., Nolan Jr, V. & Ziegenfus, C. (1994) Effects of testosterone on

424 spatial activity in free-ranging male dark-eyed juncos, *Junco hyemalis*. *Animal Behaviour*, **47**,

425 1445-1455.

426 Charmantier, A. & Gienapp, P. (2014) Climate change and timing of avian breeding and

427 migration: evolutionary versus plastic changes. *Evolutionary Applications*, **7**, 15-28.

428 Charmantier, A., McCleery, R.H., Cole, L.R., Perrins, C., Kruuk, L.E. & Sheldon, B.C. (2008)

429 Adaptive phenotypic plasticity in response to climate change in a wild bird population. *science*,

430 **320**, 800-803.

431 Chmura, H.E., Wingfield, J.C. & Hahn, T.P. (2020) Non-photoc environmental cues and avian

432 reproduction in an era of global change. *Journal of Avian Biology*, **51**.

433 Clotfelter, E.D., O'Neal, D.M., Gaudioso, J.M., Casto, J.M., Parker-Renga, I.M., Snajdr, E.A.,

434 Duffy, D.L., Nolan Jr, V. & Ketterson, E.D. (2004) Consequences of elevating plasma

435 testosterone in females of a socially monogamous songbird: evidence of constraints on male

436 evolution? *Hormones and behavior*, **46**, 171-178.

- 437 Crick, H.Q. (2004) The impact of climate change on birds. *Ibis*, **146**, 48-56.
- 438 Dunn, P.O. & Møller, A.P. (2014) Changes in breeding phenology and population size of birds.  
439 *Journal of Animal Ecology*, 729-739.
- 440 Dunn, P.O. & Winkler, D.W. (2010) Effects of climate change on timing of breeding and  
441 reproductive success in birds. *Effects of climate change on birds*, 113-128.
- 442 Duputié, A., Rutschmann, A., Ronce, O. & Chuine, I. (2015) Phenological plasticity will not  
443 help all species adapt to climate change. *Global change biology*, **21**, 3062-3073.
- 444 Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007) Adaptive versus non-  
445 adaptive phenotypic plasticity and the potential for contemporary adaptation in new  
446 environments. *Functional ecology*, **21**, 394-407.
- 447 Gienapp, P., Fior, S., Guillaume, F., Lasky, J.R., Sork, V.L. & Csilléry, K. (2017) Genomic  
448 quantitative genetics to study evolution in the wild. *Trends in Ecology & Evolution*, **32**, 897-  
449 908.
- 450 Gienapp, P., Lof, M., Reed, T.E., McNamara, J., Verhulst, S. & Visser, M.E. (2013) Predicting  
451 demographically sustainable rates of adaptation: can great tit breeding time keep pace with  
452 climate change? *Philosophical Transactions of the Royal Society B: Biological Sciences*, **368**,  
453 20120289.
- 454 Graham, J.L., Bauer, C.M., Heidinger, B.J., Ketterson, E.D. & Greives, T.J. (2019) Early-  
455 breeding females experience greater telomere loss. *Molecular ecology*, **28**, 114-126.
- 456 Greives, T.J., Fudickar, A.M., Atwell, J.W., Meddle, S.L. & Ketterson, E.D. (2016) Early spring  
457 sex differences in luteinizing hormone response to gonadotropin releasing hormone in co-  
458 occurring resident and migrant dark-eyed juncos (*Junco hyemalis*). *General and comparative*  
459 *endocrinology*, **236**, 17-23.

- 460 Halupka, L. & Halupka, K. (2017) The effect of climate change on the duration of avian  
461 breeding seasons: a meta-analysis. *Proceedings of the Royal Society B: Biological Sciences*,  
462 **284**, 20171710.
- 463 Helm, B., Van Doren, B.M., Hoffmann, D. & Hoffmann, U. (2019) Evolutionary response to  
464 climate change in migratory pied flycatchers. *Current Biology*, **29**, 3714-3719. e3714.
- 465 Hoffmann, A.A. & Sgrò, C.M. (2011) Climate change and evolutionary adaptation. *Nature*, **470**,  
466 479-485.
- 467 Ketterson, E., Nolan, V. & Sandell, M. (2005) Testosterone in females: mediator of adaptive  
468 traits, constraint on sexual dimorphism, or both? *the american naturalist*, **166**, S85-S98.
- 469 Ketterson, E.D. & Atwell, J.W. (2016) *Snowbird: integrative biology and evolutionary diversity*  
470 *in the Junco*. University of Chicago Press.
- 471 Kimmitt, A.A. (2020) Females as the gatekeepers to seasonal breeding: what we can learn by  
472 studying reproductive mechanisms in both sexes. *Integrative and Comparative Biology*, **60**, 703-  
473 711.
- 474 Kimmitt, A.A., Hardman, J.W., Stricker, C.A. & Ketterson, E.D. (2019) Migratory strategy  
475 explains differences in timing of female reproductive development in seasonally sympatric  
476 songbirds. *Functional Ecology*, **33**, 1651-1662.
- 477 Kimmitt, A.A., Sinkiewicz, D.M. & Ketterson, E.D. (2020) Seasonally sympatric songbirds that  
478 differ in migratory strategy also differ in neuroendocrine measures. *General and comparative*  
479 *endocrinology*, **285**, 113250.
- 480 Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hill, C., Hoang,  
481 A., Gibert, P. & Beerli, P. (2001) The strength of phenotypic selection in natural populations.  
482 *The American Naturalist*, **157**, 245-261.

- 483 Lande, R. & Arnold, S.J. (1983) The measurement of selection on correlated characters.  
484 *Evolution*, 1210-1226.
- 485 Manhard, C.V., Joyce, J.E. & Gharrett, A.J. (2017) Evolution of phenology in a salmonid  
486 population: a potential adaptive response to climate change. *Canadian Journal of Fisheries and*  
487 *Aquatic Sciences*, **74**, 1519-1527.
- 488 Marrot, P., Charmantier, A., Blondel, J. & Garant, D. (2018) Current spring warming as a driver  
489 of selection on reproductive timing in a wild passerine. *Journal of Animal Ecology*, **87**, 754-764.
- 490 Marrot, P., Garant, D. & Charmantier, A. (2017) Multiple extreme climatic events strengthen  
491 selection for earlier breeding in a wild passerine. *Philosophical Transactions of the Royal*  
492 *Society B: Biological Sciences*, **372**, 20160372.
- 493 Merilä, J. (2012) Evolution in response to climate change: in pursuit of the missing evidence.  
494 *BioEssays*, **34**, 811-818.
- 495 Nakagawa, S., Johnson, P.C. & Schielzeth, H. (2017) The coefficient of determination  $R^2$  and  
496 intra-class correlation coefficient from generalized linear mixed-effects models revisited and  
497 expanded. *Journal of the Royal Society Interface*, **14**, 20170213.
- 498 Newton, I. (2010) *The migration ecology of birds*. Elsevier.
- 499 Nolan, V., Ketterson, E., Cristol, D., Rogers, C., Clotfelter, E., Titus, R., Schoech, S. & Snajdr,  
500 E. (2002) Dark-eyed Junco (*Junco hyemalis*). In *The Birds of North America* 716 (A. Poole and  
501 F. Gill, Editors). *Birds of North America*, Philadelphia, PA, USA.
- 502 Nussey, D., Wilson, A. & Brommer, J. (2007) The evolutionary ecology of individual  
503 phenotypic plasticity in wild populations. *Journal of evolutionary biology*, **20**, 831-844.
- 504 Nussey, D.H., Postma, E., Gienapp, P. & Visser, M.E. (2005) Selection on heritable phenotypic  
505 plasticity in a wild bird population. *Science*, **310**, 304-306.

- 506 Phillimore, A.B., Leech, D.I., Pearce-Higgins, J.W. & Hadfield, J.D. (2016) Passerines may be  
507 sufficiently plastic to track temperature-mediated shifts in optimum lay date. *Global change*  
508 *biology*, **22**, 3259-3272.
- 509 Pinheiro, J. & Bates, D. (2006) *Mixed-effects models in S and S-PLUS*. Springer Science &  
510 Business Media.
- 511 Pyle, P. (1997) *Identification guide to North American birds: a compendium of information on*  
512 *identifying, ageing, and sexing" near-passerines" and passerines in the hand*. Slate Creek Press.
- 513 Ricklefs, R.E. (1969) An analysis of nesting mortality in birds. *Smithsonian contributions to*  
514 *zoology*.
- 515 Sih, A., Stamps, J., Yang, L.H., McElreath, R. & Ramenofsky, M. (2010) Behavior as a key  
516 component of integrative biology in a human-altered world. *Integrative and comparative*  
517 *biology*, **50**, 934-944.
- 518 Tayleur, C.M., Devictor, V., Gaüzère, P., Jonzén, N., Smith, H.G. & Lindström, Å. (2016)  
519 Regional variation in climate change winners and losers highlights the rapid loss of cold-  
520 dwelling species. *Diversity and Distributions*, **22**, 468-480.
- 521 Van Asch, M., Salis, L., Holleman, L.J., Van Lith, B. & Visser, M.E. (2013) Evolutionary  
522 response of the egg hatching date of a herbivorous insect under climate change. *Nature Climate*  
523 *Change*, **3**, 244-248.
- 524 Van Buskirk, J., Candolin, U. & Wong, B. (2012) Behavioural plasticity and environmental  
525 change.
- 526 Van Buskirk, J., Mulvihill, R.S. & Leberman, R.C. (2012) Phenotypic plasticity alone cannot  
527 explain climate-induced change in avian migration timing. *Ecology and Evolution*, **2**, 2430-  
528 2437.

- 529 Verhagen, I., Tomotani, B.M., Gienapp, P. & Visser, M.E. (2020) Temperature has a causal and  
530 plastic effect on timing of breeding in a small songbird. *Journal of Experimental Biology*, **223**.
- 531 Watts, H.E., Edley, B. & Hahn, T.P. (2016) A potential mate influences reproductive  
532 development in female, but not male, pine siskins. *Hormones and behavior*, **80**, 39-46.
- 533 Watts, H.E., Jimenez, D., Pacheco, V. & Vilgalys, T.P. (2019) Temperature-correlated shifts in  
534 the timing of egg-laying in House Finches *Haemorhous mexicanus*. *Ibis*, **161**, 428-434.
- 535 Williams, T.D. (2012) *Physiological adaptations for breeding in birds*. Princeton University  
536 Press.
- 537 Wilson, S., Norris, D.R., Wilson, A.G. & Arcese, P. (2007) Breeding experience and population  
538 density affect the ability of a songbird to respond to future climate variation. *Proceedings of the*  
539 *Royal Society B: Biological Sciences*, **274**, 2539-2545.
- 540 Wingfield, J.C., Perfito, N., Calisi, R., Bentley, G., Ubuka, T., Mukai, M., O'Brien, S. & Tsutsui,  
541 K. (2016) Putting the brakes on reproduction: implications for conservation, global climate  
542 change and biomedicine. *General and comparative endocrinology*, **227**, 16-26.
- 543 Wood, S. (2017) Package “mgcv”: mixed GAM computation vehicle with GCV/AIC/REML  
544 smoothness estimation. Version.  
545