







RESEARCH ARTICLE

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

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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. Furthermore, individual females that bred in at least 3 years 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 increase the length of the breeding season, and therefore, reproductive success.

KEYWORDS

bird, climate change, phenological shifts, phenotypic plasticity, selection, timing of breeding

1 | INTRODUCTION

Phenological shifts are one of the most documented responses to climate change (Parmesan & Yohe, 2003; Piao et al., 2019; Scheffers et al., 2016), suggesting that many species can adapt to warming temperatures (Charmantier & Gienapp, 2014; Saalfeld & 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 (Iler et al., 2021; McLean et al., 2016; Miller-Rushing et al., 2010). And yet, the relative role of microevolutionary change in response to selection and phenotypic plasticity in driving phenological shifts remains under debate (Charmantier & Gienapp, 2014).

Plastic responses allow individuals to respond more quickly to a changing climate compared to microevolutionary change in response to selection (Beever et al., 2017; Charmantier & Gienapp, 2014; Sih et al., 2010), such that evidence for plasticity has been widely reported to account for current phenological shifts observed in animals (Boutin & Lane, 2014; Charmantier & Gienapp, 2014; Crozier & Hutchings, 2014; Réale et al., 2003). 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 & 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 differs (Gavrilets & Scheiner, 1993). Phenotypic plasticity allows individuals that breed in more than one season to track or partially track optimal breeding phenology over time (de Villemeireuil 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 &

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 & Gienapp, 2014). Evolutionary adaptation can then occur if directional selection favouring earlier breeding acts on heritable traits with genetic variation (Hoffmann & 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 (Bonamour et al., 2019; Gavrilets & Scheiner, 1993).

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 (Milá et al., 2007; Nolan et al., 2002). Juncos have also served as a model songbird species for studies of ecology and evolution over the past few decades (Ketterson & 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 (Bonamour et al., 2019; Sauve et al., 2021; Visser et al., 2006).

2 | MATERIALS AND METHODS

2.1 | 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 (final protocol for the duration of the study: #12-050) [Correction added on 8 August 2022, after first online publication, the letter 'd' has been changed to 'duration of the study']. 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 to 2015 were used for this study except for 2013 due to limited research effort. Records where female ID or first egg date was unknown were removed. Female subjects that were implanted with exogenous testosterone during a separate 5-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 1–5 years of data ($\bar{x} = 1$). Annual differences in research effort (number of nests found) did not explain variation in first egg dates (see Figure 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).

2.2 | Temperature data

Between 16 November 1971 and 31 January 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 24 June 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_{mid}) for both loggers. Since the two weather stations overlapped from 1994 to 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_{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.

2.3 | 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'; Gavrillets & 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_{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_{mid}). We considered models within two ΔAICc of the top model to be competitive (Burham & Anderson, 2002). We then used the most competitive climate window for each temperature measure in our plasticity analyses.

2.4 | 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 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 3 years of our study ($n = 62$ individuals representing 190 first egg date observations, hereafter 'returning females'), we fit an 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_{mid} and T_{\max} with the LME4 package (Pinheiro & 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_m^2 and R_c^2 ; (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).

2.5 | 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 & 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 1 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_m^2 and R_c^2 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 1052 first nets from 817 females to compare GLMMs (88 climatic windows per temperature measure).

3 | RESULTS

3.1 | 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; Figure 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 (Figure 2a). This univariate LM predicted the 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 7 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_{mid} and T_{\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 ($\beta = -0.18$, $t = -1.77$, $p = 0.09$; Figure 2b). For the top T_{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 ($\beta = -0.13$, $t = -1.66$, $p = 0.11$; Figure 2c). Lastly, for the top and equivalent T_{\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 ($\beta = -0.22$, $t = -3.35$, $p < 0.01$; Figure 2d).

3.2 | Phenotypic plasticity

We used RRM 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 3 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_{mid} (T_{\min} : $\beta = -0.96$, $t = -2.06$, $p = 0.05$; T_{mid} : $\beta = -1.35$, $t = -2.29$, $p = 0.03$), whereas that for T_{\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. $R_c^2 > R_m^2$; 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

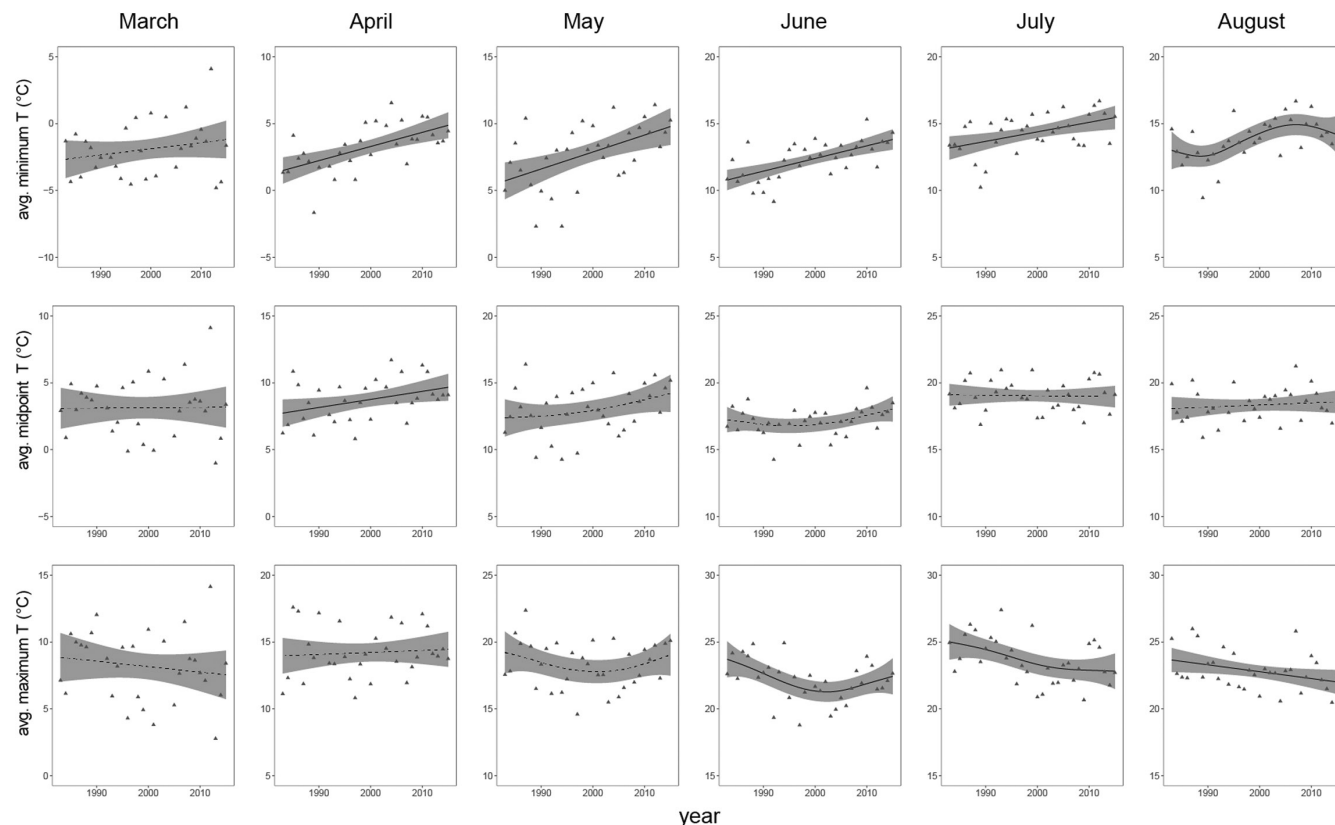


FIGURE 1 Independent relationships between year and monthly average minimum (T_{\min}), midpoint (T_{mid}) and maximum (T_{\max}) temperature in March–August from 1983 to 2015. All prediction lines and confidence bands from the GAMs are created as a function of year as a smooth term and overlaid with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

breeding phenology reaction norm for average spring temperatures (Figure 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).

3.3 | Selection analyses

We estimated overall strong negative selection on first egg date ($\beta = -0.16$, $t = -4.42$, $p < 0.001$; Figure 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_m^2 and R_c^2 , 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 ($\sigma^2 = 0.08$, $\text{LRT} = 19.06$, $p < 0.001$). 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_{mid} , all candidate GLMMs were within 2 ΔAICc of the top model, and 56% of candidate GLMMs for T_{\max} were also within 2 ΔAICc 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 (Figure 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

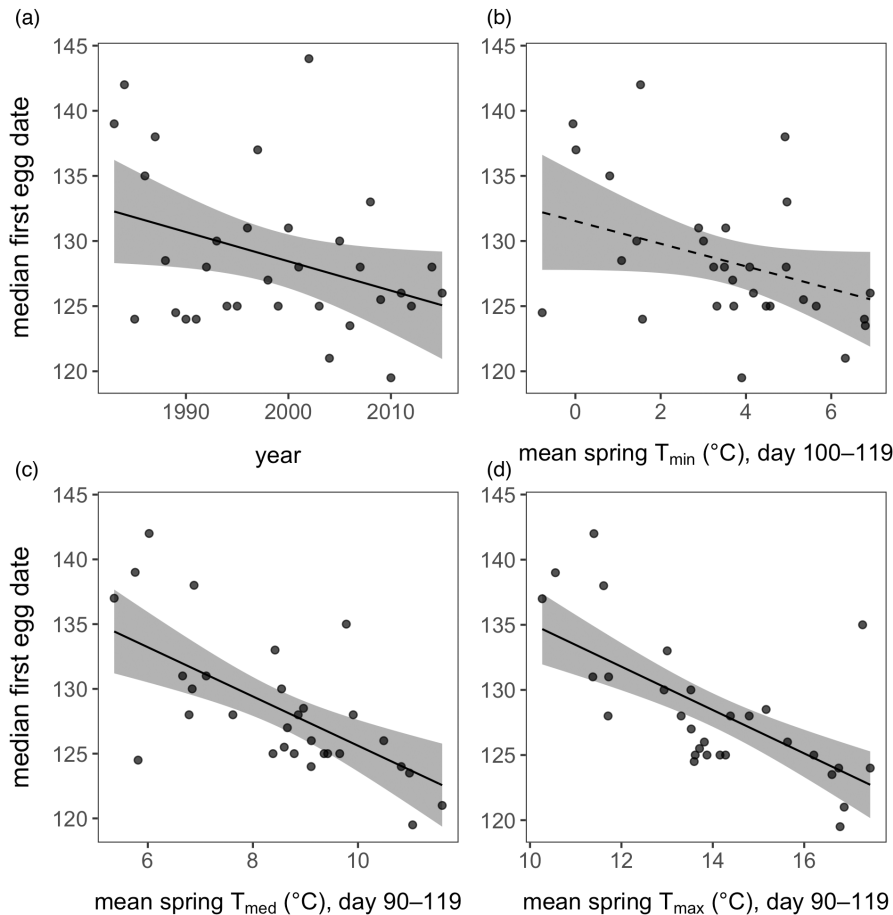


FIGURE 2 (a) Average first egg date of females is shown as a function of year only (b–d) Average first egg date is shown as a function of each average temperature identified from the sliding window analyses (T_{\min} , T_{mid} , T_{\max}) using LMs that account for year. Fitted values and 95% confidence bands from the LMs are overlaid with original data.

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; Tables S2–S4). Test statistics and p values were derived from sequential LRTs

Term	T_{\min}			T_{mid}			T_{\max}		
	σ^2	LRT	p	σ^2	LRT	p	σ^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		
R_m^2	0.04			0.04			0.02		
R_c^2	0.22			0.18			0.17		

to spring temperatures and found a net change of 7 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

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).

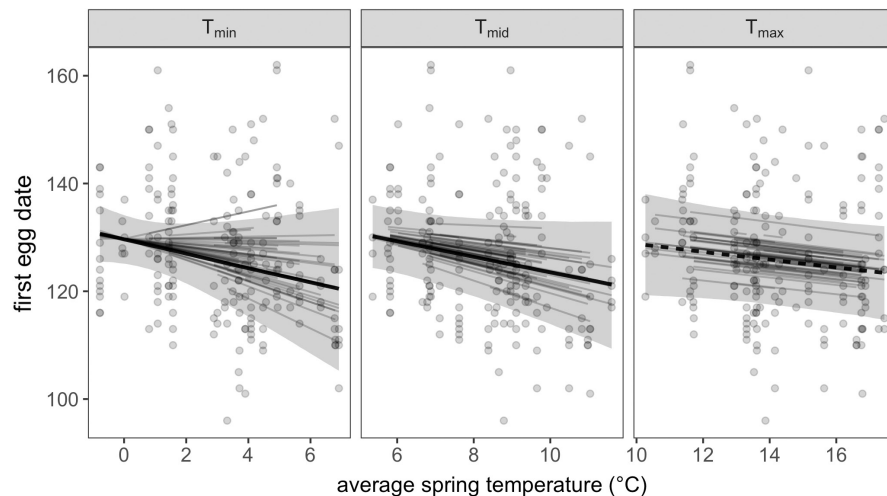
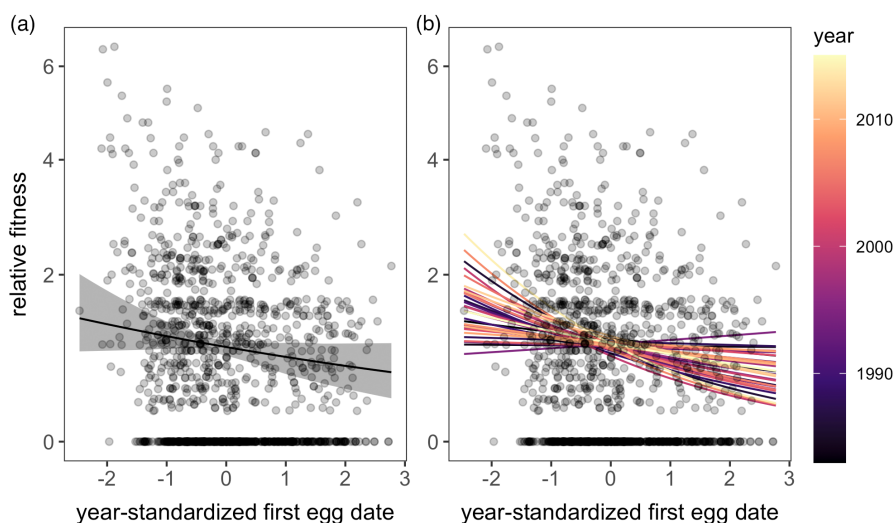


FIGURE 4 (a) Results of the GLMM predicting relative fitness as a function of first egg date after adjusting for annual total number of eggs per female and female age. Points display individual nest data. The solid line and grey band show fitted values and 95% confidence intervals (derived with a year-stratified 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.



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 to 2007 produced significantly more offspring when females initiated egg laying earlier (Gerlach, 2010). However, unlike studies of other avian species (Helm et al., 2019; Marrot et al., 2018), 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 & 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.

4.1 | 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 (Charmanier & Gienapp, 2014) or to benefit from a longer breeding season (Dunn & Møller, 2014; Møller et al., 2010). Without the 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 (Helm et al., 2019; Merilä, 2012). There is some evidence of microevolutionary changes in phenology across taxa that are likely adaptive shifts in response to climate change (Helm et al., 2019; Manhard et al., 2017; Van Asch et al., 2013). In the case of the junco, while we found strong overall selection favouring earlier breeding, and that this selection varied over time,

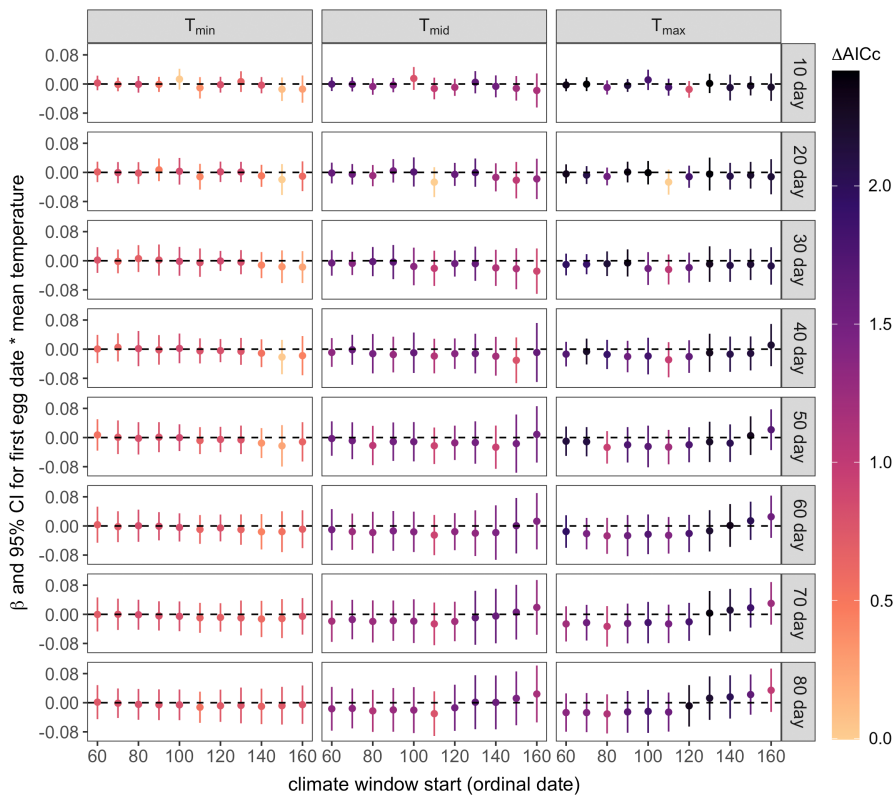


FIGURE 5 Estimated coefficient (β) and 95% confidence interval for the interaction between first egg date and mean temperature from GLMMs across all considered climate window start dates (ordinal date) and durations. Results are coloured by ΔAICc from candidate GLMMs, and the dashed line shows $\beta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

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).

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 (Beever et al., 2017; Charmantier & Gienapp, 2014; Van Buskirk et al., 2012). 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 (Duputié et al., 2015; Ghalambor et al., 2007; Gienapp et al., 2013). 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., unpubl. 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).

4.2 | Winners versus 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 (Bateman et al., 2016; Crick, 2004; 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 & 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 & 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 the 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.

4.3 | Future directions

Accurate predictions of future responses to climate change will require further consideration of mechanisms of female reproductive timing (Chmura et al., 2020; Kimmitt, 2020; Williams, 2012). 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 (Graham et al., 2019; Greives et al., 2016; 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 (Chmura et al., 2020; Wingfield et al., 2016). 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.

AUTHOR 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.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.4jOzpc8fd> (Kimmitt et al., 2022).

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