© 2018. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2018) 221, jeb185058. doi:10.1242/jeb.185058

The Company of Biologists

RESEARCH ARTICLE

Effects of temperature on the timing of breeding and molt transitions in house finches

Heather E. Watts[1,2,§], Daniela Jimenez[2,‡], Veronica Pacheco[2,‡] and Tauras P. Vilgalys[2,*,‡]

ABSTRACT

Temperature-correlated shifts in reproductive timing are now well documented in numerous bird species. However, whether temperature directly influences reproductive timing or whether its effects are mediated by an intermediate environmental cue, such as plant phenology, remains poorly understood. In this study, we investigated the direct effects of temperature on reproductive timing in house finches (*Haemorhous mexicanus*), which have a range and breeding diet not well represented in previous studies of temperature and reproductive timing. We conducted experiments with captive male house finches in which temperature was elevated within realistic ranges and the effects on the timing of preparations for reproduction, as well as on the termination of reproduction and the onset of prebasic feather molt, were examined. We found no adjustments in the timing of reproductive preparations of males in direct response to temperature. However, elevated temperature did advance the breeding–molt transition. Our results suggest that elevated temperatures in the range tested here do not directly impact physiological preparations for reproduction in male house finches, but may constrain the timing of the breeding–molt transition in this species.

KEY WORDS: Bird, Climate change, Molt, Phenology, Reproduction, Temperature

INTRODUCTION

Changes in the timing of breeding over the past century have now been documented in numerous bird species and, in many species, changes in timing have been linked to concomitant changes in temperature (reviewed in Dunn and Winkler, 2010). However, previous studies of the relationship between temperature and reproductive timing in birds have focused heavily on species that breed in Europe and on those that breed using insect-based diets. Furthermore, despite the widespread correlations between temperature and the timing of breeding, the mechanism(s) by which temperature influences reproductive timing remain poorly understood. Temperature-related shifts in reproductive timing could occur as a direct response to temperature or as a response to a temperature-sensitive intermediate factor, such as the phenology of plants or animal food items. Given that a number of species are known to adjust reproductive physiology in response to food cues (Bauchinger et al., 2009; Davies and Deviche, 2014; Dawson, 2018; Hahn, 1995; Hau et al., 2000; Ligon, 1974; Watts and Hahn, 2012), the latter hypothesis is a very reasonable alternative. Surprisingly, very few studies have experimentally tested whether birds adjust reproductive physiology or timing in direct response to realistic temperature differences similar to those observed in the field; two exceptions are studies by Perfito et al. (2005) and Visser et al. (2009). Perfito et al. (2005) found that a 4°C temperature difference influenced gonadal recrudescence in male song sparrows (*Melospiza melodia morphna*) from a mountain population, but not a coastal population. Visser et al. (2009) found that great tits (*Parus major*) adjusted the timing of the onset of egg laying in response to a 4°C temperature difference.

In this study, we aim to broaden our understanding of the relationship between temperature and reproductive timing by experimentally testing for direct effects of realistic temperature differences on the timing of both initiation and termination of reproduction in the house finch [*Haemorhous mexicanus* (Müller 1776)]. The house finch is a North American songbird that breeds on a primarily seed-based diet. Seeds make up the bulk of the diet throughout the year, including during the breeding season, and the diet of nestlings is almost entirely seed based (Badyaev et al., 2012; Beal, 1907). For example, a study of house finches in California found that the diet of nestlings was composed of 2% insects and 98% seeds, such as those of sunflower (*Helianthus* sp.), bur weed (*Amsinckia tessellate*), milk thistle (*Silybum marianum*) and poison oak (*Toxicodendron diversilobum*) (Beal, 1907). This seed-based breeding diet is in marked contrast to most species that have been studied in the context of the effects of temperature on reproductive timing. Thus, focusing on the house finch as a model, our goal was to test whether warmer temperatures, similar to those experienced in the wild, directly influence reproductive timing. Using experimental manipulations of captive house finches, we tested the effects of elevated temperatures on the timing of preparations for reproduction, as well as on the termination of reproduction and the onset of prebasic feather molt. Patterns from other species suggest that, when elevated temperatures affect reproductive timing, they generally do so by advancing the timing of initiation and/or termination of reproduction. Thus, if house finches respond directly to relatively small temperature differences to adjust reproductive timing, then we would expect to see advancements under warmer temperatures. However, if they do not respond directly to temperature or only to relatively large temperature differences, then we would expect to find no effect of our temperature manipulation on reproductive timing. Further, by testing for potential effects of temperature on the timing of initiation and termination separately, we are able to examine the effects on termination independent of any effects on initiation. We are unaware of any previous studies that have tested these effects independently.

[1]School of Biological Sciences and Center for Reproductive Biology, Washington State University, Pullman, WA 99164, USA. [2]Department of Biology, Loyola Marymount University, Los Angeles, CA 90045, USA.
*Present address: Department of Evolutionary Anthropology, Duke University, Durham, NC 27705, USA.
‡These authors contributed equally to this work

§Author for correspondence (heather.watts@wsu.edu)

H.E.W., 0000-0002-9888-825X; T.P.V., 0000-0002-0615-6888

Received 23 May 2018; Accepted 2 August 2018

Journal of Experimental Biology

Journal of Experimental Biology (2018) 221, jeb185058. doi:10.1242/jeb.185058

MATERIALS AND METHODS

We performed separate experiments to test for the effects of elevated temperature on the initiation and termination of reproduction. In house finches, as in many other temperate zone songbirds, the onset of prebasic feather molt is closely associated with the termination of breeding (Badyaev et al., 2012). Therefore, we examined the timing of prebasic molt (hereafter, molt) as an additional indicator of the termination of the breeding life history stage.

Animals

House finches were trapped in Los Angeles, CA, USA (34.0°N, 118.4°W), which is within the species' native range (Hill, 2002). All birds were after-hatch-year in age at the time of the experiments. Birds were held on a photoperiod that mimicked a naturally changing photoperiod at 34°N. Birds were given Roudybush Small Bird Maintenance Diet (Woodland, CA, USA), whole black oil sunflower seeds, water and fine grit *ad libitum*. Birds were housed in individual cages with acoustic, but not visual, access to other birds. During temperature manipulations, birds could only hear other birds belonging to the same treatment group. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Loyola Marymount University, CA, USA. Birds were captured under permits from the U.S. Fish and Wildlife Service and the California Department of Fish and Wildlife.

Historical temperature data

To assess what constitutes a realistic temperature manipulation, temperature data for the period from 1895 to 2010 were obtained from the National Oceanic and Atmospheric Administration (NOAA) Climate Divisional Database (nCLIMDIV) (Vose et al., 2014) for the climate division from which birds for this study were collected (California Division 6, South Coast Drainage). Monthly mean values for daily average, minimum and maximum temperature were used to calculate mean daily average (T_{avg}), daily minimum (T_{min}) and daily maximum temperature (T_{max}) for the spring (March–June) of each year. We determined that the difference in spring T_{avg} between the warmest and coolest years in this period was 4.7°C, the difference in T_{max} was 6.0°C and the difference in T_{min} was 4.1°C.

Initiation of breeding experiment

Experimental design

Male house finches were caught between 8 and 20 December 2014. Males were held at the control temperature regime until 6 January 2015 (day 0 of experiment), when the temperature manipulation began. Birds were randomly assigned to either the control group (n=11 birds, mean daily average: 19.2°C) or 'warm' treatment group (n=11 birds), which experienced an increase in air temperature on day 0 that was maintained for the duration of the temperature manipulation (mean daily average: 24.3°C). Schaper et al. (2012) have shown that an increase in temperature is the temperature cue to which great tits respond in adjusting the onset of egg laying. In both treatment groups, temperatures cycled across the day (Fig. 1A), with minimum, average and maximum temperatures that were approximately 5°C warmer in the treatment group compared with the control (Fig. 1B). This manipulation was designed to create an environmentally relevant temperature difference between the experimental groups based on our temperature data. The temperature manipulation continued until day 120 (7 May 2015), at which point all birds returned to the control temperature regime and the two experimental groups were no longer acoustically isolated. One bird from each experimental group died unexpectedly over the course of the experiment, so sample sizes are lower for some sampling points.

Reproductive physiology

Laparotomies were performed to measure gonadal condition of males on day –6 and again on day 62. Under general anesthesia with isoflurane, a small incision was made on the left flank of the bird and the left testis was exposed. The length of the left testis was then measured by positioning the tips of forceps at each end of the testis, pressing the tips into clay and measuring the distance between the impressions to the nearest 0.1 mm with dial calipers.

The length of the cloacal protuberance (CP) was also used as a measure of reproductive physiology, as it could be measured more frequently than testis length. CP length is an androgen-dependent trait (Deviche, 1992; Schwabl and Farner, 1989; Schwabl and Kriner, 1991). CP length was measured to the nearest 0.1 mm using dial calipers on days 0, 10, 24, 38, 80 and 107.

Finally, we measured circulating testosterone levels on days 0, 52 and 110, and following presentation of a female (days 27–32, see 'Reproductive behavior' below). Blood samples were collected during morning hours at least 2 h after lights on. Blood was collected from the alar vein into heparinized microhematocrit tubes and stored on ice until it was centrifuged to separate plasma. Plasma was collected and stored at −20°C until assayed.

Reproductive behavior

In addition to measuring reproductive physiology, we also sought to measure reproductive behavior, specifically courtship vocalization behavior. To do this, we presented each male with a female house finch (housed in adjacent cage) and recorded audio to assess vocal behavior. Female house finches were caught between 28 January and 1 February 2015 to serve as stimuli. Males and females were maintained in separate rooms, except during presentations. Audio recordings were made using a Marantz PMD 661 solid state recorder (Mahwah, NJ, USA) with a Sennheiser ME66 microphone (Wedemark, Germany) positioned immediately above the male's cage. Six females were used for presentations, with females randomly assigned to males, but balanced so that each female was presented to males from both treatment groups. Each male was given this presentation twice, once between days 27 and 32 and once again between days 69 and 77. Males were presented with a different female for the first and second presentation. All presentations occurred during morning hours.

For each presentation, the male was moved in his home cage to a new room, similar to the housing room. Birds were well habituated to captivity at this point and resumed typical behavior almost immediately. The male was given 10 min to acclimate to the room and during this time a 'pre-presentation' recording was collected. Next, a female, in her home cage, was moved into the room and positioned next to the male's cage and 10 min of 'presentation' were recorded. Finally, the female was removed, and the male was recorded for 10 min of 'post-presentation'. This relatively short testing period was used so as to be able to test all males within a few days (to minimize potential differences across time) and to limit the amount of time that each male was outside of the temperature treatment; limitations of this approach are considered in the Discussion. During the first round of presentations, a blood sample was collected from the male at the conclusion of the 'post' period in order to assess circulating testosterone level in response to the presentation. Given that circulating testosterone levels may remain relatively low in breeding males when social stimuli (e.g. breeding females, male–male competition) are absent (Goymann,

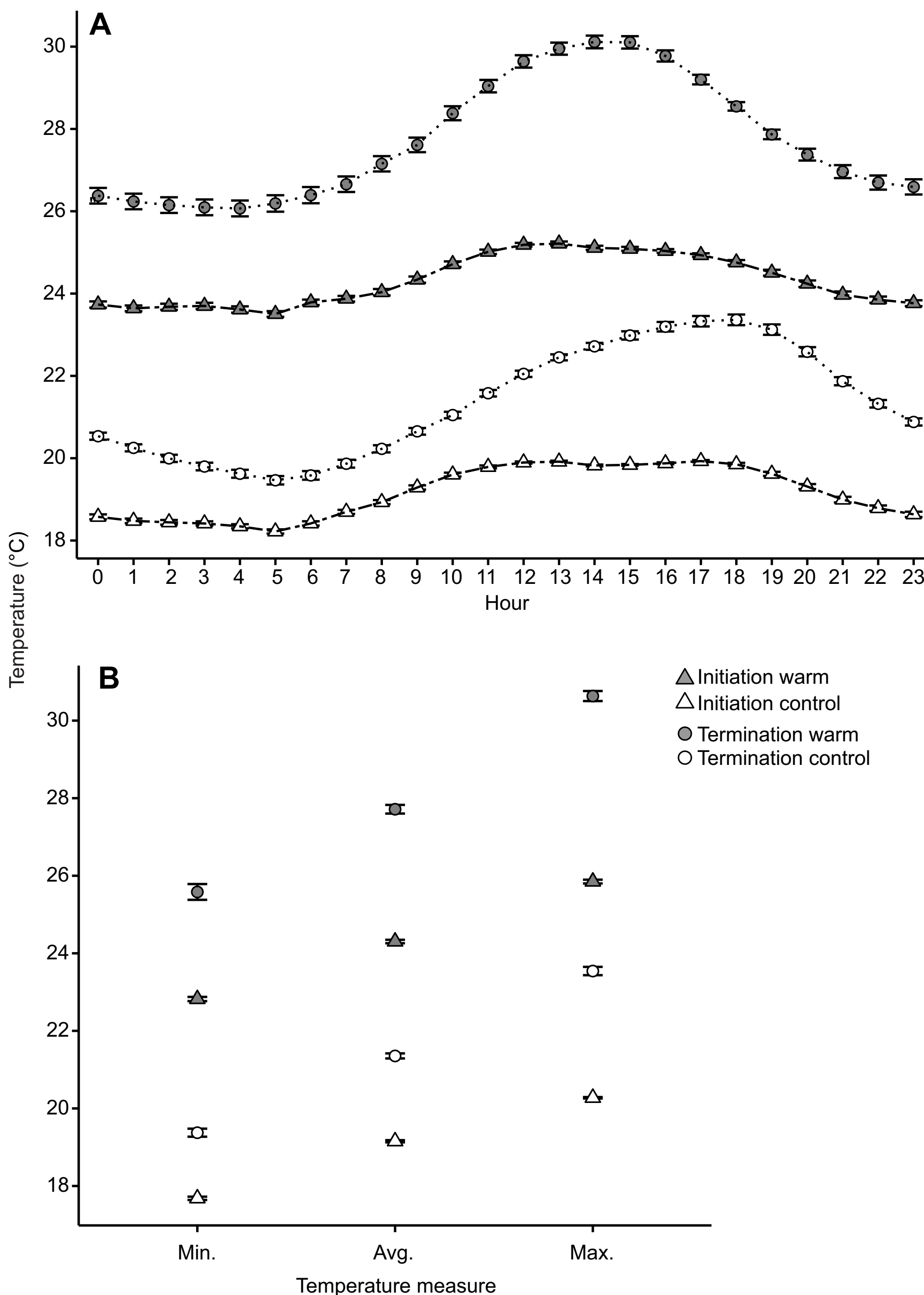

Fig. 1. Experimental temperature treatments. (A) Mean±s.e.m. hourly temperatures across the 24 h cycle and (B) mean±s.e.m. daily minimum (Min.), average (Avg.) and maximum (Max.) temperatures, in control (white symbols) and warm (gray symbols) treatments in the initiation experiment (triangles) and termination experiment (circles).

2009; Moore, 1983; Wingfield et al., 1990), the purpose of this sample was to provide an additional measure of reproductive physiology. Studies in other songbirds have found both short-term and long-term elevations in circulating testosterone levels in males when they are in the presence of females (reviewed in Goymann, 2009), although circulating testosterone levels are often low in captive birds compared to free-living birds, even in the context of courtship and breeding (Meijer and Schwabl, 1989; Wingfield et al., 1990).

Audio recordings were examined aurally and visually using Praat (http://www.praat.org, version 5.4.08) to score the total number of songs and the total number of calls (excluding alarm calls) produced in each sampling period (pre, presentation, post). Since it was not always possible to distinguish whether a vocalization was produced by the male or female during the presentation period, all vocalizations were tallied to produce a rate of vocalization by the pair. Additionally, pairs were observed to call together, producing overlapping or simultaneously calls. We recorded the rate of this behavior separately. Data are missing for one sampling point due to a failure of the audio recording device.

Other measures

Changes in corticosterone signaling have been proposed as an endocrine mechanism that may mediate the effects of temperature on reproductive timing (Lattin et al., 2016; Wingfield et al., 1992). Therefore, we were interested in whether the experimental groups differed in circulating corticosterone levels. To measure corticosterone, a single blood sample was collected from each bird

between days 89 and 97. Samples were collected 4 h after lights on and within 3 min of entering the room for the first time that day, in order to capture baseline corticosterone levels. Samples were processed and stored as described above for testosterone.

To evaluate differences in body condition between the treatment groups, body mass and fat were measured on days 0, 10, 24, 38, 80 and 107. Body mass was measured to the nearest 0.1 g using a Pesola spring scale. Subcutaneous fat was scored visually on a scale from 0 (no fat) to 5 (bulging fat) for the furcular and abdominal regions, and the values summed (Wingfield and Farner, 1978).

Termination of breeding and onset of molt experiment

Experimental design

Male house finches were caught between 5 and 10 April 2012. CP length at capture was 6.7±0.2 mm (mean±s.e.m.), indicating that reproductive development was well underway in these males. Males were held under the control temperature regime until 22 May 2012 (day 0 of experiment), when the temperature manipulation began. Birds were randomly assigned to either a control group (n=10 birds) that remained at the same temperature (mean daily average: 21.4°C) or to a ‘warm’ group (n=10 birds) that experienced an increase in temperature (mean daily average: 27.7°C). In both treatment groups, temperatures cycled across the day (Fig. 1A), with minimum, average and maximum temperatures 6–7°C higher in the warm treatment compared with the control (Fig. 1B). These temperature manipulations were similar to those used for the initiation experiment, and again targeted environmentally relevant temperature differences. These temperature regimes were maintained for the duration of the experiment, which terminated when birds were euthanized with an overdose of isoflurane on days 65 and 66 (26 and 27 July 2012).

Reproductive physiology

CP length was measured on days –8, 10, 27, 34, 41, 44, 48, 54, 58 and 63, as described above. Blood samples were collected to measure circulating testosterone levels on days –8, 10, 27 and 51. Samples were processed and stored as described for the initiation experiment. Gonadal condition was measured as length of the left testis on day 65 or 66, after the bird was euthanized. Additionally, in order to compare testis size between the experimental birds and free-living birds, seven male house finches were trapped and laparotomies performed to measure the length of the left testis. These birds were trapped at the same locations where experimental birds were caught. Data from free-living birds were collected between 12 July and 1 August 2012, corresponding to experimental days 51–71 (mean=day 65). Temperatures in this location around this time (monthly means for July 2012) were a mean minimum of 16.5°C and a mean maximum of 22.0°C, which were slightly cooler than the control temperatures.

To evaluate spermatogenesis in the experimental birds, the testes were dissected out after birds had been euthanized and immediately perfused transcardially with 4% paraformaldehyde. Testes were then post-fixed overnight, cryoprotected in 30% sucrose for 2–3 days, then flash frozen on dry ice and stored at −80°C. One testis per male, the larger of the two, was sectioned at 10 µm on a cryostat and mounted directly on to slides. Testis size varied considerably across birds; thus, we collected every other section from the smallest testes, every fourth section from the largest testes and every third section from those that were intermediate in size in order to yield a similar number of sections across each testis (∼70–120 sections per testis). The tissue was stained with hematoxylin and eosin (#MHS32 and #HT110132, Sigma-Aldrich, St Louis, MO, USA). Each section of a testis was examined at 400× magnification and the testis was given an overall score for the presence or absence of spermatids and of spermatozoa. Since many, if not most, of the testes were in regression, we were interested to determine which males were still spermatogenic.

Other measures

To measure circulating corticosterone levels, a single blood sample was collected from each bird between days 34 and 39. Samples were collected 4.5 h after lights on and within 3 min of entering the room for the first time that day. Body mass and subcutaneous body fat were measured on days –8, 10, 27, 34, 41, 48 and 63.

Determining onset of molt

The onset of prebasic molt was determined by the first day that a new growing primary wing feather was visible (termed a ‘pin’). Only cases in which feather replacement occurred symmetrically on both wings were considered primary molt. In most cases, P1 was the first feather to molt, but occasionally molt commenced with P2 or P3. Since birds were not scored for molt every day, in some cases the feather was longer than a beginning pin when it was first observed and it was necessary to estimate the date of first appearance. This was done by using estimates of feather length collected on the growing feather each time molt was scored. These lengths were used to estimate a daily growth rate for that feather, from which the day that the feather first appeared as a pin could be back calculated.

Timing of the onset of molt was determined for birds in the reproductive termination study by assessing birds for molt throughout the study. Initially molt was assessed every ∼18 days with the frequency increasing to every ∼3–5 days as birds approached molt. In this termination experiment, the temperature difference between the experimental groups was maintained for the entirety of the study period, including during the time at which birds were initiating molt. We were also interested in whether temperatures experienced earlier in the season might also influence the timing of the onset of molt, independent of the current temperature regime. To address this question, we collected additional data for the birds from the reproductive initiation experiment (described above). For these birds, the temperature manipulation ended on day 120 (7 May 2015), before any birds had begun to molt. At this point, the ‘warm’ birds were returned to the control temperature regime and all birds experienced the same temperatures for the remainder of the study. Molt was assessed in the same manner as for the termination experiment. Birds were monitored for molt through 14 August 2015 (day 220 of that experiment). For comparison across the two experiments, we report the timing of molt standardized as days since 22 May for both.

Hormone assays

Enzyme immunoassay kits from Enzo Life Sciences (Farmingdale, NY, USA) were used to measure plasma testosterone (ADI-901-065) and corticosterone (ADI-900-097). These assays have been validated for house finches (Deviche and Cortez, 2005; Valle et al., 2015).

For the testosterone assay, samples were run at a 1:15 dilution with 1% (of raw plasma volume) steroid displacement buffer (following Valle et al., 2015). Samples were run in duplicate, with samples assigned randomly across multiple plates except that all samples for a given individual were run on a single plate. The intra-plate coefficient of variation was 11.02% and the inter-plate coefficient of variation was 7.14%. The detection limit of the assay was 0.088 ng ml^{-1}. Samples below the detection limit were assigned the detection limit as a concentration.

Journal of Experimental Biology

For the corticosterone assay, samples were run at a 1:20 dilution with 2.5% (of raw plasma volume) steroid displacement buffer (following Valle et al., 2015) with a six-point standard curve ranging from 2000 pg ml^{-1} to 1.95 pg ml^{-1}. Samples were run in duplicate with each experiment run on a single plate. The intra-plate coefficient of variation was 7.89%. The average detection limit for the assay was 0.14 ng ml^{-1}. All samples were above the detection limit.

Statistical analyses

Linear mixed models were used to examine the effects of the temperature treatment on the response variables where repeated measures were collected from an individual over time (testis length in initiation experiment, CP length, body mass, body fat, testosterone). For these models, treatment, the day of the experiment, and the interaction between treatment and day were included as fixed effects and individual identity was included as a random effect [i.e. ResponseVariable~Day+Treatment+Day:Treatment+(1|ID)]. For one analysis (testosterone in initiation experiment), an examination of the data indicated a potential quadratic effect of day. Therefore, linear and quadratic effects of day were included for this analysis. Likelihood ratio tests were used to test for each main effect (by comparing the model with all main effects to the model without the effect of interest) and the interaction between treatment and day (by comparing the full model to the model with only the main effects). Models were fit with random intercepts and random slopes for individuals with respect to treatment, and the models were compared using likelihood ratio tests. In no case did inclusion of a random slope improve the model, and in three cases (testis length for initiation experiment and testosterone for both experiments) a model with random slope could not be fitted. Therefore, only random intercept models were retained in final analyses. It was necessary to log transform four response variables (testis length for initiation experiment, body mass for termination experiment, and testosterone for both experiments) to meet assumptions of the models. For response variables without repeated measures, treatment groups were compared using Welch's *t*-test (for unequal variance) or the Mann–Whitney *U*-test if samples were not normally distributed. Fisher's exact test was used to compare count data from testes histology.

To analyze the timing of molt, we used survival analyses with Cox's *F*-test to compare treatment groups. This test statistic is the most powerful option given the properties of our datasets (e.g. small sample size; Lee and Wang, 2003). For birds that experienced the temperature manipulation at termination of reproduction, the dataset included right-censored data points (i.e. birds not yet in molt). For birds that experienced the temperature manipulation at initiation of breeding, there were no censored data.

Vocalization data were analyzed statistically as the change in rate from the 'pre-presentation' period to account for baseline differences in vocalization rates between the males. Our intention was to focus on singing behavior, as song is a prominent element of courtship in this species (Thompson, 1960). However, too few instances of singing behavior were observed to analyze these data statistically. Therefore, we focused our analysis on calls. Calls are a component of courtship behavior in this species (Thompson, 1960), although our results should be interpreted cautiously since it is not clear the extent to which the calling behavior observed during presentations reflects sexual behavior. To evaluate calling during the presentation, a linear mixed model was fitted with sampling date and treatment as fixed effects and male identity as a random effect (intercept). Female identity was also included as a random effect (intercept) because each female was presented to multiple males and female vocalizations were included in tallies. We were unable to fit mixed models for the two other response variables: rate of calling together and call rate post-presentation. Therefore, we instead used the Mann–Whitney *U*-test to test for an effect of treatment on vocal behavior at each time point.

Survival analyses were carried out in Statistica (v. 10.0, TIBCO Software Inc., Palo Alto, CA, USA). All other statistical analyses were run in R (http://www.R-project.org). The package lme4 (Bates et al., 2015) was used for linear mixed models. Residual plots from models were visually inspected to check for deviations from normality and homoscedasticity. Estimates (β) for fixed effects in models are presented ±s.e.m.

RESULTS

Initiation of breeding experiment

Testis length increased significantly over the course of the experiment (Fig. 2A; day: β=0.010±0.001, χ^2_1=57.84, *P*<0.0001), but there was no significant effect of treatment (χ^2_1=0.24, *P*=0.63) and no interaction between day and treatment (χ^2_1=0.45, *P*=0.51). Similarly, CP length increased significantly over the experiment (Fig. 2B; day: β=0.0081±0.0011, χ^2_1=43.67, *P*<0.0001), but there was no significant effect of treatment (χ^2_1=0.11, *P*=0.73) or day by treatment interaction (χ^2_1=0.94, *P*=0.33). There was a significant quadratic effect of day on circulating testosterone (Fig. 2C; day^2: β=1.55±0.40, χ^2_1=12.99, *P*=0.0003), but no significant effect of treatment (χ^2_1=0.038, *P*=0.85) or day^2 by treatment interaction (χ^2_2=0.32, *P*=0.85).

We observed very little singing behavior in response to the female presentations (only two occurrences in 43 presentations). Thus, we present results on calling behavior. In fitting a model for calling during presentation, one data point was an outlier, with an extremely high calling rate. We ran the model with and without the outlier and the results were qualitatively the same, leading to the same interpretation. As the fit of the model was much better with the outlier excluded, we present those results here. There was a significant effect of sample day on calling rate, with birds calling more during the first presentation compared with the second ($\beta_{SampleDay2}$=−10.59±2.62, χ^2_1=11.63, *P*=0.0007). However, there was no significant effect of treatment on calling (χ^2_1=0.12, *P*=0.73) and no significant interaction between treatment and day (χ^2_1=0.65, *P*=0.42). There was also no significant difference between treatment groups in the rate of calling together at the first (*U*=62, *P*=0.95) or second (*U*=59.5, *P*=0.64) presentation. Additionally, there was no significant difference between treatment groups in calling during the post-presentation period in either the first (*U*=60, *P*=0.72) or second (*U*=64.5, *P*=0.47) presentation. There was no difference in testosterone levels between the treatment groups following the female presentation (Fig. 2C; *t*=0.052, d.f.=18.8, *P*=0.96) and testosterone levels were similar to those measured at other times in the study (in the absence of a female presentation).

There was a significant increase in body mass over the course of the experiment (β=0.0070±0.0019, χ^2_1=13.20, *P*=0.0003). But, there was no significant effect of treatment (χ^2_1=0.72, *P*=0.40) and no significant interaction of day and treatment (χ^2_1=0.08, *P*=0.77). For body fat, we found no significant effect of day (χ^2_1=0.86, *P*=0.35) or treatment (treatment: χ^2_1=0.52, *P*=0.47; day×treatment: χ^2_1=0.54, *P*=0.46).

There was no significant difference in circulating corticosterone levels between groups (control: 6.11±2.12 ng ml^{-1}, warm: 7.16±3.93 ng ml^{-1}, *U*=62, *P*=0.95). Three birds (two control, one warm) had unusually high corticosterone levels compared with other

Journal of Experimental Biology

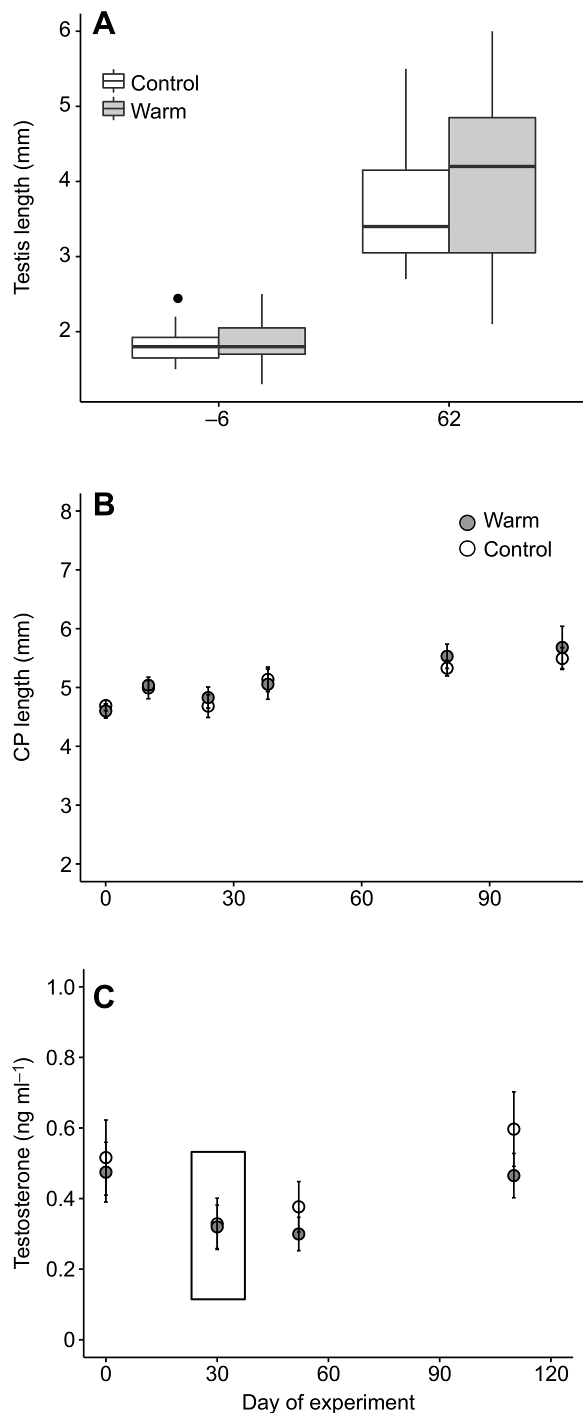

Fig. 2. Response to temperature manipulation around initiation of reproduction. Testis length (A), cloacal protuberance (CP) length (B) and circulating testosterone levels (C) in male house finches housed at control (mean 19.2°C, white symbols, n=10–11 birds depending on time point) and warm (mean 24.3°C, gray symbols, n=10–11 birds depending on time point) temperatures from January to May, when gonadal recrudescence typically occurs. The testosterone data in a box indicate samples collected following presentation of a female. There were no significant effects of treatment or day by treatment interactions on any of the dependent variables (linear mixed models, P>0.05). Data are presented as box plots with outliers shown as individual data points (A) or means±s.e.m. (B,C).

birds in the experiment. When we excluded these outliers from the analysis, we still found no significant difference between groups (U=42, P=0.84).

Termination of breeding and onset of molt experiment

The testes of most birds were smaller than typically observed in breeding males (Hamner, 1966), suggesting that testes were in regression. There was a trend for birds experiencing warmer temperatures to have smaller testes than control birds at the end of the experiment, although this did not reach statistical significance (Fig. 3A; t=1.84, d.f.=15.18, P=0.086). Testis length of experimental birds was similar to that of free-living birds sampled at the same time, with data from free-living birds being intermediate between the experimental groups (Fig. 3A). Histological examination of the testes at the end of the experiment indicated minimal spermatogenic activity in testes from both experimental groups. Some birds did still have spermatids or spermatozoa, but there were no significant differences between the experimental groups in either the presence of spermatids (control: 5/10, warm: 2/10, P=0.35) or the presence of spermatozoa (control: 3/10, warm: 2/10, P=1.0). CP length showed a significant day by treatment interaction (Fig. 3B; day×treatment: $\beta_{Day:TreatmentWarm}$=−0.0068±0.0026, χ^2_1=6.51, P=0.011; day: χ^2_1=208.62, P<0.0001; treatment: $\chi^2_1$1.04, P=0.31), with CP length declining faster in warm birds than control birds.

Circulating testosterone levels declined over the course of the experiment (Fig. 3A; day: β=−0.012±0.003, χ^2_1=17.26, P<0.0001). There was a trend for testosterone levels to be lower in the warm treatment (treatment: β_{Warm}=−0.49±0.24, χ^2_1=3.62, P=0.057; day×treatment: χ^2_1=1.54, P=0.21), but this appears to be driven primarily by an initial difference in testosterone levels between the groups prior to the start of the temperature manipulation. Given this unexpected finding, we were interested in whether the differences we observed in the onset of molt (described below) might be due to initial differences in circulating testosterone unrelated to the temperature treatments. However, this is not the case as there was no correlation between testosterone on day –8 (prior to temperature manipulation) and the date on which molt was initiated (Spearman's r=0.08, P=0.74). There was no significant difference in circulating corticosterone levels between control (5.23±0.92 ng ml^{-1}) and warm (7.16±1.62 ng ml^{-1}) birds (U=38, P=0.60).

For body mass, we found a significant day by treatment interaction (day×treatment: $\beta_{Day:TreatmentWarm}$=−0.0009±0.0002, χ^2_1=21.64, P<0.0001; day: χ^2_1=2.64, P=0.10; treatment: β=−0.032±0.030, χ^2_1=3.89, P=0.049); control birds gained body mass across the experiment, whereas warm birds showed no change or in some cases a slight decline (Fig. S1). Body fat showed an increase across the experiment (β=0.010±0.003, χ^2_1=13.81, P=0.0002), but there was no effect of treatment (χ^2_1=1.13, P=0.29) and no day by treatment interaction (χ^2_1=1.30, P=0.25).

In the termination experiment, where the temperature manipulation occurred late and was maintained as birds began to molt, there was a trend for birds in the warm group to molt earlier than control birds, although it did not reach statistical significance (Fig. 4A; $F_{12,18}$=2.33, P=0.051). For birds from the initiation experiment that were returned to the control temperature prior to molt, there was a significant effect of prior temperature treatment on the onset of molt ($F_{20,20}$=2.17, P=0.045). Birds that had been in the warm treatment molted earlier than birds that had remained at the control temperature (Fig. 4B).

DISCUSSION

We found no evidence for adjustments in the timing of reproductive preparations of male house finches in direct response to temperature. In the initiation experiment, there was no effect of temperature treatment on reproductive physiology. Similarly, we saw no effect

Journal of Experimental Biology

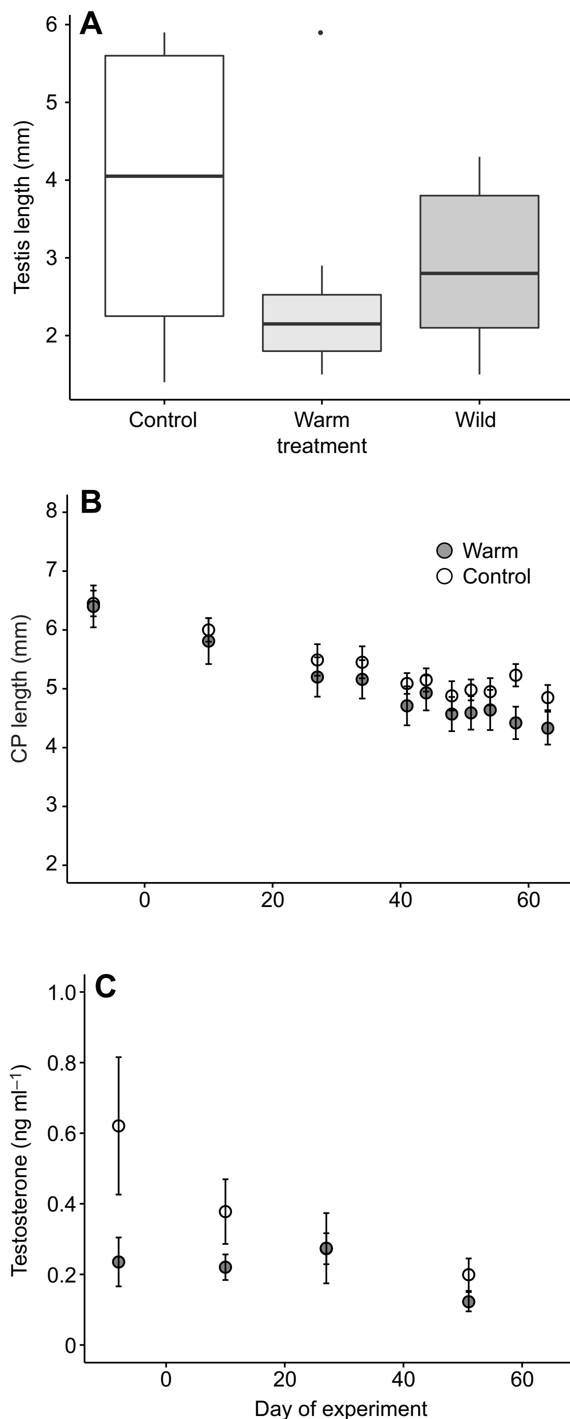

Fig. 3. Response to temperature manipulation around termination of reproduction. Testis length (A), cloacal protuberance (CP) length (B) and circulating testosterone (C) in male house finches housed at control (mean 21.4°C, white symbols, *n*=10 birds) and warm (mean 27.7°C, gray symbols, *n*=10 birds) temperatures from May to July, when the breeding–molt transition typically occurs. Testis lengths were measured at the end of the experiment and data from free-living ('Wild') birds for the same time period are shown for comparison. There is a trend for warm birds to have smaller testes than control birds (*t*-test, *P*=0.086). There was a significant day by treatment interaction effect on CP length (linear mixed model, *P*=0.011). There was a trend for testosterone to be influenced by treatment owing to differences between groups prior to temperature manipulation (linear mixed model, *P*=0.057), but there was no significant day by treatment interaction (*P*=0.21). Data are presented as box plots with outliers shown as individual data points (A) or means±s.e.m. (B,C).

on reproductive behavior. Our tests of reproductive behavior (response to female presentation) were relatively short in duration. Although we did not observe atypical behavior, birds may have been more responsive if given longer to acclimate to the testing area or if given more time with the female. Additionally, female birds were not hormonally primed and may not have been in maximal breeding condition, and therefore may not have elicited strong responses from the males. Finally, because male and female calls could not be distinguished during the presentation period, we cannot account for differences in female behavior. Consequently, our behavioral results should be interpreted cautiously. However, the behavioral results are consistent with the various physiological measures, all of which showed no response to the temperature manipulation.

Our results showing a lack of effect of temperature on male reproductive physiology contrast with a number of previous studies on other bird species that have found that higher temperatures advance and lower temperatures delay testicular recrudescence (Engels and Jenner, 1956; Jones, 1986; Lewis and Farner, 1973; Perfito et al., 2005; Silverin and Viebke, 1994; Silverin et al., 2008; Storey and Nicholls, 1982; Wingfield et al., 2003). However, except for Perfito et al. (2005), these studies all used relatively large temperature differences compared with the more realistic temperatures used in the present study. We do not know whether house finches are sensitive to greater temperature differences, but the lack of an effect of temperature in this study could also reflect variation among species in temperature sensitivity. Experiments using relatively large temperature differences have found no effect on testicular recrudescence in some species (Caro and Visser, 2009; Dawson, 2005, 2018; Silverin and Viebke, 1994; Silverin et al., 2008; Wingfield et al., 1996; Wingfield et al., 1997). Further, we know that there can be variation even among populations in temperature sensitivity (Perfito et al., 2005; Silverin et al., 2008). Given that the breeding diet of house finches is so different from that of most species previously studied, it remains to be determined whether differences in temperature sensitivity might be related to differences in breeding diet.

Although we found no effect of temperature on the initiation of breeding, we did find indications of a direct effect of temperature on the transition from breeding to molt. Our results suggest that birds that experienced an increase in temperature terminated breeding and began molt earlier than control birds. Interestingly, we observed an effect on the timing of molt even when birds experienced the temperature increase in advance of the breeding season and were maintained at the same temperature for ∼1.5 months before the onset of molt. Thus, house finches are sensitive to temperature well in advance of when they begin to molt. Similarly, Schaper et al. (2012) found that great tits (*Parus major*) adjust the timing of egg laying in response to temperature changes 1–1.5 months before egg laying. It should be noted that the 'warm' birds in this experiment also experienced a decline in temperature when they were returned to the control temperature. Thus, we cannot rule out the possibility that the temperature decline influenced the timing of molt in this treatment group, although this would still indicate a response to a temperature change occurring at least 1 month prior. Our results raise the question of why house finches would use temperature as a predictive cue to time the breeding–molt transition so far in advance. Although there is no clear answer, we speculate that perhaps elevated temperatures serve as an indicator of summer conditions and thus hasten the transition in anticipation of autumn.

Overall, our evidence that warmer temperatures advance the breeding–molt transition in house finches is consistent with studies in other species that have found that higher temperatures advance and lower temperatures delay the onset of gonadal regression and

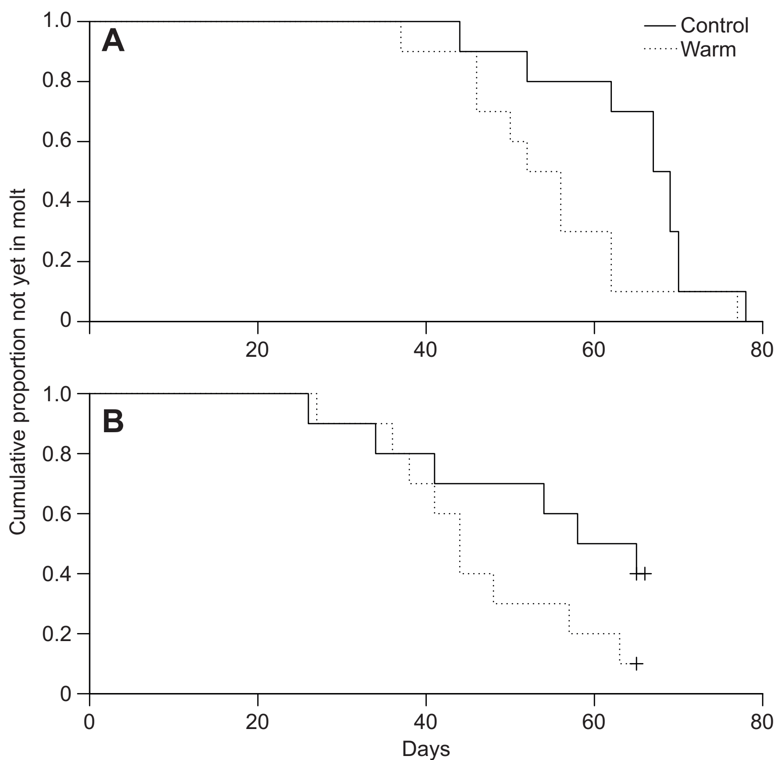

Fig. 4. Onset of molt in male house finches in control and warm treatment groups. (A) Data from birds from the initiation experiment where temperature manipulation occurred early in the year is shown (Cox's *F*-test, *P*=0.045). (B) Data from birds from the termination experiment where temperature manipulation occurred around the onset of molt is shown (Cox's *F*-test, *P*=0.051). *N*=10 birds per treatment group. Lines do not reach zero because some birds had not yet initiated molt when the experiment ended (indicated by crosses).

molt (Dawson, 2005, 2018; Silverin and Viebke, 1994; Silverin et al., 2008; Storey and Nicholls, 1982; Visser et al., 2011; Wingfield et al., 2003). In these previous studies, temperature differences between groups were established prior to gonadal recrudescence and in most cases influenced the timing of reproductive development. Consequently, it is difficult to distinguish whether temperature had a direct influence on the timing of gonadal regression and molt or whether earlier gonadal development advanced the timing of subsequent events in the annual cycle. In this study, we manipulated temperature after the onset of gonadal recrudescence and found evidence for a direct effect of temperature on the breeding–molt transition (based on CP data and consistent trends in other measures). Furthermore, when we manipulated temperature in advance of gonadal recrudescence, we found an effect on the onset of molt in the absence of any effect on gonadal recrudescence. This latter finding is very similar to what Dawson (2005) observed in starlings (*Sturnus vulgaris*), again suggesting that temperature is directly influencing the timing of the breeding–molt transition.

The mechanism(s) by which temperature influences the timing of events in the annual cycle, such as reproduction, remains poorly understood (Caro et al., 2013). Although elevated testosterone levels can delay the onset of molt (Nolan et al., 1992; Schwabl and Farner, 1989), here we found no evidence that temperature-induced differences in the timing of the breeding–molt transition resulted from differences in circulating testosterone. We also saw no evidence that temperature-induced differences in the breeding–molt transition resulted from differences in circulating corticosterone, although corticosterone is known to have inhibitory effects on reproductive function (Salvante and Williams, 2003; Wilson and Follett, 1975). Our corticosterone results should be interpreted cautiously since we collected only a single sample from each individual. Thus, individual differences in corticosterone (Cockrem and Silverin, 2002; Williams, 2008) could obscure effects of temperature. Prolactin and/or thyroid hormones remain candidate endocrine mechanisms by which temperature could influence the timing of gonadal regression and molt (Caro et al., 2013; Dawson, 2005; Wingfield et al., 2003). Intriguingly, a recent study provides evidence that ambient temperature may influence reproductive timing through effects on daily cycles of body temperature, in which case thyroid hormones are a likely endocrine mechanism to mediate this process (Dawson, 2018).

Overall, our results suggest that increasing ambient temperature may constrain the length of the breeding season for male house finches, with the transition from breeding to molt occurring earlier at higher temperatures. We found no evidence that elevated temperatures influenced male reproductive physiology at the start of the breeding season. However, temperature could still influence the timing of initiation of breeding in free-living house finches either through indirect effects via another cue, such as plant phenology, that is temperature sensitive (Thomas et al., 2010) or through direct effects on female reproduction. Given that house finches can adjust reproductive physiology in response to food availability (Hahn et al., 2005; Valle et al., 2015), and sex differences in sensitivity to temperature during gonadal recrudescence have been documented in other species (Lewis and Farner, 1973; Wingfield et al., 1997), these are both plausible scenarios.

Acknowledgements
We thank John Waggoner and Katherine Boyd for help with animal care and data collection. Kendra Sewall provided valuable input on the female presentations. We thank two anonymous reviewers for feedback on earlier versions of the manuscript.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: H.E.W.; Methodology: H.E.W.; Formal analysis: H.E.W.; Investigation: H.E.W., D.J., V.P., T.P.V.; Writing - original draft: H.E.W.; Writing - review & editing: H.E.W., D.J., V.P., T.P.V.; Supervision: H.E.W.; Project administration: H.E.W.; Funding acquisition: H.E.W.

Funding
Support for this research was provided by the National Science Foundation (IOS-1456954/1756976 to H.E.W.) and the Santa Monica Bay Audubon Society.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.185058.supplemental

References
Badyaev, A. V., Belloni, V. and Hill, G. E. (2012). House Finch (*Haemorhous mexicanus*), version 2.0. In *The Birds of North America* (ed. P. G. Rodewald). Ithaca, New York: Cornell Lab of Ornithology. https://doi.org/10.2173/bna.46
Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1-48.
Bauchinger, U., Van't Hof, T. and Biebach, H. (2009). Food availability during migratory stopover affects testis growth and reproductive behaviour in a migratory passerine. *Horm. Behav.* **55**, 425-433.
Beal, E. E. L. (1907). *Birds of California in Relation to the Fruit Industry, Part 1*. Biological Survey, Bulletin no. 30. Washington, DC: US Department of Agriculture, Biological Survey.
Caro, S. P. and Visser, M. E. (2009). Temperature-induced elevation of basal metabolic rate does not affect testis growth in great tits. *J. Exp. Biol.* **212**, 1995-1999.
Caro, S. P., Schaper, S. V., Hut, R. A., Ball, G. F. and Visser, M. E. (2013). The case of the missing mechanism: how does temperature influence seasonal timing in endotherms? *PLoS Biol.* **11**, e1001517.
Cockrem, J. F. and Silverin, B. (2002). Variation within and between Birds in Corticosterone Responses of Great Tits (Parus major). *Gen. Comp. Endocrinol.* **125**, 197-206.
Davies, S. and Deviche, P. (2014). At the crossroads of physiology and ecology: food supply and the timing of avian reproduction. *Horm. Behav.* **66**, 41-55.
Dawson, A. (2005). The effect of temperature on photoperiodically regulated gonadal maturation, regression and moult in starlings – potential consequences of climate change. *Funct. Ecol.* **19**, 995-1000.
Dawson, A. (2018). Both low temperature and shorter duration of food availability delay testicular regression and affect the daily cycle in body temperature in a Songbird. *Physiol. Biochem. Zool.* **91**, 917-924.
Deviche, P. (1992). Testosterone and opioids interact to regulate feeding in a male migratory songbird. *Horm. Behav.* **26**, 394-405.
Deviche, P. and Cortez, L. (2005). Androgen control of immunocompetence in the male house finch, *Carpodacus mexicanus* Müller. *J. Exp. Biol.* **208**, 1287-1295.
Dunn, P. O. and Winkler, D. W. (2010). Effects of climate change on timing of breeding and reproductive succes in birds. In *Effects of Climate Change on Birds* (ed. A. P. Møller, W. Fiedler and P. Berthold), pp. 113-128. Oxford: Oxford University Press.
Engels, W. L. and Jenner, C. E. (1956). The effect of temperature on testicular recrudescence in juncos at different photoperiods. *Biol. Bull.* **110**, 129-137.
Goymann, W. (2009). Social modulation of androgens in male birds. *Gen. Comp. Endocrinol.* **163**, 149-157.
Hahn, T. P. (1995). Integration of photoperiodic and food cues to time changes in reproductive physiology by an opportunistic breeder, the red crossbill, *Loxia curvirostra* (Aves: Carduelinae). *J. Exp. Zool.* **272**, 213-226.
Hahn, T. P., Pereyra, M. E., Katti, M., Ward, G. M. and MacDougall-Shackleton, S. A. (2005). Effects of food availability on the reproductive system. In *Functional Avian Endocrinology* (ed. A. Dawson and P. J. Sharp), pp. 167-180. New Delhi, India: Narosa Publishing House.
Hamner, W. M. (1966). Photoperiodic control of the annual testicular cycle in the house finch, *Carpodacus mexicanus*. *Gen. Comp. Endocrinol.* **7**, 224-233.
Hau, M., Wikelski, M. and Wingfield, J. C. (2000). Visual and nutritional food cues fine-tune timing of reproduction in a neotropical rainforest bird. *J. Exp. Zool.* **286**, 494-504.
Hill, G. E. (2002). *A Red Bird in a Brown Bag: The Function and Evolution of Colorful Plumage in the House Finch*. New York: Oxford University Press.
Jones, L. R. (1986). The effect of photoperiod and temperature on testicular growth in captive black-billed magpies. *Condor* **88**, 91-93.
Lattin, C. R., Breuner, C. W. and Romero, L. M. (2016). Does corticosterone regulate the onset of breeding in free-living birds?: the CORT-Flexibility Hypothesis and six potential mechanisms for priming corticosteroid function. *Horm. Behav.* **78**, 107-120.
Lee, E. T. and Wang, J. W. (2003). *Statistical Methods for Survival Data Analysis*, 3rd edn. Hoboken, NJ: John Wiley & Sons, Inc.
Lewis, R. A. and Farner, D. S. (1973). Temperature modulation of photoperiodically induced vernal phenomena in white-crowned sparrows (*Zonotrichia leucophrys*). *Condor* **75**, 279-286.
Ligon, J. D. (1974). Green cones of the piñon pine stimulate late summer breeding in the piñon jay. *Nature* **250**, 80-82.
Meijer, T. and Schwabl, H. (1989). Hormonal patterns in breeding and nonbreeding kestrels, Falco tinnunculus: field and laboratory studies. *Gen. Comp. Endocrinol.* **74**, 148-160.
Moore, M. C. (1983). Effect of female sexual displays on the endocrine physiology and behaviour of male White-crowned sparrows, *Zonotrichia leucophrys*. *J. Zool.* **199**, 137-148.
Nolan, V., Ketterson, E. D., Ziegenfus, C., Cullen, D. P. and Chandler, C. R. (1992). Testosterone and avian life histories: effects of experimentally elevated testosterone on prebasic molt and survival in male dark-eyed juncos. *Condor* **94**, 364-370.
Perfito, N., Meddle, S. L., Tramontin, A. D., Sharp, P. J. and Wingfield, J. C. (2005). Seasonal gonadal recrudescence in song sparrows: response to temperature cues. *Gen. Comp. Endocrinol.* **143**, 121-128.
Salvante, K. G. and Williams, T. D. (2003). Effects of corticosterone on the proportion of breeding females, reproductive output and yolk precursor levels. *Gen. Comp. Endocrinol.* **130**, 205-214.
Schaper, S. V., Dawson, A., Sharp, P. J., Gienapp, P., Caro, S. P. and Visser, M. E. (2012). Increasing temperature, not mean temperature, is a cue for avian timing of reproduction. *Am. Nat.* **179**, E55-E69.
Schwabl, H. and Farner, D. S. (1989). Endocrine and environmental control of vernal migration in male white-crowned sparrows, *Zonotrichia leucophrys gambelii*. *Physiol. Zool.* **62**, 1-10.
Schwabl, H. and Kriner, E. (1991). Territorial aggression and song of male European robins (Erithacus rubecula) in autumn and spring: effects of antiandrogen treatment. *Horm. Behav.* **25**, 180-194.
Silverin, B. and Viebke, P. A. (1994). Low temperatures affect the photoperiodically induced LH and testicular cycles differently in closely related species of tits (*Parus* spp.). *Horm. Behav.* **28**, 199-206.
Silverin, B., Wingfield, J., Stokkan, K.-A., Massa, R., Järvinen, A., Andersson, N.-Å., Lambrechts, M., Sorace, A. and Blomqvist, D. (2008). Ambient temperature effects on photo induced gonadal cycles and hormonal secretion patterns in Great Tits from three different breeding latitudes. *Horm. Behav.* **54**, 60-68.
Storey, C. R. and Nicholls, T. J. (1982). Low environmental temperature delays photoperiodic induction of avian testicular maturation and the onset of post-nuptial photorefractoriness. *Ibis* **124**, 172-174.
Thomas, D. W., Bourgault, P., Shipley, B., Perret, P. and Blondel, J. (2010). Context-dependent changes in the weighting of environmental cues that initiate breeding in a temperate passerine, the Corsican blue tit (*Cyanistes caeruleus*). *Auk* **127**, 129-139.
Thompson, W. L. (1960). Agonistic behavior in the House Finch. Part I: annual cycle and display patterns. *Condor* **62**, 245-271.
Valle, S., Carpentier, E., Vu, B., Tsutsui, K. and Deviche, P. (2015). Food restriction negatively affects multiple levels of the reproductive axis in male house finches, *Haemorhous mexicanus*. *J. Exp. Biol.* **218**, 2694.
Visser, M. E., Holleman, L. J. M. and Caro, S. P. (2009). Temperature has a causal effect on avian timing of reproduction. *Proc. R. Soc. B* **276**, 2323.
Visser, M. E., Schaper, S. V., Holleman, L. J. M., Dawson, A., Sharp, P. J., Gienapp, P. and Caro, S. P. (2011). Genetic variation in cue sensitivity involved in avian timing of reproduction. *Funct. Ecol.* **25**, 868-877.
Vose, R. S., Applequist, S., Squires, M., Durre, I., Menne, M. J., Williams, C. N., Jr., Fenimore, C., Gleason, K. and Arndt, D. (2014). NOAA's Gridded Climate Divisional Dataset (CLIMDIV). In *NOAA National Climatic Data Center*, accessed: Oct. 12, 2015.
Watts, H. E. and Hahn, T. P. (2012). Non-photoperiodic regulation of reproductive physiology in the flexibly breeding pine siskin (*Spinus pinus*). *Gen. Comp. Endocrinol.* **178**, 259-264.
Williams, T. D. (2008). Individual variation in endocrine systems: moving beyond the 'tyranny of the Golden Mean'. *Philos. Trans. R. Soc. B Biol. Sci.* **363**, 1687-1698.
Wilson, F. E. and Follett, B. K. (1975). Corticosterone-induced gonadosuppression in photostimulated tree sparrows. *Life Sci.* **17**, 1451-1456.
Wingfield, J. C. and Farner, D. S. (1978). The endocrinology of a natural breeding population of the white-crowned sparrow (*Zonotrichia leucophrys pugetensis*). *Physiol. Zool.* **51**, 188-205.
Wingfield, J. C., Hegner, R. E., Dufty, A. M., Jr and Ball, G. F. (1990). The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am. Nat.* **136**, 829-846.
Wingfield, J. C., Hahn, T. P., Levin, R. and Honey, P. (1992). Environmental predictability and control of gonadal cycles in birds. *J. Exp. Zool.* **261**, 214-231.
Wingfield, J. C., Hahn, T. P., Wada, M., Astheimer, L. B. and Schoech, S. (1996). Interrelationship of day length and temperature on the control of gonadal development, body mass, and fat score in white-crowned sparrows, *Zonotrichia leucophrys gambelii*. *Gen. Comp. Endocrinol.* **101**, 242-255.
Wingfield, J. C., Hahn, T. P., Wada, M. and Schoech, S. J. (1997). Effects of day length and temperature on gonadal development, body mass, and fat depots in white-crowned sparrows, *Zonotrichia leucophrys pugetensis*. *Gen. Comp. Endocrinol.* **107**, 44-62.
Wingfield, J. C., Hahn, T. P., Maney, D. L., Schoech, S. J., Wada, M. and Morton, M. L. (2003). Effects of temperature on photoperiodically induced reproductive development, circulating plasma luteinizing hormone and thyroid hormones, body mass, fat deposition and molt in mountain white-crowned sparrows, *Zonotrichia leucophrys oriantha*. *Gen. Comp. Endocrinol.* **131**, 143-158.