

Reproductive Allochrony in Seasonally Sympatric Populations Maintained by Differential Response to Photoperiod: Implications for Population Divergence and Response to Climate Change

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ABSTRACT: Reproductive allochrony presents a potential barrier to gene flow and is common in seasonally sympatric migratory and sedentary birds. Mechanisms mediating reproductive allochrony can influence population divergence and the capacity of populations to respond to environmental change. We asked whether reproductive allochrony in seasonally sympatric birds results from a difference in response to supplementary or photoperiodic cues and whether the response varies in relation to the distance separating breeding and wintering locations as measured by stable isotopes. We held seasonally sympatric migratory and sedentary male dark-eyed juncos (*Junco hyemalis*) in a common garden in early spring under simulated natural changes in photoperiod and made measurements of reproductive and migratory physiology. On the same dates and photoperiods, sedentary juncos had higher testosterone (initial and gonadotropin-releasing hormone induced), more developed cloacal protuberances, and larger testes than migrants. In contrast, migratory juncos had larger fat reserves (fuel for migration). We found a negative relationship between testis mass and feather hydrogen isotope ratios, indicating that testis growth was more delayed in migrants making longer migrations. We conclude that reproductive allochrony in seasonally sympatric migratory and sedentary birds can result from a differential response to photoperiodic cues in a common garden, and as a result, gene flow between migrants and residents may be reduced by photoperiodic control of reproductive development. Further, earlier breeding in response to future climate change may currently be constrained by differential response to photoperiodic cues.

Keywords: breeding phenology, gonadal development, junco, migration, reproductive allochrony, sympatry.

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Introduction

Organismal and evolutionary mechanisms that underlie divergence in seasonal strategies have important implications for both the generation and the maintenance of biodiversity (Ricklefs 2000; Ricklefs and Wikelski 2002; Wikelski and Cooke 2006; Wingfield 2012) as well as the biotic responses to environmental change (Cohen et al. 2012; Wingfield 2015). Seasonally breeding animals use a combination of predictive and supplementary environmental cues to time reproductive development with the phenology of local resource pulses. Photoperiod (day length), the primary predictive cue used by most temperate breeding animals to time seasonal changes (Bronson and Heideman 1994; Dawson et al. 2001; Bronson 2009), is robust due to the invariability of the annual orbit of the earth around the sun. Nonphotic cues (supplementary cues) such as food abundance, weather conditions, and social interactions vary from year to year and serve to fine-tune seasonal timing decisions (Charmantier et al. 2008; Bronson 2009; Visser et al. 2009; Davies et al. 2015). Numerous studies have identified general signal transduction response mechanisms by which predictive and nonphotic supplementary cues are integrated into seasonal timing decisions (Rasmussen et al. 2008; Schoech et al. 2009; Ramenofsky et al. 2012; Wingfield 2012), and the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes (and their potential interactions) are key to explaining variation in the relative importance of predictive and nonphotic cues in seasonal timing.

In general, in the temperate zone, where day length varies widely on a seasonal basis, supplementary cues are considered to induce relatively minor adjustments in phenology as compared to changes in day length (Wingfield et al. 1992).

In some situations, however, particularly in comparisons of populations residing at the same latitude and photoperiod but differing in when they reproduce, supplementary cues have been shown to substantially alter the timing of gonadal development (El-Bakry et al. 1999; Perfito et al. 2004; Wube et al. 2008; Atwell et al. 2014). Corsican blue tits (*Parus caeruleus*) provide an example. Two populations found only 25 km apart differ by 1 month in the onset of egg laying. As an ultimate explanation, this difference in reproductive timing has been attributed to female adaptation to habitat differences in optimal breeding time (Caro et al. 2009).

In other, more widely distributed temperate-zone species in which some populations migrate while others do not, migratory and sedentary populations frequently coexist during late fall, winter, and early spring under sympatry (fig. 1). Despite experiencing similar photoperiodic and supplementary cues on wintering habitats throughout a large portion of the annual cycle, there is often clear phenological divergence in spring, when individuals from sedentary populations transition into breeding, while migrants delay reproduction, prepare for migration, and complete their journeys northward before breeding. As a result, sedentary and migratory birds are allopatric during their peak breeding seasons but are seasonally sympatric during periods of overlap (heteropatry; Getz and Kaitala 1989; Winker 2010; Ketterson et al. 2015).

Situations such as these provide an excellent opportunity to examine three important questions. First, at a proximate level, do such populations differ in timing because they differ in their response to day length or to supplementary cues? Second, from an evolutionary perspective, how might the mode of regulation of timing influence gene flow between migratory and nonmigratory populations that coexist on a seasonal basis? Third, from both an ecological and an evolutionary perspective, how might the mode of regulation of timing, day length or supplementary cues, influence how distributions and patterns of gene flow will be altered by climate change?

With respect to the first question, study of seasonally sympatric populations makes it possible to examine the physiological mechanisms that allow closely related populations to adapt to different breeding environments despite identical exposure to environmental cues during much of the annual cycle. With respect to the second and third questions, if differential responses to supplementary cues are the primary cause of allochrony, a change in the environment could lead to earlier reproduction in migrants and thus a greater chance of gene flow between migratory and sedentary populations. Alternatively, if the primary cause of allochrony is differential responses to spring photoperiod, seasonal sympatry may promote population divergence but slow phenological responses to environmental change.

Here, we investigated developmental and endocrinological components of divergence in timing of reproductive de-

velopment for two seasonally sympatric subspecies of dark-eyed juncos (*Junco hyemalis*) and evaluated the association between migratory distance and timing of reproductive maturation. We asked whether reproductive allochrony observed between seasonally sympatric migrant *hyemalis* and sedentary *carolinensis* juncos results from a differential response to photoperiodic versus supplementary cues. Using stable hydrogen isotopes, we also asked whether the differential response to photoperiodic cues was affected by migratory distance. We measured morphological and physiological correlates of reproductive and migratory development across 4 weeks in early spring for birds held in a captive common garden that simulated natural photoperiod and provided ad lib. food, mild temperatures, and reduced social interactions. If supplementary cues suppress gonad development and delay reproduction in migratory juncos, we predicted convergence of seasonal timing of gonadal development and endocrine profiles of migratory and sedentary juncos in the common garden. Alternatively, if reproduction of migrants is delayed because the HPG axis of migratory and sedentary juncos responds differentially to photoperiodic cues, we predicted that timing of gonadal development and endocrine profiles should remain divergent in the common garden.

If response to photoperiod differed among migrants depending on how far their winter site was from their breeding site, we predicted that those with the longest spring migrations might be the last to exhibit gonadal developments. Thus, we also measured stable hydrogen isotope ratios in feathers grown on breeding grounds to evaluate the scope of geographic variation in breeding latitude of migrants and to test for associations between breeding latitude and the phenology of reproductive development.

Material and Methods

Study Species

Slate-colored dark-eyed juncos, small passerine birds that breed in temperate forests throughout much of North America, exhibit a continent-wide distribution across breeding and wintering ranges, including a latitudinal band in which they are observed year-round (fig. 1; Nolan 2002). In more northerly latitudes, migratory slate-colored dark-eyed juncos (*Junco hyemalis hyemalis*) breed across primarily boreal and mixed coniferous-deciduous forests of Alaska, Canada, and northern New England, migrating south in early autumn and spending the nonbreeding season throughout the central and eastern United States (fig. 1). At the southerly end of the slate-colored junco breeding range, where coniferous forests are found at higher elevations of the Appalachian Mountains, sedentary slate-colored dark-eyed juncos (*Junco hyemalis carolinensis*) remain at or near breeding sites year-round and are thus seasonally sympatric with the migratory subspecies (*J. h.*

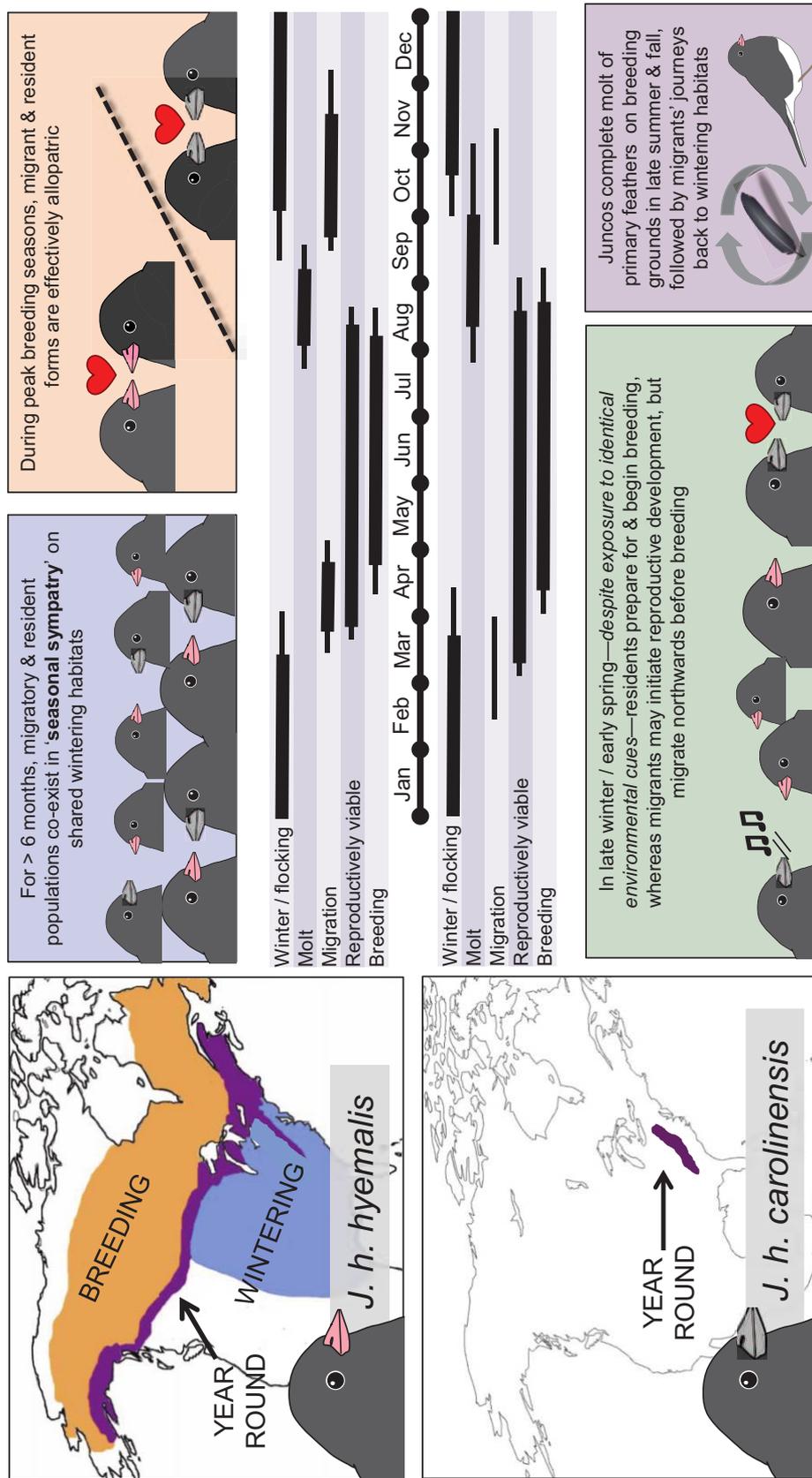


Figure 1: Annual geographic distribution of seasonally sympatric migratory and sedentary dark-eyed juncos (*Junco hyemalis*). After fall migration, migratory and sedentary juncos mix at shared wintering habitats (blue). In early spring, while still in sympatry, sedentary juncos begin preparations to breed, while migrants prepare for migration, despite exposure to identical environmental cues. Figure by J. W. Atwell.

hyemalis) from early October to late April each season (fig. 1). Throughout this period, wintering *hyemalis* and sedentary *carolinensis* juncos, which are distinguishable based on slight differences in bill and plumage coloration, are routinely observed together, often foraging in mixed flocks (Cristol et al. 2003). In late winter and early spring, when resources are still scarce and conditions harsh, sedentary juncos prepare to breed in the Appalachians, while migratory juncos delay reproduction and prepare for spring migration northward (Nolan 2002), despite exposure to similar photoperiodic and supplementary cues during the prior ~6 months.

Bird Capture and Housing

From December 4 to 12, 2013, we captured 11 migratory and nine sedentary male dark-eyed juncos at the University of Virginia's Mountain Lake Biological Station in Giles County (37.37°N, 80.52°W) using mist nets. We determined subspecies using bill coloration (*J. h. hyemalis*, pink bill; *J. h. carolinensis*, gray bill) and wing chord differences (Ketterson and Nolan 1976). Age class was determined for each bird based on wing plumage color, as dark-eyed juncos have a limited first prebasic molt, making it possible to distinguish between first-year birds and after-first-year birds (Nolan 2002; Cristol et al. 2003).

After capture, we housed birds temporarily for 1–10 days (depending on capture date) in identical outdoor aviaries at Mountain Lake Biological Station, where they were provided food (2:1 mixture of white millet and cracked corn) and water ad lib. On December 14, we transported the birds by car to Indiana University, where they were housed in mixed flocks consisting of equal proportions of migrants and residents in two climate-controlled indoor aviaries (6.4 m × 3.2 m × 2.4 m). At Indiana University, we provided water containing Nekton-S Multi-Vitamin for Birds (Arcata Pet, Arcata, CA) and food ad lib. Birds were fed a seed mix containing white millet and sunflower chips (2:1), mealworms, orange slices, and a soft diet containing ground puppy chow, hard-boiled eggs, and carrots.

On February 27, we individually housed birds in 61 × 46 × 46-cm cages and separated them into seven replicate rooms (2.5 m × 2.1 m × 2.4 m). Each room housed six birds, three migrants and three residents. Half of the birds in six rooms (three birds/room) were being held for another experiment. The seventh room contained only two birds for the current experiment (one migrant and one resident). Due to a minor injury prior to the start of the experiment, we did not include one sedentary bird in the weekly blood sampling and measurement protocols (described below); however, it recovered by the end of the experiment, so we included it in our analysis of feathers and gonad mass. Final sample sizes were 11 migrants and eight residents for weekly measurements and 11 migrants and nine residents for feather and

gonad mass comparisons. In each room, we arranged cages so that birds were visually isolated from each other. We adjusted lights in the aviary every 3 ± 1 days to track the natural seasonal changes in day length from the site of capture. Throughout the experiment, the temperature in the aviary was maintained at $16^\circ \pm 2^\circ\text{C}$.

All sampling procedures were approved by the Indiana University Institutional Animal Care and Use Committee and conducted under scientific collecting permits issued by the Virginia Department of Game and Inland Fisheries (permit 47553) and the US Fish and Wildlife Service (permit MB093279).

Blood Sample Collection

We collected small blood samples (~150 μL total) from each bird every 6 or 7 days from March 4 to 26 to measure plasma corticosterone (CORT), initial plasma testosterone, and plasma testosterone in response to a standardized "gonadotropin-releasing hormone (GnRH) challenge" protocol (testosterone post-GnRH). In brief, the GnRH challenge protocol involves collecting a blood sample 30 min after an intramuscular injection of GnRH, which is administered following the initial blood sample. This assay allows for a robust and repeatable measure of an individual male's ability to produce testosterone in response to a standardized physiological stimulus and is known to vary seasonally in relation to reproductive development in juncos (see Jawor et al. 2006 for details). Blood samples for plasma CORT and initial testosterone (~75 μL) were almost invariably taken within 3 min of entering a room, and rooms were entered only once per sampling day. We did not include blood taken after 3 min in our analysis of plasma CORT. Five minutes after entering a room (2–5 min after initial blood sample), we injected birds with 1.25 μg chicken GnRH (American peptide 54-8-23) in 50 μL of phosphate buffer solution. Thirty minutes after an injection, we collected a second blood sample (~75 μL) to assess an individual male's potential maximum testosterone. Samples were kept at 4°C until they were centrifuged (within 4 h). We collected plasma using a Hamilton syringe, and aliquots were frozen at -20°C until they were assayed.

Morphological Measurements

Each sampling day, after the last blood sample was collected from an individual, we measured subcutaneous fat and cloacal protuberance volume. Seasonal fat deposition in passerines is predicted and documented to vary seasonally in relation to transitions between both (1) wintering and breeding condition (reduced fat) and (2) wintering and migratory condition (increased fat; Clark 1979). We visually estimated an ordinal fat score (0–4) for subcutaneous fat deposition in the furcular and abdominal regions, separately, as follows:

0 = no fat present, 1 = trace amounts, 2 = half full, 3 = full, and 4 = bulging. We summed furcular and abdominal fat scores to obtain a total fat score (range 0–8).

The cloacal protuberance (CP), the terminal part of the passerine ductus deferens, is the primary location of sperm storage and is a reliable measure of breeding condition in male passerines (Wolfson 1952). Cloacal protuberance volume (V) was estimated by using the formula to calculate the volume of a cylinder ($V = \pi(\text{radius})^2(\text{height})$). Cloacal width (diameter; w , ± 0.1 mm) was measured with dial calipers, and height (h , ± 0.5 mm) was measured with a ruler, which allowed us to ensure that our measurement started flush against the body.

Gonadal Development

To investigate differences in the seasonal timing of gonadal development, at the end of the study (March 31 and April 1), we euthanized birds with isoflurane and dissected both testes, which were immediately flash frozen on dry ice and transferred to -80°C , where they were stored until weighed. To compare testis size among individuals and subspecies, we measured frozen testis mass of one testis from each bird in the lab using a digital microbalance accurate to ± 0.001 g.

Hormone Assays

Following established methods for our species (Jawor et al. 2006), we determined plasma CORT and testosterone concentrations from 10- and 20- μL aliquots, respectively, after a diethyl-ether extraction of steroids using enzyme immunoassays (CORT: 500655, Cayman Chemical; testosterone: ADI-900-065, Enzo Life Sciences). Intra- and interplate coefficients of variation were as follows: CORT: $n = 5$ plates, intraplate = $11.33\% \pm 1.8\%$ (mean \pm SE), interplate = 17.6% ; testosterone: $n = 10$ plates, intraplate = $6.33\% \pm 1.0\%$ (mean \pm SE), interplate = 15.9% . All plates had equal numbers of residents and migrants, and sampling dates were assigned equally across plates.

Hydrogen Isotopes in Feathers

On capture, we collected the outermost (distal) secondary feather from each bird's right wing to examine stable hydrogen isotope ($\delta^2\text{H}$) signatures. Because juncos complete their annual molt cycle before leaving breeding habitats or retain juvenile wing feathers throughout their first annual cycle (Nolan 2002), $\delta^2\text{H}$ of these feathers permits assignment of breeding latitude based on known patterns of isotopic variation in precipitation (Bowen et al. 2005). Feathers were stored in paper envelopes at room temperature and transported to Indiana University, where they were cleaned of oils using a 2:1 chloroform : methanol solution and al-

lowed to air-dry under a fume hood. Approximately 0.5 mg of feather tissue was clipped from the proximal end of each feather, weighed, and loaded into a 3×5 -mm pressed silver capsule. Plated capsules were shipped to the US Geological Survey Stable Isotope Laboratory in Denver. The nonexchangeable hydrogen isotope composition was measured by continuous-flow isotope ratio mass spectrometry using a Finnigan TC/EA interfaced to a Finnigan DeltaPlus XL mass spectrometer (Thermo Scientific, Bremen, Germany), using established methods (Wunder et al. 2012). The $\delta^2\text{H}$ values are reported in per mil notation (‰) relative to V-SMOW (Vienna Standard Mean Ocean Water), using internal standards (-78‰ and -172‰ , respectively) calibrated to CFS-CHS-BWB (chicken feather standard–cow hoof standard–bowhead whale baleen; Wassenaar and Hobson 2003). Benzoic acid ($\delta^2\text{H} = -61\text{‰}$) and IAEA-CH-7 ($\delta^2\text{H} = -100\text{‰}$) were also analyzed within analytical sequences with a precision of less than $\pm 4\text{‰}$.

Statistical Analyses

To analyze the effects of migratory strategy, sampling period, and the interaction between strategy and sampling period (fixed effects) on serial measures across the 4-week study (subcutaneous fat score, cloacal protuberance volume, plasma testosterone, testosterone post-GnRH challenge, and CORT), we used generalized linear mixed models, with individuals' identity included as a random factor. Age distribution was equal in migrants and residents (Fisher's exact test, $P = 1.00$; first-year residents = 3, adult residents = 5, first-year migrants = 4, adult migrants = 7); however, because first-year and adult birds are known to differ in the rate of sexual maturation (Dawson 2003), we included age as a factor in both the testosterone models and the cloacal protuberance volume model. A priori, we excluded three CORT, two baseline testosterone, and three post-GnRH testosterone outliers from statistical analyses. These samples were excluded because they were outside of the typical range we see in juncos.

We square root transformed gonad mass before all analyses to fit the data to a normal distribution. We tested for differences between migrant and resident hydrogen isotope ratios using independent sample t -tests. To analyze the effect of migratory strategy on gonad mass, we used a general linear model with age included as a covariate. To test for individual differences in timing of gonadal recrudescence associated with breeding latitude, we tested the correlation between gonad mass and hydrogen isotope values with a Pearson correlation as well as a linear regression for visualization of the relationship. Gonad mass was the only variable that we transformed. To test whether individual differences in fat score were associated with migratory distances travelled, we examined correlations between fat score and hydrogen isotope values with a Pearson correlation and lin-

ear regression for visualization of the relationship. We performed all statistical analyses using SPSS, version 22 (IBM). All reported P values are two-tailed. Data have been deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.d76mm> (Fudickar et al. 2015).

Results

Cloacal Protuberance Volume

Resident juncos had larger cloacal protuberances when compared to migrant juncos, and residents increased their cloacal protuberance volumes markedly throughout the experiment, whereas migrants did not (fig. 2a). Cloacal protuberance (CP) volumes were predicted by migratory strategy ($F_{1,15.35} = 23.941$, $P < .001$), sampling period (weeks 1–4; $F_{3,45.29} = 14.134$, $P < .001$), and the interaction between migratory strategy and sampling period ($F_{3,45.295} = 10.980$, $P < .001$). There was no effect of age ($F_{1,18.09} = 0.079$, $P = .781$), nor any interactions with age included.

Testosterone

Residents' initial plasma testosterone was higher than migrants' ($F_{1,15.29} = 14.306$, $P = .002$), and there was an increase across the experiment (sampling period: $F_{3,39.19} = 3.523$, $P = .024$) but no significant interaction between migratory strategy and sampling period ($F_{3,39.19} = 0.959$, $P = .422$; fig. 2b). We found no main effect of age on plasma testosterone or interactions between age and population or sampling period ($F_{1,15.29} = 0.429$, $P = .522$; $F_{3,39.19} = 0.490$, $P = .691$); however, there was a three-way interaction between week, age, and population ($F_{3,39.19} = 7.027$, $P = .001$). Post-GnRH plasma testosterone levels were higher in residents ($F_{1,15.41} = 13.606$, $P = .002$) and appeared to increase throughout the study more markedly in migrants, but we found no significant effect of sampling period ($F_{3,41.93} = 1.218$, $P = .315$) or any interaction between strategy and sampling period ($F_{3,41.93} = 20.966$, $P = .418$) on GnRH-induced testosterone (fig. 2c). We found no main effect of age on GnRH-induced testosterone ($F_{1,15.413} = 1.493$, $P = .240$). Interactions between age and sampling period and population were not significant ($F_{3,41.93} = 0.629$, $P = .600$; $F_{1,15.41} = 0.106$, $P = .749$). The full model including age, sampling period, and migratory strategy was not significant ($F_{3,41.93} = 1.739$, $P = .174$).

Gonads

At the end of the 4-week sampling period, migratory juncos had smaller gonads than sedentary juncos ($F_1 = 39.117$, $P < .001$). Untransformed mean resident gonad mass was 0.153 g (SE = 0.015), and migrant gonad mass was 0.046 g (SE =

0.012). The corrected model including age as a covariate was significant ($F_2 = 21.981$, $P < .001$, adjusted $R^2 = 0.688$).

Fat

Migrants exhibited more subcutaneous fat than residents throughout the study ($F_{1,17} = 51.125$, $P < .001$), and there was an interaction between strategy and sampling period ($F_{3,51} = 4.975$, $P = .004$) wherein migrants tended to increase fat while residents did not, but a main effect of sampling period was not detected ($F_{3,51} = 0.897$, $P = .449$; fig. 3a).

CORT

Residents had higher plasma CORT than migrants throughout the study ($F_{1,16.39} = 10.003$, $P = .006$; fig. 3c). Plasma CORT was significantly affected by migratory strategy but not by date. There was no main effect of sampling period ($F_{3,48.095} = 0.364$, $P = .779$) or interaction between strategy and sampling period ($F_{3,48.095} = 0.769$, $P = .517$).

Hydrogen

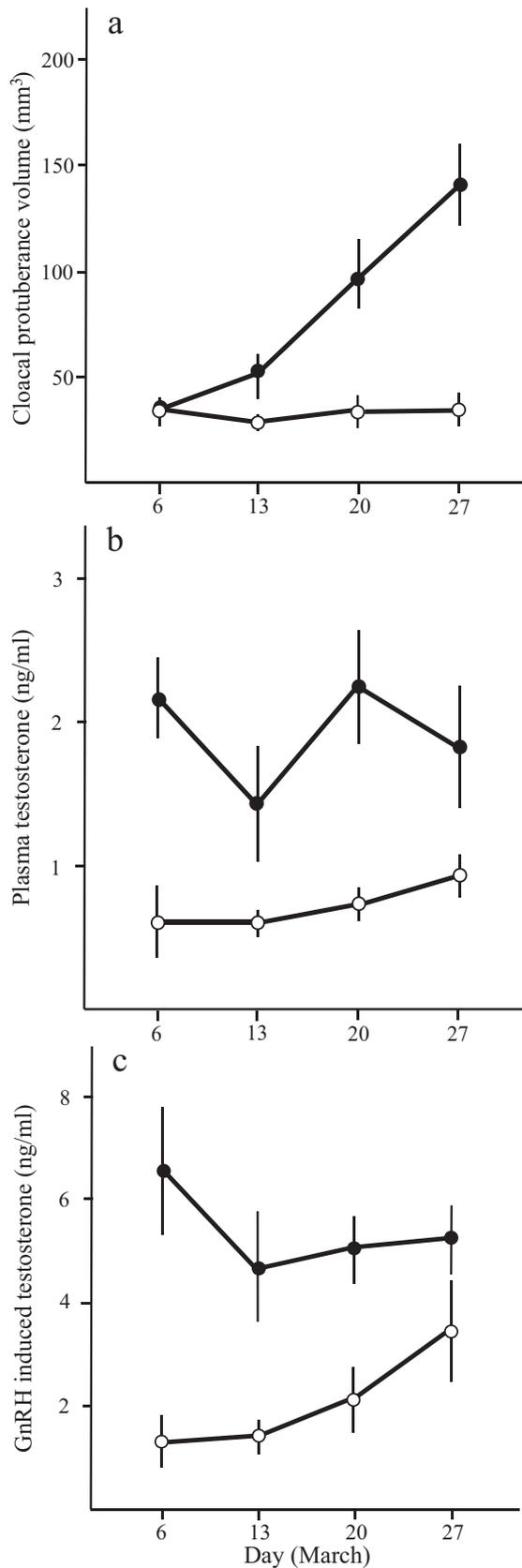
The mean hydrogen isotope composition of secondary feathers of migrants was markedly lower than that of residents ($\delta^2\text{H}$ means: migrants = -102‰ , residents = -75‰ , $t_{18} = 5.506$, $P < .001$), confirming that migrants had molted at higher latitudes. Migrants also exhibited a much wider range in hydrogen isotope compositions, indicating that they likely breed over a substantially broader latitudinal band ($\delta^2\text{H}$: migrants, range = -85‰ to -123‰ , SD = 14, SE = 4; residents, range = -66‰ to -91‰ , SD = 8, SE = 3).

Hydrogen and Fat

Pooled individual fat scores at the end of the study were correlated with $\delta^2\text{H}$ values ($r_{19} = -0.773$, $P < .001$, $R^2 = 0.597$; fig. 3b), a result driven by population differences in fat score, as this correlation was not significant within either population separately (migrant, $r_{11} = -0.282$, $P = .400$, $R^2 = 0.080$; resident, $r_8 = -0.643$, $P = .086$, $R^2 = 0.413$).

Hydrogen and Gonads

When considering all birds (populations pooled), gonad mass was significantly correlated with hydrogen isotope values ($r_{20} = 0.743$, $P < .001$, $R^2 = 0.553$), though isotopic variation was lower within the resident population. Accordingly, this result was also significant within the migrant population ($r_{11} = 0.603$, $P = .050$, $R^2 = 0.364$) but not the resident population ($r_9 = -0.660$, $P = .053$, $R^2 = 0.435$; fig. 4). The positive correlation within the migrant population provides



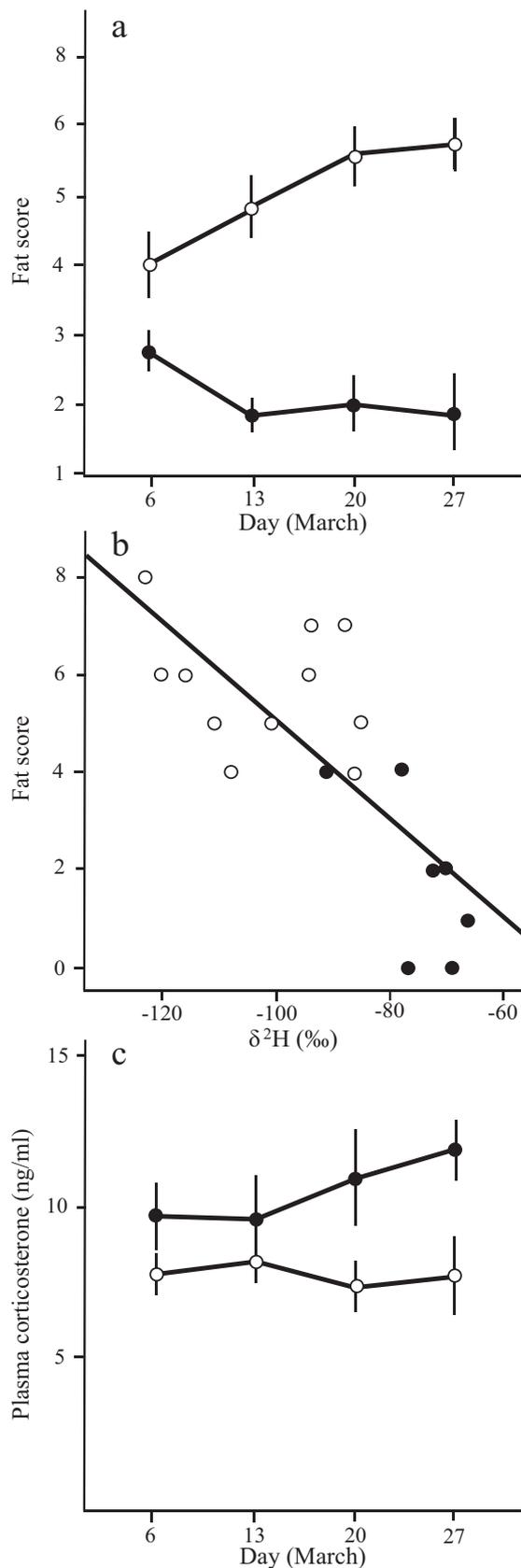
strong evidence that migrants breeding farther north had smaller testes at the end of the study.

Discussion

It is well established that photoperiodic and supplementary cues influence seasonal timing of reproduction in temperate breeding birds (Rowan 1925; Dawson et al. 2001; Williams 2012; Dawson 2015). However, the specific cues (i.e., supplementary and photoperiodic) influencing divergence in timing of reproduction in seasonally sympatric populations have received little attention despite implications for population divergence. We demonstrated a differential response to photoperiod in late winter and early spring in juncos that spend >6 months of the year together. Under identical captive conditions with ad lib. food, reduced social interactions, mild temperatures, and naturally increasing photoperiod, male migratory dark-eyed juncos significantly delayed reproductive development in the spring compared to sedentary males. Migratory male juncos had lower initial and GnRH-induced plasma testosterone levels and smaller cloacal protuberances over 4 weeks in early spring (fig. 2), and at the end of the 4-week study, migrants also had smaller testes (fig. 4). The study was undertaken in early spring, the time period during which migratory and sedentary juncos are sympatric in the wild (Nolan 2002) and thus when individuals would be most likely to interbreed, if in fact they did.

We found considerable variation in hydrogen isotope signatures of juncos' secondary wing feathers, in particular among migrant juncos, which implies broad geographic variation in breeding origins, as these feathers are grown on breeding grounds before autumnal migration (fig. 4). For migrants, hydrogen isotope values ranged nearly 40‰, which, when considered alongside an established north-south gradient in precipitation $\delta^2\text{H}$ across North America (Bowen et al. 2005; Wunder et al. 2012), indicates that they likely breed across a wide latitudinal band within the slate-colored junco breeding range. Further, we found that migrants varied in testis growth and that growth varied positively in relation to $\delta^2\text{H}$ and hence positively in relation to individuals' estimated breeding latitudes (fig. 4). We interpret this finding to strongly suggest that juncos wintering closer to their breeding sites initiated reproductive development earlier, while more northerly breeders delayed growth in response to identical photoperiod cues. Accordingly, several migrants with breeding sites that were estimated to be relatively close to

Figure 2: Male sedentary juncos (filled circles) showed larger cloacal protuberance volume (a), higher levels of plasma testosterone (b), and higher levels of testosterone in response to a gonadotropin-releasing hormone (GnRH) challenge (c) than migrant male juncos (open circles). There was a significant interaction between sampling period and cloacal protuberance. Graphs show mean \pm SE.



the wintering site had gonads that were similar in size to residents, which was also similar to testis mass of reproductively viable juncos (Bergeon Burns et al. 2014).

Continuous covariation between reproductive timing and breeding origins has not previously been described in birds held in a controlled captive environment, and this finding points to the need for additional studies about the regulation of migratory and reproductive timing. For example, are the differences in gonadal development between migrants and residents reported here fixed genetically, or do they reflect day length experienced in early development? MacDougall-Shackleton and Hahn (2007) proposed that broadly distributed species with latitudinal variation in reproductive timing could be the result of conditional plasticity, where differences are the result of the same photoperiod response system. Individual timing would vary depending on the day length experienced in a given spring—long days are later at high latitudes, so reproduction is delayed (MacDougall-Shackleton and Hahn 2007). Our results suggest that this is not the case in migratory juncos; otherwise, migrants and residents would have similar timing of reproductive maturation in captivity. The reliance of gonadal growth on a specific photoperiod provides a mechanism that could limit gene flow among variably migratory populations that encounter each other during early spring.

Previous work on populations breeding in close proximity but in heterogeneous habitat has shown adaptive differential timing in breeding independent of photoperiod (Lambrechts and Dias 1993). Interestingly, differences in timing in these populations were shown to be the result of female differences (Caro et al. 2009). In this study, we did not measure female timing, but as male juncos typically become reproductively mature before females (Nolan 2002), it is unlikely that there is overlap in reproductive timing during the period of this study.

Although we found a clear effect of photoperiod on timing of reproduction in migratory juncos, other factors can also influence gonadal development in the spring. Free-living male juncos in their first breeding season in interior Alaska have delayed gonadal maturation compared to adult males, and there is strong evidence to suggest that differences in timing are the result of social interactions and not intrinsic age differences in HPG function (DeViche et al. 2000).

Figure 3: *a*, Male migrant juncos (open circles) showed larger subcutaneous fat scores than sedentary male juncos (filled circles; mean \pm SE). There was a significant interaction between sampling period and strategy. *b*, Individual variation in subcutaneous fat on the fourth week of the experiment covaried with stable hydrogen isotope ($\delta^2\text{H}$) values of breeding latitude in all samples pooled; however, subcutaneous fat and $\delta^2\text{H}$ did not significantly covary in individual populations. *c*, Male resident juncos had higher plasma corticosterone than migrants. We found no effect of sampling period or interaction between strategy and sampling period.

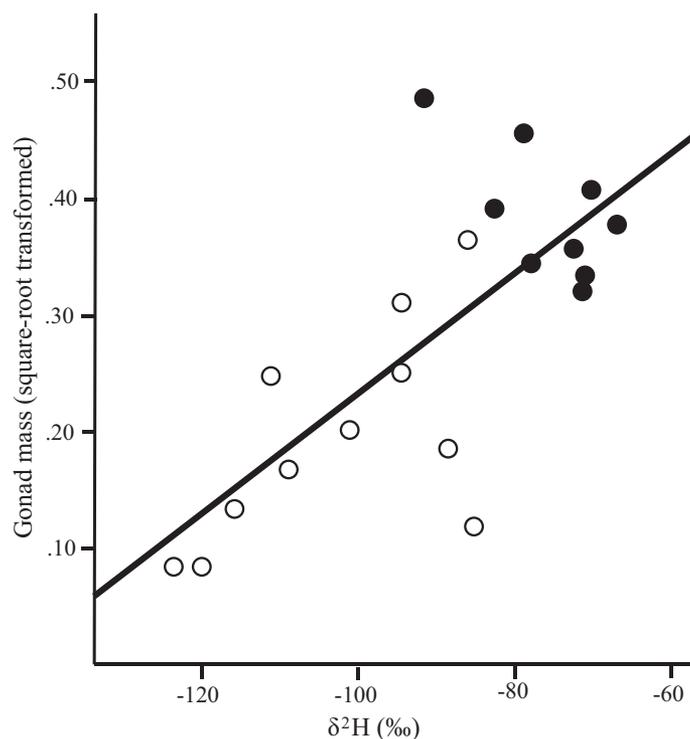


Figure 4: Male migrant juncos (open circles) had smaller testes than sedentary male juncos (filled circles) in early spring. Individual testis mass covaried significantly with stable hydrogen isotope ($\delta^2\text{H}$) values of breeding latitude in all samples pooled (solid line) and within the migrants.

Biogeographic and genetic data indicate that the migratory versus sedentary subspecies studied here are closely related and likely diverged $\sim 10,000$ – $20,000$ years ago in association with recolonization of northern latitudes following the Last Glacial Maximum (Mila et al. 2007). In this study, we found clear evidence for persistence of reproductive timing differences in the common garden, providing strong evidence for genetic or early organizational underpinnings for photoperiodic responses. In contrast, a similar comparison made in more recently diverged junco populations (~ 35 years diverged, following contemporary colonization) found convergence in reproductive development under common garden conditions (Atwell et al. 2014). This prior study found that these two populations converged in reproductive timing when held in captivity. The apparent plasticity in reproductive timing in recently diverged populations, as compared to the more fixed differences reported here, could be attributable to a difference in time since divergence. A likely explanation for the greater reliance on photoperiod reported here could be the difference in breeding latitudes from which migrants and residents derive (Silverin et al. 1993). Migratory and sedentary juncos studied by Atwell et al. (2014) breed at the same latitude and therefore experience the same pho-

toperiod year-round. Migrants in this study breed a minimum of 900 km north of sedentary juncos (Nolan 2002), where days are longer, so they may require a longer day length to reach full reproductive maturity or testis growth may proceed more slowly.

Migration is an energetically taxing stage of the annual cycle, and migrants use fat as the primary source of fuel (Jenni and Jenni-Eiermann 1998). Despite having access to the same amount of food in the common garden environment, migratory juncos put on more subcutaneous fat than sedentary juncos (fig. 3). Further, the relationship between estimated breeding latitude via feather hydrogen isotopes and subcutaneous fat stores was positive (fig. 3). Thus, a distant destination predicted not only slower gonadal growth (see above) but also greater migratory fattening. Conceivably, energy allocated to migration is gradually shifted toward gonadal growth as spring proceeds and the distance to the breeding site diminishes. It has been suggested that seasonal elevation in circulating CORT results in changes in behavior and physiology that facilitate energy mobilization and perhaps foster migratory restlessness (Holberton et al. 1996; Holberton 1999; Long et al. 2004). Surprisingly, migrants had lower circulating CORT throughout the study. Circulating

CORT has also been reported to rise as the breeding season approaches (Romero 2002), which could explain the higher levels seen in sedentary juncos here.

Conclusions

Population differences in reproductive readiness and migration in a common garden reflect the importance of the accurate timing of annual schedules. Divergence between individuals sharing a similar environment in the early spring in the timing of seasonal reproductive growth and development, which is likely common among broadly distributed species, is theorized to promote population divergence and lineage diversification (Getz and Kaitala 1989; Winker 2010). We demonstrate that population differences in photoperiodic responses can facilitate reproductive allochryony in seasonally sympatric migratory and sedentary juncos. Understanding the behavioral and physiological mechanisms that lead to population divergence should provide a greater understanding of how phenotypic variation emerges in highly mobile species.

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