

RESEARCH ARTICLE

Early elevation of testosterone advances migratory preparation in a songbird

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SUMMARY

The timing of events associated with spring migration can be an important determinant of fitness for migratory birds. The need to prepare and maintain physical condition for migration is one demand that must be met for early arrival on breeding areas, and this demand is compounded by the energetic demands of the physiological transition to breeding. Here, we examined whether migratory birds, specifically males, can adequately meet both of these demands by elevating the sex steroid testosterone early during migratory preparation. To test this, we used a captive experiment in which we photostimulated male dark-eyed juncos (*Junco hyemalis*) and manipulated circulating testosterone. We predicted males with elevated testosterone would prepare to migrate and breed faster than control males or males where the ability of testosterone to bind with receptors, or be converted to estradiol, was inhibited (testosterone inhibited). We measured migratory preparation using mass, food intake, fat deposits and nocturnal activity (*Zugunruhe*). To estimate breeding preparation, we measured the diameter of the cloacal protuberance (CP). We found that males in the testosterone treatment group began migratory preparation approximately 10 days before controls. There was no difference in the magnitude of peak migratory condition between testosterone-implanted birds and controls. Males implanted with testosterone also reached the onset of breeding preparation faster and had larger peak CP diameter compared with controls. Testosterone-inhibited birds showed no signs of migratory preparation and only a weak increase in CP diameter. These results demonstrate that early elevation of testosterone during migratory preparation could incur benefits for males in terms of the ability to depart earlier from non-breeding areas and arrive in a more advanced breeding condition. This experiment demonstrates that there may be important physiological underpinnings to known winter to breeding season carry-over effects in migratory birds.

Key words: androgen, condition, dark-eyed junco, *Junco hyemalis*, migration phenology.

INTRODUCTION

During the transition from the non-breeding to breeding life history stages, migratory animals may be forced to balance the physiological demands of preparing for and maintaining the migratory state with the demands of preparing for reproduction. In order to meet these potentially conflicting demands, migratory birds must allocate resources towards muscle anabolism and fat deposition, as well as towards gonadal growth, gamete production and initiation of reproductive behavior (Holberton and Dufty, 2005; Ramenofsky and Wingfield, 2006). For males, meeting both of these demands in a short amount of time can be challenging but crucial, especially for species that show variation in the degree of protandry, which is often a strong determinant of male fitness (Morbey and Ydenberg, 2001). Thus, hormones that enhance migratory performance, while simultaneously ensuring advanced breeding preparation upon arrival, should confer a selective advantage to those that can elevate such hormones early on in the transition between stages.

Environmental conditions during the non-breeding period of migratory birds are known to impact physical condition (e.g. Marra and Holberton, 1998; Marra and Holmes, 2001; Latta and Faaborg, 2002; Brown and Sherry, 2006; Studds and Marra, 2007; Smith et al., 2010) and the timing of migration (Marra et al., 1998; Studds and Marra, 2005; Gunnarsson et al., 2006; Bridge et al., 2010). This can ultimately lead to carry-over effects on breeding success (Marra et al., 1998; Reudink et al., 2009; Inger et al., 2010), survival

(Johnson et al., 2006; Angelier et al., 2009) and natal dispersal (Studds et al., 2008), such that non-breeding and breeding life history stages interact across geographical space. While the ecological basis for these seasonal interactions has received much attention, the physiological basis is not well understood.

For males of migratory species, the gonadal androgens, primarily testosterone and its metabolites, are crucial hormones in the transition into a breeding state (Balthazart, 1983; Ketterson and Nolan, 1999; Wingfield et al., 2001). In addition to reproductive functions, most importantly stimulating sperm production and the cloacal protuberance, testosterone plays an important behavioral role in birds as a regulator of male–male aggression associated with acquiring high quality breeding territories (Moore, 1984; Wingfield, 1984; Beletsky et al., 1990) and territory boundary advertisement (i.e. singing behavior) (Ketterson et al., 1992; De Ridder et al., 2000). Testosterone is also fundamental in the expression of secondary sex traits and courtship behaviors that influence breeding success in birds (Hegner and Wingfield, 1987; Saino and Møller, 1995; Blas et al., 2007).

Previous research has documented that testosterone plays an important organizational role in pre-migratory hyperphagia and fat deposition (reviewed in Deviche, 1995). Migratory birds prepare for migration through increased feeding (hyperphagia), which facilitates an increase in both pectoral muscle mass and fat stores (reviewed in Berthold, 2001; Holberton and Dufty, 2005). What

remains unclear is whether variation in circulating testosterone levels can lead to variation in the rate and magnitude of preparation for migration. Testosterone is widely known to have anabolic effects in vertebrates and can increase locomotion and feeding, without increasing daily energy expenditure (DEE) (Lynn et al., 2000). While testosterone can be detrimental to physical condition when maintained at high levels over long time periods, such as an entire breeding season (Ketterson et al., 1991), elevating testosterone levels during the relatively short time frame of migratory preparation could positively affect performance by increasing the rate of muscle anabolism and fat deposition. Additionally, males with high circulating testosterone during preparation for migration, and during migration itself, may have more advanced testicular development (Wingfield, 1984; Morton et al., 1990) upon arrival, and thus may be better prepared to compete for mates and territories. In this way, variation in the ability of birds to elevate testosterone prior to departure on spring migration could present a physiological basis for observed winter to breeding season carry-over effects in migratory species. Here, we began to examine this proposed mechanism by experimentally manipulating testosterone levels, under controlled conditions, early on in the transition from non-breeding to breeding.

We sought to determine whether elevated testosterone enhances migratory preparation while simultaneously enhancing breeding preparation. We used a captive experiment in which we photostimulated a group of male dark-eyed juncos (*Junco hyemalis* L.), a short- to middle-distance North American annual migrant, to simulate the transition to spring conditions. We predicted that compared with control males, those with experimentally elevated testosterone would (a) have earlier onset of migratory and breeding preparation, (b) take less time to reach peak preparation, and (c) have higher peaks in all measures of condition. We further predicted that when the ability of testosterone to bind with receptors or be converted to estradiol was inhibited, birds would prepare to migrate and breed more slowly than both testosterone-implanted birds and controls.

MATERIALS AND METHODS

Male dark-eyed juncos ($N=28$) were captured in mist nets from 15 October to 15 November 2008, in Penobscot County, ME, USA. Sex and age were determined by plumage characteristics and degree of skull ossification (Pyle, 1997). Of the 28 individuals, 12 were aged as hatching year (HY) birds. We pooled age groups for all analyses, but HY birds were evenly distributed among treatment groups. The birds were initially kept in indoor flight cages on short-day photoperiod, consistent with local winter conditions and previous studies of juncos (8 h light:16 h dark) (Dloniak and Deviche, 2001). We maintained birds on a 50:50 mixture of white millet and turkey starter, and water *ad libitum*. Vitamins and minerals were provided in the drinking water each week throughout the study period. During the last week of November, all birds were transferred to individual commercial cages (40×40×40 cm), and continued on the same photoperiod and feeding regime. All subjects were released at original capture sites in spring 2009. This study was approved by the University of Maine Institutional Animal Care and Use Committee (protocol A2009-06-05).

Measurement of dependent variables

All morphometric measurements, food intake data and blood samples were collected at least 1 h after lights came on to allow the birds to break their nocturnal fast. All activities were completed within 2 h. We used a digital scale to measure body mass (to the

nearest 0.01 g) and scored fat deposits in the furcular fossa using the following criteria: 0, no fat visible; 1, one to three thin strands of fat; 2, strands of fat merging into a continuous sheet in some places; 3, fat nearly forming a continuous thin sheet but with one or two clear areas; 4, continuous thin sheet of fat; 5, fat completely filling but not bulging from the furcular fossa; 6, fat bulging slightly from the furcular fossa. As a measure of androgen activity, and a surrogate for testis size (Wingfield, 1984; Morton et al., 1990), we recorded cloacal protuberance (CP) diameter using digital calipers (to the nearest 0.01 mm) at the structure's widest point along the coronal plane.

Each cage was equipped with one inactive, immovable perch and one active perch that rested on two microswitches (one at each end of the perch). When the active perch was deflected, an electrical circuit was completed and the event registered on an electric counter. Nocturnal migratory activity (*Zugunruhe*) was recorded as the total number of hops a bird made during the previous dark period when the perch registration system was active. The system was turned on half an hour after lights went out in the evening and turned off half an hour before lights came on in the morning. Each morning, the preceding night's activity was recorded for each bird and the mean values for all 2-night periods were used as measures of *Zugunruhe* throughout the study period.

To measure food intake, each cage contained a food cup with a plastic cover that allowed only the bird's head to reach into the cup to get seed, but prevented them from scratching seed out of the cup. We affixed plastic sheets around each cage to prevent uneaten seeds from leaving it, and then recovered and weighed the food (including food removed from the cup) to calculate each bird's daily food intake [(initial food mass – final food mass)/no. of days]. We removed any remaining hulls from eaten seeds by blowing them out of the dish. We considered CP diameter to be a measure of breeding preparation and all other variables to be measures of migratory preparation.

Experimental design

All subjects were assigned to three treatment groups, such that the groups began the study with equal means and standard errors for tarsus length. Each male received one 10 mm silastic tubing (Dow Corning®, Midland, MI, USA; i.d. 1.47 mm, o.d. 1.96 mm) implant subcutaneously under the wing (Lynn et al., 2000). Prior to implantation, the implants were punctured once with a 27 gauge needle and stored in sterile saline for 24 h. For each subject, we made a small (1–2 mm) incision in the skin under the wing with iris scissors. We then used a metal probe to loosen the skin from connective tissue, slid the implant under the skin, and sealed the incision with a liquid bandage solution (New-Skin®, Prestige Brands Inc., www.prestigebrandsinc.com). 'Testosterone-implanted' birds ($N=11$) received implants packed with testosterone propionate (product no. T-1875; Sigma-Aldrich Co., St Louis, MO, USA); 'testosterone-inhibited' birds ($N=8$) received implants packed with flutamide (blocking the ability of testosterone to bind to receptors; product no. F9397-5G; Sigma-Aldrich) and 1,4,6-androstatriene-3,17-dione (ATD; an aromatase inhibitor, preventing conversion of testosterone to estradiol; product no. A7710-1G; Sigma-Aldrich); and 'control' birds ($N=9$) received empty implants.

On day 0 (8 December 2008) we measured baseline condition (mass, fat, CP diameter) of all subjects and transferred them to long days (18 h light, 6 h dark), to stimulate the transition into migration. Because of experimental constraints, we did not keep birds on a short day photoperiod for as long as in previous studies (e.g. Dloniak and Deviche, 2001). However, all birds began the study in what

would be considered good condition (i.e. no signs of muscle atrophy and at the typical mass for non-breeding male junco, 18–19 g), and as we were at high latitude (48°N), these birds had already been exposed to short day conditions prior to capture. Further, previous research has demonstrated that temperate migrants generally break photorefractoriness in late November (e.g. Farner and Lewis, 1971), and both controls and testosterone-implanted birds showed the appropriate response to photostimulation (see Results). On day 1 we implanted all subjects with their respective treatments. On day 2, we measured condition, weighed all food dishes, and activated all perch counters. On day 4 we measured condition again and calculated the first values for food intake and activity, such that initial values for these variables represent the time interval between day 2 and day 4. All variables were subsequently measured every 2 days until the completion of the study (day 24). We removed two individuals from the study (one each on day 12 and day 20 from the testosterone-inhibited group) because their implants had fallen out and could not be replaced.

Implant validation

On day 0 (pre-implant), day 12 and day 24 we took a blood sample (50–80 µl total volume) from the brachial vein, *via* venipuncture, to validate that the implants were effective at manipulating testosterone activity. Samples were kept on wet ice until centrifuged within 1 h of collection. Plasma was stored at –20°C until assayed for plasma androgen concentration. All blood samples were collected within 8 min of technicians entering the bird room and within 3 min of the birds being removed from the cages. To determine plasma androgen concentration, we used radioimmunoassay (testosterone antibody T3-125, Endocrine Sciences/Esoterix®, Calabasas, CA, USA) following Wingfield et al. (Wingfield et al., 1992). The testosterone antibody has high specificity for testosterone but also at least 44% cross-reactivity with dihydrotestosterone (DHT). Therefore, our results represent total androgen, and are reported as such. For the testosterone-inhibited treatment, we expected to see an increase in circulating endogenous androgen, possibly higher than controls, because of the lack of negative feedback normally imposed by high androgen levels reaching the pituitary and hypothalamus. Aromatase-inhibited birds might also experience higher plasma androgen levels if they are unable to funnel the substrate into the aromatase pathway.

Statistical analysis

We used linear mixed models to test the prediction that, for all measures of migratory and breeding preparation, there would be an interaction between ‘treatment group’ and ‘day of photostimulation’. ‘Individual male’ was included as a random factor and ‘day of photostimulation’ was included as a repeated variable with a diagonal residual covariance structure and the initial baseline sample as the reference value. All models included treatment and the treatment × day of photostimulation interaction as fixed effects. Fat score was treated as an ordinal variable. Within each treatment, we examined the coefficients of all interaction pairs for each response variable to determine the points at which birds (a) first began showing evidence of migratory preparation [i.e. first day where 95% confidence intervals (CI) for coefficient did not overlap with zero] and (b) reached peak condition in a given metric (i.e. the day with the largest positive coefficient; see also Results). We used *t*-tests to compare peak means of body mass, food intake and *Zugunruhe*, and the Kolmogorov–Smirnov test for different distributions to compare peak fat scores (these two-sample tests were adequate because only two experimental groups increased in these

metrics; see Results). Differences in baseline preparation metrics, circulating androgen and peak CP diameter means were analyzed using generalized linear models (GLM) and Tukey HSD multiple-comparison tests. *Zugunruhe* (hops per bird per night) and circulating androgen were square-root transformed to normalize their residuals; the residual distributions of all other variables were normal. All analyses were done with SPSS 17.0 (SPSS Inc., 2008).

RESULTS

At the onset of the study, the three treatment groups did not differ in mass ($F_{2,25}=2.32$, $P=0.12$), food intake ($F_{2,25}=0.23$, $P=0.80$), fat ($F_{2,25}=0.74$, $P=0.49$), *Zugunruhe* ($F_{2,25}=1.49$, $P=0.25$) or CP diameter ($F_{2,25}=0.59$, $P=0.57$). Treatment groups also did not differ in circulating androgen on day 0 (pre-implant; $F_{2,25}=0.11$, $P=0.90$), but were significantly different on day 12 ($F_{2,25}=40.49$, $P<0.001$) and day 24 ($F_{2,23}=40.32$, $P<0.001$). Androgen levels for the testosterone-implanted group were the highest, followed by testosterone-inhibited, then control groups, indicating that the implants were effective (Fig. 1).

For all measures of migratory preparation (mass, fat, food intake and *Zugunruhe*), there was an interaction between treatment group and day of photostimulation (summarized in Table 1). Fig. 2 illustrates how we determined when birds in each treatment began migratory preparation and when they reached peak condition using mass as an example. Testosterone-implanted birds began migratory preparation and reached peak migratory condition earlier than controls and testosterone-inhibited birds (Fig. 3; Table 2).

With regard to breeding preparation, there was an interaction between treatment and day of photostimulation in the model of CP diameter (Table 1). Testosterone-implanted birds began preparation before the other two groups and reached peak CP diameter before testosterone-inhibited birds, although they reached peak CP diameter on the same day as controls. Controls reached both points before testosterone-inhibited birds (Fig. 3; Table 2).

Although testosterone-implanted birds reached peak preparation values earlier than controls, their peak values were not different from those of controls for mass (Fig. 3A; $t_{18}=-1.17$, $P=0.20$), food intake (Fig. 3B; $t_{18}=-1.61$, $P=0.12$), fat score (Table 2; Kolmogorov–Smirnov criterion value=0.41, $P=0.28$) or *Zugunruhe* (Table 2; $t_{18}=0.34$, $P=0.74$). Testosterone-inhibited birds did not show an increase in any of these metrics and thus had no peak values above baseline. Peak breeding preparation, as measured by CP diameter, was different between treatment groups (Fig. 3C; $F_{2,23}=62.82$, $P<0.001$), and multiple comparisons revealed that testosterone-implanted birds had larger CPs than either of the other

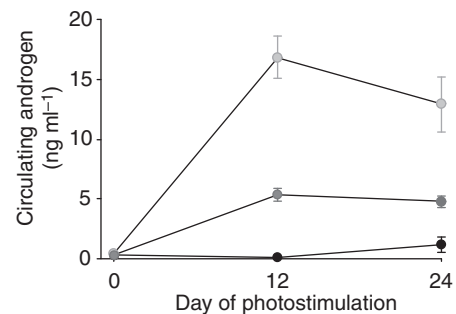


Fig. 1. Circulating androgen levels on days 0, 12 and 24 of photostimulation in each of the three implantation treatments: control (black), testosterone (light grey) and testosterone inhibited (dark grey).

Table 1. Statistical tests for the treatment × day of photostimulation interaction from linear mixed models explaining variation in metrics of migratory and breeding preparation in captive male dark-eyed juncos

| | Dependent variable | Numerator d.f. | Denominator d.f. | F | P |
|---------------------|--------------------|----------------|------------------|--------|--------|
| Migratory condition | Mass | 36 | 45.912 | 3.677 | <0.001 |
| | Fat | 36 | 50.908 | 3.505 | <0.001 |
| | Food intake | 33 | 44.718 | 3.199 | <0.001 |
| | Zugunruhe | 33 | 23.281 | 13.255 | <0.001 |
| Breeding condition | CP diameter | 36 | 45.463 | 43.635 | <0.001 |

CP, cloacal protuberance.

two groups (testosterone vs control: mean difference=1.67, lower 95% CI=1.25, upper 95% CI=2.09; testosterone vs testosterone-inhibited: mean difference=1.67, lower 95% CI=1.19, upper 95% CI=2.15) which were not different from each other (control vs testosterone-inhibited: mean difference=0, lower 95% CI=-0.49, upper 95% CI=0.49).

DISCUSSION

Several classic studies, using a combination of gonadectamy and testosterone administration, revealed that androgen activity prior to photostimulation is necessary for the onset of fat deposition, one of the major components of migratory preparation in birds (e.g. Stetson and Erickson, 1972; Schwabl and Farner, 1989). The results

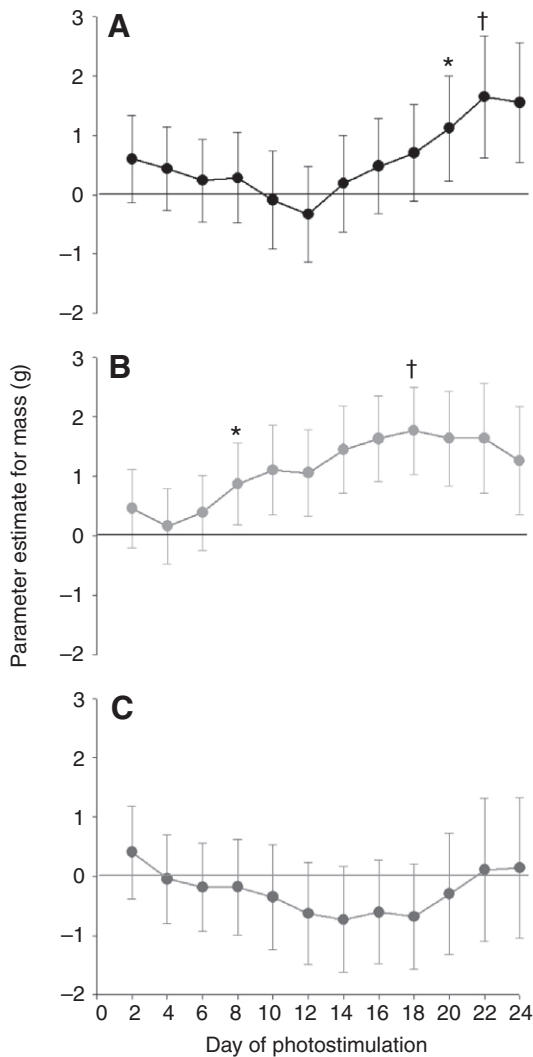


Fig. 2. Parameter estimates (±95% confidence intervals) for treatment × day of photostimulation interaction for linear mixed model explaining mass of male dark-eyed juncos in the three implantation treatments: (A) control, (B) testosterone and (C) testosterone inhibited. Day 0 was used as a baseline reference value. We considered ‘onset of preparation’ to be the first day with a significant positive coefficient (i.e. confidence intervals do not overlap zero line; denoted by *) and ‘peak preparation’ to be the largest positive coefficient (denoted by †).

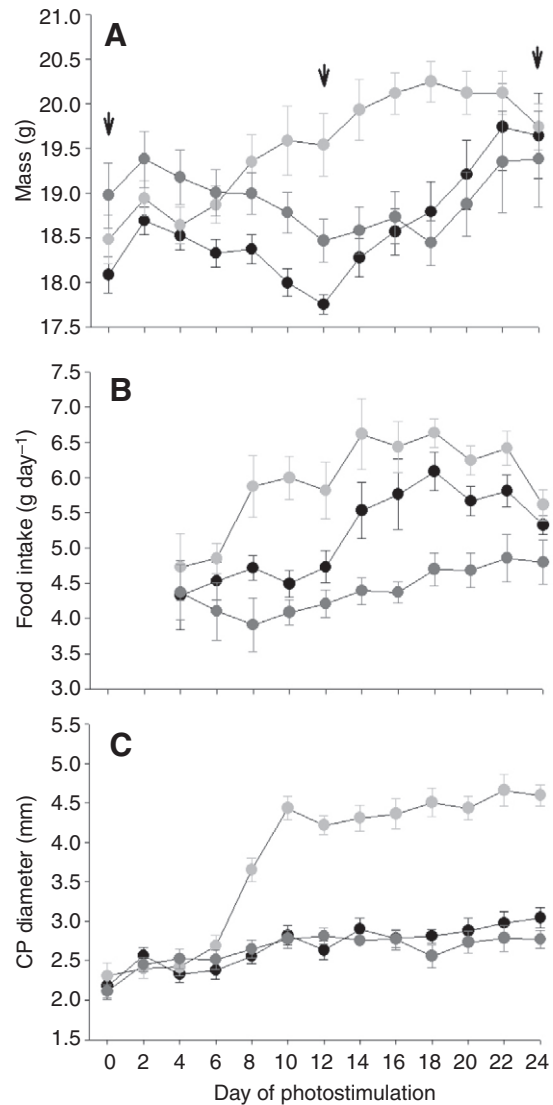


Fig. 3. Means (±s.e.) of (A) mass (arrows indicate blood sampling events), (B) food intake and (C) cloacal protuberance (CP) diameter × day of photostimulation for captive male dark-eyed juncos in three implantation treatments: control (black), testosterone (light grey) and testosterone inhibited (dark grey).

Table 2. Estimates for day of migratory (mass, food intake, fat score and *Zugunruhe*) and breeding preparation (CP diameter) onset and peak for male dark-eyed juncos in three implantation treatments

| Preparation metric | Group | Onset | Peak |
|--------------------|------------------------|--------|--------|
| Mass | Control | Day 20 | Day 22 |
| | Testosterone | Day 8 | Day 18 |
| | Testosterone inhibited | n.c. | n.c. |
| Fat score | Control | Day 20 | Day 24 |
| | Testosterone | Day 10 | Day 18 |
| | Testosterone inhibited | n.c. | n.c. |
| Food intake | Control | Day 16 | Day 18 |
| | Testosterone | Day 8 | Day 18 |
| | Testosterone inhibited | n.c. | n.c. |
| <i>Zugunruhe</i> | Control | Day 16 | Day 24 |
| | Testosterone | Day 6 | Day 12 |
| | Testosterone inhibited | n.c. | n.c. |
| CP diameter | Control | Day 12 | Day 18 |
| | Testosterone | Day 6 | Day 18 |
| | Testosterone inhibited | Day 18 | Day 20 |

'Day' refers to the day of photostimulation.

n.c., no change.

of the present study demonstrate that the timing of increased androgen activity following photostimulation can affect the timing of migratory preparation while simultaneously enhancing preparation for breeding. Male juncos implanted with testosterone began both migratory and breeding preparation, and also reached peak migratory condition, faster than controls. As further evidence of a role for androgens in migratory preparation, testosterone-inhibited males failed to show any change in these measurements by the conclusion of the study. This supports the hypothesis that, in addition to the important role of androgens in reproduction in males, androgens can also simultaneously play an important role in the timing of migration events.

Early exposure to elevated testosterone soon after photostimulation resulted in an advance in the timing of migratory and breeding preparation; however, it did not affect the magnitude of peak migratory condition. Testosterone-implanted birds did not put on greater mass or fat stores than controls. Perhaps because food was unlimited, given enough time, controls were able to reach the same upper threshold of condition as testosterone-implanted birds. This apparent upper limit suggests that at some point, the benefits of condition may be outweighed by the detrimental effects of carrying too much fat during migration (Lindström and Alerstam, 1992; Jehl, 1997). Adding the complexity of weather and food limitation to this experiment would enable future research to determine whether the external environment can bring about differences in peak condition between treatments. Our results illustrate that elevated testosterone is associated with earlier stimulation of increased activity and foraging, which in turn supports body mass gain and fat deposition. The ability of testosterone to increase activity, without increasing DEE (Lynn et al., 2000), could make it possible for birds with elevated testosterone to acquire energy stores more efficiently, i.e. while using less energy for hyperphagia than controls and testosterone-inhibited birds. The important question remaining is whether environmental variability in wild migratory populations causes natural variation in the onset of testosterone production prior to migratory departure, as has been observed in experimental studies (Bauchinger et al., 2007; Bauchinger et al., 2009) (R.L.H. and P.P.M., unpublished). This appears likely when considering the effects habitat quality can have on physical condition in wintering migratory birds (Marra and Holberton, 1998; Brown and Sherry, 2006; Studds and Marra, 2006)

and the effects of natural variation in environmental conditions on the onset of breeding development in tropical residents (Hau, 2001).

While arrival time-dependent fitness in males has long been a major component of hypotheses for the evolution of differential migration in dark-eyed juncos (Ketterson and Nolan, 1983; Holberton, 1993), to our knowledge no studies have examined the relationship between individual male arrival time and breeding success in this species. A positive relationship between early arrival and reproductive success has been observed in many other migratory bird species (e.g. Aebischer et al., 1996; Gil and Slater, 2000; Currie et al., 2000). This gap in knowledge of the role arrival time plays in male breeding success in juncos prevents us from drawing conclusions on how variation in the timing of testosterone elevation may impact fitness for this species.

A recent experimental study demonstrated that migration phenology in juncos is dependent on food availability and its effects on condition (Bridge et al., 2010). Males of other songbird species, such as the American redstart (*Setophaga ruticilla*), that winter in higher quality winter habitats arrive early at breeding sites and ultimately produce more offspring (Marra et al., 1998; Reudink et al., 2009). Further, males that arrive early have higher circulating testosterone on arrival than late arrivers (Tonra et al., in press). In this study, we observed that male juncos implanted with testosterone reached peak migratory condition approximately 4 days earlier than controls. Field studies have found that differences of about 3–5 days in mean arrival dates are enough to significantly affect the number of genetic offspring produced by a male (Reudink et al., 2009) or the probability of a male experiencing successful reproduction (Tonra et al., in press). Based on our experimental findings and previous research, it appears that variation in conditions on the wintering grounds during migratory preparation could lead to variation in circulating testosterone, which could, in turn, affect migration phenology and male breeding success.

More observational and experimental work is needed to fully elucidate the role androgens play in winter to breeding season carry-over effects. If changes in testosterone production are acting as a cue for migratory preparation, our results raise questions about why unmanipulated males do not begin elevating testosterone earlier. One potential reason could be countervailing selection on earlier arrival dates, due to increased mortality risk for early arrivers from

severe weather (Newton, 2007). Further, birds may be responding to environmental cues (such as food availability) when timing reproduction, to appropriately time later reproductive stages, such as feeding young. For example, Nager and colleagues demonstrated that supplementing food during laying in great tits (*Parus major*) advanced laying date and increased clutch size, but did not increase breeding success (Nager et al., 1997). This was likely because supplemented birds were mismatched with caterpillar outbreaks during the nestling stage. In other words, manipulating the timing of a physiological cue (testosterone) that may, in nature, respond to environmental cues [e.g. food availability (Bauchinger et al., 2009); rainfall (Hau, 2001)], produced individuals that were mismatched with their environment. Thus, future studies would benefit by looking at finer scale differences in the timing of testosterone elevation. Furthermore, our experimental administration of testosterone resulted in circulating testosterone levels that were much higher than biological levels, which presumably was enough to effectively 'flood' receptors. Control males did show an increase in circulating testosterone, concurrent with their increase in migratory preparation; however, these elevated testosterone levels were well below those of testosterone-implanted birds. Therefore, we must limit our interpretation to the overall effects of maximum binding of testosterone to all available receptors during migratory preparation. However, Soma and colleagues demonstrated that the density and number of cells with androgen receptors can change seasonally (Soma et al., 1999). Our study demonstrates that juncos have adequate receptor densities early on in the photoreceptive period to respond to increases in circulating testosterone by preparing to migrate and breed. Given the very high testosterone concentrations to which the birds were exposed, however, we do not know how naturally occurring variation among individuals in a wild population would influence variation in measures of performance. Additionally, our testosterone-inhibited treatment not only inhibited the ability of testosterone to bind to receptors but also inhibited conversion to estradiol by aromatase. Thus, our 'androgen' effects may be either direct or manifested through estrogen-dependent activities inhibited by our experimental treatments. Further, other hormones and neuropeptides such as prolactin, cholecystokinin and neuropeptide Y are known to influence migratory feeding and fattening (for a review, see Holberton and Dufty, 2005). It would be of particular interest to investigate interactions between gonadal steroids and adrenocorticosteroids such as corticosterone (CORT) as these two pathways are known to interact (Viau, 2002) and previous studies have shown that an elevation in plasma levels of baseline plasma CORT is needed to support migratory fattening (Landys et al., 2006; Holberton et al., 2007).

While our results illustrate that early elevation of circulating testosterone is enough to initiate endocrine systems associated with migratory preparation, there may be some costs associated with being exposed to high circulating testosterone, including negative effects on immune function (Casto et al., 2001; Zysling et al., 2006), condition (Ketterson et al., 1991) and survival (Dufty, 1989; Redpath et al., 2006). In fact, Redpath and colleagues demonstrated that the breeding benefits of elevated testosterone can be offset by the costs of increased overwinter mortality (Redpath et al., 2006). Our manipulations maintained higher testosterone levels, for a longer period of time, than experienced in nature. If free-living birds do maintain more appropriate, but elevated, levels for reaching peak condition (~20 days), these may be costs that males must pay to acquire the benefits of early arrival. It is more likely that individuals modulate testosterone production, maintaining higher than non-breeding levels as they transition from non-breeding to breeding

and migration, but with the ability to reduce testosterone circulation as needed in order to minimize such costs.

Taking these limitations into account, we feel our results clearly demonstrate that the role of androgens in preparing for breeding has been co-opted to facilitate the migratory events needed to reach breeding areas. Males with elevated testosterone early on during migratory preparation would be better equipped to depart early, potentially increasing individual fitness. Further, males with elevated testosterone can effectively prepare for migration while simultaneously preparing to breed (as measured by CP diameter here). Thus, increasing circulating testosterone as early as possible in the transition between life history stages (winter to breeding) would presumably result in an early arriving male (meeting the demands of migration) being in a superior condition for attracting and copulating with females (meeting the demands of the transition to breeding).

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