Experimental and observational studies of seasonal interactions between overlapping life history stages in a migratory bird

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ABSTRACT

Prior to reproduction, migratory animals are at the juxtaposition of three life history stages in which they must finish the non-breeding stage, initiate and complete migration, and prepare for the onset of breeding. However, how these stages interact with one another is not fully understood. We provide evidence that, for migratory birds that begin breeding development prior to departure from non-breeding sites, the level of breeding preparation can drive migration phenology, a critical behavioral determinant of reproductive success. Specifically, male American redstart (*Setophaga ruticilla*) plasma androgen levels, which increase in males during the period leading into migration, were positively correlated with energetic condition. We empirically tested the hypothesis that elevated androgen simultaneously supports migratory and breeding preparation in a hormone manipulation field experiment. Males with testosterone implants showed advanced preparation for migration and breeding, and ultimately departed on migration earlier than controls. It is assumed that early departure leads to early arrival at breeding areas, which increases breeding success. Collectively, our observational and experimental results demonstrate how overlapping life history stages can interact to influence important components of an individual's fitness. This highlights the critical need for understanding population processes across the full life cycle of an organism to better understand the ecological and evolutionary origins of complex life history events.

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Introduction

Endocrine control mechanisms mediate an individual's response to external conditions and thus influence the phenotype–environment interaction (Ricklefs and Wikelski, 2002). Understanding how such mechanisms regulate behavior is vital to predicting the extent to which organisms are capable of responding to short- and long-term environmental change. Ecological conditions are rapidly changing (e.g. Parmesan and Yohe, 2003; Walther et al., 2002), perhaps at variable rates in different locations used by the same individuals, as in migratory animals (e.g. Both and Visser, 2001). Some species appear limited in their ability to appropriately adjust their behaviors to match new resource optima during one or more life history stages (e.g. Both and Visser, 2001; Stenseth and Mysterud, 2002). Elucidating aspects of the endocrine system that play a role in modulating behaviors are a critical early step in determining constraints on adaptation to novel environmental conditions.

A critical component of understanding how endocrine systems may limit behavioral adaptation is determining the nature of transitions between life history stages (e.g. migration, breeding).

Defining physiological life history stages geographically, however, mischaracterizes them as entirely distinct and independent units. While an organism's life cycle can be partitioned into a finite number of distinct stages (finite state machine model; Wingfield, 2008), these stages exist on a continuum, with the development of one stage often overlapping with the completion of another (reviewed in Ramenofsky and Wingfield, 2006). Within these 'superstates' (Wingfield, 2008), components of each life history stage (e.g. endocrine mechanisms) could interact with one another, regardless of the degree of overlap. Interactions among life history stages ('seasonal interactions'; e.g. Marra et al., 1998; Runge and Marra, 2005) can influence fitness. Understanding the nature of superstates, and interactions within them, requires revealing the physiological mechanisms underlying their expression.

Migratory birds complete life history stages across several distinct geographic areas, and events at these locations can be inextricably linked. In these species, seasonal interactions are especially well documented in spring (e.g. Gunnarsson et al., 2005; Marra et al., 1998; Reudink et al., 2009) when migrants are at the nexus of non-breeding, migratory, and breeding life history stages. During this season, birds must terminate the non-breeding stage (while still interacting with their winter habitat), prepare for and complete migrations that can be thousands of kilometers long, and ultimately arrive at breeding sites ready to establish territories and reproduce. The timing of these events can be a critical determinant of fitness (e.g. Morbey and Ydenberg, 2001). Non-breeding habitat quality can influence spring departure

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and arrival schedules in migratory birds, such that individuals from superior non-breeding habitats arrive earlier to breed (e.g. Gunnarsson et al., 2006; Marra et al., 1998) and, as a result, have greater reproductive success (e.g. Gunnarsson et al., 2005; Marra et al., 1998; Reudink et al., 2009; Tonra et al., 2011a). The overlap of life history stages allows for greater flexibility in the timing of life cycle events (reduced finite stage diversity; Wingfield, 2008), such that individuals can adjust for variable conditions encountered throughout transitional periods. Interactions among stages could further facilitate flexibility in responding to a dynamic environment, perhaps optimizing important traits like arrival date and breeding readiness.

Non-breeding, migratory, and breeding stages are linked by a series of complex physiological changes in response to environmental conditions (Holberton and Dufty, 2005; Ramenofsky and Wingfield, 2006). For males, a major component of transitioning to breeding is an increase in circulating androgens, particularly testosterone (hereafter: T), which promotes development of secondary sex characteristics and breeding behavior (reviewed in Fusani, 2008), as well as sperm production (Wingfield and Farner, 1993). Androgens, including T, can also influence several important aspects of migratory condition including muscle anabolism (Fennell and Scanes, 1992), erythropoiesis (Morton, 1994), and hyperphagia (reviewed in Deviche, 1995). These activities also support the energy requirements of migratory flight. In a recent experimental study of captive dark-eyed juncos (*Junco hyemalis*), we demonstrated that pre-migratory males with experimentally elevated T began to fatten and show migratory restlessness earlier than controls and T-inhibited birds (Tonra et al., 2011b). In addition, we found that free-living migratory birds arriving earlier at a breeding site had higher circulating androgen compared to late arrivers (Tonra et al., 2011a). Collectively, these results suggest that androgen activity begins in such a way as to support the transition from the non-breeding to the migratory state, in addition to supporting breeding development. Several previous studies on migratory songbirds have demonstrated that breeding development can commence prior to departure from wintering sites (i.e. overlaps with the non-breeding and migratory stages) (e.g. Wingfield and Farner, 1978; Wingfield et al., 1992a; Gwinner et al., 1994; but see Bauchinger et al., 2007, 2008). The goal of our study was to determine how the onset of breeding physiology on the non-breeding grounds not only overlaps, but also interacts with migratory preparation.

We predicted that individuals who begin the physiological transition into a breeding state earlier are at an advantage in terms of preparing for, and departing earlier on, spring migration. To test this hypothesis that breeding and migratory life history stages interact, we used both observational and experimental approaches in free-living American redstarts (*Setophaga ruticilla*) overwintering in Jamaica, West Indies. First, to confirm that life history stages overlap in redstarts, we sampled circulating androgen from mid-winter (January through February) to late-winter (late March through April, pre-migration) to detect changes in this hormonal indicator of breeding preparation. We then examined relationships among plasma androgen levels, winter habitat type, energetic condition, and date of departure on spring migration. If breeding preparation begins prior to departure and is influenced by energetic condition, we predicted that male plasma androgen concentrations would increase as winter progressed, and that males with higher circulating androgen would be in better late-winter condition and depart earlier from winter territories. Although females also require androgens for migratory preparation (Schwabl and Farner, 1989a), they express different breeding hormone pathways in the plasma. Thus, we predicted that female plasma androgen levels would show little or no increase, compared to males, as winter progressed. Since male redstarts exhibit delayed plumage maturation and young males are subdominant (Marra, 2000), we examined patterns in young and old males separately to investigate age-related patterns.

To examine causality in our observational data, we manipulated T in older males to observe its effects on migratory and breeding preparation,

and on departure schedules. We predicted that, compared to controls, males with experimentally elevated T would not only exhibit the physiological (cloacal protuberance development) and behavioral (singing) aspects of breeding preparation earlier, but also earlier onset of migratory preparation (increased foraging rate, body mass, breast muscle, and fat stores), and, as a result, would depart earlier on spring migration. Although earlier studies have observed the overlap in breeding preparation with migration, this is the first study to attempt to experimentally alter migratory decisions through physiological manipulation in a free-living bird in this way.

Material and methods

Study site

We conducted this research at the Font Hill Nature Preserve (18°02′N, 77°57′W), St. Elizabeth Parish, Jamaica. For the observational study, birds were sampled in two mesic habitats (mangrove forest and mangrove-scrub ecotone) and one xeric habitat (second-growth scrub). Mangrove and scrub habitats are described in detail in Marra and Holmes (2001). The ecotone is a transitional habitat between mangrove and scrub, characterized by an open understory and closed canopy consisting primarily of logwood (*Haematoxylon campechianum*), white mangrove (*Laguncularia racemosa*), and young black mangrove (*Avicennia germinans*) with high abundance of epiphytes (primarily *Tillandsia* spp.), and a lack of black mangrove pneumatophores. As a result of a disparity in leaf retention between mesic and xeric habitats, the abundance of forage for redstarts (i.e. phytophagous arthropods) generally declines in xeric habitats during the dry season (late February–April), but remains constant in mesic habitat throughout the year (Studds and Marra, 2005, 2007). Redstarts defend nearly exclusive territories in all three habitat types throughout the winter period at this study site (Marra, 2000).

Observational study

We captured redstarts in mist nets either passively or using recordings of conspecific song and a stuffed decoy. Passive nets were checked approximately every 15 min. We tested for effects of capture method on circulating androgen (see Results). Birds were sampled in 2009 and 2010 during two separate periods: 1) mid- (15 Jan–28 Feb) and 2) late-winter (15 Mar–30 Apr). Birds in mid-winter are fully in a non-breeding/non-migration stage, while birds in late winter are entering the stage where migratory preparation begins. In 2009 we also sampled female redstarts to determine if potential increases in androgens were male-specific and thus more likely to be associated with breeding functions of androgen rather than solely migratory functions. Redstarts were aged as second year (young) or after second year (adult) using plumage characteristics (Pyle, 1997). Each bird was individually marked, weighed, and measured for body size following Tonra et al. (2011a), and assigned a furcular fat score (0–6) following Tonra et al. (2011b). We generated a size-corrected index of condition using residuals from a regression of body mass versus body size (first principal component of wing, tail and tarsus length; for detail see Tonra et al., 2011a; Schulte-Hostedde et al., 2005). We performed separate size corrections for each sex and age class. We measured the diameter of the cloacal protuberance (CP), following Tonra et al. (2011a; mean intra-observer repeatability = ±0.16 mm, SD = 0.11, n = 10), as a surrogate measure of testis development and, therefore, plasma T activity (e.g. Morton et al., 1990) in a subset of males (adult: 56 individuals, n = 66 samples; young: 23 individuals, n = 23 samples).

We collected a 50–80 μL blood sample within 5 min of our approach to the net and stored it as described by Tonra et al. (2011a). To determine plasma androgen concentration, we used a direct radioimmunoassay (Wingfield et al., 1992b) for T. We ran separate assays for each year's samples, run in duplicate, but all samples collected within a year were