



Tales of testosterone: Advancing our understanding of environmental endocrinology through studies of neotropical birds

I.T. Moore^{a,*}, B.J. Vernasco^a, C. Escallón^b, T.W. Small^c, T.B. Ryder^d, B.M. Horton^e

^a Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA

^b Departamento de Ciencias Básicas, Universidad de la Salle, Cra 2 No. 10-70, Bogotá, Colombia

^c Department of Biology, University of Memphis, Memphis, TN 38152, USA

^d Migratory Bird Center, Smithsonian Conservation Biology Institute, National Zoological Park, PO Box 37012, MRC 5503, Washington DC 20013, USA

^e Department of Biology, Millersville University of Pennsylvania, Millersville, PA 17551, USA

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ABSTRACT

Studies of birds have greatly advanced our understanding of how testosterone modulates complex phenotypes, specifically its role in mediating male reproductive and associated behaviors. Yet most of the foundational studies have been limited to northern latitude breeding species despite the fact that they represent only a small fraction of worldwide avian diversity. In contrast, phylogenetic, life-history, and mating system diversity all reach their apex in neotropical avifauna and yet these birds, along with more southern latitude species, remain very poorly understood from an endocrine perspective. Despite the relatively limited previous work on taxa breeding in Central and South America, empirical findings have had a disproportionately large impact on our understanding of testosterone's role in everything from geographic variation to behavioral roles and neuroplasticity. Here, we synthesize how studies of neotropical breeding avifauna have advanced our understanding of how testosterone's actions can and are associated with the broad patterns of phenotypic diversity that we see in birds. In addition, we outline how these studies can be used individually or in a comparative context to address fundamental questions about the environmental endocrinology of testosterone and to understand the diversity of roles that testosterone plays in mediating behavioral variation, reproductive strategies, and associated life-history trade-offs.

1. Introduction

The field of environmental endocrinology was initiated with foundational studies of free-living birds and other vertebrates breeding in northern temperate latitudes. From those early studies, we learned about the dynamic nature of plasma testosterone levels and how intra- and interspecific variation in circulating testosterone was greater than previously thought (Wingfield and Farner, 1976, 1978b; Wingfield and Grimm, 1977). Prior to these early studies of free-living birds, investigations of captive and domestic animals suggested that testosterone levels were simply elevated in the breeding season, declined to basal levels in the non-breeding season and were not particularly dynamic (for a review see: Soma, 2006). In contrast, studies of free-living animals, beginning in the mid-1970s, demonstrated that plasma testosterone levels can be responsive to both environmental and social cues (Wingfield and Farner, 1980). As studies of the environmental endocrinology of testosterone advanced in the late 20th and early 21st

centuries, the number of species investigated blossomed, but with a continued emphasis on higher latitude breeding species (e.g. Wingfield and Hunt, 2002).

The differences between low and mid-to-high latitude environments in terms of environmental cues, selection pressures, and associated life-histories have provided opportunities to rapidly expand our understanding of the diverse roles of testosterone action and to elucidate the proximate and ultimate drivers of testosterone variation among vertebrates. From a proximate perspective, the early ecological and behavioral studies of white-crowned sparrows (*Zonotrichia leucophrys*) documented that testosterone levels in males were socially responsive (i.e., they respond to social interactions with females and other males), vary with latitude, and are associated with life-history variation (Wingfield and Farner, 1978a,b). For example, the number of times that male testosterone levels peak during a breeding season depends on the number of breeding attempts by his mate, which in turn decreases with increasing latitude. From an ultimate perspective, studies of male dark-

* Corresponding author at: Department of Biological Sciences, 2119 Derring Hall, Virginia Tech, Blacksburg, VA 24061, USA.
E-mail address: itmoore@vt.edu (I.T. Moore).

eyed juncos (*Junco hyemalis*) have also been pivotal in understanding the fitness costs and benefits of elevated testosterone levels (Ketterson et al., 1992, 1991). Although high testosterone can facilitate increased extra-pair mating effort in male juncos, it can also reduce mate guarding and paternal care, resulting in loss of paternity and lower fledging success with their social mate, respectively (Raouf et al., 1997; Reed et al., 2006).

A critical advance in our thinking about testosterone action in free-living animals was the development of the Challenge Hypothesis (Wingfield et al., 1990). This hypothesis posits that plasma testosterone levels in males are responsive to social interactions (e.g., territorial aggression), and dependent on the degree of parental care provided by the male. Moreover, while the Challenge Hypothesis was originally developed and applied to birds, it has since been tested in members of every vertebrate class and even in humans (Hirschenhauser and Oliveira, 2006). Extensions of the original hypothesis such as the Energetics-Vocalization model have expanded the Challenge Hypothesis to include energetics and glucocorticoid hormones (Emerson, 2001). The meta-analyses and theoretical discussions noted above, as well as others, demonstrate that the Challenge Hypothesis is a dynamic, robust idea that it is still actively investigated, and continues to inform our understanding of testosterone action even if it is not applicable in all scenarios.

Another active area of testosterone research involves the hormone's role in the seasonal regulation of neuroplasticity and neurogenesis in the adult vertebrate brain. This neuroplasticity was first discovered and studied in captive breed avian systems (e.g., the canary (*Serinus canaria domestica*) and the zebra finch (*Taeniopygia guttata*)) and subsequently has been greatly expanded upon in free-living mid-latitude bird species, primarily from North America (see reviews by Brenowitz and Beecher (2005), Tramontin and Brenowitz (2000)). In the passerine brain, testosterone is the primary physiological regulator of neuroplasticity in the song control nuclei, a series of interconnected brain areas in the fore-brain that mediate the learning and expression of vocal behavior in song birds (Brenowitz et al., 1997). Briefly, as daylength increases in the spring, male birds are photostimulated which results in activation of the hypothalamic-pituitary-gonadal axis (Ball and Bentley, 2000; Dawson et al., 2001). As a result of this activation, circulating testosterone levels increase in the blood, eventually activating androgen receptors and, after conversion to estrogen, estrogen receptors in the brain (Soma et al., 2004; Tramontin et al., 2003). Both androgen and estrogen receptors are expressed in the song control nuclei and, in response to testosterone and its metabolites, these song control nuclei grow in size (Tramontin et al., 2003). This growth results from both increased neuron size as well as neurogenesis. These new neurons are incorporated into the adult brain and control the seasonal learning and expression of singing behavior. This model of the growth and incorporation of new neurons in the adult brain has been one of the best examples of adult neuroplasticity and a successful model for basic as well as applied biomedical research.

As briefly described above, and far from exhaustively, many of the most significant advances in our understanding of testosterone action have resulted from studies on temperate-breeding birds at mid-to-high latitudes. However, the majority of bird species breed in the Neotropics (Cadena et al., 2011; Melo et al., 2009; Stutchbury and Morton, 2001), and these species are substantially more diverse than temperate zone breeding taxa in regards to phylogenetics, morphology, reproductive strategies and behaviors, as well as social systems (Robinson et al., 2010). For example, the neotropical zone hosts around a third of avian biodiversity (over 3000 species) compared to just over 700 breeding species in the Nearctic (Newton, 2003). Beginning in the mid-1990's and accelerating after 2000, studies of Central and South American birds have provided us with distinct opportunities to expand our knowledge of the many roles of testosterone that were not possible given the limited diversity of north temperate species. Because this expansion of field endocrinology is occurring in parallel with studies of

natural history, behavior, and broader evolutionary patterns of diversification of neotropical birds, it is now possible to identify broad-scale patterns of variation in testosterone, behavior, and life history strategy (Hau et al., 2008). For example, relative to northern species, plasma testosterone levels in neotropical breeding male birds are often far lower and less responsive to changes in life history stages or social interactions (see next section). Similar studies in the old world tropical breeding species have provided an independent test of some of the conclusions derived from studies of neotropical breeding species (e.g., Goymann et al., 2006; Goymann and Wingfield, 2004). This paper is not intended as an exhaustive review (for a better review of the behavioral ecology of tropical birds see: Hau et al. (2008), Stutchbury and Morton (2008, 2001)), but rather here we synthesize examples of how studies of Central and South American birds, primarily those breeding in the neotropics, have challenged and expanded our understanding of testosterone action as it relates to the broader complex phenotype, and we also suggest some future avenues of research that should prove rewarding.

2. Geography and testosterone

Studies of neotropical breeding birds have identified substantial geographic variation in circulating testosterone (within and among taxa). Since the first studies of tropical breeding birds, investigators have noted their unusually low testosterone levels in comparison with northern latitude breeding species and these patterns have been supported with meta-analyses, but not always in more focused studies of individual species. In one of the first studies of hormones in a free-living neotropical breeding bird, investigators documented low circulating levels of testosterone in male bay wrens (*Thryothorus nigricapillus*) from Panama even during the height of the breeding season (Levin and Wingfield, 1992). Subsequent studies of other Central American breeding birds seemed to confirm the pattern of tropical breeding birds generally having lower testosterone than higher latitude species (Stutchbury and Morton, 2001; Wikelski et al., 2003). More recently, broader interspecific comparisons, including meta-analyses in birds, reptiles, and amphibians, have confirmed that the geographic pattern in testosterone levels is consistent and robust (Eikenaar et al., 2012; Garamszegi et al., 2008; Goymann et al., 2004). These meta-analyses have used the tropical breeding species from around the globe and not delineated them longitudinally or even more crudely by continent. The broad-scale geographical differences in testosterone levels have been attributed to the divergent selection pressures and life history strategies of tropical versus temperate-breeding birds (Stutchbury and Morton, 2001). In the first meta-analysis of testosterone levels of male tropical breeding birds it was found that testosterone was indeed lower in the tropical species, but with substantial interspecific variation (Goymann et al., 2004). Further analyses were performed to explain the variation and it was found that among the tropical species, shorter breeding seasons, seasonal territoriality (as opposed to year-round territoriality), and elevation of the population were all positively associated with circulating testosterone levels in male birds (Goymann et al., 2004). A subsequent meta-analysis further suggested that some of the effect of latitude on testosterone levels could be explained by migration and breeding synchrony (Garamszegi et al., 2008). That is, species that migrate tend to arrive on the breeding grounds and assume breeding activity more synchronously than non-migratory species and the degree of male-male conflict that arises is proposed to have selected for elevated testosterone levels in those species. A focused study directly comparing two breeding bird communities, one neotropical and one northern temperate, found that neotropical species have, on average, lower breeding season testosterone levels than northern temperate species (Hau et al., 2010). The fact that a geographical pattern in male testosterone levels is evident across all these studies despite the divergent evolutionary origins of the birds and the different laboratory and field techniques suggests that the pattern is robust and biologically

significant. One broad potential explanation is that a lack of synchronous reproductive timing, such as in non-migratory species, has resulted in decreased selection for high plasma testosterone levels at tropical latitudes (Eikenaar et al., 2012). This lack of sexual selection pressure is often associated with the prolonged, or aseasonal, breeding that is more common at tropical latitudes. Of course, these explanations only refer to the average values of only a relatively few species and not the large variation that exists among and within species.

While broad-scale meta-analyses across a wide range of avian taxa have shown geographical patterns in testosterone, those same patterns have not been evident in more focused comparative studies among closely related taxa. For example, a comparison of members of the genus *Zonotrichia* breeding from the arctic to the equator did not support the pattern of increasing circulating testosterone levels with latitude (Moore et al., 2002). That study compared both breeding baseline as well as GnRH-induced levels of the hormone and found that, if anything, the relationship between latitude and testosterone was negative with the equatorial population exhibiting the highest GnRH-induced levels of testosterone (Moore et al., 2002). As the biogeographical effects of elevation can be similar to latitude (e.g., breeding season length and synchrony), a subsequent study surveyed plasma testosterone levels among equatorial populations of rufous-collared sparrows in close proximity but spanning a wide range of elevations (range: 600–3300 m). That study also found no relationship between geography (elevation) and plasma testosterone levels (Escallón et al., 2016). A similar study on the same species that directly compared two southern temperate populations in close proximity, but differing in elevation by 2000 m also found no effect of elevation on male testosterone levels (Addis et al., 2011). Thus, it appears that focused studies of individual species do not necessarily follow the patterns predicted from broader interspecific comparisons.

Why is there such a contrast in results between the broader meta-analyses and the focused studies within the genus *Zonotrichia*? It is possible that, in some cases, lower average testosterone levels for the tropical breeding birds is a product of the “tyranny of the golden mean” (Bennett, 1987; Williams, 2008). If the breeding season is less synchronized, which is typically the case with the extended breeding seasons of tropical species, then at any given sampling period it is likely that only a few individuals will have elevated testosterone, resulting in low average values for the population even though some individuals may have quite elevated testosterone levels (e.g. Ryder et al., 2011b). Additional comparative work on groups of closely related species that span temperate and tropical zones is warranted (such as the temperate and tropical stonechats studied by Gwinner and colleagues, e.g. Goymann et al., 2006), as are more studies of tropical birds with an increased focus on the individual level of analysis and repeated sampling of individuals to establish individual endocrine profiles.

3. Challenge Hypothesis and testosterone

Although the Challenge Hypothesis was developed in temperate breeding northern latitude birds (Wingfield et al., 1990), it has since been tested across all vertebrate classes as well as across latitudes, but recent work on neotropical birds is requiring investigators to reassess aspects of it, particularly the social modulation of testosterone levels in males. Many studies across vertebrate taxa have found support for the hypothesis and its basic tenets: (1) seasonal patterns of testosterone are dependent on mating system, (2) plasma testosterone levels are socially modulated (Hirschenhauser and Oliveira, 2006; Hirschenhauser et al., 2003), and finally (3) testosterone suppresses parental behavior (e.g., offspring provisioning) in males (Lynn et al., 2005). Within the Neotropics, the Challenge Hypothesis has been tested in a variety of breeding bird species that are both closely and distantly related to northern latitude species for which the hypothesis was generated (Wingfield et al., 2014). In addition, the geographic distributions of South America species have afforded the opportunity for comparative

studies across a range of latitudes, elevations, and associated life histories that have tested the generalizations of the Challenge Hypothesis. Collectively, neotropical and southern South American breeding bird studies have provided some support for the Challenge Hypothesis, but they have also identified potential limitations regarding the extent to which its tenets are broadly applicable.

Perhaps the first direct test of the Challenge Hypothesis in a low latitude bird was conducted in Panama on male spotted antbirds (*Hylophylax n. naevioides*; Wikelski et al., 1999b). After documenting that males maintain low plasma testosterone levels during the breeding season, the investigators tested whether plasma levels of the hormone were socially modulated (Hau et al., 2000, 1999; Wikelski et al., 1999a, 2000). They found that testosterone levels did increase in response to social challenges, but only if those challenges were of long duration (i.e. 2 h; Wikelski et al., 1999b). In comparison, northern latitude temperate breeding song sparrows (*Melospiza melodia*) responded to a social challenge with an increase in testosterone over a substantially shorter temporal scale (i.e. 10 min; Wingfield and Wada, 1989). However, as the spotted antbird is from a solely tropical family of birds (Thamnophilidae) within the largely tropical suboscine clade of passerines, it is unclear if the results were due to a different environment (Neotropics) or some novel phylogenetic constraint in the way that hormones modulate behavior in this family of birds. In other aspects of their behavioral endocrinology, the spotted antbirds are similar to song sparrows. Exogenous testosterone was found to enhance territorial singing behavior; while blocking the action of the hormone suppressed the behavior during the breeding season (Hau et al., 2000). During the non-breeding season, it appears that the aggressive behaviors may be mediated by DHEA, a generally weak androgen, rather than testosterone (Hau et al., 2004; Soma et al., 2000b). Future work to continue to investigate the endocrine, as well as neuroendocrine, mechanisms mediating territorial aggression across environments and in a comparative perspective will be productive.

Viewed more broadly, empirical work on a variety of Panamanian avifauna has been unable to document direct and positive relationships between testosterone and aggression that appear to be at least somewhat generalizable from work on northern latitude species (i.e., elevated testosterone during the breeding season when males are territorial). For example, studies of song wrens (*Cyphorhinus phaeocephalus*) described males as having low plasma levels of androgens (testosterone and DHEA) during both the breeding and non-breeding seasons and in addition, androgen levels were not affected by social challenges (Busch et al., 2008). Similarly, despite having elevated testosterone levels during the breeding season relative to the non-breeding season, there is no direct relationship between testosterone and aggression in red-throated ant-tanagers (*Habia fuscicauda*; Chiver et al., 2014). Male white-bellied antbirds (*Myrmeciza longipes*) do not show either a seasonal change in testosterone levels or a testosterone response to social challenges (Fedy and Stutchbury, 2006). The buff-breasted wren (*Thryothorus leucotis*) also displayed no relationship between testosterone and aggression (Gill et al., 2008). These lack of a consistent pattern between testosterone and aggression calls into question a central tenet of the Challenge Hypothesis, that plasma testosterone levels should be positively correlated to territorial aggression.

In contrast to these studies on male neotropical breeding birds, testosterone and aggression have been more directly linked in female neotropical birds, similar to what has been documented in some northern latitude female birds (e.g. Jawor et al., 2007). For example, in the female buff-breasted wren there is evidence of a positive relationship between testosterone and aggression as territorial females had elevated testosterone after intrusions from other females (Gill et al., 2007). Similarly, female golden-collared manakins (*Manacus vitellinus*) respond to exogenous testosterone treatment with increased overall aggression, but it is worth noting that the individual components of the display are still expressed at a lower rate than in males (Chiver and Schlinger, 2017). These sex-specific differences in the relationship

between testosterone and aggression in neotropical birds suggest that there may be differential selection on hormone-behavior relationships where species exhibit year-round territory defense. Ultimately, investigating how and why testosterone modulates aggressive behaviors in sex-specific manner is one clear example of how studies of neotropical birds can advance our understanding of the social role of testosterone.

One of the larger difficulties in investigating the above question regarding testosterone and aggression is the inability to make clear phylogenetic contrasts between neotropical- and high latitude breeding species that share a recent common ancestor. Studies of the rufous-collared sparrow (*Zonotrichia capensis*) have provided an opportunity for true comparative tests of the Challenge Hypothesis across latitudes without the phylogenetic confound that has afflicted many of the other studies of neotropical species. The rufous-collared sparrow is widely distributed from southern Mexico to Tierra del Fuego and from sea level to above 4000 m. It is a congener (*Zonotrichia*) of one of the best studied North American birds in terms of reproduction and endocrinology, the white-crowned sparrow (*Z. leucophrys*). In contrast to the earlier studies of neotropical birds (described above), male rufous-collared sparrows were found to have high levels of testosterone that were comparable to their northern latitude congeners (Moore et al., 2002). However, while their plasma testosterone levels increased in response to a GnRH challenge, they were not responsive to male-male social challenges as predicted by the Challenge Hypothesis (Moore et al., 2002, 2004a). Further, aggression is not affected by administration of exogenous testosterone or by blocking the hormone's action (Moore et al., 2002, 2004a,b). These results, in which males are not responsive to territorial challenges, are geographically robust and consistent as studies of male rufous-collared sparrows from populations in both Costa Rica and Chile showed similar results (Addis et al., 2010, 2011; Wingfield et al., 2007). Finally, seasonal patterns of plasma testosterone have not followed predictions from the Challenge Hypothesis. The equatorial population exhibited elevated plasma testosterone levels during the nestling period as opposed to the predicted decline. Unexpected seasonal patterns of testosterone were documented in Costa Rican populations in which some individuals breed during a mid-rainy season dry period known as the veranillo, and those individuals displayed higher testosterone levels than did non-breeding individuals (Addis et al., 2010). In summary, these studies of the rufous-collared sparrow across much of its range and a congener of the white-crowned sparrow, have not provided phylogenetically controlled comparative support for the Challenge Hypothesis in terms of how testosterone functions in regards to male-male interactions. One possible explanation for this discrepancy is that the regulation of testosterone action could differ between species due to other factors including receptor densities and steroidogenic enzyme activity as in the Evolutionary Potential Hypothesis discussed below (Hau, 2007).

One aspect of the Challenge Hypothesis that was supported in studies of the rufous-collared sparrow is in regard to testosterone and male parental care. The Challenge Hypothesis suggests that plasma testosterone levels should decrease when a male is caring for young because the hormone is incompatible and antagonistic with paternal care of young. Indeed, in male rufous-collared sparrows, there was a negative relationship between levels of paternal care, quantified as feeding of young, and testosterone. An experimental study demonstrated a dramatic negative effect of exogenous testosterone on the rate of male feeding of offspring as males implanted with testosterone essentially completely stopped feeding their young (Lynn et al., 2009). This result is consistent with, and maybe even more dramatic than, high latitude northern species (Lynn et al., 2002, 2005) and generally supports the prediction, based on the Challenge Hypothesis, that testosterone is antagonistic to paternal care of offspring. Because rufous-collared sparrows are aggressive during parental care, the absence of a direct relationship between testosterone and male-male aggressive interactions may, in part, ameliorate potential trade-offs between parental care

and territoriality (Moore et al 2004a,b; Lynn et al., 2009).

4. Neuroplasticity and testosterone

Arguably the most important discovery of the past half century, arising from studies using birds as model systems, was the description of seasonal growth and incorporation of new neurons in the adult brain (Nottebohm, 1981). This neuroplasticity has since been documented in every vertebrate class, and it has dramatic implications for basic and applied biomedical science implication (for a review see Tramontin and Brenowitz (2000)). While the endocrine, molecular, and cellular bases of neuroplasticity have been widely investigated, the ecological and evolutionary aspects have received much less attention. Work on neotropical birds is now demonstrating that the environmental control of this neuroplasticity is much broader than previously thought.

Studies on north temperate bird species have established that neuroplasticity of the song-control system involves endocrine responses to environmental cues (Tramontin and Brenowitz, 2000; Tramontin et al., 2003). Briefly, as daylength increases in the spring, the bird becomes photostimulated and the hypothalamic-pituitary-gonadal axis is activated in males resulting in an increase in plasma levels of testosterone (Dawson et al., 2001; Deviche and Small, 2001). Testosterone, either acting through an androgen receptor or through an estrogen receptor after conversion to 17 beta-estradiol, stimulates growth of new neurons and their incorporation in the neural song control system (Brenowitz, 2004; Brenowitz and Lent, 2002; Soma et al., 1999; Tramontin et al., 2000). The potential role of environmental cues other than photoperiod in mediating neuroplasticity of the song control systems was unknown until studies of an equatorial bird that did not experience seasonal changes in day length (Small and Moore, 2009).

Initial investigations of two equatorial populations of rufous-collared sparrows, separated by 25 km and the Andean ridge, found that both displayed seasonal song production and breeding within a population but these activities were asynchronous among populations and separated by approximately four months (Moore, 2005; Moore et al., 2005). This descriptive finding presented an opportunity to investigate the environmental control of neuroplasticity independent of seasonal changes in photoperiod. Subsequent studies determined that characteristics of both the hypothalamic GnRH system and the neural song control system differed between breeding and non-breeding seasons in each of these two populations (Moore et al., 2006, 2004c). Further, because the breeding seasons of the two populations are asynchronous, the timing of the neuroplasticity was also asynchronous. These findings of asynchrony exclude the possibility that the birds are using the typical photoperiodic cues, however small they may be, to time seasonal reproductive changes as the two populations reside at the same latitude. A subsequent experimental study on captive rufous-collared sparrows found that exogenous testosterone treatment could stimulate growth of the song control system, at least in some of the nuclei, and these effects could be prevented by blocking both the androgen receptors and aromatase activity (Small et al., 2015). While testosterone treatment does stimulate singing in a variety of species of captive male birds housed alone or with other males (Boseret et al., 2006; Dloniak and Deviche, 2001; Meitzen et al., 2009; Strand et al., 2008; Tramontin et al., 1998), consistent with findings in free-living rufous-collared sparrows, testosterone treatment did not stimulate singing in these birds, indicating that the growth of the nuclei was not due to feedback from singing behavior. Together, these results suggest that testosterone regulates important aspects of the seasonal development of the song control system in equatorial rufous-collared sparrows, but it can do this without influencing, or being influenced by, singing behavior. These studies underscore that despite using the same hormonal signaling pathway as northern temperate species, local environmental cues/conditions can drive patterns of neural plasticity in the song control system (Small and Moore, 2009).

At least one other study of a South American bird has documented

neuroplasticity of the song control system that is dependent on testosterone action. In male silver-beaked tanagers (*Ramphocelus carbo*), singing behavior was described as seasonal and plasma testosterone levels were positively related to rates of dawn chorus singing (Quispe et al., 2017, 2016). More interesting, neural song control center HVC grew before an increase in plasma testosterone levels were detected. However, the investigators also found that expression of the androgen receptors in HVC was greater before plasma testosterone levels increased and this may have served as an anticipatory, and alternative, role for increased androgen action (Quispe et al., 2016). While few, these studies of neotropical birds have highlighted the importance of testosterone signaling as a mechanism mediating neuroplasticity and how seasonal changes in the song control system can be dependent on a diversity of environmental cues, not just photoperiod.

5. Complex behaviors, social organization, and testosterone

The manakins (*Pipridae*) are a family of suboscine birds that have provided good examples of how studies of neotropical breeding birds can expand our general knowledge of how testosterone modulates complex reproductive and social behaviors. The family *Pipridae* is mainly composed of polygynous species that exhibit lek breeding systems and in many manakin species males exhibit elaborate secondary sexual traits and courtship displays (for a review see: Kirwan et al. (2011)). During the breeding season, males are typically spatially aggregated in the environment and spend a large portion of the day on the lek, but relatively little time actually displaying to attract females (Barske et al., 2014; Kirwan et al., 2011). In some species, males hold individual territories within the lek, and in other species males clear courts in which to perform their displays (Schlinger et al., 2008). Multiple species have also evolved morphological specializations to perform complex courtship displays that can include acrobatic maneuvers, mechanical sound production, and, in some cases, coordinated/cooperative displays between two or more males (Kirwan et al., 2011; Prum, 1990). Given their varied and complex displays and unique social organizations, the manakins have provided some key opportunities to advance our understanding of the role of testosterone in regulating complex behavioral phenotypes.

Perhaps the best studied of the manakin species in terms of testosterone regulation of complex display behavior is the golden-collared manakin (*Manacus vitellanus*; Fuxjager and Schlinger, 2015). The courtship display of this species includes vocalizations, acrobatic maneuvers, and the production of mechanical sounds and circulating androgens are thought to activate display behaviors (Day et al., 2007; Fusani et al., 2007; Fuxjager et al., 2012, 2015). Although androgen levels are elevated at the beginning of the breeding season they become more variable (i.e., high in some males and low in others) as the breeding season progresses (Day et al., 2007). Interestingly, experimental administration of testosterone activated courtship behaviors in juveniles, females, and non-breeding males, but no dose-dependent relationship between testosterone and courtship behavior has been observed in breeding males (Day et al., 2007). Furthermore, androgen receptors have been found to be expressed in the brain, spinal cord, and in the skeletal muscles used to perform courtship displays (Fusani et al., 2007; Fuxjager et al., 2012, 2015). Blocking androgen receptors decreased the production of display behaviors among reproductively active males (Fusani et al., 2007) and specific display maneuvers were found to be shorter and more variable when peripheral androgen receptors (i.e., in skeletal muscles) were blocked (Fuxjager et al., 2013). Finally, testosterone implants changed the expression of genes that are known to support muscle function, strength, and coordination in the skeletal muscles used during display performance (Fuxjager et al., 2012). Taken together, the work on golden-collared manakins has provided a detailed understanding of the mechanistic basis for how androgenic signaling cascades can modulate neuromuscular control systems used in complex displays (Barske et al., 2011; Day et al., 2007,

2006; Fusani et al., 2014).

The relationship between androgen receptor expression and complex courtship displays has been expanded beyond just manakins. A comparative study that included seven different species of passerines (4 manakins and 3 non-manakin passerines) chosen to represent a continuum of behavioral display complexity found that species with more behaviorally complex displays express higher levels of androgen receptor mRNA in the wing muscles used during courtship (Fuxjager et al., 2015). This study, and the works described above on the golden-collared manakin, provide strong evidence to suggest that components of androgen signaling, beyond simplistic plasma levels of the hormone, can be selected upon to mediate the expression of complex display behaviors (Fuxjager et al., 2017). Given the wide diversity of courtship display behaviors exhibited by Central and South American birds, neotropical breeding species provide a wealth of opportunities for further investigation into the physiological bases of display behavior, including endocrine, neuroendocrine, and genetic mechanisms.

In addition to complex displays, some manakins have evolved male-male cooperative displays that form the basis of complex social systems and hierarchies (Kirwan et al., 2011; Prum, 1994). In the wire-tailed manakins (*Pipra filicauda*), social network theory has been used to analyze the fitness consequences of individual variation in social behavior for both floater and territory-holding males (Ryder et al., 2008). Territory-holding males that are more connected within their social network achieve higher reproductive success and, among floaters, more socially connected individuals have a higher probability of acquiring a territory (Ryder et al., 2008, 2009). In an effort to bridge social network theory and environmental endocrinology, a study of the wire-tailed manakin found that males who hold territories have higher testosterone levels than do floater males (Ryder et al., 2011b). A similar result was found by Duval and Goymann (2011) in the lance-tailed manakins (*Chiroxiphia lanceolata*) where alpha males have higher testosterone levels than lower category males. These studies have provided new and powerful evidence to help us begin to understand how individual and status-specific differences in testosterone levels shape complex social behaviors and ultimately dominance hierarchies. Future investigations relating individual hormonal variation to fitness metrics promise to provide insights on the selective pressures on those hormone levels.

6. Synthesis

At mid- to high latitudes, most passerine birds are strictly seasonal breeders and in males the rapid and coordinated increase in plasma androgen levels and the hormone's activity activates reproductive physiology, including development of secondary sexual characteristics and the song control nuclei in the brain. This rapid reproductive development in males is often accompanied by changes in behavior such as increased aggression and courtship behaviors, both of which are often aided by increased singing. Peaks in these breeding activities are associated with high plasma androgen levels, and in many species these levels decline as the birds shift into the nesting stage, especially if the males participate in parental care. In mid- to high latitude breeding populations, the above processes are quite synchronous and coordinated and this synchrony can lead to heightened periods of social conflict, which can further stimulate androgen activity as modeled by the Challenge Hypothesis. However, in many environments, particularly the Neotropics, breeding can be much less synchronous with individuals at very different reproductive stages at any given time (Class et al., 2011; Stutchbury and Morton, 2001). How breeding synchrony within a population (or the lack thereof) affects the role that androgens play in mediating complex phenotypes is an active and promising direction for future investigation.

By and large, elevated breeding season testosterone levels in temperate-breeding bird species promote aggression and mating effort by males but inhibit paternal care of offspring. In the tropics, however,

hormone-behavior relationships often do not fit this paradigm. For example, the longer breeding seasons, potentially increased costs of elevated testosterone, and slower life histories of tropical birds may select for the maintenance of lower testosterone levels year-round or, alternatively, testosterone peaks of shorter duration. Yet, due to the less synchronized timing of reproduction of many tropical species, tropical males may need to express territorial aggression, including high singing rates, outside of their own breeding period or during parental care (and with low testosterone) because surrounding males can be at different reproductive stages. This could be achieved if aggressive behavior is activated by very low levels of testosterone (e.g. Soma et al., 2000a) that are not incompatible with parental care, or if behaviors such as aggression or paternal care are dissociated from circulating testosterone control as can occur in some temperate zone species that exhibit non-breeding season territorial aggression (e.g. Canoine and Gwinner, 2002). However, some neotropical male birds exhibit relatively high testosterone levels that are comparable to those of males breeding at mid to high latitudes (e.g. DuVal and Goymann, 2011; Ryder et al., 2011b), and in some cases, they paradoxically exhibit behaviors (e.g., cooperation) thought to be incompatible with elevated testosterone. For example, territorial wire-tailed manakin males have relatively high circulating androgen levels, yet these males regularly engage in cooperative male-male courtship display and rarely exhibit overt aggression, even in a reproductive context (Ryder et al., 2011a, 2009). Disentangling these hormone-behavior relationships will require further investigation, but will ultimately lead to a better understanding of how hormone regulatory networks modulate complex behaviors.

The fact that neotropical breeding male birds exhibit hormone-behavior relationships that can differ from those seen in northern latitude birds yields exciting opportunities for investigations of how social behaviors and, more broadly, complex behavioral phenotypes are regulated by testosterone. Evidence from tropical birds suggest that variation in male reproductive physiology and behavior may be regulated by the actions of androgens targeted at very specific tissue levels, and independently of variation in plasma hormone concentrations (Canoine et al., 2007). By targeting androgen regulation at the tissue level, and independently of circulating androgen levels, tighter control can be achieved in regards to when and where testosterone influences behavior and physiology (Hau, 2007). Such targeted regulation could be achieved by a number of mechanisms, including the regulation of steroidogenic enzyme activity and steroid receptor expression, or via actions on steroid-sensitive neuropeptide systems (e.g. Canoine et al., 2007; Maney and Goodson, 2011; Soma et al., 2003). Given the wide variety of behavioral strategies exhibited by tropical male birds with diverse testosterone profiles, a myriad of prospects exist for research aimed at elucidating how testosterone signaling, including the associated neuroendocrine, molecular, and neuropeptide mechanisms, regulates the reproductive behaviors of tropical male birds.

7. Future opportunities

Above we have described some examples in which studies of neotropical breeding birds have expanded our understanding of the environmental endocrinology of testosterone. Here, we want to emphasize that these examples are just a few prime examples and not an exhaustive list of advances and opportunities and that there are inherent challenges that can be substantial (Fusani et al., 2005). It should also be emphasized that major advances were facilitated by the careful choice of focal species and populations with unique life histories and mating systems (e.g. Hau et al., 2008). It is becoming more apparent that to make transformative advances in our understanding of testosterone's role in mediating variation in complex behavioral phenotypes and, ultimately, life history strategy, investigations across species and among heterogeneous environments is not only profitable, but necessary. The Neotropics, with its wide range in elevation, latitude, and thus immense diversity of habitats and associated biodiversity, present largely

untapped opportunities to understand the diverse ways that testosterone can mediate complex phenotypes and life-history trade-offs.

The physiology-life history nexus has been presented and widely cited as a way of investigating environment-endocrine interactions (Ricklefs and Wikelski, 2002). Building upon this idea, Hau (2007) presented a productive approach for considering testosterone's action from an evolutionary perspective. She advanced two hypotheses regarding the evolutionary linkage between testosterone and male reproductive traits. The Evolutionary Constraint Hypothesis (Ketterson et al., 1999) posits that testosterone and certain male traits are perpetually linked, and as such, variation among species in testosterone-mediated traits is largely due to differences in circulating hormone levels. On the other hand, the Evolutionary Potential Hypothesis (Hau, 2007) suggests that hormone-signaling mechanisms and male traits can evolve independently. This later hypothesis is garnering support from studies of neotropical and temperate-breeding birds (e.g. Horton et al., 2010; Lynn et al., 2002; Ryder et al., 2011b), and may provide more flexibility than the former hypothesis for explaining variation in hormone-behavior relationships across species. Yet, we have much to learn about how testosterone signaling serves to mediate the male reproductive trade-offs that constrain and thus shape life-history strategies.

We propose that neotropical birds provide an obvious path forward for investigators interested in gaining a comprehensive understanding of the role that testosterone plays in mediating life-history tradeoffs as well as disentangling the evolutionary linkage between testosterone and complex male traits. More specifically, an increased focus on the neuroendocrine and genetic mechanisms underlying testosterone signaling pathways in tropical birds promises to advance our ability to explain the proximate bases of complex phenotypes. In short, the sheer diversity in reproductive behavior and life-histories among neotropical birds (Robinson et al., 2010) is an underutilized resource. Many of the species in this region are closely related to well-studied northern species but inhabit dramatically different habitats and exhibit vastly different life histories. Such species provide an especially valuable opportunity for comparative studies on the environmental endocrinology of testosterone while minimizing phylogenetic confounds.

8. Conclusions

Our understanding of the roles of testosterone in birds has been greatly expanded by studies performed on neotropical breeding birds. Yet these studies have only just scratched the surface of what is possible, especially given the massive phylogenetic and life-history diversity of species in the Neotropics. Future studies should focus on species with unique mating systems and behaviors as well as environments, such as dry tropical forests, that are very seasonal, yet have constant photoperiods. Finally, as the vast majority of the world's birds are tropical and the Neotropics are among the most biodiverse areas on earth, the future opportunities for major discoveries are limitless.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ygcen.2018.07.003>.

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