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REVIEW

Evolution of the androgen-induced male phenotype

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Abstract The masculine reproductive phenotype varies significantly across vertebrates. As a result, biologists have long recognized that many of the mechanisms that support these phenotypes—particularly the androgenic system—is evolutionarily labile, and thus susceptible to the effects of selection for different traits. However, exactly how androgenic signaling systems vary in a way which results in dramatically different functional outputs, remain largely unclear. We explore this topic here by outlining four key—but non-mutually exclusive—hypotheses that propose how the mechanisms of androgenic signaling might change over time to potentiate the emergence of phenotypical variation in masculine behavior and physiology. We anchor this framework in a review of our own studies of a tropical bird called the golden-collared manakin (*Manacus vitellinus*), which has evolved an exaggerated acrobatic courtship display that is heavily androgen-dependent. The result is an example of how the cellular basis of androgenic action can be modified to support a unique reproductive repertoire. We end this review by highlighting a broad pathway forward to further pursue the intricate ways by which the mechanisms of hormone action evolve to support processes of adaptation and animal design.

Keywords Social behavior · Endocrine system · Neuromuscular · Skeletal muscle · Birds

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Introduction

Endocrine signaling was initially identified and defined by the presence of molecules in blood that communicated information from one tissue to another (Berthold 1849). Although we now appreciate the greater complexity of endocrine signaling to include paracrine (cell-to-cell) and intracrine/autocrine (cell autonomous) signaling, it is the secretion of signaling molecules into the systemic circulation that is most widely appreciated as what defines a hormone. Of course, what gives any molecule its signaling capability is the presence of some receptor mechanism in a cell or on its membrane that transduces the external signal into an internal signal or action. Thus, if no cells have receptors for a blood-borne molecule it will not function as a hormonal signal. If one or a few cells or tissues have receptors, then the molecule will have restricted signaling functions. If many cells or tissue have receptors and/or if different types of receptors exist, then that hormone can have widespread complex functions.

Androgens are circulating steroid hormones secreted predominantly by the gonads, most abundantly by the testes, but also by the adrenals (Chang 2002). Their actions on target issues occur largely in two ways. First, they can serve as the substrate for the formation of estrogens with actions mediated by estrogen receptors. Second, androgens exert their actions via androgen receptors (AR), a relatively conserved class of receptor that, once bound to active hormone,

functions as a transcription factor to regulate cellular gene expression.

The crucial androgen function in vertebrates is to establish and promote the masculine phenotype (Chang 2002; Adkins-Regan 2005). This occurs through androgen action during early development, when these hormones establish the male reproductive system and its related structures (Baum 1979; van der Schoot 1980; Weisz and Ward 1980; Rhoda et al. 1984; Adkins-Regan 1987; Cooke et al. 1998). Upon sexual maturity, androgens then act on the juvenile condition to induce the development, form, and function of the adult male reproductive phenotype, a host of characteristics that are relatively conserved (Phoenix et al. 1959; Goy and Phoenix 1972). If we focus purely on the reproductive system, this encompasses functional testes and accessory structures, as well as behavior that can lead to successful fertilization of females. When viewed more broadly, by comparing behavior, anatomy, and physiology across individuals and higher taxa, we observe an extraordinary diversity of phenotypes that characterize how adult males differ from juvenile males and adult females, and even how individual males differ within a species. We have evidence that these masculine states result from androgen signaling, but what accounts for the enormous diversity that is observed in nature? How is evolution of the male phenotype instructed and facilitated by the effects of androgenic action throughout the body?

There are several possible mechanisms by which this variation arises, all of which are interrelated (Fig. 1). If we presume that variation in masculine phenotype quality is the product of increased androgenic activity, then the simplest explanation for variation in phenotype complexity is that this is the result of variable levels of circulating androgens, such that simpler phenotypes are the result of relatively low hormone concentration. Notwithstanding the presence and function of binding protein in blood, as the supply of reproductive androgens largely arises from the testes, we could call this the testicular androgen-phenotype hypothesis.

A second way that androgens affect phenotype emergence involves the degree to which AR is expressed in different target tissues. For example, if we focus on one tissue across individual males, we might find that some express low levels of AR, whereas others express higher levels, and these differences underlie some element of phenotypic variation from simple to complex. Across individuals or species, one male that expresses AR at similar levels to another, but expresses AR in more tissues, would possess a more complex phenotype. Collectively, we might call this overall idea the tissue AR-phenotype hypothesis.

Third, we might find that there are no differences in a tissue's AR expression, but instead in how much active hormone is available to that tissue. Local hormone concentration is primarily regulated by androgen-metabolizing enzymes (Bruchovsky and Wilson 1968; Russell and Wilson

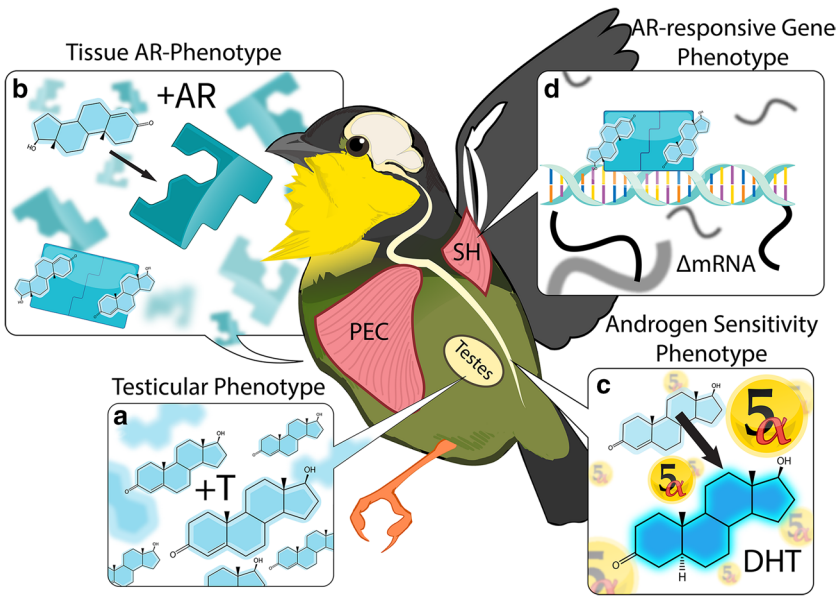

Fig. 1 Schematic representation of the different hypotheses (**a**–**d**) that posit how androgenic signaling systems can evolve to underlie species variation in the masculine phenotype. Note that none of these hypotheses are mutually exclusive of each other and that each may contribute to species differences to greater or less degrees. The boxes showing that the different hypotheses are sized according to their supposed relative impact based on the research conducted in various taxa, as well as the golden-collared manakins (pictured in the center). Abbreviations are as follows: *T* testosterone, *DHT* dihydrotestosterone, *AR* androgen receptor, *5α* 5α-reductase, *SH* scapulohumeralis caudalis muscle, *PEC* pectoralis muscle

1994; Ball and Balthazart 2006), and so altering enzyme expression is another way to alter androgenic phenotype. Thus, in this case, the features of the target tissue that are most important to androgenic signaling involves the degree to which the tissue can metabolize and processes androgenic hormones. We might call this the androgen-sensitive phenotype hypothesis.

A fourth candidate for regulation of male phenotype lies downstream from the binding of hormone and receptor, and instead relates to the extent and degree by which genes become up- or down-regulated by specific amounts of AR bound to androgen (Yoshioka et al. 2006, 2007). Under this framework, we might find that two tissues with similar levels of AR signaling capability each respond uniquely to similar levels of circulating hormone by linking AR gene regulation to altogether different suites of genes or gene networks. We might call this the AR-responsive gene-phenotype hypothesis.

Finding a model system appropriate for testing these hypotheses can be challenging. One taxon that we have used to address these ideas is a family of neotropical birds called the manakins (family: Pipridae). There are nearly 50 different species within this clade, and males of most species exhibit extraordinary masculine phenotypes (Prum 1990, 1998). As these species often exhibit polygynous mating systems, the males offer next to nothing in terms of nesting and parental care, but instead expend considerable time attempting to woo females for mating (Prum 1994). To attract females, males of most species have evolved acrobatic courtship displays that are physically intensive and often incredibly noisy (Prum 1998). Males of one species in particular—the golden-collared manakin (*Manacus vitellinus*) of Panama—have been the target of research by several labs (McDonald et al. 2001; Bostwick and Prum 2003; Stein and Uy 2006; Lindsay et al. 2015), including our own, where we have focused on understanding the hormonal and neuromuscular controls of this noteworthy masculine behavioral phenotype (Schlinger et al. 2001, 2008a, 2013; Fusani et al. 2014a). In what follows, we attempt to assess the four hypotheses described above based on research of male golden-collared manakins.

The masculine phenotype

The plumage of adult male golden-collared manakins comprised tracts of feathers that are either dull green, bright golden-yellow, or stark black (Day et al. 2006; Schlinger et al. 2008a). The legs are reddish and the golden-yellow feathers under the lower mandible are lengthened into a "beard" that the males extend, especially during courtship (Fig. 1). Given that manakins, such as many avian species, experience delayed plumage maturation (Lyon and Montgomerie 1986), both females and juvenile males are dull green throughout. If these green birds are implanted with testosterone (T) and then plucked, their feathers still regrow green (Day et al. 2006). This suggests that androgens play little role, if any, in the development of the masculine plumage phenotype as has been described for other species (Owens and Short 1995), so we will not discuss this further.

The masculine behavioral phenotype of golden-collared manakins has been well described (Schlinger et al. 2001, 2008a, b, 2013; Fusani et al. 2014a; Fuxjager and Schlinger 2015). In brief, adult males clear debris from a small patch of forest floor between small saplings that they use as an arena for courtship. In and around this court, the males jump rapidly between saplings, sometimes also jumping to the forest floor with a half-twist before springing upwards in a "helicoptering" like flight (grunt-jump display). Males also produce relatively simple "chee-poo" calls in and around their display courts. Most impressive, however, is loud snapping sounds of males made by hitting their wings above their back, causing the distal tips of their radii to swiftly collide. When jumping between saplings, a male produces a single wing-snap in midair (jump-snap display). The final manakin display, or roll-snap, is also the most remarkable: a perched male produces a chain of individual wing-snaps repeated at 55–60 Hz for ~ 300 ms. These behaviors are all the more remarkable because males congregate in groups, or leks, so the displays by multiple males amplifies the noise emanating from the forest.

This set of behavior, which comprises the golden-collared manakin masculine phenotype, is, of course, a reflection of neuromuscular systems that underlie all behavior (Fusani et al. 2014a). These neuromuscular systems may be purely sexually dimorphic—that is, present in males, but not females—or they may only be sensitive to male hormones in males. We address these concepts more fully below.

Hormone dependence of masculine courtship

There is considerable evidence that male golden-collared manakin courtship is sexually dimorphic and activated by gonadal T. First, T levels are elevated in the circulation of adult males when they are displaying, but are lower outside of the breeding season (Day et al. 2007; Fusani et al. 2007; Schlinger et al. 2008b). Moreover, T levels are basal in both females and juvenile males who do little or no courtship (Day et al. 2007). Second, treatment with T of juvenile males with naturally low circulating T levels activates the full suite of masculine courtship behaviors including claiming and clearing an arena, performing wing- and roll-snaps, and performing the jump-snap and grunt-jump displays (Day et al. 2006, 2007; Chiver and Schlinger 2017a, b). Interestingly, much of the male courtship routine appears to be highly

sex-specific as females display few if any of these behaviors naturally (Day et al. 2006, 2007), and T activates some to a minor degree and others not at all (Day et al. 2007; Chiver and Schlinger 2017a, b). Thus, the phenotype that is referred to as masculine is, in most respects, truly a male-specific set of traits which can be activated by the sex-steroid T. In all likelihood, males possess male-specific neuromuscular systems that either females lack, or that females possess in an androgen-insensitive state. Interestingly, and in contrast to these courtship behaviors, aggressive behaviors are also subject to activation by T but both males and females are sensitive to the effects of T (Chiver and Schlinger 2017a). Thus, aggressiveness is only an element of the masculine behavioral phenotype when T is present, but the masculine neural circuits underlying this form of aggression are present in both males and females in an androgen-sensitive condition.

Masculine courtship depends on the androgen receptor

Treatments that block AR function in males limit displays of masculine courtship behavior. This has been detected temporarily after administration of wild courting males with the AR antagonist flutamide (Fusani et al. 2007), but for a more sustained period after treatment with AR antagonist bicalutamide (casodex) (Fuxjager et al. 2013, 2014). Flutamide gains access to most AR in the body, so its effects could have occurred in the brain or spinal cord to block the motivation and motor output to produce courtship behaviors or in peripheral tissues such as skeletal muscles whose coordinated contractions produce actual behavior. By contrast with flutamide, bicalutamide does not cross the blood–brain barrier, suggesting its actions to inhibit courtship occurred only on peripheral tissues (Freeman et al. 1989; Furr 1989; Furr and Tucker 1996), likely skeletal muscles, such that signals to produce courtship may have been blunted directly at target musculature. Not only did AR antagonism reduce the overall rate of behavior performance, but the behaviors produced also lacked some of their motor skill. For example, after bicalutamide treatment, roll-snaps that are typically consists of 13–18 individual snaps separated by about 18 milliseconds instead consisted of only 7–9 individual snaps separated by about 23 milliseconds (Fuxjager et al. 2013). Although these results do not allow firm conclusions regarding the role for central AR in the production of golden-collared manakin male courtship, they do point to a significant role for peripheral AR, likely that in skeletal muscles.

With this basic background of golden-collared manakin behavioral endocrinology, we can now evaluate the specific hypotheses described previously regarding the possible endocrine basis for the complex courtship we observe in this species. Specifically, did golden-collared manakin males evolve complex courtship due to the evolution of attributes of their androgen signaling systems? Or, is an alternative more likely; that is, is the complexity of the masculine phenotype of the golden-collared manakin, although dependent on androgen, created and fulfilled by pre- or post-hormone-dependent processes? Did evolution use androgen signaling to direct complexity, or did evolved complexity piggy-back on a basic androgen-dependent background?

The testicular phenotype hypothesis

Given the lengthy history of research investigating hormonal control of reproduction, there are ample data to allow consideration of this hypothesis (Fig. 1a). There is no doubt that a threshold level of T is required to activate the adult male phenotype, and that supraphysiological doses of androgen can lead to some exaggerated masculine states. Nonetheless, there is also evidence that once the threshold level of T is reached, the overall concentration of T likely plays little role in further shaping dramatic phenotypic differences that are observed in nature, notably a phenomenon described years ago for behavioral differences (Grunt and Young 1952, 1953).

There are other ways in which circulating T may influence behavior. Two additional models to describe this relationship include an inverted U-function, where intermediate levels of hormones produce the most robust behavioral response, and a step-function, where individuals vary with respect to the threshold at which hormones exert their effect (Adkins-Regan 2005; Ball and Balthazart 2008). Moreover, there is experimental evidence that baseline T levels influence male phenotypes. Recent work by Rosvall and colleagues (2016a, b) investigated the mechanistic origins of differences in circulating T, highlighting the degree to which variation in gonadal expression of genes related to androgen synthesis predict population-level differences in T levels. Indeed, this work also shows that such differences are linked to marked variation in androgen-dependent masculine traits. This work highlights another layer by which evolutionary factors can drive taxonomic differences in the ability of hormonal ligand to organizing or activating suites of physiological, morphological, and/or behavioral or traits tied to reproduction.

However, for the golden-collared manakin, circulating T does not seem to be the main predictor of masculine phenotype, regardless of the model used to describe the mechanisms of action. This is consistent with the existing literature characterizing circulating T levels in male birds of various species shows that the extent of courtship has little relationship with the amount of T in blood, whereas factors such as breeding latitude, altitude or climate have a greater impact (Goymann et al. 2004, 2007; Hau 2007). For example, we

find no obvious difference in plasma T levels among golden-collared manakins, red-capped manakins (*Ceratopipra mentalis*), another manakins with a complex courtship display (Bostwick and Prum 2003), and zebra finches (*Taeniopygia guttata*), a bird with a minimally complex display (Williams 2001) (Fig. 2). This result is consistent with the idea that the overall physical complexity of these birds' courtship behavior is not related to levels of testosterone in the bloodstream. Moreover, during the breeding season, there are stark differences in circulating T levels among individual male golden-collared manakins, and this variation is far greater than that the observed individual differences in male display behavior (Fusani et al. 2007). We do not yet know whether such variation in T is related to specific elements of the birds' courtship routine or other socio-sexual relationships (Hirschenhauser and Oliveira 2006; Goymann et al. 2007). Further work is needed to fully examine these ideas.

Overall, this is not terribly surprising. All males need to have their reproductive systems functioning optimally and they need to be motivated to interact with and copulate with females. Consequently, all males must have some requisite threshold amount of androgen in blood to stimulate these attributes and to, in a sense, make them male. Indeed, extra T might prove to have detrimental effects (Marler and Moore 1988; Olsson et al. 2000; Roberts et al. 2007; Fuxjager et al. 2011), so maintaining a limited amount of this potent signal in blood could be of benefit.

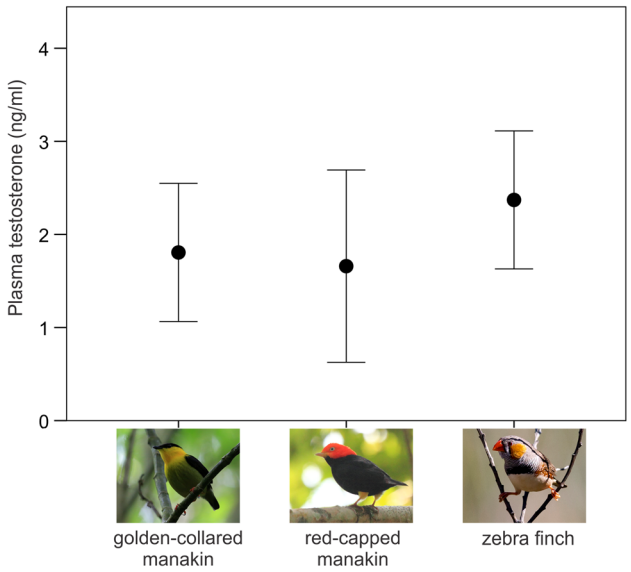

Fig. 2 Plasma testosterone (T) levels in three bird species: the golden-collared manakin (*Manacus vitellinus;* $n=12$), red-capped manakin (*Ceratopipra mentalis;* $n=4$), and zebra finch (*Taeniopygia guttata;* $n=8$). All samples represent T levels in adult male birds in reproductive condition during periods of breeding. Our analyses show that circulating T among these taxa is statistically indistinguishable (one-way ANOVA: $F_{(2,\ 32)}=0.773$, $p=0.470$). We collected these blood samples from specimens used in other analyses (Fuxjager et al. 2015), some of which have been published previously (Day et al. 2006). We measured T levels in red-capped manakins and zebra finches using commercially available enzyme immunoassay kits (Cayman Chemical; intra-assay and inter-assay CVs: 7.5 and 4.9%, respectively), whereas we obtained T levels from golden-collared manakins from Day et al. (2006)

Tissue androgen receptor-phenotype hypothesis

Our work on the golden-collared manakin provides significant support for a prominent role of AR expression levels in establishing the male behavioral phenotype. First, AR is widely expressed in brain, spinal cord, dorsal root ganglia and skeletal muscles—targets where circulating androgen could activate complex motor performance (Feng et al. 2010; Fuxjager et al. 2012; Fusani et al. 2014b). Second, AR levels seem especially elevated in some of these tissues in golden-collared manakins, such as skeletal muscle, relative to traditional androgen targets, like the testes (Feng et al. 2010). Finally, when we specifically examine levels of AR expression across different manakin species that show different levels of courtship physicality, we see a striking correlation between skeletal muscle AR levels and display complexity, especially in forelimb muscles that exert control over the wing- and roll-snap behaviors (Fuxjager et al. 2015) (Fig. 1b). This work collectively suggests that there is tight co-evolution between display physicality and muscular expression of AR. Incidentally, a more recent study in frogs echoes this point, showing that the emergence or "innovation" of a novel physical signal in frogs—the foot flag—is similarly marked by a dramatic increase in AR within the musculature that controls it (Mangiamele et al. 2016). Certainly, it appears that physical signals that make up the masculine phenotype are characterized by evolutionary exploitation of androgenic effects on the muscles that effectuate sexual behavior and reflexes.

In addition to their effect on muscle, androgens most certainly impact the behavioral phenotype by acting through the CNS as well. In manakins, we have evidence for significant AR expression in spinal cord motoneurons and DRG sensory neurons that innervate wing muscles involved in the wing-snap displays (Fuxjager et al. 2012). However, we have no direct comparison with other species regarding the degree of AR is expression in these neurons, relative to such expression in other species. Nevertheless, when examined by qPCR with low cellular resolution, we see no evidence for species differences that might relate to motoric intensity of displays.

In brain, however, we do see a unique pattern of AR expression in the golden-collared manakin relative to oscine songbirds or non-passeriform species (Fusani et al. 2014b). Oscine songbirds possess elevated AR expression in a discrete nucleus RA that is positioned within the arcopallium and which serves to provide pre-motor control of syringeal

function in the production of song (Schlinger 1997; Jarvis et al. 2005). Unlike these birds, however, manakins show increased AR expression throughout much of their arcopallium (Fusani et al. 2014b). This AR sensitivity may have evolved in neurons that participate in pre-motor control of male courtship, as the arcopallium is known to house populations of such descending neurons (Feenders et al. 2008). At the same time, while the arcopallium stands as a likely site where neural circuits have evolved elevated AR expression to give circulating androgens control over manakin courtship activity (Day et al. 2011), it is also important to recognize the role that other brain regions may play in this process. Work in songbirds, for instance, nicely illustrates that androgens can modulate singing behavior by exerting anatomically specific effects throughout the CNS (Alward et al. 2013, 2017). The manakin courtship phenotype may be anchored in a similar process, whereby androgens contribute to different facets of the bird's reproductive display by acting at multiple, potentially novel loci in the brain. Some of these are well known, given the extensive body of research that shows that hormone-dependent aspects of courtship arise from action on conserved hypothalamic structures (Schlinger 1997; Saldanha et al. 2000; Wild and Balthazart 2013; Wild and Botelho 2015). With this in mind, there is good evidence that target tissue AR levels do, in and of themselves, relate to the degree to which masculine phenotypes develop. Nevertheless, additional tissue-level factors certainly participate as well.

Androgen-sensitive phenotype hypothesis

Once T arrives at a target tissue, there are a variety of mechanisms that influence the cells' sensitivity to androgenic action. The first of these mechanisms are related to the fact that T itself typically functions as a prohormone, in that it undergoes transformation in a target tissue into either estradiol or 5α-dihydrotestosterone (5α-DHT) by the actions of two enzymes aromatase and 5α-reductase, respectively. Estradiol binds to and activates estrogen receptors (Beyer et al. 1976; Schlinger and Brenowitz 2009), whereas 5α-DHT binds to and activates AR with greater potency than T (Bruchovsky and Wilson 1968; Russell and Wilson 1994). These enzymes can exert crucial control over the actions of circulating T on a variety of tissues during both development and adulthood to craft the ultimate masculine phenotype (Ball and Balthazart 1985, 2006, 2008; Schlinger et al. 1989, 1995). Bird tissues possess a third enzyme of importance, 5β-reductase, that inactivates T by catalyzing its conversion into 5β-DHT, a largely inactive metabolite (Hutchison and Steimer 1981; Langlois et al. 2010). Thus, tissues with one or more of these enzymes can control the extent to which circulating androgens activate available AR.

The second of these mechanisms that regulate a cell's sensitivity to AR action relates to the machinery that supports AR-dependent gene expression, namely the presence of steroid receptor co-activators (e.g., SRC-1, RPL7, CPB) and co-repressors (e.g., NCoR). These proteins interact with ligand-activated AR to mediate its subsequent effect on gene expression (Xu et al. 1998; Cheng et al. 2002; Yoon and Wong 2006). Indeed, because co-activators and co-repressors guide the functional output of steroid action on a target cell, they are often thought of as "pleiotropic rheostats" for physiological output (York and O'Malley 2010). In theory, this principle can be extended to mechanisms of behavior, in that species variation in abundance of either key co-activators or co-repressors may underlie the ability of steroids to act on a given tissue. This, of course, may be a root cause of some behavioral variation, and thus is susceptible to evolution in response to selection for specific behavioral traits.

Most support for these ideas relates to the first mechanism—the biochemical control of steroid metabolism (Fig. 1c). For example, in the golden-collared manakin, we find that the skeletal muscles have little if any aromatase (Feng et al. 2010; Fuxjager et al. 2015), but have ample 5α-reductase (Types I & II) and 5β-reductase (Fuxjager et al. 2016c). We also find that these three enzymes are also expressed throughout the golden-collared manakin spinal cord (Fuxjager et al. 2016c). However, the role that these enzymes play in determining the amount of 5α-DHT available to bind to AR and how this might contribute to the male's behavioral phenotype is unclear. These enzymes were all also expressed in skeletal muscles and spinal cords of zebra finches, a species with little in the way of a physical courtship display (Williams 2001). Compared to the golden-collared manakin, zebra finches did express higher levels of 5β-reductase in both muscles and spinal cord. This suggests that zebra finches may limit androgen availability to these tissues, whereas manakins may allow more T to be available for the formation of 5α-DHT, which yields greater AR activation. In addition, when compared to females, male golden-collared manakins expressed somewhat higher levels of 5α-reductase (Type I) in their spinal cord and wing muscles, a condition not seen in zebra finches. Thus, male golden-collared manakins have the machinery present to activate circulating T in both their spinal cords and wing muscles, with some evidence for their involvement in the male behavioral phenotype. Additional experimentation to block 5α-reductase generally, or locally in specific target tissues, are needed to test the role of these enzymes in producing the male phenotype and thus to adequately test the androgen-sensitive phenotype hypothesis.

With respect to the relationship between steroid co-activators and co-repressors and behavioral variation across species, relatively little is known about this topic in any species. Indeed, considerations about how individual

variation in molecular basis of steroid action is co-opted by evolution to support processes of behavioral diversification have only been speculated about (Hau 2007). One of the few studies in birds to explore the role of co-activator function and behavior is in quail. This study illustrates that SRC-1 knockdown in reproductive active male birds suppresses androgen-dependent sexual behavior, including many of the physical elements of masculine reproduction (i.e., cloacal movements, strutting) (Charlier et al. 2005). Other work that investigates the link between steroid receptor co-activators and behavior occurs in the oscine songbird brain, particularly the neural song control system where steroids act to mediate vocal output. This evidence suggests that co-activators play an important role in regulating the brain regions important for the control of song (Auger et al. 2002; Duncan and Carruth 2011). Altogether, this work certainly suggests that regulating the abundance of steroid receptor co-activators and co-repressors in the brain, and possibly elsewhere in the body, can influence the functional effects of androgens on these tissues in a manner that influences the hormone's impact on behavioral output.

Still, there are even more avenues by which evolution can shape hormonal action. For example, tissue-specific adjustment of non-coding RNAs represents yet another epigenetic route through which the functional effects of hormone action on a target could be adjusted. From a comparative standpoint, intriguing ideas like this remain in their infancy, which future studies will need to pursue to uncover important information about the lability of steroid functioning in the natural world.

Androgen receptor responsive gene-phenotype hypothesis

Once bound to its androgenic ligand, AR acts as a transcription factor by regulating the expression of thousands of genes within target cells (Yoshioka et al. 2006, 2007; Wyce et al. 2010). These effects change how these cells, and thus the tissue that they comprise, perform their physiological tasks, which ultimately influences how behavior is controlled (Fig. 1d). It therefore follows that evolutionary change in these functional effects of androgenic action should underlie variation in hormonal control of the masculine phenotype.

In golden-collared manakins, AR differentially influences profiles of gene expression in skeletal muscles that play different roles in the biomechanical control of display behavior (Fuxjager et al. 2016b). In the *scapulohumeralis caudalis* (SH), or the dorsal wing retractor muscle that is thought to cause the wings to collide (snap) together (Fuxjager et al. 2016a), AR up-regulates many more genes compared to the *pectoralis* (PEC), which depresses (lowers) the humerus (Dial et al. 1991; Dial 1992). This indicates that androgens have a more robust effect on the muscular tissues that are more closely linked to the actuation of a masculine sexual behavior, namely wing-snapping behavior. Further corroboration of this idea comes from analyses that describe the likely function of the genes under AR control, as many are related to muscular fuel acquisition and metabolism. For example, AR appears to increase the expression of the gene that encodes apolipoprotein B (apoB), a protein that helps export lipid out of a cell. Prior studies show that muscular apoB significantly reduces triglyceride accumulation in the myocyte in a manner that can attenuate cellular insulin resistance (Bartels et al. 2014). Thus, by upregulating the production of apoB, AR might enhance muscular responsiveness to insulin stimulation, and thereby augment glucose uptake necessary to fuel demanding physical activity. Consistent with this idea is further evidence showing that AR also up-regulates the expression of the gene-encoding microsomal triglyceride transfer protein (MTP), which interacts with apoB and plays a central role in the assembly of lipoproteins before they leave the cell (Leiper et al. 1994). Thus, ensuring that the SH muscle is properly nourished so that it can support the acrobatic movements necessary for both courtship and locomotion is undoubtedly advantageous, and likely explains why evolution may have coupled AR action to the expression of these genes.

Equally intriguing is that AR also up-regulates the expression of numerous novel transcripts that appears to encode contractile filaments, which may underlie the SH's ability to exhibit some of the fastest contraction–relaxation cycling speeds documented for any vertebrate limb muscle (Fuxjager et al. 2016a). For instance, we found that testosterone induced a nearly 6000-fold increase in the expression of a novel transcript with a sequence homology to the human myosin 18A gene, as well as a nearly 1000-fold increase in the expression of another novel transcript that had a sequence homology to the myosin 5B. In humans, the myosins encoded by these genes are believed to be unconventional myosin involved in mediating actin-based cellular transport. However, this does not necessarily mean that they serve the same role in manakins, as the novel transcripts that we discovered are merely most like the myosin 18A and 5B genes (Yildiz et al. 2003; Guzik-Lendrum et al. 2013). This means that the novel myosins we uncovered may play an active role in muscle functioning, which is an intriguing idea given that the genes that encode superfast myosins remain highly elusive across the vertebrate taxa that have them (Rome et al. 1996; Elemans et al. 2004, 2008). From a comparative standpoint, this pattern of muscle-specific effects of AR action is also observed in other species, such as the zebra finch, which does not produce an elaborate wing display. However, the actual genes affected in this case encode proteins that play a markedly different role in muscular physiology. For example, AR up-regulates genes in the zebra

finch SH that are linked to general cellular homeostasis and structural maintenance, with little hint of AR-dependent specialization in performance.

The results described above imply that evolutionary modification of the mechanisms by which AR regulates the transcriptional machinery of select tissues is associated with the emergence of an adaptive sexual trait. However, this assertion leads to the simple question: what are the mechanisms that evolution affects to drive these changes over time? The answer to this question is not completely known, given that our understanding of the interface among endocrine physiology, genomics, and evolution is still in its infancy. However, one possibility is that evolutionary change to the way in which AR regulates gene expression occurs in a tissue- and/or species-specific manner. Indeed, we have found evidence of this in golden-collared manakins. These birds have more androgen response elements (AREs) within their genome than zebra finches, and they accordingly differentially express more genes in response to AR activation (Fuxjager et al. 2016b). For example, in SH, we found that 111 genes were not only differentially expressed in the manakin, compared to the zebra finch, but also responsive to androgenic treatment. Of these genes, 61 (55%) contained AREs in their promoter. By contrast, if the PEC, we found 73 genes that were regulated in a species- and androgen-dependent manner; but, in this muscle, only 31 of these genes (42.5%) contained AREs. Thus, AR appears to have a greater ability to directly regulate gene expression in the manakin SH specifically. This finding therefore points to genetic mechanisms by which programs of gene expression can be modified to potentially underlie variation in androgenic signaling capability. The results, of course, may feed up to behavioral output, as important loci of androgenic action can be modified to support the actuation of behavior. This idea is new, in that very few studies have investigated it; nonetheless, it provides another promising model for the evolution of the androgenic signaling system.

Conclusions

Here, we outline four non-mutually exclusive hypotheses that posit how the evolution of androgenic signaling systems can drive diversification in masculine phenotype (Fig. 1). Each involves methods by which the deep cellular mechanisms of androgenic action can be independently adjusted to modify how these hormones exert their effects. This results in a seemingly endless array of possible routes to facilitate variation in androgen action, which undoubtedly contributes to the exceptional diversity of androgen-mediated phenotypes observed in the natural world. We anchored this framework in our own research centered on the tropical golden-collared manakin, since this species exemplifies how specializations in androgen support the emergence of a novel, unusual behavioral repertoire—the ability to produce a stunning and magnificent courtship display that defies our conventional understanding of most animal behavior.

With this framework in mind, our paper leads to an important question: What are the main principles that inform our understanding of endocrine phenotype emergence and its contribution to evolutionary process? Although the answer is complex and difficult to address, we believe there are a few important concepts that the field of evolutionary endocrinology should consider as it moves forward:

1. Species and individual variations in circulating T levels do not always explain the extraordinary variation in masculine sexual behavior that one might expect. Certainly, there are exceptions to this notion, particularly in birds where seasonal changes in T levels play a monumental role in the activation of reproductive behavior when it is needed most—at the onset of the breeding season. This relationship is captured by the so-called "challenge hypothesis," which attempts to conceptualize when and why T levels change across the season and differ among species (Wingfield et al. 1990). This framework remains relevant to studies of behavioral endocrinology, and it continues to stimulate fascinating inquiries into the physiology and evolution of vertebrates. However, data are accumulating that are consistent with the idea that differences in circulating T levels do not always predict variation in male reproductive behavior (Goymann et al. 2004; Garamszegi et al. 2008). Meta-analyses also illustrate that the relationship between T levels and the masculine phenotype is highly complex and depend largely on the type of vertebrate in question (Hirschenhauser et al. 2003; Hirschenhauser and Oliveira 2006; Goymann et al. 2007). Thus, we need to look beyond circulating hormones to fully appreciate the links between androgenic hormone action and behavioral evolution.
2. Species and individual variations in the cellular and genetic mechanisms of androgen action may hold the key to how male phenotypes diversify. There is a growing body of work that supports this point of view, in addition to the work in the golden-collared manakin. This is particularly true with respect to recent studies in neuroendocrinology, which propose that social phenotypes are largely governed by the working of the neural social behavior network (Goodson 2005; Goodson et al. 2005; O'Connell and Hofmann 2011, 2012). The specific brain nuclei that make up the nodes of this network are all sensitive to steroid action, including that of androgenic hormones. Thus, adjusting how each of these nodes responses to androgenic action—via any of the routes posed in this review—can dramati-

cally re-shape the activational landscape of the brain that controls behavioral output (Maney et al. 2008). For example, certain nodes may express high levels of enzyme that reduce T to DHT, or that inactive T altogether, whereas receptor levels may also differ among regions. The manakin work, of course, extends these ideas to tissues outside to the brain, showing that variation in the cellular mechanisms of androgenic action within the neuromuscular system are also vital to how complex behavior is performed. Thus, there are numerous routes by which modifications to the mechanisms of androgenic signaling can accommodate and support variation in behavioral modulation.

3. *Looking beyond the CNS* Our work on manakins has identified evolved androgen-dependent actions on select muscles crucial for manakin courtship. Thus, while exploration of the brain remains a focus of much work, peripheral targets such as skeletal muscles may be the ultimate targets on which evolution acts create the great diversity of behavioral phenotypes observed in nature.

As the field of evolutionary endocrinology moves forward, these points will need to be considered more carefully, especially as researchers begin to explore hormonal control of behavioral and physiological phenomena involving diverse taxa. A major goal of our field should be to discover unifying principles that describe how cellular mechanisms change to support behavioral evolution in wild animals. Hopefully, the result will be a rich framework from which we can understand how hormones influence animal function, but also how these mechanisms are exploited by evolutionary pressures to explain the elaborate and extensive biodiversity on our planet.

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References

Adkins-Regan E (1987) Sexual differentiation in birds. Trends Neurosci 10:517–522

Adkins-Regan E (2005) Hormones and animal social behavior. Monographs in behavior and ecology. Princeton University Press, Princeton

Alward BA, Balthazart J, Ball GF (2013) Differential effects of global versus local testosterone on singing behavior and its underlying neural substrate. Proc Natl Acad Sci USA 110(48):19573–19578. doi:10.1073/pnas.1311371110

Alward BA, Rouse ML, Balthazart J, Ball GF (2017) Testosterone regulates birdsong in an anatomically specific manner. Anim Behav 124:291–298

Auger CJ, Bentley GE, Auger AP, Ramamurthy M, Ball GF (2002) Expression of cAMP response element binding protein-binding protein in the song control system and hypothalamus of adult european starlings (*Sturnus vulgaris*). J Neuroendocrinol 14(10):805–813. doi:10.1046/j.1365-2826.2002.00842.x

Ball GF, Balthazart J (1985) Neuroendocrine regulation of reproductive behavior in birds. In: Arnold AP, Etgen AM, Fahrbach SE, RT R (eds) Hormones, brain, and behavior. Academic Press, San Diego, pp 855–895

Ball GF, Balthazart J (2006) Androgen metabolism and the activation of male sexual behavior: it's more complicated than you think! Horm Behav 49(1):1–3. doi:10.1016/j.yhbeh.2005.07.008

Ball GF, Balthazart J (2008) Individual variation and the endocrine regulation of behavioral and physiology in birds: a cellular/molecular perspective. Philos Trans R Soc Lond Ser B: Biol Sci 363:1699–1710

Bartels ED, Ploug T, Størling J, Mandrup-Poulsen T, Nielsen LB (2014) Skeletal muscle apolipoprotein B expression reduces muscular triglyceride accumulation. Scand J Clin Lab Invest 74(4):351–357

Baum MJ (1979) Differentiation of coital behavior in mammals: a comparative analysis. Neurosci Biobehav Rev 3:265–284

Berthold AA (1849) Transplantation of the testes. Bull History Med 16:42–46

Beyer C, Morali G, Naftolin F, Larsson K, Perezpalacios G (1976) Effect of some antiestrogens and aromatase inhibitors on androgen induced sexual behavior in castrated male rats. Horm Behav 7(3):353–363. doi:10.1016/0018-506x(76)90040-4

Bostwick KS, Prum RO (2003) High-speed video analysis of wing-snapping in two manakin clades (Pipridae: Aves). J Exp Biol 206:3693–3706

Bruchovsky N, Wilson JD (1968) The conversion of testosterone to 5-alpha-androstan-17-beta-ol-3-one by rat prostate in vivo and in vitro. J Biol Chem 243(8):2012–2021

Chang C (2002) Androgens and androgen receptor: mechanisms, functions, and clinical applications. Springer, New York

Charlier TD, Ball GF, Balthazart J (2005) Inhibition of steroid receptor coactivator-1 blocks estroen and androgen action on male sexual behavior and associated brian plasticity. J Neurosci 25(4):906–913

Cheng ST, Brzostek S, Lee SR, Hollenberg AN, Balk SP (2002) Inhibition of the dihydrotestosterone-activated androgen receptor by nuclear receptor corepressor. Mol Endocrinol 16(7):1492–1501. doi:10.1210/me.16.7.1492

Chiver I, Schlinger BA (2017a) Sex differences in androgen activation of complex courtship behaviour. Anim Behav 124:109–117

Chiver I, Schlinger BA (2017b) Clearing up the court: sex and the endocrine basis of display-court manipulation. Anim Behav 131:115–121

Cooke BA, Hegstrom CD, Villeneuve LS, Breedlove SM (1998) Frontiers in neuroendocrinology. 19:232–362

Day LB, McBroom JT, Schlinger BA (2006) Testosterone increases display behaviors but does not stimulate growth of adult plumage in male golden-collared manakins (*Manacus vitellinus*). Horm Behav 49(2):223–232. doi:10.1016/j.yhbeh.2005.07.006

Day LB, Fusani L, Hernandez E, Billo TJ, Sheldon KS, Wise PM, Schlinger BA (2007) Testosterone and its effects on courtship in golden-collared manakins (*Manacus vitellinus*): seasonal, sex, and age differences. Horm Behav 51(1):69–76. doi:10.1016/j.yhbeh.2006.08.006

Day LB, Fusani L, Kim C, Schlinger BA (2011) Sexually dimorphic neural phenotypes in golden-collared manakins (*Manacus vitellinus*). Brain Behav Evol 77(3):206–218. doi:10.1159/000327046

Dial KP (1992) Activity patterns of the wing muscles of the pigeon (*Columbia livia*) during different modes of flight. J Exp Zool 262:357–373

Dial KP, Goslow GE, Jenkins FA (1991) The functional anatomy of the shoulder in the European starling (*Sturnus vulgaris*). J Morphol 207:327–344

Duncan KA, Carruth LL (2011) The song remains the same: coactivators and sex differences in the songbird brain. Front Neuroendocrinol 32(1):84–94. doi:10.1016/j.yfrne.2010.11.001

Elemans CPH, Spierts ILY, Muller UK, van Leeuwen JL, Goller F (2004) Superfast muscles control dove's trill. Nature 431(7005):146–146. doi:10.1038/431146a

Elemans CPH, Mead AF, Rome LC, Goller F (2008) Superfast muscles control song production in songbirds. PLoS One 3(7):e2581

Feenders G, Liedvogel M, Rivas M, Zapka M, Horita H, Hasra E, Wada K, Mouritsen H, Jarvis ED (2008) Molecular mapping of the movement associated areas in the avian brain: a motor theory for vocal learning origin. PLoS One 3(3):e1768

Feng NY, Katz A, Day LB, Barske J, Schlinger BA (2010) Limb muscles are androgen targets in an acrobatic tropical bird. Endocrinology 151(3):1042–1049. doi:10.1210/en.2009-0901

Freeman SN, Mainwaring WIP, Furr BJA (1989) A possible explanation for the peripheral selectivity of a novel non-steroidal pure antiandrogen, Casodex (ICI 176,334). Br J Cancer 60(5):664–668. doi:10.1038/bjc.1989.336

Furr BJA (1989) Casodex (ICI-176,334): a new, pure, peripherally-selective anti-androgen—preclinical studies. Horm Res 32:69–76. doi:10.1159/000181315

Furr BJA, Tucker H (1996) The preclinical development of bicalutamide: Pharmacodynamics and mechanism of action. Urology 47(1A):13–25. doi:10.1016/s0090-4295(96)80003-3

Fusani L, Day LB, Canoine V, Reinemann D, Hernandez E, Schlinger BA (2007) Androgen and the elaborate courtship behavior of a tropical lekking bird. Horm Behav 51(1):62–68. doi:10.1016/j.yhbeh.2006.08.005

Fusani L, Barske J, Day LD, Fuxjager MJ, Schlinger BA (2014a) Physiological control of elaborate male courtship: female choice for neuromuscular systems. Neurosci Biobehav Rev 46:534–546

Fusani L, Donaldson Z, London SE, Fuxjager MJ, Schlinger BA (2014b) Expression of androgen receptor in the brain of a suboscine bird with an elaborate courtship display. Neurosci Lett 578:61–65

Fuxjager MJ, Schlinger BA (2015) Perspectives on the evoltuion of animal dancing: a case study in manakins. Curr Opin Behav Sci 6:7–12

Fuxjager MJ, Foufopoulos J, Diaz-Uriarte R, Marler CA (2011) Functionally opposing effects of testosterone on two different types of parasite: implications for the immunocompetence handicap hypothesis. Funct Ecol 25:132–138

Fuxjager MJ, Schultz JD, Barske J, Feng NY, Fusani L, Mirzatoni A, Day LB, Hau M, Schlinger BA (2012) Spinal motor and sensory neurons are androgen targets in an acrobatic bird. Endocrinology 153(8):3780–3791

Fuxjager MJ, Longpre KM, Chew JG, Fusani L, Schlinger BA (2013) Peripheral androgen receptors sustain the acrobatics and fine motor skill of elaborate male courtship. Endocrinology 154(9):3168–3177

Fuxjager MJ, Heston JB, Schlinger BA (2014) Peripheral androgen action helps modulate vocal production in a suboscine passerine. Auk 131:327–334

Fuxjager MJ, Eaton J, Lindsay WR, Salwiczek LH, Rensel MA, Barske J, Sorenson L, Day LB, Schlinger BA (2015) Evolutionary patterns of adaptive acrobatics and physical performance predict expression profiles of androgen receptor—but not oestrogen receptor—in the forlimb musculature. Funct Ecol 29(9):1197–1208

Fuxjager MJ, Goller F, Dirkse A, Sanin GD, Garcia S (2016a) Select forelimb muscles have evolved superfast contractile speed to support acrobatic social displays. eLife 5:e13544

Fuxjager MJ, Lee J, Chan T, Bahn J, Chew J, Xiao X, Schlinger BA (2016b) Hormones, genes and athleticism: effect of androgens on the avian muscular transcriptome. Mol Endocrinol 30:254–271

Fuxjager MJ, Schuppe ER, Hoang J, Chew J, Shah M, Schlinger BA (2016c) Expression of 5α- and 5β-reductase in spinal cord and muscle of birds with different courtship repertoires. Front Zool 13:25

Garamszegi LZ, Hirschenhauser K, Bokony V, Eens M, Hurtrez-Bousses S, Moller AP, Oliveira RF, Wingfield JC (2008) Latitudinal distribution, migration, and testosterone levels in birds. Am Nat 172(4):533–546. doi:10.1086/590955

Goodson JL (2005) The vertebrate social behavior network: evolutionary themes and variations. Horm Behav 48(1):11–22. doi:10.1016/j.yhbeh.2005.02.003

Goodson JL, Evans AK, Lindberg L, Allen CD (2005) Neuro-evolutionary patterning of sociality. Proc R Soc B 272(1560):227–235. doi:10.1098/rspb.2004.2892

Goy RW, Phoenix CH (1972) The effects of testosterone proprionate administration before birth on the development of behavior in genetic female rhesus monkeys. In: Sawyer C, Gorski R (eds) Steroid hormones and brain function. University of California Press, Berkeley

Goymann W, Moore IT, Scheuerlein A, Hirschenhauser K, Grafen A, Wingfield JC (2004) Testosterone in tropical birds: effects of environmental and social factors. Am Nat 164(3):327–334

Goymann W, Landys MM, Wingfield JC (2007) Distinguishing seasonal androgen responses from male-male androgen responsiveness: revisiting the challenge hypothesis. Horm Behav 51(4):463–476

Grunt JA, Young WC (1952) Differential reactivity of individuals and the response of the male guinea pig to testosterone propionate. Endocrinology 51(3):237–248

Grunt JA, Young WC (1953) Consistency of sexual behavior patterns in individual male guinea pigs following castration and androgen therapy. J Comp Physiol Psychol 46(2):138–144

Guzik-Lendrum S, Heissler SM, Billington N, Takagi Y, Yang Y, Knight PJ, Homsher E, Sellers JR (2013) Mammalian myosin-18A, a highly divergent myosin. J Biol Chem 288(13):9532–9548. doi:10.1074/jbc.M112.441238

Hau M (2007) Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. Bioessays 29(2):133–144

Hirschenhauser K, Oliveira RF (2006) Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. Anim Behav 71:265–277

Hirschenhauser K, Winkler H, Oliveira RF (2003) Comparative analysis of male androgen responsiveness to social environment in birds: the effects of mating system and paternal incubation. Horm Behav 43:508–519

Hutchison JB, Steimer T (1981) Brain 5beta-reductase: a correlate of behavioral sensitivity to androgen. Science 213(4504):244–246. doi:10.1126/science.7244635

Jarvis ED, Gunturkun O, Bruce L, Csillag A, Karten H, Kuenzel W, Medina L, Paxinos G, Perkel DJ, Shimizu T, Striedter G, Wild JM, Ball GF, Dugas-Ford J, Durand SE, Hough GE, Husband S, Kubikova L, Lee DW, Mello CV, Powers A, Siang C, Smulders TV, Wada K, White SA, Yamamoto K, Yu J, Reiner A, Butler AB, Consortium ABN (2005) Avian brains and a new understanding of vertebrate brain evolution. Nat Rev Neurosci 6(2):151–159. doi:10.1038/nrn1606

Langlois VS, Zhang D, Cooke GM, Trudeau VL (2010) Evolution of steroid-5 alpha-reductases and comparison of their function with 5 beta-reductase. Gen Comp Endocrinol 166(3):489–497. doi:10.1016/j.ygcen.2009.08.004

Leiper JM, Bayliss JD, Pease RJ, Brett DJ, Scott JP, Shoulders CC (1994) Microsomal triglyceride transfer protein, the abetalipoproteinemia gene product, mediates the secretion of apolipoprotein B-containing lipoproteins from heterologous cells. J Biol Chem 269:21951–21954

Lindsay WR, Giuliano CE, Houck JT, Day LB (2015) Acrobatic courtship display coevolves with brain size in manakins (Pipridae). Brain Behav Evol 85(1):25–36
Lyon BE, Montgomerie RD (1986) Delayed plumage maturation in passerine birds: reliable signaling by subordinate males? Evolut Int J org Evolut 40(3):605–615
Maney DL, Goode CT, Lange HS, Sanford SE, Soloman BJ (2008) Estradiol modulates neural responses to song in a seasonal sonbird. J Comp Neurol 511:173–186
Mangiamele LA, Fuxjager MJ, Schuppe ER, Taylor R, Hodl W, Preininger D (2016) Increased androgenic sensitivity in the hind limb neuromuscular system marks the evolution of a derived gestural display. Proc Natl Acad Sci USA 113(20):5664–5669
Marler CA, Moore MC (1988) Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. Behav Ecol Sociobiol 23(1):21–26
McDonald DB, Clay RP, Brumfield RT, Braun MJ (2001) Sexual selection on plumage and behavior in an avian hybrid zone: experimental tests of male-male interactions. Evolution Int J org Evolution 55(7):1443–1451
O'Connell LA, Hofmann HA (2011) The vertebrate mesolimbic reward systems and social behavior network: a comparative synthesis. J Comp Neurol 519:3599–3639
O'Connell LA, Hofmann HA (2012) Evoltuion of vertebrate social decision-making network. Science 336:1154–1157
Olsson M, Wapstra E, Madsen T, Silverin B (2000) Testosterone, ticks and travels: a test of the immunocompetence-handicap hypothesis in free-ranging male sand lizards. Proc R Soc B 267(1459):2339–2343
Owens IP, Short RV (1995) Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. Trends Ecol Evol 10:44–47
Phoenix CH, Goy RW, Gerall AA, Young WC (1959) Organizing action of prenatally administered testosterone propionate on the tisues mediating mating behavior in the female guinea pig. Endocrinology 65:369–382
Prum RO (1990) Phylogenetic analysis of the evolution of display behavior in the neotropical manakins (Aves, Pipridae). Ethology 84(3):202–231
Prum RO (1994) Phylogenetic analysis of the evolution of alternative social behavior in the manakins (Aves: Pipridae). Evolut Int J org Evolution 48(5):1657–1675
Prum RO (1998) Sexual selection and the evolution of mechanical sound production in manakins (Aves: Pipridae). Anim Behav 55:977–994. doi:10.1006/anbe.1997.0647
Rhoda J, Corbier P, Roffi J (1984) Gonadal steroid concentrations in serum and hypothalamus of the rat at birth: aromatization of testosterone to 17 beta-estradiol. Endocrinology 114:1754–1760
Roberts ML, Buchanan KL, Hasselquist D, Evans MR (2007) Effects of testosterone and corticosterone on immunocompetence in the zebra finch. Horm Behav 51(1):126–134. doi:10.1016/j.yhbeh.2006.09.004
Rome LC, Syme DA, Hollingworth S, Lindstedt SL, Baylor SM (1996) The whistle and the rattle: the design of sound producing muscles. Proc Natl Acad Sci U S A 93(15):8095–8100. doi:10.1073/pnas.93.15.8095
Rosvall KA, Bergeon Burns C, Jayaratna S, Dossey EK (2016a) Gonads and the evolution of hormonal phenotypes. Integr Comp Biol 56:225–234
Rosvall KA, Bergeon Burns CM, Jayaratna SP, E.D K (2016b) Divergence along the gonadal steroidogenic pathway: implications for hormone-mediated phenotypic evolution. Horm Behav 8:1–8
Russell DW, Wilson JD (1994) Steroid 5alpha-reductase: two genes/two enzymes. Annu Rev Biochem 63:25–61. doi:10.1146/annurev.biochem.63.1.25
Saldanha CJ, Schultz JD, London SE, Schlinger BA (2000) Telencephalic aromatase but not a song circuit in a sub-oscine passerine, the golden-collared manakin (*Manacus vitellinus*). Brain Behav Evol 56(1):29–37. doi:10.1159/000006675
Schlinger BA (1997) Sex steroids and their actions on the birdsong system. J Neurobiol 33(5):619–631
Schlinger BA, Brenowitz EA (2009) Neural and hormonal control of birdsong. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT (eds) Hormones, brain, and behavior. Academic Press, San Diego
Schlinger BA, Fivizzani AJ, Callard GV (1989) Aromatase, 5alpha-reductase and 5beta-reductase in brain, pituitary and skin of the sex-role reversed Wilson's phalarope. J Endocrinol 122(2):573–581. doi:10.1677/joe.0.1220573
Schlinger BA, Amur-Umarjee S, Campagnoni AT, Arnold AP (1995) 5β-reductase and other androgen-metabolizing enzymes in primary cultures of developing zebra finch telencephalon. J Neuroendocrinol 7(3):187–192
Schlinger BA, Schultz JD, Hertel F (2001) Neuromuscular and endocrine control of an avian courtship behavior. Horm Behav 40(2):276–280. doi:10.1006/hbeh.2001.1669
Schlinger BA, Day LB, Fusani L (2008a) Behavior, natural history and neuroendocrinology of a tropical bird. Gen Comp Endocrinol 157(3):254–258. doi:10.1016/j.ygcen.2008.05.015
Schlinger BA, Fusani L, Day L (2008b) Hormonal control of courtship in male Golden-collared manakins (*Manacus vitellinus*). Ornitol Neotrop 19:229–239
Schlinger BA, Barske J, Day L, Fusani L, Fuxjager MJ (2013) Hormones and the neuromuscular control of courtship in the golden-collared manakin (*Manacus vitellinus*). Front Neuroendocrinol 34(3):143–156
Stein AC, Uy JAC (2006) Plumage brightness predicts male mating success in the lekking golden-collared manakin, *Manacus vitellinus*. Behav Ecol 17(1):41–47. doi:10.1093/beheco/ari095
van der Schoot P (1980) Effects of dihydrotestosterone and oestradiol on sexual differentiation in male rats. J Endocrinol 84:397–407
Weisz J, Ward IL (1980) Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 106:306–316
Wild JM, Balthazart J (2013) Neural pathways mediating control of reproductive behavior in male Japanese quail. J Comp Neurol 521(9):2067–2087
Wild JM, Botelho JF (2015) Involvement of the avian song system in reproductive behaviour. Biol Lett 11(12):20150773
Williams H (2001) Choreography of song, dance and beak movements in the zebra finch (*Taeniopygia guttata*). J Exp Biol 204(20):3497–3506
Wingfield JC, Hegner RE, Dufty AM, Ball GF (1990) The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am Nat 136(6):829–846
Wyce A, Bai YC, Nagpal S, Thompson CC (2010) Research resource: the androgen receptor modulates expression of genes with critical roles in muscle development and function. Mol Endocrinol 24(8):1665–1674. doi:10.1210/me.2010-0138
Xu JM, Qiu YH, DeMayo FJ, Tsai SY, Tsai MJ, O'Malley BW (1998) Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. Science 279(5358):1922–1925. doi:10.1126/science.279.5358.1922
Yildiz A, Forkey JN, McKinney SA, Ha T, Goldman YE, Selvin PR (2003) Myosin V walks hand-over-hand: single fluorophore imaging with 1.5-nm localization. Science 300(5628):2061–2065. doi:10.1126/science.1084398
Yoon HG, Wong JM (2006) The corepressors silencing mediator of retinoid and thyroid hormone receptor and nuclear receptor corepressor are involved in agonist- and antagonist-regulated

transcription by androgen receptor. Mol Endocrinol 20(5):1048–1060. doi:10.1210/em.2005-0324

York B, O'Malley BW (2010) Steroid receptor coactivator (SRC) family: masters of systems biology. J Biol Chem 285:43–50

Yoshioka M, Boivin A, Ye P, Labrie F, St-Amand J (2006) Effects of dihydrotestosterone on skeletal muscle transcriptome in mice measured by serial analysis of gene expression. J Mol Endocrinol 36(2):247–259

Yoshioka M, Boivin A, Bolduc C, St-Amand J (2007) Gender difference of androgen actions on skeletal muscle transcriptome. J Mol Endocrinol 39(1–2):119–133. doi:10.1677/jme-07-0027