

Perspectives on the evolution of animal dancing: a case study of manakins

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Elaborate physical courtship displays of many male vertebrates require extensive neuromuscular coordination dependent on hormonal activation. How neuromuscular systems evolve to perform essential natural behaviors as well as specialized behaviors required for courtship is poorly understood. We have explored neural, endocrine and muscular adaptations underlying the complex courtship displays of male golden-collared manakins, a bird of neotropical forests. We find that in addition to unique androgen-sensitivity in brain and spinal cord, these birds evolved increased expression of androgen-receptors (AR) in their skeletal musculature. Our evidence from studies of this species and others indicates that selection for elevated skeletal muscle AR expression forms the basis for the evolution of physically elaborate courtship displays.

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Current Opinion in Behavioral Sciences 2015, 6:7–12

This review comes from a themed issue on **The integrative study of animal behavior**

Edited by **Dustin R Rubenstein** and **Hans A Hofmann**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 2nd July 2015

<http://dx.doi.org/10.1016/j.cobeha.2015.06.007>

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Introduction

The spectacular ways in which animal communicate and signal one another has fascinated humans for centuries and inspired biological debate. The two founders of modern evolutionary theory, Charles Darwin and Alfred Russel Wallace, were especially stirred by the extraordinary courtship displays performed by males of many bird species [1,2]. In describing the Birds-of-Paradise, for example, Wallace [2] details their amazing dances,

employing their bodies and limbs to generate esthetically exquisite movement patterns to attract mates.

Despite the importance of our consideration of male courtship to contemporary biological thought, compared to other modes of animal communication, such as vocal acoustic production, the neural, hormonal and muscular underpinnings of physically elaborate displays are relatively poorly studied. The athleticism of such displays can involve an enormous set of the body's skeletal muscles and can make unique demands on the performance characteristics of individual muscles involving fiber size and type, energy use, contractile force and speed [3,4]. On top of this complexity, whereas vocal signaling relies predominantly on specialized muscles dedicated to sound production, rich behavioral displays utilize neuro-motor architecture that is shared with other life-sustaining functions, such as locomotion and balance. Therefore, the systems that control 'normal' movements exhibited by an animal to feed or escape a predator must also control 'spectacular' movement that is incorporated into a given display repertoire. How such behavioral phenotypes emerge requires consideration of evolutionary pressures and the proximate mechanisms through which selection acts.

Ordinarily, courtship behaviors are performed in a reproductive context; hence, the neuromotor tissues underlying the performance of these specific behaviors have likely evolved sensitivity to sex-steroids [5,6]. Thus, when the testes are engaged in spermatogenesis and the males are motivated to copulate, they also engage their neuro-motor machinery to attract females. As a consequence, it is hypothesized that the degree to which neuromuscular systems evolve sensitivity to steroids, and the manner in which steroids then exert control over neuromotor function, provides a basis for the evolution of the complex courtship displays that we observe in nature.

In what follows, we describe our research on the androgen-dependence of the neuromuscular and hormonal systems that control courtship behavior in male golden-collared manakins (*Manacus vitellinus*). These results provide data useful for evaluating the preceding hypothesis.

Androgens and the control of reproductive motor skills

There is ample evidence that testosterone (T) is the key male reproductive pro-hormone that, before or after

enzymatic conversions into more active androgenic or estrogenic metabolites, activates a host of reproductive behaviors via either androgen receptors (AR) or estrogen receptors (ER). In the periphery, AR predominates in regulating sexual motor skills and reflexes. For example, many males maintain a pool of AR-expressing motoneurons in the lumbar spinal cord that innervates AR-expressing musculature of the penis [7,8]. When reproductively active, circulating T binds to AR to drive the connectivity and functionality of this circuit dedicated for its reproductive function [9,10]. This research provides an example of a neuro-motor circuit that is vital for successful reproduction and that depends on androgenic action.

The golden-collared manakin: a model for studying androgen-dependent courtship

Golden-collared manakins are small passerine birds that inhabit the forests of Panama [11]. Males of this species court females and compete with rival males by performing a suite of intricate and complex physical maneuvers [12–14,15*,16,17]. Foremost among these is the wing-snap, whereby a male rapidly hits his wings together above his head to produce a loud firecracker-like sonation that echoes through the environment. Males also produce roll-snaps, which are the production of multiple wing-snaps at roughly 50–60 Hz. Finally, males produce a jump-snap dance, in which they rapidly leap among saplings over the forest floor and snap their wings in mid-air. These displays can be produced daily for nearly 6 months of the year [18]. Given the sheer complexity of the male's display repertoire, this species is used as a model to understand the mechanisms and evolution of physically elaborate displays.

Much of the male's display routine is androgen dependent. Circulating levels of testosterone increase in adults at the onset of the breeding season [19], and administration of testosterone to non-breeding males increases display performance both in captivity and in the wild [20,21]. Blocking AR generally with the drug flutamide decreases the production of most display moves [19]. Moreover, specifically blocking AR peripherally also reduces display output [22**]. We treated wild, actively displaying adult birds with bicalutamide, an AR antagonist that works only outside of the brain and spinal cord, thus leaving AR within the central nervous system functionally intact. We confirmed the peripheral selectivity of bicalutamide by showing that it influenced gene expression in skeletal muscles without significantly affecting gene expression in the telencephalon or hypothalamus where AR are present in manakins [23]. We then found significant reductions in male display behavior, particularly those behaviors which are motorically complex. For instance, upon analysis of the motor patterning of the roll-snap, we found that bicalutamide shortened snap trains by 40% (from ~13 to 7 snaps) and significantly increased the length and variability of the temporal spacing of snaps

within a single train; the between-snap interval was roughly 20 ms with a range of 17–22 before bicalutamide treatment, whereas after treatment, the between-snap interval was approximately 23 ms with a range of 19–29. Together, these data point to AR action on muscle itself as a modulator of fine motor function during display performance.

In keeping with the androgen-dependence of male manakin courtship, these birds have also evolved a seemingly unique endocrine phenotype, in which their neuro-motor systems are especially sensitive to androgen. Using quantitative PCR (qPCR), we have shown that adult male golden-collared manakins express significantly more AR in both their spinal cord and wing musculature, compared to other species that do not incorporate wing movements in their courtship routines, such as zebra finches (*Taeniopygia guttata*) and ochre-bellied flycatchers (*Mionectes oleagineus*) [24]. Moreover, adult males also express over double the amount of AR in their wing muscle compared to other known androgen targets in the body, such as the brain and testes [24]. Given that the contraction of the wing muscles drives display performance, these data implicate androgenic action within the wing musculature as a mediator of adaptive motor control.

We have also found considerable expression of AR in the motor and sensory neurons that are housed in the spinal cord and dorsal root ganglia (DRG) and that innervate the wing muscles responsible for lifting (the *supracoracoideus* and *scapulohumeralis caudalis*) and retracting the wing (the *pectoralis*) [25]. By injecting retrograde fluorescent tracers into these three muscles to backfill their motoneurons and performing in situ hybridization on spinal tissue to identify AR mRNA, we discovered that the backfilled α -motoneurons and DRG sensory neurons connected with the wing muscles contain abundant AR transcripts, relative to other cells within the spinal cord [25]. These data highlight at high resolution the location in the neuro-motor system at which androgens can act to affect movement. They further illustrate that the fine-tuning of behavior requires coordination of both motor and sensory systems, and androgens are positioned to exert such complementary control.

Genomic effects of AR activation in avian skeletal muscle

In separate experiments, we explored the functional effects of androgens on the wing muscles themselves. We again used qPCR to measure changes in gene expression that occur in response to testosterone treatment [26], and we found that testosterone up-regulates select genes that are known to enhance muscle function, including parvalbumin, which buffers Ca^{++} to increase muscle contraction-relaxation speed [27,28], and IGF-1, a growth factor that can increase muscle fiber size [29,30]. Interestingly, IGF-1 produced in skeletal muscle is also known

to travel in a retrograde fashion from the muscle to the spinal cord, where it maintains connectivity of spinal circuitry [31,32]. These data therefore suggest that androgens directly modulate manakin wing muscles in a way that promotes contractility, strength, and coordination, all presumably necessary for optimal display production.

Although we have identified a few androgen-dependent target genes in manakin skeletal muscle, in all likelihood androgens influence numerous gene pathways that enable the physical courtship. Indeed, we have performed transcriptomic analysis of two wing muscles in androgen-manipulated male golden-collared manakins (Fuxjager *et al.*, personal communications). These data indicate that androgens impact a wide-range of genes involved in muscle fiber contraction, metabolism and general cell maintenance. Some transcriptional regulation is shared across muscles, whereas other regulation is muscle-specific. The results point out that despite similar levels of AR expression, individual muscles respond quite differently to circulating testosterone. How skeletal muscles evolve their distinct patterns of transcriptional regulation in the face of similar circulating levels of testosterone and similar levels of AR expression remains a topic of exploration. One possibility is that individual muscles express different degrees of androgen metabolizing enzymes that provide unique levels of active androgen to individual muscles.

Androgen receptor (AR) and the evolution of display behavior

Notably, golden-collared manakins are not the only manakin species that produce complex displays. On the contrary, nearly all of the ~50 manakin species produce some type of physically elaborate display, though among genera these displays vary markedly in terms of maneuvering and movement patterns [33]. Given that AR plays an integral role in modulating motor skills necessary for the golden-collared manakin's display, we tested whether AR might be related to display behavior and overall display complexity in other manakins [34^{••}]. Thus, we used qPCR to measure AR and ER α expression in the spinal cord and main wing muscles of seven different passerine species — including golden-collared manakins, red capped manakins (*Ceratopipra mentalis*), blue-crowned manakins (*Lepidothrix coronata*), lance-tailed manakins (*Chiroxiphia lanceolata*), ochre-bellied flycatchers, pin-tailed whydahs (*Vidua macroura*), and zebra finches — that varied in terms of the motor complexity of their display. We found that birds with more motorically complex displays expressed higher levels of AR mRNA collectively in their wing muscles. This relationship was not observed in the spinal cord, nor was it observed in either tissue with respect to ER α expression. The data reveal striking co-evolution between complex displays and putative androgen sensitivity in the forelimb musculature. Importantly, these effects are specific to the

muscles and to AR expression. Therefore, because the displays are used to solicit reproductive opportunities [33], the data suggest that sexual selection may drive species variation in display capability by modifying AR levels within the skeletal muscles that directly control behavioral output. This study is one of the first to reveal an association between species variation in tissue-specific sex steroid sensitivity and behavior traits, and thus this work begins to unlock the potential pathway through which motor skills can evolve across taxa.

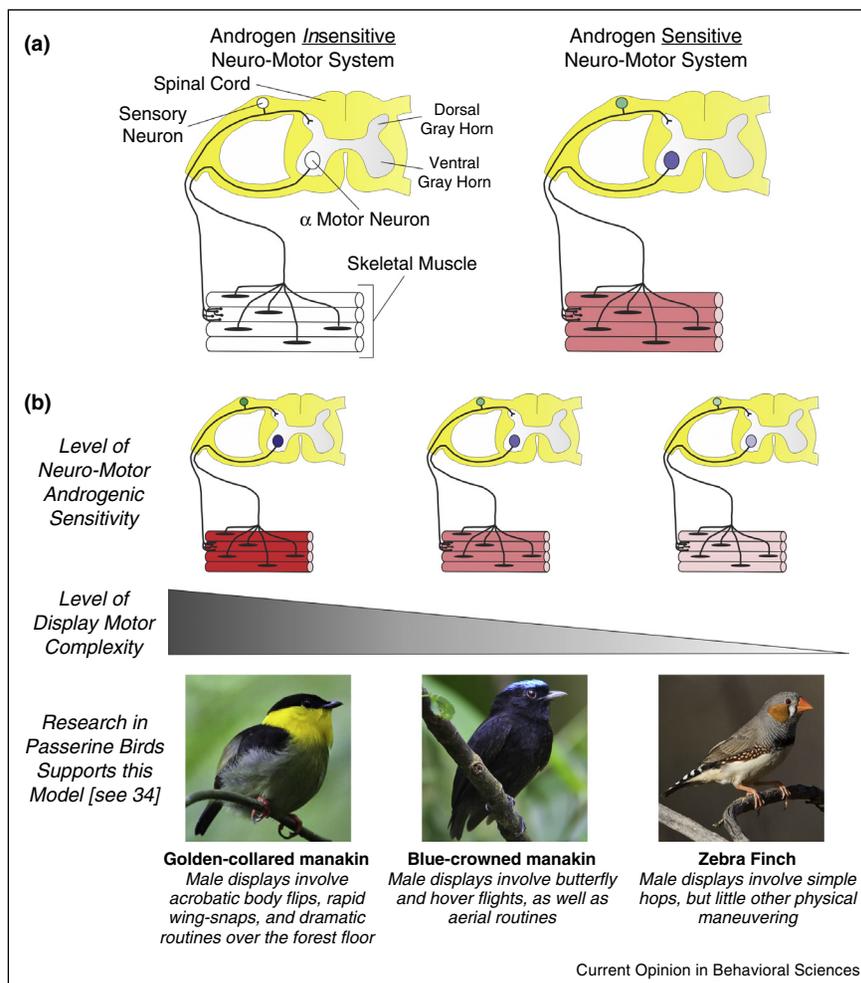
A broad model for the evolution of sexual motor skill

The manakin research described above adds to a vertebrate-wide literature that illustrates the importance of androgen signaling in helping guide male motor command in sexual contexts. Examples include strutting behavior in quail [35], dewlap extension in anoles [36,37], courtship clasp in frogs [38,39], and vocal production in birds [40[•],41] and fish [42,43]. These studies together support a model for the evolution of mechanisms that underlie physically complex courtship, whereby changing androgenic sensitivity of a given neuro-motor circuit contributes to incorporation, modification, or even removal of movement involved in reproduction and sexual behavior (Figure 1). Within this framework, we hypothesize that increasing androgenic sensitivity largely enables greater motor complexity, while decreasing androgenic sensitivity has the opposite effect. To this end, evolutionary pressures, like selection, may act differentially among the various muscles and spinal circuits, yielding different motor capability across the body. These systems undoubtedly are sculpted to work in concert and thus produce complex behavioral routines.

Although studies of golden-collared and other manakins have shed significant light on the mechanisms of physical displays and how these behaviors evolve, additional work is needed to fill additional gaps in our understanding. For example, do other hormone systems known to influence social behavior interact with the neuro-motor apparatus to help guide the production of complex courtship behavior [44^{••},45]? Also, what are the central programs that contribute to displays, and how are they coupled in the CNS [46,47[•]]?

This body of research clearly illustrates that sexual selection shapes the evolution of animal signals by influencing far more than just color patterns or vocal output. Rather, through the modification of endocrine systems, neuro-motor circuits, and select anatomical features, evolution adjusts the way in which animals move their bodies during times of reproduction. We propose a model (Figure 1) that we believe applies to a variety of species, and thus represents one mechanism by which selection alters physiology to drive the emergence of spectacular

Figure 1



Proposed model for the evolution of sexual motor skills. (A) Schematic of the peripheral neuro-motor system (*left*), which includes an α motor neuron, a muscular sensory neuron, and skeletal muscle. Studies show that all three of these cell types and/or tissues can express androgen receptor (AR) and thus are in some cases sensitive to androgenic action. This is shown (*right*) with coloration of the different neuro-motor components. In the event that a given neuro-motor circuit is or becomes androgen sensitive, sexual selection in theory may modify this sensitivity to change sexual motor ability. (B) We propose that sexual selection alters neuro-motor androgenic sensitivity as a way of enhancing physical prowess, agility, and strength in accordance with reproduction. By favoring elevated expression of AR in α motor neurons, muscular sensory neurons, and/or skeletal muscle, selection may enhance organismal performance capacity and permit the evolution of elaborate behavioral displays. In this vein, we predict that AR expression levels in the neuro-motor system are positively associated with measures of display complexity (higher AR expression is illustrated with a more intense hue within the neuro-motor structures). Fuxjager *et al.* [34**] showed such a relationship in passerine birds, and a few example species from this analysis, including the golden-collared manakin, are illustrated. Photographs of the golden-collared and blue-crowned manakins taken by Nick Athanas. Photograph of the zebra finch taken by Mat Gilfedder.

physical feats [48**,49,50]. Recent advances in the field of genomics and the availability of genomes from diverse animals will enable research on the investigation of deep molecular mechanisms that underlie such behaviors, which will, in turn, reveal the full extent of selection's power to guide these behaviors. The combination of modern genomic approaches, together with more traditional techniques, such as pharmacological manipulations or track-tracing methodology will further clarify the biological basis of complex animal courtship. Together,

these approaches will unravel some of the mystery of how and why animals like the Birds-of-Paradise evolve to perform the magnificent dances that caught the attention of Darwin and Wallace so many years ago.

Conflict of interest statement

Nothing declared.

Acknowledgements

Supported by NSF IOS-0646459 and NIH Training Grant T32 HD007228.

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