Androgens Support Male Acrobatic Courtship Behavior by Enhancing Muscle Speed and Easing the Severity of Its Tradeoff With Force

Matthew J. Fuxjager,[1] Meredith C. Miles,[1] Franz Goller,[2] John Petersen,[1] and Julia Yancey[1]

[1]Department of Biology, Wake Forest University, Winston-Salem, North Carolina 27109; and [2]Department of Biology, University of Utah, Salt Lake City, Utah 84112

Steroid hormone action in the brain regulates many animals' elaborate social displays used for courtship and competition, but it is increasingly recognized that the periphery may also be a site for potent steroidal modulation of complex behavior. However, the mechanisms of such "bottom-up" regulation of behavioral outflow are largely unclear. To study this problem, we examined how androgenic sex hormones act through the skeletal muscular system to mediate elaborate courtship acrobatics in a tropical bird called the golden-collared manakin. As part of their display, males snap their wings together above their backs at rates that are at least 2× faster than the normal wing-beat frequency used for flight. This behavior, called the roll-snap, is actuated by repeatedly activating a humeral retractor muscle—the scapulohumeralis caudalis (SH)—which produces contraction-relaxation cycling speeds similar to the "superfast" muscles of other taxa. We report that endogenous androgenic activation of androgen receptor (AR) sustains this muscle's exceptionally rapid contractile kinetics, allowing the tissue to generate distinct wing movements at oscillation frequencies >100 Hz. We also show that these effects are rooted in an AR-dependent increase to contractile velocity, which incurs no detectable cost to force generation. Thus, AR enhances SH speed necessary for courtship display performance while avoiding the expected tradeoff with strength that could otherwise negatively influence aspects of flight. Peripheral AR therefore not only sets up the muscular system to perform a complex wing display, but does so in a way that balances the functional requirements of this muscle for other life-sustaining behavior. (***Endocrinology*** **158: 4038–4046, 2017)**

Sex steroids play a prominent role in the activation of vertebrate reproductive behavior, mediating many of the elaborate social displays that animals perform (1). Most work studying this phenomenon focuses on the brain by exploring how steroids modulate sensory perception, arousal, motivation, and motor action (2–6). However, such "top-down" effects are not the only way in which steroids regulate behavior—that is, steroids may simultaneously act within peripheral tissues to regulate how the nervous system elicits behavioral outflow (7–9). These so-called "bottom-up" mechanisms of steroidal control of behavior remain unclear.

In vertebrates, skeletal muscle is the main behavioral effector in the periphery. It receives efferent commands from the central nervous system and thus actuates physical activity that constitutes an individual's behavioral repertoire. Functionally, it is well established that fast-twitch oxidative muscle contracts with either great speed or great strength, but rarely both (10–12). This means that these aspects of muscle performance are usually constrained through a tradeoff (13, 14). Thus, if selection for a certain behavioral trait requires extreme speed from a specific muscle, then it can hinder selection for other behavioral traits that require appreciable strength from the same tissue (15, 16). Overcoming these

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Abbreviations: ANOVA, analysis of variance; AR, androgen receptor; Flut, flutamide; F_{max}, maximum force; GCM, golden-collared manakin; L_0, length change; SH, scapulohumeralis caudalis; SNK, Student-Newman-Keuls; T, testosterone; V_{max}, maximal velocity.

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effects therefore requires some type of biomechanical or physiological innovation that enables the production of both fast and strong movements.

Here, we study the role of androgenic steroid hormones in regulating the dynamics of speed and strength with different performance demands to serve competing adaptive functions. Past work shows that most skeletal muscle expresses abundant androgen receptor (AR) (17), which helps modify the size and contractile machinery of a muscle fiber in response to androgenic activation (18). Such effects are frequently documented in muscles that control sexual reflexes and movements (19–24), suggesting that androgen–muscle interactions actuate reproductive behaviors. It is still not known, however, how AR action affects muscle performance as it relates to complex behavioral traits, which otherwise demand specialized motor control.

In the current study, we hypothesize that androgenic action serves as a physiological switch in the neuromuscular system, adjusting how muscles accommodate extraordinary physicality and athleticism used for sexual displays. We test this idea in a tropical bird called the golden-collared manakin (GCM; *Manacus vitellinus*) (Fig. 1a). Males of this species court females by producing rapid gestural signals in an acrobatic display (25). One of their most elaborate signals, the roll snap, occurs as the wings are hit together above the back ~60× per second (Hz) (Fig. 1b). This is driven by wing movements that are at least 2× faster than the maximum wing-beat frequency of similar sized birds during flight (26). Roll-snap production therefore likely requires modulation of the neuromuscular systems that control forelimb movement, and past work suggests that AR plays a role in this process. For example, the neuromuscular system generating the display is enriched with AR compared to other birds (27–29), and activation of these receptors changes the expression of genes that encode proteins involved in contractile dynamics (30, 31). Likewise, blocking these receptors can slow and shorten roll-snap performance (9).

We focus our study on a dorsal wing muscle called the scapulohumeralis caudalis (SH) (Fig. 1c). This tissue acts as a humeral retractor that likely helps power flight by changing the wing's shape or position (32, 33). In this regard, the SH may need to exert some level of force to help support aspects of locomotion. Yet, in the GCM, the SH is also thought to be a major actuator of the roll-snap, given that humeral retraction likely serves as the behavior's mechanical basis (25, 34). Physiological studies support this view by illustrating that this muscle is exceptionally rapid—it can generate distinct wing movements at stimulation frequencies of 100 Hz (35). These speeds are faster not only than the manakin's other wing muscles, but also than the SH of related species that do not produce wing displays for courtship but that inhabit the same environment [*e.g.*, the dusky antbird, *Cercomacra tyrannina* (35)]. Thus, in the manakin, the SH appears to exhibit performance traits that are otherwise considered mutually exclusive: strength and exceptional speed. We therefore predicted that androgenic activation of neuromuscular AR regulates SH speed-strength dynamics in a manner that promotes such performance flexibility. As such, AR activation likely maintains the rapid contractile kinetics of the manakin SH, elevating it far above the muscle speed of other species, like the dusky antbird, which inhabit the same environment but do not use the SH for rapid courtship display.

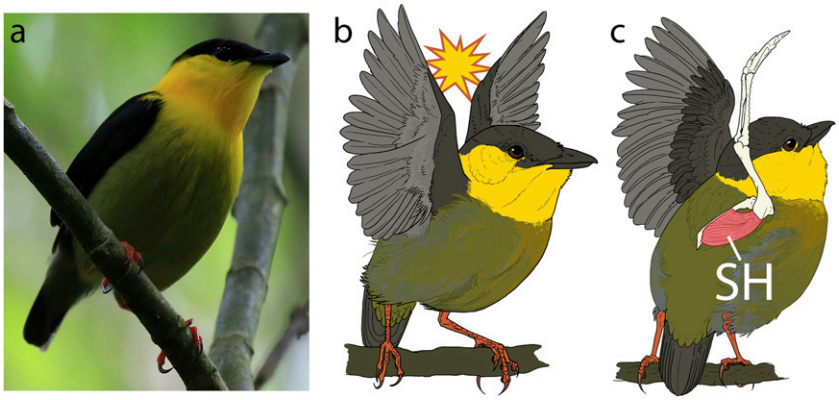

Figure 1. (a) Reproductively active adult male golden-collared manakin. Image courtesy of Seabamirum/Flickr; photograph by Tim Lenz, Creative Commons CC-BY 2.0 license, no modifications. (b) Cartoon of a manakin snapping its wings together. Kinematic analyses of this behavior show that it occurs by elevating the extended (opened) wings above the back and then repeatedly retracting the humerus to force the wrists to collide multiple times in a row (9, 25). Such movement, retraction of the humerus, is controlled by the scapulohumeralis caudalis (SH) muscle (32). (c) Position of the SH on an articulated wing; this muscle originates on the scapula and inserts on the proximal humerus.

Methods and Materials

Animals

Using mist nets, we captured reproductively mature, adult male GCMs and dusky antbirds from their breeding grounds in the forests of Gamboa, Panama, near the Smithsonian Tropical Research Institute. We immediately transported birds (manakins, n = 6; antbirds, n = 3 to 4) to our laboratory, where we either housed them or subjected them to surgeries for muscle recordings (see the following section). We performed experiments during March and April, when both species are known to actively court mates and defend territories.

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The Panamanian government, the Smithsonian Tropical Research Institute Animal Care and Use Committee, and the Wake Forest Institutional Animal Care and Use Committee approved of this work.

Hormone manipulations

GCMs were housed in captivity for 9 days [detailed descriptions in (36, 37)]. After a 48-hour habituation to such conditions, each male was implanted with a 12-mm SILASTIC tube (0.76-mm inner diameter; 1.65-mm outer diameter; Dow Corning, Auburn, MI) containing 10 mm of crystalline testosterone (T) and sealed at both ends with 1 mm of silicone sealant. Given that captive conditions can suppress circulating testosterone levels, this treatment ensured that endogenous T was clamped at levels typical of breeding males [validated (28, 36)]. Immediately following this procedure, we randomly assigned individuals to receive a second SILASTIC implant (length and dimensions the same as previously discussed) that contained either the AR antagonist flutamide (GCM-T + Flut; n = 3) or nothing (GCM-T; n = 3). Prior work in passerine birds showed that a flutamide implant of this size and diameter releases ~100 mg/d of drug (38), which in our current study equates to a daily dose of 5.5 g/kg of flutamide. This is comparable to flutamide doses used in other GCM studies that examine the effects of complete pharmacological AR inhibition, without affecting an individual's reproductive motivation, health, or gross locomotor ability (39). Similarly, the efficacy and lack of toxicity of this treatment is verified in numerous other species of passerine birds (30, 31, 38, 40–44).

Implants were administered subcutaneously along each bird's back at the base of its neck, using procedures described previously (9, 30, 31). Thus, birds in the GCM-T + Flut group maintained elevated levels of circulating T, but lacked functional AR that could detect it. By contrast, birds in the GCM-T group maintained similarly elevated levels of circulating T, which could readily act via AR. We waited 7 days after implantation (*i.e.,* hormone manipulation) before collecting muscle recording data for these birds (see the following section). This period corresponds to that in which AR inhibitors suppress manakin display behavior (9, 39). Data from the manakins were collected blindly, in that experimenters were unaware of the hormone treatment during muscle recording sessions.

Surgical preparation

All muscle recordings were collected *in situ* using techniques developed previously for both manakins, antbirds, and other small passerine species (35). We anesthetized each bird with isoflurane (2% to 4% in O_2), and then restrained it on the surgical bench. We cut a small (1-cm) incision in the skin (on the bird's back) directly above the SH, implanting the tissue with the stripped ends (1 to 2 mm) of two insulated silver wire electrodes (diameter, 0.14 mm) that were connected to a stimulator (model 2100; A-M Systems, WA). We attached a stainless-steel hook (0.1-mm diameter) to the SH muscle, which was connected to a force transducer (model FT03; Grass Technology, West Warwick, RI) via a monofilament line. The force transducer was firmly clamped to a 7-kg stand whose position was adjusted to regulate tension after each surgery. The exposed surface of the muscle was bathed in normal avian saline (0.9%) to prevent desiccation. After recordings were completed, we removed the electrodes and hook and then sutured the skin closed with Vetbond tissue adhesive. Birds were released at the site of capture after a day of postsurgery recovery.

Data collection

We connected the force transducer to an AC/DC strain gauge amplifier (model P122; Grass Technologies) to amplify (5 K to 10 K) and low-pass filter (3 kHz) the signal. Using the DC input selection, the output signal was recorded on a laptop via an A-D converter (model NI USB-6212; National Instruments, Austin, TX) with AviSoft-RECORDER (v.4.2.22). Data were analyzed with Praat software (v.5.4.21; P. Boersma and D. Weenink).

Twitch-speed measurements

Using an approach described previously (35), we measured SH twitch speeds in response to 70-, 90-, and 110-Hz stimulation frequencies. As such, we determined contraction-relaxation speeds within a stimulus period by calculating the amount of SH relaxation relative to its unstimulated length. SH relaxation (*i.e.,* from 0% to 100%) was calculated by dividing the measured degree of relaxation by that which was otherwise necessary for full recovery. We always averaged percent recovery values for the first eight stimulations for each stimulation train because this corresponds to the number of wing oscillations of the roll-snap display (9).

All stimulation trains contained 10 electrical pulses (duration: 1 ms; 0.5 to 0.8 mA). For each frequency, birds were given four to six stimulation trains spaced roughly 15 to 30 seconds apart, and the whole frequency series was administered in the order of 70 Hz to 110 Hz. To confirm that high-frequency stimulation did not damage or exhaust the SH, we compared levels of muscle recovery in response to 50-Hz trains given before and after all twitch-speed data were collected. Muscle recovery in these cases was near 100%; this did not differ between the before and after phases of the study [linear mixed model analysis of variance (ANOVA): $F_{1,45.98} = 0.15$, $P = 0.70$). This result therefore confirms that the SH was functionally intact throughout the experiment (35). Furthermore, to verify that captivity and surgical implantation procedures did not affect our measures of SH performance, we compared measurements of muscle relaxation between individuals in the GCM-T group and a group of adult male manakins (n = 3) in which twitch-speed data were obtained immediately after capture (35). We found no difference in muscle recovery between groups at either 50 Hz ($t = 0.84$, $P = 0.45$) or 90 Hz ($t = 1.0$, $P = 0.35$) stimulation frequencies, indicating that neither captivity nor the experience of implantation adversely affected SH performance.

We compared muscle-twitch dynamics using a two-way linear mixed-model ANOVA, with stimulation frequency and treatment (*i.e.,* hormone manipulation and/or species difference) as the fixed factors and stimulation train number nested within individuals as a random factor. Significant effects were followed by simple main effect *post hoc* comparisons, with Bonferroni corrections applied.

Force-velocity measurements

To generate force-velocity curves in the SH, we measured isometric contractile force and length change (L_0) in response to electrical stimulation (pulse duration, 1 ms; 0.7 mA). The bird's humerus was fixed to a static device to prevented wing movement during all recordings, such that muscular stimulation

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exclusively drove scapular flexion. We then loaded the SH via the scapula using a small pulley system, by which this bone was connected to a plastic bag that could be filled with weights via a monofilament line. Measurements were collected under the following different load conditions: no weight, bag only, and 6, 12, 18, 24, and 30 g. To ensure that the starting length of the SH was always the same, we placed a steel barrier along the medial border of the scapula to prevent it from moving medially (*i.e.*, toward the spine). The scapula could therefore only flex laterally in response to stimulation.

For these recordings, force transducer readings were calibrated to reflect L_0 (mm) using a displacement transducer (Micro Strain MDVRT 3; Lord Sensing, Williston, VT). L_0 could then be determined by its proportional relationship to the magnitude of pulse output from the force transducer during stimulation. We calculated velocity measurements under each load condition by dividing L_0 by the time (seconds) it took the SH to maximally contract, whereas we relativized force production to maximum force obtained under the "no weight" load (*i.e.*, relative max force = 1). We then used a hyperbolic-linear function to fit a curve between force and velocity under different load conditions (45), extracting estimates of maximal velocity (V_{max}) for each individual. The same data were then used to generate power curves in which the product of force and velocity was plotted as a function of contractile velocity under a given load condition. From these data, we computed the area under the curve, which reflects total power of the muscle as well as the maximum velocity at peak force production.

Overall, this setup allowed us to record measures of relative force changes and velocity in response to stimulation locally within the belly of the SH, as opposed to the entire SH. We compared V_{max}, area under the SH power curve and velocity at peak power among the treatment groups using one-way ANOVAs using Student-Newman-Keuls (SNK) *post hoc* tests after significant main effects were uncovered. We also report measures of effect size (Cohen *d*) for each pairwise comparison (46, 47), which assess standardized differences between groups and provides insight into the strength and size of the difference between two groups (*i.e.*, *d* values >0.8 represent "large" effects, whereas *d* values <0.8 represent "medium" or "small" effects) (48, 49).

Maximum force measurements

We assessed maximum force (F_{max}) by subjecting the SH to a single stimulation train of 20 pulses (0.8 mA) at 200 Hz. The amplitude of the twitch detected by the force transducer was therefore proportional to the amount of force generated by the muscle (as discussed previously); thus, we collected four to six F_{max} measurements for each individual and computed the mean of these data for analysis. Measures of F_{max} at the belly of the muscle were comparable among groups within this study. We compared measures of F_{max} across treatment groups using a one-way ANOVA and followed significant main effects with SNK *post hoc* tests.

Results

Androgens and SH contraction-relaxation cycling speed

We first tested (1) whether AR mediates contraction-relaxation cycling speeds in the manakin SH and (2) how these effects compare to the SH in the dusky antbird (Fig. 2). Our analyses show that measures of muscle-twitch speed significantly differed across these groups ($F_{2,104.3}$ = 20.63, $P < 0.001$) and among the three stimulation frequencies ($F_{2,104.3}$ = 50.81, $P < 0.001$). We also detected a significant interaction between these two factors ($F_{2,104.3}$ = 5.28, P = 0.001), indicating that the differences among hormone treatment and species varied depending on the stimulation frequency.

For manakins, our data were consistent with our previous work (35). The SH from control males was remarkably fast, relaxing around 75%, 65%, and 55% when stimulated at 70, 90, and 110 Hz, respectively (Fig. 2). We verified that this tissue generated positive work at these frequencies by noting clear, oscillatory physical movement during our stimulation regimens. This included stimulations at 110 Hz, which again cements this muscle as the fastest vertebrate limb muscle on record (35, 48, 49). To put this in context, the manakin SH is able to contract and relax at speeds that are greater than extraocular muscles (48) and on par with certain "superfast" muscles, such as the rattlesnake tail-shaker muscle (49). Importantly, *post hoc* tests showed that blocking AR significantly reduced the degree of muscle

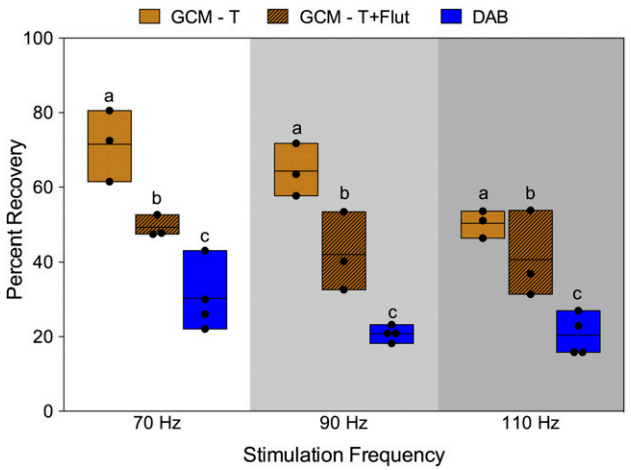

Figure 2. Twitch-speed dynamics of the SH muscle stimulated at different frequencies, including the "superfast" 110-Hz frequency. Each dot indicates the average percentage of relaxation of the SH in response to repeated stimulation at the given frequency (depicted on the horizontal axis). Individuals are clustered according to their group, which is defined by species (GCM, orange shading; DAB, blue shading) and hormone treatment (GCM-T, solid orange bin; GCM − T + Flut, hatched orange bin). Boxes depict the individual distribution for each group, with the middle line reflecting the mean value. We report significant effects of treatment (mixed-model ANOVA: $F_{2,104.3}$ = 20.63, $P < 0.001$) and stimulation frequency (mixed-model ANOVA: $F_{2,104.3}$ = 50.81, $P < 0.001$), as well as a treatment × stimulation frequency interaction (mixed-model ANOVA: $F_{2,104.3}$ = 5.28, P = 0.001). *Post hoc* tests are conducted for each stimulation frequency; differences in letters atop bars reflect significant differences per this analysis (Bonferroni corrections, $P <$ 0.05). DAB, dusky antbird.

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relaxation at each frequency (all $P < 0.05$), demonstrating that AR itself is necessary to maintain this muscle's rapid contraction-relaxation kinetics.

We also compared measures of twitch speed from both groups of manakins to those obtained from dusky antbirds, which provides important insight into the speed at which the SH is likely expected to perform if it supported only locomotion in the forest understory. Indeed, we found that the percent relaxation of the SH in the dusky antbird was significantly lower than in both groups of manakin (Fig. 2; $P < 0.05$). In fact, the antbird SH relaxed by only ~20% to 30% when stimulated at 70, 90, and 110 Hz (Fig. 2), which we confirm is insufficient to actuate repeated wing movements. Instead, the SH contractions of the antbird fused under these stimulation regimens, meaning that the tissue failed to effect individual movements at these fast cycling speeds.

Androgenic regulation of SH force-velocity dynamics

We next examined SH force-velocity dynamics in both groups of manakins and the dusky antbird (Fig. 3). Curves that describe the relationship between these two variables differed among the groups (Fig. 3a), with manakins showing a far steeper negative relationship, which indicates a generally faster muscle. However, blocking AR clearly caused the force-velocity line in this species to flatten, suggesting that contractile velocity itself is AR-dependent. At the same time, the force-velocity curve of the dusky antbird was by far the flattest, implying that this was the slowest of the tissues in our analysis.

We also used these three curves to compare V_{max} of the SH, and we found significant differences across groups ($F_{2,6} = 34.55$, $P < 0.001$) (Fig. 3b). Manakins exhibited remarkably high estimates of V_{max} compared with the antbird ($P < 0.05$); however, within manakins, blocking AR also decreased V_{max} significantly ($P < 0.05$). Meanwhile, our measures of F_{max} in the SH were statistically indistinguishable among groups ($F_{2,6} = 2.30$, $P = 0.18$) (Fig. 3c), including comparisons between manakins that had AR intact and AR inhibited. In other words, we did not uncover an appreciable reduction in force generation, despite observing a substantial increase in contractile speed.

Androgenic regulation of SH power dynamics

Finally, we examined the power dynamics of the SH in all birds to further assess whether AR-dependent changes in the force-velocity relationship affects the muscle's ability to contribute to powered flight (Fig. 4). Considering that muscle power is the product of speed and strength (power = velocity × force), we expected SH power to dramatically increase in manakins with intact AR. This is indeed what we found (Fig. 4a) because the area under the power curve differed significantly among groups ($F_{2,6} = 15.69$, $P = 0.0041$) (Fig. 4b): manakins showed the highest amount of SH power when their AR was intact ($P < 0.05$), whereas dusky antbirds showed far less area under the curve ($P < 0.05$). These groups also differed with respect to estimates of SH contractile velocity at peak power output ($F_{2,6} = 29.36$, $P < 0.001$) (Fig. 4c). Again, the manakin treatment groups significantly differed in that inhibition of AR decreases these estimates of performance ($P < 0.05$).

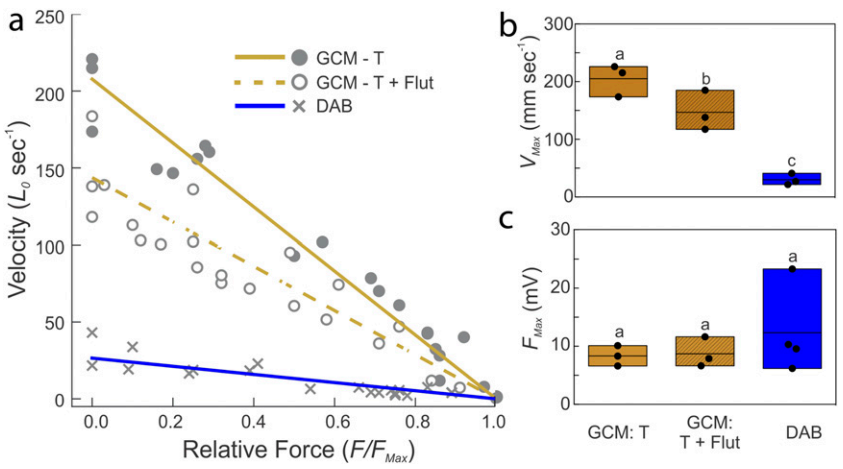

Figure 3. (a) Force-velocity curves of adult male GCMs with either free AR (GCM-T, solid orange line) or inhibited AR (GCM-T + Flut, dashed orange line), as well as adult male DABs (blue line). Note that the curves appear largely linear, which incidentally is similar to force-velocity curves generated through other techniques in passerine pectoralis and supracoracoideus (12). (b) Estimated SH V_{max} and (c) measures of F_{max} in male manakins with free AR (GCM-T, solid orange bin), inhibited AR (GCM-T + Flut, hatched orange bin), and DABs (blue bin). Dots indicate an individual's average value, whereas boxes depict the data distribution for each group (middle lines show the mean values). Analyses indicate that V_{max} (ANOVA: $F_{2,6} = 34.55$, $P < 0.001$) and F_{max} (ANOVA: $F_{2,6} = 2.30$, $P = 0.18$) values significantly differ across groups. In both graphs (b, c), differences between the letters above each bar denote significant differences between groups as detected by SNK *post hoc* tests ($P < 0.05$). Note that all pairwise comparisons in our analysis of V_{max} result in Cohen *d* values of at least 1.8, which indicates a strong effect independent of sample size (46, 47). DAB, dusky antbird.

Discussion

Our data show that androgenic activation of AR greatly increases the contraction speed of the SH muscle, transforming it into a rapid, powerful tissue capable of actuating distinct wing movements at frequencies above 100 Hz. To date, the SH muscle in the GCM is the fastest vertebrate limb muscle on record with respect to measures of twitch speed (35); our

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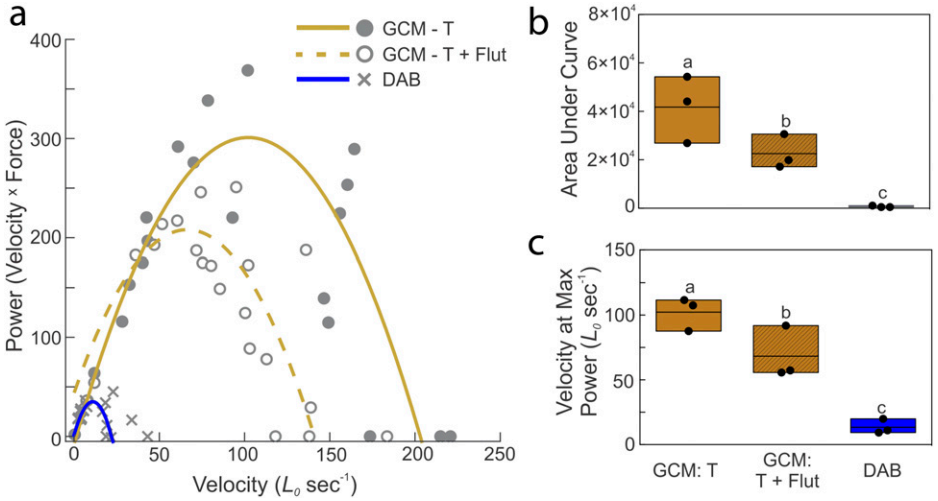

Figure 4. (a) Power curves of adult male GCMs with either intact or inhibited AR, as well as adult male DABs. (b) Values of total SH power (measured by area under the power curve) and (c) contractile velocity at maximum (peak) power production in male manakins with free AR, inhibited AR, and DABs. Dots indicate an individual's average value, whereas boxes depict the data distribution for each group (middle lines show the mean values). Measures of total power (ANOVA: $F_{2,6} = 15.69$, $P = 0.0041$) and maximum velocity at peak power (ANOVA: $F_{2,6} = 29.36$, $P < 0.001$) are significantly different between groups. In both graphs (b, c), differences between the letters above each bar denote significant differences between groups as detected by SNK *post hoc* tests ($P < 0.05$). Note that all pairwise comparisons in our analysis of V_{max} result in Cohen *d* values of at least 1.7, which indicates a strong effect independent of sample size (46, 47). DAB, dusky antbird.

findings therefore indicate that androgenic action via AR helps establish and maintain this trait. Equally important is that these effects do not depress our measures of force production, indicating that AR-dependent increases in SH speed come at little (if any) appreciable cost to strength. Accordingly, our results imply that androgenic action helps the SH alleviate a functional constraint otherwise imposed by intrinsic tradeoffs between contraction velocity and force. The result is a muscle with a highly unusual phenotype: it is primed to actuate rapid movement involved in gestural courtship signaling without encumbering aspects of locomotion that may demand a certain amount of strength.

Perhaps the most interesting finding from this study is that androgenic hormones mediate extremely fast contractility of skeletal muscle. Indeed, by blocking AR, we significantly reduce SH contraction-relaxation cycling rates and shortening velocity, thereby slowing the muscle's ability to produce repetitive movements used for roll-snap signaling. Considering that we measure SH performance *in situ* in response to direct muscle stimulation, we expect that AR influences these performance properties by acting either in the spinal cord to adjust properties of neurotransmission and/or the SH itself to alter its contractile kinetics. In GCMs, this specific muscle and its innervating motoneurons express high levels of AR compared to a host of other birds that do not perform wing-snaps (27–29). Other research confirms that AR in the SH is functional and regulates the expression of numerous genes that fuel metabolism and facilitate rapid contractility and growth (30, 31). Moreover, if AR is selectively inhibited in the SH and other wing muscles, then males produce roll-snaps that are both slower and shorter (9). This change in behavior is consistent with our current data showing that blocking AR induces a strong flattening of the SH force-velocity curve, as well as a precipitous decrease in the muscle V_{max}. To this end, our current study implies that at least some of the effects we observe are due to direct myocytic AR action. Future work, however, will be needed to tease apart the effects of AR on muscle phenotype from those on neural control, particularly in this context of androgen-dependent athleticism and acrobatics.

More broadly, our data suggest that androgenic action helps the SH balance different functional demands for both courtship and locomotion. Although the role of the SH in the latter (*i.e.*, flight) is not fully understood, studies suggest that the SH may change the wing's shape and/or position during flapping flight to maximize aerodynamic force generated by the main muscular engine, the pectoralis (50). The SH should therefore have to generate sufficient force to successfully move the wing during powered flight, by helping resist torsional stresses that are otherwise placed on it (51, 52). If, as we expect, this ability is diminished because sexual selection has pushed the tissue's performance features toward rapid contractility (13, 14), then this may be associated with a "cost" to locomotion. Based on our results, we suspect that androgenic action mitigates tension between these functional needs by easing the severity of the tradeoff between SH speed and strength. We see this effect in the manakin's flattened force-velocity curve in response to

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AR inhibition. Indeed, this may help free sexual selection to drive further elaboration of manakin display speed without grossly encumbering other life-sustaining functions related to basic locomotion (*e.g.*, foraging, predator escape). Such an ability may also be important for other taxa that simultaneously control different, and sometimes incompatible, performance traits with the same suites of muscle (34, 53–55).

At the comparative level, we find that the manakin SH is far faster than the dusky antbird SH, regardless of whether AR is blocked. This effect is not likely the result of differences in captivity and implantation surgery, because we verified in manakins that these factors have little effect on SH speed. As such, the antbird acts as a proxy for the type of SH performance we might expect if the muscle is only involved in "normal" wing movements for flight, but not high-speed courtship. Both antbirds and manakins inhabit the tropical understory, so the wing musculature of both species is likely shaped by selection to support locomotion in that environment. Thus, the faster SH of the manakin leads us to conclude that this increase in speed is probably not necessary to power flight. We attribute this gain in contractile velocity to selection for the manakin's display while also recognizing that other factors may contribute to these differences more subtly.

How exactly does AR change the SH so that it contracts quickly, but still with force? First, we suspect that AR regulates the expression of contractile filaments that possess unique biophysical properties for rapid contractile kinetics. This idea is supported by our own results, which illustrate that AR increases SH V_{max}, which is proportional to cross-bridge detachment rates that otherwise set the speed by which actin and myosin filaments slide past each other during sarcomere shortening (contraction) and lengthening (relaxation) (56). Other work similarly shows that androgens can regulate muscle fiber type (19, 21) and may even upregulate the expression of novel myosin genes in the manakin SH (31). Second, we expect that AR improves myocytic calcium handling dynamics, which in turn determines contraction-relaxation cycling speeds. Again, studies of the functional effects of androgens in the manakin muscular system show that this hormone increases the expression of genes, such as parvalbumin (30), to help buffer calcium to reduce relaxation times (57). Third, with respect to force preservation, we speculate that androgenic action triggers SH hypertrophy. Muscle fiber size (diameter) is proportional to the amount of force the tissue can generate, and it is well established that androgens increase muscle growth and mass across species (20, 22). In the manakin, androgens increase muscular expression of growth factors, such as IGF-1 (30), which likely play a role in maintaining the bird's enormous SH: the avian wing equivalent of a human bodybuilder's shoulders (58). Together, these effects may synergize in a way that helps balance decreases in force production that would result from a functional transition toward increasing muscle speed.

Conclusions

In summary, we show how the endocrine system can mediate adaptive muscle performance, transforming a forelimb muscle into a remarkably fast contractile tissue that supports production of a rapid wing display used for courtship advertisement. These effects are also achieved without appearing to encumber other performance tasks, such as locomotion, which one would typically expect to be affected through tradeoffs between especially fast muscle contractions and strength. In this regard, we highlight a physiological mechanism by which steroid hormones can act in a "bottom-up" manner to facilitate behavior by mitigating tension between opposing adaptive functional demands.

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Correspondence and Reprint Requests: Matthew J. Fuxjager, PhD, 455 Vine Street, Building 60, Department of Biology, Wake Forest University, Winston-Salem, North Carolina 27101. E-mail: mfoxhunter@gmail.com.

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