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RESEARCH ARTICLE

Androgenic modulation of extraordinary muscle speed creates a performance trade-off with endurance

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ABSTRACT

Performance trade-offs can dramatically alter an organism's evolutionary trajectory by making certain phenotypic outcomes unattainable. Understanding how these trade-offs arise from an animal's design is therefore an important goal of biology. To explore this topic, we studied how androgenic hormones, which regulate skeletal muscle function, influence performance trade-offs relevant to different components of complex reproductive behaviour. We conducted this work in golden-collared manakins (*Manacus vitellinus*), a neotropical bird in which males court females by rapidly snapping their wings together above their back. Androgens help mediate this behavior by radically increasing the twitch speed of a dorsal wing muscle (scapulohumeralis caudalis, SH), which actuates the bird's wing-snap. Through hormone manipulations and *in situ* muscle recordings, we tested how these positive effects on SH speed influence trade-offs with endurance. Indeed, this latter trait impacts the display by shaping signal length. We found that androgen-dependent increases in SH speed incur a cost to endurance, particularly when this muscle performs at its functional limits. Moreover, when behavioural data were overlaid on our muscle recordings, displaying animals appeared to balance display speed with fatigue-induced muscle fusion (physiological tetanus) to generate the fastest possible signal while maintaining an appropriate signal duration. Our results point to androgen action as a functional trigger of trade-offs in sexual performance – these hormones enhance one element of a courtship display, but in doing so, impede another.

KEY WORDS: Testosterone, Physiological constraints, Courtship displays, Manakin birds, Flutamide, Sexual selection

INTRODUCTION

Physiological trade-offs can profoundly influence phenotypic evolution (Garland, 2014; Gustafsson et al., 1995; McGlothlin et al., 2007; Roff and Fairbairn, 2007; Stearns, 1989). They arise when the presence of one trait comes at the cost of another, creating a scenario in which the expression of certain phenotypes is impossible. Thus, if selection favours the exaggeration of one of these traits, it may simultaneously constrain the other (Roff and Fairbairn, 2007; Zera and Harshman, 2001). This dynamic can play out at different scales, ranging from trade-offs between seemingly disparate traits used for different tasks (e.g. foraging versus mating; Abrahams, 1993) to separate components of a single behavioural task (e.g. behavioural speed vs accuracy; Wheatley et al., 2015). Regardless, understanding how physiological trade-offs at all levels emerge and influence phenotypic change over time is therefore an important objective of organismal biology (Stearns, 1989; Zera and Harshman, 2001). Yet, many of the mechanisms that give rise to key physiological trade-offs remain unclear despite their presence throughout much of the animal world (Drea et al., 2002; Ghalambor et al., 2004; Gilbert and Manica, 2010; Mead et al., 2017; Navarrete et al., 2011; Rome et al., 1996; Sargent et al., 1987; Sathe and Husak, 2015; Wilson et al., 2002; Young, 2006).

Performance trade-offs have some of the most profound effects on organismal evolution. Speed, for example, often comes at a cost to other locomotor vectors such as endurance (Vanhooydonck et al., 2001, 2014; Wilson and James, 2004; Wilson et al., 2002). These relationships are attributed to skeletal muscle function, particularly the relative composition of slow and fast fibre types (Rome and Lindstedt, 1998; Scales et al., 2009). In theory, when selection favours traits that require extreme muscle speed, it should also constrain the evolution of traits that depend on extreme endurance from the same muscle (the opposite is also possible). However, skeletal muscle performance is highly plastic, changing in response to factors such as temperature (Rome et al., 1996), contractile activity (Hood, 2009) and environmental stimuli (Sharples et al., 2016). It is therefore possible that these same factors may also reconfigure a skeletal muscle's trade-off landscape, which could allow selection to favour certain (otherwise constrained) performance attributes related to different behavioural traits altogether and/or different aspects of singular behaviours. Yet this idea is rarely studied, particularly in functionally relevant contexts of adaptive motor control.

Hormone signalling is one way by which physiological factors might alter muscular trade-off dynamics. Androgenic steroids, for example, regulate many aspects of muscle function (Fuxjager and Schuppe, 2018; Higham and Irschick, 2013) by acting through myocytic androgen receptor (AR) (Chambon et al., 2010). This protein is a ligand-activated transcription factor that mediates the expression of hundreds of genes (Cheung et al., 2017; Fuxjager et al., 2016a), which in turn shape the performance properties of a muscle fibre (Mooradian et al., 1987; Sheffield-Moore, 2000). For example, AR can regulate muscle fibre type composition (Chambon et al., 2010; Higham and Irschick, 2013; Klukowski et al., 2015), setting the balance between fast-twitch and slow-twitch fibres that determine the tissue's relative speed and endurance (Wilson and James, 2004; Zierath and Hawley, 2004). Similarly, androgens can increase the abundance of proteins responsible for calcium trafficking (Chambon et al., 2010; Vicencio et al., 2011) to enhance muscle contraction speeds (Nogueira et al., 2013; Syme and Josephson, 2002). Altogether, this work paints a picture in which androgens can impact muscular trade-off relationships – particularly between speed and endurance – in various ways. First,

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androgenic effects on the muscle may underlie the formation of a speed–endurance trade-off by pushing the muscle towards a performance extreme in either of these directions (i.e. trade-off exacerbation hypothesis). Alternatively, androgens might modify the molecular composition of a muscle in a way that ameliorates the severity of the speed–endurance trade-off (e.g. trade-off mitigation hypothesis). There is little work testing these possibilities to establish how androgen action might shape adaptive performance.

We test these opposing hypotheses by studying a neotropical bird called the golden-collared manakin [*Manacus vitellinus* (Gould 1843)]. Like other bearded manakins (Aves: Pipridae, *Manacus*), males court females by jumping among saplings around a small arena on the forest floor, while rapidly snapping their wings together above their back. In a display called the roll-snap, an individual male repeatedly snaps its wings around 60 times per second (Hz) (Fusani et al., 2007a; Fuxjager et al., 2013, 2017b; Miles et al., 2018) – much faster than the wing-beat frequencies used to power flight (Donovan et al., 2013). The muscle that actuates roll-snapping behaviour, the scapulohumeralis caudalis (SH) (Fuxjager et al., 2017b), is adapted to support display performance (Fuxjager et al., 2016b). For example, the SH is notably hypertrophied (Schultz et al., 2001) and exhibits extremely fast contraction–relaxation cycling kinetics (Fuxjager et al., 2016b, 2017a; Miles et al., 2018). Androgenic hormones help increase SH twitch speeds (Fuxjager et al., 2017a) and roll-snap speeds (Fuxjager et al., 2013).

Importantly, research suggests that during the mating season (i.e. peak androgen levels), SH twitch speeds are at a level that greatly exceeds the manakin's average roll-snap speed: ∼100 Hz muscle performance versus ∼60 Hz behavioural performance (Miles et al., 2018). This discrepancy is explained by the SH's propensity to quickly fatigue when it is stimulated at high frequencies. Simply put, the muscle will fully relax after the first few high-frequency stimulations, but then as fatigue sets in and the muscle cannot fully relax between contractions, muscle fusion (tetanus) emerges as the stimulation train proceeds. Accumulation of this 'rapid fatigue' presumably means that roll-snap length (number of snaps s^{-1} for a given roll-snap event) is constrained, because the SH is unable to relax enough to generate the discrete movements necessary for subsequent wing-snaps. This implies that a faster roll-snap results in a display with fewer total snaps (i.e. a shorter roll-snap), and indeed this is the case when roll-snaps are analysed at the population-level (Miles et al., 2018). As such, male manakins perform roll-snaps at a length corresponding to the highest stimulation frequency at which the SH does not exhibit any rapid fatigue (Miles et al., 2018). This behavioural trade-off is thus rooted in a muscular trade-off between speed and endurance intrinsic to the SH.

Because androgens underlie SH performance for the roll-snap, we study the role of these hormones in mediating the muscle's speed–endurance trade-off. Addressing this issue has the potential to inform our understanding of how hormone-induced muscle plasticity shapes the evolution of an adaptive performance landscape. As described above, androgens may speed up the SH to a point at which a trade-off between speed and endurance becomes evident (e.g. trade-off exacerbation hypothesis). By contrast, androgenic regulation of the SH might ameliorate the severity of a trade-off between speed and endurance that is already present in the muscle. Therefore, androgens might extend the muscle's ability to generate power over repeated cycles (e.g. trade-off mitigation hypothesis). We test these two hypotheses by combining endocrine manipulations with *in situ* recordings of SH twitch speeds in a population of wild-caught male golden-collared manakins.

MATERIALS AND METHODS

Study site, subjects and experimental design

We conducted the study in a free-living population of golden-collared manakins during the peak of their breeding season (March–April 2016). Research was performed at the Smithsonian Research Institute (STRI) in Gamboa, Panama. Adult male golden-collared manakins (n=6) were captured via passive mist netting at active leks and quickly transported back to a nearby laboratory. Housing and husbandry are described in detail elsewhere (Fuxjager et al., 2017a). The appropriate institutional animal care and use committees (IACUCs) and governmental authorities approved the methods described below. We have previously published data collected from these birds (Fuxjager et al., 2017a), but here we use these recordings to perform a novel analysis to specifically address a separate question about androgenic effects on trade-offs between muscle speed and fatigue.

Testosterone and flutamide treatment

After a 48 h habituation period in captivity, we implanted each captive bird with a 12 mm Silastic™ capsule (0.76 mm ID, 1.65 mm OD; Dow Corning, Auburn, MI) containing 10 mm crystalline testosterone. Each end (1 mm) was capped with silicone sealant, and implants were inserted subcutaneously along the nape. Testosterone implants ensured that testosterone levels remained at stable breeding levels while in captivity. Previous studies have shown that, in captivity, circulating testosterone levels fall, which leads to a substantial decrease in the frequency of mating display performance (e.g. jump-snaps; Day et al., 2007; Fuxjager et al., 2012b). Therefore, testosterone implants were given to all individuals to avoid the potential confounding effect of low testosterone levels on muscle physiology and to best approximate the levels that are seen in non-captive, actively displaying males (e.g. Fuxjager et al., 2012b). We then randomly assigned birds to one of two different treatment groups (n=3 birds per treatment group). The first group received a second implant filled with the AR inhibitor flutamide, whereas the second group received an implant that was empty (control). Previous work verifies that Silastic™ implants of this size release flutamide at a rate of ∼100 µg day^{-1} (Soma et al., 1999), providing a flutamide dose of ∼5.5 mg kg^{-1} day^{-1}. In manakins, this amount of flutamide suppresses display behaviour without having adverse effects on health or locomotion (Fusani et al., 2007b). We gave individuals 7 days to recover from the procedure and to allow flutamide to have its full physiological effect (Fuxjager et al., 2013) before assessing muscle performance.

In situ muscle recordings

Muscle twitch speeds were recorded *in situ* using a preparation for small birds described in detail elsewhere (Fuxjager et al., 2016b, 2017a; Miles et al., 2018). Briefly, birds were anesthetized with isoflurane (2–4% in O_2). We then made a small (1 cm) incision to expose the dorsal portion of the SH muscle, and then implanted the tissue with two silver wire electrodes with stripped ends that were connected to an isolated pulse electrical stimulator (Model 2100, A-M Systems, WA, USA). We connected the SH to a force transducer (model FT03; Grass Technology, West Warwick, RI, USA) by fastening a small stainless-steel hook to the belly of the muscle via a monofilament line. The force transducer's signal was amplified (5 K to 10 K) and filtered (3 kHz) with an AC/DC strain gauge amplifier (model P122; Grass Technologies), which was relayed to a laptop computer through an A-D converter (Model NI USB-6212, National Instruments, TX, USA). We applied a drop of

saline to the exposed SH to prevent desiccation while adjusting the slack in the line between the force transducer and muscle to optimize the tension necessary for sensitive recordings.

All surgical procedures and twitch recordings took place at the outside ambient temperature (≈30°C). Once we completed the recordings, we quickly removed the electrodes and hook. We sutured the incision and sealed it using Vetbond™ tissue adhesive, and then allowed the bird to recover in the surgical suite. We later removed the implants and released the birds back into the wild in the lek from which they were originally captured.

Twitch recordings were collected in AviSoft-RECORDER (v4.2.22) by experimenters who were blind to treatment group. We measured SH twitch dynamics by assessing contractile amplitudes after electrical muscle stimulation at 50, 70, 90 and 110 Hz. Each of these stimulation frequencies was administered over 3–5 trains, with 10 pulses (1 ms pulse^{-1}) at 0.5–0.8 mA per train. Stimulation trains were always separated by a brief recovery window of roughly 15–30 s. Past work verifies that this workflow does not exhaust or damage the muscle in a way that impacts twitch recordings (Fuxjager et al., 2017a; Miles et al., 2018). Nonetheless, we confirmed this by administering a second set of 50 Hz stimulation trains following the final 110 Hz stimulation train. There were no changes in percent relaxation between the first set of 50 Hz trains and the second set (paired t_5=1.000; P=0.363).

We also examined the change in relative force (ΔRF) to determine if flutamide and/or stimulation frequency had an overall effect on force production within a given stimulation train as endurance properties change. Our previous work confirms that the amplitude of the force transducer signal corresponds to relative force output from the muscle (Fuxjager et al., 2017a). Here, we define ΔRF as ΔA_1–ΔA_2, whereby ΔA_1 is the difference between baseline force and the amplitude of twitch 1, and ΔA_2 the difference between baseline force and the amplitude of twitch 8. In theory, there are three possible outcomes of our analysis: (i) if ΔRF is significantly greater than 0, then there is an increase in relative force across a simulation train; (ii) if ΔRF is not statistically different from 0, then there is no change in relative force across a stimulation train; and (iii) if ΔRF is significantly less than 0, then there is a decrease in relative force across a stimulation train.

Data collection

Muscle recordings were extracted and manually analysed using Praat software (http://www.praat.org/) as previously described (Fuxjager et al., 2017a). Percent relaxation in response to electrical stimulation was measured for the first 8 stimulations within a given train, as this corresponds to the average roll-snap length (Fuxjager et al., 2017a; Miles et al., 2018). Percent relaxation was calculated by assessing the amplitude of the muscle contraction ($Amp_{contract}$) and relaxation (Amp_{relax}) response relative to baseline [i.e. ($Amp_{contract}$−Amp_{relax})/(Baseline−$Amp_{contract}$)]. As such, baseline corresponds to the basal tension in the taut line attached to the force transducer; contraction corresponds to the increase in tension in the line due to the muscle shortening; and relaxation corresponds to the decrease in that tension in the line due to muscle lengthening.

Importantly, we did not directly measure force production as it relates to endurance or the reduction in percentage relaxation. Although force is normally an important component of endurance (fatigue is often measured as a reduction in work in response to activity), we conceptualize endurance differently herein. This difference in concept is a function of how endurance is related to a behavioural task performed over a rapid timescale (<1 s). By these measures, endurance is best captured by a muscle's ability to fully relax so that it can repeatedly actuate discrete movements (Miles et al., 2018). Still, we have previously verified in our *in situ* preparation that the amplitude of the twitch response corresponds to relative force production by the SH (Fuxjager et al., 2017a). In general, peak force slightly increases at high frequencies, whereas percent relaxation significantly decreases ($F_{1,4}$=8.494; P=0.043; see Results). This suggests that 'rapid fatigue' is characterized through the SH's inability to generate repeated movements, independent of change in peak force production.

Statistical approach

Previous studies indicate that androgenic action induces an overall decrease in percent relaxation of the SH stimulated at 70, 90 and 110 Hz (Fuxjager et al., 2017a). We therefore tested whether this effect is due to: (i) a general decrease in percent relaxation at the beginning of the stimulation train, (ii) a change in the severity of the negative relationship between percent recovery and length of the stimulation train, or (iii) both. To test the first possibility, we performed a Welch's t-test on the percent relaxation of the first twitch between treatment groups at each stimulation frequency. P-values were adjusted for multiple comparisons using the Benjamini–Hochberg correction. To test the second possibility, we used linear mixed model to test: (i) if percent relaxation across a stimulation train results in a significantly non-zero slope for each treatment group independently (Table 1) and (ii) whether blocking AR with flutamide influenced percent relaxation across a stimulation train (i.e. if flutamide changes the slope compared with the control). All models were run at 50, 70, 90 and 110 Hz. The models testing the effect of flutamide treatment on percent relaxation across a stimulation train were specified as follows: percent relaxation as a function of treatment group (control versus flutamide), twitch number and an interaction term (Treatment×Twitch number). We included individual identity as a random factor to account for non-independence of data across the technical replicates.

We also ran a 3-way repeated-measures ANOVA to test whether treatment, stimulation frequency, and repeated stimulations within a train influence force production (ΔRF, see above). Our model included these main effects, as well as all interaction terms.

Finally, using the twitch speed analyses above, we next explored how performance trade-offs between SH speed and endurance

Table 1. Summary of contraction-relaxation cycling (percent relaxation) as a function of SH twitch number at various stimulation frequencies

	Control				Flutamide			
Stimulation frequency (Hz)	*Y*-intercept	Slope	t_{92}	*P**	*Y*-intercept	Slope	t_{92}	*P**
50	99.8±1.09	−0.09±0.21	0.44	0.66	**81.8±9.87**	**0.82±0.39**	**2.08**	**0.039**
70	69.3±7.06	0.82±1.14	1.30	0.20	49.0±3.17	−0.67±0.60	1.12	0.27
90	66.5±4.82	−0.94±1.58	1.01	0.32	39.9±9.45	0.40±0.64	0.62	0.54
110	**77.1±3.26**	**−5.57±0.64**	**8.63**	**<0.0001**	**47.3±7.62**	**−2.15±0.59**	**3.64**	**0.0005**

Y-intercept and slope are presented as estimate±s.e.m. *To test if regression slope is significantly non-zero.

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(accumulation of rapid fatigue) may manifest during stimulation trains. We used a conceptual approach outlined previously (Miles et al., 2018), in which slopes of the individual models from Fig. 1 (ΔRelaxation/ΔTwitch number) are regressed against stimulation frequency (50, 70, 90, 110 Hz). If this relationship is negative, it suggests that percent relaxation of the SH begins to decrease within a stimulation train at higher stimulation frequencies. In other words, the muscle begins to show signs of tetanic fusion as a stimulation train proceeds, pointing to the presence of a trade-off between speed and endurance (Miles et al., 2018). By contrast, if this relationship is indistinguishable from zero, then muscle recovery is unchanged during a stimulation train regardless of stimulation frequency. This latter scenario does not necessarily mean that the muscle is consistently reaching full muscle relaxation in between stimulations; rather, the muscle may be fusing uniformly across the entire stimulation train. We recognize that a linear model from this analysis can theoretically produce a positive relationship between model slopes and stimulation frequency. However, this scenario would require percent recovery to increase as a function of stimulation train length, which is biologically unlikely.

For all tests, statistical significance was set as α=0.05. All statistics were performed in R (v. 3.4.4, 'Someone to Lean On') using RStudio (v. 1.0.143) with the nlme package (https://cran.r-project.org/web/packages/nlme/index.html) and GraphPad Prism (v. 8.01). R package MuMIn (https://cran.r-project.org/web/packages/MuMIn/index.html) was used to extract the conditional R^2 values for the linear mixed models presented.

RESULTS

AR activation increases SH contractile cycling speeds

We first examined how androgenic signalling influences SH recovery dynamics within stimulation trains of different frequencies. We started with 50 Hz stimulations – a frequency slightly below the species average roll-snap speed (Miles et al., 2018). Compared with controls, flutamide significantly decreased percent relaxation of the first twitch (Fig. 1A; t_{27}=4.30, P_{adj}<0.001) and changed the recovery slope between the two groups (Table 1; $F_{1,400}$=3.866, P=0.0499).

We next compared whether androgenic signalling influenced models of SH performance at higher stimulation frequencies (70 Hz, 90 Hz, and 110 Hz), reflecting the faster contraction-relaxation cycling kinetics of this tissue when it is challenged by the nervous system to generate a faster signal. At all frequencies, flutamide treatment significantly decreased percent relaxation at the first stimulation (Fig. 1B, 70 Hz: t_{21}=4.66, P_{adj}<0.001; Fig. 1C, 90 Hz: t_{21}=4.35, P_{adj}=0.002; Fig. 1D, 110 Hz: t_{20}=2.84, P_{adj}=0.040). However, at 70 and 90 Hz, flutamide did not affect percent relaxation over a stimulation train (i.e. slope) compared with the control group (Table 1; interaction for 70 Hz model, $F_{1,184}$=2.95, P=0.088; interaction for 90 Hz model: $F_{1,184}$=1.40, P=0.239). Stimulation at 110 Hz was the only exception, in that we uncovered a slope difference between treatment groups (Table 1; $F_{1,184}$=15.22, P=0.001). At this frequency, the percent relaxation slope in control birds (β=−5.56, t_{92}=8.63, P<0.00001) is significantly steeper than in flutamide birds (β=−2.15, t_{92}=3.64, P=0.0005). This interaction means that control birds show a decrease in SH relaxation within a stimulation train of 110 Hz largely because the muscle relaxes after the first few stimulations but then quickly fuses. In birds given flutamide, the SH is slower to begin with (see Fig. 1D) and then immediately fuses at this stimulation speed.

AR and relative force production in the SH

Importantly, the rapid fatigue in control animals is independent of relative force generated during a stimulation train (Fig. 2). Based on past work in manakins (Fuxjager et al., 2016b), our *a priori* prediction was that repeated contractions would lead to a significantly positive ΔRF, suggesting an increase in force production across a stimulation train. We also predicted that this effect would not differ between treatment or frequency. Consistent with this prediction, we found a significant increase in ΔRF, regardless of treatment or frequency (Twitch: $F_{1,4}$=8.494, P=0.043; Treatment: $F_{1,4}$=0.06, P=0.82; Frequency: $F_{3,12}$=1.54, P=0.25). There were no significant second-order interactions (Frequency×Treatment×Twitch: $F_{3,12}$=0.80, P=0.52) or first-order interactions (Frequency×Treatment: $F_{3,12}$=0.18, P=0.91; Treatment×Twitch: $F_{1,4}$=0.31, P=0.61; Frequency×Twitch: $F_{3,12}$=3.12, P=0.07). Together, these data suggest that relative force increases during a given stimulation train. However, flutamide treatment or stimulation frequency does not influence relative force.

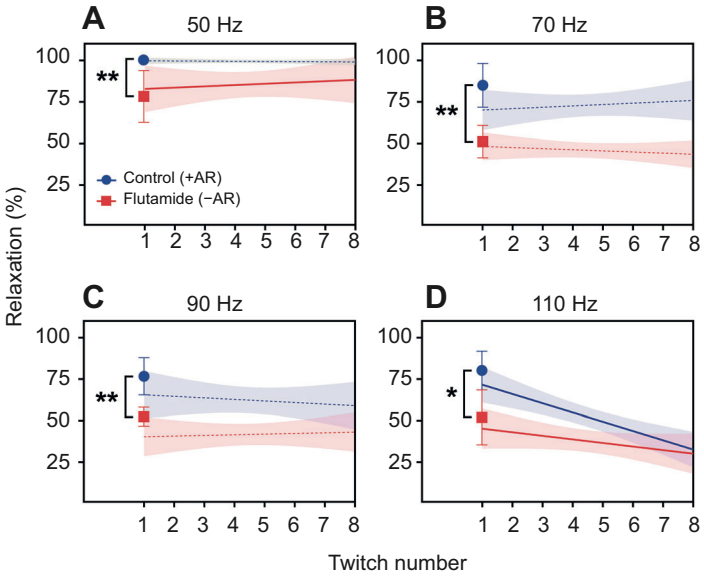

Fig. 1. Intact androgen signalling increases the SH speed. Linear regression models of percentage relaxation at stimulation frequencies of (A) 50 Hz, (B) 70 Hz, (C) 90 Hz and (D) 110 Hz in male golden-collared manakins treated with either a control capsule [Control (+AR)] or capsule containing the antiandrogen [Flutamide (−AR)]. At the highest stimulation frequency, control birds showed a more rapid decline in percentage relaxation than flutamide birds (significant interaction for Treatment×Twitch number, P=0.001). See Table 1 for full statistical summary of regression slopes. Solid lines±95% CI cloud represent a slope that significantly differs from the null model (P<0.05), while dashed lines do not meet the significance threshold. At the first twitch of each frequency, we also compared the mean±s.e.m. of each group to determine the effects of flutamide at the initiation of the stimulation train. At all stimulation frequencies there was a significant difference between groups. *P<0.05, **P<0.01.

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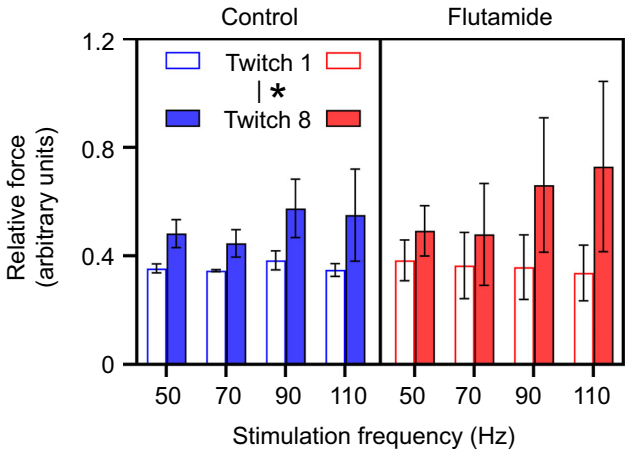

Fig. 2. Relative force of the SH increases over a given stimulation train regardless of treatment. Bar graph represents relative force production from the SH as shown through the amplitude of the first and eighth muscle twitch within a stimulation train at different frequencies and under different endocrine conditions [Control (+AR) versus Flutamide (−AR) treatment]. Data are presented as means±s.e.m.; *P<0.05, main effect of twitch (i.e. difference between relative force at the first and eighth twitch).

Androgen signalling increases speed at a cost to endurance

The aim of these analyses was to determine whether a speed–endurance trade-off occurs in the SH of control and flutamide birds, and if so, the frequency at which it arises. We therefore examined the severity of rapid fatigue accumulation (ΔRelaxation/ΔTwitch number) regressed on stimulation frequency (Fig. 3). We found that stimulation frequency negatively predicted the index of rapid fatigue in control birds (Fig. 3A, β=−0.10; t_9=3.10, P=0.015, conditional R^2=0.53). Moreover, the regression line from this model intersected the zero-slope boundary, suggesting the presence of rapid fatigue, at around 67 Hz (Fig. 3A). This is at the upper extreme of roll-snap speed in golden-collared manakins (Miles et al., 2018). On the other hand, flutamide-treated birds exhibited no relationship between stimulation frequency and the index of rapid fatigue index (Fig. 3B, β=−0.03, t_9=1.37, P=0.20, conditional R^2=0.42). SH recoveries therefore change little during a stimulation train, even at high stimulation frequencies. Instead, the SH steadily fuse as stimulation frequencies increase in the flutamide-treated animals.

DISCUSSION

Here, we explored how androgens mediate performance trade-offs between speed and endurance in golden-collared manakins. We focused on the SH, a dorsal wing muscle that actuates the bird's high-speed gestural display called the roll-snap (Fuxjager et al., 2017b). After pharmacologically blocking AR activation, we found a decrease in the SH's initial ability to contract and relax in response to stimulation trains of different frequencies. However, this same manipulation had no significant effect on the severity (slope) of the relationship between SH relaxation and length of a stimulation train, except when stimulations occurred at the highest frequency. These findings suggest that androgens act to uniformly enhance contraction–relaxation cycling kinetics of the SH; however, they have little effect on the dynamics between twitch speed and sustained performance except when stimulated at 'superfast' speeds.

With regard to force production, we found that ΔRF increases slightly during a stimulation train from the first twitch to the eighth twitch. This effect did not differ between treatment groups, which is consistent with the idea that AR has little effect on force generation within bursts of repeated activity that make up a roll-snap. Moreover, this effect did not differ among the different stimulation frequencies

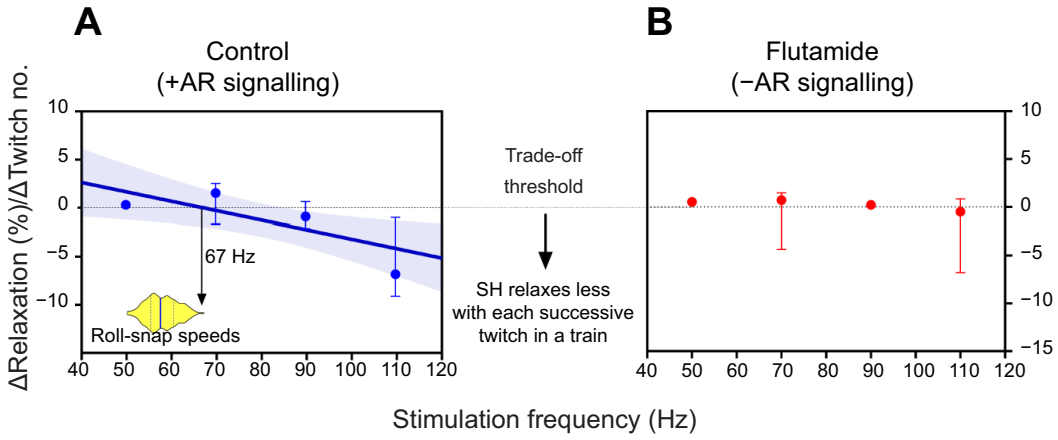

Fig. 3. Androgen signalling leads to greater percent relaxation of the SH over all stimulation frequencies but comes at a cost of endurance at the higher stimulation frequencies. Regression slopes of percent relaxation at consecutive twitches (as in Fig. 1) regressed on the different stimulation frequencies gives insight as to how the SH responds to AR signalling in terms of endurance. A slope that does not significantly differ from zero would suggest that there is no speed-endurance trade-off, as the muscle relaxation speeds would not be increasingly decreasing within a stimulation train as the muscle is stimulated at greater frequencies. However, if the regression slope is negative, this would suggest that as the stimulation frequency increases the SH experiences greater and greater rapid fatigue (a measure of endurance on a short timescale). (A) Control birds exhibited a significant linear regression slope, suggesting a performance trade-off that becomes evident at approximately 67 Hz, at which point the regression line crosses the trade-off threshold. Population variation in golden-collared manakin roll-snap speeds is represented by the yellow horizontal violin plot, whereby the solid line in the plot is the median and the dotted lines delineate the upper and lower quartile of the population. Raw roll-snap data used to construct this plot were presented in a previously published open-access paper (Miles et al., 2018), originally collected from citizen scientist recordings on the Xeno-canto online repository (www.xeno-canto.org) all under Creative Commons licenses. (B) In flutamide-treated birds, the regression slope was not significantly different from zero, suggesting that a performance trade-off is not present. However, this stems from immediate muscle fusion at the higher frequencies instead of maintaining optimal relaxation responses (see Fig. 1). In A and B, a solid line ±95% CI cloud represents a significant regression line (P<0.05), whereas no line represents a lack of a significant regression line. Superimposed dot plots are summary data presented as median±95% CI.

we administered to the muscle. Thus, the effects of 'rapid fatigue' that we quantify do not appear to be linked with a loss of force production, at least during singular brief bursts of muscle activity that are relevant to the production of roll-snap display.

We next assessed how these effects relate to the overall performance of the SH. We found that control birds showed signs of rapid fatigue, wherein the ability of the SH to fully recover within a stimulation train significantly declined as stimulation frequency increased. These findings are indicative of a trade-off between twitch speed and resistance to fatigue over short timescales (i.e. endurance). Importantly, evidence of this trade-off disappears in birds with pharmacologically blocked AR. Flutamide-treated birds instead showed increased levels of SH fusion during the entire stimulation train, implying that activation of intact AR boosts SH speed in a manner that likely induces the trade-off with fatigue. Thus, these findings support the trade-off exacerbation hypothesis, whereby androgenic action enhances the speed of this display muscle at a cost to the ability to sustain such performance. Indeed, we illustrate that this effect is likely observed through the bird's behaviour, with most roll-snap frequencies (snaps s^{-1} $event^{-1}$) occurring at the point at which the SH begins to exhibit the trade-off between speed and endurance (Miles et al., 2018).

Androgens and performance trade-offs in the SH

Many factors can constrain the available physiological space for performance modifications, with several behavioural traits requiring adaptations that shift these boundaries to modify an organism's abilities (e.g. Chapman et al., 2003). In the case of the bearded manakin, the androgenic system may serve as the evolutionary lynchpin for these effects, increasing the speed of the SH and, consequently, the bird's ability to generate a rapid roll-snap (Fuxjager et al., 2017b). Although our current study illustrates this effect at the level of the muscle, past work shows that it is also borne out behaviourally – that is, selectively blocking peripheral AR with the drug bicalutamide decreases roll-snap speeds (Fuxjager et al., 2013). Indeed, female golden-collared manakins prefer to mate with faster-displaying males, suggesting that sexual selection by female choice drives the evolution of androgenic regulation of the SH (Fuxjager et al., 2017a; Miles et al., 2018). This can occur in several ways, one of which includes an adaptive increase in the androgenic sensitivity of the SH (Fuxjager et al., 2015).

Still, our findings suggest that this endocrine adaptation has its own intrinsic limits. Androgen-mediated increases in performance hit a functional barrier (i.e. endurance) that likely constrains display duration or exacerbates the trade-off. The importance of roll-snap length to female choice is currently not known, although prior work implies that males consistently produce roll-snaps that include at least 8 snaps (Fuxjager et al., 2013; Miles et al., 2018). Thus, there may be a threshold for signal (snap) repetition or duration, below which it becomes indistinguishable from floor noise and an unattractive or ineffective display (see Mowles and Ord, 2012; Payne and Pagel, 1997 for review). If so, then current sexual selection for roll-snap speed is likely favouring the evolution of mechanisms that ameliorate the trade-off between speed and endurance. This would allow the SH to generate much faster display speeds at an appropriate length. The speed aspect of this ability is certainly already present in these birds, given that their maximum twitch times greatly exceed roll-snap performance speeds – but, individuals cannot currently sustain these high speeds for longer than two or three wing-snaps.

One facet of our dataset that we did not explore is whether the speed–endurance trade-off in the SH affects other aspects of the bird's behavioural life. We suspect that it does not, considering that there are likely few other performance-related tasks that males produce that require a >60 Hz wingbeat frequency. This likely means that the speed–endurance trade-off manifests only in the context of the roll-snap. As such, we might be looking at a behavioural phenomenon in which performance ability constrains the evolution of discrete elements that make up a singular behaviour, as opposed to different behavioural traits. However, we cannot rule out the possibility that the manifestation of this trade-off may trigger other fitness costs in some ways that are not immediately obvious. For example, even though manakin display manoeuvres are not thought to be overtly energetically expensive (Barske et al., 2011), these same moves may impose a low energetic demand that accumulates over the long term. Alternatively, engaging the muscle at the boundary of the speed–endurance trade-off throughout the breeding season and across adult life may damage the SH or other muscles that could affect other major life history traits. Such effects could impact fitness; thus, further studies are necessary to understand whether the endurance limits we describe influence other aspects of manakin life.

Muscular mechanisms of androgen-induced performance trade-offs

How do androgens increase muscle speed, and why does this effect come at a cost to endurance? Putative answers to these questions come from studies on the molecular effects of androgenic stimulation on myocytes. Androgens increase contractile speed of skeletal muscle in several ways, including increased rates of calcium release from the sarcoplasmic reticulum (Estrada et al., 2003), altered kinetics of actin–myosin crossbridge detachment (Sheffield-Moore, 2000) and modified energy allocation and/or production (Fuxjager et al., 2018; Rome et al., 1996). Although these mechanisms have not been tested in the manakin SH, past work in golden-collared manakins offers a few hints about what might be occurring. First, androgenic activation of AR increases the expression of parvalbumin in the SH (Fuxjager et al., 2012a). This protein acts as a calcium buffer, decreasing relaxation times and thus increasing twitch speeds (Muntener et al., 1995). Second, androgens increase the maximum velocity of SH contractions (V_{max}), which closely corresponds to actin–myosin detachment rates (Fuxjager et al., 2017a). Moreover, transcriptomic work indicates that androgens upregulate a variety of novel myosin isoforms that may underlie these effects (Fuxjager et al., 2016a).

The remaining question is how an androgen-mediated increase in SH speed results in a trade-off with endurance. Past work shows that the relaxation times in the manakin SH do not change over the course of a single high frequency stimulation train; however, the degree of relaxation does decrease such that rapid fatigue accumulates (Miles et al., 2018). This suggests that one factor responsible for increased fusion of the muscle is an inability to clear calcium from the myoplasm sufficiently fast. Thus, while androgens might enhance calcium buffering in the muscle (e.g. parvalbumin), the effect might not be sufficient to sustain repeated high-speed contraction–relaxation cycling. Another possibility is that other molecular elements of calcium trafficking into the sarcoplasmic reticulum, like SERCA, may be operating at their biophysical limits (Schuppe et al., 2018). If this is the case, then we predict that innovations in calcium handling are necessary to further overcome

the endurance limits of the SH muscle and allow sexual selection to drive the evolution of an even faster roll-snap.

Androgens, SH performance, and species differences in display behaviour

The findings reported herein help illustrate how hormone systems can be leveraged by evolutionary forces to support behavioural innovation. Manakin evolution is marked by strong sexual selection, with nearly all species exhibiting lek breeding systems, sexually dimorphic plumage ornamentation, and exaggerated courtship displays. Thus, changing the performance landscape to accommodate this evolution seems to involve reconfigurations of the endocrine machinery that interacts with the neuromotor system. Such effects are also documented in frogs that have evolved novel waving displays (foot flags) to communicate in noisy breeding environments (Mangiamele et al., 2016), as well as lizards that perform athletic push-up displays as a means of sexual competition (Johnson et al., 2018). These findings collectively point to androgen–muscle interactions as a common physiological conduit for the evolution of novel movement programs involved in reproduction.

Concurrently, comparative work in golden-collared manakins and one of their congeners, the white-collared manakin (*Manacus candei*), shows that the SH performance profile in the latter species looks strikingly similar to the flutamide-treated birds in our current study. In effect, white-collared manakins show no evidence of rapid fatigue in the SH when it is subjected to high frequency stimulations, but rather the muscle is unable to relax throughout the stimulation, suggesting that the muscle completely fuses (Miles et al., 2018). Behaviourally, white-collared manakins also produce a slower roll-snap than the golden-collared manakin, although roll-snap length is similar (Miles et al., 2018). Our findings are consistent with the hypothesis that androgenic modulation of the SH differentiates the roll-snap signal of golden-collared manakins from the white-collared manakin by modulating SH function. Whether androgens regulate SH performance in the white-collared manakins has yet to be tested; however, this hypothesis may help inform our understanding of how these two species diverged, particularly if the roll-snaps help facilitate assortative mating in bearded manakins. The evolution of androgenic regulation of the SH may support signal divergence in a manner that helps erect reproductive barriers that suppress gene flow between populations. One way to examine this hypothesis is to explore SH endocrine and performance phenotypes across the hybrid zone of these two species (Brumfield et al., 2001).

Conclusions

Here, we report that androgenic hormones act on the golden-collared manakin to increase contraction–relaxation cycling speeds in the SH, but in doing so bring the muscle to the edge of its performance trade-off with endurance. Overall, the results support the trade-off exacerbation hypothesis and not the trade-off mitigation hypothesis, as proposed in the Introduction. These effects can be seen in the bird's courtship display that engages this muscle – individuals appear to produce this signal as fast as they can without incurring costs to signal length that arise from fatigue (as reported elsewhere by Miles et al., 2018). These findings provide preliminary evidence for an androgen-mediated performance trade-off within a single myocytic system. However, given the small sample size herein, more studies of this nature need to be performed in manakins and other species to further support androgen-mediated performance trade-offs in isolated muscles. Nevertheless, our findings offer a framework for understanding how androgens can push physiological barriers, but in a manner that hinders other processes with potential evolutionary consequences.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: D.J.T., M.J.F.; Methodology: D.J.T., M.C.M., F.G., M.J.F.; Formal analysis: D.J.T., M.C.M., F.G., M.J.F.; Investigation: F.G., M.J.F.; Data curation: D.J.T.; Writing - original draft: D.J.T., M.J.F.; Writing - review & editing: D.J.T., M.C.M., F.G., M.J.F.; Visualization: D.J.T., M.J.F.; Supervision: M.J.F.; Funding acquisition: M.J.F.

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