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REVIEW

Insight into the neuroendocrine basis of signal evolution: a case study in foot-flagging frogs

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Abstract A hallmark of sexual selection is the evolution of elaborate male sexual signals. Yet, how the physiology of an animal changes to support a new or modified signal is a question that has remained largely unanswered. Androgens are important in regulating male reproductive behavior, therefore, selection for particular signals may drive the evolution of increased androgenic sensitivity in the neuromotor systems underlying their production. Studies of the neuroendocrine mechanisms of anuran sexual signaling provide evidence to support this idea. Here, we highlight two such cases: first, a large body of work in *Xenopus* frogs demonstrates that sexually dimorphic androgen receptor (AR) expression in the laryngeal nerves and muscles underlies sexually dimorphic vocal behavior, and second, our own work showing that the recent evolution of a hind limb signal (known as the "foot flag") in *Staurois parvus* is accompanied by a dramatic increase in androgenic sensitivity of the thigh muscles that control limb movement. Together, these examples illustrate that the evolutionary modification or gain of a sexual signal is linked with a novel pattern of AR expression in the tissues that support it. We suggest that such co-evolution of AR expression and sex-specific or species-specific signaling behavior exists across vertebrates.

Keywords Androgen receptor · Testosterone · Sexual selection · Signal evolution · Frogs

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Introduction

Sexual selection generates incredible diversity in animal signals, promoting the emergence of elaborate male displays and striking differences in courtship behavior between populations that can ultimately contribute to reproductive isolation, and thus help drive speciation (Hoskin and Higgie 2010). This diversity has led to a longstanding interest in the evolution of animal signaling systems, particularly with respect to the ways in which signal form and complexity change over evolutionary time (Wiley and Richards 1978; Ryan and Rand 1993; Endler 1993, 2015; Podos 2001; Searcy and Nowicki 2005; Bro-Jørgensen 2010). However, much of this work has neglected to investigate the physiological mechanisms that underlie signal divergence. Though previous studies have described the morphology and function of specialized courtship display structures, it remains unclear how changes in neural or neuroendocrine systems support their evolution. For example, changes to an existing signal or emergence of a completely novel one likely necessitate modification to the neuro-motor systems that underlie signal production. Do changes in hormone sensitivity in neuro-motor systems accompany the evolution of new displays? If so, do shared mechanisms support the evolution of similar courtship displays? Addressing these questions is essential to our understanding of how animal signals evolve.

We know that steroid hormones provide one avenue through which forces of selection can act to modify and elaborate male signals (Adkins-Regan 2005; Nelson 2000). In many vertebrate species, androgenic hormones, such as testosterone (T), organize the neuro-motor architecture underlying signal production and activate the expression of male signaling behavior during reproductive periods. Some well-known examples include the neuromuscular control of vocalization in frogs (Tobias et al. 1993; Zornik and

Kelley 2011), fish (Remage-Healey and Bass 2004; Bass and Remage-Healey 2008), and birds (Sartor et al. 2005; Fuxjager et al. 2014) as well as the dewlap extension in *Anolis* lizards (Holmes and Wade 2005; Holmes et al. 2007), and the gestural displays of manakin birds (Fusani et al. 2007; Schlinger et al. 2013; Fuxjager et al. 2013). Because androgens play a central role in the regulation of male gametogenesis, they are widely viewed as key integrators of an animal's reproductive physiology with its external environment (Crews and Moore 1986), producing context-appropriate behavioral phenotypes, which are highly advantageous, and thus strongly favored by selection.

To date, most of the work that investigates the relationship between male signals and the androgenic mechanisms that support their evolution has focused on levels of circulating T as the primary target of sexual selection (Wingfield et al. 1990; Hau 2007). For example, individual differences in the frequency and intensity of male sexual signaling are associated with the levels of circulating T in males of several vertebrates (reviewed in Ball 2003) and androgen levels play an important role in mediating dimorphisms in the signaling behavior of male alternative reproductive morphs (Brantley et al. 1993a, b; Lee and Bass 2005). However, selection can also act on the androgen sensitivity of target tissues to support changes in the signaling behaviors described above. Androgenic hormones readily interact with the neuro-motor system, targeting nuclei in the brain responsible for motor control, cells throughout the spinal cord, and the striated muscles that govern the control of vocalizations and physical displays (Breedlove and Arnold 1981, 1983; Jordan et al. 1991; Rand and Breedlove 1992, 1995; Brantley et al. 1993a; Wade and Buhlman 2000; Veney and Wade 2004; Remage-Healey and Bass 2004, 2006; Holmes and Wade 2005; Holmes et al. 2007; Feng et al. 2010; Fuxjager et al. 2012a, b). Levels of androgen receptor (AR) present in a tissue can influence its morphology and physiology, thus enabling similar levels of circulating T to have radically different effects on the functioning of the nervous system and effector organs that generate male signals. Selection for elaborate male courtship displays may, therefore, drive the evolution of increased expression of ARs in the neuro-motor systems underlying signal production, enabling and supporting changes in the muscles, neural circuits, or both, to generate novel motor patterns and new display elements.

In this review, we highlight our recent work on the evolution of androgenic sensitivity in the hind limb neuro-motor system in *Staurois parvus*, a frog species that uses gestural signals known as "foot flags". Compared to vocal signaling, foot flagging is relatively rare among anurans. We have demonstrated that the evolution of foot-flagging behavior in *S. parvus* males is marked by increased expression of ARs when compared to related frogs that do not foot flag, particularly in the hind limb muscles that actuate the distinctive foot movement. The independent evolution of this novel signal in multiple frog clades, primarily in species inhabiting noisy environments, provides us with a unique opportunity to explore how similar ecological conditions influence the evolution of neuroendocrine processes underlying signaling traits.

Here, we place our work in the context of what is already known about androgens and the evolution of another anuran signal, courtship vocalizations in *Xenopus* frogs. Androgenic modification of the specific nerves and muscles that control the sound-producing movements of the larynx in *Xenopus* enables males to produce rapid, trill-like vocalizations that are attractive to females. In contrast, female larynges, which cannot produce complex calls, are relatively insensitive to androgens. We argue that we can apply what we know about how androgens mediate sex-specific signaling behaviors in *Xenopus* to understanding how neuro-motor systems may change to support the evolution of an entirely new signaling modality in male *Staurois* frogs. When we do this, we find that divergent patterns of tissue-specific AR expression are associated with both the evolution of sex-specific signaling behaviors and the emergence of foot flagging, a novel sexual signal. Therefore, we hypothesize that selection for complex signaling behavior drives the evolution of androgenic sensitivity in the specific neuro-motor pathways necessary to support new motor skills.

Androgens and anuran signal evolution

The frog larynx is one of the most well-known examples of androgenic modulation of a sexually selected trait. Although in some anuran species both males and females produce vocalizations (Emerson and Boyd 1999; Boistel and Sueur 2002), in most species, male frogs display laryngeal morphology and physiology that is specialized for producing the temporally and spectrally complex vocalizations that attract females, whereas females have a reduced larynx that is usually only capable of producing simpler sounds (Kelley 1986; Tobias and Kelley 1987; McClelland and Wilczynski 1989; Tobias et al. 1991; McClelland et al. 1997; Guerra et al. 2014; but see; Preininger et al. 2016). The role of androgens in shaping the evolution of the frog larynx has been studied most extensively in the aquatic frog, *Xenopus laevis*. In this species, androgen sensitivity in the neuro-motor system controlling the larynx is sexually dimorphic, providing a mechanistic link between morphological differences in males and females and the evolution of their sex-specific signaling repertoires. For example, androgen receptor binding in the laryngeal muscle is 3–4 times higher in males during development compared to females (Segil et al. 1987; Kelley et al. 1989). This amplified androgen sensitivity in *Xenopus* males is maintained in adulthood and is specific to laryngeal muscle, which has 10–20 times more AR than other striated muscle, such as thigh muscle (Segil et al. 1987). We also see that sexual dimorphism in androgen sensitivity extends into the neural circuits responsible for the muscular contraction patterns that rapidly move the laryngeal cartilages to produce *X. laevis* males' trill vocalizations. Cell bodies in the population of motor neurons innervating the larynx have higher levels of AR in males compared to females (Perez et al. 1996), and the two primary brain nuclei that control the temporal patterning of *Xenopus* calls (n.IX–X and DTAM) are also major targets for androgens and are sexually dimorphic (reviewed in Zornik and Kelley 2011, 2017).

What are the functional effects of increased AR in tissues that support vocalization in *Xenopus* males? One effect is that activation of AR in the laryngeal muscle influences the specific growth and differentiation of male courtship structures (Sassoon and Kelley 1986; Sassoon et al. 1987). The adult male larynx has 6–7 times more muscle fibers than the female larynx (Sassoon and Kelley 1986), and male laryngeal muscles are made up of entirely fast twitch fibers, which are fatigue-resistant to enable the rapid (70 Hz) series of muscle contractions that produce trills (Sassoon et al. 1987; Tobias and Kelley 1987; Tobias et al. 1991). In contrast, laryngeal muscle fibers in females are mostly slow-twitch (Sassoon et al. 1987; Tobias et al. 1991). In addition, males have more AR-expressing motor neurons innervating the laryngeal muscles, and motor neuron soma size and dendritic extent is larger in males (Kelley 1986; Perez and Kelley 1996). AR-induced differences in motor neurons result in male and female larynges with dramatically different physiological responses that enable differences in calling behavior. A rapid train of stimulus pulses delivered via the nerve to the isolated male larynx results in a similarly rapid train of muscle contractions and 'click' sounds, while the same stimulus delivered to a female larynx results in only a single click at the beginning of the train, followed by fused muscle tension (reviewed in Zornik and Kelley 2011). Because castration reduces the rate of male trills, androgens are likely involved in maintaining the neural circuitry that enables male-specific calling patterns (Zornik and Yamaguchi 2011; Zornik and Kelley 2017), though it is not yet clear how they do so.

For some traits, sexual dimorphisms in the *Xenopus* larynx can be linked directly to downstream effects of AR activation. Androgen receptors are ligand-activated transcription factors that influence the expression of numerous other genes, including many that are related to muscle performance (Fuxjager et al. 2012a, 2016). In *X. laevis*, sex differences in laryngeal muscle fiber type are due to androgen-mediated differential expression of a larynx-specific myosin heavy chain isoform, an important contractile protein in muscle cells (Catz et al. 1992, 1995; Nasipak and Kelley 2012). Higher LM expression facilitates the male-specific developmental program of converting the larynx to an all fast-twitch muscle, via androgen-induced proliferation and differentiation of myogenic stem cells (Nasipak and Kelley 2012). Thus, both male-specific fiber number and fiber type depend on activation of the androgen receptor in laryngeal muscle.

Taken together, these findings suggest that increased androgenic sensitivity in the male neuro-motor system as a whole—including those neurons that control the specialized muscle fibers of the larynx—evolves in parallel with males' ability to produce the rapid click rate of trills that attract females to mate. In fact, androgenic modification of the central nervous system may be supportive of the changes that occur at the level of the laryngeal muscle tissue. In adult male *X. laevis*, high expression of AR in motor nuclei and spinal cord, compared to other regions of the CNS (Perez et al. 1996), may function to maintain the fast-twitch properties of the laryngeal muscle (Fischer and Kelley 1991).

How do new anuran signals evolve?

Modification of androgenic signaling systems is also likely important for the processes of signal divergence, or even the emergence of a novel signaling modality. Assessing this idea has proven difficult. Yet, one of the best ways to study the evolution of any trait is with a comparative approach, whereby species variation in signaling biology is tracked across a phylogeny (Wiens 2001). This approach is perhaps most effective when one examines the points in a phylogeny where signal structure clearly changes, or where new signals arise or disappear. Such points provide the clearest insight into the factors that support phenotypic change.

Recent comparative work in *Xenopus* accomplishes this goal nicely, highlighting how evolutionary changes in the control of laryngeal muscle contractions enable divergence in the click rate of male mating calls. In the *Xenopus* lineage, mating calls vary in complexity; they can be long trills, short bursts of clicks, or single clicks. Phylogenetic analysis suggests that the burst-type call is ancestral and the single click call (which is more similar to the calls made by *Xenopus* females) is derived, evolving independently at least twice (Tobias et al. 2011). Some of these evolutionary changes in call type appear to be due to changes in the androgen-mediated developmental trajectory of the laryngeal muscles. For example, in *X. borealis*, the evolution of single click mating calls is accompanied by the evolution of laryngeal muscles that contain mostly slow-twitch muscle fibers, which is more typical of *Xenopus* females (Leininger et al. 2015). Thus, *X. borealis* males may have lost the ancestral developmental program for dominance of fast-twitch fibers—a change that could be due to the loss of the androgen-controlled proliferation and differentiation of myoblasts in the laryngeal muscle

(Leininger and Kelley 2015). The evolution of single click calls in *X. borealis*, as well as slower trills in other *Xenopus* species (see Barkan et al. 2017), could also reflect a loss of androgen sensitivity in the brain nuclei that generate call patterns, though to our knowledge this idea has not yet been tested.

The *Xenopus* work provides insight into the physiological changes that occur when the evolution of a sexual signal requires modification of an existing neuro-motor system specialized for one particular task: vocalization. However, we can apply the same approach to understanding how motor systems are evolutionarily co-opted to support emergence of an entirely new signaling modality or sexual behavior. Of course, one possibility is to evolve a new organ dedicated specifically to the production of the selected signal; for example, this is believed to occur in birds with the evolutionary appearance of the syrinx (Clarke et al. 2016). Alternatively, existing neuro-motor systems can be modified so that they can accomplish their "normal" motor task and their "specialized" motor task associated with signal propagation. A clear example of this occurs in manakin birds, which use their wing musculature both for locomotion and for complex sexual displays (Fuxjager and Schlinger 2015). Modification of existing neuro-motor structures is a more common and more parsimonious evolutionary feat, and one way that it can occur is through the endocrine system. Thus, selection for a novel social signal—especially if it is used in reproductive contexts—may co-opt aspects of motor performance and functionality by shaping the evolution of androgen signaling mechanisms within the neuro-motor architecture already present (Fuxjager and Schlinger 2015).

Foot-flagging frogs and the neuroendocrine mechanisms that support novel signal emergence

Foot-flagging frogs are an excellent model to address the issues described above, particularly with respect to how motor mechanisms evolve to support novel signal emergence. The foot flag itself is a complex gestural signal, in which a male extends his hind limb above his head, rotates it backward in an arc to expose his white foot webbing, and then retracts the limb back to his body (Fig. 1a; Amézquita and Hödl 2004; Preininger et al. 2009, 2013; Grafe et al. 2012). The slow and deliberate movement is reminiscent of a ballerina performing an elegant oval as she moves into the 5th position. The frog's behavior, therefore, appears to be

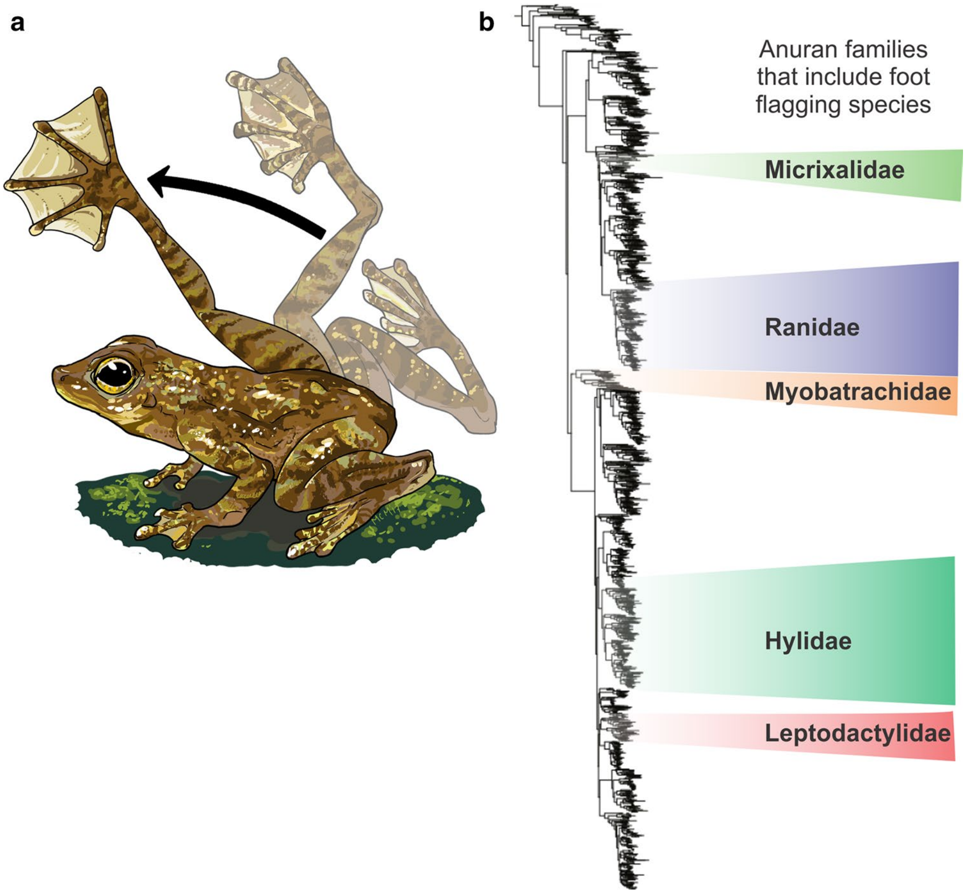


Fig. 1 **a** An individual frog, such as a male of the species *Staurois parvus*, producing a foot flag signal. The rear leg is extended above the head, rotated in an arc to expose the white foot webbing, and then retracted back to the body. Such movements are mainly controlled by muscles in the animal's thigh (Přikryl et al. 2009). **b** Phylogeny of the anurans adapted from (Pyron and Wiens 2011) on which the five families that include foot flagging species are highlighted. It is clear from this phylogeny that foot flagging behavior evolved independently only a few times (Hödl and Amézquita 2001)

defined by an ability to gracefully move an appendage in a way that is otherwise unusual for the animal.

In total, there are about 20 different anuran species that produce foot flags (Hödl and Amézquita 2001). The common link between these species is that they breed near fast flowing streams and waterfalls, where the ambient noise can easily mask efficient acoustic communication (Hödl and Amézquita 2001). Studies of foot-flagging behavior suggest that it is used to mediate male–male competition in reproductive contexts (Grafe et al. 2012). Yet, it is also possible—though largely untested—that the foot flag is used as a means of courtship. In any case, prior work collectively suggests that sexual selection by competition is a main evolutionary driver of the foot flag, particularly in contexts where novel visual signals are needed to augment social communication and advertisement in environments where the efficacy of acoustic signaling is diminished (Preininger et al. 2009; Grafe et al. 2012).

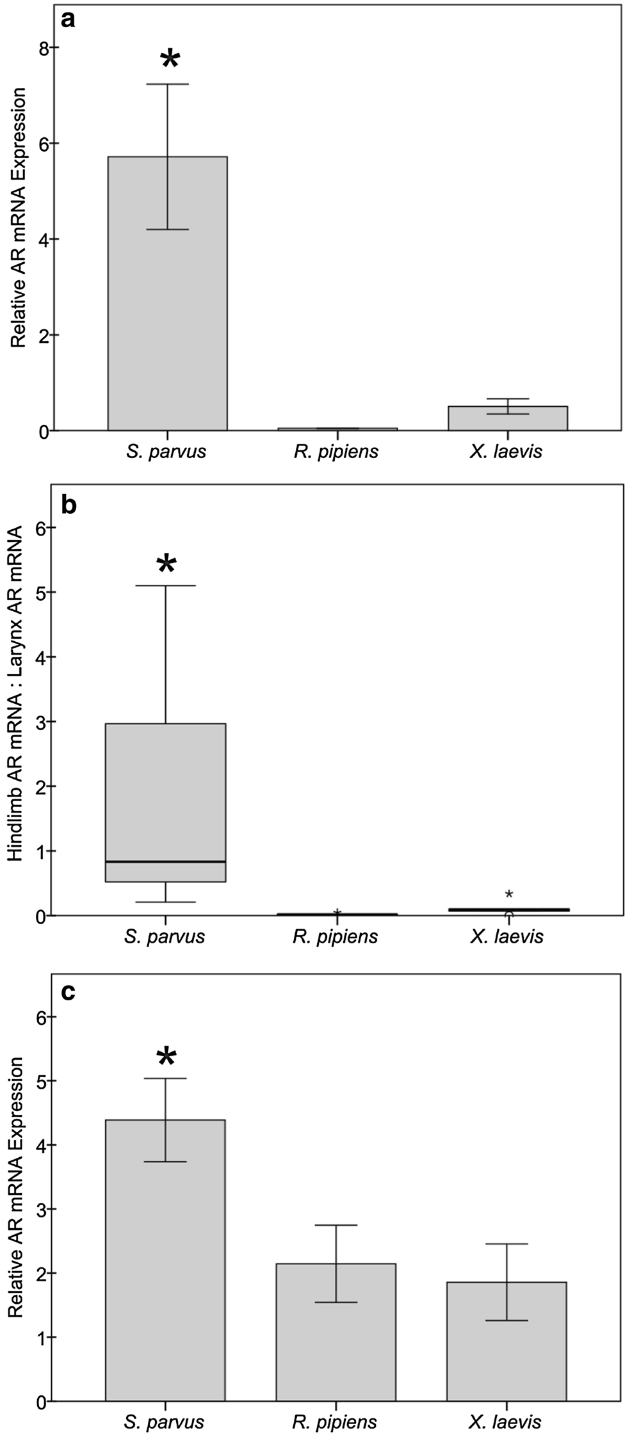
From a broader macroevolutionary perspective, foot flagging is a prime example of convergent evolution, in which the same trait independently evolved only a few different times in the anuran lineage (Fig. 1b). Moreover, foot flagging behavior is either present or absent in any given species, meaning that the sister taxa of the foot flaggers do not perform any obvious intermediate variant of the trait (Hödl and Amézquita 2001). This phylogenetic structure allows us to uncover the co-evolution of mechanisms that are linked to the ability to produce a foot flag.

We have begun to explore the link between androgenic modification of neuro-motor systems and the evolution of the foot flag in the Bornean rock frog, *Staurois parvus* (Mangiamele et al. 2016). As its name indicates, this species is found in the jungles of Borneo and, like most foot flaggers, breed around waterfalls where the abiotic noise distorts acoustic communication. We conducted our work in a semi-natural breeding facility created at the Vienna Zoo, which was designed to closely mimic the tropical rainforest habitat of Borneo (Preininger et al. 2012). The overall goal of our study was to establish whether foot-flagging behavior co-evolved with changes in the androgenic signaling systems of the frog's hind limb motor system. Thus, the first step in our experiments was to test whether androgenic steroids, such as testosterone, activated foot flagging. We found that injecting males with exogenous testosterone caused an increase in instances of foot flagging when frogs were placed in sexual contexts—that is, near a waterfall, and in the presence of a female and rival male (Mangiamele et al. 2016). At the same time, we found that this treatment neither altered locomotory behavior, nor influenced the number of calls males produced. Thus, testosterone appeared to activate foot-flagging behavior specifically, without inducing non-specific effects on the frequency of social communication behavior or other obvious changes to physical ability or performance.

Next, using the comparative approach described above, we investigated whether a species' ability to foot flag was associated with a clear change in the androgenic signaling mechanisms within the brain, spinal cord, or hind limb (thigh) skeletal muscles. We examined levels of AR mRNA expression in each of these tissues. Our results showed that foot-flagging rock frogs expressed roughly 10x more AR in their hind limb skeletal muscles—particularly those tissues that extend, rotate, and retract the foot—than the same tissues in two species of frog that do not produce foot flags (i.e., the closely related *Rana pipiens* and the more distantly related *X. laevis*) (Fig. 2a). These non-foot flagging species maintained low levels of muscle AR, which were statistically indistinguishable from each other (Fig. 2a; Mangiamele et al. 2016). This result suggests that the presence of foot flagging behavior in *S. parvus* is associated with elevated androgenic sensitivity in the skeletal musculature that controls the complex movements necessary to broadcast this signal.

Next, to establish whether co-evolution between foot flagging and AR levels was specific to the hind limb muscles, we also explored species differences in AR expression in the spinal cord and brain. Our results showed that, in the spinal cord, both *S. parvus* and its relative *R. pipiens* expressed similar amounts of AR mRNA (Mangiamele et al. 2016), and we found no differences in brain AR expression levels among the three species examined. Altogether, these results suggest that the evolutionary link between foot flagging behavior and androgenic sensitivity may be specific to skeletal muscle, as opposed to other sites in the neuromotor system. This finding notably differs from what we know about how sexual selection has driven sexual differentiation in the *Xenopus* vocal neuro-motor system, where the available evidence points to evolutionary change in the androgenic sensitivity of both laryngeal muscles and the neural circuitry that controls them as males evolved more complex calling behavior than females. It is not clear why this difference exists, however, and we should point out that other types of modifications to AR expression could occur in foot-flagging frogs. For instance, there may be specialized expression of AR in neurons or circuits dedicated to producing the display, compared to those neurons or circuits dedicated to other behavior, such as locomotion. By contrast, *Xenopus* is an aquatic frog in which the motor patterns that drive vocalization have been decoupled from those that drive respiratory movements (Zornik and Kelley 2008), so perhaps evolutionary changes in the overall androgen sensitivity of the vocal motor circuit are possible in this species because they are less likely to affect other functions. This line of questioning will become very important in future studies of gestural signaling behavior, given that our understanding of the neurobiological mechanisms that contribute to such behavior is still in its infancy.

Fig. 2 **a** Relative levels of androgen receptor (AR) mRNA expressed ▸ in the hind limb muscles of three different frog species: *Staurois parvus* (foot flagging species), and *Rana pipiens* and *Xenopus laevis* (non-foot flagging species) The asterisk (*) denotes a significant difference between groups ($p < 0.05$). **b** Ratio of AR expression in the hind limb to AR expression in the larynx. Values above 1 indicate greater expression of AR in the hind limb muscles, whereas values closer to 0 indicate greater expression of AR in the larynx muscles. Boldface line in boxplots represents average ratio value. **c** Relative AR expression among species, regardless of tissue type (hind limb muscles, brain, spinal cord, and larynx are included in the analysis). The asterisk (*) denotes a significant difference between groups ($p < 0.05$). Data are adapted from (Mangiamele et al. 2016)



For a final analysis, we attempted to further probe whether sexual selection for foot- flagging behavior drives the evolution of high AR expression in leg muscles. We calculated the ratio of AR expression within the hind limb muscle to that of the larynx. Because all species in our analysis produce vocalizations and, as discussed above, anuran larynges are functionally dependent on androgenic activation (Wetzel and Kelley 1983; Sassoon et al. 1987; Perez and Kelley 1996; Kelley 2002), we consider AR levels in the larynx a within-species index of the degree to which selection for a reproductive display influences AR levels in peripheral muscular tissue. Indeed, consistent with this idea are our results that show that AR levels in the larynx are equally high in *S. parvus, R. pipiens*, and *X, laevis*. At the same time, however, the ratio of hind limb AR to larynx AR is around 1 in *S. parvus*, but much closer to 0 in both *R. pipiens* and *X, laevis* (Fig. 2b; Mangiamele et al. 2016). In other words, AR levels in the two sets of muscle in *S. parvus* are similar, whereas AR levels are much higher in the larynx compared to the hind limb of the non-foot flagging frogs. Overall, these findings support the hypothesis that sexual selection for sexual displays drives the evolution of muscular AR in a comparable manner between the hind legs and the vocal organ.

Our studies with *S. parvus* also reveal some intriguing insight into the general evolutionary lability of androgenic signaling systems. Namely, it appears that *S. parvus* expresses more AR throughout its body, regardless of tissue, compared to other frog species that do not produce foot flags (Fig. 2c). If the primary role of increased AR in this species is to augment gestural signaling, then the observed overall difference in androgenic sensitivity among the three species might reflect constraint in the ability of non-foot flagging species to selectively adjust AR levels in a tissue-specific manner. This idea has been conceptualized previously as the "Evolutionary Constraint Hypothesis," and it posits that evolutionary change in hormone signaling mechanisms is bound by their limited flexibility. The contrasting viewpoint is conceptualized by the "Evolutionary Potential Hypothesis," which posits that different components of a given hormone signaling pathway can be modulated independently of each other (see Hau 2007 for further discussion of hypotheses). Under this hypothesis, one would predict that evolutionary pressures could increase AR in the leg muscle independently of other tissues. In the end, the answer may be somewhere in the middle: the ability to change AR expression in a tissue-specific manner is bound by forces of physiological constraint in some instance or to some degree, but not with absolution. Our data are likely consistent with this "middle ground" hypothesis, given that we see clear tissue-specific profiles of AR mRNA levels across species, as well as overall species differences in constitutive levels of AR expression. Of course, circulating hormone levels also impose a constraint on behavioral evolution. We do not yet know whether differences in circulating androgen levels correlate with display evolution, but it is possible that *S. parvus* has also evolved higher testosterone levels than non-foot flagging frogs. Further exploration of these ideas is not only warranted, but also essential to fully understanding how the evolution of hormone physiology supports processes of behavioral adaptation and diversification.

Conclusions

In summary, *Xenopus* and *Staurois* frogs provide two examples of the importance of androgenic action in the evolution of male sexual signals. In both cases, we see increased androgenic sensitivity in the tissues that control signal production in a manner that is consistent with evolutionary trends in signaling behavior. In the case of the sexually dimorphic vocalizations of *Xenopus laevis*, males have more AR expression than females in the muscles and motor neurons that control the larynx, which results in morphological and physiological divergence in the vocal organ between the two sexes. In cases in which male signals have diverged in a species' phylogeny (e.g., simplified mating calls in *Xenopus borealis*), or when a completely novel signal arises (e.g., foot flagging in *Staurois parvus*), species differences in signaling behavior are strongly associated with differences in AR expression, particularly in the muscles that control signal production. The available evidence suggests that the evolution of muscle androgen sensitivity may be a common feature of signal diversification in anurans, or at least the evolutionary changes in neuroendocrine signaling that occur in effector muscles are more similar across species (or more easily identifiable by researchers) than any accompanying neural changes. Together, these studies provide a model for understanding the physiological mechanisms that support signal evolution, whereby a new signal co-evolves with enhanced androgenic action on the particular neuro-motor structures that may serve to incorporate or elaborate the motor routines involved in generating courtship displays. We suggest that such co-evolution of AR expression and sex-specific or species-specific signaling behavior exists across vertebrates. Thus, sexual selection for dewlap extension in male *Anolis* lizard displays (see Johnson, this issue) or complex motor patterns in the male manakin wing-snap display (see Fuxjager and Schlinger, this issue) likely occurs in parallel with selection for androgenic effects on underlying neuro-motor systems.

As much as we have learned about the role of androgens in modifying sexually selected traits, many fundamental questions remain. For example, do convergent changes in AR expression underlie convergent changes in signaling behavior? We argue that *Staurois parvus* and other unrelated foot-flagging frog species are an ideal model for addressing this question. Because virtually identical foot-flagging behaviors and motor patterns have arisen independently multiple times in the anuran lineage, we can explicitly test whether selection for similar signaling behaviors in different species has resulted in similar androgenic regulation of the neuro-motor pathways that control movement of the hind leg. In addition, does male ability to perform signals correlate with AR expression in the neuro-motor system that controls signal production? If so, then perhaps individual variation in AR between males could be the physiological basis of sexual selection. Finally, what are the exact effects of AR activation on effector muscles and their innervating motor neurons that enable modification of signaling behavior? The functional effects of AR on any tissue are substantial, and thus answering this question demands a very close examination of tissue-specific regulation of gene expression and its relationship to behavioral control. One possibility is that AR modulates gene expression profiles in the muscle itself, reshaping these tissues' performance ability (Fuxjager et al. 2012a, 2013, 2016, 2017). Another possibility is that activation of muscular AR maintains and/or changes the function of spinal motor neurons that control important movement patterns, including gestural signals, via retrograde transport of neurotropic factors (Kaspar 2003; Dobrowolny et al. 2005; Verhovshek et al. 2009, 2010; Harrington and Ginty 2013). Indeed, research on motor disease illustrates that activation of muscular AR triggers the expression of such neurotropic factors, which affects the morphology and connectivity of innervating motor neurons within the spinal cord to enhance motor output (Rand and Breedlove 1995; Verhovshek et al. 2010; Verhovshek and Sengelaub 2013), often increasing physical agility and fine control of the innervated muscles (Kaspar 2003; Dobrowolny et al. 2005). Further study of these and related issues within the context of signal evolution will help us better understand not only the origin of new behavioral phenotypes but also the evolutionary lability of neuro-motor systems in general.

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