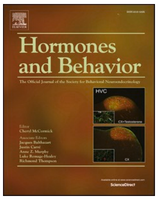
Hormones and Behavior 155 (2023) 105425

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh



Social regulation of androgenic hormones and gestural display behavior in a tropical frog

Nya Love [a], Doris Preininger [b,c], Matthew J. Fuxjager [a,*]

[a] Department of Ecology, Evolution, and Organismal Biology, Brown University, USA
[b] Department of Evolutionary Biology, University of Vienna, Austria
[c] Vienna Zoo, Vienna, Austria

ARTICLE INFO

Keywords:
Testosterone
Foot-flagging behavior
Agonistic display
Social context
Reproduction

ABSTRACT

Many animals use forms of gesture and dance to communicate with conspecifics in the breeding season, though the mechanisms of this behavior are rarely studied. Here, we investigate the hormone basis of such visual signal behavior in Bornean rocks frogs (*Staurois parvus*). Our results show that males aggregating at breeding waterfalls have higher testosterone (T) levels, and we speculate that this hormone increase is caused by social cues associated with sexual competition. To this end, we find that T levels in frogs at the waterfall positively predict the number waving gestures—or “foot flags”—that males perform while competing with rivals. By contrast, T does not predict differences in male calling behavior. In these frogs, vocal displays are used largely as an alert signal to direct a rival’s attention to the foot flag; thus, our results are consistent with the view that factors related to reproductive context drive up T levels to mediate displays most closely linked to male-male combat, which in this case is the frog’s elaborate gestural routine.

1. Introduction

Many animals court mates and compete with rivals by performing elaborate gestural displays and bizarre dance routines (Andersson, 1994). Studies that investigate such behavior often do so by looking at signal function, and by considering why such displays evolve (Beyers et al., 2010; Mowles and Ord, 2012). Far less is understood about the regulatory mechanisms that underlie gestural displays, including when they are produced and when they are not (Tobiansky and Fuxjager, 2020).

Androgenic steroids like testosterone (T) play a major role in mediating animal sexual behavior (Adkins-Regan, 2005). These hormones are largely released by the gonads into the bloodstream, where they travel around the body and act on local target tissues. Past work suggests that exogenous T can not only increase the production of sociosexual displays, but also influence how individuals produce this behavior (Alward et al., 2017; Fuxjager et al., 2013; Remage-Healey and Bass, 2006). Meanwhile, other work shows that social cues associated with reproduction (e.g., presence of receptive mates or sexual rivals) can elicit an increase in circulating T (Gleason et al., 2009; Goymann et al., 2019). Together, this research leads to the hypothesis that socially induced T surges act to mediate the production of display behaviors that help individuals attract and acquire mates. Yet, studies have rarely explored this idea, especially in the context of elaborate gestures and social dance routines. This gap is surprising given that many species use this mode of communication to navigate sociosexual interactions and that individuals appear to coordinate context-appropriate expression of this behavior.

Anurans (frogs and toads) are an excellent group of species to study this area of hormones and behavior (Emerson, 2001; Leary, 2009; Wilczynski and Chu, 2001; Wilczynski et al., 2005). For decades, researchers have shown that T plays an important role is the regulation of anuran sexual displays, both in the field and the laboratory (Moore et al., 2005). T is often elevated in males while they are actively calling (Marler and Ryan, 1996; Townsend and Moger, 1987), and it acts throughout the brain and body to increase the probability of calling behavior (Moore et al., 2005; Wilczynski et al., 2005). Of particular importance is T’s effect on select skeletal muscles, where T enhances these tissues’ ability to actuate movements that underlie reproductive behavior such as calling (Girgenrath and Marsh, 1997; Girgenrath and Marsh, 2003) and sexual clasping (Regnier and Herrera, 1993a, 1993b). It would therefore not be surprising if androgens also influenced aspects of anuran gestural signaling by acting in a similar manner (i.e., via the brain and/or muscles that govern gesture). Here, we test this idea in

---

* Corresponding author.
E-mail address: matthew_fuxjager@brown.edu (M.J. Fuxjager).

https://doi.org/10.1016/j.yhbeh.2023.105425
Received 15 June 2023; Received in revised form 30 August 2023; Accepted 31 August 2023

Staurois parvus (Bornean rock frog). This anuran species breeds year-round in streams near fast-flowing cascades or waterfalls (Grafe et al., 2012; Preininger et al., 2012). The noisy environment likely promoted the frog's elaborate "waving display" to help mediate agonistic encounters for access to mates. This visual behavior is called a foot-flag (Grafe et al., 2012), and it is produced with the hindlimbs (Fig. 1) (Anderson et al., 2021b). Field studies of *S. parvus* and other foot-flagging species show that this complex gesture functions as the primary social display among competing males, particularly at close-range (Grafe et al., 2012). While the species still calls, this particular form of display is believed to function primarily as an alert signal that directs a conspecific's attention to the subsequent foot-flagging signal (Grafe et al., 2012). Physiologically, exogenously administered T increases the probability that males foot flag (Mangiamele et al., 2016) and changes that geometry of the display to presumably appear more "dangerous" to rivals (Anderson et al., 2021a). These effects likely occur through T-dependent activation of the androgen receptor (AR), which is abundantly expressed in the frog's brain, spinal cord, and leg muscles (Mangiamele et al., 2016). In fact, if AR is blocked with the drug flutamide, then T fails to increase foot-flagging production (Smith et al., 2021). Interestingly, T does not seem to influence calling behavior in *S. parvus* (Mangiamele et al., 2016; Smith et al., 2021) like it does in several other anurans (Burmeister and Wilczynski, 2001; Penna et al., 1992; Solís and Penna, 1997; Still et al., 2019; Wada and Gorbman, 1977; Wetzel and Kelley, 1983).

With these points in mind, we hypothesize that attendance in competitive interactions at breeding sites triggers T release in adult males, which in turn increases foot-flagging behavior. We test this idea by comparing T levels in males competing at a waterfall to T levels in inactive males that have hidden themselves in nearby foliage. Using only competing males, we then test whether individual differences in T are associated with individual differences in both foot-flagging and calling behavior.

2. Methods

We studied a population of semi-wild frogs at the Vienna Zoo in Austria (>300 individuals). The original members of this population were captured from Sabah, Borneo and brought to the zoo, where they now breed prolifically. The frogs we studied inhabit a large terrarium (1.5 × 1.2 × 1.2 m) that mimics the Bornean rainforest, with small waterfalls that cascade along pristine mountain streams (relative humidity: 95–100 %; temperature: ≈25 °C, range: 22–27 °C; light-dark cycle: 12–12). All research was carried out with the appropriate permissions from the Vienna Zoo.

Fig. 1. *Staurois parvus* performing a foot-flag display.

We conducted extensive observations of male frogs throughout the enclosure between 08:00 and 16:00. This includes both actively competing individuals at the waterfall and inactive individuals in the nearby foliage (non-displaying controls). We observed each male for 10-min and recorded the number of times it performed i) foot-flags, ii) calls, iii) foot-flashes, and iv) wrestling bouts with other males. We focused our analyses on the first two behaviors, since the latter two were produced too infrequently for robust analyses.

After each observation session, we measure T in the focal frog using well described water-borne methods (Baugh and Gray-Gaillard, 2021; Rodríguez et al., 2022). This technique involves soaking an individual frog in a small sample of water, and then measuring T and its metabolites that either leach out through the skin or are excreted in urine. Assessing water-born T is common in frogs, particularly small species from which blood collection is difficult (Baugh and Gray-Gaillard, 2021). Thus, we removed each focal individual from the enclosure after its observation session, and we then soaked frogs for water collection by placing it in a small glass jar (3.8x5cm) filled with 25 ml of DI water. Frogs were always completely submerged, although we did give them a small airspace to breathe freely. We closed the jar with synthetic polyester filter foam to prevent the frog from escaping, and we kept the frog in this "bath" for 60 min. Past work suggests that soaking for this duration provides the best reflection of plasma T levels at time 0, which in our case is the moment immediately after the observation periods when we placed the focal frog in the bath (Rodríguez et al., 2022).

After this time, we removed the frog from the water, measured its weight (in g) and snout-vent length (SVL in mm), and then confirmed its relative age (adult vs. juvenile) and sex by ensuring that it had i) dark grey body coloration (as an adult would) and ii) bright-white foot webbings and light white-bluish gular area (as males would) (Stangel et al., 2015). We then placed the frog in a secondary holding terrarium until our project was completed to prevent accidental resampling.

To extract T from each water sample, we used a 20 ml sterile syringe attached to a C18 cartridge (SPE, Sep-Pak C18 Plus, 360 mg Sorbent, 55–105 μm particle size, #WAT020515, Waters corp., Milford, MA) with a flow rate of ca. 10 ml/min. We eluted cartridges with 4 ml of 96 % ethanol into an 8 ml borosilicate vial, which we stored at 4 °C. Immediately before we assayed the samples, we dried down each one with N_2 at room temperature. We then resuspended each sample with 250 μl of sample buffer from ELISA kit (see below), while incubating all samples at 4 °C overnight.

To measure T, we used a commercial enzymatic immunoassay kit (ADI-900–065; Enzo Life Sciences, USA) that is thoroughly validated to assay water-borne samples from frogs (Rodríguez et al., 2022). We began our procedure by bringing samples to room temperature and then following the kit's instructions. Samples were run in duplicate, and plates were read with a microplate reader (SpectraMax M5, Molecular Devices) at 405 nm. Assay detection limit were ≈5.6 pg/ml, and cross reactivity of the T antibody with other androgens was <15 % (see manufacturer's manual). Our average intra-assay coefficient of variation was 3.8 % ($n = 1$ plate); other validation procedures for the use of this kit are described in (Rodríguez et al., 2022).

We standardized each individual's water-borne T measurement by its body weight, controlling for variation in T due to differences in bladder and urine content (Dodd, 2010). We log transformed T levels [log(X + 1)] to better conform to assumptions of normality for parametric statistical analyses (variance were equal and residuals appeared normally distributed). We then used a two-tailed *t*-test to compare water-borne T levels from competing frogs collected at the waterfall and inactive frogs from the nearby foliage. In frogs competing at the waterfall, we used linear regression models to test whether individual differences in water-borne T predicted individual differences in the number of foot flags and calls and individual produced.

3. Results

First, we found that water-borne T levels were significantly higher in male frogs that were captured while competing at the waterfall, compared to inactive males that were captured in the foliage around the breeding area (Fig. 2A; $t_{29} = 6.02$; $p < 0.001$). Next, when we looked specifically at competing males, we found that water-borne T levels positively predicted the number of foot-flagging displays that males produce (Fig. 2B; $F_{1,13} = 5.60$, $p = 0.034$, $r^2 = 0.30$, slope $= 0.18$), but not the number of calls (Fig. 2B; $F_{1,13} = 3.31$, $p = 0.092$, $r^2 = 0.20$). As expected, inactive frogs captured in the foliage did not perform foot flags or calls.

4. Discussion

Using *S. parvus*, we show i) that social cues likely induce a positive effect on T levels, and ii) that increased T is associated with greater expression of foot-flag displays used to compete in agonistic encounters with sexual rivals. These findings therefore support the hypothesis that social context can enhance the performance of sexual gestures by triggering the release of androgenic hormones. Rarely have studies explored the hormonal basis of such display behavior, particularly in natural or semi-natural field conditions. Thus, our study is one of the few to illustrate that sex hormones play an important role in coordinating the production of complex gestural signals, which many taxa use to mediate social interactions linked to reproduction (competition, courtship, etc.).

The differences we uncover in water-borne T between males competing at the breeding waterfall and inactive frogs hiding in the foliage are quite stark, almost surprisingly so. One might expect that this difference reflects variation in reproductive condition or capacity; however, this is unlikely. All frogs in our study were adult males, and likely in full reproductive condition (see Methods). Inactive males in the foliage could easy hear (and probably observe) rivals displaying at the waterfall, and thus we suspect that the physical act of participating in male-male agonistic encounters while receiving acoustic, visual, and possibly chemical stimulation work together to elicit T release (Burmeister and Wilczynski, 2000; Chu and Wilczynski, 2001; Still et al., 2019). Male *S. parvus* may therefore stay in a somewhat constant state of reproductive condition and only experience a T surge when they choose to enter the "reproductive fray" by joining conspecific males at the waterfall. Of course, an alternate explanation is that elevated T prompts males to join the breeding site by increasing *prior* to approaching the waterfall. The cues that would elicit a T surge in this case are not abundantly clear, although we suspect that they are also likely social in nature (Burmeister and Wilczynski, 2000). Indeed, frogs that hear conspecifics calling can experience a T increase, even if these frogs are not engaging in direct competition (Burmeister and Wilczynski, 2000; Chu and Wilczynski, 2001). It is hard to imagine that cues associated with the physical environment could trigger a T surge that prompts males to join the breeding aggregation, especially in our study where frogs throughout the large home terrarium are subjected to virtually identical conditions of physical environmental (temperature, humidity, rainfall, etc.). Nonetheless, we must recognize that both situations might be at play, in that cues may initially trigger a T pulse that causes males to approach the waterfall, and then cues associated with social conflict at the breeding site further drive up T levels. Additional work is clearly warranted to disentangle the timing of the T surges that male *S. parvus* experience, the stimuli that trigger these hormone pulses, and the precise functional effect they have.

At the behavioral level, we report a positive relationship between water-borne T and foot-flagging behavior, but not calling behavior. This finding is consistent with past work in frogs showing that social cues linked to breeding can increase T levels (Burmeister and Wilczynski, 2000; de Assis et al., 2012; Mendonça et al., 1985; Rodríguez et al., 2022) and that elevated T sometimes helps increase the expression of sexual displays (Burmeister and Wilczynski, 2001; Penna et al., 1992; Solís and Penna, 1997; Still et al., 2019; Wada and Gorbman, 1977; Wetzel and Kelley, 1983). However, much of this past research focuses on calling behavior, and thus reports a T-effect on acoustic signals. In our study, socially-induced T is more likely to affect gestural communication than acoustic. We suspect that this difference is attributed to the fact that foot flagging in *S. parvus* has replaced calling as the primary social signal that mediates close-range agonistic encounters among males competing for access to mates and resources (Grafe et al., 2012). Accordingly, selection might have "swapped out" one of the major behavioral endpoints associated with socially-induced T increases, thereby favoring hormone mediation of signals that likely have the greatest chance of influencing receiver behavior. This idea is conceptually buttressed by the evolutionary potential hypothesis, which posits that the evolution of vertebrate reproductive behavior often occurs through tissue-specific changes to the independent components of hormone signaling machinery (Fuxjager and Schuppe, 2018; Hau, 2007). Elevated expression of androgen receptors, for example, often coevolves with innovations in displays behavior, particularly with respect to muscles that actuate the movements that make up these displays (Fuxjager et al., 2015; Johnson et al., 2018; Schuppe et al., 2017). Foot flagging frogs are no exception—studies show that convergence of this behavior across anurans is marked by the parallel evolution of androgen-sensitive skeletal muscles that help extend, rotate, and retract the hindlimb (Anderson et al., 2021c). Yet, while this finding reveals how selection might have tied functional effects of T action with foot flagging, we must also recognize that past work shows that the larynx of *S. parvus* also expresses substantial androgen receptor (Mangiamele et al., 2016). On its face, this would suggest that there is at least the potential for T to mediate calling behavior, which as we describe above is not something we document. One intriguing idea (which again is fully consistent with the evolutionary potential hypothesis) is that the larynx buffers effects of androgens by expressing enzymes that deactivate T, such as 5β-reductase (Fuxjager et al., 2016). Future studies are needed to further explore how such hormones systems are reconfigured to "swap"

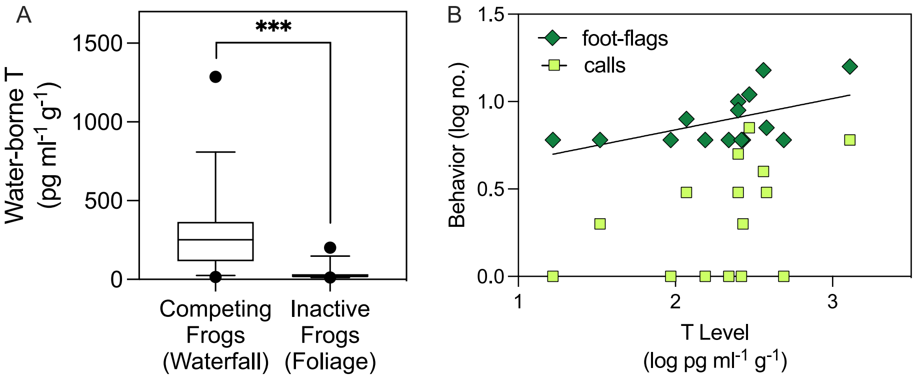


Fig. 2. (A) Boxplots of water-born T levels in males frogs competing at the waterfall and inactive males from nearby foliage (non-displaying controls). Whiskers run from 10th–90th percentiles, while the box represents the interquartile range. Statistics were run on log transformed data (see Methods), and asterisks (***) denote significant differences ($t_{29} = 6.02$; $p < 0.001$). (B) Relationships between water-borne T and behavior (foot flagging and calling). T levels only significantly predict foot-flagging behavior, as denoted by solid regression line ($F_{1,13} = 5.60$, $p = 0.034$).

around behavioral endpoints to create novel behavioral routines, including elaborate sexual displays.

T is certainly not the only hormone to likely influence foot flagging displays and other forms of reproductive behavior in *S. parvus*. Two other hormones that may be involved include vasotocin (AVT) and corticosterone (CORT), both of which influence calling in a range of anuran taxa. AVT, for instance, increases the motivation to call, while also impacting motor control of calling (Boyd, 2013; Marler et al., 1995; Semsar et al., 1998; Wilczynski et al., 2017). These effects are particularly pronounced in contexts of male-male aggression, where AVT seems to influence calling in a way that helps individuals out-compete or displace rivals (Klomberg and Marler, 2000; Semsar et al., 1998; Trainor et al., 2003). CORT, on the other hand, largely suppresses calling behavior (Leary et al., 2006; Leary et al., 2004), and it is thought that these effects are countered by those of T and/or AVT in chorusing males (Burmeister et al., 2001). We suspect that this same milieu of hormone systems—T, AVT, and CORT—has evolve to mediate foot flagging displays, just as these systems have evolved to mediate calling behavior in socially complex environments. Additional research is needed to begin assessing this idea and whether it's true, but one basic argument in support of the possibility is that the simple fact that foot flagging and calling are quite similar. In effect, both displays are little more than feats of motor control—one that regulates bizarre movements of the hindlimb, and another that regulates laryngeal and respiratory muscles. To this end, studies of the neural and endocrine basis of foot flagging promise to reveal some exciting principles about how mechanisms that underlie the production and evolution of complex sexual behavior.

In sum, our data provides compelling support for the hypothesis that social cues can regulate the production of elaborate gestural displays and dancing signals, and that such effects likely occur via androgenic hormones and their sensitivity to context. Future studies should investigate this idea further in a diversity of taxa that similarly perform seemingly bizarre sexual displays through intentional body and limb movements.

Data availability

Data will be made available on request.

Acknowledgements

We thank Nigel Anderson, Lisa Mangiamele, Vienna Zoo staff, and Rainforest House Team for logistical support, as well as Camilo Rodríguez and Virginie Canoine for guidance on hormone measurements. This work was funded by NSF (OISE-1952542 to MJF) and the Vienna Zoo.

References

Adkins-Regan, E., 2005. Hormones and Animal Social Behavior. Princeton University Press, Princeton.

Alward, B.A., Rouse, M.L., Balthazart, J., Ball, G.F., 2017. Testosterone regulates birdsong in an anatomically specific manner. Anim. Behav. 124, 291–298.

Anderson, N.K., Grabner, M., Mangiamele, L.A., Preininger, D., Fuxjager, M.J., 2021a. Testosterone amplifies the negative valence of an agonistic gestural display by exploiting receiver perceptual bias. Proc. R. Soc. B 288, 20211848.

Anderson, N.K., Gururaja, K.V., Mangiamele, L.A., Netoskie, E.C., Smith, S., Fuxjager, M.J., Preininger, D., 2021b. Insight into the evolution of anuran foot flag displays: a comparative study of color and kinematics. Ichthyol. Herpetol. 109, 1047–1059.

Anderson, N.K., Schuppe, E.R., Gururaja, K.V., Mangiamele, L.A., Cusi Martinez, J.C., Priti, H., von May, R., Preininger, D., Fuxjager, M.J., 2021c. A common endocrine signature marks the convergent evolution of an elaborate dance display in frogs. Am. Nat. 198, 522–539.

Andersson, M., 1994. Sexual Selection. Princeton University Press, Princeton.

de Assis, V.R., Navas, C.A., Mendonça, M.T., Gomes, F.R., 2012. Vocal and territorial behavior in the Smith frog (*Hypsiboas faber*): relationships with plasma levels of corticosterone and testosterone. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 163, 265–271.

Baugh, A.T., Gray-Gaillard, S.L., 2021. Excreted testosterone and male sexual proceptivity: a hormone validation and proof-of-concept experiment in túngara frogs. Gen. Comp. Endocrinol. 300, 113638.

Beyers, J., Hebets, E., Podos, J., 2010. Female mate choice based upon male motor performance. Anim. Behav. 79, 771–778.

Boyd, S.K., 2013. Vasotocin modulation of social behaviors in amphibians. In: Oxytocin, Vasopressin and Related Peptides in the Regulation of Behavior, pp. 97–109.

Burmeister, S., Wilczynski, W., 2000. Social signals influence hormones independently of calling behavior in the treefrog (*Hyla cinerea*). Horm. Behav. 38, 201–209.

Burmeister, S.S., Wilczynski, W., 2001. Social context influences androgenic effects on calling in the green treefrog (*Hyla cinerea*). Horm. Behav. 40, 550–558.

Burmeister, S., Somes, C., Wilczynski, W., 2001. Behavioral and hormonal effects of exogenous vasotocin and corticosterone in the green treefrog. Gen. Comp. Endocrinol. 122, 189–197.

Chu, J., Wilczynski, W., 2001. Social influences on androgen levels in the southern leopard frog, *Rana sphenocephala*. Gen. Comp. Endocrinol. 121, 66–73.

Dodd, C.K., 2010. Amphibian Ecology and Conservation: A Handbook of Techniques. OUP Oxford.

Emerson, S.B., 2001. Male advertisement calls: Behavioral variation and physiological processes. In: Ryan, M.J. (Ed.), Anuran Communication. Smithsonian Instituion Press, Washington DC, pp. 36–44.

Fuxjager, M.J., Schuppe, E.R., 2018. Androgenic signaling systems and their role in behavioral evolution. J. Steroid Biochem. Mol. Biol. 184, 47–56.

Fuxjager, M.J., Longpre, K.M., Chew, J.G., Fusani, L., Schlinger, B.A., 2013. Peripheral androgen receptors sustain the acrobatics and fine motor skill of elaborate male courtship. Endocrinology 154, 3168–3177.

Fuxjager, M.J., Eaton, J., Lindsay, W.R., Salwiczek, L.H., Rensel, M.A., Barske, J., Sorenson, L., Day, L.B., Schlinger, B.A., 2015. Evolutionary patterns of adaptive acrobatics and physical performance predict expression profiles of androgen receptor - but not oestrogen receptor - in the forlimb musculature. Funct. Ecol. 29, 1197–1208.

Fuxjager, M.J., Schuppe, E.R., Hoang, J., Chew, J., Shah, M., Schlinger, B.A., 2016. Expression of 5α- and 5β-reductase in spinal cord and muscle of birds with different courtship repertoires. Front. Zool. 13, 25.

Girgenrath, M., Marsh, R., 1997. In vivo performance of trunk muscles in tree frogs during calling. J. Exp. Biol. 200, 3101–3108.

Girgenrath, M., Marsh, R.L., 2003. Season and testosterone affect contractile properties of fast calling muscles in the gray tree frog Hyla chrysoscelis. Am. J. Phys. Regul. Integr. Comp. Phys. 284, R1513–R1520.

Gleason, E.D., Fuxjager, M.J., Oyegbile, T.O., Marler, C.A., 2009. Testosterone release and social context: when it occurs and why. Front. Neuroendocrinol. 30, 460–469.

Goymann, W., Moore, I.T., Oliveira, R.F., 2019. Challenge hypothesis 2.0: a fresh look at an established idea. BioScience 69, 432–442.

Grafe, T.U., Preininger, D., Sztatecsny, M., Kasah, R., Dehling, J.M., Proksch, S., Hödl, W., 2012. Multimodal communication in a noisy environment: a case study of the Bornean rock frog, *Staurois parvus*. PLoS One 7, e37965.

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

Johnson, M.A., Kircher, B.K., Castro, D.J., 2018. The evolution of androgen receptor expression and behavior in Anolis lizard forelimb muscles. J. Comp. Physiol. A Neuroethol. Sensory Neural Behav. Physiol. 204, 71–79.

Klomberg, K.F., Marler, C.A., 2000. The neuropeptide arginine vasotocin alters male call characteristics involved in social interactions in the grey treefrog, *Hyla versicolor*. Anim. Behav. 59, 807–812.

Leary, C.J., 2009. Hormones and acoustic communication in anuran amphibians. Integr. Comp. Biol. 49, 452–470.

Leary, C.J., Jessop, T.S., Garcia, A.M., Knapp, R., 2004. Steroid hormone profiles and relative body condition of calling and satellite toads: implications for proximate regulation of behavior in anurans. Behav. Ecol. 15, 313–320.

Leary, C.J., Garcia, A.M., Knapp, R., 2006. Elevated corticosterone levels elicit non-calling mating tactics in male toads independently of changes in circulating androgens. Horm. Behav. 49, 425–432.

Mangiamele, L.A., Fuxjager, M.J., Schuppe, E.R., Taylor, R., Hodl, W., Preininger, D., 2016. Increased androgenic sensitivity in the hind limb neuromuscular system marks the evolution of a derived gestural display. Proc. Natl. Acad. Sci. USA 113, 5664–5669.

Marler, C., Ryan, M., 1996. Energetic constraints and steroid hormone correlates of male calling behaviour in the túngara frog. J. Zool. 240, 397–409.

Marler, C.A., Chu, J., Wilczynski, W., 1995. Arginine vasotocin injection increases probability of calling in cricket frogs, but causes call changes characteristic of less aggressive males. Horm. Behav. 29, 554–570.

Mendonça, M., Licht, P., Ryan, M., Barnes, R., 1985. Changes in hormone levels in relation to breeding behavior in male bullfrogs (*Rana catesbeiana*) at the individual and population levels. Gen. Comp. Endocrinol. 58, 270–279.

Moore, F.L., Boyd, S.K., Kelley, D.B., 2005. Historical perspective: hormonal regulation of behaviors in amphibians. Horm. Behav. 48, 373–383.

Mowles, S.L., Ord, T.J., 2012. Repetitive signals and mate choice: insights from contest theory. Anim. Behav. 84, 295–304.

Penna, M., Capranica, R.R., Somers, J., 1992. Hormone-induced vocal behavior and midbrain auditory sensitivity in the green treefrog, *Hyla cinerea*. J. Comp. Physiol. A. 170, 73–82.

Preininger, D., Weissenbacher, A., Wampula, T., Hödl, W., 2012. The conservation breeding of two foot-flagging frog species from Borneo, *Staurois parvus* and *Staurois guttatus*. Amphibian Reptile Conserv. 5, 45–56.

Regnier, M., Herrera, A.A., 1993a. Changes in the contractile properties by androgen hormones in sexually dimorphic muscles of male frogs (*Xenopus laevis*). J. Physiol. (Cambridge) 461, 565–581.

Regnier, M., Herrera, A.A., 1993b. Differential sensitivity to androgens within a sexually dimorphic muscle of male frogs (*Xenopus laevis*). J. Neurobiol. 24, 1215–1228.

Remage-Healey, L., Bass, A.H., 2006. From social behavior to neural circuitry: steroid hormones rapidly modulate advertisement calling via a vocal pattern generator. Horm. Behav. 50, 432–441.

Rodríguez, C., Fusani, L., Raboisson, G., Hödl, W., Ringler, E., Canoine, V., 2022. Androgen responsiveness to simulated territorial intrusions in *Allobates femoralis* males: evidence supporting the challenge hypothesis in a territorial frog. Gen. Comp. Endocrinol. 326, 114046.

Schuppe, E.R., Pradhan, D.S., Thonkulpitak, K., Drilling, C., Black, M., Grober, M.S., 2017. Sex differences in neuromuscular androgen receptor expression and sociosexual behavior in a sex changing fish. PLoS One 12, e0177711.

Semsar, K., Klomberg, K.F., Marler, C.A., 1998. Arginine vasotocin increases calling-site acquisition by nonresident male grey treefrogs. Anim. Behav. 56, 983–987.

Smith, S.M., Eigerman, A.R., LeCure, K.M., Kironde, E., Privett-Mendoza, A.G., Fuxjager, M.J., Preininger, D., Mangiamele, L.A., 2021. Androgen receptor modulates multimodal displays in the Bornean rock frog (*Staurois parvus*). Integr. Comp. Biol. 61, 221–230.

Solís, R., Penna, M., 1997. Testosterone levels and evoked vocal responses in a natural population of the frog *Batrachyla taeniata*. Horm. Behav. 31, 101–109.

Stangel, J., Preininger, D., Sztatecsny, M., Hödl, W., 2015. Ontogenetic change of signal brightness in the foot-flagging frog species *Staurois parvus* and *Staurois guttatus*. Herpetologica 71, 1–7.

Still, M.B., Lea, A.M., Hofmann, H.A., Ryan, M.J., 2019. Multimodal stimuli regulate reproductive behavior and physiology in male túngara frogs. Horm. Behav. 115, 104546.

Tobiansky, D.J., Fuxjager, M.J., 2020. Sex steroids as regulators of gestural communication. Endocrinology 161, bqaa064.

Townsend, D.S., Moger, W.H., 1987. Plasma androgen levels during male parental care in a tropical frog (*Eleutherodactylus)*. Horm. Behav. 21, 93–99.

Trainor, B.C., Rouse, K.L., Marler, C.A., 2003. Arginine vasotocin interacts with the social environment to regulate advertisement calling in the gray treefrog (Hyla versicolor). Brain Behav. Evol. 61, 165–171.

Wada, M., Gorbman, A., 1977. Relation of mode of administration of testosterone to evocation of male sex behavior in frogs. Horm. Behav. 8, 310–319.

Wetzel, D.M., Kelley, D.B., 1983. Androgen and gonadotropin effects on male mate calls in South African clawed frogs, *Xenopus laevis*. Horm. Behav. 17, 388–404.

Wilczynski, W., Chu, J., 2001. Acoustic communication, endocrine control, and neurochemical systems of the brain. In: Ryan, M.J. (Ed.), Anuran Communication. Smithsonian Institution Press, Washington DC, pp. 23–35.

Wilczynski, W., Lynch, K.S., O'Bryant, E.L., 2005. Current research in amphibians: studies integrating endocrinology, behavior, and neurobiology. Horm. Behav. 48, 440–450.

Wilczynski, W., Quispe, M., Muñoz, M.I., Penna, M., 2017. Arginine vasotocin, the social neuropeptide of amphibians and reptiles. Front. Endocrinol. 8, 186.