

Hormones and acoustic communication in anuran amphibians

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Synopsis Circulating hormone levels can mediate changes in the quality of courtship signals by males and/or mate choice by females and may thus play an important role in the evolution of courtship signals. Costs associated with shifts in hormone levels of males, for example, could effectively stabilize directional selection by females on male signals. Alternatively, if hormone levels affect the selection of mates by females, then variation in hormone levels among females could contribute to the maintenance of variability in the quality of males' signals. Here, I review what is known regarding the effects of hormone levels on the quality of acoustic signals produced by males and on the choice of mates by females in anuran amphibians. Surprisingly, despite the long history of anuran amphibians as model organisms for studying acoustic communication and physiology, we know very little about how variation in circulating hormone levels contributes to variation in the vocal quality of males. Proposed relationships between androgen levels and vocal quality depicted in recent models, for example, are subject to the same criticisms raised for similar models proposed in relation to birds, namely that the evidence for graded effects of androgens on vocal performance is often weak or not rigorously tested and responses seen in one species are often not observed in other species. Although several studies offer intriguing support for graded effects of hormones on calling behavior, additional comparative studies will be required to understand these relationships. Recent studies indicate that hormones may also mediate changes in anuran females' choice of mates, suggesting that the hormone levels of females can influence the evolution of males' mating signals. No studies to date have concurrently addressed the potential complexity of hormone-behavior relationships from the perspective of sender as well as receiver, nor have any studies addressed the costs that are potentially associated with changes in circulating hormone levels in anurans (i.e., life-history tradeoffs associated with elevations in circulating androgens in males). The mechanisms involved in hormonally induced changes in signal production and selectivity also require further investigation. Anuran amphibians are, in many ways, conducive to investigating such questions.

Introduction

Hormones regulate the expression of many sexually selected traits and can thus provide important insights into the evolution of courtship signals (Wingfield and Marler 1988; Folstad and Karter 1992; Zuk 1994; Ketterson and Nolan 1994; Adkins-Regan 2005). This is exemplified by the extensive "phenotypic engineering" experiments performed by Ketterson, Nolan and colleagues in dark-eyed juncos, *Junco hyemalis* (Ketterson et al. 1992, 1996, 2001; Ketterson and Nolan 1994). Experimental elevation of androgen levels in this species increases courtship intensity and attractiveness of males (Ketterson et al. 1992, 1994; Enstrom et al. 1997; Hill et al. 1999). Males with high circulating androgen levels, however, also exhibit decreased parental care, immune responses, and survivorship (reviewed by Ketterson and Nolan 1994; Ketterson et al. 2001). Thus, although mate choice by females may exert directional selection

on hormones that mediate the expression of sexually selected traits (see Evans et al. 2006 for example of heritability of hormone levels), the costs associated with high androgen levels are expected to stabilize selection on hormone levels and correlated traits i.e., males' courtship intensity (Ketterson and Nolan 1994; Ketterson et al. 2001).

The effects of hormone levels on mate choice by females are generally not as well studied (Hutchison and Hutchison 1983; Adkins-Regan 1988; Wingfield and Marler 1988; Jennions and Petrie 1997). Hormone-related plasticity of females' mate choice could profoundly influence the evolution of males' signaling behavior (i.e., Sisneros and Bass 2003; Clement et al. 2005; Lynch et al. 2006). For example, variation in circulating hormone levels among females could effectively decrease the variance in male mating success and thus, the opportunity for sexual selection (*sensu* Heisler 1984; Andersson 1994; Jennions and Petrie 1997; Bleay and Sinervo 2007).

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Circulating hormones thus tentatively mediate dynamic relationships between senders and receivers by affecting the quality of signals by males and mate choice by females and could therefore play an important role in the evolution of sexual displays. In this review, I address the effects that hormone levels potentially have on the evolution of acoustic communication in anuran amphibians. I focus on two general areas: (1) effects of hormone levels on sexually selected components of acoustic signals and vocal performance, and (2) acoustically induced changes in the physiological state of receivers and hormonal effects on female reproductive behavior and acoustic preferences. I draw from studies on a variety of other taxa to highlight areas of research that have advanced our understanding of the neuroendocrinology of acoustic communication and to emphasize areas of research that are currently poorly understood in anuran amphibians.

Anurans as a model system

Anuran amphibians are particularly well suited for investigations related to hormonal effects on males' courtship signals. Most male anurans produce simple acoustic signals that are androgen-dependent (Moore 1983; Moore et al. 2005; Wilczynski et al. 2005) and of central importance to reproduction; vocalizations function primarily to attract mates and/or to mediate aggressive interactions among rivals, and females use acoustic signals to actively select among potential mates in choruses of males (reviewed by Andersson 1994; Gerhardt 1994; Sullivan et al. 1995; Gerhardt and Huber 2002). It is clear that particular spectral and temporal vocal characteristics are preferred by females and that some parameters of signals are under stabilizing selection (or weak directional selection) while others are under strong directional selection (Gerhardt 1991; Gerhardt and Huber 2002). Dominant frequency (DF) and pulse repetition rate (PRR), for example, are often static acoustic properties (i.e., show low levels of variation within individuals) that are subject to stabilizing selection (females generally prefer values near the mean of the population) (Gerhardt and Huber 2002). As with many other organisms, however, signal persistence (the total time spent calling) and effort (the amount of signaling per unit time) are also of central importance in mate choice (Ryan 1991; Andersson 1994; Gerhardt and Huber 2002). Vocal parameters that reflect signaling effort, such as rate and duration of calls, are often highly variable within and among individuals (i.e., dynamic) and typically under strong directional selection in anurans (Gerhardt 1991;

Gerhardt and Huber 2002). One aim of my review is to assess whether circulating hormone levels, which can fluctuate considerably among breeding male anurans (see Dupont et al. 1979; Le Boulenger et al. 1979; Licht et al. 1983; Pancak and Taylor 1983; Kühn et al. 1985; Itoh et al. 1990; Harvey et al. 1997; Romero 2002; Leary et al. 2008a, 2008b), give rise to the variation that often exists for many properties of anuran calls.

While numerous studies have determined that elevated levels of sex steroids elicit receptivity in females (reviewed by Pfaff and Schwartz-Giblin 1988; Adkins-Regan 2005; Nelson 2005), fewer studies have assessed how variation in circulating hormone levels among reproductively active females influences mate choice (Jennions and Petrie 1997; Adkins-Regan 1998). Hormonally mediated changes in mate choice by females could involve hormonal effects on motivational state and/or the ability to detect or discriminate among competing signals (Adkins-Regan 1998; Sisneros and Bass 2003). Anurans are ideal subjects for addressing these questions. Phonotactic responses of females to synthetic calls, for example, can be readily observed under controlled conditions (Gerhardt and Huber 2002) where proxies for motivational state can be measured (i.e., latencies in phonotactic responses, Lynch et al. 2005). Anurans have also served as model organisms for neurophysiological studies (Fuzessery 1988; Walkowiak 1988a, 1988b; Rose and Gooler 2007) and are thus ideal subjects in which to examine hormonal influences on sensory physiology. The second aim of this review is to examine the evidence for hormonally mediated plasticity in females' mate choice in anurans and to assess how these potential changes occur.

Hormone levels and vocalization

Overview

In addition to circulating hormone levels, selection can also act on target-tissue receptors, enzymatic pathways, and/or hormone-binding globulins to alter the expression of sexually selected traits (see reviews by Ketterson and Nolan 1999; Breuner and Orchinik 2002; Knapp 2004; Adkins-Regan 2005). However, there are currently very few, if any, data on these additional components of the endocrine system and how they relate to vocal communication in anuran amphibians. Here, I focus on potential graded relationships between circulating hormone levels and anuran calling behavior.

While it is often assumed that higher hormone levels (i.e., androgens) should promote more

attractive traits in males (e.g., increased vocal effort in anurans, Emerson 2001), hormones typically have a threshold, rather than a graded effect on target tissues. Hews and Moore (1997), Adkins-Regan (2005), and Ball and Balthazart (2008) provided thorough explanations of the problems associated with studies and with theoretical issues that revolve around presumed graded actions of hormones on courtship behavior. Nevertheless, there are several cases, mostly involving morphological attributes associated with courtship displays in birds in which graded responses to gonadal steroid levels do appear to occur (reviewed by Zuk et al. 1995; Adkins-Regan 2005; Fusani 2008; Kempenaers et al. 2008). Positive correlations have also been reported between androgen levels and vocal persistence or effort in numerous bird species (Pröve 1978; Ketterson et al. 1992; Enstrom et al. 1997; Hunt et al. 1997; Di Ridder et al. 2000; Foerster et al. 2002). I examine potential graded versus threshold effects of hormones on anuran vocalization (below) because of the importance of distinguishing between these two scenarios, especially when assessing the roles of hormone-behavior relationships in the evolution of sexually selected traits (Hews and Moore 1997; Adkins-Regan 2005).

Reviews by Moore et al. (2005) and Wilczynski et al. (2005) described the historic importance of anuran amphibians in behavioral endocrinology and the hormonal basis for sexual behavior and for production of calls. It is clear from these reviews that sex steroids, corticosterone (CORT), and arginine vasotocin (AVT) are major regulators of anuran reproductive behavior and that the endocrinology of reproductive behavior in amphibians is applicable to many other vertebrates (i.e., it is highly conserved across vertebrate taxa). I extend these previous reviews by focusing on whether variation in hormone levels potentially translates into phenotypic variation among individuals in natural populations—the raw material on which selection acts (West-Eberhard 2003). To do so requires knowledge of whether, and how, varying hormone levels affect the morphological structures and neural pathways involved in vocalization. I focus largely on sex steroids and CORT because there is very little information regarding how AVT levels fluctuate in free-living animals (for reviews see Boyd 1994a; Marler et al. 1999; Wilczynski and Chu 2001).

Androgen effects on peripheral structures

The larynx and trunk muscles are of particular importance in generating vocalization in anurans

(McAlister 1959; Schmidt 1965; Martin 1971, 1972a, 1972b; Schneider 1988; McClelland 1996; Girgenrath and Marsh 1997; Marsh 1999). Spectral characteristics of calls (i.e., dominant frequency) are predominately governed by the larynx and various temporal characteristics, including amplitude modulation (AM, responsible for PRR characteristics), can be influenced by the trunk muscles and/or the larynx (Martin 1971, 1972; Martin and Gans 1972; Girgenrath and Marsh 1997; Marsh 1999). The involvement of these structures in modulating the amplitude of vocalizations varies among anurans; in some species the amplitude of calls is actively modulated by contractions of the thoracic musculature whereas in others the amplitude of calls is passively modulated by fibrous laryngeal appendages that vibrate periodically when sufficient air pressure activates these structures (e.g., bufonids) (reviewed by Martin 1971, 1972). In some anuran species (e.g., pipids), calls are produced without respiring and the various temporal and spectral components of the calls are governed primarily by the larynx and its associated neural circuits. Hence, variation in parameters of calls could involve graded effects of hormones on one or both of these peripheral structures.

Testosterone has been shown to have a graded effect on the mass of skeletal muscle, strength of voluntary muscle and the rate of force (power) exerted by muscles in humans (Bhasin et al. 2001a, 2001b, 2005). Below, I examine the evidence that androgens exert graded effects on the musculature of peripheral structures involved in anuran vocalization and whether these effects translate into correlated changes in vocal attributes.

Larynx

Hormonal effects on anuran laryngeal morphology have been examined most extensively by Kelley and colleagues in clawed frogs, *Xenopus laevis* (Pipidae). These frogs produce “clicks” when fluid is displaced during the separation of the arytenoid discs via contraction of the laryngeal dilator muscle; production of a series of clicks (i.e., calls) is not associated with expiration or inspiration (reviewed by Kelley and Brenowitz 1992; Kelley et al. 2001). Males and females both produce clicks that differ in spectral and temporal characteristics (reviewed by Kelley and Brenowitz 1992). The larynges are sexually dimorphic and differences in calls between males and females arise, in part, from organizational effects of androgens on laryngeal morphology during a critical period of development (Sassoon and

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Kelley 1986). Males have a larger larynx (greater number and size of muscle fibers) containing fibers that are capable of contracting at high rates that reflect the click rates in the advertisement calls of males (Marin et al. 1990; Tobias et al. 1993). Most fibers in the larynx of females are only capable of contracting at slow rates that reflect the slower click rates characteristic of females' calls (Sassoon and Kelley 1986; Tobias and Kelley 1987; Tobias et al. 1993). The larynx of females can be masculinized if exposed to high androgen levels prior to six months of age; the larynx of adult females is less sensitive and not completely masculinized by androgens (Sassoon et al. 1987; Marin et al. 1990; Tobias et al. 1991a, 1991b; Potter et al. 2005). There is no reduction in the mass or change in contractile properties of the laryngeal muscle of adult males when androgen levels are low (Sassoon and Kelley 1986; Sassoon et al. 1987; Segil et al. 1987). The laryngeal morphology of males is thus permanent and largely attributable to the organizational effects of androgens (reviewed by Kelley and Brenowitz 1992; Kelley et al. 2001).

Although there is currently little evidence that the larynx is altered by acute changes in hormone levels in adult anurans, the organizational effects of androgen levels should not be overlooked. For instance, do males with higher androgen levels during the critical period develop larynges that differ from those in males with low androgen levels? If so, do these differences translate into differences among males in the quality of their calls? In humans, for example, the effects of androgens on the larynx appear to have an open-ended critical window in which organizational changes may occur. This is exemplified by adult athletes that report a long-term (permanent?) deepening of the voice (pitch) in response to androgen administration (see review by Hartgens and Kuipers 2004). As expected, the lack of exposure to androgens has the opposite effect. For example, prepubescent –17–18th century European boys were sometimes castrated to retain the high pitch of their voices (singers known as “castrati”: see Nelson 2005) illustrating the extent to which exposure to varying levels of androgens can impact the carrier frequency of the signal via organizational effects. Indirect support for similar possibilities in anurans comes from work on cricket frogs, *Acris crepitans*. In this species, there are considerable differences among populations in various characteristics of calls including dominant frequency, PRR, duration of call and number of pulses (McClelland et al. 1996, 1998). These authors found that differences in laryngeal morphology

explained much of the variation among populations in vocal attributes. For example, males from populations with lower-than-average carrier frequencies had larger larynges than did males from populations with higher-than-average frequencies (McClelland et al. 1996, 1998). Similar studies are needed to determine whether variation in hormone levels during development are responsible for these differences. To my knowledge, such studies have not been performed in any anuran species but this question warrants further investigation.

The mechanisms associated with frequency modulation (FM) of many anuran calls (e.g., the extensively studied túngara frog, *Physalaemus pustulosus*; Ryan 1985; Wilczynski et al. 1995; Ryan and Rand 2001) are not as well understood as are the mechanisms underlying AM production. Temporal changes in carrier frequency could be associated with changes in the pressure of the air passing through the larynx, in the diameter of the glottal aperture, and/or in vocal cord tension (Martin 1972; Dudley and Rand 1991). Differences in the complexity of the calls among closely related *Physalaemus* species (i.e., the addition of chucks, well known to be important in sexual selection; Ryan 1983, 1985) have been linked to morphological differences in the structure of the larynx (Drewry et al. 1982; Ryan and Drewes 1990; Boul and Ryan 2004). In particular, ablation of associated laryngeal fibrous masses in *Physalaemus pustulosus* completely abolishes the production of complex calls (Gridi-Papp et al. 2006). How hormone levels during development or in adulthood potentially influence the morphology or physiology of laryngeal structures involved in FM or complex calls is currently not known.

In many anuran species, other sexually dimorphic structures are involved in enhancing radiation of sound from the larynx. In *Rana catesbeiana*, for example, the vocal sac acts as an efficient resonator of the low-frequency spectral peak of the advertisement call while the tympanum, which is considerably larger in males, is the primary resonating structure responsible for the high frequencies characteristic of males' advertisement calls (Purgue 1997). The bimodal spectral peaks in the calls of this species correlate well with the sensitivities of the amphibian and basilar papillae, the two primary auditory sensory structures in the inner ear (Lewis and Lombard 1988), to effectively stimulate phonotaxis in females (Capranica 1966; reviewed by Fuzessery 1988; Zakon and Wilczynski 1988). Narins et al. (2001) reported that the tympanum serves a similar function in the African ranid, *Petropedetes parkeri*,

and that the sexually dimorphic fleshy tympanic protuberances in this species also vary in size among individuals and seasonally. The potential role of circulating hormone levels in mediating changes in tympanic morphology or call carrier frequencies have not been examined.

Currently, there is little direct evidence that variation in circulating sex-steroid levels among breeding males is associated with concordant morphological variation either in the larynx or in secondary structures that transmit sound from the larynx. However, there are also no studies that have tested for this relationship using varying doses of hormones. The larynx of *X. laevis*, the species that has been studied most extensively, differs from the typical anuran larynx in many respects i.e., pipids lack vocal cords (see references in Boyd et al. 1999). Comparative studies that follow the approaches of Kelley and colleagues are needed to determine whether the organizational effects of androgens on laryngeal morphology and physiology in *X. laevis* apply to other anurans i.e., whether there are acute effects of androgens in the larynx of adult males in other anuran species (see Boyd et al. 1999). In birds, for example, the syrinx can be altered throughout adulthood by circulating androgen levels; temporal changes in syrinx size of mature males corresponds to seasonal changes in androgen levels (Luine et al. 1980; Bleisch et al. 1984).

Trunk muscles

In contrast to the larynx, there are clear activational effects of androgens on the trunk muscles involved in producing vocalizations (Girgenrath and Marsh 2003). The trunk muscles of adult frogs also have androgen receptor densities that differ between males and females (Emerson et al. 1999) and sexual dimorphisms in the trunk musculature of adult frogs are well documented (Blair 1946; Marsh and Taigen 1987; Emerson et al. 1999). Girgenrath and Marsh (2003) determined that seasonal changes in androgen levels can have robust effects on the size and *in vitro* contractile properties of the oblique muscles in gray treefrogs, *Hyla chrysoscelis*. Specifically, the mass of the oblique muscles in postbreeding males (when androgen levels are low) was shown to be <50% in breeding males, while twitch duration in postbreeding males was 60% lower and the speed at which the muscles contracted was 40% lower (Girgenrath and Marsh 2003).

Such androgen-mediated activational effects on the trunk muscles could translate into differences in the calls of males with varying levels of circulating

androgens. For example, in *H. chrysoscelis* and in its allopolyploid sister species, *Hyla versicolor* (Holloway et al. 2001), contraction/relaxation cycles of the thoracic musculature (and synchronized active closing and opening of the larynx) are directly responsible for the PRR of the calls (McLister et al. 1995; Girgenrath and Marsh 1997, 1999; Marsh 1999). The various components of the calls that are important in mate choice in these species have been studied extensively and include PRR, carrier frequency, pulse shape, pulse duty cycle and the duration, amplitude and rate of calls (reviewed in Gerhardt and Huber 2002; Gerhardt 2005). It is not currently known whether the various androgen-mediated changes in muscle properties documented by Girgenrath and Marsh (2003) translate into graded changes in vocal attributes. However, the effects of androgens on muscles' contractile properties suggest that androgen levels could influence fine temporal call characteristics (i.e., pulse rise time, duty cycle, or pulse duration) or other call characteristics (i.e., call amplitude) known to be important in mate selection. To date, studies have largely neglected potential hormonal effects on fine-scale vocal parameters. Given the documented graded effects of androgens on the mass and contractile properties of skeletal muscle (Bhasin et al. 2001a, 2001b, 2005), potential graded effects of androgens on muscle physiology and vocal attributes in anurans warrant further investigation.

CORT and peripheral structures

Potential effects of CORT on the peripheral structures involved in anuran vocalization have not been studied. While high levels of CORT are involved in the mobilization of energy reserves that are supplied to muscle tissue, high CORT also probably inhibits most anabolic processes (Sapolsky 1992). High levels of CORT, for example, can inhibit androgen production (Sapolsky 1992; Wingfield and Sapolsky 2003) and have a catabolic effect on muscle tissue (reviewed by Solomon and Bouloux 2006; Schakman et al. 2008): the latter effect probably occurs only after prolonged exposure to high levels of CORT (i.e., chronic exposure). Male anurans typically exhibit dramatic elevations in levels of CORT during the breeding period (reviewed by Romero 2002) and also undergo changes in muscle morphology and physiology (reviewed by Wells 2001). Whether these changes are mediated via CORT-androgen interactions, direct effects of CORT, or both, requires further study.

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Effects of sex steroids on central vocal-motor pathways

Circulating hormone levels could also affect various sexually selected vocal attributes by acting centrally on vocal motor pathways (i.e., Schmidt 1980, 1983, 1984; Kelley 2002; Remage-Healey and Bass 2006a, 2006b). Hormonally induced changes in neuronal motor activity, however, are not necessarily required to change various vocal parameters important in mate selection. As previously discussed, some characteristics of calls could be altered by endocrinal effects on the contractile properties of peripheral muscle. Similarly, it is possible that changes in the activity of the CNS are not reflected in the output of the peripheral structures. For example, when the laryngeal nerve innervating the laryngeal dilator muscle is stimulated in female *X. laevis* at rates that reflect click rates in males, the dilator muscles of females are not capable of contracting and relaxing at the high rates characteristic of males, indicating that contractile properties of the laryngeal musculature constrains females' click rates (Tobias and Kelley 1987). Further analysis of this problem, however, indicates that sex-specific temporal patterning (i.e., click rates of males and females) originates in higher brain centers (Yamaguchi and Kelley 2000).

Current understanding of central control of vocalization in anurans stems largely from Schmidt's neurophysiological investigations in leopard frogs, *Rana pipiens*. Schmidt (1973 1974a, 1974b, 1976, 1992) described two 'semi-independent' vocal central pattern generators (CPG's); the first inclusive of the pretrigeminal nucleus and the other consisting of motor nucleus IX-X. Further studies have largely confirmed the involvement of these regions in vocalization (Wetzel et al. 1985; Kogo and Remmers 1994; Rhodes et al. 2007; Zornik and Kelley 2007). Many of the regions of the brain involved in vocal motor control have been found to concentrate androgens or androgen metabolites, including nuclei that form the CPG (i.e., pretrigeminal nucleus/dorsal tegmental area of the medulla, the reticularis inferior, and motor nucleus IX-X) as well as nuclei that provide input to the CPG and potentially modulate its activity i.e., the thalamus, the preoptic area of the hypothalamus, and the ventral striatum (Kelley 1980; Wetzel et al. 1985; Kelley and Brenowitz 1992). The thalamus, ventral striatum, and pretrigeminal nuclei also receive auditory input (Neary 1988; Wilczynski 1988) and concentrate steroid hormones (Kelley 1980), suggesting that auditory input to these regions could alter

patterns of activity in the CPG and be modulated by hormones (Aitken and Capranica 1984).

While sex steroids appear to play a central role in activating the various nuclei involved in vocalization (Schmidt 1980, 1983), little is currently known regarding the contribution of hormone levels to activity patterns of these central regions (reviewed by Kelley 2002; Bass and Remage-Healey 2008). The importance of steroid hormone levels in affecting CPG output is illustrated by the extensive research by Bass and colleagues on vocalizing batrachoidid fish (reviewed by Bass and Remage-Healey 2008). In the gulf toadfish (*Opsanus beta*), for example, males produce calls that are analogous in function to the advertisement and aggressive calls of many anurans, and temporal patterns of the calls (particularly duration of call) are affected by circulating androgen levels (reviewed in Bass and Remage-Healey 2008). Remage-Healey and Bass (2004, 2006a) isolated the vocal CPG using *in vivo* preparations to show that there are rapid effects of androgens on fictive call duration. They further demonstrated that androgenic effects on descending input to the vocal CPG can also modify CPG activity (reviewed in Remage-Healey and Bass 2006b). Potential dose-dependent effects of androgen levels have not been examined.

These findings are particularly noteworthy if applicable to anuran amphibians. First, temporal patterns of calling activity that are potentially modified by CPG's, including duration and rate of calls, and pulse-repetition rate (Bass and Remage-Healey 2008), are all known to be important in mate choice in anurans (Gerhardt and Huber 2002) with corresponding neural representations for behavioral preferences (Fuzessery 1988; Walkowiak 1988a, 1988b; Rose and Gooler 2007). Duration of call, in particular, is highly variable and under strong directional selection in anurans (Gerhardt and Huber 2002). Bass and Remage-Healey (2008) emphasized that anurans provide a promising system in which to investigate the potential effects of steroid hormones on CPG activity. Recent techniques that record fictive calling patterns in isolated brain preparations (Rhodes et al. 2007) will undoubtedly elucidate many currently unanswered questions regarding central control of vocalization in anurans. For example, are pattern generators influenced by hormone levels to produce changes in temporal properties of calls? How is CPG activity modulated to mediate changes in PRR and other call characteristics that may change considerably during transitions between advertisement and territorial calls (i.e., pacific treefrogs, *Hyla regilla*, Brenowitz et al. 2001)? Are the rhythmic

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properties of CPG's modified by hormones intrin-
sically or via descending input to these regions, or
both?

Effects of corticosterone on central vocal-motor pathways

Hormonal effects on CPG activity have generally
focused on gonadal steroids. However, glucocorticoid
receptors are also widely distributed in the anuran
brain and include many key regions important in
vocal production and/or motivational state including
the nucleus accumbens, preoptic area, striatum,
tegmental nuclei, reticular nucleus, and pretrigeminal
motor nerves (Yao et al. 2008). The potential effects
of CORT on these areas may be particularly impor-
tant in mediating vocalization given that high levels
of CORT often decrease calling activity in anurans
(Marler and Ryan 1996; Burmeister et al. 2001; Leary
et al. 2004, 2006a, 2006b). CORT levels are inversely
correlated with the duration of vocalizations in the
Great Plains toad (*Bufo cognatus*) and the effects of
CORT occur independently of changes in circulating
androgen levels (Leary et al. 2004, 2006a). It is
currently not known, however, whether CORT affects
calling behavior in anurans by acting centrally or
peripherally (for investigation of similar problem in
newts, see Lewis and Rose 2003).

The extensive work on rough skinned newts,
Taricha granulosa, by Moore and colleagues
illustrates the effects that CORT can have on the
CNS and reproductive behavior. CORT acts rapidly
on medullary motor neurons involved in amplexus
through nongenomic effects on membrane-bound
receptors (Orchinik et al. 1991; Moore and Evans
1999) and alters behavior in a graded fashion;
higher doses elicit a greater decrease in clasping
quality (Moore and Miller 1984; Lewis and Rose
2003). CORT appears to alter the secretion of AVT
from motor neurons to mediate changes in amplexic
behavior (reviewed in Moore and Evans 1999; Evans
et al. 2000). This mechanism is particularly intri-
guing because many neurons in the anuran vocal
motor pathway exhibit high AVT immunoreactivity

(Boyd 1997) and AVT administration is well-known
to affect vocal behavior in anurans (Boyd 1994b,
2006; Marler et al. 1995; Chu et al. 1998; Trainor
et al. 2003). If CORT alters the secretion of AVT
in the vocal motor pathway of anurans via similar
mechanisms, we may expect that AVT immuno-
reactivity would be higher in males with high levels
of CORT. Such studies have not been performed in
anurans. However, Marler et al. (1999) did find that
AVT immunoreactivity (primarily in the nucleus
accumbens) was higher in nonvocalizing cricket
frogs, *A. crepitans*. Whether CORT is involved in
mediating changes in behavior in *A. crepitans*,
however, is not currently known, nor is it clear
whether these differences represent increased AVT
secretion (i.e., lower AVT-immunoreactivity) in call-
ing males or whether secretion of AVT is inhibited
in noncalling males.

The various steroid-hormone relationships that
have been reported for calling and noncalling male
anurans (Table 1) suggest that there are several
potential mechanisms by which steroid hormones
could interact with AVT to alter calling behavior
(Leary et al. 2004). For example, in *P. pustulosus*
and the green treefrog, *Hyla cinerea*, experimental
elevation of CORT inhibits calling behavior but
also causes a decrease in circulating androgen levels
(Marler and Ryan 1996; Burmeister et al. 2001).
Similarly, in bullfrogs, *R. catesbeiana*, calling males
have higher circulating CORT levels and lower
androgen levels than do noncalling males, suggesting
that a decrease in androgens could also mediate
changes in calling behavior (Mendonça et al. 1985).
Hence, high CORT may alter calling behavior in
some anurans by influencing androgenic effects on
the neural circuitry underlying calling behavior.
Gonadal steroids are required for the synthesis
of AVT in vocal-control regions (Boyd 1994a); one
possibility, therefore, is that decreased levels of
androgen results in decreased AVT synthesis and
calling behavior. In contrast, CORT manipulations
in Woodhouse's toad, *Bufo woodhousii*, and the
Great Plains toad, *B. cognatus*, indicate that CORT

Table 1 Relative levels of circulating androgens and corticosterone reported for calling and non-calling male anurans

Species	Androgen level	Corticosterone level	Noncalling behavior	Citation
<i>Rana catesbeiana</i>	Lower in callers	Higher in callers	Satellite males	Mendonça et al. (1985)
<i>Eleutherodactylus coqui</i>	Higher in callers	Not measured	Males exhibiting parental care	Townsend and Moger (1987)
<i>Physalaemus pustulosus</i>	Higher in callers	No difference	Foraging males	Marler and Ryan (1996)
<i>Bufo cognatus</i>	No difference	Higher in callers	Satellite males	Leary et al. (2004)
<i>Bufo woodhousii</i>	No difference	Higher in callers	Satellite males	Leary et al. (2004)

injections do not negatively affect circulating androgen levels in transitions from calling to noncalling behavior (Leary et al. 2004). Another possibility, therefore, is that high CORT levels inhibit calling behavior by affecting the secretion of AVT via the mechanisms described for newts.

Selective pressures associated with differences in the temporal periods of breeding activity may give rise to these differences (Leary et al. 2004, 2006a). For example, in explosively breeding species (i.e., *B. woodhousii* and *B. cognatus*), reciprocal interactions between CORT and androgens are likely to have strong negative-fitness consequences because such effects may remove those individuals from choruses for a significant proportion of the breeding period (concepts of the “Emergency life-history stage” (Wingfield et al. 1998). Clearly, further comparative work is needed to determine the hormonal mechanisms involved in mediating changes in anuran calling behavior. Aside from dose-dependent effects on calling behavior in general i.e., calling versus noncalling behavior (Marler and Ryan 1996; Burmeister et al. 2001), potential graded effects of CORT on vocal parameters have not been examined.

Acoustic stimulation of the neuroendocrine axis

The stimulatory effects of males’ mating signals on the neuroendocrinology of females have been recognized for some time (reviewed by Wingfield and Marler 1988). In particular, Marshall (1936) formally articulated the idea that some courtship displays of males more effectively stimulate the reproductive neuroendocrine axis of females to elicit receptivity and synchronize reproduction, thereby driving the evolution of males’ sexual signals. Acoustic modulation of the HPG axis may be an evolutionarily conserved characteristic and critical to coordinating mating behavior in many vertebrates (Remage-Healey and Bass 2004). Stimulatory effects of conspecific, but not heterospecific, calls on testicular development and/or androgen levels has been documented in grass frogs, *Rana temporaria* (Brzoska and Obert 1980), leopard frogs, *Rana sphenoccephala* (Chu and Wilczynski 2001), and green treefrogs, *H. cinerea* (Burmeister and Wilczynski 2000, 2005). Males’ advertisement calls have also been found to stimulate receptivity and ovarian development (Rabb 1973; Lea et al. 2001) and estradiol production (Lynch and Wilczynski 2006) in female anurans. Hence, evidence suggests that the anuran HPG axis is stimulated by conspecific social signals, thereby priming the reproductive axis for sexual

readiness and coordination of reproductive activity (Wilczynski et al. 1993; Wilczynski and Chu 2001).

Acoustic stimuli are likely to modulate the anuran reproductive neuroendocrine axis via ascending input to the hypothalamus from the thalamus (that receives input from the superior olive, superficial reticular nucleus, and torus semicircularis) (Neary 1988; Wilczynski 1988; Allison and Wilczynski 1991; Wilczynski et al. 1993). Indeed, hypothalamic neurons have been shown to change their activity in response to conspecific mating calls in anurans (Wilczynski and Allison 1989; Allison 1992). However, the salient acoustic features that are selectivity filtered and relayed to the hypothalamus from the thalamus are largely unstudied (Neary 1988). Selectivity for temporal features of species-specific calls is thought to be particularly enhanced in the thalamus (Hall and Feng 1986; Endepols et al. 2003). Single unit extracellular recordings, for example, suggest that the central thalamic nucleus exhibits heightened selectivity for AM rate and pulse duration; the posterior thalamic nucleus appears to be primarily involved in spectral processing (Hall and Feng 1986, 1987). The potential effects of hormone levels on selective filtering of acoustic information transmitted to the hypothalamus have not been examined. The effects of circulating hormone levels on neural selectivity are discussed below.

Hormonal effects on peripheral and central auditory pathways

Males’ displays not only influence females’ receptivity and motivational state via changes in hormone levels but changes in hormone levels can also alter the tuning properties of neurons in the sensory pathway. For example, Sisneros et al. (2004) determined that the phase-locking characteristics (i.e., temporal coding of carrier frequency) of saccular afferent neurons in plainfin midshipman, *Porichthys notatus* (the sacculus is the primary auditory end organ in these animals), improved when nonreproductive females were treated with testosterone or estradiol; these changes reflected seasonal changes in hormone levels and auditory tuning to species-specific calls (Sisneros and Bass 2003).

Several studies on anurans have indicated that circulating hormone levels affect the tuning properties of auditory neurons (Table 2). The most commonly reported hormonal effect includes an increase in the number of neurons that are responsive to auditory stimuli during the breeding period (when gonadal steroids are presumably higher) or

Table 2 Reported hormonal effects on auditory neurons in anuran amphibians

Species	Auditory region examined	Reported major changes	Comparison	Citation
<i>Bombina bombina</i>	Torus semicircularis	Number of neurons responsive to auditory stimuli	Seasonal	Walkowiak (1980)
<i>Rana pipiens</i>	Ant. preoptic region	Number of neurons responsive to auditory stimuli	Pituitary homogenate injections	Urano and Gorbman (1981)
<i>Rana pipiens</i>	Torus semicircularis	Number of neurons responsive to auditory stimuli and spectral tuning characteristics	Intraventricular estradiol injections	Yovanof and Feng (1983)
<i>Rana pipiens</i>	Pretrigeminal nucleus	Number of neurons responsive to auditory stimuli and with complex tuning curves	Seasonal and gonadotropin injections	Aitken and Capranica (1984)
<i>Hyla chrysoscelis</i>	Torus semicircularis	Auditory thresholds and spectral tuning characteristics	Seasonal	Hillery (1980)
<i>Hyla cinerea</i>	Torus semicircularis	None	Seasonal and androgen injections	Penna et al. (1992)
<i>Rana pipiens</i>	Torus semicircularis	Spectral and temporal tuning characteristics	Seasonal	Goense and Feng (2005)

after administration of gonadotropins or gonadal steroids (Table 2). However, several investigators have also reported additional changes in the response properties of neurons during the breeding period or after hormone treatment including decreased auditory thresholds, increased number of cells with complex tuning properties (i.e., V-shaped versus W-shaped tuning curves), and/or changes in spectral and temporal tuning properties (Table 2). In general, these studies suggest that hormonally mediated changes potentially alter the response properties of neurons in the auditory pathway and thereby increase sensitivity for species-typical characteristics of calls during the breeding season. However, other studies have reported that there were no hormonal (androgen) and/or seasonal effects on sensitivity or thresholds of auditory frequency (Penna et al. 1992; Table 2). Clearly, further studies are needed to address these reported discrepancies. In particular, studies that examine the responses of single neurons before, and after, administration of hormones are needed. Recent implementation of intracellular recording techniques (Edwards et al. 2007; Leary et al. 2008) provide promising avenues for future neuroendocrinal studies aimed at examining the effects of hormones on auditory selectivity and ascertaining the mechanisms involved.

While various tuning properties of anuran auditory neurons appear to be influenced by hormone levels, it remains unclear how the tuning properties of auditory neurons relate to potential variation in females' preferences or the extent to which hormone levels fluctuate within individuals or differ among individuals during mate selection

(see D'Istria et al. 1974; Licht et al. 1983; Lynch and Wilczynski 2005 and citations within, for seasonal variation in hormone levels in female anurans). These are important considerations, however, because variation in neural tuning properties that are related to variation in circulated hormone levels among breeding females would suggest a proximate basis for plasticity in females' preferences; variability in the signal quality of males could thus be maintained if hormones affect the preferences of receivers. Such studies should provide insights into the variation in females' choices inherent in many models of the evolution in mate choice by females and maintenance of variation in signal quality of males (Andersson 1994). Concerns regarding natural variation in hormone levels are also clearly applicable to signal senders. In the following section, I examine the results from studies of whole-animal performance to address these problems.

Integrating hormonal effects and whole-animal performance

Senders

How do hormonally mediated effects on peripheral structures and/or CNS translate into measures of whole-animal performance in senders? One particularly intriguing model proposed by Emerson (2001), the "Energetics Hormone-Vocalization (EHV) model, posits that temporal changes in vocal effort will be related to circulating steroid hormone levels." The EHV model is rooted in the high energetic costs associated with calling behavior in anurans (reviewed by Wells 2001) and reciprocal interactions between

CORT and androgen levels (Greenberg and Wingfield 1987). Essentially, the energetic demands associated with calling behavior were predicted to drive temporal elevations in circulating CORT levels. Androgen levels were expected to exhibit concordant temporal fluctuations that are mediated by interactions among calling conspecifics i.e., based on the Challenge Hypothesis, (Wingfield et al. 1990) and/or through self vocal-stimulation (Cheng 1992). Temporal changes in circulating hormone levels were predicted to parallel vocal effort; high vocal effort was expected to require more energy and, thus, stimulate elevations in circulating CORT (i.e., elevations in CORT were expected to be a consequence of increased vocal effort) while varying androgen levels were expected to initiate changes in relative vocal effort (i.e., cause changes in vocal effort). Emerson (2001) predicted that CORT levels eventually reach some threshold level that inhibits androgen production and, therefore, vocalization. The model thus predicts a graded effect of androgens on vocal effort and provides a conceptual framework in which to examine the influence of circulating hormone levels on whole-animal performance.

It is currently unclear how the EHV model applies to steroid hormone profiles reported for calling and noncalling male anurans (Table 1). For example, because the model depicts temporal changes in hormone levels and vocal effort as a bell-shaped curve with calling represented on the left of the curve (when androgens and CORT are increasing) and noncalling behavior on the right (when androgens and CORT are declining), there should be no net difference in CORT or androgen levels for calling and noncalling individuals. Available data, however, demonstrate all possible scenarios for androgen levels in calling and noncalling male anurans (Table 1). Mendonça et al. (1985), for example, reported that male *R. catesbeiana* that are actively calling have lower circulating androgen levels than do noncalling males. Townsend and Moger (1987) and Marler and Ryan (1996), on the other hand, report the opposite pattern in *Eleutherodactylus coqui* and *P. pustulosus*, respectively. Yet, in *B. woodhousii* and *B. cognatus* there were no differences in circulating androgen levels for callers and non-callers (Leary et al. 2004; Table 1). Although many of these examples are often cited to illustrate the potential complexity of hormone-vocal relationships in anurans, comparisons should be interpreted with caution because they represent very different categories of noncalling behavior among the species examined (Table 1). For example, Mendonça et al. (1985) and Leary et al. (2004) compared androgen levels in calling and noncalling males

exhibiting alternative mating tactics, Townsend and Moger (1987) compared calling males to noncalling males exhibiting parental care, and Marler and Ryan (1996) compared calling males to noncalling individuals that were foraging away from the chorus (Table 1). Consideration of these behavioral differences is important because other factors could be involved in modulating hormone levels i.e., parental care is expected to be associated with decreased androgen levels (Wingfield et al. 1990). Clearly, however, calling males in natural choruses do not necessarily have higher androgen levels.

Studies that have examined CORT levels as well as androgen levels for callers and noncallers provide further insights into the potential hormonal mechanisms involved in mediating calling behavior (Table 1). For example, Mendonça et al. (1985) reported that CORT levels were higher for calling *R. catesbeiana*, suggesting that calling behavior drives elevations in CORT levels that negatively impact circulating androgen levels (as predicted by Emerson 2001). Marler and Ryan (1996) indicated that there were no differences in circulating CORT levels for calling and noncalling *P. pustulosus* but that the administration of CORT resulted in a reduction in circulating androgen levels characteristic of noncalling males. CORT levels were higher in calling *B. woodhousii* and *B. cognatus*, but in contrast to *R. catesbeiana* and *P. pustulosus*, there was no evidence that high CORT suppressed production of androgens in noncallers of either species (Leary et al. 2004). Results from administration of CORT were consistent with interpretations derived from hormone profiles in *B. woodhousii* and *B. cognatus*; elevated CORT elicited noncalling behavior independently of changes in levels of circulating androgens (Leary et al. 2004, 2006a). Such studies of hormone manipulation are particularly important because mechanistic interpretations derived from hormone profiles alone may be misleading. For example, hormone levels are not expected to be the sole determinate of vocal behavior; social interactions are well-known to influence calling behavior in anurans (reviewed in Gerhardt and Huber 2002). Male *R. catesbeiana*, for example, aggressively defend calling sites and larger males may thus prevent smaller noncalling males from calling (Howard 1978, 1984), regardless of hormonal state (studies on manipulation of hormones have not been performed in *R. catesbeiana*). Examination of natural steroid hormone levels, however, are clearly essential for determining the levels of hormones required in manipulation studies (i.e., to assure that pharmacological doses are not administered).

Other studies provide insights into the various facets of the EHV model. For example, several investigators have addressed the effects of androgen administration on calling behavior under laboratory conditions that controlled for social effects on vocal behavior. These studies provide some support for androgen-mediated effects on vocal effort. Wetzel and Kelly (1983), for example, found that administration of androgens increased the amount of time spent calling (relative to controls) and there was evidence for a dose-dependent effect of dihydrotestosterone (administered in conjunction with human chorionic gonadotropin) in *X. laevis*. Solís and Penna (1997), however, examined calling responses of androgen-manipulated frogs evoked by vocal playbacks. They found that although administration of testosterone did not cause any detectable change in vocal characteristics *per se* (i.e., number of pulses per call, call duration, PRR), it did affect the average number of calls elicited from male *Batrachyla taeniata* (Leptodactylidae) that were subject to the vocal playback stimuli. The authors suggested that elevated androgen levels increase the motivation to call although there were no differences in the latency of evoked responses. Burmeister et al. (2001), however, reported an inverse relationship between androgen level and latency to call subsequent to hormone manipulation in animals housed adjacent to natural choruses. Numerous other studies have experimentally manipulated circulating hormone levels in male anurans, but most studies were designed to examine androgen-mediated effects on reproductive behavior in general and, therefore, do not specifically examine the potential effects of androgen on vocal effort or specific characteristics of calls (see Schmidt 1966; Wada et al. 1976; Kelley and Pfaff 1978 and citations within).

A second approach has been to examine correlations between the vocal characteristics and circulating hormone levels of males engaged in calling activity in natural choruses. While this approach does not control for social factors that potentially influence reproductive behavior, it has the advantage of addressing whether variation in hormone levels is important in the context of behaving animals in their natural environment (see McClintock 1987). Few studies have examined relationships between androgen levels and the characteristics of males' calls in natural anuran choruses and the results that were obtained are contradictory. Solís (1994, cited by Solís and Penna 1997), for example, reported a positive correlation between androgen levels and the number of pulses in the calls (call duration) of chorusing *Pleurodema thaul*

(Leptodactylidae), suggesting a graded effect of androgens on vocal effort. In contrast, circulating androgen level was not an accurate predictor of vocal effort in chorusing male *B. woodhousii* (Leary et al. 2008a).

While most studies on anurans have focused on the relationship between androgen levels and vocal parameters, recent studies emphasize the importance of CORT. In *B. cognatus*, for example, males practicing alternative noncalling mating tactics appear to optimize their reproductive success by exhibiting acoustic preferences that parallel females' preferences (Leary et al. 2006b). Satellite males of this species associate with calling males producing the longest calls and females also preferred longer calls characteristic of males with associated satellites (Leary et al. 2006b). Males with, and without, associated satellites in natural choruses, however, did not differ with respect to circulating androgen levels. Rather, calling males producing the longest calls (with associated satellite males) had significantly lower CORT levels than did nearby males producing shorter calls (without associated satellites) (Leary et al. 2006b). CORT levels of chorusing males were negatively correlated with duration of call in this species and experimental elevation in CORT caused a decrease in call duration. Female anurans generally prefer mating signals that reflect greater total effort (Gerhardt and Huber 2002) and these preferences are likely to drive elevations in the circulating CORT levels of signalers (Emerson 2001; Emerson and Hess 2005; Leary et al. 2004, 2008a). Overall, these results show that while high CORT levels appear to be required to promote the mobilization of energy reserves required for increased vocal effort, in at least some species, elevations in CORT may negatively affect the quality of the signal (Leary et al. 2006b; see Husak and Moore 2008 for review of CORT effects on sexually selected traits).

Receivers

Several recent studies suggest that hormone levels in females can alter the choice of potential mates via effects on motivational state or discriminatory abilities. One of the most thoroughly studied examples involves mate selection by female túngara frogs, *P. pustulosus*. Lynch et al. (2005) determined that female receptivity (phonotaxis to conspecific calls) and permissiveness (phonotaxis to less attractive alternative calls) changed with different stages of the reproductive cycle (see also Lea et al. 2000; Bosch and Boyero 2004). Specifically, non-amplectic females (i.e., females that have not yet

selected mates) and postmated females exhibited lower receptivity and less permissiveness than did amplexic females (i.e., females that were in amplexus and subsequently removed from males for experiments on phonotaxis). Changes in receptivity and permissiveness were not, however, associated with changes in discriminatory abilities; amplexic females retained the ability to discriminate between attractive and less attractive calls (but see Lea et al. 2000 for discriminatory changes associated with reproductive stage in midwife toads, *Alytes muletensis*). Females were thus more likely to accept less attractive calls as the time to oviposition approached, yet they remain capable of discriminating among calls (Lynch et al. 2005). Temporal changes in receptivity and permissiveness were shown to be causally related to changes in circulating gonadal hormones (induced by injections of HCG) and generally increased with higher doses before leveling off at the highest dosages (Lynch et al. 2006). These investigators also showed that the experimental manipulations of hormone levels and the effects on the behaviors of females parallel those found under natural conditions (Lynch and Wilczynski 2005). These results suggest that local environmental conditions that potentially modulate hormone levels and/or delay reproduction could affect the choice of mates by females and contribute to maintaining variability in the quality of males' signals.

Conclusions

Despite considerable evidence that androgens are responsible for mediating vocal production in anuran amphibians (reviewed by Moore 1983; Moore et al. 2005; Wilczynski et al. 2005), there are substantial problems with drawing any general conclusions regarding graded effects of androgens on the relative attractiveness of males' acoustic signals. In many ways, problems associated with current models relating hormones and vocalization in anuran amphibians (i.e., the EHV model, Emerson 2001) parallel those of the immunocompetence handicap hypothesis (ICHH, Folstad and Karter 1992). The ICHH, while intended to be broadly applicable, has received most attention from researchers investigating hormonal mediation of sexually selected traits (including vocalization) in birds (reviewed by Roberts et al. 2004). Currently, however, one major criticism of the ICHH model is the lack of evidence for graded effects of hormones on sexually selected traits (Hews and Moore 1997; Roberts et al. 2004). Evidence for graded effects of androgens on vocal quality in anurans is even more

limited and the positive relationships reported for one species are not necessarily reflected in other species. Grounds on which to dismiss current models are equally problematic simply because few studies have explicitly tested for such relationships.

A central concept of the ICHH involves expected tradeoffs (i.e., decreased immune function) associated with elevations in levels of circulating androgens. This is particularly important in assessing how hormone levels potentially influence the evolution of androgen-mediated traits. The "phenotypic engineering" experiments performed on dark-eyed juncos (see Introduction) and other species (see review by Hau 1997) illustrate the insights that are often gained by such studies. Unfortunately, no such studies have been carried out on anuran amphibians (Hau 1997). Despite the general paucity of documented dose-dependent effects of hormones on vocal attractiveness in male anurans, a few studies do offer intriguing justification for further pursuing this topic. The available evidence suggests that hormone levels, when they do affect the quality of acoustic signals, are predominately affecting dynamic properties of calls that are typically under strong directional selection via female choice. Further investigations are likely to reveal interesting tradeoffs associated with changes in hormone levels and vocalization and may thus provide important insights into the evolution of acoustic signals in anurans.

An additional interesting issue stemming from the ICHH involves the potential modulatory role that nutritional state has on levels of circulating androgens and the expression of elaborate male traits; androgen-mediated effects on sexually selected traits could be reliable signals of relative nutritional status rather than of immune function (Pérez-Rodríguez et al. 2006). This may be a particularly important aspect to consider when choosing candidate anuran species in which to examine potential androgen-mediated effects on vocal quality because in some species relative nutritional state influences signal quality (see citations in Solís and Penna 1997). Importantly, supplemental feeding also elevates androgen levels in *P. pustulosus* (Marler and Ryan 1996) and body condition is positively correlated with androgen level in *B. woodhousii* (Leary et al. 2008a), suggesting that the effects of nutritional state on vocal quality documented in several anurans could be mediated via androgens.

Two findings emphasized in the current review can and, I hope, will direct future research. First, while research has focused largely on circulating androgens, recent studies suggest that stress hormones can also play a central role in mediating

vocal attractiveness. Second, the common assumption that females' choice of mates is largely static needs to be reconsidered; a role of hormones in mediating plasticity in females' choice of mates provides exciting avenues for future investigation. Studies that explicitly address hormone-behavior relationships in anurans from the perspective of senders as well as receivers are clearly needed to better apply theoretical models and understand the broader implications of hormonal influences on signal evolution. Further studies should include experimental manipulation of hormone levels and such experiments should be specifically designed to investigate dose-dependent hormonal effects. Preferably, future studies will also address experimental findings in the context of natural hormone levels and variability in signaling and choice of mates under natural field conditions. Such investigations are likely to be rewarding and initiate further integrative studies.

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