

## Elevated corticosterone levels elicit non-calling mating tactics in male toads independently of changes in circulating androgens

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### Abstract

Competition among males for a limited number of females may result in the expression of condition-dependent alternative mating tactics. In such cases, decision rules mediating mating tactic expression are likely to be influenced by a male's external as well as internal environment. For example, experimental studies with anuran amphibians (frogs and toads) indicate that changes in the social-acoustic environment alter the probability that an individual adopts a calling versus non-calling "satellite" mating tactic. However, there is considerable variation in the behavioral responses of individuals in such studies, suggesting that physiological differences among individuals play an important role in tactic expression. For instance, recent models predict that natural elevation in adrenal glucocorticoids during vocal production alters androgen production and/or neural activity to mediate transitions between reproductive tactic expression in anuran amphibians. Using corticosteroid injections, we show that elevation in circulating corticosterone levels significantly increase the probability that free-ranging male Great Plains toads (*Bufo cognatus*) and Woodhouse's toads (*B. woodhousii*) adopt a non-calling satellite tactic. Corticosterone-induced behavioral transitions occurred rapidly (<1 h) and independently of fluctuations in circulating androgen levels, suggesting a direct effect of glucocorticoids on brain centers controlling vocal production. We discuss our findings in the context of behavioral studies that alter the social-acoustic environment to examine its influence on tactic expression.

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### Introduction

Condition-dependent variation in male reproductive behavior often involves complex interactions between the organism's environment and internal physiological state (Andersson, 1994; Shuster and Wade, 2003; West-Eberhard, 2003). Hence, an understanding of individual variation in sexual behavior often requires analysis of the environmental factors that influence behavioral expression as well as the physiological parameters that alter the probability of a given behavioral response under some set of environmental conditions (Andersson, 1994; Crews and Moore, 1986; Drickamer, 1998; Drickamer and Gillie, 1998; Halliday, 1987, 1992; Sinervo and Svensson, 1998). Anuran amphibians (frogs and toads) are one taxonomic group in which a considerable

number of investigations have focused on altering the social environment to examine its influence on the expression of alternative male mating behaviors. However, less is known regarding the role that the animal's physiological state plays in the adoption of such tactics. Here, we focus on the effects of elevated glucocorticoid levels on male mating tactic expression in Great Plains toads (*Bufo cognatus*) and Woodhouse's toads (*B. woodhousii*). Elevation in circulating adrenal glucocorticoids is associated with reproductive activity for most anurans examined thus far, as well as numerous other vertebrate species (Love et al., 2004; Romero, 2002), and is a key component of recent models regarding calling behavior and reproductive tactic expression for this group (Emerson, 2001; Leary et al., 2004).

In many anuran species, males exhibit alternative "satellite" tactics wherein non-calling individuals reside in close proximity to calling conspecific males and attempt to intercept incoming females attracted to the vocalizations of the calling host

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(Halliday and Tejedo, 1995; Waltz, 1982). Individuals can alternate between the calling and non-calling satellite tactic within and between nights of chorus activity, suggesting that satellite behavior in anurans is a condition-dependent mating tactic (sensu Gross, 1986). One common approach to studying satellite behavior in anurans has been to alter the social-acoustic environment to examine its influence on behavioral expression. The working hypothesis of these studies is that information encoded within the vocalizations of conspecific males (i.e., relative attractiveness and/or social status) is used by receiver males in making mating tactic decisions. For example, Arak (1988) predicted that males should adopt satellite behavior when the mean sound pressure intensity (i.e., amplitude) of chorusing conspecifics is at least twice as great as the subject's call intensity because the probability of obtaining a mate would be greater if an "unattractive" male adopted a satellite tactic. Similarly, Waltz (1982) applied the "law of diminishing returns" to predict patterns of satellite behavior wherein mate quality and attractiveness drive behavioral expression (see also review by Luttbeg, 2004).

Currently, such models are partially supported by vocal playback experiments and satellite/caller manipulations. For example, in playback experiments of varying amplitude, 56% of 16 calling male natterjack toads (*Bufo calamita*) adopted satellite behavior when presented with a high amplitude conspecific vocal stimulus, whereas 25% of 16 calling males became satellites when subjected to a low amplitude stimulus (Arak, 1988). Conversely, satellite *B. calamita* adopted calling behavior in 20% of 10 satellite-host manipulations where the caller was removed from the vicinity of the satellite male (Arak, 1988). In the green treefrog (*Hyla cinerea*), 18% of 17 satellite males exhibited calling behavior following cessation of vocal production by the host (Perrill et al., 1978). However, in playback experiments, 63% of 8 calling male *H. cinerea* adopted satellite tactics following exposure to conspecific advertisement calls (Perrill et al., 1982), but only 13% of 24 male cricket frogs (*Acris cepitans*) adopted satellite tactics using similar experimental procedures (Perrill and Magier, 1988). Comparably variable results were obtained for *Hyla versicolor* (Fellers, 1979), *Uperoleia rugosa* (Robertson, 1986a,b), *Acris crepitans* (Wagner, 1992), *Phyllautus variabilis* (Kadadevaru and Kanamadi, 2001), and *Rana rugosa* (Park and Cheong, 2002). The responses of individuals in such studies vary considerably (across species as well as within species), suggesting that differences in physiology play an important role in observed differences in behavioral responses among individuals exposed to similar social and/or environmental conditions.

Reproductive behavior and vocal communication in anurans are known to be controlled by two chemical systems, including gonadal steroids and the neuropeptide arginine vasotocin, AVT (Marler et al., 1999; Wilczynski and Chu, 2001). Currently, few studies have documented natural variation in these systems among individual anurans practicing alternative mating tactics (i.e., Leary et al., 2004; Marler et al., 1999; Mendonça et al., 1985). For example, Mendonça et al. (1985) found that calling male bullfrogs (*Rana catesbeiana*) had significantly lower

circulating levels of androgens and significantly higher levels of circulating corticosterone than satellite males. A negative interaction between the two steroids was predicted to account for transitions between calling and satellite behavior in this species.

The potential effects of corticosteroid administration on reproductive tactic expression have not been examined in bullfrogs, but glucocorticoid administration and/or natural elevations in glucocorticoids has been found to be inversely correlated with circulating androgen levels in several other anuran species (see Burmeister et al., 2001; Licht et al., 1983; Marler and Ryan, 1996; Orchinik et al., 1982) as well as numerous reptiles, mammals, and birds (e.g., Greenberg and Crews, 1990; Greenberg and Wingfield, 1987; Knapp and Moore, 1995, 1996; Schuett et al., 1996; Wingfield and Ramenofsky, 1999). Such a relationship was combined with concepts of the "Challenge Hypothesis" (Wingfield et al., 1990) by Emerson (2001) to explain transitions between calling and non-calling activity in anuran amphibians. Emerson's (2001) "Energetic-Hormone Vocalization Model" predicted a positive relationship between androgen levels and vocal effort until the energetic demands associated with vocal production increase glucocorticoids to threshold levels that inhibit androgen production. At this stage, the individual stops vocalizing until circulating glucocorticoids are metabolized and energy reserves are restored. Subsequent to recovery from this stress response, an individual resumes calling behavior and continues this cyclic pattern throughout the breeding period.

We have proposed that elevated glucocorticoids may alter reproductive tactic expression in explosive-breeding male toads independently of fluctuations in androgen levels by altering activity of AVT-producing neurons in vocal control centers of the brain (Leary et al., 2004). Our proposed model stemmed from the lack of evidence for an inverse relationship between corticosterone and androgens in satellite and calling *B. woodhousii* and *B. cognatus* (Leary et al., 2004) and glucocorticoid–AVT relationships in the roughskin newt (*Taricha granulosa*). AVT mediates courtship behavior in *T. granulosa* and behavioral expression can be rapidly altered (within seconds to minutes) by exogenous application of corticosterone (Moore and Miller, 1984; Rose et al., 1993, 1998). Corticosterone appears to inhibit reproductive behavior in the roughskin newt by altering the secretion of AVT from neurons via a non-genomic mechanism (Moore and Evans, 1999; Orchinik et al., 1991).

In the present study, we examined tactic expression of chorusing male *B. cognatus* and *B. woodhousii* subsequent to corticosterone and saline treatment. We examined whether corticosterone administration increases the probability that free-ranging males adopt a non-calling satellite tactic under similar environmental and social circumstances. In addition, we examined whether the potential effects of corticosterone on mating tactic expression are mediated through negative effects on circulating androgen levels (i.e., Emerson, 2001) or independently of changes in circulating androgens (i.e., Leary et al., 2004).

## Materials and methods

### General information

This study used calling male *B. cognatus* and *B. woodhousii* in natural choruses in central Oklahoma (Cleveland, Grady, and Canadian counties). Male *B. cognatus* data come from three choruses comprised of 13–30 individuals and *B. woodhousii* data come from two choruses that consisted of 65–150 individuals. All procedures took place during peak periods of reproductive activity (~midnight) in June 2003 and 2004 following heavy rains. These species are explosive breeders and often complete all annual reproductive activity in 1 week or less (Krupa, 1989; Leary et al., 2004; Sullivan, 1982).

### Treatment and dosage

A total of 18 calling male toads were administered saline or corticosterone (B) in saline vehicle. Estimated dosages were based on circulating B levels and body mass measures for these species ascertained in our previous investigation (Leary et al., 2004). Crystalline B (Sigma Chemical Co., St. Louis, MO) was dissolved in ethanol (60 µg corticosterone/0.1 ml EtOH), evaporated, and resuspended in a saline solution vehicle (115 mM NaCl, 2.5 mM KHCO<sub>3</sub>, 1.0 mM CaCl<sub>2</sub>, see Propper and Dixon, 1997). Hence, toads were administered a dose of approximately 0.81 µg B/g body mass for *B. cognatus* and 0.95 µg B/g body mass for *B. woodhousii* based on the mean body mass of *B. cognatus* (73.7 g) and *B. woodhousii* (62.9 g) used in this study. Control animals were administered an equal volume (100 µl) of saline vehicle only.

### Pre-treatment procedures

Prior to treatment, focal observations were made on calling males for 10–20 min. All subjects used in this study persistently vocalized during the pre-treatment observational period. Individuals that were observed to alternate between calling and non-calling activity during the observational period were not used in this study. Individuals that persistently called for the focal time period were then captured by hand and rapidly bled at the site of capture by cardiac puncture and immediately marked with a portable tattoo device (Tattoo-A-Pet, Fort Lauderdale, FL). Approximately 100 µl of blood was obtained from each individual and the entire procedure (cardiac puncture + marking) never took more than 30 s. Individuals were then promptly returned to their respective calling site and subsequently observed. If calling behavior did not resume within 1 min, the subject was not used in this study. Individuals that resumed calling behavior within 1 min were allowed to vocalize for approximately 5 min before they were approached and given either a saline or B injection. Males of these species are not easily disturbed while they are vocalizing and, because of their relatively large body size, treatment did not require additional handling of the subject; individuals were approached while they were calling and rapidly injected intraperitoneally from the inguinal region. All subjects used in this study continued to vocalize immediately (<1 min) following injection or did not cease vocal production during injection procedures. Treatment was done in a paired fashion so that males receiving B injections were paired with males that received saline injections at approximately the same time (within 2 min).

### Post-treatment procedures

Experimental subjects were observed by field assistants with no prior knowledge of the experimental treatment (i.e., whether toads were administered B or saline). Focal observations continued for 1 h or until experimental subjects adopted satellite tactics. Males that stopped vocalizing subsequent to treatment were required to approach conspecific calling males and remain in close proximity to calling males for at least 5 min to be scored as “satellite” males. Satellite behavior in the two species differs in that *B. cognatus* typically associate with a single “host” male whereas satellite *B. woodhousii* typically patrol groups of calling males, but do not associate with a specific host (Leary et al., 2004). Males that adopted satellite tactics subsequent to treatment were approached and a second blood sample was rapidly collected if satellite behavior persisted for the required 5 min period (maximum duration of satellite behavior allowed prior to obtaining second blood sample was 15 min). The paired saline-

injected individual was also bled by cardiac puncture at approximately the same time (within 2 min). Corticosterone-treated males that did not stop vocalizing and adopt satellite tactics were bled a second time after 1 h, as were paired saline controls. The second post-treatment blood sample was obtained in 30 s or less and individuals were subsequently weighed and returned to the chorus.

Although initial blood samples and treatments were done in a paired fashion, we were unable to meet the stringent procedural criteria for all experimental subjects. Hence, 2 B-treated individuals for each species were paired with a single saline-injected control male. In these cases, the post-treatment blood sample was obtained from the saline-injected control male at the time the second B-administered individual was bled. Therefore, blood samples were not obtained at the same time for B- and saline-injected animals in these two cases, rather the second blood sample was obtained from the saline-injected male within 15 min subsequent to obtaining the second blood sample from one of the B-treated subjects. In addition, we were unable to obtain a second blood sample from one saline-injected *B. woodhousii*. Hence, pre- and post-treatment effects on hormone levels were not available for that individual. However, treatment effects on behavior are reported. Thus, we obtained pre- and post-treatment behavioral data from 18 toads (5 B and 4 saline for each species) and pre- and post-hormone levels for 17 toads (*B. cognatus*: 5 B, 4 saline; *B. woodhousii*: 5 B, 3 saline).

Precautions were taken throughout the experiment to minimize handling times, observer movement, and disturbance to the subjects. Treatment groups were also randomly assigned to assistants who assessed temporal changes in behavior so that one treatment group was not consistently designated to any assistant. Most focal observations were made under ambient moonlight, but occasionally low power LED headlamps were used. All behavioral observations were transcribed from analog tape recordings made continuously throughout the observation period.

### Radioimmunoassay and statistics

Blood samples were run in two assays to assess pre- and post-treatment steroid hormone levels. The first assay included all blood samples obtained in 2003 and the second assay contained all blood samples obtained in 2004 so that repeated measures (blood samples) obtained from a single individual were always run in the same assay. Radioimmunoassay procedures followed those described by Leary et al. (2004) and were validated in this previous investigation. Briefly, we used ether extraction followed by column chromatography prior to radioimmunoassay to measure dihydrotestosterone (DHT), testosterone (T), and B. Because the column chromatography separated the steroids from one another, we were able to use the same testosterone antibody (Wien T-3003 from Research Diagnostics, Flanders, New Jersey) to measure both DHT and T. The B antibody was purchased from ICN Laboratories (Costa Mesa, California). Mean intraassay coefficients of variation for DHT, T, and B were 5.1%, 4.5%, and 3.0%, respectively, based on 2 to 4 standards run with each assay. Interassay coefficients of variation for DHT, T, and B were 30.0%, 10.9%, and 26.7%, respectively. Although this interassay variation for DHT and B is rather high, it does not affect our conclusions because the relevant analyses used pair-wise comparisons; the variation only potentially affects the overall mean values for the treatment groups and time points.

Treatment effects on pre- and post-treatment hormone levels were analyzed using paired *t* tests. Non-parametric behavioral data were analyzed using chi-square contingency tests.

## Results

Radioimmunoassay verified that B administration significantly increased circulating B levels in *B. cognatus* ( $t_5 = -3.3$ ,  $P = 0.02$ ) and *B. woodhousii* ( $t_5 = -5.6$ ,  $P = 0.005$ ; Fig. 1). Saline injection had no significant effect on circulating B levels in *B. cognatus* ( $t_4 = -0.84$ ,  $P = 0.4$ ) or *B. woodhousii* ( $t_3 = 0.14$ ,  $P = 0.9$ ; Fig. 1).

Despite significant elevation in circulating B, there was no evidence of a decrease in total androgen levels (DHT + T) for B-

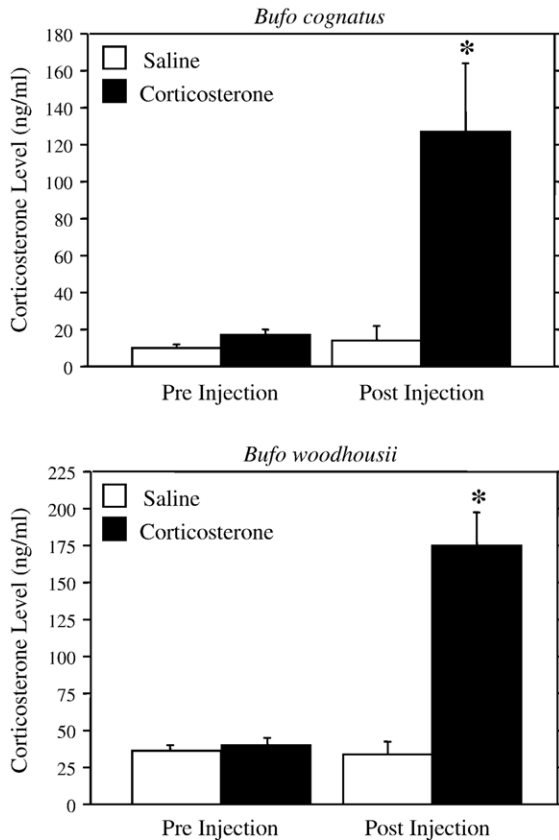


Fig. 1. Mean ( $\pm$ SE) circulating corticosterone levels in male *B. cognatus* and *B. woodhousii* before and after saline or corticosterone treatment (asterisk indicates significant difference from pre-treatment value).

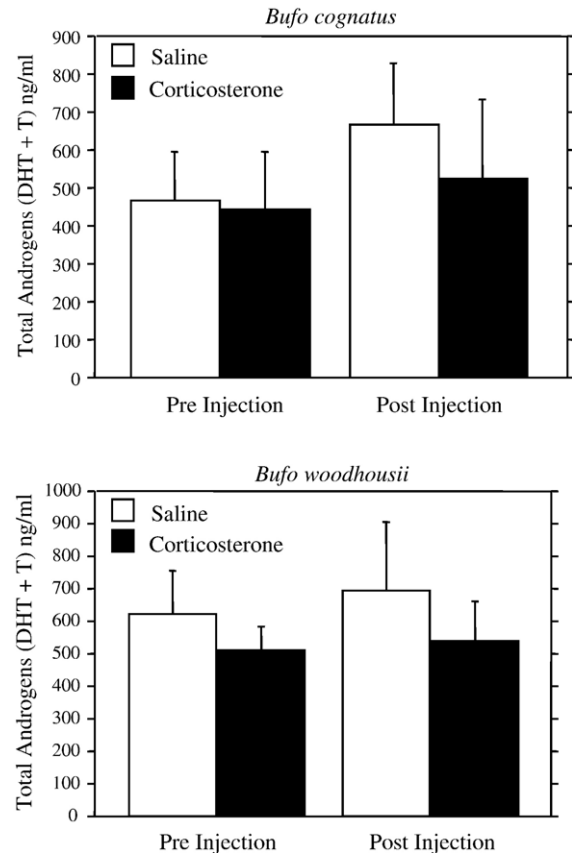


Fig. 2. Mean ( $\pm$ SE) circulating androgen levels (dihydrotestosterone + testosterone) in male *B. cognatus* and *B. woodhousii* before and after saline or corticosterone treatment.

treated *B. cognatus* ( $t_5 = -1.07$ ,  $P = 0.3$ ) or *B. woodhousii* ( $t_5 = -0.31$ ,  $P = 0.7$ ; Fig. 2). There was also no significant difference in total pre- and post-injection levels of circulating androgens for saline-injected *B. cognatus* ( $t_4 = -1.6$ ,  $P = 0.2$ ) or *B. woodhousii* ( $t_3 = -0.88$ ,  $P = 0.5$ ; Fig. 2). Only data for combined androgens (DHT + T) are presented because DHT and T are correlated in these species (Leary et al., 2004) and the results did not change when analyzed separately.

Seven of the 10 B-treated calling males adopted satellite tactics for at least 5 min within 1 h after treatment (range of time between treatment and adoption of satellite tactics = 20–52 min; mean = 41 min; Table 1). All saline-injected controls persistently vocalized throughout the duration of the experiment (Table 1). A single B-administered *B. woodhousii* continued to call for the 1 h focal period and was the only case where we were unable to obtain a second blood sample from the paired saline control male. Vocal production by this B-treated individual continued despite an increase in circulating B from 42.8 ng/ml pre-treatment to 133.9 ng/ml post-treatment, and a decrease in circulating androgens (DHT + T) from 651.7 ng/ml pre-treatment to 304.9 ng/ml post-treatment. This was the only male where B administration had a negative impact on circulating androgen levels. The two other males that did not exhibit the satellite tactic for the required 5 min time interval (1 of each species) exhibited rapid alternations between calling and satellite

behavior approximately 20–30 min post-injection. However, individuals were not scored as satellite males in these two cases because the satellite bouts were ephemeral. In other words, these individuals repeatedly stopped vocalizing and subsequently approached conspecific calling males, but not for the required 5 min period. Alternations between calling and satellite behavior in these individuals were associated with a notable increase in locomotor activity (swimming in the vicinity of calling males) when compared to the pre-injection period and to the activity patterns of control subjects. Because these individuals were not scored as satellite males, the number of individuals that we report to have switched from the calling to satellite tactic is likely to be a conservative estimate.

Table 1  
Behavior of calling male *B. cognatus* and *B. woodhousii* after injection with saline or corticosterone

| Species              | Saline |               |                       | Corticosterone |               |                       |
|----------------------|--------|---------------|-----------------------|----------------|---------------|-----------------------|
|                      | N      | Stayed caller | Switched to satellite | N              | Stayed caller | Switched to satellite |
| <i>B. cognatus</i>   | 4      | 4             | 0                     | 5              | 1             | 4                     |
| <i>B. woodhousii</i> | 4      | 4             | 0                     | 5              | 2             | 3                     |
| Totals               | 8      | 8             | 0                     | 10             | 3             | 7                     |



Because of the small sample sizes and similar results for both species, behavioral data for the two species were combined in chi-square analysis. There was a significantly greater probability that an individual adopted a satellite tactic subsequent to B injection (7 of 10 individuals) when compared to saline-injected controls (0 of 8 individuals; Pearson's  $\chi^2 = 9.16$ ,  $P = 0.002$ ).

## Discussion

Experimental elevation of circulating B increased the probability that male *B. cognatus* and *B. woodhousii* exhibited satellite tactics. Behavioral transitions occurred rapidly (<1 h) and independently of fluctuations in circulating androgen levels. Our results are consistent with the hypothesis that B acts directly on neural activity in brain vocal control centers to alter behavioral expression in these species rather than via effects on circulating androgen levels (Leary et al., 2004). Importantly, however, B injections in the present study often elevated circulating levels of B above those previously measured for these species. For example, the maximum natural circulating B level of calling males that we have measured is 75.0 ng/ml in *B. cognatus* and 104.8 ng/ml in *B. woodhousii* (unpublished data). In the current investigation, B administration resulted in circulating B levels ranging from 9.3–199.4 ng/ml for *B. cognatus* and 133–266 ng/ml for *B. woodhousii*. Nevertheless, 2 B-treated *B. cognatus* adopted satellite tactics when B levels were elevated within the known range for callers. Potential pharmacological effects of corticosterone on calling behavior in anurans are problematic in other studies as well (i.e., Burmeister et al., 2001; Marler and Ryan, 1996), largely because natural ranges of hormone levels are not known for the species investigated but post-treatment B-levels often exceeded stress-induced B levels known to occur in other anuran species. We have not measured B levels induced by stressors other than calling behavior itself in *B. cognatus* or *B. woodhousii*, but we predict that the natural plasma B levels for calling males have not yet met threshold levels that initiate satellite behavior (i.e., B levels should increase with continued vocal production, Emerson, 2001).

Our results are similar to two previous studies that also found that elevated glucocorticoids decrease the probability of calling behavior in anurans (Burmeister et al., 2001; Marler and Ryan, 1996). However, the mode of action for glucocorticoids may differ for *B. woodhousii* and *B. cognatus*. For example, B-induced effects on calling behavior appeared to be through a negative effect of B on circulating androgen levels in the túngara frog, *Physalaemus pustulosus* (Marler and Ryan, 1996) and the green treefrog, *H. cinerea* (Burmeister et al., 2001). Similarly, Mendonça et al. (1985) found evidence for an inverse relationship between glucocorticoids and androgens based on hormone profiles for calling and satellite male bullfrogs, *R. catesbeiana*. These studies contrast with our current results for *B. cognatus* and *B. woodhousii* in that non-calling tactics were adopted by males subsequent to B administration but independent of any change in circulating androgens. Concordantly, we did not find differences in natural circulating

androgens for calling and satellite male *B. cognatus* or *B. woodhousii* despite significantly higher circulating corticosteroid levels in calling males for both species (Leary et al., 2004).

Based on evidence that androgens are required for AVT synthesis (Boyd, 1994a,b), we propose that the species differences in glucocorticoid-androgen relationships represent two different pathways regulating calling behavior in anuran amphibians: a B-androgen-AVT pathway (*R. catesbeiana*, *H. cinerea*, *P. pustulosus*) versus a direct B-AVT pathway (*B. woodhousii* and *B. cognatus*). These potential mechanistic differences in vocal regulation may be related to differences in the duration of reproductive activity for the species examined. *R. catesbeiana*, *H. cinerea*, and *P. pustulosus* are prolonged-breeding species whereas *B. woodhousii* and *B. cognatus* are explosive-breeding species (Halliday and Tejedo, 1995). This proposition is based on elements of the “Emergency Life History Stage theory” (Wingfield et al., 1995, 1998) wherein explosive-breeding Arctic bird species were less likely to exhibit a reciprocal relationship between B and androgen production than more prolonged-breeding temporal species. A similar pattern may exist for anuran species that exhibit (often extreme) differences in temporal patterns of reproduction (see Halliday and Tejedo, 1995; Wells, 1977). Unfortunately, comparative data on natural steroid–hormone relationships are not currently adequate to ascertain whether such differences among anurans are related to differences in the duration of breeding activity.

The mechanism by which B induces satellite behavior in *B. cognatus* and *B. woodhousii* is currently unknown. Whether B acts on AVT-producing neurons in vocal control centers to alter AVT secretion in these species remains largely speculative at this point. Such a mechanism, however, is plausible given the available evidence. In particular, there is an extensive literature on endocrine regulation of reproductive behavior in the roughskin newt that indicates a direct inhibitory effect of glucocorticoids on AVT-producing neurons. AVT mediates courtship behavior in this species (i.e., clasping responses associated with amplexus; Moore et al., 2000; Rose et al., 1995), which can be directly and rapidly inhibited by exogenous application of B (Moore and Miller, 1984; Rose et al., 1993, 1998). Corticosterone appears to bind to membrane-bound receptors in AVT neurons and alter AVT secretion via a non-genomic mechanism (Evans et al., 2000; Moore and Evans, 1999; Orchinik et al., 1991). The mechanisms underlying reproductive behavior in newts may also be applicable to some anuran amphibians (Leary et al., 2004) and the time scale of behavioral transitions in the current investigation lends further support for potential parallel mechanistic relationships in newts and toads.

As with the motor pathways regulating amplexus behavior in newts (see above citations), vocal motor pathways in anurans include AVT-producing neurons (Boyd, 1997; Boyd and Moore, 1992; Boyd et al., 1992) and administration of AVT consistently elicits calling behavior in numerous frog and toad species (Boyd, 1994b; Burmeister et al., 2001; Chu et al., 1998; Klomberg and Marler, 2000; Marler et al., 1995; Semsar et al.,

1998; Trainor et al., 2003; Wilczynski and Chu, 2001). AVT administration has also been found to elicit calling behavior in one of our study species (*B. cognatus*, Propper and Dixon, 1997) and we have preliminary data suggesting that AVT also reverses satellite expression, consistent with the findings of Propper and Dixon (1997). For example, we have observed rapid (<10 min) elicitation of calling behavior in 4 satellite toads (2 *B. cognatus* and 2 *B. woodhousii*) following administration of 100 µg AVT injections.

It has been suggested that the vasotonic system may differ in caller and satellite Puerto Rican coqui frogs (*Eleutherodactylus coqui*) based on presumed age differences among behavioral phenotypes (Ten Eyck, 2005). However, there is no evidence that there are age differences between behavioral phenotypes in *B. cognatus* or *B. woodhousii*. In these species, satellite males are typically smaller than callers (see also Krupa, 1989; Leary et al., 2004; Sullivan, 1982), but the behavioral phenotypes do not differ in age (Leary et al., 2005). We proposed that organizational effects on neural development could occur in early ontogeny to alter activational effects of hormones in adulthood, but we also suggested that presumed differences in age based on body-size differences between behavioral phenotypes in anurans should be reconsidered.

The results of hormone manipulation studies raise important questions for studies that alter the social-acoustic environment to examine its influence on reproductive tactic expression in anurans. For example, does the variation in behavioral responses among males exposed to broadcast calls reflect differences in relative attractiveness among those individuals (as predicted by several models, see Introduction) or is the variation in behavioral responses related to differences in the physiological state of the individuals being examined? The two perspectives are, however, not mutually exclusive. For instance, it is possible that the physiological state of the individual affects call parameters and relative attractiveness, so that individuals in poor condition produce less attractive calls and are more likely to adopt a satellite tactic. For example, Robertson (1986a,b) indicated that the dominant frequency of calls produced by male *U. rugosa* in good condition was lower (and more attractive) than calls produced by the same males in poor condition. The current study and evidence that satellite males are generally in poorer condition than callers (Leary et al., 2004; Robertson, 1986a,b) are consistent with such interactive effects.

Energetic state is predicted to be one major component of tactic expression in the satellite-caller dynamic game models developed by Lucas and Howard (1995), Lucas et al. (1996), and McCauley et al. (2000). Endocrine models of vocal regulation in anurans (Emerson, 2001; Leary et al., 2004) are also consistent with these predictions. For example, negative energy balance is expected to result in activation of the hypothalamic–pituitary–adrenal (HPA) axis, resulting in an increase in circulating glucocorticoids (McEwen and Wingfield, 2003). Importantly, however, it is also well established that circulating glucocorticoid levels can be elevated in individuals that are not in a negative-energy state (Sapolsky, 1992a,b). The

potential effects of elevated glucocorticoids on calling behavior highlight that playback experiments, where individuals are often marked (i.e., freeze branded/toe clipped), must be carefully designed and executed because handling and recovery times can dramatically alter circulating glucocorticoid levels (see Kenagy and Place, 2000; Moore et al., 1991).

Given the current evidence, mating tactic expression in anurans is likely to be influenced by interactions between the male's social environment and his physiological state (see also Marler and Ryan, 1996). For instance, Trainor et al. (2003) found that gray treefrogs produced advertisement calls that were longer in duration (i.e., contained more pulses) subsequent to AVT injections, but the response occurred only when individuals were in close proximity to calling conspecific males. Likewise, the outcome of playback studies is likely to be influenced by variability in call parameters as well as the physiological condition of the subjects involved. Currently, no investigation has combined playback studies with endocrine measures to examine the potential interaction between the two factors and their relationship to mating tactic expression in anurans. The need for such integrative studies is clear if we are to fully understand the various mechanisms underlying the expression of male anuran mating tactics.

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