

Stress Hormone Is Implicated in Satellite-Caller Associations and Sexual Selection in the Great Plains Toad

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ABSTRACT: The effects of androgens on male-typical traits suggest that variation among males in circulating levels can play a major role in sexual selection. We examined whether variation in vocal attractiveness is attributable to differences in androgen levels among Great Plains toads (*Bufo cognatus*). We found that noncalling “satellite” males practicing an alternative mating tactic were more likely to associate with males producing long calls. However, callers with satellites did not have higher androgen levels than callers without satellites. Rather, callers with satellites had significantly lower corticosterone (CORT) levels than callers without satellites. A CORT manipulation experiment suggested that differences in calls for males with and without satellites were related to differences in CORT levels. Furthermore, there was a negative correlation between CORT level and call duration within most nights of chorus activity. However, the correlation was weak for the pooled data (across all nights), suggesting that local environmental and/or social factors also affect call duration. Last, we show that females preferred broadcast calls of longer duration, characteristic of males with satellites and low CORT. These results imply that satellites optimize their reproductive success by associating with males producing long calls. However, this association should negatively affect the fitness of attractive callers.

Keywords: alternative mating tactics, androgens, corticosterone, vocalization.

Males often vary more than females with respect to conspicuous coloration, morphology, and behavior (Andersson 1994), and the expression of sexually dimorphic traits

is typically mediated by organizational and/or activational effects of gonadal steroids (Phoenix et al. 1959; Moore 1991; Ketterson and Nolan 1999; Ketterson et al. 2001). The implicit role of gonadal secretions in the expression of male-typical traits is historically well recognized (Nelson 2000) and is traceable to endocrinology’s formal beginnings (Berthold 1849). However, few studies have established a direct link between natural circulating sex steroid levels and male attractiveness (reviewed by Enstrom et al. 1997), and these relationships are not always consistent (see McGlothlin et al. 2004). Such discrepancies may lie in the complexity of endocrine mediation of male-typical traits that could involve variation in hormone receptor levels (Young 1999), variation in hormone-binding globulin levels (Breuner and Orchinik 2002), and/or effects of hormones other than sex steroids (Moore and Evans 1999).

Androgens can influence the elaboration of sexually dimorphic traits via individual variation in their circulating concentrations (Ketterson and Nolan 1999; Ketterson et al. 2001). For example, vocal production in birds is a well-known sexually dimorphic, androgen-dependent behavior, and androgen administration has been shown to increase vocal attractiveness (reviewed by Ketterson et al. 2001). However, androgen-mediated effects on sexually selected traits are frequently associated with fitness trade-offs. For example, experimental elevation of androgen levels may increase male attractiveness and mate acquisition but is frequently accompanied by a decrease in parental care, immune function, and/or survivorship (reviewed by Ketterson et al. 2001).

Androgen-mediated effects on elaborate male traits have been documented in a wide variety of organisms and, hence, appear to be broadly applicable (Ketterson and Nolan 1999; Ketterson et al. 2001). In particular, the relationship between androgens and vocal attractiveness in male birds is expected to apply to other taxonomic groups where vocal production is also androgen dependent. For instance, Emerson (2001) predicted that temporal fluctuations in circulating androgen levels would be correlated with vocal effort in male anuran amphibians. Vocal effort has been shown to be positively correlated with male mat-

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ing success in anurans (reviewed in Gerhardt and Huber 2002) and is expected to alter rates of energy expenditure (Pough et al. 1992; Prestwich 1994). Given that energy mobilization is mediated by glucocorticoids (Sapolsky 1992a, 1992b; Laugero 2001; McEwen and Wingfield 2003; for review in amphibians, see Herman 1992), Emerson (2001) further predicted that an increase in androgens and vocal effort would be paralleled by an increase in corticosterone (CORT), the major glucocorticoid in anurans. Vocal production in anurans was thus predicted to be influenced by relationships between androgens and glucocorticoids: the steroids were expected to promote vocal effort until energy reserves were depleted and elevations in CORT inhibited androgen production and, hence, vocalization (Emerson 2001). Inverse relationships between glucocorticoids and androgens are well documented in a variety of vertebrates (Greenberg and Wingfield 1987; Knapp and Moore 1997; Wingfield and Ramenofsky 1999), including anurans (Emerson 2001; Leary et al. 2006). However, no studies have ascertained whether natural variation in circulating steroid hormones correlates with vocal characteristics in anuran amphibians. This is surprising, given that variation in anuran calls has been the focus of extensive research in sexual selection (Ryan 1985, 2001; Fritzsche et al. 1988; Gerhardt and Huber 2002).

Here, we investigated whether natural variation in circulating androgen and CORT levels was related to variation in vocal attractiveness in male Great Plains toads (*Bufo cognatus*). Our research stemmed from previous work related to endocrine mediation of alternative mating tactics in this species. Male Great Plains toads alternate between one of two reproductive tactics: individuals may vocalize to attract mates or they may adopt a noncalling "satellite" tactic, positioning themselves near calling conspecific males and attempting to intercept females attracted to calling males (Sullivan 1982; Krupa 1989; Halliday and Tejedo 1995; Leary et al. 2004). Behavioral transition from the calling to the noncalling satellite tactic in *B. cognatus* is correlated with an increase in circulating CORT associated with a reduction in body mass during vocal production (Leary et al. 2004). Natural CORT levels are not inversely correlated with circulating androgen levels in this species (Leary et al. 2004), and CORT injections initiate a rapid (<1 h) behavioral transition from calling to satellite behavior independent of changes in androgen levels (Leary et al. 2006).

In this study, we show that individual call differences among male Great Plains toads in natural choruses are not related to differences in circulating androgen levels. Rather, we show that circulating CORT levels may be a better predictor of vocal attractiveness. The impetus for this study arose from satellite-caller associations in this species. In particular, we build from the finding (reported

here) that satellite males in natural choruses were more likely to associate with calling males producing the longest advertisement calls with the lowest pulse rates. Through a series of studies, we then show that variation in circulating CORT levels may be a major contributor to satellite-caller associations and mate selection in the Great Plains toad. Our results suggest that variation in stress hormone levels can affect the expression of sexually selected traits that are commonly thought to be under the control of gonadal sex steroids.

Methods and Results

Vocal Attributes of Males with and without Associated Satellites

To determine whether satellite males potentially optimize their reproductive success by associating with calling males that are also preferred by females, we first examined call characteristics of males with and without associated satellites in natural choruses in central Oklahoma during peaks in reproductive activity (following heavy rains in April–June 2001–2004). We observed behavior of individual males for 15–30 min to categorize behavioral phenotype and to ensure stable satellite-caller associations. We then recorded a series of consecutive advertisement vocalizations from calling male *Bufo cognatus* with associated satellites and nearby calling males (within a 3-m radius) without satellites. Satellites were typically within 0.5 m of a particular calling male. Observations were made under ambient moonlight or with low-powered light-emitting diode headlamps. Vocal recordings were made using a Marantz PMD 222 recorder equipped with a Sennheiser ME66 condenser microphone and a Sennheiser K6-C power module.

Calls were analyzed using Canary 1.1.1 Bioacoustics software, Cornell Bioacoustics Laboratory (settings: frame length = 256 points, time = 5.752 ms, 50% overlap, fast Fourier transform size = 256 points, Hamming filter and amplitude logarithmic). We examined call parameters known to be important in mate selection in bufonids and other anurans (Gerhardt and Huber 2002) from spectrograms, spectra, and waveforms. Call parameters were measured from five to 10 consecutive calls from each individual and included call duration, pulse rate (measured from a 3-s section in the middle portion of the call), dominant frequency, and intercall duration. Call amplitude was also measured with a RadioShack digital sound pressure level (SPL) meter (model 33-2055). A 30-cm heavy gauge wire was mounted to the face of the SPL meter to control for variation in amplitude readings associated with differences in the distance between the subject and SPL meter. Calling individuals were approached, and the wire mount on the

Table 1: Mean \pm SE values for call parameters of calling males with and without associated satellites in natural choruses of *Bufo cognatus*

Call parameter	Callers with satellite		Callers without satellite		ANOVA results		
	SE	<i>n</i>	SE	<i>n</i>	<i>F</i>	df	<i>P</i>
Call duration (s)	1.6	24	1.3	35	4.94	1, 56	.03
Pulse rate (no. pulses in 3 s)		24		35			
Adjusted to mean temperature (23.2°C)	.9		.8		14.83	1, 57	.0003
Adjusted 1 SD below the mean (21.6°C)	.9		.8		3.73	1, 57	.06
Adjusted 1 SD above the mean (24.8°C)	.9		.8		33.29	1, 57	.0001
Dominant frequency (kHz)	.04	24	.03	35	.20	1, 56	.66
Intercall duration (s)	1.4	24	.9	35	.66	1, 56	.42
Amplitude (dB)	2.8	19	3.2	29	.05	1, 45	.82

SPL meter was oriented horizontal to the water substrate and placed directly in front of (almost touching) the calling male's expanded gular sac to obtain SPL readings.

Temperature effects on call parameters are well known in anurans (see Gerhardt and Huber 2002). All *B. cognatus* males at our study sites called while partly submerged in water, and water temperature was nearly identical to cloacal body temperatures. *Bufo cognatus* bred in shallow, small ephemeral pools, with consistent water temperatures within sites and within nights of data collection. Water temperature across sites and years never varied more than 5°C (range 20°–25°C), and there was no significant difference in temperature for males with and without associated satellites (ANOVA $F = 1.3$, $df = 1, 57$, $P = .25$). However, temperature was used as a covariate in initial ANCOVAs to examine potential differences in temperature effects on calls for males with and without associated satellite males. With the exception of pulse rate, there was no significant interaction (heterogeneity of slopes) for the effects of temperature on call parameters for callers with and without associated satellites ($P > .15$). Hence, the interaction term was removed from the analyses for call duration, dominant frequency, intercall duration, and amplitude, and the models were rerun. Heterogeneity of slopes for the effects of temperature on pulse rate for callers with and without associated satellites precluded interpretation of the main effects from ANCOVA (Hendrix et al. 1982). To examine potential differences in pulse rates for callers with and without associated satellites, the pulse rate was adjusted to the mean covariate value (23.2°C) and 1 SD below (21.6°C) and 1 SD above (24.8°C) the mean temperature value using pooled regressions for the effects of temperature on pulse rate for callers with and without associated satellite males (Hendrix et al. 1982; Tomkins and Simmons 2002). ANOVAs were then run on adjusted values at each temperature. Males without associated satellites outnumbered males with associated satellites, and thus statistical analyses were run on unequal numbers of callers with and without associated satellites.

Mean dominant frequency, intercall duration, and amplitude did not differ between callers with and without associated satellites (table 1). However, calling males with associated satellites produced advertisement vocalizations that were longer in duration and had lower pulse rates than those produced by nearby callers without associated satellites (table 1).

Hormone Levels of Males

We then ascertained whether calling males with associated satellites and nearby calling males without associated satellites differed with respect to circulating androgens (dihydrotestosterone + testosterone) or CORT. Blood samples were obtained by cardiac puncture from callers with and without associated satellites immediately after vocal recordings were obtained. Sample collection never took more than 30 s, and blood samples obtained in the field were kept on ice until they were returned to the lab (<4 h) and centrifuged for 12 min at 3,000 rpm. Plasma was collected and stored at -20°C until it was assayed for steroid hormones. All sampled individuals were numerically marked on the venter using a portable tattoo device (Tattoo-A-Pet, Fort Lauderdale, FL) for future identification.

We have previously described our hormone extraction, chromatography, and radioimmunoassay procedures (Leary et al. 2004), and the methods were validated in this previous investigation. Briefly, plasma samples were extracted with diethyl ether, dried under nitrogen gas, and resuspended in 10% ethyl acetate in iso-octane. Column chromatography was then used to separate dihydrotestosterone, testosterone, and CORT. Testosterone antibody was obtained from Research Diagnostics (Flanders, NJ) and used for both testosterone and dihydrotestosterone assays. The CORT antibody was purchased from ICN Laboratories (Costa Mesa, CA). Hormone sample sizes differ from those reported for call attributes because we were not able to obtain blood samples from all individuals that were recorded and/or analyses included additional individuals for

which calls were not obtained. Plasma samples were analyzed for androgen and CORT levels from three assays. Mean intra-assay coefficients of variation (CVs) for dihydrotestosterone, testosterone, and CORT were 8.7%, 4.6%, and 2.4%, respectively, on the basis of four standards run with each assay. Interassay CVs for dihydrotestosterone, testosterone, and CORT were 1.9%, 13%, and 10%, respectively.

Circulating levels of androgens (dihydrotestosterone + testosterone) did not differ for callers with satellites (mean = 311.6 ng/mL, SD = 284.9 ng/mL, $n = 39$) and callers without satellites (mean = 305.2 ng/mL, SD = 238.8 ng/mL, $n = 24$; ANOVA $F = 0.009$, $df = 1, 61$, $P = .93$). This was also true if dihydrotestosterone and testosterone were analyzed separately. However, there was a difference in circulating CORT levels between callers with and without satellite males (ANOVA $F = 6.09$, $df = 1, 61$, $P = .02$). Calling males without associated satellites had higher CORT levels (mean = 13.1 ng/mL, SD = 10.4 ng/mL, $n = 24$) than calling males with satellites (mean = 8.0 ng/mL, SD = 5.9 ng/mL, $n = 39$).

Effects of Exogenous Corticosterone on Call Parameters

To determine whether differences in call duration and pulse rate for males with and without associated satellites were causally related to differences in circulating CORT, we administered CORT or saline to calling males and measured changes in call parameters subsequent to treatment. We measured call parameters from males used in a hormone manipulation study that simultaneously addressed the related but distinct question of the effects of exogenous CORT on mating tactic expression (Leary et al. 2006). Hormone manipulation procedures are described in detail in that article. Briefly, calling male toads were administered saline or CORT in saline vehicle and subsequently recorded continuously for vocalizations (see above recording procedures). Crystalline CORT (Sigma, St. Louis, MO) was dissolved in ethanol (60 mg CORT/0.1 mL EtOH), evaporated, and resuspended in a saline solution vehicle (115 mM NaCl, 2.5 mM KHCO_3 , 1.0 mM CaCl_2 ; see Propper and Dixon 1997). Toads were administered a dose of approximately 0.81 mg CORT/g body mass on the basis of the mean body mass (73.7 g) of *B. cognatus* males used in this study. Controls were administered an equal volume (100 μL) of saline vehicle only.

Vocalizations of calling male toads were recorded in natural choruses for approximately 10–20 min before treatment. Calling individuals were then injected in the intraperitoneal cavity in a paired fashion; one male was injected with 100 μL CORT, and one male was injected with 100 μL saline. In a single case, two CORT-treated individuals were paired with a single saline-injected male.

Thus, we examined the effects of treatment from a total of nine individuals (five CORT-injected individuals and four saline-injected individuals).

Calls were continuously recorded subsequent to treatment until satellite behavior was adopted (for at least 5 min) or after 1 h (Leary et al. 2006). Males were recaptured at this time, and another blood sample was obtained. Corticosterone administration consistently elevated CORT above preadministration levels (Leary et al. 2006). Corticosterone injections had no effect on circulating androgen levels, and saline-injected controls did not show a significant change in CORT or androgen levels (Leary et al. 2006). Inter- and intra-assay CVs for individuals that were administered CORT or saline are also provided by Leary et al. (2006).

We measured call duration and pulse rate from six to 10 calls from each male at three times: immediately before treatment, within 10 min posttreatment, and approximately 30 min posttreatment. We found no overall between-groups difference in call duration (repeated-measures ANOVA, $F = 1.8$, $df = 1, 7$, $P = .21$) and no significant main effect of time on call duration ($F = 1.0$, $df = 1, 2$, $P = .36$). However, there was a significant interaction between call duration of CORT-injected and saline-injected groups (group \times time interaction: $F = 6.6$, $df = 1, 2$, $P = .009$), with CORT-treated males showing a decrease in call duration over time compared with saline-injected males (fig. 1A). There was no overall between-groups difference in pulse rate ($F = 0.1$, $df = 1, 7$, $P = .71$). Although there was a significant decrease in pulse rate over time ($F = 8.4$, $df = 1, 2$, $P = .004$), there was no significant difference in the response of CORT-injected and saline-injected groups ($F = 2.2$, $df = 1, 2$, $P = .14$; fig. 1B). The CORT or saline treatment did not affect circulating androgen levels (Leary et al. 2006). Hence, changes in vocal attributes occurred independent of changes in androgens.

Correlations between Corticosterone and Call Duration in Natural Choruses

We also assessed whether the relationship between CORT level and call duration in natural chorusing males was consistent with that found in the CORT injection study. Vocal recordings and hormone levels were available for only a subset of the individuals used in the above analyses where the two variables were examined separately.

Multiple regression analysis indicated that CORT level was not significantly correlated with call duration across all nights of chorus activity ($t = -1.21$, $df = 3, 35$, $P = .2$), nor was DHT + T ($t = 0.005$, $df = 3, 35$, $P = .9$). Temperature had a marginally significant effect on the variance in call duration across all nights of chorus activity

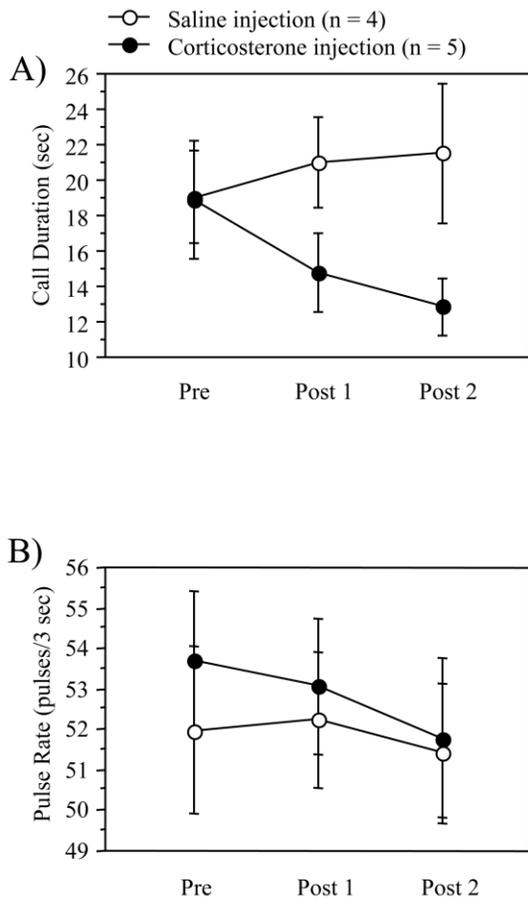


Figure 1: Interaction plots (mean \pm SE) for the effects of corticosterone and saline injections on call duration (A) and pulse rate (B) over three periods of sampling (*Pre* = immediately before treatment, *Post 1* = 10 min postinjection, *Post 2* = 30 min postinjection).

($t = -1.69$, $df = 3, 35$, $P = .09$). To visually examine the relationship between CORT and call duration, we adjusted call duration to the mean recording temperature (23.2°C) using the equation obtained from linear regression of temperature versus call duration for the pooled data across all nights. Simple regression of CORT level versus call duration (adjusted for temperature effects) indicated a weak negative correlation between the two factors ($F = 1.1$, $df = 1, 37$, $P = .2$, $r^2 = 0.029$), with the greatest variation in call duration corresponding to low CORT levels (fig. 2A).

A variety of environmental/social factors can influence call duration (reviewed in Gerhardt and Huber 2002). We thus examined the relationship between CORT level and call duration within nights of chorus activity to help control for differences in environmental and/or social factors across nights of chorus activity. We examined correlations

for nights with at least four data points and where body temperature did not vary among individuals within the sampled time frame. Four nights of calling activity met these criteria. Results indicated that CORT was negatively correlated with call duration on three of those nights (fig. 2B). Multiple regression analysis indicated that CORT was significantly correlated with call duration across these nights ($t = -2.48$, $df = 3, 16$, $P = .02$). Androgens did not contribute significantly to the variation in call duration across these nights ($t = 0.26$, $df = 3, 16$, $P = .8$), nor did temperature ($t = 1.34$, $df = 3, 16$, $P = .2$; temperature did not vary by more than 2.5°C).

Female Preference Trials

Our combined results suggest that high CORT levels play a role in the production of short duration calls and in satellite-caller associations. Although pulse rate was also significantly different for callers with and without associated satellites in natural choruses, the mean pulse rate of CORT-injected males did not differ from that of saline-injected controls and, hence, could not be attributed to differences in circulating CORT. Pulse rate is typically under stabilizing selection in anurans (i.e., values near the mean are most attractive), whereas call duration is typically under directional selection, with females preferring calls of longer duration (Gerhardt and Huber 2002). Because call duration was the only call parameter to consistently appear as an important factor, we hypothesized that satellite males potentially maximize their chances of intercepting a mate by associating with conspecific calling males with low CORT levels. This hypothesis predicts that females prefer the calls of males with low CORT levels over males with high CORT levels. To test this prediction, we performed a dual speaker playback experiment where the call durations characteristic of males with low and high CORT levels were broadcast to gravid females.

Calls broadcast from the two speakers differed with respect to call duration. Call parameters used in playback studies were based on the mean values for 24 males recorded from several choruses in central Oklahoma in 2001 (recorded at an average temperature of 24°C; pulse rate = 55 pulses/3 s, dominant frequency = 2.2 kHz, intercall duration = 9.75 s, amplitude from 31 cm = 103 dB). Differences in call duration (22.5 vs. 17.5 s) used in playback experiments were based on the mean duration from preliminary data collected in 2001 for calling males with ($n = 12$) and without ($n = 9$) satellite males, respectively. These values closely approximated differences found with the larger sample size shown in table 1.

Calls used in playback experiments were taken from a recording of a single individual male that produced calls with characteristics representative of the mean values from

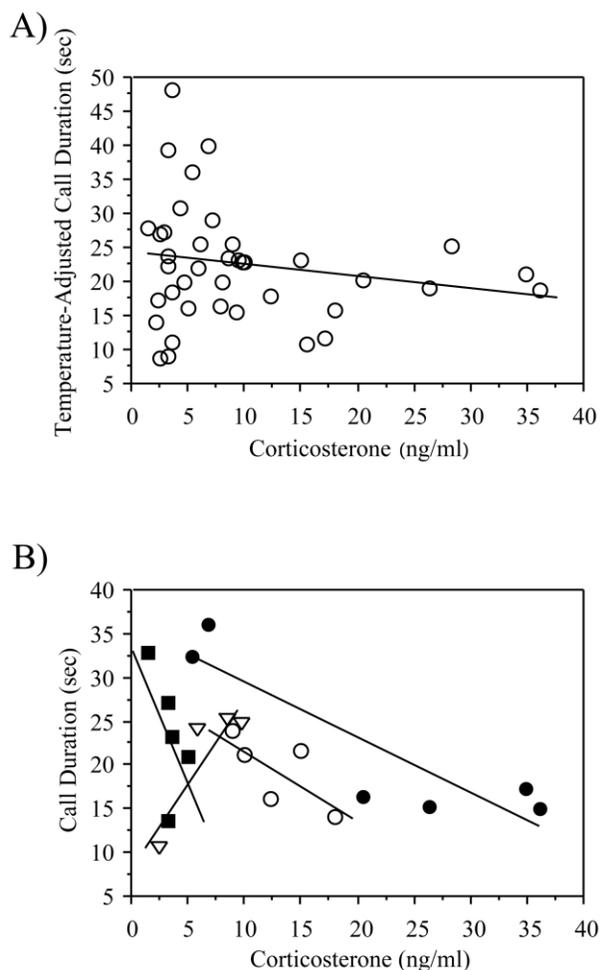


Figure 2: A, Correlation between circulating corticosterone level and call duration (adjusted to the common mean temperature of 23.2°C) across all nights of chorus activity. B, Correlations between circulating corticosterone level and call duration within four nights of chorus activity (coded by different symbols).

the 2001 data set (described above). A single call from that individual was digitized using Canary 1.2.1 software. Call duration was then altered by removing a series of pulses from the middle portion of the original call or adding pulses to the middle portion of the call while maintaining constant pulse rate/periodicity. Call duration manipulation inevitably alters other call characteristics (e.g., total number of pulses is greater for long calls versus short calls, and call rate increases for calls of short duration). However, playback stimuli were representative of differences in call duration for males with and without associated satellites (table 1) and with low and high CORT levels. The calls of Great Plains toads are simple amplitude-modulated calls that can be largely characterized by the

attributes that we measured. Hence, characteristics of the individual's call used in the playback study are likely to be representative of the population.

Digitized calls were imported into MacIntosh iTunes from Canary AIFF files, and a continuous loop of the digital file was transferred to cassette tapes used in playback trials. Gravid females collected at natural choruses were brought to the lab within 4 h after capture and run in preference trials. A female was placed under a cloth box in the center of the chamber for 5–10 min while exposed to broadcast calls. Calls were broadcast from Sennheiser accessory speakers connected to the Marantz recording equipment that were placed 3.05 m apart in a 3.2 × 2.1-m semianechoic chamber. The box was then lifted with a pulley mechanism when the onset of broadcast calls was synchronized (calls were subsequently out of synchrony because of differences in call duration). Chamber temperature was held at the mean temperature of natural chorus activity (24°C), and broadcast call amplitude was checked before trials with an SPL meter and held at a constant 103 dB at a distance of 30 cm from the speaker to the SPL meter. The amplitude of broadcast calls was based on the mean amplitude of calls acquired under field conditions at the same distance (see above).

Behavioral responses were scored under red incandescent light by an observer seated behind a blind. Positive phonotactic scores included female orientation, approach, and direct contact with the speaker. Cassette tapes were switched between speaker playback systems between successive trials to eliminate extraneous factors that potentially influence movement patterns. A total of 14 females were run in playback trials. Two females were unresponsive to calls and attempted to escape. Of the 12 females that responded, six were run in two trials, and six were run in a single trial. Statistics are reported only for the first trial for each female to avoid pseudoreplication.

All 12 females in this study responded within 5 min in playback experiments, and 100% preferred the speaker broadcasting the longer calls in all trials. Hence, females had a strong preference for the longer calls ($n = 12$ females, $\chi^2 = 12$, $P < .001$) characteristic of calling males with low CORT levels. This preference was further supported by the observation that the six females used in a second phonotaxis trial all preferred the longer duration calls in both trials.

Discussion

Males of many species engage in endurance contests, where energetically demanding behaviors must be sustained to win access to or attract mates (Andersson 1994). The increased energy expenditure associated with reproductive activity may, in turn, result in an increase in circulating

glucocorticoid levels (Romero 2002). In some cases, elevated glucocorticoids negatively affect circulating androgen levels (Moore and Jessop 2003). Under such circumstances, the expression of male-typical traits may be mediated by negative effects of glucocorticoids on androgen levels (e.g., Emerson 2001).

In the Great Plains toad, males lose weight during vocal activity, which appears to drive an increase in circulating CORT levels (Leary et al. 2004). Elevated CORT levels, in turn, mediate transitions from calling to noncalling satellite behavior independent of changes in circulating androgens (Leary et al. 2004, 2006). Hence, noncalling males practicing an alternative mating tactic appear to be recovering from recent elevations in circulating CORT incurred during vocal production. The results from this study indicate that these males may optimize their reproductive success by associating with calling males that produce long calls. This proposition was contingent on and further supported by females exhibiting a preference for calls characteristic of the mean call duration for males with satellites over the calls characteristic of males without satellites in dual speaker playback trials. Our results, however, do not unequivocally demonstrate a satellite optimization strategy; satellite-caller associations may be the result of other factors that do not necessarily entail a satellite preference for calling males with long calls. For instance, a similar pattern may occur if males with short calls are better at detecting and avoiding satellites. To clarify this possibility, we attempted to perform laboratory call preference trials with males captured while behaving as satellites in the field ($n = 4$). Unfortunately, the individuals were not amenable to such trials (i.e., they attempted to escape or hide in the acoustic chamber). Additional investigation is also required to assess whether the strong female preferences for longer calls (typical of males with associated satellites) that we found in the laboratory exist under natural chorus conditions, where the acoustic environment is more complex.

Our results link differences in call duration for males with and without associated satellites to differences in circulating levels of CORT but not androgens. For example, in addition to finding that males with associated satellites had significantly longer calls, we also found that satellites were more likely to associate with males with low CORT levels. Consistent with these findings, experimental elevation of CORT levels in calling males indicated that vocal differences for males with and without associated satellites could be associated with CORT effects on call duration. These results were also consistent with negative correlations between CORT level and call duration within most nights of chorus activity. However, this negative relationship was weak for the pooled data across all nights. The combined results thus suggest that local environmental

and/or social factors can also play a prominent role in regulating call duration independent of circulating CORT levels. For instance, agonistic encounters with other males are well known to alter call duration (reviewed in Gerhardt and Huber 2002). Another possibility for the lack of a stronger correlation between CORT level and call duration in natural choruses is that there may be physiological differences among individuals that buffer against the effects of circulating CORT on call duration (i.e., differences in binding protein levels).

The apparent link between circulating CORT levels and males with and without associated satellites is subject to the same interpretive challenges noted above for differences in call characteristics for males with and without satellite males. For instance, such patterns may be related to the effects of CORT on call duration or to some other (unrelated) factor(s). We have repeatedly observed that satellite males will follow specific calling males as they move periodically (within a night) to different locations in the pond. Hence, satellites in this species often maintain consistent associations with specific calling males. Such observations, combined with the lack of any evidence that males of this species are territorial (Sullivan 1982; Krupa 1989; Leary et al. 2004), suggest that differences in CORT levels for callers with and without associated satellites are not related to differences in the spatial distribution and/or hierarchical status of males in the chorus. Currently, the best explanation for the differences in CORT levels for callers with and without associated satellites is that CORT negatively affects call duration and that satellites use call duration when choosing among calling males.

The link between circulating CORT levels and call duration also suggests that satellite-caller associations will create dynamic fitness trade-offs for calling males. For example, the number of satellite males per calling male is known to vary considerably in Great Plains toads, with calling males having up to five associated satellites (Sullivan 1982; Krupa 1989). Hence, although calling males in the best physical condition with the lowest circulating CORT levels appear to produce the most attractive calls, males with the longest calls may also experience a reduction in reproductive success by attracting satellite males, perhaps as a diminishing returns effect (Waltz 1982; Lucas and Howard 1995; Lucas et al. 1996; McCauley et al. 2000). Our results indicate that these potential fitness trade-offs may arise from the effects of CORT on call duration and the behavior of males employing the alternative mating tactic, which, in the Great Plains toad, is also elicited by high levels of CORT (Leary et al. 2004, 2006). Because CORT levels are expected to fluctuate over the duration of the breeding period (Emerson 2001), call characteristics and satellite-caller associations are also expected to change over time. For instance, Emerson (2001) proposed that

temporal changes in CORT levels would stem from the energetic demands associated with vocal production (Bucher et al. 1982; Pough et al. 1992; Prestwich 1994; Wells et al. 1995; Wells 2001) and the established role of CORT in energy mobilization (Sapolsky 1992a, 1992b; Laugero 2001; McEwen and Wingfield 2003; for review in amphibians, see Herman 1992). Hence, the metabolic demands associated with vocal production were expected to drive an increase in circulating CORT levels over sequential nights of calling activity, but CORT levels were expected to decrease when energy reserves were restored (subsequent to periods of foraging; Emerson 2001). There is currently considerable evidence that CORT levels fluctuate over the breeding period in a variety of vertebrates (Romero 2002), and our work suggests that such temporal changes in CORT levels can result in individual temporal changes in a male's call characteristics (this article) and behavior (i.e., calling versus satellite behavior; Leary et al. 2004, 2006), as well as temporal changes in satellite-caller associations.

The current evidence we have gathered on Great Plains toads also indicates that individual differences in mating behavior are related to differences in circulating CORT levels but not to differences in circulating androgen levels (this article; Leary et al. 2004, 2006). Such CORT-mediated inhibitory effects on vocal production that occur independent of negative effects on androgen levels may be favored over a regulatory pathway that involves a reciprocal interaction between the two steroids. For instance, individuals that exhibit a reciprocal relationship between CORT and androgens may be less likely to return to the chorus and participate in mating activity on subsequent nights of breeding activity. A well-established endocrine pattern that would support such a scenario has contributed to the "emergency life-history stage" theory (Wingfield et al. 1995, 1998). Wingfield and colleagues discovered that differences in the reproductive endocrinology of various bird species were related to temporal patterns of breeding behavior. Explosive breeding species did not typically exhibit a reciprocal relationship between glucocorticoids and androgens, whereas more prolonged breeding species often exhibited such a relationship. The authors proposed that such endocrine differences could evolve, given the potential consequences associated with a reciprocal steroid-hormone relationship in explosive-breeding species; individuals that experienced a decrease in androgens were unlikely to remain in breeding condition and recover from the stress response in the allotted breeding period. However, selective pressures were predicted to be more "relaxed" in prolonged-breeding species because of the greater window of opportunity to recover and acquire mates. Consistent with predictions of the emergency life-history stage theory, Great Plains toads are an explosive breeding species

and do not exhibit a reciprocal relationship between CORT and androgens, as do prolonged-breeding anuran species (reviewed in Leary et al. 2004, 2006). Hence, a CORT-mediated inhibitory effect on calling activity that occurs independent of negative effects on androgens could maximize reproductive potential in the narrow window of opportunity to reproduce while maintaining other potential androgen-mediated mating behaviors (i.e., satellite males will still search for, intercept, amplex, and breed with females). Such a mechanism may also prevent the interruption of androgen-dependent spermatogenic processes (see Harvey et al. 1997).

If elevated glucocorticoids do not inhibit vocal production through modulatory effects on circulating androgens, how does CORT potentially alter call characteristics and vocal production? We have proposed that high CORT may elicit noncalling mating tactics in explosive-breeding toad species (*Bufo cognatus* and *Bufo woodhousii*) by altering the secretion of arginine vasotocin (AVT) in vocal control centers of the brain (Leary et al. 2004, 2006). Arginine vasotocin is well known to mediate the expression of sexual behavior in vertebrates, including vocal production in anurans (reviewed by Marler et al. 1999; Wilczynski and Chu 2001). Furthermore, work on rough-skin newts (*Taricha granulosa*) indicate that high CORT levels potentially inhibit the secretion of AVT through nongenomic pathways (reviewed by Moore and Evans 1999; Evans et al. 2000). If a CORT-mediated effect on AVT (secretion) were more sensitive than the hypothalamic-pituitary-gonadal axis to elevations in CORT, such a mechanism could prevent circulating CORT from reaching levels that would presumably inhibit androgen production (i.e., if vocal production were to persist). Alternatively, different portions of the neural vocal motor pathway could be differentially sensitive to glucocorticoids versus androgens (e.g., Remage-Healey and Bass 2004).

The various CORT-related effects on behavior, vocal production, and vocal characteristics in male toads appear to be related to energetic constraints associated with endurance contests. Mating signals are energetically expensive (Bucher et al. 1982; Pough et al. 1992; Prestwich 1994; Wells et al. 1995; Wells 2001), and male Great Plains toads exhibit a decrease in body mass when calling (Leary et al. 2004). The mobilization of energy reserves via increased CORT is likely to be required to sustain vocal activity. Elevations in CORT, however, appear to mediate a decrease in call duration that eventually leads to the adoption of the noncalling satellite tactic (Leary et al. 2006). Satellite males use a less energetically demanding mating tactic to acquire mates and, in doing so, potentially optimize their reproductive success by associating with calling males having the lowest CORT levels and producing the longest calls. Call duration has been shown to be a reliable indicator of

a male genetic quality in anurans (Welch et al. 1998), but whether differences in call duration among males is related to the ability of males to maintain low CORT levels or buffer against the effects of high CORT levels is unknown. However, satellite-caller relationships and CORT-mediated effects on vocal attractiveness may help explain how genetic variance is maintained in populations despite female preferences for particular call characteristics.

Our results do not imply that androgens play no role in mediating vocal behaviors in male toads. On the contrary, the organizational and activational roles of androgens on anuran vocal behavior are well known (Kelley 1980; Wetzel and Kelley 1983; Kelley et al. 2001; Wilczynski and Chu 2001; Moore et al. 2005). We did not manipulate androgen levels to examine potential androgen-mediated effects on vocal attractiveness. However, we do provide evidence that variation in vocal attractiveness in natural choruses of toads is not attributable to circulating androgen levels. Rather, it appears that CORT may play a direct role in altering the expression of sexually selected traits in male toads. If applicable to other species, these findings may be critical when considering the effects of androgen manipulation on sexually selected traits. For example, androgen administration often results in an increase in glucocorticoid levels (Ketterson et al. 2001). Hence, it may be important to consider not only the potential effects of glucocorticoids on androgens but also the potential direct effects of glucocorticoids on sexually selected traits.

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