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Variation in Testosterone and Corticosterone in Amphibians and Reptiles: Relationships with Latitude, Elevation, and Breeding Season Length

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ABSTRACT: Latitudinal variation in life-history traits has been the focus of numerous investigations, but underlying hormonal mechanisms have received much less attention. Steroid hormones play a central role in vertebrate reproduction and may be associated with life-history trade-offs. Consequently, circulating concentrations of these hormones vary tremendously across vertebrates, yet interspecific geographic variation in male hormone concentrations has been studied in detail only in birds. We here report on such variation in amphibians and reptiles, confirming patterns observed in birds. Using phylogenetic comparative analyses, we found that in amphibians, but not in reptiles, testosterone and baseline corticosterone were positively related to latitude. Baseline corticosterone was negatively related to elevation in amphibians but not in reptiles. For both groups, testosterone concentrations were negatively related to breeding-season length. In addition, testosterone concentrations were positively correlated with baseline corticosterone in both groups. Our findings may best be explained by the hypothesis that shorter breeding seasons increase male-male competition, which may favor increased testosterone concentrations that modulate secondary sexual traits. Elevated energetic demands resulting from greater reproductive intensity may require higher baseline corticosterone. Thus, the positive relationship between testosterone and corticosterone in both groups suggests an energetic demand for testosterone-regulated behavior that is met with increased baseline glucocorticoid concentrations.

Keywords: testosterone, corticosterone, latitude, elevation, amphibians, reptiles, breeding season.

Introduction

Life-history traits have been of great interest to ecologists and evolutionary biologists for decades, and geographic

variation in those traits has received much attention. For example, tropical birds have long been known to produce smaller clutches than higher-latitude species. Recently, investigators have started to address such patterns using experimental techniques, but from an ultimate (Ghalambor and Martin 2001) and not a proximate perspective. So while we are gaining some understanding of the ultimate reasons for geographic variation in life-history traits, we have a poorer understanding of the proximate mechanisms that underlie this variation (Ricklefs and Wikelski 2002). Hormones are likely associated with geographic variation in life-history traits, as hormones, especially steroids, are ubiquitous among vertebrates and are known to mediate many reproductive variables that may vary geographically. As expected, there is tremendous variation in circulating plasma concentrations of steroid hormones across vertebrates. Much of the variation can be explained by differences between the sexes and among vertebrate classes. However, even within a sex and a vertebrate class, there is substantial variation. For example, comparative meta-analyses of bird species, probably the best-studied group in this regard, have reported ranges of both baseline corticosterone and breeding-season testosterone concentrations that span 2 orders of magnitude (Goymann et al. 2004; Garamszegi et al. 2008; Bókony et al. 2009; Hau et al. 2010). Testosterone and glucocorticoids (corticosterone in reptiles, amphibians, and birds, cortisol in many mammals and fish) are the hormones that have received the most attention in comparative studies of free-living vertebrates. Over the past 3 decades, much of the focus on plasma testosterone variation has been associated with social factors such as male-male and male-female interactions, paternal care, and differences in mating systems (Wingfield et al. 1990; Hirschenhauser et al. 2003; Hirschenhauser and Oliveira 2006). Over this same period, much of the focus on plasma glucocorticoid variation has

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been associated with social interactions, seasonal changes, and environmental stressors (Sapolsky et al. 2000; Romero 2002; Moore and Jessop 2003; Romero et al. 2009). More recently, however, geography has been suggested as an important large-scale factor that influences the social factors typically proposed to explain variation in plasma hormone concentrations in birds (Goymann et al. 2004; Garamszegi et al. 2008; Bókony et al. 2009; Hau et al. 2010).

For plasma testosterone concentrations, recent studies in male birds have tried to explain interspecific variation during the breeding season. Across bird species, male testosterone appears to be negatively correlated with the length of the breeding season (Goymann et al. 2004; Garamszegi et al. 2008; Hau et al. 2010). For vertebrates in general and birds in particular, breeding seasons are typically shorter at higher latitudes and elevations (Ricklefs 1969; Morrison and Hero 2003). Consistent with this pattern, testosterone has been found to be positively correlated with latitude (Garamszegi et al. 2008) and elevation (Goymann et al. 2004), and tropical birds tend to have lower testosterone levels than temperate species in general (Goymann et al. 2004). Similar geographic influences have been proposed for baseline (samples obtained immediately upon capture) plasma concentrations of glucocorticoids. Accordingly, in a comparison of tropical and temperate bird species, baseline corticosterone concentrations were negatively related to breeding-season length (Hau et al. 2010). Further, peak plasma corticosterone concentrations, those elicited in response to capture and handling (typically 30–60 min after capture), in birds were positively related to latitude (Bókony et al. 2009).

These links between circulating steroid hormone concentrations and large-scale geographic factors suggest that breeding-season length, as determined by latitude and/or elevation, may strongly influence concentrations of testosterone and baseline corticosterone, at least in males. But why should breeding-season length be an important determinant of testosterone and corticosterone concentrations in male birds? Testosterone is typically involved in male-male competition over mates and territorial aggression (Wingfield et al. 1990; Hirschenhauser et al. 2003). Competition among males for access to females or territories is likely higher when breeding within a population is more synchronized as a result of a short breeding season (Andersson 1994; Shuster and Wade 2003). This could explain why testosterone concentrations have been linked to latitude, elevation, and breeding-season length (Goymann et al. 2004; Garamszegi et al. 2008). At baseline concentrations, the primary function of corticosterone is thought to be the regulation of uptake, storage, and mobilization of energy (Landys et al. 2006 and references therein). Hau et al. (2010) proposed that males of species with shorter breeding seasons experience greater repro-

ductive intensity, requiring mobilization of more energy reserves on a daily basis and thus higher baseline corticosterone concentrations.

While advances have been made in describing and understanding geographic variation in testosterone and glucocorticoid concentrations across species, our knowledge is at present restricted to male birds. Here we greatly expand this knowledge by reporting on geographic variation in male testosterone and baseline corticosterone concentrations in two other vertebrate classes: amphibians and reptiles. Because for many amphibian and reptile species accurate information on the length of the breeding season is unavailable, our primary focus was on variation in testosterone and baseline corticosterone concentrations in relation to latitude and elevation of the study site. Nevertheless, for a subset of amphibian and reptile species for which we had breeding-season-length data, we also investigated the relationships between breeding-season length and hormone concentrations. Latitude and elevation have been shown to explain numerous life-history characteristics in both amphibians and reptiles. For example, both latitude and elevation have been documented to be negatively related to breeding-season length and positively related to adult body size, clutch size, and age at reproductive maturity (e.g., Fitch 1970; Niewiarowski 1994; Morrison and Hero 2003; Angilletta et al. 2004; Leaché et al. 2010). Therefore, effects of latitude and elevation on breeding-season length are probably at least comparable to those in birds. In this study, we tested the following predictions. First, we expected that in both reptiles and amphibians, baseline steroid hormone concentrations would be positively related with latitude as well as with elevation. Second, we expected that in both reptiles and amphibians, breeding-season length would be negatively related with baseline steroid hormone concentrations.

Methods

Data Set

We searched the primary literature published until October 2010 and used unpublished data kindly provided upon request by several researchers; all data are deposited in the Dryad repository (<http://dx.doi.org/10.5061/dryad.4s0mg>). We compiled data on the highest reported average circulating plasma testosterone and baseline corticosterone concentrations in male reptiles and amphibians found for a given species, as described below. The highest average hormone concentrations used in this study usually, but not necessarily, came from within the breeding season. Restricting the analyses to include only hormone concentrations from within the breeding season did not change

our results in any significant way. Many studies presented a single average based on samples collected during the (sometimes supposed) breeding season. Other studies, however, presented multiple averages based on monthly sampling or on biologically relevant time periods, for example, prereproductive, reproductive, and postreproductive periods. When multiple averages were presented, we always used the highest average in our analyses. For species for which concentrations of both hormones were available, these concentrations often, but not always, came from the same study and study site. When exact hormone concentrations were not given in the text, we estimated concentrations from the graphs in the paper or obtained values from the authors upon request. Minimum sample size was set to 3 individuals for the calculation of highest average hormone concentration. In our data set, we included only hormone concentrations of free-living adult males, in their native habitat and unmanipulated, that were sampled immediately upon capture (Romero and Reed 2005). Several papers compared hormone concentrations between male amphibians that were in amplexus with or mounted on a female and those that were not. Because most other papers did not present such data, in all instances we used the hormone concentration of the males that were not in amplexus with or mounted on a female. Similarly, when hormone concentrations were presented for populations in which males follow different reproductive strategies (e.g., vocalizing, satellite, or sneaker frogs) within a species, we used the hormone concentration of the males that followed the strategy most commonly observed in other amphibians or reptiles (vocalizing in amphibians and territory defense in reptiles). We note that this approach loses some resolution, but the focus of this study was not to examine variation among alternative reproductive strategies. Further, for species that had alternative reproductive strategies, the differences in hormone concentrations between different morphs was small compared to the differences in hormone concentrations among species used in our analyses. When hormone concentrations obtained from the same study and study site were presented for two or more color morphs (lizards), we averaged the values of the different morphs before analyses. A handful of studies report only corticosterone concentrations pooled for males and females. Because in these studies corticosterone concentrations were specifically reported to not be different between the sexes, these pooled data were included in our analyses and, if anything, make our analyses more conservative.

Not all studies used the same analytical methods to measure hormone concentrations. First, most studies used radioimmunoassay procedures (some from kits), but 8 of the 163 studies reporting hormone concentrations used in our analyses were measured with an enzyme-linked im-

munosorbent assay procedure. Excluding these studies from the analyses did not change the results in any significant way. Second, only about half of the studies separated different androgens from one another using chromatography or some other means. Because some antibodies show considerable cross-reactivity with androgens other than testosterone, studies without separation might overestimate testosterone concentrations. Comparison of models with the Akaike Information Criterion revealed that models including the factor “yes/no separation of androgens” provided a better fit to the data, especially in amphibians (table 1). Therefore, to account for its effect on androgen concentrations, we entered this factor into all conventional regression analyses (see below) on testosterone. Because we are not interested in the size of the effect this separation factor has on testosterone concentrations, in the results we do not present its parameter estimates. For simplicity, we refer to all androgen concentrations as “testosterone concentration” in the remainder of the text.

Whenever possible, we obtained latitude and elevation of the study sites from the papers that were used to extract data on hormone concentrations. Such information was either obtained from the text or derived from descriptions of study sites, by means of a detailed geographical map from Google Earth (<http://www.google.com/earth/index.html>). When the description of the study site was not detailed enough, authors were contacted for the necessary information. When elevation was not reported, a digital elevation model from the Shuttle Radar Topography Mission (<http://www2.jpl.nasa.gov/srtm/>) was used to estimate the elevation. Length of the breeding season, as defined by the authors, was obtained from the papers that we used to extract data on hormone concentrations, and

Table 1: Comparison of multivariate models for male testosterone concentrations in amphibians and reptiles

Model	AIC _c	Δ _i	ω _i
Amphibians (<i>n</i> = 37):			
Latitude + elevation + separation	66.84	0	.78
Latitude + elevation	69.43	2.59	.22
Reptiles (<i>n</i> = 57):			
Latitude + elevation + separation	74.35	0	.61
Latitude + elevation	75.28	.93	.39

Note: Separation = whether androgens were separated before assay was included as a factor; AIC_c = Akaike Information Criterion corrected for small sample size; Δ_i = AIC_c difference between the best model and each candidate model; ω_i = Akaike weight (a relative estimate of the probability that a given model is actually the best model in the set of models).

for some populations it was kindly provided by the researchers upon request.

Data Analysis

Data were analyzed with both conventional regression analysis and regressions controlled for the phylogenetic nonindependence of data. Separate analyses were performed for reptiles and amphibians. The phylogenetic distances among lepidosaurs (tuataras, lizards, and snakes), turtles, and crocodylians are extremely large. Therefore, phylogenetic analysis with a composite phylogeny including all turtles, crocodylians, and lepidosaurs is inappropriate and potentially misleading without branch lengths to account for the potential amount of evolution to have occurred since their divergences. Hence, phylogenetically corrected regressions for reptiles were performed on lepidosaurs only. To be able to compare these results to those obtained with conventional regressions, additional conventional regressions were performed on lepidosaurs alone. Latitude was expressed in all analyses as absolute latitude rounded to the nearest degree (interaction terms between latitude and hemisphere were never significant, indicating that the patterns we observed are similar in both hemispheres). Elevation was expressed in kilometers and breeding-season length in months. To obtain normally distributed residuals, we log-transformed hormone concentrations before all analyses. Probabilities are two-tailed in all tests.

Conventional Regression. We analyzed the effects of the latitude and elevation of the study site and their interaction on male hormone concentrations with multiple regression. Because the interaction between latitude and elevation was significant in none of the analyses, interaction terms were dropped from all models (including independent contrasts analyses). We also determined whether male testosterone concentrations were correlated with male baseline corticosterone concentrations for those species that had both available from the same study site. Sometimes, data on a species' hormone concentrations were available from different studies or collected at different sites in the same study. Using species-average hormone concentrations would reduce variation in the data set. We therefore decided to include multiple hormone concentrations of a single species as long as the latitudes or elevations of the study sites used for hormone sampling were not identical. To correct for the resulting pseudoreplication, we entered species as a random factor into all models. Regressions and correlations were run with a normal error distribution, in MlwiN 2.02 (Rasbash et al. 2004).

Phylogenetically Corrected Regression. Common ancestry

makes species averages nonindependent of each other (e.g., Felsenstein 1985), so we performed the same sets of analyses described above with phylogeny taken into account, except that "species" was not entered as a random factor. In addition, using bivariate analyses, we assessed the strength of the relationships between the length of the breeding season and the factors latitude, elevation, and male testosterone and baseline corticosterone concentrations. Because there is no published phylogeny that includes all of the taxa we studied, we created a composite phylogeny of squamates (lizards and snakes; the tuatara is the sister taxon to squamates) with higher-order relationships from Townsend et al. (2004, 2011), and we set branch lengths equal to 1 because divergence times among the different species are unclear. Because there is some uncertainty as to the phylogenetic position of snakes within the squamates, we performed analyses with two separate phylogenetic hypotheses (Townsend et al. 2004). The first analysis placed snakes as the sister taxon of Lacertiformes, and the second placed snakes within a clade that includes Anguimorpha and Iguania (Townsend et al. 2004). However, results did not differ between the two phylogenetic placements of snakes, so for simplicity we present only results from the former placement. We were also able to obtain phylogenetic relationships of populations within *Sceloporus occidentalis* (J. Archie, unpublished data) and *Uta stansburiana* (Corl et al. 2010). We created a composite amphibian phylogeny based on that of Pyron and Wiens (2011), and we set branch lengths equal to 1. All phylogenies are presented in the appendix, available online, and have been deposited in TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S12782>). We then used the PDAP:PD TREE module (Garland et al. 1999; Midford et al. 2005) in Mesquite, version 2.72 (Maddison and Maddison 2009), to calculate standardized, phylogenetically independent contrasts (Felsenstein 1985), which we used to perform the same regression analyses explained above. Regressions and correlations of standardized contrasts were performed in SPSS 18.

Results

In amphibians, both testosterone concentration (fig. 1A) and baseline corticosterone concentration (fig. 1B) were positively related to latitude of the study site (table 2). The effect of elevation of the study site on male hormone concentrations was less clear than the effect of latitude. In amphibians, testosterone was unrelated to elevation, whereas baseline corticosterone showed a trend to be negatively related to elevation (table 2). When data were controlled for phylogenetic nonindependence, the patterns remained similar: both testosterone and baseline corticosterone concentrations were positively related to latitude

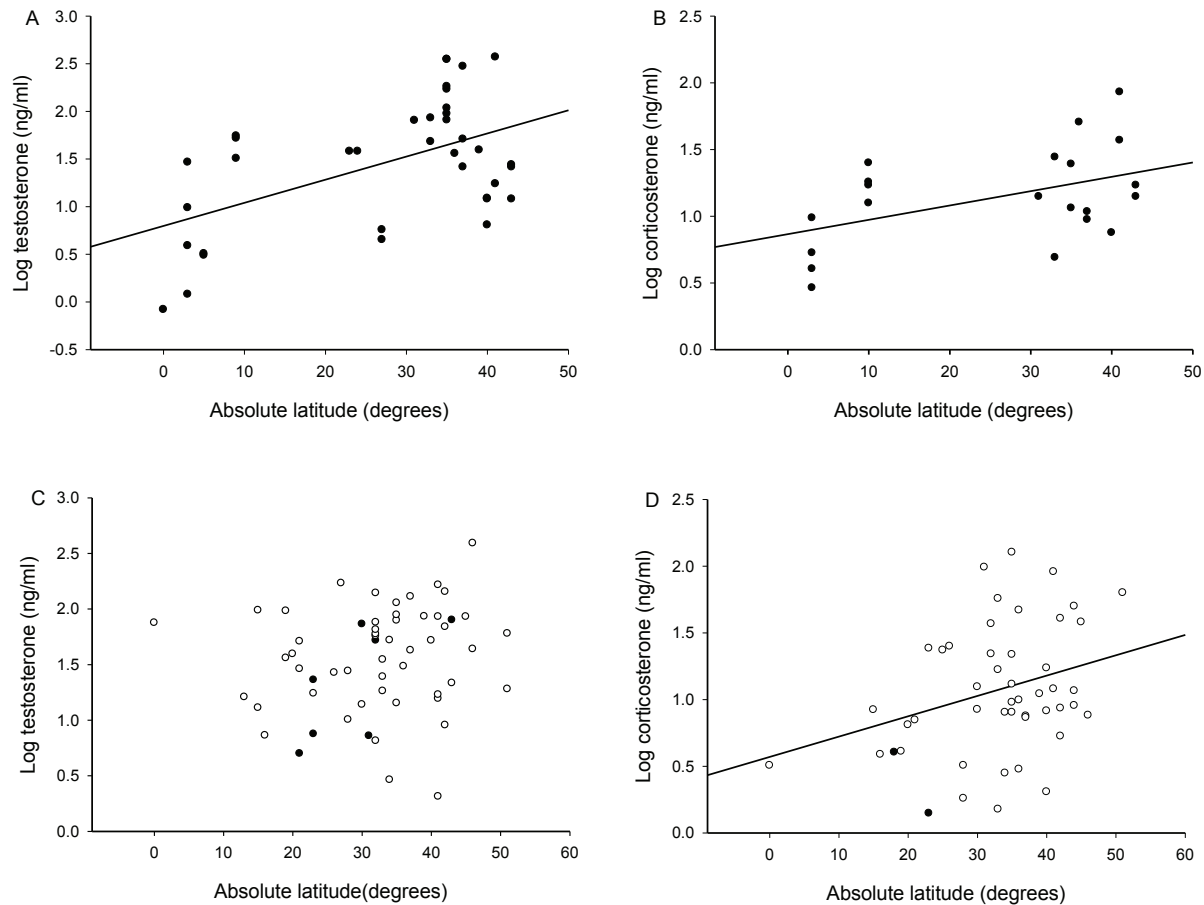


Figure 1: Relationships between absolute latitude and male plasma concentrations of testosterone (A, C) and baseline corticosterone (B, D) in amphibians (A, B) and reptiles (C, D). Open circles represent lepidosaurs, and filled circles represent turtles and crocodiles. Several species' hormone concentrations are depicted more than once because data were available from different studies or collected at different sites (with respect to latitude and elevation) in the same study. Regression lines are drawn only for significant relationships. Note that hormone concentrations are on a logarithmic scale.

(table 3). The only real change was that the nonsignificant negative trend between baseline corticosterone and elevation became statistically significant (table 3). To facilitate visualization of the large-scale latitudinal pattern in male steroid hormone concentrations, we plotted, as an example, testosterone concentrations of male amphibians on a world map (fig. 2).

To provide evidence that the observed relationships between latitude and hormones were not driven by interlaboratory variation (some laboratories had data available only from particular geographical regions, e.g., either tropical or temperate), we looked at latitudinal patterns of hormones within the amphibian genus *Bufo*. This is the most thoroughly studied genus in our data set with respect to latitude, and it has been studied by numerous different laboratories. Despite the relatively small phylogenetic dis-

tances among the *Bufo* species, hormone concentrations within this genus showed a spectacularly large range (testosterone: 1.2–370 ng/mL; baseline corticosterone: 4–85 ng/mL), and relationships with latitude (fig. 3) were consistent with the patterns we observed in the full amphibian data set. This suggests that the relationships between latitude and hormones in amphibians are real and not merely an artifact of particular laboratories working in particular geographic regions.

In reptiles, testosterone concentration was not significantly related to latitude (fig. 1C; table 2), but baseline corticosterone concentration was positively related to latitude (fig. 1D; table 2). Limiting the analyses to include only lepidosaurs did not change the results in any significant way (table 2). In reptiles, testosterone was positively related to elevation, but baseline corticosterone was un-

Table 2: Relationships between male plasma hormone (testosterone and baseline corticosterone) concentrations and absolute latitude and elevation of the study site in amphibians and reptiles

Group, hormone	Latitude			Elevation			<i>n</i>
	$\beta \pm SE$	χ^2	<i>P</i>	$\beta \pm SE$	χ^2	<i>P</i>	
Amphibians:							
Testosterone	.026 ± .006	16.51	<.001	.098 ± .169	.34	.56	37
Corticosterone	.013 ± .004	9.36	.002	-.321 ± .168	3.66	.056	21
Reptiles:							
Testosterone	.008 ± .006	2.046	.15	.203 ± .085	5.72	.017	57
Corticosterone	.018 ± .007	6.36	.012	.002 ± .153	.001	.99	48
Lepidosaurs:							
Testosterone	.006 ± .006	1.0	.32	.186 ± .087	4.53	.033	50
Corticosterone	.015 ± .007	4.57	.033	-.019 ± .152	.02	.90	46

Note: Separate regressions were performed for lepidosaurs (see “Data Analysis” for rationale). Summaries are derived from the mixed modeling procedure in MlwiN (Rasbash et al. 2004).

related to elevation (table 2). When data were controlled for phylogenetic nonindependence within lepidosaurs, the positive relationships between baseline corticosterone and latitude and between testosterone and elevation were no longer significant (table 3), so that neither latitude nor elevation were significantly related to hormone concentrations.

Bivariate analyses on the relationships between breeding-season length and both hormones and geography (using data controlled for phylogenetic nonindependence) showed that testosterone was negatively correlated with breeding-season length in both amphibians and lepidosaurs (table 3). Baseline corticosterone was negatively related to breeding-season length in amphibians but not in lepidosaurs (table 3). Latitude was negatively correlated with breeding-season length in amphibians in both the testosterone data set (Pearson’s $r = -0.86$, $P < .001$, $n = 24$) and the corticosterone data set ($r = -0.94$, $P < .001$, $n = 14$). Latitude was not correlated with breeding-season length in lepidosaurs in either the testosterone data set ($r = -0.14$, $P = .39$, $n = 42$) or the corticoste-

rone data set ($r = -0.11$, $P = .63$, $n = 23$). Elevation was not correlated with breeding-season length for amphibians or lepidosaurs in any of the data sets (all $P > .19$).

Male testosterone concentration was positively correlated with male baseline corticosterone concentration in both amphibians and reptiles (table 4; fig. 4). These relationships remained intact after phylogenetic nonindependence of data was controlled for (amphibians: Pearson’s $r = 0.70$, $P = .001$, $n = 18$; lepidosaurs: $r = 0.54$, $P = .007$, $n = 24$).

Discussion

Examining large-scale patterns of hormone concentrations in free-living vertebrates can offer insight into how factors such as geography may influence physiology and life history beyond the local factors, such as density or sexual selection, that are traditionally studied. Further, as hormones are thought to mediate many life-history traits, we can develop an understanding of the mechanisms behind

Table 3: Relationships between phylogenetically independent contrasts for male plasma hormone (testosterone and baseline corticosterone) concentrations and absolute latitude and elevation of the study site, and breeding-season length in amphibians and lepidosaurs

Group, hormone	Latitude				Elevation				BS length		
	<i>b</i>	<i>t</i>	<i>P</i>	<i>n</i>	<i>b</i>	<i>t</i>	<i>P</i>	<i>n</i>	<i>r</i>	<i>P</i>	<i>n</i>
Amphibians:											
Testosterone	.50	2.94	.006	36	-.19	-1.11	.28	36	-.56	<.01	24
Corticosterone	.66	3.50	.003	20	-.54	-2.84	.011	20	-.65	.01	14
Lepidosaurs:											
Testosterone	-.09	-.61	.55	49	.21	1.46	.15	49	-.40	<.01	42
Corticosterone	.12	.82	.42	45	.18	1.17	.25	45	-.12	.59	23

Note: Latitude and elevation were analyzed with multiple regressions, and *b* is the standardized regression slope. Breeding-season (BS) length was analyzed in separate bivariate analyses, and *r* is Pearson’s correlation coefficient.

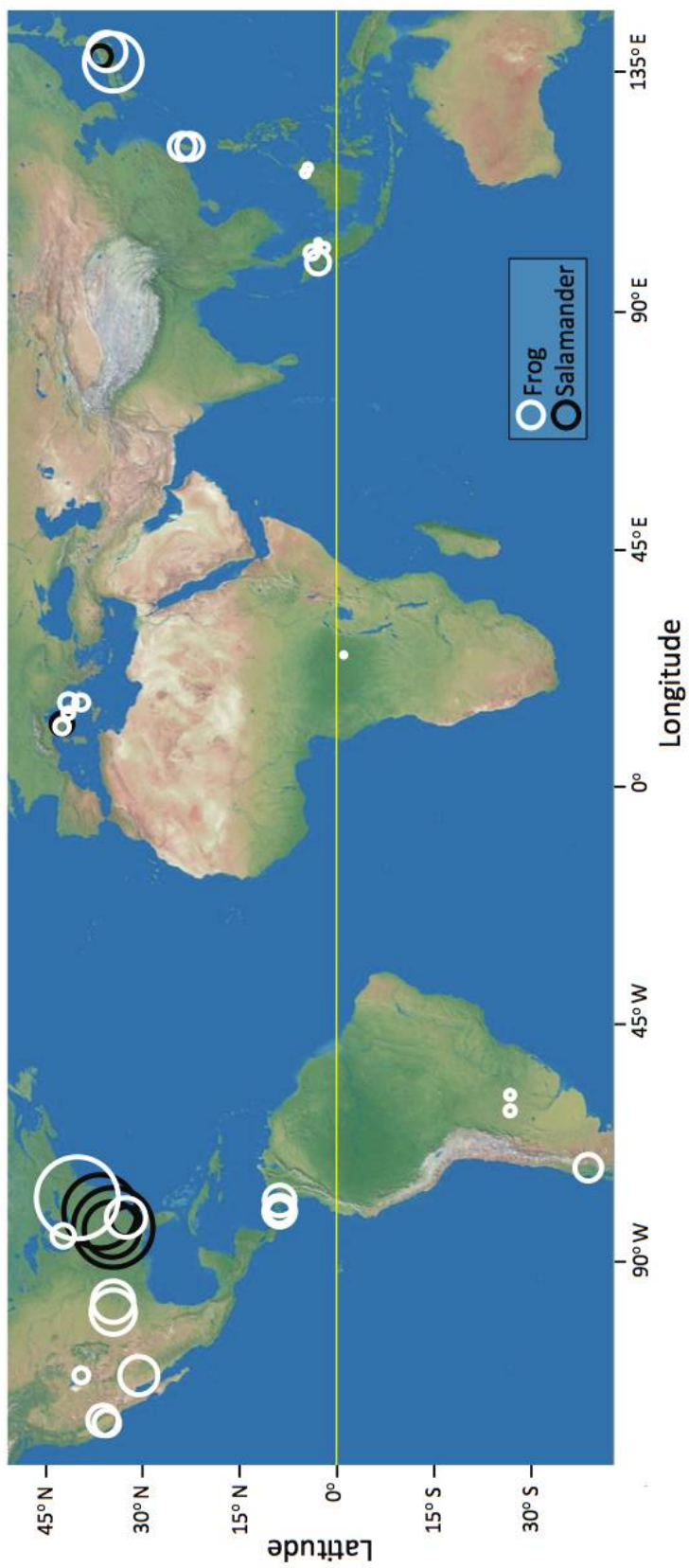


Figure 2: Bubble plot of all available testosterone concentrations in male amphibians on a world map. Bubble size is equivalent to untransformed testosterone concentrations.

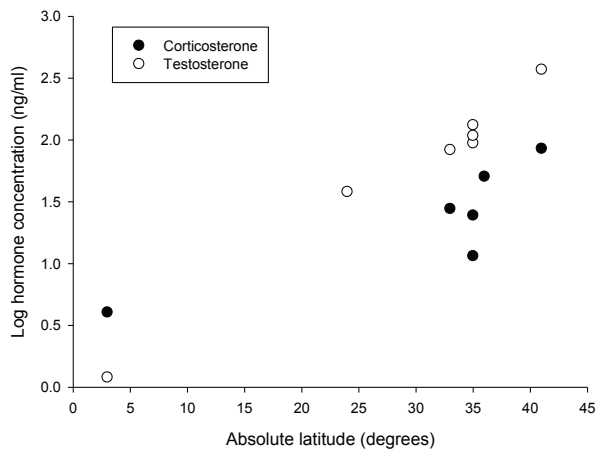


Figure 3: Relationships between absolute latitude and male plasma concentrations of testosterone and baseline corticosterone in toad species belonging to the genus *Bufo*. For *Bufo bankorensis* and *Bufo japonicus*, testosterone concentrations were available from two study sites. However, for each of these species, we plotted the average testosterone concentration of the two sites because the latitudes of the sites were either identical or differed by less than 1° (elevation did differ between sites). Note that hormone concentrations are on a logarithmic scale.

large-scale patterns of life-history traits. We found that across amphibian species, both circulating testosterone and baseline corticosterone concentrations are positively related to latitude and that baseline corticosterone concentrations are negatively related to elevation. We found no significant relationships between hormone concentrations and either of these geographical factors in lepidosaur reptiles. However, testosterone concentration was negatively related to breeding-season length in both amphibians and lepidosaurs. Breeding-season length was negatively related to baseline corticosterone concentrations in amphibians but not in lepidosaurs. Our findings on the variation in steroid hormone concentrations in amphibians and reptiles match the findings of similar comparative studies in birds. In birds, testosterone was found to be positively correlated with latitude (Garamszegi et al. 2008) and negatively correlated with breeding-season length (Goymann et al. 2004), and baseline corticosterone has been shown to be negatively correlated with length of the breeding season (Hau et al. 2010). In another comparative study on birds, baseline corticosterone and latitude were uncorrelated in bivariate analysis but appeared positively related in multivariate analysis (Bókony et al. 2009). These large-scale patterns between geography and physiology appear to be clear, but what drives the relationships (or lack thereof) between latitude (or breeding-season length) and male steroid hormone concentrations? Several mutually

nonexclusive explanations have been proposed on which we elaborate here.

Goymann et al. (2004) and Garamszegi et al. (2008) proposed that shorter breeding seasons at higher latitudes intensify male-male competition over access to territories and mates, because breeding in the population is more synchronized and there are fewer reproductive bouts per year. Because testosterone is important in many vertebrate species for mate attraction, mate guarding, and territorial aggression (e.g., Marler and Moore 1988; Wingfield et al. 1990; Wilczynski and Yang 2002; Hirschenhauser et al. 2003), male testosterone concentrations may be higher at higher latitudes because of increased selective pressures on male secondary sexual characteristics and behaviors mediated by testosterone. Another reason why male-male competition may be more intense at higher latitudes is that selection for female choosiness may be stronger at high latitudes. At higher latitudes, reproductive events are fewer, and female investment in each reproductive event (number and size of eggs) is larger than that at low latitudes (e.g., Fitch 1970; Niewiarowski 1994; Böhning-Gaese et al. 2000; Morrison and Hero 2003). Mistakes in mate choice may thus be more costly (in terms of reduced fitness) to females breeding at high latitudes. As a result, to attract a (very choosy) female, males at high latitudes may have to invest more in such traits as coloration, singing, calling, and displaying to receptive females, where the intensity of each is, in general, positively associated with testosterone concentrations (e.g., Enstrom et al. 1997; De Ridder et al. 2000; Sinervo et al. 2000; Oliveira 2004). It is important to note, however, that while some evidence for female mate choice exists in anuran amphibians, in reptiles such evidence is generally lacking (Sullivan and Kwiatkowski 2007), which makes the above-proposed mechanism less likely in this group. Intensification of male-male competition with increasing latitude may also explain the positive relationship between latitude and baseline corticosterone concentration that we observed. At baseline concentrations, corticosterone is thought to serve primarily metabolic functions, preparing and adjusting organismal functions to predictable variations in energetic

Table 4: Bivariate relationships between baseline male plasma testosterone and baseline corticosterone concentrations in amphibians and reptiles

Group	$\beta \pm SE$	χ^2	<i>P</i>	<i>n</i>
Amphibians	1.108 ± .258	18.42	<.001	19
Reptiles	.31 ± .15	4.14	.042	26
Lepidosaurs	.324 ± .162	4.01	.045	25

Note: A separate correlation was performed for lepidosaurs (see “Data Analysis” for rationale). Summaries are derived from the mixed modeling procedure in MlwiN.

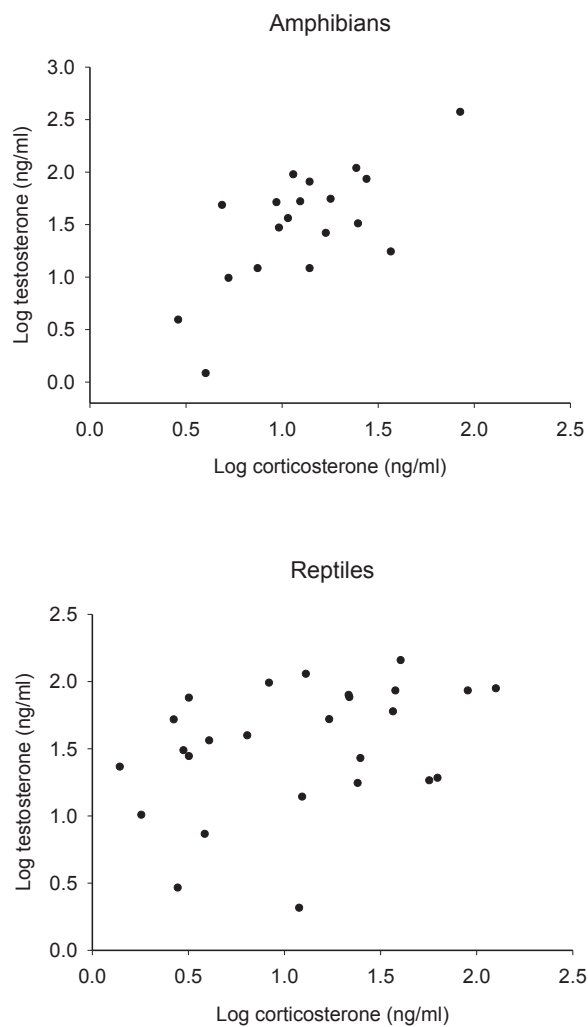


Figure 4: Relationships between male plasma concentrations of testosterone and baseline corticosterone in amphibians (*top*) and reptiles (*bottom*). For each data point, samples for testosterone and corticosterone were collected at the same study site (same latitude and elevation) and, with one exception, published in the same paper. A few species' hormone concentrations are depicted more than once because data were available from different studies. In the bottom panel, the only nonlepidosaur, the turtle *Chelonia mydas*, is represented by the leftmost data point. Note that hormone concentrations are on a logarithmic scale.

demands during the day and the year (Landys et al. 2006 and references therein). Behaviors associated with male-male competition, such as mate attraction, mate guarding, and territorial aggression, are all energetically demanding (Sullivan and Walsberg 1985; Marler et al. 1995; Emerson and Hess 2001; Husak et al. 2007). Therefore, at high latitudes with increased male-male competition, males may have to sustain higher baseline corticosterone concentrations than males at lower latitudes.

Previously, patterns of testosterone concentrations in males have been explained by the challenge hypothesis (Wingfield et al. 1990). This hypothesis has been powerful because it relies on the nature of social interactions as a way to explain and predict observed seasonal patterns of testosterone in males across all vertebrate classes. The challenge hypothesis posits that testosterone will increase above those concentrations necessary for reproduction because of male-male competition and male-female courtship. This hypothesis was expanded to include a role for glucocorticoids in the energetics-vocalization model, proposed by Emerson (2001) to explain the observed relationships between calling behavior, testosterone, and corticosterone in frogs. This model posits that in times of high male-male competition, testosterone concentrations will increase to support calling behavior and glucocorticoid concentrations will follow to mobilize the energy supplies necessary to support the calling behavior. Thus, the model predicts positive relationships between male calling behavior and testosterone as well as between concentrations of testosterone and baseline glucocorticoids. These relationships hold until glucocorticoid concentrations surpass a threshold, beyond which they suppress testosterone concentrations, and the animal stops calling and proceeds to replenish fuel reserves. Once energy supplies are renewed, glucocorticoid concentrations return to baseline, and the animal returns to calling. From this point, the pattern can begin again, and testosterone and glucocorticoid concentrations again begin to rise. This pattern seems to explain the hormonal relationships in explosively breeding amphibians particularly well. Explosive breeders have a small window of time during which all mating occurs, which will intensify male-male competition and speed up depletion of energy reserves, as compared to prolonged breeders (Halliday and Tejedo 1995). Although the temporal pattern of breeding is variable in anurans, many temperate-zone ranids and bufonids breed explosively in early spring, whereas many tropical and subtropical anurans breed more or less continuously during long rainy seasons (Hartel et al. 2007; Wells 2007). This possible bias in explosive breeding toward temperate regions may have contributed to the positive relationships we observed between latitude and both testosterone and baseline corticosterone concentrations in males. Nevertheless, tropical anurans are known to display a remarkable diversity of breeding patterns (Wells 2007), and systematic studies of how latitude may explain length of breeding are needed.

In their comparative study of birds, Hau and colleagues (2010) showed that testosterone and baseline corticosterone concentrations were uncorrelated. They reasoned that this suggests that concentrations of these hormones vary independently among species in response to environmen-

tal and life-history traits. We, however, observed a very strong positive correlation between testosterone and baseline corticosterone concentrations across amphibian species, covering a large range of latitudes. It is not unlikely that in amphibians these hormones covaried in a manner consistent with a modified interpretation of Emerson's (2001) energetics-vocalization model. That is, a truncated breeding season would result in increased testosterone, which in turn would require increased glucocorticoids to meet energetic demands associated with calling. This model may be less applicable to birds, as the relative energetic demands of vocalization are much higher for amphibians (frogs) than for birds (Emerson 2001; Ward et al. 2003). Another possibility is that we do not see a positive relationship between testosterone and corticosterone in birds because testosterone is much more sensitive to social interactions and thus more variable at any given sampling time in birds than in amphibians and reptiles. Testosterone does not seem to respond to social interactions in many reptiles (Husak et al. 2009 and references therein), and thus plasma concentrations may be near maximum concentrations throughout the breeding season in reptiles and amphibians, in contrast to birds.

There are several alternative explanations for the hormone-environment patterns we describe here. First, prolonged elevated concentrations of testosterone may incur costs that potentially reduce fitness (reviewed in Wingfield et al. 2001). Therefore, at low latitudes with extended breeding seasons, testosterone levels may be lower to diminish these costs. Alternatively, increased testosterone concentrations (e.g., during social interactions) may be so short in duration that they are rarely observed. Second, irrespective of the length of the breeding season, testosterone could be immunosuppressive in some, but not all, species (Folstad and Karter 1992). While this idea is controversial, the immunosuppressive effect of testosterone has received some empirical support with regard to parasite levels, especially in reptiles (e.g., Roberts et al. 2004; Cox and John-Alder 2007). When parasite loads are higher in the tropics, as, for example, in the eastern water skink *Eulamprus quoyii* (Salkeld et al. 2008), maintaining high plasma testosterone may be too costly because of its immunosuppressive effects. However, we note that this general scenario assumes that the risk of parasitism is correlated with latitude, a relationship that requires empirical testing. Nevertheless, this is a testable hypothesis that would explain why testosterone concentrations may be lower at low latitudes. Third, the preparative hypothesis proposes that animals will be better able to cope with predictable exposure to challenging conditions by circulating high baseline corticosterone concentrations (Romero 2002). If animals at higher latitudes are more likely to be subjected to predictable stressors (e.g., adverse, cold

weather), then one would predict higher baseline corticosterone concentrations at those higher latitudes. Finally, variables that we did not analyze but that vary geographically and are linked to male steroid hormones (e.g., metabolic rate; Miles et al. 2007) could have contributed to the patterns we observed.

Several substantial questions are raised by our results. First, why are the patterns, especially those of baseline corticosterone, so much clearer for amphibians than for reptiles? One possibility is the energetics aspect of the relationships. Hare et al. (2007) showed that several New Zealand lizard species have relatively low energetic costs of locomotion, presumably because these animals adapted to relatively cool summers. If such energy-saving mechanisms are widespread in reptiles living in cool-temperate environments (e.g., at high latitudes or elevations), this could have obscured relationships between glucocorticoids and geography in this group. Also, reptiles may be better able to mitigate energetic demands of cool-temperate environments through behavioral thermoregulation than amphibians, which may be constrained by water-balance maintenance. Again, this could have masked relationships between glucocorticoids and geography in reptiles. Finally, calling in frogs is thought to be one of the most energetically costly courtship behaviors in vertebrates (e.g., Sullivan and Walsberg 1985; Emerson 2001). That factor, along with the possibility of a bias for explosive breeding toward temperate regions (Hartel et al. 2007; Wells 2007), might drive the relationships between testosterone, glucocorticoids, and latitude in amphibians. A second potential explanation for the discrepancy in findings between amphibians and reptiles is that amphibians display marked variation in degree of parental care (Duellman and Trueb 1986; Heatwole and Sullivan 1995; Wells 2007), whereas lepidosaurs show less variation (Fox et al. 2003 and references therein). The larger variation in amphibians in the degree of paternal care may have resulted in divergent selection on male baseline corticosterone concentrations. At baseline, the primary function of corticosterone is to regulate the uptake, storage, and mobilization of energy (e.g., Sapolsky et al. 2000; Landys et al. 2006). Caring for offspring is energetically demanding, and (species-specific) variation in the degree to which males engage in care may be reflected in male baseline corticosterone concentrations. A final possibility is that we simply do not have sufficient data (i.e., lack of low-latitude reptile studies) or that the data were originally collected in such a variety of ways that existing patterns did not emerge. In this regard, it is worth noting that the significant patterns we describe here are, in fact, probably stronger than we observed. The reason for this is that most of the studies in our data set sampled hormones in a single breeding season, while average male steroid hormone concentrations in a popula-

tion may fluctuate between years (e.g., Moore et al. 2001; Schuett et al. 2005; Vitousek et al. 2008) as a result of variation in social (e.g., breeding density) or environmental factors (e.g., weather or food availability). The resulting sampling error may very well have weakened or masked geographical patterns in hormone concentrations. The fact that we were able to describe significant relationships between the static geographical variable latitude and male steroid hormone concentrations suggests that the relationships between environment (latitude in particular) and hormone concentrations are extremely strong.

Second, why did we find, against our predictions, that in amphibians testosterone is unrelated to, and baseline corticosterone is negatively related to, elevation and yet support our prediction that both hormones are positively related to latitude? For testosterone, one possibility is that we simply did not have the resolution to detect an effect of elevation on testosterone concentrations. The only comparative study reporting a relationship between elevation and testosterone (Goymann et al. 2004) had a considerably larger proportion of high-elevation species (>1,000 m) in their data set than did we. The negative relationship between baseline corticosterone and elevation in amphibians is unclear, especially since we found no relationship between elevation and breeding-season length. Future studies will help us to understand whether the underlying mechanism for this relationship is due to energetics associated with breeding-season length, which we may not have detected with our small sample size, or to energetics associated with thermoregulatory efficiency at high elevations.

Third, why did inclusion of phylogeny in our analyses change the results for reptiles, whereas previous studies on interspecific variation in testosterone and corticosterone concentrations in birds concluded that phylogenetic signal was very weak or absent (Garamszegi et al. 2008; Bókonyi et al. 2009; Hau et al. 2010)? Why this is the case in lepidosaur reptiles will require further comparative data across disparate clades, but one explanation could be related to the general differences in mating systems between lizards and snakes. Whereas lizards are documented to have a wide variety of social organizations and mating systems (e.g., Stamps 1983; Fox et al. 2003), snakes are not known to be territorial and instead tend to have mating strategies that involve mate searching (e.g., Arnold and Duvall 1994; Rivas and Burghardt 2005). This would suggest that factors other than geography may be more important for determining testosterone concentrations in reptiles. Nevertheless, in lepidosaurs we did find a negative relationship between testosterone and breeding-season length as well as a positive relationship between testosterone and baseline corticosterone concentrations, which matches our findings for amphibians. In the case of lepidosaurs, however, breeding-season length was not cor-

related with latitude (or elevation), which explains the lack of a relationship between latitude (or elevation) and hormone concentrations. Thus, increased testosterone in reptiles associated with increased breeding-season length likely also requires increased glucocorticoids to meet energetic demands associated with aggressive and courtship behavior mediated by testosterone.

Despite large physiological differences (e.g., endothermic vs. ectothermic) and distant phylogenetic relatedness between groups, our results agree with previous findings in birds. This indicates that breeding-season length and its association with latitude in some, but not all, taxa may strongly influence male vertebrate steroid hormone concentrations. The fact that testosterone and baseline corticosterone concentrations were positively correlated across species in both classes suggests that, at least in these ectothermic groups, the energetic demands of acquiring mates with secondary sexual traits regulated by testosterone are met with increased baseline corticosterone. Such interspecific physiological correlations may be more widespread than once thought, and future comparative studies will be able to test the generality of the relationships we found as well as to reveal the exact selective forces that result in such correlations. Finally, we have added to our understanding of the hormonal mechanisms behind some of the life-history traits that have long been known to vary with latitude.

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