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Life history and environment predict variation in testosterone across vertebrates

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Endocrine systems act as key intermediaries between organisms and their environments. This interaction leads to high variability in hormone levels, but we know little about the ecological factors that influence this variation within and across major vertebrate groups. We study this topic by assessing how various social and environmental dynamics influence testosterone levels across the entire vertebrate tree of life. Our analyses show that breeding season length and mating system are the strongest predictors of average testosterone concentrations, whereas breeding season length, environmental temperature, and variability in precipitation are the strongest predictors of within-population variation in testosterone. Principles from small-scale comparative studies that stress the importance of mating opportunity and competition on the evolution of species differences in testosterone levels, therefore, likely apply to the entire vertebrate lineage. Meanwhile, climatic factors associated with rainfall and ambient temperature appear to influence variability in plasma testosterone, within a given species. These results, therefore, reveal how unique suites of ecological factors differentially explain scales of variation in circulating testosterone across mammals, birds, reptiles, amphibians, and fishes.

Endocrine systems act as an important interface between environmental stimuli and organismal function by coordinating physiological homeostasis among body systems (Flatt et al.

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2005; Amdam et al. 2007; Lema and Kitano 2013). Steroid hormones are key to this regulation, simultaneously shaping growth, morphology, and social behavior (Ketterson and Nolan 1992; Zera et al. 2007). Testosterone is perhaps the most widely studied steroid hormone—it either directly or indirectly modulates the expression of sexually dimorphic traits and sex-specific behavior in males, and it mediates environmental influences on reproductive physiology (Wingfield et al. 2001; Hau 2007). Yet despite these important physiological and behavioral roles, circulating levels of testosterone (as well as other hormones) are notorious for their extreme variability within individuals over time, among individuals in a population, and across species. Such variability in concentrations is not surprising given the role of steroid hormones in developmental processes and in responding to the environment; however, a defining goal of evolutionary endocrinology is to uncover the general factors that predict this variability not only within populations, but also among species (Williams 2008).

Currently, several large-scale environmental factors that influence average testosterone levels have been identified. Latitude, for example, is consistently a strong positive predictor of mean testosterone levels in males within the major vertebrate groups (Wingfield and Farner 1980; Hau 2001; Goymann et al. 2004; Garamszegi et al. 2008; Eikenaar et al. 2012). However, latitude itself is also a proxy for numerous abiotic and biotic factors that covary with geography. Higher latitudes are associated with lower mean temperatures, more pronounced seasons, and shorter breeding seasons, but precipitation patterns do not vary linearly with latitude due to continental effects and atmospheric circulation within seasons (Adler et al. 2017). Latitude also covaries with key life-history traits (Gotelli and Pyron 1991; Morrison and Hero 2003; Londoño et al. 2015; Varpe 2017), including several associated with testosterone levels such as the degree of male parental care (Martin et al. 2000; Goymann and Landys 2011; Londoño et al. 2015). In total, these patterns suggest the hypothesis that climate places coarse bounds on steroid hormone signaling and some aspects of life history (e.g., breeding duration, adult size), while other life-history traits (e.g., mating system, social organization) fine-tune reproductive endocrinology to match the specific selective pressures in a population (Moore et al. 2019). A robust analysis that explores the large-scale factors that drive testosterone levels could disentangle these distinct correlates of latitude, partitioning the explanatory power of each.

Although environmental factors are likely important predictors of testosterone concentrations, it has not previously been possible to test this in a large evolutionary framework due to a lack of aggregated data at a large phylogenetic scale. We amassed such a dataset to directly test how environmental characteristics influence testosterone levels across the vertebrate tree of life (Vitousek et al. 2018). The magnitude of variation in testosterone levels, as well as average testosterone levels within a population, are strongly determined by the ability of individuals to use environmental cues for successful reproduction (Hau 2001; Hau et al. 2010). In temperate regions, seasonal changes in photoperiod provide predictable cues that enable animals to match breeding with food availability, generally resulting in synchrony among individuals in reproductive state and thus higher testosterone levels (Hau 2001; Moore et al. 2005; Dawson 2008). In the tropics, on the other hand, weak photoperiodic cues force a reliance on other environmental cues to time reproduction, which can lead to asynchrony in reproductive readiness among individuals, especially if environmental conditions are spatially or temporally variable (Hau 2001). Consequently, many tropical species have more flexible control of breeding (Hau et al. 2000; Goymann and Helm 2015). In short, species at low latitudes should have more among-individual variability in the endocrine regulators of reproduction (e.g., male testosterone) at a given time of the breeding period compared to temperate species due to increasing variation in important environmental cues. Several studies have revealed relatively consistent predictors of mean testosterone levels, but none have included locality-specific environmental data from the populations sampled to consider what factors explain either average testosterone levels or among-individual variability in testosterone levels across diverse vertebrate species (Goymann et al. 2004; Hirschenhauser and Oliveira 2006; Garamszegi et al. 2008; Eikenaar et al. 2012).

Here we test how different life-history traits and environmental factors explain species differences in mean male testosterone during the breeding period, as well as within-species variability in male testosterone. We used HormoneBase, an open-access database of circulating hormone levels in free-living vertebrates (Vitousek et al. 2018), to conduct a phylogenetically informed analysis of data from 1139 sampling periods in 280 populations of 230 species across all vertebrate groups (fishes [jawless, bony, and cartilaginous], amphibians, reptiles, birds, and mammals). We combined hormone data of males during their breeding periods with life-history data obtained from the primary literature (Johnson et al. 2018; Vitousek et al. 2019), as well as temperature and precipitation data for each study location during the season of hormone data collection obtained from the Climatic Research Unit Time-Series Data (Harris et al. 2014). We then compared candidate models to explain mean testosterone and testosterone variation (using the coefficient of variation of testosterone levels from each population). We used phylogenetically informed Markov Chain Monte Carlo generalized mixed model comparisons to determine whether an environmental, life history, or omnibus model with all variables (Table 1) best fits the hormone data.

Table 1. Comparison of candidate models of mean testosterone levels and variation in testosterone levels.

Mean Testosterone			Testosterone Variation		
Model	**DIC**	**ΔDIC**	**Model**	**DIC**	**ΔDIC**
Omnibus	**3203.5**	**0**	**Omnibus**	**2007.5**	**0**
Life history	3206.4	2.9	Life history	2010.1	2.6
Environmental	3213.3	9.8	Environmental	2015.5	8.0
Intercept only	3217.5	14.0	Intercept only	2016.6	9.1

Note. All models included "Hormone laboratory identity," Species, and Population ID as fixed effects.

Materials and Methods

HORMONE DATA

Data on circulating testosterone levels were obtained from HormoneBase (Vitousek et al. 2018), a publicly available database of circulating plasma steroid hormone levels across vertebrate populations. All data are from adults in free-living populations in which data from each sex were recorded separately. Our analysis used data on mean testosterone concentrations and their corresponding coefficients of variation (CV) for males obtained during their breeding season. For the mean testosterone measure, we used the highest mean testosterone levels that were reported during the breeding period for each population. If multiple populations of the same species were reported within the breeding period, we included the highest mean for each population (and when available, its corresponding CV), accounting for species identity (see below). Thus, each CV represented variation among males within the population at the time when reported testosterone was highest. Analyses were conducted on a rigorously filtered dataset (details in Supporting Information Methods) that included 1139 measures of mean testosterone concentrations and 963 measures of testosterone variation (CV within a population). The discrepancy in sample sizes between analyses of mean and CV is because not all studies that reported mean testosterone levels reported variation. Because of available data in the database, the testosterone variation dataset had fewer species than for mean testosterone levels. Thus, the filtered data set for mean testosterone levels included 31 species of amphibians, 31 species of fishes, 23 species of mammals, 66 species of reptiles, and 79 species of birds, and for testosterone variation included 25 species of amphibians, 26 species of fishes, 21 species of mammals, 60 species of reptiles, and 70 species of birds. Although 11-keto-testosterone (KT) is perhaps a more biologically active androgen than testosterone in some fishes, we used testosterone levels for three reasons: (1) testosterone and KT levels are significantly correlated across 31 fish species ($P < 0.004$, Supporting Information Fig. S1), (2) this approach allowed the same androgen to be examined across all species, eliminating potential issues related to binding affinity differences between testosterone and KT, and (3) using testosterone levels gave us a larger sample size of fish species.

PHYLOGENETIC TREE

A species-level phylogenetic tree of the species included in HormoneBase was constructed as described previously (described and visualized in Johnson et al. 2018; Vitousek et al. 2019). Briefly, taxonomy was matched between HormoneBase and lineage-specific trees for fishes (Rabosky et al. 2013), amphibians (Pyron and Wiens 2011; Eastman et al. 2013), mammals (Bininda-Emonds et al. 2007), squamates (Pyron et al. 2013), turtles (Jaffe et al. 2011), and birds (Jetz et al. 2012). We dropped tips from the HormoneBase tree using the *ape* package (Paradis and Schliep 2018) in R version 3.4.3 (R Core Team 2017) to match species in the filtered dataset described above.

ENVIRONMENTAL DATA

We obtained data on mean monthly temperature and precipitation for each study location from the Climatic Research Unit Time-Series Data (CRU-TS; Harris et al. 2014; Johnson et al. 2018), which includes data on monthly variation in 0.5 degree grids across the globe. We then calculated the 51-year average of seasonal mean temperature and precipitation, and of inter- and intraseasonal variation (standard deviation) in temperature and precipitation for all study locations. This timeframe includes the time over which most HormoneBase data were collected (1965-2015). We grouped seasons in 3-month intervals (March-May, June-August, September-November, December-February) and restricted our environmental analyses to the season in which samples were collected so that each study was assigned the single relevant season in which data were collected (3-month intervals specified above). Since HormoneBase includes information on the months in which data were collected, we were thus able to match environmental data and hormone data. If months were not specified in the original article, we used data from the corresponding season sampled. Hormone samples collected in multiple seasons were assigned to the first season if sampled in two consecutive seasons, or the middle season if sampled in three consecutive seasons. We excluded studies that pooled samples across all four seasons or multiple nonconsecutive seasons, as well as studies that did not specify the month or season of sample collection.

Because measures of environmental variation (inter- and intraseasonal variation in temperature and precipitation) were correlated (see Supporting Information), we chose to focus our analyses on a subset of uncorrelated variables that we predicted were most likely to influence testosterone: intraseason variation in temperature and precipitation. Although this approach somewhat limits our ability to explore the role of other environmental factors in shaping testosterone regulation, it circumvents concerns associated with the use and interpretation of principal components in phylogenetic comparative analyses (Uyeda et al. 2015; Adams and Collyer 2018). Latitude and elevation are both coarse proxies for combinations of environmental parameters, so were not included in our final candidate models (see Supporting Information).

LIFE-HISTORY DATA

We compiled life-history data from primary literature and public databases on all species contained in the HormoneBase database as described elsewhere (Johnson et al. 2018; Vitousek et al. 2019). Whenever possible, we included data that were specific to the sampled populations, but when not available, we included data from other populations of the species. Traits included male body mass, breeding season length, degree of sociality during the breeding season (classified as solitary or not for this analysis), mating system (polygynous, monogamous, or other [cooperative, parasitic, polyandrous]), whether males exhibit parental care or not, and whether oviparous or viviparous. Male body mass was not correlated with mean testosterone levels ($P = 0.84$) or with variation in testosterone levels ($P = 0.97$) using phylogenetically informed techniques described below, so it was not included in analyses.

STATISTICAL ANALYSIS

We used phylogenetically informed Markov Chain Monte Carlo generalized mixed models to compare candidate models of mean testosterone levels and within-population variation (CV) in testosterone levels using the MCMCglmm package (Hadfield 2010) in R version 3.4.3 (R Core Team 2017). This is the same approach as some previously published uses of HormoneBase data (Vitousek et al. 2019; Injaian et al. 2020). Hormone data and other continuous variables were natural log-transformed. We added a constant (+30) to seasonal mean temperature to ensure that all values were greater than zero before transformation. Candidate models used a Gaussian distribution, and initially used uninformative inverse Gamma priors (v = 1, nu = 0.002). We ran models for 1,000,000 iterations with a burn-in of 50,000 and a thin of 200. We fit each model three times to confirm model stability. Finally, we reran each model with highly informative priors (nu = 1) to confirm that prior specification was not impacting results. Generalized linear mixed models included "species" (the matrix of phylogenetic relatedness) as a random effect. Measured levels of hormone concentrations can differ among laboratories conducting the assays (Fanson et al. 2017), so we also included "hormone lab identity" as a random effect (Vitousek et al. 2019). Single-hypothesis models (environment, life history; Table 1) were competed against an omnibus model that contained all fixed effects, as well as an intercept-only model.

Results

The best candidate model for mean testosterone levels of breeding males was the Omnibus model (Table 2). Breeding season length and mating system type were the only statistically significant life-history variables in the Omnibus model, and within-season temperature variation was the only significant environmental variable. Shorter breeding seasons were associated with higher mean testosterone levels (Fig. 1A), and males with mating systems other than social monogamy or polygyny (i.e., cooperative, parasitic, forms of polyandry) had lower testosterone levels. Furthermore, higher mean testosterone levels were associated with greater within-season temperature variation.

The best-fit model for within-species variation in testosterone was the omnibus model that included all life-history and environmental variables (Table 2), but significant predictor variables differed from the mean testosterone analysis. Testosterone was more variable within populations that have longer breeding seasons (Fig. 1B) and that are in environments with low mean seasonal temperatures and high intraseasonal precipitation variability (Fig. 1C). Thus, variability in testosterone levels matched variability in precipitation, both within and among seasons, but not variability in temperature.

Discussion

Our analysis of hundreds of species across all vertebrate groups, combined with temperature and precipitation data from the sites where hormones were sampled, as well as life-history traits, provides the most powerful test yet of large-scale predictors of testosterone levels. We found that breeding season length is important for both aspects of testosterone signaling, where shorter breeding seasons are associated with higher mean testosterone levels and lower variation in testosterone. The type of mating system in a population was also important for mean testosterone levels. These results combined emphasize the likely importance of mating opportunities and competition in driving testosterone

Table 2. **Predictors of testosterone signaling. The best fit models of mean testosterone levels in free-living vertebrates and variation in testosterone within populations.**

Mean testosterone (Omnibus model)	Posterior Mean	Lower 95% CI	Upper 95% CI	Effective sample size	*P* MCMC
(Intercept)	2.262	−1.075	5.881	4975	0.190
Male parental care	−0.243	−0.745	0.216	4975	0.320
Mating system polygynous	0.083	−0.369	0.510	4975	0.731
Mating system other	**−0.722**	**−1.220**	**−0.191**	**4975**	**0.007**
Viviparity	−0.352	−1.209	0.397	4975	0.395
Breeding season length	**−0.125**	**−0.185**	**−0.069**	**4975**	**<0.001**
Sociality	0.343	−0.124	0.820	4975	0.153
Temperature	−0.249	−0.797	0.386	4975	0.399
Precipitation	−0.007	−0.053	0.038	4975	0.781
Within-season temperature variation	**0.222**	**0.036**	**0.424**	**4975**	**0.026**
Within-season precipitation variation	0.087	−0.023	0.209	4396	0.150
Testosterone variation (Omnibus model)					
(Intercept)	**5.150**	**3.323**	**6.999**	**4975**	**<0.001**
Male parental care	0.189	−0.093	0.469	4684	0.191
Mating system monogamous	−0.066	−0.370	0.238	4975	0.678
Mating system polygynous	−0.260	−0.554	0.067	4975	0.105
Viviparity	0.057	−0.286	0.428	4975	0.731
Breeding season length	**0.052**	**0.014**	**0.089**	**4975**	**0.006**
Sociality	−0.182	−0.446	0.062	4639	0.157
Temperature	**−0.429**	**−0.855**	**−0.001**	**4555**	**0.049**
Precipitation	−0.017	−0.055	0.020	4487	0.360
Within-season temperature variation	−0.004	−0.141	0.144	4975	0.949
Within-season precipitation variation	**0.129**	**0.038**	**0.219**	**4975**	**0.008**

Note. Fixed effects are shown in bold where the 95% confidence interval of the mean does not cross zero.

concentrations across the entire vertebrate lineage (mammals, birds, reptiles, amphibians, and fishes; Goymann et al. 2019). Nevertheless, future studies that also include testosterone levels of females and resultant offspring will help to better understand the constraints placed on the evolution of testosterone signaling (Rosvall et al. 2020). At high latitudes, where breeding seasons are shorter, and temperatures likely vary more, many vertebrate species have less time to prepare for, and successfully carry out, breeding before resources are gone (Hau 2001; Goymann et al. 2004; Moore et al. 2019). Thus, males in these environments will have highly synchronous breeding with intense competition and simultaneously high testosterone levels to facilitate interactions with rival males and potential mates (Ketterson and Nolan 1992; Wingfield et al. 2001; Hau 2007; Goymann et al. 2019). We note that we detected no significant correlation between mean testosterone levels and degree of sociality, presence of parental care, or reproductive mode across our wide sample of vertebrates. Our results generally support previous smaller-scale comparative studies (Hirschenhauser and Oliveira 2006; Garamszegi et al. 2008; Eikenaar et al. 2012), revealing that the pattern is generalizable across vertebrates.

The applicability of the link between sociosexual factors (breeding season length and mating system) and species variation in testosterone is notable for several reasons. Foremost, this relationship suggests that principles of evolutionary endocrinology developed in certain vertebrate clades also apply to other major (distantly related) lineages. For example, we find that breeding season length and mating system similarly appear to influence testosterone levels in groups of ocean fishes. Such taxa often have seasonal breeding due to seasonality of primary production (Merrett 1987), or they engage in seasonal migrations (Mauchline 1988; Varpe 2017). Fish also show a range of mating systems, with decades of research showing that their reproductive design is shaped by intense sexual selection. In this way, it makes sense that testosterone systems in fish evolve accordingly to the same principles that govern their evolution in birds or mammals.

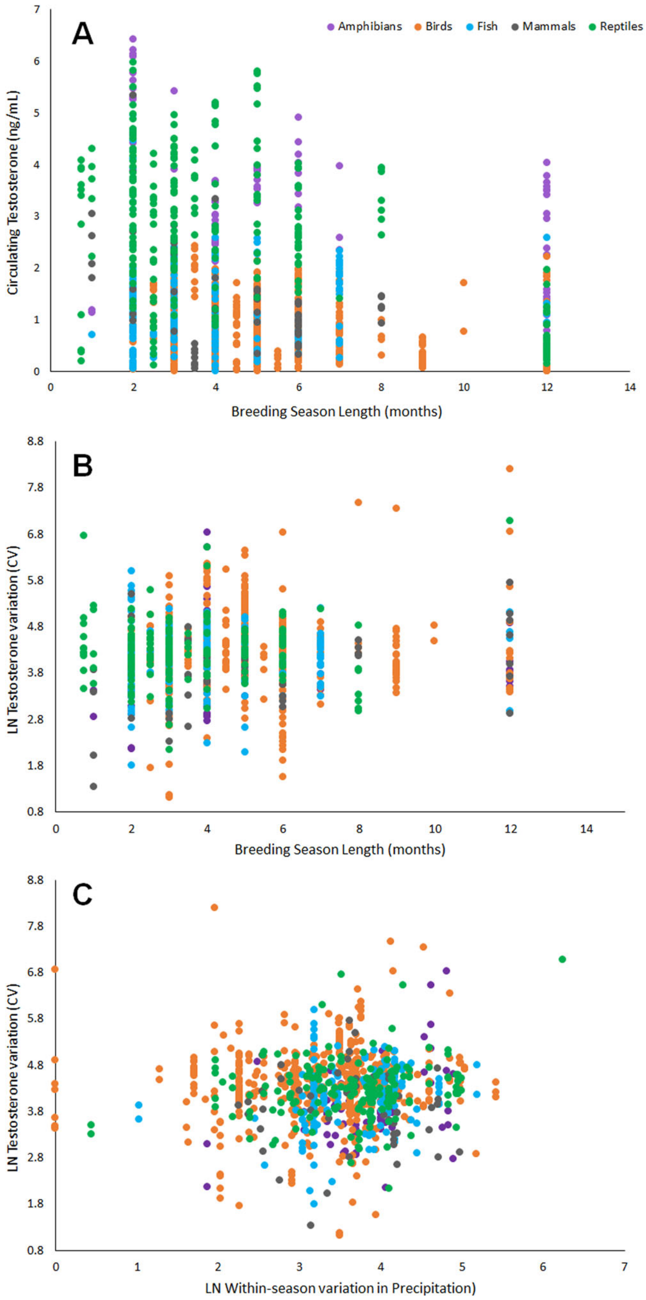

Figure 1. Testosterone signaling is related to life-history traits and environmental variability. Mean testosterone levels decrease with increasing breeding season length (A), but testosterone variability increases with increasing breeding season length (B) and increases with increasing variability in precipitation (C). Figures show mean T levels and testosterone variation that are natural-log transformed as described in the text.

Of course, at the same time we must recognize that the available data on testosterone in fishes are still largely lacking, particularly compared to the high numbers of mammal and bird taxa in our study. We would certainly benefit from a much larger sample of fish species, which dominate vertebrate diversity, to truly generalize across all terrestrial and aquatic vertebrates. Regardless of these considerations, our study provides an important platform to begin thinking about common forces that shape how androgenic systems evolve from species to species across the entire vertebrate tree of life.

On the other hand, within-species variation in testosterone was best explained by breeding season length and environmental factors. To our knowledge, ours is the first study to test for predictors of testosterone variability at such a large phylogenetic scale. Across all major vertebrate groups, our results support the proposition that reproduction should be synchronous in short breeding seasons and when resources are predictably high, but synchrony should be low when resources are variable (Wingfield and Farner 1980; Martin et al. 2000; Hau 2001). Warmer seasonal temperatures were associated with lower variability in testosterone, which may be due to abiotic or biotic mechanisms. Higher average environmental temperatures may increase the chance that more individuals will maximize testosterone production, especially in ectotherms. Comparative studies suggest that, at least in some taxa, extra-pair paternity rates are higher in warmer temperatures, perhaps due to sperm competition (Johnsen and Lifjeld 2003). This suggests that there may be selection on males to have less variable testosterone levels due to higher competition, regardless of breeding synchrony (Stutchbury and Morton 1995). Alternatively, species from warmer regions may use subtler photoperiodic cues to time their reproduction with available food resources (Hau 2001; Moore et al. 2019). Subsequently, those species should have more flexibility in the control of their reproduction and rely more on other environmental cues (Hau et al. 2000). Within-season variability in precipitation at the population level was significantly and positively correlated with variability in testosterone levels. High precipitation variability may result in unpredictable food resources for reproduction, and thus, more difficulty in matching breeding with those food resources. This suggests that the ability to predict important environmental events, such as rainfall, determines the extent to which individuals activate their reproductive axis and synchronize reproduction (Hau et al. 2000, 2004; Hahn et al. 2008; Shine and Brown 2008). We note again that the generalizability of these patterns to fishes, especially pelagic species, needs confirmation with future studies.

Our detection of a strong environmental signal in testosterone variability despite data coming from multiple laboratories and across decades of research suggests a strong relationship among these variables. In short, even though hormone levels can vary tremendously among individuals (Kempenaers et al. 2008; Williams 2008; Taff and Vitousek 2016; Miles et al. 2018), large-scale climatic factors appear to govern variability among and within species. As global climate change continues, altered patterns of rainfall and temperature may have heretofore unknown consequences to vertebrate reproductive physiology and homeostasis, as well as other body systems that are functionally linked by testosterone.

AUTHOR CONTRIBUTIONS

All authors aggregated the data. J.F.H. and M.N.V. analyzed the data. J.F.H., M.J.F., M.N.V., and M.A.J. wrote the article. All authors contributed to the text.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Relationship between 11-ketotestosterone levels and testosterone levels across 31 fish species (38 populations).