10-2018

# Illuminating Endocrine Evolution: The Power and Potential of Large-Scale Comparative Analyses

Maren N. Vitousek

Michele A. Johnson
*Trinity University*, mjohnso9@trinity.edu

Jerry F. Husak



## Repository Citation

Vitousek, M. N., Johnson, M. A., & Husak, J. F. (2018). Illuminating endocrine evolution: The power and potential of large-scale comparative analyses. *Integrative and Comparative Biology, 58*(4), 712-719. https://doi.org/10.1093/icb/icy098

Integrative and Comparative Biology

*Integrative and Comparative Biology*, volume 58, number 4, pp. 712–719
doi:10.1093/icb/icy098

*Society for Integrative and Comparative Biology*

SYMPOSIUM INTRODUCTION

# Illuminating Endocrine Evolution: The Power and Potential of Large-Scale Comparative Analyses

Maren N. Vitousek,[1,*,†] Michele A. Johnson[‡] and Jerry F. Husak[§]

*Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA; †Cornell Lab of Ornithology, Cornell University, 159 Sapsucker Woods Road, Ithaca, NY 14850, USA; ‡Department of Biology, Trinity University, San Antonio, TX 78212, USA; §Department of Biology, University of St. Thomas, 2115 Summit Avenue, St. Paul, MN 55105, USA

From the symposium "Understanding the Evolution of Endocrine System Variation through Large-scale Comparative Analyses" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2018 at San Francisco, California.

[1]E-mail: mnv6@cornell.edu

**Synopsis** Hormones are central mediators of genotype–phenotype and organism–environment interactions. Despite these important functions, the role of selection in shaping hormonal mediators of phenotype remains poorly understood. Thanks to decades of work by endocrinologists, circulating hormone levels have been measured in a diversity of organisms. Variation in other endocrine traits and mediators (e.g., receptor expression and binding globulins), and the hormonal response to standardized challenges (e.g., restraint, pharmacological challenges) are also increasingly measured in both captive and free-living populations. Large-scale comparative analyses of the multitude of available endocrine data represent a particularly promising approach to addressing the function and evolution of these key phenotypic mediators, and their potential to serve as indicators of disturbance. Several recent phylogenetic comparative analyses and meta-analyses have begun to reveal the power and potential of these approaches to address key questions in integrative biology. Here we highlight two recent developments that are facilitating such analyses: increasingly powerful and flexible phylogenetic comparative methods, and the release of two endocrine trait databases—HormoneBase (currently 474 species) and the Wildlife Endocrinology Information Network (currently 25 species)—that contain compiled measures of endocrine traits across vertebrates. Increasingly comprehensive comparative analyses of endocrine data could provide insight into many interesting questions, including how rapidly changing environments are impacting phenotypes, why endocrine traits differ so remarkably within and across populations, and the evolution of plasticity. The endocrine system mediates interactions between genotypes and phenotypes, and between organisms and their environment. Environmentally induced hormonal responses regulate phenotypic flexibility across timescales by altering physiological state, gene expression, and epigenetic marks. A staggering diversity of phenotypic traits are mediated by hormones from early development through senescence. Through their actions on behavior, hormones also exert widespread influence over how organisms interact with their biotic and abiotic environments. Because hormones are responsive to the environment, there has long been interest in their use as biomarkers of exposure to challenges. More recently, increasing attention has been paid to the potential for within and among-population variation in endocrine regulation or responsiveness to serve as indicators of resistance or resilience to future challenges, or measures of evolutionary potential.

## Evolutionary endocrinology: the importance of variation

Despite the many crucial roles of hormone systems, their evolution remains poorly understood. Because hormones mediate a diversity of fundamental biological processes, selection acting on the heritable components of hormonal traits might be expected to constrain circulating hormone levels around one or more fitness optima (Ketterson and Nolan 1999; Hau 2007). Yet empirical data reveal

Advance Access publication July 18, 2018

that hormone levels are remarkably variable, not only among species, but also within populations, sexes, and life history stages (Williams 2008; Miles et al. 2018). Why do some females in a population have substantially higher plasma estradiol levels than others during the same process of egg production? Similarly, why are plasma testosterone levels an order of magnitude greater in some species than in other species, when testosterone mediates the same basic reproductive processes in each?

Determining how selection shapes patterns of hormone secretion across broad taxonomic scales requires an integration of the traditionally disparate approaches of evolution and physiology. Characterizing variation in heritable traits and determining their fitness effects is a cornerstone of evolutionary biology, but physiological research has historically focused on comparing mean trait values between and among groups of organisms (e.g., sexes, life history stages, or species), without addressing the causes and consequences of the often dramatic inter- and intra-individual variation in these traits (Williams 2008).

In recent years, however, the nascent field of evolutionary endocrinology has begun to address the causes and consequences of this variation from an evolutionary perspective (Dufty et al. 2002; Zera et al. 2007; Lema 2014; Wada and Sewall 2014; Bonier and Martin 2016; Cox et al. 2016a). The presence of heritable variation in circulating hormone levels has been confirmed by both artificial selection studies and experiments in natural populations (Satterlee and Johnson 1988; Pottinger and Carrick 1999; Evans et al. 2006; Jenkins et al. 2014; Stedman et al. 2017), and a rapidly growing literature addresses relationships among natural variation in hormone levels, hormone-mediated traits, and fitness (Breuner et al. 2008; Bonier et al. 2009a; Hau et al. 2016). At the same time an increasing number of studies are using evolutionary approaches to gain new insights into the substantial within-individual variation in endocrine traits, including estimating endocrine repeatability (Taff et al. 2018), quantifying the relative amount of within and among-individual variation in hormone levels (Hau et al. 2016), and using a reaction norm approach to quantify individual differences in endocrine flexibility and its phenotypic and fitness consequences (reviewed in Taff and Vitousek 2016). These and other approaches have provided insight into the potential for endocrine traits and their context-dependent flexibility to be shaped by selection. Nevertheless, our understanding of when and how selection actually operates on these key mediators of phenotype, and how endocrine variation influences population-level processes (e.g., persistence, divergence, colonization), are still in their infancy.

Large-scale comparative analyses are a particularly promising approach to addressing the puzzle of endocrine system variation (Bókony et al. 2009; Bonett 2016; Bonier and Martin 2016). Circulating hormones have been measured in free-living populations across vertebrate taxa, providing a rich resource on which to base large-scale comparative analyses. Continuing improvements in phylogenies have enabled much greater resolution of relatedness matrices within lineages, and made it possible to conduct phylogenetically informed analyses across these lineages over broad taxonomic scales (Bininda-Emonds 2014; Garamszegi and Gonzalez-Voyer 2014; Johnson et al. 2018). At the same time, advances in phylogenetic comparative methods now enable the incorporation of within-species variation using Bayesian approaches (Hadfield 2010; Revell 2012; Burkner 2017), and the implementation of increasingly powerful meta-analytic approaches (Viechtbauer 2010; de Villemereuil and Nakagawa 2014). Together, these advances have set the stage for comparative analyses of the function and evolution of hormones—and their variation within species—on much broader taxonomic scales than has previously been possible.

## Species differences in circulating hormones: signatures of selection?

The often dramatic differences in hormone concentrations among species were historically regarded as having little functional significance, but a growing number of phylogenetic comparative analyses have shown patterns consistent with selection shaping hormone levels across taxa (Hau et al. 2010; Swanson and Dantzer 2014; Jessop et al. 2016). Phylogenetic comparative analyses conducted within several vertebrate groups have revealed some striking consistencies in geographic and ecological patterns in circulating hormone levels. For example, in all vertebrate classes studied to date, testosterone levels are higher in populations with shorter breeding seasons (Goymann et al. 2004; Garamszegi et al. 2008; Hau et al. 2010; Eikenaar et al. 2012), glucocorticoid concentrations are greater at higher latitudes (Bókony et al. 2009; Hau et al. 2010; Eikenaar et al. 2012; Jessop et al. 2013), and higher insulin-like growth factor levels are associated with faster-paced life histories (Swanson and Dantzer 2014; Lodjak et al. 2018).

These intriguing patterns support the potential for among-species variation in endocrine traits to reflect

signatures of selection. Furthermore, they suggest that selection may favor similar endocrine profiles in species inhabiting similar environments, or facing similar life history constraints, resulting in the convergent evolution of endocrine phenotypes. However, broad characterizations of environment and life history could also mask variation in the specific selective pressures facing different populations and species. For example, a positive relationship between glucocorticoids and latitude could be generated by selection favoring higher glucocorticoids in environments that are colder, have more short-term variation, greater seasonal unpredictability, higher predation risk, require greater reproductive investment, or any number of other factors. An important goal for future research is to combine existing conceptual frameworks of endocrine function with macroevolutionary patterning in endocrine traits to develop and test hypotheses about how specific selective pressures shape the evolution of endocrine phenotypes.

### Variation within species: challenges and opportunities

The evolutionary causes and consequences of the striking variation in circulating hormones within populations—which can reach two orders of magnitude even within life history stages and sexes—are particularly poorly understood (Kempenaers et al. 2008; Williams 2008). Endocrine traits are strongly influenced by environmental factors, exhibiting both developmental plasticity and reversible phenotypic flexibility at temporal scales ranging from months (e.g., seasonal increases in testosterone during reproduction) to minutes (e.g., heightened glucocorticoid secretion during a stress response). Yet despite the significant influence of environmental and social context on hormone levels (Goymann et al. 2004; Gesquiere et al. 2011), consistent individual differences in hormones are often observed in free-living individuals, even across life history stages and years (Cockrem 2013; Taff et al. 2018). For this variation to evolve through selection it must have a heritable component; at least within glucocorticoids, the presence of low to moderate heritability in circulating levels has been confirmed in a number of species (Pottinger and Carrick 1999; Evans et al. 2006; Jenkins et al. 2014; Stedman et al. 2017). Circulating testosterone levels also show low to moderate heritability in the few species in which it has been studied (reviewed in Cox et al. [2016b]); very little is known about the heritability of other endocrine traits in natural populations. The potential for selection to operate on hormone systems to shape phenotypic differentiation is also supported by findings of adaptive divergence in several components of hormone signal systems between populations and incipient sympatric species (Kitano et al. 2010, 2011), and by genetic accommodation of the endocrine mediators of phenotypic plasticity among species (Kulkarni et al. 2017).

In order to determine the evolutionary consequences of hormonal variation within populations, it is necessary to distinguish within- from among-individual variation, and to understand how each of these types of variation influence evolutionary trajectories. In recent years, there has been substantial progress in beginning to differentiate among-individual and within-individual variation in other plastic traits (Westneat et al. 2015), but few studies to date have partitioned these sources of variation in endocrine traits in a way that they can be directly compared (but see e.g., Fürtbauer et al. 2015; Lendvai et al. 2015). Such comparisons will be facilitated by the increasing prevalence of the reaction norm approach—in which hormonal trait expression is repeatedly measured in individuals over a natural or experimentally induced environmental gradient, or in response to a standardized stimulus (Williams 2008; Cockrem 2013; Wada and Sewall 2014; Taff and Vitousek 2016). Because endocrine trait expression at any one time represents the outcome of multiple simultaneous reaction norms, characterizing individual differences in the endocrine response to specific environmental factors or stimuli, and determining their evolutionary consequences, will be challenging. Nevertheless, the evolution of endocrine reaction norms remains a promising area of research.

Another approach that has received much less attention—despite a wealth of available data—is investigating why some populations show more hormonal variation than others (Guindre-Parker 2018). Within-population variation in heritable traits is often considered a measure of genetic diversity; greater genetic variation in a population may render it more robust to disturbance or better able to colonize new habitats (Kolbe et al. 2004). As hormone levels are highly plastic traits, measurements of within-population variation are not independent from variation within individuals (Westneat et al. 2015). Nevertheless, analyses of patterns of within-population variation are providing insight into evolutionary potential in other plastic traits. For example, a recent analysis of the behavioral response to the threat of predation (a classically labile trait) found that environmental factors significantly predicted the relative amount of within- vs. among-population variance in birds (Garamszegi and Møller 2017). Comparative analyses of within and

among-population variation in endocrine traits could reveal novel patterns, and help to generate new hypotheses about the drivers of this variation.

### Do consistent individual differences in hormones drive the differential expression of phenotypic traits that affect fitness?

Decades of experimental studies have illuminated the role of hormones in mediating a multitude of phenotypic traits, including those central to reproduction, aggression, and the response to stress (Zera and Harshman 2001; Wingfield and Sapolsky 2003; Adkins-Regan 2005; Hau 2007). However, the rapidly growing literature on the links among circulating hormones, other phenotypic traits, and fitness has produced contrasting results. In some free-living populations, individual variation in hormone levels covaries with the expression of putatively hormone-mediated behavioral and physiological traits (Ouyang et al. 2011; Vitousek et al. 2014), yet in other studies no such relationship is apparent (Husak et al. 2007; Garamszegi et al. 2012). Likewise, natural variation in circulating steroid hormones can predict survival and reproductive success, but—as is the case for many phenotypic traits—the nature of trait–fitness relationships differs across populations and contexts (Angelier et al. 2009, 2016; Bonier et al. 2009b; Patterson et al. 2014; Vitousek et al. 2018b). For example, testosterone levels positively predict male aggression across species of *Anolis* lizards on some Caribbean islands, but the opposite pattern is seen on other islands (Husak and Lovern 2014). The importance of context-dependency was also highlighted by a recent phylogenetically informed meta-analysis of glucocorticoid–fitness relationships across vertebrates (L. A. Schoenle et al., in preparation). Both baseline and stress-induced glucocorticoid levels showed a relatively consistent negative relationship with reproductive success, but the presence and strength of glucocorticoid–survival relationships were strongly influenced by pace of life (L. A. Schoenle et al., in preparation). These results highlight the importance of developing new conceptual frameworks that explicitly incorporate context dependence over different temporal scales to generate testable predictions about when and how endocrine variation will influence fitness across environments and life histories.

## Resources and considerations for large-scale comparative analyses

While substantial data on circulating hormone levels and other endocrine traits are available in the literature, large-scale comparative analyses are often limited by the significant effort required to aggregate and standardize these data. Two new publicly-available databases of hormone data provide valuable resources for researchers interested in endocrine traits and how they vary across taxa. HormoneBase, a recently released database, contains data on circulating hormone levels and their variation in steroid hormones (currently glucocorticoids and androgens) across vertebrates (Vitousek et al. 2018a). This freely-available database, which represents the collaborative efforts of 14 endocrinologists, evolutionary biologists, and data technology specialists, includes measures of the mean, variation, and range of plasma hormone levels from free-living populations (currently >6500 entries from 474 species). Entries are accompanied by a variety of additional information, including data on sampling location, time and methodology, sex, life history stage, assay methods, and the identity of the endocrine laboratory from which the measures originated (see Johnson et al. 2018; Vitousek et al. 2018a). HormoneBase also accepts uploads of new data.

A second publicly available resource, the Wildlife Endocrinology Information Network (WEIN) is a developing searchable data network that contains information on circulating steroid hormones in zoo and wildlife animals (currently 25 species). Most of the current WEIN data are fecal hormone concentrations in mammals, but WEIN also accepts data from other species, and measured hormone concentrations in other biological matrices, including urine, plasma, saliva, hair, and blow spray. This resource was developed predominantly to provide reference values for conservation and management purposes, but as it continues to grow it could provide an increasingly valuable resource for comparative analyses, particularly by providing data from species that are challenging to sample under natural conditions.

Because hormone concentrations are relatively easy to measure, including in many free-living populations, many more data are currently available on circulating hormone levels in non-model organisms than on variation in other components of endocrine system function. Yet the downstream effects of circulating hormones depend not only on their concentrations but also on the integrated function of multiple components of hormone signaling systems (e.g., receptor expression, binding globulins, intracellular signaling pathways, cofactors) (Adkins-Regan 2008), which also vary within and among individuals (Wingfield 2018). The number of studies addressing other endocrine traits in non-model organisms has increased substantially in recent years. Similarly, the

probing of integrated measures of endocrine system responsiveness and performance through pharmacological challenges (e.g., ACTH, GnRH, and dexamethasone challenges) has grown increasingly common, as various analyses suggest that such measures may provide important insights into endocrine function and evolution (McGlothlin et al. 2010; Romero and Wikelski 2010; Needham et al. 2017). Before long, sufficient data on many endocrine traits may be available to support large-scale comparative analyses; however, there is currently no standardized repository for many types of endocrine data. The development of such a database—or the expansion of an existing database to support other data types—may prove particularly fruitful for the field of comparative endocrinology.

## Novel approaches and future directions

A recent symposium at the annual meeting of the Society for Integrative and Comparative Biology highlighted several exciting new approaches that have begun to use the wealth of existing data to illuminate endocrine evolution across the vertebrate tree of life. Studies of macroevolutionary patterning in androgen and glucocorticoid concentrations have begun to reconstruct the ancestral states of these traits, and to test whether different taxonomic groups have similar or distinct evolutionary optima (M. J. Fuxjager et al., in preparation). Large-scale phylogenetic comparative analyses are providing insight into whether hormones scale with metabolic rate across tetrapods (Francis et al. 2018). The incorporation of rich resources for fine-scale environmental and trait data (Johnson et al. 2018), coupled with new phylogenetic comparative methods, are enabling the determination of which selective pressures shape endocrine traits in consistent ways over broad taxonomic scales (e.g., across vertebrates), and which pressures differ in their direction or strength according to other aspects of phenotype or environment. Phylogenetically informed analyses of the reactive scope of populations across seasons (e.g., seasonal scope in birds: Casagrande et al. 2018) or environmental gradients have particularly high potential to yield insights into the evolution of phenotypic flexibility.

The potential for circulating glucocorticoid levels and other endocrine measures to be used as indicators of exposure or resilience to anthropogenic and other challenges has been of interest to biologists and conservationists for some time (Walker et al. 2005; Wikelski and Cooke 2006; Wingfield et al. 1997). But while endocrine measures have in some cases provided key insights into the presence or nature of environmental disturbances (e.g., Creel et al. 2009; Ouyang et al. 2015; Kleist et al. 2018), decades of research within and across populations have yielded inconsistent patterns among exposure to challenges, hormones, and fitness (Bonier et al. 2009a; Dantzer et al. 2014; Schoenle et al. 2018). Large-scale phylogenetic comparative analyses may shed new light on the use of endocrine measures as tools to assess or predict the impacts of various kinds of disturbance—including exposure to toxins, urbanization, and large-scale anthropogenic changes (e.g., light and sound exposure). Similarly, analyses of the relationship between hormonal variation and population status (e.g., increasing/declining, expanding/contracting: Martin et al. 2018) could also provide information relevant to conservation, biogeography, or population patterns (e.g., divergence and speciation: Garamszegi et al. 2018). Together, these novel approaches to endocrine analyses—and many others that will no doubt be developed in the coming years—represent promising new directions.

Moving forward, we see particularly strong potential in addressing the evolutionary causes and consequences of hormonal variation within and among populations. The HormoneBase database, which both provides population-level information and explicitly incorporates variation within populations, may be a valuable resource for such analyses. Looking further into the future, as data on individual reaction norms accumulate, comparative analyses of how endocrine flexibility varies across populations and species, and of the relationships among endocrine flexibility, phenotypic flexibility, and fitness outcomes, will likely yield important new insights. Likewise, as transcriptomic studies increase in prevalence, comparative approaches can help to illuminate variation in the downstream effects of hormones on gene expression networks. This is an exciting time for large-scale hormone analyses; we hope that over the coming years such approaches will together provide a much fuller picture of the function and evolution of these central mediators of phenotype.

## Acknowledgments

We are grateful to the symposium participants and members of the HormoneBase consortium for valuable feedback, and to the sponsoring organizations, including the Company of Biologists, and SICB's DAB, DCE, DCPB, and DEE divisions.

## Funding

This work was supported in part by the National Science Foundation (IOS-1457251 to MNV and IOS 1257021 to MAJ).

## References


Adkins-Regan E. 2005. Hormones and animal social behavior. Princeton, New Jersey: Princeton University Press.

Adkins-Regan E. 2008. Do hormonal control systems produce evolutionary inertia? Philos Trans R Soc B Biol Sci 363:1599–609.

Angelier F, Holberton RL, Marra PP. 2009. Does stress response predict return rate in a migratory bird species? A study of American redstarts and their non-breeding habitat. Proc R Soc B Biol Sci 276:3545–51.

Angelier F, Wingfield JC, Tartu S, Chastel O. 2016. Does prolactin mediate parental and life-history decisions in response to environmental conditions in birds? A review. Horm Behav 77:18–29.

Bininda-Emonds ORP. 2014. An introduction to supertree construction (and partitioned phylogenetic analyses) with a view toward the distinction between gene trees and species trees. In: Garamszegi LZ, editor. Modern phylogenetic comparative methods and their application in evolutionary biology. Berlin, Heidelberg: Springer. p. 49–76.

Bókony V, Lendvai ÁZ, Liker A, Angelier F, Wingfield JC, Chastel O. 2009. Stress response and the value of reproduction: are birds prudent parents? Am Nat 173:589–98.

Bonett RM. 2016. Analyzing endocrine system conservation and evolution. Gen Comp Endocrinol 234:3–9.

Bonier F, Martin PR. 2016. How can we estimate natural selection on endocrine traits? Lessons from evolutionary biology. Proc R Soc B Biol Sci 283:20161887.

Bonier F, Martin PR, Moore IT, Wingfield JC. 2009a. Do baseline glucocorticoids predict fitness? Trends Ecol Evol 24:634–42.

Bonier F, Moore IT, Martin PR, Robertson RJ. 2009b. The relationship between fitness and baseline glucocorticoids in a passerine bird. Gen Comp Endocrinol 163:208–13.

Breuner CW, Patterson SH, Hahn TP. 2008. In search of relationships between the acute adrenocortical response and fitness. Gen Comp Endocrinol 157:288–95.

Burkner PC. 2017. brms: an R package for Bayesian multilevel models using Stan. J Stat Softw 80:1–27.

Casagrande S, Garamszegi LZ, Goymann W, Donald J, Francis CD, Fuxjager MJ, Husak JF, Johnson MA, Kircher B, Knapp R, et al. 2018. Do seasonal glucocorticoid changes depend on reproductive investment? A comparative approach in birds. Int Comp Biol published online (https://doi.org/10.1093/icb/icy022).

Cockrem JF. 2013. Individual variation in glucocorticoid stress responses in animals. Gen Comp Endocrinol 181:45–58.

Cox RM, McGlothlin JW, Bonier F. 2016. Evolutionary endocrinology: hormones as mediators of evolutionary phenomena: an introduction to the symposium. Integr Comp Biol 56:121–5.

Cox RM, McGlothlin JW, Bonier F. 2016. Hormones as mediators of phenotypic and genetic integration: an evolutionary genetics approach. Integr Comp Biol 56:126–37.

Creel S, Winnie JA, Christianson D. 2009. Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. Proc Natl Acad Sci U S A 106:12388–93.

Dantzer B, Fletcher QE, Boonstra R, Sheriff MJ. 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? Conserv Physiol 2:cou023–18.

de Villemereuil P, Nakagawa S. 2014. General quantitative genetic methods for comparative biology. In: Garamszegi LZ, editor. Modern phylogenetic comparative methods and their application in evolutionary biology. Berlin, Heidelberg: Springer. p. 287–303.

Dufty AM, Clobert J, Møller AP. 2002. Hormones, developmental plasticity and adaptation. Trends Ecol Evol 17:190–6.

Eikenaar C, Husak J, Escallón C, Moore IT. 2012. Variation in testosterone and corticosterone in amphibians and reptiles: relationships with latitude, elevation, and breeding season length. Am Nat 180:642–54.

Evans MR, Roberts ML, Buchanan KL, Goldsmith AR. 2006. Heritability of corticosterone response and changes in life history traits during selection in the zebra finch. J Evol Biol 19:343–52.

Francis CD, Donald J, Fuxjager MJ, Goymann W, Hau M, Husak JF, Johnson MA, Kircher B, Knapp R, Martin LB, et al. 2018. Metabolic scaling of stress hormones in vertebrates. Int Comp Biol published online (https://doi.org/10.1093/icb/icy063).

Fürtbauer I, Pond A, Heistermann M, King AJ. 2015. Personality, plasticity and predation: linking endocrine and behavioural reaction norms in stickleback fish. Funct Ecol 29:931–40.

Garamszegi LZ, Donald J, Francis CD, Fuxjager MJ, Goymann W, Hau M, Husak JF, Johnson MA, Kircher B, Knapp R, et al. 2018. Species-specific means and within-species variance in glucocorticoid hormones and speciation rate in birds. Int Comp Biol published online (https://doi.org/10.1093/icb/icy086).

Garamszegi LZ, Gonzalez-Voyer A. 2014. Working with the tree of life in comparative studies: how to build and tailor phylogenies to interspecific datasets. In: Garamszegi LZ, editor. Modern phylogenetic comparative methods and their application in evolutionary biology. Berlin, Heidelberg: Springer. p. 19–48.

Garamszegi LZ, Hirschenhauser K, Bókony V, Eens M, Hurtrez-Boussès S, Møller AP, Oliveira RF, Wingfield JC. 2008. Latitudinal distribution, migration, and testosterone levels in birds. Am Nat 172:533–46.

Garamszegi LZ, Møller AP. 2017. Partitioning within-species variance in behaviour to within- and between-population components for understanding evolution. Ecol Lett 20:599–608.

Garamszegi LZ, Rosivall B, Rettenbacher S, Markó G, Zsebők S, Szöllősi E, Eens M, Potti J, Török J. 2012. Corticosterone, avoidance of novelty, risk-taking and aggression in a wild bird: no evidence for pleiotropic effects. Ethology 118:621–35.

Gesquiere LR, Learn NH, Simao MCM, Onyango PO, Alberts SC, Altmann J. 2011. Life at the top: rank and stress in wild male baboons. Science 333:357–60.

Goymann W, Moore IT, Scheuerlein A, Hirschenhauser K, Grafen A, Wingfield JC. 2004. Testosterone in tropical birds: effects of environmental and social factors. Am Nat 164:327–34.

Guindre-Parker S. 2018. Types of evolutionary studies and what they can teach us. Int Comp Biol (doi:10.1093/icb/icy048).

Hadfield JD. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J Stat Softw 33:1–22.

Hau M. 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. BioEssays 29:133–44.

Hau M, Casagrande S, Ouyang JQ, Baugh AT. 2016. Glucocorticoid-mediated phenotypes in vertebrates: multilevel variation and evolution. Adv Study Behav 48:41–115.

Hau M, Ricklefs RE, Wikelski M, Lee KA, Brawn JD. 2010. Corticosterone, testosterone and life-history strategies of birds. Proc R Soc B Biol Sci 277:3203–12.

Husak JF, Irschick DJ, Meyers JJ, Lailvaux SP, Moore IT. 2007. Hormones, sexual signals, and performance of green anole lizards (*Anolis carolinensis*). Horm Behav 52:360–7.

Husak JF, Lovern MB. 2014. Variation in steroid hormone levels among Caribbean *Anolis* lizards: endocrine system convergence? Horm Behav 65:408–15.

Jenkins BR, Vitousek MN, Hubbard JK, Safran RJ. 2014. An experimental analysis of the heritability of variation in glucocorticoid concentrations in a wild avian population. Proc R Soc B Biol Sci 281:20141302.

Jessop TS, Lane ML, Teasdale L, Stuart-Fox D, Wilson RS, Careau V, Moore IT, Adkins-Regan E, Michalakis Y. 2016. Multiscale evaluation of thermal dependence in the glucocorticoid response of vertebrates. Am Nat 188:342–56.

Jessop TS, Woodford R, Symonds MRE. 2013. Macrostress: do large-scale ecological patterns exist in the glucocorticoid stress response of vertebrates? Funct Ecol 27:120–30.

Johnson MA, Francis CD, Miller ET, Downs CJ, Vitousek MN. 2018. Detecting bias in large-scale comparative analysis: methods for expanding the scope of hypothesis-testing with HormoneBase. Int Comp Biol published online (doi: 10.1093/icb/icy045).

Kempenaers B, Peters A, Foerster K. 2008. Sources of individual variation in plasma testosterone levels. Philos Trans R Soc B Biol Sci 363:1711–23.

Ketterson ED, Nolan V. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. Am Nat 154:S4–25.

Kitano J, Kawagishi Y, Mori S, Peichel CL, Makino T, Kawata M, Kusakabe M. 2011. Divergence in sex steroid hormone signaling between sympatric species of Japanese threespine stickleback. PLoS One 6:e29253.

Kitano J, Lema SC, Luckenbach JA, Mori S, Kawagishi Y, Kusakabe M, Swanson P, Peichel CL. 2010. Adaptive divergence in the thyroid hormone signaling pathway in the stickleback radiation. Curr Biol 20:2124–30.

Kleist NJ, Guralnick RP, Cruz A, Lowry CA, Francis CD. 2018. Chronic anthropogenic noise disrupts glucocorticoid signaling and has multiple effects on fitness in an avian community. Proc Natl Acad Sci U S A 115:E648–57.

Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB. 2004. Genetic variation increases during biological invasion by a Cuban lizard. Nature 431:177–81.

Kulkarni SS, Denver RJ, Gomez-Mestre I, Buchholz DR. 2017. Genetic accommodation via modified endocrine signalling explains phenotypic divergence among spadefoot toad species. Nat Commun 8:993.

Lema SC. 2014. Hormones and phenotypic plasticity in an ecological context: linking physiological mechanisms to evolutionary processes. Integr Comp Biol 54:850–63.

Lendvai ÁZ, Giraudeau M, Bókony V, Angelier F, Chastel O. 2015. Within-individual plasticity explains age-related decrease in stress response in a short-lived bird. Biol Lett 11:20150272.

Lodjak J, Mänd R, Mägi M. 2018. Insulin-like growth factor 1 and life-history evolution of passerine birds. Funct Ecol 32:313–23.

Martin LB, Francis CD, Vitousek MN, Donald J, Fuxjager MJ, Goymann W, Hau M, Husak JF, Johnson MA, Kircher B, et al. 2018. IUCN conservation status does not predict glucocorticoid concentrations in reptiles and birds. Int Comp Biol (doi: 10.1093/icb/icy102).

McGlothlin JW, Whittaker DJ, Schrock SE, Gerlach NM, Jawor JM, Snajdr EA, Ketterson ED. 2010. Natural selection on testosterone production in a wild songbird population. Am Nat 175:687–701.

Miles MC, Vitousek MN, Husak JF, Johnson MA, Martin LB, Taff CC, Zimmer C, Lovern MB, Fuxjager MJ. 2018. Standing variation and the capacity for change: are endocrine phenotypes more variable than other traits? Int Comp Biol published online (doi: 10.1093/icb/icy062).

Needham KB, Dochtermann NA, Greives TJ. 2017. Consistent individual variation in day, night, and GnRH-induced testosterone concentrations in house sparrows (*Passer domesticus*). Gen Comp Endocrinol 246:211–7.

Ouyang JQ, de Jong M, Hau M, Visser ME, van Grunsven RHA, Spoelstra K. 2015. Stressful colours: corticosterone concentrations in a free-living songbird vary with the spectral composition of experimental illumination. Biol Lett 11:20150517.

Ouyang JQ, Sharp PJ, Dawson A, Quetting M, Hau M. 2011. Hormone levels predict individual differences in reproductive success in a passerine bird. Proc R Soc B Biol Sci 278:2537–45.

Patterson SH, Hahn TP, Cornelius JM, Breuner CW. 2014. Natural selection and glucocorticoid physiology. J Evol Biol 27:259–74.

Pottinger TG, Carrick TR. 1999. Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. Gen Comp Endocrinol 116:122–32.

Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things): phytools: r package. Methods Ecol Evol 3:217–23.

Romero LM, Wikelski M. 2010. Stress physiology as a predictor of survival in Galapagos marine iguanas. Proc R Soc B Biol Sci 277:3157–62.

Satterlee DG, Johnson WA. 1988. Selection of Japanese quail for contrasting blood corticosterone response to immobilization. Poult Sci 67:25–32.

Schoenle LA, Zimmer CZ, Vitousek MN. 2018. Understanding context-dependence in glucocorticoid–fitness relationships: the role of the nature of the challenge, the intensity and frequency of stressors, and life history. Int Comp Biol (doi: 10.1093/icb/icy046).

Stedman JM, Hallinger KK, Winkler DW, Vitousek MN. 2017. Heritable variation in circulating glucocorticoids and endocrine flexibility in a free-living songbird. J Evol Biol 30:1724–35.

Swanson EM, Dantzer B. 2014. Insulin-like growth factor-1 is associated with life-history variation across Mammalia. Proc R Soc B Biol Sci 281:20132458.

Taff CC, Schoenle LA, Vitousek MN. 2018. The repeatability of glucocorticoids: a review and meta-analysis. Gen Comp Endocrinol 260:136–45.

Taff CC, Vitousek MN. 2016. Endocrine flexibility: optimizing phenotypes in a dynamic world? Trends Ecol Evol 31:476–88.

Viechtbauer W. 2010. Conducting meta-analyses in R with the metafor package. J Stat Softw 36:1–48.

Vitousek MN, Johnson M, Donald J, Francis C, Fuxjager M, Goymann W, Hau M, Husak J, Kircher B, Knapp R, et al. 2018a. HormoneBase, a population-level database of steroid hormone levels across vertebrates. Sci Data 5:180097.

Vitousek MN, Taff CC, Hallinger KK, Zimmer C, Winkler DW. 2018b. Hormones and fitness: evidence for trade-offs in glucocorticoid regulation across contexts. Front Ecol Evol 6:1–14.

Vitousek MN, Jenkins BR, Safran RJ. 2014. Stress and success: individual differences in the glucocorticoid stress response predict behavior and reproductive success under high predation risk. Horm Behav 66:812–9.

Wada H, Sewall KB. 2014. Introduction to the symposium-uniting evolutionary and physiological approaches to understanding phenotypic plasticity. Integr Comp Biol 54:774–82.

Walker BG, Boersma PD, Wingfield JC. 2005. Field endocrinology and conservation biology. Integr Comp Biol 45:12–8.

Westneat DF, Wright J, Dingemanse NJ. 2015. The biology hidden inside residual within-individual phenotypic variation: the biology of residual phenotypic variance. Biol Rev 90:729–43.

Wikelski M, Cooke SJ. 2006. Conservation physiology. Trends Ecol Evol 21:38–46.

Williams TD. 2008. Individual variation in endocrine systems: moving beyond the "tyranny of the Golden Mean." Philos Trans R Soc B Biol Sci 363:1687–98.

Wingfield JC. 2018. Environmental endocrinology: insights into the evolution and diversity of regulatory mechanisms in life cycles. Int Comp Biol (doi:10.1093/icb/icy081).

Wingfield J, Hunt K, Breuner C, Dunlap K, Fowler GS, Freed L, Lepson J. 1997. Environmental stress, field endocrinology, and conservation biology. In: Clemmons JR, Buchholz R, editors. Behavioral approaches to conservation in the wild. London: Cambridge University Press. p. 95–131.

Wingfield JC, Sapolsky RM. 2003. Reproduction and resistance to stress: when and how. J Neuroendocrinol 15: 711–24.

Zera AJ, Harshman LG. 2001. The physiology of life history trade-offs in animals. Annu Rev Ecol Syst 32:95–126.

Zera AJ, Harshman LG, Williams TD. 2007. Evolutionary endocrinology: the developing synthesis between endocrinology and evolutionary genetics. Annu Rev Ecol Evol Syst 38:793–817.