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SYMPOSIUM

Hormones as Mediators of Phenotypic and Genetic Integration: an Evolutionary Genetics Approach

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Synopsis. Evolutionary endocrinology represents a synthesis between comparative endocrinology and evolutionary genetics. This synthesis can be viewed through the breeder's equation, a cornerstone of quantitative genetics that, in its univariate form, states that a population's evolutionary response is the product of the heritability of a trait and selection on that trait ($R = h^2S$). Under this framework, evolutionary endocrinologists have begun to quantify the heritability of, and the strength of selection on, a variety of hormonal phenotypes. With specific reference to our work on testosterone and corticosterone in birds and lizards, we review these studies while emphasizing the challenges of applying this framework to hormonal phenotypes that are inherently plastic and mediate adaptive responses to environmental variation. Next, we consider the untapped potential of evolutionary endocrinology as a framework for exploring multivariate versions of the breeder's equation, with emphasis on the role of hormones in structuring phenotypic and genetic correlations. As an extension of the familiar concepts of phenotypic integration and hormonal pleiotropy, we illustrate how the hormonal milieu of an individual acts as a local environment for the expression of genes and phenotypes, thereby influencing the quantitative genetic architecture of multivariate phenotypes. We emphasize that hormones are more than mechanistic links in the translation of genotype to phenotype: by virtue of their pleiotropic effects on gene expression, hormones structure the underlying genetic variances and covariances that determine a population's evolutionary response to selection.

Introduction

Hormones are agents of biological coordination, orchestrating the (co)expression of genes and phenotypes in response to both intrinsic and extrinsic cues. Hormones circulate systemically, signal diverse cells and tissues, and regulate nearly all aspects of the phenotype, including behavior, morphology, physiology, and life history. As such, researchers working at the intersection of endocrinology and evolutionary biology have increasingly emphasized the importance of understanding (1) the genetic basis of endocrine phenotypes (Pavitt et al. 2014; Iserbyt et al. 2015), (2) the strength and form of natural selection on endocrine phenotypes (McGlothlin et al. 2010; Patterson et al. 2014), and (3) the evolutionary implications associated with hormonal regulation of other phenotypes under selection (Ketterson and Nolan 1999; McGlothlin and Ketterson 2008; Williams 2012). Each of these topics is central to the emerging synthesis between comparative endocrinology and evolutionary genetics (Zera et al. 2007).

In this article, we review recent progress and identify promising areas for new research along these three related fronts, using the breeder's equation as a conceptual framework for developing connections between endocrinology and quantitative genetics. We draw heavily from our own research on birds and lizards while focusing specifically on the steroid hormones corticosterone (CORT) and testosterone (T) as components of the interrelated hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes. Our main goals are to (1) highlight the advantages and challenges of synthesizing comparative endocrinology with evolutionary

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genetics, (2) illustrate and propose methodological solutions to some of these challenges, and (3) call attention to the broader evolutionary significance of hormones as mediators of phenotypic and genetic correlations that shape the evolutionary trajectories of a multitude of behavioral, physiological, morphological, and life-history traits.

The breeder's equation as a conceptual framework

Evolutionary change in a phenotypic trait under selection can be separated into two components: selection itself, which arises from differential survival and reproduction, and inheritance, the transmission of parental phenotypes to the next generation. The effects of these components on evolutionary change are represented by the univariate breeder's equation:

$$R = h^2 S, \quad (1)$$

where R is the per-generation evolutionary response of a quantitative trait to selection (the change in the phenotypic mean of the trait across generations), h^2 is the narrow-sense heritability (the proportion of phenotypic variance in the trait attributable to additive genetic variance among individuals), and S is the selection differential for that trait (the covariance between phenotype and relative fitness). These parameters are specific to the population under study, and both h^2 and S can vary substantially across space and time due to differences in the genetic makeup of a population and the dynamic influence of the environment it encounters.

As its name implies, the breeder's equation was developed as a predictive tool to guide artificial selection by animal breeders. Although useful in this context, the breeder's equation makes several assumptions that may limit its predictive power in wild populations (Morrissey et al. 2010, 2012). Importantly, it assumes a causal effect underlying the phenotype-fitness covariance that defines S, a limitation that can be exacerbated by the exclusion of any additional traits or environmental factors that affect fitness and are correlated, causally or otherwise, with the focal trait (Wade and Kalisz 1990; Morrissey et al. 2010; Dantzer et al. 2016). A partial solution lies in the multivariate expansion of the breeder's equation to include multiple traits, which can be expressed as:

$$\mathbf{R} = \mathbf{G}\mathbf{P}^{-1}\mathbf{S}, \quad (2)$$

where $\mathbf{R}$ is a vector of responses to selection on multiple traits, $\mathbf{G}$ is a matrix describing the additive genetic variances and covariances of these traits, $\mathbf{P}$ is a matrix describing the phenotypic variances and covariances of these traits, and $\mathbf{S}$ is a vector of selection differentials on these traits (Lande 1979). Here, $\mathbf{G}\mathbf{P}^{-1}$ is a multivariate analog of h^2. Alternatively, we may write:

$$\mathbf{R} = \mathbf{G}\boldsymbol{\beta}, \quad (3)$$

where $\boldsymbol{\beta}$ is a vector of selection gradients ($\boldsymbol{\beta}$), which represent the partial effects of each trait on fitness. This form is particularly useful because selection gradients can be estimated as partial regression coefficients of relative fitness on each trait (Lande and Arnold 1983). Nonetheless, this multivariate approach still assumes that all traits causally influencing fitness have been included, which is unlikely for most studies of wild populations.

Recent theoretical and empirical work suggests that the most reliable approach to predicting the evolutionary response to selection is to directly measure the additive genetic covariance between phenotype and fitness (i.e., the Robertson-Price identity, Morrissey et al. 2010; 2012). Empirically, this approach is probably beyond the scope of most existing datasets for endocrine phenotypes, which tend to separately address either the genetic basis or the fitness correlates of phenotypic variation in endocrine traits, analogous to the separate terms of h^2 and S in the breeder's equation. For this reason, the breeder's equation provides a more useful conceptual framework for synthesizing studies of the genetic basis (h^2) and fitness consequences (S or $\boldsymbol{\beta}$) of variation in endocrine phenotypes, and for identifying aspects of this framework that may prove particularly challenging for endocrine phenotypes. Likewise, the multivariate breeder's equation provides a conceptual backdrop for exploring the roles of hormones in coordinating patterns of phenotypic and genetic integration ($\mathbf{P}$ and $\mathbf{G}$) that shape the evolutionary responses of entire suites of behavioral, physiological, and morphological phenotypes.

Heritability of endocrine phenotypes

Endocrine phenotypes are inherently complex and, depending upon the question at hand, could be quantified as concentrations of hormones, metabolites, binding proteins, or enzymes involved in hormone synthesis and degradation, as densities of hormone receptors in a particular tissue, or using more holistic measures, such as the hormonal output of an endocrine axis in response to stimulation (e.g., challenge of the HPG axis with gonadotropin-releasing hormone, GnRH, or territorial intrusion; challenge of the HPA axis with

adrenocorticotropic hormone, ACTH, or acute stress) or the response of a tissue or organism (e.g., growth, behavior, gene expression) to an endocrine manipulation (e.g., treatment with exogenous hormone or its antagonist, ablation of the source of endogenous hormone). Nonetheless, most of what we know about the heritability of endocrine phenotypes comes from point estimates of circulating hormone levels, because the relative ease with which this phenotype can be measured is well suited to the large numbers of individuals (typically hundreds) required for robust estimates of heritability. Consequently, an important caveat to our current understanding of the genetic basis of endocrine phenotypes is that the majority of published data address circulating hormone levels, whereas selection presumably acts on more integrated aspects of endocrine axis function that also encompass factors such as binding globulins, receptor densities, and binding affinities (Bergeon Burns et al. 2014, 2013; Patterson et al. 2014; Rosvall et al. 2016).

Methodologically, estimates of h^2 for endocrine traits can be obtained using parent-offspring regression (Mills et al. 2009) or analogous approaches (Iserbyt et al. 2015), by partitioning the additive genetic portion of phenotypic variance using information on relatedness obtained from controlled breeding experiments (Bates et al. 1986), clinical twin studies (Bartels et al. 2003), captive-born litters (King et al. 2004), and pedigreed wild populations (Pavitt et al. 2014), or by deriving the realized h^2 after experimentally applying S (i.e., artificial selection) and measuring R (Robison et al. 1994; Zera and Zhang 1995). In addition to requiring large sample sizes, analyses of heritability (and of selection, see below) are only informative when phenotypes are measured with a high level of repeatability, which may be a general concern for point estimates of hormone levels that often vary temporally within individuals (Bonier et al. 2009b; Ouyang et al. 2011a; Pavitt et al. 2015; Zera 2016).

Despite this caveat, point estimates of T levels are often repeatable within individuals (Pelletier et al. 2003; van Oers et al. 2011; While et al. 2010), and heritable within populations (Supplementary Table S1). Formal estimates of h^2 for plasma T levels are available from production-oriented studies of domestic pigs (Bates et al. 1986; Lubritz et al. 1991; Robison et al. 1994), clinical studies of humans (e.g., Travison et al. 2014), and evolutionary studies of captive mammals, birds, and reptiles (King et al. 2004; Mills et al. 2009; Iserbyt et al. 2015). In general, these studies indicate fairly substantial heritability of circulating T levels (Supplementary Table S1). Comparable data from wild populations are scarce, though studies of free-living red deer (*Cervus elaphus*) reveal low but significant heritability of circulating T levels in neonates (Pavitt et al. 2014). Additive genetic variance for plasma T has also been confirmed as an evolutionary response to artificial selection for high or low T levels (Robison et al. 1994; Walker et al. 2004; Mills et al. 2012; Mokkonen et al. 2016) and as an indirect response to artificial selection on correlated traits such as immune function (Mills et al. 2010) and exploratory behavior (van Oers et al. 2011). Whereas phenotypic levels of T typically differ between the sexes, genetic variation in plasma T can be positively correlated between the sexes, such that males with high T levels have sisters with relatively high T levels (Pavitt et al. 2014; Iserbyt et al. 2015), though this is not always the case (Ketterson et al. 2005).

Plasma CORT levels are also repeatable within individuals, at least over some time scales and under some circumstances, though not under many others (Romero and Reed 2008; Bonier et al. 2009b; Ouyang et al. 2011a; Rensel and Schoech 2011). Baseline levels of corticosterone are moderately heritable in some studies of birds (Jenkins et al. 2014), as are baseline levels of cortisol in mammals and fish (Fevolden et al. 1999; Bartels et al. 2003; Federenko et al. 2004; Kadarmideen and Janss 2007). Stress-induced changes in CORT levels may provide more informative phenotypes than baseline levels, and these holistic measures of stress response are also often heritable (Satterlee and Johnson 1988; Fevolden et al. 1999; Tanck et al. 2001; Federenko et al. 2004; Evans et al. 2006; Jenkins et al. 2014). In barn swallows, stress-induced levels of CORT exhibit more additive genetic variance than do baseline levels, suggesting greater potential to evolve in response to selection, and these two measures are also genetically independent of one another (Jenkins et al. 2014). Both baseline and stress-induced levels of CORT (or its metabolites) have also been shown to evolve as a correlated response to artificial selection on other traits, such as behavior and performance (Stöwe et al. 2010; Garland et al. 2016). As a general conclusion, both baseline and induced levels of circulating T (Supplementary Table S1) and CORT (Supplementary Table S2) tend to exhibit moderate heritability in a variety of vertebrate taxa. This indicates that at least some components of endocrine phenotypes—circulating hormone levels—tend to exhibit genetic variation and thus have the potential to respond to natural selection.

Selection on endocrine phenotypes

The univariate selection differential (S) for a trait can be quantified as the covariance between phenotype and relative fitness (individual fitness divided by population mean fitness). It is useful to estimate S using values of the trait that are standardized to a population mean of zero in unit variance (SD), so that S measures the number of standard deviations by which selection favors an increase or decrease the trait mean. An example of this approach using baseline levels of T and CORT in male eastern fence lizards, *Sceloporus undulatus*, is shown in Fig. 1 (John-Alder et al. 2009). Because S includes both direct selection on the trait of interest and indirect selection on any correlated traits, multiple regression can be used to obtain partial regression coefficients ($\boldsymbol{\beta}$, multivariate selection gradients) that partition out the direct effects of selection on individual traits from indirect selection acting via other traits in the analysis (Lande and Arnold 1983). Despite the analytical simplicity of this approach, its widespread adoption by evolutionary biologists (see reviews by Cox and Calsbeek 2009; Kingsolver et al. 2001; Kingsolver and Pfennig 2007; Siepielski et al. 2009), and considerable interest among endocrinologists in the adaptive significance of hormonal phenotypes (Bonier et al. 2009a; Ouyang et al. 2011b; Dantzer et al. 2016), formal estimates of phenotypic selection on levels of T and CORT (or any other hormone) are surprisingly rare (Supplementary Table S3).

The paucity of formal selection estimates for circulating hormone levels may relate, at least in part, to the close association of S and $\boldsymbol{\beta}$ with the breeder's equation and its goal of predicting evolutionary response. Field endocrinologists, who are acutely aware of the plasticity of circulating T and CORT levels in response to myriad intrinsic and extrinsic cues (Bonier et al. 2009a; 2009b; Goymann 2009; Kempenaers et al. 2008; Moore and Jessop 2003), may be duly skeptical about the potential to predict R from even the most robust estimates of S or $\boldsymbol{\beta}$ in wild populations. For endocrine phenotypes, two interrelated concerns deserve mention, each of which is a special case of a more general issue in selection analyses (Mitchell-Olds and Shaw 1987; Wade and Kalisz 1990). First, the inherent plasticity of circulating hormone levels, which is itself adaptive, means that the hormonal phenotype of an individual at any given time may yield an unreliable measure of the phenotype under selection over a longer interval (Williams 2008). Similar concerns are often encountered in selection analyses of behavioral phenotypes (Brodie 1993; Brodie and Russell 1999). In extreme

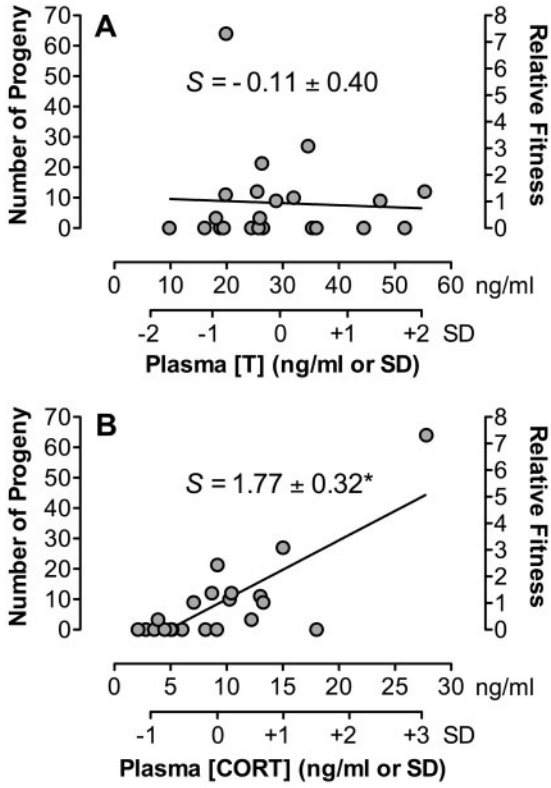

Fig. 1 Selection on circulating plasma testosterone (**A**) and corticosterone (**B**) levels in male eastern fence lizards (*Sceloporus undulatus*). Fitness was quantified as the number of offspring sired based on DNA fingerprinting (left axes). For selection analyses, hormone levels were standardized to a mean of zero in unit variance (SD, bottom axes) and relative fitness was calculated for each individual by dividing number of offspring sired by the population mean number of offspring sired. Selection differentials (S) were derived from the slopes of OLS regressions of relative fitness on standardized phenotypes. Redrawn from John-Alder et al. (2009) with permission from Oxford University Press.

instances, this endocrine plasticity can give rise to dramatically different correlations between fitness and circulating CORT depending on when in the breeding cycle the hormonal phenotype is assayed (Bonier et al. 2009b; Ouyang et al. 2013). Second, estimates of S and $\boldsymbol{\beta}$ will be biased by any unmeasured phenotypes or environmental effects that influence both fitness and the phenotype of interest (Mitchell-Olds and Shaw 1987; Price et al. 1988; Wade and Kalisz 1990). This may be particularly common in situations where intrinsic (e.g., energetic state) or extrinsic (e.g., local environmental quality) factors simultaneously alter hormone levels and, either independently or via their effects on hormone secretion, also influence fitness. Dantzer (2016) provides an example of this issue as it applies to correlations between CORT and fitness in red squirrels, *Tamiasciurus hudsonicus*. In Fig. 2, we illustrate these

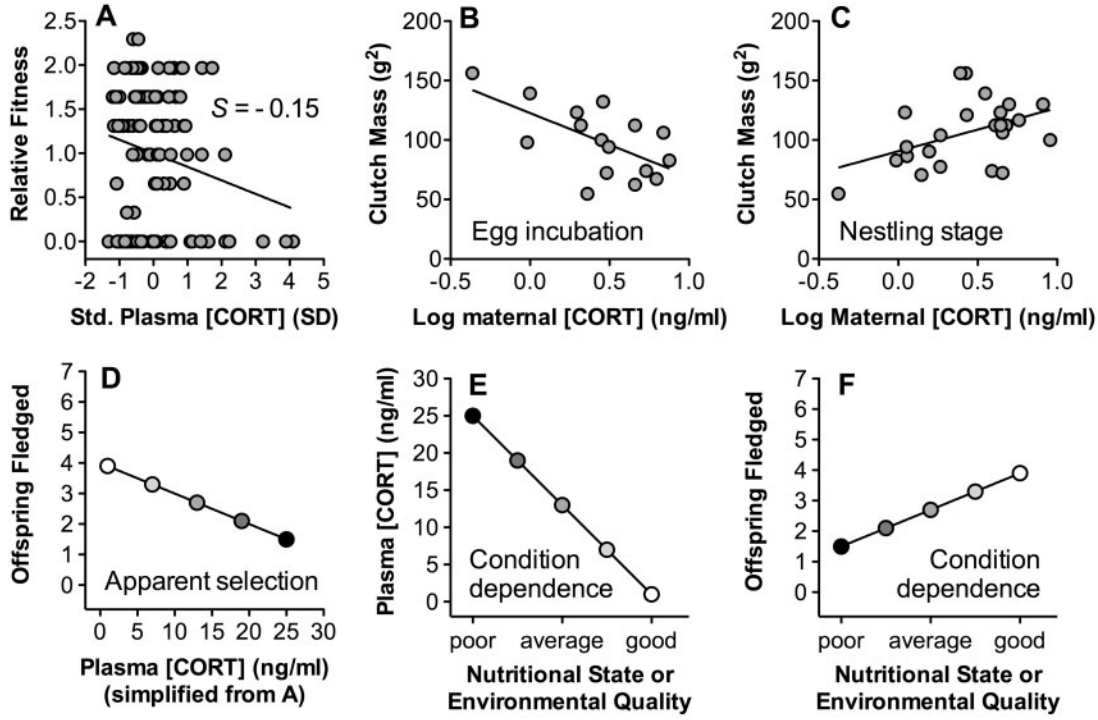

Fig. 2 Potential complications associated with measurement of selection on hormone levels, as illustrated by data from female tree swallows (*Tachycineta bicolor*). (**A**) Negative correlation between number of offspring fledged (expressed as relative fitness) and standardized variance in maternal CORT levels suggests significant directional selection for lower CORT. However, due to adaptive plasticity in hormonal secretion, opposing correlations with reproductive output are observed when maternal CORT is assayed early in the breeding season during egg incubation (**B**), versus later in the breeding season during the nestling stage (**C**). This presumably indicates that mothers with the lowest initial stress levels invest the most in reproduction, which causes an adaptive increase in their CORT levels, thereby complicating inferences regarding selection on CORT levels. Apparent selection on endocrine phenotypes (**D**) can also arise from confounding intrinsic (e.g., nutritional state) or extrinsic factors (e.g., environmental quality) that affect both CORT levels (**E**) and reproductive success (**F**), as shown here with hypothetical data. Redrawn in part from Bonier et al. (2009) with permission from Elsevier.

two concerns regarding (1) adaptive plasticity in hormone levels, and (2) unmeasured phenotypes and environmental effects, with data from female tree swallows, *Tachycineta bicolor* (Bonier et al. 2009b).

In light of these concerns, how might studies of selection be conducted so as to provide greater insight into the adaptive significance of endocrine phenotypes? One partial solution, as encapsulated in the multivariate breeder's equation, is to measure correlated phenotypes and environmental effects, which can then be included in multivariate analyses to derive selection gradients ($\boldsymbol{\beta}$) for endocrine phenotypes (Mills et al. 2007; McGlothlin et al. 2010; Patterson et al. 2014). Nonetheless, there is no magic in a multiple regression, and the statistical partitioning of direct and indirect selection will often be difficult for traits that are highly collinear (Mitchell-Olds and Shaw 1987; Fairbairn and Preziosi 1996). A second approach is to infer the relationship between hormones and fitness by experimentally altering circulating hormone levels or blocking their action, as frequently done for T and CORT in wild populations (Reed et al. 2006; Love and Williams 2008; John-Alder et al. 2009). The advantage of this approach is that it can be used to test for causal effects of hormones on fitness while randomizing any confounding phenotypic or environmental factors with respect to hormonal treatment. However, inferences about selection on natural phenotypic variance and covariance may be tenuous when based on manipulations that deliberately and dramatically alter these parameters (Fusani 2008; McGlothlin et al. 2010). Third, if assessing the potential for an evolutionary response is indeed the goal, then the quantification of h^2 in wild populations can, at least in principle, address the extent to which plasticity in endocrine phenotypes obscures (or not) the exposure of additive genetic variance to selection. Although studies of the heritability of endocrine phenotypes in wild populations are sorely needed (Supplementary Tables S1 and S2), we propose that a promising path forward for analyses of

selection in the wild will be to quantify endocrine phenotypes in more holistic ways that capture their inherently dynamic and integrated properties.

Examples of this approach come from recent analyses of selection on both baseline and experimentally induced variation in circulating T and CORT levels (McGlothlin et al. 2010; Patterson et al. 2014). In male dark-eyed juncos, *Junco hyemalis*, standardized injections of GnRH stimulate the HPG axis to produce elevated T levels that are repeatable within individuals (Jawor et al. 2006) and correlated with those induced by social stimuli (McGlothlin et al. 2008). Whereas both survival and reproductive success generate uniformly weak selection on baseline T levels, these same components of fitness generate moderate directional selection ($\boldsymbol{\beta}$) and strong stabilizing selection (γ) on GnRH-induced T levels (Fig. 3). Thus, a combination of multivariate selection analyses and dynamic measures of endocrine phenotypes revealed patterns of selection that were not evident from baseline T levels alone (McGlothlin et al. 2010). Likewise, Patterson et al. (2014) subjected white-crowned sparrows, *Zonotrichia leucophrys*, to handling stress to derive several measures of stress response for free (unbound) and total CORT, each of which demonstrated higher repeatability that did baseline levels of free and total CORT. Multivariate analyses including these endocrine phenotypes, along with several morphological and energetic variables, reveal a range of weak to moderate selection via survival and reproductive success on both baseline and stress-induced CORT levels (Patterson et al. 2014). Moving forward, we suggest that evolutionary endocrinologists should build on these approaches by explicitly treating endocrine phenotypes as reaction norms or state-dependent traits (Williams 2008), an emerging framework in quantitative genetic studies of adaptive phenotypic plasticity (Nussey et al. 2005; Kingsolver et al. 2015; Nussey 2015).

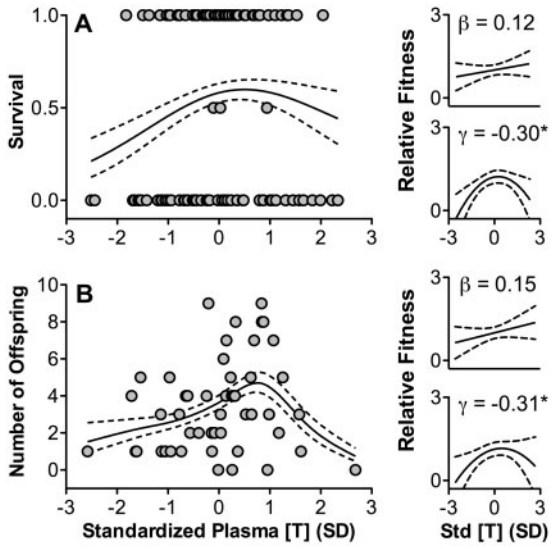

Fig. 3 Selection on GnRH-induced plasma T levels in male dark-eyed juncos, *Junco hyemalis*. Large panels on the left present cubic splines (±SE) illustrating fitness surfaces for individual survival (**A**) and reproductive success (**B**) as a function of GnRH-induced plasma T levels standardized to a mean of zero in unit variance. The three symbols with survival of 0.5 are means of individuals with the same hormonal phenotype. Small panels on the right illustrate directional selection gradients ($\boldsymbol{\beta}$, top panels) and quadratic selection gradients (γ, bottom panels) derived from partial correlation coefficients from linear ($\boldsymbol{\beta}$) or quadratic (γ) regressions of relative fitness (individual fitness values in left panels divided by population mean fitness) on standardized T levels. The form and magnitude of selection is remarkably similar for each component of fitness. Redrawn from McGlothlin et al. (2010) with permission from University of Chicago Press.

Phenotypic integration and the multivariate breeder's equation

Whereas the univariate breeder's equation provides a basic conceptual framework for exploring the heritability of and selection on endocrine phenotypes, the multivariate breeder's equation can be used to illustrate the broader evolutionary significance of hormones as mediators of phenotypic and genetic integration for diverse traits. The concept of phenotypic integration has a long history in evolutionary biology (reviewed by Murren 2012), and can be operationally defined by the structure of covariance or correlation between traits (Armbruster et al. 2014), which is contained in **P**, the phenotypic variance-covariance matrix in the multivariate breeder's equation. Phenotypic variance provides the raw material upon which selection acts, but the covariance between phenotypes determines the extent to which selection can act independently (or not) on multiple traits (Lande and Arnold 1983). Although phenotypic and genetic variances and covariances are used to predict evolutionary change, for simplicity we refer primarily to phenotypic and genetic correlations, which are analogous to phenotypic and genetic covariances, but are more intuitive because they are scaled to total variance so that they are bounded between −1 and 1 and correspond empirically to correlation coefficients.

Though correlations between traits may reflect constraints due to trade-offs or shared developmental and genetic pathways (Arnold 1992; Murren 2012), they may also arise adaptively, due to correlational selection for co-expression of particular trait combinations (Brodie 1992; Sinervo and Svensson 2002;

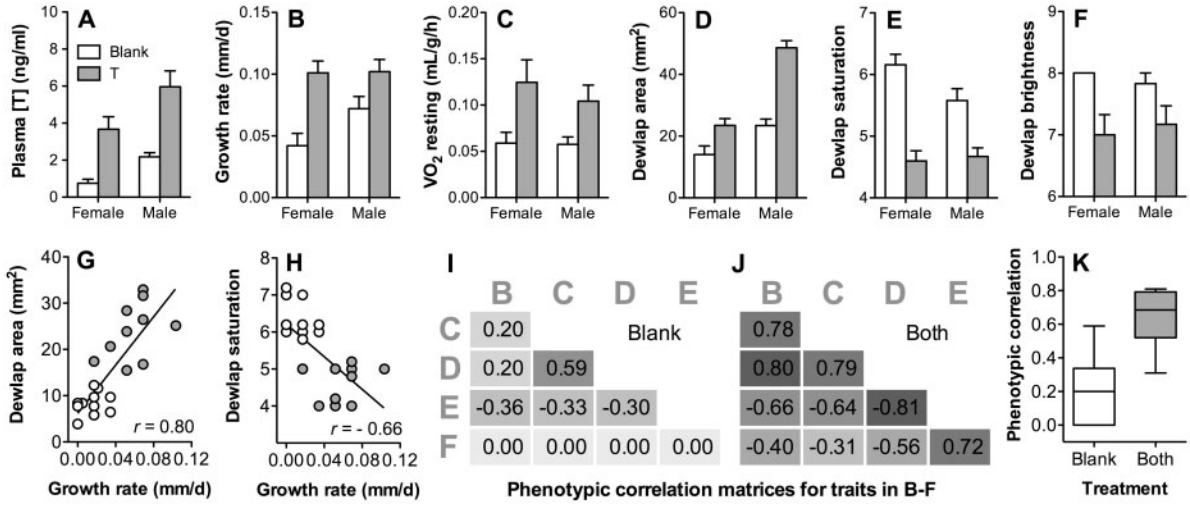

Fig. 4 Phenotypic integration mediated by testosterone (T) in the brown anole. Treatment of juvenile males and females with exogenous T elevates circulating T levels relative to blank-implanted controls (**A**) and reveals stimulatory effects of T on growth (**B**), resting metabolic rate (**C**), and dewlap size (**D**), as well as inhibitory effects on dewlap saturation (**E**) and brightness (**F**). Data are means ± SE. See Cox et al. (2015) for details. These effects of T generate positive phenotypic correlations between traits that respond similarly to T (**G**) and negative correlations between traits that respond in opposite directions (**H**). Experimentally enhanced variation in plasma T therefore results in phenotypic integration, as evident from comparison of phenotypic correlation matrices for the traits in panels (B)–(F) when measured across females in the blank-implanted group (**I**) versus females in the blank-implanted and T-implanted groups (**J**). Intensity of shading corresponds to the degree of correlation. Box-and-whisker plots (**K**) show median, 25–75 interquartile, and minimum/maximum for the $n = 10$ values of r reported in (I) and (J).

McGlothlin et al. 2005; McGlothlin and Ketterson 2008). The latter is presumably true of many examples of hormonal pleiotropy in which diverse phenotypes are influenced by a single hormone (Flatt et al. 2005; McGlothlin and Ketterson 2008; Ketterson et al. 2009). Given the growing appreciation of the dual significance of hormonal pleiotropy in facilitating adaptive phenotypic integration while also potentially constraining the independent evolution of integrated traits (Finch and Rose 1995; Ketterson and Nolan 1999; Hau 2007; Adkins-Regan 2008; McGlothlin and Ketterson 2008; Hau and Wingfield 2011; Williams 2012), it is somewhat surprising that no study to date has directly quantified the effects of hormones on statistical patterns of phenotypic integration. Therefore, although the concept of hormonal pleiotropy is widely accepted, the extent to which hormones actually shape **P** and thereby alter the trait combinations available to selection remains largely conjectural.

To test this principle, we derived phenotypic correlation matrices for a suite of morphological and physiological traits that we have previously shown to be influenced by administration of exogenous T to juvenile brown anole lizards, *Anolis sagrei* (Cox et al. 2015; summarized in Fig. 4A–F). We found that, whereas T-mediated traits such as growth, resting metabolic rate, and the size and coloration of the dewlap are generally uncorrelated with one another in juvenile females with uniformly low circulating T levels (mean $r = 0.20 \pm 0.06$ SE), they are weakly correlated in juvenile males with slightly more variable T levels (mean $r = 0.35 \pm 0.08$), and highly correlated when assessed across a broader range of T levels encompassing females with naturally low and experimentally elevated T levels (mean $r = 0.65 \pm 0.06$). This sharp increase in the overall degree of phenotypic integration (Fig. 4I–K) is directly attributable to the pronounced effects of exogenous T on the expression of "male-typical" phenotypes in females (Fig. 4G and H). As such, this example provides an illustrative proof-of-concept in which the effects of T are causally established, though it does not directly address the relevance of natural variation in plasma T (and in trait responsiveness to T) to phenotypic integration in males. Testing the role of natural variation in endocrine phenotypes with respect to statistical patterns of phenotypic integration represents an important avenue for progress in evolutionary endocrinology, particularly when combined with multivariate analyses of phenotypic selection.

Genetic (dis)integration by hormonal regulation of gene expression

Though it is generally accepted (if infrequently demonstrated) that hormonal pleiotropy can structure phenotypic integration and thereby shape **P**, what

is less widely appreciated is that hormones can also influence the underlying patterns of genetic variance and covariance that comprise **G** in the multivariate breeder's equation. This point is of particular significance because **G** describes the underlying genetic architecture through which selection is translated into evolutionary response (Arnold 1992). To explore this principle in its simplest form, we can set aside the multivariate complexity of **G** and focus instead on the specific case of a single between-sex genetic correlation (r_{mf}), which quantifies the extent to which additive genetic effects (A) for a given trait are correlated between males and females:

$$r_{mf} = \frac{cov(A_m, A_f)}{\sqrt{(V_{Am} V_{Af})}}. \quad (4)$$

where V_{Am} and V_{Af} are additive genetic variances within each sex. High values of r_{mf} imply a strong genetic constraint on the evolution of sexual dimorphism because any selection on heritable variation in one sex should produce a correlated evolutionary response in the opposite sex. Consequently, the evolution of sexual dimorphism is expected to proceed via the gradual reduction of r_{mf} for shared traits (Lande 1980, 1987), a prediction that is supported by comparative evidence (Poissant et al. 2010) and artificial selection experiments (Delph et al. 2011). Interestingly, r_{mf} is not a static property of a trait, but tends to decrease as ontogeny progresses and sexual dimorphism develops (Poissant and Coltman 2009). This developmental process of "genetic disintegration" is of particular interest in the context of evolutionary endocrinology because it likely often corresponds to maturational increases in the circulation of sex steroids such as T, which are known to activate many phenotypic sexual dimorphisms (e.g., Cox et al. 2015; Cox et al. 2005; Cox et al. 2009). Fisher (1958) was the first to recognize this association, proposing that the ontogenetic emergence of sex-specific genetic variance could be mediated by "secretions of the sexual glands" (Poissant and Coltman 2009).

To explore this idea, we recently characterized the ontogeny of sexual size dimorphism, its quantitative genetic architecture, and genome-wide patterns of sex-biased gene expression in the brown anole, *Anolis sagrei* (R. M. Cox, C. L. Cox, J. W. McGlothlin, D. C. Card, A. L. Andrew, and T. A. Castoe, in preparation). Males and females of this species hatch at nearly identical sizes, but males grow to be over 30% longer and nearly three times as massive as females by adulthood (Cox et al. 2009; Cox and Calsbeek 2010). This gradual development

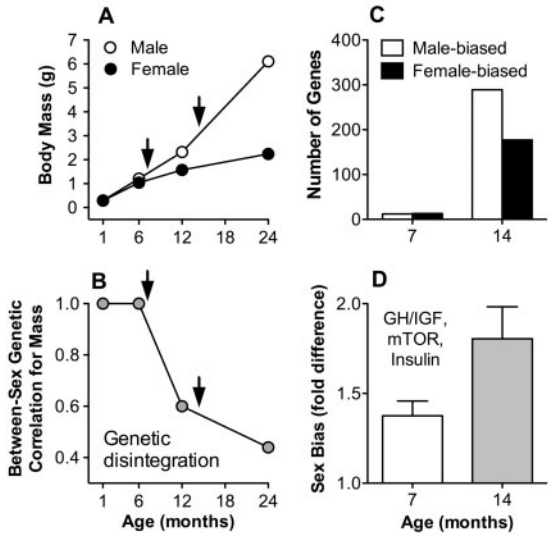

Fig. 5 In the brown anole, *Anolis sagrei*, the development of sexual size dimorphism in mean body mass (**A**) is mirrored by an ontogenetic breakdown of the between-sex genetic correlation for body mass (**B**) and a sharp ontogenetic increase in the number of significantly sex-biased genes in the liver (**C**). An ontogenetic increase in mean (±SE) sex-biased gene expression is particularly evident across 101 genes in the interrelated growth hormone/insulin-like growth factor (GH/IGF), mechanistic target of rapamycin (mTOR), and insulin signaling pathways that regulate growth, metabolism, and cell proliferation (**D**). Arrows in (A)–(B) indicate the timing of transcriptome sequencing for (C)–(D). Statistical significance of sex-biased gene expression at each age in panel (C) was assessed from $n = 4$ individuals per sex at $P < 0.05$ following correction for transcriptome-wide false-discovery rates.

of sexual size dimorphism during ontogeny is mirrored by the ontogenetic breakdown of r_{mf} for body size (Fig. 5A and B), suggesting that many of the genes that initially contribute to size variation in similar ways in both sexes become increasingly sex-specific in their expression as ontogeny progresses. In line with this view, gene expression in the liver (which integrates growth and energetics) becomes increasingly sex-biased with age, whether viewed across the entire transcriptome (Fig. 5C) or with respect to specific endocrine pathways related to growth, energetics, and cell proliferation (i.e., the growth hormone/insulin-like growth factor pathway, the mechanistic target of rapamycin pathway, and the insulin signaling pathway; Fig. 5D).

Work currently underway suggests that this developmental breakdown of r_{mf} via sex-biased gene expression may be orchestrated in large part by hormonal pleiotropy. For example, of the 466 genes conservatively identified as being significantly sex-biased during the period of maximal growth

divergence between the sexes (Fig. 5C), 92 genes (20%) were also found to be significantly responsive to exogenous testosterone in a separate experiment on juvenile females (C. L. Cox, D. C. Card, A. L. Andrew, T. A. Castoe and R. M. Cox, in preparation). Across the entire hepatic transcriptome, as well as within the GH/IGF, mTOR, and insulin signaling pathways mentioned above, the direction and magnitude of sex-biased gene expression also predicts the direction and magnitude of transcriptional responsiveness to T (C. L. Cox et al., in preparation). In other species, pleiotropic effects of T on gene expression differ between sexes (Peterson et al. 2013, 2014), which could further increase the potential for sex-specific expression of genetic variance and covariance. Collectively, these findings suggest that pleiotropic effects of T on gene expression can result not only in the production of sexually dimorphic phenotypes, but also in the "genetic disintegration" of males and females with respect to additive genetic covariance for shared phenotypes. Though it remains to be tested, we predict that the pleiotropic effects of sex-biased hormones such as T also promote unique patterns of genetic integration within each sex (i.e., sex-specific **G** matrices), analogous to the role of T in promoting phenotypic integration (Fig. 4).

Implications of phenotypic and genetic integration by hormones

Our review of heritability and selection estimates for circulating levels of T and CORT supports the general conclusion that heritable variation in endocrine phenotypes is, with several important caveats, often under selection in wild populations. However, our main goal has been to call attention to the larger significance of hormones in structuring the evolutionary trajectories of the numerous traits they regulate. First, we illustrated how pleiotropic effects of T can alter patterns of phenotypic correlation (Fig. 4). These results imply that the phenotypic variance in hormone levels across a population, whether genetic or environmental in nature, will influence the trait combinations that are available to selection. Second, we used the specific case of a between-sex genetic correlation to illustrate how hormones such as T can, by virtue of their pleiotropic effects on gene expression, alter patterns of genetic correlation (Fig. 5). A key implication of this result, and one that represents an exciting avenue for future research, is that hormonal regulation of sex-biased gene expression may permit traits mediated by T (and by other sex-biased hormones) to evolve more rapidly in response to sex-specific selection.

Our results call attention to the fact that genetic variances and covariances are not immutable properties of a genome, but changing parameters that describe the translation of genotype to phenotype at a particular time, in a particular environment. By virtue of their systemic circulation and pleiotropic effects on transcription, hormones can be viewed as key features of the local environments in which genes reside and are translated into phenotypes. As such, differences in hormonal phenotypes among individuals should shape the population-level patterns of phenotypic (**P**) and genetic integration (**G**, r_{mf}) that mediate the translation of selection into evolution. Viewed in this light, hormones play an interactive role in the evolutionary process by responding directly to selection (e.g., for higher or lower circulating levels), while simultaneously influencing the evolutionary response to selection for the many other traits they adaptively regulate. When couched in this framework of evolutionary genetics, studies of endocrine mechanisms are thus uniquely situated to provide key insights into the nature of genotype-to-phenotype mapping and the evolution of complex traits.

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Supplementary data

Supplementary data available at *ICB* online.

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References

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

Armbruster WS, Pélabon C, Bolstad GH, Hansen TF. 2014. Integrated phenotypes: understanding trait covariation in plants and animals. Philos Trans R Soc Lond B Biol Sci 369:20130245; DOI: 10.1098/rstb.2013.0245.

Arnold SJ. 1992. Constraints on phenotypic evolution. Am Nat 140:S85–107.

Bartels M, Van den Berg M, Sluyter F, Boomsma DI, de Geus EJC. 2003. Heritability of cortisol levels: review and simultaneous analysis of twin studies. Psychoneuroendocrinology 28:121–37.

Bates RO, Buchanan DS, Johnson RK, Wettemann RP, Pent RW, Hutchens LK. 1986. Genetic parameter estimates for reproductive traits of male and female littermate swine. J Animal Sci 63:377–85.

Bergeon Burns CM, Rosvall KA, Hahn TP, Demas GE, Ketterson ED. 2014. Examining sources of variation in HPG axis function among individuals and populations of the dark-eyed junco. Horm Behav 65:179–87.

Bergeon Burns CM, Rosvall KA, Ketterson ED. 2013. Neural steroid sensitivity and aggression: comparing individuals of two songbird subspecies. J Evol Biol 26:820–31.

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.

Brodie E. D., III. 1992. Correlational selection for color pattern and antipredator behavior in the garter snake. Thamnophis ordinoides. Evolution 46:1284–98.

Brodie E. D., III. 1993. Consistency of individual differences in anti-predator behaviour and colour pattern in the garter snake, Thamnophis ordinoides. Anim Behav 45:851–61.

Brodie E. D., III, Russell NH. 1999. The consistency of individual differences in behaviour: temperature effects on antipredator behaviour in garter snakes. Anim Behav 57:445–51.

Cox RM, Calsbeek R. 2009. Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. Am Nat 73:176–87.

Cox RM, Calsbeek R. 2010. Sex-specific selection and intraspecific variation in sexual size dimorphism. Evolution 64:798–809.

Cox CL, Hanninen AF, Reedy AM, Cox RM. 2015. Female anoles retain responsiveness to testosterone despite the evolution of androgen-mediated sexual dimorphism. Funct Ecol 29:758–67.

Cox RM, Skelly SL, Leo A, John-Alder HB. 2005. Testosterone regulates sexually dimorphic coloration in the eastern fence lizard, *Sceloporus undulatus*. Copeia 2005:597–608.

Cox RM, Stenquist DS, Calsbeek R. 2009. Testosterone, growth, and the evolution of sexual size dimorphism. J Evol Biol 22:1586–98.

Dantzer B, Westrick S, van Kesteren F. 2016. Relationships between endocrine traits and life histories in wild animals: insights, problems, and potential pitfalls. Integr Comp Biol.

Delph LF, Steven JC, Anderson IA, Herlihy CR, Brodie IIIED. 2011. Elimination of a genetic correlation between the sexes via artificial correlational selection. Evolution 65:2872–80.

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.

Fairbairn DJ, Preziosi RF. 1996. Sexual selection and the evolution of sexual size dimorphism in the water strider. Aquarius remigis. Evolution 50:1549–59.

Federenko IS, Nagamine M, Hellhammer DH, Wadhwa PD, Wüst S. 2004. The heritability of hypothalamus pituitary adrenal axis responses to psychosocial stress is context dependent. J Clin Endocrinol Metab 89:6244–50.

Fevolden SE, Røed K, Fjalestad K, Stien J. 1999. Poststress levels of lysozyme and cortisol in adult rainbow trout: heritabilities and genetic correlations. J Fish Biol 54:900–10.

Finch CE, Rose MR. 1995. Hormones and the physiological architecture of life history evolution. Quart Rev Biol 70:1–52.

Fisher R. 1958. The genetical theory of natural selection. New York: Dover Publications, Inc.

Flatt T, Tu M-P, Tatar M. 2005. Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. BioEssays 27:999–1010.

Fusani L. 2008. Endocrinology in field studies: problems and solutions for the experimental design. Gen Comp Endocrinol 157:249–53.

Garland TJ, Meng Z, Saltzman W. 2016. Hormones and the evolution of complex traits: insights from artificial selection on behavior. Integr Comp Biol.

Goymann W. 2009. Social modulation of androgens in male birds. Gen Comp Endocrinol 163:149–57.

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

Hau M, Wingfield JC. 2011. Hormonally-regulated trade-offs: evolutionary variability and phenotypic plasticity in testosterone signalling pathways. In: Flatt T, Heyland A, editors. Mechanisms of life history evolution. Oxford, UK: Oxford University Press. p. 349–61.

Iserbyt A, Eens M, Müller W. 2015. Sexually antagonistic selection during parental care is not generated by a testosterone-related intralocus sexual conflict–insights from full-sib comparisons. Sci Rep 5:17715.

Jawor JM, McGlothlin JW, Casto JM, Greives TJ, Snajdr EA, Bentley GE, Ketterson ED. 2006. Seasonal and individual variation in response to GnRH challenge in male dark-eyed juncos (Junco hyemalis). Gen Comp Endocrinol 149:182–9.

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 Lond B Biol Sci 281:20141302; DOI: 10.1098/rspb.2014.1302.

John-Alder HB, Cox RM, Haenel GJ, Smith LC. 2009. Hormones, performance and fitness: natural history and endocrine experiments on a lizard (*Sceloporus undulatus*). Integr Comp Biol 49:393–407.

Kadarmideen HN, Janss LLG. 2007. Population and systems genetics analyses of cortisol in pigs divergently selected for stress. Physiol Genom 29:57–65.

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

Ketterson ED, Atwell JW, McGlothlin JW. 2009. Phenotypic integration and independence: hormones, performance, and response to environmental change. Integr Comp Biol 49:365–79.

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

Ketterson ED, Nolan V Jr, Sandell M. 2005. Testosterone in females: mediator of adaptive traits, constraint on sexual dimorphism, or both? Am Nat 166:S85–98.

King RB, Cline JH, Hubbard CJ. 2004. Heritable variation in testosterone levels in male garter snakes (*Thamnophis sirtalis*). J Zoology 264:143–7.

Kingsolver J, Diamond S, Gomulkiewicz R. 2015. Curve-thinking: Understanding reaction norms and developmental trajectories as traits. In: Martin LB, Ghalambor CK, Woods HA, editors. Integrative organismal biology. Hoboken, NJ: John Wiley & Sons, Inc. p. 39–54.

Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P. 2001. The strenght of phenotypic selection in natural populations. Am Nat 157:245–61.

Kingsolver JG, Pfennig DW. 2007. Patterns and power of phenotypic selection in nature. BioScience 57:561–72.

Lande R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. Evolution 33:402–16.

Lande R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–307.

Lande R. 1987. Genetic correlations between the sexes in the evolution of sexual dimorphism and mating preferences. In: Bradbury JW, Andersson MB, editors. Sexual selection: testing the alternatives. Chichester: John Wiley and Sons. p. 83–94.

Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. Evolution 37:1210–26.

Love OP, Williams TD. 2008. The adaptive value of stress-induced phenotypes: effects of maternally derived corticosterone on sex-biased investment, cost of reproduction, and maternal fitness. Am Nat 172:E135–49.

Lubritz D, Johnson B, Robison OW. 1991. Genetic parameters for testosterone production in boars. J Anim Sci 69:3220–3224.

McGlothlin JW, Jawor JM, Greives TJ, Casto JM, Phillips JL, Ketterson ED. 2008. Hormones and honest signals: males with larger ornaments elevate testosterone more when challenged. J Evol Biol 21:39–48.

McGlothlin JW, Ketterson ED. 2008. Hormone-mediated suites as adaptations and evolutionary constraints. Philos Trans R Soc Lond B Biol Sci 363:1611–20.

McGlothlin JW, Parker PG, Nolan VJr, Ketterson ED. 2005. Correlational selection leads to genetic integration of body size and an attractive plumage trait in dark-eyed juncos. Evolution 59:658–71.

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.

Mills S, Grapputo A, Jokinen I, Koskela E, Mappes T, Oksanen T, Poikonen T. 2009. Testosterone-mediated effects on fitness-related phenotypic traits and fitness. Am Nat 173:475–87.

Mills SC, Grapputo A, Jokinen I, Koskela E, Mappes T, Poikonen T. 2010. Fitness trade-offs mediated by immunosuppression costs in a small mammal. Evolution 64:166–79.

Mills SC, Grapputo A, Koskela E, Mappes T. 2007. Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices. Proc R Soc Lond B Biol Sci 274:143–50.

Mills SC, Koskela E, Mappes T. 2012. Intralocus sexual conflict for fitness: sexually antagonistic alleles for testosterone. Proc R Soc Lond B Biol Sci 279:1889–95.

Mitchell-Olds T, Shaw RG. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. Evolution 1149–61.

Mokkonen M, Koskela E, Mappes T, Mills S. 2016. Evolutionary conflict between maternal and paternal interests. Integr Comp Biol.

Moore IT, Jessop TS. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Horm Behav 43:39–47.

Morrissey MB, Kruuk LEB, Wilson AJ. 2010. The danger of applying the breeder's equation in observational studies of natural populations. J Evol Biol 23:2277–88.

Morrissey MB, Parker DJ, Korsten P, Pemberton JM, Kruuk LEB, Wilson AJ. 2012. The prediction of adaptive evolution: empirical application of the secondary theorem of selection and comparison to the breeder's equation. Evolution 66:2399–410.

Murren CJ. 2012. The integrated phenotype. Integr Comp Biol 52:64–76.

Nussey DH. 2015. The ecological and evolutionary importance of variation in life-history reaction norms. In: Martin LB, Ghalambor CK, Woods HA, editors. Integrative organismal biology. Hoboken, NJ: John Wiley & Sons, Inc. p. 23–38.

Nussey DH, Postma E, Gienapp P, Visser ME. 2005. Selection on heritable phenotypic plasticity in a wild bird population. Science 310:304–6.

Ouyang JQ, Hau M, Bonier F. 2011a. Within seasons and among years: when are corticosterone levels repeatable? Horm Behav 60:559–64.

Ouyang JQ, Sharp P, Quetting M, Hau M. 2013. Endocrine phenotype, reproductive success and survival in the great tit, *Parus major*. J Evol Biol 26:1988–98.

Ouyang JQ, Sharp PJ, Dawson A, Quetting M, Hau M. 2011b. Hormone levels predict individual differences in reproductive success in a passerine bird. Proc R Soc B Biol Sci 282:2537–45.

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

Pavitt AT, Walling CA, Möstl E, Pemberton JM, Kruuk LEB. 2015. Cortisol but not testosterone is repeatable and varies with reproductive effort in wild red deer stags. Gen Comp Endocrinol 222:62–68.

Pavitt AT, Walling CA, Pemberton JM, Kruuk LEB. 2014. Heritability and cross-sex genetic correlations of early-life

circulating testosterone levels in a wild mammal. Biol Lett 10:0140685; DOI: 10.1098/rsbl.2014.0685.

Pelletier F, Bauman J, Festa-Bianchet M. 2003. Fecal testosterone in bighorn sheep (*Ovis canadensis*): behavioural and endocrine correlates. Can J Zool 81:1678–84.

Peterson MP, Rosvall KA, Choi J-H, Ziegenfus C, Tang H, Colbourne JK, Ketterson ED. 2013. Testosterone affects neural gene expression differently in male and female juncos: a role for hormones in mediating sexual dimorphism and conflict. PLoS ONE 8:e61784.

Peterson MP, Rosvall KA, Taylor CA, Lopez JA, Choi J-H, Ziegenfus C, Tang H, Colbourne JK, Ketterson ED. 2014. Potential for sexual conflict assessed via testosterone-mediated transcriptional changes in liver and muscle of a songbird. J Exp Biol 217:507–17.

Poissant J, Coltman DW. 2009. The ontogeny of cross-sex genetic correlations: an analysis of patterns. J Evol Biol 22:2558–62.

Poissant J, Wilson AJ, Coltman DW. 2010. Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations. Evolution 64:97–107.

Price T, Kirkpatrick M, Arnold SJ. 1988. Directional selection and the evolution of breeding date in birds. Science 240:798–9.

Reed WL, Clark ME, Parker PG, Raouf SA, Arguedas N, Monk DS, Snajdr E, Nolan V Jr, Ketterson ED. 2006. Physiological effects on demography: a long term experimental study of testosterone's effects on fitness. Am Nat 167:667–83.

Rensel MA, Schoech SJ. 2011. Repeatability of baseline and stress-induced corticosterone levels across early life stages in the Florida scrub-jay (*Aphelocoma coerulescens*). Horm Behav 59:497–502.

Robison OW, Lubritz D, Johnson B. 1994. Realized heritability estimates in boars divergently selected for testosterone levels. J Anim Breed Genet 111:35–42.

Romero LM, Reed JM. 2008. Repeatability of baseline corticosterone concentrations. Gen Comp Endocrinol 156:27–33.

Rosvall KA, Bergeron Burns CM, Jayaratna SP, Dossey EK, Ketterson ED. 2016. Gonads and the evolution of hormonal phenotypes. Integr Comp Biol.

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

Siepielski AM, DiBattista JD, Carlson SM. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. Ecol Lett 12:1261–76.

Sinervo B, Svensson E. 2002. Correlational selection and the genomic architecture. Heredity 89:329–38.

Stöwe M, Rosivall B, Drent PJ, Möstl E. 2010. Selection for fast and slow exploration affects baseline and stress-induced corticosterone excretion in Great tit nestlings, *Parus major*. Horm Behav 58:864–71.

Tanck MWT, Vermeulen K-J, Bovenhuis H, Komen H. 2001. Heredity of stress-related cortisol response in androgenetic common carp (*Cyprinus carpio* L.). Aquaculture 199:283–94.

Travison T, Zhuang W, Lunetta K, Karasik D, Bhasin S, Kiel D, Coviello A, Murabito J. 2014. The heritability of circulating testosterone, oestradiol, oestrone and sex hormone binding globulin concentrations in men: the Framingham Heart Study. Clin Endocrinol 80:277–82.

van Oers K, Buchanan KL, Thomas TE, Drent PJ. 2011. Correlated response to selection of testosterone levels and immunocompetence in lines selected for avian personality. Anim Behav 81:1055–61.

Wade MJ, Kalisz S. 1990. The causes of natural selection. Evolution 44:1947–55.

Walker S, Robison OW, Whisnant CS, Cassady JP. 2004. Effect of divergent selection for testosterone production on testicular morphology and daily sperm production in boars. J Anim Sci 82:2259–63.

While GM, Isaksson C, McEvoy J, Sinn DL, Komdeur J, Wapstra E, Groothuis TGG. 2010. Repeatable intra-individual variation in plasma testosterone concentration and its sex-specific link to aggression in a social lizard. Horm Behav 58:208–13.

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

Williams TD. 2012. Hormones, life-history, and phenotypic variation: opportunities in evolutionary avian endocrinology. Gen Comp Endocrinol 176:286–95.

Zera A. 2016. Evolutionary endocrinology of hormonal rhythms: JH titer circadian polymorphism in *Gryllus firmus*. Integr Comp Biol.

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

Zera AJ, Zhang C. 1995. Evolutionary endocrinology of juvenile hormone esterase in *Gryllus assimilis*: direct and correlated responses to selection. Genetics 141:1125–34.