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Hormones and phenotypic plasticity: Implications for the evolution of integrated adaptive phenotypes

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Abstract It is generally accepted that taxa exhibit genetic variation in phenotypic plasticity, but many questions remain unanswered about how divergent plastic responses evolve under dissimilar ecological conditions. Hormones are signaling molecules that act as proximate mediators of phenotype expression by regulating a variety of cellular, physiological, and behavioral responses. Hormones not only change cellular and physiological states but also influence gene expression directly or indirectly, thereby linking environmental conditions to phenotypic development. Studying how hormonal pathways respond to environmental variation and how those responses differ between individuals, populations, and species can expand our understanding of the evolution of phenotypic plasticity. Here, we explore the ways that the study of hormone signaling is providing new insights into the underlying proximate bases for individual, population or species variation in plasticity. Using several studies as exemplars, we examine how a 'norm of reaction' approach can be used in investigations of hormone-mediated plasticity to inform the following: 1) how environmental cues affect the component hormones, receptors and enzymes that comprise any endocrine signaling pathway, 2) how genetic and epigenetic variation in endocrine-associated genes can generate variation in plasticity among these diverse components, and 3) how phenotypes mediated by the same hormone can be coupled and decoupled via independent plastic responses of signaling components across target tissues. Future studies that apply approaches such as reaction norms and network modeling to questions concerning how hormones link environmental stimuli to ecologically-relevant phenotypic responses should help unravel how phenotypic plasticity evolves [*Current Zoology* 59 (4): 506–525, 2013].

Keywords Hormone, Endocrine, Reaction norm, Developmental plasticity, Pleiotropy, Ecology, Network

The development and expression of an organism's phenotype can be influenced by a variety of factors including the type of environmental conditions experienced throughout life. Environmental experience can shape a variety of traits such as morphology (Lively, 1986; Mittelbach et al., 1999; Nijhout, 2003; Lema and Nevitt, 2006), patterns of behavior (Keller and Ross, 1993; Emlen, 1997; Carroll and Corneli, 1999; Maruska and Fernald, 2013), and the expression of life history characters such as when to become sexually mature and reproduce (Morgan and Christy, 1994; Sultan, 2000; Dawson, 2008), or even what sex to be (Warner, 1984; Crews, 2003; Godwin et al., 2003). Such environmentally-induced variation in an organism's phenotype is termed *phenotypic plasticity* (West-Eberhard, 2003). The extent to which a given organism exhibits plasticity depends on the environmental conditions experienced, the genetic composition of that individual organism, and the specific phenotypic character in question (Schlicting and Pigliucci, 1998; Pigliucci, 2001).

There has been interest among biologists in partitioning the contributions of genes and environment to phenotypic variation ever since Wilhelm Johannsen coined the distinction between genotype and phenotype in the early 1900's (Johannsen, 1911). Hypotheses about the relative importance of genotype and environment in phenotypic expression have been fervently contended for years under the umbrella of the 'nature vs. nurture debate' (e.g., Johnston, 1987). However, studies on the development of phenotypes over the past few decades have established that phenotypic plasticity itself has a genetic basis, and that plasticity can evolve (e.g., Pigluicci, 2001; Pigluicci, 2005; West-Eberhard, 2003).

Accordingly, considerable interest has now been directed toward understanding *why* phenotype plasticity evolves, including what environmental conditions lead plasticity to evolve, what conditions might result in the loss of adaptive plasticity, and what broader significance plasticity holds for evolutionary diversification (de Jong, 2005; Crispo, 2007; Pfennig et al., 2010). *How* pheno-

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typic plasticity evolves, however, has received less attention. Understanding *how* phenotypic plasticity evolves presents several challenges, as it requires both identifying the causative links between environment and phenotype and exploring how genotype-environment interaction changes across generations or varies among taxa. In some scenarios of developmental plasticity, the environmental interactions that shape plastic phenotypic responses occur earlier in development than the resulting phenotypic outcome. Depending on the duration of this temporal separation, the mechanistic details of how environments influence phenotypes differently among populations or species may be difficult to distinguish, ultimately making the identification of evolutionary change in causative interactions formidable.

A foothold toward understanding *how* phenotypic plasticity evolves, however, can be found by recalling that the essential elements of phenotypic plasticity arise from the network of interactions that connect an organism to its environment. Understanding the evolution of plastic responses to environmental variation therefore requires thinking within the context of those interactions, including the organism's experiences with both current environmental conditions and conditions earlier in life, and the physiological and behavioral mechanisms that mediate cell- or tissue-specific gene expression changes in response to environmental experience. Studying the evolution of phenotypic plasticity thereby necessitates thinking broadly about the molecular, physiological and behavioral mechanisms that mediate how environmental variation shapes ontogeny, including focused investigations on each of the following: 1) identifying the network of interacting molecular and physiological pathways, including feedback loops, that link an organism to its environment, 2) distinguishing existing variation among populations or species in the environmental sensitivity and function of these pathways, and 3) exploring how genetic and epigenetic differences underpin any such pathway variation to enable evolutionary changes in phenotypic plasticity between populations or over time.

In this article, we address how studies of phenotypic plasticity from an endocrinology perspective can inform these critical questions about *how* phenotypic plasticity evolves. Hormones can be viewed as developmental links between an organism's genetics and its environment via their role in transducing environmental input – whether external to the organism (e.g., temperature, social interaction) or internal (e.g., metabolic state, a signal from another tissue or cell) – into changes in cell function or gene expression. Accordingly then, endocrine systems have been a focus for studies on the physiological basis of phenotypic plasticity for years, and there are now many examples where hormones have been shown either to have organizing effects by shaping trajectories of phenotypic development, or activational effects by inducing repeatable switches in phenotype depending on current environmental conditions (Table 1) (Moore, 1991; Dufty et al., 2002; Hatle, 2003; Nijhout, 2003). In this article, we go beyond the existing literature on the hormonal basis for phenotypic plasticity to consider how studies of hormone-mediated plasticity in an ecologically relevant context have the potential to provide new insights into how environmental cues regulate the network of molecular and cellular pathways that link organismal development to experience, and how that environmental regulation evolves.

1 Endocrine Systems as Multidimensional Regulatory Networks

To consider the role of hormones in the evolution of phenotypic plasticity, it is helpful to first review how endocrine systems conjoin environmental conditions to an organism's physiology and behavior. Hormones are secreted from an endocrine gland or tissue in response to a change in environmental stimulus, whether external to the organism (e.g., photoperiod, temperature, social challenge, pheromone) or internal (e.g., hydromineral balance, glucose availability, another hormone). The secreted hormone then travels though the organism via systemic circulation until it reaches a peripheral target tissue, where, if it is a polypeptide hormone, it binds a membrane receptor to induce changes in intracellular levels of second messenger molecules such as cytosolic cAMP, inositol triphosphate (IP_3) or Ca^{+2}, or – if it is a steroid (e.g., estrogens, androgens, glucocorticoids, ecdysteriods) or thyroid hormone – it may bind either a intracellular nuclear receptor to up- or down-regulates gene transcription directly, or a membrane or cytoplasmic intracellular receptor to have nongenomic actions on target cells (Bassett et al., 2003; Zhu et al., 2003; Hammes and Levin, 2007; Davis et al., 2008; Cheng et al., 2010). In either scenario, the hormone's action in peripheral tissues is a change in cell function or gene expression that, ultimately, induces a change in the organism's physiology, behavior, or development. Thus, hormones can be viewed as the molecular connectors between an organism's environmental experiences and its genome (Pigluicci, 2001; West-Eberhard, 2003).

Changes in the concentration of circulating hormone (e.g., blood hormone titer) clearly hold a central place in mediating any hormone's effects, so suitably much attention has been paid to how hormone concentrations evolve between populations or species from different environments (Dufty et al., 2002; Bradshaw, 2007; Zera et al., 2007). However, endocrine signaling pathways are complex, and important steps in endocrine pathways are often neglected in ecological studies even though consideration of these components is vital for understanding the evolution of hormone-mediated traits (Fig. 1) (Ball and Balthazart, 2008; Williams, 2008). For instance, steroid hormones, thyroid hormones and many peptide hormones attach to hormone binding proteins (HBPs), which facilitate transport of these hormones in blood circulation. It is now recognized that many HBPs may be multifunctional and not only transport hormones, but also regulate hormone access to receptors, mediate alternate signaling mechanisms for hormone responses, and sometimes even function independently as signaling factors themselves (Breuner and Orchinik, 2002; Duan, 2002; Mohan and Baylink, 2002; Hammond, 2011).

Likewise, the type and relative abundance of different receptors expressed in a target tissue will shape the response of that tissue to a hormone. Evolutionary diversification of endocrine-mediated phenotypes has been shown to result from selection on genetic variation in receptor genes either in the coding region or in the 5′ flanking promoter region, which regulates tissue expression patterns of the receptor (Hoekstra et al., 2006; Phelps, 2010). Alternatively, it is known that many hormone receptors have diversified following a full or partial genome duplication event in a taxonomic lineage (Thornton, 2001; Larhammar et al., 2009; Daza et al., 2012); once duplicated, one copy of the receptor can accumulate mutations, eventually leading to two functionally distinct receptor types.

Evolutionary changes in endocrine pathways can also occur via mutations in other components of the endocrine signaling pathway. For instance, conversion enzymes are common components of steroid and thyroid hormone signaling systems, and play a major role in regulating the forms and levels of these hormones both in systemic circulation and in peripheral tissues (Miller,

Table 1 Examples where endocrinology has been used to study mechanisms of phenotypic plasticity in an ecologically-relevant context

Plastic Character	Hormone(s)	Taxon/Taxa	Reference
Life History			
Timing of metamorphosis	cortiocotropin-releasing hormone (CRH); thyroid hormones	spadefoot toads	Denver, 1998; Denver, 2013
Sex determination	sex steroids	reptiles	Crews, 1996
Sex change	sex steroids; arginine vasotocin (AVT)	fishes	Kroon and Liley, 2000; Godwin et al., 2000
Egg size, egg number	follicle-stimulating hormone (FSH)	lizards	Sinervo and Licht, 1991a,b
Migration timing	thyroid hormones, cortisol, growth hormone (GH), insulin-like growth factor (IGF)	salmonid fishes	Dittman and Quinn, 1996; McCormick et al., 1998; McCormick, 2009
Reproductive mode	juvenile hormone (JH)	pea aphid	Ishikawa et al., 2012
Larval stage development	transforming growth factor-β (TGF-β); insulin/IGF	nematode	Sommer and Ogawa, 2011
Behavior			
Sexual behaviors, social status	arginine vasotocin (AVT); sex steroids	fishes	Semsar et al., 2001; Miranda et al., 2003; Lema and Nevitt, 2004b; Oliveira, 2009
Reproductive behaviors	gonadotropin-releasing hormone (GnRH)	birds	MacDougall-Shackleton et al., 2009
Social dominance phenotype	gonadotropin-releasing hormone (GnRH); sex steroids	fishes	Hofmann, 2006; Maruska and Fernald, 2010, 2013; Fernald and Maruska, 2012
Morphology			
Horn size, body size (also reproductive tactics)	juvenile hormone (JH)	dung beetle	Emlen and Nijhout, 1999; Emlen, 2000
Wing eyespots, wing coloration	ecdysteroids	butterflies	Brakefield et al., 1998; Nijhout, 2003
Wing polymorphism	juvenile hormone (JH), ecdysteroids	crickets	Zera, 2006
Body shape, fin development	thyroid hormones (THs)	fishes	Lema and Nevitt, 2006
Leaf morphology	abscisic acid	waterclover	Lin and Yang, 1999
Skeletal growth, muscle development	androgen steroids	birds	Navara and Mendonça, 2008

2008; Orozco et al., 2012). Conversion enzymes transform steroid and thyroid hormones into more or less active forms (Fig. 1) and are commonly expressed at varying levels in different target tissues, thereby providing a mechanism for peripheral regulation of hormone effects. Hormone signaling pathways may also involve membrane transporters such as monocarboxylate transports (MCTs) and the organic anion transporter

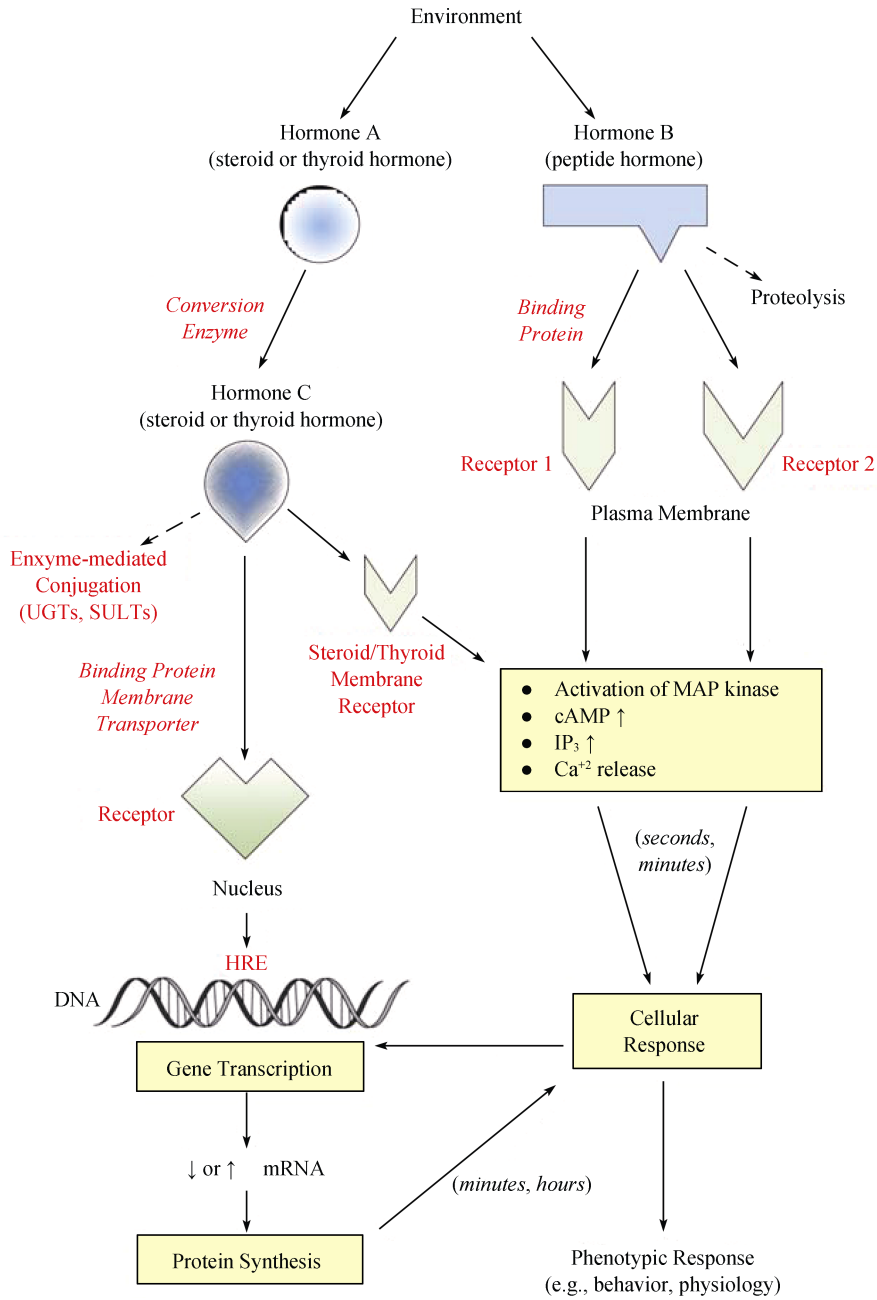

Fig. 1 Schematic representation of how endocrine signaling pathways link environmental conditions to phenotypic responses

Two typical pathways are shown: the 'Hormone A' pathway represents a steroid hormone or thyroid hormone signaling pathway, while the 'Hormone B' pathway represents a polypeptide signal pathway. The perception of an environmental cue stimulates endocrine tissues to release hormones, which are transported in the blood by serum binding proteins to target tissues in the organism's periphery. In the case of steroid or thyroid hormones, the hormone may be metabolized within target tissues to more or less active forms by conversion enzymes and then bind receptors locally, or be released back into blood circulation. If the signal is a steroid or thyroid hormone, the hormone might bind an intracellular receptor, which then interacts with hormone response element (HRE) sequences in the *cis*-regulatory region of loci to up- or down-regulate gene transcription. If the hormone signal is a polypeptide hormone, steroid hormone or thyroid hormone, the hormone may instead bind receptors located within the plasma membrane of target cells and activate a G-protein or phosphorylation mediated cascade of intracellular changes. Hormone signals can also be degraded enzymatically by conjugation or proteolysis. Key steps in the signaling pathways where genetic variation could alter hormone signal function are shown in red text.

proteins (OATPs), which facilitate the uptake of thyroid hormones, steroid hormones, prostaglandins and select polypeptide hormones into cells of peripheral tissues (Visser et al., 2008; van der Deure et al., 2010; Svodoba et al., 2011). And, several enzymes function in the hydroxylation or conjugation of hormones, either to enable excretion of excess hormone, facilitate deactivation by catabolic processes (Sembdner et al., 1994), or even generate alternate forms of active hormone signal (Staswick, 2009). In the vertebrate liver, for instance, steroid and thyroid hormones can be hydroxylated to metabolites with lower or no activity by cytochrome P450 enzymes, or conjugated for excretion through the addition of glucouronide or sulfate moieties by glucuronosyltransferase (UGT) and sulfotransferase (SULT) enzymes (Strott, 1997; James, 2011).

By thinking more broadly about hormone signaling, we begin to see that endocrine pathways are structured as complex, multidimensional networks comprised of several interacting components (e.g., hormones, HBPs, receptors, conversion enzymes, membrane transporters) (Fig. 1). With respect to endocrine-mediated phenotypic plasticity, however, most studies to date have focused on environmentally-mediated changes in hormone levels, and very few studies have taken into account these additional components of endocrine signaling (but see, Zera et al., 2007; McCormick, 2009). But, is it possible that environmental regulation of hormone signaling will include changes in several of these other components? In order to fully understand how endocrine systems respond to environmental variation and contribute to phenotypic plasticity, we need to think beyond just environmental alteration of hormone production, and begin considering how environmental conditions affect the network of components that comprise a hormone signaling pathway. To do this, one must make quantitative measures of several components in the hormone pathway, including blood hormone titers, protein or transcript expression levels of receptors, conversion enzymes, HBPs, or membrane transporters, or the activity of conversion enzymes or conjugation enzymes. When taken together, such values will provide a multidimensional picture of the hormone signaling network, and when measured in several different environments, can provide a clearer window into the environmental responsiveness, or plasticity, of endocrine systems themselves.

2 Physiological Reaction Norms for Hormone Signaling Endophenotypes

Efforts to study endocrine networks to understand how phenotypic plasticity evolves promptly encounter a common problem in studying evolutionary change in environmentally-responsive traits: how does one both visualize the environmental responsiveness of a complex hormone pathway and assess how that responsiveness differs between genotypes, populations or species? One of the simplest approaches to look at how phenotypic variants of any trait both respond to environmental conditions and vary with genotype is the 'norm of reaction.' A 'norm of reaction' is a graphical function that depicts the environment-phenotype relationship of a specific genotype under differing environmental conditions (Stearns, 1989; Schlichting and Pigliucci, 1998; Pigluicci, 2001). Reaction norms are typically plotted as lines connecting trait values for a genotype (or collection of genotypes from distinct populations or species) measured across a range of environments (Fig. 2). Depending on the study system and hypothesis, these environments might be a single parameter of interest (e.g., temperature, social density) or a set of complex, natural habitats that vary from each other in several ways.

The norm of reaction approach was first introduced by Woltereck (1909) in a study of the morphological responses of *Daphnia* species to environmental conditions of temperature and nutrient levels. Since that time, the norm of reaction approach has become one of the most tractable methods for comparing phenotypic response functions over the same scope of environmental variation (Schlichting and Pigliucci, 1998). In cases when the phenotype of an individual can be sampled repeatedly, the reaction norm approach can be used to study the phenotypic response profile of a single individual to environmental variation over time. Alternatively, this approach can be applied to compare variation in the plasticity of different genotypes – whether asexual clones, family lines, or populations – over a similar range of environmental conditions. Such comparisons can quickly reveal whether the genotypes under study exhibit genetic variation for that phenotypic character, plasticity in phenotypic expression, or genetic variation for plasticity itself (Fig. 2) (Carrol and Corneli, 1999; Piglucci, 2001).

The reaction norm approach reveals several key points that are necessary to consider when investigating evolution of environment-phenotype relationships (Pigliucci, 2001). First, the shape or slope of a reaction norm reveals the presence or absence of plasticity in phenotype over the range of environments examined (Fig. 2). Although commonly oversimplified in presentation as straight lines when traits are quantified in only

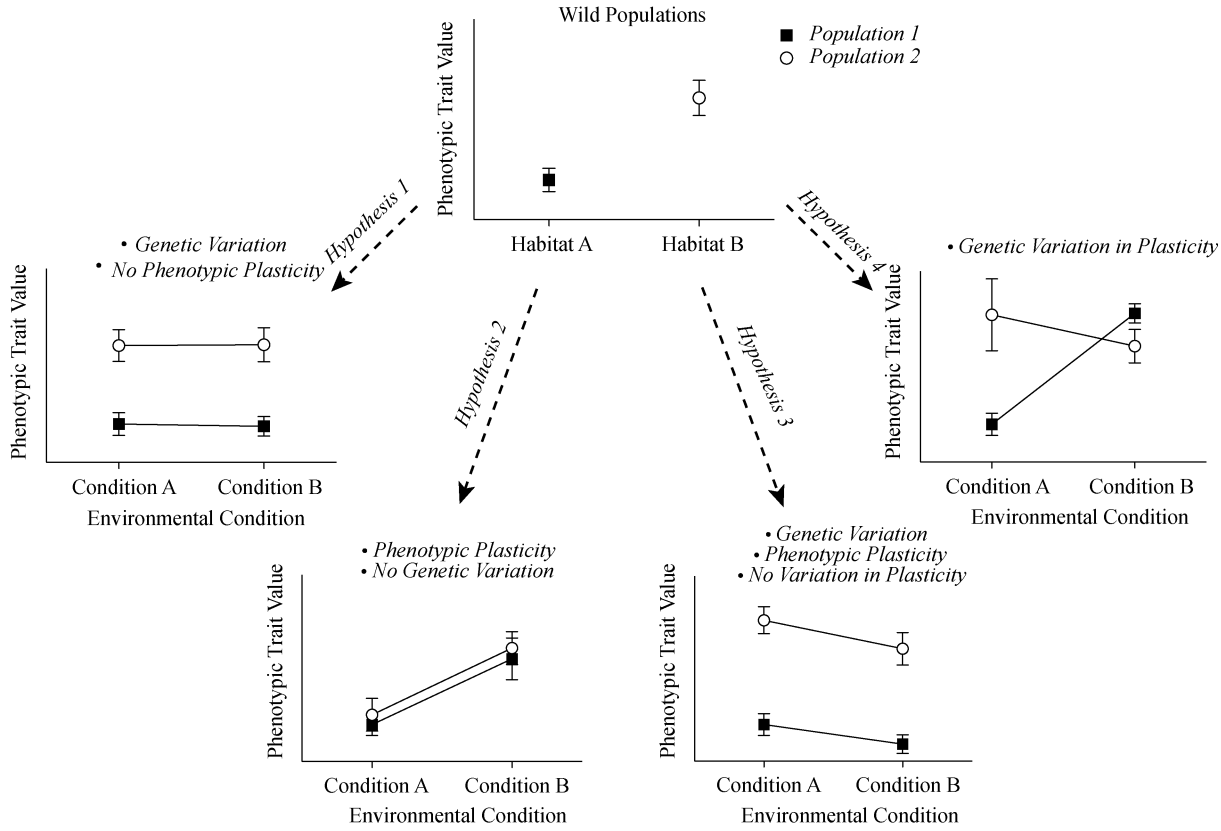

Fig. 2 Illustration of how reaction norms can be used to identify sources of phenotypic variation

When an observation of phenotypic variation is made among individuals, populations, or species for the first time, the source(s) of that variation are often unknown. A reaction norm diagram describes how the phenotype produced by a given genotype (or set of genotypes from a population) varies over a range of environmental conditions, with each phenotype resulting from interactions among all of the organism's genes (genotype) and the environment (external and internal) experienced prior to that point in development. This idealized set of reaction norms illustrates how a reaction norm approach can be used to distinguish hypotheses for the sources of phenotypic variation. Although shown as straight lines here, reaction norms typically present themselves as straight lines only when phenotypes are measured in two environments. More commonly, reactions norms vary in shape and slope, so that the share of phenotypic variation that can be ascribed to genotypic variation and environmental variation will depend on the range of environments examined.

two environments – such as in the hypothetical examples presented in Fig. 2 – reaction norms are rarely straight but rather vary in both shape and slope among environments as a result of the complexities of gene interaction, environmental variation, and developmental processes that construct phenotypes during ontogeny (Stearns, 1989). Second, the range of environmental conditions tested in any study can in itself be insightful. If reaction norms are measured over a range of environmental conditions broader than the environments where the populations currently live, new phenotypes not presently expressed might be revealed. Although some investigators may consider such conditions ecologically inconsequential, measuring reaction norms in conditions beyond those currently experienced has the potential to reveal information about how the expression of the phenotypic character is regulated by environmental cues, as well as how novel traits arise developmentally under environmental conditions experienced by ancestors in the past, or by descendants in the future.

Although not typically applied to endocrine systems, when used to simultaneously examine several of the components that comprise any endocrine pathway (described in the previous section), the norm of reaction approach can be powerful for visualizing whether individual components of a hormone pathway respond differently to shifting environmental conditions (Cockrem and Silverin, 2002; Williams, 2008; Oostra et al., 2011). For example, in a recent study exploring the endocrine underpinnings of intraspecific behavioral variation in bicolor damselfish *Stegastes partitus*, Schrandt and Lema (2011) used a reaction norm approach to investigate how distinct components of the endocrine response to environmental stressors might differ among individuals occupying ecologically divergent habitats. Bicolor damselfish live on coral reefs in close association with

the benthic substratum where the fish establish stable territories and use holes as nests and shelters from predators. On the coral reefs of Curaçao in the Caribbean Sea, this fish exhibits spatial variation in the frequencies of aggression, shelter use and courtship behaviors in accordance with variation in the physical structure of the reef (Schrandt et al., 2012). Fish occupying areas of the reef comprised almost entirely of dead coral rubble are more aggressive, court more frequently, and use shelters more often than fish living in habitats containing live coral (Fig. 3A) (Schrandt and Lema, 2011; Schrandt et al., 2012). When the plasma cortisol response to an acute stressor (20 min capture and confinement stress) was examined in male and female fish from these habitats, only a marginal difference in cortisol reactivity was evident that might explain the habitat-associated variation in behavior (Fig. 3C,D). In teleost fishes, the primary site of cortisol production is the interrenal glands (equivalent of the mammalian adrenal glands), and the secretion of cortisol is controlled by pituitary secretion of ACTH, which is itself regulated by peptide hormones such as corticotropin-releasing

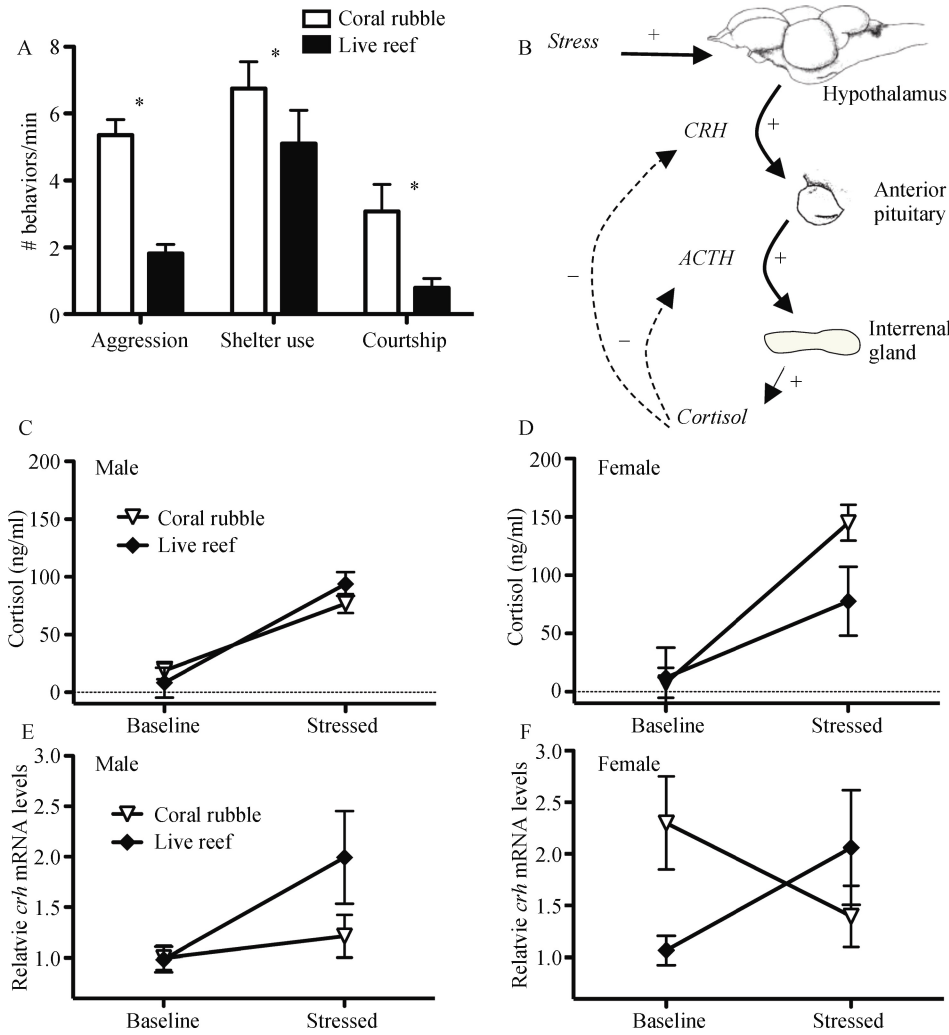

Fig. 3 Reaction norms might differ for each component of an endocrine signaling network

A study of intraspecific behavioral variation in a coral reef fish, the bicolor damselfish *Stegastes partitus*, identified significant variation in aggression, substratum shelter use, and courtship behaviors between live coral and dead coral rubble areas of a reef (**A**), and this behavioral variation was hypothesized to be associated with variation in hormone responses to stress, including the hypothalamic-pituitary-interrenal (HPI) axis endocrine response (**B**). Cortisol hormone responses from baseline (unstressed) to stressed condition (after 20 min of capture and holding stress) in both male (**C**) and female (**D**) damselfish revealed increased cortisol titers with stress ($P < 0.0001$ in each sex separately), but no statistically significant difference in the response in males from the different habitats (rubble or live reef) ($F_{1,53} = 0.960$, $P = 0.332$) (**C**). Although not statistically significant ($F_{1,29} = 2.763$, $P = 0.107$), the data suggest that females from different habitats may vary in cortisol titers following exposure to an acute stressor. Examination of the response of corticotrophin-releasing hormone (*crh*) mRNAs in the brain, however, revealed habitat-associated variation in the response to stress in females (**F**), but not males (**E**). The response of urotensin1 and CRH binding protein mRNAs exhibited a similar pattern of habitat-dependent plasticity in females, while CRH receptor type 1 and type 2 mRNAs did not (data not shown, see Schrandt and Lema, 2011). All data are shown as mean ± *SEM*, and are adapted from Schrandt and Lema (2011).

hormone (CRH) and urotensin-1 produced in the hypothalamus (Mommsen et al, 1999; Pankhurst, 2011). However, both CRH and urotensin-1 also act locally within the brain to have neuromodulatory and behavioral effects, and variation in brain concentrations of CRH and urotensin-1 have been linked to changes in locomotor activity and feeding behaviors in fish (e.g., Bernier and Craig, 2005; Lowry and Moore, 2006). Looking in the brain of these same damselfish revealed significant, sex-specific variation in gene transcript responses of *crh* (Fig. 3D, E), as well as urotensin-1 (*uroten1*) and CRH binding protein (*crh-bp*) (data not shown, see Schrandt and Lema, 2011) linked to habitat. Interestingly, the relative abundance of gene transcripts encoding CRH receptor type 1 (*crh-r1*) and CRH receptor type 2 (*crh-r2*) did not exhibit habitat-associated responses to acute stress in these same females (data not shown, see Schrandt and Lema, 2011), suggesting that the environmental differences between these habitats have led to intraspecific variation in some components of CRH-related protein hormone signaling in the brain, but not in receptor regulation.

Additional work is needed on these damselfish to determine which nuclei in the brain are generating these habitat- and sex-associated differences in *crh* and *uroten1* mRNA transcription responses, as well as whether these transcriptional responses link to variation in hypothalamic-pituitary-interrenal (HPI) axis-mediated cortisol production, or to behaviors mediated directly by CRH or urotensin1 acting within the brain itself. Nonetheless, this example of environment-associated variation in hormonal responses to acute stress illustrates how each component of an endocrine signaling pathway can respond differently to environmental variation, and how those differences might vary even within a single population, either between sexes or in other ways such as between developmental stages. With respect to understanding the evolution of hormone-mediated phenotypes, this means that whenever possible multiple components of the multidimensional endocrine phenotype – and not just hormone levels – should be examined simultaneously in individuals exposed to a range of environments to avoid overlooking key components where plastic or evolutionary changes in endocrine signaling might have occurred. It is also important to recognize that because many hormone systems interact with each other, the environmental condition of interest will not always be external to the organism (e.g., temperature, rainfall), but could also be internal, such as systemic or tissue levels of another hormone (Williams, 2008).

Examining norms of reaction for several components of a given hormone pathway should increase the likelihood of identifying components that show heritable variation in environmental responsiveness. We might then consider each component a separate 'endophenotype,' as each component may be underpinned by different genes – and thus different degrees of genetic or epigenetic variation – and could show a distinct plastic response. This term 'endophenotype' was coined by John and Lewis (1966) in a study of geographic variation in insects to refer to genetic and physiological attributes that were "…not the obvious and external but the microscopic and internal…" (p. 720). An 'endophenotype' could therefore refer to any measurable component of an organism that is not apparent solely by observing the organism's morphological, behavioral or life history phenotype, but provides a key source of variation along the pathway from underlying genotype to emergent phenotype (Gottesman and Gould, 2003). While the concept of an 'endophenotype' has not yet been adopted by evolutionary endocrinologists, the term has become commonplace in the study of heritable, physiological indicators for complex behavioral socialization disorders such as schizophrenia and attention-deficit/hyperactivity (Hasler et al., 2004; Flint and Munafò, 2007; Phelps, 2010). Studies in psychiatric genetics are now identifying hormonal endophenotypes for behaviors in humans, such as variation in CRH challenge activation of the HPA axis as a marker of depression (Steimer et al., 2007), the identification of single-nucleotide polymorphisms (SNPs) in the CRH-R1 receptor associated with alcohol dependence (Chen et al., 2010), and the role of serotonin receptor variation in impulsive aggressive behaviors (Zouk et al., 2007). Given the complex, multidimensional structure of hormone signaling pathways and that evolutionary change could occur in any component (e.g., receptor, conversion enzyme, binding protein, etc.) of a pathway, the concept of an endophenotype has utility for describing the various component(s) of an endocrine pathway that could underlie the evolution of hormone-mediated phenotypic plasticity. In the context of hormone-mediated plasticity, any component of a hormone pathway that meets the following parameters could be considered an endophenotype:

(1) Variation in the endocrine signaling component needs to be associated with the plastic phenotypic response of interest (e.g., plasticity in behavior, morphology, life history, etc.).

(2) Variation in the environmental response of the

endocrine signaling component should be detectable by experimental exposure of the organism to a variety of environments (e.g., 'norm of reaction'), potentially even before the plastic phenotypic response is observed.

(3) Variation in the endocrine signaling component needs to be heritable (e.g., has a genetic or epigenetic basis).

Under these criteria, any hormone pathway component exhibiting differential environmental regulation as a result of underlying variation in genotype would be considered an endophenotype for plasticity in the traits mediated by that hormone. And, since the goal of understanding the evolution of hormone-mediated plasticity requires distinguishing heritable variation in environmental responsiveness within endocrine regulatory networks, the identification of endophenotypes with the above characteristics could be a major step toward pinpointing the mechanistic foundations for individual-, population- or species-level variation in hormone-mediated phenotypic plasticity.

3 Identifying Heritable Variation in Hormone Endophenotypes Related to Phenotypic Expression

Comparative and evolutionary endocrinologists interested in understanding how hormone-mediated phenotypes evolve are now focusing their efforts on understanding two key components of such evolution: 1) how environmental conditions alter phenotypic development and expression via shifts in the hormone regulatory network, and 2) what role genetic and epigenetic variation in hormones, hormone receptors, and other components of endocrine networks play in organismal adaptation. Hormones hold a central role in the mediation of phenotypic plasticity because the structure of hormones and their receptors are often encoded in genes, *and* because the mode of action of hormonal effects on cells frequently occurs via changes in patterns of gene expression (Fig. 1) (Pigluicci, 2001; Dufty et al., 2002). Given the advances in genomic, proteomic and metabolomic technologies over the last decade, our understanding of the evolution of endocrine regulation is likely to progress rapidly as these new approaches are applied to the study of variation in hormone signaling and, specifically, hormone-mediated plastic phenotypes (Aubin-Horth and Renn, 2009; Kitano et al., in press). Here, we outline several research avenues that need to be addressed by future studies, including how genetic and epigenetic variation in endocrine-associated genes contributes to variation in endocrine networks underlying plasticity, and how the regulation of molecular and cellular pathways can be coupled or decoupled across multiple tissues to produce integrated phenotypic responses.

3.1 Heritable variation in environmentally-induced hormone production

To date, the majority of studies on hormone signal variation have focused on how the response of circulating hormone levels to environmental changes varies between individuals, populations, and species and, subsequently, how any such variation in hormone titers affects phenotypic variation (Bradshaw, 2007; Zera et al., 2007; Williams, 2008). Such studies have contributed important findings about how hormones link environment and phenotype to regulate suites of traits and fashion an integrated phenotype. For instance, rainbow trout *Oncorhynchus mykiss* artificially selected for high responding or low responding post-stressor plasma cortisol titers exhibit correlated changes in other traits including behavioral dominance propensity, feeding and locomotion during social stress, and brain monoaminergic metabolism (Øverli et al., 2005). Evidence such as this for hormone-influenced trait associations supports the idea that selection on systemic hormone levels can lead to correlated changes in several traits. Such pleitropic actions of hormones have been hypothesized to be an evolutionary constraint, wherein selection on any single hormone-mediated trait has the potential to alter other characters regulated by that same hormone (Sinervo and Svensson, 1998; Ketterson and Nolan, 1999; McGlothlin and Ketterson, 2008; Hau, 2007). However, whether and when such contraints exist remains in question (e.g., Roberts et al., 2004; Ketterson et al., 2009), and the use of 'phenotypic engineering' approaches – where hormone titers are experimentally altered to reveal both the suite of phenotypic traits regulated by that hormone and the relative fitness consequences of variation in circulating hormone – continue to provide important insights into the fitness trade-offs associated with systemic hormone variation (Ketterson et al. 1996; Ketterson and Nolan, 1999; Cox et al., 2009).

Part of the difficulty in quantifying such fitness trade-offs likely emerges from the complexity of the endocrine regulatory network. Circulating hormone levels are often variable within an individual, as many hormones vary in secretion diurnally, or with age or reproductive status (e.g., Norris, 2007; Williams, 2008). Quantifying a hormone at a single time point and as-

suming that single value to be representative of an individual's endocrine status may thus obscure key dynamics of hormone signaling in that individual. Rather, it may be the activation profile (e.g., change from baseline to elevated) of the hormone axis that is under selection as individuals cope with environmental challenges (Wingfield, 1994; Ketterson et al., 2009), but that profile would not be apparent from a single hormone titer measurement. This method of looking at the hormone response profile is commonly used in studies of glucocorticoid production resulting from HPA/HPI axis activation in vertebrates (Wingfield, 1994; Romero et al., 2008; Schrandt and Lema, 2011; Romero, 2012), as well as in studies of androgen responses in social challenge scenarios (e.g., Goymann et al. 2007; Goymann 2009; Dijkstra et al., 2012).

The approach of quantifying hormone activation profiles should be applicable to many hormone systems. The environmental cues relevant to titer changes will vary depending on the hormone, as will the relevant time scale of the hormone response. Depending on the nature of the plastic phenotype of interest, it might be important to quantify a hormone response profile over short-term, acute (minutes or hours) or longer-term, developmental (days, weeks or years) time scales, or both. For example, a recent study of males of two genera of East African cichlid fishes (*Pundamilia* spp. and *Mbipia* spp.) found evidence for evolutionary divergence in circulating testosterone and cortisol concentrations between the genera (Dijkstra et al., 2012). This evolutionary difference in steroid hormone levels, however, was only apparent in males tested under conditions of prolonged (1 week) social interaction with other males, and not in socially-isolated males or males experiencing a territorial intrusion challenge by another male (Dijkstra et al., 2012; see also Dijkstra et al., 2011). As a second example, variation in levels of the thyroid hormone thyroxine (T_4) was recently identified as an underpinning for divergence in migratory behavior among threespine stickleback fish (*Gasterosteus aculeatus*) ecotypes (Kitano et al., 2010; Kitano and Lema, 2013). This ecotypic divergence in the thyroid hormone signaling network extends beyond T_4 levels in these fish, as SNP variation in the thyroid-stimulating hormone β-subunit 2 (*tshβ2*) locus and relative *tshβ2* transcript abundance in the pituitary both segregated closely with ecotype (Kitano et al., 2010). What is more, however, is that the magnitude of the variation in *tshβ2* gene expression among ecotypes is influenced by photoperiod, so that ecotypes exhibit dramatic differences in pituitary *tshβ2* mRNA levels on short day photoperiod (8 hrs light:16 hrs dark), but considerably reduced variation on long day photoperiod (16 hrs light: 8 hrs dark) (Kitano et al., 2010).

As these examples illustrate, observing the hormone phenotype under only a single environmental condition could overlook ecologically-important dynamics in hormone production. The use of reaction norms can therefore be a useful approach for how hormone production responds to a range of environmental conditions, and help evaluate whether divergence in hormone responses has occurred. One clear example where such a reaction norm approach has been applied in an evolutionary context comes from work on the arginine vasotocin (AVT) neuropeptide system in allopatric populations of pupfish (Lema, 2006). AVT is produced in the preoptic area (POA), and either secreted locally in the brain where it functions as a neuromodulator or into systemic circulation via the neurohypophysis. AVT regulates a variety of functions in fish, including aggressive behaviors, hydromineral balance and HPI stress responses (Semsar et al., 2001; Lema and Nevitt, 2004b; Balment et al., 2006), and the AVT produced in different regions of the POA (e.g., parvocellular, magnocellular) has been linked to different physiological and behavioral functions (e.g., Lema, 2006; Greenwood et al., 2008). Some pupfishes occupy isolated habitats that differ widely in environmental conditions such as salinity, temperature, and social density, and select populations were found to have diverged in how their neural AVT systems responds to environmental salinity (Fig. 4; Lema and Nevitt 2004a; Lema, 2006).

3.2 Tissue-specific changes in hormone levels

The findings from this study of pupfish above illustrate a second important point about evolutionary changes in endocrine regulatory networks: hormone systems can evolve in a tissue-specific or brain region-specific manner. In those populations of pupfish, different AVT neural groups in the POA were discovered to have evolved distinct plastic responses to environmental conditions like salinity (Fig. 4), indicating that selection can act in a highly specific manner on hormonal endophenotypes, and even distinguish between adjacent brain nuclei producing the same hormone if those hormone-producing cells have dissimilar functions (Lema, 2006). Although the prevalence of such nuclei-specific evolution is still unknown, evidence for tissue specificity implies that evolution can dislink environmentally-regulated hormone production and secretion pathways across tissues. Tissue variation

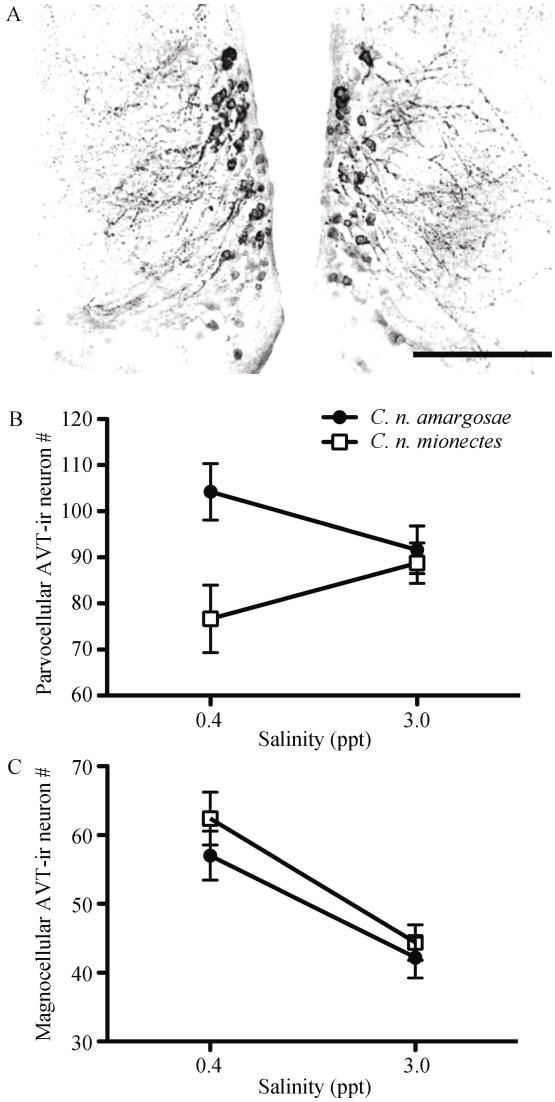

Fig. 4 Example of tissue-specific evolutionary change in plasticity of an endophenotype detected using reaction norms

Plasticity in neural arginine vasotocin (AVT) endophenotype has evolved between two allopatric populations of *Cyprinodon nevadensis* pupfish. In fishes, arginine vasotocin (AVT) peptide hormone is produced in neurons in the preoptic area (POA) of the hypothalamus, as shown in this representative image of AVT-immunoreactive neuronal bodies and fibers in the POA of a male pupfish (**A**). Quantification of AVT-ir neuron number in fish that experienced environmental salinities of either 0.4 ppt or 3.0 ppt from 15 days post fertilization until sexual maturity revealed evolutionary divergence in developmental plasticity in parvocellular POA ($F_{1,61}$ = 4.430, P =0.039) (**B**), but not magnocellular POA ($F_{1,64}$ = 0.064, P = 0.802) (**C**) AVT-ir endophenotype between the pupfish populations. Scale bar in (**A**) represents 100 μm. All data are shown as mean ± *SEM*. Results adapted from Lema (2006).

in genetic or epigenetic control of gene expression might allow selection to acts on hormone pathways with tissue specificity, so that plasticity in hormone production might evolve in one tissue of brain nucleus, but not in an adjacent tissue or nucleus (e.g., Lema, 2006; Rosvall et al., 2012).

It is also important to consider that variation in paracrine or autocrine signaling might be central to the evolutionary diversification of endocrine function. Although paracrine and autocrine hormonal function is still largely unexplored in comparative animal systems, technical advances in the ability to detect mRNA transcripts at even low abundance is providing evidence that many hormones may be produced in tissues (e.g., CRH production and CRH-R expression in skin; mRNAs encoding *tshβ* hormone in gonadal tissues; or AVT polypeptide and mRNAs in the gonad) beyond the major endocrine organs where they were discovered and have been lengthily studied (Slominski et al., 2006; Lema et al., 2009; Ramallo et al., 2012). The functional significance of hormone production in these non-traditional tissues remains largely unexpounded.

3.3 Heritable variation in other hormone signaling components

Variation in other components (e.g., receptors, conversion enzymes, membrane transporters, etc.) of hormone signaling at the level of peripheral target tissues is also likely to play an important role in the evolution of endocrine-mediated adaptive plasticity. Accumulating evidence from a variety of taxa suggests that the regulatory responses of receptors and conversion enzymes in peripheral tissues can – but don't always – occur in a tissue-specific manner (Table 2). If commonplace, such tissue-specific regulation of hormone receptors, binding proteins, conversion enzymes or other target tissue components of a hormone signaling network could generate phenotypic change in a tissue-specific manner, effectively bypassing any pleiotropic constraints that might accompany evolved changes in systemic hormone titers (Hau, 2007; Zera et al., 2007; McGlothlin and Ketterson, 2008; Ketterson et al., 2009).

Studies on the evolution of these target tissue components of hormone signaling are sparse, likely due to the difficulty in measuring the characters of hormone receptors, degradation and conversion enzymes, and binding proteins. However, with the increasing availability of genomic and proteomic techniques in comparative endocrinology (Kitano et al., in press), there are now several examples where the examination of these target tissue components is providing new insight into how hormone-mediated phenotypic plasticity evolves. For instance, spadefoot toads are now a model for hormone regulation of developmental plasticity, as environmentally-induced changes in CRH and TH sig-

Table 2 Select studies demonstrating tissue-specific responses of hormone concentration, hormone receptors, or conversion enzymes to environmental or systemic hormone variation

Endocrine Component	Experimental Manipulation	Measure*	Tissue(s)	Taxon	Reference
Tissue Hormone Concentration					
estradiol levels	territorial intrusion	1	brain regions	White-crowned sparrow *Zonotrichia leucophrys*	Charlier et al., 2011
corticosterone & dehydroepiandrosterone (DHEA)	territorial intrusion; seasonal variation	1	blood; brain regions; liver; pectoral muscle	Song sparrow *Melospiza melodia*	Newman and Soma, 2011
insulin-like growth factor-1 (IGF-1)	treatment with a catabolic cytokine	2	liver; heart; gastrocnemius	rat *Rattus rattus*	Lang et al., 2001
Hormone Receptors					
thyroid hormone receptors	exogenous T_3 or methimiazole goitrogen	2	brain; liver; gonad	fathead minnow *Pimpehales promelas*	Lema et al., 2009
thyroid hormone receptors	exogenous T_4, T_3 or methimazole goitrogen	2	liver; pituitary; cerebral cortex; cardiac and leg muscles	domestic duck *Anas platyrhynchos*	Bishop et al., 2000
growth hormone (GH) receptor	GH transgenic, GH antagonist transgenic	2	liver; pituitary gland	mouse *Muscus mus*	Iida et al., 2004
V1a-type AVT receptors	socially-induced sex change	2	hypothalamus; gonad	bluehead wrasse *Thalassoma bifasciatum*	Lema et al., 2012
Conversion Enzymes					
androgen metabolizing enzymes	collection at different stages of breeding season	3	brain regions	Lapland longspur *Calcarius lapponicus*	Soma et al., 1999
aromatase enzyme	simulated territorial intrusion	3	brain regions	White-crowned sparrows *Zonotrichia leucophrys*	Charlier et al., 2011
iodothyronine deiodinase enzymes	exogenous T_3 or methimiazole goitrogen	2	brain; liver; gonad	striped parrotfish *Scarus iserii*	Johnson and Lema, 2011
angiotensin-converting enzyme	exogenous angiotensin II	2,3	lung; heart; kidney	rat *Rattus rattus*	Metsärinne et al., 1996
3β-HSD enzyme	simulated territorial intrusion; seasonal variation	3	brain regions	Song sparrow *Melospiza melodia*	Pradhan et al., 2010

Measure codes: [1] hormone titer; [2] relative gene transcript (mRNA) abundance; [3] enzyme activity

naling will shift the timing of metamorphosis in response to a drying aquatic habitat (Denver, 1998; 2013). Spadefoot toad species vary, however, in this plasticity (Buchholz and Hayes, 2002), and species have been found to differ in whole body, tail and liver TH content, and in the sensitivity and responsiveness of tail metamorphosis to T_3 (Buchholz and Hayes, 2005). Recent work by Hollar and coworkers (2011) revealed that these species differences in metamorphic sensitivity to THs may be related to species variation in the abundance of TH receptor isoform-α (*trα*) in the tail, suggesting that regulatory changes in peripheral TH receptor expression may contribute to evolutionary divergence in metamorphic plasticity. In a second example of peripheral regulation of plasticity, it has been demonstrated that genetic variation in the activity of juvenile hormone (JH) esterase can generate evolutionary change in the wing polymorphisms of the cricket *Gryllus firmus* (Zera and Huang, 1995; 1999; Zera, 2006).

Examples where evolutionary changes in peripheral hormone regulation have been linked to divergence in plasticity are presently few in number but will likely increase as emerging genomic, transcriptomic and proteomic methods facilitate studies of peripheral hormone regulation in an evolutionary context (Zera et al., 2007; Kitano et al., in press). In the interim, we might look to studies of inter- and intraspecific variation in peripheral hormone regulation to get a picture of how such evolutionary changes may occur. Such studies reveal that even closely related taxa can vary in hormone signaling mechanisms in peripheral tissues. For example, in a now classic example of the hormonal basis for behavioral evolution, several species of voles that differ in mating system and associated social behaviors were observed to vary in brain distribution patterns of the V1a-type receptor for arginine vasopressin (AVP) (Insel et al., 1994; Wang et al., 1997; reviewed in Insel and Young, 2000; Phelps, 2010). These species differences in receptor expression result from variation in the length of a microsatellite located in the 5′ flanking *cis*-regulatory region of the V1a-type receptor (*avpr1a*) locus. Insertion of the *avpr1a* gene from the prairie vole *Microtus ochrogaster* which is highly affiliative and monogamous, into a transgenic mouse, a species typically polygynous, shifted both the pattern of brain V1a receptor distribution and behavior toward that of the monogamous vole (Young et al., 1999; Lim et al., 2004). Along the same line, work with two sympatric species of threespine stickleback in Japan revealed significant variation in plasma sex steroid profiles linked to variation in steroidogenic enzyme expression in the gonads (Kitano et al., 2011). These stickleback species differ in mating behaviors and have different migration routes while at sea. This finding of significant variation in gonadal conversion enzyme expression between incipient sympatric species speaks to the sensitivity that hormonal signaling components can show to ecological variation.

Variation in peripheral endocrine signaling is not limited to species differences, but also has been observed among individuals of the same species. The white-crowned sparrow *Zonotrichia leucophrys*, for example, exhibits latitudinal variation in plasma corticosteroid-binding capacity and tissue glucocorticoid receptor-like binding, but not baseline or stressed total (HBP bound and free) corticosterone levels (Breuner et al., 2003). Latitudinal variation in HPA stress reactivity in this species thus appears mediated not by changes in the production and secretion of corticosterone, but rather by variation in other signaling components such as blood binding protein capacity. In a separate study involving another songbird, the dark-eyed junco *Junco hyemalis*, Rosvall and colleagues (2012) found that individual variation in aggression in both males and females was positively associated with variation in the relative abundance of transcripts encoding androgen receptor, estrogen receptor and aromatase enzyme proteins in the ventromedial telencephalon. Taken as a whole, the findings of studies such as these suggest that individuals and populations can vary significantly in how hormones are transported, enzymatically converted, and transduced by receptors into intracellular responses in peripheral tissues. If heritable, such endophenotype variation should be subject to selection in dissimilar environments.

Given the accumulating evidence that peripheral target tissues can exhibit independent patterns of regulation in hormone production, conversion enzyme activity and receptor expression (Table 2), perhaps the key question should no longer be whether such components can be differentially regulated in target tissues, but rather under what ecological conditions of selection does regulation of the same peripheral signaling component (e.g., the same receptor) become coupled or decoupled across tissues. How does genetic and epigenetic variation mediate that decoupling, and how do the environmental conditions that an organism experiences shape the strength of correlated expression of those signaling components (Stearns et al., 1991)? If the expressional regulation of peripheral signaling components can be independent among target tissues – and therefore should

be considered different endophenotypes in each tissue – do these endophenotypes also vary in environmentally-mediated expression (plasticity)? And, what role might such plasticity play in the evolution of target tissue responses to hormones?

4 Emerging Approaches to Study Hormone Regulatory Networks and Phenotypic Plasticity

4.1 Application of genomic and transcriptomic methods

Future studies of the evolution of hormone-mediated phenotypic plasticity will benefit greatly from the application of new methodologies, including the advances of genome biology (Aubin-Horth and Renn, 2009; Kitano et al., in press). Such genomic methodologies will be essential for providing both qualitative and quantitative information on the many peripheral components (e.g., HBPs, transporters, conversion enzymes) that comprise any endocrine signaling network. Recent advances such as next-generation sequencing (NGS) and RNA-seq methods now enable genomes or transcriptomes from even non-model organisms to be sequenced quickly. In the past, one of the primary limitations on the study of endocrine signaling in non-model species – which typically are the species of evolutionary interest – is that the diversity and structure of protein components in a non-model species' hormone network were unknown. With the completion of more sequenced genomes each year, the full diversity of receptor, enzyme, and transporter proteins involved in endocrine signaling should become better illuminated. As described above, quantitative assessment of how expression of these peripheral components is regulated will be key to evaluating constraints and trade-offs in the evolution of hormone-mediated phenotypic plasticity (Kitano et al., in press).

Emerging genomic methods are also providing insights into possible mechanisms for tissue-specific gene regulation in endocrine signaling networks. Studies examining epigenetic mechanisms of gene regulation such as CpG methylation, histone acetylation, and miRNAs are already generating important revelations about plasticity in hormone signaling (Zhang and Ho, 2011). For instance, recently it was found that rats exposed to exogenous T exhibit elevated CpG methylation in the promoter region of the AVP gene – but decreased methylation in the gene encoding estrogen receptor α – in cells located in the bed nucleus of the stria terminalis (BST), a brain region linked to social aggression (Auger et al., 2011). Similarly, both maternal care and repeated exposure to stressors can alter DNA methylation patterns that contribute to altered HPA axis-mediated stress reactivity as adults (Champagne and Curley, 2009) and across generations (Crews et al., 2012). The study of epigenetic regulation is in its infancy in endocrinology, and even more so in ecology (e.g., Bossdorf et al., 2008). And yet, because variation in gene expression underlies the development and expression of phenotypic variation (e.g., Whitehead and Crawford, 2006), studies into the epigenetic basis for phenotypic plasticity are likely to make significant contributions toward understanding how hormone-mediated plasticity occurs in dissimilar environments (e.g., Snell-Rood et al., 2013).

4.2 Network and path analysis modeling approaches

As the use of genomic, transcriptomic and proteomic approaches becomes more common, a clear need is emerging for the application of mathematical methods that can analyze and model the information contained within the large data sets that characteristically result from these '-omic' approaches. To be relevant to questions about phenotypic plasticity, such mathematical methods need to represent the real-world complexity of organism-environment interaction, such as the integrative structure of the endocrine network and varied environmental inputs. Evolutionary endocrinologists need not "reinvent the wheel" for these models, as several current modeling and statistical methods exist that should help establish relationships among components of an endocrine signaling network and facilitate quantification of the direction and strength of relationships within the broader physiological network (Ament et al., 2012; Cohen et al., 2012). Such network models are currently being used in other scientific fields of inquiry, but are only beginning to be applied in studies of comparative endocrinology (e.g., Oldfield et al., 2013; O'Connell and Hofmann, 2012). In genome biology, for instance, network modeling is already enabling predictive ability for identifying genetic loci where perturbations will be most deleterious for health and for informing which nodes to target for the development of new pharmaceuticals (e.g., Tu et al., 2012; Zhu et al., 2012).

The application of network modeling to questions in evolutionary endocrinology and phenotypic plasticity could be powerful for understanding the complex network of interactions that connect organismal phenotypes to environmental conditions. The first step in such an approach is to map the network of environment-hormone-trait interactions for a system of interest (Fig.

5). Such network models can be constructed using a variety of methods including predicted causal relationship derived from published reductionist studies on just a few of the network components (e.g., hormone A upregulates receptor B). When such relationships are not known, multiple network models can be constructed and then tested for 'best-fit' using statistical methods such as path analysis and structural equation modeling (Shipley, 2000). These methods allow for inferential testing of causal relationships among multiple variables that interact in complex patterns. While statistical approaches like path analysis have been applied in several scientific fields such as epidemiology, neuroscience, and ecology (e.g., Hoke et al., 2005; Schrandt et al., 2012; for more explanation, see Wright, 1984), to our knowledge, they have been absent from comparative endocrinology.

One of the strengths in this approach is that the scope of the model (i.e., number of variables included) can be expanded or restricted according to the research questions being addressed (Sultan and Stearns, 2005). Initial applications of these methods may only need to represent the integrative structure of the hormone network, but might later be extended to account for developmental changes in sensitivity to environmental input, denote known changes in hormonal pathways due to previous hormone levels (organization-activation), or characterize broader impacts that plastic changes in any single component might have for the functioning of the overall network. Given that endocrine regulatory networks are complex systems than link genetic variation to environmental variation, a fuller understanding of both the causal structure of these hormone pathways and the effects that change in any pathway component will have for plasticity in hormone effects could be greatly enhanced by the use of network models and meta- analysis approaches (Ament et al., 2012; Cohen et al., 2012).

5 Conclusions

To understand *how* phenotypic plasticity evolves, it is important to recall that plasticity emerges from the network of relationships whereby an organism interacts with its environment. Understanding the evolution of phenotypic plasticity therefore requires the study of how

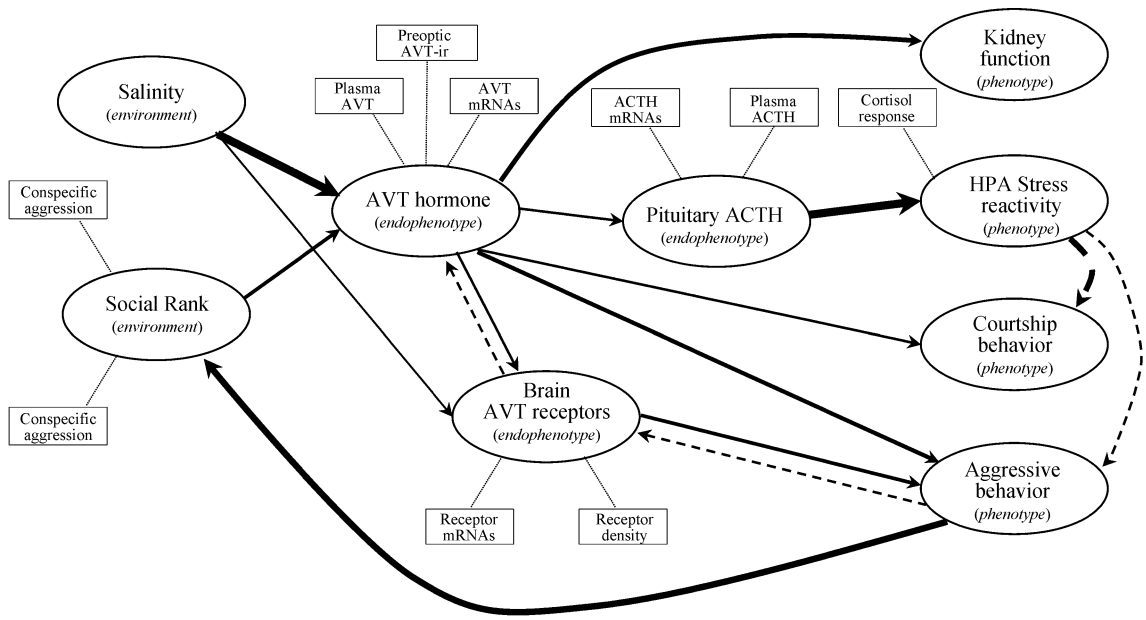

Fig. 5 Illustration of a hypothetical path analysis model for arginine vasotocin (AVT) hormone regulation of kidney function, hypothalamic-pituitary-interrenal (HPI) stress reactivity, and behaviors by two environmental conditions: salinity and social status/rank

This path analysis model represents a structural hypothesis about the causal relationships among the variables shown as ovals. In this model, these variables include environmental condition, endophenotypes, and other components of organismal phenotype. Each arrow is a 'path' in the model for a hypothesized association among the variables; solid arrows represent positive associations, while dashed arrows represent negative associations. The thickness of each arrow indicates the strength of that association. The boxes represent quantitative measures used in structural equation models to generate a composite value for each endophenotype variable. Although the model shown here is hypothetical, models such as this one could be used to quantitatively infer causal associations among the variables and test statistically the structure of the model, the strength of each associations, and whether the structure and strength of associations remain similar for organisms experiencing differing environments.

organisms incorporate those conditions during development, including the physiological and behavioral mechanisms that underpin coordinated phenotypic responses to environmental experience. Hormone chemical messengers influence phenotypes by regulating cellular function and gene expression in response to changes in the internal or external conditions. Hormones can therefore be viewed as a developmental link between an organism and its environment. Investigations of the hormonal bases for *how* genotype-environment interaction occurs can therefore provide significant insights into the evolution of phenotypic plasticity, especially if these studies are conducted in multiple populations or species.

Given the complex structure of endocrine signaling networks, it should be expected that evolutionary changes in hormone-regulated plasticity involve several network components, especially since emerging evidence indicates that environmental conditions can alter the expression of receptors, conversion enzymes, membrane transporters and binding proteins in cell- or tissue-specific ways. The challenge for researchers striving to understand the evolution of phenotypic plasticity will be to develop methods to quantify each component in an endocrine network, identify genetic or epigenetic variation in each of these components, and examine how any such variation generates differences in component expression in dissimilar environments. Overcoming these challenges will require a greater degree of communication and collaboration across research laboratories, to combine 'phenotypic engineering' approaches using exogenous hormones or manipulated environments with new methods for hormone measurement, transcriptome and proteome profiling in multiple tissues, analysis of genetic and epigenetic variation, and network modeling to integrate multidimensional data sets. Evolutionary endocrinology is at the crossover to a period of rapid discovery given the variety of genomic tools now available for studying phenotypic variation in non-model species (e.g., Denver et al., 2009), and our understanding of *how* phenotypic plasticity evolves should advance rapidly as these tools are increasingly applied in ecologically relevant contexts.

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References

Ament SA, Blatti CA, Alaux C, Wheeler MM, Toth AL et al., 2012. New meta-analysis tools reveal common transcriptional regulatory basis for multiple determinants of behavior. Proc. Natl. Acad. Sci. USA 109: E1801–E1810.

Aubin-Horth N, Renn SCP, 2009. Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. Mol. Ecol. 18: 3763–3780.

Auger CJ, Coss D, Auger AP, Forbes-Lorman RM, 2011. Epigenetic control of vasopressin expression is maintained by steroid hormones in the adult male rat brain. Proc. Natl. Acad. Sci. USA 108: 4242–4247.

Ball GF, Balthazart J, 2008. Individual variation and the endocrine regulation of behavior and physiology in birds: A cellular/molecular perspective. Phil. Trans. R. Soc. B 363, 1699–1710.

Balment RJ, Lu W, Weybourne E, Warne JM, 2006. Arginine vasotocin a key hormone in fish physiology and behaviour: A review with insights from mammalian models. Gen. Comp. Endocrinol. 147: 9–16.

Bassett JHD, Harvey CB, Williams GR, 2003. Mechanisms of thyroid hormone receptor-specific nuclear and extra nuclear actions. Mol. Cell. Endocrinol. 213: 1–11.

Bernier NJ, Craig PM, 2005. CRH-related peptides contribute to stress response and regulation of appetite in hypoxic rainbow trout. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289: R982–R990.

Bishop CM, McCabe CJ, Gittoes NJL, Butler PJ, Franklyn JA, 2000. Tissue-specific regulation of thyroid hormone receptor mRNA isoforms and target gene proteins in domestic ducks. J. Endocrinol. 165: 607–615.

Bossdorf O, Richards CL, Pigliucci M, 2008. Epigenetics for ecologists. Ecol. Lett. 11: 106–115.

Bradshaw D, 2007. Environmental endocrinology. Gen. Comp. Endocrinol. 152: 125–141.

Brakefield PM, Kesbeke F, Bernhardt P, 1998. The regulation of phenotypic plasticity of eyespots in the butterfly *Bicyclus anynana*. Amer. Nat. 152: 853–860.

Breuner CW, Orchinik M, 2002. Plasma binding proteins as mediators of corticosrteroid action in vertebrates. J. Endocrinol. 175: 99–112.

Breuner CW, Orchinik M, Hahn TP, Meddle SL, Moore IT et al., 2003. Differential mechanisms for regulation of the stress response across latitudinal gradients. Am. J. Physiol. Regul. Integr. Comp. Physiol. 285: R594–R600.

Buchholz DR, Hayes TB, 2002. Evolutionary patterns of diversity in spadefoot toad metamorphosis (Anura: Pelobatidae). Copeia 2002: 180–189.

Buchholz DR, Hayes TB, 2005. Variation in thyroid hormone action and tissue content underlies species differences in the timing of metamorphosis in desert frogs. Evol Dev. 7: 458–467.

Carroll SP, Corneli PS, 1999. The evolution of behavioral norms of reaction as a problem in ecological genetics. In: Foster SA, Endler JA ed. Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms. New York: Oxford Univ. Press, 52–68.

Champagne FA, Curley JP, 2009. Epigenetic mechanisms mediating the long-term effects of maternal care on development. Neurosci. Biobehav. Rev. 33: 593–600.

Charlier TD, Newman AEM, Heimovics SA, Po KWL, Saldanha

CJ et al., 2011. Rapid effects of aggressive interactions on aromatase activity and oestradiol in discrete brain regions of wild male white-crowned sparrows. J. Neuroendocrinol. 23: 742–753.

Chen ACH, Manz N, Tang YQ, Rangaswamy M, Almasy L et al., 2010. Single-nucleotide polymorphisms in corticotropin releasing hormone receptor 1 gene (CRHR1) are associated with quantitative trait of event-related potential and alcohol dependence. Alchol. Clin. Exp. Res. 34: 988–996.

Cheng S-Y, Leonard JL, Davis PJ, 2010. Molecular aspects of thyroid hormone actions. Endocr. Rev. 31: 139–170.

Cockrem JF, Silverin B, 2002. Variation within and between birds in corticosterone responses of great tits *Parus major*. Gen. Comp. Endocrinol. 125: 197–206.

Cohen AA, Martin LB, Wingfield JC, McWilliams SR, Dunne JA, 2012. Physiological regulatory networks: ecological roles and evolutionary constraints. Trends Ecol. Evol. 27: 428–435.

Cox RM, Stenquist DS, Henningsen JP, Calsbeek R, 2009. Manipulating testosterone to assess links between behavior, morphology and performance in the brown anole *Anolis sagrei*. Physiol. Biochem. Zool. 82: 686–698.

Crews D, 1996. Temperature-dependent sex determination: The interplay of steroid hormones and temperature. Zool. Sci. 13: 1–13.

Crews D, 2003. Sex determination: Where environment and genetics meet. Evol. Dev. 5: 50–55.

Crews D, Gillette R, Scarpino SV, Manikkam M, Savenkova MI et al., 2012. Epigenetic transgenerational inheritance of altered stress responses. Proc. Natl. Acad. Sci. USA 109, 9143–9148.

Crispo E, 2007. The Baldwin effect and genetic assimilation: Revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. Evolution 61: 2469–2479.

Davis PJ, Leonard JL, Davis FB, 2008. Mechanisms of nongenomic actions of thyroid hormone. Front. Neuroendocrinol. 29: 211–218.

Dawson A, 2008. Control of the annual cycle in birds: Endocrine constraints and plasticity in response to ecological variability. Phil. Trans. R. Soc. B 363: 1621–1633.

Daza DO, Sundstrom G, Bergqvist CA, Larhammar D, 2012. The evolution of vertebrate somatostatin receptors and their gene regions involves extensive chromosomal rearrangements. BMC Evol. Biol. doi:10.1186/1471–2148–12–231

de Jong G, 2005. Evolution of phenotypic plasticity: Patterns of plasticity and the emergence of ecotypes. New Phytol. 166: 101–118.

Denver RJ, 2013. Neuroendocrinology of amphibian metamorphosis. Curr. Topics Dev. Biol. 103: 195–227.

Denver RJ, 1998. Environmental stress as a developmental cue: Corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. Horm. Behav. 31: 169–179.

Denver RJ, Hopkins PM, McCormick SD, Propper CR, Riddiford L et al., 2009. Comparative endocrinology in the 21st century. Integr. Comp. Biol. 49: 339–348.

Dijkstra PD, Verzijden MN, Groothuis TGG, Hofmann HA, 2012. Divergent hormonal responses to social competition in closely related species of haplochromine cichlid fish. Horm. Behav. 61: 518–526.

Dijkstra PD, Wiegertjes GF, Forlenza M, van der Sluijs I, Hofmann HA et al., 2011. The role of physiology in the divergence of two incipient cichlid species. J. Evol. Biol. 24: 2639–2652.

Dittman AH, Quinn TP, 1996. Homing in Pacific salmon: Mechanisms and ecological basis. J. Exp. Biol. 199: 83–91.

Duan C, 2002. Specifying the cellular responses to IGF signals: roles of IGF-binding proteins. J. Endocrinol. 175: 41–54.

Dufty AM, Clobert J, Møller AP, 2002. Hormones, developmental plasticity and adaptation. Trends Ecol. Evol. 17: 190–196.

Emlen DJ, 1997. Alternative reproductive tactics and male dimorphism in the horned beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). Behav. Ecol. Sociobiol. 141: 335–341.

Emlen DJ, Nijhout HF, 1999. Hormonal control of male horn length dimorphism in the horned beetle *Onthophagus taurus*. J. Insect Physiol. 45: 45–54.

Emlen DJ, 2000. Integrating development with evolution: A case study with beetle horns. BioScience 50: 403–418.

Fernald RD, Maruska KP, 2012. Social information changes the brain. Proc. Natl. Acad. Sci. USA 109(Suppl. 2): 17194–17199.

Flint J, Munafò MR, 2007. The endophenotype concept in psychiatric genetics. Psychol. Med. 37: 163–180.

Godwin J, Sawby R, Warner RR, Crews D, Grober MS, 2000. Hypothalamic arginine vasotocin mRNA abundance variation across sexes and with sex change in a coral reef fish. Brain Behav. Evol. 55: 77–84.

Godwin J, Luckenbach JA, Borski RJ, 2003. Ecology meets endocrinology: Environmental sex determination in fishes. Evol. Dev. 5: 40–49.

Gottesman II, Gould TD, 2003. The endophenotype concept in psychiatry: Etymology and strategic intentions. Am. J. Psychiatry 160: 636–645.

Goymann W, 2009. Social modulation of androgens in male birds. Gen. Comp. Endocrinol. 163: 149–157.

Goymann W, Landys MM, Wingfield JC, 2007. Distinguishing seasonal androgen responses from male-male androgen responsiveness: Revisiting the challenge hypothesis. Horm. Behav. 51: 463–476.

Greenwood AK, Wark AR, Fernald RD, Hofmann HA, 2008. Expression of arginine vasotocin in distinct preoptic regions is associated with dominant and subordinate behaviour in an African cichlid fish. Proc. Roy. Soc. B Biol Sci. 275: 2392–2402.

Hammes SR, Levin ER, 2007. Extranuclear steroid receptors: Nature and actions. Endocr. Rev. 28: 726–741.

Hammond GL, 2011. Diverse roles for sex hormone-binding globulin in reproduction. Biol. Reprod. 85: 431–441.

Hasler G, Drevets WC, Manji HK, Charney DS, 2004. Discovering endophenotypes for major depression. Neuropsychopharmacology 29: 1765–1781.

Hatle JD, 2003. Physiology underlying phenotypic plasticity and polyphenisms: Introduction to the symposium. Integr. Comp. Biol. 43: 605–606.

Hau M, 2007. Regulation of male traits by testosterone: Implications for the evolution of vertebrate life histories. BioEssays 29: 133–144.

Hoekstra HE, Hirschmann RJ, Bundey RA, Insel PA, Crossland JP, 2006. A single amino acid mutation contributes to adaptive beach mouse color pattern. Science 313: 101–104.

Hofmann HA, 2006. Gonadotropin-releasing hormone signaling in behavioral plasticity. Curr. Opin. Neurobiol. 16: 343–350.

Hoke KL, Ryan MJ, Wilczynski W, 2005. Social cues shift functional connectivity in the hypothalamus. Proc. Natl. Acad. Sci. USA 102: 10712–10717.

Hollar AR, Choi J, Grimm AT, Buchholz DR, 2011. Higher thyroid hormone receptor expression correlates with short larval periods in spadefoot toads and increases metamorphic rate. Gen. Comp. Endocrinol. 173: 190–198.

Iida K, Del Rincon JP, Kim D-S, Itoh E, Nass R et al., 2004. Tissue-specific regulation of growth hormone (GH) receptor and insulin-like growth factor-1 gene expression in the pituitary and liver of GH-deficient (*lit/lit*) mice and transgenic mice that overexpress bovine GH (bGH) or a bGH antagonist. Endocrinology 145: 1564–1570.

Insel TR, Wang ZX, Ferris CF, 1994. Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. J. Neurosci. 14: 5381–5392.

Insel TR, Young LJ, 2000. Neuropeptides and the evolution of social behavior. Curr. Opin. Neurobiol. 10, 784–789.

Ishikawa A, Ogawa K, Gotoh H, Walsh TK, Tagu D et al., 2012. Juvenile hormone titer and gene expression during the change of reproductive modes in the pea aphid. Insect Mol. Biol. 12: 49–60.

James MO, 2011. Steroid catabolism in marine and freshwater fish. J. Steroid Biochem. Mol. Biol. 127: 167–175.

Johannsen W, 1911. The genotype conception of heredity. Am. Nat. 45: 129–159.

John B, Lewis KR, 1966. Chromosome variability and geographic distribution in insects. Science 152: 711–721.

Johnson KM, Lema SC, 2011. Tissue-specific thyroid hormone regulation of gene transcripts encoding iodothyronine deiodinases and thyroid hormone receptors in striped parrotfish *Scarus iseri*. Gen. Comp. Endocrinol. 172: 505–517.

Johnston TD, 1987. The persistence of dichotomies in the study of behavioral development. Dev. Rev. 7: 149–182.

Keller L, Ross KG, 1993. Phenotypic plasticity and "cultural transmission" of alternative social organizations in the fire ant *Solenopsis invicta*. Behav. Ecol. Sociobiol. 33: 121–129.

Ketterson ED, Nolan V Jr, Cawthorn MJ, Parker PG, Ziegenfus C, 1996. Phenotypic engineering: Using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. Ibis 138: 70–86.

Ketterson ED, Nolan V Jr., 1999. Adaptation, exaptation, and constraint: A hormonal perspective. Am. Nat. 154: S4–S25.

Ketterson ED, Atwell JW, McGlothlin JW, 2009. Phenotypic integration and independence: Hormones, performance, and response to environmental change. Integr. Comp. Biol. 49: 365–379.

Kitano J, Lema SC, Luckenbach JA, Mori S, Kawagishi Y et al., 2010. Adaptive divergence in the thyroid hormone signaling pathway in the stickleback radiation. Curr. Biol. 20: 2124–2130.

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

Kitano J, Lema SC, 2013. Divergence in thyroid hormone levels between juveniles of marine and stream ecotypes of the threespine stickleback *Gasterosteus aculeatus*. 15: 143–153.

Kitano J, Ishikawa A, Lema SC, in press. Integrated genomics approaches in evolutionary and ecological endocrinology. In Landry C, Aubin-Horth N ed. Ecological Genomics: Ecology and the Evolution of Genes and Genomes. Springer Press.

Kroon FJ, Liley NR, 2000. The role of steroid hormones in protogynous sex change in the blackeye goby *Coryphotpterus nicholsii* (Teleostei: Gobiidae). Gen. Comp. Endocrinol. 118: 273–283.

Lang CH, Nystrom GJ, Frost RA, 2001. Tissue-specific regulation of IGF-1 and IGF-binding proteins in response to TNFα. Growth Horm. IGF Res. 11:250–260.

Larhammar D, Sundström G, Dreborg S, Daza DO, Larsson TA, 2009. Major genomic events and their consequences for vertebrate evolution and endocrinology. Ann. New York Acad. Sci. 1163: 201–208.

Lema SC, 2006. Population divergence in plasticity of the AVT system and its association with aggressive behaviors in a Death Valley pupfish. Horm. Behav. 50: 183–193.

Lema SC, Nevitt GA, 2004a. Variation in vasotocin immunoreactivity in the brain of recently isolated populations of a Death Valley pupfish *Cyprinodon nevadensis*. Gen. Comp. Endocrinol. 135: 300–309.

Lema SC, Nevitt GA, 2004b. Exogenous vasotocin alters aggression during agonistic exchanges in male Amargosa River pupfish *Cyprinodon nevadensis amargosae*. Horm. Behav. 46: 628–637.

Lema SC, Nevitt GA, 2006. An ecophysiological mechanism for morphological plasticity in pupfish and its relevance to conservation efforts for endangered Devils Hole pupfish. J. Exp. Biol. 209: 3499–3509.

Lema SC, Dickey JT, Schultz IR, Swanson P, 2009. Thyroid hormone regulation of mRNAs encoding thyrotropin β-subunit, glycoprotein α-subunit, and thyroid hormone receptors α and β in brain, pituitary gland, liver, and gonads of an adult teleost *Pimephales promelas*. J. Endocrinol. 202: 43–54.

Lema SC, Slane MA, Salvesen KE, Godwin J, 2012. Variation in gene transcript profiles of two V1a-type arginine vasotocin receptors among sexual phases of bluehead wrasse *Thalassoma bifasciatum*. Gen. Comp. Endocrinol. 179: 451–464.

Lim MM, Wang Z, Olazabal DE, Ren X, Terwilliger EF et al., 2004. Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene. Nature 429: 754–757.

Lin B-L, Yang W-J, 1999. Blue light and abscisic acid independently induce heterophyllous switch in *Marsilea quadrifolia*. Plant Physiol. 119: 429–434.

Lively CM, 1986. Predator-induced shell dimorphism in the acorn barnacle *Chthamalus anisopoma*. Evolution 67: 858–864.

Lowry CA, Moore FL, 2006. Regulation of behavioral responses by corticotropin-releasing factor. Gen. Comp. Endocrinol. 146: 19–27.

MacDougall-Shackleton SA, Stevenson TJ, Watts HE, Pereyra ME, Hahn TP, 2009. The evolution of photoperiod response systems and seasonal GnRH plasticity in birds. Integr. Comp. Biol. 49: 580–589.

Maruska KP, Fernald RD, 2010. Behavioral and physiological plasticity: Rapid changes during social ascent in an African cichlid fish. Horm. Behav. 58, 230–240.

Maruska KP, Fernald RD, 2013. Social regulation of male reproductive plasticity in an African cichlid fish. Integr. Comp. Biol.

(doi: 10.1093/icb/ict017)

McCormick SD, Hansen LP, Quinn TP, Saunders RL, 1998. Movement, migration, and smolting of Atlantic salmon *Salmo salar*. Can. J. Fish. Aquat. Sci. 55(Suppl. 1): 77–92.

McCormick SD, 2009. Evolution of the hormonal control of animal performance: Insights from the seaward migration of salmon. Integr. Comp. Biol. 49: 408–422.

McGlothlin JW, Ketterson ED, 2008. Hormone-mediated suites as adaptations and evolutionary constraints. Phil. Trans. Roy. Soc. B 363: 1611–1620.

Metsärinne KP, Helin KH, Saijonmaa O, Stewen P, Sirviö ML et al., 1996. Tissue-specific regulation of angiotensin-converting enzyme by angiotensin II and losartan in the rat. Bloos Press. 5: 363–370.

Miller WL, 2008. Steroidogenic enzymes. Endocr. Dev. 13: 1–18.

Miranda JA, Oliveira RF, Carneiro LA, Santos RS, Grober MS, 2003. Neurochemical correlates of male polymorphism and alternative reproductive tactics in the Azorean rock-pool blenny *Parablennius parvicornis*. Gen. Comp. Endocrinol. 132: 183–189.

Mittelbach GC, Osenberg CW, Wainwright PC, 1999. Variation in feeding morphology between pumpkinfish populations: Phenotypic plasticity or evolution? Evol. Ecol. Res. 1: 111–128.

Mohan S, Baylink DJ, 2002. IGF-binding proteins are multifunctional and act via IGF-dependent and -independent mechanisms. J. Endocrinol. 175: 9–31.

Mommsen TP, Vijayan MM, Moon TW, 1999. Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish. 9: 211–268.

Moore MC, 1991. Application of organization-activation theory to alternative male reproductive strategies: A review. Horm. Behav. 25: 154–179.

Morgan SG, Christy JH, 1994. Plasticity, constraint, and optimality in reproductive timing. Ecology 75: 2185–2203.

Navara KJ, Mendonça MT, 2008. Yolk androgens as pleiotropic mediators of physiological processes: A mechanistic review. Comp. Biochem. Physiol. A 150:378–386.

Newman AEM, Soma KK, 2011. Aggressive interactions differentially modulate local and systemic levels of corticosterone and DHEA in a wild songbird. Horm. Behav. 60: 389–396.

Nijhout HF, 2003. Development and evolution of adaptive polyphenisms. Evo. Dev. 5: 9–18.

Norris DO, 2007. Vertebrate Endocrinology. Burlington, MA, USA: Elsevier Academic Press.

O'Connell LA, Hofmann HA, 2012. Social status predicts how sex steroid receptors regulate complex behavior across levels of biological organization. Endocrinology 153: 1341–1351.

Oldfield RG, Harris RM, Hendrickson DA, Hofmann HA, 2013. Arginine vasotocin and androgen pathways are associated with mating systems variation in North American cichlid fishes. Horm. Behav. (doi: 10.1016/j.yhbeh.2013.04.006)

Oliveira RF, 2009. Social behavior in context: Hormonal modulation of behavioral plasticity and social competence. Integr. Comp. Biol. 49: 423–440.

Oostra V, de Jong MA, Invergo BM, Kesbeke F, Wende F et al., 2011. Translating environmental gradients into discontinuous reaction norms via hormone signalling in a polyphenic butterfly. Proc. Roy. Soc. B 278: 789–797.

Orozco A, Valverde-R C, Olvera A, García-G C, 2012. Iodothyronine deiodinases: A functional and evolutionary perspective. J. Endocrinol. 215: 207–219.

Øverli Ø, Winberg S, Pottinger TG, 2005. Behavioral and neuroendocrine correlates of selection for stress responsiveness in rainbow trout: A review. Integr. Comp. Biol. 45: 463–474.

Pankhurst NW, 2011. The endocrinology of stress in fish: An environmental perspective. Gen. Comp. Endocrinol. 170: 265–275.

Pfennig D, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD et al., 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends Ecol. Evol. 25: 459–467.

Phelps SM, 2010. From endophenotypes to evolution: Social attachment, sexual fidelity and the *avpr1a* locus. Curr. Opin. Neurobiol. 20: 795–802.

Pigliucci M, 2001. Phenotypic Plasticity: Beyond Nature and Nurture. Baltimore: Maryland, USA: Johns Hopkins University Press.

Pigliucci M, 2005. Evolution of phenotypic plasticity: Where are we going now? Trends Ecol. Evol. 20: 481–486.

Pradhan DS, Newman AEM, Wacker DW, Wingfield JC, Schlinger BA et al., 2010. Aggressive interactions rapidly increase androgen synthesis in the brain during the non-breeding season. Horm. Behav. 57: 381–389.

Ramallo MR, Grober MS, Cánepa MM, Morandini L, Pandolfi M, 2012. Arginine vasotocin expression and participation in reproduction and social behavior in males of the cichlid fish *Cichlasoma dimerus*. Gen. Comp. Endocrinol. 179, 221–231.

Roberts ML, Buchanan KL, Evans MR, 2004. Testing the immunocompetence handicap hypothesis: A review of the evidence. Anim. Behav. 68: 227–239.

Romero LM, 2012. Using the reactive scope model to understand why stress physiology predicts survival during starvation in Galápagos marine iguanas. Gen. Comp. Endocrinol. 176: 296–299.

Romero LM, Meister CJ, Cyr NE, Kenagy GJ, Wingfield JC, 2008. Seasonal glucocorticoid responses to capture in wild free-living mammals. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294: R614–622.

Rosvall KA, Bergeon Burns CM, Barske J, Goodson JL, Schlinger BA et al., 2012. Neural sensitivity to sex steroids predicts individual differences in aggression: Implications for behavioural evolution. Proc. R. Soc. B 279: 3547–3555.

Schlichting CD, Pigluicci M, 1998. Phenotypic Evolution: A Reaction Norm Perspective. Sunderland, Massachusetts, USA: Sinauer.

Schrandt NM, Lema SC, 2011. Intraspecific variation in stress reactivity of a demersal fish associates with spatial variation in coral reef structure. Mar. Ecol. Prog. Ser. 443: 153–166.

Schrandt NM, Hardy KM, Johnson KM, Lema SC, 2012. Physical habitat and social conditions across a coral reef shape spatial patterns of intraspecific behavioral variation in a demersal fish. Mar. Ecol. 33: 149–164.

Sembdner G, Atzorn R, Schneider G, 1994. Plant hormone conjugation. Plant Mol. Biol. 26: 1459–1481.

Semsar K, Kandel FLM, Godwin J, 2001. Manipulations of the AVT system shift social status and related courtship and aggressive behavior in the bluehead wrasse. Horm. Behav. 40: 21–31.

Shipley B, 2000. Cause and Correlation in Biology: A User's

Guide to Path Analysis, Structural Equations and Causal Inference. Cambridge, UK: Cambridge University Press.

Sinervo B, Licht P, 1991a. Proximate constraints on the evolution of egg size, egg number and total clutch mass in lizards. Science 252: 1300–1302.

Sinervo B, Licht P, 1991b. The physiological and hormonal control of clutch size, egg size and egg shape in *Uta stansburiana*: Constraints on the evolution of lizard life histories. J. Exp. Zool. 257: 252–264.

Sinervo B, Svensson E, 1998. Mechanistic and selective causes of life history trade-offs and plasticity. Oikos 83: 432–442.

Slominski AT, Botchkarev V, Choudhry M, Fazal N, Fechner K et al., 2006. Cutaneous expression of CRH and CRH-R: Is there a "skin stress response system?" Ann. New York Acad. Sci. 885: 287–311.

Snell-Rood EC, Troth A, Moczek AP, 2013. DNA methylation as a mechanism of nutritional plasticity: Limited support from horned beetles. J. Exp. Zool. B Mol. Dev. Evol. 320: 22–34.

Sommer RJ, Ogawa A, 2011. Hormone signaling and phenotypic plasticity in nematode development and evolution. Curr. Biol. 21: R758–R766.

Staswick P, 2009. Plant hormone conjugation: A signal decision. Plant Signal Behav. 4: 757–759.

Stearns SC, 1989. The evolutionary significance of phenotypic plasticity: Phenotypic sources of variation among organisms can be described by developmental switches and reaction norms. Bioscience 39: 436–445.

Stearns S, de Jong G, Newman B, 1991. The effects of phenotypic plasticity on genetic correlations. Trends Ecol. Evol. 6: 122–126.

Steimer T, Python A, Scholz PE, Aubry JM, 2007. Plasma corticosterone, dexamethasone (DEX) suppression and DEX/CRH tests in a rat model of genetic vulnerability to depression. Psychoneuroendocrinol. 32: 575–579.

Strott CA, 1997. Steroid sulfotransferases. Endocrinol. Rev. 17: 670–697.

Sultan SE, 2000. Phenotypic plasticity for plant development, function and life history. Trends Plant Sci. 12: 537–542.

Sultan SE, Stearns SC, 2005. Environmentally contingent variation: Phenotypic plasticity and norms of reaction. In: Hallgrímsson B, Hall BK ed. Variation: A Central Concept in Biology. Burlington, MA, USA: Elsevier Academic Press, 303–332.

Svoboda M, Riha J, Wlcek K, Jaeger W, Thalhammer T, 2011. Organic anion transporting polypeptides (OATPs): Regulation of expression and function. Curr. Drug Metabol. 12: 139–153.

Thornton JW, 2001. Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. Proc. Natl. Acad. Sci. USA 98: 5671–5676.

Tu Z, Keller Mp, Zhang C, Rabaglia ME, Greenawalt DM et al., 2012. Integrative analysis of a cross-loci regulation network identifies App as a gene regulating insulin secretion from pancreatic islets. PLoS Genet. 8: e1003107.

van der Deure WM, Peeters RP, Visser TJ, 2010. Molecular aspects of thyroid hormone transporters, including MCT8, MCT10, and OATPs, and the effects of genetic variation in these transporters. J. Mol. Endocrinol. 44: 1–11.

Visser WE, Friesema ECH, Jansen J, Visser TJ, 2008. Thyroid hormone transport in and out of cells. Trends Endocrinol. Metabol. 19: 50–56.

Wang Z, Young LJ, Liu Y, Insel TR, 1997. Species differences in vasopressin receptor binding are evident early in development: Comparative anatomic studies in prairie and montane voles. J. Comp. Neurol. 378: 535–546.

Warner RR, 1984. Mating behavior and hermaphroditism in coral reef fishes. Amer. Sci. 72: 128–136.

West-Eberhard MJ, 2003. Developmental Plasticity and Evolution. New York: Oxford Univ. Press.

Whitehead A, Crawford DL, 2006. Variation within and among species in gene expression: Raw material for evolution. Mol. Ecol. 15, 1197–1211.

Williams TD, 2008. Individual variation in endocrine systems: Moving beyond the 'tyranny of the Golden Mean'. Phil. Trans. R. Soc. B 363: 1687 –1698.

Wingfield JC, 1994. Modulation of the adrenocortical response to stress in birds. In: Davey KG, Peter RE, Tobe SS ed. Perspectives in Comparative Endocrinology. Ottawa, Canada: National Research Council, 520–528.

Woltereck R, 1909. Weitere experimentelle Untersuchungen über Artveränderung, speziell über das Wesen quantitativer Artunterschiede bei Daphnien. Verhandlungen der deutschen zoologischen Gesellschaft 19: 110–173.

Wright S, 1984. Diverse uses of path analysis. In: Chakravarti A, ed. Human Population Genetics. New York: Van Nostrand Reinhold Co. 1–34.

Young LJ, Nilsen R, Waymire KG, MacGregor GR, Insel TR, 1999. Increased affiliative response to vasopressin in mice expressing the V1a receptor from a monogamous vole. Nature 400: 766–768.

Zera AJ, 2006. Evolutionary genetics of juvenile hormone and ecdysteroid regulation in *Gryllus*: A case study in the microevolution of endocrine regulation. Comp. Biochem. Physiol. A 144: 365–379.

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

Zera AJ, Huang Y, 1995. Evolutionary endocrinology of juvenile hormone esterase in *Gryllus assimilis*: Direct and correlated responses to selection. Genetics 141: 1125–1134.

Zera AJ, Huang Y, 1999. Evolutionary endocrinology of juvenile hormone esterase: Functional relationship with wing polymorphism in the cricket *Gryllus firmus*. Evolution 53: 837–847.

Zhang X, Ho S-M, 2011. Epigenetics meets endocrinology. J. Mol. Endocrinol. 46: R11–R32.

Zhu Y, Rice CD, Pang Y, Pace M, Thomas P, 2003. Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. Proc. Natl. Acad. Sci. USA 100: 2231–2236.

Zhu J, Sova P, Xu Q, Dombek KM, Xu EY et al., 2012. Stitching together multiple data dimensions reveals interacting metabolomics and transcriptomic networks that modulate cell regulation. PLoS Biol. 10: e1001301.

Zouk H, McGirr A, Lebel V, Benkelfat C, Rouleau G et al., 2007. The effect of genetic variation of the serotonin 1B receptor gene on impulsive aggressive behavior and suicide. Am. J. Med. Genet. Part B 144B: 996–1002.