SYMPOSIUM

Hormones and Phenotypic Plasticity in an Ecological Context:
Linking Physiological Mechanisms to Evolutionary Processes

Sean C. Lema

Biological Sciences Department, Center for Coastal Marine Sciences, California Polytechnic State University, San Luis Obispo, CA 93407, USA


1E-mail: slema@calpoly.edu

Synopsis  Hormones are chemical signaling molecules that regulate patterns of cellular physiology and gene expression underlying phenotypic traits. Hormone-signaling pathways respond to an organism’s external environment to mediate developmental stage-specific malleability in phenotypes, so that environmental variation experienced at different stages of development has distinct effects on an organism’s phenotype. Studies of hormone-signaling are therefore playing a central role in efforts to understand how plastic phenotypic responses to environmental variation are generated during development. But, how do adaptive, hormonally mediated phenotypes evolve if the individual signaling components (hormones, conversion enzymes, membrane transporters, and receptors) that comprise any hormone-signaling pathway show expressional flexibility in response to environmental variation? What relevance do these components hold as molecular targets for selection to couple or decouple correlated hormonally mediated traits? This article explores how studying the endocrine underpinnings of phenotypic plasticity in an ecologically relevant context can provide insights into these, and other, crucial questions into the role of phenotypic plasticity in evolution, including how plasticity itself evolves. These issues are discussed in the light of investigations into how thyroid hormones mediate morphological plasticity in Death Valley’s clade of pupfishes (Cyprinodon spp.). Findings from this work with pupfish illustrate that the study of hormone-signaling from an ecological perspective can reveal how phenotypic plasticity contributes to the generation of phenotypic novelty, as well as how physiological mechanisms developmentally link an organism’s phenotype to its environmental experiences.

Introduction  Phenotypic variation is generated by the complex orchestration of interacting factors that contribute to an organism’s development. Research into “phenotypic plasticity”, defined as the production of different phenotypes by a genotype exposed to different environmental conditions (West-Eberhard 2003; Pigliucci et al. 2006), has taught us that the environment an organism experiences during its life is one of these factors. While it is now recognized that many phenotypic traits exhibit plasticity, there remain gaps in our understanding of the role of phenotypic plasticity in evolution, including how plasticity itself evolves. These gaps stem from the conceptual disjunction between plasticity, which is a developmental process that occurs within the lifetime of individuals, and evolutionary processes that happen in populations over generations. Such gaps are evident in the challenges to understand whether plasticity facilitates adaptive evolutionary change by contributing novel phenotypes under altered environmental conditions (Behera and Nanjundiah 2004; West-Eberhard 2005a, 2005b; Ghalambor et al. 2007; Scoville and Pfender 2010), how environmentally malleable traits become canalized (Matsuda 1982; Pigliucci et al. 2006), whether plasticity ever results in maladaptive traits (Ghalambor et al. 2007; Morris and Rogers 2013), and how phenotypic integration is affected when one or more traits exhibits plasticity (Hau 2007; Lema and Kitano 2013).
The study of hormonally mediated phenotypic plasticity is providing fundamental insights into these queries about the role of plasticity in evolution. Hormones are chemical signaling molecules that regulate cellular physiology, development, behavior, morphology, and life history. Hormones themselves are synthesized and secreted from select glands or cell groups in response to stimuli either external (e.g., photoperiod and social interactions) or internal (e.g., osmotic balance and another hormone) to the organism, and then act locally or systemically to alter the physiological state or expression of genes in cells expressing receptors for that hormone. Ultimately, an activated hormone receptor either up-regulates or down-regulates gene transcription directly, or alters levels of intracellular second-messenger molecules (e.g., cAMP, IP3, and Ca2+) to change cellular state, which itself may also affect patterns of gene expression. Hormonal systems therefore function as operational links between an organism’s external environment and its internal developmental and physiological processes, including its genetic and epigenetic composition (Ketterson et al. 2009; McCormick 2009; Lema and Kitano 2013).

Since the importance of hormones in mediating phenotypic plasticity has been discussed elsewhere, both broadly and in the context of specific animal systems (Dufty et al. 2002; Williams 2008; Lema and Kitano 2013; Maruska and Fernald 2013; Middlemis Maher et al. 2013), this article will instead focus on how the study of hormonally mediated plasticity can bridge gaps in understanding plasticity’s importance for evolution. Studies of hormonally mediated plasticity in an ecologically relevant context are revealing how commonly environmentally induced changes in phenotype occur in wild populations, how correlated traits respond both developmentally to environmental variation and evolutionarily to selection, whether such correlations might constrain evolution, and how the genetic and molecular mechanisms that underlie plastic responses may themselves limit patterns of phenotypic differentiation. Hormonal systems also mediate the developmental stage-specific malleability of many traits, and studies of the endocrine underpinnings for stage-specific plasticity can provide insights into how environmental experience at different developmental stages contributes to patterns of phenotypic variation among individuals or populations.

**Using hormones to establish plasticity’s role in evolutionary divergence**

How important is phenotypic plasticity in guiding adaptation? This question remains largely unanswered, even though it has been a subject of interest ever since the psychologists James Baldwin and Conwy Lloyd Morgan and paleontologist Henry Fairfield Osborn independently theorized in the late 1800s that environmentally induced phenotypic responses might have a directing role in evolution (Morgan 1896; Osborn 1896; Baldwin 1896). Since the initial proposal of these ideas, theories for how environmentally elicited phenotypes might facilitate evolutionary change have progressed through several iterations (West-Eberhard 2003, 2005a; Pigliucci et al. 2006; Pfennig et al. 2010). Despite differences in the details of these theories (Hall 2001), the fundamental question of what role phenotypic plasticity plays in evolution remains of interest because plasticity can developmentally generate rapid phenotypic responses and phenotypic novelties under shifting environmental conditions (West-Eberhard 2005a, 2005b).

Phenotypic plasticity might promote divergence in populations as any individual variation in environmentally induced phenotypes should be subject to selection (West-Eberhard 2005a; Pfennig et al. 2010), and we might expect to be able to see evidence for plasticity’s role in quickening divergence reflected in the patterns of evolution in recently differentiated sister taxa or local populations (Fig. 1). Given this consideration, how might the study of hormonal mechanisms of plasticity provide support for phenotypic plasticity promoting evolutionary divergence? In one of the first efforts to link environmental variation, hormone-signaling, and phenotypic responses in the study of evolution, Matsuda (1982) pointed out: “It should be borne in mind that it is the physiology of animals, including their hormonal activity, that is first and inevitably affected by environmental changes.” (p. 733). Endocrine mechanisms function to help maintain physiological status within specific limits (e.g., homeostasis) and respond when the organism experiences changes in its environment or internal status. Changes in hormone-signaling are thus one of the first responses that organisms exhibit under environmental change.

Investigating the links between environment, hormones, and phenotype in an ecologically relevant context can therefore aid in the detection of plasticity-mediated phenotypic change in wild populations. Expanding on the illustrations of plasticity’s effect on divergence (Fig. 1), studying hormonally mediated plasticity in two recently isolated populations or sister taxa—in which one of the populations or taxa has a derived character state putatively associated with a plastic response—provides a tractable approach to establish evolutionary divergence.
initiated by plasticity without an initial genetic change (Hall 2001). As long as the populations or taxa are identified before any phenotypic change might become canalized (e.g., genetic assimilation), it should be possible to test whether the initial phenotypic divergence was mediated by plasticity (Fig. 2). Reciprocal transplants, common-garden experiments, or the rearing of individuals from both populations under controlled laboratory conditions representing each population's respective environment can be used to test whether environmental conditions restore the derived character to the original character state, as well as whether the original value of the character can be developmentally shifted to the derived character via exposure to the novel environment (Hall 2001).

With hormonally mediated plasticity, however, it is also possible to manipulate hormone-signaling pathways to “engineer” plastic phenotypic responses (Fig. 2). This requires identifying endocrine-signaling pathways that might underlie the plasticity, and then modulating those pathways at the appropriate stages of development via exogenous hormones, receptor agonists or antagonists, conversion enzyme inhibitors, and other tools of experimental endocrinology to effectively “engineer” hormonally mediated phenotypic responses (e.g., Ketterson et al. 1996; Ketterson and Nolan 1999). This ability to identify and manipulate the hormonal mechanisms linked to plasticity-mediated phenotypic divergence provides a tractable tool to identify scenarios in which

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**Fig. 1** Phenotypic plasticity might alter patterns of evolutionary divergence. (A) A Brownian-motion simulation illustrates population divergence in a quantitative character over time. The mean character value of the population is indicated by the solid line, and boundaries for the range of variation of characters in the population are shown by the dotted lines. (B) Phenotypic plasticity may generate rapid phenotypic divergence when environments change, such as when individuals from one population establish a population in a new habitat. If conditions in that new habitat are sufficiently dissimilar from the original habitat, plasticity may generate novel values of traits that are outside the range of that character in the ancestral population. (C) Even when a single population experiences a rapidly changing environment, plasticity could generate novel character-states outside the phenotypic range prior to the environmental change. In such a scenario, the population might exhibit a “phenotypic skip”, whereby novel phenotypes are expressed without a gradual transition through intermediate character states.

**Fig. 2** Phenotypic plasticity under hormonal regulation allows for additional experimental tests to help identify plasticity’s contribution to the generation of phenotypic change during early stages of evolutionary divergence. Here, two populations of the same species have recently diverged. Population 1 expresses the original character state, and Population 2 has a derived character state hypothesized to be due to a plastic response to a new environment (Habitat 2). Experimental approaches, such as common garden rearing or reciprocal transplants can be used to demonstrate a role for plasticity in phenotypic differentiation. Plastic responses mediated by hormones provide additional opportunities to examine how the shift from ancestral to novel phenotype occurred by “engineering” plastic responses via the use of exogenous hormone, receptor agonists or antagonists, enzyme inhibitors, or other endocrine approaches.
plasticity has facilitated evolutionary divergence (Matsuda 1987). Advances in next-generation sequencing technologies now allow for the characterization of transcriptional differences and epigenetic variation among populations, individuals, or tissues. Future studies that combine the hormonal “engineering” of phenotypes with assessments of changes in transcriptional or epigenetic variation promise to provide new insights into when phenotypic divergence is driven by environmentally initiated shifts in development, rather than by mutation or other changes in genetic structure (Aubin-Horth and Renn 2009; Kitano et al. 2014).

Hormones, correlations of traits, and integrated phenotypes

Traits that comprise an organism’s phenotype can be positively or negatively correlated in expression due to linkage disequilibrium, a common structural or developmental origin, or a shared molecular or physiological regulatory mechanism. Cases in which the correlation results from multiple traits being influenced by a common regulating gene are referred to as pleiotropy. Because hormones typically act on several tissues at a time to coordinate multiple traits, suites of traits regulated by the same hormone have themselves been referred to as instances of “hormonal pleiotropy” (Ketterson and Nolan 1999; McGlothlin and Ketterson 2008; Ketterson et al. 2009).

Such shared endocrine control of traits could result from selection coupling two or more traits under a common hormonal regulatory mechanism because the association between traits conveys positive fitness-consequences (McGlothlin and Ketterson 2008). Under such a scenario, hormonal pleiotropy would have evolved because it had a fitness-benefit in a given environment. Alternatively, such constraints could instead be indicative of a functional trade-off when hormones affect a trait that has energetic or developmental consequences for other phenotypic attributes (Lessells 2008). For example, the development of exaggerated “horns” in scarab beetles is mediated by insulin and other growth-factors associated with nutritional experience during larval life (Emlen 2010; Snell-Rood and Moczek 2012). Experimental manipulations in some beetle species have revealed developmental trade-offs between investment in growth of the horn and size of the testes; these trade-offs are presumed to be mediated by limits in allocation of resources during development (Moczek and Nijhout 2004; Simmons and Emlen 2006).

It has been suggested that hormonal pleiotropy might result in evolutionary compromises, so that selection on any single hormonally mediated trait could produce disadvantageous changes in other traits regulated by that same hormone (Ketterson and Nolan 1999; Wingfield et al. 2001; Hau 2007; Adkins-Regan 2008; Lessells 2008). However, as with pleiotropy involving genes (Stearn 2000), it is important to differentiate between the pleiotropic roles of hormones (i.e., the multitude of traits established to be regulated by a given hormone) and the pleiotropic effects of a change in the hormone-signaling pathway (e.g., a change in concentration of circulating hormone altering several traits concurrently) when considering potential evolutionary constraints in hormonally mediated phenotypes. While this distinction may at first seem inconsequential, it is central to understanding how hormones mediate complex, integrated phenotypes. A given hormone may regulate multiple traits, but the pleiotropic impacts of a change in that hormone-signaling pathway—for instance, an environmentally induced increase in the secretion of that hormone, or the administration of exogenous hormone to experimentally elevate hormone concentration—may only impact some of those traits in any given developmental stage, sex, or environmental context. This discrepancy between the current pleiotropic role of a hormone and the effects of a change in that hormone system emerges from the complex structure of endocrine-signaling pathways and the dynamics of dose–response relationships generated by the interactions of hormones and receptors.

Hormone-signaling as multidimensional regulatory pathways

Hormone-signaling pathways are structured with several interacting components that act from the synthesis and release of the hormone to the hormone’s effect on target cells. The identity of these components varies but commonly includes hormone-binding proteins that transport the hormone, plasma membrane transporters that facilitate the movement of hormones across a target cell’s plasma membrane, conversion enzymes that alter the chemical structure of the hormone to more or less active forms, and receptors that vary in affinity and intracellular signaling mechanisms. Beyond these elements, hormone-signaling pathways are modulated by other regulatory components such as arrestins that can alter internalization of receptors or even redirect the intracellular second-messenger profile of an activated receptor (Oakley et al. 2012),
transcriptional enhancers such as short (typically 50–200 bp nucleotides) non-coding enhancer RNAs that can shift cell-specific and tissue-specific transcriptional responses to hormone–receptor activation (e.g., Wang et al. 2011; Rubinstein and de Souza 2013), and coregulators and repressors that modulate hormonally mediated transcription of genes.

Investigations that have quantified expression of non-hormonal components (e.g., receptors, membrane transporters, and conversion enzymes) of endocrine-signaling pathways are scarce compared with studies that measured hormone concentration. Nevertheless, expression changes in any of these non-hormonal components might be expected to impact the functioning of the hormone pathway and, ultimately, the hormone–phenotype relationship. Indeed, the number of different components within any endocrine signaling pathway seems to point toward the possibility that endocrine-signaling pathways might exhibit considerable flexibility in regulation between cell or tissue types, sexes, or developmental stages, and even more so if signaling components in an endocrine pathway exhibit independent regulatory control by environmental conditions (Lessells 2008; Lema and Kitano 2013).

Several studies now provide evidence that individual components of an endocrine pathway can exhibit differential, tissue-specific expression responses to environmental changes both external (e.g., photoperiod and social interactions) and internal (e.g., hormone concentration) to the organism (e.g., Soma et al. 1999; Charlier et al. 2011; Johnson and Lema 2011; Muzzio et al. 2014; reviewed by Lema and Lema 2013). Although more research is needed in this area to establish the prevalence and identify of molecular mechanisms for “tissue-specific” regulation, such regulation is likely important to evolutionary diversification. For example, study of two populations of the pupfish *Cyprinodon nevadensis* from habitats that differ in salinity (Big Spring and Amargosa River habitats in Fig. 5) revealed that neighboring arginine vasotocin (AVT) neuron groups in the preoptic area (POA) of the hypothalamus had evolved different plastic responses to environmental salinity (Lema 2006). Parvocellular POA AVT neurons in these populations showed divergent responses to salinity, while neighboring magnocellular POA AVT neurons responded similarly across populations. While this is only one example, this finding demonstrates that evolution can alter hormone production in one grouping of cells but not an adjacent grouping, and illustrates how such evolutionary precision may only be apparent when organisms are examined under particular environmental conditions (reviewed by Lema and Kitano 2013).

Evolutionary constraints present in some environments may therefore not be present under other conditions due to plasticity in hormone-transport, hormone-metabolism, and peripheral tissue sensitivity of tissue within endocrine-signaling pathways. It is thus possible that the environment itself—via the triggering of differential expression responses in the components that comprise any hormone-signaling pathway—could decouple and recouple associations among hormonally mediated traits. Environmental variation is already known to alter the magnitude and direction of correlations of traits when those correlations are not hormonally mediated (Stearns et al. 1991). If that is the case with correlated, hormonally mediated traits as well, then the environmental context wherein correlations are examined will be fundamental to understanding the scenarios under which evolutionary constraints emerge from hormonal pleiotropy.

**Implications of non-linear, non-monotonic action of hormones**

Physiological concentrations of hormones in circulation or in intercellular fluids are typically very low, and the endocrine system is tuned to respond to these low concentrations via high-affinity receptors expressed in tissue-specific patterns. The ability of hormones to function at such low concentrations occurs in part because of non-linear relationships among the concentration of hormone, the number of receptors occupied by the hormone on a target cell, and the biological effect of that hormone–receptor’ occupancy (Vandenberg et al. 2012). Such non-linear relationships allow small changes in hormone concentration at the lower end of the physiological range to have considerable effects on cellular function, and maximal responses of target tissues often occur before saturation of receptor occupancy (Welshons et al. 2003).

These non-linear relationships among hormone concentration, receptor occupancy, and cellular effect can generate dose–response curves that are non-monotonic (Vandenberg et al. 2012). A monotonic hormone–response curve could be linear (Fig. 3A) or non-linear (Fig. 3B), but in either case has an unchanging slope sign over the range of hormone concentrations. In comparison, non-monotonic response curves exhibit a change in the sign of the curve’s slope and may be U-shaped, inverted U-shaped (Fig. 3C), or show a more complex pattern (Fig. 3D). Such non-monotonic dose–response
curves are thought to be common in endocrine-signaling for several reasons: (1) the simultaneous action of a single hormone on multiple subtypes of receptors (and subsequent competition for ligand among receptors), (2) the expression of different subtypes of receptors in different cells, even within the same tissue or organ, (3) the down-regulation, internalization, and inactivation/desensitization of receptors, (4) the types and ratios of cofactors (e.g., coactivators and corepressors) present in cells, and (5) negative feedback loops prevalent in endocrine axes (Kohn and Melnick 2002; Smith and O'Malley 2004; Li et al. 2007; reviewed by Vandenberg et al. 2012). Different target tissues may also exhibit different response curves. For example, in one study of an estrogen’s effects on female mice, 17β-estradiol had monotonic dose–response effects on uterine mass, coverage of uterine glandular epithelium, and coverage of uterine myometrium, but non-monotonic inverted U-shaped responses on duct-extension and number and density of terminal end buds in the mammary gland (Vandenberg et al. 2006).

To date, evidence for non-monotonic dose–responses of hormones comes largely from studies into the endocrine disrupting effects of chemicals (Vandenberg et al. 2012). In such studies, testing a range of hormone concentrations is routine; yet, there is also evidence for non-monotonic response curves in studies of hormonal function in behavioral or ecological contexts other than the effects of chemical contaminants (e.g., Norton and Wira 1977; Huggard et al. 1996; Sheehan et al. 1999; Navara et al. 2005; Ribeiro et al. 2009). For instance, corticosterone regulation of locomotor activity follows inverted U-shaped response curves in Adélie penguins (Pygoscelis adeliae) (Spée et al. 2011) and white-crowned sparrows (Zonotrichia leucophrys) (Breuner and Wingfield 2000). Likewise, AVT induces changes in aggression that follow an inverted U-shaped dose–response profile in damselfish (Stegastes leucostictus) (Santangelo and Bass 2006).

These non-linear, non-monotonic dose–response relationships may be fundamental to understanding the dynamics of flexibility and constraint in hormonally mediated, correlated traits. Non-monotonic dose–response curves may themselves generate non-monotonic correlations of traits that shift sign and strength, depending on hormone concentration (Fig. 4). The shape of a hormone dose–response curve could also be altered by environmental context. An example of such an environmental effect can be seen in the studies of Breuner and Wingfield (2000), who found that the behavioral response of white-crowned sparrows to corticosterone varied with photoperiod; intermediate doses of corticosterone increased activity under a long-day photoperiod but had no effect during a short-day photoperiod. If the dose–responses of hormones can themselves be plastic, then the shape of any relationship of correlated traits might also change with environmental conditions (Fig. 4B). Such plasticity in hormonal dose–responses may loosen any evolutionary constraints that arise from hormones regulating multiple traits. Plasticity therefore provides a mechanism that links the evolutionary decoupling and recoupling of hormonally mediated trait expression to environmental context.

**Dynamics of hormonally mediated plasticity: Lessons from Death Valley’s pupfishes**

My laboratory studies hormonal mechanisms of phenotypic plasticity in pupfishes (genus *Cyprinodon*)
inhabiting the Death Valley region of California and Nevada, USA. Death Valley is one of the hottest and driest geographic regions globally, with summer air temperatures routinely exceeding 49°C and rainfall averaging less than 5 cm annually; yet, this region is home to a clade of pupfishes that provides one of the clearest illustrations of allopatric speciation in North America (Miller 1948, 1950).

Pupfish are hypothesized to have dispersed into the drainages that empty into Death Valley ~3–2 Ma (Knott et al. 2008). Since that time Death Valley has sustained pupfish in alternating pluvial freshwater lakes, saline lakes, or marshes, as climatic conditions in the region oscillated during the repeated glaciations of the Pleistocene (Forester et al. 2005). Following the last glacial maximum ~21,000 years ago, the lakes and rivers of the Death Valley region largely have disappeared under an increasingly hot and arid climate, so that pupfishes today inhabit a scattering of remote habitats including groundwater-fed springs, saline marshes, and remnants of the Amargosa River (Fig. 5). Hydrological studies suggest that these populations have been isolated for at most 18,000–20,000 years, and likely less for populations among which limited gene flow may occur during atypically wet winters or after heavy rainfall.

Death Valley’s remnant aquatic habitats therefore represent “islands in a sea of desert” with a clade of pupfishes (C. nevadensis, C. diabolis, and C. salinus) occupying environments that vary in physical (e.g., salinity, temperature, and water flow) and social (e.g., conspecific density) conditions (Soltz and Naiman 1978). Microsatellite and neutral nuclear DNA markers indicate incomplete sorting of haplotypes in this clade, and genetic drift and bottleneck events have contributed to genetic divergence among populations (Duvernell and Turner 1998, 1999; Martin and Wilcox 2004). Nonetheless, the pupfishes...
in Death Valley exhibit extensive phenotypic variation with differences in morphology, behavior, life-history patterns, and physiology evident among populations (Miller 1948; Hirshfield et al. 1980; Soltz and Hirshfield 1981; Lema 2006).

These phenotypic differences appear to result in part from plastic responses of morphology, behavior, and physiology to the dissimilar habitats (Lema 2008). Pupfish in Death Valley, and outside this region, exhibit extensive plasticity in morphology and behavior (e.g., Sweet and Kinne 1964; Kodric-Brown 1981; Lema and Nevitt 2006; Collyer et al. 2007), and there is evidence that some populations in Death Valley have evolved differences in behavioral and physiological plasticity to environmental conditions (Hirshfield et al. 1980; Soltz and Hirshfield 1981; Lema 2006). The fishes in this desert region therefore provide an opportunity to explore how hormonally mediated phenotypic plasticity facilitates evolutionary adaptation and diversification in response to disparate environments.

**Thyroid hormone, morphological plasticity, and rapid divergence of populations**

Taxonomic designations of pupfishes in the Death Valley region are based on morphological variation in body shape, scale counts, and structure of the fins (Wales 1930; Miller 1948; LaBounty and Deacon 1972). Incontestably, the most unique of these taxa is the Devils Hole pupfish *C. diabolis*, which lives in what may be the most restricted habitat of any vertebrate in the world: Devil’s Hole. Devil’s Hole is a warm (~33–34°C) aquatic system that is the only native habitat of *C. diabolis* (Riggs and Deacon 2002). Big Spring is a stable, groundwater-fed thermal spring (~27.3°C, 0.4 ppt salinity) that is occupied by *C. n. mionectes*. Amargosa River is a variable desert stream where daily temperatures can fluctuate over 25°C (annual range: 0.4°C to >42°C) and salinities vary (0.2–8.3 ppt) (Lema and Nevitt 2004; S. Lema unpublished data). Photographs of all habitats and of *C. n. mionectes* and *C. n. amargosa* by S. Lema. Photograph of *C. diabolis* provided by T. Baugh.
only breed on a shallow rock shelf located at the southern end of the habitat. The *Cyprinodon diabolis* pupfish that occupy Devil’s Hole live in extreme conditions of warm water (~33.5°C), low dissolved oxygen (avg. of 2.7–3.0 mg l⁻¹), and a low availability of food (Bernot and Wilson 2012). Perhaps accordingly, the morphology of *C. diabolis* is also exceptional among Death Valley’s pupfishes. *Cyprinodon diabolis* is characterized by a small body size (typically <20 mm in standard length [SL]), large relative head size (proportion of head length to SL) and eye size (proportion of eye diameter to SL), and the absence of pelvic fins (Wales 1930; Miller 1948; Williams 1977). The behavior of *C. diabolis* in Devil’s Hole is also distinctive; males do not exhibit the overt aggressive behaviors observed with the defense of reproductive territories common to other pupfishes in Death Valley (Wilcox and Martin 2006).

During the 1960s, pumping of groundwater in the vicinity of Devil’s Hole was dropping the water level and uncovering the shallow rock shelf used for breeding. This conservation predicament led to a legal challenge considered by the U.S. Supreme Court in 1976, which decided in a landmark ruling to stop the groundwater pumping (Deacon and Williams 1991). Despite a partial recovery of the water level in Devil’s Hole once local pumping ceased (Riggs and Deacon 2002), this crisis prompted construction of three artificial refuge habitats to establish additional populations of *C. diabolis* as a source for reintroducing fish into Devil’s Hole should the endemic population be extirpated (Baugh and Deacon 1988).

Even though the artificial refuges were constructed to mimic the physical and biotic conditions of Devil’s Hole (Sharpe et al. 1973), the morphology of *C. diabolis* in the first refuge constructed—the Hoover Dam refuge—was found to have diverged only 4 years after introducing the fish (Williams 1977). Refuge *C. diabolis* were larger, exhibited a reduced relative head size, and had a greater body depth (Williams 1977). *Cyprinodon diabolis* in the other refuges were subsequently also found to differ from *C. diabolis* in Devil’s Hole, with 32% and 48% of refuge adults exceeding the maximum size of Devil’s Hole fish (Wilcox and Martin 2006). Refuge fish were also found to defend reproductive territories and exhibit aggressive behaviors uncommon to *C. diabolis*, but typical of pupfish in other habitats in Death Valley (Wilcox and Martin 2006). Later investigations even identified ~14% of *C. diabolis* in one artificial refuge, the Hoover Dam refuge, having developed either one or both of the paired pelvic fins (B. Hobbs 2006, personal communication).

Environmental conditions in the refuges differed from those in Devil’s Hole; the refuges were cooler and more variable in temperature, had higher dissolved oxygen (3.5–5.9 mg l⁻¹), and contained a greater abundance and different composition of algae and invertebrates (Wilcox and Martin 2006; Karam et al. 2012). To examine whether plastic developmental responses to these altered conditions contributed to the morphological change of *C. diabolis* in the refuges, Lema and Nevitt (2006) reared pupfish under a range of availabilities of food and at temperature conditions that mimicked the differences between the refuges and Devil’s Hole (see also Lema 2008). Because *C. diabolis* is a federally endangered species, Lema and Nevitt (2006) raised closely related *C. n. amargosae* pupfish from 15 days post-fertilization (dpf) until 141 dpf under feeding regimes of 100% ration, 50% ration, or 20% ration so as to generate variation in growth rate (Fig. 6A). The growth rate of the low-ration treatment was chosen to mimic rates of growth observed for *C. diabolis* in Devil’s Hole (James 1969). This variation in availability of food and in growth affected the development of body shape, so that slowly growing fish exhibited a larger relative head size (Fig. 6B) and eye size, and only ~14% of the fish in the low-growth treatment developed pelvic fins compared with 78% of fish at 100% ration (Fig. 6C) (Lema and Nevitt 2006).

Lema and Nevitt’s (2006) finding that low food and reduced growth affected the development of the paired pelvic fins suggests that the larval-to-juvenile morphological transition can be altered by environmental factors. The paired pelvic fins generally function as trimming foils during swimming and first develop when fish transition from a larva with a finfold to the morphology of juveniles (Yamanoue et al. 2010). This transition could be viewed as analogous to metamorphosis in amphibians in that the morphological transformation is mediated in part by thyroid hormone (TH) (Brown 1997). Supporting this idea, Lema and Nevitt (2006) found that whole-body levels of thyroxine (T₄) appeared reduced in pupfish from the treatment resulting in low growth (Fig. 6D), although comparisons in this case are confounded by the variation in body size intentionally generated by limiting food rations. Experimentally inhibiting endogenous TH production by treating *C. n. amargosae* larvae pharmacologically with a goitrogen (methimazole or potassium perchlorate [KClO₄]) also shifted morphological development toward a larger relative head size (Fig. 6E) and eye size, a reduced body depth, and reduction in growth of the pelvic fin (Fig. 6F) (Lema and Nevitt 2006). While the overall
effect of experimentally reducing endogenous TH was to shift development toward the Devil’s Hole C. diabolis phenotype, these TH-mediated changes in morphology occurred without any reduction in growth. This finding implies that alterations in body shape and in development of the pelvic fin can be decoupled from effects on growth and body size via alterations in TH signaling.

Lema and Nevitt (2006) also found that even small increases in the temperature at which pupfish larvae were reared increased relative eye size, decreased body depth, and inhibited development of the pelvic fins. This thermal effect was evident both as an increase in the percentage of fish with no pelvic fins when reared at higher temperatures and as a decrease in the mean number of pelvic fins on each fish across all food rations (Fig. 6G) (Lema and Nevitt 2006). Notably, the range of temperature variation examined in this study only spanned from $32.5^\circ$C to $34.5^\circ$C, which encompasses the mean temperature experienced by C. diabolis in Devil’s Hole ($\sim33.5^\circ$C) (Bernot and Wilson 2012; Karam et al. 2012).

The plastic responses of morphology observed in C. n. amargosae by Lema and Nevitt (2006) suggest that Death Valley’s pupfishes retain a latent ability to shift morphology in environments that affect energy balance and growth, and implicate TH as a hormonal regulator of the plasticity. It is likely that these observations of morphological plasticity provide a partial explanation for the morphological changes that occurred when C. diabolis were transferred to artificial refuges as a conservation strategy. While the small population of C. diabolis precludes testing that hypothesis directly, the results with C. n. amargosae indicate that phenotypic plasticity may play a fundamental role during the initial stages of evolutionary divergence in this allopatric clade of pupfish.
populations. In this case, plasticity appears to have contributed to a shift in discrete phenotypic attributes—the complete loss of pelvic fins—without having to transition through intermediate phenotypes (e.g., a gradual reduction in pelvic fins).

Even so, our understanding of environmentally induced morphological plasticity in Death Valley’s pupfishes is certainly incomplete. Recent findings in zebrafish (*Danio rerio*) suggest that the role of THs in metabolism in teleost fish might vary at low and high temperatures due to changes in peripheral sensitivity to THs (Little et al. 2013). Also, recent work in another pupfish, *C. variegatus*, revealed that the thermal experience of adults can alter the growth-rate response of offspring to temperature, suggesting that there can be cross-generational transfer of plastic responses of growth via material effects or other epigenetic mechanisms (Salinas and Munch 2012). It is also important to note that other environmental factors, such as low dissolved O₂, may contribute developmentally to the phenotypes of *C. diabolis* in Devil’s Hole. While no study has yet examined the developmental effects of hypoxia on pupfish, hypoxia (dissolved O₂ < 2 mg l⁻¹) has been shown to reduce circulating concentrations of T₃ in sexually mature carp (*Cyprinus carpio*) (Wu et al. 2003).

As my laboratory continues to examine mechanisms of TH-mediated plasticity in Death Valley’s pupfishes, we conjecture whether plasticity could facilitate recurrent patterns of morphological divergence in habitats with elevated temperature or with restricted food. Supporting this possibility, we recently identified a population of *C. n. amargosae* pupfish in which ~33% of the population lacks one or both of the paired pelvic fins (S. Lema, unpublished data). This population occupies a thermal spring where the water emerges from the ground at ~47°C and then cools gradually as the water flows down the spring’s outflow channel and enters a shallow marsh. Remarkably, the absence of pelvic fins in 33% of fish from this population appears to have occurred only within the past 4 years, after a culvert allowing water in the spring’s outflow channel to flow under a nearby road was cleared, altering the thermal profile of the habitat and increasing the relative area of habitat with water temperatures > 35°C. While we continue to explore the contribution of phenotypic plasticity to the recent morphological change in this particular population, our early data suggest that environmentally induced variation in sensitivity of peripheral tissues to THs may be a contributing factor (S. Lema, unpublished data).

**Closing perspectives**

Taken as whole, these investigations into TH-mediated plasticity in Death Valley’s pupfishes illustrate how the phenotypes that result from plasticity are an outcome of the integrated developmental responses of the organism to multiple environmental factors. In this particular case, the environmental factors of high temperature and low availability of food have overlapping—but distinguishable—affects on TH signaling and, ultimately, morphology. Reduced availability of food acts to depress the endogenous production of THs, while temperature has no apparent direct effect on TH concentrations, but may act peripherally to alter sensitivity of tissues to THs. When these environmental conditions covary, however, they can interact to jointly affect signaling pathways of TH and shape morphological outcomes of the larva-to-juvenile transition (Fig. 7). This mechanism of hormonally mediated plasticity appears to be able to generate a “phenotypic skip” (see Fig. 1C) in morphology for the pupfishes of Death Valley, if a population experiences a rapid change in temperature or in abundance of food.
This example with pupfish, however, is just one illustration of how the study of hormonal mechanisms holds promise for informing the role of plasticity both in the initial stages of divergence of populations and in the evolution of co-segregating traits. As studies of endocrine mechanisms of plasticity with other hormones and other animal systems accumulate, we are likely to gain a much clearer understanding of the physiological and genetic mechanisms that make specific traits environmentally malleable at a given developmental age, how that malleability contributes to phenotypic variation among individuals, populations, and species, and how environmental variation may have developmental influences on the coupling and decoupling of correlated traits. Hormone-signaling pathways hold a central place in directing the complex orchestration of phenotypic development, and the study of hormonally mediated plasticity promises to contribute to a richer understanding of developmental evolution.

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