An Integrative Endocrine Model for the Evolution of Developmental Timing and Life History of Plethodontids and Other Salamanders

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An Integrative Endocrine Model for the Evolution of Developmental Timing and Life History of Plethodontids and Other Salamanders

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In recent years, many molecular endocrine mechanisms that regulate tissue morphogenesis have been detailed in laboratory amphibian models. However, most of these pathways have not been examined across more closely related species to understand how deviations in endocrine pathways may have contributed to amphibian diversification. The timing of metamorphosis and maturation vary extensively across plethodontid salamanders (including direct developing, biphasic, and paedomorphic species), making them an ideal system for analyzing the evolution of endocrine mechanisms in a phylogenetic context. Recent phylogenetic-based reconstructions concluded that ancestral plethodontids were likely direct developers, and free-living larval periods were independently derived multiple times within this family. Furthermore, within one clade (Spelerpini) there have been multiple independent transitions from biphasic (metamorphic) to paedomorphic developmental modes. This inspires the question: What endocrine/developmental mechanisms govern these life history transitions? Prior endocrine models for the evolution of direct development and paedomorphosis have been largely based on the ontogenetic timing of thyroid hormone release and/or thyroid hormone responsiveness of target tissues. Here I review endocrine pathways that influence metamorphosis and maturation in laboratory amphibians (Clawed frog and Axolotl) and other species, and develop a model that integrates prior thyroid hormone-based patterns with other endocrine axes. This integrated framework incorporates developmental shifts that result from plasticity or evolution in the timing of hatching, metamorphosis, and maturation, and can be used to test mechanistic changes that underlie life history variation of plethodontids and other salamanders.

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amphibians. The hypothalamus plays a central role in mediating the influence of environmental factors on endogenous hormone levels, which are important determinants of how phenotypes and life histories are manifested. Below I provide an overview of the relevant aspects of three endocrine systems (thyroid, corticosteroid, and reproductive), which individually and in concert are major drivers of amphibian development and life histories.

Thyroid hormone and amphibian metamorphosis.—Amphibians have historically been important models for our understanding of thyroid hormone function, ever since Gudernatsch (1912) precociously metamorphosed tadpoles of *Rana temporaria* by feeding them mammalian thyroid glands. Subsequently, metamorphosis was successfully induced with thyroid hormone for many amphibian larvae. These included treatments with both 3,5,3-triiodothyronine (T3) and the less active form 3,5,3′,5′-tetraiodothyronine (T4; thyroxine). Notable exceptions included some obligately paedomorphic salamanders (discussed under larval form paedomorphosis below). Several studies also showed that plasma levels of T3 and T4 increase during metamorphosis (e.g., Regard et al., 1978; Suzuki and Suzuki, 1981; Alberch et al., 1986; Norman et al., 1987), and ablation of the thyroid gland can prevent metamorphosis (Allen, 1916, 1918; Hoskins and Hoskins, 1919). Metamorphosis can be recovered in thyroidecтомized larvae by treatment with TH (Allen, 1932; Etkin, 1935; Hanaoka, 1966). Taken together, these studies demonstrate that thyroid hormone is necessary for amphibian metamorphosis.

The release of T3 and T4 from the thyroid gland is regulated by the hypothalamic-pituitary-thyroid (HPT) axis (Fig. 2). Thyrotropin-releasing hormone (TRH) from the hypothalamus causes the release of thyroid-stimulating hormone (TSH) from the anterior pituitary, which stimulates the thyroid gland to release T3 and T4 into the circulatory system. However, TRH appears to only activate the release of TSH in adult metamorphosed amphibians (Darras and Kühn, 1983; Denver, 1988; Jacobs et al., 1988). In contrast, hypothalamic corticotropin-releasing factor (CRF; discussed with corticosteroids below) appears to regulate the release of TSH in larval amphibians (Denver and Licht, 1989; Denver, 1993, 1997, 2009, 2013; Boorse and Denver, 2002). Plasma TSH levels are undetectable in early stages of tadpole development, but elevate prior to metamorphic climax (Dodd and Dodd, 1976). Treatment of larval amphibians and paedomorphic salamanders with TSH has been shown to stimulate thyroid activity and induce metamorphosis (Dent and Kirby-Smith, 1963; Norris et al., 1973; Norman and Norris, 1987). Further evidence of the central role of this axis comes from experiments that have inhibited metamorphosis by ablating the anterior pituitary (Kollros, 1961; Hanaoka, 1966).

At target tissues (e.g., tailfin, external gills, brain), T3 and T4 interact directly with the nuclear transcription factors TRα and TRβ (thyroid hormone receptor alpha or beta, collectively TRs), which form heterodimers with retinoid X receptor (RXR) and bind to thyroid hormone response elements (TREs) in the genome (Ranjan et al., 1994; Buchholz et al., 2006; Das et al., 2009). Unliganded TRs are bound to corepressor complexes, which inhibit gene expression (Buchholz et al., 2003; Sato et al., 2007). Whereas, when T3 and T4 are bound to TRs they recruit co-activators, which can alter the expression of genes regulated by thyroid hormone (Paul et al., 2005). T3 and T4 are known to upregulate and downregulate hundreds of genes in target tissues of amphibians during metamorphosis (Brown et al., 1996; Denver et al., 1997; Das et al., 2006, 2009; Page et al., 2008, 2009). Among these, TRs are autoregulated by T3 and T4 and, at least for TRβ, this upregulation is direct (Machuca and Tata, 1992; Ranjan et al., 1994; Das et al., 2009). In other words, T3 and T4 can further accentuate their effects by producing more...
receptors for the hormone to bind. Dominant negative TRβ transgenic tadpoles do not metamorphose (Buchholz et al., 2004), which further supports the necessity of TH and functional TRs. The expression patterns of TRs are related to metamorphic timing and life histories in Spadefoot Toads (Hollar et al., 2011) and Oklahoma Salamanders (Aran et al., 2014; discussed more below).

It should also be noted that TH activity can be altered in target tissue via deiodinases, which are a class of enzymes that can add or remove iodine to produce more or less active forms of the hormone (Becker et al., 1997; Brown, 2005; St. Germain et al., 2009). For example, type-2 deiodinase converts T4 into the more active T3 with the removal of an iodine atom. The transcriptional actions of TH and associated regulatory mechanisms ultimately govern tissue transformation and metamorphosis of larval amphibians.

Corticosteroids and interactions with thyroid hormone.—Corticosteroids are regulated through the hypothalamic-pituitary-interrenal (HPI) axis and are important mediators of “environmental stress” in vertebrates (Denver, 2009, 2013; Carr, 2010; Crespi et al., 2013; Fig. 2). CRF from the hypothalamus regulates adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary, and ACTH stimulates the production and release of corticosteroids (glucocorticoids and mineralocorticoids) from the interrenal glands. Corticosteroids can regulate transcription in target tissues by binding to corticoid receptor (CRs, including GRs or MRs) or thyroid hormone receptors (TRs). Sufficient increases in these hormones can drive the transformation of target tissues and metamorphosis, as depicted here with Eurycea tynerensis (see text for details).

**Fig. 2.** Consensus of general endocrine pathways for amphibian metamorphosis via the HPT and HPI axes. The pathway depicts Corticotropin Releasing Factor (CRF) from the hypothalamus as the regulator of both Adrenocorticotropic Hormone (ACTH) and Thyroid Stimulating Hormone (TSH) from the pituitary. These pituitary hormones stimulate the release of corticosteroids from the adrenocortical cells of the interrenal gland, and T₃ and T₄ from the thyroid gland, respectively. Gene expression is regulated in target tissues (e.g., tailfin) by these hormones through binding to their respective nuclear receptors: corticoid receptors (CRs, including GRs or MRs) or thyroid hormone receptors (TRs). Sufficient increases in these hormones can drive the transformation of target tissues and metamorphosis, as depicted here with Eurycea tynerensis (see text for details).
Middlemis Maher et al., 2013). However, during amphibian metamorphosis, corticosteroids increase endogenously (Krug et al., 1983; Carr and Norris, 1988; Denver et al., 1998; Chambers et al., 2011) and have been shown to alter the effects of thyroid hormone on morphogenic changes (Frieden and Naile, 1955; Kobayashi, 1958; Kikuyama et al., 1983; Kühn et al., 2004; Bonett et al., 2010). Most importantly, there is significant cross talk between the HPI and HPT axes at both the neuroendocrine level and in target tissues. For example, during metamorphosis the anterior pituitary expresses CRF type 2 receptor, which allows hypothalamic CRF to directly stimulate the release of TSH (Manzon and Denver, 2004; Okada et al., 2007; see also above). In target tissues, corticosteroids can enhance the effects of thyroid hormone through several avenues. Transcriptional regulatory mechanisms driven by corticosteroids include the regulation of genes that code for thyroid hormone converting enzymes (e.g., type-2 deiodinase; Bonett et al., 2010) or for transcription factors that upregulate thyroid hormone receptor expression (e.g., Krüppel-like factor 9, KLF9, formerly known as BTEB1; Bagamasbad et al., 2008; Bonett et al., 2009). More complex interactions between corticosteroids and thyroid hormone occur at the level of gene regulation, including additive, synergistic, and inhibitory effects (Bonett et al., 2010; Kulkarni and Buchholz, 2012). For example, corticosteroids and thyroid hormone have greater than additive (synergistic) effects on transcription of thyroid hormone receptors, deiodinases, and KLF9 (Kühn et al., 2005; Bonett et al., 2010; Kulkarni and Buchholz, 2012; Bagamasbad et al., 2015). These regulatory effects may provide a link between corticosteroids and the rapid morphogenic changes in late stage larvae, which could expedite their departure from a “stressful” larval environment such as a crowded, drying pond.

Reproductive hormones.—Understanding the timing of reproductive development is critical for reconstructing the evolution of maturation with respect to other events (e.g., metamorphosis) and also because reproductive hormones can both stimulate and inhibit other endocrine pathways. It also is a pivoting point for shifting resource allocation between growth/morphogenesis and reproduction (Steams, 1992).

Sex steroids (estrogens and androgens) can function in a similar manner as corticosteroids, by binding to nuclear transcription factors (estrogen and androgen receptors) to regulate gene transcription. There is also significant crosstalk between the hypothalamic-pituitary-gonadal (HPG) axis and HPT axes. This topic has been extensively reviewed (Hayes, 1997; Duarte-Guterman et al., 2014), and most research has focused on the effects of endocrine disruptors on reproductive development and sex ratios. The most relevant aspects for this review are the influence of thyroid hormone on gonadal development (Flood et al., 2013) and sex steroids on the inhibition of metamorphosis. Thyroid-less and thyroid-suppressed (goitrogen treated) tadpoles and larval salamanders can still develop gonadal germ cells (Allen, 1918; Wakahara, 1999; Yamaguchi et al., 1996; Kanki and Wakahara, 1999; Rot-nikickeic and Wassersug, 2004), and this may be mediated through an increase in TSH (Kanki and Wakahara, 1999). The ability of larval salamanders to develop gonads without an increase of TH (which could stimulate metamorphosis) provides the potential for larval form paedomorphism. At the same time, both estrogens and androgens can inhibit tadpole metamorphosis (Gray and Janssens, 1990; Hogan et al., 2008), but data for salamanders are limited. If this mechanism is common among all amphibians, then the acceleration of maturation and the production of sex steroids into larval stages could permanently displace metamorphosis (Ryan and Semlitsch, 1998).

ENDOCRINE BASIS OF HETEROCHRONY IN PLETHODONTIDS AND OTHER SALAMANDERS

Shifts in the timing of developmental events (heterochrony) such as hatching, metamorphosis, and maturation have occurred extensively among salamanders and probably most extremely among plethodontids. These have resulted in three major developmental-based life histories: direct development, biphasic, and paedomorphic. Studies that examine the endocrine basis of these developmental patterns in plethodontids and other amphibians are discussed immediately below.

**Direct development.**—Direct development is widespread in plethodontids and has been considered an important contributor to their diversification, especially in the neotropics (Wake and Hanken, 1996). Direct development is likely the ancestral condition for the family and has been maintained in most major lineages except for spelerpines, one clade of desmognathines, and *Hemidactylium* (Bonett et al., 2014b; Fig. 1). The molecular mechanisms that underlie direct development are compelling, because somatic morphogenesis is accelerated so rapidly, and at such a small body size, that metamorphosis occurs completely within the egg (Dent, 1942; Wake and Hanken, 1996; Kerney et al., 2012; Bonett et al., 2014b). Surprisingly, there have been only a few molecular endocrine studies on direct developing plethodontids, although there is additional information that can be derived from the many studies on direct developing frogs.

Dent (1942) proposed, and partially tested, thyroid hormone-based mechanisms to explain direct development in plethodontids. He proposed that direct developers either had precociously developed pituitary and thyroid glands (to trigger early metamorphosis) or that the tissues of direct developers had an increased sensitivity to thyroid hormone. Histological and morphological examination of *Plethodon cinereus* showed that the pituitary and thyroid gland develop at relatively early embryonic stages (Dent, 1942). Furthermore, he observed morphological changes to the thyroid gland that signified secretory activity, and were coincident with several morphogenic changes such as gill resorption. These changes in embryos of *P. cinereus* (between stages XXII and XXIII) are similar to those that occur during early metamorphosis of *Europyce bislineata*, which have a multi-year larval period (Wilder, 1925; Dent, 1942).

The mechanisms of direct development in the frog *Eleutherodactylus coqui* have been investigated much more extensively (reviewed in Elinson, 2013), and this species shares some important similarities with *P. cinereus*. For example, *E. coqui* also develop their thyroid system embryonically, well before species with free-living tadpoles, and peaks of thyroid activity are associated with metamorphic changes (Hanken et al., 1997; Jennings and Hanken, 1998). CRF is important for metamorphosis in *E. coqui* and likely operates by directly stimulating TH release from the thyroid gland (Kulkarni et al., 2010; Elinson, 2013). However, while treatment of embryos of *E. coqui* with the goitrogen methimazole prevents complete metamorphosis, many frog-
let features still develop (Callery and Elinson, 2000). This suggests that either thyroid hormone is provided maternally in yolk, or other hormones (such as corticosterone) are driving metamorphosis. The potential influence of corticosteroids on metamorphosis of direct developing amphibians makes sense from a phylogenetic perspective, because all direct developing lineages were originally derived from biphastic ancestors, which likely had plastic larval periods. This may also explain the variation in metamorphic timing of biphastic species such as Desmognathus quadramaculatus that typically have an aquatic larval period of several months (Danstedt, 1975) but can complete metamorphosis after less than one month when raised strictly on wet surfaces without free-standing water (Noble and Evans, 1932; see also Marks and Collazo, 1998 for further discussion about D. aeneus). This shows that some biphastic plethodontids can effectively exhibit direct development under some conditions.

Our experiments on hatching Eurycea tynerensis, a species that has minimally a several month larval period (or larval form paedomorphosis), showed that treatment with exogenous thyroid hormone upregulates TRβ in tail tissue and significantly advances nasal capsule development. Exogenous corticosterone alone has no gross developmental effects, but in combination with thyroid hormone has strong synergistic effects on TRβ expression and nasal capsule development (Jackson et al., unpubl.). However, other tissues that normally transform during metamorphosis of Eurycea, such as the hyobranchial apparatus, do not seem to be sensitive to thyroid hormone or corticosterone treatment at such early stages of development (Jackson et al., unpubl.). As with direct developing frogs (Elinson, 2013), this indicates that other hormones may be necessary for the transformation of some tissues, or local differences in tissue sensitivity determine whether or not metamorphosis can be completed prior to hatching.

**Biphastic.**—Biphastic life histories occur in spelerpines, one clade of desmognathines, and Hemidactylium (Fig. 1). Perhaps the most interesting aspect of biphastic life cycles of plethodontids is that they are likely independently derived from direct developing ancestors (Chippindale et al., 2004; Bonett et al., 2014b; Fig. 1). This suggests that not only was there potentially a major endocrine-based acceleration of larval development in ancestral plethodontids (or earlier) to achieve direct development, but there may have been multiple endocrine-based decelerations in the “re-evolution” of larval forms in desmognathines, spelerpines, and Hemidactylium. If these are indeed independent reversals to freeliving aquatic larvae, then their origins may be based on different mechanisms. These may have involved delays in the development of the HPT axis or decreasing sensitivity of tissues to thyroid hormone and/or other endocrine signals.

Larvae of biphastic species/populations of spelerpines have been transformed using both T3 and T4 (Alberch et al., 1985; Rose, 1995a, 1995b, 1996; Aran et al., 2014). Hormone dosage and the age/age of larvae influence the timing of transformation of skeletal elements (Rose, 1995a, 1995b). Treatment of larvae from multiple metamorphic populations of E. tynerensis with exogenous T3 upregulates TRα and TRβ expression in tailfin tissue (Aran et al., 2014).

Hickerson et al. (2005) found that metamorphic progress of first year larvae of Desmognathus quadramaculatus was not influenced by a low dose of T4 (up to 4.8 nM) administered over approximately two months. We found that similar doses (1 to 5 nM) of the more active hormone T3 were highly influential on metamorphic changes of larval D. ocoee, D. santeetlah, and D. trimleyorum over periods of three to five months (Robison et al., unpubl.).

Alberch et al. (1986) performed radioimmunoassays for T3 and T4 on a series of E. bislineata sampled from across metamorphosis. They found the common pattern of increased concentrations of plasma T3 and T4 in metamorphic individuals, but neither hormone was detectable in some specimens that were clearly in the process of metamorphosis. This suggested that hormone levels may fluctuate during metamorphosis, or that other hormones are used in the process.

The lengths of larval periods of biphastic plethodontids vary extensively among and within species (Petranka, 1998; AmphibiaWeb, 2015). Several ecological studies have shown that factors such as temperature, hydroperiod, and stream order can further influence metamorphic timing (Voss, 1993; Beachy, 1995; Camp et al., 2000; Freeman and Bruce, 2001; Bruce, 2005; Hickerson et al., 2005) but food availability does not (e.g., O’Laughlin and Harris, 2000; Hickerson et al., 2005). While variation and plasticity indicate that endocrine processes play a major role in regulating metamorphic timing (Rose, 2005; Denver, 2009, 2013; Crespi et al., 2013), there have been relatively few published studies that simultaneously evaluate the effects of environmental and endocrine factors on biphastic plethodontids (Hickerson et al., 2005).

Temperature is known to influence the rate of larval growth, development, and the timing of metamorphosis of plethodontids, with low temperatures delaying metamorphosis (Hickerson et al., 2005). An important endocrine facet of this effect, which has been generally underexplored in salamanders, is the potential for low temperatures to inhibit TH-induced metamorphosis, as seen in studies with larvae of Hynobius (Moriya, 1983). Integrative studies are needed to assess how environmental factors and natural stressors influence TH regulated gene expression programs to control metamorphic timing of biphastic plethodontids.

**Larval form paedomorphosis.**—Shifts from biphastic to larval form paedomorphic developmental modes have occurred many times in salamanders, and nine of the ten salamander families include larval form paedomorphic species. In plethodontids, larval form paedomorphosis is restricted to several independent instances in the Spelerpini, which are associated with aquatic subterranean habitats and arid climate regimes (Bonett et al., 2014a; Fig. 1). These include isolated instances of paedomorphosis in primarily metamorphic clades (e.g., E. cirtigera; McEntire et al., 2014), clades with highly variable life histories among populations (e.g., E. tynerensis, Bonett and Chippindale, 2004, 2006; Emel and Bonett, 2011; Gyrinophilus, Niemiller et al., 2008), divergent paedomorphic lineages (e.g., E. subfluvicola; Steffen et al., 2014), and primarily paedomorphic clades (Edwards Plateau Eurycea; Chippindale et al., 2000). While most instances of paedomorphosis are derived, there is strong phylogenetic support that life history is reversible in spelerpines. In particular, the Edwards Plateau clade of Eurycea likely includes a derived instance of metamorphosis after several million years of paedomorphosis (Bonett et al., 2014a, 2014b).

Obligately paedomorphic salamanders, which never naturally metamorphose and also do not completely transform with thyroid hormone treatment, include all species in the families Amphiumidae (Kobayashi and Gorbman, 1962), Cryptobranchidae (Noble and Farris, 1929), Proteidae (Swin-
thyroid gland functioned normally, but they exhibited lower
by adult paedomorphic
paedomorphic and metamorphic plethodonids have not
within a very narrow geographic area in the western Ozark
E. tynerensis
may occur among populations of
facultative and obligate paedomorphosis
sensitivity in some populations (Aran et al., 2014). This
tions, with respect to both thyroid hormone receptor (TR
Furthermore, larvae from paedomorphic populations also
variants appear to be a primary determinant of metamor-
Approximately half of the populations in the western clade of Eurycea tynerensis are paedomorphic (Emel and Bonett, 2011). Most populations include only a single life history mode, and larval form paedomorphosis is associated with chert streamed substrate (Bonett and Chippindale, 2006; Emel and Bonett, 2011), which permits access to stable, subsurface aquatic environments (Treglia et al., unpubl.). There are a relatively small number of populations of E. tynerensis that are known to exhibit both paedomorphosis and metamorphosis. A few of the variable populations are facultatively paedomorphic, based on the fact that paedo-
morphic females layed fertilized eggs in the lab, but then completed metamorphosis in subsequent years. By compar-
ison, other variable populations are composed of sympatric, but genetically distinct, paedomorphic and metamorphic populations (Bonett et al., unpubl.). In addition, some paedomorphic populations of E. tynerensis have been main-
tained in the lab (at the University of Tulsa) for several years
without spontaneous metamorphosis, whereas larvae from metamorphic populations raised under the same conditions always metamorphose eventually. These observations suggest
that variability in metamorphic timing among populations is
not completely driven by environment. Larval E. tynerensis from metamorphic populations are more sensitive to exog-
ous T3 treatment than larvae from paedomorphic popu-
lations, with respect to both thyroid hormone receptor (TRα or TRβ) upregulation and metamorphosis (Aran et al., 2014).
Furthermore, larvae from paedomorphic populations also
differ in their sensitivity to T3, with extremely reduced
sensitivity in some populations (Aran et al., 2014). This
indicates that both facultative and obligate paedomorphosis
may occur among populations of E. tynerensis occurring
within a very narrow geographic area in the western Ozark Plateau.

Direct comparisons of circulating hormone levels between
paedomorphic and metamorphic plethodonids have not
been conducted. Analyses of the assimilation of radioiodine
by adult paedomorphic E. tynerensis showed that their
thyroid gland functioned normally, but they exhibited lower
levels of thyroid activity compared to other vertebrates (Dundee and Gorbman, 1960). Using similar methods, Dent
and Kirby-Smith (1963) found that paedomorphic Gyrtinophi-
lus puleucus exhibited relatively low levels of thyroid activity,
but activity was considerably higher in individuals that
showed signs of spontaneous metamorphosis. These studies
documented general patterns of vertebrate endocrinology,
but it is difficult to evaluate their evolutionary significance
without direct comparisons to related metamorphic species.

INTEGRATIVE ENDOCRINE MODEL FOR SALAMANDER LIFE HISTORY EVOLUTION

The extensive variation in metamorphic timing among amphibians and the pervasive influence of thyroid hormone,
as well as changes in the thyroid gland itself, lead several
investigators to suggest that variations in the HPT axis may
be a driver of heterochronic evolution (Lynn, 1936, 1942;
Dent, 1942, 1968; Rose, 1995a, 1995b, 1996; Hanken et al.,
1997; Elinson, 2013; Johnson and Voss, 2013). Most recently,
Johnson and Voss (2013) developed a salamander life history
model (based largely on Ambystoma), which synthesized
commonly identified differences associated with paedomor-
phosis versus metamorphosis. This model showed that
compared to metamorphosis, paedomorphosis is associated
with greater habitat stability, lower TH responsiveness, and
larger body size (Johnson and Voss, 2013).

Here I provide an extension to previous TH-based life
history models by incorporating additional endocrine and
developmental processes that influence plasticity of meta-
orphic timing and maturation (Fig. 3). This integrated
model details where the three primary salamander life
history categories (direct development, biphasic, and paedo-
mporphosis) occur in this spectrum and shows how plasticity
can result in variation in their expression. It also shows how
similar life histories can be derived from different endocrine
and developmental processes. The model can be used as a
framework for both comparative endocrine analyses and
endocrine manipulation experiments.

Both TH responsiveness and the timing/amount of endogenous TH release can influence the timing of meta-
 morphosis; therefore, I considered both processes in this
model (Fig. 3A). Timing of metamorphosis is generally
positively correlated with TH release and negatively correlat-
ed with TH responsiveness. In other words, early TH release and high TH responsiveness are related to early metamor-
phosis (e.g., direct development), and late TH release and low
TH responsiveness are related to late metamorphosis (e.g.,
long larval periods and paedomorphosis). The evolution of
genetic-based differences in “baseline” TH responsiveness/
release could move a lineage along the diagonal between
direct development and paedomorphosis, presumably always
passing through a biphasic life history.

At the same time, many other factors such as environ-
mental stressors, temperature, and reproductive maturity
described above) can significantly influence the timing of
amphibian metamorphosis for a given genotype (or species).
These factors typically work through molecular endocrine
processes to influence metamorphic timing, but the magni-
tude and direction of these effects can vary across species, life
histories, and stages (described above). Nevertheless, these
effects, individually or in combination, can push metamor-
photic timing to its theoretical minimum or maximum for a
given genotype. I depict this plasticity along the “baseline”
TH diagonal of the model, and show how plasticity of a given genotype could result in a life history shift (Fig. 3A).

The model also shows two primary events that transect the ontogeny of nearly all salamanders: hatching from an oviparous egg and maturation. Both of these events likely have “baselines” for a given genotype (or species), but may also be influenced by plasticity. Evolutionary or plasticity-based accelerations or decelerations of hatching and maturation could also result in major life history shifts (Fig. 3B, C).

The following describes pathways to transitions between major life history categories, as well as permanent transitions to paedomorphosis (obligate paedomorphosis) and the lack of transitions in some clades.

Transitions between direct development and biphasic.—When metamorphosis is temporally proximal to hatching, then genetic changes to baseline TH responsiveness/release or plasticity effects could shift a lineage from direct development to biphasic or vice versa through accelerations or decelerations (Fig. 3A) in metamorphic timing. Also, genetic or plasticity effects that cause hatching time to shift before (Fig. 3B) or after (Fig. 3C) metamorphosis could also result in transitions between these life histories.

Transitions between biphasic and paedomorphic.—When metamorphosis is proximal to maturation, genetic changes to baseline TH responsiveness/release or plasticity effects could shift a lineage from biphasic to facultatively paedomorphic, or the reverse. The transitions could occur through deceleration (neoteny) or acceleration of metamorphic timing (somatic morphogenesis). These transitions can also be achieved by acceleration (progenesis; Fig. 3B) or deceleration (Fig. 3C) of maturation relative to metamorphosis (Alberch et al., 1979). Both mechanisms have been demonstrated in salamanders, sometimes even within a single species (Denoël and Joly, 2000). The only phylogenetic test of these alternatives (neoteny vs. progenesis) was performed on Edwards Plateau Eurycea, where paedomorphosis was likely derived through neoteny (Bonett et al., 2014b). However, given the extensive crosstalk between endocrine pathways, these processes may not be completely independent in all cases. For example, the loss of thyroid function should cause a truncation of somatic development (neoteny), but may simultaneously speed up reproductive development (progenesis) as shown in frogs and salamanders (Gray and Janssens, 1990; Wakahara, 1994; Hogan et al., 2008; see discussion of reproductive hormones above).

Transitions to obligate paedomorphosis.—Obligate paedomorphs are likely derived from facultatively paedomorphic ancestors, but are instead infinitely paedomorphic (Fig. 3). They present an unusual case and may completely deviate from the model in several ways. Once TH is decoupled from tissue transformation, the level or timing of TH release does not need to be low or late to prevent metamorphosis. In other words, in terms of transformation, the TH responsiveness of the decoupled tissues (e.g., external gills of Necturus; Safi et al., 2006; Vlaeminck-Guillem et al., 2006) will be nonexistent, so the level and ontogenetic release time of circulating TH could fall anywhere on the spectrum compared to other salamander life histories. It should be noted that some, but not all, tissues remain responsive in some
obligate paedomorphs, such as *E. rathbuni* and *E. wallacei* (Dundee, 1957, 1962), so the model should be specified as a reference to either metamorphic timing of a particular tissue or a whole animal. Another unique attribute of obligate paedomorphosis is that since metamorphosis never occurs, environmental effects (e.g., via “stress hormones”) on the non-responsive tissues should also be nonexistent. Environmental factors could, however, still affect tissues that metamorphose, such as the external gills of *Amphiuma* that resorb just after hatching.

**Lack of certain transitions in some clades.**—Larval form paedomorphosis evolved several times among the spelerpines, but is completely unknown for some spelerpine clades, as well as *Desmognathus* and *Hemidactylium* (Fig. 1). This is surprising given that many of these biphilic clades have larvae that develop in permanently aquatic habitats. Considering the origins of their biphilic life histories in the context of the life history model presented here may provide some clarity. Phylogenetic reconstructions show that biphilic *Desmognathus* and *Hemidactylium* (as well as spelerpines) are independently derived from direct developing ancestors (Chippindale et al., 2004; Bonett et al., 2014b). This means that they were derived from ancestors with highly accelerated metamorphic timing, and even though they currently exhibit free-living larval forms, the ontogenetic timing between metamorphosis and maturation may be too long to be bypassed through plasticity of either event. For example, *Hemidactylium* and many *Desmognathus* have larval periods of less than several months, but take minimally two years to reach maturation, so plasticity in these events would have to be collectively shifted by more than a year for maturation to precede metamorphosis. It is also possible that a developmental mechanism prevents maturation from occurring without metamorphosis, such as in frogs. The *Desmognathus quadramaculatus/marmoratus* clade is a unique example that may be an informative test case. These species have long larval periods (two to three years) and minimally mature one year later, but always metamorphose even though *D. marmoratus* remain permanently aquatic as adults. Therefore, *D. marmoratus* accomplishes “ecological paedomorphosis” by retaining their aquatic larval ecology without a complete truncation of somatic development.

**Requirements for synthesis and future research**

Much of the data on the timing of hatching, metamorphosis, and maturation for plethodontids was collected from the 1950s through the 1980s. However, our knowledge of phylogenetic relationships and species diversity were still rudimentary at the time, limiting evolutionary interpretations of life history data. Tremendous progress has been made on plethodontid phylogeny and species diversity over the last decade, but now much more life history data are needed to make accurate and comprehensive estimates of the evolution of developmental timing. An added complication is the potential effect of plasticity in developmental timing, which must be separated from genetic-based, geographic variation. Therefore, measurements of salamanders raised under common laboratory conditions are required to obtain baseline estimates of developmental timing.

Several plethodontids have been treated with T3 or T4 (Kezer, 1952; Dundee, 1957, 1962; Dent and Kirby-Smith, 1963; Rose, 1996; Hickerson et al., 2005; Aran et al., 2014), but more experiments are needed that include a common dose and duration to make comparative evaluations of sensitivity. There have been relatively few analyses of thyroid gland activity (Dent, 1942; Dent and Lynn, 1958; Dundee and Gorbman, 1960; Dent and Kirby-Smith, 1963), and no studies have directly compared related species that exhibit different life histories. Also, there has been little published data on transcriptional differences among plethodontid life histories (Aran et al., 2014; Jackson et al., unpubl.). Such studies are critical for establishing endocrine correlates for metamorphic timing (e.g., TH responsiveness and TH release).

Similarly, several studies have examined a diversity of environmental effects on metamorphic timing of different species (Noble and Evans, 1932; Voss, 1993; Beachy, 1995; Camp et al., 2000; Freeman and Bruce, 2001; Hickerson et al., 2005), but there is very little data on the effects of stress hormones on metamorphic timing of plethodontids. Studies are needed to systematically compare individual factors among species to establish the degree of the variance around baseline metamorphic timing. In particular, it will be informative to test species with baseline metamorphic timing (or TH sensitivity) that are near the transitions of life history categories. This will help to establish the degree of plasticity required for major life history transitions and determine whether some taxa are unable to make such transitions.

Finally, molecular endocrine experiments that examine the ontogeny of hormone release and the effects of hormones on transcription in target tissues are needed to determine whether patterns of laboratory models are generalizable across plethodontids. It will also ultimately permit phylogenetic tests of how the evolution of endocrine pathways have influenced plethodontid life histories.

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