

Repeated ecological and life cycle transitions make salamanders an ideal model for evolution and development

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Abstract

Observations on the ontogeny and diversity of salamanders provided some of the earliest evidence that shifts in developmental trajectories have made a substantial contribution to the evolution of animal forms. Since the dawn of evo-devo there have been major advances in understanding developmental mechanisms, phylogenetic relationships, evolutionary models, and an appreciation for the impact of ecology on patterns of development (eco-evo-devo). Molecular phylogenetic analyses have converged on strong support for the majority of branches in the Salamander Tree of Life, which includes 764 described species. Ancestral reconstructions reveal repeated transitions between life cycle modes and ecologies. The salamander fossil record is scant, but key Mesozoic species support the antiquity of life cycle transitions in some families. Colonization of diverse habitats has promoted phenotypic diversification and sometimes convergence when similar environments have been independently invaded. However, unrelated lineages may follow different developmental pathways to arrive at convergent phenotypes. This article summarizes ecological and endocrine-based causes of life cycle transitions in salamanders, as well as consequences to body size, genome size, and skeletal structure. Salamanders offer a rich source of comparisons for understanding how the evolution of developmental patterns has led to phenotypic diversification following shifts to new adaptive zones.

KEYWORDS

adaptive zones, biphasic, direct development, metamorphosis, paedomorphosis, traits

1 | INTRODUCTION

Organisms that exhibit repeated transitions between environments and life cycles provide an opportunity for investigating developmental novelty and diversity. Despite retaining many basic tetrapod features, salamanders have explored a wide range of niches resulting in many highly aberrant traits.¹⁻⁴ In some cases, repeated invasions of the same environment have resulted in

phenotypic convergence.^{2,4-6} However, the degree of morphological convergence varies as well as the developmental pathway to arrive at a given outcome. There are multiple levels of inquiry for understanding the potentially great diversity of developmental mechanisms in salamanders including:

1. How do fundamental mechanisms of salamander development work?

2. How do mechanisms change (both genetically and plastically) to give rise to novel phenotypes in response to colonizing new environments?
3. Do repeat ecological invasions that produce similar phenotypes result from the same developmental mechanisms (developmental convergence)?
4. What factors promote or constrain developmental diversification in different ecological settings across life stages?

Salamanders exhibit many traits that vary with respect to developmental timing (e.g., heterochrony).⁷⁻⁹ These include shifts in the timing of hatching, growth, metamorphosis, and maturation. Therefore, life cycle shifts, which can result from different mechanisms (causes), are themselves examples of developmental diversification. Once a lineage has shifted to a new adaptive zone (e.g., aquatic to terrestrial) it is then subject to different selective pressures that favor developmental alterations to other traits (consequences).

Research on classical laboratory model amphibian species such as African Clawed Frogs (*Xenopus*),¹⁰⁻¹² the Tiger Salamander complex (*Ambystoma tigrinum* and *mexicanum*),¹²⁻¹⁴ and several genera of newts (*Cynops*, *Notophthalmus*, and *Pleurodeles*)¹⁵⁻¹⁹ have provided important insights into fundamental developmental mechanisms that can inform our understanding of wild species. However, patterns and structures altered by the repeated ecological and life cycle transitions modes may harbor a wealth of mechanistic nuance. The solution, of course, is to expand the number of representative species studied developmentally in the laboratory.²⁰

Here we describe transitions in life cycle and ecology across the history of salamanders that likely promote developmental diversification. We further discuss multiple axes of endocrine regulation that are likely causes of life cycle transitions as well as interrelated consequences to body size, genome size, and the skeleton. Salamanders provide a promising source for exploring developmental diversity, and an ideal framework for testing the relationships between ecology, evolution, and development.

1.1 | Life cycle evolution

Salamanders have undergone repeated transitions in life cycle modes to match the invasion of diverse environments (Figure 1). A *biphasic* life cycle with an aquatic larval stage followed by metamorphosis into a more terrestrial form is phylogenetically widespread among salamanders.²⁷ This life cycle mode facilitates the utilization of alternative resources and particularly temporary aquatic habitats.²⁸⁻³¹ However, there have been two

significant developmental deviations, each of which coincide with life cycle simplifications that occur in stable habitats. At least some species in nine of the 10 salamander families exhibit *larval form paedomorphosis*, whereby adults retain a primarily aquatic lifestyle and some aquatic larval traits, specifically a larval gill structure.^{27,31} In some species, larval form paedomorphosis is obligate, meaning individuals do not naturally metamorphose; at least completely. This is most evident in four salamander families (Amphiumidae, Cryptobranchidae, Proteidae, and Sirenidae), which include only obligately paedomorphic species. These families appear to have independently shifted to larval form paedomorphosis in the Mesozoic (Figure 1).^{32,33} The family Plethodontidae also includes many independently derived paedomorphic lineages.³⁴ Paedomorphosis can also be facultative, where developmental plasticity dictates whether an individual reproduces in its “larval form” or metamorphoses into a terrestrial phenotype.^{9,35} Facultative paedomorphosis is most common in the families Salamandridae, Ambystomatidae, and Plethodontidae, although the latter two families also include obligately paedomorphic species.⁹

At the opposite extreme is *direct development* where pre-metamorphic development is completed inside of the egg.³⁶ This is the most common mode of development in the largest family of salamanders (Plethodontidae) and appears to have permitted their diversification into a wide range of terrestrial habitats, particularly in the neotropics.³⁷ Ancestral state reconstructions show that direct development is most likely the ancestral mode for this family, but this requires two or three independent reversals to a biphasic life cycle.^{4,38} A phylogenetically more restricted, but nonetheless intriguing developmental pattern is *viviparity*, which occurs in a clade of salamandrids (genera *Lyciasalamandra* and *Salamandra*). This involves the retention of developing embryos in the mother until “live birth” of free-living offspring.³⁹⁻⁴² The retention, duration, and developmental rates vary among species and populations leading to the birth of aquatic larvae (*larviparity*) or fully metamorphosed terrestrial juveniles (*pueriparity*). Ecologically, the former mode is similar to biphasy and the latter to direct development.

1.2 | Ecological evolution

The life cycle modes described above coarsely match with major transitions between aquatic-to-terrestrial, completely-aquatic, and completely-terrestrial ecologies. Salamanders occur in a wide range of aquatic habitats. Some species only inhabit water for part of their life cycle such as when breeding or during larval development, while others remain aquatic across ontogeny. In general, more environmentally stable aquatic environments promote larval form paedomorphosis.^{43,44} These include

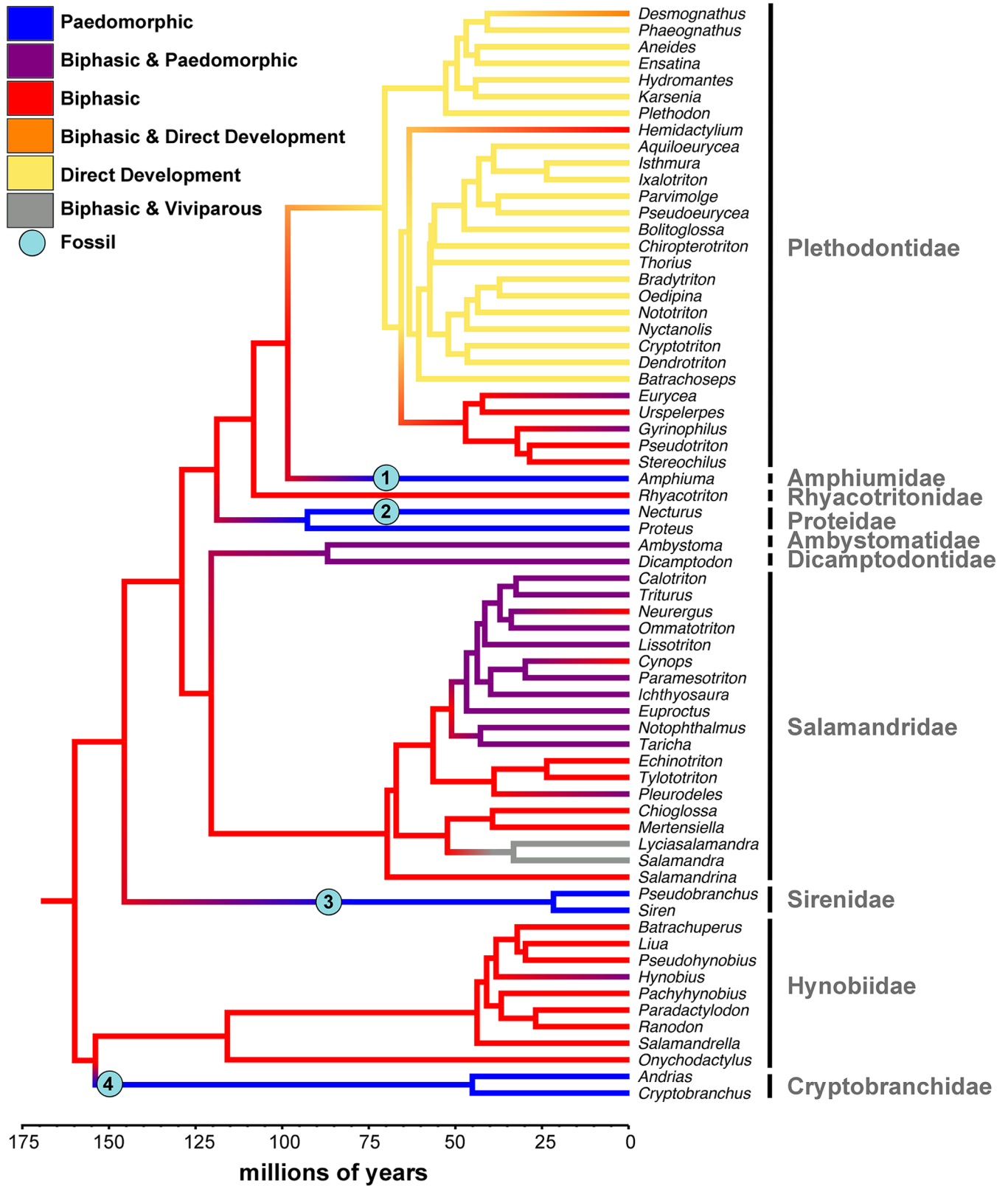


FIGURE 1 Time calibrated phylogeny of salamanders⁴ collapsed to 66 named genera. Major relationships are also supported by several studies.^{21,22} Life cycle categories are reconstructed to generic level variation. Genera with variable life cycles include some biphasic species with either facultative or obligate paedomorphs, direct developers, or viviparous species. Fossils indicate minimum origin for paedomorphosis in several obligately paedomorphic lineages: (1) *Proamphiuma cretacea* (~70 MYA)²³; (2) *Paranecturus garbanii* (~70 MYA)²⁴; (3) *Habrosaurus prodilatus* (~85 MYA)²⁵; *Chunerpeton tianyiensis* (~150 MYA)²⁶

permanent ponds and lakes,⁴⁵ subterranean aquifers,³⁴ permanent mountain streams, and springs with continued access to groundwater.^{46,47} This is not always the case though, particularly in ponds that can intermittently dry up.^{48,49} Also, many species of biphasic salamanders and newts develop in permanent, seemingly stable, water bodies but still metamorphose and move onto land, with some dispersing to other water bodies.⁵⁰ There are few species of biphasic salamanders and newts that metamorphose but remain completely aquatic.^{51,52} At the population and individual levels, environmental circumstances that necessitate remaining in the water may be common, but not persistent enough to drive a life cycle shift. While obligately paedomorphic lineages are primarily aquatic, some species can intermittently traverse land and aestivate out of water.^{53,54}

Adults of biphasic species often live under rocks, logs, or underground outside of their aquatic breeding habitats.⁵⁵ In long-term aquatic breeders such as newts, the active part of life may be, in some cases, mostly in the aquatic habitat due to environmental constraints on land.⁵⁶ In contrast, being freed from a need to spend part of their larval life in water, direct-developing species have diversified extensively into a variety of terrestrial microhabitats, particularly in the neotropics.³⁷ These radiations range from highly subterranean to arboreal species, with microhabitats invaded multiple times by divergent lineages.^{34,57,58}

2 | DEVELOPMENTAL CAUSES OF LIFE CYCLE TRANSITIONS

Reproductive and somatic development are partially decoupled in salamanders, so they are able to shift in timing somewhat independently. This permits maturation while maintaining a larval form,⁸ and also means there are multiple heterochronic pathways to arrive at paedomorphosis.⁵⁹⁻⁶¹ Through somatic alterations salamanders can achieve larval form paedomorphosis by slowing their rate of larval development (*neoteny*) or delaying metamorphosis (*postdisplacement*). Alternatively, through gonadal alteration salamanders can reproduce earlier in a paedomorphic state (*progenesis*; a.k.a. *hypomorphosis*).^{60,62} Phylogenetic reconstruction of reproductive timing and metamorphosis for at least one radiation of *Eurycea* from the Edwards Plateau of Central Texas shows that the evolution of paedomorphosis results from neoteny.³⁸ Whereas mesocosm experiments of Mole Salamanders (*Ambystoma talpoideum*) show that paedomorphosis can be the result of early reproduction.⁸ Through skeletochronology it has been demonstrated that both progenesis and neoteny can occur within a single species of Alpine Newt (*Ichthyosaura alpestris*), which

shows the lability of these pathways.⁶³ There are also potentially multiple pathways to direct development.⁶⁴

The release of systemic endocrine signals can permit coordinated transformation of diverse tissues throughout the organism. This is in part how hormones determine vertebrate life stages, by regulating growth, metamorphosis, and reproduction.⁶⁵ Environmental and endogenous signals are mediated through the hypothalamus and pituitary to stimulate the thyroid gland (HPT axis), interrenal glands (HPI axis), and gonads (HPG axis). These systems have been studied in great detail over the last century, particularly as they relate to amphibian metamorphosis and maturation. However, much of the research has focused on laboratory model species (*Xenopus* and *Ambystoma tigrinum* and *mexicanum*).¹² In recent years there have been many detailed reviews on the endocrinology of amphibian metamorphosis and reproduction.^{64,66-69} Here we more generally discuss these systems in reference to their potential responsibility for major life cycle changes as well as their intimate interconnectivity.

2.1 | Thyroid hormone axis

The thyroid gland is nested in the throat and produces an essential hormone for vertebrate development, thyroid hormone (TH). Treatment of mid to late-stage tadpoles and larval salamanders with thyroid hormone can initiate precocial metamorphosis,^{70,71} and thyroid-ectomies can prevent transformation of frog tadpoles.⁷²⁻⁷⁵ Furthermore, the timing of appearance and size of the thyroid gland appears to be related to amphibian metamorphosis.⁷⁶⁻⁷⁸ The active variants of thyroid hormone, T₄ (thyroxine) and the more potent T₃ (3,3',5-triiodothyronine), increase in circulation during amphibian metamorphosis.⁷⁹⁻⁸² Inside of target tissues TH interacts with nuclear receptors to regulate gene expression.

Salamanders of the *Ambystoma tigrinum* complex (Tiger Salamanders, Axolotls and relatives) vary in their sensitivity to thyroid hormone, with moderate effect QTLs identified that are associated with delayed metamorphosis and increased body size.⁸³ This was the basis for a proposed TH-sensitivity model for predicting metamorphosis vs. larval form paedomorphosis.⁶⁸ This model showed paedomorphosis is associated with stable aquatic environments, reduced TH-sensitivity, and large body size. This model was since expanded to include direct developers, obligate paedomorphs, and alternative pathways to life cycle transitions.⁶⁴

There have been many tests of TH-sensitivity across salamanders over the past century conducted at varying levels of crudeness.⁸⁴⁻⁹⁰ From these tests it is clear that deeply divergent obligate paedomorphs do not transform

their “larval form” tissues when treated exogenously with thyroid hormone (Amphiumidae⁸⁷; Cryptobranchidae⁸⁵; Proteidae^{88,89}; and Sirenidae⁸⁴). The obligately paedomorphic species *Necturus maculosus* has functional TH signaling, but TH appears to be dysregulated from larval tissue transformation.^{89,91} This may be the case for some tissues of other obligate paedomorphs as well. There is also variation in tissue and transcriptional responsiveness to TH among populations of Oklahoma Salamanders (*Eurycea tynerensis*).⁹⁰ Among relatively shallowly divergent populations, some paedomorphic *E. tynerensis* showed limited responsiveness, while other populations had almost no response over the same treatment duration.⁹⁰ This suggests that obligate paedomorphosis can evolve rapidly.

There is very little data on the endocrinology of direct-developing salamanders, but it has been shown that some, such as *Plethodon cinereus*, develop both pituitary and thyroid gland early in embryonic development.⁷⁶ Therefore direct developers should have early release of TH, which would be consistent with early transformation.^{64,76} Direct developers may also be more sensitive to thyroid hormone. Another alternative is to evolve direct development by delaying hatching time until after transformation. This event can serve as a toggling point between direct development and biphasic life cycles. There have been no analyses to rigorously evaluate these possibilities across species.

While TH is clearly important in regulating transitions between paedomorphosis and biphasic life cycles, other mechanisms (discussed below) can alter these outcomes. These include environmental influences that operate through the stress axis to change the effects of TH on metamorphosis, and shifting reproductive timing (e.g., progenesis⁶⁴).

2.2 | Stress axis

Larval amphibians are subject to a variety of environmental stressors that can signify a deteriorating aquatic environment.^{67,92} These stressors include reduced water levels,⁹³ crowding,⁹⁴ and interactions with predators.⁹⁵ Perceived stress can be mediated through the hypothalamus by Corticotropin Releasing Factor (CRF), which can signal to the pituitary to release Adrenocorticotrophic Hormone (ACTH). In circulation, ACTH signals the release of “stress” hormones such as corticosterone and cortisol (collectively corticosteroids, CORT) from the interrenal glands.^{67,92}

Corticosteroids have diverse effects on physiology and development including synergistic increases in metamorphic rate.^{67,96,97} This is known to operate through at least

two mechanisms: (1) At some developmental stages CRF can instigate the pituitary to release thyroid stimulating hormone (TSH), which stimulates the release of thyroid hormone.⁶⁷ So, stress can directly regulate TH-based metamorphosis. (2) Corticosterone can work in concert with TH to enhance expression of immediate early genes to potentially initiate metamorphosis.^{67,96,98} For example, TH and CORT can synergistically upregulate the gene encoding the transcription factor krüppel-like factor 9 (*klf9*), which, among other functions, is important for central nervous system development.^{98,99} More recently it is appreciated that corticosteroids may be essential for amphibian metamorphosis.^{100–102}

Our understanding of the endocrine mediation of environmental stress is largely based on frogs.⁶⁷ Much investigation is needed to determine whether stress-induced metamorphosis of biphasic salamanders is driven by similar mechanisms. Thus far this seems to be the case, because some of the presumed ecological stressors (e.g., pond drying) have been identified as determinants of developmental trajectories of larvae to paedomorphosis or metamorphosis⁴³ or paedomorphic adults to metamorphose.^{35,103} There is clear variation in the rate at which larval salamanders transform when stressed. The larvae of pond-dwelling biphasic ambystomatids and salamandrids tend to metamorphose rapidly,^{35,103} whereas many stream breeding plethodontids are often slower.¹⁰⁴ It has been suggested that some stream-dwelling larvae have reduced metamorphic sensitivity to stressors.¹⁰⁴

Experiments that have explicitly evaluated the endocrinology of potential stressors in salamanders are limited. CRF has been shown to accelerate the metamorphosis of Tiger Salamander (*A. tigrinum*) larvae. Simultaneous treatment of axolotls with thyroxine and a synthetic corticosteroid (dexamethasone) has been shown to synergistically increase metamorphic changes¹⁰⁵ and expression of thyroid hormone converting enzymes, deiodinases.¹⁰⁶ These examples provide evidence that the response to environmental stressors is at least somewhat similar to anuran tadpoles, but the lack of stress sensitivity and loss of metamorphosis in paedomorphs suggest alterations to this system in some lineages. More work is needed in salamanders to determine the relative importance of stress responses, and the interaction between corticosteroids and TH in driving life cycle evolution.

2.3 | Reproductive axis

There are many environmental cues that instigate vertebrate reproduction. Seasonally breeding amphibians rely on signals such as rainfall, photoperiod, and temperature to trigger migrations and courtship behaviors.^{107–110} For

some species, artificially changing photoperiod and temperature during the quiescent period can induce testicular^{111–113} and ovarian maturation.^{114,115} The effects of environmental cues, such as photoperiod and temperature, are modulated through the pineal gland that produces melatonin. Arginine vasotocin along with melatonin are important modulators of courtship. An alternative pathway to courtship and gamete release is through GnRH released from the hypothalamus. GnRH stimulates gonadotrophs to synthesize luteinizing hormone (LH) and follicle-stimulating hormone (FSH) that act on the testes and ovaries, generally before the breeding season.⁶⁹ Resource acquisition and lipid stores are also important reproductive triggers, and in a given season can determine whether breeding is initiated or foregone.¹¹⁶ The protein leptin is released from adipose tissues. In frogs it serves as a signal of satiety and sufficient reserves to complete metamorphosis,¹¹⁷ but there is little information on the potential interaction with reproduction in amphibians. Leptin gene sequences and limited-expression data are only available for a couple species of salamanders.¹¹⁸

HPT and HPG axes also interact which impacts gonadal maturation as well as TH and steroid synthesis.^{119,120} TH appears to be more important in male reproductive development, and can influence testicular maturation and testosterone production.¹¹⁹ Interestingly, larval amphibians can still produce gonadal germ cells without the presence of TH,^{121–123} which allows for the development of mature larvae (paedomorphs) without an increase in TH that could trigger metamorphosis.⁶⁴ In addition to this, sex steroids can interact with the TH axis and inhibit metamorphosis.^{124,125} Through this mechanism accelerated gonadal development and production of sex steroids as a larva could inhibit metamorphosis.⁶⁴ In other words, progenesis can instigate early maturation in a larval form at a small body size, and at the same time prevent metamorphosis. However, facultative paedomorphs can overcome this, particularly progenetic male newts, by still maintaining the potential for metamorphosis.^{35,126} The variation in TH sensitivity and differential interactions with stress and reproductive axes make salamanders a compelling model for understanding how well-coordinated endocrine networks are maintained and evolve.

3 | DEVELOPMENTAL CONSEQUENCES OF LIFE CYCLE TRANSITIONS

Major shifts in ecology and life cycle, especially between environments as disparate as aquatic and terrestrial, can dramatically alter selective pressures on a variety of traits. New developmental patterns may be correlated with life cycle transitions, but are not necessarily the

cause. Shifts in ecology are expected to result in phenotypic divergence, but other ontogenetic, developmental, and temporal factors can promote or limit optimization.^{127,128} For example, experiencing different selective regimes across ontogeny can influence macroevolutionary patterns. Traits that conflict across ontogeny may be constrained to a single solution that is optimal for both situations.⁴ Whereas, traits that metamorphose between selective regimes can potentially express different optima at each stage, with stages phenotypically divergent from each other.^{128,129} It is important to note that metamorphosis is not constraining, it is an evolutionary decoupling process and potentially permits different optima for each stage.¹²⁸ Similarly, if a trait originates at metamorphosis then it would also not be expected to be constrained by a multiphasic life cycle. In contrast, traits that persist across stages (different selective regimes), and do not metamorphose, are the ones that we would expect to be constrained. These can be liberated by life cycle simplification.⁴

The time that a lineage spends under a given selective regime may also determine the degree to which its traits are optimized. Rapidly transitioning between adaptive zones may not allow sufficient time for them to be optimized to either.^{4,130} Plasticity can lead to rapid phenotypic changes triggered by a global regulator, such as in facultative paedomorphosis, but these changes are not necessarily canalized. Here we discuss how three interrelated components of salamander form (body size, genome size, and elements of the skeletal system) have likely been impacted by salamander life cycle evolution. These downstream consequences highlight further developmental diversification spawned from salamander ecological and life cycle transitions.

3.1 | Body size evolution

The larval period is an opportunity for rapid resource accumulation and growth. Body size tends to plateau after maturation when resources are primarily reallocated to reproduction, which can have long-term consequences on fecundity. For biphasic salamanders, extending the length of the larval period is correlated with increased adult body size.^{131,132} Salamanders show repeated shifts in adult body size that is in part correlated with life cycle evolution, but the relationship appears to differ among clades and is sometimes confounded by alternative developmental pathways.

The relationship between the length of larval period and adult body size is best displayed in plethodontid salamanders.^{131,133} A classic example is the dusky salamanders of the genus *Desmognathus* that show an association between length of larval period and adult body size along

an aquatic to the terrestrial gradient. The smallest species of *Desmognathus* are terrestrial direct developers, and the largest are highly aquatic and have larval periods that can extend for several years. Most of the radiation consists of species with short to medium length larval periods and intermediate body sizes that dwell at varying distances from the edge of streams.^{133,134} It is most likely that ancestral plethodontids were direct developers or had a very short larval period,³⁸ and the lengthy larval periods in large *Desmognathus* and unrelated large biphasic plethodontids (e.g., *Gyrinophilus*) were independently derived and actually occur in parallel.^{38,135}

The body size and age structure of amphibians are inherently linked to environmental conditions, with colder habitats associated with a longer developmental time to maturity and consequently increased longevity as shown in newts (Salamandridae¹³⁶). In such cold environments, aquatic gilled larvae overwinter before metamorphosis¹³⁷ producing larvae that reach sizes above the usual threshold for metamorphosis. In some cases this can extend to paedomorphosis.¹³⁸⁻¹⁴⁰ Depending on the developmental pathway, a paedomorph could reach sexual maturity early at a smaller body size (progenesis) or forgo metamorphosis and reach maturity at a typical body size (neoteny).⁶³ There are large variations among populations in these mechanisms calling for comparative analyses of somatic vs. gonadal development across situations.^{8,141,142} Progenesis allows for reproduction before drying could occur,^{36,126} permitting the colonization of under-used trophic niches.¹⁴³ The smaller the progenetic individuals, the more trophically distant they can be from the metamorphosed adults. This allows for use of alternate food resources while keeping similar energy inputs. Progenetic individuals have yet lower immediate reproductive outputs than the metamorphosed adults due to the positive correlation between size and fecundity.¹⁴⁴ Altogether, despite these size-dependent costs, the ultimate benefits of progenesis could promote a progenetic development that links with both the instability and complexity of aquatic habitats. Moreover, individual growth patterns were also modeled as driving larval development through alternative routes. Not only do productive aquatic conditions favor fast-growing larvae to mature as paedomorphs, but in some circumstances, detrimental growth could impose larvae to make the “best from a bad lot” in the perspective of costly metamorphosis.^{7,139}

Superficially, it seems reasonable that extrapolating the length of a larval period to permanently aquatic paedomorphosis would result in extreme increases in body size, and overall it does.¹⁴⁵ Four obligately paedomorphic families (Amphiumidae, Cryptobranchidae, Proteidae, and Sirenidae) include the most gigantic species of salamanders, with records over 1.6 m long. Overall permanent

commitments to an aquatic lifestyle may permit a significant expansion to maximum adult body size.¹⁴⁵ However, body size has actually been dynamic in these families since their origins in the Mesozoic. Most instances of gigantism are derived with subsequent reversals to small size.³³ A macroevolutionary perspective may obscure the mechanisms that precipitate the relationship between the larval environment and adult body size. Understanding developmental shifts in body size require assessment of changes in cell proliferation and cell size, which is commonly correlated with genome size.¹⁴⁶

3.2 | Genome size evolution

Genome size is known to impact many cellular processes including metabolic rate,¹⁴⁷⁻¹⁵² developmental timing,^{153,154} and developmental rate.¹⁵⁴⁻¹⁵⁶ Salamanders show extraordinary diversity in genome size,¹⁵¹ and include some of the largest genomes known in vertebrates (e.g., the mudpuppy *Necturus maculosus* has a haploid genome size of 80 to 95 pg, about 25 times larger than in humans^{147,157}). Life cycle evolution is linked with genome size in salamanders (Figure 2).^{130,154,156,159,160} Time-limited developmental windows constrain genome size evolution and have led to macroevolutionary correlations between life cycle complexity and larger genome size.^{130,156} Meaning, direct-developing and paedomorphic salamanders have significantly higher optimal genome sizes compared to biphasic species.¹³⁰ The life cycle of a biphasic amphibian is interrupted with an abrupt morphogenic shift (metamorphosis) that often corresponds with major habitat transitions. The need for rapid development in ephemeral environments has been suggested as a reason why some biphasic amphibians maintain a relatively small genome size.^{161,162} At the same time, a lower developmental rate due to increased genome size may facilitate transitions to simple life cycles (direct developers and paedomorphs).^{130,163,164} In salamanders, simpler life cycles permit genomic expansion which could have potential physiological advantages or could be a drift-related process caused by relaxed selection^{165,166} or reduced mutational hazard.¹⁶⁷ Direct developing frogs do not show increases in genome size,^{154,168} however they still transform at a very rapid rate inside of the egg (as fast as a couple of weeks in frogs compared to at least a couple of months in direct-developing salamanders).¹³⁰

There is likely a connection between genome size and body size. Some of the largest obligately paedomorphic salamanders also have the largest genomes.^{130,145} Within direct-developing bolitoglossines there is a correlation between genome size and body size, suggesting some degree of interaction. However, it is worth noting that bolitoglossines vary extensively in both of these traits.¹⁶⁹

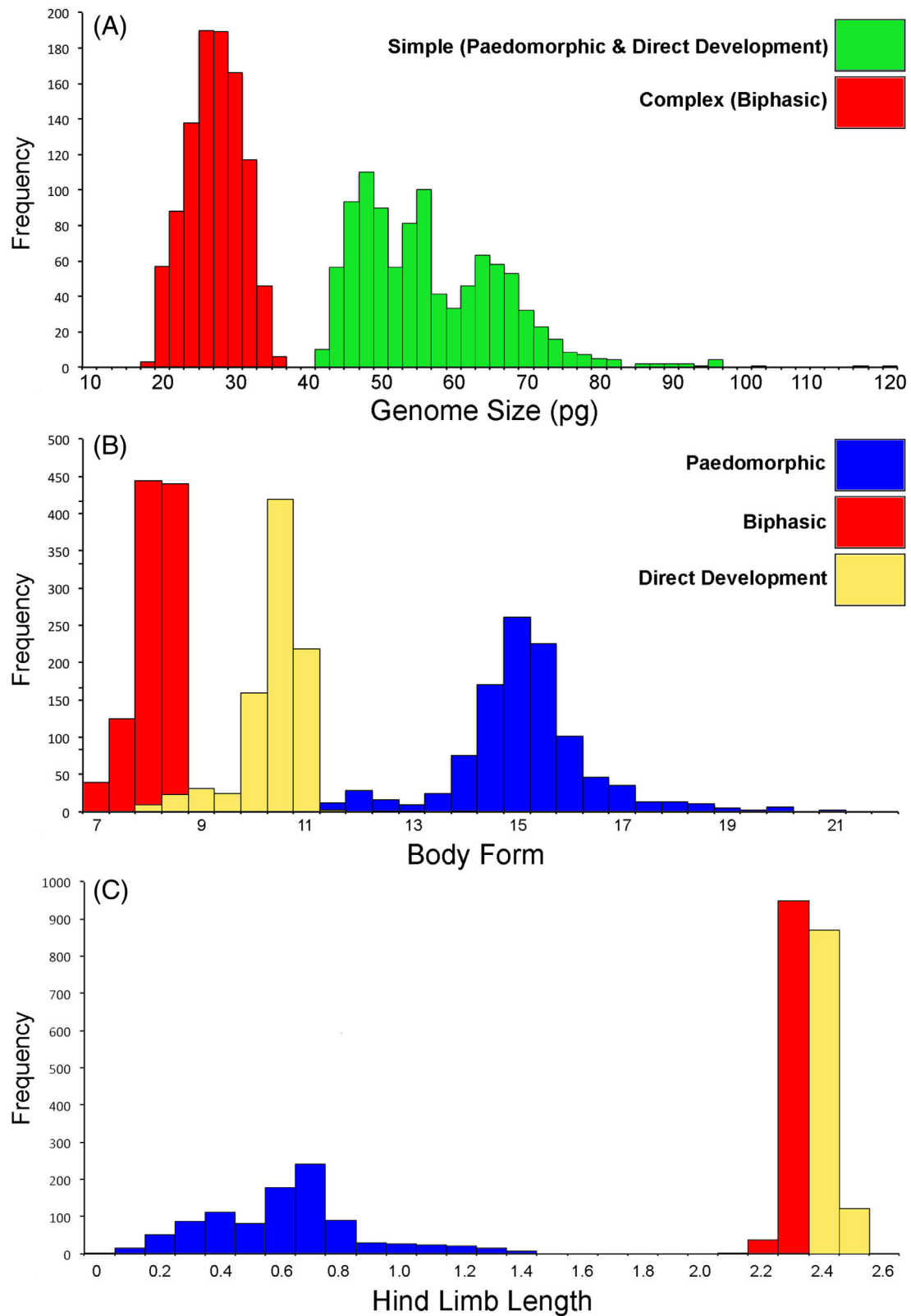


FIGURE 2 Best fit evolutionary models of salamander genome size A,¹³⁰ body form B,⁴ and relative hind limb length C.¹⁵⁸ Genome size best fits a life cycle complexity model where collectively simple life cycles (paedomorphic and direct-developing) have substantially higher optimal genome size than biphasic species, A.¹³⁰ Obligate paedomorphs exhibit a wide range of body forms including many elongate species, B, with short or no hind limbs, C. Direct developers also commonly have more elongate body forms than biphasics, but to a lesser extent. Direct developers also have the highest constraint on hind limb length. Genome size is measured as haploid in picograms. Body form is snout to vent length divided by body width, and relative hindlimb length is standardized by body width. Frequency for each trait is the optimal estimates of each group based on 1000 stochastic character maps^{4,130,158}

Genome size constrains the lower bound of cell size,^{154,160,170-172} providing a theoretical lower limit on body size. This could pose a major limitation on organismal complexity in some direct-developing and paedomorphic species that have become miniaturized in clades with ancestrally large genomes.^{155,169,173,174} Biological size has been cited as a better measurement when considering the relationship between genome size, body size, and developmental constraints.^{174,175} Unfortunately, biological size has been challenging to quantify across salamanders to date.¹⁶⁹

The relationship between genome size and regeneration has been recently described as “paradoxical” because genome size expansion slows down many aspects of physiology, growth, and development,¹⁴⁷⁻¹⁵⁵ and there is a negative correlation between genome size and regeneration time.¹⁵⁵ Genomic expansion also results in a reduced rate of cellular differentiation. This is hypothesized to maintain “younger” stem cell populations, which should have a high capacity for tissue regeneration.^{176,177} However, slower regeneration rates are observed in obligately paedomorphic lineages with the highest degrees of extreme genome expansions, suggesting that large genomes aid the regeneration process, but it may take a long time.¹⁷⁷ Another complicating factor is life cycle stage. Regeneration speed is shown to decrease after metamorphosis in salamanders¹⁷⁸⁻¹⁸⁰ and is almost completely lost in frogs after metamorphosis.¹⁸¹⁻¹⁸³ Comparative studies may further enlighten mechanisms of regeneration.²⁰ It would be ideal to integrate information about genome size, life cycle, and ecology to investigate the evolution of stem cell populations and regenerative capabilities in salamanders. The repeated shifts in these interrelated patterns across the history of salamanders suggest that there may be a variety of factors, both aiding and limiting regeneration. Genome size’s relationship with regeneration has aspects reflective of being both a cause and consequence, making it difficult to elucidate its role.

3.3 | Skeletal evolution

The skeletal system is a fundamental component of the vertebrate form, and both life cycle and ecology can impact its diversity. The conservation of some skeletal elements can enlighten fundamental mechanisms of tetrapod development and evolution.^{158,184-187} At the same time, some salamander lineages exhibit phenotypic divergence resulting in highly aberrant body forms making for an intriguing system to explore both constraint and convergence acting upon development.²⁻⁴

Elements of vertebral and appendicular skeletal systems are largely maintained from the larval period

through metamorphosis and into adulthood. As such, both are subject to potentially conflicting constraints due to the disparate environments faced during ontogeny in biphasic salamanders.^{4,158,188} Across salamanders, the average and rate of vertebral number evolution increases in obligately paedomorphic lineages relative to biphasic and direct-developing salamanders.⁴ Notably elongate paedomorphic lineages include the Amphiumidae, Sirenidae, Proteidae of the genus *Proteus*, and some plethodontids.⁴ Limb length and numbers of digits also show a higher rate of evolution in obligate paedomorphs, with a trend toward a decrease in both digit number and limb size associated with an increase in vertebral number (Figure 2).^{145,158} Even a single polymorphic lineage (*E. tynerensis*) show signatures of increased vertebral number in paedomorphic populations, demonstrating that life cycle transitions can apply strong selective pressure and rapidly impact skeletal evolution.¹⁸⁸ However, facultatively paedomorphic lineages have patterns of vertebral evolution that more closely resemble biphasic salamanders,⁴ a consequence of frequently shifting between environments.¹³⁰

Whether losing an aquatic life cycle stage (direct development) increases the rate of evolution is trait dependent. Direct developers have a significantly higher rate of body form evolution compared to biphasic salamanders, but a significantly lower rate of limb evolution. However, in both cases there appears to be a stronger constraint lifted when losing a terrestrial life cycle stage compared to an aquatic one.⁴ Interestingly, most genera in the largest clade of direct developers (bolitoglossines) have a fixed number of trunk vertebrae (at 14), but still exhibit considerably high rates of trunk form evolution.⁴ Among other direct developers there are several genera with high and variable numbers of trunk vertebrae (e.g., *Plethodon*, *Batrachoseps*, *Oedipina*, and *Phaeognathus*). The same occurs in salamandrids with intraspecific variation in most taxa.¹⁸⁹ This highlights the multiple pathways to expanding axial length, through adding vertebrae or lengthening them.^{4,5,190} The lack of variation in numbers of vertebrae across a few hundred species of bolitoglossines, that otherwise vary widely in form and habitat, indicates a strong developmental constraint on somitogenesis. Changing vertebral proportions may represent a path of the least resistance to trunk evolution.⁴

Mechanisms of vertebral column and limb development and evolution have been analyzed extensively and are reviewed elsewhere.^{20,191-194} Salamanders exhibit major patterning differences compared to other tetrapods, and even show interclade variation.^{184,195} The vast majority of the mechanistic work has been in *Ambystoma*¹⁹⁶⁻¹⁹⁸ and newts^{18,199} with an emphasis on regeneration (Reviewed in References 187,192,200,201). Given the potential for developmental variation and the repeated evolution of body elongate and limb reduced phenotypes,

it is likely that lineages use different molecular pathways to accomplish at least some convergent phenotypic outputs. For example, elongation due to adding vertebrae probably involves a different mechanism compared with elongation of individual vertebrae as shown in other vertebrates.^{4,5,202,203} The same could be true for different degrees of a similar morphological change. Has the 2-fold increase in the number of vertebrae in sirenids evolved via the same general mechanism as a 4-fold increase in amphiumids?⁴

Limb reduction in salamanders ranges from loss of a single wrist or ankle element to loss of limbs. It has long been hypothesized that loss of appendicular elements could be the result of fewer cells in a developing limb bud.²⁰⁴ In species with large genomes (and larger cells), also having small limb buds could be a powerful developmental constraint leading to loss of elements.^{164,204} Furthermore, salamanders exhibit notable variation in the timing of limb development and digital patterning compared with other tetrapods lineages, despite the investigation of just a few major lineages (reviewed in Reference 184). A range of different developmental mechanisms related to timing, molecular evolution, or physical constraints may contribute to the patterns of limb and vertebral column evolution, which should be a fruitful avenue of comparative studies.

Diversification of the cranial skeleton categorically follows the limbs, but for a different reason. The highest rate of skull evolution is in paedomorphic lineages, followed by biphasics, and the lowest is in direct developers.²⁰⁵ The skull is a collection of bones that can either transform or appear entirely during metamorphosis. Overall skull disparity among life cycles is best explained by differential metamorphosis in paedomorphic and biphasic species.²⁰⁵ In contrast, many direct developers feed with a ballistic tongue, which is thought to developmentally canalize skull shape.²⁰⁵ Additionally, many aspects of the cranial skeleton are persistent across development making a valuable system for testing the impacts of life cycle transitions on differentially metamorphic traits. Overall, the effect of salamander life cycle shifts on skeletal evolution provides a strong system for understanding convergence and developmental/ecological constraints across phylogeny.

4 | CONCLUSIONS

Ancestral state reconstructions on the salamander phylogeny demonstrate repeated ecological and life cycle transitions that provide a robust system to understand developmental diversity and mechanisms. Developmental variations can be the direct cause of a transition or a

downstream consequence. This can provide a more comprehensive understanding of the evolution and constraint on developmental mechanisms that are fundamental to tetrapods. The inherent challenge to analyzing the evolution of major developmental traits in salamanders is disentangling ecology, life cycle, endocrine system, genome size, and body size since they are tightly intercorrelated. Resolving this will entail collecting more comprehensive data on genomic, transcriptomic, proteomic, and other developmental parameters covering key phylogenetic transitions. Some aspects of these traits are dynamic (e.g., expression, hormone circulation, growth rate, and developmental timing), which need to be collected both in controlled and natural settings across ontogeny. Given that collecting such data across even a handful of species can be a major undertaking, it would be useful to establish common environmental conditions for raising salamanders in the lab to facilitate the compilation of comparative data.

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Ronald Bonett: Writing-review & editing. **Nicholus Ledbetter:** Writing-review & editing. **Alexander Hess:** Writing-review & editing. **Madison Herrboldt:** Writing-review & editing. **Mathieu Denoël:** Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interests with regard to this review.

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