# 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]
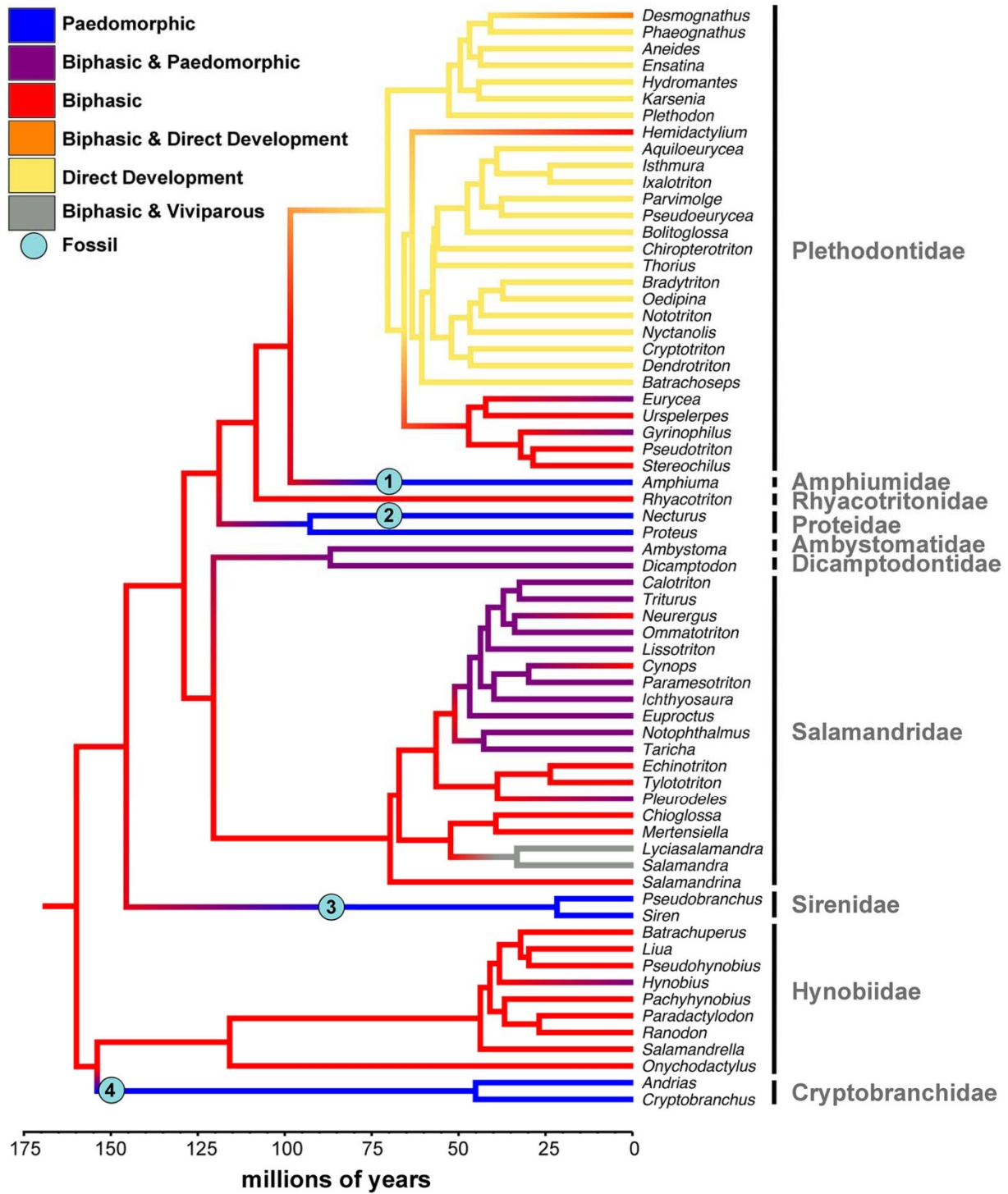
**Figure 1.** Time calibrated phylogeny of salamanders[4] collapsed to 66 named genera. Major relationships are also supported by several studies.[21, 22] Life cycle categories are reconstructed to generic level variation. Genera with variable life cycles include some biphasic species with either facultative 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]

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 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, $T_4$ (thyroxine) and the more potent $T_3$ (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 (Amphiumidae[87]; Cryptobranchidae[85]; Proteidae[88, 89]; and Sirenidae[84]). 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., progenesis[64]).

## 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 Adrenocorticotropic 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 potently 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 metamorphosis[43] 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 changes[105] 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 (Salamandridae[136]). In such cold environments, aquatic gilled larvae overwinter before metamorphosis[137] 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 developmental 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]
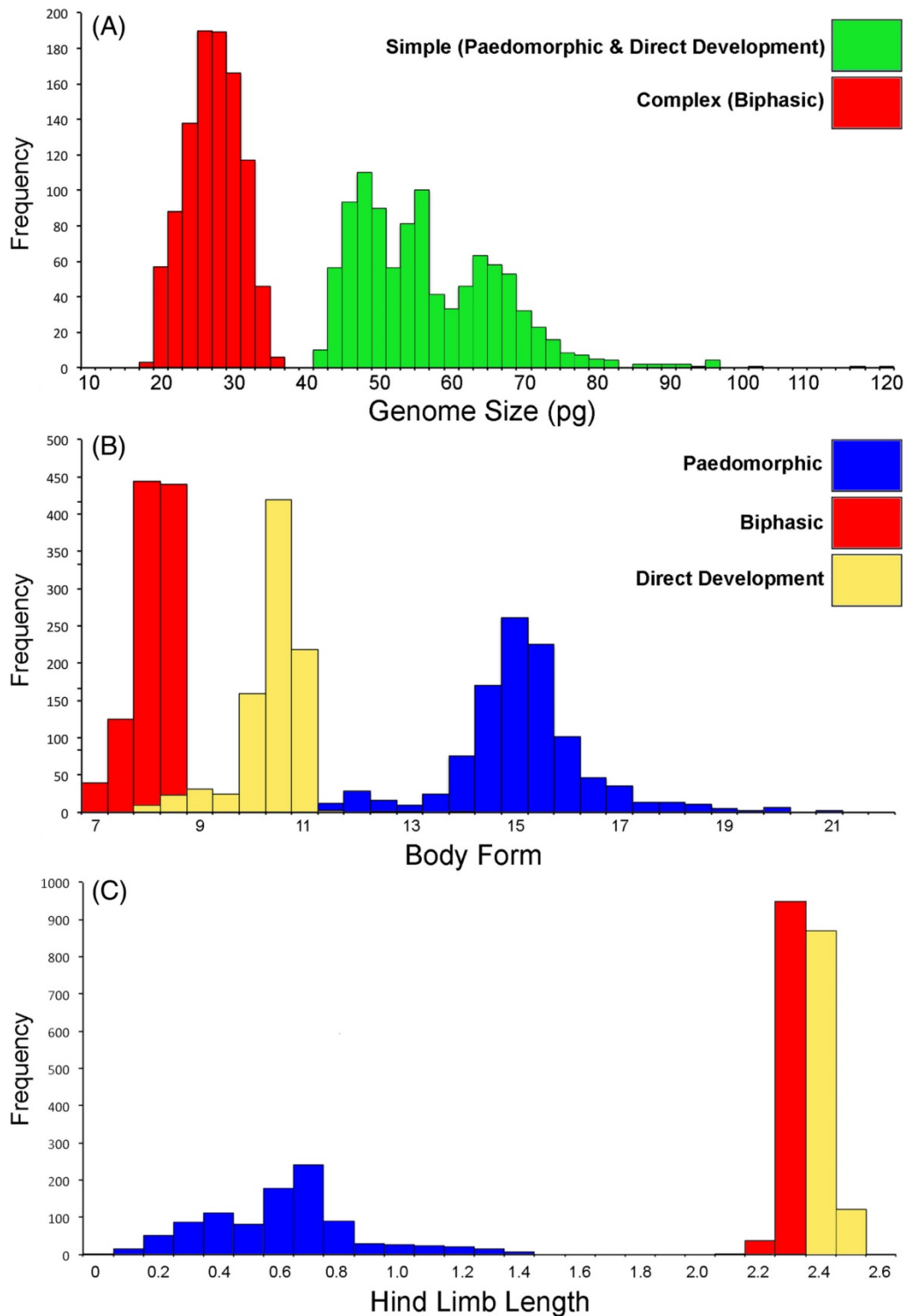
## 3.2. Genome size evolution

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] 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 salamanders[178-180] 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.

**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, 158, 130]

### 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, 188, 158] 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.[5, 190, 4] 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, 202, 203, 5] 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.

### AUTHOR CONTRIBUTIONS

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.

**REFERENCES**

1.      Wake DB. Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. Mem S California Acad Sci. 1966; 4: 1-111.

2.      Wake DB. Homoplasy: the result of natural selection, or evidence of design limitations? Am Nat. 1991; 138: 543-567.

3.      Weins JJ, Bonett RM, Chippindale PT. Ontogeny discombobulates phylogeny: paedomorphosis and higher-level salamander relationships. Syst Biol. 2005; 54: 91-110.

4.      Bonett RM, Blair AL. Evidence for complex life cycle constraints on salamander body form diversification. PNAS. 2017; 114: 9936-9941.

5.      Parra-Olea G, Wake DB. Extreme morphological and ecological homoplasy in tropical salamanders. PNAS. 2001; 98: 78887891.

6.      Wiens JJ, Chippindale PT, Hillis DM. When are phylogenetic analyses misled by convergence? A case study in Texas cave salamanders. Syst Biol. 2003; 52: 501-514.

7.      Whiteman HH. Evolution of facultative paedomorphosis in salamanders. Q Rev Biol. 1994; 69: 205-221.

8.      Ryan TJ, Semlitsch RD. Intraspecific heterochrony and life history evolution: decoupling somatic and sexual development in a facultatively paedomorphic salamander. PNAS. 1998; 95: 5643-5648.

9.      Denoël M, Joly P, Whiteman HH. Evolutionary ecology of facultative paedomorphosis in newts and salamanders. Biol Rev. 2005; 80: 663-671.

10.     Morvan-Dubois G, Demeneix BA, Sachs LM. *Xenopus laevis* as a model for studying thyroid hormone signalling: from development to metamorphosis. Mol Cell Endocrinol. 2008; 293: 71-79.

11.     Beck CW, Belmonte JCI, Christen B. Beyond early development: *Xenopus* as an emerging model for the study of regenerative mechanisms. Dev Dyn. 2009; 238: 1226-1248.

12.     Shi YB. Animal metamorphosis. New York, USA: Academic Press; 2013.

13.     Voss SR, Epperlein HH, Tanaka EM. *Ambystoma mexicanum*, the axolotl: a versatile amphibian model for regeneration, development, and evolution studies. Cold Spring Harb Protoc. 2009; 8: pdb-emo128.

14.     Ziermann JM, Diogo R. Cranial muscle development in the model organism *Ambystoma mexicanum*: implications for tetrapod and vertebrate comparative and evolutionary morphology and notes on ontogeny and phylogeny. Anat Rec. 2013; 296: 1031-1048.

15.     Vlaskalin T, Wong CJ, Tsilfidis C. Growth and apoptosis during larval forelimb development and adult forelimb regeneration in the newt (*Notophthalmus viridescens*). Dev Genes Evol. 2004; 214: 423-431.

16.     Kurosaka H, Takano-Yamamoto T, Yamashiro T, Agata T. Comparison of molecular and cellular events during lower jaw regeneration of newt (*Cynops pyrrhogaster*) and west African clawed frog (*Xenopus tropicalis*). Dev Dyn. 2008; 237: 354-365.

17.     Hayashi T, Yokotani N, Tane S, et al. Molecular genetic system for regenerative studies using newts. Develop Growth Differ. 2013; 55: 229-236.

18.     Kumar A, Gates PB, Czarkwiani A, Brockes JP. An orphan gene is necessary for preaxial digit formation during salamander limb development. Nat Commun. 2015; 6: 8684.

19.     Suzuki M, Hayashi T, Inoue T, et al. Cas9 ribonucleoprotein complex allows direct and rapid analysis of coding and noncoding regions of target genes in *Pleurodeles waltl* development and regeneration. Dev Biol. 2018; 443: 127-136.

20.     Joven A, Elewa A, Simon A. Model systems for regeneration: salamanders. Development. 2019; 146: dev167700.

21.     Roelants K, Gower DJ, Wilkinson M, et al. Global patterns of diversification in the history of modern amphibians. PNAS. 2007; 104: 887-892.

22.     Hime PM, Lemmon AR, Lemmon ECM, et al. Phylogenomics reveals ancient gene tree discordance in the amphibian tree of life. Syst Biol. 2021; 70: 49-66.

23.     Estes R. The fossil record of amphiumid salamanders. Breviora. 1969; 322: 1-11.

24.     DeMar DG Jr. A new fossil salamander (Caudata, Proteidae) from the upper cretaceous (Maastrichtian) Hell Creek formation, Montana, USA. J Vertebr Paleontol. 2013; 33: 588-598.

25.     Gardner JD. Revision of *Habrosaurus* Gilmore (Caudata; Sirenidae) and relationships among sirenid salamanders. Paléo. 2003; 46: 1089-1122.

26.     Gao K-Q. Shubin NH. 2003. Earliest known crown-group salamanders. Nature. 2003; 422: 424-428.

27.     Duellman WE, Trueb L. Biology of Amphibians. New York: McGraw–Hill; 1986.

28.     Istock CA. The evolution of complex life cycle phenomena: An ecological perspective. Evolution. 1967; 21: 592-605.

29.     Wilbur HM, Collins JP. Ecological aspects of amphibian metamorphosis. Science. 1973; 182: 1305-1314.

30.     Wilbur HM. Complex life cycles. Annu Rev Ecol Syst. 1980; 11: 67-93.

31.     Laudet V. The origins and evolution of vertebrate metamorphosis. Curr Biol. 2011; 21: R726-R737.

32.     Holman JA. Fossil Salamanders of North America. Bloomington, IN: Indiana University Press; 2006.

33.     Bonett RM, Trujano-Alvarez AL, Williams MJ, Timpe EK. Biogeography and body size shuffling of aquatic salamander communities on a shifting refuge. Proc R Soc B. 2013; 280: 20130200.

34.     Bonett RM, Steffen MA, Lambert SM, Wiens JJ, Chippindale PT. Evolution of paedomorphosis in plethodontid salamanders: ecological correlates and re-evolution of metamorphosis. Evolution. 2014; 68: 466-482.

35.     Mathiron AGE, Lena J-P, Baouch S, Denoël M. The 'male escape hypothesis': sex-biased metamorphosis in response to climatic drivers in a facultatively paedomorphic amphibian. Proc R Soc B. 2017; 284: 20170176.

36.     Wake DB, Hanken J. Direct development in the lungless salamanders: what are the consequences for developmental biology, evolution and phylogenesis? Int J Dev Biol. 1996; 40: 859-869.

37.     Wake DB. Adaptive radiation of salamanders in middle American cloud forests. Ann Mo Bot Gard. 1987; 74: 242-264.

38.     Bonett RM, Steffen MA, Robison GA. Heterochrony repolarized: a phylogenetic analysis of developmental timing in plethodontid salamanders. EvoDevo. 2014; 5: 27.

39.     Greven H. Larviparity and pueriparity. In: Sever DM, ed. Reproductive Biology and Phylogeny of Urodela. Vol 1. Plymouth, UK: Science Publishers, Inc.; 2003.

40.     Buckley D, Alcobendas M, García-París M, Wake MH. Heterochrony, cannibalism, and the evolution of viviparity in *Salamandra salamandra*. Evol Dev. 2007; 9: 105-115.

41.     Buckley D. Evolution of viviparity in salamanders (Amphibia, Caudata). eLS 2012.

42.     Velo-Antón G, Santos X, Sanmartín-Villar I, Cordero-Rivera A, Buckley D. Intraspecific variation in clutch size and maternal investment in pueriparous and larviparous *Salamandra salamandra* females. Evol Ecol. 2015; 29: 185-204.

43.     Semlitsch RD. Paedomorphosis in *Ambystoma talpoideum*: effects of density, food, and pond drying. Ecology. 1987; 68: 994-1002.

44.     Denoël M, Ficetola GF. Heterochrony in a complex world: disentangling environmental processes of facultative paedomorphosis in an amphibian. J Anim Ecol. 2014; 83: 606-615.

45.     Denoël M, Ficetola GF, Sillero N, et al. Traditionally managed landscapes do not prevent amphibian decline and the extinction of paedomorphosis. Ecol Monogr. 2019; 89: e01347.

46.     Bonett RM, Chippindale PT. Streambed microstructure predicts evolution of development and life history mode in the plethodontid salamander, *Eurycea tynerensis*. BMC Biol. 2006; 4: 1-12.

47.     Emel SL, Bonett RM. Considering alternative life history modes and genetic divergence in conservation: a case study of the Oklahoma salamander. Conserv Genet. 2011; 12: 1243-1259.

48.     Healy WR. Population consequences of alternative life histories in *Notophthalmus v. viridescens*. Copeia. 1974; 1974: 221-229.

49.     Denoël M. Seasonal variation of morph ratio in facultatively paedomorphic populations of the palmate newt *Triturus helveticus*. Acta Oecol. 2006; 29: 165-170.

50.     Cayuela H, Valenzuela-Sánchez A, Teulier L, et al. Determinants and consequences of dispersal in vertebrates with complex life cycles: a review of pond-breeding amphibians. Q Rev Biol. 2020; 95: 1-36.

51.     Grayson KL, Bailey LL, Wilbur HM. Life history benefits of residency in a partially migrating pond-breeding amphibian. Ecology. 2011; 92: 1236-1246.

52.     Liang F, Changyuan Y. Amphibians of China. Vol 1. Beijing, China: Science Press; 2016.

53.     Etheridge K. The energetics of estivating sirenid salamanders (*Siren lacertina* and *Pseudobranchus striatus*). Herpetologica. 1990; 46: 407-414.

54.     Smith ME, Secor SM. Physiological responses to fasting and estivation for the three-toed *Amphiuma* (*Amphiuma tridactylum*). Physiol Biochem Zool. 2017; 90: 240-256.

55.     Jarvis LE. Terrestrial ecology of juvenile great crested newts (*Triturus cristatus*) in a woodland area. Herpetol J. 2016; 26: 287-296.

56.     Denoël M, Joly P. Adaptive significance of facultative paedomorphosis in *Triturus alpestris* (Amphibia, Caudata): resource partitioning in an alpine lake. Freshw Biol. 2001; 46: 1387-1396.

57.     Blankers T, Adams DC, Wiens JJ. Ecological radiation with limited morphological diversification in salamanders. J Evol Biol. 2012; 25: 634-646.

58.     Baken EK, Adams DC. Macroevolution of arboreality in salamanders. Ecol Evol. 2019; 9: 7005-7016.

59.     Gould SJ. Ontogeny and Phylogeny. Cambridge, MA: Harvard University Press; 1977.

60.     Alberch P, Gould SJ, Oster GF, Wake DB. Size and shape in ontogeny and phylogeny. Paleobiology. 1979; 5: 296-317.

61.     McKinney ML, KJ MN. Heterochrony. The Evolution of Ontogeny. New York: Plenum Press; 1991.

62.     Bonett RM. In: Nuño de la Rosa L, de la Müller G, eds. Heterochrony. Evolutionary Developmental Biology. Springer International Publishing; 2018: 1-14.

63.     Denoël M, Joly P. Neoteny and progenesis as two heterochronic processes involved in paedomorphosis in *Triturus alpestris* (Amphibia: Caudata). Proc R Soc B. 2000; 267: 1481-1485.

64.     Bonett RM. An integrative endocrine model for the evolution of developmental timing and life history of plethodontids and other salamanders. Copeia. 2016; 104: 209-221.

65.     Norris DO, Carr JA. Vertebrate Endocrinology. New York, USA: Academic Press; 2020.

66.     Buchholz DR, Paul BD, Fu L, Shi Y-B. Molecular and developmental analyses of thyroid hormone receptor function in *Xenopus laevis*, the African clawed frog. Gen Comp Endocrinol. 2006; 145: 1-19.

67.     Denver RJ. Stress hormones mediate environment-genotype interactions during amphibian development. Gen Comp Endocrinol. 2009; 164: 20-31.

68.     Johnson CK, Voss SR. Salamander paedomorphosis: linking thyroid hormone to life history and life cycle evolution. Curr Top Dev Biol. 2013; 103: 229-258.

69.     Vu M, Trudeau VL. Neuroendocrine control of spawning in amphibians and its practical applications. Gen Comp Endocrinol. 2016; 234: 28-39.

70.     Gudernatsch JF. Feeding experiments on tadpoles: I. the influence of specific organs given as food on growth and differentiation. A contribution to the knowledge of organs with internal secretion. Rouxs Arch Dev Biol. 1912; 35: 457-483.

71.     Allen BM. The response of *Bufo* larvae to different concentrations of thyroxin. Anat Rec. 1932; 54: 45-64.

72.     Allen BM. Extirpation of the hypophysis and thyroid glands of *Rana pipiens*. Science. 1916; 44: 755-757.

73.     Allen BM. The results of thyroid removal in the larvae of *Rana pipiens*. J Exp Zool. 1918; 24: 499-519.

74.     Hoskins ER, Hoskins MM. Growth and development of amphibia as affected by thyroidectomy. J Exp Zool. 1919; 29: 1-69.

75.     Hanaoka Y. Uptake of 131I by the thyroid gland during metamorphosis in *Xenopus*. J Fac Sci, Hokkaido Univ, Ser 6. 1966; 16: 106-112.

76.     Dent JN. The embryonic development of *Plethodon cinereus* as correlated with the differentiation and functioning of the thyroid gland. J Morphol. 1942; 71: 577-601.

77.     Hanken J, Jennings DH, Olsson L. Mechanistic basis of life-history evolution in anuran amphibians: direct development. Am Zool. 1997; 37: 160-171.

78.     Elinson RP. Metamorphosis in a frog that does not have a tadpole. Curr Top Dev Biol. 2013; 103: 259-276.

79.     Regard E. Cytophysiology of the amphibian thyroid gland, through larval development and metamorphosis. Int Rev Cytol Suppl. 1978; 52: 81-118.

80.     Suzuki S, Suzuki M. Changes in thyroidal and plasma Iodine compounds during and after metamorphosis of the bullfrog, *Rana catesbeiana*. Gen Comp Endocrinol. 1981; 45: 74-81.

81.     Alberch P, Gale EA, Larsen PR. Plasma T4 and T3 levels in naturally metamorphosing *Eurycea bislineata* (Amphibia; Plethodontidae). Gen Comp Endocrinol. 1986; 61: 153-162.

82.     Norman MF, Carr JA, Norris DO. Adenohypophysial-thyroid activity of the tiger salamander, *Ambystoma tigrinum*, as a function of metamorphosis and captivity. J Exp Zool. 1987; 242: 55-66.

83.     Voss SR, Kump DK, Walker JA, Shaffer HB, Voss GJ. Thyroid hormone responsive QTL and the evolution of paedomorphic salamanders. Heredity. 2012; 109: 293-298.

84.     Noble GK. The 'retrograde metamorphosis' of the Sirenidae; experiments on the functional activity of the thyroid of the perennibranchs. Anat Rec. 1924; 29: 100.

85.     Noble GK, Farris EJ. A metamorphic change produced in *Cryptobranchus* by thyroid solutions. Anat Rec. 1929; 42: 59.

86.     Kezer J. Thyroxine-induced metamorphosis of the neotenic salamanders *Eurycea tynerensis* and *Eurycea neotenes*. Copeia. 1952; 4: 234-237.

87.     Kobayashi H, Gorbman A. Thyroid function in *Amphiuma*. Gen Comp Endocrinol. 1962; 2: 279-282.

88.     Švob M, Musafija A, Frank F, et al. Response of tail fin of *Proteus anguinus* to thyroxine. J Exp Zool. 1973; 184: 341-343.

89.     Safi R, Vlaeminck-Guillem V, Duffraisse M, et al. Pedomorphosis revisited: thyroid hormone receptors are functional in *Necturus maculosus*. Evol Dev. 2006; 8: 284-292.

90.     Aran RP, Steffen MA, Martin SD, Lopez OI, Bonett RM. Reduced effects of thyroid hormone on gene expression and metamorphosis in a paedomorphic plethodontid salamander. J Exp Zool B Mol Dev Evol. 2014; 322B: 294-303.

91.     Vlaeminck-Guillem V, Safi R, Guillem P, Leteurtre E, Duterque-Coquillaud M, Laudet V. Thyroid hormone receptor expression in the obligatory paedomorphic salamander *Necturus maculosus*. Int J Dev Biol. 2004; 50: 553-560.

92.     Crespi EJ, Williams TD, Jessop TS, Delehanty B. Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? Funct Ecol. 2013; 27: 93-106.

93.     Gomez-Mestre I, Kulkarni S, Buchholz DR. Mechanisms and consequences of developmental acceleration in tadpoles responding to pond drying. PLoS One. 2013; 8: e84266.

94.     Glennemeier KA, Denver RJ. Role for corticoids in mediating the response of *Rana pipiens* tadpoles to intraspecific competition. J Exp Zool. 2002; 292: 32-40.

95.     Middlemis Maher J, Werner EE, Denver RJ. Stress hormones mediate predator-induced phenotypic plasticity in amphibian tadpoles. Proc R Soc B. 2013; 280: 20123075.

96.     Bonett RM, Hoopfer ED, Denver RJ. Molecular mechanisms of corticosteroid synergy with thyroid hormone during tadpole metamorphosis. Gen Comp Endocrinol. 2010; 168: 209-219.

97.     Kulkarni SS, Buchholz DR. Beyond synergy: corticosterone and thyroid hormone have numerous interaction effects on gene regulation in *Xenopus tropicalis* tadpoles. Endocrinology. 2012; 153: 5309-5324.

98.     Bagamasbad PD, Bonett RM, Sachs L, et al. Deciphering the regulatory logic of an ancient, ultraconserved nuclear receptor enhancer module. Mol Endocrinol. 2015; 29: 856-872.

99.     Bonett RM, Hu F, Bagamasbad P, Denver RJ. Stressor and glucocorticoid-dependent induction of the immediate early gene Krüppel-like factor 9 (KLF9): implications for neural development and plasticity. Endocrinology. 2009; 150: 17571765.

100.    Sachs LM, Buchholz DR. Insufficiency of thyroid hormone in frog metamorphosis and the role of glucocorticoids. Front Endocrinol. 2019; 10: 287.

101.    Shewade LH, Schoephoerster JA, Patmann MD, Kulkarni SS, Buchholz DR. Corticosterone is essential for survival through frog metamorphosis. Endocrinology. 2020; 161: bqaa193.

102.    Sterner ZR, Shewade LH, Mertz KM, Sturgeon SM, Buchholz DR. Glucocorticoid receptor is required for survival through metamorphosis in the frog Xenopus tropicalis. Gen Comp Endocrinol. 2020; 291: 113419.

103.    Denoël M. How do paedomorphic newts cope with lake drying? Ecography. 2003; 26: 405-410.

104.    Beachy CK, Ryan TJ, Bonett RM. How metamorphosis is different in Plethodontids: larval life history perspectives on lifecycle evolution. Herpetologica. 2017; 73: 252-258.

105.    Kühn ER, Groef BD, Grommen SVH, der Greyton SV, Darras VM. Low submetamorphic doses of dexamethasone and thyroxine induce complete metamorphosis in the axolotl (*Ambystoma mexicanum*) when injected together. Gen Comp Endocrinol. 2004; 137: 141-147.

106.    Kühn ER, Groef BD, der Greyton SV, Darras VM. Corticotropin-releasing hormone-mediated metamorphosis in the neotenic axolotl *Ambystoma mexicanum*: synergistic involvement of thyroxine and corticoids on brain type II deiodinase. Gen Comp Endocrinol. 2005; 143: 75-81.

107.    Salthe SN, Mechan JS. Reproductive and courtship patterns. In: Lofts B, ed. Physiology of the Amphibia. Vol 2. New York and London: Academic Press; 1974.

108.    Griffiths RA. Newts and Salamanders of Europe. London: T. & A.D. Poyser Natural History; 1996.

109. Canavero A, Arim M. Clues supporting photoperiod as the main determinant of seasonal variation in amphibian activity. J Nat Hist. 2009; 43: 2975-2984.

110. Dervo BK, Bærum KM, Skurdal J, Museth J. Effects of temperature and precipitation on breeding migrations of amphibian species in southeastern Norway. Scientifica. 2016; 2016: 2016.

111. Werner JK. Temperature-photoperiod effects on spermatogenesis in the salamander *Plethodon cinereus*. Copeia. 1969; 1969: 592-602.

112. Paniagua R, Fraile B, S'aez FJ. Effects of photoperiod and temperature on testicular function in amphibians. Histol Histopathol. 1990; 5: 365-378.

113. Delgado MJ, Alonso-G'omez AL, Alonso-Bedate M. Role of environmental temperature and photoperiod in regulation of seasonal testicular activity in the frog, *Rana perezi*. Can J Phys. 1992; 70: 1348-1352.

114. Horseman ND, Smith CA, Culley DD Jr. Effects of age and photoperiod on ovary size and condition in bullfrogs (*Rana catesbeiana* Shaw) (Amphibia, Anura, Ranidae). J Herpetol. 1978; 12: 287-290.

115. Saidapur SK, Hoque B. Effect of photoperiod and temperature on ovarian cycle of the frog *Rana tigrina* (Daud.). J Biosci. 1995; 20: 445-452.

116. Fitzpatrick LC. Life history patterns of storage and utilization of lipids for energy in amphibians. Am Zool. 1976; 16: 725-732.

117. Bender MC, Hu C, Pelletier C, Denver RJ. To eat or not to eat: ontogeny of hypothalamic feeding controls and a role for leptin in modulating life-history transition in amphibian tadpoles. Proc R Soc B. 2018; 285: 1-10.

118. Boswell T, Dunn IC, Wilson PW, Joseph N, Burt DW, Sharp PJ. Identification of a non-mammalian leptin-like gene: characterization and expression in the tiger salamander (*Ambystoma tigrinum*). Gen Comp Endocrinol. 2006; 146: 157-166.

119. Flood DEK, Fernandino JI, Langlois VS. Thyroid hormones in male reproductive development: evidence for direct crosstalk between the androgen and thyroid hormone axes. Gen Comp Endocrinol. 2013; 192: 2-14.

120. Duarte-Guterman P, Navarro-Martín L, Trudeau VL. Mechanisms of crosstalk between endocrine systems: regulation of sex steroid hormone synthesis and action by thyroid hormones. Gen Comp Endocrinol. 2014; 203: 69-85.

121. Wakahara M. Spermatogenesis is extraordinarily accelerated in metamorphosis-arrested larvae of a salamander, *Hynobius retardatus*. Experientia. 1994; 50: 94-98.

122. Kanki K, Wakahara M. Precocious testicular growth in metamorphosis-arrested larvae of a salamander *Hynobius retardatus*: role of thyroid-stimulating hormone. J Exp Zool. 1999; 283: 548-558.

123. Rot-Nikcevic I, Wassersug RJ. Arrested development in *Xenopus laevis* tadpoles: how size constrains metamorphosis. J Exp Biol. 2004; 207: 2133-2145.

124. Gray KM, Janssens PA. Gonadal hormones inhibit the induction of metamorphosis by thyroid hormones in *Xenopus laevis* tadpoles in vivo, but not in vitro. Gen Comp Endocrinol. 1990; 77: 202-211.

125. Hogan NS, Duarte P, Wade MG, Lean DR, Trudeau VL. Estrogenic exposure affects metamorphosis and alters sex ratios in the northern leopard frog (*Rana pipiens*): identifying critically vulnerable periods of development. Gen Comp Endocrinol. 2008; 156: 515-523.

126. Denoël M, Drapeau L, Oromi N, Winandy L. The role of predation risk in metamorphosis versus behavioural avoidance: a sex-specific study in a facultative paedomorphic amphibian. Oecologia. 2019; 189: 637-645.

127. Schluter D, Price TD, Rowe L. Conflicting selection pressures and life history trade-offs. Proc R Soc B. 1991; 246: 11-17.

128. Moran NA. Adaptation and constraint in the complex life cycles of animals. Annu Rev Ecol Syst. 1994; 25: 573-600.

129.     Sherratt E, Vidal-García M, Anstis M, Keogh JS. Adult frogs and tadpoles have different macroevolutionary patterns across the Australian continent. Nat Ecol Evol. 2017; 1: 1385-1391.

130.     Bonett RM, Hess AJ, Ledbetter NM. Facultative transitions have trouble committing, but stable life cycles predict salamander genome size evolution. Evol Biol. 2020; 47: 111-122.

131.     Bruce RC. Size-mediated tradeoffs in life-history traits in dusky salamanders. Copeia. 2013; 2013: 262-267.

132.     Phung TX, Nascimento JCS, Novarro AJ, Wiens JJ. Correlated and decoupled evolution of adult and larval body size in frogs. Proc R Soc B. 2020; 287: 20201474.

133.     Hairston NG Sr. Species packing in *Desmognathus* salamanders: experimental demonstration of predation and competition. Am Nat. 1986; 127: 266-291.

134.     Kozak KH, Larson A, Bonett RM, Harmon LJ. Phylogenetic analysis of ecomorphological divergence, community structure, and diversification rates in dusky salamanders (Plethodontidae: Desmognathus). Evolution. 2005; 59: 2000-2016.

135.     Kozak KH, Mendyk RW, Wiens JJ. Can parallel diversification occur in sympatry? Repeated patterns of body-size evolution in coexisting clades of north American salamanders. Evolution. 2009; 63: 1769-1784.

136.     Miaud C, Guyetant R, Faber H. Age, size, and growth of the alpine newt, *Triturus alpestris* (Urodela: Salamandridae), at high altitude and a review of life-history trait variation throughout its range. Herpetologica. 2000; 56: 135-144.

137.     Grossenbacher K. Untersuchungen zur Entwicklungsg eschwindigkeit der Larven von *Triturus a. alpestris* (Laurenti 1768), *Bufo B. bufo* (Linnaeus 1758) und *Rana t. temporaria* (Linnaeus 1758) aus Populationen verschiedener Hoehenstufen in den Schweizer Alpen [PhD Thesis]. Bern: Universität Bern; 1979.

138.     Fasola M. Resource partitioning by three species of newts during their aquatic phase. Ecography. 1993; 16: 73-81.

139.     Whiteman HH, Wissinger S, Denoël M, Mecklin C, Gerlanc N, Gutrich J. Larval growth in polyphenic salamanders: making the best of a bad lot. Oecologia. 2012; 168: 109-118.

140.     Denoël M. On the identification of paedomorphic and overwintering larval newts based on cloacal shape: review and guidelines. Curr Zool. 2017; 63: 165-173.

141.     Ivanović A, Cvijanović M, Denoël M, Slijepčević M, Kalezić ML. Facultative paedomorphosis and the pattern of interand intraspecific variation in cranial skeleton: lessons from European newts (*Ichthyosaura alpestris* and *Lissotriton vulgaris*). Zoomorphology. 2014; 133: 99-109.

142.     Denoël M, Ivanović A, Džukić G, Kalezić ML. Sexual size dimorphism in the evolutionary context of facultative paedomorphosis: insights from European newts. BMC Evol Biol. 2009; 9: 278.

143.     Lejeune B, Bissey L, Didaskalou EA, Sturaro N, Lepoint G, Denoël M. Progenesis as an intrinsic factor of ecological opportunity in a polyphenic amphibian. Funct Ecol. 2021; 35: 546-560.

144.     Denoël M, Drapeau L, Winandy L. Reproductive fitness consequences of progenesis: sex-specific payoffs in safe and risky environments. J Evol Biol. 2019; 32: 629-637.

145.     Wiens JJ, Hoverman JT. Digit reduction, body size, and paedomorphosis in salamanders. Evol Dev. 2008; 10: 449-463.

146.     Gregory TR. Nucleotypic effects without nuclei: genome size and erythrocyte size in mammals. Genome. 2000; 43: 895-901.

147.     Licht LE, Lowcock LA. Genome size and metabolic rate in salamanders. Comp Biochem Physiol B. 1991; 100B: 83-92.

148.     Vinogradov AE. Nucleotypic effect in homeotherms: body-mass-corrected basal metabolic rate of mammals is related to genomic size. Evolution. 1995; 49: 1249-1259.

149. Vinogradov AE. Nucleotypic effect in homeotherms: body-mass independent resting metabolic rate of passerine birds is related to genome size. Evolution. 1997; 51: 220-225.

150. Gregory TR. A bird's-eye view of the C-value enigma: genome size, cell size, and metabolic rate in the class Aves. Evolution. 2002; 56: 121-130.

151. Gregory TR. The Evolution of the Genome. New York, USA: Elsevier; 2005.

152. Starostova Z, Kubicˇka L, Konarzewski M, Kozłowski J, Kratochvíl L. Cell size but not genome size affects scaling of metabolic rate in eyelid geckos. Am Nat. 2009; 174: E100-E105.

153. Jockusch EL. An evolutionary correlate of genome size change in plethodontid salamanders. Proc R Soc B. 1997; 264: 597-604.

154. Gregory TR. Genome size and developmental complexity. Genetica. 2002; 115: 131-146.

155. Sessions SK, Larson A. Developmental correlates of genome size in plethodontid salamanders and their implications for genome evolution. Evolution. 1987; 41: 1239-1251.

156. Wake DB, Marks SB. Development and evolution of plethodontid salamanders: a review of prior studies and a prospectus for future research. Herpetologica. 1993; 49: 194-203.

157. Brown DD, Dawid IB. Specific gene amplification in oocytes. Science. 1968; 160: 272-280.

158. Ledbetter NM, Bonett RM. Terrestriality constrains salamander limb diversification: implications for the evolution of pentadactyly. J Evol Biol. 2019; 32: 1-11.

159. Martin C, Gordon R. Differentiation trees, a junk DNA molecular clock, and the evolution of neoteny in salamanders. J Evol Biol. 1995; 8: 339-354.

160. Sessions SK. Evolutionary cytogenetics in salamanders. Chromosom Res. 2008; 16: 183-201.

161. Goin OB, Goin CJ, Bachmann K. DNA and amphibian life history. Copeia. 1968; 1968: 532-540.

162. Lertzman-Lepofsky G, Mooers AØ, Greenberg DA. Ecological constraints associated with genome size across salamander lineages. Proc R Soc B. 2019; 286: 20191780.

163. Roth G, Nishikawa KC, Wake DB. Genome size, secondary simplification, and the evolution of the brain in salamanders. Brain Behav Evol. 1997; 50: 50-59.

164. Wake DB, Wake MH, Specht CD. Homoplasy: from detecting pattern to determining process and mechanism of evolution. Science. 2011; 331: 1032-1035.

165. Lynch M, Conery JS. The origins of genome complexity. Science. 2003; 302: 1401-1405.

166. Itgen MW, Prša P, Janža R, et al. Genome size diversification in central American bolitoglossine salamanders (Caudata: Plethodontidae). Copeia. 2019; 107: 560-566.

167. Mohlhenrich ER, Mueller RL. Genetic drift and mutational hazard in the evolution of salamander genomic gigantism. Evolution. 2016; 70: 2865-2878.

168. Liedtke HC, Gower DJ, Wilkinson M, G'omez-Mestre I. Macroevolutionary shift in the size of amphibian genomes and the role of life history and climate. Nat Ecol Evol. 2018; 2: 17921799.

169. Decena-Segarra LP, Bizjak-Mali L, Kladnik A, Sessions SK, Rovito SM. Miniaturization, genome size, and biological size in a diverse clade of salamanders. Am Nat. 2020; 196: 634-648.

170. Cavalier-Smith T. Coevolution of vertebrate genome, cell, and nuclear sizes. In: Ghiara G, ed. Symposium on the Evolution of Terrestrial Vertebrates. Mucchi: Modena; 1991: 51-86.

171. Beaulieu JM, Leitch IJ, Patel S, Pendharkar A, Knight CA. Genome size is a strong predictor of cell size and stomatal density in angiosperms. New Phytol. 2008; 179: 975-986.

172.    Mueller RL. Genome biology and the evolution of cell-size diversity. Cold Spring Harb Perspect Biol. 2015; 7: a019125.

173.    Roth G, Rottluff B, Grunwald W, Hanken J, Linke R. Miniaturization in plethodontid salamanders (Caudata: Plethodontidae) and its consequences for the brain and visual system. Biol J Linn Soc. 1990; 40: 165-190.

174.    Hanken J, Wake DB. Miniaturization of body size: organismal consequences and evolutionary significance. Annu Rev Ecol Evol Syst. 1993; 24: 501-519.

175.    Levy DL, Heald R. Biological scaling problems and solutions in amphibians. Cold Spring Harb Perspect Biol. 2016; 8: a019166.

176.    Grigoryan EN, Radugina EA. Behavior of stem-like cells, precursors for tissue regeneration in Urodela, under conditions of microgravity. Stem Cells Dev. 2019; 28: 423-437.

177.    Sessions SK, Wake DB. Forever young: linking regeneration and genome size in salamanders. Dev Dyn. 2020; in press.

178.    Ferner JW. A review of marking techniques for amphibians and reptiles. Society for the Study of Amphibians and Reptiles; 1979.

179.    Ott JA, Scott DE. Effects of toe-clipping and PIT-tagging on growth and survival in metamorphic *Ambystoma opacum*. J Herpetol. 1999; 33: 344-348.

180.    Monaghan JR, Stier AC, Michonneau F, et al. Experimentally induced metamorphosis in axolotls reduces regenerative rate and fidelity. Regeneration. 2014; 1: 2-14.

181.    Polezhayev LW. The loss and restoration of regenerative capacity in the limbs of tailless amphibia. Biol Rev. 1946; 21: 141-147.

182.    Goss RJ. Principles of Regeneration. New York, NY: Academic Press; 1969.

183.    Muneoka K, Holler-Dinsmore G, Bryant SV. Intrinsic control of regenerative loss in *Xenopus laevis* limbs. J Exp Zool. 1986; 240: 47-54.

184.    Fröbisch NB, Shubin NH. Salamander limb development: integrating genes, morphology, and fossils. Dev Dyn. 2011; 240: 1087-1099.

185.    Pierce SP, Clack JA, Hutchinson JR. Three-dimensional limb joint mobility in the early tetrapod *Ichthyostega*. Nature. 2012; 486: 523-526.

186.    Pierce SP, Hutchinson JR, Clack JA. Historical perspectives on the evolution of tetrapodomorph movement. Integr Comp Biol. 2013; 53: 209-223.

187.    Brockes JP, Gates P. Mechanisms underlying vertebrate limb regeneration: lessons from the salamander. Biochem Soc Trans. 2014; 42: 625-630.

188.    Bonett RM, Phillips JG, Ledbetter NM, Martin SD, Lehman L. Rapid phenotypic evolution following shifts in life cycle complexity. Proc R Soc B. 2018; 285: 20172304.

189.    Colleoni E, Denoël M, Padoa-Schioppa E, Scali S, Ficetola GF. Rensch's rule and sexual dimorphism in salamanders: patterns and potential processes. J Zool. 2014; 293: 143-151.

190.    Reece JS, Mehta RS. Evolutionary history of elongation and maximum body length in moray eels (Anguilliformes: Muraenidae). Biol J Linn Soc. 2013; 109: 861-875.

191.    Caldwell MW. "without a leg to stand on": on the evolution and development of axial elongation and limblessness in tetrapods. Can J Earth Sci. 2003; 40: 573-588.

192.    Dwaraka VB, Voss SR. Towards comparative analyses of salamander limb regeneration. J Exp Zool. 2021; 336: 129-144.

193.    Dequéant ML, Pourquié O. Segmental patterning of the vertebrate embryonic axis. Nat Rev Genet. 2008; 9: 370-382.

194.    Duboc V, Logan MP. Regulation of limb bud initiation and limb-type morphology. Dev Dyn. 2011; 240: 1017-1027.

195.    Buckley D, Moln'ar V, Németh G, Petneh'azy O, Vörös J. 'Monster…-omics': on segmentation, re-segmentation, and vertebrae formation in amphibians and other vertebrates. Front Zool. 2013; 10: 17.

196.    Torok MA, Gardiner DM, Izpisúa-Belmonte JC, Bryant SV. Sonic hedgehog (shh) expression in developing and regenerating axolotl limbs. J Exp Zool. 1999; 284: 197-206.

197.    Han MJ, An JY, Kim WS. Expression patterns of Fgf-8 during development and limb regeneration of the axolotl. Dev Dyn. 2001; 220: 40-48.

198.    Christensen RN, Weinstein M, Tassava RA. Expression of fibroblast growth factors 4, 8, and 10 in limbs, flanks, and blastemas of *Ambystoma*. Dev Dyn. 2002; 223: 193-203.

199.    Imokawa Y, Yoshizato K. Expression of Sonic hedgehog gene in regenerating newt limb blastemas recapitulates that in developing limb buds. PNAS. 1997; 94: 9159-9164.

200.    Stocum DL, Dearlove GE. Epidermal-mesodermal interaction during morphogenesis of the limb regeneration blastema in larval salamanders. J Exp Zool. 1972; 181: 49-61.

201.    Garza-Garcia AA, Driscoll PC, Brockes JP. Evidence for the local evolution of mechanisms underlying limb regeneration in salamanders. Integr Comp Biol. 2010; 50: 528-535.

202.    Ward AB, Mehta RS. Axial elongation in fishes: using morphological approaches to elucidate developmental mechanisms in studying body shape. Integr Comp Biol. 2010; 50: 11061119.

203.    Bajard L, Morelli LG, Ares S, Pécréaux J, Jülicher F, Oates AC. Wnt-regulated dynamics of positional information in zebrafish somitogenesis. Development. 2014; 141: 1381-1391.

204.    Alberch P, Gale EA. A developmental analysis of an evolutionary trend: digital reduction in amphibians. Evolution. 1985; 39: 8-23.

205.    Fabre AC, Bardua C, Bon M, et al. Metamorphosis shapes cranial diversity and rate of evolution in salamanders. Nat Ecol Evol. 2020; 4: 1129-1140.