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How Metamorphosis Is Different in Plethodontids: Larval Life History Perspectives on Life-Cycle Evolution

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Abstract

Plethodontid salamanders exhibit biphasic, larval form paedomorphic, and direct developing life cycles. This diversity of developmental strategies exceeds that of any other family of terrestrial vertebrate. Here we compare patterns of larval development among the three divergent lineages of biphasic plethodontids and other salamanders. We discuss how patterns of life-cycle evolution and larval ecology might have produced a wide array of larval life histories. Compared with many other salamanders, most larval plethodontids have relatively slow growth rates and sometimes exceptionally long larval periods (up to 60 mo). Recent phylogenetic analyses of life-cycle evolution indicate that ancestral plethodontids were likely direct developers. If true, then biphasic and paedomorphic lineages might have been independently derived through different developmental mechanisms. Furthermore, biphasic plethodontids largely colonized stream habitats, which tend to have lower productivity than seasonally ephemeral ponds. Consistent with this, plethodontid larvae grow very slowly, and metamorphic timing does not appear to be strongly affected by growth history. On the basis of this, we speculate that feeding schedules and stress hormones might play a comparatively reduced role in governing the timing of metamorphosis of stream-dwelling salamanders, particularly plethodontids.

Keywords

Corticosterone; Direct development; Growth; Larva; Paedomorphosis; Thyroid hormones

There are more species of plethodontid salamanders than of all other salamander families combined (Frost 2016). The diversification of plethodontids during the Cenozoic has been manifested by both lineage proliferation and morphological disparity (Rabosky and Adams 2012; Shen et al. 2016). Plethodontids also exhibit a diversity of life-cycle strategies that include: (1) a biphasic life cycle with an aquatic larval stage followed by metamorphosis into a more-terrestrial adult, (2) larval-form paedomorphosis through the loss of metamorphosis and the terrestrial adult stage, and (3) direct development with transformation to a terrestrial form before hatching. Biphasic life cycles occur in three

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different clades (the subfamily Spelerpinae, *Hemidactylium* and some *Desmognathus*). Several spelerpine lineages express obligate paedomorphosis (Bonett et al. 2014a,b), but facultative paedomorphosis is uncommon. Direct-developing lineages collectively represent the greatest diversity of salamanders (Wake and Hanken 1996), and range from western North America into South America, with relict species in the Mediterranean and Asia (Dunn 1926; Wake 1966; Min et al. 2005). Lineages with a biphasic life cycle and larval-form paedomorphosis are restricted to eastern North America, especially within the Appalachians, Interior Highlands (Ozark and Ouachita Mountains), and Edwards Plateau (central Texas).

Classical interpretations of plethodontid relationships (Wilder and Dunn 1920; Dunn 1926; Wake 1966) were based on the notion that ancestral life cycles were biphasic, and subsequent life-cycle evolution only occurred through simplification (to direct development or larval-form paedomorphosis). Analyses based on molecular phylogenies have indicated that life-cycle evolution is reversible, however, with complex life cycles secondarily derived from direct-developing and paedomorphic ancestors (Titus and Larson 1996; Chippindale et al. 2004; Mueller et al. 2004; Bonett et al. 2014a,b). With regard to direct development, there are either (1) multiple episodes of direct development from biphasic ancestors, or (2) re-evolution of free-living larvae from direct-developing ancestors, including the possibility that a direct-developing life cycle could be the ancestral life cycle for plethodontids (Chippindale et al. 2004; Mueller et al. 2004). Indeed, ancestral-state reconstruction suggests the possibility that the ancestral plethodontid was direct developing (Bonett et al. 2014b). Also, over a much shorter time frame there is evidence of a reversal from paedomorphosis to a biphasic life cycle in one clade of *Eurycea* from the Edwards Plateau of central Texas (Bonett et al. 2014a).

The possibility that larval periods have been re-evolved from direct-developing ancestors now seems probable (Wake 2009; Bonett et al. 2014a,b). Regardless of the directionality, life-cycle transitions can be explained by heterochrony (i.e., that the timing of metamorphosis, maturation, and hatching is shifted, with the result being a new life-cycle mode; Ryan and Bruce 2000; Bonett et al. 2014b). What remains unresolved is the source of the susceptibility to express significant life-cycle evolution. Ecology, phylogeny, and endocrinology can shed light on how changes in developmental timing have produced the unusual larval forms of plethodontids. By drawing on both classic and recent work in several disparate fields, we postulate several explanations for the unusual patterns of metamorphosis in plethodontids. In particular, we describe aspects of metamorphic biology that differ more in plethodontids than in any other group of amphibians. Specifically, we discuss (1) the natural history of the larval period, (2) experiments on larval growth and metamorphosis, and (3) what is known about the endocrinology of plethodontid metamorphosis. We discuss how these observations fit with the most likely path of life-cycle evolution in this family.

How Is the Larval Life History Different in Plethodontids?

The two principal ways in which the larval life history of plethodontids is different from other amphibians are that plethodontid larvae grow slowly and the larval period is very long (Bruce 1980; Voss 1993). There is an abundant literature on growth in larval amphibians (e.g., Alford 1999; Harris 1999). In their first 30 d of larval life, tadpoles can experience a

40-fold increase in mass (Beachy et al. 1999; Beachy 2001), and larval ambystomatids can increase mass by 25–125 times during this period (Clarkson and Beachy 2015; Ihli and Beachy 2016). In contrast, larval plethodontids do not even double their mass during this period, even when fed ad libitum (Beachy 1995a; O’Laughlin and Harris 2000). Field observations verify the idea that larval growth is slow in plethodontids. For example, both desmognathan larvae (e.g., Beachy 1995a; Bruce 2016a,b) and spelerpine larvae (Bruce 1980; Voss 1993) grow less than 1 mm/mo. These low rates of larval growth also appear to hold for paedomorphic spelerpines (Niemiller et al. 2016; Bendik 2017).

It is possible that these low growth rates are determined by the larval habitat. Most plethodontid larvae occupy streams. These habitats are often characterized by substantially lower productivity (principally just allochthonous leaf fall) than temporary pools and eutrophic lakes (Hynes 1970; Wetzel 1983). Several species of larval plethodontids use productive temporary pools for larval development, yet these species (e.g., *Eurycea quadridigitata* [Semlitsch 1980], *Hemidactylium scutatum* [Blanchard 1923], and *Pseudotriton montanus* [Bruce 1978]) still do not exhibit the remarkable rapid larval growth seen in syntopic amphibians (e.g., tadpoles and larval ambystomatids). In addition, even in growth experiments where larval growth history is directly manipulated (e.g., Alford and Harris 1988), plethodontids fail to grow rapidly. In experiments where at least one growth treatment is to provide ad libitum food, the slowest-growing ambystomatid, *Ambystoma maculatum*, grows an order of magnitude faster than the fastest-growing plethodontid, *Hemidactylium* (Ihli and Beachy 2016; O’Laughlin and Harris 2000; Fig. 1). Interestingly, *Hemidactylium* larvae fed ad libitum (O’Laughlin and Harris 2000) did not grow substantially faster than the stream-dwelling larval *Desmognathus ocoee* fed ad libitum (Beachy 1995b). This indicates that genetics (rather than environment alone) shapes the major differences in larval growth of plethodontids compared with pond-dwelling salamanders from other families.

In addition, larval periods are generally longer in plethodontids than in other amphibians (Beachy and Bruce 1992; Ruben et al. 1993). The long larval period has been associated with several other life-history attributes, including large genome size (Sessions and Larson 1987), long generation times (Hairston 1987), low metabolic scope (Feder 1983), and delayed maturation (Beachy 1995a; Ryan and Bruce 2000; Beachy and Bruce 2003). In any case, the longest larval periods in plethodontids covary with delays in maturation age and consequent large adult body size (Bruce 2016a). This is most clearly seen in the desmognathan plethodontids: the largest, *Desmognathus quadramaculatus*, has the longest larval period (up to 48 mo) and the smallest, *D. wrighti* and *D. aeneus*, are direct developers. Although the spelerpines do not exhibit direct development, the largest species, *Gyrinophilus porphyriticus*, has a larval period that may extend to 60 mo (Bruce 1980). It is important to note that other salamanders with mountain brook (stream-dwelling) larvae from other families such as *Rhyacotriton* also have extended multiyear larval periods (36 to 48 mo; Nussbaum and Tait 1977). Explicit macroevolutionary analyses to test the ecological associations of larval durations have yet to be performed. In fact, the cause–effect relationships involved in the pattern of life-history associations among egg size, larval growth, larval period, juvenile growth, age at metamorphosis, age at maturation, and adult body size among salamanders remain unresolved (Bruce 2016b).

How Is Metamorphosis Different in Plethodontids?

Another remarkable pattern is that plethodontid larvae of several species have been shown not to vary in metamorphic timing when grown at different rates (Table 1). When exposed to variable growth schedules (via different feeding treatments), amphibian larvae can metamorphose at different times (Alford and Harris 1988; Beachy et al. 1999; Beachy 2001). Every kind of animal, except for plethodontids, treated in similar growth experiments will metamorphose at different times (Table 1). Generally, larvae grown rapidly metamorphose early (e.g., Hensley 1993; Leips and Travis 1994; Beachy et al. 1999), but there are cases (restricted to the spadefoots, *Spea* and *Scaphiopus*) where rapidly growing larvae have metamorphosed later than slow growers (Newman 1988, 1998; Morey and Reznick 2000; Boorse and Denver 2004). This last observation is noteworthy given that spadefoot tadpoles inhabit the most ephemeral of pools and metamorphose to inhabit a hostile terrestrial habitat. The emphasis on taking advantage of larval growth opportunity, if available, in this case behaves as optimality theory predicts (Wilbur and Collins 1973; Alford and Harris 1988). Alternatively, the fixed-rate model describes patterns of metamorphic timing that are independent of environmental factors such as growth. In other words, the age of metamorphosis is fixed regardless of growth rate or body size. Larvae of some species show transitions between growth rate-dependent and fixed-rate patterns during ontogeny (Hentschel 1999; Rose 2005).

The timing of metamorphosis is not affected by larval growth in *Desmognathus ocoee* (Beachy 1995b), *Hemidactylium scutatum* (O’Laughlin and Harris 2000), *D. quadramaculatus* (Hickerson et al. 2005), and *Eurycea wilderae* (C.K. Beachy, personal observation). These studies include representative species in all three lineages with biphasic life cycles (i.e., *Hemidactylium*, *Desmognathus*, and the Spelerpinae). Even *H. scutatum*, which has secondarily invaded the more productive pond larval habitat, follows this pattern (O’Laughlin and Harris 2000). This apparent decoupling of growth rate and metamorphic timing is thus far unique to plethodontid larvae, and we suggest that this might be associated with their historical patterns of life-cycle evolution. If ancestral plethodontids were direct developers, then biphasic lineages are derived decelerations of an ancestrally nonfeeding form. Direct developers transform into a fully terrestrial salamander within the egg, and with yolk as a sole resource. Perhaps during their history as direct developers, feeding-based signals (e.g., those produced by fat reserves such as leptin; Crespi and Denver 2006) were lost, played a reduced role in stimulating morphogenesis, or were overridden by other processes. Therefore, the fixed-rate metamorphic pattern predominates. Metamorphosis of the re-evolved free-living larvae of some biphasic plethodontids might be governed by a signaling system more similar to their direct-developing ancestors. A wide range of manipulation and comparative experiments could test this hypothesis.

Is the Metamorphic Response to Thyroid Hormones and Corticosterone Different in Plethodontids?

In amphibians, metamorphosis is regulated by the hypothalamus–pituitary–thyroid (HPT) axis via the production of thyroid hormones (THs), specifically thyroxine (T₄) and

triiodothyronine (T_3 ; Rose 2005, 2009; Brown and Cai 2007; Bonett 2016). Most of what is known has been derived from work with frogs, principally *Xenopus* and *Rana* (e.g., Buchholz et al. 2007), although recent work with ambystomatid salamanders (especially the axolotl, *Ambystoma mexicanum*) has also added to this information (Boorse and Denver 2002; Page et al. 2008, 2009). T_3 is the more active form that regulates transcription and morphogenesis; the conversion of T_4 to the more active T_3 is controlled by deiodinases in the cytoplasm of target tissues. The role of deiodinases has been intensively explored in frogs (e.g., Brown 2006), but not in salamanders (but see Galton 1992). Because simply immersing most larval amphibians in THs induces transcription and morphogenesis, this hormone is an excellent tool for research programs in vertebrate developmental biology.

The influences of TH on plethodontid metamorphosis have been investigated in many species (Table 2; recently reviewed in Bonett 2016), but mechanistic details are limited, especially compared with the metamorphic models of *Xenopus* and axolotl. In most of these cases, TH treatment was only performed to determine if a species could be induced to metamorphose (e.g., Kezer 1952; Dundee 1957, 1962). The early TH treatments were often based on crude doses, limited replication, small sample sizes, and no statistics. Furthermore, it is difficult to compare the responses of different species and life histories because the studies were based on different (1) durations of exposure, (2) TH variants (i.e., T_3 or T_4), (3) concentrations of TH, (4) laboratory conditions, and (5) the assay to determine TH efficacy (e.g., transcriptional response in a target tissue vs. complete metamorphosis by the individual; Table 2). Nevertheless, these early studies demonstrate that several species of *Eurycea* can be at least partially transformed with thyroid hormones. However, not all exogenous treatments of larval plethodontids have produced transformation. A study of young larval *Desmognathus quadramaculatus* treated with T_4 failed to metamorphose (Hickerson et al. 2005).

Analyses of plasma hormones in *Eurycea bislineata* have shown an increase in the number of individuals with elevated levels of circulating TH during natural metamorphosis (Alberch et al. 1986). However, neither T_3 nor T_4 was detected in many individuals that were in the process of metamorphosis. The reason for the lack of TH detection in some individuals was discussed (Alberch et al. 1986), but has not been resolved. Exogenous T_4 treatments of larval *E. bislineata* have demonstrated size- and dose-dependent responses in cranial development (Rose 1995a,b, 1996). *Eurycea bislineata* showed more abrupt changes in the transformation of cranial components compared with larvae of nonplethodontids. One interpretation is that other families have a more gradual increase in plasma TH concentration throughout larval development that is associated with a gradual cranial metamorphosis (Rose 1996, 1999). More recently, a study has shown T_3 sensitivity differences in transcription and metamorphic timing among biphasic and paedomorphic populations of *E. tynerensis* (Aran et al. 2014). It is tempting to suggest an important regulatory role for deiodinases that could account for differences in metamorphic competence among structures, species, and life histories. Perhaps some tissues in premetamorphic-age plethodontid larvae do not produce deiodinases at sufficient concentrations to allow for T_4 to induce metamorphosis early.

The hypothalamus–pituitary–interrenal (HPI) axis can provide a mechanism of detection of environmental stressors (e.g., habitat desiccation). The HPT and HPI axes interact in

complex ways to influence development, growth, and metamorphosis (Kulkarni and Buchholz 2012). For example, the release of corticosterone (CORT) from the interrenal glands can alter the effects of TH on morphogenesis and synergistically influence the rate of metamorphosis (Kikuyama et al. 1983; Bonett et al. 2010). The utility of cross-talk between these axes allows aquatic larvae under environmental stress to accelerate its transition to land. However, most studies that have evaluated the influence of the HPI axis on amphibian metamorphosis have been based on pond-dwelling species.

Analyses of CORT in plethodontids have largely dealt with physiological (rather than developmental) responses in paedomorphic adults (Gabor et al. 2016) and terrestrial adults (Wack et al. 2013; Thomas et al. 2017). Recent experiments indicate synergistic influences of CORT on T₃-induced metamorphosis in *Eurycea tynnerensis*, (R.M. Bonett, personal observations). We predict, however, that a metamorphic response to CORT will be less potent in stream-dwelling compared with pond-dwelling larvae from seasonally ephemeral environments. Most larval plethodontids have multiyear stream-dwelling larvae, even though many live in environments with strong seasonal variation. It is necessary that metamorphosis is not triggered under moderate stress (e.g., brief temperature or water-level changes). A reduced reliability on the stress axis in stream-dwelling plethodontids (compared with other salamanders) might also explain the relative paucity of plethodontids that exhibit facultative life cycles. The context of a direct-developing ancestor and the evolution of a stream-dwelling larval ecology can provide useful framework for designing comparative experiments and further testing the developmental drivers of plethodontid metamorphosis.

Models for Plethodontid Life History and Plasticity

Ryan and Bruce (2000) provided a model of life-cycle evolution in the spelerpine plethodontids that emphasized heterochronic shifts in three key life-history events: hatching, metamorphosis, and maturation. The importance was that the timing of these events can be either accelerated or decelerated, and these episodes of heterochrony in one of these events can be considered either independent or dependent of each of the others. Although this model was focused on the spelerpines (i.e., *Eurycea*, *Gyrinophilus*, *Pseudotriton*, and *Stereochilus*), it can work equally well to understand life-cycle diversity across Plethodontidae.

Bonett (2016) extended this model by incorporating what is known about the endocrinology of amphibian metamorphosis. The transitions between metamorphic and direct-developing life cycles are predicted to be based on genetic changes in baseline TH release or responsiveness (Bonett 2016). Other endocrine axes might also play a role in influencing variation around baseline HPT patterns (Bonett 2016), but these factors have yet to be tested. Furthermore, it is reasonable to consider that endocrine patterns have been optimized to match diverse larval habitats. Determining the relative importance of HPT and HPI regulation of metamorphic timing among lineages with different ecologies and life-history modes will enlighten our understanding of the developmental patterns that have shaped plethodontid life-cycle evolution.

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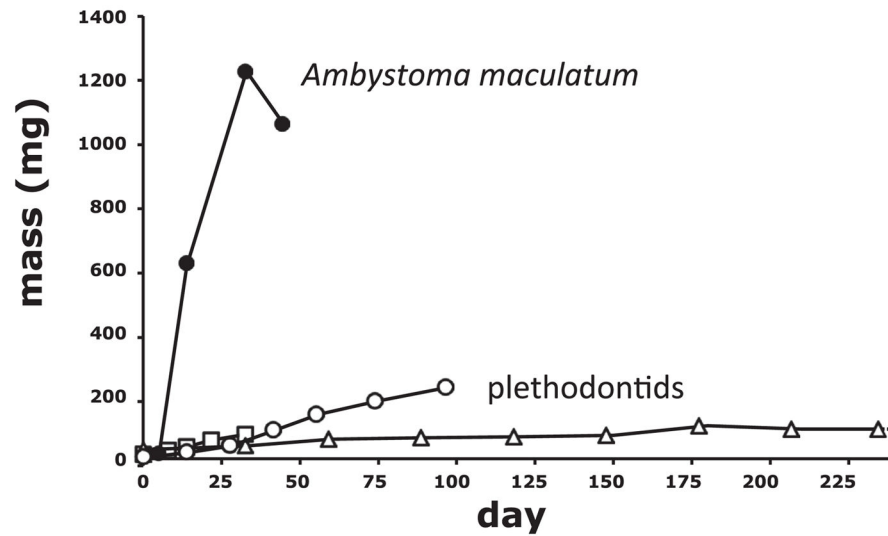


Fig. 1.

Larval growth profiles of several amphibians. Growth profiles compare the larval growth and metamorphosis of the plethodontid salamanders *Desmognathus ocoee* (triangles), *Eurycea quadridigitata* (open circles), and *Hemidactylum scutatum* (squares) with the ambystomatid salamander, *Ambystoma maculatum*. Each profile begins at first mass taken after hatching, and terminates at the mean time and size at metamorphosis in each taxa. *Ambystoma maculatum* has the slowest growth for any larval ambystomatid. In contrast, *E. quadridigitata* and *H. scutatum* have the fastest growth for any larval plethodontid. The profiles presented here are those taken from fastest-growing treatment groups in each experiment. Growth treatments were initiated when hatchling larvae were placed in individual containers and fed prey items ad libitum. Profiles begin with the first measurement of mass and terminate at the mean time of metamorphosis, save for *E. quadridigitata*, where the experiment was terminated before the completion of metamorphosis. The profiles are taken from Beachy (1995b) for *D. ocoee*, T.J. Ryan and B. Rothermel (personal observations) for *E. quadridigitata*, O’Laughlin and Harris (2000) for *H. scutatum*, and Ihli and Beachy (2016) for *A. maculatum*.

Table 1

Growth experiments on various taxa wherein growth of individual larvae was manipulated and individual responses including metamorphic timing were recorded and evaluated.

Taxon	Growth treatment affected metamorphic timing?	Reference
Caudata		
Plethodontidae		
<i>Desmognathus quadramaculatus</i>	No	Hickerson et al. 2005
<i>Desmognathus ocoee</i>	No	Beachy 1995b
<i>Hemidactylum scutatum</i>	No	O'Laughlin and Harris 2000
<i>Eurycea wilderae</i>	No	C.K. Beachy, personal observation
Ambystomatidae		
<i>Ambystoma maculatum</i>	Yes	Ihli and Beachy 2016
<i>Ambystoma tigrinum</i>	Yes	Ihli and Beachy 2016
<i>Ambystoma mexicanum</i> ²	Yes	Clarkson and Beachy 2015
<i>Ambystoma talpoideum</i>	Yes	Ryan and Semlitsch 2003
Salamandridae		
<i>Salamandra infraimmaculata</i>	Yes	Marburg 2009
<i>Neureergus microspilotus</i>	Yes	Vaissi and Sharifi 2016
Anura		
Bufonidae		
<i>Anaxyrus americanus</i>	Yes	Beachy 2001
<i>Anaxyrus woodhousei</i>	Yes	Alford and Harris 1988
Hylidae		
<i>Hyla versicolor</i>	Yes	Beachy et al. 1999
<i>Hyla chrysoscelis</i>	Yes	Audo et al. 1995
<i>Hyla squirella</i>	Yes	Beck 1997
<i>Hyla gratiosa</i>	Yes	Leips and Travis 1994
<i>Hyla cinerea</i>	Yes	Leips and Travis 1994
<i>Pseudacris crucifer</i>	Yes	Hensley 1993
Pelobatidae		
<i>Spea hammondi</i>	Yes	Morey and Reznick 2000; Denver et al. 1998
<i>Scaphiopus couchii</i>	Yes	Newman 1989, 1998; Morey and Reznick, 2000
<i>Spea intermontana</i>	Yes	Morey and Reznick, 2000
Pipidae		
<i>Xenopus laevis</i>	Yes	McCoy et al. 2007
Arthropoda		
Crustacea		
<i>Mesocyclops edax</i>	Yes	Twombly 1996
<i>Balanus glandula</i>	Yes	Hentschel and Emlet, 2000
<i>Petrolisthes cabrilloi</i>	Yes	Howard and Hentschel 2005
Insecta		
<i>Aedes aegypti</i>	Yes	Zeller and Koella 2016

Taxon	Growth treatment affected metamorphic timing?	Reference
<i>Oncopeltus fasciatus</i>	Yes	Blakley 1981
<i>Oncopeltus cingulifer</i>	Yes	Blakley 1981
<i>Lestes viridis</i>	Yes	Rolff et al. 2004

^a *Ambystoma mexicanum* are obligately paedomorphic. Larvae in this experiment were induced to metamorphose by immersion in 5 nM T₄.

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Table 2

Summary of studies on plethodontids treated with thyroid hormone (TH). T₃ = triiodothyronine; T₄ = thyroxine.

Taxon	TH	Dosage (nM)	Duration (d)	Metamorphic response?	Reference
Larvae					
<i>Desmognathus quadramaculatus</i>	T ₄	1.2–4.8	62	No	Hickerson et al. 2005
<i>Eurycea bislineata</i>	T ₄	50	1–2	Yes	Alberch et al. 1985
<i>Eurycea bislineata</i>	T ₄	0.05	84–168	No	Rose 1995a
<i>Eurycea bislineata</i>	T ₄	0.5	42	Partial	Rose 1995a
<i>Eurycea bislineata</i>	T ₄	5–50	21	Yes	Rose 1995a
<i>Eurycea tynerensis</i>	T ₃	25	21	Yes	Aran et al. 2014
Paedomorphs					
<i>Eurycea neotenes</i>	T ₄	1200–2400	19	Yes	Kezer 1952
<i>Eurycea rathbuni</i>	T ₄	1200–12,000	53	Yes	Dundee 1957
<i>Eurycea tynerensis</i>	T ₃	25	21	No	Aran et al. 2014
<i>Eurycea tynerensi</i>	T ₄	2400	17	Yes	Kezer 1952
<i>Eurycea wallacei</i>	T ₄	600–12,000	15–25	Yes	Dundee 1962
<i>Gyrinophilus palleucus</i>	T ₄	2400	120–470	Yes	Dent and Kirby-Smith 1963
Direct developers					
<i>Plethodon cinereus</i>	T ₄	6000–12,000	9	Yes	Dent 1942