J. Zool., Lond. (1996) **239,** 253–284

An endocrine-based model for developmental and morphogenetic diversification in metamorphic and paedomorphic urodeles

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(*Accepted 3 May 1995*)

(With 7 figures in the text)

Mechanistic interpretations of the diversity in urodele cranial ontogenies have focused largely on the primary distinctions of metamorphic versus paedomorphic forms and obligate versus facultative expressions of the latter. These distinctions, however, do not address the underlying spectrum of developmental and morphogenetic patterns in thyroid hormone (TH)-mediated tissues. This study integrates empirical and comparative observations on TH-mediated remodelling to formulate a new endocrine-based model to explain cranial diversification within and between metamorphic and paedomorphic urodeles. The dose-dependent remodelling induced by TH in a metamorphic urodele, the hemidactyliine plethodontid *Eurycea bislineata*, is compared against ontogenetic and phylogenetic variation in the same remodelling across Urodela. Immersion of *Eurycea* larvae in a T_4 concentration within the range of plasma T_4 levels found in natural *Eurycea* metamorphs results in rapid, synchronous, and complete metamorphic tissue responses as in natural plethodontid development. In contrast, lower doses produce gradual, incomplete remodelling patterns that bear greater resemblance to nonplethodontid development. A large proportion of remodelling events shows a strong correspondence between their sensitivity to TH in *Eurycea* and both their range of occurrence and developmental sequence in nonplethodontids. Also, the morphogenesis exhibited by certain tissues at low TH, although aberrant for plethodontids, is similar to natural development in nonplethodontids. These findings suggest that the widespread dissociation evinced by urodele cranial ontogenies may owe more to variable thyroid activity than previously realized. In particular, the abruptly metamorphic ontogeny of plethodontids and the varying degrees of metamorphic remodelling exhibited by facultative and obligate paedomorphs would seem to be explained more parsimoniously by specific changes in the profile of TH activity than by independent changes in individual tissue sensitivities. The corollary, that tissue sensitivity is largely conserved in urodeles, raises important implications for understanding character evolution, homology, and dissociation in metamorphic systems.

Introduction

Perennibranchiation—the retention of larval gills in sexually mature urodeles—plays a major role in the diversification of their morphology (Duméril, 1865; Kollmann, 1885; Gadow, 1903; Gould, 1977; Raff & Kaufman, 1983; McKinney & McNamara, 1991). However, despite over 70 years of research, the developmental basis of this phenomenon remains unresolved. Mechanistic investigators have focused largely on the activity of the neuroendocrine axis, with the tacit assumption that perennibranchiation and the more general phenomenon of nontransformation or larval paedomorphosis (a term coined by Collazo & Marks, 1994 for adult urodeles that retain

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a number of larval features) represent only one or a few types of ontogenetic transformation. However, a survey of the morphological literature quickly reveals that urodele paedomorphosis and metamorphosis are both very much relative phenomena, pertaining to broad, almost continuous, ranges in cranial ontogeny and morphogenesis. The aim of this paper is to summarize this variation and to present an empirically based model that accounts for a significant proportion of the developmental variation within, as well as between, metamorphic and paedomorphic urodeles.

By analogy with anurans, metamorphic development in urodeles is attributed to tissues responding at successively higher threshold concentrations of plasma thyroid hormone (TH) (Kollros, 1961; Etkin, 1964; Kaltenbach, 1968). Paedomorphic development in urodeles has been traditionally attributed to change in one or both of two primary parameters, tissue sensitivity and thyroid activity. The former term refers to the threshold or minimum concentration of plasma TH required to initiate a morphogenetic response for each tissue; the latter term is used here to refer to the levels of plasma TH produced during larval ontogeny. Facultative paedomorphs[1], which are exclusive to primitively metamorphic families, e.g. Ambystomatidae, Salamandridae, and Plethodontidae, are characterized by full sensitivity to TH in tissues that remodel in confamilials (Keller, 1946; Kezer, 1952; Snyder, 1956; Dent & Kirby-Smith, 1963; Prahlad & Delanney, 1965; Dent, 1968; Tompkins, 1978) and low thyroid activity (Dundee & Gorbman, 1960; Rosenkilde, 1985; Kühn & Jacobs, 1989). Failure to metamorphose is usually attributed to a regulatory block at the hypothalamic or hypophysial level of the neuroendocrine axis, although low temperature and elevated prolactin may also be involved (reviews by Dodd & Dodd, 1976; White & Nicoll, 1981; Larras-Regard, 1985; Matsuda, 1987; Kühn & Jacobs, 1989). Genetic analyses implicate multiple loci that may differ both between and within species (Shaffer, 1993). Obligate paedomorphs, such as cryptobranchids, sirenids, and proteids, are characterized by low thyroid activity (Norris & Platt, 1973), low tissue sensitivity (Noble, 1924; Gutman, 1926; Durand & Vandel, 1968; Svob *et al.*, 1973), low genetic variation (Shaffer & Breden, 1989), large cell size and DNA content (Cavalier-Smith, 1978; Horner & MacGregor, 1983; Szarski, 1983), and, to a lesser degree, low metabolic rate (Duke & Ultsch, 1990; Licht & Lowcock, 1991). Whereas low genetic variation and low metabolic rate are more likely to be consequences than causes of perennibranchiation (Shaffer & Breden, 1989; Licht & Lowcock, 1991), high DNA content and large cell size correlate with, and may be causal for, a slower developmental rate (Horner & MacGregor, 1983; Sessions & Larson, 1987). However, it is not clear that this effect would be manifest throughout larval development, the duration of which varies substantially in urodeles in response to both ecological and adaptive factors (Horner & MacGregor, 1983). Since the loss of either tissue sensitivity or thyroid activity is sufficient to preclude metamorphosis, it is difficult to resolve which of these parameters may have played the primary role (Dent, 1968; Larsen, 1968).

The aim of this paper is to integrate empirical and comparative data on TH-mediated remodelling in order to evaluate the respective roles of thyroid activity and tissue sensitivity in

[1] Though well entrenched in the literature, the term facultative paedomorphosis has drawn recent criticism on the grounds that an intraspecific polymorphism by definition is not an evolutionary relationship, i.e. a difference between ancestral and descendent species (Reilly, 1994). Further, of the species commonly regarded as facultative paedomorphs, some are genetically fixed for their developmental truncation, e.g. *Ambystoma mexicanum*, while others express the trait as a temporary, environmentally induced delay in some or all metamorphic remodelling, e.g. *A. talpoideum* and *Notophthalmus viridescens* (Reilly, 1994). It is still not clear how many (if any) species have a genetically-controlled capacity to truncate its development permanently in response to environmental cues.

urodele evolution. More specifically, the potential for variation in thyroid activity alone to account for differences in cranial ontogeny is estimated by comparing the range in developmental sequences and morphogenetic patterns that are empirically inducible in a metamorphic model with the natural diversity exhibited by other metamorphic and paedomorphic urodeles. The remodelling induced at three thyroxine (T_4) doses in larvae of the hemidactyliine plethodontid, *Eurycea bislineata* (Rose, 1995*b*) is compared against natural development in representative species of all urodele families except amphiumids. The highest dose, which lies within the range of natural plasma T_4 levels measured in *Eurycea* metamorphs (Alberch, Gale & Larsen, 1986), produces remodelling that is similar and exclusive to natural plethodontid development. In contrast, the remodelling produced by lower doses exhibits several unexpected similarities to natural remodelling in nonplethodontids. These similarities suggest that certain trends in urodele ontogeny—the shift to an abrupt metamorphosis in plethodontids and the progressive reduction of metamorphic remodelling in paedomorphic urodeles—may be explained by specific changes in thyroid activity. They also offer insight into the developmental control of character evolution and dissociation in TH-mediated systems.

Materials and methods

The 21 skeletal and 5 soft tissue remodelling events surveyed in this study are listed in Table I. Natural remodelling of skeletal tissues in *Eurycea bislineata* has already been described from 238 embryonic, larval, metamorphic, and postmetamorphic specimens (Rose, 1995*a*). TH-induced remodelling in these tissues has been described from larvae immersed in 5×10^{-9}, 5×10^{-10}, and 5×10^{-11} M L-thyroxine (3,5,3′,5′-tetraiodo-L-thyronine or T_4) (N = 44, 53, and 41, respectively; Rose, 1995*b*). The natural and induced remodelling of soft tissues described here derives from sectioned specimens examined in these studies. This paper describes only that remodelling which is consistently induced in the 3 smaller size-age classes of Rose (1995*b*); these classes include first year larvae collected in the autumn and spring (1Autumn and 1Spring), and second and third year larvae collected in the autumn (2Autumn), at least half a year before the first occurrence of metamorphosis in this species.

Representative species of other urodele families were selected on the basis of literature sources and specimens available for scoring the 26 remodelling events listed above. Skeletal references are listed in Tables III and IV; skin references are from Grant, 1930 and Noble, 1931; additional references for

TABLE I

Remodelling events scored in ***Eurycea bislineata***

Morphogenetic process	Endochondral bones	Dermal bones	Cartilages	Soft tissues
Formation	Footplate and stylus of the columella, Orbitosphenoid, Os thyroideum	Maxilla, Septomaxilla, Prefrontal, Nasal, Parasphenoid tooth patch, Extension of frontal over nasal capsule, Extension of vomer into anterior shelf	Nasal capsule, Pterygoid process, Radials, Lingual, Adult ceratobranchial	Jacobson's organ, Skin glands
Resorption		Pterygoid, Coronoid	Larval ceratobranchials, Scleral cartilage, Urohyal	Leydig cells, Gills, Tail fin

Jacobson's organ are Seydel, 1895; Anton, 1908; Bruner, 1914; and Jurgens, 1971. The specimens examined include 51 *Ambystoma maculatum* (SVL (snout-vent length) = 7.5–43.1 mm), 6 *Dicamptodon ensatus* (SVL = 25–57 mm), 22 *Pseudobranchus striatus* (SVL = 32–79 mm), 10 *P. axanthus* (SVL = 41–96 mm), 3 *Pseudobranchus* of unknown species (SVL = 81–90 mm), 16 *Siren intermedia* (12 of TL (total length) = 16–85 mm and 4 of SVL = 83.8–137.1 mm), 10 *Notophthalmus viridescens* (SVL = 16–38 mm), 8 *Cryptobranchus alleganiensis* (4 of TL = 27–39 mm, 3 of SVL = 20–24 mm, and 1 postmetamorphic specimen of unknown size), 3 *Hynobius retardatus* (TL = 50–53 mm), 1 *H. nigrescens* (SVL = 65 mm), 4 *Necturus alabamensis* (SVL = 23.2–29.0 mm), 5 *N. maculosus* (SVL = 40.1–72.8 mm), and 5 *Haideotriton wallacei* (SVL = 16–27 mm) which had been treated previously with thyroxine (Dundee, 1962). See **Appendix A** for collecting locations or catalogue numbers, sample sizes, and stages of specimens prepared as whole-mounts and histological slides. All specimens prepared for this study were fixed in 10% neutral-buffered formalin. Whole-mounts were cleared with trypsin and KOH and stained with Alcian blue and Alizarin red for cartilage and bone, respectively (Hanken & Wassersug, 1981). Histological specimens were sectioned transversely at 5 or 10 μm and stained with a quadruple connective tissue stain (Hall, 1986); previously prepared histological specimens had been stained in haematoxylin-eosin.

Most remodelling events are scored as the simple loss or gain of a character, although certain events in certain urodeles require some clarification of character homology and/or elaboration of scoring criteria (see **Results**). In the absence of common staging criteria for all urodeles, the timing and duration of individual remodelling events in natural development are expressed relatively, on the basis of size and stage differences between specimens. When appropriate, events are grouped into a larval period (following completion of the larval hyobranchium, but prior to gill loss) and metamorphic period (simultaneous with gill loss and the move to land, except for *Hynobius nebulosus* and *Pleurodeles waltlii* which lose their gills but may remain aquatic).

Results

Homologization of remodelling events

The second basibranchial ossification in proteids (Wilder, 1903; Marche & Durand, 1983) and sirenids (Hilton, 1946) is homologized here with the os thyroideum of metamorphic urodeles; this element is lacking in most salamandrids (Hilton, 1947) and both cryptobranchid genera (Edgeworth, 1923*a*). Though the stylus and footplate of the columella are generally present as distinct parts of one cartilage, these elements are scored as distinct ossifications only when each part is known to ossify from a distinct centre. The stylus and footplate of the columella are indistinguishably fused with the otic capsule in salamandrids, and hence treated as absent in this group (Kingsbury & Reed, 1909; Larsen, 1963; Monath, 1965).

This paper uses Parsons's (1967) definition of Jacobson's organ or vomeronasal organ as an outpocketing of the nasal sac, rather than an outpocketing plus sensory epithelium. The ventrolateral diverticulum that forms this organ in adult *Eurycea* is present in the larva as a small lateral recess of the nasal sac. Its metamorphic development is marked by a substantial size increase, change in proportions, and the development of a sulcus maxillopalatinus, a lateral outpocketing of the nasal sac at the level of the choana (Wilder, 1925). This development is scored in induced *Eurycea* and other metamorphic forms by the presence of the sulcus maxillo-palatinus, and in paedomorphic forms by the presence of a prominent ventrolateral (ventromedial in sirenids) diverticulum.

This paper follows Jurgens' (1971) interpretation of, and terminology for, the lateral fenestration of the nasal capsule in urodeles (see p. 105 and fig. 107 in Jurgens, 1971). The three large foramina shared by most metamorphic urodeles are the narial foramen, the lateral fenestra which lies immediately posterior to the narial foramen, and the incisura ectochoanalis

which is posteroventral to the lateral fenestra and may be separated from the basal fenestra by ectochoanal cartilage.

Urodeles exhibit variation in several remodelling events in the palate and hyobranchium. Though the pterygoid process of the palatoquadrate generally chondrifies as a short process that runs anteriorly off the ascending process of the palatopterygoid (Edgeworth, 1925), this process in cryptobranchids and some hynobiids extends further anteriorly to fuse with either the trabecula or antorbital process (Edgeworth, 1923*b*). The anterior connection, which is always resorbed later in larval development, is generally interpreted as a primitive feature that has been lost by most groups (Edgeworth, 1925). Hence, this study scores only formation of the posterior part of the process. This paper also follows Huxley (1874), Wiedersheim (1877), Parker (1877, 1882), Winslow (1896) and Wilder (1903) in regarding the pterygoid process as absent in sirenids and proteids, although Edgeworth (1925) and Bourque (1939) identify one in *Siren*, and Holmgren (1949: fig. 10) indicates one in *Necturus* at 20 mm TL.

Whereas plethodontids lack a distinct palatine bone and resorb the entire pterygoid bone at gill loss, nonplethodontids bear either separate or fused palatine and pterygoid bones (Larsen, 1963; both conditions are referred to here as a palatopterygoid) and generally resorb only the palatine portion, although sirenids resorb the anterior two-thirds of the pterygoid. All three resorptions are scored here as homologous events at the level of a palatopterygoid bone resorption.

Though most metamorphic urodeles develop a transverse vomerine tooth row, plethodontids, salamandrids, and some hynobiids develop a longitudinal tooth row and the former two groups extend this tooth row into a parasphenoid tooth patch (Larsen, 1963; Regal, 1966). However, the tooth row develops from the posteromedial edge of the vomer in plethodontids and from the posterolateral edge in salamandrids; hence the resulting tooth patches are not considered homologous (Regal, 1966). Although much shorter, the hynobiid tooth row forms in the same way as the plethodontid tooth row and may be homologous on this basis (Larsen, 1963; Regal, 1966).

Since the choanal notch of cryptobranchids forms posterior to the vomerine tooth row, it is considered not homologous with the choanal notches that form anterior to this tooth row in other urodeles (Larsen, 1963).

The lingual and radial cartilages of *Eurycea* are homologized with the anterior and posterior radials of most metamorphic nonplethodontids, and the single pair of anterior hyobranchial processes in hynobiids is homologized with anterior radials. These determinations are based on the relationship of these cartilages to the hyoid arch. The anterior pair of processes in ambystomatids derive from hypophyals, whereas the posterior pair arises *de novo* (Reilly & Brandon, 1994). The single pair of processes in hynobiids also derive from hypophyals (Tsusaki, 1922; Edgeworth, 1935). The lingual in *Eurycea* chondrifies from procartilage that is initially continuous with the medial ends of the ceratohyals, whereas the radial condensations arise *de novo*; this is particularly well demonstrated by an induced specimen described in Rose (1995*b*). Though these observations contradict Edgeworth's (1935) claim that posterior radials derive from hypohyals and anterior radials and lingual arise *de novo*, they are supported by most workers on ambystomatids (e.g. Theron, 1952; Papendieck, 1954; Reilly & Brandon, 1994) and salamandrids (e.g. Stadtmüller, 1936).

Distribution of remodelling events

Whereas metamorphic nonplethodontids undergo most of the remodelling events scored in this study, obligate paedomorphs exhibit a progressive reduction of events (Table II). Further,

TABLE II

Remodelling events listed cumulatively by taxon and tissue sensitivity in ***Eurycea***

Taxon	T_4 dosage		
	5×10^{-11} M	5×10^{-10} M	5×10^{-9} M
Necturus	Chondrification of nasal capsule, Ossification of footplate,[1] stylus[1] and os thyroideum[2,3]	Formation of skin glands	
Pseudobranchus	Ossification of nasal and orbitosphenoid	Resorption of pterygoid bone[3], Formation of Jacobson's organ	
Siren		Ossification of maxilla	Loss of Leydig cells
Cryptobranchus	Ossification of anterior extension of frontal	Ossification of prefrontal, Chondrification of pterygoid process	Resorption of gills
Andrias			Resorption of larval ceratobranchials
[Ambystomatids (A), Salamandrids (S), Hynobiids (H) Dicamptodontids (D) Rhyacotritonids (R)]		Formation of parasphenoid tooth patch (H)[3] Resorption of coronoid (A, S, H, R) Ossification of anterior extension of vomer (A, S, H, D, R)	Resorption of scleral cartilage (S, H) Ossification of septomaxilla (A, H, D, R), Chondrification of radial and lingual cartilages (A, S, D, R)[3], Resorption of urohyal and tail fin (A, S, H, D, R),
Plethodontids			Chondrification of adult ceratobranchial

[1] Does not occur in *Pseudobranchus* and salamandrids
[2] Does not occur in cryptobranchids and some salamandrids
[3] See text for a discussion of character homology

the distribution of the events in obligate paedomorphs bears some correspondence to the minimum dose required to elicit their morphogenesis in *Eurycea*. *Necturus*, which undergoes the least remodelling, exhibits five events, four of which (nasal capsule chondrification and the ossification of three endochondral bones) are inducible at 5×10^{-11} M in *Eurycea*. *Pseudobranchus* exhibits two more events inducible at this dose, plus two events inducible at 5×10^{-10} M (pterygoid resorption and formation of Jacobson's organ). *Siren* exhibits another event inducible at 5×10^{-10} M (maxilla ossification), plus one inducible at 5×10^{-9} M (Leydig cell loss). *Cryptobranchus* exhibits five more events covering the range of tissue sensitivity and *Andrias* exhibits an additional event inducible at 5×10^{-9} M (larval ceratobranchial resorption). The remaining events, almost all of which either are inducible at 5×10^{-9} M or need 5×10^{-9} M to be completed (see below), are restricted to metamorphic urodeles. Of those inducible at 5×10^{-9} M, chondrification of the posterior radials does not occur in hynobiids, septomaxilla ossification does not occur in salamandrids (Larsen, 1963), scleral cartilage resorption does not occur in ambystomatids or *Rhyacotriton* (Cloete, 1961; Srinivasachar, 1962) and is incomplete in some hynobiids and salamandrids (Stadtmüller, 1929), and resorption of the second ceratobranchial is incomplete in some hynobiids (Tsusaki, 1992; Edgeworth, 1923*a*; Cox & Tanner, 1989).

Some correspondence between range of occurrence and tissue sensitivity is also evinced by the

remodelling of larval paedomorphs in metamorphic families. These include various hemidactyliine plethodontids, the hynobiid *Onychodactylus japonicus*, the ambystomatids *Ambystoma talpoideum*, *A. mexicanum*, *A. rivularis*, and *A. altamirani*, and the salamandrids *Notophthalmus viridescens* and *Triturus vulgaris*. Whereas no hemidactyliine paedomorphs are known to exhibit any postembryonic remodelling beyond larval endochondral ossifications (Rose, 1995*a*, and references therein), all of the remaining paedomorphs additionally develop at least the nasal skeleton and maxilla. *Ambystoma mexicanum* also retains the shift from larval to adult haemoglobin, which is more sensitive than most, if not all, morphological events accompanying gill loss (Ducibella, 1974*a*, *b*; Tompkins & Townsend, 1977). *Ambystoma talpoideum* also exhibits partial resorption of the palatopterygoid (Reilly, 1987) and *A. rivularis* and *A. altamirani* exhibit a partially remodelled vomer plus a partially completed adult hyobranchium (Reilly & Brandon, 1994). The two salamandrid paedomorphs complete all skull remodelling except in the vomer and palatopterygoid (Reilly, 1986, 1987; Marconi & Simonetta, 1988) and a variable amount of hyobranchial remodelling (Noble, 1929; Reilly, 1987). Hence, most of the differences among nonplethodontid paedomorphs concern remodelling events that respond at 5×10^{-10} M or higher in *Eurycea*. Interestingly, whereas *A. rivularis* and *A. altamirani* fail to complete the anterior, largely progressive aspects of hyobranchial remodelling, *N. viridescens* paedomorphs fail in the posterior, largely regressive aspects of this remodelling (cf. Reilly & Brandon, 1994 with Noble, 1929 and Reilly, 1987).

Developmental pattern of remodelling events

The sequence and relative duration of remodelling events induced in *Eurycea* at 5×10^{-9} M bears a strong resemblance to natural development in this species (see Table III and Fig. 1). In induced larvae, all 26 events are completed over the second, third, and fourth weeks of treatment. In natural development, all events except ossification of the four larval endochondral bones occur in a brief metamorphic period at 2–3 years of age. In contrast, patterns induced at the lower two doses in *Eurycea* bear greater similarity to nonplethodontid development, with a large proportion of remodelling events showing some correspondence between their tissue sensitivity in *Eurycea* and their timing of occurrence in nonplethodontids.

The first skeletal event induced in *Eurycea* at 5×10^{-11} M, chondrification of the nasal capsule, begins soon after embryonic development in almost all nonplethodontids and continues throughout most of the larval period; only sirenids appear to initiate this event in synchrony with embryonic skull chondrifications. Chondrification of the pterygoid process, which has a sensitivity of 5×10^{-10} M in *Eurycea* and does not occur in proteids and sirenids, generally occurs midway or late in the larval periods of other urodeles (Fig. 1, plus Stadtmüller, 1924 on *Salamandra salamandra*). Most of the six dermal ossifications, which are also first inducible in *Eurycea* at 5×10^{-10} M, likewise appear at larval stages in most nonproteid urodeles (Table III); *Pleurodeles waltlii* appears to be unusual for salamandrids as both *Notophthalmus viridescens* (Reilly, 1986) and *Triturus vulgaris* (Erdmann, 1933) acquire all six bones before gill loss. Resorptions of cartilage, which show a sensitivity of 5×10^{-9} M in *Eurycea*, occur at the stage of gill loss in *Andrias* and metamorphic forms, though this response is consistently expressed only by the posterior two larval ceratobranchials and the urohyal.

The natural developmental sequence of nasal capsule, pterygoid process and dermal ossifications, and eventually cartilage resorption is also exhibited in the two metamorphic families not depicted in Fig. 1 and Table III. Of the six *Dicamptodon* (Dicamptodontidae) larvae examined in

TABLE III

Ossification sequences

T-4 induced sequences in *Eurycea bislineata* (Plethodontidae)[1]

5×10^{-9} M		5×10^{-10} M		5×10^{-11} M	
Endochondral	Dermal	Endochondral	Dermal	Endochondral	Dermal
Os thyroideum	Maxilla	{Footplate,	Frontal ext	Footplate	Frontal ext
Orbitosphenoid	Frontal ext	Orbitosphenoid}	Nasal	Orbitosphenoid	Nasal
Footplate	Vomer ext	Os thyroideum	Vomer ext	Os thyroideum	
Stylus	Septomaxilla	Stylus	Maxilla	Stylus	
	Prefrontal		Prefrontal		
	Nasal		±Septomaxilla		

Natural sequences for representatives of each urodele family[9]

Plethodontidae	Ambystomatidae	Hynobiidae	Salamandridae	Cryptobranchidae	Sirenidae	Proteidae
Eurycea bislineata[2]	*Ambystoma maculatum*[3]	*Hynobius* sp.[4]	*Pleurodeles waltlii*[5]	*Cryptobranchus* and *Andrias*[6]	*Siren intermedia*[7]	*Necturus maculosus*[8]
Footplate	**Os thyroideum**	{Maxilla,		{Nasal,	{**Os thyroideum,**	**Footplate/stylus**
Orbitosphenoid	Maxilla	Nasal}	**Orbitosphenoid**	Maxilla,	Nasal}	**Os thyroideum**
Os thyroideum	Prefrontal	{Prefrontal,	{Maxilla,	Prefrontal}	**Orbitosphenoid**	
Stylus	**Footplate/stylus**	Septomaxilla}	Nasal,	Frontal ext	Maxilla	
Maxilla	{Frontal ext,	Vomer ext	Prefrontal}	{**Orbitosphenoid,**		
{Frontal &	Septomaxilla,	{**Os thyroideum,**	{Vomer ext	**Footplate/stylus**}		
Vomer ext}	**Orbitosphenoid**}	**Footplate/stylus**}	Frontal ext?}			
Septomaxilla	Nasal	**Orbitosphenoid**?				
Prefrontal	Vomer ext					
Nasal						

[1] When considered separately, induced endochondral and dermal bones follow clearly defined modal sequences. When these bones are grouped together, a modal sequence is usually not evident, possibly as a result of the disparate timing of endochondral and dermal ossifications in natural *Eurycea* development.

[1–8] See **Appendix C** for source material and references for ossification sequences in confamilials.

[9] Endochondral bones are listed in bold print; horizontal lines separate bones in larval, metamorphic, and postmetamorphic periods.

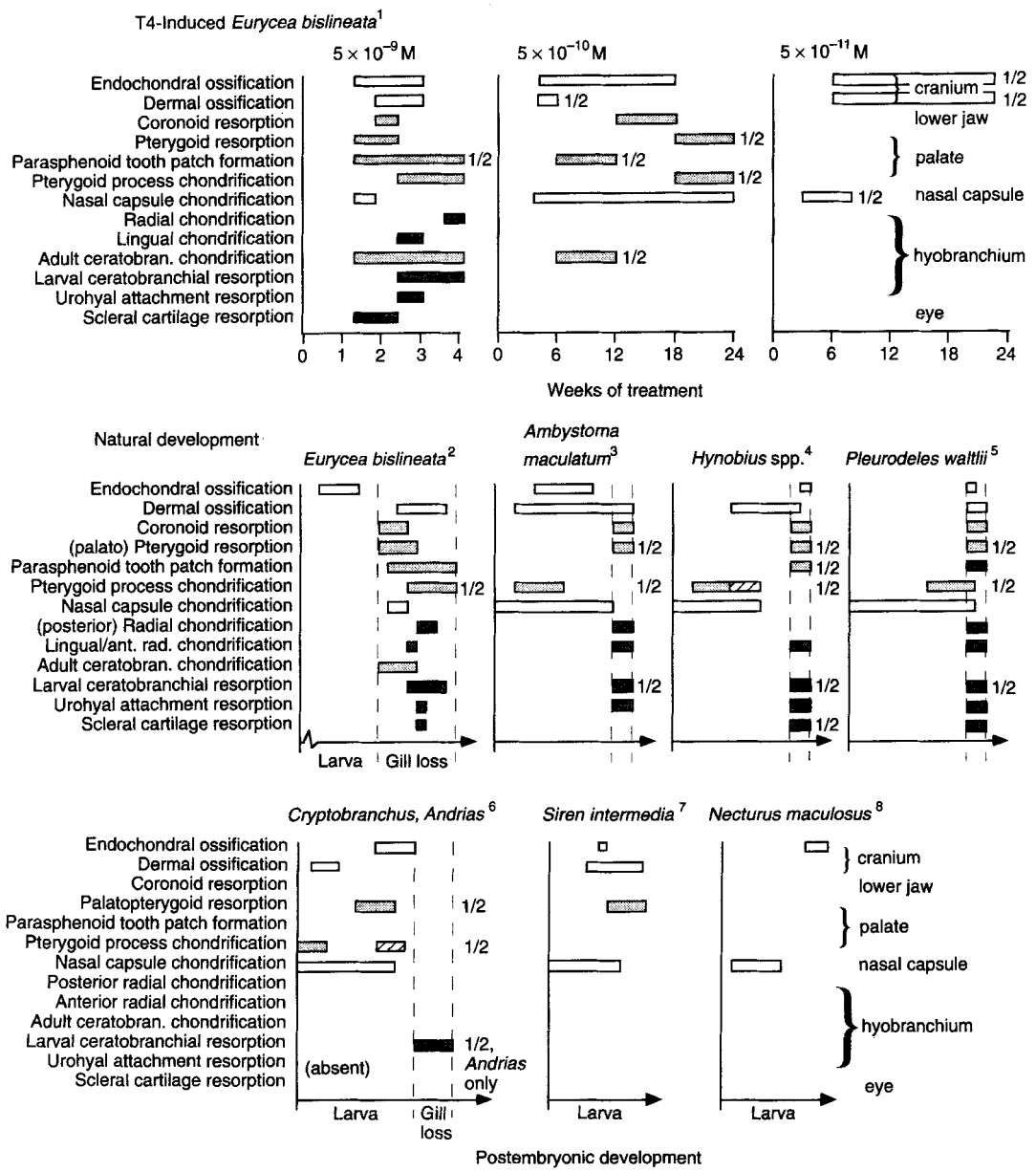

FIG. 1. Developmental patterns of skeletal remodelling in T_4-induced and untreated *Eurycea* and representative nonplethodontids. Clear, light and dark stipple indicate tissue sensitivities of 5×10^{-11}, 5×10^{-10}, and 5×10^{-9} M T_4, respectively; cross-hatching indicates a resorption event not found in *Eurycea*; vertical lines separate events into larval and metamorphic periods; boxes for endochondral and dermal ossification cover the periods over which each type of bone appears in sectioned specimens; see Table III for modal ossification sequences and **Appendix C** for footnotes 1–8. The induced patterns shown are from the 1Spring size class of Rose (1995*b*; N = 24, 23, and 10 for the three doses, respectively); timings of onset at the lowest dose were not available so 1Autumn data were used instead. 1/2 indicates partial formation or resorption; absent means that the element involved in a particular resorption event does not form.

this study, the two at 24–25 mm SVL exhibit an incipient nasal capsule; the two at 42–43 mm show a fully chondrified pterygoid process, plus a prefrontal, frontal extension, maxilla, footplate ossification, orbitosphenoid and os thyroideum; and the two at 54–57 mm have an ossified columella, but no sign of any cartilage (or bone) resorption. Five of the six larvae do not exhibit a coronoid bone, though one sectioned specimen of 42 mm reveals an unpaired, edentate splint of bone in the expected location of this bone. The nasal in *Dicamptodon* appears at metamorphosis, followed by the variably present septomaxilla (Larsen, 1963). *Rhyacotriton* (Rhyacotritonidae) resembles *Dicamptodon* in lacking a coronoid and developing the nasal cartilage, pterygoid process, and some dermal ossifications at larval stages; the latter include the maxilla, followed by the prefrontal and frontal extension (Srinivasachar, 1962; Worthington & Wake, 1971). The septomaxilla arises at metamorphosis and is followed by the variably present nasal (Worthington & Wake, 1971; Wake, 1980).

Soft tissue remodelling also shows some correspondence between tissue sensitivity in *Eurycea* and developmental timing. Skin glands and Jacobson's organ, which have a sensitivity of 5×10^{-10} M in *Eurycea*, arise at larval stages in *Siren intermedia*, *Pseudobranchus striatus*, *Ambystoma maculatum*, *Notophthalmus viridescens*, and *Hynobius retardatus*. Leydig cells, which have a sensitivity of 5×10^{-9} M in *Eurycea*, disappear after skin glands appear in *S. intermedia* and *Rhyacotriton* larvae (see Worthington & Wake, 1971: fig. 3). Leydig cell loss is synchronous with the other soft tissue events of this sensitivity (gill loss and tail-fin resorption) in *A. maculatum*, *N. viridescens*, and *H. retardatus*.

The few remodelling events not to show a correspondence between tissue sensitivity in *Eurycea* and developmental timing in nonplethodontids include the two resorptions of dermal bone and the formations of the parasphenoid tooth patch and adult ceratobranchial. These events have a sensitivity of 5×10^{-10} M in *Eurycea*, but are generally synchronous with events induced at 5×10^{-9} M, including gill loss, in nonplethodontids. However, pterygoid resorption does occur prior to gill loss in at least one nonplethodontid (cryptobranchids) (Fig. 1). In addition, although the adult ceratobranchial of *Eurycea* shows incipient morphogenesis at 5×10^{-10} M, its formation as a distinct cartilage requires 5×10^{-9} M and does not occur outside plethodontids. Formation of a tooth row homologous to the plethodontid parasphenoid tooth patch is limited to a few species of hynobiids (see below).

Morphogenetic pattern of remodelling events

Whereas all skeletal morphogenesis induced in *Eurycea* at the highest dose resembles natural development in this species (Rose, 1995*a*), the morphogenetic patterns exhibited by certain tissues at lower doses bear a closer resemblance to nonplethodontid development. Further, the differences in sensitivity within these tissues tend to match the sequence and extent of morphogenesis in the same tissues of nonplethodontids.

In natural metamorphosis and at 5×10^{-9} M, the nasal capsule of *Eurycea* appears as an anterolateral extension of the trabecular crest which expands to form the nasal tectum (Fig. 2a, e); all other parts of the capsule chondrify soon afterwards as extensions of the nasal tectum, trabecular horn and lamina orbitonasalis (Fig. 2b, f). Likewise, the maxilla, septomaxilla, prefrontal, nasal, parasphenoid tooth patch, and anterior extensions of the adult vomer and frontal all begin to ossify and attain their adult shapes within the same relatively brief period.

Three departures from these morphogenetic patterns are evident in *Eurycea* treated at 5×10^{-11} M and 5×10^{-10} M. First, the nasal tectum develops from a discrete centre of

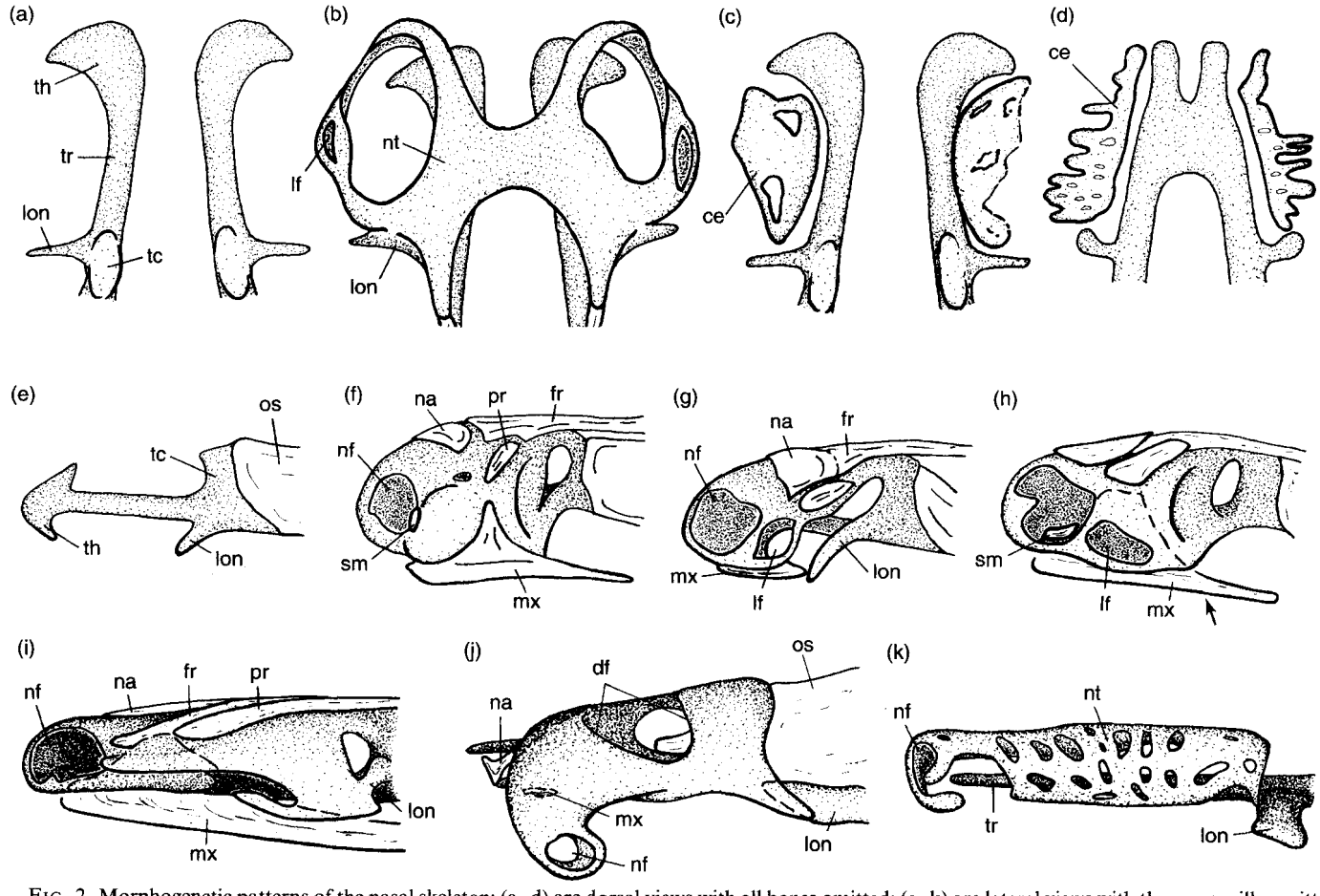

FIG. 2. Morphogenetic patterns of the nasal skeleton: (a–d) are dorsal views with all bones omitted; (e–k) are lateral views with the premaxilla omitted; the facial process of the maxilla is dashed in h and i to show underlying cartilage, the maxilla and nasal are dashed in j where deep to cartilage; figures are not drawn to scale. (a) Larval *Eurycea*, (b) early metamorphic *Eurycea*, (c) larval *Eurycea* after six weeks at 5×10^{-11} M T_4, (d) larval *Necturus*, (e) larval *Eurycea*, (f) late metamorphic *Eurycea*, (g) larval *Eurycea* after 18 weeks at 5×10^{-10} M T_4, (h) postmetamorphic *Ambystoma*, the arrow indicates the posterior limit of the larval-stage maxilla, (i) postmetamorphic *Cryptobranchus*, (j) postlarval *Siren*, (k) postlarval *Necturus*. Abbreviations: ce, columna ethmoidalis; df, dorsal fenestra; fr, frontal; lf, lateral fenestra; lon, lamina orbitonasalis; mx, maxilla; na, nasal; nf, narial foramen; nt, nasal tectum; os, orbitosphenoid; pr, prefrontal; sm, septomaxilla; tc, trabecular crest; th, trabecular horn; tr, trabecular. (a–c), (e–g) Redrawn from Rose (1992), (d) redrawn from Higgins (1920*b*), (h) from specimens examined in this study, (i–k) modified from Jurgens (1971).

TABLE IV

Distribution of morphogenetic patterns across urodeles

Taxon ± treatment	Morphogenetic features[1]						
	1) Columna ethmoidalis	2) Lateral fenestra	3) Incisura ectochoanalis & basal fenestra	4) Lamina orbitonasalis & nasal tectum	5) Maxilla	6) Choanal notch	7) Parasphenoid tooth patch
Eurycea at 5×10^{-11} M T_4[2]	Present	Not evident	Not evident	Separate	Absent	Not evident	Not evident
Eurycea at 5×10^{-10} M T_4[2]	Present	Incomplete or permanent	Connected	Separate or fused	Short	Open	Half length
Plethodontidae[3]	Absent	Temporary	Separate	Fused	Full	Closed	Full length
Ambystomatidae	Present[4,5,27]	Permanent[10,11,27]	Connected[10,11,27]	Fused[4,5,10,11,27]	Short (L)[18,27] or short (P)[25,32]	Closed[10,11,18,27]	Not longitudinal[20]
Rhyacotritonidae	?[29]	Temporary?[28,29]	Connected[28,29]	Fused[28]	Short (L)[29,30]	Closed[28,29]	Not longitudinal[20]
Dicamptodontidae	?[27]	Permanent[17]	Connected[17]	Separate[17]	Short (L, P)[20,27]	Closed[17]	Not longitudinal[20]
Hynobiidae	Present[7]	Incomplete[13,15], permanent[16] or temporary[7,14]	Connected[13]	Fused[13]	Short (L)[20,24] or short (P)[13,20]	Open[20,27] or closed[13]	Half length[20,27]
Salamandridae	Present[6]	Incomplete[6] or permanent[12,26,27]	Connected[6,31] or separate[12,26]	Fused[6,12,26,31]	Short (L)[19,26,27] or short (P)[19,31]	Open[19–22,27,31]	Not homologous
Cryptobranchidae	Present[5,9]	Not evident[17]	Not evident[17]	Fused[17,23,27]	Short (L)[23,27]	Not homologous	Not evident[20]
Sirenidae	Absent[27]	Not evident[17,27]	Not evident[17]	Separate[17,27]	Absent or vestigial[20,27]	Not evident[20]	Not evident[20]
Proteidae	Present[5,8]	Not evident[5,17]	Not evident[17]	Separate[17]	Absent	Not evident[20]	Not evident[20]

[1] 1) The columna ethmoidalis arises as a separate cartilage or the nasal tectum arises as an extension of the trabecular crest. 2) The lateral fenestra is either incomplete (still connected with the incisura ectochoanalis), or evident as a permanent (larval and postmetamorphic stages) or temporary (larval stages only) opening in the lateral wall of the nasal capsule. 3) The incisura ectochoanalis and basal fenestra either remain continuous with each other or become separated by the ectochoanal cartilage. 4) The lamina orbitonasalis either fuses with, or remains separate from, the nasal tectum. 5) The posterior process of the maxilla either remains short in induced specimens, larval stages (L) and larval paedomorphs (P) or develops fully with the other two processes. 6) The choanal notch of the vomer forms either a broad, open arc or a closed, semicircular arc. 7) Parasphenoid tooth patches arising from the posteromedial edge of the vomer extend either halfway down the cultriform process or all the way into the base of the parasphenoid. 'Not evident' means indiscernible owing to incomplete nasal capsule formation (2, 3) or an unchanged larval condition (6, 7); see text for nonhomologous features. [2–32] See **Appendix D** for references and species names.

chondrification at both doses (Fig. 2c). This centre occurs naturally as the first stage of capsule formation in all nonplethodontids except sirenids and is known as the columna ethmoidalis (Fig. 2d, Table IV).

Second, subsequent development of the nasal capsule is limited to a lateral expansion of the columna ethmoidalis at 5×10^{-11} M and a recognizable, but incomplete, capsule at 5×10^{-10} M (Fig. 2g). Regions that fail to chondrify at 5×10^{-10} M include the posteroventral wall, which normally fuses with the lateral portion of the lamina orbitonasalis; the lateral wall, which normally fills in the lateral fenestra; and the ectochoanal cartilage, that part of the floor which normally separates the incisura ectochoanalis from the basal fenestra. These regions are generally the last parts of the capsule to form in nonplethodontids and they often remain incomplete (Table IV). The lateral fenestra (Fig. 2h) and the gap between the incisura ectochoanalis and basal fenestra persist as adult features in various metamorphic forms, the posterior and lateral walls and floor remain incomplete in most paedomorphic forms, especially *Necturus* (Fig. 2i–k), and the lamina orbitonasalis fails to fuse with the nasal capsule in sirenids and proteids (Fig. 2j, k).

Third, although the six dermal bones and parasphenoid tooth patch all begin to form at 5×10^{-10} M, four of these elements develop either incompletely or inconsistently at this dose. The posterior process of the maxilla either fails to develop or appears as a small bump that does not extend beyond the lamina orbitonasalis; the facial process is also absent or shorter and narrower than in natural postmetamorphs (Fig. 2g). The septomaxilla does not appear when the lateral portion of the nasal capsule, particularly the border between the narial and lateral fenestrae, remains incomplete (Fig. 2g). The parasphenoid tooth patch forms in a more anterior position than normal, with teeth and vomerine bone limited to the anterior half of the cultriform process of the parasphenoid (Fig. 3c). Lastly, the anterior extension of the vomer is stunted and the choanal notch remains broad and open (Fig. 3c).

These morphogenetic states, which are not encountered naturally in *Eurycea* or at 5×10^{-9} M,

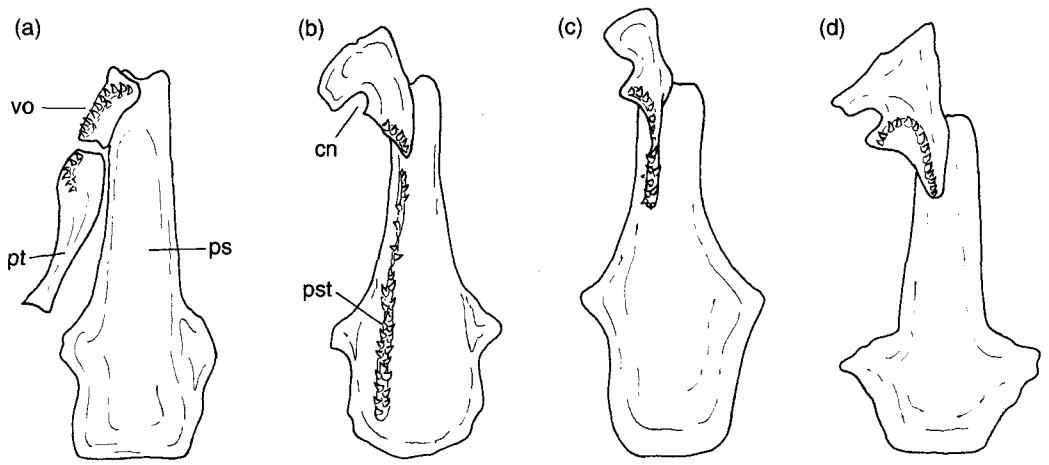

FIG. 3. Morphogenetic patterns of the bony palate: ventral views not drawn to scale: (a) larval *Eurycea*, (b) late metamorphic *Eurycea*, (c) larval *Eurycea* after 12 weeks at 5×10^{-10} M T_4, (d) postmetamorphic *Salamandrella*. Abbreviations: cn, choanal notch; ps, parasphenoid; pst, parasphenoid tooth patch; pt, pterygoid; vo, vomer. (a–c) Redrawn from Rose (1992), (d) modified from Larsen (1963).

all resemble intermediate or terminal stages of morphogenesis in nonplethodontids (Table IV). The posterior process of the maxilla remains short in ambystomatids, salamandrids and cryptobranchids until the stage of gill loss, when it lengthens to become level with the lens of the eye (Fig. 2h). The posterior process is also terminally stunted in the larval paedomorphs of metamorphic families listed above. The septomaxilla is missing in salamandrids in conjunction with their laterally elongated narial fenestra, the parasphenoid tooth patch reaches only halfway down the cultriform process in those hynobiids that develop one (Fig. 3d), and the choanal notch remains open in numerous metamorphic forms and some paedomorphs of metamorphic families (Table IV, Reilly & Brandon, 1994).

Discussion

Comparison of the cranial remodelling induced at three different TH doses in *Eurycea bislineata* with natural remodelling across Urodela yields two basic results. First, a TH dose that approximates the natural level of thyroid activity in metamorphic-stage *Eurycea* elicits an assemblage of tissue responses that is similar in content, sequence and morphogenetic pattern to natural remodelling in *Eurycea* and other metamorphic plethodontids. Second, lower TH doses in *Eurycea* elicit progressively fewer tissue responses that exhibit a developmental sequence and morphogenetic patterns more similar to natural development in nonplethodontids. Additionally, the TH sensitivity of tissue responses in *Eurycea* appears to correlate with the range of occurrence of the homologous responses in nonplethodontids, as well as with their developmental timing relative to other tissues or other parts of the same tissue. More sensitive tissue responses (i.e. those inducible at lower TH) tend to occur across a greater range of nonplethodontids, as well as earlier in nonplethodontid ontogenies. These correspondences lead to several testable hypotheses regarding the developmental mechanisms underlying diversification in urodele cranial ontogeny.

Diversification of urodele cranial ontogenies

Previous discussions of amphibian metamorphosis have implicated two primary control parameters in the evolution of TH-mediated development: the sensitivity to TH of individual tissues and the profile of thyroid activity (Raff & Kaufman, 1983; Hanken & Hall, 1988; Hanken & Summers, 1988; Raff & Wray, 1989). Three additional factors may also affect metamorphic remodelling through a global truncation or retardation of development: selection for decreased adult size (Trueb & Alberch, 1985; Hanken, 1989), increase in DNA content and/or cell size (Horner & MacGregor, 1983; Sessions & Larson, 1987), and cooler climatic conditions (Wake, 1980). However, the extensive dissociation that is evidenced within and among remodelling events in urodele crania clearly requires more than a single mechanism of diversification based on paedomorphic truncation or a global loss of thyroid activity/tissue sensitivity. Rather, the continuous but nonarbitrary nature of this dissociation suggests continuous variance in one or both hormonal parameters. This study finds that a significant proportion of the dissociation that is expressed by the 26 remodelling events surveyed here can be generated empirically by varying thyroid activity. Of the events excluded from this study, almost all either arise embryonically or occur in fewer than four of the nine urodele families considered here (**Appendix B**). These findings suggest that urodele cranial ontogenies have diversified largely by change in the rate or level of thyroid activity and that the intracranial distribution of tissue sensitivity in urodeles has been largely conserved in their evolution.

Metamorphic urodeles exhibit at least two distinct patterns of metamorphic remodelling. As demonstrated by primitive desmognathines and the majority of hemidactyliine plethodontids including *Eurycea* (Wake, 1966; Rose, 1995*a*, *b*), almost all postembryonic cranial remodelling in metamorphic plethodontids is expressed in a brief metamorphic period at one or more years of age. In contrast, other metamorphic urodeles generally initiate postembryonic remodelling soon after hatching and sustain it throughout the greater part of the larval period. In addition, the content, sequence, and morphogenesis of this remodelling in nonplethodontids is matched by the low dose remodelling induced in *Eurycea*. One interpretation of these observations is that plethodontids and nonplethodontids utilize two different profiles of thyroid activity to control their postembryonic development (Fig. 4). The gradual pattern of nonplethodontid development suggests a steady increase in thyroid activity throughout larval and metamorphic stages (Fig. 4a), i.e. the profile inferred by Etkin (1964; White & Nicoll, 1981) and confirmed by Weil (1986) for anurans. Conversely, the very abrupt pattern of plethodontid development suggests negligible thyroid activity throughout the larval period, followed by an abrupt rise at the onset of metamorphosis (Fig. 4b), a profile that agrees with the plasma TH data currently available for *Eurycea*. Alberch *et al.* (1986) report nondetectable levels prior to internal remodelling and a rapid increase at its onset. This profile also offers a means of reconciling the high tissue sensitivity of some skeletal elements in *Eurycea* with the relatively late onset of their remodelling in natural development (Rose, 1995*b*).

The progressive reduction of metamorphic remodelling in larval paedomorphs is also consistent with change in the shape of the TH profile. Ranked by the number of remodelling events that show TH dependence in *Eurycea*, the obligate paedomorphs include *Andrias*, *Cryptobranchus*, *Siren*, *Pseudobranchus*, and *Necturus*. The progressive loss of remodelling events implied by this ranking is consistent with increasing attenuation of a gradual TH profile as described above for metamorphic nonplethodontids (Fig. 4). Cryptobranchid development is consistent with minor attenuation at metamorphic stages (Fig. 4c); larval and metamorphic stages are still distinguishable and the only events lost—aspects of hyobranchial remodelling and cartilage resorption—are relatively insensitive to TH. Sirenid development, with its indistinct and incomplete metamorphic phase, suggests greater attenuation throughout larval and metamorphic stages. Proteid development, which accomplishes little more than an incomplete nasal capsule, suggests an almost flat profile of thyroid activity (Fig. 4d).

Regarding the larval paedomorphs in metamorphic families, larval development of the nasal skeleton and maxilla in *Ambystoma mexicanum* is similar to metamorphic congenerics (cf. Keller, 1946 and Bonebrake & Brandon, 1971), which suggests that paedomorphosis in this species may be caused by attenuation of the TH peak required for gill loss. This suggestion is supported by empirical data. *A. mexicanum* exhibits a steady increase in plasma TH during early development, followed by a five-fold decrease after completion of the hind limbs (Rosenkilde, 1985). The variable completion of additional medium and low sensitivity remodelling events in other ambystomatid and salamandrid paedomorphs may be explained by variability in the level or timing of attenuation in these families.

While the observations presented here demonstrate a plausible role for thyroid activity in urodele cranial diversification, they do not exclude a contribution by change in tissue sensitivity. However, an argument for tissue sensitivity as the primary parameter of evolutionary change encounters several shortcomings when applied to the major shifts in cranial ontogeny described above. For the purposes of illustration, this argument shall assume that thyroid activity has remained relatively constant in urodele evolution and is expressed in either a gradual or abrupt

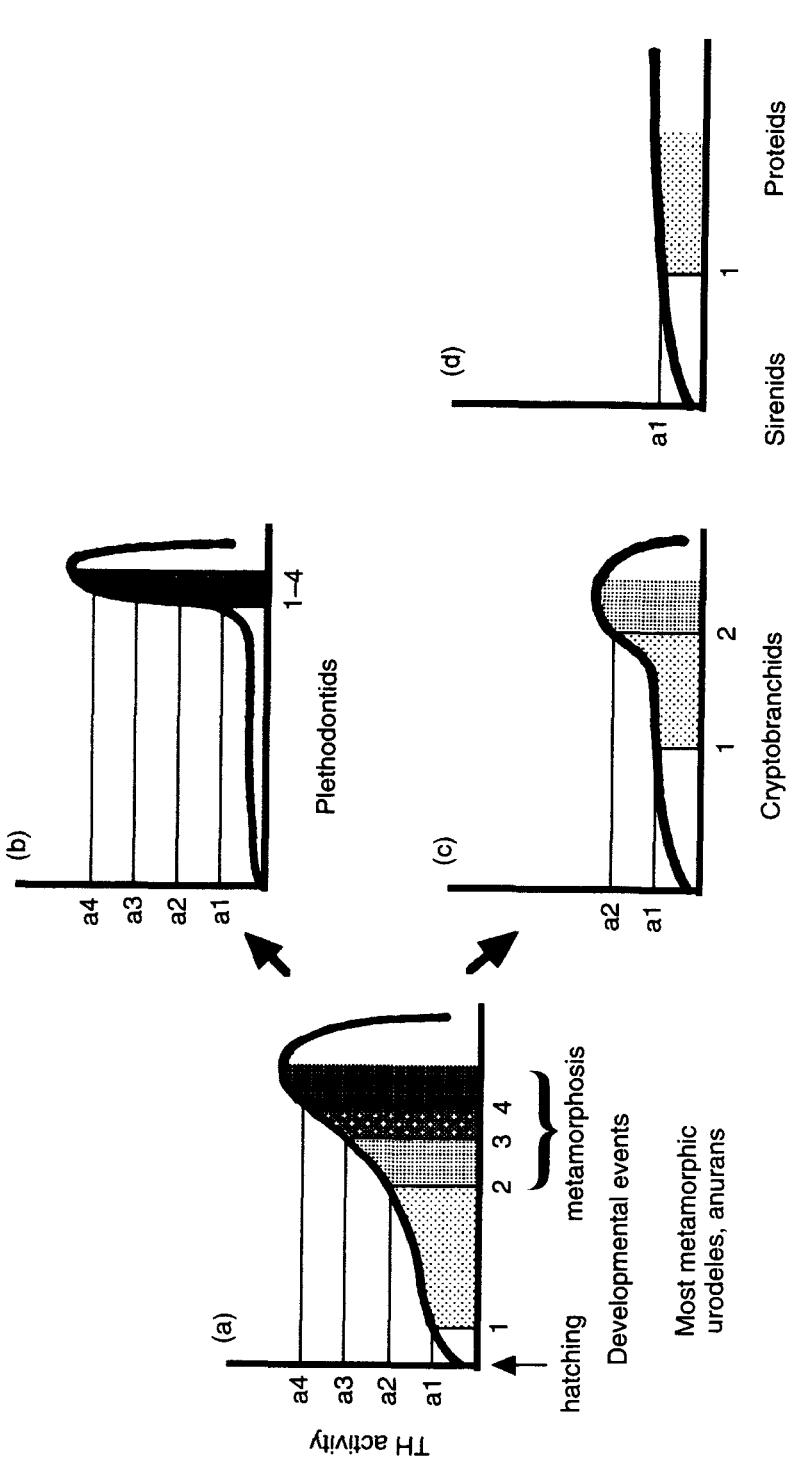

FIG. 4. The transformations in profile of TH activity that are proposed to explain major shifts in urodele cranial ontogeny. The temporal differences between plethodontid and nonplethodontid ontogenies suggest that plethodontids evolved an abrupt profile with negligible TH activity at larval stages. The progressive reduction of metamorphic remodelling in larval paedomorphs suggests increasing levels of attenuation in thyroid activity.

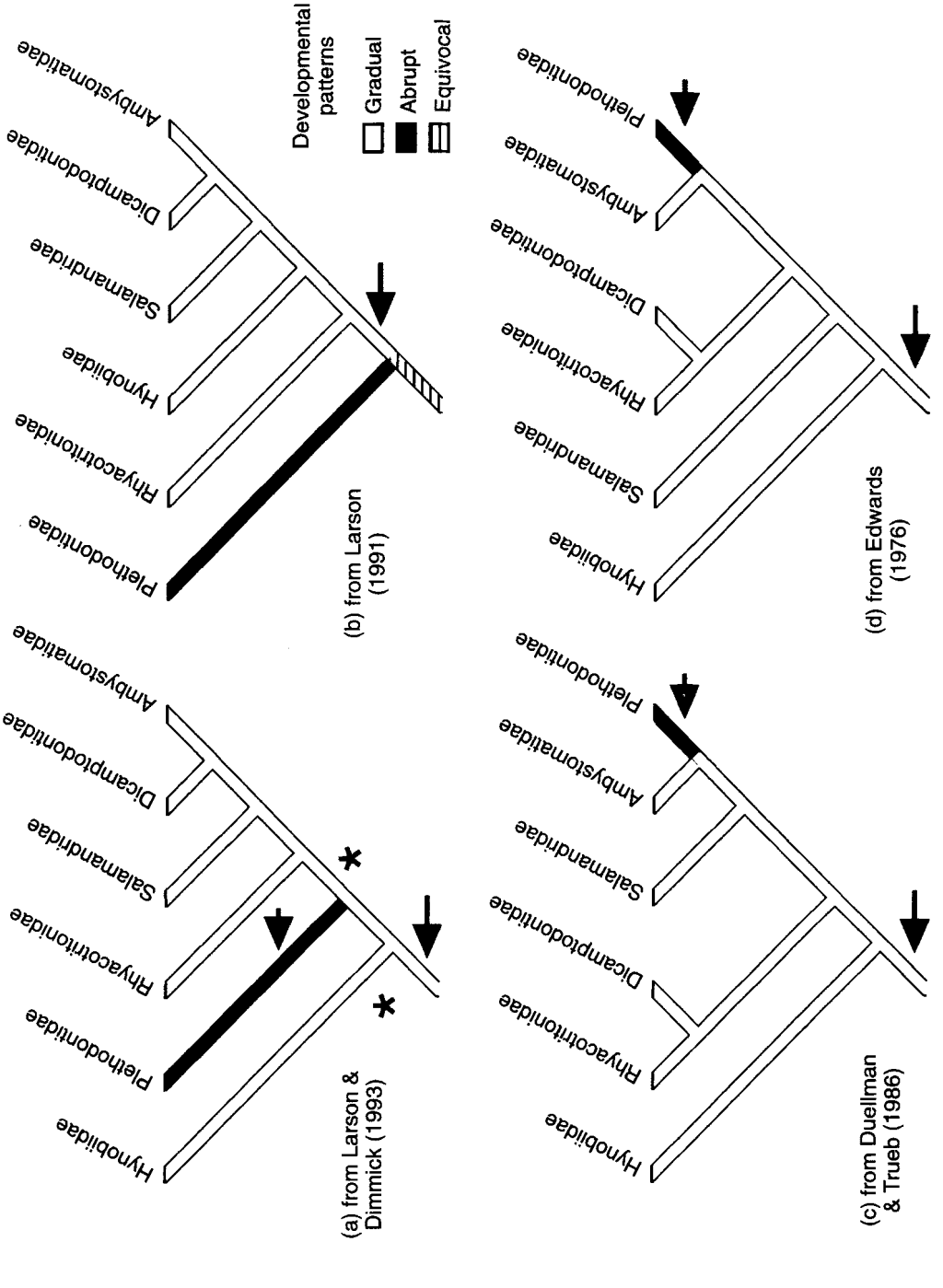

FIG. 5. Phylogenetic reconstructions of Urodela (metamorphic clades only) showing the distribution of abrupt and gradual metamorphic cranial ontogenies. On the basis that an abrupt profile of TH activity is conserved in urodeles and that TH dependence in all cranial remodelling is ancestral for urodeles, three of four interpretations (a, c, d) would require either the loss of TH dependence by tissues that are remodelled prior to gill loss in ancestral urodeles (long arrow) plus its subsequent gain in plethodontids (short arrow), or two losses of this TH dependence within urodeles (asterisks in a).

profile (Figs 4a and b, respectively). If a gradual profile is assumed, the abruptly metamorphic pattern of plethodontids would require that all their metamorphic cranial tissues be relatively insensitive to TH, a condition that is clearly refuted by the data presented here. The alternative assumption of an abrupt profile is not compatible with the gradual metamorphic ontogenies of nonplethodontids unless one postulates that all of their remodelling events prior to gill loss are TH-independent. Though this postulation cannot be ruled out, it is not parsimonious. Of the two metamorphic out-groups for urodeles, at least one (anurans) requires TH for almost all postembryonic remodelling of the cranium (Kollros, 1961). On the basis that this condition is ancestral for urodeles, three of four interpretations of urodele phylogeny (Fig. 5a, c–d) would require at least two losses of TH dependence by larval-stage remodelling in nonplethodontid clades or one loss in ancestral urodeles and a subsequent gain in plethodontids. The postulation is also refuted by empirical data. Preliminary experiments on the ambystomatids *Ambystoma maculatum* and *A. mexicanum* indicate that much of the cranial remodelling that occurs prior to gill loss/completion of the hindlimbs is TH-inducible (Rose, In prep.); the level of TH activity at this stage in *A. mexicanum* (Rosenkilde, 1985) further suggests that this TH-inducibility can be equated with TH dependence. In summary, changes in tissue sensitivity alone cannot parsimoniously account for the transition between plethodontid and nonplethodontid ontogenies, though they may well explain variation within each pattern such as the timing differences in dermal ossification between *Pleurodeles* and other salamandrids.

In order to explain the progressive reduction of metamorphic remodelling in obligate paedomorphs, the assumption of constant thyroid activity would require that the loss of tissue sensitivity by paedomorphs be consistent with the distribution of tissue sensitivity in metamorphic urodeles; less sensitive tissues are expected to be more prone to lose their sensitivity than more sensitive tissues. Although loss of tissue sensitivity without a reduction in thyroid activity is not incompatible with our understanding of the molecular mechanisms involved, this particular scenario receives equivocal support from the tissue sensitivity data available for obligate paedomorphs. The only remodelling to exhibit tissue sensitivity (i.e. be inducible) in cryptobranchids and sirenids is Leydig cell loss (Noble & Farris, 1929; Noble & Richards, 1930), which has low sensitivity in *Eurycea*. In contrast, the only events inducible in the obligate plethodontid paedomorphs are maxilla and septomaxilla ossification in *Typhlomolge* (Dundee, 1957) and coronoid resorption in *Haideotriton* (Dundee, 1962; though reinspection of this material by the present author also revealed pterygoid resorption); these events have intermediate and low sensitivity in *Eurycea*. However, as Dundee stained only for bone, it is possible that more sensitive events in cartilage and soft tissue are also inducible in these forms.

Resolution of the relative importances of thyroid activity and tissue sensitivity to urodele cranial evolution may be approached by a systematic survey of these two control parameters across urodeles. However, several caveats do apply. First, profiles of TH activity documenting intra-stage variation may have to be limited to species with larvae large enough to provide sufficient plasma for detecting TH without pooling samples. Second, there is no guarantee that the TH profiles of obligate paedomorphs would bear any relevance to their cranial ontogenies, since their thyroid activity may have evolved an alternative function following the loss of tissue sensitivity (Larsen, 1968). Third, the gradual developmental patterns of nonplethodontids implies higher thyroid activity at larval stages than in *Eurycea*, in which case endogenous TH would have to be controlled by thyroid removal or goitrogenic drugs in order to measure tissue sensitivity.

The fourth and potentially most important caveat is that cranial diversification may also derive

from variation in secondary parameters such as additional hormones and tissue interactions that directly mediate the interaction of plasma TH with peripheral tissues. Corticosteroids exert a direct positive effect upon regressive remodelling (Kaltenbach, 1958; Gray & Janssens, 1990) and prolactin exerts a direct negative effect upon both progressive and regressive remodelling (Tata, Kawahara & Barker, 1991). At least one of these effects is enforced at the level of TH receptor expression, which is the earliest detectable response to TH and appears to be the primary mechanism by which tissue sensitivity is coupled to thyroid activity (Yaoita & Brown, 1990; Kawahara, Baker & Tata, 1991; Kanamori & Brown, 1992). Prolactin blocks up-regulation of the TH receptor, thereby preventing subsequent TH-dependent gene expression (Baker & Tata, 1992). In addition, epithelial-mesenchymal interactions are known to determine the site-specific remodelling of the epithelia and connective tissues in the anuran tail (Niki *et al.*, 1982, 1984; Kinoshita, Sasaki & Watanabe, 1986) and intestine (Ishizuya-Oka & Shimozawa, 1992, 1994), of the tympanum in anurans (Helff, 1928, 1931), and possibly also of some dermal bones in urodeles (Medvedeva, 1959, 1960, 1986). At least one of these interactions is controlled by a diffusible factor (Niki *et al.*, 1984), which by analogy to nonmetamorphic inductions may also control TH receptor expression (Heuberger *et al.*, 1982). However, the potential for additional hormones and inductive factors to influence the diversification of urodele metamorphic patterns depends ultimately on their capacity to regulate TH-dependent remodelling on an individual tissue basis. Though locally produced inductive factors may pose a more viable option than hormones in this regard, there is still little evidence for epithelial-mesenchymal interactions in metamorphic skull remodelling (Summers & Hanken, 1988).

Phylogenetic distribution of urodele cranial ontogenies

How are the different types of cranial ontogeny distributed in urodele phylogeny and what might their phylogenetic distribution reveal about urodele evolution? Regarding gradual versus abrupt metamorphic patterns, the resemblance of low dose remodelling in *Eurycea* to natural development in the majority of nonplethodontids suggests that low dose remodelling is atavistic to *Eurycea*. Also, low dose remodelling induced in larvae of the ambystomatids *Ambystoma maculatum* and *A. mexicanum* remains consistent with natural development in these species (Rose, In prep.). These observations favour plethodontids evolving from an ancestral condition comparable to other metamorphic urodeles, rather than vice versa (Fig. 4). This direction is maintained by at least two of the four phylogenetic reconstructions that have been proposed for urodeles (Fig. 6). Duellman & Trueb (1986) and Edwards (1976) both use morphological data to place plethodontids within a clade bearing gradual metamorphosis as a sympleisiomorphy.

However, Larson (1991) and Larson & Dimmick (1993), using ribosomal RNA and morphological data, place Plethodontidae and Amphiumidae as sister groups linked by early fusion of the premaxilla. Amphiumids are unique in their highly developed skull ossification, lack of ceratobranchial resorption and early loss of gills (Hay, 1890; Ultsch & Arceneaux, 1988). This combination of events does not accord with either the cranial ontogenies of other paedomorphic forms or the low dose remodelling in *Eurycea*. Thus, it is difficult to infer an ancestral cranial ontogeny for plethodontids and amphiumids and to account for the divergence required between them on the basis of thyroid activity.

With regard to larval paedomorphosis, the emerging consensus on lissamphibian monophyly (Szarski, 1962; Parsons & Williams, 1963; Trueb & Cloutier, 1991*a*, *b*) and the prevalence of TH mediation in anuran and possibly caecilian development (Klumpp & Eggert, 1934) support the

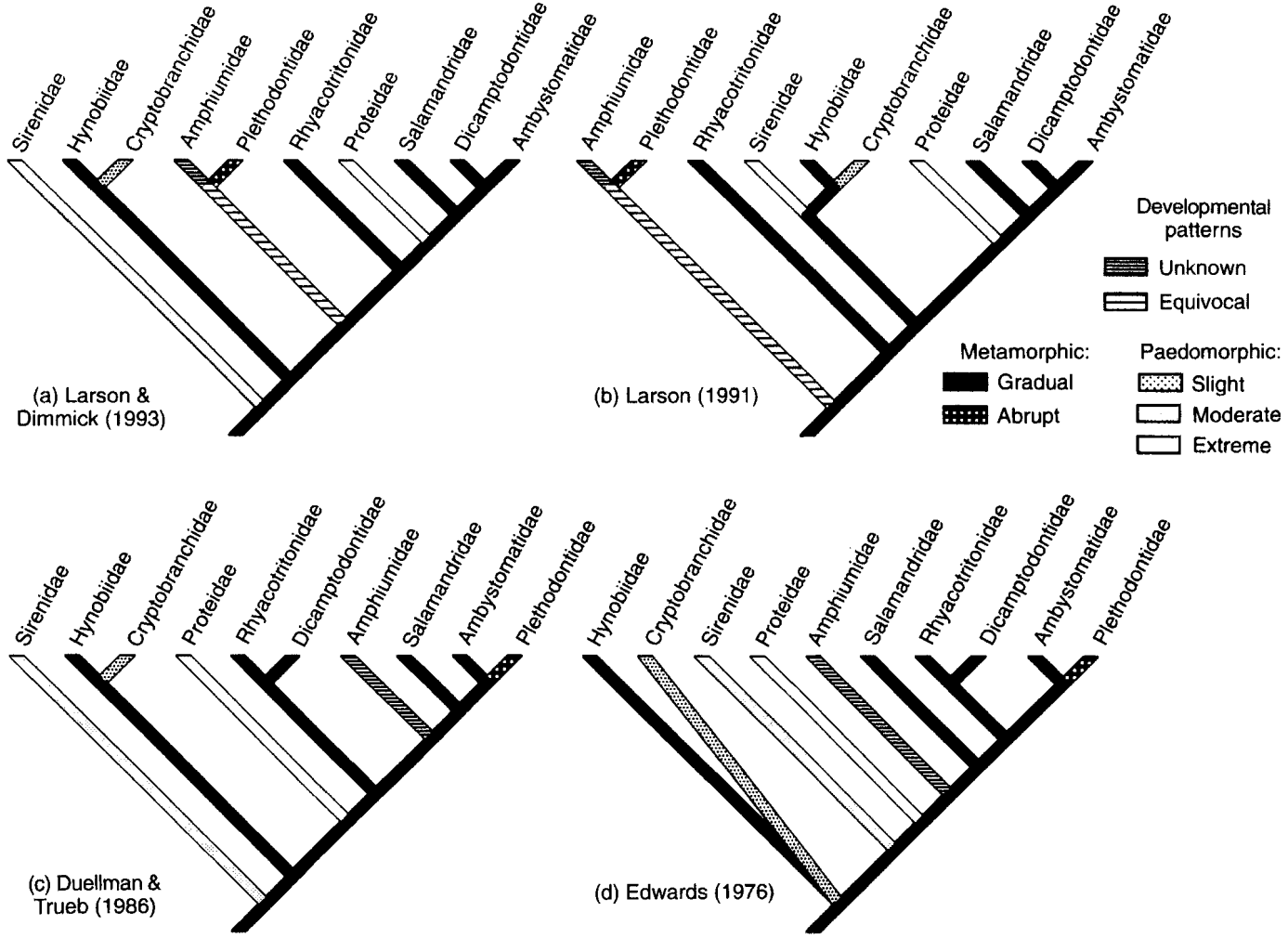

FIG. 6. Phylogenetic reconstructions of Urodela showing the distribution of different grades of metamorphic and paedomorphic cranial ontogeny. A gradual metamorphic ontogeny is inferred as ancestral by out-group comparison with anurans. However, since the gradual metamorphosis of anurans involves TH-dependent development of the limbs as well as the cranium, only the overall pattern of TH-dependent development can be considered as ancestral to urodeles.

generally accepted view of urodeles as a primitively metamorphic clade. This raises the question of how the various occurrences of obligate paedomorphosis (amphiumids excluded) relate phylogenetically and whether they represent progressive stages of a single evolutionary trend. In no phylogenetic reconstructions are any two obligate paedomorphs related as sister taxa, meaning that their transformations from ancestral metamorphic patterns most likely occurred as separate events. The earliest reconstruction, Edwards (1976), holds that the paedomorphic clades arose sequentially during the early evolution of the order, which raises the intriguing possibility that early urodeles were subject to ecological conditions more conducive to aquatic life, or alternatively, a less canalized program of thyroid activity.

Conserved tissue sensitivity

The corollary of diversification by change in thyroid activity is that TH-dependent remodelling in urodeles is more conserved phylogenetically, but more plastic developmentally, than previously thought. That the distribution of tissue sensitivities evinced by *Eurycea* is common to nonplethodontids is presently supported only by the improbability that this distribution would otherwise correspond so closely to the natural development of so many distantly related species. However, the possibility that *Eurycea*'s tissue sensitivities are in fact a relic of the ancestral urodele condition has profound implications for issues of character evolution, homology, and dissociation.

A generalized distribution of tissue sensitivities predicts that morphological characters would be lost or reduced on the basis of tissue sensitivity to TH, in contrast to a generalized ontogenetic trajectory which predicts loss or reduction on the basis of ontogenetic timing (e.g. Wake, 1980). Though these two factors are probably correlated and even causally related in most urodeles, plethodontids demonstrate that tissue sensitivity alone does not determine ontogenetic timing or the likelihood of evolutionary loss. Unlike other urodeles, plethodontids express the most sensitive tissue response (nasal capsule formation) synchronously with almost all other post-embryonic remodelling, and plethodontids alone have repeatedly lost this tissue response in larval paedomorphs (Wake, 1966; Yeatman, 1967; Rose, 1995*a*). Thus, evolutionary loss appears to depend ultimately (but not exclusively) on the interaction of thyroid activity and tissue sensitivity, and not on either parameter alone.

A generalized distribution of tissue sensitivities may also yield developmental criteria for inferring the homology and polarity of TH-mediated characters. This argument embraces de Quieroz's (1985) position that characters be viewed as ontogenetic transformations rather than instantaneous morphologies. If tissue sensitivity is an evolutionarily conserved parameter, then a morphological feature (or suite of features) that expresses a range of sensitivities among its component parts or successive phases of morphogenesis is bound to a conserved set of morphogenetic (or developmental) patterns, the actual expression of which (i.e. the character state) is controlled by the profile of TH activity. Trivial demonstrations of this premise are provided by morphogenesis of the nasal capsule and the overall development of cranial cartilages. The most sensitive part of the nasal capsule (tectum) is predicted to form in the absence of, prior to, or in synchrony with, other parts (depending on the rate and extent of TH increase), but never after them. Likewise, cranial cartilages that form in response to TH are predicted to form in the absence of, prior to, or in synchrony with, the remodelling of cartilages in resorption. Both predictions are borne out by the urodele ontogenies surveyed here.

How this approach may help resolve problems of homology can be illustrated with the

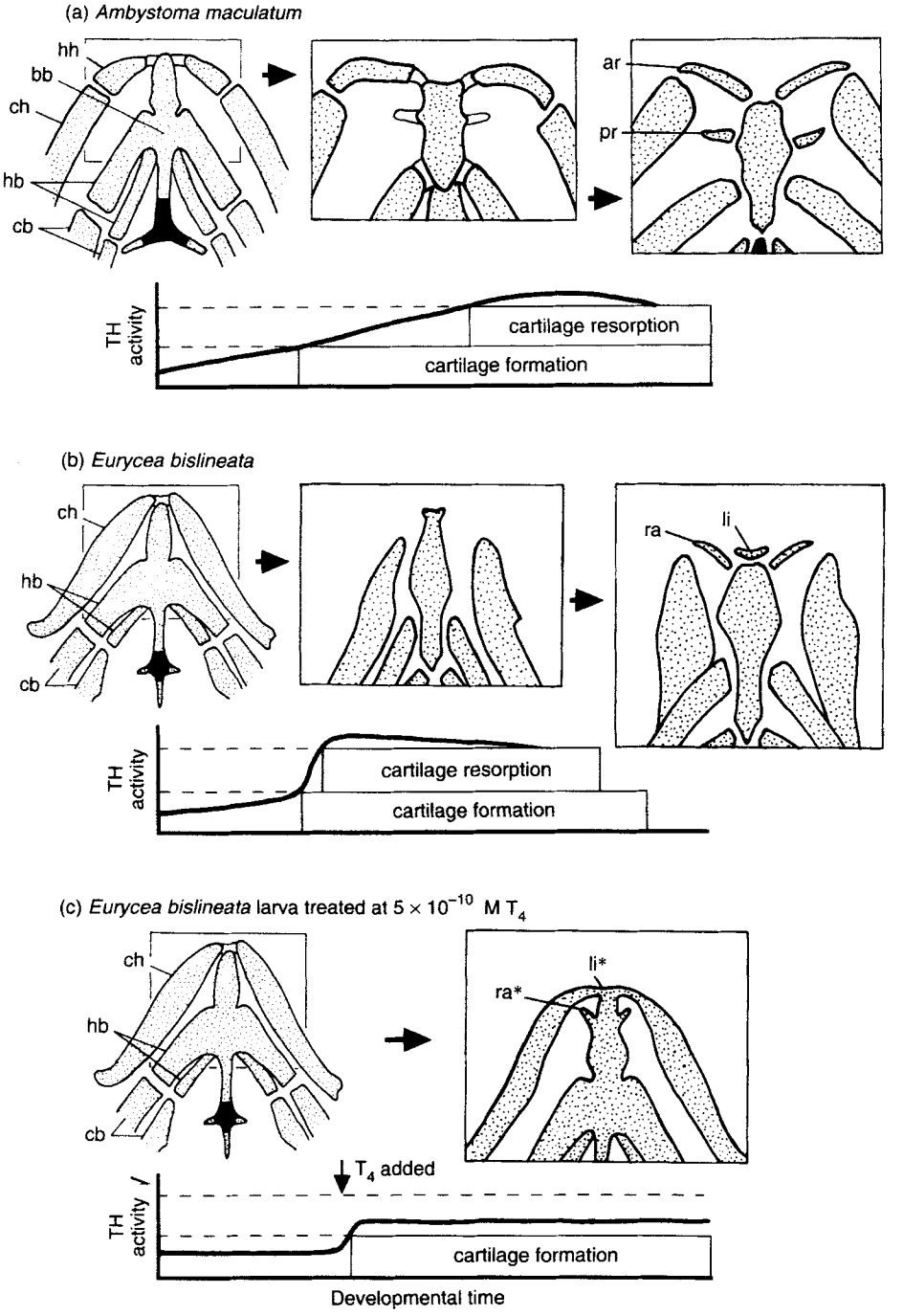
(a) Ambystoma maculatum
hh
bb
ch
hb
cb
ar
pr
TH activity
cartilage resorption
cartilage formation
(b) Eurycea bislineata
ch
hb
cb
ra
li
TH activity
cartilage resorption
cartilage formation
(c) Eurycea bislineata larva treated at 5 × 10−10 M T4
ch
hb
cb
li*
ra*
T4 added
TH activity
cartilage formation
Developmental time

FIG. 7

metamorphic remodelling of the anterior hyobranchium (Fig. 7). Most metamorphic urodeles form two pairs of radial cartilages, the more anterior of which is clearly derived from the hyoid arch (Fig. 7a). However, plethodontids form one pair of radial cartilages plus an anteromedian lingual plate; both elements appear to arise *de novo* after the ceratohyals have become detached from the basibranchial plate (Fig. 7b). Conversely, in one *Eurycea* specimen treated at 5×10^{-10} M, which is above the threshold for cartilage formation but below that for cartilage resorption, the lingual chondrified from the prechondrogenic connection between ceratohyal and basibranchial cartilages, while the radials arose *de novo* (Rose, 1995*b*; Fig. 7c). These relationships suggest that the lingual is actually a highly modified anterior radial and the single radial is a posterior radial. This example further illustrates the potential of empirically derived morphogenesis to illuminate aspects of morphogenetic pattern that have become obscured by dissociation of the underlying morphogenetic processes—in the case of the plethodontid lingual, by the presumed synchronization of progressive and regressive remodelling.

Comparative analyses of empirically derived morphogenesis may also provide a means of inferring character polarity, as alluded to previously in the discussion of developmental patterns. Briefly, the induction of a low dose remodelling pattern in *Eurycea*, that is aberrant for plethodontids but similar to nonplethodontid development, suggests that low dose remodelling in *Eurycea* is atavistic and that the state of abrupt remodelling exemplified by plethodontids is derived from the more gradual pattern exemplified by ambystomatids, rather than vice versa. This interpretation, which receives a posteriori support from at least two phylogenetic reconstructions, is based on the principle that derived states may be induced to revert to primitive states under experimental, environmental or genetic perturbation (Hall, 1984). Certainly, the list of alternative TH perturbations, e.g. eliminating TH activity at larval stages, that may be applied to transform an ambystomatid developmental pattern into a 'plethodontid' one has not been exhausted and, even if it had, one still cannot conclude that ambystomatids lack the potential to express a 'plethodontid' pattern. Despite this caveat, the ability empirically to transform a plethodontid pattern into an 'ambystomatid' one demonstrates two things: first, the evolutionary feasibility of an 'ambystomatid'-to-'plethodontid' transformation and second, the potential of the chosen developmental variable (in this case, rate of TH activity) to effect this transformation. The same reasoning may be applied to infer the polarity of character states in individual tissues, e.g. the 'ambystomatid' and 'plethodontid' states of maxilla morphogenesis (Table IV). However, there must be agreement among all polarities inferred from the same TH perturbation in order to maintain the assumption of conserved tissue sensitivity.

Another consequence of conserved tissue sensitivity may be the recurring pattern of

FIG. 7. Morphogenetic patterns of the anterior hyobranchium: ventral views not drawn to scale; white, procartilage; stipple, cartilage; black, bone. (a–b) late larval, midmetamorphic, and early postmetamorphic specimens; (c) a 1Autumn larva after 12 weeks of T_4. The accompanying profiles of TH activity and thresholds for cartilage formation and resorption have been inferred from the results presented in this study. The early onset of cartilage formation in *Ambystoma maculatum* (a) reveals that the anterior radial forms from the hypohyal and the posterior radial arises *de novo*. In the natural morphogenesis of *Eurycea bislineata* (b), the procartilaginous connection between ceratohyal and basibranchial is resorbed before the appearance of either radial or lingual. However, the dissociation of cartilage formation from cartilage resorption in induced development (c) reveals that the lingual emerges in continuity with the ceratohyal while the radial forms *de novo*. Abbreviations: ar, anterior radial; bb, basibranchial; cb, ceratobranchial; ch, ceratohyal; hb, hypobranchial; hh, hypohyal; li, lingual; pr, posterior radial; ra, radial; * denotes incipient formation. (a) Drawn from specimens examined in this study, (b) from specimens in Rose (1995*a*), (c) from a specimen in Rose (1995*b*).

dissociation associated with larval paedomorphosis. Considering the morphological characters standardly used for urodele systematics, Larson (1991) found no evidence for parallel evolution of character sets in paedomorphic lineages. In contrast, the present study finds that TH-dependent characters with relatively conserved morphogenesis in metamorphic urodeles appear to have undergone co-ordinate transformations in paedomorphic lineages. The remodelling events considered here exhibit a pattern of loss that is generally consistent with their TH sensitivity in *Eurycea* (Table II).

Conserved tissue sensitivity may further underlie the basic tendency of urodeles to evolve larval paedomorphosis. The low sensitivity of cartilage resorption events, which primarily involve the hyobranchium, predisposes this cranial region to resist transformation more than the nasal or palatal regions. Only a small attenuation of thyroid activity is needed to eliminate ceratobranchial reduction (and gill loss) and thus sustain the larval modes of gill breathing and suction feeding into adulthood. Conversely, the sensitivity data available for anuran cartilages (Hanken & Summers, 1988) indicate relatively high values, making it less likely that hyobranchial remodelling could become dissociated from other cranial remodelling in this group. Such an internal constraint is supported by the high incidence of urodeles retaining a larval hyobranchium and the almost total absence of this phenomenon in anurans; the few exceptions to the latter are thought to reflect abnormal thyroid glands or iodine-deficient environments (Dent, 1968; Wassersug, 1975). Tissue sensitivity data for caecilians, which resemble anurans in lacking larval paedomorphs (Taylor, 1968), may help to resolve this issue.

Whether constant or variable, tissue sensitivity clearly bears significant implications for understanding character evolution, homology, and dissociation in TH-mediated systems. More empirical analysis is needed to resolve the evolutionary lability of this parameter and to isolate developmental factors controlling the direction and extent of its evolutionary change.

Thanks to D. Auth, University of Florida; E. Gilland; H. A. Dundee, Tulane University; E. McGhee, University of Georgia at Athens; J. Reiss; and J. Rosado, Museum of Comparative Zoology for the loan of specimens. Thanks also to B. K. Hall, W. Bemis, and two anonymous reviewers for comments on various drafts of this manuscript.

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Appendix A: Specimens examined in this study

Ambystoma maculatum: 20 sectioned and 31 whole-mounted specimens covering larval, metamorphic, and postmetamorphic stages (from lab-raised clutches collected in western MA); *Dicamptodon ensatus*: three sectioned and three whole-mounted larvae (collected by John Reiss in Santa Cruz Co., CA); *Pseudobranchus striatus*: four sectioned and 18 whole-mounted specimens (UF51511, UF51514–18, UF51528–9, UF61453–4, UGAMNH 21282, UGAMNH 21287, UGAMNH 21289, UGAMNH 21291–92, UGAMNH 21303, UGAMNH 21305–8, MCZ A 107677); *P. axanthus*: 10 whole-mounted specimens (UF3088:2–6, 8, 10–13); *Pseudobranchus* species unknown: three whole-mounts (provided by Ed Gilland); *Siren intermedia*: 12 sectioned specimens (CUMSC; Bourque, 1939) and four whole-mounts (Charles Sullivan Co., Nashville, TN); *Notophthalmus viridescens*: 10 whole-mounted specimens covering larval, metamorphic, eft and adult stages (collected in eastern MA); *Cryptobranchus alleganiensis*: four sectioned larvae (CUMSC), three whole-mounted larvae (provided by John Reiss), and one whole-mounted postmetamorph (MCZ 108565); *Hynobius retardatus*: three sectioned larvae and metamorphs (CUMSC); *H. nigrescens*: one whole-mounted postmetamorph (MCZ 22512); *Necturus alabamensis*: four whole-mounts (MCZ A107681–2, A108631, A108637); *N. maculosus*: five whole-mounts (MCZ 16259–63); *Haideotriton wallacei*: five whole-mounts (TU 21064–21068; Dundee, 1962). Abbreviations: CUMSC, Cornell University Microscope Slide Collections; MCZ, Museum of Comparative Zoology; TU, Tulane University; UF, University of Florida; UGAMNH, University of Georgia at Athens Museum of Natural History.

Appendix B: Skeletal remodelling events not covered by this study

The endochondral and dermal bones (or portions thereof) that are scored in this survey are limited to those that remodel well after embryogeny and have demonstrable TH dependency in *Eurycea*. This excludes the appearances of most dermatocranial elements (the parasphenoid, squamosal, dentary, prearticular, parietal, frontal, pterygoid, coronoid, vomer, and premaxilla), which occur approximately at hatching, plus the first pedicellate teeth and certain endochondral bones (exoccipital, quadrate, prootic, and opisthotic), which occur soon afterwards (Rose, 1995*a*). Early development in nonplethodontids is generally similar (references in text, plus Greven, 1989), although ossification of the quadrate, prootic and/or opisthotic may overlap with some of the ossifications scored here (e.g. Corsin, 1966; Bonebrake & Brandon, 1971; Reilly, 1986). Metamorphic events that are common to most urodeles but excluded by scoring difficulties include the transition from unicuspid, conical teeth to bicuspid, bladed teeth and shape changes of the ceratohyal and basibranchial plate which involve both progressive and regressive remodelling (Smith, 1920; Greven, 1989; Rose, 1995*a*).

Skeletal remodelling events that are excluded on the basis of their absence (or unrecognizability) in plethodontids include: formation of a distinct operculum, which arises in cartilage at the stage of gill loss in most ambystomatids, *Dicamptodon*, and some hynobiids, and before gill loss in salamandrids and *Ambystoma mexicanum* (Larsen, 1963; Monath, 1965; and references therein); fusion of part of the palatine to the preorbital process of the vomer, which occurs during gill loss in ambystomatids and possibly some salamandrids, depending on the author (Larsen, 1963; Clemen, 1979); ossification of the lacrimal, which appears at a similar stage as the septomaxilla in hynobiids and *Rhyacotriton* and before the septomaxilla in *Dicamptodon*

(Worthington & Wake, 1971; Larsen, 1963); ossification of the articular, which is the last bone to appear in *Siren intermedia* (Bourque, 1939) and *Pseudobranchus striatus* (specimens in this study) and appears during or after gill loss in ambystomatids, *Rhyacotriton*, and salamandrids (Cloete, 1961; Srinivasachar, 1962; Bonebrake & Brandon, 1971; Reilly, 1986, 1987); ossification of bones that are restricted to one or a few species, e.g. the angular, quadratojugal, basioccipital, mentomeckelian, and sclerotic (de Beer, 1937); changes in the shape and orientation of the palatopterygoid bone which occur at gill loss in all metamorphic nonplethodontids and cryptobranchids (Aoyama, 1930; Larsen, 1963); fusion of the first hypobranchial and first ceratobranchial, which occurs at gill loss in hynobiids and cryptobranchids (Tsusaki, 1922; Edgeworth, 1923*a*; Aoyama, 1930); modifications of the hypohyal, which occur at gill loss in cryptobranchids (see previous references); and hyobranchial calcifications and ossifications (other than the os thyroideum) which usually arise at or after gill loss, though they are also common in larval paedomorphs and large larvae, e.g. *Dicamptodon* and *Gyrinophilus*.

Appendix C: Footnotes 1–8 for Fig. 1 and Table III

[1] From the size-age classes 1Autumn, 1Spring, and 2Autumn of Rose (1995*b*); the number indicates the year class of the larva and the season its time of collection. Larvae were sampled at regular intervals and prepared as whole mounts and for histology. Endochondral sequences are from 1Autumn and 1Spring classes only, as these features were already formed in 2Autumn larvae at the start of treatment.

[2] From 198 untreated larvae and metamorphs sampled from two populations and prepared as whole mounts and for histology (Rose, 1995*a*). See Rose (1995*a*) for *Hemidactylium scutatum* and *Gyrinophilus porphyriticus*.

[3] From specimens in this study, plus Winslow (1896), Terry (1906), and Higgins (1920*a*, *b*). See Keller (1946) for T_4-induced *Ambystoma mexicanum* and Bonebrake & Brandon (1971) and Reilly (1987) for *A. texanum* and *A. talpoideum*, respectively.

[4] Incomplete material necessitated a combination of observations on two similar species; from *Hynobius retardatus* specimens in this study, plus Tsusaki (1922), Edgeworth (1923*a*, *b*), and Stadtmüller (1929), on *H. nebulosus*, and Fox (1959) on *H. nebulosus* (early larval stages) and *H. retardatus* (late larval stages). See Chung (1932) for *H. leechi*, Okutomi (1936) and Larsen (1963) for *Onychodactylus japonicus*, and Lebedkina (1960) and Larsen (1963) for *Salamandrella keyserlingii*.

[5] From Medvedeva (1963), Eyal-Giladi & Zinberg (1964), and Corsin (1966). See Stadtmüller (1924) for *Salamandra maculosa* (*salamandra*), Erdmann (1933) for *Triturus vulgaris*, and Reilly (1986) for *Notophthalmus viridescens*.

[6] This pattern pertains to both *Cryptobranchus alleganiensis* and *Andrias japonicus*; from *C. alleganiensis* specimens in this study, plus Aoyama (1928, 1930) and Fox (1954) on *A. japonicus*, Higgins (1920*a*, *b*) and Jurgens (1971) on *C. alleganiensis*, and Parker (1882) and Edgeworth (1923*a*, *b*) on both.

[7] From specimens in this study, plus Bourque (1939), Hilton (1946), Altig (1965) and Reilly & Altig (In press). See Parker (1882), Noble (1929) and Larsen (1963) for *Siren lacertina*, and Hilton (1946) and Larsen (1963) for *Pseudobranchus striatus*. Noble (1929) reports a similar size range (40–60 mm TL) for pterygoid resorption in *S. lacertina* as that found here for *S. intermedia* (45–70 mm). Ossification of the columella has been reported only in large specimens (392, 405 mm, SVL) of *S. lacertina* (Larsen, 1963). *P. striatus* and *P. axanthus* both lack the maxilla,

and the smallest specimens available for each species already show complete pterygoid resorption.

[8] From specimens in this study, plus Platt (1897), Winslow (1896), Eycleshymer & Wilson (1910), Reed (1915), Higgins (1920*b*), and Shumway & Webb (1932). See Parker (1877) and Marche & Durand (1983) for *Proteus anguinus*.

Appendix D: Footnotes continued from Table IV

[2] Rose (1995*b*).

[3] Rose (1995*a*) for *Eurycea bislineata*, *Hemidactylium scutatum*, *Gyrinophilus porphyriticus*, and *Pseudotriton ruber*, plus Rose (1995*b*) for *E. bislineata* treated at 5×10^{-9} and 5×10^{-8} M T_4.

[4] Terry (1906) for *Ambystoma maculatum*.

[5] Higgins (1920*b*) for *Ambystoma maculatum*, *Necturus maculosus*, or *Cryptobranchus alleganiensis*.

[6] Eyal-Giladi & Zinberg (1964) for *Pleurodeles waltlii*.

[7] Fox (1959) for *Hynobius nebulosus*.

[8] Platt (1897) for *Necturus maculosus*.

[9] Fox (1954) for *Andrias japonicus*.

[10] Theron (1952) for *Ambystoma maculatum*.

[11] Papendieck (1954) for *Ambystoma macrodactylum*.

[12] Jarvik (1942) for *Salamandra salamandra*.

[13] Ryke (1950) for *Onychodactylus japonicus*.

[14] Chung (1931) for *Hynobius nebulosus* and *Onychodactylus fischeri* (predicted for the latter since larval stages were not examined).

[15] Chung (1931, 1932) for *Hynobius leechii*.

[16] Chung (1931) for other hynobiids (reviewed by Ryke, 1950 and Jurgens, 1971).

[17] Jurgens (1971) for *Necturus maculosus*, *Siren lacertina*, *Dicamptodon ensatus*, or *Cryptobranchus alleganiensis*.

[18] Bonebrake & Brandon (1971) for *Ambystoma texanum*.

[19] Reilly (1986) for transformed and paedomorphic *Notophthalmus viridescens*.

[20] Larsen (1963) for *Taricha granulosa*, *Salamandrella keyserlingii* and paedomorphic *Onychodactylus japonicus*; *Pseudotriton striatus* and *Siren lacertina*; or transformed and paedomorphic *Dicamptodon ensatus*.

[21] Corsin (1966) for *Pleurodeles waltlii*.

[22] Wintrebert (1922) for *Salamandra maculosa* (*salamandra*).

[23] Aoyama (1930) for *Andrias japonicus*.

[24] Parker (1879) for paedomorphic *Onychodactylus* (species unknown).

[25] Keller (1946) for transformed and paedomorphic *Ambystoma mexicanum*.

[26] Stadtmüller (1924) for *Salamandra maculosa* (*salamandra*).

[27] Specimens of *Notophthalmus viridescens*, *Ambystoma maculatum*, *Cryptobranchus alleganiensis*, *Dicamptodon ensatus*, *Hynobius nigrescens* or *Siren intermedia* and *Pseudobranchus striatus* examined in this study.

[28] Cloete (1961) for *Rhyacotriton olympicus*.

[29] Srinivasachar (1962) for *Rhyacotriton olympicus*.

[30] Worthington & Wake (1971) for *Rhyacotriton olympicus*.

[31] Marconi & Simonetta (1988) for transformed and paedomorphic *Triturus vulgaris*.

[32] Reilly & Brandon (1994) for *Ambystoma rivularis* and *A. altamirani*.