

## Phylogenetic and evolutionary perspectives on an enigmatic organ: the balancer of larval caudate amphibians

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### Summary

The balancer is a larval appendage whose taxonomic distribution is limited to approximately half of the species found in three of the ten families of salamanders. These organs are small projections from the region of the jaw joint that are present before limbs develop; they apparently function in mechanical support and chemical adhesion. Balancers are typically associated with species that are pond breeding, as opposed to stream breeding, and are therefore assumed to be adaptive in still water and non-functional in flowing water. However, many exceptions to this generalization exist. We explain this unusual distribution by combining morphological and natural history data with a phylogenetic analysis. Because the balancer has been little studied, we summarize the literature on morphology, development, variation, function, ontogenetic fate, ecological distribution, and homology. Using immunohistochemical methods we find that the balancer is a complex organ. The balancer contains a layer of type II collagen that may function as a non-bony skeleton and is innervated by the ramus mandibularis of the fifth cranial nerve. We hypothesize that balancers evolved as a synapomorphy of advanced salamander families, after the divergence of the Sirenidae, and that they have been lost independently many times. Homoplasy is unidirectional involving loss of the balancer alone, according to this hypothesis.

### Introduction

The caudate balancer is a paired, larval organ that is slender and rod-like with slightly bulbous tips.

Each balancer projects laterally and posteroventrally from a point slightly posteroventral to the eye of the hatchling, dorsal to the jaw joint. It is a morphological novelty of Caudata (Amphibia), putatively without homologue (Lieberkind 1937). Balancers are found in a limited number of caudate taxa, and only in mid- to late embryonic and early hatchling stages of development. The organ has largely escaped the recent attention of herpetologists and evolutionary morphologists. In this paper we review the literature on balancer morphology and report new, comparative immunohistochemical data. We further provide a comprehensive summary of current knowledge about development, variation, function, ontogenetic fate, ecological distribution, and homology of the balancer. We then present a phylogenetic analysis of the distribution of balancers, evaluating their origin and evolution in light of our understanding of morphology and life history.

Balancers are typically found in those caudate species that possess a pond-type larval morphology and life history (Noble 1931). These organs are thought to confer physical support and stability of a limbless larva at rest (e.g., Pough *et al.* 1998; but see below). To explain its patchy taxonomic distribution, Noble (footnote in Harrison 1925) proposed a working hypothesis that "the balancer develops in all forms where it can function," implying that this organ may function in a still pond, but not in a stream or swift current. Noble also acknowledged some exceptions, and "the absence of balancers in some pond-type larvae (e.g., sirenids) is unexplained at present" (Duellman and Trueb 1986, p. 157). Further, "the curious absence of a functional

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balancer in at least two races of *Ambystoma tigrinum* does not appear explainable at this time" (Salthe and Mecham 1974, p. 455). Comments such as these suggest that a phylogenetic analysis of balancer evolution is needed.

Balancers were first described by Spallanzani (1784), who mistook them for "smaller bundles of gills" (p. 59). These organs have been identified by various terms, including *crochets de Rusconi* (van Bambeke 1880), *Kieferfortsatz* and *Fortsatz des Kieferbogens* (Maurer 1888), *Saugnäpfe* (Wiedersheim 1875), *Rusconi'sche Häkchen* (Wunderer 1910), *Stützorgane* (Egert 1913), *Balanzierstange* (Murayama 1928), balancing-sticks (Oyama 1929b), *Haftfaden* (Mangold 1931), and *Haftorgane* (Lieberkind 1937). The term "balancer" has been credited erroneously to Clarke (1880) who, in fact, stated that the term was already in use, and noted that its origin derived from resemblance of the organs to the balancers (i.e., halteres) of dipteran insects.

## Morphology

The general morphology of balancers shows remarkable constancy. While anatomical studies of balancers have been conducted in only a few species, taxonomic diversity has been well represented: *Ambystoma jeffersonianum* (Anderson and Kollros 1962), *A. maculatum* (Latta 1919; Harrison 1925), *A. opacum* (Latta 1919; Anderson and Kollros 1962), *A. tigrinum* (Nicholas 1924b), *Hynobius nigrescens* (Murayama 1928), *Notophthalmus viridescens* (Bell 1907), *Pleurodeles waltl* (Fox 1985; Lieberkind 1937), *Triturus alpestris* (Rollhäuser-ter Horst 1977), *T. cristatus* (Lieberkind 1937), and *T. vulgaris* (Maurer 1888; Egert 1913; Lieberkind 1937).

Balancers are vascularized by branches of the hyomandibular artery and jugular vein, which together form a single loop within each organ (Harrison 1925; Maurer 1888). Each balancer is lined by two layers of epithelial cells, an outer cuboidal layer and an inner columnar layer, which cover the basement membrane (Harrison 1925; i.e., the basement lamella of Fox 1985). Filaments of collagen (Fox 1985) compose the basement membrane of the balancer and lack the high degree of orientation found in the skin (Anderson and Kollros 1962). Below the basement membrane lies an undifferentiated mesenchyme matrix (Harrison 1925; Anderson and Kollros 1962). A branch of the fifth cranial nerve supplies the balancer (Harrison 1925), and the balancer epithelium is more extensively innervated than the surrounding tissue (Fox 1985).

The bulbous tip of the balancer contains many small secretory cones, each emanating from a single epithelial cell, that excrete a sticky mucous [*Am-*

*bystoma maculatum* (Harrison 1926); *Hynobius nigrescens* (Murayama 1928); *Triturus alpestris* (Greil 1906)]. Bell (1907) may have observed these same structures, which he called thick, sucker-like projections, in *Notophthalmus viridescens*. Investigation of the cells of secretory cones by electron microscopy has revealed an extensive, granular endoplasmic reticulum and well developed Golgi complexes that are involved in the synthesis of a muco-proteinaceous substance (Fox 1985).

Dunn (1923a) and Noble (1927) reported the occurrence of "double balancers" in *Hynobius naevius*. These reports are second hand accounts of Tago's (1907) original study published in Japanese; they have never been verified. In contrast, Oyama (1929a) reported a complete absence of balancers in *H. naevius*. The photographs of *H. naevius* in Obara (1984) also show no balancers, standard or doubled, in the embryos and hatchlings. According to M. Matsui (pers. comm.), the species Tago studied was in fact *H. tokyoensis*, which has typical balancers. Perhaps the Japanese term for "paired balancers" was erroneously translated as "double balancers." Noble (1927, p. 37) also reported that double balancers were found once in *Ambystoma*, but he provided no evidence or citation for this claim.

## Immunohistochemical Analysis

Balancers of *Ambystoma gracile* (Ambystomatiidae) and *Taricha torosa* (Salamandridae) were studied using whole mount immunohistochemistry. Eggs of the two species were collected in naturally occurring ponds in Del Norte and Monterey counties, California, respectively, allowed to hatch in the laboratory, and the larvae fixed in Dent fixative (one part dimethyl sulfoxide in four parts 100% methanol). Following standard techniques (Klymkowsky and Hanken 1991), whole mount preparations were made using two kinds of antibodies: type II collagen (II<sub>B</sub>3/15A4; Linsenmayer and Hendrix 1980) which stains for dense collagen matrices, and alpha-tubulin (6-IIB-1; Piperno and Fuller 1985) which stains for peripheral nerves.

Balancers are closely similar in the two species. They are attached to the side of the head, just behind and below the eyes, and are directed posteriorly in the preserved specimens. They are well stained by type II collagen, especially near their base but also distally (Figs. 1 and 2). There is a relatively swollen basal region that is apparently an outgrowth of the outer surface of the developing palatoquadrate cartilage, immediately above the posterior end of Meckel's cartilage. Also visible at this stage of development are incomplete but paired Meckel's cartilage, trabecula, suspensorium, otic capsule and parts of the hyobranchial apparatus, as



are proximal elements of the forelimb and shoulder girdle. The surface of the balancer appears to be fuzzy, especially distally, in these whole-mounts. The apparent fuzziness may be due to secretory cones protruding from the surface near the tip (Harrison 1925). Proximal portions of the balancer are densely stained. This stained region is relatively thick and completely encircles the base of the balancer. Although it is thinner distal to the base, the densely stained material is clearly present throughout the length of the organ as a distinct layer, which is a continuation of the basement membrane (the balancer membrane of Harrison 1925). The rigidity of the balancer is likely due to this thick layer, which may serve as a compact, non-bony skeleton. Harrison (1925, p. 264 and see fig. 49) found that a severed balancer with its associated epithelium and mesenchyme removed by digestive enzymes retains its shape and appears similar to "a long narrow test-tube." However, type II collagen does not necessarily serve a skeletal function, and other factors may contribute to the balancer's rigidity.

Balancers in both species are supplied by a distinct nerve that branches from the ramus mandibularis of the fifth cranial (trigeminal) nerve. The balancer nerve is well developed and branches extensively (Figs. 3 and 4). The main nerve forms a long loop within the balancer that fades into small branches as it returns to the base of the organ.

Whole mount immunohistochemistry enables three-dimensional visualization of structures previously visible only by using histological methods involving sectioning. The innervation is especially clear using this approach, and in general our results fully support Harrison's (1925) observations concerning the morphology of the organ.

## Development

The classic study of balancer development was conducted on *A. maculatum* by Harrison (1925), whose results we briefly summarize. Internal, visible development of the balancer starts at Harrison's stage (H) 33, when the inner ectodermal layer thickens as its cells take on a columnar shape. The balancer first appears exteriorly in H 34 as a faint rounded bulge on the mandibular arch, near the palatoquadrate cartilage. At H 36, microvilli and a few cilia appear on the surface of the now cylindrical balancer. Cilia are not found on balancers of older stages (Fox 1985). Secretory cones develop at H 38, accompanied by elongation of the balancer through change in cell shape. The balancer is fully developed at H 40–41. The balancer is lost by H 46. At this point each forelimb possesses three digits.

By transplanting tissues between individuals of different ages, Harrison (1925) showed that the life-

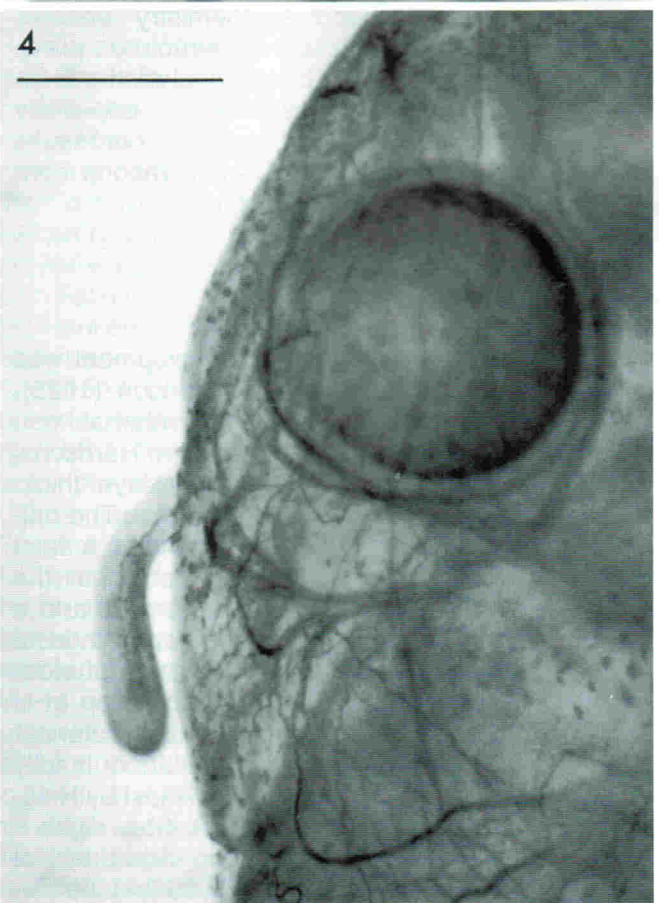
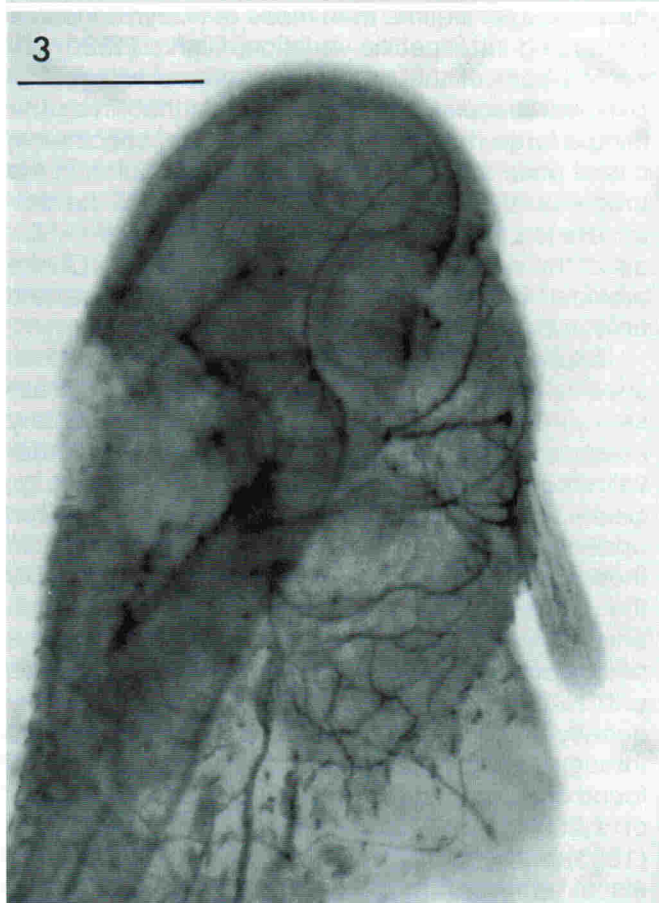
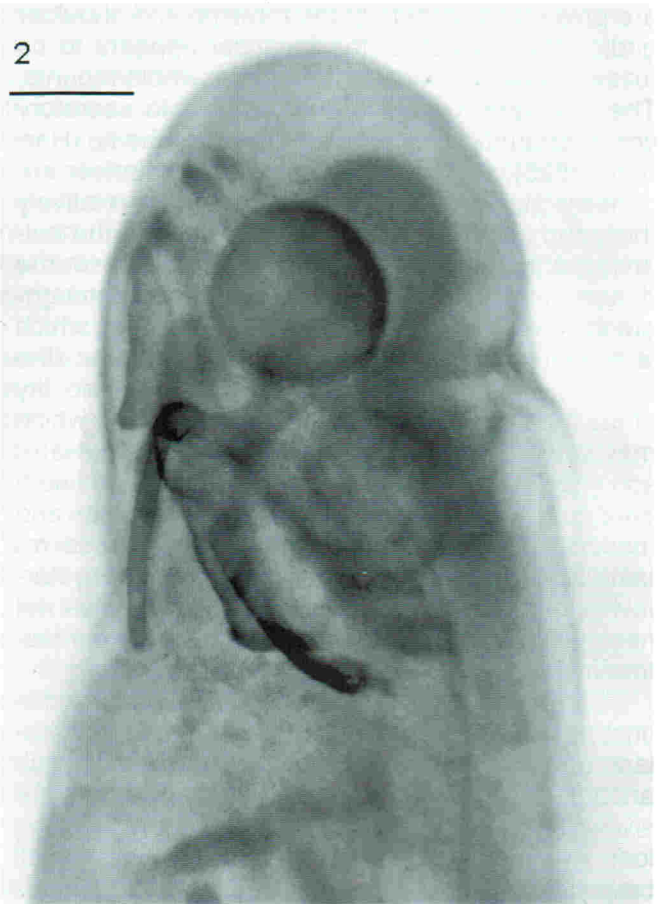
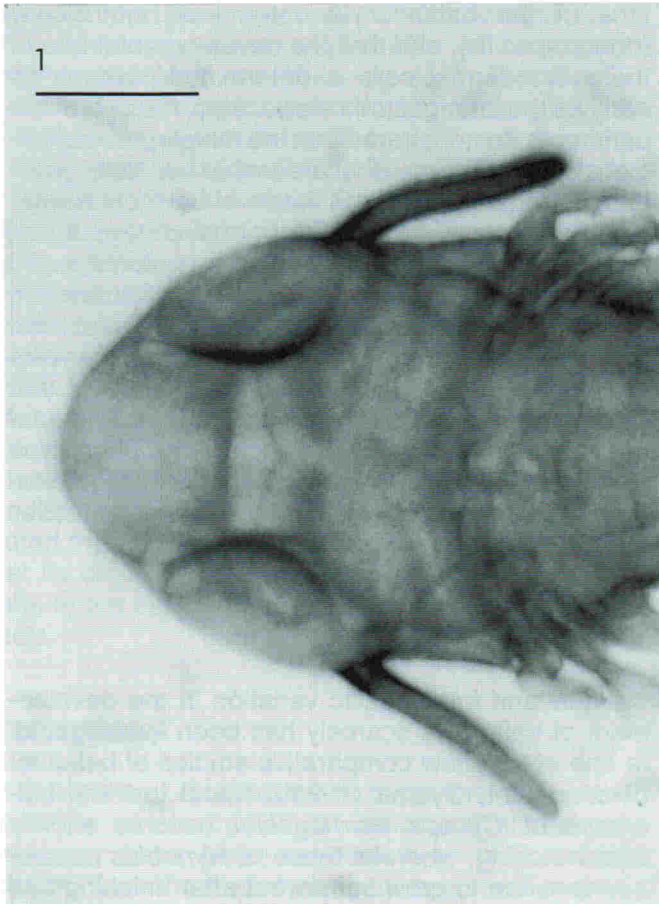
time of the balancer is determined intrinsically (donor specific), and that the developmental fate of these ectodermal cells is determined perhaps as early as the later gastrula stage. Also, transplant experiments demonstrated that the developmental origin of the balancer is ectodermal, i.e., only ectoderm is required for the initiation of balancer formation. Further evidence of ectodermal control of balancer development was revealed by heterospecific transplantation experiments between *Triturus vulgaris*, a species possessing balancers, and *Ambystoma mexicanum*, a species without balancers (Mangold 1931). These experiments showed that transplanted mandibular arch tissue from late gastrula or neurula embryos developed the phenotype of the donor, but not of the host species. Regeneration of balancers is limited or nonexistent (Bell 1907).

## Variation

Inter- and intraspecific variation in the development of balancers scarcely has been investigated. In one of very few comparative studies of balancer development, Oyama (1929b) found that the balancers of *Cynops pyrrhogaster* become shorter after hatching, whereas those of *Hynobius nebulosus* continue to grow somewhat after hatching and have a longer lifetime than those of *C. pyrrhogaster*. Regarding intraspecific variation, Clarke (1880) observed both of the preceding patterns within *Ambystoma maculatum*. Clarke claimed that "in examining a large number of [*A. maculatum*] specimens, it is at once seen that there is great variation in the progress of development. The position of the balancers too, varies considerably in different individuals of the same age (p. 115)." Unfortunately, Clarke provided no data to back up this provocative and unique claim.

Balancers are not always fully developed in species that possess them, nor are they always absent in all species said to lack them. Rudimentary balancers are described as part of the normal development of certain salamander species, e.g., *Salamandra atra* (Wunderer 1910) and *Echinotriton andersoni* (Utsunomiya and Utsunomiya 1977). In these species the balancers first appear at roughly the same stage in ontogeny as functional balancers, but they grow to only a fraction of the expected size and are quickly resorbed. Individuals with rudimentary balancers may also occur infrequently in a species that otherwise lacks balancers throughout development. Y. Misawa (pers. comm.) found one unpaired yet well-developed balancer out of 1,200 embryos of *Hynobius kimurae*. Maurer (1888) reported one paired balancer in 60 individuals of *Ambystoma mexicanum*. Rudimentary bal-







ancers have also been reported in *A. tigrinum*. Nicholas (1924a, b) reported rudimentary balancers in as many of 56 of 61 individuals of *A. tigrinum* from Wisconsin or North Dakota (exact source locality unknown), whereas Harrison (1925) found none in *A. tigrinum* from Illinois, Kansas, Minnesota, and perhaps North Dakota. Lastly, Twitty (1936) reported rudimentary balancers of various sizes in 42% of *Taricha rivularis* larvae.

## Function

Five nonexclusive functions of balancers have been proposed. The first three involve structural support. First, balancers keep the limbless larva upright while it rests on the substrate (Egert 1913). Second, balancers also function in preventing the larva from sinking into the sediment, and concomitantly serve to elevate the head and gills above the substrate into a zone where the water is cleaner (Clarke 1880). Latta (1919) tested Clarke's hypothesis by amputating the balancers of *Ambystoma maculatum*. He observed that when approaching the substrate these larvae "sink into the ooze which so cover the gills that respiration is necessarily hindered." These first two functions are the most widely cited (e.g., Duellman and Trueb 1986; Pough *et al.* 1998). Third, Clarke (1880) pointed out that not only are the gills elevated by the balancers, but so too is the pericardial region. He suggests that this is an adaptation to reduce interference to the heartbeat from the substrate.

The fourth proposed function of balancers is adhesion. This function may be more important than is

recognized currently. The distal, bulbous ends of balancers, as mentioned above, are covered with tiny mucous secreting cones, and Fox's (1985) investigation of balancer ultrastructure lends further indirect support to the hypothesized mucous-secreting and adhesion function of balancers. Harrison (1925) found that "when a needle is applied to this region, it will stick." Bell (1907) observed that "the balancers are made use of at once for holding to the sides and bottom of the dish and for supporting the body" (and pers. obsv.). Similarly, Latta (1919) noted that larvae use their balancers to hang on blades of grass, etc. Balancers may also be used to "adhere" from below to the water's surface. Captive *Salamandrella keyerlingii* larvae were observed "hanging" vertically by their balancers while feeding at the water's surface, before diving back down to the bottom of the aquarium (E. Vorobyeva, pers. comm.). We believe that the adhesion function gains support from the morphology of the organ and the presence of secretory cones.

Adhesion appears to us to be the one function that would be present in hypothesized ancestral rudimentary structures when the organ made its first evolutionary appearance.

Lastly, balancers could function as mechano- or chemo-sensory organs. This idea had previously been dismissed by Maurer (1888) who noted an absence of any differentiated sensory cells in the balancer epithelia of *Triturus vulgaris*. Fox (1985), however, has argued for the possibility that balancers indeed have a sensory function. He described the balancer of *Pleurodeles waltl* as being extensively innervated by non-myelinated neurites. We have confirmed the presence of extensive innervation of

**Fig. 1.** Dorsal view of the head and anterior trunk of a larval salamander, *Taricha torosa*, stained with antibody to type II collagen. This is a 10.0 mm (total length) larva, stage 37 or 38 (Twitty and Bodenstein 1948), collected from Bottle Pond, Hastings Natural History Reservation, Monterey Co., CA. Strong staining of the balancer membrane, especially basally, indicates that there is a skeletal component to the relatively rigid balancers. Note the lightly stained otic capsules and the even more lightly stained olfactory capsules. Scale bar equals 0.5 mm.

**Fig. 2.** Lateral view of the left side of the head and anterior trunk of a larval salamander, *Ambystoma gracile*, stained with antibody to type II collagen. This is a fresh hatchling, 15.3 mm (total length), from eggs collected from a small pond near the lower reaches of Wilson Creek, Del Norte Co., CA. It is approximately stage 43, using the normal table for *Ambystoma maculatum* (Harrison 1969). Note the deeply stained balancer and its connection to the distal end of the suspensorium, just above the articulation with Meckel's cartilage. Note also the weakly stained otic capsule, the developing trabecular cartilages, parts of the hyobranchial apparatus, and elements of the developing forelimb. Scale bar equals 0.5 mm.

**Fig. 3.** Lateral view of the right side of the head and anterior trunk of a larval salamander, *Taricha torosa*, stained with antibody to alpha-tubulin. Same data as for Fig. 1. Note the fine nerves in the balancers. These nerves are branches of the ramus mandibularis of the trigeminal nerve. Outlines of the developing brain and the fourth ventricle are also visible. Scale bar equals 0.5 mm.

**Fig. 4.** Lateral view of the left side of the head of a larval salamander, *Ambystoma gracile*, stained with antibody to alpha tubulin to reveal peripheral nerves. Same data as Fig. 2, except this approximately stage 42 (Harrison 1969) and is 16.2 mm (total length). Note the fine nerve supplying the balancer, which forms a loop. This nerve is a branch of the ramus mandibularis of the trigeminal nerve. The eye is well developed in this individual, as are all of the cranial and peripheral nerves. Patches of epithelial cilia are also stained. Scale bar equals 0.5 mm.



the balancer (see Figs. 3 and 4). Whether all the above functions are exhibited, or are adaptive in all larvae that possess balancers, is not known.

## Ontogenetic Fate

Balancers are ephemeral organs; they always are lost well before metamorphosis. Three modes of balancer disappearance have been recorded: 1) they may gradually shrivel and be resorbed (autolysis); 2) they may simply break off due to injury or structural weakness; and 3) they may break off due to an inward, constricting growth of ectoderm at the base (autotomy), with or without the formation of what has been described as an internal "epithelial plug." Harrison (1925) observed an external constriction at the base of the balancer concomitant with the formation of a so-called epithelial plug, preceding autotomy of the balancer in *Ambystoma maculatum*. Anderson and Kollros (1962) showed that constriction without plug formulation precedes balancer loss in *A. jeffersonianum*. Accordingly, balancers may be lost by a combination of modes.

Oyama (1929b) compared the loss of balancers in *Cynops pyrrhogaster* and *Hynobius nebulosus*. In the former species, balancer loss occurs via autolysis, involving the inward growth of the epithelial plug that loosely constricts blood flow to the balancer and promotes internal disintegration and resorption. In the latter species, however, balancer loss is via autotomy, in which the epithelial plug grows tightly into the base of the balancer, cutting off blood flow completely, and causing the balancer to decay and fall off. *Hynobius nigrescens* (Maruyama 1928) exhibits a mode of balancer loss similar to that of *H. nebulosus*. The balancers of *Notophthalmus viridescens* (Bell 1907), *Ambystoma maculatum*, and *A. opacum* (Latta 1919) are reportedly lost by autotomy. Lieberkind (1937) found that autolysis was the mode of balancer loss in *Pleurodeles waltl*, *Triturus cristatus*, and *T. vulgaris*. A superficial view of autolysis is illustrated for *T. helveticus* in Gallien and Bidaud (1959). Fox (1985) described what may be small primary lysosomes involved in balancer autolysis in *P. waltl*.

Of the eleven species summarized above, one finds three cases of balancer loss by autotomy in ambystomatids, two cases of autotomy in hynobiids, and in salamandrids one case of autotomy and five cases of autolysis. The occurrence of different modes of balancer loss across species may exhibit a phylogenetic pattern, as suggested by Salthe and Mecham (1974), but more data are needed. Furthermore, different modes may occur within a single species. Autolysis necessarily involves a shrinking and weakening of the balancer, thus increasing the likelihood that the balancer will simply break off (Fox

1985). Perhaps one mode serves as a backup for another. For example, when Kollros (1940) experimentally increased the life span of the balancers of *Ambystoma maculatum* beyond the normal period of autotomy, these organs were lost via resorption. Clarke (1880), who also described a constriction and concomitant reduction of blood flow at the base of the balancers of *A. maculatum*, observed a larva actively breaking off its senescing balancers.

## Ecological Distribution

Salamander larvae have been categorized based upon morphology and life history as pond, stream, and mountain-brook types (Valentine and Dennis 1964; see also Duellman and Trueb 1986; Salthe 1969; and references therein). In an archetypal pond-type species, newly hatched larvae possess a pair of balancers, long gill rami and fimbriae, and limbs still in early stages of development. The anterior limit of the caudal fin in these larvae is greater, and the caudal musculature is less developed, than in stream- and mountain brook-type larvae. Embryonic and larval growth exhibit an exaggerated anterior to posterior gradient as compared to stream-type and mountain-brook-type larvae. Older pond-type larvae also typically possess longer toes and a lateral line system. In addition to these morphological differences, pond-type species also exhibit differences in life history mode: total clutch sizes are larger and eggs are often laid in clusters that are exposed, ova are smaller and possess melanin in the animal hemisphere, hatching is precocial, and parental care of clutches is not observed. Stream and mountain brook larvae lack balancers, have short gills, have a fin restricted to the tail, and hatch with well developed limbs.

While balancers generally occur in those species whose larvae lack functional forelimbs upon hatching, it may be more accurate to say that balancers are found in those species whose larvae *begin feeding* before or concomitant with the development of functional limbs. Some caudate species completely lack functional appendages upon hatching, having no balancers or adequately developed limbs. Among these species, there may exist a previously unreported correlation between the absence of balancers and the presence of a well provisioned yolk supply. A key difference between these hatchlings and the typical pond-type larval life history, is that upon hatching these species are still utilizing their maternally provisioned yolk supply and are not required to obtain their own energy. This suggests that the occurrence of balancers may be more tightly correlated with the state of limb development not at the time of hatching, but at the time of first feeding.



Examples of this novel larval life history strategy include the following. Within Hynobiidae, *Hynobius formosanus*, *H. kimurae*, *H. naevius*, and *H. sonani* all lack balancers and appear well provisioned with yolk (Oyama 1929a; Kakegawa *et al.* 1989; Matsui 1993). Size of gills, anterior extent of the tail fin, and the steep anteroposterior gradient of development in these species seem intermediate between pond and stream types. The larvae hatch out without functional limbs or balancers, but they still possess a large amount of yolk. The yolk supply remains until the forelimbs have developed into functional appendages and the larvae begins feeding.

Such observations are not limited to the hynobiids. Among the salamandrids, *Echinotriton* hatchlings are well provisioned with yolk (Utsunomiya and Utsunomiya 1977, plate II). *Chioglossa lusitana* hatchlings also lack a balancer and limbs and seem well endowed with yolk (Figs. 5 and 6 in Gonçalves 1962). The same may be true of an undescribed species of *Pachytriton* whose development is reported by Thiesmeier and Hornberg (1997). Further evidence of this correlation is found in species of the plethodontid tribe Hemidactyliini. The somewhat pond-type hatchlings of *Stereochilus* are apparently without functioning limbs, and "when resting the larvae lie on the bottom on their sides," and "the mouth is apparently unopened at hatching, and much yolk material is present" (Schwartz and Etheridge 1954).

## Homology

The observation that balancers develop from ectoderm that is associated with the mandibular arch (based on patterns of innervation) suggests that, *contra* Noble (1931; p. 23), they are not evolutionary homologues of the adhesive organs of Anura (Lieberkind 1937), which develop from the hyoid arch. For the same reason, balancers cannot be considered serial homologues of the gills (Latta 1919). Thus, balancers are a novelty of Caudata, though not all species have them. The question of whether balancers arose singly, and are all homologues, or multiply, representing one or more parallelism within Caudata, is discussed below.

## Phylogenetic Distribution

In addition to the ecological component of the distribution of balancers there is a historical component as evidenced by the phylogenetically non-random distribution of pond-type larvae without balancers. Here we present the first phylogenetic analysis of the distribution of balancers and evaluate their evolutionary history in light of what is known

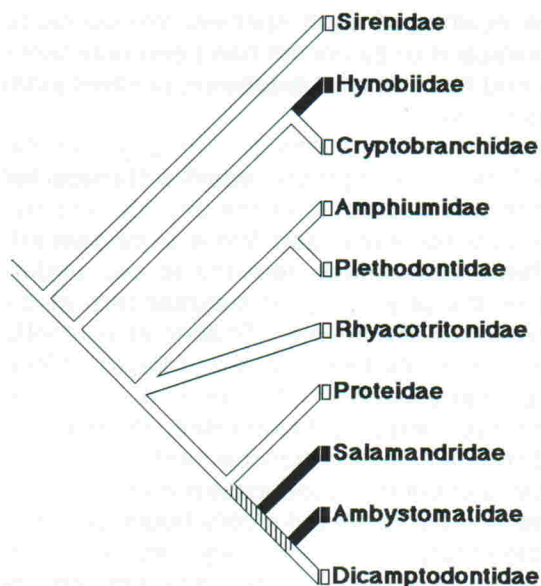
about the ecology of each species. We conclude that the evolution of balancers has been non-parsimonious and has involved extensive, unidirectional homoplastic loss.

There are two limitations to our phylogenetic analyses. First, a phylogenetic analysis of character evolution is only as reliable as the phylogenetic hypotheses on which it is based. While significant advances have been made recently to our understanding of the phylogeny of salamanders at diverse taxonomic levels (e.g., Shaffer *et al.* 1991; Larson and Dimmick 1993; Titus and Larson 1995, 1996; Jackman *et al.* 1997), further work is needed on several taxa, e.g., the Hynobiidae, Plethodontidae, and certain salamandrids. Second, knowledge of balancer presence or absence is not recorded for many species. Because balancers begin development in the embryo and are quickly lost, their presence or absence is often not mentioned in species descriptions and natural history accounts. The Appendix provides a compilation of data from the literature (and a few previously unpublished observations) concerning the presence or absence of balancers in members of the three families in which they occur. The possibility exists that the larvae of a species may lack balancers, while the embryo may possess rudimentary balancers that are resorbed before hatching or parturition.

Balancers are known from three caudate families: Hynobiidae, Salamandridae, and Ambystomatidae (Peters 1964; see Fig. 5). Balancers are absent in the phylogenetically basal Sirenidae, yet larvae of this family typically display a pond-type life history and adults retain larval morphology and live only in pond habitats. Hatchlings of *Pseudobranchius axanthus* have extensive dorsal and ventral fins although, unlike most pond-type species, they already possess three digits (Goin 1947). The newly hatched larva of *Siren lacertina* exemplifies a pond-type morphology (Goin 1947), except for its lack of a balancer. The same holds true for *S. intermedia*, whose larva nerver shows even a vestigial balancer (Noble and Marshall 1932). As Noble (footnote in Harrison 1925) pointed out, "This little fellow was in sad need of a balancer for he could lie only on his side." Noble recognized but could not account for this notable exception to this functional or phylogenetic hypotheses of the taxonomic distribution of balancers. The absence of a balancer in the pond-type larvae of Sirenidae, as in the case of pond-type plethodontids, suggests that this organ does not follow a strictly ecological or functional distribution.

Stream-type larvae are found throughout the families Cryptobranchidae, Dicamptodontidae (Noble 1925, 1927), and Rhyacotritonidae (Good and Wake 1992). The absence of balancers in these taxa is expected, concomitant with the absence of pond-type larvae, and no balancers have been recorded in the





**Fig. 5.** This phylogenetic hypothesis of family level relationships in the order Caudata is derived from the strict consensus tree of Larson and Dimmick (1993), in which the relationships of Rhyacotritonidae are unresolved. The occurrence of balancers within families is shown in black. Hatched lines indicate that the ancestral character state in this lineage remains unresolved under the criterion of parsimonious evolution (i.e., minimizing the total number of character state changes). The balancer or its homologue is unknown outside this clade. References are given in Appendix.

literature. Species of *Amphiuma* (Amphiumidae) lay their eggs on land, larvae hatch at an advanced state of development, complete with functional limbs (Hay 1888; Weber 1944), and balancers are not known within this group (G. K. Noble, footnote in Harrison 1925). Although he did not publish a developmental series, Latta (1919) reported having found no balancer among several series of sections of embryos and larvae of *Amphiuma* sp. The existence of rudimentary balancers in this family should not be ruled out until complete developmental sequences are available for all species.

Balancers are unknown within Proteidae. Complete developmental sequences are available for *Necturus maculosus* (Eycleshymer and Wilson 1910) and *Proteus anguinus* (Briegleb 1962; Durand 1971). However, the existence of rudimentary balancers within other species of *Necturus* should not be ruled out until complete developmental sequences are available. *N. maculosus* usually breeds in streams but often breeds in lakes as well (Bishop 1941). This species displays an intermediate larval morphology (Bishop 1941, fig. 7; Smith 1911, fig. 2). Relative to a stream-type larval morphology, the hatchling of this species displays rather

poorly developed limbs but well developed gills. Similar to a stream-type larva, however, the antero-dorsal limit of the caudal fin stops at the pelvis. Relative to *N. maculosus*, the hatching of *Proteus* appears more pond-type with its poorly developed limbs and anteriorly extensive dorsal fin, but has reduced gills (Briegleb 1962; Durand 1971).

The plethodontids include a small number of species possessing pond-type larvae, yet balancers are unknown within this group. We have been unable to substantiate the claim of rudimentary balancers in plethodontids (Duellman and Trueb 1986, p. 158). Plethodontids with pond-type larvae are limited to the tribe Hemidactyliini and include *Hemidactylium scutatum* (Noble 1931, p. 59), *Stereochilus marginatus* (Schwartz and Etheridge 1954; see above) and *Eurycea quadridigitata* (Duellman and Trueb 1986). Larvae of *Hemidactylium* show a number of pond-type characteristics, such as large gills and a strong anterior-posterior gradient of development, but they hatch with forelimbs fully developed (Bishop 1919). Another plethodontid known to hatch before the development of functional appendages is *Pseudotriton montanus floridanus*. In captivity these hatchlings rest "usually on their backs or sides" (Goin 1947), but otherwise conform to a stream-type morphology.

Noble (1925) interpreted the absence of balancers in plethodontids by invoking evolutionary history. He pointed out that balancers are lacking in any pond-type species which have been derived from terrestrial or mountain-brook forms. Pond-type characteristics of *Hemidactylium* and *Eurycea*, such as increased clutch size and enlarged caudal fin and gill rami, represent a derived condition within Plethodontidae (Noble 1931), or a reversal within Caudata (Dunn 1923a; Larson and Dimmick 1993, if one assumes that sirenids represent the ancestral condition).

## Caudata

Mapping the occurrence of balancers on the family level caudate phylogeny motivates several hypotheses of their evolution (Fig. 5). In light of several phylogenetic hypotheses of the relationships of salamander families in which sirenids are the basal extant taxon of Caudata (Duellman and Trueb 1986; Larson and Dimmick 1993), coupled with the observation that sirenids conform to an otherwise archetypal pond-type morphology, we interpret the probable absence of balancers in sirenids (and all outgroup taxa) as evidence that these organs evolved after the split between Sirenidae and the common ancestor of all other extant caudates. It is of course possible that sirenids lost balancers, since we argue that loss has been common (see



below), but given that sirenids use the only habitat in which balancers appear to function, we consider loss to be unlikely.

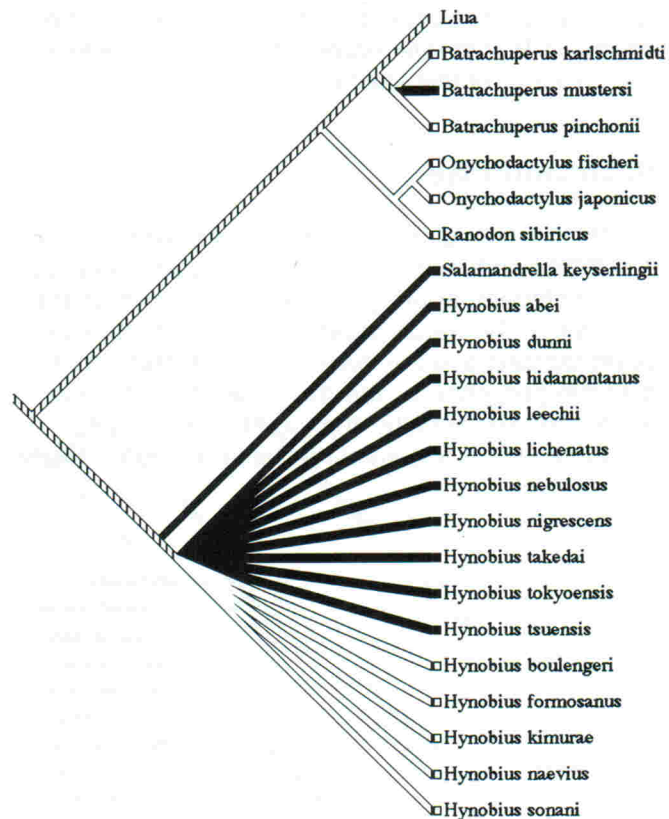
There are two equally parsimonious hypotheses (i.e., minimizing the amount of evolution necessary) concerning the evolution of balancers: they evolved three times (ancestors of the hynobiids, the salamandrids, and the ambystomatids), or twice (once in the ancestor of Hynobiidae and once in a common ancestor of Salamandridae, Dicamptodontidae, and Ambystomatidae) with subsequent loss in the dicamptodontid ancestor. It is difficult to evaluate which of these two hypotheses is more likely, unless one argues that losses should be more likely than gains, in which case the latter scenario would be favored.

We argue, however, that the evolution of balancer organs involved more homoplasy than *a priori* assumptions of parsimonious character evolution suggest. Balancers are complex structures and are a unique morphological feature without homologue outside of Caudata. These observations lead us to believe that balancers did not originate more than once within the lineage, but more likely evolved one time and have experienced numerous secondary losses. Thus, we hypothesize that the evolution of balancer organs at the family level consisted of a single origin and from four to six subsequent losses. Even if the relationships among the taxa of less well-supported nodes were altered to minimize homoplasy in balancer evolution, given a single origin of this character one would have to hypothesize an independent loss occurring in the ancestor of each of the taxa Cryptobranchidae, Dicamptodontidae, and Proteidae.

## Hynobiidae

The most basal taxon that possess balancers is the eurasian family, Hynobiidae (Fig. 5), but not all hynobiids have balancers. The ancestral condition for the family is ambiguous (Fig. 6), but we hypothesize that balancers were present. Noble (1927) believed Hynobiidae to be the most basal of all caudates, whose common ancestor was a pond-type species, and from this stock the stream-breeding taxa evolved secondarily. Subsequent data suggest that sirenids are the basal caudates (Fig. 5), and if this family is assumed to represent the ancestral caudate condition, then the hynobiid ancestor was still most likely a pond-type species, like that of sirenids, but with the addition of a balancer.

If the presence of balancers is plesiomorphic for Hynobiidae, then one might weight character state gains over losses and hypothesize that the loss of balancers across genera represents independent evolutionary events, likely correlated with the adaptation of each lineage to a stream or mountain-



**Fig. 6.** This phylogeny of hynobiid salamanders represents the hypothesis of generic level relationships by Zhao *et al.* (1988), with the addition of those species for which data on the presence or absence of balancers are available. Intrageneric relationships are unresolved. Species known to possess a balancer are represented in black. Data are unavailable for *Liua*. References are given in Appendix.

brook habitat, as Noble (1927) suggested. Indeed, *Ranodon* and *Onychodactylus* are mountain-brook forms (Dunn 1923b), as are most species of *Batrachuperus* (Liu 1950). *Batrachuperus mustersi* provides an interesting exception that Reilly (1983) classified as an intermediate form between mountain-brook and stream types. This species has balancers at hatching, but they are soon lost (Nawabi 1965). Its tail fin is shortened relative to its congeners, but the gills are larger, clutch size larger, ova smaller, and larvae tend to live in pools (Reilly 1983).

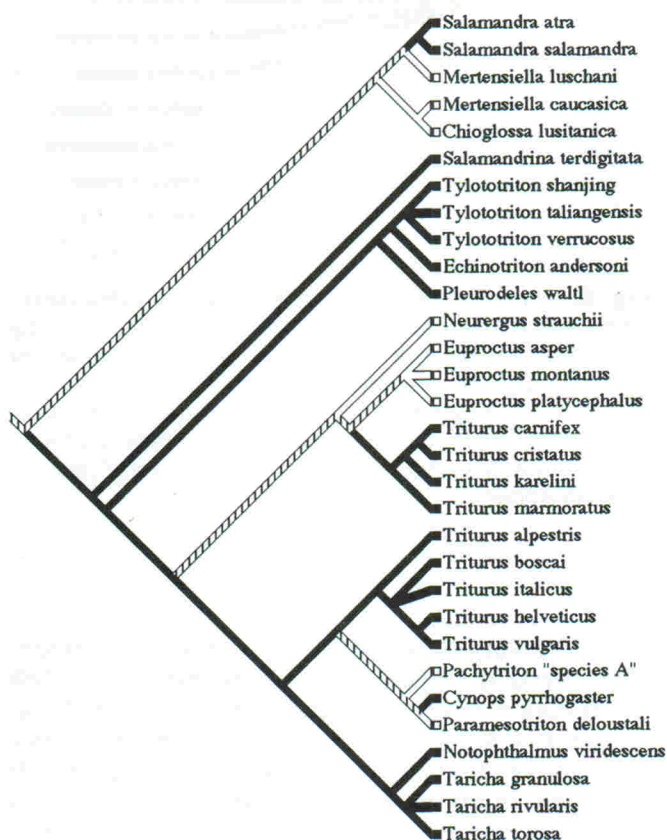
Alternatively, one could make no assumptions of the outgroup character state, in which case the balancer evolved independently at least three times, once in each genus in which it is found. Evidence against this hypothesis is the observations (Y. Misawa, pers. comm.) of a single, unpaired balancer on one in 1,200 embryos of *Hynobius kimurae* from Kyoto, Japan. We interpret this anomalous balancer



as an atavism, suggesting that the absence of balancers in *H. kimurae* represents a secondary loss in the ancestor of this species or lineages.

## Salamandridae

Optimization of the occurrence of balancers, both functional and rudimentary, on the given salamandrid phylogeny again yields an ambiguous character state in the basal lineage (Fig. 7). There is no *a priori* reason based on parsimony to assume that either the presence or the absence of a balancer is ancestral. Historically, the pond-type condition is presumed to be ancestral for Salamandridae. Noble



**Fig. 7.** This hypothesis of relationships within the family Salamandridae is based on Titus and Larson (1995), with the addition of congeneric species for which data on the presence or absence of balancers are available. Relationships among additional *Triturus* spp. are based on Macgregor *et al.* (1990) and the occurrence of chromosome 1 syndrome. *Echinotriton* is placed with *Tylotriton*. The presence of either functional or rudimentary balancers is shown in black. Hatched lines indicate that the ancestral character state in this lineage remains unresolved under the criterion of parsimonious evolution (i.e., minimizing the total number of character state changes). References are given in Appendix.

(1925, 1927) believed the pond-type life history of *Pleurodeles* and *Tylotriton* represented the basal condition for the family (cf. Wake and Özeti 1969), and likened these genera to the life histories of pond-type hynobiids and ambystomatids. Noble regarded these two families together as comprising the sister clade to the salamandrids. However, more recent hypotheses place Hynobiidae more basal within the order (Fig. 5). Therefore, we must look for other evidence concerning the ancestral condition of salamandrid life history.

If a balancer-possessing, pond-type morphology is ancestral for Salamandridae, the six salamandrid lineages lacking balancers represent independent losses of the balancer (Fig. 7). This view may be regarded as the conventional wisdom. Evidence in favor of this scenario includes the following. In at least four of these six lineages, the absence of the balancer is associated with a stream-type morphology that includes reduced or absent lungs. The stream-type condition is thought to be derived since the presence of lungs is ancestral. For example, *Euproctus* larvae are of the mountain-brook form (Noble 1925) and have reduced or rudimentary lungs (Wilder and Dunn 1920). Similarly, *Chioglossa* is a lungless mountain species (Wilder and Dunn 1920). *Neurergus strauchii* is also regarded as a mountain brook-type species (Schmidtler and Schmidtler 1975), although variation among subspecies exists (Steinfartz 1995). Lastly, *Mertensiella caucasica* appears to be a stream-type species as well (Schultschik 1994), and is sometimes ovoviparous.

As further evidence of the presence of balancers being ancestral for Salamandridae, three lineages (*Salamandra salamandra*, *Taricha rivularis*, *Echinotriton*) within the family possess rudimentary balancers. These under-developed organs are thought to give evidence of a pond-type ancestor because species having rudimentary balancers possess larval morphologies intermediate between pond- and stream-type categories, and are thought to have evolved from a pond-type ancestor (Riemer 1958 for *Taricha*). *Neurergus strauchii* spp. may represent a similar case because it lacks a balancer, whereas its three congeners all have pond-type larvae and may possess balancers (Schmidtler and Schmidtler 1975; Steinfartz 1995).

Alternatively, application of strict parsimony criteria – minimizing the overall number of character state changes with gains otherwise less likely than losses – results in six independent character state changes within the modified salamandrid phylogeny (Fig. 7), consisting of two independent acquisitions and four secondary losses. Under this scenario, balancers evolved independently in *Salamandra* and in the most recent common ancestor (MRCA) of the newts, with secondary losses in the ancestors



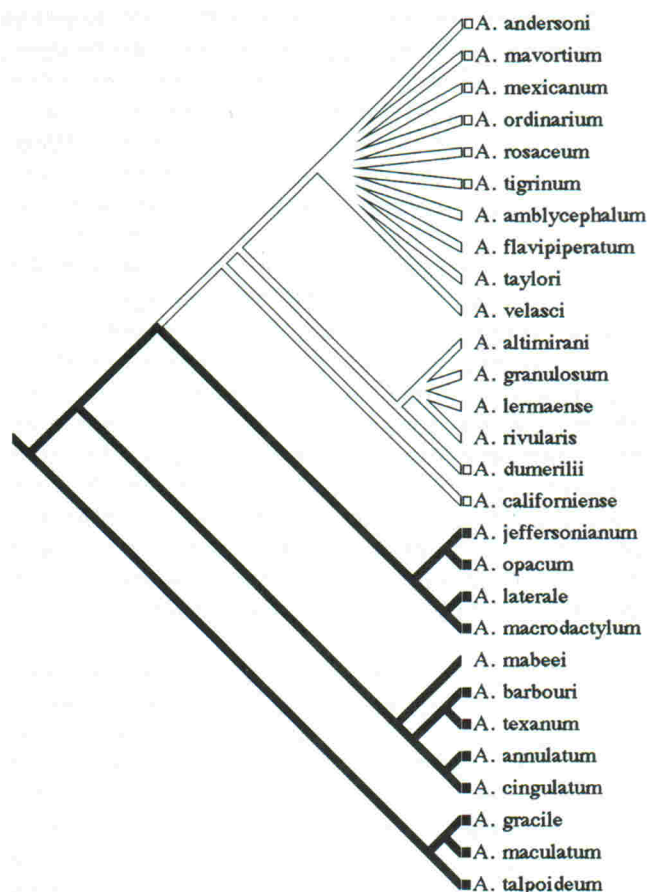
of *Neurergus*, *Euproctus*, *Pachytriton*, and *Parame-sotriton*. This scenario requires only one more evolutionary step than the hypothesis that balancers are ancestral for the whole of Salamandridae. Evidence against this hypothesis includes the presence of rudimentary balancers in the intrauterine embryos of *Salamandra* (Wunderer 1910), which suggests that this genus evolved from a pond-type ancestor (Noble 1925, 1927). The same could be true of *Chioglossa* and *Mertensiella*, but complete developmental sequences are unavailable for these species (Appendix).

Among caudate species, *Salamandrina terdigitata* appears unique in that it possess a pond type larval morphology (Griffiths 1996), including well-developed balancers (Lessona 1874; Lanza 1983; *contra* Duellmann and Trueb 1986), yet it is associated with stream habitats. This species has been reported as living and breeding in mountain streams (Wilder and Dunn 1920), as well as living in quiet backwaters and pools (Salthe and Mecham 1974). According to Lanza (1983), *S. terdigitata* occurs in mountain streams, but in weakly or slowly moving water in pools at the base of small rapids or torrents. This species possesses more or less pond-type larvae that live in mountain streams, but in pond-like sections of those streams.

We support the traditional view that the presence of balancers is ancestral for Salamandridae, and that these organs have been lost six or more times independently, concomitant with an evolutionary transition to the stream habitat. The persistence of rudimentary balancers in some species might suggest a more recent transition than occurred in those descendent lineages completely lacking these organs. This idea may be tested by estimating times since divergence between species that possess rudimentary balancers and their most closely related sister taxon with fully developed balancers. These values should tend to be smaller than the estimated times since divergence between those species that completely lack a balancer and their most closely related sister species that shows a fully developed balancer.

## Ambystomatidae

Phylogenetic analysis of the taxonomic distribution of balancers in ambystomatids reveals a remarkably simple pattern. Basal lineages within this family all have balancers, whereas all species in the more nested taxon that includes *Ambystoma andersoni* and *A. californiense* (the *A. tigrinum* clade) apparently lack balancers (Fig. 8). The presence of balancers is very likely ancestral for Ambystomatidae, and a secondary loss of the balancer occurred just once in the MRCA of tiger salamanders. We



**Fig. 8.** This phylogenetic hypothesis of relationships within the family Ambystomatidae is based on Shaffer *et al.* (1991), Shaffer and McKnight (1996), and the Tree-of-Life website. The presence of balancers is shown in black. The absence of balancers in *A. tigrinum* is based on Harrison (1925). References are given in Appendix.

support the conclusion from our parsimony analysis that the absence of balancers in *A. tigrinum*, *A. mexicanum*, *A. ordinarium*, *A. andersoni*, *A. rosaceum*, *A. dumerillii*, and *A. californiense* (Appendix) is the product of a single evolutionary event.

Despite the simple phylogenetic history of balancers in ambystomatids, this group presents the greatest enigma. With the exception of *Ambystoma ordinarium*, the larvae of tiger salamanders possess an otherwise paradigmatic pond-type natural history. The hatchlings are not known to possess large yolk supplies, as observed in the balancerless *Echinotriton* and certain *Hynobius* species. Why, then, do these species lack balancers and/or have failed to re-evolve them? A phylogenetic perspective of ambystomatid balancers simplifies this question greatly, but in the absence of known correlates, it does not suggest an explanation.



Curiously, two species within this balancerless clade breed and lay eggs in streams. *Ambystoma rosaceum* breeds in slow-moving mountain streams, but, like *Salamandrina*, shows no larval adaptations to this habitat (Anderson and Webb 1978). *Ambystoma ordinarius*, on the other hand, breeds in fast-moving mountain brooks, and, like *Taricha rivularis*, has evolved a larval morphology intermediate between pond- and stream-types (Anderson and Worthington 1971). Hatchlings of this species have smaller gills and a fully developed tail fin that regresses quickly to a "stream condition." Ovum size is small as in pond-type species, but clutch size is reduced and limb development is accelerated similar to a stream-type species. In accordance to the phylogenetic hypothesis above, however, the lack of balancers in these two ambystomatid species should not be regarded as an adaptation to the stream habitat, *contra* Anderson and Worthington (1971), but as the ancestral condition of this lineage.

*Ambystoma barbouri* offers a second example of a species that has colonized running water but retains a balancer (J. Petranksa, pers. comm.). Differences between the intermediate stream-type *A. barbouri* and its sister taxon, the pond-type *Ambystoma texanum*, include the following. *A. barbouri* lays its eggs singly on the undersides of rocks in streams, clutch size is two times smaller, eggs are two to three times larger, and larvae hatch roughly two Harrison stages later than those of *A. texanum* (Kraus and Petranksa 1989; Petranksa 1982). Phylogenetic considerations allow the assignment of a "typical" *Ambystoma* pond-type morphology and life-history to the ancestor of *A. barbouri* (Fig. 8). Therefore, in the evolution of *A. barbouri* from its more pond-type ancestor, numerous life history characters have approached stream-type states while the balancer persists. This "phylogenetic inertia" of the balancer supports our contention, based on morphological considerations, that these organs are not phenotypically plastic.

## Overview and Directions for Further Research

Simple observation of the phylogenetic distribution of balancers suggests that these organs have undergone numerous independent acquisitions (Figs. 5 and 6) and losses (Figs. 7 and 8). We argue, however, that the most likely evolutionary history of the balancer is less parsimonious. By combining morphological and ecological data with the phylogenetic data, we conclude that the balancer is a synapomorphy of the derived salamander families, i.e. this organ evolved only once, subsequent to the split of the sirenid lineage from the an-

cestor of all other extant caudates (Fig. 5), and that all subsequent character state evolution has involved only the repeated, homoplasious loss of the balancer.

While it is difficult to quantify morphological complexity, we believe that the balancer, and its component genetic and biochemical machinery, are sufficiently complex to make unlikely the possibility that these organs evolved more than once. The ecological distribution of balancers is widely consistent with our hypothesis of a single origin with several subsequent instances of loss. We note that the reduction or absence of balancers in any derived (i.e., non-sirenid) taxon is correlated with the evolution of a derived larval life history mode of one kind or another, with the singular exception of the tiger salamanders. This observation holds both within and between families. Phylogenetic arguments favor a pond-type life history mode as being ancestral for both Salamandridae and Ambystomatidae. The evidence is less strong for Hynobiidae, but any hypothesis of evolutionary history within this group remains speculative pending a rigorous phylogenetic systematic study of the group. Also needed are more data on the occurrence of balancers, especially in embryonic development.

Critical to any hypothesis of the evolution of balancers is a general understanding of their functional morphology and ecological role. While no data exist on how variation in balancer morphology may be related to fitness, the organs do keep larvae upright, slightly elevated, and allow them to adhere to surfaces. However, such observations do not *a priori* reveal in what functional and ecological contexts these organs evolved, are adaptive, or are lost. The existence of six taxa (Sirenidae, Hemidactyliini, *Hynobius*, *Chioglossa*, *Echinotriton*, and *Ambystoma*) in which hatchlings may lack both a balancer and functional limbs raises questions concerning the supposed functional consequences to larval survival of having no anterior appendages. Clearly, a caudate larva without appendages may survive outside the ovum. Of these six taxa, however, only one, the tiger salamanders (*A. tigrinum* clade), has an ancestor hypothesized both to have balancers and to lack functional limbs at the time of first feeding. The presence of balancers, therefore, may be more tightly correlated with the extent of larval development at feeding rather than at hatching. If this correlation has biological reality, the evolutionary significance of balancers might be found by looking at their functional role while feeding (recall the above observations by E. Vorobyeva), not while resting.

This study of balancer evolution raises the larger issue of the evolution of pond and stream type morphologies and natural histories, as well as the more general question of whether the pond/stream dichotomy is, in fact, valid (e.g., the unusual case of



*Salamandrina terdigitata*). With the production of more complete and robust phylogenetic hypotheses for groups of caudates, we are in a better position to address questions concerning the roles of ecology and phylogeny in promoting or constraining the evolution of complex and integrated traits (i.e., clutch size, ovum size, balancers, gills, timing of developmental events along anterior-posterior axis, parental care, etc.). A detailed, comparative analysis of whole suites of traits involved in the evolutionary transition between so called pond and stream type life histories would reveal the order of character state changes and the nature and strength of correlations among traits. Such analyses may suggest possible mechanisms of evolutionary change. To address such questions, one might compare the evolutionary transitions found in such pairs of taxa as *Ambystoma barbouri* and *Taricha rivularis*, various genera of salamandrids, or salamandrids and hynobiids. One might also compare the evolution of pond type traits in the plethodontids, *Eurycea*, *Hemidactylium*, and *Stereochilus*.

For example, one hypothesis for the evolution of balancers might be that salamander populations may experience an increase in fecundity selection, leading to an increase in clutch size, which indirectly causes a decrease in ovum size (Salthe 1969). Smaller ova would necessitate greater nutritional self-sufficiency on the part of hatchlings and require them to hatch and feed at an earlier, limbless stage of development. The limited maternal energetic investment given to pond type larvae also causes an increase in the anterior-posterior gradient of development (Schmalhausen 1925). Thus, an ancestral lineage of pond breeding salamanders that happened to evolve an energetically economical way of making forelimbs might be at a selective advantage. We hypothesize that such a complex and serendipitous event never happened in the ancestor of sirenids, but did happen in the lineage leading to all other caudates.

Our challenge to this hypothesis is the fact that limbs are not a functional requirement for pond living, as evidenced by, for example, *Pseudobranchius* (Noble, in Harrison 1925), *Stereochilus* (Schwartz and Etheridge 1954), *Hynobius naevius* (Oyama 1929a), and *Ambystoma californiense* (Twitty 1941). In streams, limblessness may not be an option for a

feeding larva, although non-feeding limbless larvae may be found in streams (Kakegawa *et al.* 1989). Therefore, a well-supplied ovum is perhaps a constraint on stream or mountain-brook breeding species, whose larvae cannot achieve self-sufficiency until at least the forelimbs are functional. Certainly, more data are needed concerning the apparent correlation between hatchling yolk supply, forelimb development, and the time of first feeding.

Finally, any phylogenetic hypothesis for the evolutionary history of balancers in the salamander lineage reveals a great deal of homoplasy, a phenomenon that is rampant in this group (Wake 1991). We believe that homoplasy is all in the same direction – loss of balancers. This argument is based on the fact that balancers are complex organs, and that the exact combination of factors making up the organ would be difficult to duplicate. How likely is it that a relatively complex organ will evolve identical structure independently within a single phylogenetic lineage? There are some remarkable similarities in function between the eye of some mollusks and those of vertebrates, but many fundamental differences suggest that they are analogues, as phylogenetic analysis reveals. In the present case we are unable to find any significant differences in structure among the taxa having the organ, and accordingly conclude that homoplastic loss of balancers has been extensive.

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## Appendix

Summary of the taxonomic distribution of balancers within the three caudate families known to possess them. Within families, species are grouped by presence of standard balancers, rudimentary balancers, and absence of balancers, then arranged alphabetically. \* conflicting accounts; + complete developmental sequence known for this species without balancers; ♂ paternal hybrid parent; ♀ maternal hybrid parent.

### Hynobiidae

#### • Balancers present in:

- \**Batrachuperus mustersi* (Nawabi 1965)
- Hynobius abei* (Sato 1943)
- H. dunni* (Sato 1943)
- H. hidamontanus* (M. Matsui, pers. comm.)
- H. leechii* (Sato 1943)
- H. lichenatus* (Sato 1943)
- \**H. naevius* (Tago 1907; contested by Oyama 1929a)
- H. nebulosus* (Oyama 1929b)
- H. nigrescens* (Murayama 1928; Okada 1933)
- H. takedai* (M. Matsui, pers. comm.)
- H. tokyoensis* (Sato 1943)
- H. tsuensis* (Sato 1943)
- Salamandrella keyerlingii* (Shitkov 1895)

#### • Balancers absent in:

- Batrachuperus karlschmidti* (Liu 1950, hatchling shown in fig. 13)
- B. pinchonii* (Y. Misawa, pers. comm.)
- Hynobius boulengeri* (Sato 1934, 1943)
- H. formosanus* (Kakegawa et al. 1989)
- H. kimurae* (Matsui 1993)
- H. naevius* (Oyama 1929a; Obara 1984, figs. 2 and 3)
- H. sonani* (Kakegawa et al. 1989)
- Onychodactylus fischeri* (B. Thiesmeier, pers. comm.)
- +*O. japonicus* (Iwasawa and Kera 1980)
- Ranodon sibericus* (Snitnikov 1913, cited by Noble in Harrison 1925)

### Salamandridae

#### • Balancers present in:

- Cynops pyrrhogaster* (Oyama 1929b)
- Notophthalmus viridescens* (Bell 1907)
- Pleurodeles waltl* (Lieberkind 1937)
- Taricha granulosa* (Twitty 1936)
- T. granulosa* ♂ × *T. rivularis* ♀ (Twitty 1936, ex situ fertilization)
- T. torosa* (Twitty 1936)

- T. torosa* ♂ × *T. rivularis* ♀ (Twitty 1936, ex situ fertilization)
- Triturus alpestris* (Epperlein and Junginger 1982; van Bambeke 1880)
- T. boscai* (García-París 1985)
- T. cristatus* (Lieberkind 1937)
- T. carnifex* (Fox 1955)
- T. italicus* (Lanza 1983)
- T. karelini* (Fox 1955)
- T. helveticus* (Gallien and Bidaud 1959; van Bambeke 1880)
- T. marmoratus* (Salvador 1985)
- T. vulgaris* (Maurer 1888)
- Tylostotriton taliangensis* (B. Thiesmeier, pers. comm.)
- T. verrucosus* (Ferrier 1974; Shrestha 1989)
- Salamandrina terdigitata* (Lessona 1874)

#### • Rudimentary balancers present in:

- Echinotriton andersoni* (Utsunomiya and Utsunomiya 1977)
- Salamandra atra* (Wunderer 1910)
- S. salamandra* (Wunderer 1910)
- \**Taricha rivularis* (Twitty 1936; 42% freq., size variable but reduced)
- T. rivularis* ♂ × *T. torosa* ♀ (Twitty 1936, size of balancer = 0%–66% of std.)
- T. rivularis* ♂ × *T. granulosa* ♀ (Twitty 1936, size = 50%–66% of std.)

#### • Balancers absent in:

- Chioglossa lusitanica* (Gonçalves 1962)
- +*Euproctus asper* (Gasser 1964)
- E. montanus* (B. Thiesmeier, pers. comm.)
- E. platycephalus* (Alcher 1980)
- Mertensiella caucasica* (Schultschik 1994)
- M. luschni antalyana* (Özeti 1979)
- Neureergus strauchii* (Schmidtler and Schmidtler 1975)
- Pachytriton labiatum* (B. Thiesmeier, pers. comm.)
- Paramesotriton deloustali* (Rehák 1984, see figs. 3 and 4)
- \**Taricha rivularis* (Riemer 1958)

### Ambystomatidae

#### • Balancers present in:

- Ambystoma annulatum* (Trapp 1959, cited in Hutcherson et al. 1989)
- A. barbouri* (J. Petranka, pers. comm.)
- A. cingulatum* (Palis 1995)
- A. gracile* (Henry and Twitty 1940)
- A. jeffersonianum* (Harrison 1925; Anderson and Kollros 1962)
- A. laterale* (J. P. Bogart, pers. comm.)
- A. macrodactylum croceum* (Anderson 1967)
- A. maculatum* (Clarke 1880)
- A. opacum* (Anderson & Kollros 1962)
- A. taploideum* (Volpe and Shoop 1963)
- A. texanum* (Brandon 1961)

#### • Rudimentary balancers reported in:

- \**A. mexicanum* (Maurer 1888, 1 in 60)
- \**A. tigrinum* (Nicholas 1924b, N. Dakota, 56 of 61)
- A. tigrinum* × *A. laterale* hybrid (Bogart et al. 1987, balancers absent or 1 side only)
- A. tigrinum* × *A. texanum* hybrid (Bogart et al. 1987, balancers absent or 1 side only)

#### • Balancers absent in:

- A. andersoni* (Krebs and Brandon 1984)
- A. californiense* (Twitty 1941)
- A. dumerillii* (Brandon 1972)
- A. mavortium* (Webb and Roueche 1971)
- +*A. mexicanum* (Schreckenbach and Jacobson 1975)
- \**A. opacum* (Noble 1931, p. 51, "rarely absent")
- A. ordinarium* (Anderson and Worthington 1971)
- A. rosaceum* (Anderson and Webb 1978)
- A. tigrinum* (Harrison 1925; Bogart et al. 1987)

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