Chapter 5
Homoplasy, a Moving Target

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5.1 Background

My doctoral dissertation dealt with evolutionary diversification of a lineage of salamanders, the Lungless Salamanders (family Plethodontidae), the largest salamander clade, then with 173 species and now with ~443 species (AmphibiaWeb 2014; Wake 1966). Aquatic larvae are characteristic of salamanders, including many plethodontids, but most plethodontids have direct development—an encapsulated embryo passes through a gilled phase but hatches as a miniature of the adult. Some plethodontids spend their entire lives as gilled aquatic forms, in essence larvae that ultimately mature sexually while remaining larvamorphs. I was struck by the extent of homoplasy in the clade. For example, gilled forms had achieved sexual maturity in several different clades. While homoplasy of life history might be expected in salamanders, other kinds of homoplasy should not have been more common than in other taxa. Many features had evolved homoplastically: projectile tongues, autotomy planes in tails, interdigital webbing, increases in numbers of vertebrae accompanied by body and tail elongation, and fifth toe loss (as well as other traits). Larval reproduction, termed neoteny in salamanders, was well known in other salamander families (e.g., axolotls, mudpuppies, olms, and sirens). I came to understand that neoteny, a particular kind of paedomorphosis—the appearance of embryonic or youthful traits of ancestors in later, adult stages of descendants—took many forms in this lineage. I envisioned metamorphosis as being something more than simply the transformation of a larva into an adult. It was a span of time during which development was accelerated for many features, with diverse outcomes. When direct development evolves, metamorphosis (in the sense of an identifiable transformation from a gilled aquatic organism to a fully terrestrial one) is

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abandoned and selection is relaxed; some traits develop as in metamorphosing species, others develop more slowly or more rapidly. Some traits never appear. Thus, loss of metamorphosis is in effect an enabling event that leads to new opportunities through a kind of mixing and matching, which can produce innovation, but may also promote homoplasy. This relaxed or differential metamorphosis led to heterochrony and heterotopy, changing both the relative timing of different developmental events and the spatial relationships of morphological traits during development.[1] I saw such phenomena as opportunities for evolutionary experimentation.

Skip forward a decade. My research on homoplasy had progressed. I focused special attention on the convergent evolution of projectile tongues in salamanders, and showed that the most extreme kinds had evolved independently three times in plethodontids alone (e.g., Lombard and Wake 1977). Differential metamorphosis had played an important role. For example, loss of a functional larval stage had enabled the extreme specialization of very long tongues because intervening developmental stages did not have to function as parts of suction-generating mechanisms in larvae. Then Steve Gould's book *Ontogeny and Phylogeny* appeared (Gould 1977). A neotenic salamander, the famous Mexican Axolotl, was on the cover and its story was an important part of the book. Axolotls were mysterious organisms when first studied by scientists in the nineteenth century. They resembled giant salamanders but had gills as adults and their classification was uncertain. Then the French biologist Duméril raised some in the laboratory. Whereas the parents remained larval throughout life, the offspring metamorphosed into salamanders. Axolotls were seen as an example of evolutionary reversal, a violation of Dollo's Law (see below) and one of the three modes of homoplastic evolution (the other two are convergence and parallelism).

The mid-1970s was a time of intellectual ferment from which Evo-devo emerged. At Berkeley we enjoyed the presence of François Jacob, who was appointed a Hitchcock Professor, one of our most distinguished visiting professorships. Jacob delivered a memorable lecture on what he termed "tinkering" (derived from the French term, *bricolage*), which struck a chord with faculty and students, and led to much discussion (Jacob 1977). Jacob emphasized the contingent nature of evolution and argued that exact convergence was unlikely (e.g., eyes of squids and mammals are remarkably similar in some respects but very different in others).[2] It was just at this time that I was asked to write a review of Gould's 1977 book, and I distributed a draft to some graduate students and faculty members for their feedback (Wake 1978). As a result, I decided to offer a graduate seminar course, together with my colleague George Oster (a mathematical biologist with a background in physics), using *Ontogeny and Phylogeny* supplemented by articles such as Jacob's

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[1] I used the term "differential metamorphosis" for this mode of evolution. Although the term did not "take," I use it here to refer to the phenomenon.

[2] Gould, too, argued in favor of contingency, as in his famous metaphor about how replaying the tape of life would have a very different outcome (Gould 1989).

(whose views continue to resonate; cf. Bock and Goode 2007). That course enrolled several graduate students who went on to publish important research in Evo-devo, including Pere Alberch, Jacques Gauthier, Ed Guerrant, and Jim Hanken (see Hanken, Chap. 4, this volume). We quickly focused on what we considered to be the central issue in Gould's book, his clock-face model of heterochrony. We found the presentation to be metaphorical and imprecise, but also inspirational. When the seminar finished several of us wrote up our thoughts in a more formal style than Gould had used. We sent a draft to Gould and invited him to join us as a co-author. He liked what we had done and did not take it as criticism (which was not our intent—his "model" had stimulated us to go further), but instead responded positively and offered numerous suggestions for clarification and examples to illustrate our points. Pere Alberch, then a mid-career graduate student, took the lead in developing the paper and he was the first author, with Gould, Oster, and me appearing in alphabetical order. The manuscript was published in *Paleobiology* (Alberch et al. 1979) and it became one of my most cited papers. Our intent was to produce a quantitative method for describing how heterochronic changes in ontogeny translate into patterns in phylogeny. We were more concerned with structuralist dynamics than historical contingencies at this point. We envisioned integrating development with evolutionary ecology to examine morphological evolution. "Tinkering" was inherent in the method, made explicit in control parameters that modified "ontogenetic trajectories," a concept we introduced.

5.2 Origins of the Dahlem Conference

Pere Alberch conducted his thesis research in the framework of the burgeoning field of evolutionary developmental biology (e.g., Alberch 1980; Alberch and Alberch 1981) and in the spring of 1978 Oster took him to a Gordon Conference, where he met Lewis Wolpert. Pere, a native of Barcelona, went directly from New England to his home, and from there he wrote me a letter (July 8, 1978).

> The Gordon Conference was very interesting since I had the opportunity to meet a lot of people in a field [Theoretical Biology] that was new for me. The most important event was to meet Lewis Wolpert. He was very interested in our paper and we had a long discussion about the role of development in evolution. He also believes that 'the next major breakthrough in biology will involve the integration of development in evolutionary theory' and the product of this discussion is that we put him in contact to Gould to organize a small meeting, probably in Germany, where the topic will be evolution and development. We will try to bring together the developmental biologists that like Wolpert are interested in general principles, with evolutionists and comparative anatomists. A small list of people who will be invited has been elaborated. . .

This is the kernel that eventually became the Dahlem conference of 1981. Alberch later recorded his own impressions of these discussions (Alberch 1995, reprinted in Rasskin-Gutman and De Renzi 2009).

While I do not remember the details of what took place next, I know that Lewis Wolpert approached Silke Bernhard, the long-time organizer of Dahlem conferences in Berlin. I was invited to an organizational meeting chaired by John Bonner at Princeton, I believe in the fall of 1980. I do not remember everyone who participated but I think all who constituted the Program Advisory Committee were present: John Bonner, Eric Davidson, Gary Freeman (see Freeman, Chap. 10, this volume), Steve Gould, Henry Horn, George Oster, Helmut Sauer, David Wake, and Lewis Wolpert. It was a positive experience for me, and out of this meeting came the basic invitation list for the subsequent conference. Later some "younger German scientists" were invited to participate in the conference, including Günter Wagner (see Wagner, Chap. 15, this volume), who, although an Austrian, was doing a post-doc in Germany, and Christiane Nüsslein-Volhard, who was an active participant in the conference discussions.

5.3 The Dahlem Conference on Evolution and Development

The original conference took place in May 1981 at the Europa Center located in West Berlin. I remember it as stimulating and rewarding. Dahlem conferences were well organized, or, more to the point, *rigidly* organized. Participants were arranged in four groups, and most discussions took place within the group and intergroup meetings. A couple of journalists were present. Manuscripts (subsequently published in the book) were distributed ahead of time and were intended to be foci of discussion, but the group meetings tended to more or less ignore these and develop their own "personalities." Interaction was less widespread than I had anticipated, but adequate; the more molecularly oriented group (Group I) treated the occasion as an opportunity for detailed research discussions. I felt that the other three groups reacted more in the spirit of what the organizers hoped would happen. I agree with the assessment of Alberch (1995) that dialogue between developmental, cellular, and molecular biologists, on the one hand, and ecologists, systematists, and population geneticists, on the other, was premature; little common ground was found.

It is important to remember the intellectual context in which the conference took place. I have already mentioned Gould's book, Jacob's talk and publication, and my own work with Alberch, Gould, and Oster. Other important background ideas came from Susumu Ohno's stimulating book *Evolution by Gene Duplication* (1970) and King and Wilson's (1975) postulation that changes in gene regulation were more important than genetic mutations for major steps in evolution, including human evolution. It was an exciting period of discovery in genetics as it related to development, and one had the impression that we were on the brink of something big. Nüsslein-Volhard and Wieschaus (1980) had completed their massive mutation screen in *Drosophila* and that work was enthusiastically discussed. Walter Gehring had been invited although he was unable to attend, but Klaus Sander, Antonio

García-Bellido, and Peter Lawrence ensured that homeotic genes and genetic compartments were much discussed. *Hox* clusters were as yet known only in *Drosophila*. Discovery of the homeobox and the idea of a genetic toolbox were still a few years off (McGinnis et al. 1984; Scott and Weiner 1984; reviewed in Lawrence 1992). There was a sense that we were on the verge of major research breakthroughs that would establish a field of study—what later became Evo-devo.

The conference made a lasting impression on me. While I cannot say that it changed my personal research direction in any profound way, it reinforced the trajectory of my research and left me far better informed than before and put me in touch with central workers in the area of research combining evolution and development.

5.4   Pere Alberch—An Early Force in Evo-devo Research

Pere Alberch was a strong presence at the meeting. He had moved from my lab directly to an assistant professorship at Harvard, without a post-doc, on the strength of his promise and some noteworthy papers (Alberch et al. 1979; Alberch 1980). Pere was one of the early figures in Evo-devo research and his lab was an exciting place for graduate students and post-docs (such as Neil Shubin, John Reiss, Annie Burke, Chris Rose and Gerd Müller). He wrote important papers on limb and tongue evolution, topics close to my own research interests, and in retrospect his creative work is seen as seminal and prescient; he has been credited as a central figure in the origin of Evo-devo (Reiss et al. 2009; Rasskin-Gutman and De Renzi 2009). His star burned brightly, but he and his research were difficult to categorize—not sufficiently molecular, not sufficiently herpetological, not mainline development—and he was denied tenure at Harvard. He returned to his native Spain where he assumed a professorial position and directorship of the Museo Nacional de Ciencias Naturales in Madrid, a position he held with distinction for about 10 years. He was about to move to Valencia, Spain, to assume a professorship and head a new program in Evo-devo in 1998 when he died of heart failure in his sleep at the age of 43 (Wake 1998).

5.5   Homoplasy—A Key Concept in Evo-devo Research

A primary motivation for my interest in the relation between development and evolution was my struggle with homoplasy, the evolution of similarity (morphological, in the case of my research) in independent lineages. Historically homoplasy had been variously termed convergence, parallel evolution, and evolutionary reversal. Convergence is straightforward and readily detectable if one uses an appropriate definition (e.g., false resemblance resulting from different developmental pathways in different phylogenetic lineages). Parallelism, in contrast, has always

caused problems ("a sort of intermediate case between "true" and "false" resemblance" (Eldredge and Cracraft 1980; see also Hall 2008; Arendt and Reznick 2007; Abouheif 2008). Generally, parallel evolution is considered to be that kind of homoplasy in which similar developmental genetic mechanisms are deployed to produce similar morphological outcomes. Reversal is even more difficult. There have been many reported refutations of Dollo's famous "law"—that organs lost in the course of evolution cannot be regained (see discussion below).

I mentioned that my doctoral dissertation dealt with comparative morphological evolution in a large family of salamanders, the Plethodontidae or Lungless Salamanders (about two-thirds of the living species of salamanders are included in this taxon), in which homoplasy of many sorts seemed rampant (Wake 1966). In the intervening years it has only become more evident that homoplasy is ubiquitous. I saw the developing field of Evo-devo, combined with new methods and approaches in phylogenetic systematics (e.g., Fink 1982), as the way to study the causes of homoplasy. What immediately became evident is that studying homoplasy is not easy because one must first confront the question of homology, a topic that has been under nearly continual discussion among evolutionary biologists for more than a century and a half. The problems of exactly what constitutes homology conceptually and how to define it practically have been so extensively studied that it may seem like folly to attempt to say anything new.

I taught a course in evolutionary biology at Berkeley for 30 years. The students were advanced undergraduates and first-year graduate students who already had been exposed, or even inculcated, in population genetic approaches to the subject. Therefore, I focused more on conceptual issues, including species, homology, phylogeny, individuation, integration, and the like; I also considered the evolution of morphology, which got us into Evo-devo, species formation, and related topics. I have long felt that evolutionists spend too much time worrying about old words.[3] The terms often predated Darwin, or were formulated without even a loose notion of evolution. (Species is a case in point.) I agree with Dobzhansky's famous aphorism: "Nothing in biology makes sense except in the light of evolution." The vast majority of biologists accept that species are the outcome of evolutionary processes. But most biologists, even many evolutionary biologists, treat species as if they are essentially biblical species! They are seen as "real" entities that have arisen in some manner akin to "birth" ("speciation"!), even though for most taxa, especially vertebrates, species are usually outcomes of the subdivision or fragmentation of pre-existing species, and achieve "reality" through the extinction of intermediates and the passage of time. As a consequence, the phylogenetic reconstruction of species relationships is fraught with peril. Homology is even more problematic. Its roots are ancient (Panchen 1999, economically summarizes the history of the idea; see also Laubichler 2000); the key to any modern understanding of the term is the idea that all life is connected—that the trait of interest is inherited,

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[3] Admittedly, I have contributed to these discussions, having devoted some effort in sorting out terms related to heterochrony (Alberch et al. 1979).

and often transformed—and that in the course of phylogenesis a homologue is the *same thing* in different species, even in all of its evolutionary manifestations. In contrast, what we today call homoplasy is the *appearance of sameness* in traits found in different species that are not homologues. How can we know the difference?

Aristotle and Belon used different kinds of logic to conclude that whales were mammals and fish, respectively (Panchen 1999). Today we consider a robust "natural" phylogeny to be a necessity prior to any homology assessment. Increasingly, homology assessment is but a technical detail (although a critically important one) in cladistic procedure. But I fear that with regard to the concept of homology, we evolutionary biologists have made a mountain out of a molehill. The homology debate is a distraction from the larger questions of how morphology evolves, why it stays the same, why it gives the appearance of having re-evolved in different species, and how we arrive at an integrated science of form that incorporates phylogeny with the genetics of development and morphogenesis in a truly evolutionary framework (Wake 1999). There has been one history of life (in particular at the level of multicellular organisms, in which lateral gene transfer is so rare that we can ignore it), and it is a genealogical necessity that a trait is "the same thing" as it is transmitted from parent to offspring. Thus, by hierarchical translation it also becomes a phylogenetic necessity. The sole reason for the existence of homology is evolution and phylogeny. Some think homology is profoundly important, but I fear it is simply a trivial outcome of history. What is not trivial is how the morphology that is the same in different species is generated and how "the same thing" evolves into diverse manifestations during phylogenesis; this is the domain of Evo-devo. In particular I find it promising, even fascinating, to employ homoplasy heuristically. One can postulate that similar structures ought to have similar developmental genetic and morphogenetic foundations, whether they are homologues or homoplasies. This opens up abundant avenues of research (Wake et al. 2011).

But, is it easy to determine what is homoplastic and what is homologous? This question is in the realm of phylogenetics, which is given too little attention in Evo-devo research. Gould (1977) and Alberch et al. (1979) clarified the distinctions between the different kinds of heterochrony in terms of developmental and evolutionary processes, but it was Fink (1982) who made it clear that phylogenetic discipline was a necessary component in such studies. Heterochrony is involved in homoplastic evolution—a vivid example being the repeated appearance of reproductively mature larvamorph taxa in different salamander lineages. Alberch et al. assumed that relevant analysis would be done in a phylogenetic context, while Fink reformulated their approach in terms of phylogenetics and showed how to detect heterochronic ontogenetic processes in nature. Fink appropriately insisted on the necessity of the prior existence of phylogenies "to which process analysis can be applied." He went on to observe that while convergence can often be detected with a minimal phylogeny (eyes of cephalopods and vertebrates, and fins of whales and fish, come to mind), parallel evolution can be detected, "if at all," only with the sophisticated analysis of large datasets. His observation is even more relevant for the most troublesome of the three forms of homoplasy—evolutionary reversals.

Dollo's Law on the irreversibility of evolution is viewed by some as a simple statement about the low probability of the recurrence of a trait based on its being the result of the integration of a large number of parts. While "laws" in biology have little credence, what might be called Dollo's *maxim* or *generalization* has been difficult to overturn despite much effort, especially with respect to organs. The recent debate concerning claims of evolutionary reversal is instructive. Goldberg and Igic (2008) issued a general refutation of claims of evolutionary reversal, arguing that the accompanying phylogenetic analysis was usually insufficiently rigorous. A case in point was the re-evolution of digits in a South American lizard *Bachia*, an attenuated snake-like lizard with greatly reduced limbs. The claim was that some species derived from ancestors that had lost particular digits had regained them (Kohlsdorf and Wagner 2006). Goldberg and Igic found the phylogenetic argument unconvincing. In response, the original authors recruited a phylogenetic analyst and reasserted their position (Kohlsdorf et al. 2010). I remain unconvinced, in part because of the still somewhat equivocal phylogenetic analysis. More importantly, I do not find the argument for reversal convincing because no member of *Bachia* loses *all* of its digits, and digits are reiterated serial elements; as long as the developmental genetic mechanisms underlying digital production in general are retained, I do not consider this a case of reversal in Dollo's sense. Rather, it falls into the zone called mesoevolution (Abouheif 2008), studies between microevolution and macroevolution that explicitly focus on issues such as deep homology and its relation to the evolution of development.

Homoplasy was a problem for me in 1966. While I think I made some progress after the Dahlem conference (Wake 1991), homoplasy remains a challenge today. But substantial progress has occurred in a couple of areas. Our understanding of the genetic foundations of development and morphogenesis were in their infancy at the time of the Dahlem conference. Baguñà (2009) has nicely summarized major steps in these areas from the time of the Dahlem conference to the present, starting with the discovery of the homeobox, the recognition that regulatory systems are widely conserved across taxa, the discovery of the important role of gene duplication in vertebrate phylogeny, the findings that *cis*-regulatory modules evolve by mutation, co-option and reshuffling, the understanding that there is a kind of molecular toolbox for development, the formulation of the concept of gene regulatory networks, sequencing of whole genomes, and the dawn of the age of genomics, and subsequently phylogenomics. We now live in an age of experimental Evo-devo.

But progress was made in another area as well—phylogenetics (nicely summarized by Felsenstein 2004), where there have been high levels of research activity up to the present. For morphologists the key development was the emergence of the field of cladistics, which democratized systematic procedures and took the field out of the hands of specialists (taxonomic "authorities"). Soon it became clear that we morphologists needed help, partly because homoplasy proved to be even more common than many of us had thought possible. Starting in the late 1960s, the stirrings of a new field of molecular phylogenetics began to emerge. By the time of the Dahlem conference, use of allozymes in systematics was common, but one could not readily develop phylogenies from such data. Various indirect methods of

estimating differences in DNA were employed (immunological approaches, DNA hybridization), and those of us working in this area awaited the discovery of methods of direct sequencing of DNA, which became available by the late 1980s. There has been a veritable explosion of approaches to combine DNA sequence data and other data (principally morphological) to generate increasingly robust phylogenetic hypotheses. A renewed focus on homoplasy has been one major result. When such approaches are extended from living to fossil taxa, the impact of homoplasy becomes ever clearer. Wiens et al. (2010) studied squamate reptiles and showed how molecular data change interpretations of fossils, concluding that "parallel adaptations to a burrowing habitat in multiple lineages seem to erase the historical signal" and lead the most sophisticated analyses of morphological data from fossil and living taxa to give the wrong answer. While phylogenies are essential for homology and homoplasy assessment, phylogenetic methods may fail when the data are inadequate or when the analysis is at a scale where genealogy has not yet translated into phylogeny, such as detecting parallel evolution in loss of armor in different sublineages within a single stickleback species where geneology is so young (and intraspecific) that it has not yet translated into phylogeny (Goldberg and Igic 2008). Especially troubling are instances where several different phylogenetic methods give statistically significant results, which are determined to be incorrect when new data are added (Wiens et al. 2010).

5.6 Hierarchical Issues and Levels in the Assessment of Homoplasy

If one accepts the definition of morphological parallelism as deriving from the same developmental genetic framework, then it is but a short step to the argument that the trait in question is a homologue at a deeper hierarchical level (Hall 2008). However, I insist that what determines homoplasy is phylogeny *at the focal level of analysis*. The *Mc1r* gene in vertebrates is associated with pelage color in mammals and skin color in lizards. The same mutation appears to be responsible for pale fur in some desert rodents and pale skin in two only distantly related lizards that live in the White Sands of New Mexico (Manceau et al. 2010; Rosenblum et al. 2009). Certainly this is a case of homoplasy, qualifying as parallelism at the level of the apparently bleached hair and skin of the taxa involved (by the definition above). But at the level of the gene it may be the same thing, a homologue. The mutations in these homologous genes are likely independent, but more research is needed to trace the gene through its convoluted evolutionary history over more than 150 Ma! The gene might be the "same" in many molecular traits, but it will be very difficult to reject the hypothesis that it is an independent invention in lizards and mice, and maybe even in the two lizards. Rigorous testing would require an enormous amount of work and is probably impossible (from a phylogenetic perspective) because of the extinction of relevant taxa. We may have to take it at face value from molecular

biology that this gene is indeed the same thing, and for the purposes of making progress in Evo-devo research we might as well accept its homology because we have no reason (at present) not to do so.

What has been called "deep homology" is the recognition that structures such as eyes of vertebrates and cephalopods (Piatagorsky 2008) and appendages of arthropods and vertebrates (Shubin et al. 2009) share some developmental genetic systems in common, which are derived from common ancestors and deployed in different organismal and evolutionary contexts. In this sense, the clearly homoplastic organs (because ancestors of both groups lacked fully formed eyes, as well as multipart appendages) share some remotely similar features due to their extremely ancient common ancestry. These examples simply reinforce my point (Wake 1999) that homology is really nothing but the outcome of common ancestry expressed in many different ways, and to lose any sleep over "the homology problem" is to take it too seriously.

5.7   Homoplasy Since Dahlem

Pere Alberch conducted his doctoral research on the largest clade of salamanders, the genus *Bolitoglossa* of Middle and South America. In several papers (Alberch 1981; Alberch and Alberch 1981) he developed a theme that I had introduced earlier (Wake 1966; Wake and Brame 1969): that the fully webbed hands and feet of members of this genus were paedomorphic and that they represented embryonic stages of ancestors transformed into adult stages of descendants. Embryonic salamanders have tiny pads out of which digits emerge as development proceeds. Pere showed that there were two very different kinds of webbing. Some species were indeed paedomorphic, with poorly developed digits that were reduced to tiny dots of bone terminally; these were generally miniaturized species and can be considered to have retained embryonic pads, although of larger size, into adulthood. There was no evidence that these tiny pads (which superficially appear to be webbed) served any special adaptive function. In contrast, the webbing spreading between the elongating digits in generally larger species, which had grown out of the basal pad phalanges, were well developed. Pere showed (1981) that species with large webbed hands and feet were capable of producing suction, and reasoned that in such species these structures were adaptations for arboreal locomotion. Researchers had noted that upland species generally had individuated digits with relatively little webbing and often were terrestrial, whereas species in the lowlands, whether large or small, were fully padded or webbed and were arboreal (Wake and Lynch 1976). *Bolitoglossa* is widespread and it seemed that ancestral unwebbed species had given rise, repeatedly, in different parts of the vast range of the clade, to species that had invaded the lowlands by developing pads or webs, or both, and becoming arboreal (because terrestrial lowland habitats in the tropics seem saturated with other forms of life, some quite hostile to salamanders).

Jaekel and Wake (2007) re-evaluated this problem and showed that all species of *Bolitoglossa*, whether webbed or not, follow a similar developmental trajectory shared only by one other species, a large-footed, webbed member of a cave-dwelling species of the distantly related genus *Chiropterotriton*. They concluded that webbing was functionally ineffective (except in *Chiropterotriton*) because the area of the pad or webbed appendage was insufficient to make a difference in the ability of an organism to locomote or even cling to an above ground surface. There are seven clades within *Bolitoglossa*, based on molecular phylogenetic analysis (Parra-Olea et al. 2004). Padded species, nearly fully webbed species, or both (with intermediates) occur in all seven clades, but species with well-individuated digits are found in only three clades. The implications of these diverse findings are that the ancestral *Bolitoglossa* likely was a small, paedomorphic form with padded hands and feet,[4] and the free-digited species of the uplands have been derived independently in different upland areas from southern Mexico into Colombia. This kind of homoplasy—reversal to a digited condition found in out-group taxa—was not previously considered likely. The reversal is not "perfect" in that the free digits develop differently than those of out-group taxa. I do not consider this example to constitute a rejection of Dollo's Law because even fully padded miniature species of *Bolitoglossa* have rudiments of digits; but it is a vivid example of homoplasy. At the level of the organ and organism, this example may not constitute a reversal. At the level of population biology and ecology it is one, because free digits are functional and hence likely to prove adaptive (relative to the postulated padded hands and feet of ancestors) for terrestrial, and some kinds of scansorial salamanders.

Following the 1981 Dahlem conference I focused intently on homoplasy, which at the time was generally thought to be evidence of natural selection. I felt this was too narrow a focus and developed the argument that another realm of explanation, one focused on phenotype generation, offered an alternative perspective (Wake 1991). I contrasted functionalist (externalist, adaptationist) with structuralist (internalist, generative) "ways of seeing" (e.g., Wake and Larson 1987), trying to avoid conflating explanations for the generative evolution of the form and explanations for the adaptive evolution of form, attempting to achieve an integration of both through phylogenetic analysis. My focus remained plethodontid salamanders, whose phylogenetic history and patterns of life history evolution I thought I understood thoroughly. The desmognathine plethodontids evidently had the most generalized larvae, adapted to life in rapidly flowing streams. Two different lineages of miniaturized desmognathines evolved terrestriality with encapsulated embryos that hatch as miniatures of the terrestrial adults (Wake 1966). Desmognathines constituted one of two major clades. The second included three major clades, one of which had aquatic larvae that were mainly but not exclusively stream-adapted, whereas the other two had evolved direct terrestrial development,

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[4] E.g., all species of *Bolitoglossa* have incompletely developed tarsal elements (Alberch and Alberch 1981; Wake 1991).

either from a common ancestor with the trait or perhaps independently. However, new molecular evidence in the last decade turned this picture upside down (Mueller et al. 2004; Chippindale et al. 2004; Vieites et al. 2007). Desmognathines now are recognized as being deeply nested within an otherwise direct-developing clade, so an evolutionary reversal has occurred. While this reversal has profound ecological implications, it is doubtful that Dollo would regard it as a refutation of his "law" because encapsulated embryos differ relatively little from free-living larvae during early development (Kerney et al. 2011) so no "organ" was lost and regained. These examples highlight only some of the problems associated with attempted refutations of Dollo's Law, which in my eyes has as much validity as it did 30 years ago.

Another Evo-devo issue in my research at the time of the Dahlem conference was the homoplastic evolution of highly projectile tongues in plethodontid salamanders (Lombard and Wake 1977, 1986). According to the 1966 phylogenetic hypothesis, there was extensive homoplasy, with freely projectile tongues evolving three times and somewhat less extensively projectile tongues evolving three additional times. The key factor was loss of lungs, which meant that prior functional constraints were relaxed and extreme specialization possible. The three most specialized instances followed different biomechanical pathways in developmentally different lineages, using one of two options. The first of these was associated with direct-developing species, where the tongues developed directly with no need to function in larvae. The second option, less biomechanically efficient, was the result of a kind of "compromise" necessitated by the constraint that the larval tongue act to wave the gills and produce suction for feeding in the aquatic medium. New insights were obtained from extensive developmental neurobiological studies in the late 1980s and 1990s (summarized in Roth and Wake 2001). The question was revisited when the new phylogenies were obtained and the homoplasy was discovered to be even more extreme than previously believed. The two clades with the most extreme tongue projection capabilities, formerly considered to be fairly close relatives, now were determined to be only distantly related; hence the convergence was even more impressive (Mueller et al. 2004; Wake et al. 2014).

Following the Dahlem conference I undertook studies of brain evolution in salamanders with my long-time collaborator Gerhard Roth of Bremen, Germany. It had long been recognized that salamanders has simple brains, organized poorly and seemingly primitive. However, by integrating across hierarchical levels and using modern phylogenetic analysis, we recognized that the brains of salamanders were secondarily simplified, giving a false impression of simplicity and showing what might be considered as a return to what can be inferred for ancestral patterns of tetrapod brain organization. We showed that this secondary simplification is the outcome of a cascade of events: (1) genome size increase; (2) consequent cell size increase; (3) consequent increase in cell cycle time (particularly important in these ectotherms); and, (4) inhibition of cell migration because of large cell sizes and metabolic issues (reviewed by Roth and Wake 2001). The end result of this cascade is apparent embryonic or juvenile anatomy that is not simple in function but paedomorphic in appearance. Such homoplastic evolution occurred independently in the three orders of living amphibians, as well as in the distantly related lungfishes (Roth et al. 1993).

Perhaps the clearest example of the difference between convergence and parallelism in my own work relates to body form evolution in plethodontid salamanders. A common homoplasy in salamanders, as also in lizards, is the elongation and attenuation of the body in fossorial and semifossorial species. The simplest developmental solution to the problem of how to accomplish body and tail elongation is an increase in the number of vertebrae, accomplished early in development by delaying the offset signal for segment formation. Often individual species display local and geographic variation in the numbers of trunk vertebrae, sometimes varying by three or more vertebrae within a single population. Many of these species show patterns of geographic variation in vertebral numbers. This phenomenon was studied by one of my former students, who showed that both environmental and genetic factors affected adult vertebral numbers (Jockusch 1997). The radiation of a bolitoglossine clade of plethodontid salamanders in the tropics offers a sharp contrast. One nested clade, *Oedipina*, displays variation in trunk vertebral number, with from 18 to 23 being found among its 38 species, most showing some intraspecific variation; the remaining 252 species of tropical bolitoglossines have a fixed number of trunk vertebrae (14), with extremely rare variation. One small clade, *Lineatriton* (three species, recently synonymized with the genus *Pseudoeurycea*) is unique among caudate amphibians in being slender and attenuate, superficially resembling species of *Oedipina*. It has accomplished this in an entirely different manner—its 14 trunk vertebrae are each elongated, with shortened ribs (Wake 1991). The common homoplasy is a classic example of parallel evolution enabled by inherent variation in vertebral number and is subject to selection as part of a process of adaptation. I long thought the convergent situation in *Lineatriton* was unique, but molecular analysis revealed that the three species constituted another instance of parallel evolution; two species were close relatives of one clade within the large genus *Pseudoeurycea* (50 species), but one was closely related to a separate clade within that genus (Parra-Olea and Wake 2001). This complicated example of homoplasy thus involves two distinct developmental modes producing a common morphotype and ecotype, each deployed repeatedly.

A final example of extreme homoplasy in salamanders takes us back to Steve Gould's (1977) book, with an axolotl on the cover and an extended discussion of paedomorphosis in salamanders inside. When nineteenth century biologists attempted to classify sexually mature, aquatic, gilled amphibians (such as the axolotl), they were initially stumped and tried a number of options. When captive-bred axolotls suddenly metamorphosed it became apparent that they were salamanders and the term neoteny became linked with axolotls. As group after group was studied it was recognized that different salamander taxa displayed an array of paedomorphic states that had evolved homoplastically. These paedomorphic species became nearly impossible to classify and many arguments ensued over the years. Wiens et al. (2005) explicitly dealt with this problem by producing phylogenetic hypotheses based on treating all salamanders equally, regardless of the developmental stage of adults. They conducted analyses using molecular characters alone, and molecular plus morphological characters, concluding that the inclusion of traits related to paedomorphosis consistently gave the wrong

answer. Pervasive, organism-wide paedomorphosis can produce many homoplasies all at once. Only with the advent of molecular data have comparative biologists gained tools to examine the extent and implications of homoplasy. Studies of other taxa have reached similar conclusions: "convergent evolution acting on groups of characters in concert—can lead to highly supported but erroneous phylogenies" (Holland et al. 2010, 433).

5.8   The Future of Homoplasy in an Evo-devo Context

Homoplasy offers exceptional opportunities to students of Evo-devo (Wake et al. 2011). Why does evolution tend to "run in grooves" within clades, following avenues of least resistance to reach the same endpoint again and again? What determines the limitations on form? Why don't forms evolve to fill the potential morphospace, instead giving rise to similar forms repeatedly? How does integration across several hierarchical levels occur, for example from increased genome sizes within cells to repeated patterns of evolution at the level of whole organisms? For many years, functionalist (selectionist, adaptationist) approaches to such problems have been emphasized. Is it not time to rationalize or integrate structuralist perspectives in a more positive and definitive manner?

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