Early Animal Evolution: Emerging Views from Comparative Biology and Geology

Andrew H. Knoll, *et al.*

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Early Animal Evolution: Emerging Views from Comparative Biology and Geology

Andrew H. Knoll[1]* and Sean B. Carroll[2]

The Cambrian appearance of fossils representing diverse phyla has long inspired hypotheses about possible genetic or environmental catalysts of early animal evolution. Only recently, however, have data begun to emerge that can resolve the sequence of genetic and morphological innovations, environmental events, and ecological interactions that collectively shaped Cambrian evolution. Assembly of the modern genetic tool kit for development and the initial divergence of major animal clades occurred during the Proterozoic Eon. Crown group morphologies diversified in the Cambrian through changes in the genetic regulatory networks that organize animal ontogeny. Cambrian radiation may have been triggered by environmental perturbation near the Proterozoic-Cambrian boundary and subsequently amplified by ecological interactions within reorganized ecosystems.

Near the mouth of the Kotuikan River, northern Siberia, steep cliffs punctuate the boreal forest. At river level, carbonate rocks record the deposition of lime muds in a shallow seaway some 545 million years ago (Ma). Fossils are rare in these rocks, and those that can be found are simple. The only trace fossils are meanders of small worm-like creatures preserved on bedding surfaces. About 3 m above this level, there is an abrupt shift to shore-face sandstone; volcanic breccias to the east date this horizon at 544 ± 1 Ma (*1*). Above the sandstone ledge, variegated shales form a steep shoulder above which rises a wall of limestone and dolomite. The shales record a flooding event, but as sediments accumulated the sea grew shallower, so that overlying carbonates reflect marine environments progressively closer to the shore. Near the top of the cliff, an erosional surface records subaerial exposure—short-lived, as the sea soon encroached again (*2*).

Beginning at the level of the sandstone bench, the rocks contain small skeletal fossils (*3*). In the lowermost beds, there are only a few forms, cones of calcite little more than a millimeter long. But as one ascends the cliff, the abundance and variety of these fossils increase. So, too, do the abundance and behavioral complexity of preserved tracks, trails, and burrows. Nearly 120 m above river level, rocks estimated to be about 530 million years (m.y.) old contain more than 80 skeletal taxa. Some, like those found along the river, have a threefold symmetry that differentiates them from most animals alive today. Others, however, include spiral shells that are recognizably molluscan and, a bit higher, arthropods and the bivalved shells of brachiopods.

This pattern is recorded in greater or lesser detail in rocks of comparable age throughout the world. It marks the unfolding of animal diversity popularly known as the Cambrian explosion. The broad outline of Cambrian diversification has been known for more than a century, and for almost that long scientists have debated its biological interpretation and possible causes. Beginning with Darwin (*4*), some have argued that the appearance of explosive evolution is illusory—that the apparently rapid diversification of animals reflects massive record failure below the Proterozoic-Cambrian boundary (*5*). Others have accepted the reality of Cambrian radiation and ascribed it either to causes intrinsic to animal biology or to extrinsic (environmental) triggers (*6*).

The debate about Cambrian evolution may be old, but only in the past decade have the data necessary to weigh conflicting hypotheses begun to emerge, issuing from a broad range of disciplines that includes not only paleontology and stratigraphy, but also geochemistry, molecular systematics, and developmental genetics. It is becoming increasingly clear that understanding the Cambrian explosion requires that considerations of the Cambrian fauna and the environments that shaped it be complemented by new perspectives on the late Proterozoic world. To understand what actually transpired during or what may have enabled the Cambrian explosion, we must consider what animals, developmental and genetic mechanisms, and ecosystems were in place before it. Here, we review recent fossil, phylogenetic, embryological, and paleoenvironmental discoveries that are spawning new ideas about early animal evolution.

Paleontology and the Pattern of Animal Diversification

The earliest Cambrian remains are not the oldest record of animals. Cambrian faunas were preceded by the so-called Ediacaran biota, preserved as impressions, casts, and molds in uppermost Proterozoic rocks around the world (*7*). Centimeter-scale, radially symmetrical impressions arguably formed by diploblastic animals occur in rocks more than 600 m.y. old (*8*). Radially symmetric fossils also predominate in younger Ediacaran faunas (575 to 544 Ma) (Fig. 1A), but they are joined by a wider diversity of morphotypes (Fig. 1D) as well as trace fossils made at least in part by bilaterian animals (Fig. 1F) (*9*). [Reports of older bilaterian traces (*10*) require confirmation of age and interpretation. The unambiguous, abundant, and continuous record of bilaterian traces begins only near the end of the Proterozoic Eon.]

Although the stratigraphic distribution of Ediacaran fossils is clear enough, their biological interpretation remains controversial, providing what amounts to a paleontological Rorschach test. Several distinct body plans are represented (*11*). Most radially symmetric fossils plausibly represent polypoid organisms or the inflated holdfasts of colonial, diploblastic animals—mostly unrepresented in the modern fauna. More complex fossils include a range of forms built of repeated, tube-like units (Fig. 1D). In a stimulating, if controversial proposal, Seilacher (*12*) grouped such fossils into a clade that he christened the Vendobionta and viewed as an extinct experiment in multicellular organization. Others have questioned this interpretation, assigning various forms to colonial diploblasts or to stem members (*13*) of several bilaterian clades (*11, 14, 15*). It is genuinely difficult to map the characters of Ediacaran fossils onto the body plans of living invertebrates. Long viewed as the principal problem of interpreting Ediacaran assemblages, this difficulty increasingly appears to be their central point. Much opinion supports the broad view that both extinct diploblastic-grade animals and bilaterian stem groups [for example, the mollusk-like *Kimberella* (*15*)] are represented. Trace fossils record a modest diversity of (mostly) simple bilaterians (*16*). Crown group protostomes or deuterostomes may also lurk in Ediacaran-aged rocks but at present, evidence of such animals remains equivocal.

Early Cambrian fossil assemblages are distinctly different. Although at least some

[1]Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA. [2]Howard Hughes Medical Institute, Laboratory of Molecular Biology, University of Wisconsin–Madison, 1525 Linden Drive, Madison, WI 53706, USA.

*To whom correspondence should be addressed.

Ediacarans survived into the Cambrian (*17*), the Cambrian fauna consists predominantly of stem and crown group members of extant bilaterian phyla, along with diverse sponges and rarer cnidarians and ctenophores (*18*). Mineralized skeletons are widely distributed, if formed by a minority of Cambrian species. Trace fossils independently record a notable expansion of behavioral complexity within bilaterian animals (*19*).

New windows on Proterozoic biology. The Burgess Shale has achieved almost iconic status among fossil assemblages, and for good reason: it preserves a remarkably detailed record of Cambrian diversification. Burgess fossils postdate the beginning of the Cambrian Period by as much as 40 million years, however, so the recent documentation of Burgess-like preservation in somewhat older shales is welcome. Especially in the Chengjiang (China) and Sirius Passet (Greenland) faunas (*18, 20*), superbly preserved fossils show that body plans of Burgess complexity already existed late in the Early Cambrian—still as much as 20 to 25 million years after the start of the period. Although not widely appreciated, carbonaceous shales in some latest Proterozoic successions also preserve compressed macroscopic fossils in minute detail (Fig. 1B). Particularly in China, these deposits contain diverse algae and, perhaps, simple animals (*21*). But, despite exceptional preservation, these assemblages are not known to include arthropods or any of the other bilaterian complexities that characterize taphonomically comparable Cambrian shales.

A similar picture is emerging from calcified fossils in terminal Proterozoic carbonates. It has been known for nearly 30 years that the problematic animal *Cloudina* formed a weakly mineralized skeleton by the enzymatic precipitation of calcite in an organic matrix (*22*). Grotzinger and colleagues (*23*) have now shown that *Cloudina* was only one of a moderately diverse group of animals that formed preservable skeletons in the latest Proterozoic ocean (Fig. 1C). Calcified fossils are abundant and well preserved in carbonate platform settings, where they are commonly associated with microbial reefs. Sponges, cnidarian-grade animals, and bilaterians may all be represented in this assemblage, but like Ediacaran casts in interbedded sandstones and organic compressions in contemporaneous shales, these fossils bear only a limited similarity to the diverse protostomes and deuterostomes preserved in younger rocks.

Postmortem replication by phosphate minerals is responsible for some of the best Cambrian fossil preservation, and exquisitely phosphatized remains have recently been found in terminal Proterozoic rocks, as well (*24*). The fossils include diverse protists, anatomically preserved algae, and, remarkably, spheroidal fossils interpreted as early cleavage stages of animal embryos (Fig. 1E). Preserved embryos display egg case ornamentation and an unusual cleavage geometry found today among the arthropods, consistent with other paleontological inferences (*11, 14, 15*) that bilaterian cladogenesis began well before the Cambrian. At present, however, the adult body plan associated with these fossils remains unknown, limiting phylogenetic inference. Terminal Proterozoic phosphorites do not include the fossils of crown group bilaterians that are so obvious in their Cambrian counterparts.

Thus, while paleontological discoveries shed increasing light on biological diversity just before the Cambrian explosion, new fossils have not extended bilaterian crown group morphologies deeply below the Proterozoic-Cambrian boundary. They do, however, allow us to reject the episodically popular view that the lack of recognizable crown group bilaterians in Proterozoic rocks can be attributed to a dearth of suitable rocks or preservational opportunities (*5*). Latest Proterozoic sedimentary rocks are widely distributed and, commonly, well preserved and exposed. They are full of body and trace fossils that reflect the complete spectrum of fossilization mechanisms responsible for the succeeding Cambrian record. Crown group bilaterians may yet be demonstrated in terminal Proterozoic rocks, but the diversification of crown groups was principally a Cambrian event. Cambrian diversification thus reflects an evolutionary milestone regardless of the length or character of earlier animal history.

Stratigraphy, geochronology, and a new sense of evolutionary pattern. Fossils can be assembled into meaningful evolutionary patterns only when ordered with respect to time and environment. Among the most important recent advances, therefore, is the establishment of a robust geochronology for Proterozoic and Cambrian evolution. This advance has two components. First, it has been recognized that the isotopic compositions of C and Sr in carbonate rocks and organic matter vary greatly through this time interval. These variations provide a means of correlating terminal Proterozoic and Cambrian rocks accurately among many localities (*25, 26*). Organic-walled microfossils provide additional (and independent) biostratigraphic constraints (*27*). Second, high-precision U-Pb dates on zircons in volcanic rocks have provided accurate ages of sedimentary successions that contain both fossils and a record of Sr and C isotopic shifts. Together, these provide an unprecedented sense of the timing of early animal diversification (*1, 23, 28*).

In consequence, an expanded view of terminal Proterozoic-Cambrian evolutionary history is emerging. Independent of any molecular clock considerations, fossils of red algae and other protists indicate that the major radiation of eukaryotic life—a divergence that includes the animals (*29*)—began no later than 1200 to 1000 Ma (*30*). Multicellularity and clade divergence came early to several algal groups and may have done so in the animal clade as well, but at present the fossil record is silent on these issues. The widespread preservation of millimeter-scale lamination in strata older

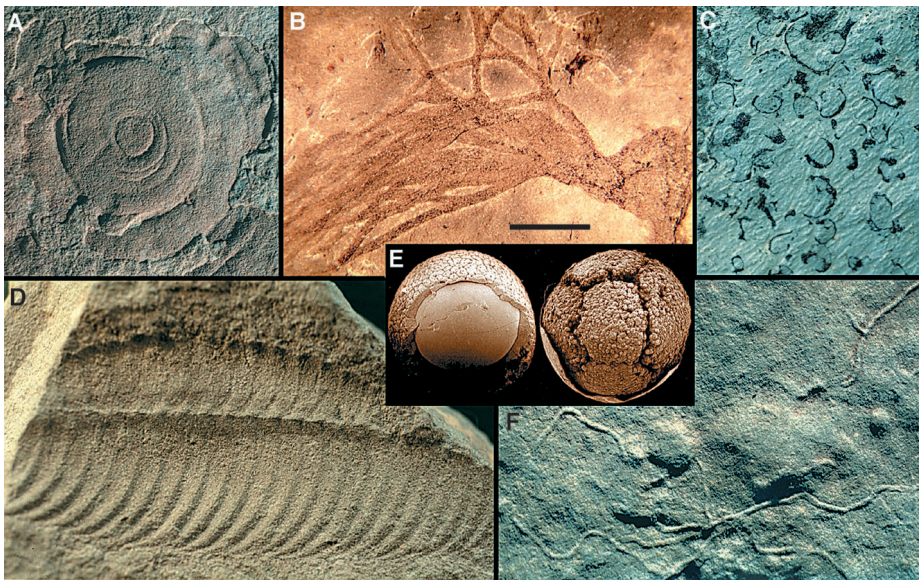

Fig. 1. The nature of the terminal Proterozoic fossil record. (**A**) *Ediacaria*, a radially symmetrical cast preserved on the underside of a sandstone bed, Rawnsley Quartzite, South Australia. (**B**) Macroscopic alga preserved as a carbonaceous compression in shales of Doushantuo Formation, China. (**C**) Calcified fossils in limestones of the Nama Group, Namibia. (**D**) *Pteridinium*, a frondose Ediacaran fossil consisting of three vanes built of repeating units (two visible in specimen) that are joined along a central axis. (**E**) Phospatized animal egg and early cleavage-stage embryo, Doushantuo Formation. (**F**) Simple trace fossils of bilaterian animals, Rawnsley Quartzite. Bar = 2.5 cm for (A), 3 mm for (B), 1.5 cm for (C) and (D), 250 μm for (E), and 2 cm for (F).

than 600 m.y. suggests that any pre-Ediacaran metazoans must have been rare, small, or gossamer forms unlikely to be preserved. Should evidence of such organisms be found, it will likely occur as phosphatized remains in Neoproterozoic carbonate rocks (*24*).

Paleontologists have come to terms with the relatively short time frame of Ediacaran biology. Diverse Ediacaran assemblages from Australia, northern Russia, and Namibia were all deposited within the last 15 to 20 million years of the Proterozoic Eon. Less completely assimilated are the implications of new radiometric dates for Cambrian evolution. Although most Ediacaran fossils have no post-Proterozoic record, they were not immediately succeeded in lowermost Cambrian rocks by diverse crown group bilaterians. Earliest Cambrian assemblages contain few taxa, and the diversity of trace and body fossils grew only over a protracted interval (*3, 26, 31, 32*). Hyoliths and halkierids (extinct forms thought to be related to mollusks), true conchiferan mollusks and, perhaps, chaetognaths enter the record during the first 10 to 12 million years of the Cambrian, but crown-group fossils of most other bilaterian phyla appear later: the earliest body fossils of brachiopods, arthropods, chordates, and echinoderms all post-date the beginning of the period by 10 to 25 million years (Fig. 2) (*32*). Trace fossils suggest earlier appearances for some groups, notably arthropods (*31*), but the observation remains that the Early Cambrian contains considerable time for the assembly and diversification of crown group morphologies.

From this overview, we can begin to appreciate the Cambrian explosion as a biological event. It does not represent the origin of life or of the animal clade. Nor, given the the presence of mollusks near the beginning of the Cambrian and probable stem bilaterians in Ediacaran rocks, does it appear to represent the initial divergence of major animal clades. Rather, the Cambrian explosion records the radiation of bilaterian animals (and sponges) with modern body plans (*33*)—the diversification of crown groups within clades that diverged earlier (*34*). Equally important, Cambrian fossils record the initial assumption by animals of the prominent ecological and biogeochemical roles they play in modern ecosystems (*31*).

The Genealogy and Genetics of Animal Ancestors

Biological explanations for the patterns observed in the early fossil record must address both historical and mechanistic questions. What are the phylogenetic relationships among animal ancestors and their modern descendants? And, because morphological diversity is the product of genetic differences in development, what developmental genetic mechanisms governed the origin and diversification of various forms? Progress in both systematics and developmental genetics has revolutionized our perspective on animal relationships and provided new hypotheses about early animal evolution (*35*).

Metazoan phylogeny: Redrawing the tree. Constructing an accurate picture of metazoan relationships has been challenging, and there are many alternative pictures of animal phylogeny. With most of the 35 or so modern metazoan phyla represented or inferred by the end of the Cambrian, the age, rate of divergence, and number of branches in this tree impose both technical and theoretical limitations on its resolution (*36–38*). Nonetheless, new molecular phylogenies based on 18*S* ribosomal RNA sequences suggest that the Bilateria should be reorganized into three great clades: the deuterostomes, lophotrochozoans, and ecdysozoans (Fig. 2) (*39*). This phylogeny, which has received some independent support (*40*), overturns traditional trees, nesting groups once viewed as primitive, such as the flatworms and nematodes, well within the two protostome branches (Fig. 2) (*34, 41*). More recently, it has been suggested that acoel flatworms could represent the earliest extant bilaterians (*42*), a clade that branched off the Bilateria before the radiation of the three major clades. These new views of the metazoan tree have important implications for the ancestry and evolution of Bilaterian animals (*43*).

One implication of the new bilaterian phylogeny is that early-diverging groups within derived clades may display ancestral and derived characters in combination. This appears to be the best explanation for the long-standing difficulties concerning the phylogeny of the lophophorates. These animals have been asserted to be basal deuterostomes on mor-

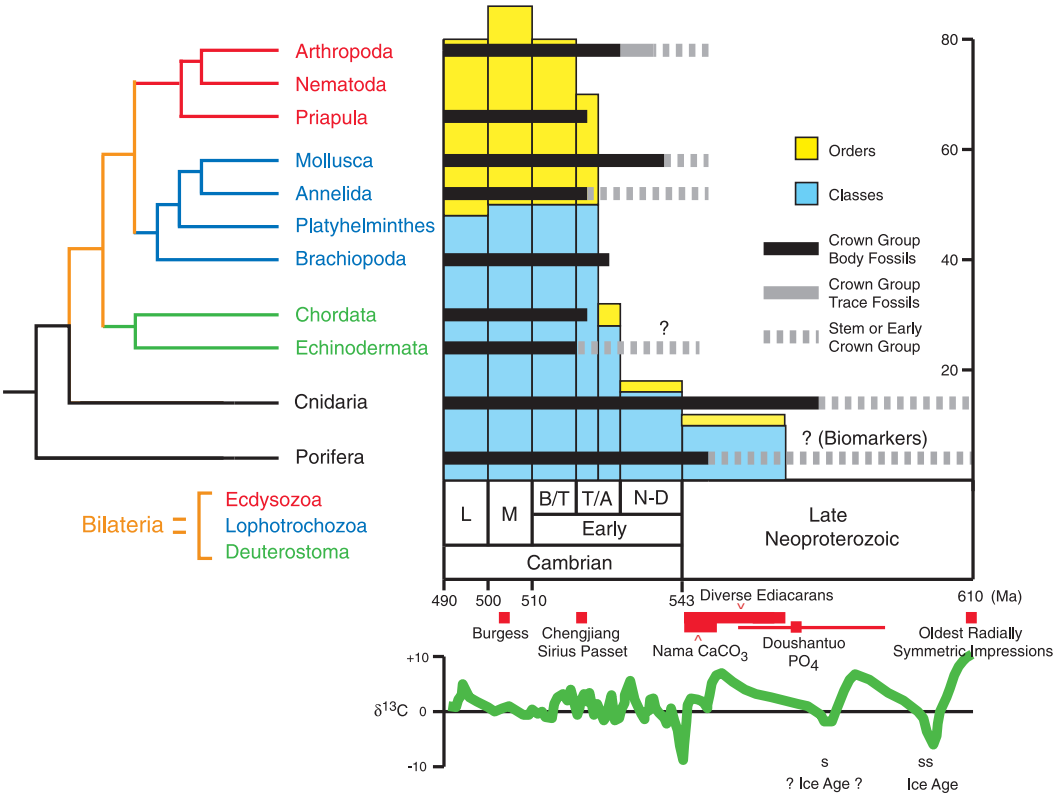

Fig. 2. Animal diversity across the Proterozoic-Cambrian transition. Phylogeny based on (*39, 40*); time scale and diversity history of animal classes and orders from (*1, 23, 27*). First appearances of crown and possible stem representatives of phyla from references cited in text, as well as (*119*) for the proposed Ediacaran stem echinoderm and (*120*), for biomarker molecules ascribed to poriferans. Carbon isotopic record for terminal Proterozoic and Cambrian carbonates from references cited in text, plus (*121*).

phological grounds (*44*). Yet, molecular phylogenies (*45, 46*) and, more recently, *Hox* gene characters (*40*), place them within the protostomes. Debate has focused on which set of observations is correct, but it is now possible to rationalize both morphological and molecular data (Fig. 3). Similarly, because priapulids display radial cleavage, yet also molt and possess *Hox* genes characteristic of ecdysozoans (*40*), this group may have diverged first among the Ecdysozoa (Fig. 3).

Clues about Urbilateria: Rocks, Hox, and molecular clocks. The three-branched bilaterian tree (*34, 39*) provides no extant candidates for the sort of animal that could represent the hypothetical last common ancestor of protostomes and deuterostomes, dubbed Urbilateria (*47*). Even if the recent basal placement of acoel flatworms is confirmed, they may represent some stage of early bilaterian evolution, but not the last common ancestor of the three great clades. Our approach to Proterozoic animal ancestors, therefore, relies on phylogenetic inferences drawn from extant animals about gene content, development, and potential morphological features. Given a picture of Urbilateria, we can then probe more deeply to identify some of the innovations that must have occurred early in the evolution of bilaterians by comparing these Urbilaterian features with those of diploblastic animals (for example, Cnidaria). This, in turn, makes it possible to examine innovations that accompanied the radiation of bilaterians into major clades and their ultimate diversification within the Cambrian.

The shared genetic regulatory repertoire of protostomes and deuterostomes reveals that Urbilateria possessed a sizable array of intercellular signaling systems (transforming growth factor–B, Wnt, hedgehog, Notch, epidermal growth factor receptor) and a large, diverged complement of transcription factor gene families (*Hox*, *Pax*, bHLH, zinc finger, and various homeobox-type genes such as the *Distal-less*, *engrailed*, and *LIM*-type genes). How might the existence of these shared developmental regulatory genes relate to potential morphological features of Urbilateria? As progress has been made in understanding the developmental regulatory mechanisms underlying embryonic axis formation, the patterning of the rostral-caudal axis, and organogenesis in both protostomes (primarily the arthropod *Drosophila melanogaster*) and deuterostomes (primarily vertebrates), a number of remarkable similarities have emerged. These include: the deployment of an extensive cluster of *Hox* genes along the major rostral–caudal axis (*48, 49*); the deployment of ParaHox cluster in different anteroposterior regions of the gut (*50*); the organization of the dorsal/ventral axis by the homologous transforming growth factor–β protein family members Dpp/BMP and their interacting ligands Sog/Chordin (*47*); the utilization of similar genetic machinery for the formation of appendages (*Dll*) (*51, 52*), eyes [*Pax-6*/*eyeless*; (*53, 54*)], and mesodermal derivatives including muscles (*55–57*) and heart [*tinman*/*NKX*. 2.5; (*58*)]; and the periodic expression of the *engrailed*-related (*59*) and *hairy*/*her1* (*60, 61*) genes in selected taxa. These developmental genetic similarities have led to the hypothesis that Urbilateria not only possessed all of the genes shared between arthropods and chordates, which is certain, but also some of the morphological characters or structures that these genes regulate, such as photoreception organs, appendages, a heart, and some type of metamerism (*47, 62, 63*).

While the existence of such features in early bilaterians cannot be confirmed without direct fossil evidence, it seems unlikely that each of these genetic functions in regulating similar processes evolved convergently in protostomes and deuterostomes, especially in light of emerging evidence that similarities extend beyond individual genes to interacting networks of developmental regulators (*52, 64–66*) and to key structural genes they regulate [for example, opsins (*64*)]. Yet, at the same time, it is difficult to imagine highly complex eyes, appendages, or hearts in ancestral bilaterians. One possibility, that dismisses convergence but takes a more moderate view of the anatomical potential, is that stem bilaterians possessed some differentiated but primitive forerunners of these structures: perhaps a simple outgrowth of the body wall or a circumoral tentacular feeding structure rather than a modern locomotory appendage, a contractile muscle regulating hemacoel fluids rather than a modern heart, and a simple photoreceptor complex rather than an optically sophisticated eye. The development of these structures could constrain genetic evolution enough to conserve the functions of regulatory genes across the Bilateria without requiring modern morphologies in ancestral animals.

Conjectures about how *Urbilateria* generated their primary embryonic axes, germ layers, body cavity, and bilateral symmetry depend upon inferences about which mechanisms observable today are primitive and which are derived. The three-clade bilaterian tree opens the door to viewing the basic developmental characteristics of deuterostomes as ancestral features of bilaterians rather than deuterostome synapomorphies (*43*). Urbilateria may, thus, have been small, bilate-

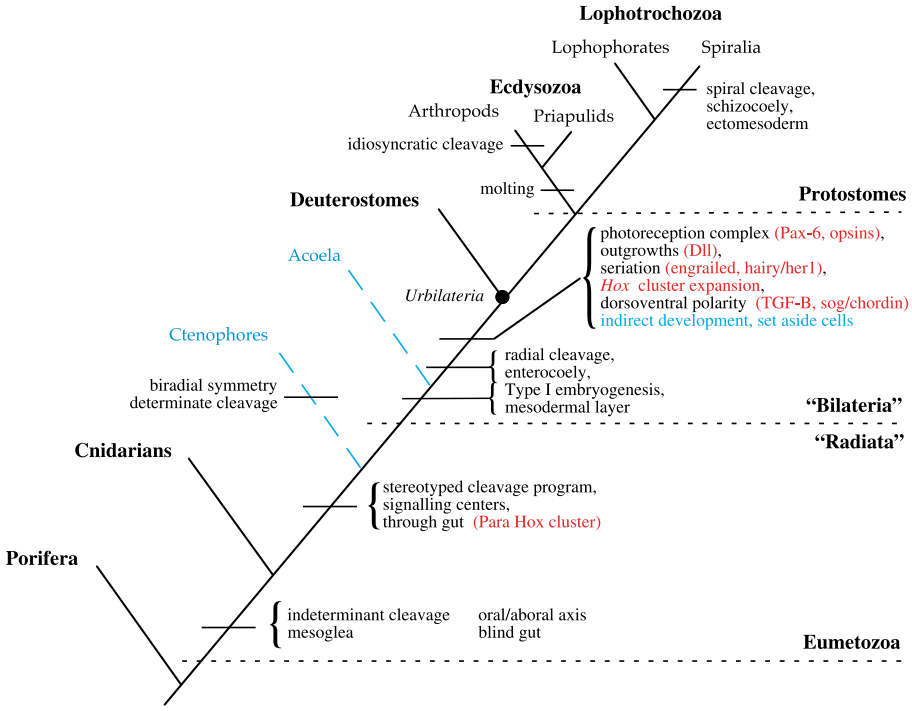

Fig. 3. Anatomical, developmental, and genetic innovations in the evolution of *Bilateria*. Inferences about the evolution of bilaterian features have been drawn from comparisons of developmental mechanisms and genetics among sponges, cnidarians, ctenophores, and selected protostomes and deuterostomes and mapped onto one presumed phylogeny [figure modified from (*69*)]. We note that the relationships among lower metazoans and bilaterians and the evolution of particular characters (highlighted in blue) are both uncertain and of central importance. Characteristics shared between protostomes and deuterstomes are deduced to have existed in some form in their last common ancestor, Urbilateria. The evolution of gene functions in controlling specific developmental features are shown in red. Figure based on references (*34, 39, 40, 50, 52, 63, 69*), and text.

rally symmetrical creatures with radial cleavage, coelomic cavities formed by the outpouching of the archenteron (enterocoely), and anterior tentacular appendages formed by coelomic outpouching.

Thus, at the genetic, morphological, and developmental levels, Urbilateria were fairly sophisticated, not flat amorphous worms. Paleontology indicates that these ancestors lived before the Cambrian—at least 560 Ma and possibly more than 600 Ma. Molecular clock estimates are controversial and yield disparate results (*5, 67, 68*), but even those estimates most nearly concordant with paleontological data suggest a prostome-deuterostome split at least 100 m.y. before bilaterians appear in the fossil record (*67*). Collectively, then, available data suggest that two substantial periods of bilaterian evolution preceded the Cambrian: the evolution of the bilaterian stem lineage leading to Urbilateria and the subsequent diversification of the three major bilaterian clades. We must understand what happened during these two intervals to delimit what eventually transpired in the Cambrian.

The Diploblast-Bilaterian gap: Pre-Ediacaran genetic and developmental innovation. Comparisons of bilaterians to other metazoans, particularly at the anatomical and genetic levels, suggest that there was a tremendous amount of developmental evolution within the internode between the last common ancestor of diploblastic animals such as the Cnidaria and Urbilateria (*50, 69*). The discontinuity between cnidarian and bilaterian body plans as well as the probable complexity of Urbilateria suggests that the evolution of bilaterian characters must have required many innovations, including a third germ-layer, bilateral symmetry, a centralized nerve cord, a through gut, various primitive organs, and the genetic systems to organize and pattern these features (Fig. 3). It is not clear to what degree any extant taxon represents an intermediate in the anatomical or genetic evolution of bilaterian organization. The phylum Ctenophora (comb jellies) has been proposed as a sister group to the Bilateria (*70, 71*), and the acoel flatworms as an early branch of the Bilateria (*42*). Both groups possess characters that can place them within the Cnidaria-Urbilateria internode (Fig. 3). However, the phylogenetic placement of the ctenophores remains controversial, and other data variously suggest close relationships to cnidarians, placozoans (*72*), or even deuterostomes (*44*). Similarly, the placement of the acoels awaits further examination.

Whatever the phylogenetic relationship among bilaterians and the diploblastic phyla, there was clearly a significant expansion in genomic information within the internode that connects the bilaterian clade to other branches. For example, surveys of *Hox* gene diversity in cnidarians have suggested that the last common ancestor of cnidarians and bilaterians had as few as one [probably two, (*73–76*)] ortholog of bilaterian *Hox* genes, only one ancestral *Pax* homeobox gene (*77*), a smaller array of intercelluar signaling molecules, and generally fewer transcription factor types than we know existed in Urbilateria. A limited survey of ctenophores also did not reveal an expanded *Hox* family (*78*). The differences in genome content and regulatory gene family size and diversity between extant diploblastic animals and bilaterians appear much greater than those known among any bilaterian taxa (except chordates). At the *Hox* gene level alone, it is certain that at least seven genes had evolved by duplication and were functionally diverged in Urbilateria, far more genes than existed in the common ancestor of diploblasts and bilaterians (*40*). It seems inescapable then that the phylogenetic internode leading to Urbilateria represents a substantial period of pre-Ediacaran history. Moreover, we can speculate that a spectrum of intermediate and divergent body plans could have existed that represented different stages of the developmental and genetic evolution of Bilateria. These predicted body plans provide both a challenge and search images for paleontology.

The role of the expanding systems of signaling proteins and transcription factors in the evolution of stem bilaterians requires consideration of the primitive mode of bilaterian development, a question which has recently received considerable attention (*43, 69, 79–84*). It has been argued that most bilaterians, except (ironically) insects and vertebrates, share mechanisms of embryonic specification termed type I embryogenesis that are distinct from the much less ordered styles of embryonic development in diploblastic organisms (*80*). In type I development, a series of 10 or so divisions produces cell lineages which give rise to particular cell types that are generally invariant within species. The position and fate of these lineages are specified by short-range interactions among adjacent or nearby cells. The shared bilaterian signaling systems and cell-type specific transcription factors appear sufficient to generate a small feeding animal composed of the basic gut, muscle, neural, and ectodermal cell types found in all bilaterians. Based on the widespread occurrence of type I embryogenesis in extant phyla, Davidson *et al.* (*80*) argued that this is the ancestral mode of embryogenesis that operated in early microscopic bilaterians.

The short-range regulatory interactions that operate in type I embryogenesis are not sufficient, however, to generate the macroscopic body plans characteristic of most modern bilaterians. Therefore, the evolution of larger body plans must have involved further developmental innovations. Davidson *et al.* (*80*) proposed that the key innovation in the origin of macroscopic body plans was the evolution of so-called "set-aside" cells. These cells are released from the constraints on proliferation and specification imposed by type I processes and become organized into the adult bilaterian by regional pattern-forming mechanisms. Furthermore, it has been argued that maximal indirect development, in which the adult develops from an imaginal rudiment of a larva, is a general, widespread, and ancestral mode of bilaterian development (*82*). This view has been challenged, based on concerns that the widespread occurrence of planktotrophic larvae may be convergent (*33, 81, 83–85*).

Evidence to support the view that type I embryogenesis is primitive and was sufficient for micrometazoan development has recently emerged from analysis of *Hox* gene utilization in embryos that give rise to maximal indirectly developing larvae. In modern bilaterians, highly conserved *Hox* gene products sculpt the morphology of adult bodies regulating diverse sets of target genes in a region-specific manner along the rostral-caudal axis. However, in the development of the echinoderm pluteus-stage larva, only two of the conserved set of ten deuterostome *Hox* genes are deployed (*86*). This demonstrates that the generation of a free-living feeding bilaterian may not require a cluster of *Hox* genes. Similarly, *Hox* genes are largely not required for embryogenesis in the nematode *Caenorhabditis elegans* (*87*), nor are they deployed during polychaete annelid larval development (*88*). If type I embryogenesis is indeed primitive, these observations could indicate that the evolution of *Hox* gene diversity occurred in concert with the set-aside progenitor cells to generate adult body patterns in stem bilaterians. If, on the other hand, the earliest bilaterians developed directly from embryos to adults (as, for example, extant acoels do), then the restrictions imposed by type I embryogenesis must have been circumvented before the evolution of set-aside cells. In either scenario, all the extant developmental regulatory mechanisms for generating macroscopic animals would have been put in place, setting the stage for the bilaterian radiation. Such a view does not require that adult body plans evolved independently in all bilaterian phyla; in fact, the portrait of Urbilateria painted in previous paragraphs makes that unlikely.

Urbilateria branches out: Proterozoic cladogenesis. The extent of bilaterian radiation at the dawn of the Cambrian is uncertain. However, fossils as well as molecular clock estimates suggest that the protostome-deuterostome divergence, the separation of the two protostome clades, and some radiation within these clades, predated the Cambrian. Thus, the developmental characters that distinguish major clades would have also evolved by this time. Spiral cleavage, molting, and other features associated with specific protostome clades must already have been in place (Fig. 3).

While there is now a better prospect for inferring sister group relationships among extant taxa, the origins of the various bilaterian body plans remain obscure. We can group priapulids with arthropods or annelids with mollusks, but the morphological transitions that gave rise to the phyla cannot be extrapolated from their living descendants. It will require a far richer Proterozoic and earliest Cambrian fossil record to solve the problem paleontologically. And the keys to the origin of body plans are not likely to be the sort of genetic innovations that occurred in the bilaterian stem lineage; protostomes and basal deuterostomes have fairly comparable batteries of developmental regulatory genes (*40, 49*). Rather, the differences in body plans are likely to have arisen at the level of the regulatory networks that organize pattern formation. This idea is illustrated most clearly by studies on the diversification of one of the most successful bilaterian body plans: the arthropods.

Cambrian diversification: So many arthropods, so little time. Arthropods are the most abundant Cambrian fossils, and by the time of the Burgess Shale, they had evolved a number of different body plan designs and many variations upon these themes (*18, 89–91*). The sister group to the arthropods, the lobopodans, is also well known, which has inspired efforts to synthesize a picture of arthropod stem-lineage evolution from lobopodan-like ancestors (*92*). The modern descendants of these forms, including the chelicerates, crustacea, myriapods, insects, and onychophora illustrate all the elements of the body plan diversity apparent in Cambrian assemblages, including the major differences in tagmosis, segment and appendage number, and limb morphology (Fig. 4, right). Investigations into the developmental mechanisms that specify differences among extant arthropods (and onychophorans) can thus provide a window on the developmental processes underlying the Cambrian radiation.

The most obvious trend in the evolution of the onychophoran-arthropod clade has been the evolution of increased segment diversity from a generally homonomous condition in basal forms to various heteronomous forms in Cambrian and recent taxa. Lobopodans had only a few appendage types and a homonomous trunk, whereas arthropods are more distinctly tagmatized and possess a wider array of appendage types. Since segment and appendage diversity in the highly derived insects is regulated by *Hox* genes (*93*), most work has focused on the roles these genes play in the development and evolution of arthropod diversity. Three general points have emerged from comparative studies of *Hox* gene organization and expression in chelicerates (*94, 95*), crustacea (*96–98*), myriapods (*99*), insects (*100*), and onychophora (*99*). First, the entire onychophoran-arthropod clade possesses essentially the same set of *Hox* genes that pattern the main body axis (Fig. 4; left) (*99*). Thus, Cambrian and recent diversity evolved around an ancient and conserved set of *Hox* genes. Second, the increase in segment diversity is correlated with changes in the relative domains of *Hox* gene expression along the main body axis (Fig. 4, right). This result is illustrated by the evolution of appendage diversity among the crustacea in which the modification of thoracic limbs into feeding appendages correlates precisely with the modification of *Hox* gene expression (*96*). And third, changes in the morphology of homologous appendages are correlated with changes in the array of genes that are regulated by the same *Hox* gene (*101*).

The mechanisms underlying the evolution of developmental changes in the arthropods, while generally limited thus far to inferences about *Hox* genes, may, nonetheless, have some general explanatory power. First, the conservation of the *Hox* gene family both within and among phyla suggests that most body plan evolution arose in the context of very similar sets of *Hox* genes, and thus was not driven by *Hox* gene duplication. Second, the trend toward the evolution of heteronomous body plans is apparent in other groups, such as annelids. Third, in other derived groups that have been examined such as the vertebrates, the correlation between diversification of *Hox* gene expression patterns and the evolution of anterior-posterior patterning along the body axis applies (*102*). These observations suggest that bilaterian body plan diversification has occurred primarily through changes in developmental regulatory networks rather than the genes themselves, which evolved much earlier.

The Environmental Context of Animal Diversification

If assembly of the developmental toolkit was not by itself the trigger for the Cambrian explosion, can we identify environmental events that could have released the morphogenetic poten-

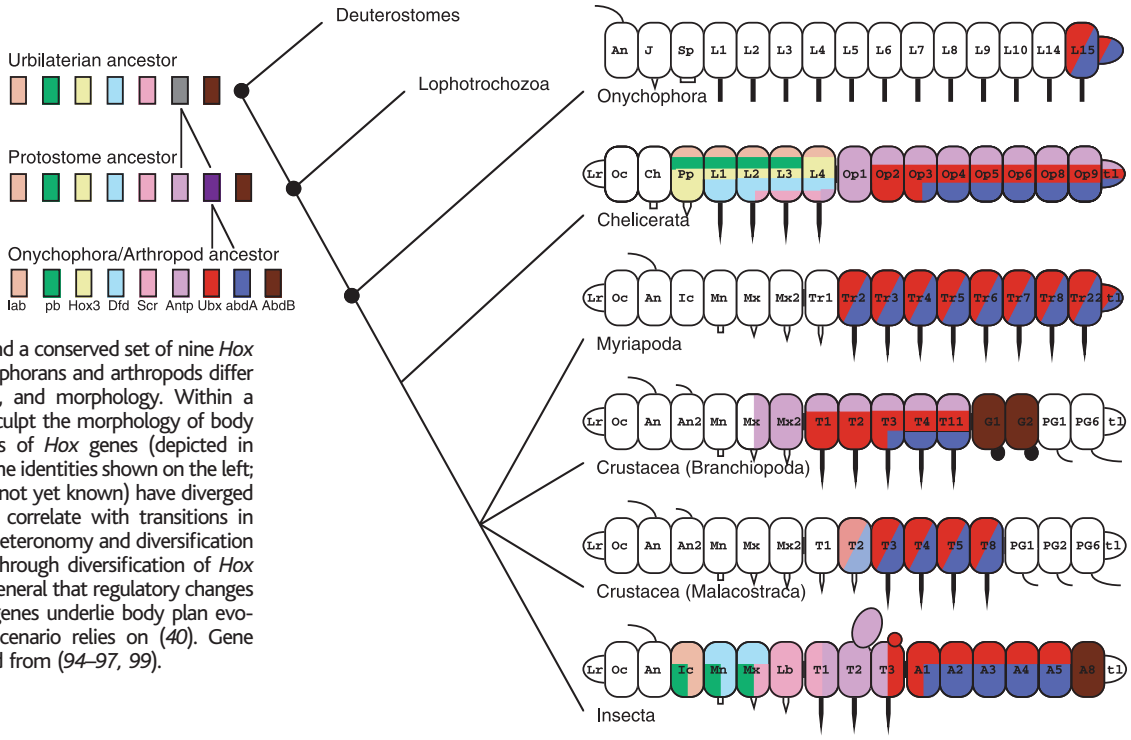

Fig. 4. Evolution of the onychophoran-arthropod clade and *Hox* gene regulation. (Left) The last common ancestor of protostomes and deuterostomes possessed at least seven diverged *Hox* genes. The common ancestor of the protostomes possessed an additional central class gene that gave rise to the *Ubx* and *abdA* genes in the onychophoran-arthropod ancestor. The diversity of this clade has evolved around a conserved set of nine *Hox* genes. (Right) Modern onychophorans and arthropods differ in segment number, identity, and morphology. Within a species, different *Hox* genes sculpt the morphology of body parts. The relative boundaries of *Hox* genes (depicted in colors corresponding to the gene identities shown on the left; many expression patterns are not yet known) have diverged among arthropods but often correlate with transitions in appendage morphology. The heteronomy and diversification of the arthropod body plan through diversification of *Hox* gene regulation illustrates in general that regulatory changes in conserved developmental genes underlie body plan evolution. *Hox* gene evolution scenario relies on (*40*). Gene expression data is summarized from (*94–97, 99*).

tial of these genes? The most likely candidate for such an environmental gate keeper is molecular oxygen (*103*). Simple biophysical considerations relate maximum body size to oxygen availability in animals that obtain oxygen by diffusion, a likely prospect in early metazoans (*104*). (The relationship applies principally to bilaterian animals with potentially thick muscles and mesodermally derived internal organs; diploblasts can become macroscopic by limiting metabolically active cells to a thin superficial layer that may be complexly folded to increase surface area). A late Proterozoic increase in atmospheric oxygen levels could, thus, reconcile the known fossil record with molecular inferences that call for an extended interval of metazoan prehistory, played out by small animals unlikely to be preserved (*91, 105*).

Biogeochemical investigations of Neoproterozoic rocks reveal patterns of stratigraphic variation that stand out against the backdrop of the past 2 billion years. In particular, unusually strong positive excursions in the carbon isotopic composition of carbonate minerals and organic matter indicate that rates of organic carbon burial in the late Proterozoic ocean basins were episodically high (*25, 106*). Insofar as the burial of photosynthetically derived organic matter provides a mechanism for oxygen build-up in the atmosphere and oceans (*107*), carbon isotopic data provide direct empirical support for the oxygen facilitation hypothesis. The biogeochemical record of sulfur independently suggests that oxygen increased in the late Proterozoic (*108*).

Oxygen would not in and of itself have caused animals to evolve. Rather, it would have removed an environmental barrier to the evolution of large, metabolically active animals. Canfield (*109*) has recently modeled earlier Proterozoic oceans as moderately oxic at the surface but anoxic below the mixed layer, making predictions that can be tested against observations of Proterozoic sedimentary rocks. If confirmed, this model suggests that regardless of genetic potential, large bilaterian animals could not have diversified before the late Proterozoic Eon.

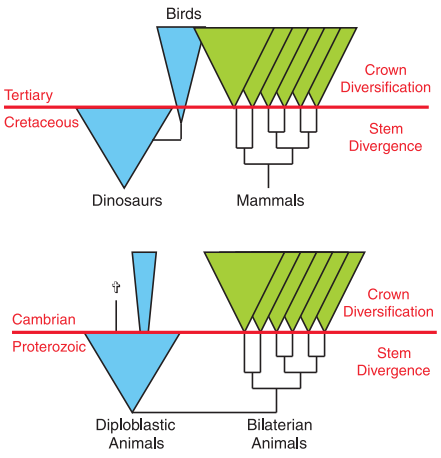

Fig. 5. Comparison of evolutionary dynamics across the Proterozoic-Cambrian and Cretaceous-Tertiary boundaries. The history of terrestrial vertebrates, in which mammals radiated only after dinosaurs suffered mass extinction, may provide a framework for understanding the successive radiations of Ediacarian-grade and crown-group bilaterian animals.

Late Proterozoic carbon isotopic profiles display strong negative as well as positive excursions. Negative excursions are specifically associated with the major ice ages that mark immediately pre-Ediacaran time. Much research is currently focused on this unusual coupling of climate and biogeochemistry (*110*), and both paleoceanographic models and clustered phytoplankton extinctions (*111*) suggest that these ice ages had a severe impact on the biota—potentially applying brakes to early animal evolution (*24, 110*). All diverse Ediacaran fossil assemblages post-date the last major Proterozoic ice age.

Although environmental change can thus help explain why large animals could flourish late in the Proterozoic Eon, it does not appear to illuminate Cambrian diversification per se. Possibly, once rising oxygen levels removed physical barriers to the evolution of large size, no other extrinsic drivers were required; morphological innovations such as complex nerve nets or sensory, locomotory, and feeding appendages made the Cambrian explosion inevitable. This may be, but one other recent biogeochemical discovery strongly suggests that evolution received a further nudge from the environment.

In the past few years, evidence has accumulated for a remarkable perturbation in the carbon cycle close to the Proterozoic-Cambrian boundary. Globally distributed sedimentary successions document a strong (7 to 9 per mil) but short-lived negative excursion in the carbon-isotopic composition of surface seawater at the stratigraphic breakpoint between Ediacaran-rich fossil assemblages and those that document the beginning of true Cambrian diversification (*112, 113*). The causes of this event remain uncertain, but the only comparable events in the more recent Earth history coincide with widespread extinction—for example, the Permo-Triassic crisis, when some 90% of marine species disappeared, is marked by an excursion similar to but smaller than the Proterozoic-Cambrian boundary event (*114*). An earliest Cambrian increase in bioturbation shuttered the taphonomic window on Ediacaran biology. Thus, while Chengjiang and Sirius Passet fossils indicate that Ediacarian-grade organisms were not ecologically important by the late Early Cambrian, biostratigraphy admits the possibility that Ediacarans were eaten or outcompeted by Cambrian animals. It is biogeochemistry that lends substance to the hypothesis that Ediacaran and Cambrian faunas are separated by mass extinction.

It is possible, then, that the evolution of early animals parallels the better resolved history of land vertebrates (Fig. 5). Mammals diverged early in the Mesozoic Era, and a moderate diversity of mammalian stem groups lived for millions of years in dinosaur-dominated ecosystems. Only with the demise of the dinosaurs did mammals radiate to produce the crown group diversity seen in Tertiary and modern faunas. Perhaps Ediacaran animals are the "dinosaurs" of the terminal Proterozoic oceans, simple but successful organisms that placed ecological constraints on bilaterian evolution. In this view, end-Proterozoic environmental perturbation created ecological opportunity, facilitating the simultaneous diversification of multiple clades of bilaterian survivors (*113*).

In reorganized Cambrian ecosystems, interactions among organisms further catalyzed biological diversification. Predation likely facilitated the evolution of skeletonized invertebrates (*115*), and, despite their earlier appearance, algae record a Cambrian explosion of phytoplankton diversity that parallels that of animals. Such observations bring a new ecological dimension to the story (*27, 116*), requiring that Cambrian diversification be seen as an ecosystem-wide phenomenon that affected protists, sponges and bilaterians alike. As noted above, morphological diversification in the Cambrian oceans reflects changes in gene regulation. Therefore, the role of end-Proterozoic environmental disruption—like oxygen before it—would have been to introduce a new selective landscape in which these novel variants could persist. As new body plans evolved, the biological components of environment, themselves, became principal features of this new landscape.

Conclusions

We cannot yet claim to have solved the major questions of early animal evolution. But we have reached a point where pathways to understanding are becoming clearer. Testing hypotheses about the identity, age, complexity, and diversity of early animals and the environments in which they lived has become an interdisciplinary exercise, and we see four areas that are potentially the most productive pursuits. First, we need to mine the late Proterozoic record for bilaterian fossils and paleoenvironmental insights. Second, to understand the assembly of the bilaterian tool kit, investigations into the developmental genetics (and phylogeny) of sponges, cnidarians, and ctenophores are crucial. Third, the origin of key developmental characters (for examples, mesoderm, spiral cleavage, set aside cells) may be elucidated by broadening the base of comparative developmental biology

to less well-studied taxa such as mollusks, polychaete annelids, and invertebrate deuterostomes (*117*). And finally, understanding the molecular developmental basis of evolutionary change is crucial. The architecture and evolution of the genetic regulatory networks that pattern animals must be elucidated in much greater depth if we are to reconstruct the genetic history of the major transitions in animal evolution.

We can see clearly now that intrinsic and extrinsic hypotheses are not really alternative ways of explaining animal diversification. There were certainly intrinsic catalysts of early animal evolution. The assembly and regulatory diversification of the genetic toolkit for animal development undoubtedly underpin Proterozoic and Cambrian evolution. And the evolution of complex appendages, organs, and sophisticated nervous and musculo-skeletal structures must have facilitated diversification (*118*). Yet, extrinsic events also helped to shape early animal evolution altering environments in ways that doomed some clades and created opportunity for others.

Discipline-bound intrinsic or extrinsic explanations of early animal history fail not so much because they are wrong as because they are incomplete. The Cambrian explosion—the stratigraphic pattern seen in those cliffs along the Kotuikan River—is the historical product of the interplay between genetic possibility and environmental opportunity, amplified by ecological interactions to extend across all of biology.

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REVIEW

The Evolution of Dinosaurs

Paul C. Sereno

The ascendancy of dinosaurs on land near the close of the Triassic now appears to have been as accidental and opportunistic as their demise and replacement by therian mammals at the end of the Cretaceous. The dinosaurian radiation, launched by 1-meter-long bipeds, was slower in tempo and more restricted in adaptive scope than that of therian mammals. A notable exception was the evolution of birds from small-bodied predatory dinosaurs, which involved a dramatic decrease in body size. Recurring phylogenetic trends among dinosaurs include, to the contrary, increase in body size. There is no evidence for co-evolution between predators and prey or between herbivores and flowering plants. As the major land masses drifted apart, dinosaurian biogeography was molded more by regional extinction and intercontinental dispersal than by the breakup sequence of Pangaea.

During the past 30 years, intensified paleontological exploration has doubled recorded dinosaurian diversity (*1*) and extended their geographic range into polar regions (*2*). Exceptional fossil preservation has revealed eggshell microstructure (*3*), nesting patterns and brooding posture among predators (*4*), and epidermal structures such as downy filaments and feathers (*5*, *6*). Analysis of bone microstructure and isotopic composition has shed light on embryonic and posthatching growth patterns and thermophysiology (*7*). Footprint and track sites have yielded new clues regarding posture (*8*), locomotion (*9*), and herding among large-bodied herbivores (*10*). And the main lines of dinosaurian descent have been charted, placing the aforementioned discoveries in phylogenetic context (*11*).

Department of Organismal Biology and Anatomy, University of Chicago, 1027 East 57th Street, Chicago, IL 60637, USA.

The most important impact of this enriched perspective on dinosaurs may be its contribution to the study of large-scale evolutionary patterns. What triggers or drives major replacements in the history of life? How do novel and demanding functional capabilities, such as powered flight, first evolve? And how does the breakup of a supercontinent affect land-based life? The critical evidence resides in the fossil record—in the structure, timing, and geography of evolutionary radiations such as that of dinosaurs.

Early Dinosaurs: Victors by Accident

Did dinosaurs outcompete their rivals or simply take advantage of vacant ecological space? The ascendancy of dinosaurs on land transpired rather rapidly some 215 million years ago, before the close of the Triassic. Herbivorous prosauropods and carnivorous coelophysoid ceratosaurs spread across Pangaea, ushering in the "dinosaur era": a 150-million-year interval when virtually all animals 1 m or more in length in dry land habitats were dinosaurs.

Dinosaurs, the descendants of a single common ancestor, first appeared at least 15 million years earlier but were limited in diversity and abundance (Fig. 1). Well-preserved skeletons discovered recently in 230-million-year-old rocks (mid-Carnian in age) provide a glimpse of a land radiation already underway (*12*). The most fundamental adaptations for herbivory and carnivory among dinosaurs had already evolved. A novel means for slicing plant matter, utilizing inclined tooth-to-tooth wear facets, is fully developed in the meter-long herbivore *Pisanosaurus*, the oldest known ornithischian (Fig. 1, left; Fig. 2, node 1; Fig. 3A, feature 4). Jointed lower jaws and a grasping hyperextendable manus for subduing and eviscerating prey are present in the contemporary predators *Eoraptor* and *Herrerasaurus*, which are the oldest well-preserved theropods (Fig. 1, right; Fig. 2, node 41; Fig. 3B, features 11 and 12).

Traditional scenarios for the ascendancy of dinosaurs that invoke competitive advantage (*13*) have difficulty accommodating the substantial temporal gap (15 million years or more) between the initial radiation of dinosaurs and their subsequent global dominance during the latest Triassic and Early Jurassic (*14*). Opportunistic replacement of a diverse array of terrestrial tetrapods (nonmammalian synapsids, basal archosaurs, and rhynchosaurs) by dinosaurs is now the most plausible hypothesis (*11*, *14*, *15*). This pattern is broadly similar to the replacement of nonavian dinosaurs by therian mammals at the end of the Cretaceous. Recent