

The River of Life: A Genetic Perspective on Macroevolution

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*So where the river flows
Everything will live.
—Ezekiel 47:9*

Abstract

The genetic mechanisms that might have triggered the macroevolutionary events throughout the geological record, the sudden and spectacular radiations of certain clades both in the seas and on land, remain elusive. The patterns of the fossil record document the emergence of nine major macroevolutionary events or dynasties: (1) eucaryans, (2) metazoans, (3) embryophytes, (4) angiosperms, (5) craniates, (6) gnathostomes, (7) teleosts, (8) neognaths, and (9) eutherians. Such big leaps in evolution required enrichment and expansion of gene pools. A synthesis in paleontology, genetics, and developmental biology recognizes several genetic mechanisms that might be linked to macroevolutionary events. In this paper I hypothesize that macroevolution occurs via macrogenesis, a different set of genetic mechanism than the point mutation of microevolution. These macrogenesis agents are powerful facilitators of genome evolution in living organisms that include horizontal gene transfer (HGT), endosymbiosis, hybridization, and polyploidization for generating variation. They create new body plans, morphological innovation, phenotypic diversity, and taxon richness. The primary strategy employed here is to look for correlations between a macroevolutionary event and its likely macrogenesis agent. Although geographic isolation is not necessary for macrogenesis, its effect in small isolated peripheral populations is intense as they can cause genetic changes of great magnitude and variety in a drastic evolutionary acceleration. Among various agents of macrogenesis, the most prevalent mechanism appears to be gene duplication in supplying raw material for macroevolution. Macroevolution can be analyzed as a three-step process, interspersed with intervals of millions of years: (1) origin of a higher clade by macrogenesis; (2) an interim stabilizing process called phylogenetic fuse; and (3) rapid cladogenesis or evolutionary explosion, mediated by developmental genetics. Delay between origination of higher taxa and their subsequent phylogenetic fuse and explosive evolution indicates that macroevolutionary pattern depends on survival and adaptation of newly acquired genes, biological potential, regulatory gene networks, and ecological opportunity. The tempo of macroevolution is episodic, fast during the origination of a clade and its eventual cladogenesis, which are separated by a slow pace of phylogenetic fuse regime. Macrogenesis changes the topology of *The Tree of Life*, which is a powerful metaphor in Darwinian evolution to portray the divergence of life from a common ancestor. Recent molecular phylogeny has produced a three-trunked tangled tree of ancient microbes at the deepest level of phylogeny—bacteria, archaea, and eucarya—where the branches recurrently fuse and divide like a river, driven by horizontal gene transfer and endosymbiosis. The topology of the tangled tree continues upward in the multicellular level, where the tree branches fuse recurrently during the macroevolutionary explosion events by macrogenesis. A tree reconstructed from combined phylogeny of genes and fossils cannot fully represent the patterns and processes of history of life. A better metaphor to portray the branching and fusion pattern of macroevolution of life would be *The River of Life*—a braided river system in preference to a conventional tree, because the branches of a river form a network by lateral anastomoses, while each branch of a tree stands free.

Introduction

This year, 2009, marks the 200th anniversary of the birth of Charles Darwin, the father of evolution, who inspired new generations of naturalists to explore the glorious diversity and complexity of life. His insights of evolution have withstood 150 years of scrutiny, but his theory has broadened and melded with genetics, molecular biology, and paleontology. One of the most wonderful mysteries in nature is biodiversity, the astonishing variety of life on Earth that inspired Darwin (1859) to develop his theory of natural selection. Speciation is among the most fundamental events in the history of life. The effort to understand how life diversified from its bacterial beginning to the present day is based on studies of two distinct mechanisms of speciation: microevolution and macroevolution. Darwin was mainly concerned about microevolution (evolution at or below the species level) of eucaryans via unilineal decent where vertical gene transfer (VGT) from generation to generation creates variation, the raw material for

natural selection. He was also puzzled about macroevolution, a large-scale rapid evolutionary event above the species level as documented in the fossil record that seemed to contradict his phyletic gradualism hypothesis of microevolution. Macroevolution, a major evolutionary process by which new and higher taxonomic categories (such as families, orders, classes, kingdoms or even domains) arose suddenly and maintained their independent trajectories, still remains a perplexing and challenging problem in evolutionary biology since the time of Darwin, because we do not know its genetic cause and consequences.

The concept of macroevolution comes from the perception that the paleontological record lacks fossils that show the transition between major taxonomic groups. These fossils would be expected if microevolutionary steps bridged the transition. Paleontologists have long known that higher taxa usually appear suddenly in the fossil record without smooth transitional forms between ancestor and descendant. The lack of transitional fossils could be due to imperfect geological record as Darwin (1859) maintained, or to the fact that major higher taxa occur abruptly by major genetic reorganization. Many groups of higher taxa above the prokaryote level such as—eucarya, embryophytes, angiosperms, metazoans, craniates, gnathostomes, teleosts, neognaths, and placentals—appeared suddenly in the fossil record and are not preceded by continuous transitional stages; their sudden appearances had intrigued and puzzled paleontologists for more than a century. Simpson (1944) made a pioneering stride in our understanding of tempo and mode of macroevolution. He observed that macroevolutionary ‘explosions’ in the fossil record were preceded by a greatly extended but obscure period of earlier history, producing some gaps in the fossil record. These gaps are not artifacts but real phenomena requiring special explanation other than microevolution. Simpson concluded that the patterns of macroevolution in the fossil record are unique in style, qualitatively different from the patterns seen in the data of geneticists in microevolution.

Unlike microevolution, which can be observed both in nature and in the laboratory by analyses of population genetics and physiology, study of macroevolution was quite inaccessible through the classical genetic methods because it occurs over intervals that far exceeded the human lifespan, and was not incorporated during the Modern Synthesis. Macroevolution is generally based on morphological characters, the study of phenotypes that are preserved in the fossil record. For example, when we study macroevolution, say the origin of birds from theropods, or hominin evolution from chimpanzees, we interpret their phenotypes—their anatomical changes and acquisition of new characters from fossil evidence. Paleontological data do not allow an inference to be made about changes in a species’ genetic pool. As such genetic mechanisms of macroevolution remain largely speculative because of their historic origin (Simpson, 1944; Mayr, 1963).

The relationship between micro- and macroevolution is a highly contentious issue that goes back to the earliest days in evolutionary biology. Darwin (1859) believed that microevolution alone couldn’t explain macroevolution; there might be a biological filter or discontinuity between these two processes, possibly because of imperfection of fossil record. Darwin believed that as more and more transitional fossils would be found, microevolutionary processes could explain some of the macroevolutionary events. Now we know that Darwin was right at least for the origins of three major groups of vertebrates that show gradual evolution in a long series of small changes: tetrapods from sarcopterygian fish, mammals from therapsids, and birds from coeluroaurian dinosaurs. Modern Synthesis (Dobzhansky, 1941; Mayr, 1942) implies that macroevolution occurs by the same molecular and Mendelian mechanisms as microevolution. Microevolution leads to macroevolution by the same process—natural selection and adaptive

improvement—as has been observed within speciation, but the process is operating at a higher taxonomic level over a much longer geological period. Thus the difference between micro- and macroevolution, according to Modern Synthesis, is history, scale, and hierarchy. Macroevolution represents microevolution extrapolated over long geologic periods. In contrast, most paleontologists believe that special factors that are not evident at the species level are responsible for macroevolutionary events, but the genetic mechanisms remain elusive. They claim that microevolution and macroevolution are two separate and disconnected processes: microevolution cannot give rise to macroevolution (Simpson, 1944; Stanley, 1979; Gould, 2002).

Two major macroevolutionary variables are species richness of the clade (number of lineages) and their diversity of form (disparity) (Vrba and Eldredge, 2005). Current debate about macroevolution is hinged on the nature of mechanism, whether it is triggered environmentally or genetically to create such a rapid diversity and disparity of life. Both external and internal triggers for macroevolution, or a combination of both processes, have been proposed. The external triggers include climate change, continental drift, mass extinction, adaptive radiation, ecologic opportunity, species selection, and species sorting (Vrba and Eldredge, 2005).

Punctuated Equilibrium and Species Sorting

The widespread neglect of the role of speciation in macroevolution continued until Eldredge and Gould (1972) proposed the theory of punctuated equilibrium. They reinterpreted the fossil record at its face value; they claimed that the great majority of species originate in geological moments in the fossil record and then persist in stasis throughout their long durations with little or no change. The fossil record demonstrates the widespread occurrence of stasis in many species in continuous stratigraphic sequences over long time period. Gould and Eldredge (1977) proclaimed stasis is a real pattern in the fossil record. They proposed a new view of speciation pattern, a punctuation model that contrasted with the traditional phyletic gradualism hypothesis of neo-Darwinism. According to their theory, the intermediate stages in the evolution of organisms, the so called ‘missing links,’ might not appear in the fossil record because the transitional organisms were short-lived, extremely unstable species, which quickly evolved into stable species. They proposed that natural selection could fine-tune organisms during periods of stasis, but allopatric speciation (Mayr, 1942) had to account for punctuated change. They formalized the tempo of evolution on two steps: the origin of a species occurs in the fossil record suddenly (punctuations) and then persists for millions of years without apparent change (equilibrium or stasis). In many cases, evolutionary innovations seem to arise at the time of a new species. As a result, morphological evolution in some groups seem to consist of long periods of stasis that are occasionally punctuated by speciation events that appear instantaneously in the geological record. The crucial difference between punctuated equilibrium and phyletic gradualism of neo-Darwinism concerns the rate of evolution at, and between, speciation events; in punctuation mode, evolution proceeds relatively rapidly at the time of splits, whereas in phyletic gradualism, evolution has a similar range of rates at the two events (Gould and Eldredge, 1977; Gould, 2002).

Stanley (1979) extended the concept of punctuated equilibria in formulating species selection hypothesis where selection can operate at the level of an entire species population, rather than individuals, to explain macroevolution, which is decoupled from microevolution. In his species selection hypothesis, two evolutionary processes that arise by interaction of intrinsic biological traits with the environment, may cause a clade’s species richness: quantum speciation and

extinction, the net effect of which gives rise to the net rate of diversification. Furthermore, genetic recombination is crucial to the process of quantum speciation (Simpson, 1944; Goldschmidt, 1958) that creates new body plans and behavior patterns rapidly with genetic rearrangements. According to this view, quantum speciation is a real phenomenon in the punctuational scheme of evolution and a source of great variability for macroevolution via species selection. Species selection is the natural unit of large-scale evolution and is responsible for proliferation of species that have lower extinction and higher speciation rates. It is the alternative interpretation of the macroevolutionary process offered by paleontologists to expand the natural selection to higher taxonomic levels and provides another reason why macroevolution and microevolution are uncoupled (Gould, 2002). Species selection requires the three same preconditions as individual selection: variation, heritability, and differential reproduction. Within a species, natural selection favors one character in one species and another character in a second species. Species selection over longer periods may cause the species with one of the characters to proliferate because it may result from the greater longevity of species or from different rates of speciation. Further refinement of the concept, however, has led to the essentially philosophical distinctions species selection and species sorting. Species sorting is a neutral description of differential birth and death of a species (Vrba and Gould, 1980). The fossil record often reveals species sorting, meaning that some lineages rapidly diversify into new species whereas others decline. When two or more species compete, the differential survival that sorts out the winners and losers may be due to intrinsic species properties rather than to natural selection on individuals and populations.

Ecological Opportunity

After the origin of a higher clade, the number of species in it usually increases; so, too, do the number of genera, families, and other taxonomic levels. Why, for example, did the neognathous birds and placental mammals remain a minor group in the Late Cretaceous for millions of years after their origin and then radiate rapidly in the Early Tertiary? The general consensus is that until the Paleocene the ecological niches later to be occupied by neognaths and placentals were already filled by pterosaurs and dinosaurs respectively. Thus both neognaths and placentals radiated as the ecological replacements of pterosaurs and dinosaurs respectively. Jablonski (1991) has integrated the dynamics of ecology, climate changes, plate tectonics, and mass extinctions in macroevolutionary history to bridge the gap between microevolution and macroevolution. Like Darwin, he believes that these two evolutionary processes are complementary and not mutually exclusive. He emphasized that many macroevolutionary patterns are difficult to interpret without invoking positive or negative interactions (such as competition, predation, parasitism, and mutualism) with species in other clades. Organisms may have the opportunity to invade a new adaptive zone simply because it is empty, or vacated by mass extinction, or created by plate interactions, without any significant changes in their adaptive ability. Alternatively, changes in structure, physiology, or behavior may give organisms the facility to evolve within a previously unavailable adaptive zone.

Genetic Mechanisms of Macroevolution

Although the patterns of macroevolutionary radiations in the geological record have been extensively studied (Simpson, 1944; Stanley, 1979; Gould, 2002; Vrba and Eldredge, 2005), the earlier histories that preceded the adaptive radiations—the origin of higher taxa followed by a long period of calm and stabilizing process—have not received quite the same attention. The genetic mechanisms of macroevolution have been explored earlier, but these hypotheses encountered harsh criticisms and rejections (Goldschmidt, 1940; Ohno, 1970; Stebbins, 1970). Goldschmidt (1940) proposed a ‘hopeful monster’ theory to explain the sudden appearance of a clade or a new structure by a single macromutation. He believed that macroevolution is qualitatively different from microevolution within a species, and is based on a totally different kind of genetic and developmental repatterning. Goldschmidt was the first to make explicit links between organismal complexity and gene duplication. Gene duplication can occur by a variety of mutational mechanisms: polyploidy, the loss or gain of particular chromosomes, and the duplication of large chromosome parts. Goldschmidt studied the development of gypsy moth caterpillar and found that variants of these insects appeared quite abruptly and discontinuously. He suggested that these changes might be due to small changes in regulatory genes resulting in macromutation that gave an organism an advantage. This hopeful monster could establish a new taxon abruptly rather than gradually, which would become the first member of an entirely new taxon, perhaps an order, or even a phylum. Goldschmidt’s hopeful monster was a single animal, a full-blown bizarre creature that served as a progenitor of a new higher taxon leading to a viable and adaptive form. The main reason for inventing these macromutations by Goldschmidt is that some features of animals and plants can hardly be imagined as arising by gradual steps. According to this view, micromutations take place by natural selection of small genetic variants, but higher taxa evolve by different process—via hopeful monsters. If a new macromutation appeared to give an organism a great advantage, this hopeful monster could establish a new taxon abruptly rather than gradually.

Hopeful monster theory was rejected by neo-Darwinian paradigm, because single macromutations that produce drastic alterations of developmental patterns would have a negative adaptive value by themselves and would be eliminated by natural selection (Dobzhansky, 1941; Mayr, 1942; Simpson, 1944). However, the theory has been revived in recent years as the creative force of regulatory genes began to unfold as predicted by Goldschmidt before they were actually discovered. There are many examples in the history of vertebrate evolution where macromutation might have occurred to produce a new body plan and higher taxa. For example, Frazetta (1970) proposed that the bolyerine snakes in Mauritius Island such as *Casarea* originated from the boa constrictors, but they have a unique feature: the maxillary bone is hinged in the middle. Frazetta points out that it is impossible to imagine a gradual transition from solid boid maxillary bone to the hinged bolyerine maxilla. Frazetta argued that *Casarea* is a good example of hopeful monster that evolved by macromutation with large phenotypic consequences. Similarly, the Late Miocene dwarf rhino *Teleoceras* appeared suddenly in the fossil record due to single mutations among small interbreeding populations as seen in domestic sheep (Stanley, 1979). A number of evolutionists are now convinced that macroevolutionary change is more than just microevolution extrapolated upward. In their view, evolutionary novelty, emergence of new body plans, and macromutation to form new taxon is fundamentally different from the processes that control microevolution. Gould (1980) called this macroevolutionary change the ‘Goldschmidt break’ in honor of the iconoclastic geneticist who first proposed it.

Like Goldschmidt, several other geneticists such as Ohno (1970) and Stebbins (1974) also emphasized that without expanding gene pools in a taxon by various known mechanisms such as polyploidy and hybridization, the origin of higher taxa in plants and animals cannot be explained by microevolutionary processes such as point mutation and natural selection. As the genotype produces phenotype, they argued that morphological innovation stems from new genes.

However, the defenders of Modern Synthesis largely ignore the role of polyploidy and hybridization as a viable means of speciation in animals. They argue that there are many natural barriers to interspecific hybridization—geographical, ecological, behavioral, and anatomical barriers—that normally prevent contact between an egg of one species and sperm of another. Even if these barriers are circumvented, hybridization is considered as a rare and maladaptive process in animals because hybridizing individuals produced too few fertile progeny to generate functional novelty and new species (Mayr, 1982). New genetic evidence suggests that that polyploid and hybrid speciation are not only common in plants, but also prevalent among animals; they produced two main types of speciation—polyploid speciation and recombinational speciation (the diploid form of hybrid speciation)—which give rise to new lineages and are considered as main engines of macroevolution and diversification (Grant and Grant, 1992; Mallet, 2005; Arnold, 2006 Schwenk et al., 2008). This renewed interest in exploring the role of genetic factors in mediating macroevolutionary change could restore a more balanced view of evolution and its causes, away from the strictly adaptationist view of Modern Synthesis.

Macrogenesis

Changes in the genetic information stored in the genome are the ultimate basis of evolution. Like the organisms, genomes themselves also evolve gradually by mutations. They can also increase their numbers and sizes abruptly by horizontal gene transfer from other species or by gene duplication. Early genomes in prokaryotes were small, but today's living eucaryans, animals, and plants have large number of genes with diverse functions. Thus the progressive increase in genome size through time is a major trend in the evolution of life. An important, though infrequent, process of gene enrichment is addition of genes or genomes from other organisms. More frequently, increase in gene numbers come from gene duplication.

In classical Darwinian evolution, new species arise via vertical gene transfer (VGT) with gradual modification. In this process genome-size does not change; it simply modifies gradually through point mutation from generation to generation. Darwin's insistence that evolution is entirely gradual encountered a great deal of resistance by his contemporary biologists such as Nägeli, Kölliker, Mivart, and Galton, who used a term 'macrogenesis' for abrupt, discontinuous, or saltational evolution by large-scale genetic changes. Contemporary paleontologists likewise used macrogenesis to describe the sudden origin of a new clade in the fossil record and on the total absence of any intermediate types. T. H. Huxley, in an 1859 letter to Charles Darwin, chided him for adhering to the motto *Natura non facit saltum* (Nature does not make leaps), but for Darwin change has to be gradual so that organisms can adapt. As macrogenesis became incompatible with gradual natural selection, Modern Synthesis rejected the concept (Mayr, 1982). Macrogenesis, which fell into disfavor by Modern Synthesis, is now ripe for reassessment.

Although genes are the origin of variation—the raw material of the evolutionary process—gene pools are finite in a species and provide little stimulus for creating macroevolutionary novelty, phenotypic complexity, and biodiversity. In this paper, various means of enriching and

expanding gene pools such as horizontal gene transfer (HGT), endosymbiosis, hybridization, and polyploidy for generating variation that were not used by Modern Synthesis to formulate microevolution, are explored here to explain macroevolution. Here I resurrect the term 'macrogenesis' in a broader genetic context to include these various evolutionary mechanisms that increase the gene number. Since the roles of these agents in the evolution of bacteria, eucarya, and angiosperms are well known among living groups, I speculate that macrogenesis might be responsible for triggering macroevolution as seen in the fossil record.

The questions of the origin of macroevolutionary events are mostly historical in nature and we can seldom pinpoint definitely, as in any historical science, the proximate cause for that event. We don't have DNA from the fossil record to test this hypothesis, and we cannot identify or isolate for molecular analysis the genes responsible for macroevolutionary bursts. But we know these macrogenesis agents operate on living organisms to give rise to new species. So in most cases the ideas about what sorts of genetic mechanisms were involved in macroevolution is only speculation, but the speculation is based on the principle of uniformitarianism—present-day processes have operated throughout geologic time. We can ask whether current evolutionary and ecological theory is sufficient to explain the history of macroevolution. If historical events, inferred from paleontological and phylogenetic studies, are compatible with our theory of genetic mechanisms, then we are justified in accepting the current hypothesis as a working model until further evidence falsifies it in favor of another. Since the potential role of regulatory genes in mediating dramatic phenotypic transformations is now well known (Carroll 2005), I speculate that the increased number of regulatory genes that results from expanded gene pools by macrogenesis provides the raw material for cladogenesis. The point about macrogenesis hypothesis presented here is that it is an extension or application of already existing paradigm of genetics, a strong evolutionary force for rapid speciation.

The mode of gene transfer in macrogenesis is bimodal, where both VGT and HGT are involved. In contrast, microevolution by point mutation is unimodal, only by VGT. Macrogenesis occurs via a different set of mechanisms including HGT, endosymbiosis, hybridization, and polyploidization and are diverse. HGT, endosymbiosis, and hybridization violate the simple branching pattern of a conventional tree of life by fusion of lineages; polyploidization does not; it follows the VGT pattern. What they have common in all macrogenesis processes are that they are facilitators for gross changes in genomes. In that way they are qualitatively different than minor changes in point mutation of microevolution. Macrogenesis at one stroke changes the overall genome in qualitative ways and has created new lineages in the history of life. Macrogenesis is used here as a descriptor for an internal mechanism for macroevolution that expands and enriches genetic pools quickly. The emergence of higher taxa mostly comes about through macrogenesis. Macrogenesis is Goldschmidt's long-sought mechanism, his dream for hopeful monsters that arose saltationally, by a mode of major genetic enrichment different in kind from the alterations that yield microevolution.

However, macrogenesis is different from both point mutation of microevolution or Goldschmidt's macromutation, because these mechanisms are genetically inherited change via VGT. Both lead to a pattern of relationships among taxa that is akin to a branching pattern of a tree. In contrast, macrogenesis accommodates both VGT and HGT. It is an extension and elaboration of the HGT mechanism that includes not only endosymbiosis and hybridization but also a VGT mechanism that includes polyploidy. Macrogenesis thus creates a complex and reticulated pattern of relationships among taxa, quite different from the tree of life.

In recent years genomes of many species have been sequenced that provide unprecedented information about the phylogeny and evolution of a clade. Genetic data often reveals the process of ancient macrogenesis events such as timing of origin of a clade and its likely mechanisms such as HGT, polyploidy (gene duplication), and hybridization. Similarly paleontological data reveal the pattern of macroevolution: the timing of the origin of a higher clade, its subsequent phases of stabilization for many millions of years at low diversity, and its eventual cladogenesis. Combining two sets of evidence from genes and fossils, a better picture for the patterns and processes of macroevolution can be inferred.

One of the prominent patterns in macroevolution as preserved in the fossil record is that new higher taxon appears in the fossil record suddenly and then persist for million years with little change and then suddenly bursts into explosive speciation or cladogenesis when the ecological opportunity opens up (Simpson, 1944). Unlike the punctuated equilibrium theory (Eldredge and Gould, 1972), which operates on two stages—punctuation and stasis—the new macroevolutionary model proposed here occurs on three levels: punctuated origin of a higher clade, followed by slow and long stabilization process that culminates into an evolutionary explosion by rapid cladogenesis.

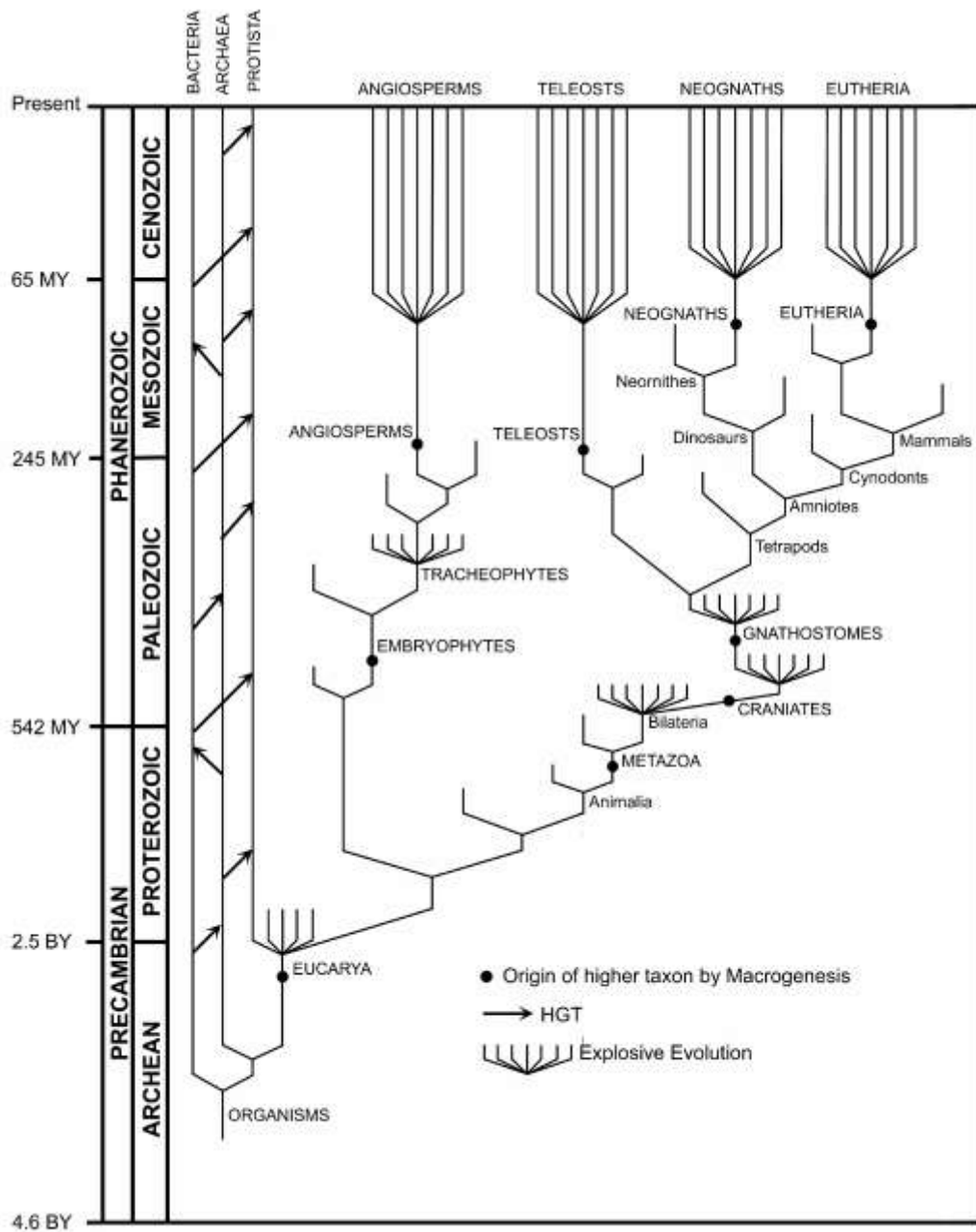


Figure 1. Major macroevolutionary pulses in the history of life as documented in the paleontological record. Three successive regimes of macroevolution are recognized: origin if a higher clade, stabilization or phylogenetic fuse, and cladogenesis or explosive evolution. Macrogenesis agents (such as HGT, endosymbiosis, hybridization, and polyploidy) trigger the origination of higher taxa (shown by solid circles) such as eucarya, embryophytes, angiosperms, metazoans, craniates, gnathostomes, teleosts, neognaths, and placentals. Macrogenesis event is often followed by a prolonged period of stabilization or phylogenetic fuse (shown by the vertical line between the origin and cladogenesis). When ecological opportunity opens up, stabilization leads to intense cladogenesis or explosive evolution (shown by radiating cluster), splitting higher taxa into lower taxa, creating phenotypic complexity, biodiversity, and species richness.

Three-gear Engine of Macroevolution

A literal reading of paleontological data provides evidence of change versus stability of the frequency distribution of phenotypes in a phyletic lineage. The fossil record of a major clade shows three distinct regimes of macroevolution in ascending order of timeline like a three-gear engine: (1) sudden emergence of a higher taxon with a new ground body plan; (2) a prolonged period of stabilizing process with initial branching of basal lineages; and (3) an explosive evolution and intense cladogenesis (Fig. 1). There appears to be extended time lags, millions of years, between intervals of three macroevolutionary processes: origin, stabilization, and cladogenesis. Here I offer some plausible genetic mechanisms for each of these macroevolutionary regimes. A general formalization of these macrogenesis processes remains challenging, but approaches drawing on neontological and paleontological data for a single set of clades appear promising. I hope to stimulate the necessary integration across hierarchical levels for future study.

1. Origin of a higher clade—Macroevolutionary bursts in the fossil record are recognized by disparity and species richness (Vrba and Eldredge, 2005). A central goal of evolutionary biology is our understanding how changes in genotypes may produce changes in phenotypes. One of the most striking features of macroevolution is the apparently sudden appearance of genotypic complexity leading to new body plans and morphological innovations such as organelles and nucleus in eucarya, multicellularity in plants and metazoans, flowers in plants, development of head and jaws in vertebrates, mobility of premaxilla-maxilla in teleosts, placenta in placentals, flexible joints between pterygoid and palatine in neognaths, and plethora of many other innovations in these lineages. Macrogenesis is a causal evolutionary theory that explains macroevolution by increasing genome size, which could be modulated adaptively in response to cellular and organismal needs. I propose acquiring new genomes by macrogenesis is the proximate cause for triggering the macroevolutionary pulse, the origin of a higher clade; its effect or aftermath cascades into two sequential regimes: stabilization and explosive evolution.

It is generally believed that environmental barriers do not play a role in macroevolution where different selection pressures operate on species populations that are in contact along a common border. Mayr (1942, 1963) was the first author to develop a detailed model of the connection between peripatric speciation and macroevolution. He has emphasized that the most rapid evolutionary change does not occur in widespread, populous species, but in small founder populations. Owing to continuing peripatric speciation, there is a steady, highly opportunistic production of new species. In this most isolated peripheral location, two or more related species living in one place could merge or hybridize, transfer genes across species barrier, or change genomes simply by doubling of genes or chromosomes within a species. Living in an entirely different physical as well as biotic environment, such small population may undergo evolutionary bottleneck and would have unique opportunities to enter new niches and to select adaptive pathways. As Mayr (1982) pointed out a drastic reorganization of the gene pool is far more easily accomplished in a small founder population than in widespread, populous species. Some components of macrogenesis such as polyploidy and hybridization are speciation in action where new taxon can form quickly during a human lifetime, as many horticulturists know when they create new hybrids or new varieties. Macrogenesis acts as the founders of

macroevolutionary lineages and plays a principal role in creating species richness and biodiversity.

The widespread occurrence of paleopolyploidy among multicellular organisms suggests that gene duplication is the most prevalent and important mechanism of macrogenesis related to macroevolution, as Ohno (1970) has predicted almost four decades ago. In both plant and animal lineages, large portions of genes were generated by gene duplication. The primary evidence that gene duplication has played a vital role in the evolution of higher taxa is the widespread existence of paralogous gene families in a lineage, often found clustered in a genome (Gregory, 2005). Gene duplication has probably contributed to the evolution of gene networks in such a way that sophisticated expression networks could be established as seen in the follow-up cladogenesis process producing phenotypic diversity and species richness (Wagner, 1994).

2. Phylogenetic fuse—The molecular mechanisms by which macrogenesis act for speciation can be deleterious to many individuals by harmful mutations. Most of them are doomed to rapid extinction but a few may make evolutionary inventions and may be beneficial to a lineage to undergo adaptive radiation. The phylogenetic fuse stage is the incubation period of macroevolution, whether it would succeed or not by natural selection, whether it could speciate or become extinct. This stabilization stage has been identified recently as ‘phylogenetic fuse’ before the event of evolutionary explosions or cladogenesis (Cooper and Fortey, 1998). The immediate results of macrogenesis are often unstable and new genome undergoes a series of stabilizing secondary processes reflecting the cascading consequences of changes in genome size; bulk DNA exerts a strong causative influence on cell size and division rate that would have potential to produce new body plans, increase of body size, phenotypic complexity, and change in developmental and metabolic rate (Gregory, 2005). The environment might change from the earlier condition under which origination took place to a later condition when radiation was favored. However, the ‘fuse’ is within the genome.

The conservation and maintenance of enriched genes acquired by macrogenesis over long evolutionary time periods are crucial for survival of the newly evolved lineage. The new genes must overcome substantial hurdles before they become genetically stabilized by natural selection and reproductively isolated from the parental ones. Any organismal trait that reduces the probability of sterility and extinction will increase in frequency of the founder population. The newly acquired genes occur in an individual can be fixed or lost in a population, similar to a point mutation. Various mechanisms for stabilizing the population with newly acquired genes by macrogenesis agent, especially by gene duplication have been discussed (Hurles, 2004; Zhang, 2003; Gregory, 2005). Fixation within the population results in a minority cases. After fixation, one gene is inactivated (degradation) or assumes a new function (neofunctionalization), or the expression pattern of the original is partitioned between two duplicates as one promoter is silenced in each duplicate in a complementary manner (subfunctionalization) (Fig. 2).

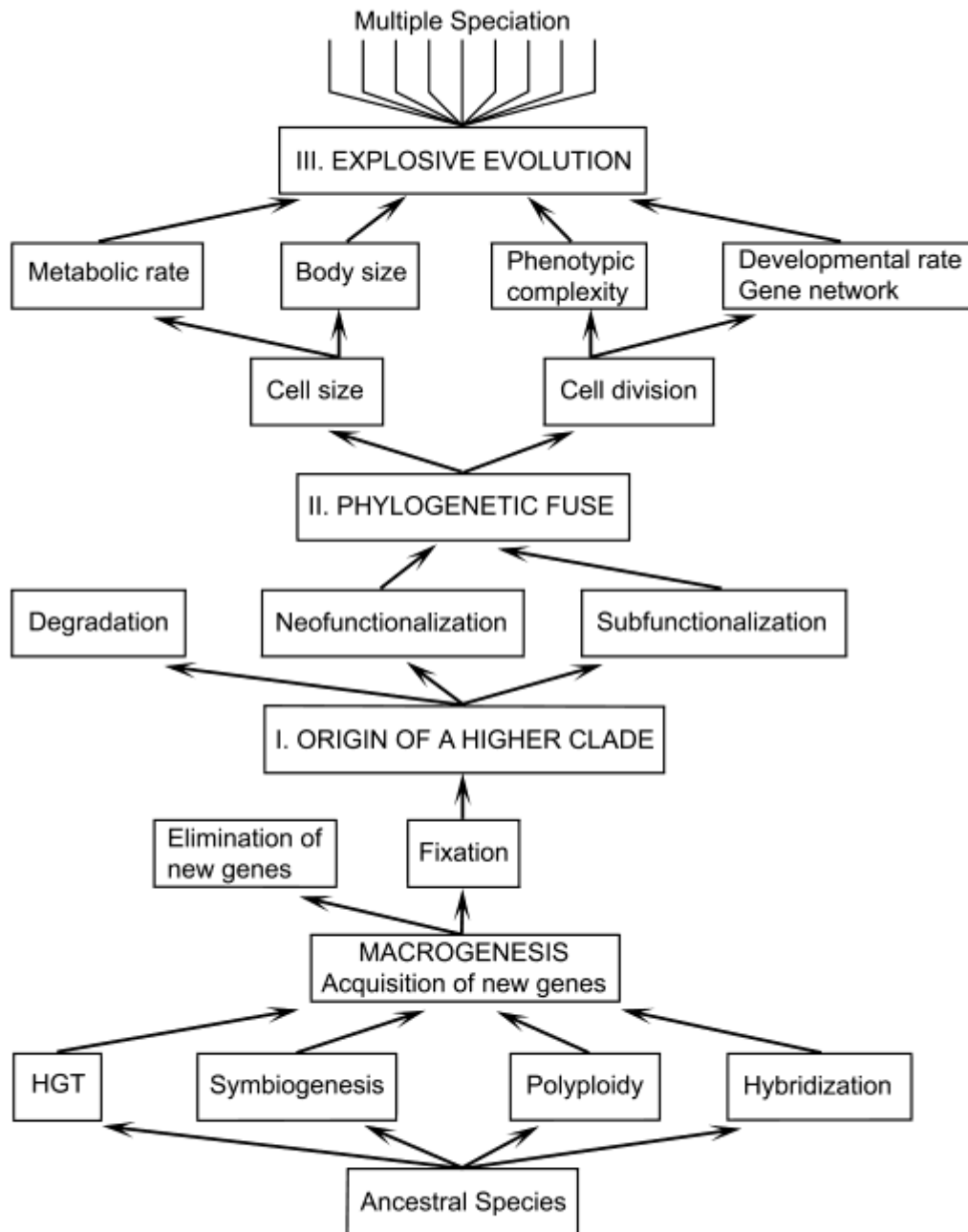


Figure 2. A three-gear engine of macroevolution; the first gear is the enrichment of gene pools by various macrogenesis agents leading to new body plans and origin of higher taxa. The immediate results of macrogenesis are often unstable; the second gear is the secondary stabilization process that influences on cell size and division rate leading to initial diversification. The third gear of macroevolution is cladogenesis; genome size may be linked to one or more organization level such as heterochrony, phenotypic complexity, body size, and metabolic rate. When

an ecological opportunity opens up, intense cladogenesis occurs via smaller genomic ‘aftershocks’ resulting in taxon richness.

Once a new higher taxon is originated by macrogenesis with increasing and expanding gene pools, increasing body size would follow the event of increasing genome. In many taxa, variation in body size is primarily a product of differences in cell number rather than cell size. Macrogenesis provides an internal mechanism for the origin of body plans, morphological innovations with increase in phenotypic complexity.

Delay between the origin of higher taxa and their subsequent cladogenesis suggests increased organizational complexity potential alone was not sufficient to trigger biodiversity. Evolutionary potential will be realized soon after environmental opportunities opened up. In this three-gear engine of macroevolution, macrogenesis triggers the origin of a clade with enrichment of genomes, stabilization or phylogenetic fuse is the adjustment and survival of this potential by natural selection with few sprouting of lineages, but cladogenesis is the implementation of the potential with sudden explosion of adaptive radiation when the right ecology is available. It appears from the fossil record that there is a long time lag between the origin of a higher clade and its subsequent explosive event—a period of stabilization, adaptation, and initial branching of basal clades. For example, the metazoans first appeared during the Late Proterozoic, whereas its explosive evolution took place in the Early Cambrian, almost 100 million years later. Here I list similar time intervals between origin, stabilization, and cladogenesis among all major clades (Table 1, Fig. 1). The time lag could be explained by adaptations for stabilizations as well as availability of environmental opportunity. If the newly acquired genes survive over time, they may acquire novel functions.

Table 1. Estimated Duration of Macroevolutionary Regimes of Selected Major Clades

Rank/Clade	Origin of a Clade	Phylogenetic Fuse	Explosive Evolution
1. Domain: Eucarya	~2.1 By	1900-1300 Ma	1300-720 Ma
2. Kingdom: Metazoa	>635 Ma	635-542 Ma	530-515 Ma
3. Subkingdom: Embryophyta	~470 Ma	470-400 Ma	400-360 Ma
4. Class: Angiosperm	~160 Ma	160-109 Ma	109-65 Ma
5. Subphylum: Craniata	>530 Ma	530-444 Ma	444-360 Ma
6. Infraphylum: Gnathostomata	>430 Ma	430-390 Ma	390-360 Ma
7. Infraclass: Teleostei	>225 Ma	225-100 Ma	100-65 Ma
8. Superorder: Neognathae	~125 Ma	125-65 Ma	65-37 Ma
9. Cohort: Eutheria	~125 Ma	125-65 Ma	65-37 Ma

Paleontological data suggest that the cladistic root of higher taxon is stable for a period of some million years and then explodes into multiple phylogenetic lines. I hypothesize that the initial macrogenesis created a new genome that was stressed to work together. This induced a cellular stress response perhaps identical to heat response. Over expression of these chaperoning proteins allows many others to fold normally whereas otherwise they could not. There is a lot more room for silent genetic change under conditions of Heat Shock Protein (HSP) overexpression. A tremendous amount of silent diversity builds up until the shut-off of HSP expression it becomes available for selection, called ‘Evolutionary Capacitor’ (Queitsch et al., 2002). Thus the macrogenesis events may be a trigger for HSP overexpression and its shutoff

many generations later (millions of years) opens the floodgates of selection on the until-hidden diversity leading to cladogenesis.

For the macroevolutionary event to continue, natural selection has to act on acquired genomes of the newly evolved population in a way that confers genetic continuity of the lineage. How the new taxon formed by macrogenesis is not fully understood among living organisms. Several conditions, particularly among plants, might give us some clue how a new polyploid could form a viable population by self-fertilization, vegetative propagation, higher fitness than the diploid progenitor species, or niche separation from the diploid progenitor species (Schwenk et al., 2008). Selection here can also be internal and will often be, i.e. for genomes that function well and stable. Often, higher gene doses by macrogenesis event may reduce fitness rendering an evolutionary dead end. In some cases, the gene increase may not have any advantages, so that natural selection may not to preserve these newly acquired genes. Sometimes, however, the new set of genes may gain new functions. During the phylogenetic fuse regime, various selection processes would operate for millions of years that would determine whether the final expression of macroevolution, the explosive evolution of a clade would take place or not. Whereas botanists traditionally stressed the diversity-generating potential of macrogenesis (Stebbins, 1974), zoologists traditionally saw it as a process that limit diversification because it reduces fitness and promotes sterility (Mayr, 1963). Some macrogenesis events such as gene duplication might be favorable in many cases because they produce genes that could be altered without disadvantage to the organism (Haldane, 1939, Ohno, 1972). In addition, organisms with multiple copies of genes would be less prone to harmful mutations.

3. Explosive evolution— The third regime of macroevolution is explosive evolution or cladogenesis, which is identified in the fossil record by increases in taxon richness, increase in rate of character acquisition, and accelerated phenotypic evolution. Many new species probably arise via smaller genomic ‘aftershocks’ mediated by heterochrony and regulatory networks. Most likely, high frequency mutator strains are unstable and change rapidly into more stable derivatives (Magnasco and Thaler, 1996). Cladogenesis, the macroevolutionary bursts are consequent to changes in the rate and type of generation of diversity (Fig. 1). Gene duplication contributes in a major way to the evolution of developmentally relevant gene networks, which are key to cladogenesis (Wagner, 1994). Although the potential of cladogenesis begins to develop soon after the macrogenesis event, its timing of triggering may be dependent on availability of the optimum ecology for diversification. It happens when an adaptive zone, a set of similar niches, opens up and the lineage has physical, evolutionary, and ecological access to it.

Establishment of a stable population also requires ecological divergence. After the stabilization has progressed to a stage with two or more incipient species, the third level of a macroevolutionary event would facilitate further adaptive radiation or cladogenesis. A single or small group of newly evolved species rapidly diversifies into a large number of descendant species that occupy a wide variety of ecological niches (Jablonski, 1991). Evolutionary explosion or cladogenesis can occur with the rearranging and resorting of acquired genes by heterochrony, which is achieved by modifications of development and ecological opportunity.

Cladogenesis or evolutionary explosion is mediated by developmental history—heterochrony and expressions of regulatory genes. Once new genes are acquired by macrogenesis, heterochrony can create phylogenetic disparity and phenotypic diversity in a lineage by shuffling and expressing these regulatory genes to change the sequence of developmental timing. This allows large-scale evolution to take advantage of the changes already encoded in embryology

and development, a short-cut route for major phenotypic diversity in a lineage. Heterochrony has long been invoked as an important agent of macroevolution (Gould, 1977, 2002; McKinney and McNamara, 1991). Genetically it could be due to point mutations or to gross chromosomal rearrangements via macrogenesis. There are three basic heterochronic factors—changes in growth rate, changes in offset timing, and changes in onset timing—that vary between ancestor and descendant. Many evolutionary novelties can be explained by heterochrony where slight perturbations in ontogenetic trajectories in ancestor can be amplified through time to produce phenotypic complexity in descendant. Heterochrony does not require a great rearrangement of genome or its enrichment to develop a new body plan, but generates new body plan simply by altering the timing of development. Like new genes, heterochrony provides the raw material for natural selection to work on and operates from micro- to macroevolutionary levels. Identifying heterochrony in the fossil record requires ontogenetic information (growth series) on size and shape of both ancestral and presumed descendant forms. There are many examples in the fossil record where heterochrony might have played important roles in the evolution of higher taxa such as gametophytes, trilobites, cephalochordates, tetrapods, urodeles, anurans, dinosaurs, birds, mammals, and hominins (McNamara, 2001; Chatterjee in press).

Since the homeotic genes organize the body and the number of homeotic genes has increased over time due to gene duplication events, changes in number and function of these genes were responsible for two distinct events in macroevolution as organisms diversified: origin of a higher taxon and its subsequent cladogenesis. Origin of higher taxon is mediated by changes in homeotic gene numbers, whereas its subsequent cladogenesis is controlled by changes in homeotic gene expression.

During evolution, novel phenotypes emerge through changes in gene expression, but the genetic basis is poorly understood. Over the last two decades, it has been shown that many of the differences among organisms are not due the presence or absence of specific genes, but due to changes in the regulation of expression in time and space of these highly regulatory genes (Shubin and Marshall, 2000; Carroll, 2005). Although macrogenesis may be the trigger for macroevolution by enriching the gene pool, homeotic genes may be responsible for cladogenesis and evolutionary explosion. Homeotic genes found nearly in all multicellular organisms regulate the fundamental development of the body plan and how major organ systems develop. The amazing diversity of vertebrates—whether the first tetrapods from the lobe-fin fish, or the first birds from the maniraptoran theropods—is the result of the flexibility of the small number of homeotic genes (especially the *Hox* genes) that appeared in the gnathostomes by gene duplication four hundred million years ago (Holland, 1997; Shubin and Marshall, 2000). The switching of the shifting zones of *Hox* gene expression that eventually gave rise to higher taxa of diverse groups of vertebrates. Thus the intrinsic mechanisms of cladogenesis may be linked to both heterochrony and its genetic basis of the expression of the regulatory genes.

In recent times, a correlation between morphological transformations by heterochrony and the underlying molecular mechanisms by homeotic-mediated activation began to emerge. An increasing number of experiments have shown how shifts in relative timing of onset and offset of particular homeotic genes—genetic heterochrony—may produce significant phenotypic changes such as paired appendages or digit developments during vertebrate limb evolution (Smith, 2003; Sakamoto et al., 2009). This causal linking between molecular genetics of homeotic gene expression with macroevolutionary process provides an underlying mechanism for rate or timing of developmental events in heterochrony (Wagner, 1994). Thus genetic heterochronies can produce phenotypic patterning and major morphological changes. The genotypic homeotic gene

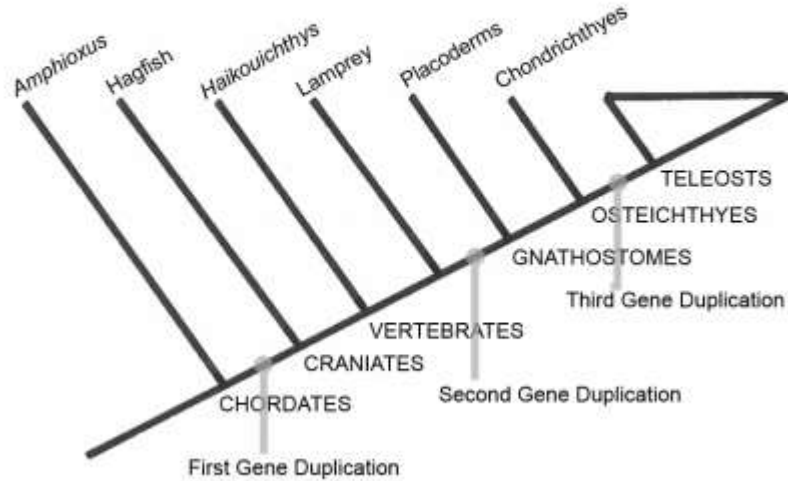
expression and the phenotypic heterochrony appear to be a unified process, a unique mechanism of macroevolution without acquiring new genomes. The intense cladogenesis event of macroevolution soon after the macrogenesis event is mediated by heterochrony and shifting of the coordinates of the tool kit-genes, particularly where the *Hox*-genes are expressed, by genetic switches. The huge number of possible combinations of genetic tool kits acting on vast arrays of evolutionary switches might have triggered the explosion and diversity of life throughout the geologic history (Shubin and Marshall, 2000; Carroll, 2005).

Methodology

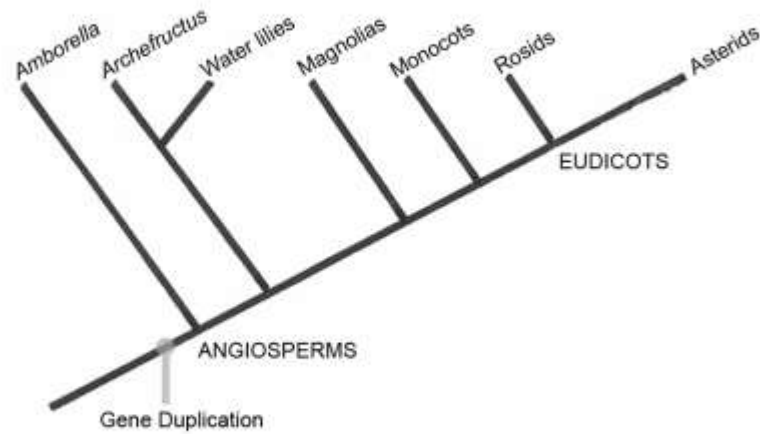
Resolving the timing, pattern, and tempo of macroevolution represents one of the controversial debates in evolutionary biology in recent times. Both the quality of the fossil record and the reliability of molecular clock are focal issues in current debates about the timing of origin of major clades. Molecular data have the potential to provide an independent test of gaps in the fossil record, especially the earliest representatives of taxa in a clade. Few paleontologists agree that the fossil record is inadequate. They argue that where fossils are dense in a lineage, fossil-based estimates are reliable. But they also acknowledge that there are many examples where fossil record is inadequate to reconstruct the macroevolutionary pattern. On the other hand, molecular clock almost always provides older divergence time than fossil-based estimate. Paleontologists criticize molecular clock on the grounds that variation in the rate of molecular evolution could affect the date of estimates. The theoretical foundation originally proposed for the clock, namely the neutrality theory of molecular evolution that predicts that the rate of molecular evolution is constant over time, is found to be untenable and more robust statistics are being employed in recent times. This raises the question of how reliable the molecular clock is (Donoghue and Smith, 2004). Thus both molecular clocks and fossil-based estimates have limitations and both methods should be refined; they should be used concurrently to check and crosscheck the timing and duration of major events in the history of life.

There may be other reasons for conflicts between two processes. When the fossil record and molecular data give conflicting views for calibrating these macroevolutionary events such as placental mammals (Bininda-Emonds et al., 2007), neognathous birds (Copper and Penny, 1997), or angiosperms (Soltis et al., 2009), it is likely that in most cases these three distinct macroevolutionary events—origin of a clade, phylogenetic fuse, and explosive evolution—that span millions of years are lumped together. In reality, the divergence time of molecular clock is actually the timing of the origin of a clade. When we say hominins diverged from chimpanzees about 7 million years ago, it is also the timing of the origin of the hominin clade. The earliest hominin fossil such as 7 million-year-old *Sahelanthropus* from Chad actually supports this assertion. Once we untangle these three major events of macroevolution and identify prolonged periods of phylogenetic fuse separating the timing of the origin of a clade from the explosive evolutionary phase, molecular and fossil data become more congruent and meaningful (Table 1).

Witmer (1997) suggested the Extant Phylogenetic Bracket (EPB) Approach where the living and fossil taxa could be combined in a phylogeny to estimate paleobiological inferences. I have applied EPB approach combining gene and fossil evidence to infer the timing of major macrogenesis events in a clade as shown here in fish and angiosperms (Fig. 3).



A



B

Figure 3. The Extant Phylogenetic Bracket Approach (Witmer, 1997) to reconstruct macrogenesis events. A, Three rounds of gene duplication events in fish; the first round gave rise to craniates with the acquisition of skull, the second round to gnathostomes with jaws, and the third round to teleosts with flexible premaxilla-maxilla. B, macroevolution of angiosperms with gene duplication as revealed by the most primitive extant flowering plant *Amborella*.

Macroevolutionary Pulses in the Fossil Record

Evolution on a large scale unfolds as a succession of dynasties of increasing size and complexities, like much of human history; each dynasty has introduced fundamentally new evolutionary entities. The fossil record provides a unique perspective on the approximate timing and consequences of evolutionary dynasties by macrogenesis. The transitions for the origin of these new clades can be complemented and reconstructed from the study of living counterparts on biological and molecular evidence. Evolutionary novelties of higher taxa are the raw material of evolution, sorted and molded by selection and other forces. Interestingly, some of the dynasties in the history of life—evolution of eucaryan cells, multicellularity of animals, development of skull and jaws in vertebrates, flexibility in the upper jaw in teleosts, the invasion of lands by plants, the origin of angiosperms, the origin of neognathous birds and placental mammals—all were possibly triggered by macrogenesis. Macrogenesis may provide an intrinsic mechanism for macroevolution—the origin of higher taxa during geological time, which had puzzled paleontologists for more than a century. It produces such a rapid chain-effect of origin of higher clades and subsequent explosive evolution that new taxon will soon be drastically discontinuous with its ancestral mutators. The founder population is ideally suited for a rapid ecological shift to invade a new adaptive zone representing both structural and ecological discontinuity from the ancestral groups (Jablonski, 1991).

The sudden appearance of higher taxon in the fossil record implies rapid origination of major clades. The origination is followed by a prolonged period of cryptic evolution, the stabilization or phylogenetic fuse, which in turn is followed by evolutionary explosion—the cladogenesis—often prompted by ecological opportunity. Macroevolution is a time-dependent process and is unidirectional: origin of a clade → phylogenetic fuse → explosive evolution. The originations of these higher taxa by macrogenesis, and their subsequent two phases—phylogenetic fuse and explosive evolution—are discussed below in a temporal context as they appear in the geological record; their appearances in the geologic records are calibrated with the available evidence of molecular evidence to provide a new view of the major events in macroevolution in the history of life.

The range of body forms found across the plant and animal kingdoms are strikingly discontinuous, which is reflected in the appearance of higher taxa in the fossil record. The nine most important dynasties in the history of life include: (1) eucaryans, (2) metazoans, (3) embryophytes, (4) angiosperms, (5) craniates, (6) gnathostomes, (7) teleosts, (8) neognaths, and (9) eutherians. The origins of these nine major events of macroevolutions are possibly triggered by macrogenesis agents and have undergone radical change in developmental mechanism to create explosive evolution and biodiversity. This is a preliminary attempt for combining fossil and molecular data in understanding the mechanisms of macroevolution. As more data are available from genes and fossils, a comprehensive picture of patterns and processes of macroevolution will emerge. Nine major macroevolutionary events in the history of life and their regimes—origin of a clade, phylogenetic fuse, and explosive evolution—are discussed below in a phylogenetic/temporal framework to explain their pattern and process (Fig. 1).

Long-term directional changes appear to pervade the macroevolutionary history that produced organisms of increasing size and complexity through time. The origins of major novelties and higher taxa need to be evaluated in terms of temporal scale and hierarchy (Gould, 2002; Jablonski, 2007). In this study, another macroevolutionary trend of hierarchy at different temporal scale is recognized. An interesting pattern of the evolution of a clade rank begins to

emerge during the macroevolutionary pulses, when plotted against the temporal scale: the highest rank (such as *Domain* Archaea) appears during the Precambrian time, which is followed by relatively higher ranks (*Kingdom* Metazoa, *Subkingdom* Embryophyta, *Subphylum* Craniata, and *Infraphylum* Gnathostomata) during the Paleozoic, then moderately high ranks (*Class* Angiosperm and *Infraclass* Teleostei) during the Mesozoic, and finally the lowest ranks (*Cohort* Eutheria and *Superorder* Neognathae) during the Tertiary radiations (Table 1). This trend of decreasing rank may indicate that phenotype variation decreases through geologic time to elaborate morphological complexity. Ideally, a higher clade includes all descendants of a single progenitor species. These higher clades listed above (from Domain to Superorder, orders, families and genera) are aggregates of species united by a common descent from an ancestor in which the characteristics of the group are first evident.

1. Domain: Eucarya—The oldest eucaryan fossils such as *Grypania* are known from banded iron formations (BIF) in Michigan dated at 2.1 billion years, but have also been described from 1.4 billion-year-old sediments from China and Montana (Han and Runnegar, 1992). These ancient protists had organelles and distinct nuclei indicating their eucaryan affiliation. Molecular clock estimate suggests that the first eucaryans probably evolved around 1600 Ma (Yoon et al., 2004). The temporal appearance of fossil record suggests that eucaryans must have evolved from some prokaryote ancestors, but we do not know the details of how the first eucaryans evolved, because the key events took place in the interior of the cells. The fossil record reveals nothing about how eucaryans evolved from prokaryote ancestors. The answer comes from the symbiotic relationships of some living microbial cells that give some idea of how this transition may have occurred.

A nucleus and other organelles are defining features of eucaryan cells. It is generally believed that the nucleus may have evolved by infoldings of the plasma membrane, whereas other organelles through symbiogenesis. A novel example of acquiring new and entire genomes can be found in eucarya by fusion of two or more microbes, called endosymbiosis (Margulis, 1981). Early in the history of life, cells became food for each other. In some cases engulfed but undigested bacterial cells developed an uneasy partnership with the host cells and lived symbiotically. Over time, the engulfed bacteria and host cells fused together and evolved into a new kind of organism, the eucarya. Margulis (1981) championed the modern version of symbiotic origin for the early eucarya, in which mitochondria and chloroplasts were descended from free-living bacterial cells. By swallowing or being invaded by ancestral bacteria, the original host cell (possibly archaea, similar to thermophiles) became complex organisms with symbiotic merger, allowing them to accomplish far more complex functions. Eucaryan cells thus originated as communities of different kinds of cooperating bacteria that had joined together in a definite order for food or protection with the original host cell: first respiratory bacteria (such as purple nonsulfur bacteria) and then photosynthetic bacteria (such as cyanobacteria) to form mitochondria and chloroplasts, respectively. Finally, the nucleus evolved after the bacteria and archaea merged to form a chimera, the symbiotic ancestor of amitochondriate protists. This bacteria-archaea chimera was invaded by karyomastogont symbiont, consisting of a nucleus, a rhizoplast, and an undulipodium to form an integrated cell that insured the mobility of the eucaryan cell (Margulis et al., 2005). Surprisingly, mitochondria and chloroplasts make proteins with their own set of biochemical pathways, which are different from those used by the rest of the eukaryan cell. Moreover, they can multiply only by dividing into daughters, thus indicating their prokaryote heritage. Such symbiogenesis (Margulis, 1981), the merging of different

organisms into collectives, is a major source of evolutionary change on early Earth. Early first symbiotic mergers of three or more prokaryote cells such as archaea, purple nonsulfur bacteria, and cyanobacteria possibly led to the evolution of complex eukaryan cells. Endosymbiosis unites different species of cells into one organism. The rapid evolution of eucaryans probably reflects their much higher frequency of genetic recombination by meiosis with development of sexuality.

The geological record of eucaryans begins in the Proterozoic rocks well before the emergence of animals, but the identifications of these fossils are difficult and often controversial because of their soft body and microscopic size. Molecular clock estimates commonly suggest an earlier Proterozoic origin and later Proterozoic diversification of eucaryans (Yoon et al., 2004). If we accept the origin of eucaryan cells in the geological record around 2.1 billion years ago (Han and Runnegar, 1992), the phylogenetic fuse stage of stem group of protists probably occurs from 1800-1300 Ma with complex ultrastructure, regular ornamentation, and cylindrical processes. It was followed by explosive evolution stage documenting the divergence of major eucaryan clades such as acritarchs around 1300-720 Ma (Knoll et al., 2006).

Multicellularity

Multicellularity has emerged at least dozen times during the Late Proterozoic from single-celled protists. Plants, animals, and fungi are the most spectacular and complex achievements of this kind. The origin of multicellularity involves two stages: colonial lifestyle of unicellular organisms followed by macrogenesis event such as gene duplication to expand the gene pool. The colonial theory was first proposed by Haeckel in 1874 to explain the multicellularity of organisms. Many of these protists live in colonial life. There are essentially two ways to make multicellular organisms from single cells: either a single cell divides or its offspring stick together, or several solitary cells aggregate together to form a colony. Single-celled organisms divide to form copies of themselves, called clones, which tend to differentiate into a colony, a group of organisms all deriving from the same parent but differing one from another from the location. Cells at the exterior of the clone group have more exposed surface from the interior cells and began to assume different functions. Multicellularity arose by fusion and integration of these colonial organisms, mediated by development, into one multitasking function unit, where distinct types of cells, tissues, and organs can be differentiated each within the complex bodies of multicellular organisms.

The transition from single cell to multicellular life did not evolve in one evolutionary leap. There are four requirements for multicellularity: (1) cohesion among cells, (2) communication between cells, (3) a genetic program to control cell differentiation during development, and finally (4) apoptosis to remove cells that had outlived their usefulness or become a liability (Knoll, 2003, 2009). To form a multicellular organism, the single cells in a colony must be somehow bound together to form an integrated structure. First, they evolve the ability to form loose cooperative communities, called biofilms as protection from environments. Over time these organisms became dependent on each other and merge together to perform multiple functions. The second step in the development of a multicellular organism is the establishment of a pattern of cells that can differentiate along different pathways and more sophisticated method of coordination among them. It would require the increased ability of genomes to regulate and coordinate gene expression. It is likely that during the emergence of multicellularity new method of reproduction with gametes evolved that insured better gene propagation. By collaboration and by division of labor it becomes possible by multicellular

organisms to exploit resources that no single cell could utilize so well. The third step would be to coordinate the activity of cells by sending messages to each other. They also had to cooperate to develop their body plan. Finally apoptosis, a form of cell death develops, in which a programmed sequence of events leads to elimination of old cells, unnecessary cells, and unhealthy cells without releasing harmful substances into the surrounding area.

In reconstructing the origin of multicellularity, one can imagine a ground state in the form of a colony of identical cells. Most colonies of single-celled organisms are clonal, i.e. derived from a single cell whose daughters stick together. This process does not increase the gene pool. One way to increase diversity in multicellular organisms is gene duplication. The search for genetic changes that accompanied the origin of multicellularity is still speculative. It is believed that key ancestral gene duplication occurred near the base of both plants (Becker and Martin, 2009) and animals (Holland, 1998) that led to multicellularity of both lineages. These increases in the gene complexity at the basal multicellular organisms are the product of evolutionary innovation, tinkering, and expansion of genetic material.

There appears to be two-stage increase in the size of multicellular organisms during the Late Proterozoic-Early Paleozoic eras: first, the primitive microscopic stage formed by macrogenesis (polyploidy) to form the multicellular cells; and second, more derived megascopic or 'metacellularity' stage (McMenamin, 1998) to make the organisms large. The pattern and timing of the rise of microscopic multicellular life is not well established in the fossil record, but the radiation of megascopic multicellular organisms is better constrained from the fossil record. Metazoans appeared first in the fossil record, followed by fungi, and the embryophytes.

It is generally believed that multicellularity evolved independently around 900 million years ago along shorelines of Rodinia that would give rise to land plants, fungi, and metazoans respectively. Metazoans were first to radiate along the coastal regions soon after the warming the seas around 600 million years ago soon after the Varanger deglaciation. Sudden bloom of oxygen and the ability to extract it by mitochondria were key factors in the rise of megascopic size in metazoans. Since large Vendian metazoans maintained similar shallow marine habitats from their microscopic ancestors, the major changes were centered on warmer climatic and availability of oxygen. Large size enabled metazoans to swim and crawl faster toward food and away from danger (Knoll, 2003).

Plants and fungi, on the other hand, faced adaptive threshold as they moved from water to air that involved new structures and new ways of life. Megascopic size allowed plants to become physically large, to have roots in the ground, to have leaves in the air, to develop vascular system for transporting water and nutrients between the roots and the leaves. Because of this adaptive threshold barrier, the proliferation of fungi and plants would occur much later than the origin of their microscopic multicellularity stage, perhaps during the Silurian Period with the formation of the ozone layer when land was safe for colonization.

An intrinsic genetic system is developed in multicellular organisms to arrange cells in three-dimensional space and to specify their fate accordingly. The genes responsible for coding this information are called homeotic loci. In plants, the most important loci belong to a family called the *MADS*-box genes. In animals, the key homeotic loci are called the *Hox* genes (Carroll, 2005). These homeotic loci were responsible for the subsequent cladogenesis of land plants and metazoans. Two major but independent macroevolutionary events of multicellular organisms include metazoans and embryophytes.

2. Kingdom: Metazoa—The origin of metazoans—the multicellular animals—represents one of the pivotal transitions in life’s history. Darwin (1869) was puzzled by the seemingly sudden appearance of animals in the Early Cambrian fossil record, beginning some 543 million years ago, and considered it a major challenge to his theory, because he believed that evolution was a slow, gradual process. He argued intuitively that the Cambrian explosion was preceded by a lengthy period of gradual evolution for which the geological record is missing. Recent fossil and molecular evidence has finally come to Darwin’s defense especially with the discovery of Ediacaran fauna, which followed the end of the second Cryogenian ice ages around 635 million years ago. The Ediacaran fauna represents a long Proterozoic phylogenetic fuse or stabilization process before the Cambrian explosion, an extended period of metazoan diversification (Cooper and Fortey, 1998). The phylogenetic fuse is a genome that is poised at a high level of potential energy, trapped in local minima of a glassy state.

The possible mode of the origin of metazoans draws heavily on the comparative study of existing animals and their protozoan relatives. Colonial lifestyle of protists and endosymbiosis appear to be major driving forces in the origin of multicellularity in animals from colonial protozoans. The mechanisms by which some recent protozoans such as choanoflagellates form colonies, establish cell polarity, and reproduce provide crucial insights into the transition to multicellularity in metazoans. Haeckel (1866) suggested that the earliest metazoans must have been microscopic organisms similar in development and morphology to the embryos of modern animals. Because all animals start as a single fertilized cell that divides and multiplies, Haeckel argued, some unicellular organisms divided and formed a group of cells that did not disperse, but clustered together to form a colony. Division and adhesion is characteristic of multicellular forms of aquatic origin. Eventually, some cells in the colony became specialized to perform specific functions, such as reproduction, respiration, and food gathering. Molecular data indicate that the single-celled protozoans, such as choanoflagellates, appeared about one billion years ago and are the closest relatives to animals (Gregory, 2005). The modern choanoflagellates live in the oceans, some solitary, and some in colonies. They reproduce by budding off new individuals, which then stay together to form a compound animal or colony rooted to the seafloor. The colonial choanoflagellates illustrate a possible route from single-celled protozoans through clusters of independent cells to metazoans made of interdependent cells. Each choanoflagellate cell carries a flagellum (or tail) at one end that beats to generate a feeding current that enables the organisms to capture bacteria from water and ingest as food. Choanoflagellates also possess genes that make proteins essential to multicellular life. This suggests that one-celled organisms were genetically equipped for making animals. *Sphaerocea*, a modern spherical colony of thousands of individual choanoflagellates, may be similar to that of the earliest evolutionary link between the protists and animals. The cells of this spherical colony come into close contact and form occluding junctions between them similar to some animal cells, but each cell is functionally independent of the others. From this body plan, the crucial next stage in the development of multicellularity would have been the infolding of the outer surface to form a hollow cup, the blastula—thus creating a protoanimal that is fully integrated and mimics the embryonic stage of a sponge. *Trichoplax* a tiny ameba-like marine animal that in its adult form resembles a flattened blastula may be similar to the earliest animals of the Late Proterozoic (Margulis and Dolan, 2002). A sponge is a simple multicellular variation on this theme that indicates the next level of organization. The ‘collar cell’ or choanocyst of a modern sponge functions very similarly to the single cell of a choanoflagellate. Likely, the ancestors of animals would mimic the embryonic stage of a sponge-like organism that represents the

minimalist design of an animal. Eventually the colonial choanoflagellate-like protozoans possibly underwent gene duplication to evolve into multicellular animals (Holland, 1998). Sponges, the basal metazoans evolved by extending the choanoflagellate way of life to large size and sophisticated packaging.

Metazoans probably evolved sometime in the Late Proterozoic, but these microscopic metazoans are not preserved in the fossil record. Animals certainly diversified and enlarged during the Ediacaran time (635-542 Ma) with an excellent fossil record all over the world. With the help of the fossil record, we can reconstruct three distinct levels of macroevolution of early metazoans: (1) the punctuation origin before the Marinoan glaciation (>635 Ma); (2) the phylogenetic fuse phase during the Ediacaran period (635-542 Ma); and the (3) cladogenesis during the Cambrian explosion ((530-515 Ma). Molecular fossils found in an oil field in Oman are the oldest evidence yet of animals, pushing back the rise of the metazoans before the Marinoan glaciation (>635 Ma) (Love et al., 2009). We can proxy this time for the punctuation event, the first level of a macroevolutionary process that led to the origination of metazoans.

The second regime of metazoan macroevolution is represented by the Ediacaran fauna (635-542 Ma), the oldest known large animals preserved in the fossil record. This is the phylogenetic fuse (Cooper and Fortey, 1998) when animals began to experiment in various body plans and became large by ‘metacellularity,’ by clonal and colonial development characteristics of the larger protozoans today (McMenamin, 1998). Some Ediacaran animals such as vendobionts are puzzling and their affinity remains poorly resolved; others can be allocated to basal groups of metazoans such as sponges, corals, mollusks, annelids, and arthropods (Narbonne, 1998).

The Cambrian explosion represents the third level of metazoan macroevolution in the form of extensive cladogenesis. Once metazoans first appeared in the Ediacaran fauna, they underwent extensive cladogenesis in the Early Cambrian period as documented by the Chengjiang fauna of China (530 Ma) and the Burgess Shale fauna (515 Ma) of Canada. Cambrian explosion documents the greatest elaboration of evolutionary novelties that has ever occurred in the history of life and includes shelly bilaterians such as arthropods, mollusks, brachiopods, and echinoderms, as well as fish-like chordates and vertebrates (Conway Morris, 1998; Hou et al., 2004). The appearance of such a large range of body plans and life strategies at the base of the Cambrian in an apparently short space of geological time has intrigued paleontologists for many decades. Both intrinsic and extrinsic triggers have been proposed to explain the sudden explosion of metazoans. The genetic framework for the differentiation of many cell types and the development of complex body plans that started during the Ediacaran reached their acme during the Early Cambrian. It is generally believed that the evolution of the developmental control mechanisms that included the genetic tool kit—the *Hox* gene cluster—was a key ingredient in the Cambrian explosion that led to an assortment of new body plans (Valentine et al., 1996; Carroll, 2005). Shifting zones of *Hox* gene expression is one of the mechanisms that contributed to an increase in the number of cell types in Cambrian animals that likewise led to the development of more and more complex body plans.

Cladogenesis of metazoans at the base of the Cambrian was ecologically driven. The breakup of Rodinia into smaller landmasses produced an increase in favorable habitats such as continental shelf edges and shallow seas for proliferation of metazoans. Other ecological factors might be the acquisition of biomineralized skeletons and the origination of complex life strategies such as predation (Conway Morris, 1998). It took almost 100 millions for early metazoans to exploit the ecological opportunities and diversify. There is a time lag between three events of macroevolution of metazoans: the origination of metazoans (> 635 Ma), its

stabilizing phase during the Ediacaran (635-542 Ma), and its cladogenesis at Early Cambrian (530-515 Ma).

3. Subkingdom: Embryophyta—The origin and early evolution of land plants (embryophytes) in the mid-Paleozoic era was one of the important events in biological evolution. Macroevolutions and adaptive radiations among land plants occurred at several taxonomic levels in different geologic time. Two of the most notable ones were unique events in the history of life, one in Paleozoic, and the other in Mesozoic, similar to the Cambrian explosion of animal diversity. The first was the radiation of terrestrial plants from aquatic ancestors in the Early Devonian, about 400 Ma. The second radiation of plant evolution was the Cretaceous explosion of flowering plants about 80 Ma. Ancient polyploidy such as gene duplication probably triggered both these events of macroevolution.

The fossil record suggests that single-celled algae termed acritarchs become increasingly conspicuous around Proterozoic rocks. The earliest known multicelled alga, *Bangiomorpha*, lived 1.2 billion years ago in Canada and provides a key datum point for constraining complex multicellularity in the fossil record (Butterfield, 2000). Differential spore/gamete formation certainly makes *Bangiomorpha* one of the earliest practitioners of sexual reproduction. Plants colonized land near the close of the Silurian period when the ozone shield was in place that protected the land from deadly ultraviolet light from the Sun. If *Bangiomorpha* were truly the first multicellular algae that evolved 1.2 billion years ago, it took almost 800 million years for embryophytes to become large and colonize land. Apparently this transition from water to land was an extremely difficult one for plants until the right ecological opportunity opened up.

The origin of land plants from unicellular freshwater algae such as Chlorophyta (green algae) can be reconstructed from the combined evidence of fossil record and developmental study of living groups of algae. Some terrestrial multicellular algae such as *Coleochaete* bear morphological similarities to a fossil plant *Parka* from the Early Devonian deposits of Scotland (Taylor and Taylor, 1999). *Parka* may be a relic of ancient progenitors of land plants. Most likely the earliest land plants had a fusion origin by endosymbiosis when a colony of unicellular algae lived together by symbiotic association, became sessile, and began to follow an evolutionary direction toward higher plants.

Green algae (phylum Chlorophyta) are of special interest because they are believed to be ancestors of plants. They share with plants photosynthetic pigments, cell wall structure, and starch. From unicellular green algae like *Chlamydomonas*, one can reconstruct how the early multicellular land plants might have originated from fusion evolution by three successive stages: (1) the formation of colonial chlorophytes such as *Volvox* from symbiotic association of unicellular algae such as *Chlamydomonas*; some species of *Volvox* have developed a true ‘division of labors’ among different types of cells, a functional precursor to multicellularity; (2) the repeated division of nuclei with no cytoplasmic division, as seen in multinucleate filaments of *Bryopsis*; and (3) the formation of true multicellular forms, as in *Ulva* or sea lettuce that has developed an alternation of generations in which the gametophytes and sporophytes resemble one another closely (Campbell, 1996). It is likely that multicellular plants evolved from colonial unicellular algae such as *Volvox* by gene duplication to increase the gene pool (Becker and Martin, 2009).

The first phase of macroevolution of land plants, the origin, is represented by the emergence of non-vascular plants or bryophytes with the development of embryos that allowed the descendants of green algae to adapt life on land. These early plants were small and soft,

confined to moist environments. They possibly lacked morphological differentiation of roots, stems, and leaves, and were ecologically restricted to moist, fresh water environments. The earliest records of embryophytes are known mainly from the spore assemblages in the form of microfossils representing the durable components such as tetrahedral reproductive spores of non-vascular plants or bryophytes. These spores have been recovered from the Middle Ordovician to Early Silurian deposits (470 to 430 Ma) in many parts of the world. They resemble to those of living mosses, liverworts, and hornworts, suggesting that plants invaded land at this time. Reproduction in these groups had to occur in a film of water, restricting these groups to moist, marginal aquatic habitats (Kenrick and Crane, 1997). In contrast, molecular clock estimate for the origin of land plant is much older, around 700 Ma (Wang et al., 1999), a time when land remained virtually uninhabited without any sign of colonization. This is why fossil-based estimate for the origin of embryophytes is preferred here. Currently the most reliable estimate for the time of origin of land plant remains the minimum estimate of Middle Ordovician (470 Ma) provided by the early land plant dispersed microfossil record. Recent genomic study reveals that the origin of land plants from charaophycean ancestors is marked by gene duplication (Peterson et al., 2009). The timing of the origin of embryophytes by macrogenesis event could be calibrated from the fossil record around at 470 Ma and that represents the first regime of macroevolution of embryophytes.

The second phase of macroevolution of embryophytes is represented by the stabilization of early tracheophytes or vascular plants on land with increased body size. The fossil record for early land plant megafossils is relatively poor and highly biased. Unequivocal land plant megafossils of tracheophyte affinity are first recognized in the fossil record from the mid-Silurian through Early Devonian (430 to 400 Ma) sediments of Europe, Bolivia, Australia, and China (Kenrick and Crane, 1997). A major adaptive breakthrough for life on land, before the evolution of roots and leaves, was the origin of vascular tissue because it allowed the transport of food and water. These early tracheophytes lived in low, wet, marshy bogs of fresh water with underground rhizome, but they had managed to raise their stem above the water into an upright position. Some of these plants began to develop branched leaves and sporangia. One of the oldest known vascular plants is *Baragwanathia* from the Upper Silurian Victoria, Australia. The plant is about 2.5 cm in diameter. This phylogenetic fuse phase is represented in the fossil record the Rhynie Chert Beds of Scotland, a Middle Devonian hot spring bog (Kenrick and Crane, 1997). The timing of phylogenetic fuse and early diversification of land plants as calibrated from the fossil record ranges from 470 Ma to 400 Ma.

The third phase of macroevolution of land plants, the cladogenesis took place during the Late Devonian (400 to 360 Ma). Tracheophytes did so by evolving a variety of structural adaptations, which include the alteration of generations with gametophytes and sporophytes, female and male sexual organs, a cuticle, desiccation resistant haploid spores, development of vascular tissues for the transport of food and water, and roots to collect water and nutrients from the soil and to help anchor the plant in the ground (Kenrick and Crane, 1997). Plant size increased sufficiently to assume tree size. Tracheophytes began to diversify rapidly during Late Devonian and expand in well-drained habitats leading to all major plant groups such as lycophytes, trimerophytes, horsetails, ferns, progymnosperms, and seed ferns. These plants carpeted the soil, gripped it with their roots, and grew to tree proportions, The appearance of seed, another innovation in reproduction, liberated tracheophytes from their dependence on moist conditions and allowed them to invade drier habitats. Many of these woody trees became tall with large lateral branches to form forest canopy for the first time over the landscape.

4. Class: Angiosperm—The second wave of macroevolution of land plants occurred during the origin and explosive evolution of angiosperms that have dominated the terrestrial ecosystems since the Cretaceous. Stebbins (1974) recognized that hybridization and polyploidy played important roles in the origin of diversification of flowering plants. It is generally believed that 100% of angiosperms have polyploidy in their ancestry. The single characteristic that seems to distinguish angiosperms from gymnosperms is double fertilization and the consequent development of a polyploidy endosperm. In an attempt to estimate the timing of angiosperm origin various authors have used a different molecular data. Analysis of the complete genome sequence of *Arabidopsis* suggested two ancient polyploidy events during the early origin and diversification of angiosperms. The first genome duplication event appears to have occurred during the Late Jurassic or Early Cretaceous (180-140 Ma), a period during which the angiosperms originated and rose to ecological dominance; the second round of gene took place during the Late Cretaceous (109-66 Ma) leading to extensive cladogenesis (Soltis et al., 2009). These two whole-genome duplication events in early angiosperms were crucial for the creation of many developmental and regulatory genes found in extant angiosperm genomes.

The earliest angiosperm fossil *Archaeofructus* comes from the Early Cretaceous (~125 Ma) Liaoning deposits of China. It is a water-dwelling weed with no petals but shows the earliest development of closed carpel with seeds inside (Sun et al., 2002). *Archaeofructus* is placed in water lilies, the basal group of angiosperms indicating their aquatic origin. Thus there appears to be a discrepancy between the molecular clock and fossil evidence for the origin of angiosperms; molecular estimates (180-140 Ma) are older than the oldest fossils 125 Ma), suggesting that older fossil remain to be discovered from the Late Jurassic-Early Cretaceous interval in future to make both estimates compatible (Soltis et al., 2009). The origin of angiosperms may be older than our current fossil-based estimates indicate and is calibrated around 160 Ma averaging molecular estimates.

Besides gene duplication, other macrogenesis agents such as HGT were also common during the early evolution of flowering plants. With the help of DNA sequences, a rare tropical shrub called *Amborella* appears to be a basal group of angiosperm. Found only on New Caledonia, an island in the South Pacific, *Amborella*, a diminutive plant with creamy flowers and red fruit, had gone unnoticed by most botanists. Recent study suggests that its reproductive structure, the embryo sac represents a transitional stage between gymnosperms and angiosperms. It has been suggested that widespread horizontal transfer of mitochondrial genes from diverse plant donors to the basal flowering plant *Amborella* and other angiosperms might have led to their origin and diversity (Bergthorsson et al., 2003, 2004). It is speculated that about 135 million years ago, non-flowering plants—perhaps similar to today's pines—gave rise to *Amborella*-like flowering plants.

Once the angiosperm clade was established in the Early Cretaceous, it began to stabilize during the mid-Cretaceous time with initial radiation of some basal lineages. The second phase of angiosperm macroevolution, the stabilization event is documented in the fossil record fossils in the Potomac Group of Maryland (~100 Ma). By the end of the Cretaceous (~70 Ma), angiosperms underwent extensive cladogenesis, when most flowering plant families had evolved that had replaced conifers and ferns as the dominant terrestrial plants. The third phase of macroevolution of angiosperms, the explosive evolution might be triggered by the second round of gene duplication around that time (109-66 Ma) (Soltis et al., 2009) that gave the angiosperm genome opportunities to try out new floral shapes, new chemical attractants, and to exploit new

niches (Fig. 3B). This radiation produced approximately 97% of all angiosperm species.

Regulatory genes have also played important roles in producing the variety of flowers—the hallmark trait of angiosperms—as well as species diversity. Flower morphology depends on the expression of homeotic genes such as *MADS*-box gene family; these loci specify which floral organs appear in different locations and each locus includes a DNA-binding region called *MADS* domain (Coen, 1999). Not only the regulatory genes, but also the symbiotic relations between insects and angiosperms have contributed enormously to their success and speciation. As the Cretaceous ended with mass extinction, major groups of gymnosperms suffered at this crisis, but angiosperms not only survived, they rebounded from the crisis and increased their diversity in each succeeding period of the Cenozoic down to the present day. Polyploidization may have contributed to the survival and propagation of angiosperms during or following the KT extinction event because additional copies of the genomes such plants possessed allowed them to more readily adapt to the changing catastrophic environmental conditions (Fawcett et al., 2009). Today angiosperms are the most dominant and species-rich plant group in the history of life, comprising about 350,000 living species.

Expansion of *Hox*-clusters in Vertebrate Evolution

The most primitive vertebrates are fish, and some of the earliest fish remains are found in Early Cambrian (~530 Ma) during the Cambrian explosion that produced most groups of multicellular animals. Molecular biological studies have shown that the cephalochordate such as amphioxus (or lancelet) with a distinctive notochord is the closest living invertebrate relative of vertebrates. The next step above the amphioxus in complexity is represented by the craniates such as hagfishes, with a well-defined head and a rudimentary brain. Lampreys, though superficially similar to hagfishes, are more advanced than craniates and generally regarded as the stem vertebrates that have developed segmented cartilages protecting notochord. Hagfishes and lampreys are the only living groups of jawless craniates that provide critical insights into early vertebrate evolution. They are but a tiny relict of a huge radiation of armored jawless vertebrates, called ostracoderms that flourished in the Paleozoic.

The earliest known fossils of the jawless fish come from the Early Cambrian Chengjiang fauna of China (~530 Ma). These small and delicate soft-bodied fish show early stages of vertebrate phylogeny like their living counterparts: *Cathaymyrus* probably represented a cephalochordate similar to amphioxus, *Myllokunmingia* was an early craniate like hagfishes, and *Haikouichthys* is identified as a stem vertebrate like the living lampreys (Hou et al., 2004).

Four decades ago, Ohno (1970) proposed that great leaps in early vertebrate evolution—such as transition from invertebrate to vertebrate— could occur only if whole genomes were duplicated. He predicted that duplication of regulatory genes and their control regions provided the raw material for macroevolution. There are two ways in which gene duplication could generate macroevolution. Either one member of the duplicate gene pair could take on new function, or two duplicate genes could divide the multiple functions of ancestral gene between them. Ohno focused on the importance of gene redundancy in allowing forbidden mutations to occur that could impart to new functions in proteins. Like Goldschmidt, Ohno was also discredited for his iconoclastic proposal. Recent study of developmental genes tends to support Ohno's gene duplication theory. Recent synthesis suggests that three distinct pulses of gene duplication in the metazoan evolution took place during the evolution of fish leading to morphological innovation and new body plans (Holland, 1997). *Hox* genes are the molecular architects for the animal body plan and are expressed in different regions of developing embryo.

Duplication of *Hox* genes and their developmental regulatory networks triggered these macroevolutionary pulses. Fish show the most extensive polyploidy among vertebrates. Fish evolution has been punctuated by three episodes of widespread gene or genome duplication leading to innovations of new structures, body plans, and phenotype complexity: the craniates with a head, the gnathostomes with jaws, and the teleosts with flexible premaxilla-maxilla joint along with acquisition of myriads of new characters (Fig. 3A). These events can be calibrated by molecular phylogeny and fossil record. Each pulse of macroevolution was followed by extensive cladogenesis leading to species diversity. The first major macrogenesis stabilizes then pulses of lesser macrogenesis lead to the diversification of new species. Three major macroevolutionary events in fish include craniates, gnathostomes, and teleosts.

5. Subphylum: Craniata—In chordates such as in amphioxus, the dozen or so *Hox* genes are clustered on a single chromosome. In contrast, the *Hox* genes in gnathostomes such as sharks, bony fish, amphibians, reptiles, birds, and mammals are arranged as four separate clusters, each located on a different chromosome (Holland, 1997; Holland and Garcia-Fernández, 1996). Three levels of macroevolution can be identified in the macroevolution of craniates. The first round of gene duplication led to the development of the head in the craniates such as hagfish or lampreys. With the discovery of Chengjiang biota, this first round gene duplication could be constrained around 530 Ma, which is congruent with molecular estimates (Hedges, 2001). The origin of craniates is considered here around 530 Ma.

After the initial gene doubling, the phylogenetic fuse continued throughout the rest of the Cambrian and Ordovician (530-444 Ma); first set of genes went on performing its original role, while the duplicate set was co-opted to evolve new novelties, such as the development of a head. With the acquisition of head, the agnathans underwent an explosive evolution mediated by the *Hox*-genes (Holland, 1997), representing the third phase of macroevolution; it is exemplified by the diverse fossils of ostracoderms represented by more than 600 species during the Silurian and Devonian periods (444-359 Ma).

6. Infraphylum: Gnathostomata—The evolutionary history of fish continued with innovation of a new body plan—the appearance of jaws as seen in heavily armored placoderms. The evolution of jaws was a major advance among primitive vertebrates. Gnathostomes—the jawed vertebrates—could chew food and become active predators, thus opening new ecological niches. Gnathostomes include extinct placoderms, as well as sharks, bony fish, and tetrapods. It is generally believed that the second round of gene duplication took place during the origin of gnathostomes such as placoderms, with the innovation of jaws and many other features (Holland and Garcia-Fernández, 1996). Like the origin of craniates, three levels of macroevolution are apparent in the origin of gnathostomes. The appearance of the first placoderms such as *Silurolepis* at the beginning of Silurian Period (~430 Ma) might be used as the minimum age for the origin of jaws, the tentative timing for the second round of gene duplication. Molecular estimate for the origin of gnathostome is much older, even before the Cambrian (~559 Ma) (Kumar and Hedges, 1998). Thus the fossil-based estimate for the origin of gnathostomes (~430 Ma) is preferred here.

The origin of gnathostome was followed by a period of stabilization when the early placoderms began to diversify in Early Devonian (430-390 Ma). They underwent an extraordinary cladogenesis during the Late Devonian (390-360 Ma) that allowed them to conquer

every aquatic ecological niche. They suddenly dwindled at the close of the Devonian Period and became extinct in the Early Carboniferous.

These two events of gene duplications have shaped the genomes of all vertebrates. Genetic complexity apparently reached a plateau after jaws appeared in early fishes. Since then, four *Hox* clusters have remained essentially stable in all gnathostomes including sharks, bony fish, amphibians, reptiles, birds, and mammals (Holland, 1997). Thus, more genes are not the crucial factor for the subsequent evolution of higher vertebrates from early gnathostomes, but shifting, shuffling, and networking of existing genes that gave rise to new body plans (Shubin et al., 2009).

7. Infraclass: Teleostei—A third round of fish-specific gene duplication took place in the Late Permian (~320 Ma) in actinopterygians lineages before the diversification of teleosts (Vandepoele et al., 2004). With almost 24,000 species, teleosts are the most diverse and successful groups of vertebrates and their phenotypic complexity and taxon richness have all been linked to gene duplication. The origin of teleosts shows three levels of macroevolution. The earliest known teleosts are the Late Triassic (~225 Ma) pholidophorids representing the initial radiation of basal groups. This leaves a long gap between the molecular estimates (320 Ma) to the oldest fossils confidently assigned to teleosts. We choose fossil-based estimate for the origin of teleosts.

The stabilization process, the phylogenetic fuse of teleosts, spans from the Late Triassic to Early Cretaceous (225 to 100 Ma), when several basal groups of teleosts began to diversify. It was followed by explosive evolution in the Late Cretaceous (~100 to 65 Ma) when they replaced the holosteans. Teleosts diversified throughout the Cenozoic and are the most successful group of vertebrates today. The key innovation of teleosts that led to their success lies in the flexible joint between premaxilla and maxilla that allows them to capture and manipulate prey more efficiently. Other features include symmetrical tails, overlapping scales, specialized fins, and short jaws. *Hox* genes mediated the phenotypic complexity of teleosts after cladogenesis (Gregory, 2005). Many groups of teleosts such as salmonids, catostomids, and cyprinids show evidence of more recent evidence polyploidy, but their long-time effects are unknown.

Tetrapods

Vertebrates reached an evolutionary milestone in the Late Devonian (~400 Ma) with the first appearance of tetrapods that used limbs instead of fins to propel their body forward, an adaptation that eventually would lead them to conquer the continents and to open up a new terrestrial realm. The transition from water to land required major structural changes in the tetrapod evolution such as the modification of paired lobefins of rhipidistian fish into early tetrapod limbs during the Late Devonian, which are represented by excellent intermediate fossils (Shubin et al., 2009). The tetrapod appendages that evolved gradually from rhipidistian fish are attributed to the modification of regulatory circuits of *Hox*-genes (Carroll, 2005; Shubin et al., 2009). *Hox* genes in developing embryos retooled fins into the earliest limbs that could walk on land.

Once the first tetrapods evolved, they radiated into a great variety of sizes, shapes, and ways of life, continually modifying and improving their locomotory skill. Later this innovation of limb was modified into an enormous variety of shapes and sizes, with functions ranging from running, climbing, flying, digging, and swimming. Changes in the timing or level of expression in the pattern-forming genes in the *Hox* complex are responsible for the adaptive changes of

limbs (Shubin et al., 2009). Among living amphibians such as frogs and salamanders, polyploidy is common but the time of acquisition of ancient genomes in early tetrapod evolution is poorly understood (Gregory, 2005). Very little is known about macrogenesis event during the fish-tetrapod transition, but the adaptive radiation of tetrapods into about 40 families in the Carboniferous by cladogenesis is well known in the fossil record. Two major macroevolutionary events within tetrapods include neognathous birds and eutherian mammals.

8. Superorder: Neognathae—Neognaths are the dominant flying birds around us consisting of more than 9,500 species, which are characterized by specialized palatal structure showing a flexible joint between the palatine and the pterygoid, and a modified ankle structure where the ascending process runs up in front of the tibia (Chatterjee, 1997). Estimates for the timing of origin of neognathous birds have been based directly on the known fossil record or on molecular clocks. The most complete neognaths fossils include a loon-like *Polarornis* (Chatterjee, 2002), and a duck-like *Vegavis* (Clarke et al., 2007), both recovered from the latest Cretaceous sediments (~65 Ma) of Antarctica just below the KT boundary. Molecular studies of birds (Cooper and Penny, 1997) suggest somewhat earlier date for the origination of neognaths around Early Cretaceous (~125 Ma). Part of the discrepancy may result from the lack of definite neognathous fossil record in the Early Cretaceous, although fragmentary remains are known, which may fill up the gap between 125 and 65 Ma. If we calibrate the timing of the origin of neognaths is around 125 Ma on the basis of molecular clock, the phylogenetic fuse, the second level of macroevolution for the neognaths, might have ranged from 125 to 65 Ma during the Cretaceous period, when some basal groups began to radiate. The most dramatic examples of ecologically driven cladogenesis include the adaptive radiations of neognathous birds in the Early Tertiary (65-37 Ma), the third level of macroevolution. The KT extinction may have been an important factor for the radiation of neognaths, which occupied the vacant niches of pterosaurs. The basal lineages of neognaths survived the KT boundary and rebounded in the Early Tertiary. The adaptive radiation of neognaths in the Tertiary ecosystem was composed of a variety of wing designs and was probably mediated by the timing or level of expression in the pattern forming genes or sequences in the *Hox*-gene complex (Carroll, 2005). Both fossil record and genetic study reveals that morphological and chromosomal evolution of birds has considerably slowed down since the Miocene (Prager and Wilson, 1975).

Did any of the macrogenesis events such as polyploidy or interspecific hybridization trigger the origin of neognaths during the Cretaceous? Recent work suggests that hybridization is widespread in birds; about 10% of non-marine species of birds regularly hybridize (Grant and Grant, 1992). I speculate that the origin of neognaths is linked to an ancient macrogenesis event, probably hybridization during the Cretaceous, although the molecular evidence to support this view is not very robust. I predict this macrogenesis event from the phylogenetic signature of the cladogenesis of neognaths in the Early Tertiary. Hybridization, though a common mode of speciation in plants, is also increasingly implicated in the origin of some animal species such as *Lonicera* fly, butterfly, minnow, birds, and mammals (Schwenk et al., 2008). Hybridization (allopolyploidy) between species can allow alleles from one genetic background to integrate into another to form new gene pools if favored by selection and a new lineage, thus providing a key to quantum evolution among certain animal species. It confers several evolutionary advantages and represents a source of novel evolutionary trajectories. The extra genomes are thought to provide a buffering effect for deleterious alleles and gene mutations.

9. Cohort: Eutheria—The origin and diversification of eutherian (or placental) mammals mimic the history of neognaths and shows three levels of macroevolution: origin, stabilization, and adaptive radiation. The oldest eutherian mammal *Eomaia* is known from the Early Cretaceous (~125 Ma) of China (Ji et al. 2002), which is consistent with the molecular divergence time of eutherians from marsupials (Kumar and Hedges, 1998). This is paralleled by the study of Bininda-Emonds et al. (2007) that more than 40 lineages of modern mammals have survived from the Cretaceous, some 100-80 Ma. The conflict between molecular dating and fossil record could be resolved if we assume that molecular dating indicates the punctuation event, not the cladogenesis regime. The key innovation was the appearance of a highly developed placenta through which the developing embryo receives nourishment and gas exchange from the mother and forms a connecting link with her own bloodstream. After fertilization occurs, a eutherian mammal begins to develop the embryo in the uterus, a hollow organ in the mother's abdomen. Another organ, called the placenta, attaches the embryo to the uterus wall. Placentals give birth to fairly well developed offspring. The emergence of placental structure relied largely on co-option of ancient genes. Once the basic placenta was formed, duplication event during the Early Cretaceous (~125 Ma) led to the rise of eutherian mammals with new and highly varied reproductive strategies (Knox and Baker, 2008). During the Cretaceous, placentals began to stabilize for nearly 60 million years with few basal radiations, the second level of macroevolution. As the nonavian dinosaurs died out at the end-Cretaceous extinction, it lifted major impediment to the ecological expansion of placental mammals. The Tertiary radiation of placentals is the third level of macroevolution. The Paleocene (65-56 Ma) marks the first phases of the radiation of the placental mammals that continued throughout the Paleogene.

Tempo of Macroevolution

On the basis of fossil record it is quite apparent that evolutionary rates fluctuate in space and time. They vary greatly from group to group, and even among closely related lineages. Differences in rates of evolution are among the reasons for great biodiversity. Some groups hardly change at all over long periods of time. Others change very rapidly over short periods of time. Simpson (1944) recognized three different rates or tempos of evolution in the fossil record: tachytelic for 'fast' evolving lineages such as artiodactyls; horotelic, the standard rate of neo-Darwinian evolution, typical of most Phanerozoic animals and plants; and bradytelic for slow morphologic evolution such as living fossils. However, punctuated equilibrium and macrogenesis reverse our traditional perspective for tempo of evolution.

Macroevolution of multicellular organisms, as interpreted from Figure 1, tends to occur in transient, episodic forms very rapidly, short in duration such as the origination of higher taxa, similar to the punctuated mode of speciation (Gould and Eldredge, 1972). Sudden increased of genome pools would seem a sufficient condition for rapid evolution. The driving force of macroevolution, according to this new emerging theory, is to be found not in the chance events of random mutations, but in life's inherent tendency to create novelty, in the spontaneous emergence of increasing complexity and order by macrogenesis. The punctuated origin of higher taxa is followed by a prolonged period of stabilization or phylogenetic fuse with initial radiation of basal members in a slow pace of evolution (Cooper and Fortey, 1998). Unlike the stasis in punctuated equilibrium, stabilization is a prolonged period of slow evolution, a period of

incubation before the phylogenetic bursts. Stabilization is followed by intense cladogenesis resulting in explosive evolution of branching pattern in a tachytelic mode.

The bacterial fossil record, though fairly extensive, was not particularly informative to decipher the tempo of evolution; bacterial morphologies are too simple to tell much about rate of evolution and has been interpreted as extremely slow pace of evolution (Schopf, 1994). In reality, microbial evolution by HGT is an example of macroevolution in rapid pace with increased mutation rate (Woese, 1987).

The Tangled Tree of Life

The Tree of Life—the community of all organisms on this planet, both living and extinct—is the central concept of Darwinian evolution. Darwin (1859) suggested that all organisms are connected through time and all descended from a common ancestor—all the way back to bacteria. He based his evolutionary theory on two fundamental ideas—chance variation, later to be called random mutation, and natural selection. He presented a metaphorical tree that showed how species change through time. The diagram illustrates several branching patterns of evolution from an ancestral species to illustrate the expected outcome of descent with modification, and he explained how this might happen according to the principle of divergence (Fig. 4). Darwin recognized that species not only evolve but also divide. As species evolve, they split and diversify through time, increasing morphological divergence like the branching pattern of a tree. Most of the branches have been pruned by extinction, but few reach right to the top today—these are living species that create biodiversity. All organisms, Darwin proclaimed, belong to different branches of the same genealogical tree. The tree is a visual representation that shows how species are related by descent from a common ancestor. The shape of the tree reflects the shape of evolution through time. The tree was based on vertical inheritance of traits from parent to offspring across successive generations. The Tree of Life as an icon is elegant and powerful, conveying the essence of Darwinian evolution. From an unknown common ancestor in the remote past, life diversified over time by successive divisions of boughs, limbs, branches, and twigs.

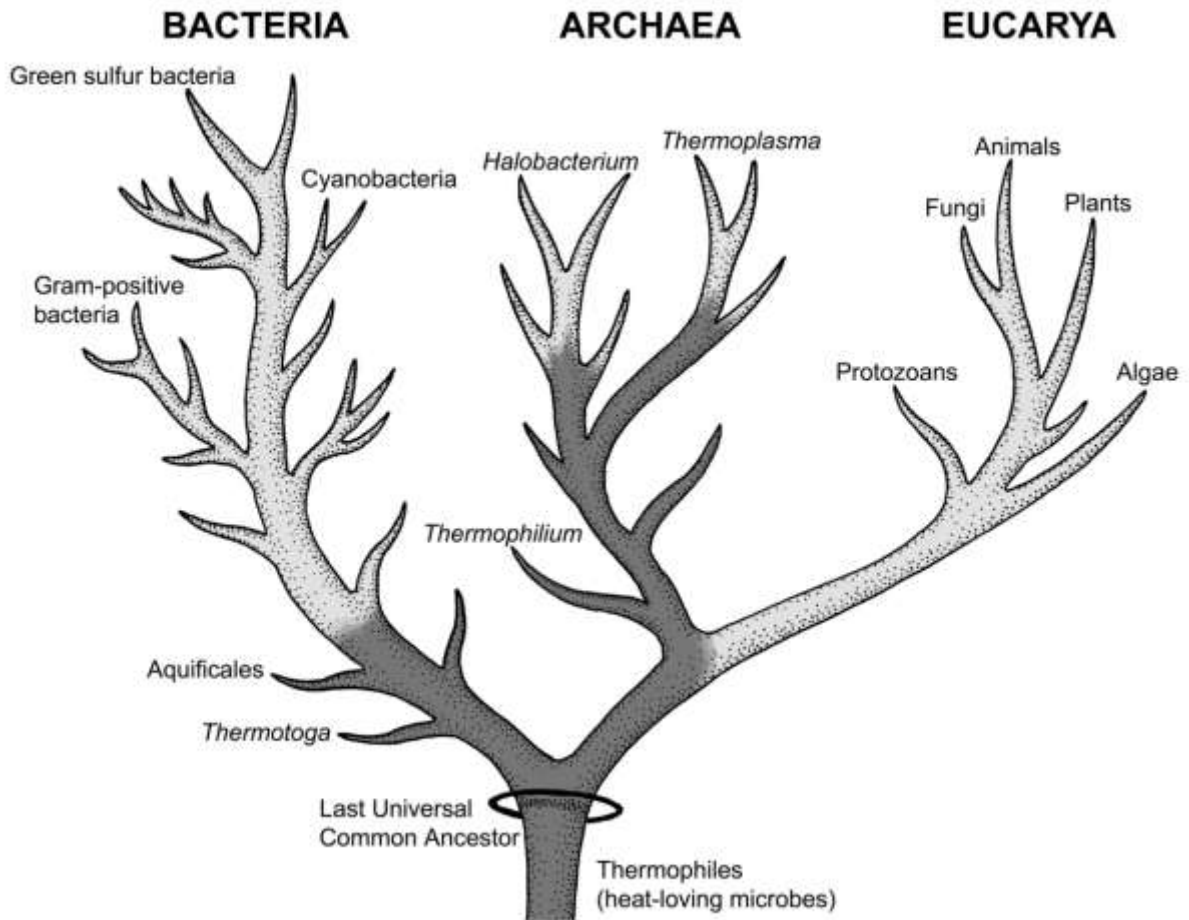


Figure 4. The genomic (ribosomal RNA) Tree of Life, proposed by Woese (1998) showing three domains or branches of life, Bacteria, Archaea, and Eucarya. Thermophiles, the heat-loving microbes (shown by gray stippled) lie in the domains of both bacteria and archaea. The hotter the microbe’s natural environment, the closer it is to the base of the tree, the last universal common ancestor. *Thermotoga*, one of the most primitive bacteria discovered recently is believed to be most similar to one of the Earth’s first living cells (modified from Monastersky, 1998 and Woese, 1998).

Darwin left to other biologists to figure out what real evolutionary trees looked like. The first responder was Ernst Haeckel, Darwin’s ardent supporter in Germany, who introduced the term phylogeny or the pattern of descent, which corresponded strictly to Darwin’s theory of common descent. Haeckel (1866) published a series of phylogenetic trees, grouping all known animals and plants as general guides to relationships among organisms. All of his genealogical trees branched continuously upward and outward, forming a cone, thus depicting the diversity of life from a common ancestral trunk. In one of his famous trees with real bark and gnarled branches, Haeckel captioned it the *Pedigree of Man*, in which humans occupy the apex of a symmetrical evolutionary tree, whereas the simplest organisms such as bacteria came off a main trunk near its base (Fig. 5). Haeckel’s depiction of evolutionary tree was a great one at that time, linking bacteria, invertebrates, vertebrates, and mammals to humans in a single genealogy. Since

then, more and more living and fossil species have been discovered and incorporated to fill the missing branches of the universal tree. Ever since Darwin taxonomists are drawing and redrawing the tree for a particular group of organisms by comparing their anatomy and physiology. As a result thousands of phylogenetic trees have been constructed separately that appeared in myriad of books and literatures but mostly they were not joined together to show the topology of the Tree of Life encompassing all living and extinct species on Earth.

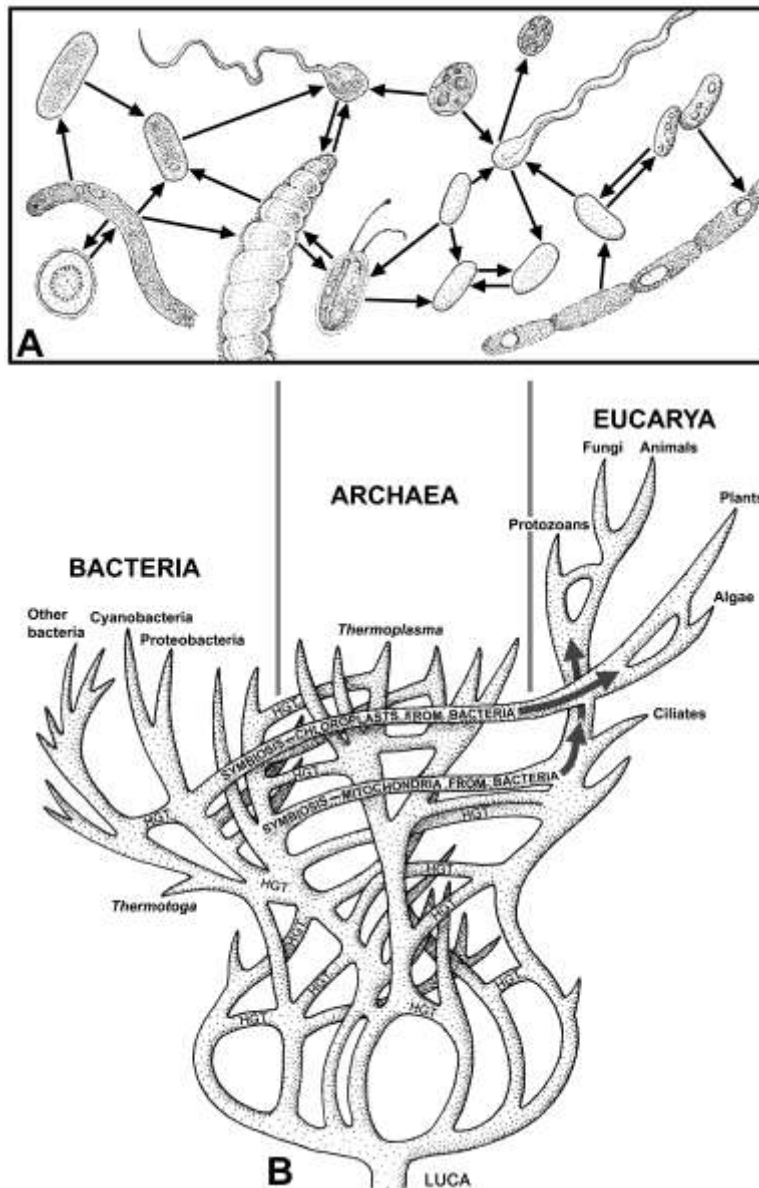


Figure 5. A, Prokaryotes share genetic information by horizontal gene transfer (HGT) breaking the species barrier. B, Modifications of Gene Tree by macrogenesis agents. Horizontal gene transfer (HGT) and endosymbiosis contradict the very concept of Darwin’s Tree of Life, where the branches of the tree merge and fuse. The Last Common Ancestor (LUCA) similar to extant thermophiles could be used to for rooting the tree of life (modified from Doolittle, 2000).

For a long time phylogeny was based on morphology: the study of structural characters such as shape, size, or features that an organism might possess. The topology of the tree has been modified through decades, as the relationships among different organisms are better understood when molecules and fossils are combined to reconstruct phylogeny and the missing branches are inserted with the discovery of new taxa. Traditionally organisms are classified into two major groups on the basis of cell structure: prokaryotes and eukaryotes. Prokaryotes are single-celled organisms that lack nuclei. In contrast, eukaryotes may be single or multicellular forms and possess a membrane-bound nucleus containing well-defined chromosomes, membrane-bounded organelles such as chloroplasts and mitochondria, and a cytoskeleton.

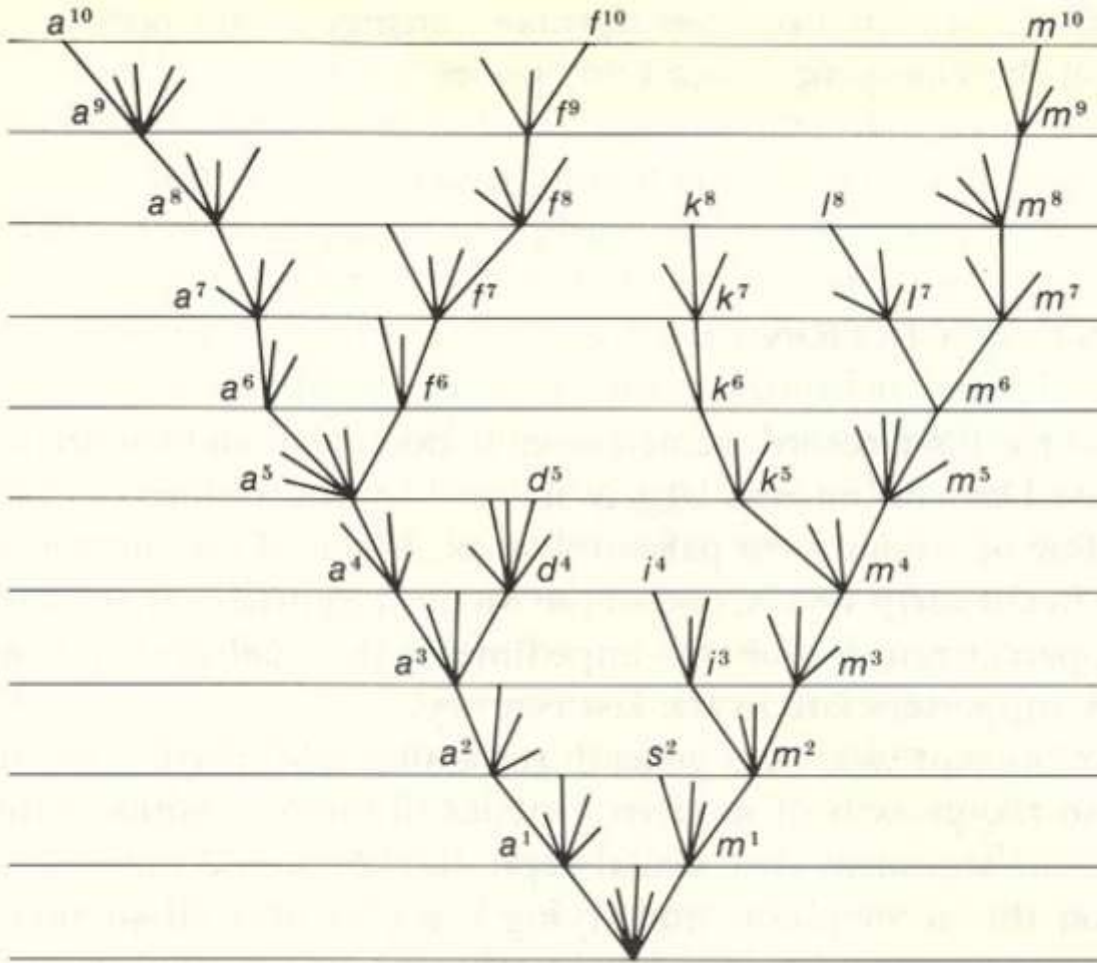


Figure 6. A reproduction of the segment of Darwin’s hypothetical evolutionary tree, the only figure included in his book *On the Origin of Species*. The tree captures Darwin’s view of evolution as a branching, tree-like process. The horizontal line represents morphological divergence, whereas the vertical line represents time. The original species gives rise to a fan of lineages that represent diverging population. Lines that reach to the top of the diagram represent extant or surviving varieties. Lines that end farther down are lineages that have gone extinct.

Molecular phylogeny recognizes three major groups or domains of organisms at the deepest level of the universal tree of life, represented by three main branches: Bacteria, Archaea, and Eucarya (Woese, 1998, 2002). All organisms are related by a common descent from a single form of life, represented by the tree's trunk at the bottom (Fig. 6). This tree was constructed using slow-evolving ribosomal RNA genes. This new realignment suggests that two fundamental divisions occur among early microbes—bacteria and archaea, which are traditionally lumped into bacteria. The new domain archaea, though appear as bacteria and behave like bacteria, actually branched off from bacteria a long time ago, and has a unique genetic identity that makes it a sister taxon of eucarya. Archaea are more different from bacteria genetically than humans are from mosquitoes or worms. Both bacteria and archaea lack nuclei and lumped together in prokaryotes.

The combined evidence of fossils and genes can be used to reconstruct the pattern and process of early history of life. The fossil record of early microbes is fragmentary and tantalizing. Bacteria, the simplest surviving forms of life, first appeared in the fossil record about 3.5 billion years ago (Schopf, 1994; 1999). Archaea might have evolved somewhat later as they seem closer to eucarya than bacteria. Using the fossil record as a guide, the root of the tree probably represents a solitary bacterium, which is now termed the Last Universal Common Ancestor (LUCA) of all living things that lived around 3.5 to 3.8 billion years ago. Both molecular phylogeny and the oldest fossil evidence suggest that the LUCA might be similar to thermophiles—the heat-loving bacteria—that thrive in hot, dark submarine hydrothermal vent systems, the oldest ecosystems on Earth (Monastersky, 1998; Nisbet and Sleep, 2001). Bacterial thermophiles could be used for rooting the tree of life (Fig. 6).

In neo-Darwinian evolution, new species arise exclusively via vertical gene transfer (VGT). New genome sequences reveal unexpected connections between the three domains of life by HGT and contradict the very concept of a Tree of Life Darwin envisioned (Doolittle, 2000). Bacteria can exchange genes with totally unrelated species, which stymies efforts to work out their evolutionary history based on individual gene phylogenies (Fig. 7A). Microbes' use of both HGT and VGT explain conflicting results in constructing genetic trees of evolutionary relatedness. HGT played an important role in the diversification of bacteria and archaea. Similarly, early eucaryan cells received both chloroplasts and mitochondria from two different sets of bacteria and acquired a combination of genomes through symbiotic mergers (Margulis, 1981). Genes hopped from branch to branch as early organisms either stole genes from their food or swapped DNA with their neighbors. In this revised gene tree, the root of the tree is not represented by a single cell but by a community of primitive cells. Such horizontal transfers involve the delivery of single genes, or whole suites of genes, freely crossing these contemporary domains, breaking the species barrier. Above the prokaryote level, a massive form of gene transfer comes from the acquisition of new genomes by endosymbiosis that turns out to be the primary cause for the origin of eucarya (Margulis, 1981; Margulis and Sagan, 2002). Because of HGT and endosymbiosis, establishing the Tree of Life through molecular phylogeny becomes more challenging because the three main branches tend to fuse (Fig. 7B). The role of HGT and endosymbiosis in evolution has raised fierce debate about the relevance of the Tree of Life. The origin and diversifications of embryophytes, metazoans, gnathostomes, craniates, teleosts, angiosperms, neognaths, and eutherians by macrogenesis are not exactly tree-like either, but show fusion of branches.

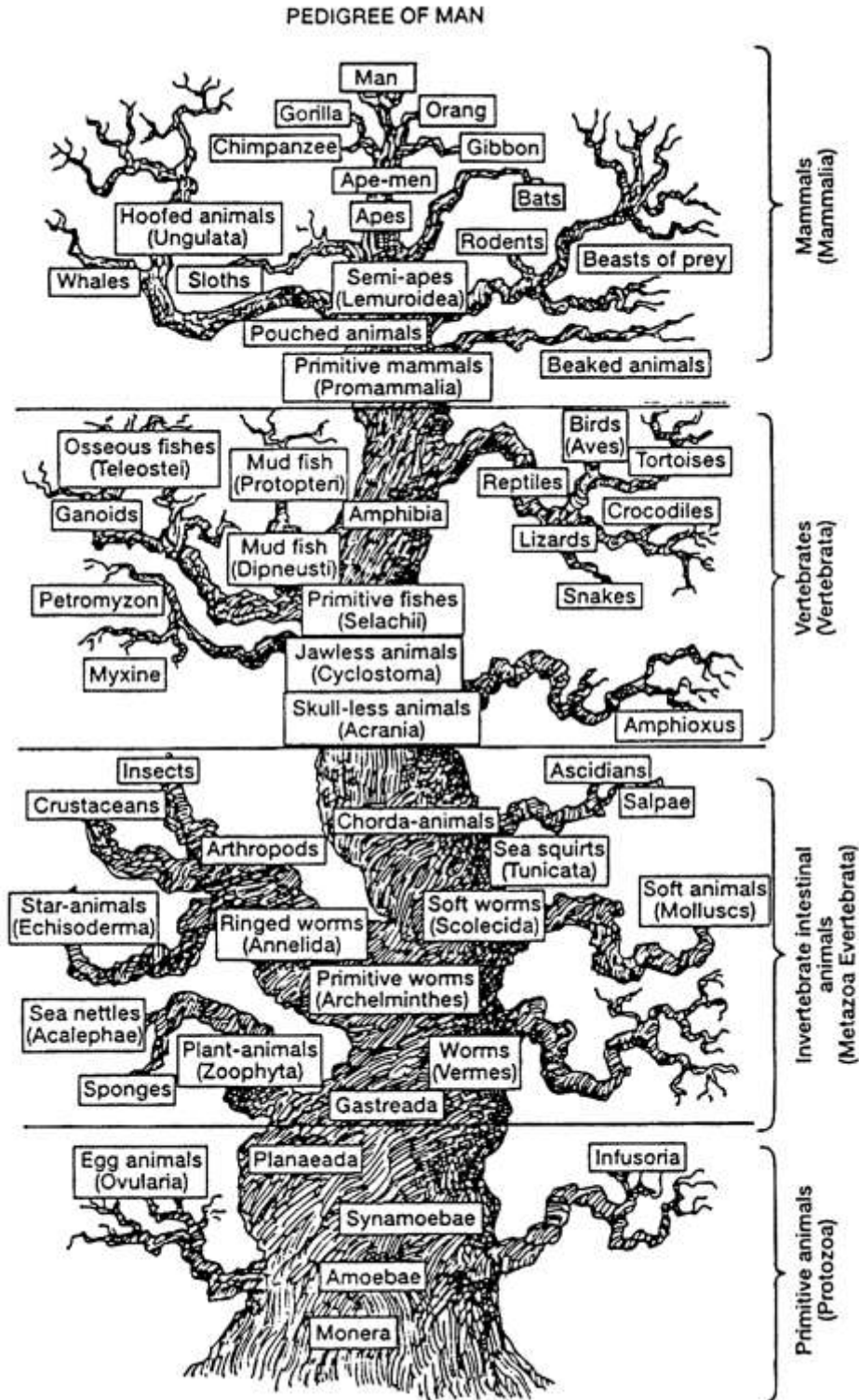


Figure 7. Ernst Haeckel first proposed the Tree of Life in 1866 that displays the branching pattern of species. In this tree, Haeckel showed the evolution of humans via four successive stages of animals namely bacteria, invertebrates, vertebrates, and mammals.

In recent times, there has been attempt to assemble all life into a universal Tree of Life to reconstruct the evolutionary relationships of Earth's taxonomic diversity—including all living and extinct forms. Ironically, if biologists do ever succeed in reconstructing the evolutionary origins of all living things, it will look more complicated and fundamentally different from Darwin's vision of Tree of Life. In a typical geometry of a tree, the branches are permanently separated without subsequent joining; they do not fuse. In contrast, in the new tree of life, as emerged from molecular phylogeny, a conflicting image begins to appear. Lineages do branch as they evolve as Darwin suggested; however, sometimes the branches join back together, contrary to Darwin's worldview. New genome sequences reveal unexpected connections between the three domains of life—bacteria, archaea, and eucarya by horizontal gene transfer (HGT) and contradict the very concept of a Tree of Life Darwin envisioned (Doolittle, 2000). Similar connections of tree branches persist in many lineages of plants and animals.

It thus appears that the nice, neat, nested hierarchy of a diverging tree branches does not truly represent the pattern and complexity of evolution. Evolution shows alternate pattern of fusion and branching lineages. If horizontal gene transfer and endosymbiosis were rampant in the evolution of life, some molecular biologists argue that the time has arrived to uproot the Tree of Life (Doolittle, 2000; Pennesi, 1999). If so, is there any alternative imagery to portray the biodiversity, combining both extinct and extant life?



Figure 8. The River of Life. Image of the Rakaia River, one of the largest braided river systems in New Zealand flowing from a series of glaciers that mimics the evolution of life in a deeper level of phylogeny. Some of the early clades such as bacteria, archaea, eucarya, embryophytes, and metazoans are plotted in this braided river system to show the anastomizing pattern of history of life created by macrogenesis, which is quite different from the branching pattern of tree of life envisioned by Darwin (courtesy of Robert E. Ford).

The River of Life

The wonder of evolution is manifested not only in branching, as Darwin proposed in his metaphor of the Tree of Life, but also is represented by truncations and mergers. Because of horizontal gene transfer among the three branches of life—bacteria, archaea and eucarya—endosymbiotic origin of eucarya, and recurrent hybridization and polyploidy in plant and animal lineages—a new metaphor, *River of Life*, more closely resembles the pattern of evolution than does a *Tree of Life*. In the Tree of Life the tree branches are permanently separated without subsequent joining; they do not fuse, and therefore the metaphor is too simplistic to represent the complexity of evolution by fusion and merger. In contrast, the interplay of the drainage pattern of a river from its head to its ultimate destination in the ocean evokes a more apt analogy to the evolution of life.

As the life radiated into three domains—bacteria, archaea and eucarya—they made unexpected connections and genetic exchanges in the early evolution of life like a braided river (Fig. 8). The early evolution of microbes during the Precambrian period mimics the beginning of a river from its head dominated by the braided river system, where the branches join and separate. The same braided drainage pattern continues during the origin of craniates, gnathostomes, teleosts, angiosperms, neognaths, and eutherians (Fig. 9).

The River of Life provides a road map for history of life, its branching and fusion order reflecting the successive radiation of biological diversity. In its course, certain rivers dry up like the extinction of a lineage, while others again ramify and lead to new possibilities. The coastline where the river empties into the ocean is the present time frame representing the biodiversity around us, the great spectacle of life on Earth. Our own species, *Homo sapiens*, lies on a tiny brook of humanity, off to the side of the great River of Life.

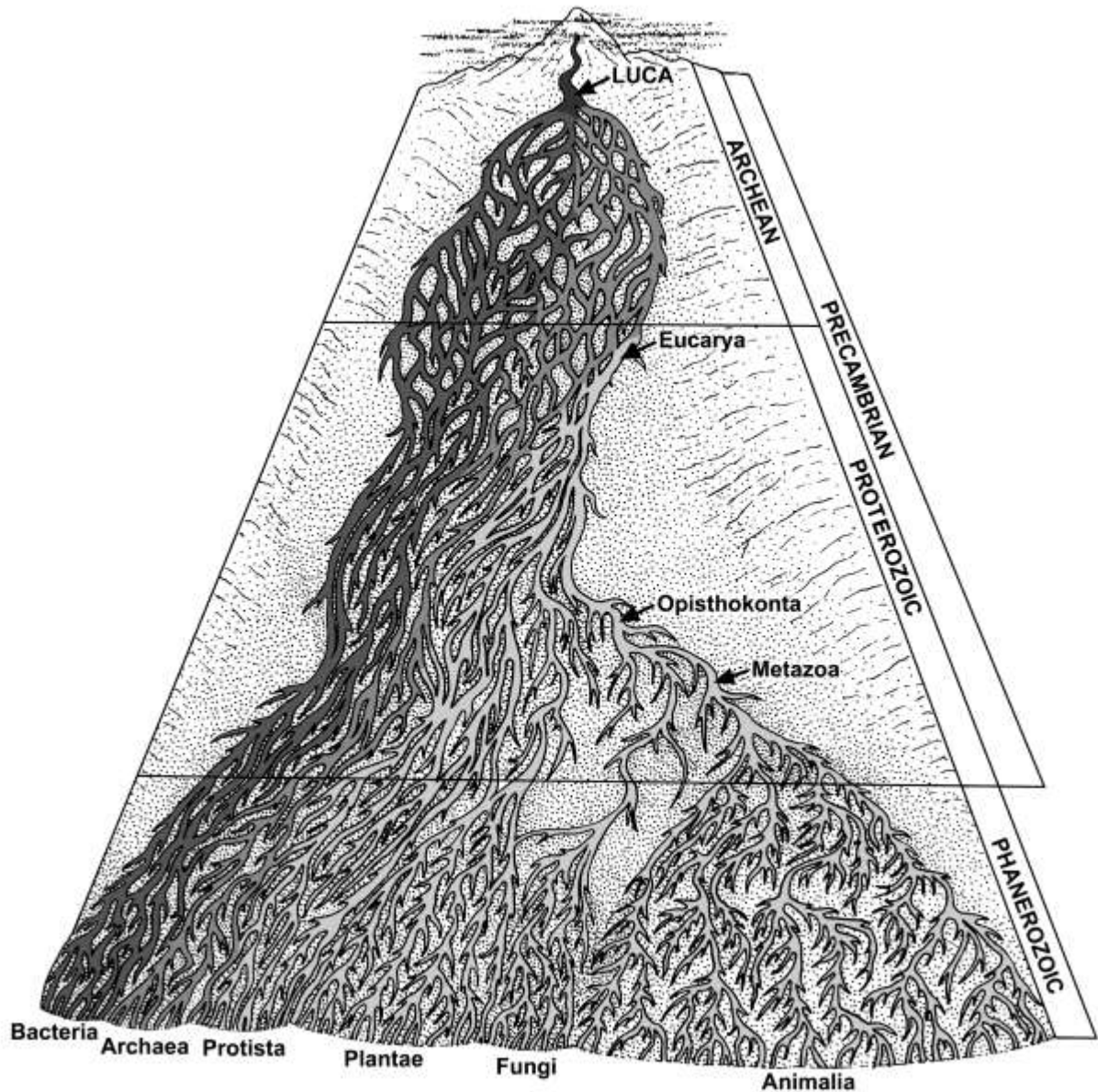


Figure 9. The evolution of life more closely resembles the drainage pattern of a complex braided river system than a branching tree, because the branches fuse and divide. The Archean microbial life originating from LUCA (last universal common ancestor) mimics the braided pattern of a river showing the rampant lateral gene transfer among three domains of life—Bacteria, Archaea, and Eucarya. Multicellularity arose independently from different kinds of protists to give rise to embryophytes, fungi, and metazoans. During the origination of higher taxa, many branches fuse recurrently by macrogenesis, but branch out again during cladogenesis. The coastline represents the present time line with astonishing biodiversity in the delta fronts.

Conclusion

Assessing the genetic causes for macroevolution is one of the major challenges to our understanding of large-scale biodiversity patterns. Various macrogenesis agents such as HGT, endosymbiosis, hybridization, and polyploidy enrich and expand gene pools among living organisms. It is likely that these agents also operated in the geologic past to trigger macroevolution in different clades and create biodiversity. Recurrent pulses of macrogenesis had produced organisms of increasing genetic complexity and size through time and created directionality in the history of life. The driving force of evolution, according to the emerging new theory, is to be found not only in the chance events of random mutation at the lower level, but also in life's inherent tendency to create novelty, in the spontaneous emergence of increasing complexity and order at higher level. Time and again, macroevolution brings the diversity of nature, whereas microevolution stabilizes the species with fine-tuning. Thus macroevolution and microevolution are uncoupled analogous to two opposing flowing streams meeting at the confluence of a species; both processes are responsible for speciation. Macroevolution is a top-down process cascading from higher to lower taxonomic levels such as (Domain→Kingdom→Order→Family→Genus→Species); in contrast, microevolution is a bottom-up process, where the direction of evolution is from lower to higher hierarchical levels (Gene→Population→Species). Species is the confluence in the hierarchy of life, Darwin's biological filter that can originate either by top-down process or bottom-up process. Like microevolution, macroevolution also takes place by the same Darwinian external processes—natural selection and adaptive improvement at any hierarchical level where there is heritable variation by virtue of interaction with the environment.

The theory of macrogenesis implies a radical shift of perception in evolutionary thought from traditional neo-Darwinian mechanism. In this process, large quantities of genes, even the entire genomes, could be transferred or fused across the species barrier, a short cut and rapid evolutionary mechanism for origination of higher clades. Macrogenesis explains the reason for hierarchy of life: why a domain is divided into kingdoms, a kingdom into phyla, a phylum into orders, an order into families, and family into genera, and a genus into species. After the origin of higher taxon, the number of species in it usually increases; so, too, do the number of genera, families, and other taxonomic levels. Macrogenesis is a powerful driving force for biodiversity. Cladogenesis triggers entirely new and expansive evolutionary trajectories that create phenotypic complexity, biodiversity, and species richness. In the history of life, macroevolution is more powerful than microevolution to create biodiversity.

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