Evolutionary molecular genetic clocks—a perpetual exercise in futility and failure

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Since its first use in the early 1960s, molecular genetic clock methodologies assume evolution and deep-time calibrations taken from paleontology. In addition, the following problems plague its use: 1) different genes/sequences give widely different evolutionary rates, 2) different taxa exhibit different rates for homologous sequences, and 3) divergence dates commonly disagree with paleontology despite being calibrated by it. Because the molecular clock idea is directly tied to the neutral model theory of evolution, recent discoveries in full codon utility and pervasive genome-wide biochemical functionality negate its foundational premise.

As stated in a recent review of evolutionary theory: "A molecular clock is now a standard assumption in almost every study of molecular evolution."[1] The molecular clock has had a major influence in nearly all biological disciplines, as well as causing much grief and dissension for the fields of paleontology and geochronology.[2,3] The basic premise is that informational macromolecules such as proteins and DNA sequences evolve at rates that can be measured and calibrated by evolutionary estimates provided by paleontology.[4,5] The increasing popularity of this methodology, particularly over the past decade, and its use in evolutionary systematics is illustrated by the yearly number of research publications documented in NCBI's PubMed database (figure 1). While there were only a handful of such papers in the late 1960s and 1970s, current trends indicate that these types of reports may soon top over 100 publications per year.

When the molecular clock was first being developed, much hope was invested in the technique with the idea that it would ultimately allow the construction of a unified evolutionary tree of life marked by historically accurate deep-time points. However, instead of resolving the tree of life, the past five decades of molecular clock research has produced nothing but discordance and confusion within the evolutionary community.[3,6] In fact, in a recent interview this year, human evolutionary geneticist David Reich of Harvard stated: "The fact that the clock is so uncertain is very problematic for us" and "It means that the dates we get out of genetics are really quite embarrassingly bad and uncertain."[7]

A history of molecular clocks

The idea of a molecular clock was first introduced in the early 1960s at a time when molecular biology was still in its infancy and DNA sequencing would not be realized for another decade. According to evolutionary biologist and historian Sean Carroll, it was Zuckerkandl and Pauling who first proposed a "new picture of evolution that was invisible to paleontologists and taxonomists—a picture of molecules ticking off evolutionary time without affecting how organisms looked, behaved, or functioned".[8] Zuckerkandl, the post-doctoral scientist of Linus Pauling, stated in an interview 50 years later that "it seemed natural to wonder whether the succession of changes that were obviously taking place through evolution, and not exclusively by any means but to a large extent were then known as attributable primarily to exchanges of individual bases in genes or amino acids in corresponding proteins".[9]

Zuckerkandl began analyzing enzymatically digested hemoglobin protein fragments from gorillas, chimpanzees, rhesus monkeys, orangutans, cows, pigs, and an assortment of fish.[10] By comparing fragments, he found that human and gorilla globin proteins only differed by one or two amino acids. Using the estimated time of the divergence of horses and humans (taken from paleontology), which they put at 130 Ma, and the number of differences between their α-chains, they calculated a time of about 14.5 Ma for each amino acid change. Zuckerkandl and Pauling then used this 14.5 Ma value as the standard rate of one change in each alpha globin chain. By multiplying the number of changes by 14.5, then dividing by 2 because the changes occurred across the two lineages, they estimated that the difference between the gorilla and human globin chains indicated their last common ancestor lived from 7.3 to 14.5 Ma ago.

They then averaged these two numbers to obtain 11 Ma, a value that was much earlier than that proposed by paleontologists at the time, which was 11 to 35 Ma. These claims stirred up a heated and contentious controversy with the traditional proponents of the modern Darwinian synthesis of the day. One of the most prominent and vocal skeptics was Ernst Mayr, a leading expert on speciation and systematics, plus George Gaylord Simpson, a prominent

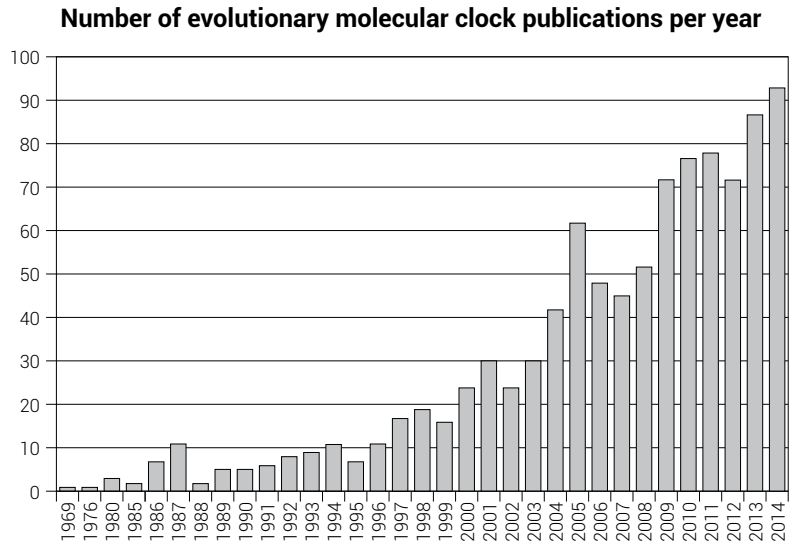

Figure 1. Number of publications listed by PubMed at NCBI per year using the combined search terms 'evolutionary' + 'molecular' + 'clock'.

paleontologist. They were two of the chief architects of the so-called Modern Synthesis of evolutionary theory that emerged in the 1940s.[8] Simpson and Mayr inferred from the fossil record that evolution occurred erratically, and some creatures didn't seem to evolve at all. Thus, the idea that it worked like a steadily ticking clock was not readily accepted.

Already, basic presuppositional problems with the molecular clock method should be noted outside of the controversy it caused within the evolutionary community. Namely, it assumes evolution and depends on deep-time calibrations from paleontology.[6,11] As noted, Zuckerkandl and Pauling used the estimated time of divergence of horses and humans to develop their rate of sequence substitution. And as we shall see later, while the statistical applications of this assumption have become increasingly more sophisticated, the basic restraint of evolutionary paleontology has always been a key component of molecular clocks from their first inception.

At about the same time as Zuckerkandl and Pauling's research, immunological techniques (relative differences between taxa in protein precipitate using human antibodies) were also being applied to studying protein relatedness within an evolutionary clock scenario and used for human serum proteins, cytochrome c, and fibrinopeptides.[12–15] However, Zuckerkandl and Pauling strongly pushed the idea of a molecular evolutionary clock based on biological sequence and formalized their ideas further in 1965 stating: "Anyone who recognizes the value of the immunological approach for estimating phyletic distance with certain limits should find it impossible to deny that the comparison of amino acid sequences is potentially an even better tool. It is only potentially less equivocal, more accurate, suited for absolute instead of only relative evaluations, and able to extrapolate from the present to the past."[16]

While immunological based techniques continued to be applied for a number of years thereafter, the idea of using biological sequences became more important as continuing ideas about selection, neutral changes, and fixation began taking shape in the contentious esoteric cauldron of evolutionary debate at the time. Indeed, some of the first discussions of the idea of the neutral theory of evolution are contained in Zuckerkandl and Pauling's 1965 paper in which they state: "The changes in amino acid sequence, will, however, be limited almost exclusively to the functionally nearly neutral changes."[16] The high evolutionary rates reported by Zuckerkandl and Pauling along with their ideas about amino acid substitution rates was the first topic addressed in Mootoo Kimura's popular 1968 paper (Kimura was one of the initial key proponents of the neutral theory).[17]

The neutral theory was developed by Kimura in large part as a solution to Haldane's dilemma which Kimura noted by stating: "... the calculation of the cost based on Haldane's formula shows that if new alleles produced by nucleotide replacement are substituted in a population at the rate of one substitution every 2 yr, then the substitutional load becomes so large that no mammalian species could tolerate it."[17] His solution to this problem stated: "the very high rate of nucleotide substitution which I have calculated can only be reconciled with the limit set by the substitutional load by assuming that most mutations produced by nucleotide replacement are almost neutral in natural selection".[17] However, other prominent researchers, who were developing similar ideas at the time (e.g. Jack King and Thomas Jukes), soon noted that Kimura's estimates of per-genome substitution rates could be exaggerated.[18,19] At present, a variety of competing hypothetical evolutionary paradigms exist that propose different levels of neutrality and selection in genome evolution.[20–23]

Nevertheless, the basic idea of the neutral theory provided a strong rationale for the molecular clock, even though the earlier research for a molecular clock slightly predated the formal development and promulgation of neutral theory. The basic model postulates that neutral sites in the genome are not under selection, and that the rate of evolution/substitution at a neutral site is the same as the rate of mutation.[20,24] As we will show later, recent findings of pervasive biochemical

function across the human and other metazoan genomes directly threaten the validity of this concept.

Rate heterogeneity problems

A major difficulty that evolutionists have had with the clock method is its original assumption that molecules accumulated differences at a regular rate purely as a function of evolutionary time. From the fossil record, paleontologists inferred that the rate of evolution varied greatly, sometimes enormously, while many species remained unchanged for long periods of time (referred to as 'stasis').[25] In fact, much to the chagrin of paleontologists, biologists have proposed that the clock hypothesis could even be used for "determining evolutionary events of the remote past for which the fossil and other evidence is lacking or insufficient".[4]

In the early era of molecular clock research, prior to the genomics revolution, the focus was largely on relatively recent evolutionary events alleged to have occurred during hominid evolution.[12,13,26] As the DNA sequencing advanced, more ambitious efforts to ascertain divergence dates among diverse animal phyla and even for the major kingdoms of living organisms was undertaken.[27,28] However, it soon became apparent that wide rate differences existed between phylogenies based on genetic analyses and those obtained strictly by the fossil record. As noted by Ho *et al.*: "Rates of microevolutionary change [within species], measured between successive generations, were found to be far higher than rates of macroevolutionary change inferred from the fossil record."[29] Venkatesh *et al.* concluded that analyses "of molecular sequences have given conflicting models even when large data sets were used".[30]

The most empirical way to measure genetic change is by determining DNA base substitutions observed between generations of pedigree lines. These have been shown to greatly exceed the more speculative and spurious rates achieved from paleontology.[6] Some researchers have attempted to correct this conflicting empirical data with the hypothetical effects of selection.[31] The other method of determining genetic change is purely hypothetical and based on comparing homologous sequences between diverse taxa. This could be called the phylogenetic method, while the former could be termed the biochemical method. When either method is used, the data is typically calibrated with deep time.

With either method of determining the genetic clock rate, the prospects of achieving any type of evolutionary concordance has been dismal. As noted in a recent review, the author stated that the estimation of divergence dates "is a perilous exercise fraught by artifacts which become progressively more severe for events further in the past" and, "These difficulties are intrinsic to the dating of ancient divergence events and are reflected in the large discrepancies between estimates obtained with different approaches."[32]

Alongside the progression of such studies over the years, has been the growing recognition that rate variation (also termed rate heterogeneity) contradicted the foundational premise of the molecular clock. To combat this enigma, analyses now include sophisticated models that incorporate rate heterogeneity across the different lineages.[33] However, rate variation is an important evolution-negating problem that cannot simply be swept under the rug with sophisticated statistical models. It must be fully understood to appreciate the deep fundamental problems that exist in evolutionary molecular clock research.

The underlying components of rate variation are multifactorial and include gene sequence effects, lineage effects, and residual effects (the difference between the observed value and the *estimated/predicted* value).[34] For the purposes of this study we will focus on gene and lineage effects since the large levels of residuals common to such studies are largely the result of the inconvenient fact that the data by its nature contains many statistical outliers, primarily because evolutionary assumptions don't fit real world biology.

The influence of gene or genomic region effects are notorious and plague nearly all studies done in molecular clocks.[34] This inconvenient fact was widely popularized by evolutionist Francisco Ayala at the beginning of the genomics revolution when he noted "... every one of the thousands of proteins or genes of an organism is an independent clock, each ticking at a different rate".[35] In illustrating this concept, Ayala noted the glaring example of molecular clock disparity for the superoxide dismutase (SOD) and glycerol-3-phosphate dehydrogenase (GPDH) genes that encode key metabolic proteins found in a diversity of animals. In regards to these genes, Ayala states: "Drosophila flies and mammals (which have longer generation time and lesser population numbers than occur in Drosophila) evolve at the same SOD rate, but mammals evolve five times faster in the case of GPDH." He then notes that one of the key problems with this discrepancy is that the "intra-cellular role of scavenging oxygen radicals would seem likely to have remained the same through time and across lineages over the last 650 My [Ma]". His conclusion is "that we are left with no predictive power and no clock proper".

Another significant problem is that while some genes differ widely in their sequence characteristics, others exhibit little change across a wide variety of life forms. For this reason, histone genes are never used because they would generate a molecular clock and divergence dates in complete contradiction to those obtained from studies of other less conserved genes.[36] In another of many examples, the motor protein myosin 2 is structurally identical in turkeys and

scallops despite the 600 Ma of evolution that separates the two life forms.[37]

In a recent study among primates evaluating differences in conserved CpG islands across the genome, Kim *et al.* reported: "Our conclusion that different regions of genomes follow different molecular clocks should be considered when inferring divergence times using molecular data and in phylogenetic analysis."[38] They also determined that heterogeneous genomic molecular clock sites across the different primate genomes were of a "markedly different nature, reflecting differences in their molecular origins". Even more remarkably, the authors showed that two different types of genomic clocks operated in these regions in which they "demonstrate that the two types of mutations [replication origin and methylation origin] follow statistically different molecular clocks". They go on to say: "Methylation-origin mutations accumulate relatively constantly over time, while replication-origin mutations scale with generation-times."

Rate variations exist not only for different genes and genomic regions, but also among lineages, including major metazoan ones. Peterson *et al.* pointed out this discrepancy, stating that "comparative genomic analyses suggest that a significant rate difference exists between vertebrates and dipterans, because the percentage difference between the genomes of mosquito and fly is greater than between fish and mouse, even though the vertebrate divergence is almost twice that of the dipteran".[39] As an author of one evolutionary review paper stated: "Differences in the rate of evolution across the major groups of life are dramatic."[40]

Even within a more restricted group of organisms that have somewhat similar molecular machinery, such as mammals, rate variation among taxa can be large. The most popularized of these is the discrepancy between rodents (murid rodents in particular) which have an elevated substitution rate compared to apes and humans, which have a decreased rate.[41] This has been famously termed "the hominid slowdown" by evolutionists. Interestingly, in an even more restricted sense, just among different bat taxa, rates were also found to vary widely.[40]

So what is the biochemical cause of this rate variation among different animal taxa? The level of discrepancy in the dating of a single set of organisms caused by lineage effects can often be as high as twenty-fold.[42] While many factors contribute to it, an evolutionary answer has been elusive. In a comprehensive study that analyzed mutation rates in a diverse set of 44 homologous genes for 2,108 nodes on the mammalian super-tree, the researchers stated: "Despite concerted effort, the reasons underlying any global lineage-specific differences remain unclear, with explanations invoking or refuting any, or all, of the differences in cellular DNA proofreading and repair mechanisms, body size, mass-specific metabolic rate, and/or (genomic) generation time."[40]

Codons—not so redundant after all

In the protein coding regions of genes, three consecutive DNA letters form what is called a codon, and each codon corresponds to a specific amino acid in a translated protein. An early noticed aspect of codons is that of apparent redundancy where the first two bases are non-negotiable, but the third base can vary. The variation in the third base was termed 'wobble' and codon variability was considered redundant. In effect, it was assumed that different codon variants for a given amino acid were functionally equivalent.

When alleged codon redundancy was discovered, scientists were interested in the possible evolutionary role that mutations in the third base might play.[43] In the emerging dogma of the day, mutations that did not alter the encoded amino acid of a codon (synonymous) would ultimately have no effect on the resulting protein sequence and thus, have no effect on cellular functionality, organismal fitness, or selective evolutionary processes. They were ideal candidates for neutral sites of evolution.

Neutral model proponent Masatoshi Nei stated in 2005: "Because of degeneracy of the genetic code, a certain proportion of nucleotide substitutions in protein-coding genes are expected to be silent and result in no amino acid substitution."[21] Nei *et al.* re-affirmed this widely held belief in a 2010 paper followed by his book, *Mutation Driven Evolution*, published in 2013.[22,23] However, in recent years, evidence for multi-role functionality at the codon's third position has been rapidly mounting.

Organisms across the spectrum of life show large variability in their particular preferences for the use of different codons that encode the same amino acid.[44–46] In one interesting study, 50 randomly selected genes were chosen from four diverse prokaryotes and five diverse eukaryotes (including humans) and the level of codon preference was found to not only vary among taxa, but also vary widely between genes even within an organism's own genome.[44] This intra-genome variation for codon preference was more recently confirmed in an extensive study among insect taxa.[46] As noted in a recent review of the subject, such complicated scenarios of codon usage represent "features that are difficult to explain through mutation alone".[44]

Early on it was known that changes in the third base of codons do affect the functional effectiveness of the cell because of the enormous interconnectivity of cellular biochemistry. An example is that a specific codon code is tied to the tRNA production system, and a change in a codon thus impinges upon the effectiveness of the protein translation apparatus. The tRNA production levels are 'set' for the original code, and changes cause a tRNA supply imbalance.[47] More recently, it was discovered that tRNAs are re-used in the translation process and that codon sequence, especially

at the third base, plays a large role in this recycling system.[48] This is especially true for genes that are highly and rapidly expressed to improve translation efficiency.

One of the largest problems for the idea of redundancy, however, has been the discovery in recent years of dual codes found in codons. In complex eukaryotic genomes, it has been widely demonstrated that protein-coding exons contain a variety of signals (e.g. splice sites, editing sites, miRNA binding sites, mRNA turnover signals, etc) other than just the information delineating amino acids.[49] It was also recently demonstrated in a genome-wide study in humans that transcription factors commonly clamp onto specific sites encoded within exons inside genes.[50] While one set of codons specifies the order of amino acids for a protein, the very same sequence specifies where transcription factors are to bind to regulate transcription.[51] More specifically, it was determined that about 14% of the codons inside 87% of human genes are occupied target sites for transcription factors. These dual-function codon sites in exons have been labeled 'duons'.

The evolutionary implications for the preponderance of dual codes in codons, particularly as it relates to the neutral model, immediately became obvious to the scientific community. Several researchers in a recent review recognized this problem and stated: "How widespread is the phenomenon of 'regulatory' codes that overlap the genetic code, and how do they constrain the evolution of protein sequences?"[52]

In addition to the discovery of duons, it has also been recently documented that the third base of the codon plays a key biochemical role during protein translation. During translation, periodic pausing occurs while the protein is being produced and directed out of a tunnel in the ribosome.[53,54] The sequence specified in codons affects the rate of pausing in the ribosome, and is critical to the folding of proteins into their proper three-dimensional shape which occurs during the process of exiting the ribosome. Because the translation and the initial folding of the protein are linked together, the processes are called 'co-translational'. A recent study has shown that the third base is key to telling the ribosome when to pause and how to regulate the rate at which the protein is being made, which ultimately determines the folding of the protein into its proper three-dimensional shape.[55] Not only does a codon provide the alphabet for which amino acid to add in a protein, but it provides important information needed to regulate its folding. The researchers state: "These dual interpretations enable the assembly of the protein's primary structure while also providing important folding controls via pausing of the translation process."

What was once thought only to be meaningless redundancy and fodder for neutrally evolving sequence, has now been proven to be exactly the opposite. Clearly codons are information rich features containing multiple overlaying languages and sets of instructions for different systems in different parts of the cell. In fact, the researchers of the most recent protein folding study go on to say: "The functionality of codonic redundancy denies the ill-advised label of 'degeneracy'."[55] They add: "Redundancy in the primary genetic code allows for additional independent codes. Coupled with the appropriate interpreters and algorithmic processors, multiple dimensions of meaning and function can be instantiated into the same codon string."[55]

Genes are networked

Another problem with the molecular clock is that genes do not function as single entities, but rather are part of complex, highly interconnected genomic networks. This concept was recently demonstrated when scientists observed the effects of 550 sequentially inhibited genes on the overall fitness of nematodes over eight generations.[56] Fitness is defined here as the ability of a population of organisms to grow and reproduce over time compared to a control population that did not have the mutation. In the majority of cases, the disruption of single genes reduced the fitness of the nematode populations. This was an effect that kept increasing with successive generations. Theoretically, this would have eventually led to extinction. As a result, the researchers concluded that almost every gene tested was essential to survival of the nematode. And because the mutant worms' fitness decreased over successive generations, the researchers also concluded that even single mutations negatively impact entire gene networks. The researchers wrote: "In contrast to previous estimates, we find that, in these multigeneration population assays, the majority of genes affect fitness, and this suggests that genetic networks are not robust to mutation. Our results demonstrate that, in a single environmental condition, most animal genes play essential roles."

Compounding the evolutionary problem of interconnected genes is the fact that the boundaries of what constitutes a gene have become blurred as we begin to unravel the complexities of the genome. What was once thought to be a single gene can instead be a nest of different genes due to the fact that introns can contain genes, genes can overlap, and many genes have antisense counterpart genes located on the opposing strand.[57–60] Also, regulatory sequences such as promoters and enhancers that can control and regulate several genes (even bi-directionally), can be located at long distances away from the genes they control (up to a million bases), or even be found inside neighbouring genes, and are themselves often transcribed to produce products that participate in gene regulation and/or chromatin modification.[61–64] In addition to protein coding genes, it is now widely understood that up to twice as many long noncoding RNA genes exist in the

genome that are coregulated and networked with protein coding genes.[65–69]

Taking into account this level of interaction and complexity and applying it to standard evolutionary clock analyses is essentially beyond reason at this point. One of the leading researchers attempting to do so has been Michael Lynch. In his view, "Although numerous investigators assume that the global features of genetic networks are moulded by natural selection, there has been no formal demonstration of the adaptive origin of any genetic network" and "the mechanisms by which genetic networks become established evolutionarily are far from clear".[70] So what sort of model does Lynch propose to explain the origination of complex genetic networks? Something akin to the neutral mutation-driven model on a grand scale where genomes just somehow mystically evolve through random genetic drift. He states: "... many of the qualitative features of known transcriptional networks can arise readily through the non-adaptive processes of genetic drift, mutation and recombination, raising questions about whether natural selection is necessary or even sufficient for the origin of many aspects of gene-network topologies."

Interestingly, several years after Lynch published this hypothetical paper, he produced several other papers showing how random mutation on a genomic scale was actually counterproductive to evolution. One paper described an extensive population genetics study in water fleas, a complex eukaryote, and found that genetic lines with high germline spontaneous mutation rates generally had lower levels of fitness.[71] Thus, an increase in spontaneous mutation rate, the alleged engine of evolution, actually lowered the ability of the organism to both survive and reproduce. Another report by Lynch showed how the organismal mutation rate decreases with both genome size and effective population size among both prokaryotes and eukaryotes.[72] And yet another report showed that while the total amount of transposable elements, intron size, and other noncoding DNA—alleged candidate regions for neutral model evolution—all increased with genome size, the recombination rate decreased.[73]

So if the amount of alleged junk DNA is increasing with genome size, the fodder of neutral model evolution, why is the mutation rate not scaling accordingly? Clearly, the answer is that nearly all of the genome is serving some undiscovered functional purpose, even in those genes that appear to be extensively populated with retroelements as is often observed in many plant species.

Complex, genome-wide, biochemical functionality following multiple lines of combinatorial evidence associated with pervasive transcription, long noncoding RNA functionality, complex patterns of chromatin modification, and genome-wide co-regulation data, is now being widely documented in both plants and animals.[63,69,74–79] Thus, the amount of genomic landscape not under functional selective constraint (as the evolutionist would view it) is diminishing rapidly as research progresses.

Molecular clock discrepancies with paleontology

In a recent paper, the authors state: "Major disparities are recognized between molecular divergence dates and fossil ages for critical nodes in the Tree of Life."[80] In this same paper the authors specifically documented huge disparities between paleontology and molecular clock dates for 67

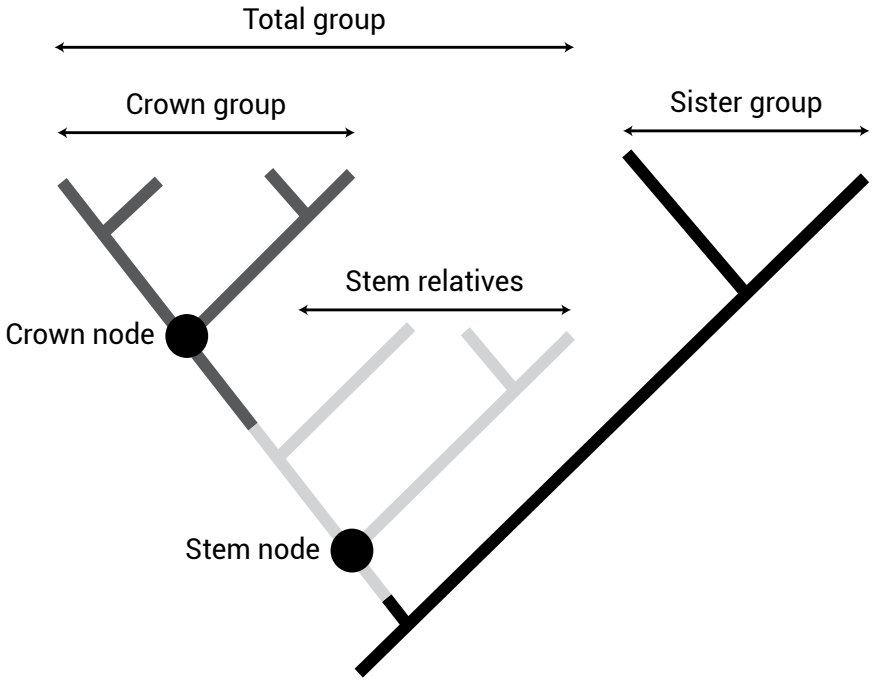

Figure 2. Basic evolutionary terminology of inferred phylogenetic relationships used in calibrating and developing models for molecular clocks. A crown group is the most recent common ancestor located at the crown node of a living clade, which would also include all the living and extinct descendants of that ancestor. A stem node represents the inferred evolutionary divergence of all members of a monophyletic group descended from a common ancestor, including extinct lineages alleged to have diverged below crown groups, called stem relatives.

clades of birds. They found that "for Aves, discord between molecular divergence estimates and the fossil record is pervasive across clades and of consistently higher magnitude for younger clades" and "These divergence estimates were, on average, over twice the age of the oldest fossil in these clades."

In another very recent and an even more taxonomically broad study among placental mammals, researchers examined a complex matrix of morphological characters in combination with a large nuclear DNA sequence dataset and total discordance with the fossil record was the end result.[81] These conflicting results were accomplished despite the fact that two very different relaxed clock models were used to account for extensive rate heterogeneity, including one that was constrained using the current evolutionary consensus for placental phylogeny. The authors stated that the end result of the effort was to "retrieve implausibly ancient, Late Jurassic-Early Cretaceous estimates for the initial diversification of Placentalia (crown-group Eutheria)" and that "These dates are much older than all recent molecular and palaeontological estimates." To try and force their results to fit the evolutionary paradigm, they added even stronger deep-time constraints and stated: "Enforcing additional age constraints on selected internal divergences results in only a slight reduction of the age of Placentalia."

The frustration that this common discrepancy has caused the evolutionary community was recently voiced in a paper written by paleontologists in which the authors stated:

> "As paleontologists who frequently collaborate with geochronologists, we expect that molecular 'timetrees' will help fill gaps in the fossil record. However, we find molecular divergence-age estimates ('dates') difficult to evaluate, and not only because many results differ strikingly from the fossil data. Molecular dates are extremely sensitive to placements of calibrating fossils at stem vs crown nodes and to choices of methods and calibration scenarios."[3]

Figure 2, in addition to illustrating the concept of evolutionary phylogeny, depicts the difference between stem and crown nodes.

Interestingly, this discrepancy between the two dating systems (genetic vs paleontology) has led some biologists to propose the idea that somehow molecular clock rates not only vary between genes and lineages, but that they must also vary across deep evolutionary timescales.[82] Because the two systems (genetic clocks and paleontology) do not consistently agree with each other, this brings us to the main issue of molecular clock theory—calibration and the overriding assumption of evolution.

Clock calibration—the crux of the matter

When evolutionists ascertain time in a phylogenetic tree, they routinely insist that "the molecular clock needs to be calibrated".[34] In other words, clock calibration is standard operating procedure and is based on the presupposition of evolution and deep time, and has been implemented since the first studies published by Zuckerkandl and Pauling. However, as noted in a recent review, "uncertainty in the fossil or geological information used to construct calibrations is

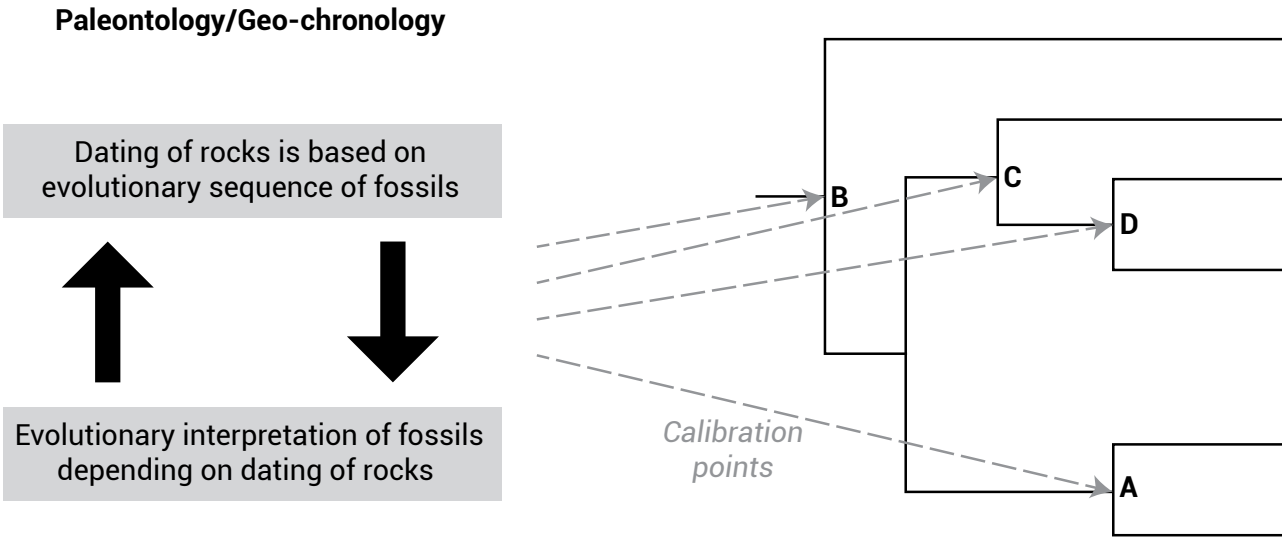

Figure 3. Basic illustration of the circular reasoning employed in paleontology and the points (nodes) in an evolutionary phylogeny to which they are employed. Possible points of calibration and constraint according to guidelines at timetree.org could include a minimum constraint (hard bound) that would be the oldest fossil in a group or a maximum constraint that could be a probability distribution of the fossiliferous rocks related to the time in question.

rarely trivial".[34] Of course, creationists have long pointed out the serious problems with using fossils and geochronologic dating systems, unequivocally showing that they cannot be used as a valid basis of ancient time determination or as proof for macroevolution in a cohesive tree-of-life continuum.[83–88]

As stated in a recent evolutionary review: "In all molecular-dating analyses, the single most important component is the choice of calibrations."[89] Molecular clock calibration is generally accomplished in one of two ways: by setting the rate to an alleged known value taken from paleontology or by constraining the age at one or more nodes in the phylogeny using the alleged age of fossils and/or a hypothetical geochronologic event.[6,34,90] See figures 2 and 3, which show the various types of points (nodes) in a phylogenetic tree that are 'calibrated' by evolutionary assumptions. Because agreeable rate estimates are typically hard to come by, the latter approach is usually employed when many diverse taxon are used over a large amount of evolutionary time, particularly when there are substantial rate differences among the lineages—a pervasive anomaly described earlier.

Typically, the earliest fossil in a lineage is used to infer time of divergence for that lineage from its alleged sister lineage.[91] The supposed age constraint can be applied in several ways, of which the easiest is to fix the node's age to a single point value. Of course, this methodology ignores the inherent uncertainty in the evolutionary-based calibration of the geochronologic age(s) associated with both radiometric dating and taxonomic assignment. Thus, many studies attempt to account for this uncertainty by allowing the age of the phylogenetic node to vary within chosen limits.[6,34]

A survey of deep-divergence studies by molecular evolutionists Dan Graur and William Martin critiqued one particular study in which the authors "claim to be 95 percent certain that their divergence date for certain animal groups falls within a 14.2-billion-year range—more than three times the age of the earth and a clearly meaningless result".[5] Graur and Martin further document the absurdity of the problem by stating that these scientists "inferred ostensibly precise molecular-clock dates for speciation events ranging from the divergence between cats and dogs to the early diversification of prokaryotes". As Graur and Martin noted, divergence-time estimates are often based on a single calibration point and tenuous methodology stating that the "calibration point that is both inaccurate and inexact—and in many instances inapplicable and irrelevant—has been used to produce an exhaustive evolutionary timeline that is enticing but totally imaginary". They concluded that many molecular clock estimates "look deceptively precise" but, given the many problems with this technique, their "advice to the reader is: whenever you see a time estimate in the evolutionary literature, demand uncertainty!"

However, despite the inherent evolutionary uncertainties associated with calibrating the molecular clock, the overriding problem is the illogical circular reasoning surrounding the whole process (figure 3). Molecular genetic clocks are calibrated by fossils that are themselves calibrated by their sedimentary rock formations and the sedimentary rock formations are calibrated by the fossils. Ultimately, the presupposition and assumption of evolution is the overriding paradigm—calibrating everything. Even honest evolutionists will occasionally admit to this, as explained by Schwartz and Maresca in their 2007 paper titled: "Do molecular clocks run at all? A critique of molecular systematics":[92]

> "Although molecular systematists may use the terminology of cladism, claiming that the reconstruction of phylogenetic relationships is based on shared derived states (synapomorphies), the latter is not the case. Rather, molecular systematics is (largely) based on the assumption, first clearly articulated by Zuckerkandl and Pauling (1962), that degree of overall similarity reflects degree of relatedness. This *assumption* derives from interpreting molecular similarity (or dissimilarity) between taxa *in the context of a Darwinian model of continual and gradual change* [emphasis added]."

Summary

The paradigm of an evolutionary molecular genetic clock employs the multiple alignment of biological sequences combined with a variety of sophisticated statistical models to estimate rates of evolution among diverse taxa that creationists would consider to be completely separate created kinds. Since its first inception and use in the early 1960s, standard molecular clock methodologies routinely use deep-time calibrations taken from paleontology and assume macroevolution based on a grand tree of life. In addition to this presuppositional bias, the following problems still plague the use of the molecular clock: 1) different genes/sequences give widely different evolutionary rates (even among genes within the same genome); 2) different taxa exhibit widely different rates of change for seemingly homologous sequences; and 3) clock-derived divergence dates commonly disagree with paleontology despite the fact that deep-time calibrations are incorporated into the evolutionary clock models. Furthermore, because the molecular clock idea is directly tied to the neutral model theory of evolution, recent discoveries in full codon utility and pervasive genome-wide biochemical functionality, present serious obstacles to the evolutionary necessity of a large fraction of the genome being 'junk'.

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