Molecular clock

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Dating events in the past using modern genetic evidence remains one of the key goals of molecular anthropology. Accurate estimates of when past events occurred are crucial to place them in their appropriate anthropological context, as referenced by related subject areas, such as archaeology and linguistics. Importantly, however, dating extends beyond simply pinning times on events. Uncertainty in genetic dating often lies at the heart of arguments around contentious aspects of human history, notably the timing of the first modern human expansions out of Africa (see OUT OF AFRICA HYPOTHESIS). Dates matter, and inaccurate dating limits how we interpret and understand human history.

The discovery of the molecular clock, and the role that molecular evidence can play in assigning dates to past events, can be traced back to the 1960s. Zuckerkandl and Pauling (1962) proposed that DNA acquires mutations in a regular fashion, allowing sequence divergence to be used as a proxy for time. Sarich and Wilson (1967) applied this logic just five years later to conclude that humans and chimpanzees diverged approximately five million years ago, overturning the widespread belief that humans and our nearest primate species are only distantly related. Contemporary fossil evidence from Africa, notably the discovery of the *Australopithecus afarensis* specimen "Lucy," appeared soon afterwards and supported this claim. In part due to these early successes, molecular dating has since flourished, although the approaches used today are vastly more complex than the initial forays of the 1960s.

Mathematical theory tells us that molecular dating is, and always will be, an uncertain science. In an ideal world, the ages of specific genetic lineages within populations and species, and how these ages vary across the genome, would be known with complete precision. A branch of mathematics known as coalescent theory has largely made this possible. One of its most important results was deriving equations that describe the mean and variance of the time back to the most recent common ancestor (commonly termed TMRCA). However, these equations show that the variance of molecular date estimates is always large relative to their means—in other words, genetic dates always have considerable uncertainty. A related conclusion is that the dates of individual genes can vary widely, even if they represent the same prehistoric event. This is particularly a problem for studies that employ only a single region of the genome for dating, such as mitochondrial DNA (see MITOCHONDRIAL DNA) or the Y chromosome (see CHROMOSOME), because dates for these regions can vary widely simply due to random effects. This is one reason why molecular anthropology is now turning rapidly to genome-scale data. Often viewed as a large homogeneous whole, the human genome actually comprises thousands of small genetic regions, each unlinked from the next by recombination, and thus representing an independent historical record that can be used to reconstruct the past. Apart from those studies that specifically aim to analyze a single gene or a sex-biased process, modern genetic studies are largely moving away from the mitochondrial DNA and Y chromosome, turning instead to genome-scale information. These studies now typically look at thousands of markers (see GENETIC MARKERS (SNPs/SATELLITES/STRs/RFLPs)) across many chromosomes simultaneously, thus allowing researchers to "average across" histories recorded at different parts of the genome. This process returns much more robust dating estimates than those available previously.

Other strong confounders arise when reconstructing dates from molecular data. Individual genes and lineages evolve at different rates (reviewed in Ho 2014), and hence these regions of the genome change at variable speeds. Some genetic regions are strongly constrained (such as the ubiquitin gene, which is almost identical in mammals and worms), while other regions are free to change much more quickly. The fast

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rate of the mitochondrial DNA control region is one of its key benefits, partly explaining its widespread use for dating events over the very recent timescale of human history. That genes evolve at different rates has been known at least since the early 1960s. This gene-specific variation demands a careful choice of genetic region when studying different aspects of human history, and, consequently, a range of loci are employed today precisely to study evolutionary processes at different timescales.

Importantly, rates may also vary among different human lineages, even for the same gene. It was originally believed that genes evolve according to a "strict" molecular clock, where all lineages change at some fixed and regular rate, much like the ticking of a metronome. There is now greater awareness that rates actually vary with generation time, metabolic rate, and the accuracy of DNA repair mechanisms. These factors are particularly influential at deep time depths, but also affect dating of younger events, such as those within species. For this reason, contemporary methods typically employ a "relaxed clock," where mutation rates are allowed to vary among lineages and different branches in a gene tree can evolve at different speeds. Although there is still active and vigorous debate about which relaxed clock methods perform best, there is a general consensus that relaxed clocks are preferable to strict clocks, even for dating recent events.

Different regions of the genome evolve at substantially different rates, even within a single lineage. This behavior is most well studied for mitochondrial DNA: the control region evolves faster than the coding regions, and the redundancy of the genetic code means that rates vary considerably between first, second, and third codon positions. Patterns of mutation can be even more nuanced. Mutations are typically clustered along the genome, rather than occurring randomly, perhaps due to localized failures of DNA repair. Mutation rates are also strongly correlated with the age of the father (but not the mother), mostly because there is a marked difference in the number of cell divisions required to produce eggs (approximately 31 cell divisions regardless of age) versus sperm (approximately 400 cell divisions at age 30 and 650 at age 40). At present, molecular dates are seldom calculated taking these complex determinants of mutation rates into consideration, but new tools are being designed to accommodate them, especially as genome sequencing continues to produce large genetic datasets apace.

An important characteristic of all molecular dating methods is that they return only the order and relative ages of events. Real chronological dates must be determined through the process of calibration. Calibrated dates are usually obtained by rescaling a tree with a known mutation rate, but unfortunately, mutation rates are never known with perfect accuracy, thus increasing the uncertainty of molecular dating. Three key methods have been developed to determine mutation rates: (1) phylogenetic comparisons, (2) archaeologically dated events, and (3) pedigrees. Each has benefits and disadvantages, and, as explained below, these methods often return different estimates of the mutation rate.

One of the most commonly used calibration points is the divergence time between humans and chimpanzees (Prüfer et al. 2012) (see PHYLOGENETICS). Mutation rates are a function of sequence divergence, which can be calculated accurately, versus the time since humans and chimpanzees diverged, which remains uncertain. The human–chimpanzee divergence event is often thought to have occurred around 6 million years ago, but molecular evidence supports values anywhere from 4 to 8 million years ago, while the fossil record places the split 6 million years ago or older, mostly due to a lack of clarity around which fossils are direct human ancestors versus sister taxa. Mutation rates calculated from phylogenetic comparisons typically assume that humans, chimpanzees, and intermediate forms have broadly similar generation intervals and that the human–chimpanzee split occurred relatively quickly. Both of these assumptions have been questioned. Nevertheless, mutation rates based on this phylogenetic comparison are still among the most widely used today.

Another approach favors much younger calibration points, typically archaeologically dated events, such as the arrival of modern humans in Australia (see AUSTRALIA, HUMAN EVOLUTION) or the first settlement of the Americas. Because these events are younger, they are often known with more precision than older events, such as human–chimpanzee divergence. Conversely, many of these calibration points remain highly

contentious in their own right, and they are therefore of questionable use for molecular dating. For instance, both early and late dates have been proposed for the settlement of Australia and the Americas. If the wrong age were used as a calibration point, any subsequent dating would be biased. Even if the date of an historic event is well accepted, making a clear connection between the event and genetic lineages can still be highly problematic. An alternative, and increasingly practical approach, uses ancient genomes (see ANCIENT DNA) as known calibration points. Sequence divergence between modern and ancient genomes is easily calculated, and many archaeological remains can be robustly dated, at least within the radiocarbon limit. Calibrations against ancient genomes are already used extensively with nonhuman systems and now show considerable promise for dating events in molecular anthropology too.

The final calibration method uses direct estimates of mutation rates from human pedigrees. Sequence divergence is calculated for individuals separated by a known number of generations, such as parent–child pairs. These must be corrected for the human generation interval, which varies widely between different global communities, but averages about 30 years (Fenner 2005). Originally, the major limitation was observing enough new mutation events to estimate the mutation rate accurately. However, with rapid decreases in sequencing costs, this problem has now largely been overcome. Identifying false positive and false negative mutations remains an important concern, because these can inflate or decrease the true mutation rate. Nevertheless, pedigree methods are rapidly supplanting many earlier approaches used for estimating mutation rates.

Applying a single mutation rate, even if it is known accurately, still cannot account for rate variation among different lineages. Some of the more popular molecular dating programs instead infer a gene tree and then fit it to multiple calibration points, which might be archaeologically dated events, ancient genomes, or other temporally well-known features. Often analyzed in a Bayesian statistical setting, calibration points are frequently provided as tight priors on particular nodes in a gene tree, thereby explicitly building uncertainty into the calibration date rather than assuming it is an exactly known value. This approach has recently been applied to linguistic variation observed across the Indo-European language family, thus showing its utility beyond genetics. Increased accuracy is gained by using multiple calibration points, but as an added benefit, molecular dates inferred in this way are often robust to one or a few calibration points that are inaccurate or even wrong.

Intuitively, it might superficially be thought that all three calibration methods should yield the same mutation rate. In practice, however, large systematic differences have been observed between the different approaches. This discrepancy is most well understood for mitochondrial DNA. Pedigree studies have estimated a mutation rate for the noncoding control region of around 7.95×10^{-7} mutations/base pair/year ($4.30 \times 10^{-7} - 10.2 \times 10^{-7}$), while phylogenetic rates instead fall around 2.14×10^{-7} mutations/base pair/year ($1.15 \times 10^{-7} - 3.60 \times 10^{-7}$). These two estimates are quite different; the pedigree rate is 3.7 times higher than the phylogenetic rate, and, surprisingly, the two distributions do not even overlap.

This difference appears to result from a behavior known as the "time dependency" of molecular rates (Ho and Larson 2006). Rates not only differ between the pedigree and phylogenetic methods, but also seem to change regularly through time, appearing to decline exponentially from fast rates in the present to slow rates in the past. This pattern probably arises because not all changes that we observe are selectively neutral. Many of the genetic regions studied in molecular anthropology, such as the mitochondrial DNA control region, are assumed to evolve neutrally, and under neutral theory, the mutation rate is determined only by the rate of spontaneous mutation, independent of population size. In practice, most parts of the genome are subject to at least some selection pressure: either purifying selection, which removes disadvantageous mutations that arise in the population; or positive selection, which sweeps advantageous mutations to higher frequency. Consequently, most mutations that occur within a population are transient and quickly lost through drift or selection. Pedigree studies count these mutations, but only a few survive long enough to contribute to sequence divergence at older time depths, such

as between humans and chimpanzees. In other words, pedigree studies measure the true mutation rate, while phylogenetic studies measure the substitution rate (that is, fixed differences between species). This distinction likely explains at least part of the apparent discrepancy between pedigree and phylogenetic rates.

Moreover, mutations are only independent of population size if they are neutral or at least nearly neutral. When selection is acting, changes in population size can also alter the observed mutation rate. Even for neutral markers, population demography—founder events, bottlenecks, and population structure—can alter the shape of gene trees, and hence the rate at which mutations arise and persist in a population. Unfortunately, these demographic features were all common in human prehistory, thus adding yet more uncertainty to molecular dating estimates.

With rapid advancements in DNA sequencing technologies, genome-wide data at population scales is becoming more common in molecular anthropology (see GENOMES). Studies of mutation rates are consequently moving away from mitochondrial DNA, and instead focusing on the nuclear chromosomes. Genome-wide data—initially short regions sequenced across the genome, but now often complete genome sequences—are increasingly available. Phylogenetic rates for autosomal regions, calibrated by fossil evidence for the divergence between humans and orangutans, or humans and macaques, typically fall around 1×10^{-9} mutations/base pair/year. In contrast, pedigree studies return rates around 13×10^{-9} mutations/base pair/generation ($9.7 \times 10^{-9} - 18.5 \times 10^{-9}$), or assuming a human generation interval of 30 years, 0.43×10^{-9} mutations/base pair/year ($0.32 \times 10^{-9} - 0.62 \times 10^{-9}$). A 2.3-fold discrepancy is again observed between these two rates, but in striking contrast to the mitochondrial DNA results, the pedigree rate is now lower than the phylogenetic rate. When calibrated against a range of primate divergences (in descending order: macaques, orangutans, gorillas, and chimpanzees), a strong trend of lower mutation rates toward the present is observed, a process that has been termed the "hominoid slowdown" (Goodman 1961). Superficially, it appears that mutation rates have decreased during the evolution of humans and the great apes. There is still considerable debate about whether this finding is true, with alternatives such as errors in fossil divergence estimates, higher effective population sizes, longer generation intervals, false negatives leading to missing mutations, and the sheer complexity of the mutation process all being proposed. Regardless, the pattern is a reminder that there is still much to learn about mutation processes, even in extremely well-studied species like humans.

New forms of molecular dating have even recently been developed that do not require mutation rates at all. A key feature of human history is extensive admixture (see ADMIXTURE) between long separated groups, which on intermarriage produce offspring with chromosomes from each of the parent populations. Recombination creates mosaic chromosomes with ancestry blocks from each parent population, and as recombination events accumulate over time, these ancestry blocks fragment to become progressively smaller. The time of population admixture can be inferred from the distribution of block sizes, assuming that the recombination rate is known. While this technique is currently limited to dating admixture events, it emphasizes the potential for new ways of molecular dating to be discovered. Nevertheless, any new methods are unlikely to provide a simple panacea to the issues raised above. Like mutation rates, recombination rates are increasingly found to be variable between species, populations, and individuals. A growing body of evidence also shows that recombination rates can change over time. While new dating methods are always welcome, attempts to date past events using patterns of recombination simply emphasize the fact that many sources of uncertainty in molecular dating are generic, and therefore will likely require common solutions.

Molecular dating has come a long way from the strict molecular clocks and relatively simple metrics of the 1960s. Complex analyses are now routine, key sources of uncertainty have been identified, and, in many cases, reasonable practical solutions have been developed to address them. Nevertheless, dating past events using molecular evidence is by no means a solved problem. Ongoing research shows that we know less about mutation rates than perhaps we once thought; new sources of variation are being

discovered, and old sources are being revisited in the light of new genomic information. Uncertainty at the heart of molecular dating, especially around mutation rates, is causing considerable upheaval in the interpretation of major anthropological questions. Many of these fundamentals of molecular dating are areas of active research and are likely to remain so for the foreseeable future.

SEE ALSO: Population structure

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