Human Evolution: Turning Back the Clock

The timing of human evolution can be inferred from DNA sequence comparisons, but this requires an accurate estimate of the mutation rate. While recent data suggested a lower rate and a longer timeline, a new study reinstates the previous timeline.

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How long ago did the deepest divergences in the human family tree take place? Are Neandertals and Denisovans the descendants of the *Homo heidelbergensis* fossils found in Eurasia? Did the first anatomically modern humans leave Africa as recently as 60 thousand years ago, (kya) or as early as 130 kya? Genetic analysis offers the promise of augmenting archaeological and paleontological analysis in addressing these questions. However, an accurate interpretation of genetic data often requires knowing a conceptually simple number: the mutation rate — the rate at which DNA sequences accumulate differences through time. Previous estimates of the mutation rate have recently been challenged by analysis of complete genome sequences of parents and their offspring to directly observe a per-generation mutation rate [1]. These pedigree-based estimates imply a rate that is about half of what was previously thought. This lower rate would require pushing back the time estimate for key population splits in human evolutionary history. For example, pedigree-based rates indicate that the common ancestor of all modern humans lived 250–300 kya [2], much older than traditional estimates of 120–200 kya [3]. In this issue of *Current Biology*, Fu and colleagues [3] take a different approach, and estimate the evolutionary rate of human mitochondria using DNA recovered from human fossil bones that have been radiocarbon dated. Their conclusions are in general concordance with the traditional timeline of recent human evolution.

The rate at which DNA sequence changes through time is often called the molecular clock and has traditionally been inferred using what are known as phylogenetic methods (Figure 1): DNA sequences are compared between two or more species to determine the proportion of positions along the sequence at which the two species differ. Humans and chimpanzees, for example, differ at about 1.37% of their nuclear genome [4]. How long did evolution take to accumulate this amount of sequence divergence? A well-dated fossil that clearly lies on one lineage or the other can establish a time by which divergence must have happened at the latest, thereby yielding a rate of sequence change per unit time.

In practice, there are several well-known uncertainties inherent to this approach [5]. First, the fossil or geological calibration must be correctly dated and interpreted. Within primates, the ape–old world monkey split [6], the split of orangutans from other apes [7] and the 7 million year old *Sahelanthropus* fossil, often cited as the earliest hominin fossil [8], have all been used as calibration points. Yet, the precise ages of these fossils and their phylogenetic placement are not free of uncertainty. Second, one must assume that the molecular clock was, as it were, ticking at a constant speed since the lineages diverged. This assumption, however, may not hold for all primate lineages. Some primate genomes, including apes, show evidence for a rate that has slowed recently [9]. The extent to which this is due to lineage-specific effects, such as longer generation times, is difficult to discern. Finally, the observed divergence itself can be difficult to measure due to multiple mutations occurring at the same position in the genome, and failure to correctly identify and align orthologous sequences.

In theory, high-throughput sequencing within known pedigrees provides a way to avoid the uncertainties of the phylogenetic approach. As reviewed in Scally and Durbin [2], several recent studies have directly compared the genomes of parents and their children, counting the number of *de novo* mutations that arise in a single generation (Figure 1B). Several interesting features have been observed with this approach: for instance, the mutation rate from the father, but not the mother, is positively correlated with age [10], and the per-generation mutation rate is much lower than was previously estimated using phylogenetic methods [1].

Taken at face value, a lower mutation rate has profound implications for timing key events in human history, as more time will be required for the observed number of mutations to occur [2,11]. For example, the degree of genetic divergence or allele sharing that exists between currently living humans of African versus non-African ancestry can be used to infer when humans dispersed out of Africa [12,13]. A slower rate of DNA sequence change implies that this happened more than 100 kya, longer ago than was traditionally thought. Importantly, this earlier dispersal would allow for the possibility that the anatomically modern human remains found at Skhul in Isreal were part of the main human out-of-Africa dispersal and not an unassociated preamble to it.

Sequence comparison between humans and Neandertals allows an estimate of the population split that led to these groups as well [14]. A slower rate implies that the previous estimate of 270–435 kya may be too recent, and the split may have instead occurred 400–600 kya [2]. The older date would more easily allow for the possibility that *H. heidelbergensis* or other archaic hominins in Eurasia could, in fact, be the ancestors of Neandertals — a point of abiding uncertainty [15].

An innovative approach to estimating evolutionary rates has emerged with the growing number of DNA sequences isolated from fossil remains. In particular, organisms that lived within the last 40,000 years — the range for which reliable radiocarbon dates can be obtained with reasonable precision — can provide both a measure of DNA sequence divergence and a time calibration [16]. This approach, which has been variously called ‘tip-calibration’, ‘external calibration’, or ‘branch shortening’, relies on the assumption that mutations will arise approximately constantly

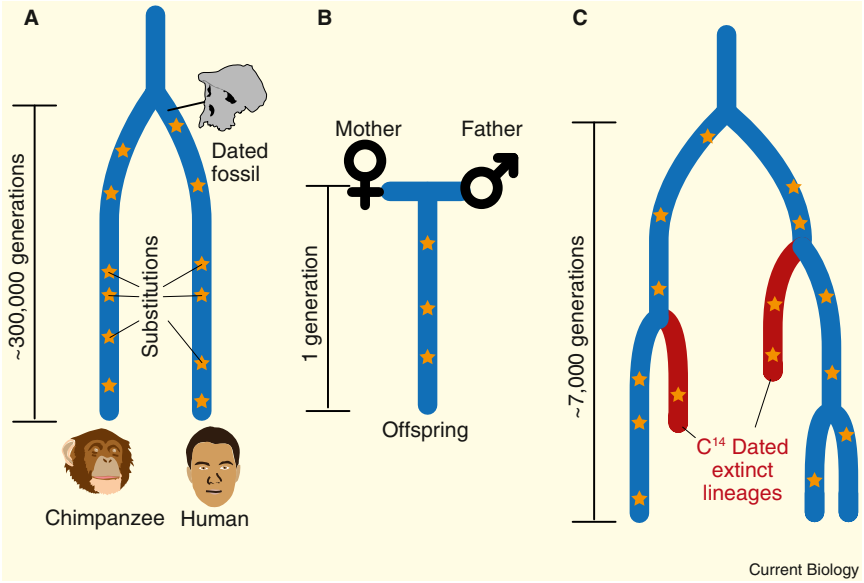

Figure 1. Calibration schemes for inferring the rate of molecular evolution.

(A) Pairwise comparison of DNA sequence between species, humans and chimpanzee, for example, yields a measure of sequence divergence. This divergence can be converted to a rate of change per year using a well-dated fossil that lies at the base of one lineage or the other. (B) Alternatively, per-generation rates of sequence change can be inferred with sequence data across a multi-generational pedigree. Inexplicably, these rates have been observed to be slower that phylogenetic-inferred rates, in humans. (C) As demonstrated by Fu *et al*. [2], mitochondrial DNA recovered from well-dated human fossil material can be used to calibrate a rate of sequence change within the human mtDNA tree. They infer the time of the most recent common ancestor of the human mtDNA tree to be 157 kya (120–197).

through time along each lineage in a genealogy. Lineages leading to ancient samples, therefore, will be missing whichever mutations would have happened between the time when the organism lived (the age of the sample) and the age of the youngest sample in the tree (often the present day) (Figure 1C). As evolution is a stochastic process, not all data sets that include ancient sequences can be expected to be temporally informative, especially at loci that may be subjected to bouts of strong selection, including mitochondrial DNA [17]. However, when the age difference between the oldest and youngest sequences is large relative to the evolutionary rate, this approach can provide remarkably consistent estimates of evolutionary timescales [18].

Fu and colleagues use this approach to infer an evolutionary rate and associated timescale within the well-resolved human mitochondrial (mtDNA) phylogeny [3]. They combine complete mtDNA sequences of dozens of extant humans with complete or nearly complete mtDNA sequence from ten ancient samples that fall within the phylogeny of modern humans. Importantly, the bones from which the DNA was extracted range in age from 700 to 40,000 years, and the ages of each of these are known with high confidence. Using two approaches to estimate the amount of sequence evolution that is missing from these ancient lineages, they infer a mutation rate within the human mtDNA phylogeny that is remarkably consistent with previous, phylogeny-based estimates [19]. They go on to calculate that mtDNA haplotypes that are unique to non-Africans diverged from the most closely related haplogroup present within Africa (L3) around 78.3 kya (62.4–94.9). While this is not a direct estimate for the time of human dispersal out of Africa, it provides an upper limit. Importantly, this upper limit is more consistent with previous estimates using nuclear genome data and the faster, phylogeny-informed mutation rates [20].

These results lead to new questions, some of which may soon be possible to answer. Regardless of which time-scale of human evolution is correct, why is it that measurements of the per-generation mutation rate are lower than phylogenetic rates? Possible answers include scenarios in which both measurements are correct. For example, averaging over the millions of years of a phylogeny may obscure the dynamics that occur at much shorter, i.e. generational, time-scales. High-quality nuclear genome sequence from well-dated archaic humans that fall within the human family tree may provide a way to address this question.

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