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NEWS AND COMMENTARY

Clock debate: when times are a-changin'

Time dependency of molecular rate estimates: tempest in a teacup

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Evolutionary geneticists and molecular anthropologists rely on solid knowledge to underpin the molecular dating that is used in their interpretation of molecular variation between or within species. In particular, they require reasonable calibration of mutation rates and a model of a molecular clock that is not too elusive. Ho *et al*. (2005) have apparently shaken the confidence of the field, by claiming an exponential decay law that is at odds with the conventionally assumed linear relationship between genetic differences and time. However, this study was beset with several problems (Bandelt *et al*., 2006; Emerson, 2007), which have not been resolved convincingly (Ho *et al*., 2007).

Three kinds of mitochondrial DNA (mtDNA) data sets (along with their calibration points) were selected by Ho *et al*. (2005) to bear witness to an exponential decay law, spanning a range from one generation time (of pedigrees) to more than 25 million years ago. One series employed the first hypervariable segment of the human mtDNA control region, which has been popular with molecular anthropologists as a multi-purpose marker—but which does not contain sufficient phylogenetic information for tens or, let alone, hundreds of thousands of years. Thus, Ho *et al*. (2005) took a teacup, so to speak, of mud—mainly constituting very old data of problematic quality—from a lake of thousands of partial and complete human mtDNA sequences that were available in 2005.

The seemingly stark contrast between a 'phylogenetic rate' for long-term evolution and a 'pedigree rate' has always been the main impetus for postulating a decay law (Bandelt *et al*., 2006). The pedigree rate for mtDNA is, however, not well-defined since every individual harbours a whole spectrum of closely related variants, typically including a single predominating mtDNA sequence. Most mutations seem to emerge and proliferate through a phase of heteroplasmy over the course of several generations. This within-individual variation means that it is quite hazardous to infer an instance of mutation between the mtDNA samples of mother and child, at least, in the absence of single-cell analysis. The best way forward may, therefore, be to follow a conservative strategy of screening large pedigrees with multiple generations: the careful study by Sigurdarðóttir *et al*. (2000) settled on a rate that is about twice as fast as the conventional (phylogenetic) rate but has a wide confidence interval. This discrepancy is not really alarming and certainly does not support claims that the 'pedigree rate' is an order of magnitude faster.

Much of the dispute between Emerson (2007) and Ho *et al*. (2007) centres around the question of how the data should be subjected to recently developed Bayesian methods. This issue simply underscores that much more care is needed when those tools are applied to real data, and it is not even clear whether much is gained from a Bayesian approach, in this case. There is often a striking imbalance between the poor choice of data and models and the enormous efforts that are undertaken to treat the data with fashionable software. This is somewhat reminiscent of the innocent application of coalescent methods to tiny data sets such as the Nuu-Chah-Nulth data set in the early 1990s (Lundstrom *et al*., 1992). Curiously, the same data set has still been adduced by Ho *et al*. (2005, 2007) for their decay law, although with unsustainable hypotheses leaving out phylogeographic insight.

In fact, the Nuu-Chah-Nulth mtDNA tree encompassing lineages from all major Native American mtDNA haplogroups (A2, B2, C1, D1 and X2a) probably has, at its deepest level, perhaps 20 ky (thousand years) of East African history, then roughly 20 ky of southern coastal Asian plus some 20 ky of (North-) East Asian history and finally a mere 20 ky of Beringian and American history—given conventional rate estimates and a series of calibration points (Kivisild *et al*., 2006a, and references therein). Phylogeography thus dictates that most parts of the entire mtDNA tree of Amerind populations evolved outside the Americas. It is therefore understandable that artificially squeezing time estimation for the deeper parts produces rate estimates that are not consistent with archaeological traces of modern humans out of Africa more than 50 ky ago. Thus, the conventional time estimates based on human mtDNA do not require a drastic rethink but do need additional systematic precision, which for example could allow us to distinguish more reliably between an event that was 20 ky ago from one that was 15 ky ago.

What then is the remaining evidence for strong time dependence of mtDNA mutation rates after the exchange of arguments and reanalyses by Emerson (2007) and Ho *et al*. (2007)? Not much. The approach of Ho *et al*. (2007) was to 'take four published mutation rate estimates at face value'. Well, this is the crux of the matter: one should never take any result in this field at face value in view of omnipresent laboratory artefacts and ill-applied methodology (Bandelt *et al*., 2006). For instance, substitution rate and divergence rate have occasionally been confounded in this context. Many published pedigree analyses seem to suffer from an ascertainment bias and a thin layer of sequencing errors, which have a large effect on the estimated rate via extrapolation.

An important aspect that really deserves further investigation is that the pattern of synonymous and non-synonymous coding-region mutations does not appear to be uniform across different levels of the mtDNA phylogeny (Elson *et al*., 2004; Kivisild *et al*., 2006b; Howell *et al*., 2007). Evidence seems to point to the presence of purifying selection, in that young terminal branches show a relative excess of non-synonymous mutations. On the other hand, some frequent synonymous mutations in the coding region as well as many transitions in the control region may come close to saturation in the deepest parts of the human mtDNA phylogeny (Bandelt *et al*., 2006), leaving aside occasional violations of standard model assumptions. Careful bookkeeping of a large number of reliable complete mtDNA sequences sampled across all major sections of the mtDNA phylogeny is indispensable for any progress in our understanding of the mutational process. A distinction between several categories of sites and mutations (Howell *et al*., 2007; Ingman and Gyllensten, 2007) is mandatory

before any sort of time dependence of estimated rates can be formulated and evaluated.

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