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## NEWS AND COMMENTARY

Molecular clock debate

# Time dependency of molecular rate estimates for mtDNA: this is not the time for wishful thinking

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We must respond to the recent Commentary by our colleague Dr Bandelt (Bandelt, 2008). In brief, he is highly critical of the concept that rate estimates of mtDNA sequence evolution are time dependent and fit an exponential decay model (Ho *et al*., 2007). This is a complex issue, but we argue that many of Dr Bandelt's arrows miss the target.

Dr Bandelt believes that human mtDNA sequences have evolved according to some simple clock-like process that allows highly accurate and reliable time estimates for coalescent events during human evolution (a molecular 'stopwatch'). In his Commentary, he calls for greater precision of the mtDNA clock than is feasible, such that one can accurately differentiate events that occurred 15 ky ago from those that occurred 20 ky ago. This wishful thinking ignores two realities. Firstly, there is a steady accumulation of reports that human mtDNA does not evolve in a clock-like manner (Howell *et al*., 2007 and references therein). Sequence sets that 'pass' a robust clock test are the exception. Secondly, we lack adequate calibration points for accurate time estimates, even if there were a human mtDNA clock (Pulquério and Nichols, 2007).

The criticism is made that Ho *et al*. (2007), analyzed human mtDNA hypervariable region I sequences, and—according to the current view of Dr Bandelt—this segment of the control region does not contain sufficient phylogenetic signal for robust analysis. However, time dependent rate variation is also seen in analyses of the mtDNA coding region (Ho *et al*., 2007; see also further comments below).

Dr Bandelt again takes the opportunity to dismiss the discrepancy between mtDNA rate estimates from pedigree analyses and those from phylogenetic analyses. We disagree that the pedigree rate is not well defined. It has an explicit operational definition and is, in fact, more empirical and less model-dependent than phylogenetic rate estimates (Howell *et al*., 2003). Dr Bandelt also makes the unsubstantiated charge that pedigree analyses '…seem to suffer from ascertainment bias and…sequence errors…'. We cannot find evidence for either and the issues he raises have been addressed previously (Howell *et al*., 2003). On the other hand, it is Dr Bandelt who has concluded that many mtDNA sequence sets, often used for phylogenetic analyses, contain a high proportion of errors.

Despite his criticisms, Dr Bandelt eventually admits that there is a discrepancy in the rate estimates, but that it is not an order of magnitude. Our meta-analysis confirmed that the pedigree rate was less than one set of phylogenetic rates by an order of magnitude (Howell *et al*., 2003). A more significant problem is that phylogenetic rate estimates vary widely, something that should trouble 'stopwatchers'. It is worth noting at this point that a three- to fourfold pedigree/phylogenetic discrepancy has been observed for rate estimates of the Y chromosome microsatellite sequences (Zhivotovsky *et al*., 2006 and references therein). Our disagreements with Dr Bandelt on these technical issues are important, but they should not detract from the point of general significance: the pedigree rate of substitution is significantly less than the molecular rate of mtDNA mutation but greater than the 'zone' of phylogenetic rate estimates. Why would there be any difference between rate estimates, if there is a simple mtDNA molecular clock?

Selection has been a major force acting on mtDNA evolution and this finding has profound implications for the operation of an mtDNA clock. Some investigators have suggested a role for positive selection, but it is not discussed further, because such a role has failed to obtain support. Instead, here, we focus on purifying (negative) selection and its consequences for the rate of sequence evolution. While purifying selection operates at the level of the germline (Stewart *et al*., 2008), it does not act instantaneously, and, instead, a substantial proportion of slightly deleterious mutations are lost continuously from the mtDNA gene pool over a prolonged period of time (Elson *et al*., 2004; Kivisild *et al*., 2006; Howell *et al*., 2007; Elson *et al*., submitted for publication). As a result of this selection acting throughout the human mtDNA phylogenetic tree, relatively more mutations have been lost in older branches (for example, mtDNAs from Africans) than in younger branches (for example, mtDNAs from Europeans). Dr Bandelt also refers to these results in his Commentary, but he does not 'connect the dots' and point out that the continuous loss of mtDNA mutations on a similar timescale as human evolution will necessarily result in time-dependent rates of substitution.

It must be emphasized, finally, that the case for a 'slow' process of purifying selection, one that leads to time-dependent rates, does not rely on measurements of substitution rates in pedigrees. The latest example is the impressive study of fish mtDNA evolution (Burridge *et al*., 2008) where time-dependent rates are observed and , at least in part, due to the operation of purifying selection. However, pedigree rates do offer us an important insight to the early phase of selection (and other important evolutionary processes such as random genetic drift and bottlenecks) and this is why we must note here our disagreements with Dr Bandelt.

Our comments must also include the caution that there is much that we do not understand about the sequence evolution of human mtDNA. (a) The decay curve of mtDNA substitution rates needs greater precision (see especially Burridge *et al*., 2008). (b) Purifying selection appears to play a major role but the issue of positive selection remains unresolved. (c) Random genetic drift is also a prominent feature of human mitochondrial genetics, largely due to the germline bottleneck (Cree *et al*., 2008). There is a conundrum, because, according to standard population genetic models, drift tends to minimize or diminish the effects of purifying selection. (d) It is remarkable that the mtDNA control and coding regions do not appear to have evolved in lockstep (Howell *et al*., 2007), and the reasons for this 'decoupling' warrant investigation.

For more than a decade, Dr Bandelt has been wholehearted in his efforts to

simplify mtDNA evolution and, especially, to champion the use of simple mtDNA clocks. It is our contrary view, based both on our research and that of many other groups, that mtDNA evolution is not clock-like and that the evidence for time-dependent rates should not be dismissed. When it comes to mtDNA, one should not use a sundial as a stopwatch.

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## Editor's suggested reading

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