Additional File 2 (Supplementary Background)

Supplementary Background. Mitochondrial Replication and Transition Biases.

The mt genome has long been believed to replicate asymmetrically [1], which strongly contributes to the substantial difference in mutation rates and nucleotide composition biases between strands [2-6]. During replication under the classical model, the synthesis of the nascent heavy strand initiates at the origin of heavy strand replication (O_H), within the control region (CR). This has been reviewed extensively elsewhere [2, 7], but in brief, after two thirds of the nascent heavy strand is synthesized, the synthesis of the nascent light strand starts at the origin of light strand replication (O_L), a short secondary-structure-forming segment located within the tRNA cluster (the WANCY region) between the NADH dehydrogenase subunit 2 (ND2) and Cytochrome C oxidase subunit 1 (COX1) genes. The strand-asymmetric replication mechanism has been thought to expose different regions of the parental heavy strand to varying amounts of time in the single-stranded state during replication (D_{ssH}; [6]), depending on the distances of the regions from the O_H and O_L. Variation in this strand-asymmetric replication process appears to have contributed substantially to variation in substitution rates among genes [2, 7, 8].

Controversy has arisen recently over the classical mt genome replication mechanism, mostly concerning the asymmetry of the process, the role of the putative origin of light strand replication, and whether the replicating DNA spends substantial amounts of time single-stranded [9-11]. Although newly proposed models of replication that embody these concerns are directly at odds with the genetic data, one of us has hypothesized

(Pollock, *in review*) that most of the biochemical and genetic data are compatible with a reconciled model of mt genome replication, which retains most critical features of the classical model except for single strandedness. Regardless of the final reconciliation, here we take a neutral position on the biochemical issue of single-strandedness by referring to the time that a gene or nucleotide is predicted to spend in an asymmetric mutagenic state (T_{AMS}), rather than the predicted duration of time that the heavy strand spends single-stranded (D_{SSH}); the calculation of T_{AMS} is, however, identical to that for D_{SSH} [6, 7, 12].

Cytosine →Uracil deaminations are common in single-stranded DNA, while Adenine → Hypoxanthine deaminations are less common [13, 14]. These two deaminations lead to mutations (Cytosine→Thymine and Adenine→Guanine, or C→T and A→G) that appear to account for most of the asymmetry in synonymous substitutions found in vertebrate mt genome [7, 8, 12, 15-19]. C→T and A→G mutations on the heavy strand during replication apparently lead respectively to G→A and T→C substitutions (and G and T deficiencies) on the light strand. Most protein-coding genes (all but ND6) use the heavy strand as a template; thus, the mutation biases observed in the light strand parallel the biases in most protein-coding gene transcripts. Faith and Pollock [7] found that, in vertebrates, T→C light strand substitutions at four-fold and two-fold redundant 3^{rd} codon positions increase linearly with increasing T_{AMS}. In contrast, G→A light strand substitutions increase rapidly but quickly reach a maximal level. Consequently, T→C substitutions and the resultant C/T nucleotide frequency gradient are good predictors of T_{AMS}.

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