

will hopefully lead to rapid progress for other species.

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Response to Kondrashov

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The 'mutational deterministic' hypothesis proposes that a high genomic rate of deleterious mutation (U) might maintain sexual reproduction. Our recent work casts doubt on this, as we estimate a low U for sexually reproducing species with short generation times. Following criticism by Kondrashov, here we defend our methods for estimating U and challenge the mutational deterministic hypothesis.

The rates and effects of deleterious mutations are indeed important issues, not least because they are central to the understanding of why sexual reproduction is maintained in so many species. Sexual reproduction seems to be favoured over asexual reproduction when the number of deleterious mutations per diploid genome per generation (U) exceeds ~ 1 event per generation¹, or ~ 2 events if newly arising asexual populations are not assumed to be mutation free² and finite population sizes³ are taken into account. It has therefore been suggested that high genomic rates of deleterious mutation might maintain sexual reproduction, a theory known as the mutational deterministic (MD) hypothesis.

Recently, we estimated U for point mutations in protein-coding sequences for a range of taxa, on the

basis of molecular divergence at synonymous sites between nine pairs of species⁴. We observed a linear relationship between U and the estimated generation time. Estimates of U for species with short generation times were as low as 0.05, whereas U for species with long generation times, such as long-lived mammals, were above two. We argued that the low estimates of U for protein-coding sequences in taxa with short generation times cast doubt on the MD hypothesis as a general explanation for the maintenance of sex.

Kondrashov's principal criticism of our work is that we have 'ignored selection acting on synonymous coding sites'. This would indeed be a serious problem if it were so, since selection has the potential to depress the substitution rate significantly, and hence reduce the estimate of the mutation rate. However, we did not ignore selection on synonymous codon use in the datasets for which there is evidence of selection, namely the *Drosophila* datasets. To remove the potential effects of selection on the synonymous substitution rate (K_s), we regressed the K_s value for each gene against a measure of synonymous codon bias, taking the Y-axis intercept as the estimate of the substitution rate in the absence of selection: this was the estimate of the nucleotide mutation rate. For one of the *Drosophila* datasets, the regression was significant and gave a corrected estimate of the substitution rate that was ~50% greater than the uncorrected estimate. For the other datasets, we found no evidence of a correlation between K_s and codon bias.

We believe our estimates to be accurate for several reasons. First, the evolution of synonymous codon use is well understood in *Drosophila*. Selection is apparently weak⁵, and weak selection is predicted to have little effect on the rate of synonymous substitution⁶. Second, an estimate of the nucleotide mutation rate in *Drosophila*, obtained under an explicit model of synonymous codon selection, was very similar to ours⁷. And third, a recent analysis that corrects for the effects of codon bias on the estimate of K_s , indicates that K_s is not correlated to the level of codon bias⁸ and, hence, that selection does not affect the synonymous substitution rate (the

correlation we observed was probably caused by a downward bias in the substitution rate estimate in genes with high codon bias).

The evolution of synonymous codon use in mammals and birds is not yet fully understood. Although there is some evidence that either selection or biased gene conversion (BGC) is acting upon synonymous codon use in mammals⁹, it seems unlikely that selection or BGC can explain the variation in our genomic mutation rate estimates in mammals and birds, because most birds and mammals have very similar levels of codon-usage bias; we therefore infer that they are subject to similar levels of selection or BGC. The exception is rodents, which actually show lower levels of synonymous codon bias than primates¹⁰, suggesting that selection (or BGC), is weaker in rodents than primates. Why then, as Kondrashov points out, are rates of visible mutation similar in humans and mice, given that the DNA-sequence-based estimates differ by tenfold? It seems probable that this is due to biased sampling of the genes used to measure visible mutation rates¹¹. The genes for which rates of visible mutation have been estimated are those that produce visible mutations at appreciable rates. Most loci do not produce a visible phenotype when mutated so have not been included in the sampling, whereas the most mutable loci have mutation rates that approach 10^{-4} . Given this variation, a valid comparison of visible mutation rates between mouse and humans will need to be for homologous loci.

We believe that our estimates of genomic deleterious point mutation rates in protein-coding sequences are soundly based, and most likely imply that the MD hypothesis is incorrect as a general explanation for the maintenance of sexual reproduction. Only two possibilities could rescue the hypothesis: (1) the vast majority of deleterious mutations occur outside protein-coding sequences (however, this does not appear to be the case in *Caenorhabditis*¹², for example), or (2) transposable elements cause large numbers of deleterious mutations in both sexual and asexual species (yet asexuality might suppress the deleterious effects of transposable elements⁴).

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