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Quantifying the genomic decay paradox due to Muller's ratchet in human mitochondrial DNA

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Summary

The observation of high mitochondrial mutation rates in human pedigrees has led to the question of how such an asexual genetic system can survive the accumulation of slightly deleterious mutations caused by Muller's ratchet. I define a null model to quantify in unprecedented detail the threat from extinction caused by Muller's ratchet. This model is general enough to explore the biological significance of Muller's ratchet in various species where its operation has been suspected. For increased precision over a wide range of parameter space I employ individual-based simulations run by evolution@home, the first global computing system for evolutionary biology. After compiling realistic values for the key parameters in human mitochondrial DNA (mtDNA) I find that a surprisingly large range of biologically realistic parameter combinations would lead to the extinction of the human line over a period of 20 million years – if accepted wisdom about mtDNA and Muller's ratchet is correct. The resulting genomic decay paradox complements a similar threat from extinction due to mutation accumulation in nuclear DNA and suggests evaluation of unconventional explanations for long-term persistence. A substantial list of potential solutions is given, including compensatory back mutations, mutation rate heterogeneity and occasional recombination in mtDNA. Future work will have to explore which of these actually solves the paradox. Nonetheless, the results presented here provide yet another reason to minimize anthropogenic increase of mutation rates.

1. Introduction

(i) *Mitochondrial biology*

Mitochondria have always been very important for eukaryotes ever since they crossed the line from endosymbiont to organelle (Gray *et al*., 1999; Kurland & Andersson, 2000; Dyall *et al*., 2004; Timmis *et al*., 2004). The size of sequenced mitochondrial DNA (mtDNA) varies substantially among the kingdoms of life, with extremes such as 6 kbp in malaria-causing *Plasmodium* and 480 kbp in rice (some plants even exceed that: Burger *et al*., 2003). In plants, very slow sequence evolution is combined with a large degree of structural flexibility and frequent recombination (Wolfe *et al*., 1987; Palmer & Herbon, 1988; Knoop, 2004). In animals, mtDNA is generally about 15–20 kbp long and rarely rearranged; most vertebrates share the same arrangement of 37 genes that is known from the 16·57 kbp of human mtDNA (Boore, 1999). These genes contain 13 protein-coding genes that play a central role in achieving the main purpose of mitochondria, which is to supply the cell with energy (Saraste, 1999); all other genes help encode the non-standard translation machinery of mitochondria. Production of these 13 proteins in mitochondria seems to have functional reasons (Allen, 2003; Timmis *et al*., 2004) that include improved redox control (Allen, 2003) and problems with the import of large hydrophobic proteins (von Heijne, 1986; Claros *et al*., 1995; Daley *et al*., 2002). The central role of these proteins in producing the cell's energy suggests that there

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is strong selection for keeping mtDNA close to its optimum, as functional deviations are likely to affect the agility and therefore the fitness of animals. Indeed, evidence for non-neutral evolution was found in mtDNA (Ballard & Kreitman, 1994; Rand & Kann, 1998; Gerber *et al*., 2001) and the large number of hereditary diseases in humans caused by deviant mtDNA suggests a similar conclusion (Wallace, 1999; MITOMAP, 2006).

(ii) *Mitochondrial population genetics*

Experimental ease led to the frequent use of animal mtDNA in phylogenetic studies (Brown *et al*., 1979; Strauss, 1999; Gerber *et al*., 2001; Arbogast *et al*., 2002) and it is usually treated as a neutrally evolving haploid maternally inherited genome (Yaffe, 1999; Birky, 2001) to avoid complications during the analysis. Our understanding of the evolution of this molecule lacks a treatment of the combined effects of (i) its multi-level population genetics (Birky *et al*., 1983; Takahata & Slatkin, 1983; Bergstrom & Pritchard, 1998; Birky, 2001; Rand, 2001), (ii) heteroplasmy (Rand, 2001), (iii) mutation rate heterogeneity (Pääbo, 1996; Meyer *et al*., 1999; Arbogast *et al*., 2002; Hagelberg, 2003; Galtier *et al*., 2006), (iv) selection (Ballard & Kreitman, 1994; Rand & Kann, 1998; Gerber *et al*., 2001) and (v) possible existing very low rates of recombination (Awadalla *et al*., 1999; Birky, 2001; Ladoukakis & Zouros, 2001; Innan & Nordborg, 2002; Gandolfi *et al*., 2003; Hagelberg, 2003; Rokas *et al*., 2003; Knoop, 2004; Kraytsberg *et al*., 2004; Piganeau *et al*., 2004; Tsaousis *et al*., 2005; Galtier *et al*., 2006).

(iii) *Human mtDNA*

Humans show no signs of recombination of mtDNA in the germline so far (Innan & Nordborg, 2002), despite an intense search for such evidence. The closest species that show signs of recombination belong to the Old World monkeys (Piganeau *et al*., 2004), which diverged from the human line about 23 or 31 million years (Myr) ago (Takahata & Satta, 1997; Glazko & Nei, 2003). If the evolutionary line leading to humans has evolved for such long periods of time without recombination, then the possibility exists that the fittest lines of descent in mtDNA could be lost stochastically due to the accumulation of slightly deleterious mutations. This process is called Muller's ratchet (Muller, 1964; Felsenstein, 1974).

(iv) *Muller's ratchet*

Deleterious mutation accumulation due to Muller's ratchet is usually discussed as a potential cause for the evolution of recombination (Maynard Smith, 1978; Kondrashov, 1993; Barton & Charlesworth, 1998; West *et al*., 1999), the degeneration of Y chromosomes (Charlesworth, 1978; Charlesworth & Charlesworth, 2000) or the extinction of asexual genetic systems (Gabriel *et al*., 1993; Lynch *et al*., 1993). While Muller's ratchet is relatively easy to explain as a problem (Haigh, 1978; Maynard Smith, 1978), its mutation-accumulating behaviour is notoriously hard to understand quantitatively (Gabriel *et al*., 1993; Higgs & Woodcock, 1995; Baake & Gabriel, 1999; Gordo & Charlesworth, 2000*b*; Stephan & Kim, 2002; Rouzine *et al*., 2003). The stochastic theory of Muller's ratchet is related to the deterministic theory of quasi-species error thresholds (Nowak & Schuster, 1989; Wagner & Krall, 1993; Higgs, 1994; Baake & Gabriel, 1999; Anderson *et al*., 2004; Wilke, 2005).

(v) *The principle of Muller's ratchet*

The main effect of Muller's ratchet is the accumulation of slightly deleterious mutations (SDMs). This occurs if (i) recombination is absent, (ii) population size is finite, (iii) slightly deleterious mutation rates are high and (iv) purifying selection is too weak to remove all new deleterious mutations. In such populations there is a substantial probability that all fittest individuals (the fittest class) will eventually acquire a slightly deleterious mutation and therefore go extinct. This event is called a 'click' of the ratchet. After each click all previously second-fittest individuals become the new class of fittest individuals, because they carry only one additional deleterious mutation, while all other individuals carry more deleterious mutations. A population will accumulate mutations with a characteristic rate that depends on effective population size, deleterious mutation rate and selection coefficient. If these mutations decrease reproductive capacity, they can lead to the extinction of the population. Muller (1963) was the first to consider the possibility of extinction exclusively due to the ratchet, but his later main paper dismissed this possibility, largely emphasizing the disadvantage of asexuals that compete with sexual species (Muller, 1964). Consequently, extinction was frequently considered only in the context of competing lines. However, there is no reason why mutation accumulation might not lead to the extinction of a whole species, even in the absence of competition, if deleterious mutations frequently decrease the absolute reproductive capacity as stated by the mutational meltdown theory (Lynch & Gabriel, 1990; Lynch *et al*., 1993). If Muller's ratchet can lead to extinctions, then it might help to explain the features that we observe in surviving species today.

This potential biological importance continues to attract attention.

(vi) *Muller's ratchet may degrade mtDNA*

Several studies suggest that mtDNA could be an unstable genetic system that might cause the extinction of its host (Hastings, 1992; Frank & Hurst, 1996; Lynch & Blanchard, 1998; Gemmell & Allendorf, 2001). MtDNA seems to be particularly susceptible to the action of Muller's ratchet (Takahata & Slatkin, 1983; Gabriel *et al*., 1993; Howell *et al*., 1996; Lynch, 1996, 1997; Bergstrom & Pritchard, 1998). This potential threat of extinction is independent of the proposed role of Muller's ratchet in the loss of genes during the early stages of mitochondrial adaptation after endosymbiosis (Berg & Kurland, 2000; Kurland & Andersson, 2000). However, it remains unclear whether a simple model of Muller's ratchet actually predicts a threat of extinction under the biologically relevant parameter space for either human mtDNA or many of the other biological systems where the operation of Muller's ratchet has been suspected (Charlesworth, 1978; Chao, 1990; Duarte *et al*., 1992; Rice, 1994; Schartl *et al*., 1995; Andersson & Hughes, 1996; Escarmis *et al*., 1996; Lynch, 1996, 1997; Moran, 1996; Fraile *et al*., 1997; Berg & Kurland, 2000; Kurland & Andersson, 2000; Butlin, 2002; Shoemaker *et al*., 2004). As an exception to this rule, recent work has used simulations to assess the importance of Muller's ratchet in the evolution of neo-Y chromosomes in *Drosophila* (Gordo & Charlesworth, 2000*a*, *b*; Bachtrog, 2004).

(vii) *Simulations of Muller's ratchet*

The frequent lack of quantification of hypotheses involving Muller's ratchet in particular organisms is remarkable in the light of the extensive simulation efforts that have significantly advanced our understanding of how accumulation of deleterious mutations *generally* affects fixation of mutations (Higgs & Woodcock, 1995; Charlesworth & Charlesworth, 1997) and neutral diversity (Higgs & Woodcock, 1995; Gordo *et al*., 2002) and how it is *generally* affected by issues such as synergistic epistasis (Melzer & Koeslag, 1991; Kondrashov, 1994; Butcher, 1995; Schultz & Lynch, 1997), quantitative genetic variation (Wagner & Gabriel, 1990), distributions of mutational effects (Butcher, 1995; Schultz & Lynch, 1997; Gordo & Charlesworth, 2001*a*), back mutations (Antezana & Hudson, 1997*a*; Bergstrom *et al*., 1999; Gordo & Charlesworth, 2001*a*; Rouzine *et al*., 2003), advantageous mutations (Schultz & Lynch, 1997; Rouzine *et al*., 2003; Bachtrog & Gordo, 2004), small effective population size (Lynch & Gabriel, 1990; Lynch *et al*., 1995*a*, *b*), limited reproductive capacity (Lynch & Gabriel, 1990; Melzer & Koeslag, 1991; Gabriel *et al*., 1993; Lynch *et al*., 1993, 1995*b*; Schultz & Lynch, 1997), ploidy (Charlesworth *et al*., 1993*b*, *c*; Lynch *et al*., 1995*a*; Antezana & Hudson, 1997*a*, *b*; Charlesworth & Charlesworth, 1997), recombination (Pamilo *et al*., 1987; Bell, 1988; Charlesworth *et al*., 1993*c*; Lynch *et al*., 1995*a*; Antezana & Hudson, 1997*b*; Schultz & Lynch, 1997), outcrossing (Charlesworth *et al*., 1993*c*; Lynch *et al*., 1995*a*; Schultz & Lynch, 1997), multi-level population genetics (Bergstrom & Pritchard, 1998; Bergstrom *et al*., 1999; Rispe & Moran, 2000) and metapopulation structure (Bergstrom & Pritchard, 1998; Bergstrom *et al*., 1999; Gabriel & Burger, 2000; Higgins & Lynch, 2001).

(viii) *Previous work is difficult to apply to human mtDNA*

An important previous attempt to predict extinction times due to high mutation rates in mtDNA focused on selection within the population of mtDNA molecules within each individual, but neglected selection among individuals that carry these populations of mtDNA molecules (Gabriel *et al*., 1993). Such results cannot predict extinction time of a large population of individuals due to loss of vital mtDNA, since important lineage sorting effects are neglected.

It is well known that Muller's ratchet will always click, provided that selection coefficients are sufficiently small (Gessler, 1995). However, if mutational effects are too small, then extinctions resulting from fitness degradation will require more than several billion years. Thus extrapolations from simulations with effective population sizes that are larger than those of humans can be used to infer the operation of Muller's ratchet (Gordo & Charlesworth, 2000*a*), but this cannot produce realistic estimates of extinction times. This situation is unsatisfactory, especially since reasonable estimates of the important parameters of the ratchet in human mtDNA are now available and the existence of such a threat of extinction should be of considerable general interest. The absence of quantitative rigour for investigation of specific alleged examples of the operation of Muller's ratchet has seriously hampered progress (Butlin, 2002): Should mutation accumulation due to Muller's ratchet operate too slowly to lead to extinction within the known time of existence of an evolutionary line, then we can safely consider Muller's ratchet to be irrelevant for species survival, although it may still shape genomic sequences (Gordo & Charlesworth, 2001*b*). However, if extinction is expected, then further research should evaluate the various biological processes that might keep such an evolutionary line alive.

(ix) *Muller's ratchet is difficult to quantify*

There have been numerous attempts to predict the rate of mutation accumulation from Muller's ratchet using computer simulations and analytical approximations (e.g. Haigh, 1978; Pamilo *et al*., 1987; Bell, 1988; Melzer & Koeslag, 1991; Gabriel *et al*., 1993; Lynch *et al*., 1993; Stephan *et al*., 1993; Butcher, 1995; Gessler, 1995; Higgs & Woodcock, 1995; Antezana & Hudson, 1997*b*; Charlesworth & Charlesworth, 1997; Prügel-Bennett, 1997; Baake & Gabriel, 1999; Gordo & Charlesworth, 2000*b*; Stephan & Kim, 2002; Rouzine *et al*., 2003). However, no single approach works well over the whole range of biologically interesting parameters; many methods are not easily automated and some are mathematically or computationally too demanding for most biologists with a special interest in a particular model system. Up to now, the most accurate analytical approximations (Gordo & Charlesworth, 2000*b*) are Stephan's diffusion approximation (Stephan *et al*., 1993; Stephan & Kim, 2002) and Gessler's quantitative genetics approach (Gessler, 1995), each in their corresponding range of parameter combinations. The most reliable approach is therefore to combine the most accurate analytical approximations with computer simulations, although the latter require excessive computing times for exploring the full range of biologically realistic parameter combinations. Combining these complexities with frequent uncertainties about parameter values and the absence of a clearly defined null hypothesis makes it easy to understand why the risk of extinctions from Muller's ratchet is rarely quantified.

(x) *Aim of this work*

Here, I construct a null model that allows quantification of the threat of extinction from deleterious mutation accumulation due to Muller's ratchet. I apply this model to human mtDNA using the best parameter estimates that are currently available. To predict the time between clicks of Muller's ratchet, I use analytical approximations combined with individual-based computer simulations. Since simulations are very demanding, they are distributed globally using evolution@home, the first global computing system for evolutionary biology (Loewe, 2002*a*, *b*, 2006). Simulation results are stored in a database that is continually expanded by new computations to increase its statistical power and the variety of parameter combinations it contains. Using these data it is possible to reject the hypothesis that the fitness-degrading effects of Muller's ratchet in human mtDNA can be neglected over a timeframe of 20 million years. Potential solutions of the resulting genomic decay paradox are discussed.

2. Methods

(i) *Analytic approximations*

To predict the effective mean time between clicks of Muller's ratchet, T_{cl}, the quantitative genetics approach of Gessler (1995) and the diffusion-approximation-based approach of Stephan & Kim (2002) were employed in their respective appropriate ranges ($N_0 < 1$ and $N_0 \geqslant 1$) with

$$N_0 = N_{\mathrm{e}} * \exp(-U_{\mathrm{sdm}}/|s|), \tag{1}$$

where N_{e} is the effective population size, N_0 is the size of the fittest class at deterministic equilibrium, i.e. the number of individuals with the least mutations, and U_{sdm} is the genomic mutation rate per generation for mutations with deleterious selection coefficient s. For $U_{\mathrm{sdm}}/|s| > 500$ the simple expectation $T_{\mathrm{cl}} = 1/U_{\mathrm{sdm}}$ was used. Automatic computation was implemented in the statistical programming language 'R' (http://www.r-project.org/). The abruptness of the analytic approximations around the limits of their applicability is an artefact that is not found in simulations.

The double integrations required for the diffusion approximation results were computed using the Newton–Cotes method (Simpson's rule: see Bronstein & Semendjajew, 1991) and an adaptive, recursive integration algorithm that gave more accurate results if more recursions were allowed. For high-precision results, the diffusion approximation was integrated from 0 to N_0 and from N_0 to 1 with a maximum of 10 recursive iterations, where each recursion halves the integration interval that it computes. The corresponding integrations for Fig. 1 took almost half a year of CPU time on a 2·4 GHz Xeon and are denoted as the 'precise' lines there. As a shortcut, one may integrate only from 0 to N_0 with 5 recursive iterations and then double the value, because the population will spend approximately half the time above and half the time below the equilibrium expectation N_0. This gives much better results than integrating the whole range with less accuracy (i.e. less recursive iterations) and takes only hours to days. The resulting extinction times are denoted as the 'fast' lines in Fig. 1. The two types of lines are plotted on top of each other in Fig. 1 to demonstrate the virtually non-existent difference between the two computational approaches for the purpose of this study (extremely careful inspection shows that the only detectable difference is at the lower part of the rising right part of the curve; there the lower black curve denotes the 'precise' and the upper coloured

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curve the 'fast' computations). Combining these results with computations at other numerical integration step widths indicates that, intuitively speaking, the shape of the corresponding integrals is rather benign below N_0, meaning that large numerical integration steps still lead to reasonably accurate results, whereas the shape of the integrals immediately above N_0 is extremely unpleasant for many parameter combinations, meaning that the actual size of the best class is most of the time immediately above N_0, and much less often at higher values. Computing these integrals with too little accuracy therefore easily led to gross overestimations of the time spent above N_0 and correspondingly exaggerated click times.

(ii) *Parameter combinations used*

To assess sensitivity of results regarding changes in parameters, the most credible intermediate value is given along with the minimum and maximum. Two parameters are encoded in Figs. 1–3 by the colour of the plotted line (U_{sdm}) and by the x-axis (s) and are discussed separately below. The other parameters are as follows. The maximal reproductive capacity (female offspring per mother that reach sexual maturity), $R_{\max}$, was assumed to be 10 (8–12) daughters, much larger than the 7 children per woman born in Niger (CIA, 2005). Generation time, T_{gen}, was assumed to be 15 (10–20) years (see www.animalinfo.org; Takahata & Satta, 1997; Elango *et al*., 2006) and the effective population size, N_e, to be 15 800 (5000–50 000) females (Ayala, 1995; Takahata & Satta, 1997; Harpending *et al*., 1998). It is important to consider the larger long-term N_e, since this will govern mutation accumulation for the longest part of evolutionary history. The time during which the evolutionary line leading to humans has evolved without recombination, T_{age}, was assumed somewhat arbitrarily to be 10 (5–20) Myr, which is on the same order of magnitude as divergence between humans and Old World monkeys, the closest outgroup to humans that shows evidence for recombination (Piganeau *et al*., 2004).

(iii) *Simulations*

Individual-based simulations were computed by Simulator005r6 of evolution@home (Loewe, 2002*a*, *b*) on more than 300 computers of voluntary participants from the general public around the globe. See Loewe (2006) for updates and a list of parameters observed by Simulator005. Each symbol in Figs. 2, 3 and 5 denotes one independent stochastic simulation with a different random seed using U and s as specified (shape, abscissa) and intermediate parameters of $R_{\max}$ and T_{gen} to compute the extinction time T_{ex} in years. N_e was constant at 5000, 10 000 or 50 000 individuals (hence the occasional huge variability in T_{ex} at some s). Simulations were run until either 500 clicks had been observed or the population went extinct or the global computing participant terminated the application or a predetermined final generation was reached (10^5–10^8 generations, depending on N_e).

The biological model employed does not differ significantly from that used in other simulations (Gabriel *et al*., 1993; Gessler, 1995) and comparison of output (e.g. with Stephan & Kim, 2002) showed similar T_{cl} values. In short, after initializing random number generators, a population of N_e asexual females $= K$ (environmental capacity) was created that evolved in discrete generations with the event order mutation > selection > reproduction > death. An individual's Wrightian fitness W was

$$\mathrm{W} = (1+s)^n \tag{2}$$

for n mutations as counted by a variable in each individual, where each mutation has the same unconditionally deleterious effect s, which is negative to denote deleterious mutations. New mutations and offspring were Poisson distributed with mean U and $W * R_{\max} * C_{\text{dd}}$, respectively. The density-dependent culling factor C_{dd} is defined as

$$C_{\text{dd}} = \min\left(1,\ \frac{K}{N_{\text{e},\,t-1} R_{\max} \overline{W_{t-1}}}\right), \tag{3}$$

where $\overline{W_{t-1}}$ is the average fitness in the previous generation $t-1$. This density regulation mechanism keeps N_e fluctuating around K with variance K. After each generation the individuals with the least mutations were counted to determine whether a click had occurred.

(iv) *Computation of extinction time*

As soon as fitness falls below $1/R_{\max}$, the parental generation will no longer be able to replace itself and mutational meltdown will lead to extinction in a few generations (Lynch *et al*., 1993). Thus, assuming hard selection, C_{mm}, the number of clicks needed for mutational meltdown to start is given by

$$C_{\text{mm}} = \log(1/R_{\max})/\log(1+s), \tag{4}$$

where s is negative. Thus, the extinction time T_{ex} is given by

$$T_{\text{ex}} = C_{\text{mm}} * T_{\text{cl}} * T_{\text{gen}}, \tag{5}$$

where T_{gen} is the average time between generations and the maximal reproductive capacity $R_{\max}$ includes only offspring that actually reach maturity after viability selection.

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3. Null model for quantifying the threat of extinction from Muller's ratchet

(i) *How to quantify the threat of extinction*

The null model builds upon a variant of Haigh's model of Muller's ratchet (Haigh, 1978), modified to allow for variable population sizes and extinctions. The evolutionary line investigated consists of a single random mating population of effective size N_e with a complete lack of recombination, a complete lack of back mutations (infinite sites) and with discrete generations of generation time T_{gen}. While N_e is constant in Haigh's model, the population density regulation mechanism used here makes N_e fluctuate like a Poisson variate with expectation N_e before mutational meltdown. Each new offspring accumulates new deleterious mutations as given by a Poisson variate with an expectation of U_{sdm} new slightly deleterious mutations per genome. All mutational effects are computed multiplicatively and share the same slightly deleterious selection coefficient s throughout a single simulation (negative to indicate the disadvantage). From N_e, U_{sdm} and s a mean effective click time (T_{cl}) of the ratchet is computed analytically or by simulation. After enough clicks mutational meltdown is known to occur (Lynch & Gabriel, 1990; Gabriel *et al*., 1993; Lynch *et al*., 1993, 1995*a*, *b*). It starts when a population can no longer produce enough offspring to replace all parents, because their maximal effective reproductive capacity R_{max} is too small to compensate for loss of fitness due to accumulation of deleterious mutations. This condition allows us to estimate C_{mm}, the number of clicks needed for mutational meltdown to begin. Multiplying C_{mm} and T_{cl} gives a good estimate of the extinction time T_{ex}, because the duration of demographic processes including mutational meltdown can be neglected on the resulting timescales.

Using these foundations, I propose a null model to quantify the threat of Muller's ratchet based on key properties of the U-shaped plot (Gabriel *et al*., 1993) of extinction time against selection coefficient (see Fig. 1 for an example). The U-shape of this plot reflects the fact that very deleterious mutations do not contribute to extinctions because they never fix, whereas nearly neutral mutations do not contribute to extinctions because the known history of life on earth is too short to allow accumulation of enough damage to cause extinction. Only intermediate mutational effects may cause extinctions. The null hypothesis states that Muller's ratchet cannot cause extinctions. The null model will reject a potential threat of extinction from Muller's ratchet if there are no intermediate mutational effects that can cause extinction in the known time of existence of the species considered. The choice of this U-shaped plot reflects the fact that we usually have an idea of the order of magnitude of the other parameters, but we know least about s; s is also the only parameter that is certain to vary over many orders of magnitude as it includes effectively neutral and effectively lethal mutations. To quantify the threat from Muller's ratchet T_{ex} is plotted against s for the most probable combinations of U, N_e, R_{max} and T_{gen}. Then the use of an age of the evolutionary line (T_{age}) allows derivation of the following key quantities:

1. The most critical range of selection coefficients, s_{cm}, denotes the range of mutational effects that minimizes the time to extinction. Practically, this includes the fastest extinction times ($T_{ex,min}$) and all other values of T_{ex} within a factor of 2 to account for statistical noise in simulations and other sources of ruggedness at 'the bottom of the U' of the U-shaped plot (see Figs. 1–3 for examples).
2. The critical range of selection coefficients, s_c, denotes mutational effects that could lead to extinction within T_{age}. While exact values for T_{age} are hard to estimate (evolutionary lines are always older than the species they generate), it is easy to see that Muller's ratchet will pose no threat of extinction when predicted extinction times are excessively large.

This approach allows the exclusion of any threat of extinction from Muller's ratchet whenever there is no s_c ($T_{ex,min} \gg T_{age}$). On the other hand, if s_c is present and stable in the light of the uncertainties about the other parameters, then other biological processes must be invoked to explain population persistence. This assumes equal multiplicative effects of mutations.

(ii) *Influence of the distribution of mutational effects*

How does a distribution of deleterious mutational effects on fitness (DDME) affect the generality and power of the null model of quantifying extinctions due to Muller's ratchet? First, any exclusion of a threat of extinction will not be affected, as a DDME will reduce the fraction of mutations within s_{cm} relative to a model where all mutations have effects in s_{cm}. T_{ex} will be minimal when all probability mass of a DDME is concentrated under s_{cm}. Increasing variance of the DDME will slow down loss of fitness as some mutations are more likely to be removed by selection and others will cause less damage (Lynch & Gabriel, 1990; Gabriel *et al*., 1993; Lande, 1994; Schultz & Lynch, 1997). Therefore acceptance of the null hypothesis is robust to a DDME, as long as all deleterious mutations are included in U_{sdm}.

Second, computation of the U-shaped plot with a DDME is not really helpful. Increasing the width of the DDME will decrease maximal threat of extinction

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(longer $T_{ex,min}$), but also widen any s_c, because T_{ex} will increase for critical s_{cm} (the DDME will 'move' some mutations out of s_{cm}) and it will lower T_{ex} for non-critical s (the DDME will 'move' some mutations into s_{cm}). For real data, any attempt to estimate the DDME will lead to confidence intervals for shape *and* location estimates. Then integrated simulations for that specific range of DDMEs will be of much more interest than a U-shaped plot with an arbitrarily fixed shape and many location parameters that are known to be unrealistic in the x-axis.

Third, computation of the U-shaped plot without a DDME allows fast assessments of potential threats from any particular DDME. A simple estimation of U_{sdm} for effects in s_{cm} for a given DDME is enough for a quick, conservative assessment of the threat from Muller's ratchet. Just multiply the total non-synonymous mutation rate U_{tot} by the fraction of the probability mass f_{sdm} located in the s_{cm} to get an estimate for effective U_{sdm}. Use this corrected U_{sdm} to read T_{ex} from the plot. This will be an upper limit, if very small and very large mutational effects also contribute to the threat of extinction from Muller's ratchet. Therefore, this method is conservative in accepting the hypothesis that Muller's ratchet poses a threat of extinction in a given situation. Such an approach reduces the DDME to only one relevant parameter, f_{sdm}, which may be easier to estimate than a fully parameterized DDME.

Fourth, previous results have shown that Muller's ratchet with a mixture of two mutational effects has the same behaviour as a ratchet with an effect as large as the harmonic mean of the mixture (Gordo & Charlesworth, 2001*a*). This suggests collapsing DDMEs into a single mutational effect given by the harmonic mean of the distribution in the effectively selected range ($|N_e s| > 1$) and may allow inclusion of more mutations than using f_{sdm} as described above. Results from these two approaches do not differ substantially.

4. Best parameter estimates

This section reviews the literature to find the best estimates for the mutational parameters that determine the speed of Muller's ratchet in human mtDNA.

(i) *Deleterious mutation rates in mtDNA*

To estimate U_{tot} we need the total number of mutations at sites that can harbour significant deleterious effects, excluding synonymous sites and non-functional intergenic sequences. I assume that about 30% of the 11 259 bp in human mitochondrial proteins can be ignored for our purposes, since they are either silent (i.e. encode the same amino acids) or nonsense mutations (i.e. change the reading frame; this is assumed to be deleterious enough to ensure immediate selective removal). From the remaining 5294 bp in mtDNA, 1118 bp belong to the D-loop that is frequently assumed to evolve neutrally. This leaves 4176 bp for RNA genes and intergenic and regulatory sequences, which I arbitrarily assume to consist of about 50% potentially deleterious sites. Thus any point mutation rate average will have to be multiplied by about 10 000 sites (4176 bp * 50% + 11 259 bp * 70%) that may contribute to driving Muller's ratchet. I neglect indels in this discussion, assuming that their mutational effects are usually so strongly deleterious that they are deterministically removed by purifying selection.

One cannot rely on simple sequence comparisons between species, as these reflect substitution rates, which underestimate actual mutation rates due to multiple hits and removal of deleterious mutations. (This approach would suggest values of e.g. $U_{tot} \approx 0{\cdot}0034$ potentially dangerous mutations per generation per mtDNA genome based on substitution rates at *all* mtDNA sites excluding the control region (Ingman *et al*., 2000) or $U_{tot} \approx 0{\cdot}010$ from 1765 differences that accumulated at 3602 silent sites (Ingman *et al*., 2000; Piganeau & Eyre-Walker, 2003) in the last 5 Myr, assuming $T_{gen} = 20$ years and no further corrections.) Muller's ratchet can lead to unexpected extinctions when it fixes a small fraction of mutations with relatively large slightly deleterious effects, simply because more mutations occur than selection can possibly remove (e.g. see effects from $s = 0{\cdot}1\%$ to $s = 3\%$ in Figs. 1–3). A corresponding risk of extinction does not show in substitution rates; the selection coefficients of completed substitutions are unknown. It is therefore important to obtain independent estimates of the intergenerational (per meiosis) effective substitution rate in the germline. Treating such a rate as though it were a haploid intergenerational mutation rate allows ignoring the complicated multi-level-population genetics at lower levels of selection (only the overall result of lower-level processes is considered).

The following methods suggest that intergenerational mutation rates are higher than traditional phylogenetic estimates in the coding region. They complement similar observations in the control region of humans (Howell *et al*., 2003) and in the whole mitochondrial genome of the worm *C. elegans* (Denver *et al*., 2000).

(a) *Pedigree studies*

Sequencing relatives with known pedigrees yields $U_{tot} \approx 0{\cdot}014$ (based on 4 mutations in 2772 kbp sites * meioses), although more screening is necessary to arrive at more robust estimates for the coding

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region (Howell *et al.*, 2003). This mutation rate appears to be a tenth of what is estimated for the control region, where pooled data (Parsons *et al.*, 1997; Parsons & Holland, 1998; Howell *et al.*, 2003) are more reliable and suggest $1{\cdot}5*10^{-5}$ mutations per site per generation from 36 mutations in 2329 kbp sites*meioses. All pedigree rates may need to be corrected because of the way that the data were obtained. On one hand rates may have to be halved to obtain a lower limit, since many pedigree studies count heteroplasmies as full substitutions (Howell *et al.*, 2003) although roughly equal numbers of heteroplasmies and full substitutions are observed (Sigurdardottir *et al.*, 2000; Howell *et al.*, 2003). On the other hand, if many heteroplasmies are below the detection limit they will not show up in pedigree studies but still make a contribution to overall substitution rates. In any case the true value is probably not outside the frame set by counting only full substitutions (about 50% of classical pedigree rate) and full substitutions together with newly observed heteroplasmies (100% of classical pedigree rate).

(b) *Segregating sites*

One can assume complete neutrality of silent mutations and $N_e = 10\,000$ for nuclear genes as the long-term human effective population size for the last several hundred thousand years (Harpending *et al.*, 1998). This allows use of Watterson's expectation for the number of segregating neutral sites S found in n randomly sampled sequences (Watterson, 1975). Watterson's formula estimates $U_{tot} = S/(a*4*N_e)$, where $a = 1+1/2+1/3+\ \ldots\ +1/(n-1)$ and N_e is a quarter of the nuclear value for haploid maternally inherited mtDNA. Thus, the 320 silent polymorphisms (Piganeau & Eyre-Walker, 2003) at the 3602 silent sites of mitochondrial proteins observed in $n = 53$ humans (Ingman *et al.*, 2000) suggest $U_{tot} \approx 0{\cdot}020$. This value might be a slight underestimate, since it assumes no reduction of diversity from Muller's ratchet (see Section 7 and Gordo *et al.*, 2002). However, the number of segregating sites is much less sensitive to the diversity-reducing side-effects of Muller's ratchet than the mean number of pairwise differences between randomly sampled sequences (Gordo *et al.*, 2002). Therefore the number of segregating sites might lead to meaningful mutation rate estimates, although we do not know yet how to correct for all the diversity-reducing side-effects of Muller's ratchet. Another reason why Watterson's formula might result in slight underestimates of U_{tot} is the assumption of neutrality of synonymous sites, since weak selection at these sites reduces observations of S from their expected value under complete neutrality.

(c) *Scaling pedigree rates*

If the mutation rate in the coding region is indeed lower than in the control region, this should lead to a proportional decrease in S, suggesting that the fraction of S in the coding region (320 segregating out of 3602 silent sites from Ingman *et al.*, 2000; Piganeau & Eyre-Walker, 2003) and in the control region (141 segregating out of 1118 sites from Ingman *et al.*, 2000) can be used to scale the mutation rate observed in the control region. This results in $U_{tot} \approx 0{\cdot}11$, which may have to be halved to correct for observed heteroplasmies but has the advantage of being independent of assumptions about values of N_e, if there is no weak selection on any of these sites. It is unclear how the result would have to be corrected in the presence of weak selection, as this will depend on the relative strengths of selection in those two regions, but no radical changes are expected. This approach suggests a surprisingly small difference between the mutational pressure in the control region and in the coding region – remarkably similar to the situation in *C. elegans*, where no difference between mutation rates in control region and coding region was observed (Denver *et al.*, 2000). However, the huge differences between worms and mammals suggest caution when discussing this as support for high mutation rates in mammals. A report with similar findings in humans has too little statistical power to be significant (Howell *et al.*, 1996).

(d) *Deleterious mutations in the control region*

Mutations observed in pedigrees (but not in phylogenies) may be removed by selection in the long term (Parsons *et al.*, 1997; Howell *et al.*, 2003). If yet unknown functional structures lead to such selective removal for 90% of all mutations observed in the control region, then mutational pressure is increased by about 0·015 deleterious mutations per generation. Again this value may have to be halved to correct for heteroplasmies.

(ii) *The distribution of deleterious mutational effects in mtDNA*

While general information on the DDME is sparse, mtDNA and humans have recently been the target of a number of studies (Nachman, 1998; Eyre-Walker *et al.*, 2002; Nielsen & Yang, 2003; Piganeau & Eyre-Walker, 2003). Results suggest that a significant amount of the probability mass of the distribution of mutational effects on fitness is in the area of $|N_e s| = 1$ for primates (Eyre-Walker *et al.*, 2002; Nielsen & Yang, 2003; Piganeau & Eyre-Walker, 2003). Thus potential assumptions about the fraction of the probability mass with critical slightly deleterious

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mutational effects, f_{sdm}, include values from 90% (if most mutations have the most dangerous effect) down to about 10%, assuming that each order of magnitude from $s=-10^{0}$ to -10^{-10} occurs approximately equally often as one may be tempted to guess, when looking at nuclear genes in primates (see fig. 2 in Eyre-Walker *et al.*, 2002). More specifically, the published DDMEs for human mtDNA (Nielsen & Yang, 2003; Piganeau & Eyre-Walker, 2003) allow estimation of f_{sdm} for the critical selection coefficients as determined from Fig. 3 (i.e. critical selection coefficients in the range of $-0{\cdot}0002$ to $-0{\cdot}008$, see Results). Selection coefficients need to be scaled by N_e, to allow comparison with the reported DDME parameters. To convey a feeling for the influence of N_e values, 2500 and 25 000 shall be used as medium-term and long-term N_e for mtDNA. Then diversity data (Piganeau & Eyre-Walker, 2003) suggest that the fraction of critical mutations is (assuming no advantageous back mutations):

$f_{sdm}=15\%$ ($N_e=2500$; $a=0{\cdot}39$, $N_es=-700$)

$f_{sdm}=35\%$ ($N_e=25000$; $a=0{\cdot}39$, $N_es=-700$),

where a is the shape parameter of the Gamma distribution assumed and N_es determines the location parameter expressed as the arithmetic mean. Including advantageous back mutations (Piganeau & Eyre-Walker, 2003) suggests

$f_{sdm}=16\%$ ($N_e=2500$; $a=0{\cdot}47$, $N_es=-410$)

$f_{sdm}=43\%$ ($N_e=25\,000$; $a=0{\cdot}47$, $N_es=-410$).

Estimates based on the variation in the rate of substitutions between sites in primate mtDNA (Nielsen & Yang, 2003) led to a group of DDMEs with similar likelihoods and shapes similar to a Normal distribution. These results suggest approximately

$f_{sdm}=98\%$ ($N_e=2500$; $a=3{\cdot}22$, $N_es=-2{\cdot}6$)

$f_{sdm}=7\%$ ($N_e=25\,000$; $a=3{\cdot}22$, $N_es=-2{\cdot}6$; please note that the bulk of the probability mass is immediately below the lower border resulting in a very fast-turning ratchet that will just need a bit longer to accumulate enough damage to cause extinction).

While these numbers do show the huge variability that is associated with current DDME estimates, they clearly suggest that a considerable fraction of all mutations falls in the critical range of selection coefficients s_c as defined above.

(iii) *Most probable mutation rate for critical deleterious effects*

Combining all this information suggests that U_{sdm} probably ranges somewhere between 0·01 and 0·1 new critically deleterious mutations per genome per generation in human mtDNA. If corrections for heteroplasmy are necessary, then these values have to be halved (see discussion of pedigree studies above). Circumstantial evidence suggests that the current lowest conceivable limit ($U_{sdm}<0{\cdot}001$ from $U_{tot}=0{\cdot}014*f_{sdm}=5\%$) does not apply to human mtDNA, because it appears unlikely that most mutations are just deleterious enough to be removed completely but not deleterious enough to be noticed by the medical community (Naviaux, 2004). To understand this, consider the following: (i) The ratio of non-synonymous to synonymous mutations fixed in mtDNA is small (Piganeau & Eyre-Walker, 2003) suggesting that most amino-acid-changing mutations probably have deleterious effects of $s>1/N_e$. If they had smaller effects they would appear in phylogenetic studies. (ii) N_e for human mtDNA is probably around 2500 (see above). (iii) Most deleterious mutations probably have fitness effects of less than 10%, because it appears unlikely that the medical mtDNA research community would not have noticed larger effects much earlier (Naviaux, 2004). If (i)–(iii) are combined with the result from Fig. 2 that the critical region of selection coefficients in human mtDNA encompasses effects of about $1/N_e$ up to more than one order of magnitude above that value, then very little space is left on the scale of mutational effects for a full DDME that does not lead to extinctions from Muller's ratchet. It would be interesting to check whether this narrow range of mutational effects (around a few per cent) is particularly frequent in mtDNA, as would be expected if selection were to explain the discrepancy between rates in pedigrees and phylogenies without increasing the threat from Muller's ratchet.

(iv) *Most mutations in mtDNA are probably under hard selection*

Hard selection is assumed throughout this work (Lynch *et al.*, 1993), since soft selection does not allow for extinctions by definition. One may question this assumption by suggesting that there will always be some quantitative traits that can compensate for any real loss of fitness in mtDNA (Wagner & Gabriel, 1990). Further discussion may appear futile, as we cannot determine the compensatory limits of such traits due to gaps in our knowledge of the complex regulatory networks that determine fitness. However, recent work on the relationship between performance of mitochondria and fertility challenges this view (Gemmell & Allendorf, 2001). Basic mammalian biology dictates that survival of a species depends on the ability of sperm to fertilize eggs, which is influenced by many factors (see references in Kilgallon & Simmons, 2005). At least in humans, one of these

factors is the quality of mitochondria, as they determine the speed of sperm on its way towards the egg (Moore & Reijo-Pera, 2000; Ruiz-Pesini *et al.*, 2000*a*, *b*; Jansen & Burton, 2004). This appears to be a race against time, since (i) sperm are killed by mild acidity, (ii) a healthy human vagina has a pH of 4·0 to 4·5 and (iii) ejaculate acts as an alkaline buffer for several hours only (Olmsted *et al.*, 2000). Thus it appears possible that a decline in mitochondrial functionality may actually drive a population to extinction (Gemmell & Allendorf, 2001), especially since male-only functions are not protected by selection in the maternally inherited mtDNA (Frank & Hurst, 1996). Since even a small reduction in mitochondrial power output might affect male fertility, but only reductions of 80% or more lead to clinical disease (presumably affecting female fitness), an appreciable fraction of these male-specific deleterious mutations may in effect accumulate freely (see references in Gemmell & Allendorf, 2001). That fraction of mutations that cannot accumulate freely due to effects that affect females too can still be fixed by Muller's ratchet. Thus empirical reasons seem to support the assumption of hard selection. Since sperm mobility appears to be correlated with mtDNA in other species too, there is no reason to believe that this problem is confined to humans (Gemmell & Allendorf, 2001).

5. Results

To determine the U-shaped plots of extinction time, T_{ex}, against selection coefficient s for humans (Fig. 1), two of the best analytic approximations available (Gessler, 1995; Stephan & Kim, 2002) were employed together with globally distributed individual-based simulations (Loewe, 2002*a*, *b*). Fig. 1 shows expectations from analytic approximations, where the upper coloured lines are computed by the simplified diffusion approximation described in Section 2. Comparing this fast method with the computationally challenging more precise approach (lower black lines) shows that the fast method is accurate enough for our purposes. The quality of the analytic approximations employed here deteriorates at the limits of their range of applicability (around $N_e * \exp(-U_{sdm}/|s|) = 1$; see the abruptness of the lines); unfortunately, this coincides roughly with the biologically most interesting range of selection coefficients s_{cm} that lead to minimal extinction times. Fig. 2 adds individual-based simulation results to increase precision in this critical region of parameter space. A test of the null hypothesis that Muller's ratchet cannot lead to extinctions in human mtDNA requires investigation of the limits of the underlying input parameters to avoid conclusions based on one particular parameter combination that may or may not be a good representative of reality.

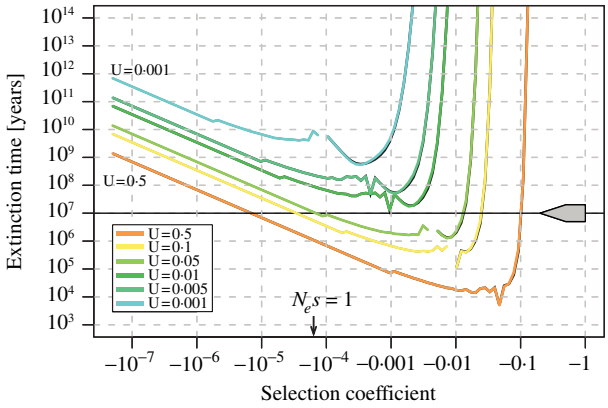

Fig. 1. Analytic predictions of extinction times of the human line due to accumulation of slightly deleterious mutations in mtDNA. The upper grey arrow denotes the assumed age of the line, including limits. The lower arrow marks the border with effective selective neutrality for the effective population size used. The lines represent the analytic predictions of the extinction time for different deleterious genomic mutation rates (U_{sdm}) and intermediate values of N_e, T_{gen} and R_{max}. Discontinuities in the analytical lines denote transitions in the computational method. From right to left: (i) Stephan's diffusion approximation, where 'fast' and 'precise' computations are plotted on top of each other ('fast' is represented by the upper lines in colour and 'precise' by the barely visible lower lines in black; see Section 2 for details) (ii) Gessler's quantitative genetics approximation and (iii) use of the simple equation $1/U_{sdm}$ to predict click time. Note that the numerical inaccuracies introduced by using the 'fast' method can be neglected for most purposes. See Section 2 for parameter values and Fig. 4 for a draft of the most important features of this U-shaped plot of extinction time over selection coefficient.

Therefore we need to determine the size of the region of biologically realistic parameter values that lead to a threat of extinction from Muller's ratchet. Fig. 3 restricts mutation rates to the upper and lower credible limits for humans while including the other sources of variability for predictions of T_{ex}. Such variability can come from:

1. *Stochasticity*. Each simulation gives a single measurement of mean effective click time based on all the clicks observed in that simulation. This mean is used to predict T_{ex} and thus each simulation produces a separate symbol. Small symbols give lower limits, as they indicate unsuccessful attempts to observe clicks of Muller's ratchet. For most parameter combinations differences are smaller than the symbol, because many clicks are observed in most simulations. Since extinction requires many clicks, the high variability of effective click time observed in simulations effectively cancels out.
2. *Different effective population sizes*. To produce precise U-shaped plots would have meant that Fig. 3 consisted of a whole series of similar plots,

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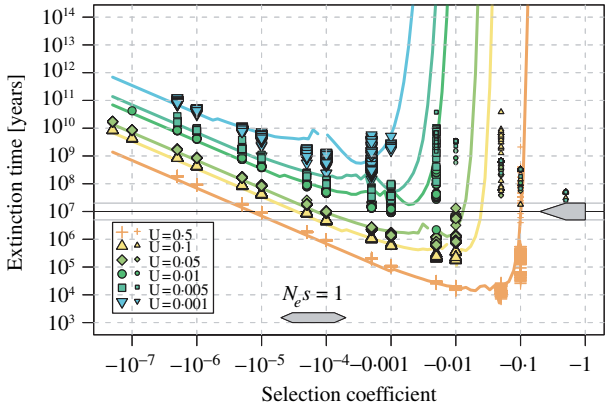

Fig. 2. Simulations of extinction times of the human line due to accumulation of slightly deleterious mutations in mtDNA. This figure builds on Fig. 1. The lower grey bar marks the border with effective selective neutrality for the effective population sizes used. Each symbol denotes an independent simulation with a N_e somewhere in the given limits assuming intermediate values of T_{gen} and R_{max}. Large symbols indicate valid T_{ex} estimates; small symbols indicate lower limits (no clicks were observed). Each type of symbol represents different effective population sizes at once to convey a feeling for variability. This is important when interpreting results close to the wall of background selection: the ratchet may operate in small populations, but not in large populations. This plot contains 23 222 simulations that required a total of 10·3 years of computing time. See Section 2 for parameter values and Section 5 for a more detailed explanation of this plot.

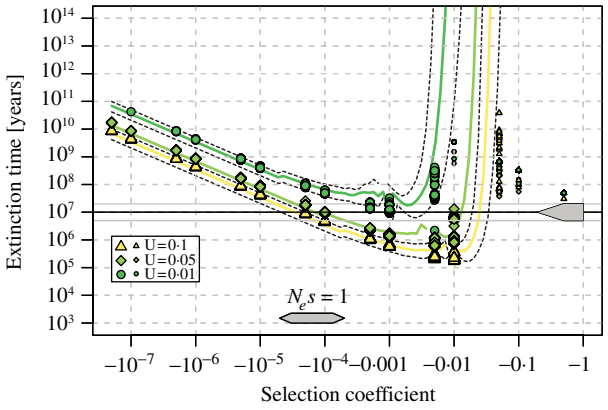

Fig. 3. Most credible estimates of extinction times of the human line due to accumulation of slightly deleterious mutations in mtDNA. This figure builds on Figs. 1 and 2. The upper grey arrow denotes the assumed time during which mtDNA in the human evolutionary line evolved without recombination. The lower grey bar marks the border with effective selective neutrality for human effective population sizes. Unbroken lines represent analytic predictions of the extinction time for intermediate values of N_e, T_{gen} and R_{max} assuming different deleterious mutation rates (U_{sdm}, see colour code or shape of associated symbols). The upper and lower credible limits for mutation rates are about $U_{sdm}=0{\cdot}1$ and $U_{sdm}=0{\cdot}01$ and the corresponding lines are surrounded by dashed lines to provide a feeling for the variability of extinction time estimates (the dashed lines use the corresponding upper and lower limits of N_e, T_{gen} and R_{max}). Symbols denote simulation results (see Fig. 2) and details about parameter values used here can be found in the text. See Section 5 for a more detailed explanation of U-shaped plots like this one and Fig. 4 for a draft of the most important features.

each one based on a different N_e. However, since these plots are almost identical, layering them makes it easier to determine what variability in T_{ex} is caused by different realistic values of N_e. This is exactly what was done in Figs. 2, 3 and 5, all corresponding symbols denoting separate simulations where different values of N_e are plotted on top of each other.

3. *Other variation*. To make the plots easier to understand, each simulation is used to predict only one expected value for T_{ex} based on average values for T_{gen} and R_{max}. The black dashed lines in Fig. 3 show the corresponding upper and lower limits of T_{ex} for the upper (yellow) and lower (green) credible intergenerational deleterious mutation rates of mtDNA. Variability at the left border of the plot is due mostly to T_{gen} and R_{max}, whereas variability at the upper border is mostly due to N_e. This makes it easy to assess variability in T_{ex} due to T_{gen}, R_{max} and N_e, because all lines show similar behaviour. See Section 2 for parameter values.

These conventions make it easy to determine whether a particular set of input parameters will lead to extinction from Muller's ratchet under the null model. To compute a formal probability for extinction is not necessarily needed. It would require a complicated integration over all input parameter combinations that occur in nature and their respective frequencies. This has to be distinguished from the probabilities that certain parameter combinations that we regard as realistic do actually occur in nature. In the absence of such complicated computations it is up to biologists to assess the credibility of parameter combinations that lead to extinctions. One result from investigating simulations with many different input parameter combinations is that interpolation between different parameters is easily possible for Muller's ratchet, once its main patterns are understood (see Fig. 4). This means that it is relatively easy to read 'between the lines' as long as the grid of upper and lower next parameter combinations puts solid boundaries to such efforts.

For example one of the most probable parameter combinations in humans, assuming $U_{sdm}=0{\cdot}03$, suggests $s_{cm}\approx -0{\cdot}001$ to $-0{\cdot}007$ at $T_{ex,min}\approx 3$ Myr and $s_c\approx -0{\cdot}0002$ to $-0{\cdot}008$ at $T_{age}\approx 10$ Myr. Considering all possible variation in the standard null model of Muller's ratchet described above suggests that a surprisingly large range of biologically realistic parameter combinations should have led to extinction of the evolutionary line leading to humans within 20 Myr.

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This was not obvious from the fact that Muller's ratchet must have been operating in mtDNA, as current knowledge of the variability of mitochondrial biology suggests a debate about the biological significance of extinction times like $T_{ex,min} \approx 100$ Myr that are predicted for the lowest credible limits of mutation rates in mtDNA. Widely used phylogenetic substitution rates cannot be applied here, but would lead to $T_{ex,min}$ on the order of the age of the Earth.

The extinctions predicted by the most likely parameter combinations suggest that there are some serious problems, either with our standard assumptions about what is important for mtDNA or with the simple model of Muller's ratchet employed here. Given the nature of these results a debate is needed about where the null model deviates from reality.

6. Potential solutions for the genomic decay paradox

Similar unexpected extinctions have been predicted earlier by computation of the combined mutational load of many sites in nuclear DNA (Crow, 1993, 2000; Kondrashov, 1995*c*; Eyre-Walker & Keightley, 1999; Sunyaev *et al*., 2001) that may or may not be specific to humans (e.g. Gilad *et al*., 2003; Keightley *et al*., 2005). Small effective population sizes are believed to increase the risk of extinction (Lande, 1994; Kondrashov, 1995*c*; Lynch *et al*., 1995*a*) and have received particular attention from conservation geneticists (Frankham *et al*., 2002). Further discussion may benefit from formally defining this phenomenon:

A genomic decay paradox exists in an evolutionary line if the apparent age of that line is greater than the best mechanistic predictions of extinction time that are currently available for the corresponding ancestral state of that line.

Detection and discussion of such genomic decay paradoxes can be expected to stimulate further research that increases the quality of predictions, either by increasing the accuracy of parameter observations or by improving the mechanistic models of evolution used for predictions. A considerable number of potential solutions exist for nuclear genomic decay paradoxes, which possibly include (i) prevalence of anti-mutators in the past (e.g. Mukai *et al*., 1985; Kondrashov, 1995*b*; Schaaper, 1998), (ii) smaller effective genome sizes than currently assumed (Drake *et al*., 1998), (iii) DDMEs with lower densities of critical selection coefficients than currently assumed (Kondrashov, 1995*c*), (iv) soft selection acting on most sites (Kondrashov, 1995*a*, *c*), (v) molecular modulation of mutational effects (Gabriel *et al*., 1993; Hurst & McVean, 1996; Moran, 1996; Boerner *et al*., 1997; Partridge & Barton, 2000; Krakauer & Plotkin, 2002; Queitsch *et al*., 2002), (vi) cancer (Lichtenstein, 2005), (vii) quantitative traits that compensate deleterious effects on fitness (Wagner & Gabriel, 1990; Estes & Lynch, 2003), (viii) quasi-truncating selection (Crow & Kimura, 1979; Crow, 1993, 1997), (ix) general high frequencies of adaptive mutations (Whitlock, 2000; Sawyer *et al*., 2003; Bierne & Eyre-Walker, 2004), (x) reservoirs of non-decayed genes that slow down evolution (e.g. Liu & Tabashnik, 1997), (xi) repair of broken genes by horizontal gene transfer (Ochman *et al*., 2000), (xii) gene conversion among multiple-copy genes (Ohta, 1989), (xiii) a different N_e for particular genomic decay situations (Kondrashov, 1995*c*; Whitlock & Barton, 1997), (xiv) spatial population structure (Whitlock, 2002; Glemin *et al*., 2003), (xv) long-term lineage sorting (Lynch & Blanchard, 1998) or group selection possibly due to cultural evolution (Szathmáry & Maynard Smith, 1995; Feldman & Laland, 1996), (xvi) sexual selection for good genes (Koeslag & Koeslag, 1993; Whitlock, 2000; Agrawal, 2001; Brooks & Kemp, 2001; Siller, 2001; Foerster *et al*., 2003; Leroi, 2003), (xvii) inbreeding avoidance (Pusey & Wolf, 1996) or purging of deleterious alleles by inbreeding (Frankham *et al*., 2001; Visscher *et al*., 2001; Keller & Waller, 2002; Glemin, 2003), (xviii) appropriate changes in the environment (Szafraniec *et al*., 2001), (xix) harsh selection in the past or during early stages of development, and (xx) the possibility that the equilibrium mutational load is never reached (Kondrashov, 1995*c*). Finally, yet unknown potential solutions in addition to the list discussed in more detail below, or any combination of these, might solve the genomic decay paradox. Some of these 'potential solutions' are mere verbal arguments that need to be quantified properly before it becomes clear whether they possess any potential to slow down genomic decay at all. Please note that none of the potential solutions discussed here is part of the simple null model of Muller's ratchet presented above. They rather suggest the construction of more advanced null models, until the paradox can be resolved and our quantitative models can be regarded as conflict-free *and* specific at the same time. The genomic decay paradox in mtDNA calls specifically for further investigations of the following potential solutions.

(i) *Back mutations*

One of the underlying assumptions of the standard model of Muller's ratchet used above is the complete absence of back mutations. As argued by Maynard Smith, back mutations must occur in the few individuals that belong to the best class and specifically repair the damage of a deleterious mutation, suggesting that they can be safely ignored (Maynard Smith, 1978, p. 35). However, this reasoning assumes an

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infinite number of potentially deleterious sites, something that may not be reasonable in a genome as small as mtDNA, if the fraction of sites fixed for a deleterious allele increases over time. The resulting back mutations could restore the original function and therefore considerably increase extinction times (Lande, 1998; Whitlock & Otto, 1999). Assuming that every deleterious mutation can always be undone by a back mutation will considerably increase the probability of generating back mutations and eventually lead to equilibrium as observed in a number of simulations (Antezana & Hudson, 1997*a*; Bergstrom *et al*., 1999; Rouzine *et al*., 2003). This situation is similar to models of codon bias evolution (McVean & Charlesworth, 2000) and one would consequently not expect Muller's ratchet to lead to any extinctions, since back mutations easily compensate any potential long-term damage. However, several details of this scenario are unrealistic enough to doubt this as a sufficient solution to the paradox in mtDNA.

First, careful inspection of Fig. 3 shows that there is a small but significant range of relatively large selection coefficients within s_{cm} that make the ratchet click infrequently. These effects contribute similar amounts towards fitness decay as much more frequent clicks from less harmful mutations and could lead to extinction in humans, well before back mutations can arise with reasonable frequencies (e.g. $U_{sdm} = 0{\cdot}05$ and $s = -0{\cdot}01$ change only about 2% of mtDNA before extinction, suggesting negligible back mutation rates), unless lower mutation rates and peculiar shapes of the DDME reduce U_{sdm} to the point where the threat vanishes.

Second, it is doubtful, whether back mutations still have their full compensatory effects after a major part (e.g. 20%) of mtDNA has changed. If other compensatory mutations adjusted structures around the original SDM, then a back mutation that may be beneficial or at least neutral, could easily become deleterious (Kondrashov *et al*., 2002; Loewe, in prep.). If this is combined with the reasonable assumption that any biological structure could always be just a little bit worse, then one can expect equilibrium to build up between deleterious and compensatory back mutations. Once this equilibrium is reached, mutation accumulation will be as fast as in a simpler ratchet model without compensatory mutations, since each new SDM will destroy the possibility for repair of another SDM by compensatory back mutations (Loewe, in prep.). Thus the main effect of compensatory back mutations in this context is to increase the number of deleterious mutations that must be accumulated before extinction can occur, assuming that the original genome was free from SDMs. Obviously the equilibrium level of deleterious mutations will be much lower in recombining genomes. If we assume that mitochondria recombined for the most part of their history and stopped only 'recently' (see below), then it will depend on the exact details whether compensatory back mutations can solve the paradox by significantly increasing C_{mm}, the number of SDMs needed for extinction.

Thus, the existence of back mutations does not seem to rule out the threat of extinction. The following argument suggests that the first possibility raised above cannot be neglected. Parameter combinations requiring SDM saturation of mtDNA for extinction usually also accumulate SDMs at about the same rate as neutral mutations, predicting unity as the ratio of non-synonymous to synonymous mutations (see Fig. 5 and discussion of divergence below). The fact that this ratio is far less than unity in mtDNA suggests that many mutations have rather strong effects on mtDNA (Piganeau & Eyre-Walker, 2003) and it would be surprising if extraordinarily few effects would be within s_{cm}, where back mutations can be neglected in any model.

(ii) *Compensatory mutations at the molecular level*

High frequencies of advantageous mutations can always stop genomic decay (Schultz & Lynch, 1997; Bachtrog & Gordo, 2004), but it is unclear to what extent mtDNA can still be optimized easily, as it is probably close to its evolutionary optimum. Therefore, most beneficial mutations can be expected to compensate for deleterious mutations directly at the molecular level. These compensatory mutations go beyond repair by simple back mutations, by adjusting the molecular structure to truly accommodate the originally deleterious mutation (Kondrashov *et al*., 2002; Kern & Kondrashov, 2004). Compensatory mutations might turn out to be much more common than assumed above (Hartl & Taubes, 1996; Whitlock & Otto, 1999; Kern & Kondrashov, 2004; Poon & Chao, 2005; Poon *et al*., 2005) and have been hypothesized to stop the decline of fitness (Escarmis *et al*., 1999; Poon & Otto, 2000; Whitlock, 2000; Whitlock *et al*., 2003). Future studies will have to show whether their frequency is indeed high enough to stop decay. These mutations that mechanistically compensate for the molecular damage of a deleterious mutation are independent of compensatory mutations at unrelated quantitative trait loci (Wagner & Gabriel, 1990).

(iii) *Mutational hotspots*

The arguments above assume that deleterious mutation rates are uniformly distributed across the mitochondrial genome. However, extreme rate heterogeneity among sites could solve the genomic decay paradox. In this scenario, most SDMs observed in pedigrees would come from a minority of relative

hotspots, while most sites have effective mutation rates low enough to be compatible with long-term survival (e.g. on the order of 10^{-7} per site per generation). Muller's ratchet will easily fix SDMs at mutational hotspots, but these sites also have a high probability of acquiring back mutations. If most functionality is encoded in sites with low mutation rates, then Muller's ratchet is easily reduced to being irrelevant. The key question is the extent of mutation rate heterogeneity among sites in the evolutionary lineage of humans. Data for the D-loop with its well-known hypervariable regions show considerable heterogeneity (Meyer *et al*., 1999; Arbogast *et al*., 2002) and the situation in the coding region seems to be similar (Galtier *et al*., 2006). It is important to consider the heterogeneity of mutation rates before selection and not the heterogeneity of substitution rates, i.e. mutation rates modulated by fixation probabilities (Pesole & Saccone, 2001; Meyer & von Haeseler, 2003). Additional factors that might contribute to mutation rate heterogeneity could include mechanisms of mismatch repair (Mason & Lightowlers, 2003; Mason *et al*., 2003), asymmetrical mutation bias caused by mtDNA replication (Faith & Pollock, 2003; Raina *et al*., 2005) and heterogeneities in particular mutational transitions (Samuels *et al*., 2003).

(iv) *Mutator lineages*

Even if mutation rates turn out to be rather homogeneous among sites, rate heterogeneity among individual lines of descent could solve the paradox. Thus, Muller's ratchet would eliminate lines of descent with high mutation rates yet allow population persistence because lines with low mutation rates readily replace them. If mutator lines arise frequently and their presence is not noticed in studies that measure mutation rates (by averaging over mutators and non-mutators), then the relevant rate (i.e. that of the non-mutators) is much lower than the average inferred rate. Such a scenario might be suggested by the observation that mutations in DNA polymerase γ frequently cause mitochondrial disorders (Longley *et al*., 2005). Indeed, rate heterogeneity among lines can be observed between animal populations (Zhang & Ryder, 1995), animal species (Gissi *et al*., 2000; Arbogast *et al*., 2002) and closely related plant species (Cho *et al*., 2004; Parkinson *et al*., 2005), assuming that other forces can be neglected in these examples. However, it is unclear where the lower limit of the mutation rate might be in mtDNA and how it is influenced by molecular mechanisms of mismatch repair (Mason & Lightowlers, 2003; Mason *et al*., 2003) and general trade-offs related to the cost of replication fidelity (Kondrashov, 1995*b*; Furio *et al*., 2005). It is also possible that mutation rates in the human line have been much lower in the distant past, if findings of slow neutral mtDNA evolution in sharks (Martin *et al*., 1992) or plants (Wolfe *et al*., 1987; Palmer & Herbon, 1988; Knoop, 2004) can be applied here.

(v) *Bimodal distributions of mutational effects*

While current evidence suggests that the DDME has much probability mass in the critical range of selection coefficients, it should be noted that methods for estimating DDMEs are rather new and future research might lead to different conclusions. The paradox can be solved by a bimodal DDME, where many mutations with large effects are completely removed by selection and some with very small effects accumulate, but never combine to a noticeable threat (Kondrashov, 1995*c*). However, epidemiological studies in modern societies suggest that most mutations might not be deleterious enough to exclude operation of the ratchet, since the incidence of genetic forms of mitochondrial disease are between 1 in 2000 and 1 in 5000 live births (Naviaux, 2004). This means that most non-synonymous mutations in mtDNA seem to have effects that are too small to be noticed by the medical community. It would be surprising if most of these effects cluster in the probably very narrow band of mutational effects that are deleterious enough to stop the ratchet (see Fig. 3, e.g. $s > 1\%$, depending on U_{sdm}) but small enough to escape the attention of the medical community (e.g. $s < 10\%$?). More studies are needed; these will also have to consider the possibility that environmental conditions might have been more severe in the past, leading to the selective removal of a larger fraction of mutations over most of the history of the human line.

(vi) *Recombination stopped recently*

Recombination is known to be very effective at stopping decay due to Muller's ratchet (Muller, 1964; Felsenstein, 1974; Pamilo *et al*., 1987; Bell, 1988; Charlesworth *et al*., 1993*c*; Lynch *et al*., 1995*a*; Antezana & Hudson, 1997*b*; Schultz & Lynch, 1997). Therefore this work makes the simplifying assumption that genomic decay in mtDNA would be insignificant if there were enough recombination. Apparently there is no recombination of mtDNA in the human line today (Innan & Nordborg, 2002), but that does not mean that there was no recombination in the past. In fact, mitochondrial recombination might occur in more species than previously assumed (Piganeau *et al*., 2004; Tsaousis *et al*., 2005). The closest primates with some evidence for recombination in mtDNA are the Old World monkeys *Papio papio*, *Macaca nemestrina* and *Mandrillus sphinx* (Piganeau *et al*., 2004). Old World monkeys are

believed to share a common ancestor with humans about 23 or 31 Myr ago (Takahata & Satta, 1997; Glazko & Nei, 2003). It therefore seems reasonable that the human line might have lived the last 20 Myr without recombination, but there is currently no reason to assume the absence of recombination before that. While the decay paradox disappears if recombination of mtDNA stopped only very recently in the human line, it might be impossible to test this hypothesis.

(vii) *Rare recombination still happens*

While conventional wisdom believes that there is no recombination in mtDNA, recent years have seen a considerable debate about the potential existence/frequency of rare recombination events even in human mtDNA (Awadalla *et al*., 1999; Birky, 2001; Ladoukakis & Zouros, 2001; Innan & Nordborg, 2002; Gandolfi *et al*., 2003; Hagelberg, 2003; Rokas *et al*., 2003; Knoop, 2004; Kraytsberg *et al*., 2004; Piganeau *et al*., 2004; Tsaousis *et al*., 2005; Galtier *et al*., 2006). While there is no conclusive evidence of recombination of mtDNA in the human germline (Innan & Nordborg, 2002; Galtier *et al*., 2006), future work may find enough recombination to stop decay, as only very little recombination is necessary to do so (see references above).

(viii) *Multi-level population genetics*

Many mtDNA molecules exist per mitochondrion, many mitochondria per cell and many cells compete for entering the germline. It has been argued that the resulting multi-level population genetics of mtDNA plays a pivotal role in stopping its genomic decay (Takahata & Slatkin, 1983; Bergstrom & Pritchard, 1998). In this context, a process known as oocyte atresia is of special importance (Jansen & de Boer, 1998; Krakauer & Mira, 1999, 2000; Jansen & Burton, 2004; Johnson *et al*., 2004; Stearns, 2005). During this process human females produce large amounts of oocytes only to destroy them again during further development. Production peaks about 3 months after conception when 7 million oocytes are present in the embryo, but continues well after birth (Johnson *et al*., 2004). At birth or puberty only 1 million or a few thousand oocytes survive, respectively (Stearns, 2005). Since only about 0·005% of the oocytes formed are allowed to continue to ovulation, this provides an excellent opportunity to extensively test the functional integrity of mtDNA and remove defective genomes with very small costs. Indeed molecular mechanisms have been found that allow mitochondria to contribute to the induction of programmed cell death (Green & Reed, 1998; Green, 2005). It has been argued that oocyte atresia occurs to stop Muller's ratchet (Krakauer & Mira, 1999, 2000; Stearns, 2005) and that mitochondria act as guardians of the genetic quality of the male contribution to the human zygote (Giannelli, 2001). Almost certainly mutation accumulation in mtDNA is slowed down by oocyte atresia. However, the mutation rates employed in Fig. 3 have been measured between generations and therefore reductions in U_{sdm} from oocyte atresia have already been factored in. Observatios show that oocyte atresia cannot remove all severely deleterious mutations from mtDNA (McFarland *et al*., 2002), implying that its purifying effects on less deleterious mutations might be even weaker. While the presumably staggering speed of genomic decay without oocyte atresia is irrelevant for the current genomic decay paradox, other multi-level population genetics processes might well contribute towards a solution (Birky *et al*., 1983; Takahata & Slatkin, 1983; Blackstone, 1995; Otto & Orive, 1995; Bergstrom & Pritchard, 1998; Otto & Hastings, 1998; Rispe & Moran, 2000; Birky, 2001; Paulsson, 2002). The extremely small bottleneck of mtDNA between generations (Jenuth *et al*., 1996; Jansen & de Boer, 1998; Jansen & Burton, 2004) seems to play an important role by increasing the efficacy of selection by increasing the variance in fitness among individuals (Bergstrom & Pritchard, 1998). However, it is not clear whether corresponding effects are enough to counteract the ratchet.

(ix) *Epistasis*

The multiplicative fitness model used in this work assumes that mutations do not interact with each other (no epistasis). This seems reasonable as a first approximation, despite numerous examples of interactions between mutations that either increase or decrease the effects of 'second' mutations relative to the 'first' mutations, which corresponds to synergistic or antagonistic epistasis, respectively (Wolf *et al*., 2000). It has been argued that synergistic epistasis may stop the accumulation of deleterious mutations (Crow & Kimura, 1979; Melzer & Koeslag, 1991; Crow, 1993, 1997, 2000; Kondrashov, 1994; Whitlock & Bourguet, 2000). However, this critically depends on the absence of a DDME, as mutation accumulation will only stop if synergistic interactions increase all mutational effects up to the point where selection against them is strong enough to prevent their accumulation (Butcher, 1995; Schultz & Lynch, 1997). This point is almost never reached with most current DDME models. It is also not clear whether biological systems exhibit enough synergistic epistasis to apply such models. Empirical observations of epistasis on a large scale (i.e. as in Elena & Lenski, 1997; Sanjuan *et al*., 2004; Segre *et al*., 2005) typically suggests that about half of all epistatic interactions

are synergistic and half are antagonistic. More precise data are needed for building a solid foundation for detailed analyses of long-term fitness consequences of complex metabolic networks. In addition, other approaches may be used to infer the importance of epistasis for reducing mutational load (Rice, 1998; Peck & Waxman, 2000). Since a large number of nuclear genes are devoted to mitochondrial maintenance (Contamine & Picard, 2000) and epistatic interactions can also occur between nuclear and mitochondrial mutations (Johnson *et al*., 2001; Zeyl *et al*., 2005), one might hypothesize that nuclear genes compensate for problems in mitochondria. This has been shown for some deleterious mtDNA mutations in cultured human cells (Manfredi *et al*., 2002; Kolesnikova *et al*., 2004). As nuclear DNA can recombine, it might be faster in responding to problems with mitochondria than mtDNA, assuming sufficiently high mutation rates at the corresponding nuclear loci. However, the apparent functional requirement for some genes to be synthesized inside mitochondria moves them out of the reach of nuclear help (von Heijne, 1986; Claros *et al*., 1995; Daley *et al*., 2002; Allen, 2003; Timmis *et al*., 2004) and it is not clear how many exceptions to this rule exist (as reported e.g. by Manfredi *et al*., 2002; Kolesnikova *et al*., 2004). Epistatic interactions caused by RNA-editing have also been invoked to explain the persistence of mtDNA (Boerner *et al*., 1997).

(x) *A reservoir of nuclear copies*

It is well known that complete genes are frequently transferred from mtDNA to the nucleus in many species (Thorsness & Fox, 1990; Thorsness & Weber, 1996; Adams *et al*., 2000; Berg & Kurland, 2000; Kurland & Andersson, 2000; Bensasson *et al*., 2001) including humans (Ricchetti *et al*., 2004). Therefore we cannot categorically exclude the formal possibility that a few of the many nuclear copies of mtDNA genes might occasionally make their way back into the mitochondrial genome, although this has never been observed and is certainly more than 10^5 times less frequent than transfer in the other direction (Thorsness & Fox, 1990; Thorsness & Weber, 1996). Since mutations typically accumulate much slower in nuclear DNA, such transfers might occasionally restore some mtDNA functions to better ancestral states. Support for such speculation might come from the existence of techniques for the transformation of mtDNA (Klinner & Schafer, 2004; Sato *et al*., 2005), the rescue of a mtDNA deficiency by transfer of the corresponding gene to the nucleus (Manfredi *et al*., 2002), the observation of the results of transfer of nuclear sequences into mtDNA in plants (Knoop, 2004) and a possible similar transfer in a soft coral (Pont-Kingdon *et al*., 1998). Rare examples of paternal leakage of mtDNA (Schwartz & Vissing, 2003; Schwartz & Vissing, 2004) further suggest that, even in humans, sources of mtDNA other than the maternal oocyte are occasionally available. Such paternal leakage has been hypothesized to contribute towards stopping Muller's ratchet in parthenogens (Schartl *et al*., 1995; Beukeboom & Vrijenhoek, 1998). Nevertheless, the overwhelming lack of evidence for gene transfer from the nucleus to mtDNA in vertebrates makes this scenario appear one of the most unlikely solutions to the paradox in humans. Paternal leakage does not help here, since there is no reason to believe that paternal mtDNA has lower mutation rates than maternal mtDNA. The evidence for gene transfer into mtDNA cited above comes from species where mtDNA is under greatly relaxed selection; none of these species requires fast, energetically expensive movements. The fact that some eukaryotes such as yeast can live completely without mitochondria (Bernardi, 2005) underscores the huge difference in functional demands on mtDNA among different kingdoms of life. A final problem with the nuclear reservoir hypothesis is the high probability that nuclear copies accumulate serious damage over time, since they are not under purifying selection (Huang *et al*., 2005). Thus, unless transfer from the nucleus into mtDNA happens frequently enough to escape crippling frameshift mutations, it cannot contribute to long-term survival.

7. Discussion

(i) *Can molecular signatures be found?*

Based on signatures left in sequences, it is very difficult to distinguish the operation of Muller's ratchet from operation of its sister processes weak selection Hill–Robertson effect and background selection (see review in Charlesworth & Charlesworth, 2000). Assuming the absence of recombination and that the distribution of mutational effects spans a wide range of selection coefficients will cause the simultaneous operation of all three processes as shown in Fig. 4: Muller's ratchet does not click in the parameter space of background selection, since selection is too strong and all new mutations are always removed. This region of parameter space is clearly separated from the parameter space of Muller's ratchet by the wall of background selection (Charlesworth *et al*., 1993*a*, 1995; Gordo & Charlesworth, 2001*a*). As $|s|$ decreases below the location of this wall, extinction times switch almost abruptly from effectively infinite to their minimal values, where Muller's ratchet operates slowly and few large mutations contribute most to genomic decay. Close to the wall, extinctions can occur well before back mutations have a chance to

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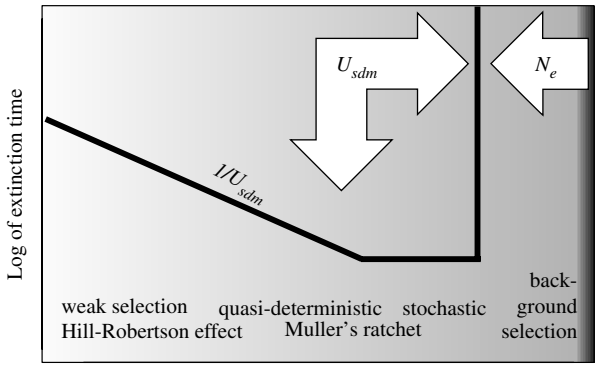

Fig. 4. Simplified overview over the U-shaped plot. The various shades of grey mark the various parameter spaces that are relevant to quantify the threat of extinction from Muller's ratchet. From left to right: white neutrality; very light grey, weak selection Hill–Robertson effect; light and medium grey, quasi-deterministic and stochastic operation of Muller's ratchet, respectively; dark grey, background selection (reviewed in Charlesworth & Charlesworth, 2000). The two most important parameters determining the operation of the ratchet are the deleterious mutation rate U_{sdm} (makes the valley deeper and wider) and the population size N_{e} (opposes the effect of U_{sdm} that makes the valley wider), assuming that most of the distribution of mutational effects is fixed by the biology of an organism. To simplify quantification of Muller's ratchet even further, one may want to concentrate on the location of the wall of background selection (Charlesworth *et al*., 1993*a*, 1995) and on the depth of the valley that represents the most critical range of selection coefficients (s_{cm}). This plot is called U-shaped plot because of the shape it takes on linear scales.

appear at significant frequencies (e.g. $U=0{\cdot}05$ and $s=-0{\cdot}01$ change only about 2% of mtDNA before extinction). As $|s|$ decreases further, the speed of the ratchet increases until it changes from stochastic to quasi-deterministic, when the equilibrium size of the best class $N_{\mathbf{0}}=N_{\mathrm{e}}*\exp(-U_{\mathrm{sdm}}/|s|)$ falls below 1 (Gessler, 1995). Further decrease in $|s|$ leads to faster accumulation of less deleterious mutations, which increases the chance of back mutations and compensatory mutations gradually entering the parameter space of the weak selection Hill–Robertson effect. At $U/|s|\geqslant 500$ substitution speed can be approximated by $1/U$ and is hard to distinguish from conventional neutral drift models.

Whether the excess of low-frequency polymorphisms found in human mtDNA (Ingman *et al*., 2000) stems from Muller's ratchet (Gordo *et al*., 2002), selective sweeps or population expansions (Ingman *et al*., 2000) is hard to say. Comparisons of the stability of tRNA molecules suggest that relatively more SDMs do get fixed in mtDNA than in the nucleus (Lynch, 1996, 1997). The two recent independent estimates of the distribution of mutational effects for human mtDNA discussed above agree that most effects are slightly above $1/N_{\mathrm{e}}$ (Nielsen & Yang, 2003; Piganeau & Eyre-Walker, 2003) suggesting appreciable frequencies of critical selection coefficients. Therefore many mutational effects are probably not large enough or small enough that operation of the ratchet can be excluded or regarded as irrelevant, respectively. Thus current molecular data do not appear to be in conflict with operation of Muller's ratchet in mtDNA, and it would be surprising if Muller's ratchet did not contribute significantly to the excess of low-frequency polymorphisms observed in human mtDNA.

(a) *Comparing synonymous and non-synonymous sites*

Fig. 5 further explores the impact of Muller's ratchet on molecular divergence data using the following simple approximation. Simulator005 cannot predict fixation rates for mutations directly, since it does not keep track of the position of individual mutations in the genome. However, previous studies have shown that Muller's ratchet fixes approximately one new mutation for each click (Higgs & Woodcock, 1995; Charlesworth & Charlesworth, 1997). Thus predictions of click time allow the rate of divergence to be inferred. Selection at non-synonymous sites does not affect the fixation rate at linked neutral sites (Birky & Walsh, 1988). The reasonable assumption that synonymous loci are effectively neutral allows us to compute an approximation for $K_{\mathrm{A}}/K_{\mathrm{S}}$, where K_{A} and K_{S} are the divergence rates at non-synonymous and synonymous sites, respectively. The resulting inferred rate of fixation relative to the neutral value is plotted in Fig. 5 and can be easily compared with observations of $K_{\mathrm{A}}/K_{\mathrm{S}}$ reported in the literature.

Such observations of $K_{\mathrm{A}}/K_{\mathrm{S}}$ in mitochondria comparing humans and chimpanzees suggest a ratio of about 7·5% (Pesole *et al*., 1999; Saccone *et al*., 2000) or 5% (as computed from table 1 in Piganeau & Eyre-Walker, 2003). This implies that more than 90% of all non-synonymous mutations are removed by selection, which is supported by the high level of significance found in McDonald–Kreitman tests of mtDNA (Weinreich & Rand, 2000; Piganeau & Eyre-Walker, 2003). Comparing these results with the predictions in Fig. 5 strongly suggests that the majority of selection coefficients must be substantially larger than $1/N_{\mathrm{e}}$. These observations clearly allow the exclusion of a very fast turning ratchet that accumulates essentially harmless mutations. If all non-synonymous mutations were so rare in the population that they could not be observed, then their inferred effects would all be in the evolutionary safe background selection range and the existence of intermediate critical selection coefficients could be excluded, but this is not the case. Thus Fig. 5 leaves us somewhere between a 'harmless', but improbable,

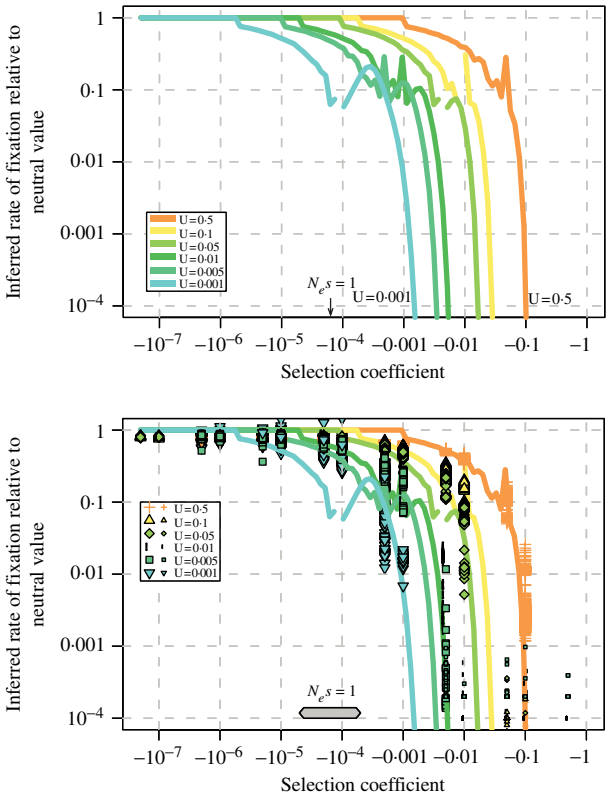

Fig. 5. Predicted reduction in divergence rates in the human mtDNA line due to Muller's ratchet. This figure uses the same effective click time prediction mechanisms as Fig. 2. The inferred rate of fixation of deleterious mutations relative to the rate for neutral mutations (*y*-axis) is computed by dividing $1/U_{\mathrm{sdm}}$ by the predicted effective click time. The resulting fraction gives a rough idea of the fraction of non-synonymous mutations to synonymous mutations fixed in protein sequences (K_A/K_S) if all mutations had the same effect and mutational bias and selection for codon bias are assumed to be absent. The upper plot uses the click time prediction methods from Fig. 1 (omitting the 'precise' lines) and is shown to facilitate navigation through the lower plot that includes simulation results from Fig. 2 as well. See Figs. 1 and 2 for more details.

bimodal DDME and a unimodal DDME with a significant fraction of critical selection coefficients. Existing evidence (Nielsen & Yang, 2003; Piganeau & Eyre-Walker, 2003) supports the latter, suggesting that Muller's ratchet might drive mtDNA to extinction. It is intuitively easy to see why at least some mutations have to be in the intermediate range by comparing divergences and diversities. The ratio of non-synonymous to synonymous diversity as estimated from the corresponding fractions of segregating sites is about 13·4% (as computed from table 1 in Piganeau & Eyre-Walker, 2003). Since this ratio of diversities is higher than the corresponding ratio of divergences, there must be some mutations that are deleterious enough to be removed in the long term but not deleterious enough to be removed immediately (i.e. not to contribute to diversity). These mutations clearly belong to some intermediately deleterious range (Hasegawa *et al.*, 1998).

The fact that results presented here did not simulate realistic distributions of mutational effects does not significantly affect conclusions, as previous results show that the effects of Muller's ratchet from two classes of similar selection coefficients are equivalent to the effects of a ratchet with constant selection coefficients that are as large as the harmonic mean of the effects of the two classes (Gordo & Charlesworth, 2001*a*). This means that the most realistic distributions of mutational effects can be approximated by a constant selection coefficient somewhere in the critical range (it has to be larger than $1/N_e$ to be effectively selected and it will be biased to the smaller values, since the harmonic mean is always biased towards the smaller values of a distribution).

(b) *Predicting diversity at synonymous sites*

The reduction of neutral diversity (π, mean number of pairwise differences between randomly sampled sequences) by recurrent deleterious mutations at linked sites is well known from background selection theory (Charlesworth *et al.*, 1993*a*, 1995; Nordborg *et al.*, 1996). It follows naturally that mutations with weaker effects capable of driving Muller's ratchet should reduce linked neutral diversity as well (Gordo & Charlesworth, 2001*a*; Gordo *et al.*, 2002). This effect might be used to infer the operation of Muller's ratchet by comparing π at neutral mtDNA sites with expectations based on (i) extrapolations from the nuclear genome after correcting for differences in mutation rate and mode of inheritance and (ii) predictions of the relative reduction of π at neutral sites in mitochondria that is expected from Muller's ratchet (see Fig. 6 and Gordo *et al.* (2002) for methods).

Applying such reasoning to human mtDNA suggests an expectation of π in mitochondria without Muller's ratchet is at least 0·008 (assumptions: $1{\cdot}0 \times 10^{-6}$ is the lower limit for the average total mutation rate for any effects in the coding region of mitochondria; π in human nuclear DNA is 0·0008 (Takahata & Satta, 1997; Reich *et al.*, 2002); the average nuclear mutation rate in humans is $2{\cdot}5 \times 10^{-8}$ per generation; N_e is 10^4; generation time is 20 years; the human–chimpanzee divergence started 5 Myr ago (Nachman & Crowell, 2000); diversity in mtDNA is 1/4 of autosomal values, since inheritance is effectively haploid and maternal only). This prediction is about 3 times higher than the observed value of $\pi = 0{\cdot}0028$ for the coding region of human mtDNA (Ingman *et al.*, 2000) and supports the notion that Muller's ratchet actively reduces π. To determine the precise degree of this reduction in π requires

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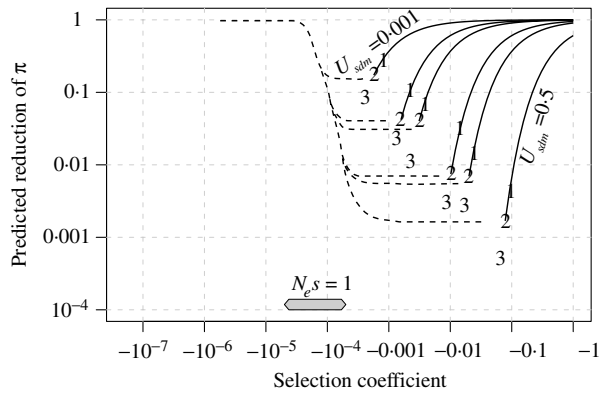

Fig. 6. Estimated reduction in diversity in the human mtDNA line due to Muller's ratchet. Diversity in this plot is defined as π, the mean number of pairwise differences between randomly sampled sequences, not as the fraction of segregating sites. If Muller's ratchet were not operating, diversity at synonymous sites would not be reduced. If the ratchet operates, diversity (and corresponding estimates of N_e from synonymous sites) will be reduced by the factor shown in the plot. Diversity reduction for the lines in the right part of the figure is predicted by $\exp(-|U_{sdm}/s|)$, which was shown to hold approximately until $N_e s$ $\exp(-|U_{sdm}/s|) \approx 1$ (Gordo *et al.*, 2002). The numbers 1, 2 and 3 on this line indicate where this approximation breaks down, depending on assumed values of $N_e = 5000$, 15 800 and 50 000, respectively. Gordo *et al.* (2002) found in simulations that diversity reduction for less deleterious mutations after this point stayed approximately at the same level until it vanished again when mutational effects approached effective neutrality ($N_e s \approx 1$). To indicate this behaviour in this plot, extensions (dashed lines) were manually drawn for $N_e = 15\,800$. The actual lines in the left part of the figure are purely fictional and have no computational basis. Since the more sophisticated equations in Gordo *et al.* (2002) are based on the diffusion method, they could extend predictions towards smaller $|s|$ only for a small distance on the log scale. The mutation rates are the same as in Figs. 1, 2 and 5.

better estimates of actual mutation rates, as the upper limit for the average mutation rate in mtDNA used in this study implies a 30-fold difference between observed and predicted diversities. Comparing these values with Fig. 6 shows that Muller's ratchet could easily have led to the corresponding reductions in π. This approach cannot be used for diversity as estimated from the number of segregating sites (θ), since Muller's ratchet leads to an excess of rare variants (Gordo *et al.*, 2002) that is actually observed in mtDNA (compare with data in Piganeau & Eyre-Walker, 2003).

Why has such evidence for the operation of Muller's ratchet not been noticed earlier? A common simplifying assumption is to neglect the removal of deleterious mutations by purifying selection. This assumption suggests that the long-term substitution rate of $1{\cdot}70 \times 10^{-8}$ per site per year in the coding region of mtDNA (Ingman *et al.*, 2000) can be used to infer actual mutation rates per generation simply by multiplying by generation time (20 years). The resulting expected diversity in mtDNA of 0·00272 ($= 0{\cdot}0008/2{\cdot}5 \times 10^{-8} * 1{\cdot}7 \times 10^{-8} * 20 * 1/4$) is almost exactly the same as the observed value of 0·0028 (Ingman *et al.*, 2000). It is also interesting to note that a careful analysis of Fig. 2 almost certainly excludes a genomic decay paradox for such a low mutation rate. The problem is that current populations seem to mutate faster.

To assess the true importance of Muller's ratchet in mtDNA, we need much more robust estimates of the mutation rate in human mtDNA, especially regarding heterogeneity among sites and among populations. Support for a high fraction of critical selection coefficients may also come from observations of phylogenetic networks, which suggest that amino acid replacements observed in the periphery of such networks are more deleterious than those in the central parts due to purifying selection (Moilanen & Majamaa, 2003), and from potential associations between clinical disorders and haplogroups (Herrnstadt & Howell, 2004). More refined methods can be expected to produce a clearer picture of Muller's ratchet in action.

(ii) *Uses of the U-shaped plot*

The U-shaped plot of extinction time over selection coefficients (Figs. 1–4) has several important applications. It points to potentially critical parameter combinations that are consistent with eventual population extinction and can stimulate research to obtain more precise estimates. These can be expected to solve genomic decay paradoxes at least on some occasions. Furthermore, such plots help make the genomic decay paradox explicit to prompt investigation of processes that may solve it.

(a) *Application to other species*

U-shaped plots can be used to quantify claims of the operation of Muller's ratchet in asexual genetic systems – something that is long overdue (Butlin, 2002). This work led to the generation of the largest comprehensive overview of the parameter space of Muller's ratchet that is currently available (central database: $>10^5$ simulations, >80 years CPU time). Therefore production of U-shaped plots for a particular system is no longer prohibitively complex (see http://www.evolutionary-research.net/).

(b) *Most important parameters*

The broad overview of T_{ex}–U_{sdm}–s–N_e–T_{gen}–R_{max} parameter space presented here allows an assessment of parameters that most influence the threat of extinction from Muller's ratchet. Fig. 2 shows that the

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mutation rate has the strongest influence: An increase in U_{sdm} shifts the curve towards lower extinction times and increases the range of critical selection coefficients by moving the wall of background selection. Assuming that we cannot change most mutational effects makes effective population size the second most important parameter. While a large population size cannot stop the ratchet for lower selection coefficients, it can narrow the critical range s_c by moving the wall of background selection towards smaller mutational effects. All other parameters act only as scaling factors and do not influence the operation of the ratchet.

(c) *Simplified picture*

Careful inspection of Figs. 1–3 suggests that quantification of the threat of extinction by Muller's ratchet may be further simplified. As drafted in Fig. 4, the key results are given by (i) the location of the wall of background selection, (ii) the range of critical selection coefficients s_c and (iii) the minimal extinction time. All other selection coefficients can be approximated by $1/U_{sdm}$ or infinity as click time for selection coefficients below or above s_c, respectively.

(iii) *Conclusion*

We need more precise estimates of the basic parameters in mtDNA, including the mutation rate in the coding region, the extent of mutation rate heterogeneity, potential low levels of recombination and the distribution of deleterious mutational effects. These may allow more detailed predictions of the molecular signatures (Charlesworth & Charlesworth, 2000) of decaying populations and may elucidate the solutions to the genomic decay paradox in human mtDNA, if combined with models that include more details like rate heterogeneity, different mutational effects (Butcher, 1995), rare recombination (McVean & Charlesworth, 2000) and spatial structure (Higgins & Lynch, 2001). It will be thrilling to see which of the various potential solutions actually solves the genomic decay paradox in mtDNA.

A more practical conclusion from this work is independent of such uncertainty and strongly supported by existing population genetic theory (Muller, 1950; Lynch *et al*., 1999): The anthropogenic increase in mutation rates should be minimized, since mutation rates are a key factor in determining the speed of accumulation of genetic damage. Indeed, the parameter that largely determines the impact of Muller's ratchet is the very same parameter that humans have most control over. While mutation rates cannot be reduced below natural spontaneous levels, much man-made increase can certainly be avoided by reducing pollution and mutagenic lifestyle. Causative agents include (i) mutagens in food (Joint Institute for Food Safety and Applied Nutrition, 2005), (ii) some medical X-ray practices (Giles, 2004), (iii) military operations that develop and use M(utagenic)-weapons (e.g. Cyranoski, 2002; Hambling, 2003; Hande *et al*., 2003), (iv) polluted air (Samet *et al*., 2004; Somers *et al*., 2004), (v) unsafe nuclear power stations (Ellegren *et al*., 1997; Moller *et al*., 2005) and (vi) environmental pollution by pesticides and other mutagenic chemicals (Aitken *et al*., 2004). Halving mutagenesis just from airborne particles (Samet *et al*., 2004; Somers *et al*., 2004) increases extinction times by a factor of 2 for most mutational effects and makes extinctions from Muller's ratchet impossible for others (see Figs. 1–4).

There are many causes that can drive populations to extinction (Frankham *et al*., 2002; Groom *et al*., 2005) and many reasons for keeping mutation rates as low as possible, including cancer and hereditary diseases (Sunyaev *et al*., 2001; Yampolsky *et al*., 2005). Muller's ratchet provides yet another reason: the health of future generations. Genetic counselling has no power against most mutations with evolutionary critical effects and the same is true even for very optimistic scenarios of future gene therapies. Therefore a reduction in the anthropogenic increase of mutation rates is by far the best option for fighting genomic decay.

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