Reproductive life history may modulate selection on the germline mutation rate

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Abstract

A long lifespan is a potential liability from the point of view of mutation accumulation, allowing more time for cancer-causing mutations to accumulate in somatic tissues. In practice, however, long-lived organisms do not seem to suffer more cancer mortality than short-lived organisms, a pattern known as “Peto’s Paradox.” One suggested resolution to Peto’s Paradox is that long lifespans may facilitate the evolution of better somatic DNA repair. This comes into some conflict with the predictions of the drift-barrier hypothesis of mutation rate evolution, which infers that germline DNA repair efficacy should be best in short-lived organisms with high genetic diversity and efficient natural selection. Here, we show that this conflict can be resolved by modeling the tendency of long generation times to increase the strength of selection against mutator alleles that affect clocklike mutational processes. By considering the empirical joint distribution of generation time and effective population size, we show that stronger selection against mutator alleles in long-lived organisms is likely to be partially but not completely offset by their tendency to have low effective population sizes. This model resolves a conflict between the predictions of theories of germline and somatic mutation rate evolution, leading to the prediction that both germline and somatic DNA repair should be most efficient in long-lived organisms despite their lower genetic diversity.

Significance Statement

All cells accumulate mutations due to DNA damage and replication errors. Mutations affecting the germline can harm future offspring, while mutations in other tissues can threaten longevity. Previous theories have predicted that somatic DNA repair should be most effective in long-lived organisms, but that germline DNA repair should instead be most effective in organisms with high genetic diversity that tend to be short-lived. We model how reproductive age should affect germline mutation rate evolution and show that this may reverse the prediction that high-diversity species should have more efficient selection for effective germline DNA repair. By demonstrating how population size *and* lifespan likely shape selection on mutagenesis, we are able to harmonize predictions about germline and somatic mutation rate evolution.

Introduction

A species’ life history strategy has a profound impact on its physiology and evolutionary trajectory (1–6). Some organisms invest in producing large numbers of offspring and achieving rapid boom-time population growth, while other organisms invest in producing smaller numbers of offspring that are each expected to live out long, reproductively successful lifespans (7, 8). The choice of life history strategy clearly affects the relative utility of producing large numbers of gametes versus investing in parental care (9–11), but life history can also affect the evolution of traits that are less obviously related to reproduction. One of these traits is the mutation rate

(12–15), which affects individual reproductive success and longevity but also modulates the evolvability and long-term genetic health of populations (16, 17).

Reproductive life history is known to have a short-term proximate impact on the mutation rate: in humans, for example, the children of older parents tend to have a higher germline mutation burden than children whose parents are younger at their time of birth (17–19). Over longer periods, life history strategy may also influence the strength of selection acting on the mutation rate–in long-lived organisms that reproduce late in life and practice extended parental care, there is likely to be stronger selection against somatic mutations that might lead to cancer than there would be in a species that tends to reproduce early in life and die of exposure at a young age. Such selection has been put forward as an explanation for Peto's paradox: the finding that long-lived organisms are not notably more likely to die of cancer than short-lived organisms whose DNA must avoid oncogenic mutations for a longer period of time (20, 21).

Though it is less obvious that the germline mutation rate should be subject to different selective pressures in long-lived versus short-lived organisms, there are at least two indirect reasons why this could be the case. One reason is the potential for pleiotropy between germline and somatic mutagenesis: if long-lived species evolve more effective DNA repair mechanisms to prevent somatic mutations and cancer, these should also be available for use in the germline–it has conversely been observed that DNA repair defects cause pleiotropic increases to germline and somatic mutation rates in humans (22, 23). Another factor is that fast-reproducing, short-lived organisms tend to have larger effective population sizes (24), and larger population sizes may enable more efficient selection for low mutation rates (25).

In a population with effective population size N_e, alleles can routinely fluctuate in frequency by as much as $\sim 1/N_e$ each generation (26). These random allele frequency changes reduce the efficiency of natural selection acting on weakly deleterious mutations with selection coefficients $s < 1/N_e$, since such subtle selective effects will tend to be overpowered by genetic drift. The threshold $1/N_e$ is sometimes called the "drift barrier" because below this threshold, drift is an effective barrier against the action of natural selection. About a decade ago, Lynch identified this drift barrier as a potential determinant of mutation rate variation among species, arguing that most "mutator alleles" that increase the mutation rate likely have relatively weak negative effects on population fitness, meaning that population size is likely to have a large impact on their evolutionary fate (27, 28).

The drift-barrier hypothesis is empirically supported by a robust negative correlation between N_e and the germline mutation rate, which has been observed among the major domains of life as well as within smaller clades such as animals and vertebrates (29, 30).

Despite the robustness of this anticorrelation, there are factors that complicate its interpretation, particularly in multicellular organisms. One complication is that long-lived species tend to have lower effective population sizes than short-lived species (24), leading to diverging predictions between the germline and the soma. Compared to mice, a relatively short-lived species with a large effective population size, long-lived species like humans and naked mole rats have lower somatic mutation rates per year (31, 32) and higher germline mutation rates per generation (33). At face value, this fits the drift-barrier-based prediction that mice should have stronger selection against germline mutator alleles and also fits the gerontological prediction that they should have weaker selection against somatic mutator alleles. However, it is not clear why there DNA repair mechanisms that evolve to protect the soma should be so ineffective in the germline and vice versa.

The potential significance of an inverse correlation between N_e and generation time was recognized as early as Tomoko Ohta's 1977 formulation of the nearly neutral theory (34), though this correlation was not empirically reported until 1993 (24). Ohta postulated that a relationship between N_e and generation time was required to reconcile the nearly neutral theory (which predicted a constant mutation rate per generation) with Zuckerkandl and Pauling's influential "molecular clock" hypothesis (35), which found substitution rates to be roughly constant per year in early comparative protein data. Ohta proposed that mutations occur at a constant rate per generation across species, but that a lower proportion of mutations tend to get fixed as substitutions in short generation time species if their large population sizes caused a higher proportion of weakly deleterious variation to be removed by purifying selection. This higher rate of purifying selection could have the effect of equalizing substitution rates between species with short generation times and high mutation rates per year and species with long generation times and low mutation rates per year. Although higher resolution data eventually revealed that neither the mutation rate per generation nor the substitution rate per year is truly invariant among species (36, 37), this historical dialogue illustrates how the inverse correlation between N_e and generation time may act to equalize rates of mutation accumulation between species with very different life history strategies.

In the context of the drift-barrier hypothesis, the association of small N_e with long generation time means that organisms with small N_e tend to have more time to accumulate mutations within the span of a generation, making it difficult to disentangle how much of the anticorrelation between N_e and mutation rate might be driven by generation time rather than selection against mutator alleles. In a recent study of vertebrate mutation rate variation, Bergeron et al. (29) noted that the mutation rate per generation was positively correlated with

generation time as well as negatively correlated with N_e. However, they also noted that generation time and N_e were negatively correlated with each other, making it difficult to identify which variable (if any) is most likely to be a true causal driver of vertebrate mutation rate variation. Wang and Obbard (30) were able to statistically resolve some of this nonidentifiability in a larger, more taxonomically diverse dataset, for which they determined that N_e still explained additional mutation rate variation after controlling for generation time.

In this manuscript, we propose a model in which generation time exerts both short-term and long-term effects on the germline mutation rate. While the short-term effect of generation time on the mutation rate is well known, no longer-term effect of generation time is generally incorporated into the drift-barrier model of selection against mutator alleles. We argue that the strength of selection against mutator alleles likely does depend on generation time due to the clocklike dynamics of mutation accumulation, which have been recently estimated using a combination of germline and somatic mutation measurements. When we assume that most mutations accumulate at a constant rate per year throughout germline development, as suggested by empirical studies, we predict that clocklike mutator alleles should be most effectively selected against in long-lived organisms with modest effective population sizes, thereby resolving the seeming inconsistency between the original drift-barrier hypothesis and the lifespan-dependence of somatic mutation rates.

Model and Results

About 90% of human germline mutations have been attributed to a process known as SBS5 in the Catalog of Somatic Mutations in Cancer (COSMIC) database, while the remaining 10% are mostly attributed to a process known as SBS1 (38, 39). Both SBS1 and SBS5 have been classified as “clocklike” mutational signatures based on the correlation of their exposures with age in tumor data, though they are not clocklike with respect to the same time unit. Although the etiology of SBS5 is not known, it appears to accumulate at roughly a constant rate per year in sperm, ova, and somatic cells (40). In contrast, SBS1 is dominated by CpG>TpG transitions caused by deamination of methylcytosine. While SBS1 was once thought to accumulate at a constant rate per year based on phylogenetic substitution data (41), de novo mutation data have since suggested that the load of SBS1 actually scales with the number of cell divisions, with little to no activity in post-mitotic cells but commensurately high activity in cells that are rapidly dividing (39, 40). The clocklike nature of these processes explains the

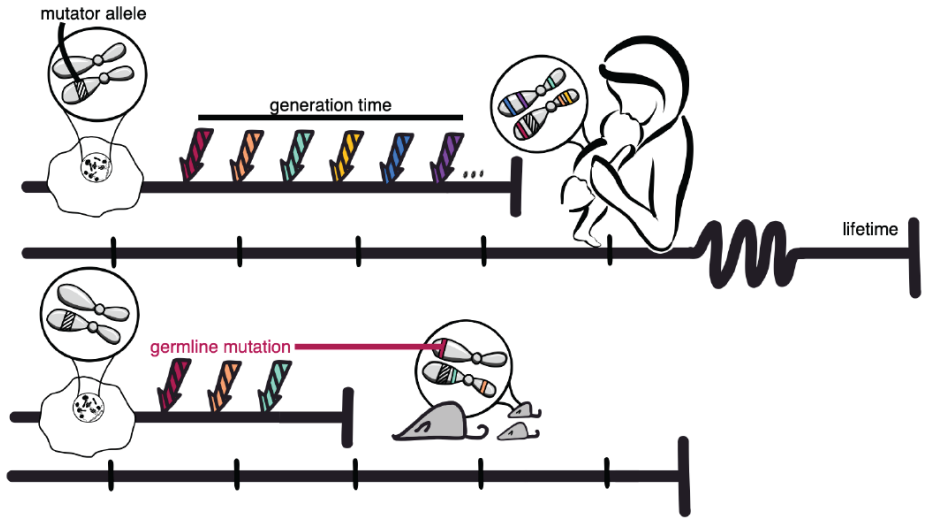
mutagenic effect of generation time within species, since longer generations tend to span both more time and more cell divisions than shorter generations, leaving more time for SBS1 and SBS5 to accumulate.

The clocklike accumulation of SBS1 and SBS5 is somewhat unique among the signatures identified so far in human cancers–other cancer signatures likely accumulate at rates driven by more complex variables like environmental mutagen exposure or the appearance of oncogene mutations (40). That being said, clocklike mutation accumulation appears to be the norm in the germline (33, 42, 43). It is possible that some mutator alleles might create a constant number of mutations per generation, if for example they only cause mutations during the especially mutagenic early embryonic cell divisions (33, 44, 45). However, given that 90% of mutations are attributed to SBS5, we postulate that most germline mutator alleles are likely to alter the rate of accumulation of mutations per year.

To make this assumption concrete, we consider a clocklike germline mutator allele that is expected to generate k mutations per year, for example by slightly damaging a DNA repair gene or a polymerase proofreading domain. Assume that the fitness effects of these extra mutations are randomly sampled from the distribution of fitness effects (DFE) of new mutations in the population, and that $E[s]$ is the DFE mean. If the generation time is g years, then on average the mutator allele will cause each offspring to be born with $k*g$ extra mutations, which will have a total fitness impact of $k*g*E[s]$ (**Figure 1**). This implies that the fitness effect of a clocklike germline mutator allele will be proportional to generation time. The upshot of this scaling is that the distribution of mutator allele fitness effects should be shifted toward more deleterious values in species with long generation times.

Under the nearly neutral theory, germline mutator alleles will thus only be efficiently selected against in a diploid species if $k*g*E[s] > 1/(2N_e)$. As in the original drift-barrier hypothesis, the threshold for efficient selection will be highest in small-N_e populations, but the long generation times of small-N_e populations will mean that mutator selection coefficients will also tend to be larger in these populations, potentially helping them clear the drift barrier just as often as in large-N_e populations.

A. The fitness effect of a clocklike mutator allele should be proportional to generation time



B. A summary of causal relationships among the variables of the clocklike drift barrier model

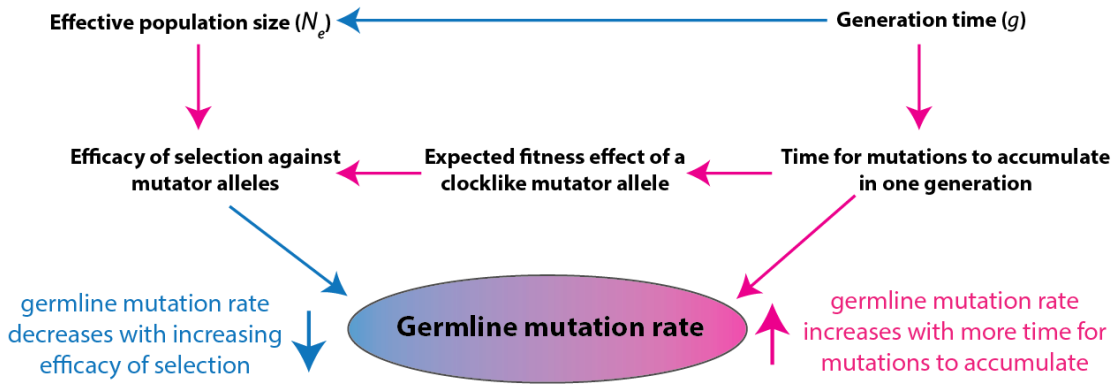


Figure 1: A model of germline mutation rate variation as a function of generation time, effective population size, and genetic variation that impacts the mutation rate measured per year. A. Here, we compare the effects of identical molecular changes occuring in some human DNA repair gene as well as its mouse homolog. If these mutator alleles produce the same number of germline mutations per year, the human allele will produce a greater mutation burden per generation compared to the mouse allele, leading to a greater expected fitness cost and a larger negative selection coefficient in the long-generation-time species. *Figure credit: Natalie Telis.* **B.** This diagram summarizes the multiple ways that generation time can affect the mutation rate, including its direct impact on the number of mutations that accumulate in a generation and its other impacts on the effective population size and the efficiency of natural selection. Pink arrows indicate positive correlations (an increase in the upstream variable causes an increase in the downstream variable), and blue arrows indicate negative correlations (an increase in the upstream variable causes a decrease in the downstream variable).

To illustrate the impact of this generation time correction, we consider a comparison between humans and mice. Humans have an effective population size around 10,000, whereas mouse N_e estimates range from 500,000 to 2,000,000 (46, 47). Assuming a mouse N_e of 1,000,000, the classical drift-barrier model predicts that any mutator allele with selection coefficient between 10^{-4} and 10^{-6} should be nearly neutral in humans but efficiently selected against in mice, leading us to predict that mice have more efficient DNA repair. But this calculation neglects the fact that mice have a generation time of ~4 months, about 100-fold smaller than the human generation time of ~30 years. If mice and humans have a similar DFE overall, then each clocklike mutator will have a 100-fold lower fitness effect in mice compared to humans. This effectively cancels out the 100-fold difference between mouse and human effective population sizes, leading us to predict that mutators with fitness effects $k*E[s]$ less than $1/(g*2N_e)$ will be nearly neutral, a threshold that equals about $3\text{x}10^{-6}$ in both species. If the mouse N_e is on the lower end of the predicted range, then we will actually predict that mice will select against mutator alleles less effectively than humans. This appears consistent with the fact that mice have higher mutation rates per year than humans in both the germline and the soma, but is also consistent with the fact that mice have a lower germline mutation rate per generation, since the shorter length of the mouse generation more than compensates for the faster rate of mutation accumulation per year.

To study the behavior of our Clocklike Drift-Barrier model in a broader set of species, we reanalyzed a large dataset of mutation rate and effective population size estimates that was recently compiled by Wang, et al. (30) and was used to test the predictions of the DBH (**Figure 2**). We restricted our analysis to vertebrates to focus on species with a diversity of generation times but a similar mode of reproduction. **Figure 2A** confirms the drift-barrier model prediction that N_e should be negatively correlated with the mutation rate per generation in these species, accounting for phylogenetic non-independence (PGLS slope = -0.455, $p = 3.3\text{x}10^{-12}$). Bergeron, et al. (29)who generated most of these mutation rate estimates, found that this negative correlation holds across a variety of N_e estimation methods, though its exact magnitude varies. In contrast, **Figure 2B** shows that when the mutation rate is measured per year instead of per generation, its correlation with N_e disappears (PGLS slope = -0.018, $p = 0.87$).

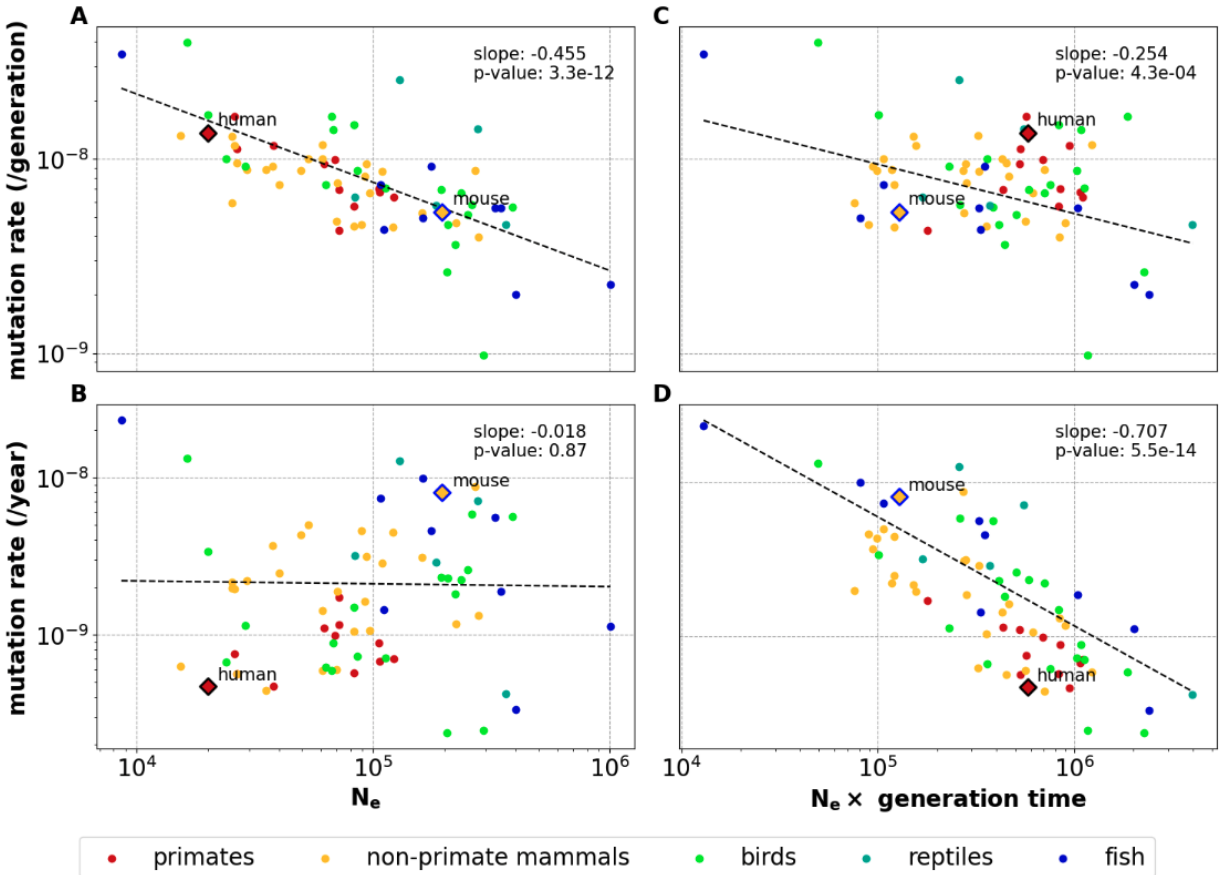


Figure 2: Mutation rates measured per generation and per year have different dependencies on N_e and generation time. A. The per-generation mutation rate is negatively correlated with N_e in a diverse set of species. After controlling for phylogenetic inertia, the correlation remains significantly negative (PGLS slope = -0.455, $p = 3.3\text{x}10^{-12}$). **B.** When these mutation rates are rescaled by generation time to yield mutation rates per year, their correlation with N_e is not significant after correction for phylogenetic inertia (PGLS slope = -0.018, $p = 0.87$). **C.** The mutation rate per generation is negatively correlated with the parameter $g*N_e$ that determines the strength of selection against clocklike mutator alleles in the context of our clocklike drift-barrier model (PGLS slope = -0.254, p-value = $4.3 \text{ x } 10^{-4}$). **D.** The mutation rate per year shows an even more robust negative correlation with the clocklike mutator selection parameter $g*N_e$ (PGLS slope -0.707, p-value = $5.5\text{x}10^{-14}$). Note that while the mutation rate per generation is high in humans and other primates compared to shorter-lived mammals such as the mouse, this trend reverses for mutation rates calculated per year, which are lower in humans and other primates than in mouse.

In panels **2C** and **2D**, we move on to explore how the mutation rate depends on N_e*g, since our theoretical argument implies that N_e*g should determine the efficiency of selection against clocklike mutator alleles. Although N_e*g is not significantly correlated with the mutation rate measured per generation (**Figure 2C**), it is significantly negatively correlated with the mutation rate per year (**Figure 2D**), a trend consistent with our prediction that species with large

N_e*g values are able to select most efficiently against clocklike mutators and reduce their per-year mutation rates compared to species with lower N_e*g values.

What are the species with high values of N_e*g? If all we know is that N_e and g are inversely correlated, this gives us no information about whether the mutation rate should increase or decrease with increasing g – the overall trend depends on the rate at which N_e decreases with increasing g. In **Figure 2D**, we can see that the species with high values of N_e*g are clearly species with high values of g and fairly low values of N_e, such as the primates. We can understand why this is the case by better quantifying the correlation between N_e and g. Following the lead of Chao and Carr, who previously noted that log(N_e) appears to be a linear function of log(g) (24), we estimated the slope α of log(N_e) regressed against log(g) in the large dataset compiled by Wang and Obbard (**Figure 3**).

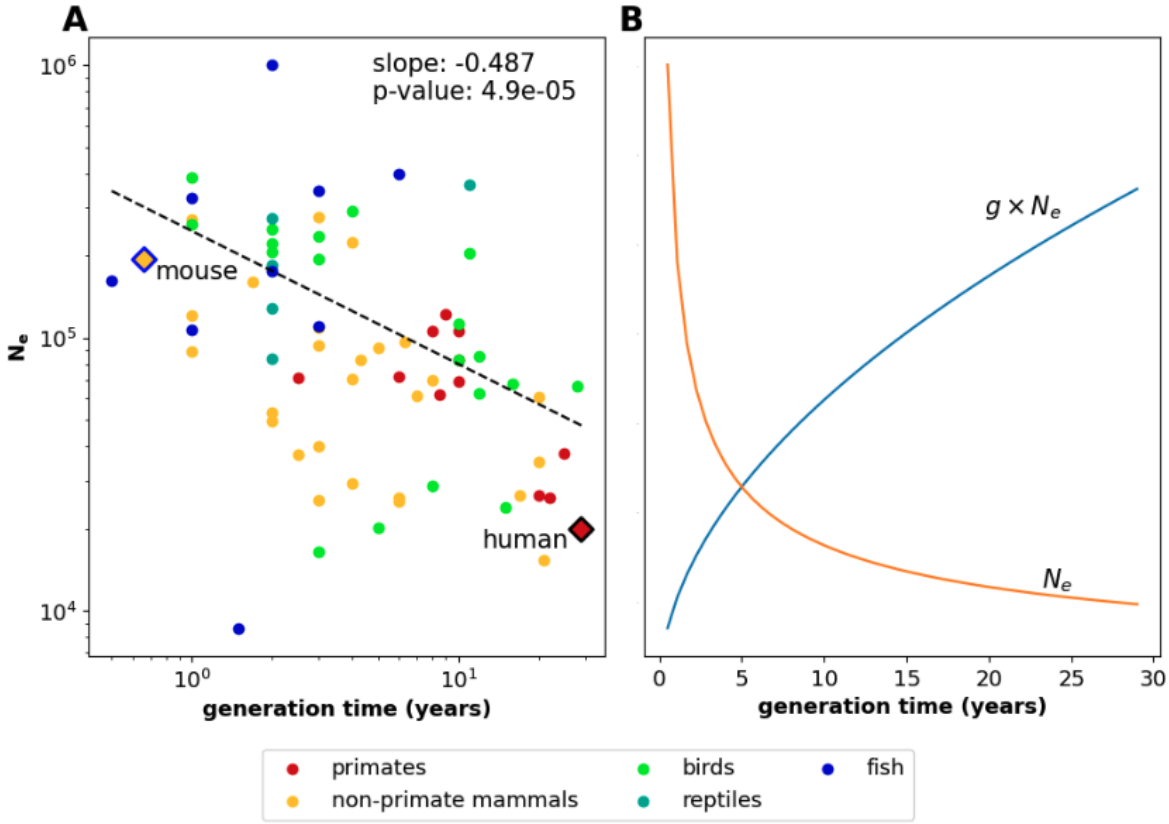


Figure 3: The relationship among N_e, generation time, and the strength of selection against clocklike mutator alleles. A. The empirical inverse correlation between log(N_e) and log(g) in the set of species analyzed by Wang and Obbard. We estimate a slope of -0.487 based on a PGLS regression. **B.** Variation of N_e and N_e*g with g, extrapolated from the regression line in panel A and converted from log scale to natural scale. Each curve has been visualized using an arbitrary y-axis scaling, but together they illustrate that N_e*g increases with increasing g even as N_e decreases.

The linear relationship $\log(N_e) = \alpha*\log(g) + \log(C)$ (where C is an arbitrary constant) implies that $N_e = C * g^{\alpha}$, which in turn implies that $N_e*g = C *g^{1+\alpha}$. This expression might increase or decrease with increasing g depending whether α is greater or less than -1, so knowing the value of α is key to deciding whether species with long or short generation times are likely to have the lowest mutation rates. We estimate that $\alpha \approx -0.487$ based on a PGLS regression of $\log(N_e)$ against $\log(g)$ in vertebrate species, remarkably close to the value of $\alpha = -0.5$ that Kimura and Ohta originally proposed to reconcile the nearly neutral theory with the molecular clock model. This implies that $g^{1+\alpha} = g^{1-0.487} = g^{0.513}$, which is an increasing function of g, thus explaining why the species with high values of N_e*g are the species with long generation times and modest effective population sizes. An important implication of the fact that N_e*g increases with increasing g is that long-lived species with relatively small population sizes should have the lowest mutation rates per year, which is consistent with observations and predictions about somatic mutation rate evolution but at odds with the prediction that small-N_e species have the lowest efficiency of selection against germline mutator alleles.

Using the data plotted in **Figures 2** and **3**, we have derived a model of how the mutation rate evolves forward in time after a perturbation of a species' generation time, and a schematic is pictured in **Figure 4**. As a baseline for comparison, **Figure 4A** shows how the germline and somatic mutation rates behave under the original drift-barrier model, fluctuating beneath a critical threshold due to the random drift of mutator alleles but then equilibrating to new levels after a change in effective population size. In contrast, **Figure 4B** shows how the germline and somatic mutation rates behave in the context of the clocklike drift-barrier model. We illustrate what happens when the generation time lengthens and N_e subsequently contracts to maintain the inverse relationship pictured in **Figure 3A.** Although the lengthening of generation time and the reduction of N_e have opposite effects on the mutation rate measured per year, these effects are qualitatively the same in the germline and the soma and cause the mutation rate to settle at a lower level than it was when the parameter N_e*g was smaller.

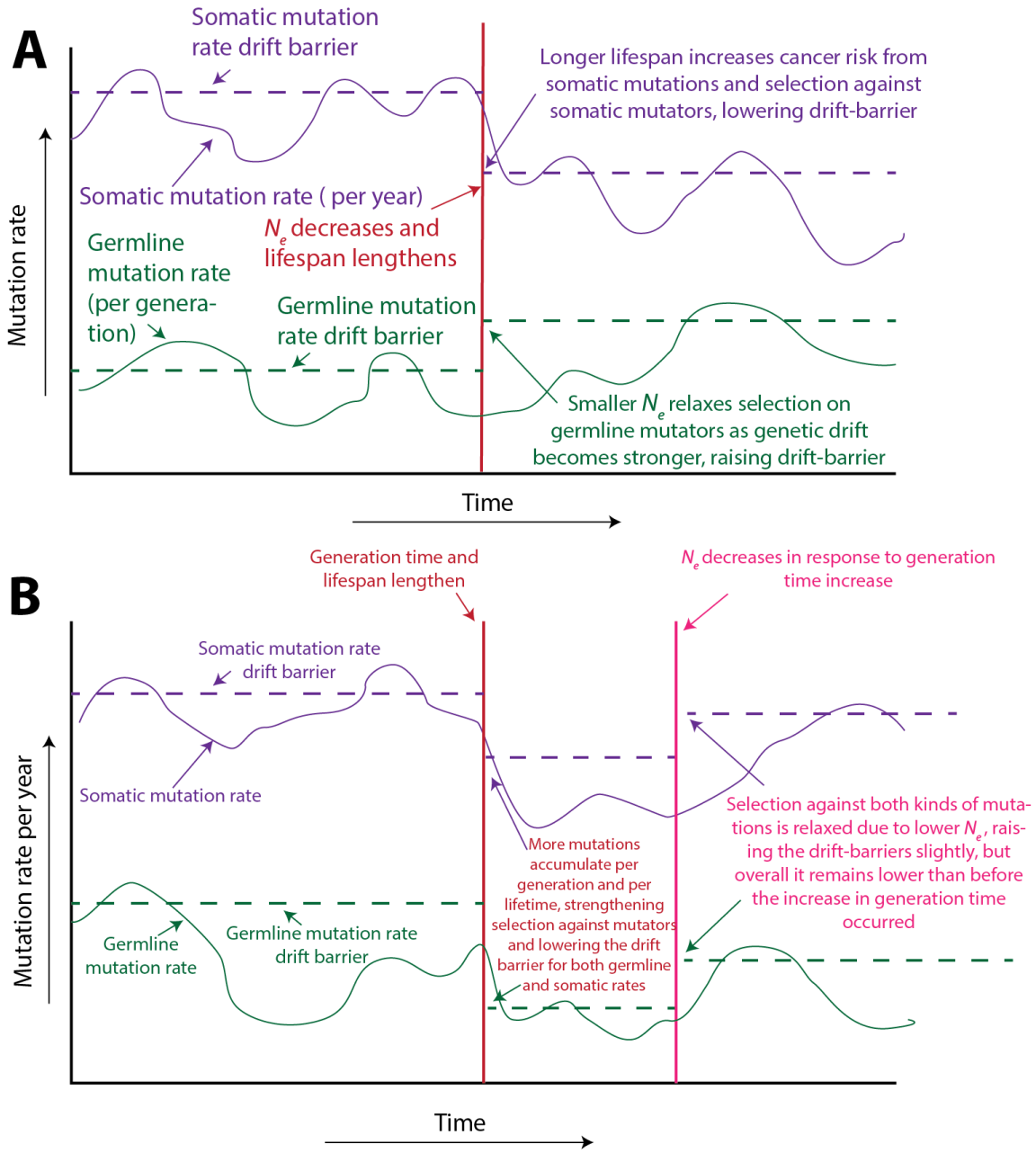


Figure 4: Forward-time comparison of the original drift-barrier model with the clocklike drift-barrier model. A. An illustration of how germline and somatic mutation rates fluctuate beneath the drift barrier: the threshold above which extra mutations are deleterious to be efficiently selected against (dashed lines). If the generation time increases and N_e commensurately decreases as predicted by **Figure 3A**, the germline drift-barrier threshold is predicted to increase while the somatic drift-barrier threshold will likely decrease due to lifespan effects. **B.** In the context of the clocklike drift-barrier hypothesis, the generation time increase and commensurate N_e decrease should have effects on the mutation rate that are opposite but not equal and result in both the germline and somatic mutation rates measured per year settling at lower levels than they were set at when $g*N_e$ was smaller.

Discussion

The model we propose in this paper is fundamentally a nearly neutral drift-barrier model in that it explains germline mutation rate variation as a function of the efficiency of selection against mutator alleles. Like the original drift-barrier model, we predict that most species' mutation rates should be higher than the minimum biophysically achievable mutation rate, and that mutator allele turnover will cause the mutation rate to oscillate above and below the lowest rate that a population is able to maintain over the long term. We also follow the lead of the drift-barrier model in assuming that higher mutation rates are always somewhat deleterious, neglecting any potential beneficial effects of high mutation rates (for example, if they facilitate adaptation to new environments (48, 49)).

Where our model differs from the original drift-barrier model is that it incorporates a key principle of somatic mutation rate evolution to better understand the dynamics of germline mutation rate evolution. This key principle is that the strength of selection on DNA repair is likely amplified by a long lifespan, an idea that is supported by empirical inverse correlations between lifespan and per-year somatic mutation rates (31, 50). Although total lifespan is not directly relevant to selection on the germline mutation rate, reproductive lifespan is extremely relevant since longer generation times result in more opportunities for each offspring to accumulate deleterious mutations. Taking generation time into account, we predict that long-lived organisms should have lower mutation rates per year in both the germline and the soma compared to shorter-lived organisms with larger effective population sizes.

Our model implies that generation time changes should perturb the germline mutation rate in two ways: an immediate effect followed by a delayed effect. For example, the immediate effect of a generation time increase will be to raise the mutation rate *per generation* by increasing the time window for mutations to occur, but if the longer generation time is maintained, the mutation rate per generation may creep back downward due to intensification of selection against germline mutator alleles that have become more deleterious as a result of the lengthening of generation time. (Note that **Figure 4B** depicts the trajectory of the mutation rate measured *per year* which should decrease as a result of both the immediate and delayed generation time effects). Body size changes appear to have similar immediate and delayed effects on the somatic mutation rate: when body size decreases rapidly, it appears to decrease cancer incidence (e.g. small dogs tend to be less cancer-prone than larger dogs (51, 52)), but this association mostly disappears over longer evolutionary timescales, likely due to selection for better cancer suppression in large-bodied species (53).

Since generation time is clearly a trait that can vary within species and change quickly as a result of changes in environment or social behavior, we should not always expect DNA repair efficacy to reflect a species' current reproductive timelines. In humans, post-industrial demographic transitions have likely elongated generation times compared to what they were for most of human history (54), and current longer generation times have not had much time to impose additional selective pressure on germline mutation rates. Effective population size also varies and changes over time as populations migrate into new environments and experience booms and busts (55, 56). In both cases, it will take time for mutation rates to equilibrate, and this lag time is expected to create some discrepancies between empirical and predicted mutation rates.

Generation times have been generally increasing for several million years along the hominoid lineage, as evidenced by the fact that they are longer in great apes compared to monkeys and longer still in humans compared to great apes (57–59). This hominoid generation time slowdown has clearly been accompanied by a decrease in the per-year mutation rate, which is consistent with a lengthening of the generation time combined with a commensurate increase in the per-generation mutation rate, but it is not clear what proportion of this decrease has been driven by the fast-acting generation time effect as opposed to slower-acting genetic changes affecting the DNA repair efficacy (60–63). As early as 1981, Morris Goodman noted the existence of this hominoid slowdown, and that generation time changes might explain it without the need to invoke any molecular changes to the mutagenic process (59). More modern comparisons between mutation rates in different primate species have sometimes found that life history changes are enough to explain the differences, but other primate studies have concluded that the fast-acting generation time effect is not enough to explain the observed variation (63, 64). One study proposed that the hominoid slowdown might be explained by selection to reduce somatic mutation rates in longer-lived great apes (65), and our model suggests that the same dynamics might be explained by stronger selection against clocklike mutator alleles in later-reproducing species.

We expect that the best way to test our proposition more robustly will be to carry out the kind of experiment conceived in **Figure 1** by introducing known human mutator alleles such as *POLE* and *POLD1* defects (23, 66) into the homologous DNA repair genes of faster-reproducing species such as mice. This would allow us to test whether these variants cause fewer deleterious mutations per generation in mice compared to humans, as we predict that the shorter murine generation time should allow these variants to produce fewer mutations in the span of a single generation. Since germline mutator variants of the DNA repair gene *MUTYH*

have already been identified in both humans and mice (23, 67), further study of these variants may also give us insight into whether humans' longer generation time acts to amplify any deficits in *MUTYH*'s ability to repair damage caused by reactive oxygen species. As we discover more of the genetic variants that drive mutation rate variation within and between species, it will be important to directly measure whether their effects tend to scale with generation time. If it turns out that most germline mutator alleles actually have effects that do not scale with generation time, the original drift-barrier model will likely make the most accurate predictions about relative DNA repair efficacy in small-N_e versus large-N_e species.

An additional assumption we have made that will be important to revisit in the future is that the expected fitness cost of a typical new deleterious mutation does not vary across species. There is evidence that mutations may actually be more deleterious in low N_e species compared to high N_e species (68), which could have the effect of further intensifying selection against mutator alleles in low N_e species. Before making this extrapolation, however, it will be important to investigate whether mutation rate variation or generation time variation might confound inferences of DFE differences among species. When we start modeling the variation of population genetic parameters such as mutation rate, generation time, and population size, these non-equilibrium dynamics may interact in unexpected ways and lead to surprising changes in our understanding of patterns of genetic diversity and divergence.

Methods

We used the nucleotide diversity (π) and mutation rate (μ) data compiled by Wang and Obbard to estimate N_e for each species via the formula $N_e = \pi/(4 \cdot \mu)$ (30)(see Data and Code Availability). We then performed all PGLS regressions using the R library caper (69). Additionally, we used Pagel's λ (70) to quantify the amount of phylogenetic signal in the dataset, which was estimated using maximum likelihood (ML). λ is a scaling parameter applied to internal branch lengths in the phylogenetic tree, and is typically a value between 0 and 1. $\lambda = 1$ means that the traits being regressed against one another appear to have evolved according to a Brownian motion evolutionary model and is interpreted as strong evidence for phylogenetic signal in the dataset, whereas $\lambda = 0$ suggests that the traits evolved completely independently of the phylogenetic tree structure. See Supplementary Table 1 for detailed numerical regression results.

Competing Interest Statement

The authors declare no competing interests.

Data and Code Availability

The mutation rates, nucleotide diversity, generation time data, and phylogenetic tree utilized in our calculations were originally compiled by Wang and Obbard (30) and are all publicly available at https://github.com/Yiguan/mutation_literature. The code we used to perform this paper's analysis is available at https://github.com/harrispopgen/clocklike-DBH.

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References

1. Stearns SC. 1976. Life-history tactics: a review of the ideas. *Q. Rev. Biol.* 51(1):3–47
2. Giesel JT. 1976. Reproductive strategies as adaptations to life in temporally heterogeneous environments. *Annu. Rev. Ecol. Syst.* 7(1):57–79
3. Pianka ER. 1976. Natural selection of optimal reproductive tactics. *Am. Zool.* 16(4):775–84
4. Goodman D. 1979. Regulating reproductive effort in a changing environment. *Am. Nat.* 113(5):735–48

5. Stearns SC. 2000. Life history evolution: successes, limitations, and prospects. *Naturwissenschaften*. 87(11):476–86
6. Wright J, Solbu EB, Engen S. 2020. Contrasting patterns of density-dependent selection at different life stages can create more than one fast–slow axis of life-history variation. *Ecol. Evol.* 10(6):3068–78
7. Staps M, van Gestel J, Tarnita CE. 2019. Emergence of diverse life cycles and life histories at the origin of multicellularity. *Nat. Ecol. Evol.* 3(8):1197–1205
8. Van de Walle J, Fay R, Gaillard J-M, Pelletier F, Hamel S, et al. 2023. Individual life histories: neither slow nor fast, just diverse. *Proc. R. Soc. B Biol. Sci.* 290(2002):20230511
9. Podolsky RD. 2004. Life-history consequences of investment in free-spawned eggs and their accessory coats. *Am. Nat.* 163(5):735–53
10. Boivin G, Jacob S, Damiens D. 2005. Spermatogeny as a life-history index in parasitoid wasps. *Oecologia*. 143(2):198–202
11. White CR, Alton LA, Bywater CL, Lombardi EJ, Marshall DJ. 2022. Metabolic scaling is the product of life-history optimization. *Science*. 377(6608):834–39
12. Anna B-H, Berlin S, Smith NGC, Mosller AP, Ellegren H. 2003. Life history and the male mutation bias. *Evolution*. 57(10):2398–2406
13. Bromham L. 2011. The genome as a life-history character: why rate of molecular evolution varies between mammal species. *Philos. Trans. R. Soc. B Biol. Sci.* 366(1577):2503–13
14. Krašovec R, Richards H, Gifford DR, Hatcher C, Faulkner KJ, et al. 2017. Spontaneous mutation rate is a plastic trait associated with population density across domains of life. *PLOS Biol.* 15(8):e2002731
15. Chintalapati M, Moorjani P. 2020. Evolution of the mutation rate across primates. *Curr. Opin. Genet. Dev.* 62:58–64
16. Crow JF. 1997. The high spontaneous mutation rate: Is it a health risk? *Proc. Natl. Acad. Sci.* 94(16):8380–86

17. Lynch M. 2010. Rate, molecular spectrum, and consequences of human mutation. *Proc. Natl. Acad. Sci.* 107(3):961–68
18. Wong WSW, Solomon BD, Bodian DL, Kothiyal P, Eley G, et al. 2016. New observations on maternal age effect on germline de novo mutations. *Nat. Commun.* 7(1):10486
19. Goldmann JM, Veltman JA, Gilissen C. 2019. De Novo Mutations Reflect Development and Aging of the Human Germline. *Trends Genet.* 35(11):828–39
20. Drenos F, Kirkwood TBL. 2005. Modelling the disposable soma theory of ageing. *Mech. Ageing Dev.* 126(1):99–103
21. Nunney L, Muir B. 2015. Peto's paradox and the hallmarks of cancer: constructing an evolutionary framework for understanding the incidence of cancer. *Philos. Trans. R. Soc. B Biol. Sci.* 370(1673):20150161
22. Robinson PS, Thomas LE, Abascal F, Jung H, Harvey LMR, et al. 2022. Inherited MUTYH mutations cause elevated somatic mutation rates and distinctive mutational signatures in normal human cells. *Nat. Commun.* 13(1):3949
23. Sherwood K, Ward JC, Soriano I, Martin L, Campbell A, et al. 2023. Germline de novo mutations in families with Mendelian cancer syndromes caused by defects in DNA repair. *Nat. Commun.* 14(1):3636
24. Chao L, Carr DE. 1993. The molecular clock and the relationship between population size and generation time. *Evolution*. 47(2):688–90
25. Lynch M, Ackerman MS, Gout J-F, Long H, Sung W, et al. 2016. Genetic drift, selection and the evolution of the mutation rate. *Nat. Rev. Genet.* 17(11):704–14
26. Ohta T. 1992. The nearly neutral theory of molecular evolution. *Annu. Rev. Ecol. Syst.* 23(1):263–86
27. Sung W, Ackerman MS, Miller SF, Doak TG, Lynch M. 2012. Drift-barrier hypothesis and mutation-rate evolution. *Proc. Natl. Acad. Sci.* 109(45):18488–92
28. Lynch M. 2012. Evolutionary layering and the limits to cellular perfection. *Proc. Natl. Acad.*

Sci. 109(46):18851–56

29. Bergeron LA, Besenbacher S, Zheng J, Li P, Bertelsen MF, et al. 2023. Evolution of the germline mutation rate across vertebrates. *Nature*. 615(7951):285–91
30. Wang Y, Obbard DJ. 2023. Experimental estimates of germline mutation rate in eukaryotes: a phylogenetic meta-analysis. *Evol. Lett.* 7(4):216–26
31. Milholland B, Dong X, Zhang L, Hao X, Suh Y, Vijg J. 2017. Differences between germline and somatic mutation rates in humans and mice. *Nat. Commun.* 8(1):15183
32. Zhang L, Dong X, Tian X, Lee M, Ablaeva J, et al. 2021. Maintenance of genome sequence integrity in long- and short-lived rodent species. *Sci. Adv.* 7(44):eabj3284
33. Lindsay SJ, Rahbari R, Kaplanis J, Keane T, Hurles ME. 2019. Similarities and differences in patterns of germline mutation between mice and humans. *Nat. Commun.* 10(1):4053
34. Ohta T. 1977. Extension to the neutral mutation random drift hypothesis. *Mol. Evol. Polymorph.*, pp. 148–67
35. Zuckerkandl E, Pauling L. 1965. Evolutionary divergence and convergence in proteins. In *Evolving Genes and Proteins*, pp. 97–166
36. Britten RJ. 1986. Rates of DNA Sequence Evolution Differ Between Taxonomic Groups. *Science*. 231(4744):1393–98
37. Martin AP, Palumbi SR. 1993. Body size, metabolic rate, generation time, and the molecular clock. *Proc. Natl. Acad. Sci.* 90(9):4087–91
38. Moore L, Cagan A, Coorens THH, Neville MDC, Sanghvi R, et al. 2021. The mutational landscape of human somatic and germline cells. *Nature*. 597(7876):381–86
39. Spisak N, Manuel M de, Milligan W, Sella G, Przeworski M. 2023. Disentangling sources of clock-like mutations in germline and soma. bioRxiv 556720. **https://doi.org/10.1101/2023.09.07.556720**
40. Alexandrov LB, Jones PH, Wedge DC, Sale JE, Campbell PJ, et al. 2015. Clock-like mutational processes in human somatic cells. *Nat. Genet.* 47(12):1402–7

41. Moorjani P, Amorim CEG, Arndt PF, Przeworski M. 2016. Variation in the molecular clock of primates. *Proc. Natl. Acad. Sci.* 113(38):10607–12

42. Thomas GWC, Wang RJ, Puri A, Harris RA, Raveendran M, et al. 2018. Reproductive longevity predicts mutation rates in primates. *Curr. Biol.* 28(19):3193-3197.e5

43. Wang RJ, Raveendran M, Harris RA, Murphy WJ, Lyons LA, et al. 2022. De novo mutations in domestic cat are consistent with an effect of reproductive longevity on both the rate and spectrum of mutations. *Mol. Biol. Evol.* 39(7):msac147

44. Sasani TA, Pedersen BS, Gao Z, Baird L, Przeworski M, et al. 2019. Large, three-generation human families reveal post-zygotic mosaicism and variability in germline mutation accumulation. *eLife*. 8:e46922

45. Harland C, Charlier C, Karim L, Cambisano N, Deckers M, et al. 2017. Frequency of mosaicism points towards mutation-prone early cleavage cell divisions in cattle. bioRxiv 079863. **https://doi.org/10.1101/079863**

46. Baines JF, Harr B. 2007. Reduced X-Linked Diversity in Derived Populations of House Mice. *Genetics*. 175(4):1911–21

47. Halligan DL, Oliver F, Eyre-Walker A, Harr B, Keightley PD. 2010. Evidence for Pervasive Adaptive Protein Evolution in Wild Mice. *PLOS Genet.* 6(1):e1000825

48. Bedau MA, Packard NH. 2003. Evolution of evolvability via adaptation of mutation rates. *Biosystems*. 69(2):143–62

49. Payne JL, Wagner A. 2019. The causes of evolvability and their evolution. *Nat. Rev. Genet.* 20(1):24–38

50. Cagan A, Baez-Ortega A, Brzozowska N, Abascal F, Coorens THH, et al. 2022. Somatic mutation rates scale with lifespan across mammals. *Nature*. 604(7906):517–24

51. Grüntzig K, Graf R, Boo G, Guscetti F, Hässig M, et al. 2016. Swiss Canine Cancer Registry 1955–2008: Occurrence of the Most Common Tumour Diagnoses and Influence of Age, Breed, Body Size, Sex and Neutering Status on Tumour Development. *J. Comp.*

Pathol. 155(2):156–70

52. Boddy AM, Abegglen LM, Pessier AP, Aktipis A, Schiffman JD, et al. 2020. Lifetime cancer prevalence and life history traits in mammals. *Evol. Med. Public Health*. 2020(1):187–95
53. Tollis M, Schiffman JD, Boddy AM. 2017. Evolution of cancer suppression as revealed by mammalian comparative genomics. *Curr. Opin. Genet. Dev.* 42:40–47
54. Reher DS. 2011. Economic and Social Implications of the Demographic Transition. *Popul. Dev. Rev.* 37:11–33
55. Gutenkunst RN, Hernandez RD, Williamson SH, Bustamante CD. 2009. Inferring the Joint Demographic History of Multiple Populations from Multidimensional SNP Frequency Data. *PLOS Genet.* 5(10):e1000695
56. Li H, Durbin R. 2011. Inference of human population history from individual whole-genome sequences. *Nature*. 475(7357):493–96
57. Goodman M. 1985. Rates of molecular evolution: The hominoid slowdown. *BioEssays*. 3(1):9–14
58. Goodman M, Tagle DA, Fitch DHA, Bailey W, Czelusniak J, et al. 1990. Primate evolution at the DNA level and a classification of hominoids. *J. Mol. Evol.* 30(3):260–66
59. Yi SV. 2013. Morris Goodman's hominoid rate slowdown: The importance of being neutral. *Mol. Phylogenet. Evol.* 66(2):569–74
60. Li W-H, Tanimura M. 1987. The molecular clock runs more slowly in man than in apes and monkeys. *Nature*. 326(6108):93–96
61. Li W-H, Ellsworth DL, Krushkal J, Chang BH-J, Hewett-Emmett D. 1996. Rates of Nucleotide Substitution in Primates and Rodents and the Generation–Time Effect Hypothesis. *Mol. Phylogenet. Evol.* 5(1):182–87
62. Elango N, Thomas JW, NISC Comparative Sequencing Program, Yi SV. 2006. Variable molecular clocks in hominoids. *Proc. Natl. Acad. Sci.* 103(5):1370–75
63. Scally A, Durbin R. 2012. Revising the human mutation rate: implications for understanding

human evolution. *Nat. Rev. Genet.* 13(10):745–53

64. Besenbacher S, Hvilsom C, Marques-Bonet T, Mailund T, Schierup MH. 2019. Direct estimation of mutations in great apes reconciles phylogenetic dating. *Nat. Ecol. Evol.* 3(2):286–92

65. Thomas GWC, Hahn MW. 2014. The human mutation rate Is increasing, even as It slows. *Mol. Biol. Evol.* 31(2):253–57

66. Robinson PS, Coorens THH, Palles C, Mitchell E, Abascal F, et al. 2021. Increased somatic mutation burdens in normal human cells due to defective DNA polymerases. *Nat. Genet.* 53(10):1434–42

67. Sasani TA, Ashbrook DG, Beichman AC, Lu L, Palmer AA, et al. 2022. A natural mutator allele shapes mutation spectrum variation in mice. *Nature*. 605(7910):497–502

68. Huber CD, Kim BY, Marsden CD, Lohmueller KE. 2017. Determining the factors driving selective effects of new nonsynonymous mutations. *Proc. Natl. Acad. Sci.* 114(17):4465–70

69. Orme D, Freckleton RP, Thomas GH, Petzoldt T, Fritz SA, Isaac N. 2013. CAPER: comparative analyses of phylogenetics and evolution in R. *Methods Ecol. Evol.* 3:145–51

70. Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature*. 401(6756):877–84