The evolution of mutation rates in the light of development and cell-lineage selection

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Abstract

Mutation rates drive the pace and potential of evolutionary change. However, to better understand the evolutionary implications of mutation rates, there is a need to uncover the causes of their diversfification. In multicellular organisms, all mutations first arise in a single cell in a developmental context. Whether a mutation enters a population's gene pool can therefore depend on developmental events that affect the likelihood of mutant cell lineages of producing gametes. For this reason, the evolution of mutation rates in populations is governed not only by changes in the rates at which mutations occur at the molecular level, but also by changes in developmental features of organisms and how mutations impact cellular fitness during development. We present a theoretical framework that, supported by empirical data from mammals and experiments on the fruit fly, demonstrate how generational mutation rates can be shaped by changes in developmental parameters and intraorganismal selective processes even when molecular mutation rates are presumed constant. Our model highlights how the diversity of mutation rates observed across animals may be the byproduct of organismal development rather than the result of direct selection against mutator alleles. As such, development not only introduces phenotypic biases, it also shapes the rates and trajectories of genetic diversity and is thus at the core of evolutionary theory.

Introduction

How genetic variation arises within a population shapes evolutionary trajectories (Agashe et al. 2023; Cano and Payne 2020; Cano et al. 2022; Martin et al. 2024; Nei 2013; Simpson 1953; Stoltzfus and Yampolsky 2009; Yampolsky and Stoltzfus 2001). The rates of mutations in a population may affect how promptly adaptive genotypes may be encountered, while also defining how deleterious mutations may burden populations. Thus, mutation rates are closely tied to the pace and potential of evolutionary change. Although classical models presumed mutation rates to be relatively constant across the tree of life (Kimura 1969; Zuckerkand and Pauling 1965), advances in molecular biology have progressively challenged this view (Britten 1986; Gillespie 1991; Jukes and Holmquist 1972; Kimura 1987). The depth and granularity at which we can now explore genetic diversity suggest that mutation rates are an

evolvable trait, varying not only between clades (Bergeron et al. 2023; Lynch et al. 2023; Wang and Obbard 2023), but also within species (Harris 2015) and populations (Sniegowski et al. 1997), underscoring their dynamic role in shaping genetic diversity.

The diversification of mutation rates can be shaped by the evolution of mutator alleles, defined as "any genomically encoded modifier of the mutation rate" (Lynch et al 2016). These alleles are typically considered to operate at the molecular scale, altering the balance between DNA damage and repair through variations in genes involved in these processes (Beichman et al. 2024; Britten 1986; Lynch et al. 2016; Lynch et al. 2023; Sniegowski et al. 2000; Spisak et al. 2024). Mutator alleles that promote hypermutation may be favored under strong adaptive pressures (Sniegowski et al. 1997; Taddei et al. 1997), while those causing excessive deleterious mutations would be selected against (Kimura 1967; Lynch 2010). Because most mutations tend to be deleterious, the mutation rates of species are thought to be dominated by the efficacy of selection in purging alleles that increase the rates of mutations (Lynch 2010). This is why the so-called drift-barrier hypothesis posits that the efficiency of selection against mutator alleles depends on population size. Larger populations can better counteract genetic drift, resulting in lower mutation rates, whereas smaller populations experience weaker selection against mutators and, thus, higher mutation rates (Lynch 2010; Lynch et al. 2016; Sung et al. 2012). This view suggests that mutation rates evolve primarily through changes in molecular mechanisms constrained by selection.

However, mutation rates may also evolve through non-molecular causes. In multicellular organisms, new mutations arise in a single cell in the organismal context of each parent. For a mutation to enter the population's gene pool, it must propagate within the organism, meaning developmental traits can shape the likelihood of heritable mutations. For instance, the timing of germline determination (Buss 1983), and differences in gamete production between sexes or species can influence mutation rates (Baer et al. 2007; Bergeron et al. 2023). Moreover, mutations that affect the fitness of cells relative to their sisters may bias their own heritability if they occur on the germline lineage (Extavour and García-Bellido 2001; Haig 2023; Hastings 1989; Majic and Payne 2023; Otto and Orive 1995; Tseng et al. 2022). While evolutionary implications of intraorganismal selection of cell lineages is well-explored in relation to tumors (Caulin and Maley 2011; Erten and Kokko 2020; Sulak et al. 2016), its implications for germline evolution remain underexplored. Importantly, these intraorganismal selective dynamics at the cellular level could influence the mutation rates at the population level.

In this work, we propose that differences in population-level mutation rates across species may not solely result from molecular changes mediated by mutator alleles. Instead, they could arise from the interplay of developmental and life-history parameters interplaying with intraorganismal selection on mutant cells. Using analytical models, data analysis and experiments in *Drosophila,* we demonstrate

how developmental parameters can affect the rate of new mutations in a population, even when molecular mutation rates remain constant. This perspective has important conceptual implications. Unlike adaptationist explanations like the drift-barrier hypothesis, our model highlights how developmental processes may inadvertently govern mutation rates, positioning them as a byproduct of organismal development rather than direct selection. Furthermore, this model broadens the role of organismal development in population genetics: it not only introduces phenotypic biases, but it also shapes the rates and trajectories of genetic diversity, thus placing development at the core of evolutionary theory.

Results

A theoretical framework to assess how developmental features influence mutation rates

To distinguish between the molecular mutation rate, μ_{mol}, and the mutation rate per generation at the population level, μ_{pop}, we revisited the theoretical framework of Otto & Orive (1995), who explored how the mutation load of a population is affected by intraorganismal selection within individuals (see Methods). Conceptually, development is modeled as a branching tree of cell lineages, with μ_{pop} partly determined by the fraction of cells (M_i) carrying a mutation at locus i at the end of development (Figure 1). M_i is a function of:

i) a molecular variable μ_{mol}, which is the mutation probability per cell per locus per cell division,

ii) developmental parameters such as the duration of development (τ), and the rate of cell divisions (C), and

iii) the fitness effect of mutations on cells (β_i), where $\beta_i < 1$ indicates deleterious effects, $\beta_i = 1$ neutrality, and $\beta_i > 1$ beneficial effects.

With an estimation of M_i and assuming that germline determination is independent of mutational events, it is then possible to calculate μ_{pop} at locus i as the probability of sampling a mutant cell among the total pool of cells, which include all M_i mutated cells as well as all cells that completed development without mutations, N_i. Such that:

$$\mu_{pop_i} = \frac{M_i}{M_i + N_i}$$

This formalization allows us to assess how developmental features can influence μ_{pop}, even if the molecular mutation rate μ_{mol} is constant.

101

102 Developmental parameters can account for the diversification of mutation rates

103

104 Under neutral conditions (β_i = 1), evolutionary lineages that alter either C or τ, also alter the expected
105 μ_{pop} for locus i (Fig. 1B). To evaluate whether developmental parameters may explain interspecific
106 differences in μ_{pop}, we analyzed estimates of μ_{pop} (Bergeron et al., 2023) for a number of amniote
107 species. Focusing on the mammalian clade, which has the most comprehensive phylogenetic sampling
108 (Fig. 1C, Table S1) and developmental information (Parr et al. 2014), we focused on gestation time as
109 a proxy for developmental time (τ), as well as on the body mass (as a proxy for total cell count,
110 proportional to $2^{C\tau}$ (Alberch et al. 1979). As predicted by the model, the μ_{pop} of mammals positively
111 correlated with the proxy τ (Fig. 1D, Pearson's $\rho = 0.49$, $p = 0.0025$).

112

113 To more directly address the question of whether developmental parameters could account for the
114 diversification of μ_{pop}, we compared these variables between sister clades in our phylogeny by
115 calculating the ratio of μ_{pop} and body mass (Methods). We found that the majority of the comparisons
116 between relatively recently diverged species yielded a correlation such that the larger of both species
117 tended to have higher μ_{pop} (Fig. 1E). For example, the μ_{pop} of domestic cats (*Felis catus*) is ~2 times
118 lower than that of tigers (*Panthera tigris*) and ~2.5 times lower than that of leopards (*Panthera pardus*),
119 while the μ_{pop} of walruses (*Odobenus rosmarus*) is ~1.95 times that of Antarctic fur seals (*Arctocephalus*
120 *gazella*). Body size likely reflects germline size (Kenagy and Trombulak 1986), which scales with the
121 total number of cells. However, if the number of cells in the germline of a species is disproportionate
122 relative to its body size, we would expect that developmental heterochronies in the gonads would affect
123 the expected values of μ_{pop}. This is what happens in the comparison between chimpanzees and humans,
124 where μ_{pop} and body mass coefficients are misaligned ($\frac{\mu_{pop_{chimpanzee}}}{\mu_{pop_{human}}} = 0.93$, $\frac{mass_{chimpanzee}}{mass_{human}} = 1.17$,
125 Fig. 1E). As previously suggested (Wong 2014), we presume this may be because even though body
126 mass is greater in humans, chimpanzee testes are considerably larger (Harcourt et al. 1981).

127

128 If developmental parameters affecting body mass are indeed affecting μ_{pop}, then the diversification of
129 developmental parameters may offer a non-molecular explanation for observed diversification patterns
130 of mutation rates. As mentioned above, according to the drift-barrier hypothesis, the negative
131 correlation existing between the generational mutation rates of species and their population sizes could
132 be due to the efficacy of selection against alleles affecting μ_{mol} (Lynch 2010; Lynch et al. 2016).
133 However, the relationship between population size and mutation rates could be the neutral byproduct
134 of changes in developmental parameters affecting body size, rather than the selective purging of mutator
135 alleles. This is because mammals follow a global relationship between body size and population density

136 described by the so-called Damuth's rule (Damuth 1981). Considering that body mass is proportional
137 to the total number of cells in a body, then τ and C are parameters affecting not only μ_{pop}, but also the
138 demographic properties of a population. Therefore, simply considering the predicted population size
139 for different values of $C\tau$ based on this proportionality produces an inverse relationship between the
140 expected population density of a species and its mutation rate (Fig. 1F), as would also be expected from
141 the drift-barrier hypothesis. Thus, the differences in mutation rates observed in populations of different
142 sizes may be the result of changes in developmental parameters that, because of ecological and energetic
143 reasons, may also relate to changes in population sizes (Yeakel et al. 2018).
144
145 Overall, these results support the notion that in order to explain differences in μ_{pop} between species, it
146 is not necessary to summon changes at the level of μ_{mol} or natural selection acting directly on
147 generational mutation rates.

148 <u>The selective environment of the embryo and its effect on mutation rates</u>

149 So far, we have been considering the case in which mutations are selectively neutral at the cellular level.
150 In other words, we have been considering the case where $\beta = 1$. However, if we take into account how
151 mutations may impact their own heritability by affecting cellular fitness, a selective regime in the
152 embryo or body of an organism can then also affect μ_{pop} without changes in μ_{mol}. Cellular fitness during
153 germline lineages could be affected by various processes, including the determination of the germline
154 cell fate, migration of cells, and proliferation. Mutations on genes involved in these processes may end
155 up under- or over-represented in the germline of an organism (Fig. 2A). In our model, varying the
156 selective effect β of a mutation on a cell can drastically change the effective μ_{pop} even when molecular
157 mutation rates are constant, such that higher values of β imply higher μ_{pop} (Fig. 2B).
158
159 To explore this point, we considered the *Drosophila melanogaster* gene *trithorax-related (trr)*, which
160 influences cell proliferation (Kanda et al. 2023). We generated mutant clones by inducing mitotic
161 recombination through the FLP/FRT technique (Golic and Lindquist 1989) on flies that carried both a
162 sub-functional allele of *trr* (TrrCA, Gandara et al. (2022)) on an unmarked chromosome and a wild-
163 type version of the gene (TrrWT) on a chromosome marked with a *red fluorescent protein, mRFP* (see
164 Methods). As a result, within a single embryo, some cells are homozygotic for TrrCA, homozygotic for
165 TrrWT, or heterozygotic with a copy of each allele (Fig. 2C). Because an impaired *trr* activity increases
166 the rates of cell proliferation (Kanda et al. 2023), we then expected that $\beta_{trrCA/trrCA} > \beta_{trrWT/trrCA} >$
167 $\beta_{trrWT/trrWT}$ and, therefore, that the TrrCA allele would be overrepresented in the germline and thus have
168 a higher heritability than TrrWT. Consistent with this hypothesis, when mosaicism was induced, a
169 median of ~55% of offspring carried the mutant allele, a fraction that was significantly higher than the

170 cases when mosaicism was not induced on the TrrCA/TrrWT heterozygotes or when it was induced in
171 the lines with two copies of TrrWT (Fig. 2D).
172
173 The way in which μ_{pop} is affected by β also depends on the parameters C and τ (Fig. 2B). For higher
174 values of β, higher values of $C\tau$ tend to produce larger differences in μ_{pop} relative to neutral mutations
175 where $\beta = 1$. On the other hand, for lower values of β, mutation rates are more homogeneous regardless
176 of $C\tau$ and they tend to the same value of μ_{mol}. Our model, therefore, suggests that the selective
177 consequences of mutations may be fine-tuned by evolving developmental parameters that either buffer
178 or enhance competition between different genotypes in the germline of organisms. Specifically, our
179 model predicts that organisms of greater size and slower development should be more efficient in
180 purging mutations that are deleterious for cells and more prone to facilitate the fixation of mutations
181 that are beneficial to cells. Another relevant insight from this model is that even mild consequences for
182 cellular fitness may have major consequences for the rate at which new mutations enter the gene pool
183 of populations. For example, in the case of $C\tau = 20$ (representing 2^{20} cells, roughly the number of
184 ovarian follicles an infant is born with (te Velde and Pearson 2002) or half of the number of germ cells
185 in the ovary of cows by the third trimester of pregnancy (Silva-Santos et al. 2011)), a mere 1% increase
186 ($\beta = 1.01$) or decrease ($\beta = 0.99$) in cell fitness may result in a ~7% change in μ_{pop} (Fig. 2B).
187
188 The effect of mutations on cellular fitness is expected to vary across genes, depending on their
189 involvement on cellular processes, and even across loci within genes, depending on their relevance for
190 gene function. To explore how this may affect genome-wide mutation rate estimations, we adjusted our
191 developmental model such that the overall genomic mutation rate would be proportional to the fraction
192 of sites that have either deleterious ($F_{\beta<1}$), neutral ($F_{\beta=1}$) or advantageous ($F_{\beta>1}$) fitness effects on
193 cells (Methods). Moving from a case in which mutations on all sites are neutral for cellular fitness
194 ($F_{\beta=1} = 1$,), to a case in which all mutations are strongly deleterious ($F_{\beta=0} = 1$), there is a steady drop
195 in the expected μ_{pop}, tending towards μ_{mol} as most mutations become deleterious (Fig. 2E). Notably,
196 variations in the ratio of $F_{\beta=1}$ and $F_{\beta=0}$ vary the relationship between μ_{pop} and developmental
197 parameters, in a way that organisms with smaller C or τ have a higher μ_{pop} for higher $F_{\beta=1}$. These results
198 may help explain the most extreme cases that deviate from the pattern described above about how the
199 larger of two sister species tend to have a larger μ_{pop} (points for which the ratio between the μ_{pop} of two
200 species is smaller than 1 in Fig. 1F). Even though tigers and dwarf musk deer (*Moschus berezovskii*)
201 are considerably larger than leopards and goats (*Capra hircus*) respectively, they also have a
202 considerably smaller μ_{pop} (Table S1). In the context of our framework, this could be because populations
203 of tigers and musk deer have pronounced heterozygosity deficiencies (Armstrong et al. 2024; Fan et al.

2019) which would exacerbate the susceptibility of cells to deleterious mutations, thus augmenting $F_{\beta<0}$.

Considering cases in which most mutations are either neutral or deleterious for cellular fitness would be in accordance with what is expected (Kimura 1967). In contrast, scenarios in which many mutations tend to increase cellular fitness are presumably rare. However, our model does suggest that even if infrequent, mutations with strong beneficial effects on the fitness of cells can drastically influence the genome-wide estimations of μ_{pop}. Depending on the developmental parameters $C\tau$, this is true if mutations that increase cellular fitness are 1 in 1000 ($F_{\beta>1} = 0.001$), or even 1 in a million ($F_{\beta>1} = 1x10^{-6}$) (Fig.2F). Thus, the difference in the fitness effect of mutations at the cellular level may strongly affect the interpretation of comparisons between mutation rates of different species or genes. These findings suggest that variation in developmental parameters, rather than molecular mutation mechanisms alone, can explain interspecific differences in mutation rates.

Selection in the mature germline stem cell niche

In addition to embryonic development, the lifetime of organisms could also shape μ_{pop}. This could take place through a greater accumulation of new mutations before reproduction (Baer et al. 2007; Witt et al. 2023; Zhu et al. 2024), but also because of the dynamic competition between germline stem cells of different genotypes. During an individual's lifetime, germline stem cell niches can undergo a cellular turnover. In mice, for example, spermatogenic stem cells sometimes cease to be stem cells and are replaced by neighboring stem cells, keeping the number of stem cells relatively constant (Nakagawa et al. 2007). Although the repopulation of these cleared niches by neighboring cells could be a stochastic process (Klein et al. 2010), mutations that affect the rate of stemness loss (either through death or differentiation) might eventually create a mosaic of spermatic stem cells with an overall higher fitness. Thus, a selective cell turnover might impact the repertoire of mutations that a single individual can introduce into a population's gene pool.

To incorporate the effect of selection during the lifespan of an organism, we built on our developmental model described above to include a maturation and reproductive phase (Fig. 3A, Methods). In this adapted version, at the end of development, there is a final and constant pool of stem cells that can produce gametes, $M_{t=0}$ of which are mutated. These cells can undergo a turnover, either because of a death-birth dynamic in the stem cell niche or because of differentiation. This turnover of cells in the stem cell niche would occur at a rate d over an arbitrary unit of time t subdividing the total generational time l, and a new selective variable β_l which describes the fitness effect of mutation on cells related to stem cell turnover during the lifetime of an organism. In this model, in the case when there is no turnover of cells during the lifetime of an individual ($d = 0$) or when mutations are neutral ($\beta_l = 1$), μ_{pop} only

238 changes due to the mutational accumulation of new mutations. Whenever there is a turnover of cells
239 and mutations do affect the proliferative capacities of cells, a positive effect of mutations on the fitness
240 of cells increases the overall μ_{pop} while deleterious mutations decrease it closer to μ_{mol} (Fig. 3B). This
241 effect is more pronounced the longer it takes for an individual to reproduce (Fig. 3C).
242
243 The intraorganismal selective process over the lifespan of a mature organism may interplay with the
244 intraorganismal selective process taking place earlier during development to shape an effective μ_{pop}
245 (Fig. 3D). For example, mutations on genes involved in basic cellular functions would likely have
246 fitness consequences for cells at any stage of an individual's life, thus affecting cell proliferation during
247 development, but also the rates of germline stem cell turnover ($\beta > 1$, $\beta_l > 1$). On the other hand, there
248 may be genes that are irrelevant for cellular fitness in the germline stem cell niche that may be important
249 during development ($\beta \neq 1$, $\beta_l = 1$) and vice versa ($\beta = 1$, $\beta_l \neq 1$). That would be the case of mutations
250 on the *chimno* mutants in *Drosophila*, which have been reported to evict wild-type cells from the stem
251 cell niche of the testis (Tseng et al. 2022). It is also possible that the selective regime during
252 development and maturity point in opposite directions, which is what we observed in the case of the
253 TrrCA mutation presented above: even though mosaic females introduced in Fig. 2C produced a
254 progeny that was biased in favor of the *trr* mutant allele right after pupation, as those same females
255 aged, the biased shifted in favor of TrrWT (Fig. 3E), suggesting that for this allele $\beta > 1$ and $\beta_l < 1$. On
256 the other hand, as expected, TrrWT retained a $\beta = \beta_l = 1$ through the development and lifespan of each
257 female fly (Fig. 3F). This again reinforces the notion that the estimation of global genome-wide
258 mutation rates may lead to biased interpretations of the molecular rates because of the functional
259 consequences of mutations on different loci. More generally, our results suggest that the overall
260 expected μ_{pop} is likely the composite outcome of the selective effect of mutations at the cellular level
261 during development and maturity, as well as with life history traits.
262
263 **Discussion**
264 Mutations and Their Developmental Contexts
265 Mutations arise in organismal contexts that vary across individuals and species. As our models
266 demonstrate, these contexts significantly influence how genetic variation is produced and incorporated
267 into gene pools. Therefore, differences in population-level mutation rates may not solely result from
268 changes in molecular mutation rates but could arise from evolutionary shifts in developmental
269 parameters. These include the rate of cell proliferation, developmental duration, germline differentiation
270 modes, germline dynamics during maturity, and—crucially—selective dynamics at the cellular level.
271 This perspective underlines the importance of distinguishing between molecular mutation rates and
272 population-level or generational mutation rates (Baer et al. 2007; Kimura 1987).
273

Because for evolutionary models what ultimately matters is the mutation rate at the level of populations, we propose an expansion of the definition of "mutator alleles" (Lynch et al. 2016) beyond molecular mechanisms that affect DNA repair and damage. Instead, mutator alleles may also include genes that influence developmental processes, life history traits, or organismal physiology. By highlighting the interplay between development and selection at the cellular level, we provide a broader framework for understanding the diversification of mutation rates.

Challenges in Deciphering Drivers of Mutation Rate Diversification

Disentangling whether a particular developmental parameter has driven the diversification of mutation rates between species may be challenging. Developmental variables often correlate with one another and with other biological traits that influence mutation rates. For instance, generation time is a well-known determinant, with longer lifespans allowing more cell divisions and thus a greater accumulation of mutations (Baer et al. 2007). While generation time differences between species like mice and humans can explain disparities in mutation rates (Wu and Li 1985), our model suggests that development may also contribute via differences in body size and developmental tempo, with humans exhibiting slower developmental rates compared to mice (Rayon et al. 2020).

Confounding factors such as molecular mutation rate adjustments further complicate interpretations. Molecular rates might evolve to mitigate the burden of deleterious mutations, particularly somatic mutations that can cause cancer (Cagan et al. 2022; Lynch 2008; Zhu et al. 2024). Notably, Zhu et al. (2024) showed that while the embryonic mutation rate is proportional to the generation time, the gamete mutation rate is inversely proportional. This finding aligns with the drift-barrier hypothesis, where embryonic mutation rate modifiers are constrained by population size, while gamete mutation rate modifiers are constrained by the product of population size and generation time. Interestingly, our intra-organismal selection model predicts similar trends, albeit through a different mechanism: longer-lived species not only accumulate more mutations but also allow more efficient elimination of deleterious variants and greater retention of beneficial ones due to extended selective dynamics during development.

Reconciling Molecular and Developmental Contributions

While we propose that developmental and life history traits play a significant role in mutation rate diversification, we do not dismiss the evolutionary relevance of molecular mechanisms. Alterations to baseline molecular mutation rates are well-documented (Sasani et al. 2022) and likely coexist with intra-organismal selection and developmental influences (Majic and Payne 2023; Xia et al. 2020). However, we do argue that it is more parsimonious to attribute mutation rate diversification to developmental variations accompanying population divergence than to assume frequent adjustments of mutation rate in response to changing population sizes. This is consistent with our findings and supported by evidence

that diversification in mutation spectra, often attributed to molecular changes, primarily occurs between distantly related lineages (Beichman et al. 2023) and may also be influenced by environmental factors (Alexandrov et al. 2016) or cell cycle dynamics (Spisak et al. 2024).

Ultimately, the causes of mutation rate diversification are multifaceted. They may arise from molecular mechanisms, life history strategies, developmental modes, or combinations of these factors in species-specific ways. It remains an open question whether one of these sources consistently dominates the evolutionary diversification of genetic variability. Future studies should account for the developmental contexts in which mutations arise and the ecological interactions between cells.

Considerations about the place of development in evolution

The role of development in evolution is increasingly recognized, but much of the discussion has focused on how developmental systems constrain or drive phenotypic evolution (Arthur 2001; Galupa et al. 2023; Majic et al. 2022; Maynard Smith et al. 1985). Our findings emphasize an additional role: by influencing mutation rates, development can shape the evolutionary pace and direction at both phenotypic and genotypic scales.

In the conceptual framework of genotype space (Wright 1932), populations could, in principle, explore the space equally in all directions (Fig. 4A). However, mutational biases—such as the well-documented transition versus transversion bias—skew this exploration, affecting the spectrum of adaptive substitutions available to populations (Cano et al. (2022), Fig. 4B). Beyond molecular biases, developmental processes can further constrain or promote specific directions of exploration (Fig. 4C) and influence the breadth of genotype space sampled within a generation (Fig. 4D). This has quantitative and qualitative implications for the genetic diversity of populations. Quantitively, populations of larger organisms like rheas, for example, can explore a broader portion of genotype space compared to those of smaller organisms like gulls, which have a considerably smaller generational mutation rate (Bergeron et al. 2023). While qualitatively, this broader exploration may occasionally introduce deleterious variants, many of these—particularly those disrupting basic cellular functions or germline potential—are more efficiently pruned in larger species due to their developmental and intraorganismal selective dynamics. Thus, organisms are not merely vehicles for genes, as major evolutionary paradigms suggest (Dawkins 1976). Instead, development actively participates in shaping genotypic changes arising in the gene pools of populations and, by extension, the evolutionary trajectories of species.

Methods

Developmental model for estimation of μ_{pop}

We define the generational mutation rate μ_{pop} as the probability of sampling a mutant cell among all cells in an organism that are capable of producing gametes at the time of reproduction of an individual. To calculate this probability, it is necessary to first calculate M_i, which represents the total number of cells that are expected to carry a mutation at a given locus i. We adapted a model from Otto & Orive (1995), wherein M_i is a function of the molecular mutation rate μ_{mol}, the total developmental time τ, the rate of cell divisions during development C, and a factor β_i, which represents the effect of a mutation on the fitness of a cell carrying a mutation on locus i. At developmental time x, one would expect $2^x(1-\mu_{mol})^{x-1}$ cells to not carry any mutations. Each of those cells can mutate with a probability given by μ_{mol} and the mutated lineage will then undergo $\beta_i(C\tau - x)$ cell divisions more until the conclusion of development. Thus, the number of mutant cells resulting from mutations at time x is:

$$2^x(1-\mu_{mol})^{x-1} * \mu_{mol} * 2^{\beta_i(C\tau - x)}$$

Summing over all x gives us M_i at the conclusion of development:

$$M_i = \sum_{x=1}^{C\tau} 2^x(1-\mu_{mol})^{x-1} * \mu_{mol} * 2^{\beta_i(C\tau - x)}$$

For estimating μ_{pop} it is also necessary to calculate the expected number of cells that do not undergo mutations at locus i through the course of development i.e., N_i. This can be calculated as:

$$N_i = 2^{C\tau}(1-\mu_{mol})^{C\tau}$$

The total number of cells at the end of development is $(M_i + N_i)$ and thus μ_{pop_i} approximates the probability of sampling a cell mutated at locus i from all cells in the pool of gametes, such that

$$\mu_{pop_i} = \frac{M_i}{M_i + N_i}$$

To estimate μ_{pop} across the entire length of a genome, as a first order approximation, we assumed that no cell obtains mutations at more than one locus during the entire course of development. In other words, we assume $\mu_{mol}{}^2 \approx 0$. This assumption would hold for very small μ_{mol}. Using this

simplification, we can estimate μ_{pop} across the entire length of a genome by averaging μ_{pop} across all sites with different values of β_i. In our model, we considered $F_{\beta i=f}$ to be the fraction of the total sites in a genome that have a $\beta_{i=}f$, giving:

$$\mu_{pop} = \sum_{f} \mu_{pop_i}(\beta_i = f) \cdot F_{\beta i=f}$$

<u>Expansion of the model to account for maturation and time to reproduction</u>

To consider the role of lifetime of an organism in the calculation of $\mu_{pop,}$ we considered the pool of cells resulting from the developmental process to be constant in number. Immediately at the end of development, there are $M_{t=0}$ cells in the germline stem cell niche that are mutated and $N_{t=0}$ that are not. During an organism lifetime *l,* germline stem cells can undergo a turnover (due to differentiation or death) at a rate *d* over an arbitrary unit of time *t*. The rate at which mutant cells replace lost cells during this turnover is given by a selective variable β_l. As in the case of the developmental selective parameter β_i, when $\beta_l < 1$ mutations are deleterious, when $\beta_l = 1$ mutations are neutral and when $\beta_l > 1$ mutations are advantageous to cells. In our model, during the lifetime of an organism before reproduction, it is possible for the total pool of mutated cells to decrease either due to reverse mutations (which would occur at a low rate, $M_{t-1}\mu_{mol}$) or loss due to death or differentiation (dM_{t-1}). Furthermore, mutant cells can increase in proportion whenever non-mutant cells in the stem cell niche mutate ($N_{t-1}\mu_{mol}$) or whenever mutant cells proliferate to replenish the stem cell niche after the loss of sibling cells. The number of mutated cells at time *t* during the lifetime of an organism can thus be calculated as,

$$M_t = M_{t-1} + N_{t-1}\mu_{mol} - dM_{t-1} - M_{t-1}\mu_{mol} + \frac{\beta_l d(M_{t-1} + N_{t-1})M_{t-1}}{\beta_l M_{t-1} + N_{t-1}}$$

And the number of non-mutant cells would be given by

$$N_t = N_{t-1} + M_{t-1}\mu_{mol} - dN_{t-1} - N_{t-1}\mu_{mol} + \frac{d(M_{t-1} + N_{t-1})N_{t-1}}{\beta_l M_{t-1} + N_{t-1}}$$

407 Comparison of μ_{pop} for different mammalian species

408 To corroborate the predictions of our model we considered estimations of the μ_{pop} of each of 36 species
409 of mammals. We considered the μ_{pop} of each species to be the mean of the number of de novo mutations
410 per base pair across different numbers of parent-offspring trios reported by Bergeron et al (2023). We
411 collected the other biological parameters used, namely the body mass of each species and the gestation
412 time, from the Encyclopedia of Life (Parr et al. 2014). The phylogenetic relationships we considered
413 were based on recent phylogenetic assessments of mammals (Foley et al. 2023), while the estimated
414 divergence times were collected for various sources listed in Table S1.

415 Damuth's rule: estimation of population density

416 For organisms of a given mass $m = m_{cell}\, 2^{C\,\tau}$, where $m_{cell} = 10^{-15} kg$ is the mass of a single cell
417 (Sender et al. 2016) and $2^{C\tau}$ is the number of cells, we computed the population density (*D*) using
418 Damuth's rule i.e., $D = K \times m^{-3/4}$, where *K* is a constant of proportionality and equals $10^{4.23}$
419 (Damuth 1981). The value of μ_{pop} was calculated using the expression derived above.

420 Flippase assay to assess cell competition in fly germlines

421 We developed heat shock-induced mitotic clones in fruit flies following a standard protocol (Golic and
422 Lindquist 1989). Briefly, a fly line (# 31418 from the Bloomington Drosophila Stock Center) containing
423 a red fluorescent protein (mRFP), a flippase-encoding gene induced by a heat-shock promoter (hsFLP)
424 and a FRT site for the binding of flippases was crossed with another line carrying a single-amino acid
425 substitution in the *trithorax-related* gene (*trr*) and the w^{1118} allele for the gene white. We thus created a
426 trr^{CA},w^{1118},FRT,hsFLP and a w^{1118},FRT,hsFLP lines. For our experiments we crossed these lines with
427 the parental mRFP,FRT,hsFLP lines and we exposed the heterozygotic eggs collected during a window
428 of four hours to a heat shock of 37°C for one hour.
429
430 We collected mosaic virgin females that developed from these heat-shocked embryos as well as virgin
431 females from embryos that were not heat-shocked (control). Each of these virgins were individually
432 paired to two w^{1118} males and we then counted the fraction of offspring that were positive for red
433 fluorescence, which are the ones that inherited the mRFP+ allele and not the mRFP- (trr^{CA},w^{1118}) allele.
434

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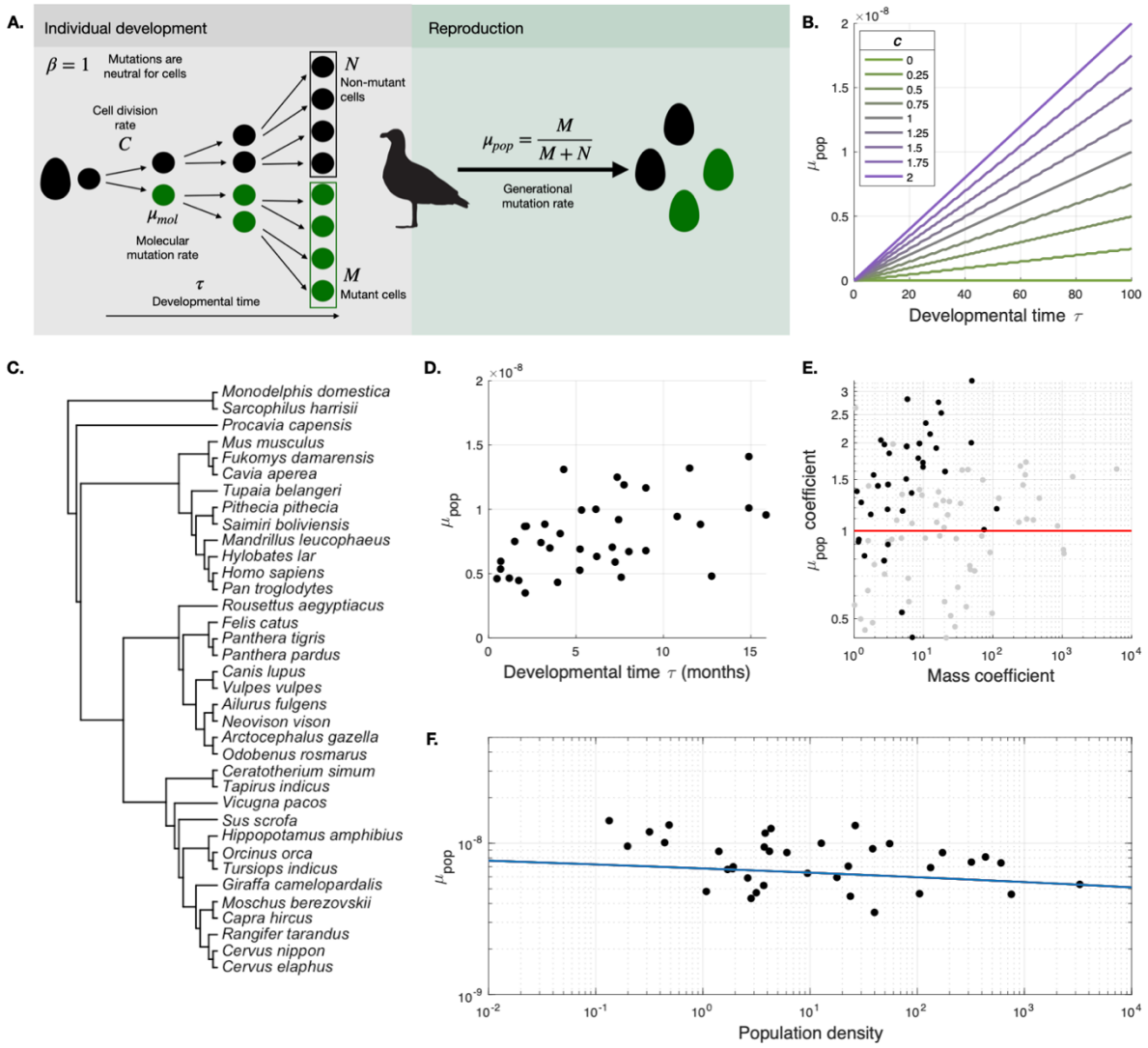

Figure 1. Changes in developmental parameters can causally explain diversification of mutation rates. (A) Illustrated summary of the core model consisting of an individual developmental phase and a reproductive phase. In the developmental phase a lineage starting from a single cell goes through successive cell divisions at a rate C over a developmental time τ. During development, mutations can occur at a rate μ_{mol}. Cell proliferation could be affected by the fitness effect of mutations on cells β. For $\beta = 1$ mutations are neutral. At the end of development, during the reproductive phase, the fraction of germline-capable cells that carry mutations define the rate at which new alleles are added to the gene pool μ_{pop}. (B) Population-level mutation rate μ_{pop} at a single locus as a function of τ. Each curve represents a different value of c. μ_{mol} was held constant at a value of 1×10^{-10} mutations per cell division, approximating empirical estimates for human and mouse (Milholland et al. 2017). (C) Phylogeny of mammals for which estimations for μ_{pop} are available (Bergeron et al. 2023). (D) μ_{pop} for each mammalian species in (C) as a function of their gestation time, a proxy for τ. (E) Coefficient between the μ_{pop} of sister species as a function of the coefficient of the mass of sister species. Notice how, for the vast majority of comparisons between younger branches μ_{pop} coefficients are above 1 (red line), which means that larger clades tend to have higher mutation rates, as predicted by our model. (F) Datapoints represent the expected relationship between the predicted μ_{pop} of each of the studied mammalian species and their expected population density based on their body mass according to Damuth's rule (Methods). The blue line represents the prediction of this relationship based on our developmental model for μ_{pop}.

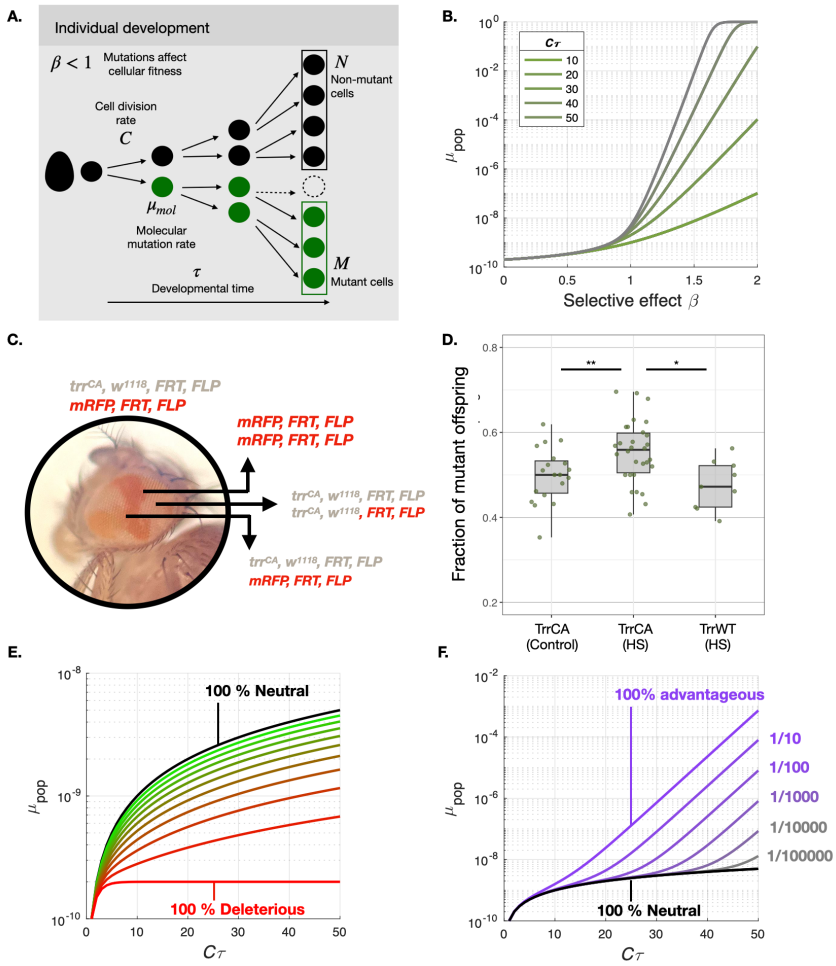

Figure 2. The impact of mutations on cell fitness during development can repercuss on mutation rates. (A) In the model introduced in Fig. 1A, when $\beta \neq 1$, mutations can affect the total number of mutated cells at the end of development M. (B) Population-level mutation rate μ_{pop} as a function of the effect of mutations on the fitness of cells β. Each curve represents a different value of developmental time τ with a constant value for $C = 1$. (C) Induced mitotic recombination can generate mosaic organisms with cells with different genotypes. The shown specimen corresponds to a female *Drosophila melanogaster* on which mosaicism can be easily observed in the eyes. Red, white, and orange patches represent each of the possible genotypes thanks to the w^{1118} allele. (D) Fraction of offspring carrying the mRFP(-) allele from crossing a female of interest with a w^{1118} male. TrrCA refers to females of the trr^{CA},w^{1118} genotype, while TrrWT refers to the w^{1118} genotype that is wild-type for *trr*. "HS" refers to females that developed from embryos that were heat-shocked, while "Control" females developed from embryos that were not heat-shocked. Each point represents the fraction of offspring from a different mother. * indicates $p < 0.05$, ** indicates $p < 0.005$ using a Wilcoxon test. (E) Population-level mutation rate μ_{pop} as a function of developmental parameters $C\tau$. Each curve represents a different value proportion of neutral or deleterious mutations. (F) Population-level mutation rate μ_{pop} as a function of developmental parameters $C\tau$. Each curve represents a different proportion of mutations that are advantageous for the cell ($\beta = 1.5$) in the context of an otherwise fully neutral sequence.

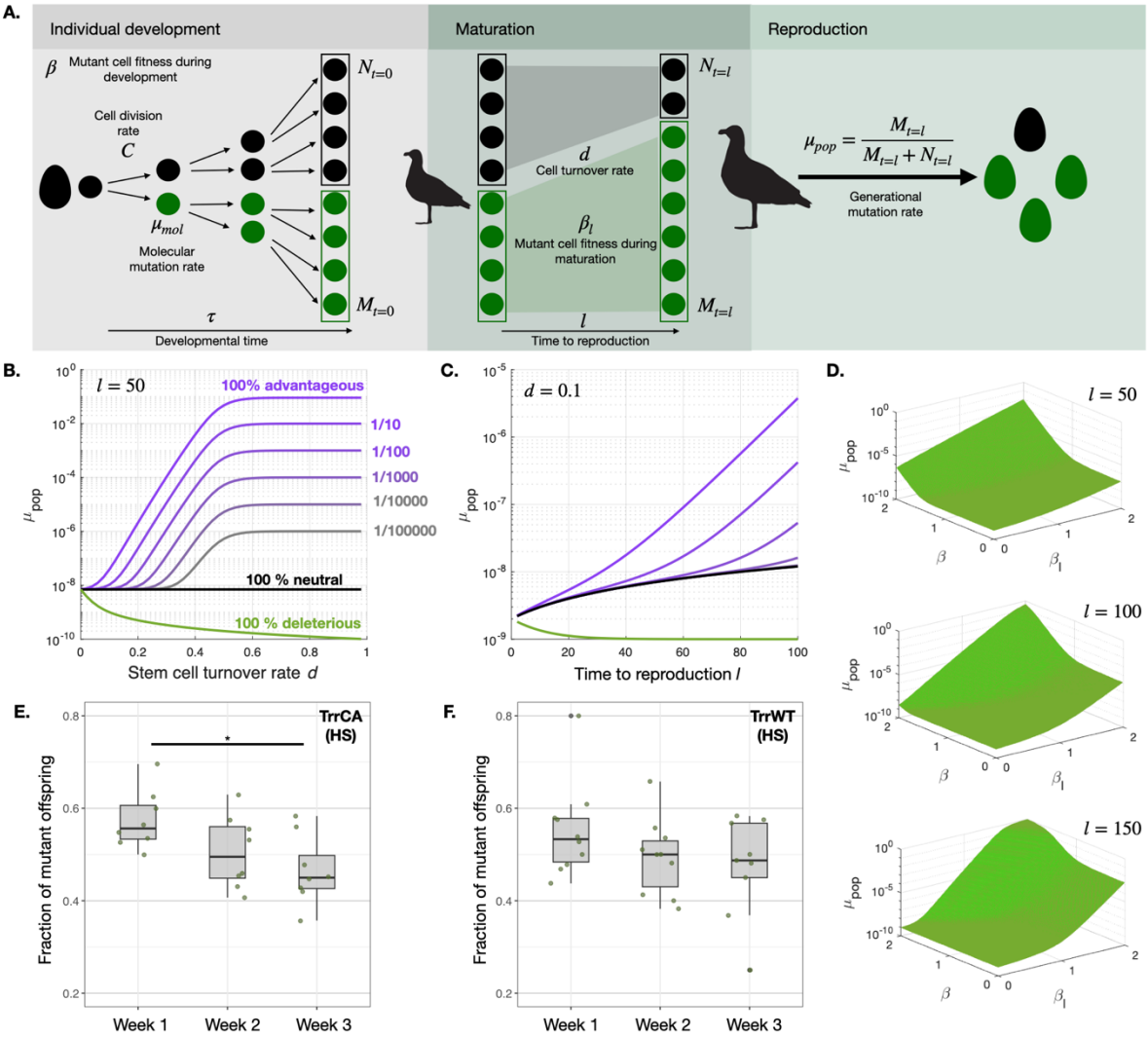

Figure 3. Selection within the germline stem cell niche can also impact mutation rates over an organism's lifetime. (A) Illustrated summary of the model integrating a maturation phase before reproduction. Adding this phase incorporates parameters such as the time to reproduction of an organism l, a turnover rate of germline stem cells d, and a cellular fitness variable for the stem cell niche β_l. (B) Population-level mutation rate μ_{pop} as a function of the rate of stem cell turnover d for a constant generation time of $l = 50$. Each curve represents a different proportion of mutations that are advantageous for the cell ($\beta_l = 1.5$) in the context of an otherwise fully neutral sequence (gray to purple lines), a fully neutral case (black) or a fully deleterious case (green). (C) Population-level mutation rate μ_{pop} as a function of generation time l for a constant rate of stem cell turnover $d = 0.1$. The different lines follow the same color code as in (B). (D) μ_{pop} as a function of cellular fitness variable for the stem cell niche β_l for different values of l and the fitness effect of mutation on cells during development β. (E and F) Fraction of offspring carrying the mRFP(-) allele from the same set of mothers reproducing at the beginning of weeks one, two or three post-hatching. TrrCA refers to females of the trr^{CA},w^{1118} genotype, while TrrWT refers to the w^{1118} genotype that is wild-type for *trr*. "HS" refers to females that developed from embryos that were heat-shocked. * indicates $p < 0.05$.

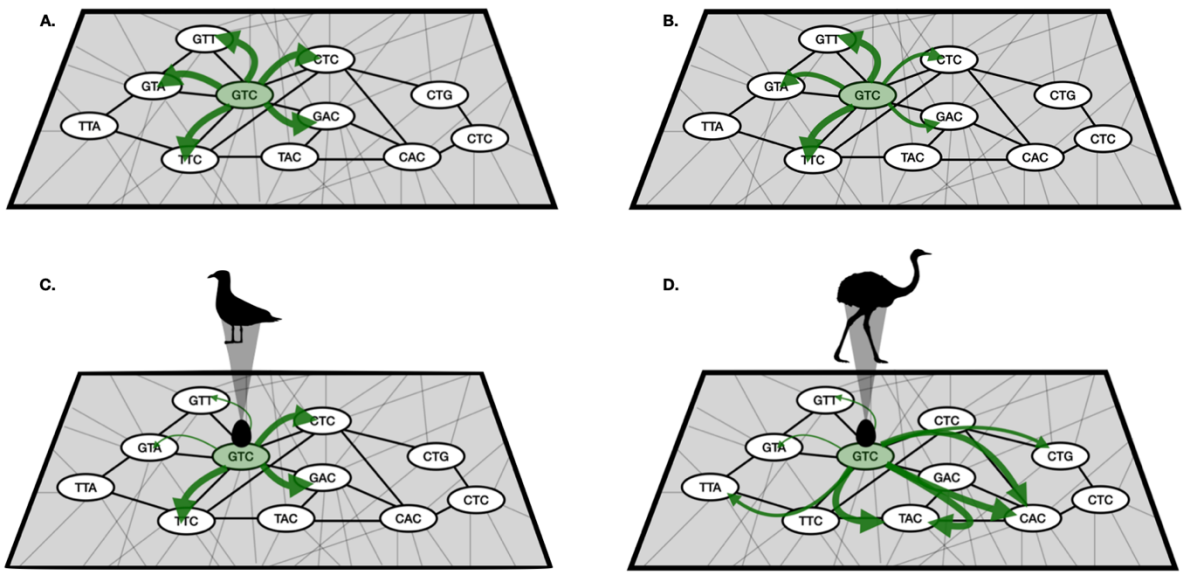

Figure 4. How development can influence the trajectories and pace of genotypic evolution. In a theoretical model of evolution, it could be considered that given a starting genotype, GTC, all mutational steps are equally likely (A). However, if molecular realism is taken into account there are certain mutations that are more likely than others, as represented by arrows of different thickness goingt to CTC and GAC. (B). Furthermore, as we argue in this work, development can act as a selective filter for mutations which increases or decreases the likelihood of mutational steps depending on how a mutation affects the cell lineage it belongs to (C). Developmental and life-history differences can also impact how far mutational steps can take an organism. As per our model, larger organisms should be capable of more broadly exploring genotypic spaces within a generation (D).

669 **Table S1. Empirical parameters for 36 mammalian species.**

670 Bergeron et al (2023) estimated the number of de novo mutations in parent-offspring trios of each of the
671 studied species. With those estimations we calculated μ_{pop} as the average of all replicates reported. The mass
672 and τ (gestation time) values were collected from the Encyclopedia of Life (Parr et al 2014). Branch ages
673 representing the estimated divergence time from the closest relative in the phylogeny (Fig. 1C) were gathered
674 from several sources indicated by the superindeces in the Branch Age column: [1](Foley et al. 2023), [2](Wang
675 2008) [3](Rossiter et al. 2020) [4](Hassanin et al. 2003) [5](Johnson et al. 2006) [6](Law et al. 2018) [7](Gilbert et al.
676 2006).

Species	μ_{pop}	Mass (grams)	τ (months)	Branch age (my)
Vicugna pacos	9.44 x10^{-9}	60000	10.79	66[1]
Cavia aperea	8.67 x10^{-9}	442.8	2.07	56[1]
Rousettus aegyptiacus	8.11 x10^{-9}	135.5	4.1	80[1]
Saimiri boliviensis boliviensis	6.90 x10^{-9}	615	5.23	37.3[1]
Felis catus	3.49 x10^{-9}	2884	2.09967	33.9
Pan troglodytes	1.25 x10^{-8}	49896	7.36	7.246[1]
Sarcophilus harrisii	5.95 x10^{-9}	8109.3	0.7	160[1]
Canis lupus familiaris	8.68 x10^{-9}	32183.3	2.16	33[2]
Tursiops truncatus	8.83 x10^{-9}	209791	12.12	19.5[3]
Mandrillus leucophaeus	6.33 x10^{-9}	18250	6.19	37.71[1]
Vulpes vulpes	4.46 x10^{-9}	5603.75	1.73	33[2]
Hylobates lar	7.05 x10^{-9}	5930	7.08	27[1]
Giraffa camelopardalis	1.01 x10^{-8}	932324.5	14.89	45[4]
Capra hircus	5.26 x10^{-9}	61000	5.22	27.8[1]
Hippopotamus amphibius	1.19 x10^{-8}	1417490	7.75	61[1]
Homo sapiens	1.17 x10^{-8}	58540	9	7.246[1]
Procavia capensis	9.20 x10^{-9}	3029.3	7.44	100[1]
Panthera pardus	8.84 x10^{-9}	52038.3	3.22	10[5]
Neovison vison	4.64 x10^{-9}	834.67	1.19	33[6]
Fukomys damarensis	7.41 x10^{-9}	88.5	3	56[1]
Mus musculus	5.35 x10^{-9}	10.1	0.69	66[1]
Moschus berezovskii	1.00 x10^{-8}	12477	6.1413	27.8[1]
Monodelphis domestica	4.60 x10^{-9}	66.5	0.49	160[1]
Orcinus orca	1.41 x10^{-8}	4300000	14.89	19.5[3]
Sus scrofa	4.32 x10^{-9}	86250	3.95	60[1]
Ailurus fulgens	1.31 x10^{-8}	4900	4.3	33[6]
Rangifer tarandus	5.90 x10^{-9}	94744.67	7.24	20.2[7]
Cervus elaphus yarkandensis	6.71 x10^{-9}	165016	8.03	6.8[7]
Ceratotherium simum simum	9.56 x10^{-9}	2591659.5	15.87	61[1]
Pithecia pithecia	9.94 x10^{-9}	1900	5.32	37.3[1]
Arctocephalus gazella	6.78 x10^{-9}	143500	9	33.9[1]
Tupaia chinensis belangeri	7.50 x10^{-9}	200	1.5	77.69[1]
Cervus nippon	4.71 x10^{-9}	74750	7.59	6.8[7]
Tapirus indicus	4.80 x10^{-9}	296250	12.76	61[1]
Panthera tigris	6.99 x10^{-9}	141132	3.51	10[5]
Odobenus rosmarus	1.32 x10^{-8}	825000	11.5	33.9[1]

677