Reproductive mode and the evolution of mutation rate†

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Abstract

The mutation rate is a fundamental force in evolution, modulating patterns of genetic variation, adaptation, and genome evolution throughout the tree of life. As such, many attempts have been made to explain its variation across species. Currently, the dominant theory of mutation rate evolution is the drift-barrier hypothesis. However, although this framework has received wide support across taxa, a substantial fraction of variation in mutation rate remains unexplained, and a major challenge persists in ruling out confounding factors in phylogenetic comparisons. In this review, we propose that transitions in reproductive mode may also be a major factor governing the evolution of mutation rate, through their effects on recombination rates, genetic linkage, and effective population size. We synthesize theoretical predictions and empirical evidence bearing on these effects, highlighting major gaps in our understanding of how reproductive mode influences mutation rate evolution. We argue that progress in this area is now primarily constrained by the need for more realistic theoretical modeling, experimental evolution work, and direct, comparable empirical estimates of mutation rate across diverse taxa and reproductive strategies. Expanding such estimates across taxa will be essential for testing theory and for understanding the extent to which reproductive modes modulate mutation rate evolution.

Keywords

Drift-barrier hypothesis, Sexual reproduction, Asexual reproduction, Facultative sexuality, Self-fertilization, Selfing, Outcrossing

1 Introduction

Mutations are the ultimate source of all genetic variation. As such, the rate at which mutations occur plays a central role in shaping evolutionary dynamics across the tree of life. By controlling how many new variants enter populations each generation, the mutation rate—here used in its broad sense as how frequently spontaneous genomic changes arise between generations—determines the extent to which there is raw material upon which natural selection, genetic drift, and other evolutionary forces can act. Accordingly, mutation rate influences all evolutionary processes, from molecular evolution to macroevolutionary patterns of diversification (Box 1).

The central role of mutation rate in evolution makes the wide taxonomic variation in mutation rate at least superficially surprising. For example, the base-substitution mutation rate (u) per site, per generation in an organism varies by more than three orders of magnitude in eukaryotes. Indeed, we observe mutation rates of $u = 1.2 \times 10^{-8}$ in humans (Kong et al. 2012; Lindsay et al. 2019; Bergeron et al. 2023), 7.4×10^{-9} in the mustard-family plant *Arabidopsis thaliana* (Yang et al. 2015), 9.6×10^{-10} in the unicellular green alga *Chlamydomonas reinhardtii* (Ness et al. 2015), and 7.6×10^{-12} in the micronucleus of the ciliate *Tetrahymena thermophila* (Long et al. 2016).

Understanding the evolutionary forces that drive variation in mutation rate is a fundamental question in evolutionary biology that has been the subject of intense theoretical and empirical investigation for decades. One of the most influential and far-reaching theories for mutation rate evolution is the drift-barrier hypothesis (DBH, detailed in sections 4.1–4.2) (Lynch 2010, 2011; Sung, Ackerman, et al. 2012). While this theory has received wide support from diverse organisms, a substantial fraction of mutation rate variation is not explained by this framework (Lynch 2010; Sung, Ackerman, et al. 2012; Lynch et al. 2016, 2023; Bergeron et al. 2023; Wang and Obbard 2023; Weinstein and Roy 2026). There also remain important concerns that factors other than the selection–drift balance central to the DBH may have confounding effects, and that these could exert direct causal influences on mutation rate (Weinstein and Roy 2026).

Box 1. Evolutionary consequences of mutation rate variation.

The mutation rate governs the influx of new genetic variation into populations, providing the raw material on which evolutionary processes can act. Higher mutation rates cause the introduction of more mutations into populations each generation, increasing the supply of variants that may contribute to standing polymorphisms within populations (Wright 1931; Kimura and Ohta 1971b; Lande 1975). The direction and strength of selection acting on these variants will vary depending on their effects on organismal fitness, which themselves can be context-dependent. Ultimately, the interaction between mutation, genetic drift, and natural selection can influence, in either direction, the total genetic variation within populations.

The mutation rate is thus a primary determinant of the rate of molecular evolution and of patterns of genetic divergence between lineages. Classical work (Kimura and Ohta 1971a) has shown that for effectively neutral mutations, whose fate is governed almost entirely by genetic drift, the rate of substitution equals the mutation rate. Similarly, for adaptive mutations, whose fate is determined in part by natural selection, the rate of substitution is proportional to the mutation rate.

Given its central role in determining the supply of new mutations, the mutation rate further interacts with processes like the mode of reproduction. Theory indeed predicts that the long-term evolutionary benefits of sexual reproduction relative to asexual reproduction can depend on the rate at which new deleterious mutations arise (Kondrashov 1988; Charlesworth 1990).

While mutation rate is a crucial parameter in evolutionary biology, it is also a key factor in human health. For example, cancer cells often display higher mutation rates than the normal somatic cells from which they descend (Loeb et al. 1974; Bielas et al. 2006), and elevated mutation rates appear to facilitate the evolution of resistance to antibiotic combinations in bacteria (Gifford et al. 2023). Understanding the factors that influence mutation rate evolution is, therefore, not only of fundamental interest but also has practical implications for medicine and public health.

In this review, we propose that an important source of this unexplained variation may be found in the diversity of reproductive modes that exist across and even within many lineages (Barrett et al. 2007; Chen and Wiens 2021). We explore this hypothesis by reviewing both theoretical predictions and preliminary empirical evidence on the effects of reproductive modes on mutation rates and by discussing the implications of these findings for understanding how and why mutation rate evolution varies so widely among species.

2 Drivers of mutation rate evolution

2.1 Mutator and antimutator alleles

Because new mutations are more often deleterious than beneficial, DNA-based organisms have evolved sophisticated mechanisms that reduce their mutation rate by ensuring DNA replication fidelity and repairing DNA damage (Friedberg et al. 2006). Mutations in the genes underlying these processes can result in either increases or reductions in mutation rate, generating *mutator* and *antimutator* alleles, respectively (Drake et al. 1969; Miller 1996; Kunz et al. 1998; Herr et al. 2011; Fijalkowska et al. 2012). Collectively, mutator and antimutator alleles are often referred to as mutation-rate *modifier* alleles. While we focus primarily on DNA-based organisms in this review, the general principles discussed here also extend to RNA viruses.

Direct observations of mutation rate evolution have been reported in experimental populations (Chao and Cox 1983; Mao et al. 1997; Giraud et al. 2001; Loh et al. 2010; Wielgoss et al. 2013; Fitzsimmons et al. 2018; Liu and Zhang 2021). For example, a population of *Escherichia coli* adapting to a constant environment evolved a frameshift mutation in the *mutT* gene after ~25,000 generations that increased mutation rate 150-fold (i.e., a mutator allele) (Barrick et al. 2009; Wielgoss et al. 2013). Then, within ~10,000 generations, the population evolved two independent alleles in the *mutY* gene, each of which halved the mutation rate (i.e., antimutator alleles) (Wielgoss et al. 2013). This example demonstrates that both mutator and antimutator alleles can readily arise and spread in evolving populations.

It is important to note that although much of the literature on mutation rate evolution has focused on canonical mutation rate genes (e.g., those encoding DNA polymerases or mismatch-repair proteins), many other genetic pathways can also influence mutation rates. For example, in a mutation accumulation (MA) experiment in the budding yeast *Saccharomyces cerevisiae*, many lines evolved either lower or higher mutation rates than their progenitor (Liu and Zhang 2021). Among the mutations associated with this variability, alterations in the *PSP2* gene, a regulator of autophagy that promotes autophagy-related protein translation (Yin et al. 2019), were found to be more frequent in MA lines that evolved lower mutation rates. Furthermore, deleting *PSP2* altogether caused a 42% reduction in

mutation rate (Liu and Zhang 2021). This study also highlights that mutation rate evolution encompasses a broad spectrum of mutational processes in addition to base substitutions, including insertions, deletions, and structural mutations like inversions or translocations. Indeed, in the ancestral strain of yeast used in the experiment, indels accounted for only 16% of detected mutations, but that proportion rose to $\gtrsim 80\%$ in two MA lines that had evolved higher mutation rates (Liu and Zhang 2021).

Transposable elements, along with the genes that regulate their activity, provide another example of modifier alleles outside the spectrum of base-substitution mutation (Chao et al. 1983). Transposable element insertions directly generate mutations and can further promote genomic instability. Because transposable elements are regulated by multiple silencing mechanisms like DNA methylation, histone modification, splicing inhibition, and RNA-based pathways (reviewed in Almeida et al. 2022), mutations affecting these pathways can indirectly increase or decrease mutation rates by altering transposable element activity. However, such effects are comparatively difficult to measure, likely contributing to the relative paucity of studies incorporating these mutation sources into broader models of mutation rate evolution. Nevertheless, recent work suggests extensive natural variation in the epigenetic silencing of transposable elements, with consequences for rates of transposition and, thus, mutation rate evolution (Dubin et al. 2015; Sasaki 2022).

2.2 Evolutionary processes driving mutation rates

The genes that drive mutation rate evolution are, like all genes, shaped by both stochastic and deterministic evolutionary processes. Sturtevant (Sturtevant 1937) was perhaps the first to predict that multiple forms of natural selection are likely to act on mutation-rate modifier alleles. The main processes currently proposed to underlie mutation rate evolution are summarized in figure 1 and described in more detail in subsections (i)–(iv) below. While some of these processes act directly on the mutation rate, others act indirectly through their effects on other processes.

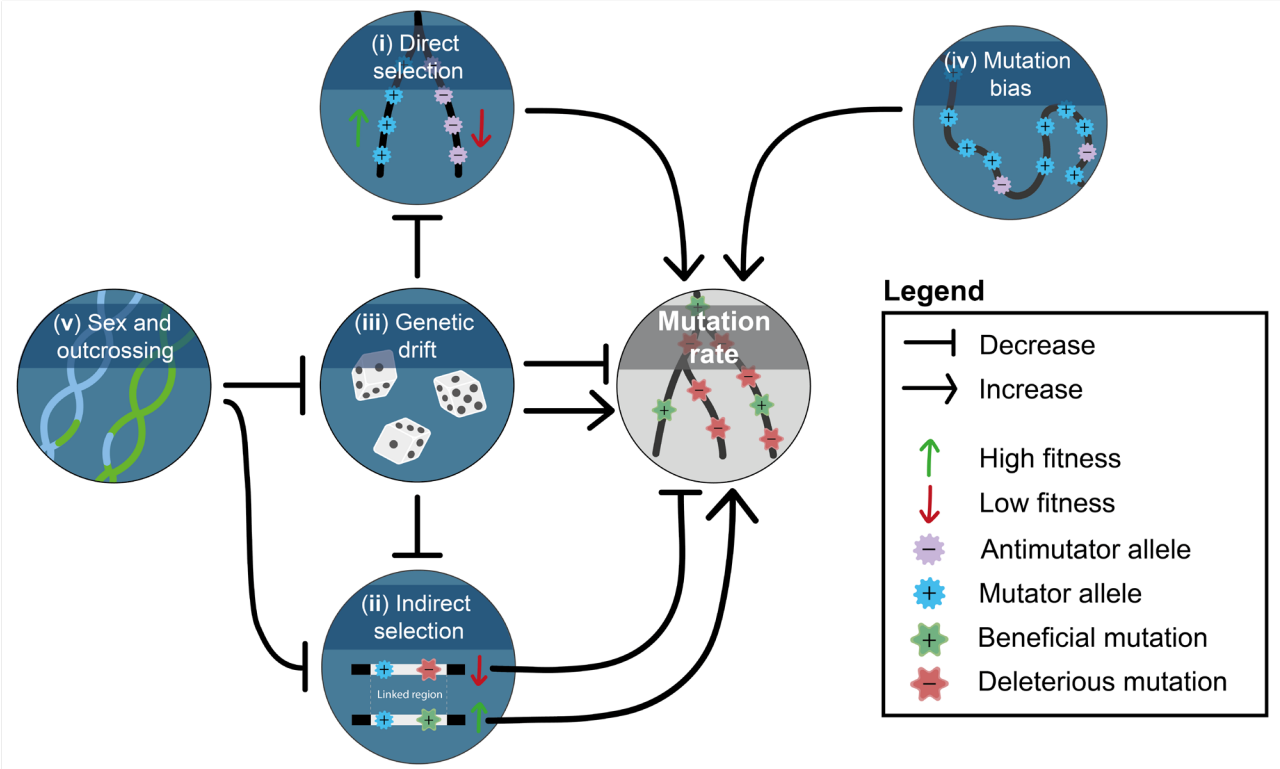

Figure 1. Processes shaping mutation rate evolution within populations. Mutation rate is influenced by multiple evolutionary processes acting within populations, including natural selection (i–ii) and genetic drift (iii). Selection may either act directly on mutator and antimutator alleles (i) or indirectly via linkage disequilibrium between a mutation-rate modifier allele and subsequent mutations affecting fitness (ii; illustrated here using a mutator allele as an example). Direct selection is believed to favor higher mutation rates because high fidelity of replication and repair efficiency are thought to be physiologically costly, whereas indirect selection may favor either mutator or antimutator alleles depending on the fitness consequences of the mutations they generate or prevent from occurring, with upward and downward arrows denoting increases and decreases, respectively, in modifier allele fitness. Similarly, because genetic drift (iii) acts stochastically, it may lead to either increases or decreases in mutation rate. Another process influencing mutation rate is mutation bias (iv), expected to act primarily to increase mutation rate. In this review, we consider the extent to which reproductive modes (v) also influence the evolution of mutation rates. This effect is illustrated using sex and outcrossing (in contrast to obligate asexuality), which are expected to weaken indirect selection by reducing linkage disequilibrium. On the other hand, sex and outcrossing will decrease the effects of genetic drift by alleviating the effects of selective interference, thereby increasing the efficacy of selection indirectly. A fuller discussion of how reproductive modes—including facultative asexuality and self-fertilization—influence these processes is provided in section 3. Selection acting at higher levels of organization (vi) is not shown. Illustration: Joëlle Lafond.

(i) Direct selection

Direct selection on mutator and antimutator alleles is believed to be an important contributor to mutation rate evolution (figure 1–i). For example, DNA replication fidelity is thought to be physiologically costly, for instance by requiring the production of additional repair proteins or slowing the replication of DNA. Accordingly, mutator alleles might be favored if they lessen

costs associated with mechanisms such as DNA replication fidelity, DNA repair efficiency, or transposable element silencing (Kimura 1967; Dawson 1998; Sniegowski et al. 2000). One empirical study has confirmed the existence of this trade-off between replication fidelity and fitness among eight clones of vesicular stomatitis virus (an RNA virus) carrying single amino acid substitutions in the RNA polymerase (Furió et al. 2005). Data on HIV-1 (another RNA virus) revealed a trade-off between the accuracy of the reverse transcriptase and its polymerization speed (Furió et al. 2006). However, empirical evidence for such costs of fidelity or other forms of direct selection on modifiers of mutation rate remain limited, with RNA viruses providing the only well-supported examples to date (Furió et al. 2005, 2006; Fitzsimmons et al. 2018). There is therefore a clear need for more studies explicitly designed to evaluate the direct fitness consequences of mutator and antimutator alleles in diverse DNA-based organisms.

(ii) Indirect selection

Mutation rates can also be shaped by indirect selection arising from linkage disequilibrium (LD) between a mutation-rate modifier allele and subsequent mutations with effects on fitness (figure 1–ii). LD measures the extent to which particular alleles at different loci are found together more or less often than expected by chance in a population. If there is LD between two loci in a population, selection acting on one locus can cause the frequency of an allele at the other locus to increase, even if that allele is neutral or deleterious, a phenomenon known as hitchhiking (Maynard Smith and Haigh 1974). If mutation-rate modifier alleles are themselves neutral, and all mutations that influence fitness are deleterious, then variation in mutation rate within a population will, over time, generate *negative* LD between mutation-rate modifier loci and fitness loci. In other words, mutator alleles will tend to co-occur with a greater deleterious mutation load than antimutator alleles. This negative LD will cause indirect selection against mutator alleles and for antimutator alleles (Sturtevant 1937; Kimura 1967; Leigh 1970, 1973; Dawson 1998; Johnson 1999b). Conversely, beneficial mutations can create *positive* LD between mutation-rate modifier loci and fitness loci, reversing the direction of selection on mutation-rate modifier alleles (Sturtevant 1937; Johnson 1999a; Tenaillon et al. 1999; André and Godelle 2006; Raynes et al. 2018).

The extent of LD is closely tied to the rate of recombination in an organism. Because recombination breaks up associations between mutation-rate modifiers and the mutations they generate, it reduces the opportunity for modifiers to hitchhike with beneficial mutations or be selected against through their association with deleterious mutations. Therefore, indirect selection on mutation-rate modifiers is expected to be weakest in organisms with high recombination rates (Kimura 1967; Leigh 1970; Tenaillon et al. 2000).

(iii) Genetic drift

Mutation rates are also influenced by genetic drift, stochastic fluctuations in allele frequencies caused by finite population size (figure 1–iii). The strength of genetic drift depends on N_e, the effective size of a population—that is, the number of individuals in an idealized population evolving according to the Wright–Fisher model (Ewens 2004) that would show the same rate of allele frequency change due to drift as the focal population (Wright 1931). As N_e decreases, random sampling effects become stronger, and selection becomes less effective at governing allele frequencies. Because the strength of selection—direct or indirect—acting on mutation-rate modifier alleles is expected to be weak (Sturtevant 1937; Kimura 1967), the evolution of mutation rate is likely to be strongly affected by genetic drift, especially in small populations.

(iv) Mutation bias

Mutation rates may further be shaped by mutation bias towards either increasing or decreasing mutation rate (figure 1–iv). Such biases could take one of two non-mutually exclusive forms: mutator mutations could occur with either higher or lower probability than antimutator mutations, or mutator mutations could have either larger or smaller effects on average than antimutator mutations. While there is little evidence that either kind of bias in either direction actually exists, there is likely an inherent asymmetry in the generation of modifier alleles (i.e., a bias of the first type), whereby alterations that produce mutators arise more readily than those that give rise to antimutators, for two reasons. First, mutator alleles involve molecular disruptions such as loss-of-function mutations (Miller 1996; Kunz et al. 1998; Friedberg et al. 2006; Fijalkowska et al. 2012), which are thought to be more common than other kinds of mutations because there are so many different ways to disrupt the function of a gene (e.g., frameshifts, nonsense and missense mutations, deletions). Second,

once a particular loss-of-function mutation has occurred, there are far fewer ways to reverse its effect and restore function. Indeed, certain kinds of loss-of-function mutations, such as whole-gene deletions, are unlikely to be reversible by a single mutation. This logic might explain why theoretical analyses of the evolution of mutation rate often assume the operation of a mutation bias towards increased mutation rate (Taddei et al. 1997; Tenaillon et al. 1999; Tenaillon et al. 2000; Gerrish et al. 2007; Sloan and Panjeti 2010; Desai and Fisher 2011; Lynch 2011; Jain and Nagar 2013). On the other hand, many antimutator mutations have been isolated even though antimutators are believed to be more difficult to detect than mutators (Schaaper 1998; Kunz et al. 1998; Herr et al. 2011; Liu and Zhang 2021).

(v) Reproductive modes

The preceding paragraphs outlined the major processes influencing mutation rate evolution: natural selection, genetic drift, and mutation bias. The relative influence of these evolutionary forces depends on factors such as recombination rate, effective population size (N_e), population structure, the mutation rate itself, the genomic architecture of the organism (e.g., ploidy, number and size of chromosomes), and the genetic architecture of mutation rate and fitness (e.g., number of genes determining each trait and their distribution in the genome, degree of pleiotropy between the two traits, dominance, epistasis). These factors are not fixed, but instead vary widely across biological systems. A key axis of this variation lies in life-history strategy, and in particular, in whether and how organisms exchange and combine genetic material. Because reproductive modes influence recombination, segregation, and N_e—and thereby modulate all the processes highlighted in this section—, we propose that they are likely a decisive driver of mutation rate evolution (figure 1–v).

(vi) Higher-level selection

Natural selection on mutation rate might also operate at levels of organization higher than the individual organism, such as the deme (local interbreeding population) or species (Jablonski 2008). Kimura (Kimura 1967) proposed that populations with high mutation rates may have higher rates of adaptation than populations with low mutation rates because of an increased supply of beneficial mutations. For example, in a long-term evolution experiment with *E. coli*, 3 out of 12 replicate populations evolved an approximately 100-fold higher mutation rate within 8,500 generations. As a result, these 3 populations adapted more quickly than the 6

populations that retained the ancestral mutation rate over the following 40,000 generations (Wiser et al. 2013). Alternatively, populations with high mutation rates may be more likely to go extinct (Gabriel et al. 1993; Gerrish et al. 2007, 2013; Bull et al. 2007). As an example, Zeyl and colleagues allowed 12 populations of *S. cerevisiae* with genetically elevated mutation rates to evolve and found that two of them went extinct within 2,900 generations (Zeyl et al. 2001).

3 Population genetic consequences of different reproductive modes

Across biological systems, the extent to which the transmission of genomes from one generation to the next involves genetic exchange between individuals spans a continuum from obligate asexuality or clonality to obligate sexuality, encompassing intermediate forms such as facultative sexuality or self-fertilization. In the following sections, we outline how these reproductive modes shape genome transmission and composition, as well as how they influence the effective population size, N_e. It should be noted that the predictions presented below focus on the direct population-genetic consequences of reproductive mode and do not account for variation in other properties that may covary with them, including life-history traits, ecology, geographic distribution, or the strength and direction of sexual selection. These factors may themselves influence patterns of genomic diversity and evolution in many ways, independently of the effects discussed here.

Sexual reproduction is the most common mode of reproduction across eukaryotes (Bell 1982) and is typically associated with extensive effective recombination during gametogenesis and the combination of genetic material from different individuals through outcrossing. Although local reductions in recombination permit hitchhiking and the build-up of LD, genome-wide recombination generally maintains low LD between loci, thereby reducing the efficiency of indirect selection (figure 1, v ⟊ ii). At the same time, because high recombination rates limit selective interference (see paragraph below) (Hill and Robertson 1966; Felsenstein 1974; Nordborg et al. 1996; Desai and Fisher 2011; Charlesworth 2012), sexual reproduction (indirectly) enhances the efficacy of direct selection (figure 1, v ⟊ iii ⟊ i).

In contrast to obligate sexuality, obligate asexuality eliminates or severely reduces recombination, which causes every locus to be linked and increases genome-wide LD. Under these conditions, selection acts on entire genomes rather than on individual loci, and different mutations cannot be independently combined or separated. This LD thus generates selective interference among loci (Hill and Robertson 1966; Felsenstein 1974; Comeron et al. 2008): beneficial alleles arising in different genetic backgrounds compete with one another (clonal interference (Fisher 1930; Muller 1932; Gerrish and Lenski 1998)), while deleterious alleles reduce the probability that linked advantageous alleles spread (background selection, reviewed in (Charlesworth 2012)). Further, without recombination, genomes cannot shed accumulated deleterious mutations, leading to their irreversible buildup (Muller's ratchet (Muller 1964; Felsenstein 1974; Haigh 1978)). Background selection also results in lower N_e, which in turn causes drift to become a stronger force in shaping evolution (Charlesworth 2012). This contrasts with sexual reproduction and outcrossing, in which genetic backgrounds are continuously broken apart and reassembled, preventing genomes from acting as persistent evolutionary units across generations (figure 1, v ⟊ iii). Taken together, the increased genome-wide LD and stronger drift under obligate asexuality reduce the efficacy of all forms of natural selection.

Many organisms exhibit facultative sexuality, whereby they can reproduce both sexually and asexually (Kondrashov 1997; Hartfield 2016). For example, gall wasps of the family Cynipidae typically have one sexual generation and one asexual generation every year. *Daphnia* water fleas reproduce asexually when conditions are favorable, but switch to sexual reproduction when conditions deteriorate (Decaestecker et al. 2009). In facultative sexuals with high rates of asexual reproduction, LD and selective interference are expected to be elevated relative to obligate sexuals, but not to the same extent as under obligate asexuality. Facultative sexuality, if it is synchronous (i.e., if most individuals in the population reproduce in the same way at a given time (Kokko 2020)), generates temporal variation in recombination and N_e, producing cyclical shifts in LD and the efficacy of selection across the genome (Hill and Robertson 1966; Lynch and Gabriel 1983; Colegrave 2002). During asexual phases, persistent LD increases selective interference and hitchhiking, elevating the fixation probability of deleterious alleles. When sexual reproduction resumes, recombination reduces LD, restores selective independence among loci, and strengthens selection against

deleterious alleles. These alternating regimes generate threshold-dependent dynamics in which the fixation of deleterious alleles depends on recombination frequency, resulting in increased temporal and among-lineage variance relative to obligate sexual or asexual reproductive modes (Raynes and Sniegowski 2014). The extent to which synchronous and asynchronous facultative sexuality lead to similar evolutionary outcomes is unknown (Kokko 2020).

Self-fertilization (or selfing), whereby a single individual provides both gametes that form a zygote, is often facultative, with individuals capable of reproducing through both selfing and outcrossing, sometimes depending on environmental or demographic conditions (Hartfield 2016). Across generations, selfing increases homozygosity because offspring can inherit identical alleles from their unique parent at any given heterozygous locus. As a result, recessive deleterious alleles are more likely to be expressed in homozygous form, which can enhance the efficacy of selection but may also reduce fitness through inbreeding depression (Charlesworth and Willis 2009). Increasing homozygosity also reduces the effective recombination rate because, although meiosis and crossing over still occur, genetic exchange increasingly takes place between identical rather than polymorphic sites, generating few new allele combinations (Charlesworth and Wright 2001). As a result, LD is increased because loci behave as though they are tightly linked despite ongoing recombination (Glémin et al. 2006). Highly selfing populations therefore experience reduced effective recombination and elevated LD, leading to selective dynamics that may approach those of obligate asexuals, although the effects of obligate asexuality and complete LD remain much more extreme. These highly selfing populations may also exhibit reduced N_e, both due to fewer effective number of independent gametes, increased LD and frequent bottleneck events. For these reasons, genetic drift and LD are expected to be major forces influencing highly selfing populations, whereas populations with low selfing rates retain evolutionary dynamics closer to those of sexual outcrossing systems.

4 Theoretical predictions for mutation rate evolution across reproductive modes

The patterns outlined in section 3 suggest that reproductive modes should exert important influences on mutation rate evolution. In particular, differences in recombination, LD, and N_e imply that the strength and efficacy of selection on mutation-rate modifiers may vary across reproductive systems. Specifically, in low-recombination systems and highly self-fertilizing lineages, linkage will increase associations between modifier alleles and the mutations with which they co-occur. These associations will on the one hand strengthen indirect selection on mutators and antimutators, and on the other hand reduce selection efficiency due to selective interference and drift (Kimura 1967; Gervais and Roze 2017).

Theoretical models provide an important framework for predicting how these processes may influence mutation rate evolution. In particular, the drift-barrier hypothesis (DBH)—one of the most influential frameworks for understanding mutation rate evolution (Lynch 2010, 2011; Sung, Ackerman, et al. 2012; Lynch et al. 2016, 2023)—is grounded in the interaction between indirect selection and genetic drift. These factors, as discussed above, are also directly influenced by reproductive modes (figure 1–v), thereby establishing a direct connection between reproductive modes and the processes underlying the DBH.

Despite this conceptual overlap, current DBH formulations incorporate variation in reproductive mode in limited ways. In what follows, we first describe the DBH framework (section 4.1), then examine how reproductive modes are integrated into its current formulation (section 4.2), and identify the main challenges of further integration (section 4.4). We also review the predictions and comparative evidence for the DBH (section 4.3), and conclude with a discussion of transposition rate evolution and how it may be similarly shaped by reproductive modes (section 4.5).

4.1 The drift-barrier hypothesis

The DBH proposes that mutation rates evolve under the combined influences of indirect selection caused by deleterious mutations, genetic drift, and a mutation bias towards an increased mutation rate (Lynch 2010, 2011; Lynch et al. 2016). As described above, it is widely believed that there is a mutation bias towards higher mutation rates. In the absence of selection, this mutation bias would be expected to cause mutation rate to increase over time.

Because the vast majority of mutations affecting fitness are deleterious, indirect selection is expected to favor antimutator alleles that reduce mutation rate. As the mutation rate declines, the fitness benefits of further reducing the mutation rate diminish. Eventually, the selective advantage conferred by antimutators becomes so small that it is overwhelmed by the power of genetic drift and mutation bias, preventing further reduction in mutation rate and resulting in an equilibrium mutation rate. The mutation rate at which natural selection becomes ineffectual—known as the *drift barrier* (Lynch 2010, 2011; Lynch et al. 2016)—is determined by the effective size of the population, N_e. The DBH is expected to apply to all classes of mutations, including insertions and deletions of all sizes, gene duplications, mitochondrial and plastid mutations, and mutations caused by transposable elements, as long as their rates can be modulated through the effects of modifier alleles.

4.2 Integrating reproductive modes into the DBH framework

Because reproductive modes affect genome structure and evolution (section 3), they alter the forces described in section 2.2 and therefore also influence the framework of the DBH. To illustrate this, we first consider the general formula for the strength of indirect selection acting on a mutation-rate modifier allele under the DBH in a diploid organism (equation 4.1). We assume that the modifier allele itself does not have a direct effect on fitness. The selection coefficient of a modifier allele in a heterozygous state (s_m) depends on three factors. First, the change in genome-wide rate of deleterious mutations (ΔU_d) caused by the modifier relative to the population mean ($\Delta U_d > 0$ for a mutator; $\Delta U_d < 0$ for an antimutator). Second, the reduction in fitness caused by a single deleterious mutation in a heterozygous state ($s_d < 0$), that is, the strength of purifying selection. Third, the average number of generations ($\bar{t}$) the association between a new deleterious mutation and the modifier allele persists. Every generation, this association is destroyed by recombination at a rate r (the recombination frequency between the two loci) and by selection at a rate $|s_d|$. The persistence time can therefore be approximated as $\bar{t} \approx 1/(r - s_d + rs_d)$ (Kimura 1967; Lynch 2011). Together, these factors determine the strength of indirect selection acting on the modifier according to the following expression (Kimura 1967; Lynch 2008, 2011; Lynch et al. 2016):

$$s_m \approx \Delta U_d \, s_d \, \bar{t} \approx \frac{\Delta U_d \, s_d}{r - s_d + rs_d} \tag{4.1}$$

Equation 4.1 predicts that indirect selection will favor antimutator alleles ($s_m > 0$) and impede mutator alleles ($s_m < 0$). As the recombination rate r depends crucially on the reproductive mode of an organism, so does equation 4.1. For an obligately asexual organism with no recombination ($r = 0$) we have

$$s_m^{\text{asex}} \approx -\Delta U_d \tag{4.2}$$

Thus, in asexual lineages, the strength of indirect selection on a modifier depends solely on the number of deleterious mutations it introduces relative to the population mean and not on the fitness consequences (s_d) of those mutations. Conversely, for an obligate sexual organism, if we assume free recombination between fitness and modifier loci ($r \approx 0.5$), a new deleterious mutation will tend to disassociate from a mutation-rate modifier allele in just $\bar{t} \approx 2/(1 - s_d)$ generations so equation 4.1 becomes

$$s_m^{\text{sex}} = \frac{2\,\Delta U_d\, s_d}{1 - s_d} \tag{4.3}$$

Equations 4.2 and 4.3 show that free recombination reduces the strength of indirect selection on a modifier allele by a factor of $\sim 1/(2|s_d|)$ (e.g., a factor of 6.6 for $s_d = -0.07$; see figure 2).

According to equation 4.1, natural selection will favor antimutator alleles and cause mutation rates to decline. However, as the mutation rate declines, the ΔU_d associated with new antimutators must also decline in magnitude, which will in turn cause selection for antimutators to become weaker. The DBH notes that selection for an antimutator will only be effective (i.e., overcome genetic drift) if

$$s_m > \frac{1}{2N_e} \tag{4.4}$$

where N_e is the effective size of the population (Kimura 1983; Lynch 2011). Because the change in the deleterious mutation rate caused by an antimutator is limited by the current mutation rate of the organism ($-U_d < \Delta U_d < 0$), equation 4.4 defines an approximate drift barrier for the evolution of the mutation rate. The DBH predicts that, in the presence of a mutation bias towards higher mutation rate, the mutation rate will evolve to an equilibrium mutation rate determined by the effective population size, N_e (Lynch 2011). This equilibrium is stable: if the mutation rate rises above the equilibrium, indirect selection will bring it down; if it drops below the equilibrium, mutation bias and drift will push it back up.

4.3 Predictions of the DBH

The central prediction of the DBH is that there should be a negative correlation between mutation rate and N_e among organisms (Lynch 2010; Sung, Ackerman, et al. 2012; Lynch et al. 2016, 2023). In populations with high N_e, the drift barrier is lower because natural selection is more effective, and so mutation rates are expected to be lower. Conversely, in populations with low N_e the drift barrier is higher because genetic drift and mutation bias dominate, meaning that mutation rates are expected to be higher. Similar predictions can be made if, instead of (or in addition to) an upward mutation bias, there is a cost of fidelity (i.e., upward direct selection) (Lynch 2010).

This central prediction of a negative correlation between mutation rate and N_e appears to be well-supported across the tree of life (Lynch 2010; Sung, Ackerman, et al. 2012; Lynch et al. 2016, 2023; Krasovec et al. 2020; Bergeron et al. 2023; Wang and Obbard 2023). For example, a recent study estimated the base-substitution mutation rate, u, and the harmonic mean of N_e over the past 1 million years for 55 species of vertebrates and found a statistically significant negative correlation between the two variables (Bergeron et al. 2023). The correlation held after taking into account the phylogenetic nonindependence of the species (Bergeron et al. 2023). One problem with this comparative evidence for the DBH, however, is that both u and N_e are themselves correlated with other traits. In vertebrates, for example, u was strongly positively correlated with both generation time and age at sexual maturity and negatively correlated with the number of offspring per generation (Bergeron et al. 2023). Weinstein and Roy (Weinstein and Roy 2026) used phylogenetic path analysis to test if N_e has an independent effect on u after correcting for the fact that both N_e and u are correlated with multiple life history traits. They found support for a model where generation time independently influences both N_e and u, while N_e has no independent effect on u, contrary to the prediction of the DBH. These results highlight the need for more data in more systems and for evolution experiments to test the DBH and alternative hypotheses more directly.

Another prediction of the DBH is that, for species that can exist in different ploidy states, mutation rates should be lower in the ploidy state that occurs most frequently in a population because DNA replication and repair mechanisms employed most often are expected to experience stronger indirect selection (Melde et al. 2022). This prediction has

been verified in two species of yeast. Budding yeasts *S. cerevisiae* exist primarily as diploids and these show a lower u than haploids with the same genetic background (Sharp et al. 2018). By contrast, fission yeast *Schizosaccharomyces pombe* exist primarily as haploids, which show a lower u than diploids with the same genetic background (Bao et al. 2026).

The DBH also provides explicit predictions about how mutation-rate evolution should vary across different reproductive modes. If we assume that parameters N_e and s_d are identical across reproductive modes, equations 4.2–4.4 indicate that under the DBH, asexual populations are expected to evolve a lower mutation rate than sexual populations. Although selfing populations are not identical to obligate asexual lineages, we expect a similar pattern because their effective recombination rate remains lower than that of outcrossing sexual populations (Gervais and Roze 2017). However, as we argue in section 4.4(iv), the assumption that N_e and s_d are comparable across reproductive modes may not hold in natural populations because reproductive modes themselves influence these parameters. Whether the strength and efficiency of selection on mutation-rate modifier alleles is stronger or weaker in asexual and selfing populations than in sexual outcrossing populations will thus depend in important ways on both the strength of selection and the extent of selective interference.

The predictions of the DBH for different reproductive modes have not been subjected to systematic comparative tests. However, the prediction that asexuals should show lower mutation rates than sexuals appears to be contradicted by the finding that the lowest estimates of u in any organism were obtained from facultatively sexual species of ciliates—four species within the *Paramecium aurelia* species complex (Sung, Tucker, et al. 2012; Long et al. 2018) and *Tetrahymena thermophila* (Long et al. 2016)—even after taking into account the correlation between N_e and u.

4.4 Limitations of the current drift-barrier framework

Despite the effort that has been made to incorporate reproductive modes into the DBH (Lynch 2011; Lynch et al. 2016) and other models (Kimura 1967; Kondrashov 1995; Dawson 1998; Tenaillon et al. 2000; Gervais and Roze 2017) of the evolution of mutation rate, we still lack a comprehensive understanding of their evolutionary consequences for at least four reasons (see (i)–(iv) below).

(i) Reproductive modes take many forms

DBH predictions often oversimplify reproductive modes by considering only the two extremes—obligate asexuality or obligate sexuality. This perspective overlooks three important facts: 1) asexual reproduction can take many different forms, with different evolutionary genetic consequences (e.g., mitotic or meiotic parthenogenesis (Kondrashov 1997; Neiman et al. 2014)), 2) many organisms reproduce via facultative sexuality or facultative self-fertilization (Kondrashov 1997; Hartfield 2016), and 3) transitions between reproductive modes over evolutionary time can further shape mutation rate dynamics.

Intuitively, the evolutionary consequences of facultative sexuality should be intermediate between those of obligate sexual and asexual reproduction (Green and Noakes 1995). Lynch (Lynch 2011) incorporated this intuition into the DBH by arguing that facultative sexuals with a probability ϕ of being generated by sexual reproduction and a recombination rate r between loci determining mutation rate and fitness should evolve like obligate sexuals with a rescaled recombination rate of ϕr. However, rescaling r in an obligate sexual is not always an appropriate way to model facultative sex. Figure 2 shows the outcome of invasion analyses of antimutator mutations in populations experiencing different reproductive modes. These results confirm the DBH predictions in equations 4.2 and 4.3: obligate asexuals fix an antimutator allele with a probability 6.4-fold higher than obligate sexuals, which is not significantly different from the theoretical expectation of a 6.6-fold difference ($P_{\text{fix}} \approx 2s_m$ for either obligate mode of reproduction). The results also show that rescaling the recombination rate in an obligate sexual is not appropriate to model facultative sexuality in diploids (figure 2). A facultative sexual population where individuals are generated sexually every 100 generations ($\phi = 1\%$) shows a probability of fixation of an antimutator indistinguishable from an asexual population and 3-fold higher than an obligate sexual with rescaled r. This is because only $\sim 60\%$ of the effect of sexual reproduction is caused by crossover; the remaining 40% is caused by segregation (see rescaled r with $\phi = 0$ in figure 2). These results suggest another way by which facultative sexuality could enhance the ability of natural selection to reduce mutation rate: although the antimutator P_{fix} is identical for obligate asexuals and facultative sexuals with $\phi \lesssim 2\%$, in the former case the allele fixes in a heterozygous state whereas in the latter case it fixes in a homozygous state and, therefore, results in a larger reduction in the mutation rate. Similarly, highly selfing lineages share

certain features with asexuals (low effective recombination, high linkage), but their rapid loss of heterozygosity makes them fundamentally different from obligate sexuals and asexuals, suggesting that the evolutionary consequences of facultative selfing cannot be captured simply by rescaling the effective recombination rate.

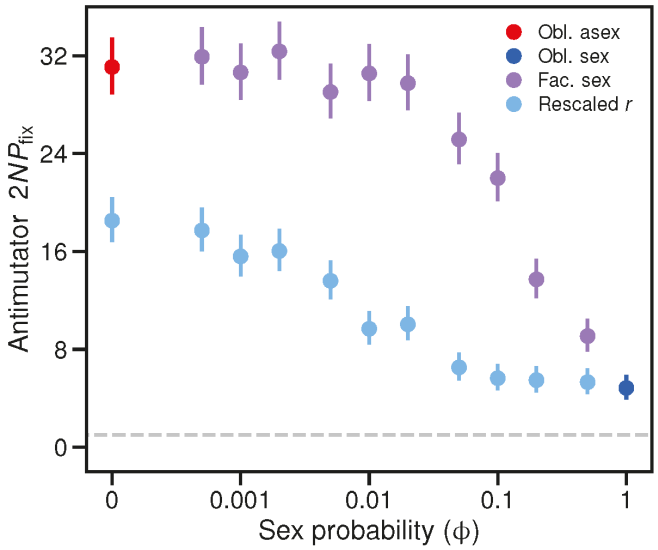

Figure 2. Facultative sexuality increases the probability of fixation (P_{fix}) of an antimutator allele relative to obligate sexuality with a rescaled recombination rate. Simulations were conducted on diploid populations of $N = 2000$ individuals. Fitness was determined by $L = 50$ loci; alleles acted additively within and multiplicatively among loci. Deleterious mutations occurred with a genomic rate of $U_d = 0.02$ per generation and caused a reduction in fitness of $s_d = -0.07$ in a heterozygote (medians of empirical estimates of both U_d and s_d from 24 studies on 14 species of DNA-based organisms (Olofsson et al. 2023)). The antimutator allele reduced U_d by $\Delta U_d = -0.005$ in a heterozygote and was codominant. No other mutator or antimutator mutations were allowed. Sex involved random mating and free recombination between adjacent loci ($r = 0.5$). Facultative sexuals reproduced sexually every $1/\phi$ generations. "Rescaled r" refers to obligate sexuality with $r = \phi/2$. Values are normalized fixation probabilities calculated based on 10^5 invasion simulations. Error bars are 95% confidence intervals. The dashed line shows the neutral expectation ($2NP_{fix} = 1$). See electronic supplementary material, Methods 2, for more details.

(ii) Extrapolating from short-term predictions may not work

The DBH predictions summarized in equations 4.2–4.4 are based on the short-term evolutionary consequences of the appearance of an antimutator allele. These predictions are then used to infer the long-term evolution of the mutation rate. This extrapolation can be problematic because the mutation rate is influenced by many loci (see section 2.1). Accordingly, short-term predictions can only capture long-term behaviour if these mutation rate loci evolve under a strong selection/weak mutation regime (SSWM) (Gillespie 1984) where most of the time a population contains a single mutation-rate genotype and new mutation-rate modifier alleles arise and fix independently of each other. The SSWM

assumption is questionable because indirect selection on antimutator alleles tends not to be strong (especially in sexuals, equation 4.3), and the rate of mutation towards antimutator alleles is not necessarily low (i.e., weak) (Liu and Zhang 2021).

If the SSWM assumption is not met for mutation-rate modifier loci, then there will be genetic variation at multiple modifier loci, reducing the ability of equations 4.2–4.4 to predict long-term evolution in ways that relate to reproductive mode. In asexual populations, mutator alleles are indirectly deleterious and, therefore, could experience Muller's ratchet (Muller 1964; Felsenstein 1974; Haigh 1978), which would drive long-term increases in the mutation rate. Similarly, indirectly beneficial antimutator alleles arising in different individuals would experience clonal interference (Fisher 1930; Muller 1932; Gerrish and Lenski 1998), blunting the ability of natural selection to reduce the mutation rate. Any amount of sexual reproduction, including selfing, reduces the intensity of these types of selective interference (Felsenstein 1974; Comeron et al. 2008) and, therefore, has the potential to cause long-term reductions in the mutation rate under the DBH framework.

(iii) Some mutations *are* beneficial

Existing DBH theory ignores the role of beneficial mutations in mutation rate evolution. Although beneficial mutations are relatively rare, mutator alleles can nevertheless spread by hitchhiking (Maynard Smith and Haigh 1974) with the beneficial mutations they help to generate in large asexual populations (Johnson 1999a; Tenaillon et al. 1999; André and Godelle 2006; Raynes et al. 2018), implying that equations 4.2 and 4.3 do not tell the whole story. Mutator hitchhiking could drive the evolution of higher mutation rates in asexual lineages than predicted under the DBH. Conversely, in sexual populations recombination continuously reshuffles alleles among genetic backgrounds, breaking up LD and weakening the hitchhiking effects that can strongly favor mutator alleles in asexual populations (Tenaillon et al. 2000).

(iv) Reproductive modes influence how selection operates on the mutation rate in complicated ways

Critically, reproductive modes influence three parameters (r, N_e, and s_d), all of which in turn affect mutation rate evolution under the DBH (equations 4.1–4.4). A further complication is that these parameters are treated as fixed in the theoretical models underpinning the DBH,

whereas changes in reproductive mode are expected to alter several of them simultaneously and in interdependent ways. For example, transitions from obligate sexuality to obligate asexuality are expected to increase both the rate and fitness effects of new mutations, U_d and s_d (Gardner and Kalinka 2006; Azevedo et al. 2006; Misevic et al. 2006; Whitlock et al. 2016). Background selection against deleterious mutations tends to reduce long-term N_e by a factor of $\exp(U_d/s_d)$ (Charlesworth 1994). Changes in reproductive mode can also modulate N_e in other ways that depend on demographic parameters and population structure (Orive 1993; Yonezawa 1997; Balloux et al. 2003; Yonezawa et al. 2004). The evolution of reproductive mode may also influence the evolution of mutation rate indirectly because reproductive mode often co-evolves with other traits. Relevant examples come from asexual reproduction, which is often associated with hybridization and polyploidy, (Bierzychudek 1985; Neiman et al. 2014), and which themselves might influence the evolution of mutation rate. By contrast, sexual selection, which can only operate in sexually reproducing organisms, may select for increased mutation rate because sexual selection allows the evolution of higher mean population fitness at relatively higher mutation rates (Møller and Cuervo 2003; Baur and Berger 2020; Roberts and Petrie 2022).

(v) Conclusion

Taken together, these considerations highlight that current attempts to integrate reproductive modes into the DBH framework provide only a partial view of their influence on mutation rate evolution. It is important to note that these limitations do not necessarily undermine the DBH as a conceptual framework, but instead underscore the need to expand it to incorporate more realistic features, including the effects of facultative sexuality and selfing, selective interference, departures from SSWM, beneficial mutations, sexual selection, and changes in key parameters N_e and s_d.

4.5 Alternative theoretical perspectives: transposition rate evolution

In complement to the DBH, other theoretical approaches have addressed mutation rate evolution from related but distinct perspectives. In particular, the dynamics of transposable elements offer an alternative angle through which to examine how reproductive modes may influence mutational processes.

As with the global mutation rate framework, theoretical models predict that lineages with high LD—such as asexual or highly selfing populations—should experience stronger indirect selection for reduced rates of transposition, mediated through both host genome silencing mechanisms and element self-regulation (Charlesworth and Langley 1986; Almeida et al. 2022). Furthermore, transposable elements may be likely to be in the SSWM regime (Charlesworth and Langley 1989), further leading to the expectation of reduced transposition in asexual and selfing lineages.

However, these expectations depend critically on the evolutionary timescale. Recently evolved asexual and selfing lineages are expected to inherit active transposable elements from their sexual or outcrossing ancestors, where transposition dynamics are shaped by recombination and transmission between individuals (Dolgin and Charlesworth 2006). Transitions in reproductive mode are therefore likely to disrupt this balance in multiple ways. In sexual organisms, recombination interacts closely with the transposition landscape, with transposable elements typically accumulating in regions of low recombination (Kent et al. 2017). A shift to reduced or absent recombination is thus expected to alter these dynamics, potentially leading to transient increases in transposition rates and transposable element copy number. At the same time, outcrossing allows transposable elements to spread between individuals, enabling their proliferation even at a cost to host fitness (Dolgin and Charlesworth 2006; Hickey 1982). Following a transition to asexuality, this between-host transmission is largely lost (apart from horizontal transfers), while the efficiency of purging deleterious insertions is reduced due to processes such as Muller's ratchet. As a result, inherited active elements may continue to transpose and accumulate within lineages, generating a rapid increase in deleterious insertions that can contribute to genome degradation and elevate extinction risk (another form of higher-level selection, section 2.2(vi)).

Consequently, many newly formed asexual lineages are likely to go extinct rapidly, whereas persisting lineages are expected to display reduced transposable element activity, with predominantly inactive or low-proliferation copies (Doolittle et al. 1984). Those asexual lineages that exceed a critical persistence timescale may therefore reflect lineages in which selection for reduced transposition has successfully taken hold, rather than the typical outcome of transitions to asexuality.

5 Empirical evidence

Despite strong theoretical expectations that reproductive modes should influence the evolution of the mutation rate (section 4), empirical evidence remains inconclusive. It is important to note that mutation rates can be estimated empirically in two fundamentally different ways: either *indirectly*, through comparative genomic divergence, or *directly*, through mutation accumulation or parent-offspring studies. These two classes of methods provide complementary but fundamentally different types of information, and they differ in their strengths and limitations for inferring the underlying mutation rate.

5.1 Indirect estimates of mutation rate

Many studies have relied on indirect genomic indicators to infer patterns related to mutation rate, particularly the rate of substitution at synonymous sites (d_S). If synonymous mutations are selectively neutral, d_S provides a direct estimate for the mutation rate to neutral alleles (Kimura and Ohta 1971). When comparing two lineages that differ in reproductive mode, d_S over each branch since the lineages split approximates the mutation rate associated with each reproductive mode. This can be done by either comparing the focal lineages to an equally distant outgroup or using a phylogeny-aware framework. Empirical studies using these approaches have had mixed outcomes, but broadly suggest that estimates of d_S from asexual or selfing lineages are similar to or higher than those from sexual outcrossing lineages (table 1). Indeed, all six comparisons yielding statistically significant differences support the same qualitative pattern: asexual or selfing lineages exhibit higher d_S values than their sexual outcrossing counterparts, contrary to the DBH predictions. However, the studies summarized in table 1 vary widely in their d_S estimates, the number of genes analyzed, and the lineages examined. While much more work is needed to draw firm conclusions, these data are consistent with important roles for selective interference, mutator hitchhiking, and/or reduced N_e on mutation rate evolution in selfing and asexual lineages.

Table 1. Values of d_S across closely related eukaryotes differing in reproductive mode. Numbers in parentheses in the species column indicate the number of different species or lineages studied, when applicable. The d_S values shown are typically medians or means (labeled "a" or "b", respectively, when known) calculated over many genes and/or many lineages. Pairs of values shown in bold are statistically significantly different ($P < 0.05$), whereas those shown in italics are not. The approach column shows whether d_S was computed using pairwise comparisons to an outgroup ("pairs") or direct comparisons within a phylogeny ("phyl"). All estimates were obtained using nuclear genes, except those for *Daphnia* which used mitochondrial genes (Paland and Lynch 2006). Methods for data generated in this table can be found in the electronic supplementary material, Methods 1.

group	species	reproductive mode	d_S	genes	approach	reference
aphids	*Rhopalosiphum maidis*	asexual	**0.035**[a]	255	phyl	Ollivier et al. 2012
	Rhopalosiphum padi	facultative sexual	**0.021**[a]			
	Acyrthosiphon kondoi	asexual	*0.041*[a]			
	Acyrthosiphon pisum	facultative sexual	*0.046*[a]			
ascomycetes	*Neurospora africana*	selfing	0.079	2 789	phyl	Gioti et al. 2013
	Neurospora crassa	outcrossing	0.178			
crustaceans	*Trichoniscus pusillus*	asexual*	**7.22**[a]	11 136	pairs	Yarbrough and Chandler 2024
	Hyloniscus riparius	sexual	**6.60**[a]			
	Daphnia pulex (9)	asexual	*0.0032*[a]	13	phyl	Paland and Lynch 2006
	Daphnia pulex (9)	sexual	*0.0043*[a]			
evening primroses	Onagraceae spp. (11)	asexual†	**0.0295**[a]	1	phyl	Hersch-Green et al. 2012
	Onagraceae spp. (11)	sexual	**0.0206**[a]			
mustards	*Arabidopsis thaliana*	selfing	**0.0805**[b]	12 994	pairs	Payne and Alvarez-Ponce 2018
	Arabidopsis lyrata	outcrossing	**0.0667**[b]			
	Capsella orientalis	selfing	**0.0340**[b]	17 335	phyl	this study
	Capsella grandiflora	outcrossing	**0.0325**[b]			
nematodes	*Caenorhabditis briggsae*	selfing	0.759[a]	7 846	phyl	Cutter et al. 2008
	Caenorhabditis remanei	outcrossing	0.635[a]			
rotifers	bdelloid spp. (3)	asexual	*1.12*[b]	1	pairs	Welch and Meselson 2001
	monogont spp. (4)	sexual	*1.37*[b]			
stick insects	*Timema* spp. (5)	asexual	**0.0016**[a‡]	5 329	phyl	Bast et al. 2018
	Timema spp. (5)	sexual				

* Triploid lineage.
† Permanent translocation heterozygosity (functionally asexual).
‡ $\Delta d_S = d_S^{\text{asex}} - d_S^{\text{sex}}$.

However, we should be careful in our interpretation of such data. The assumption that synonymous mutations evolve neutrally is frequently violated. Synonymous changes can influence many processes, including codon usage, mRNA stability, splicing, protein folding, and translational efficiency (Hershberg and Petrov 2008). Consequently, d_S may at least partially reflect the fixation of mutations under mutation–selection–drift balance rather than the underlying mutation rate. In addition, even if d_S is made up almost entirely of neutral

709 mutations, since it captures only a subset of mutational processes (e.g., excluding
710 fitness-affecting mutations), it may not be representative of the whole mutational profile of an
711 organism. Therefore, comparisons between species with distinct reproductive modes will
712 only be meaningful if the proportion of mutations captured by d_S out of all mutations has not
713 evolved differently in the species considered. Another important limitation for mutation rate
714 estimation via d_S concerns the relationship between divergence time and the number of
715 generations elapsed. Estimates of d_S implicitly assume that comparable lineages have
716 experienced similar numbers of generations since divergence, an assumption that is often
717 violated. Indeed, changes in reproductive mode are frequently associated with shifts in life-
718 history traits such as generation time. For example, the selfer *Arabidopsis thaliana* is an
719 annual species, whereas the outcrosser *A. lyrata* is a short-lived perennial, which may
720 contribute to some of the differences in substitution rates observed in table 1 without
721 invoking differences in reproductive mode. To explore this possible effect, we added a new
722 analysis of d_S comparing two annual species with contrasting mating systems, the self-
723 incompatible and outcrossing *Capsella grandiflora* and the highly selfing *C. orientalis*. This
724 species pair also demonstrates a trend towards elevated d_S in the selfing lineage, providing a
725 stronger line of evidence that selfing could indeed be involved. Finally, the timing of
726 transitions in reproductive mode does not necessarily coincide with species divergence. If
727 shifts in reproductive mode occur more recently than lineage splitting, comparisons based on
728 d_S may have limited power to detect mutation rate differences associated with reproductive
729 mode. Together, these considerations highlight that, while d_S provides a useful first-order
730 approximation of mutation rate, it remains subject to several important limitations.

731 In addition to substitution-based metrics such as d_S, patterns of transposable
732 element content and activity provide an alternative, genome-wide indicator of mutational
733 processes and have been extensively studied using a range of approaches and metrics
734 (Glémin et al. 2019). Comparisons of transposable element abundance and diversity can be
735 used to test the prediction that transposition rates differ among species with different
736 reproductive modes. However, it is important to note that transposable element profiles in a
737 genome reflect the combined effects of transposition, natural selection, and genetic drift. A
738 recent review (Glémin et al. 2019) reports a general tendency toward increased transposable
739 element diversity, expression, transposition rate, and abundance in sexual or outcrossing

organisms relative to asexual or selfing ones, although substantial variation exists across taxa and studies.

Taken together, these indirect approaches—ranging from substitution-based measures such as d_S to genome-wide signatures of transposable element dynamics—provide valuable but incomplete insights into mutation rate evolution. Although they represent the majority of available empirical data for comparisons across reproductive modes, their interpretation is complicated by the confounding effects of selection, demographic history, and life-history variation. As a result, while these approaches can reveal coarse trends, they cannot provide rigorous and unambiguous comparisons of underlying mutation rates.

5.2 Direct approaches to measuring mutation rate

While indirect metrics can be informative about differences in divergence across many generations, direct approaches are critical to isolate the mutation rate itself without confounding factors. Such estimates remain rare, particularly those based on comparisons of closely related lineages that differ in reproductive mode and are examined under comparable experimental conditions.

Even when such data are available, comparisons across studies are often complicated by methodological differences between the two main approaches used to estimate mutation rates. In mutation accumulation experiments, a number of initially genetically identical populations are allowed to evolve at low N_e (e.g., by subjecting populations to periodic single-individual bottlenecks or sib-mating) for multiple generations. The low N_e means that selection is ineffectual and that drift will result in fixation or loss of most mutations. Mutation accumulation experiments yield relatively stable estimates of mutation rate by averaging mutational input over many generations. However, this approach is only feasible in organisms with short generation times that can be readily cultured in the laboratory. By contrast, the parent–offspring approach estimates mutation rates within a single generation by sequencing parents and their offspring, and capturing most mutations except those subject to strong viability selection. This method can be applied across a much broader range of organisms, but it faces its own limitations: because each offspring carries only a small number of new mutations, even a single undetected sequencing error can distort

the estimate. This limitation necessitates the sequencing of large numbers of progeny to mitigate such effects (Lynch 2016). Thus, while mutation-accumulation and parent-offspring methods are the best-available strategies for estimating mutation rates, they are limited in how widely they can be applied across taxa and the extent to which estimates can be compared.

As summarized in table 2, only a handful of studies provide direct contrasts of mutation rate between related species with varying reproductive modes, and even these come with important confounding factors that must be considered. A good example comes from a comparison of facultatively sexual and obligately asexual genotypes of *Daphnia pulex*, in which the mutation rate of the latter was estimated to be approximately twice that of the former. At first glance, this pattern seems to suggest that the reduced N_e in asexual lineages overwhelms the expected increased indirect selection, but this would require further parameterization and modeling to assess further. A contrasting pattern is observed in *Caenorhabditis*, where the outcrossing species, *C. remanei*, has a mutation rate roughly twice that of two closely related selfing species, *C. elegans* and *C. briggsae*. Although this outcome aligns with the expected increase in indirect selection on mutation rate in selfing lineages, the comparison remains difficult to interpret because the outcrossing species also has a larger N_e than the selfers. Disentangling these effects would require explicit parameterization of the model to jointly account for differences in LD and N_e (Jovelin et al. 2003; Cutter, Félix, et al. 2006; Cutter, Baird, et al. 2006; Cutter 2006; Teterina et al. 2023). Finally, the comparison between asexual *Propanagrolaimus* sp. and sexual but selfing *Panagrolaimus* sp. is difficult to interpret because the species differ in ploidy level and, therefore, may differ in the strength of selection on deleterious mutations, s_d.

Table 2. Base-substitution mutation rates ($\times 10^{-9}$ per site per generation) in closely related lineages with different reproductive modes. All estimates were obtained through mutation accumulation (MA) experiments. Strain names are in parentheses.

species	MA lines	reproductive mode	mutation rate	reference
Daphnia pulex	4	asexual	10.18	Keith et al. 2016
	3	facultative sexual	4.33	
Caenorhabditis remanei (SP8)	6	outcrossing	2.98	Chen et al. 2023
C. briggsae (HK104)	7	selfing	1.23	Denver et al. 2012
C. briggsae (PB800)	6	selfing	1.44	
C. elegans (N2)	7	selfing	1.33	
C. elegans (PB306)	5	selfing	1.62	
Panagrolaimus sp.	6	asexual	0.58	Villegas et al. 2024
Propanagrolaimus sp.	6	selfing	0.89	

5.3 Current empirical limitations

Altogether, existing empirical comparisons suggest that reproductive modes may alter mutation rates, but direct estimates remain few in number and often lack the statistical power needed for rigorous comparisons. Both direct and indirect estimates are often confounded by differences in N_e, ploidy, genomic composition, and generation time, making it difficult to isolate the contribution of reproductive mode *per se*. Most of these studies also rely on single pairwise contrasts between lineages, which limits broader interpretation. Across-study variation in the methodologies used as well as in data filtering and quality-control threshold may introduce additional noise (Bergeron et al. 2022; Manuel et al. 2026). Moreover, most approaches and studies focus on only a subset of mutational processes. Consequently, estimates obtained using any single method may not fully capture the overall mutational profile of a lineage, and integrating evidence across multiple approaches may therefore provide a more complete picture. Therefore, despite these important case studies, we still lack an empirical foundation for assessing how reproductive modes and reproductive mode transitions influence mutation rate evolution across organisms.

6 Conclusions

Explaining the extensive variation in mutation rates across the tree of life remains a central goal of evolutionary biology. While the drift-barrier hypothesis provides a powerful framework linking mutation rate evolution to genetic drift and indirect selection, we here make the case that reproductive modes substantially modulate the evolutionary forces on mutation-rate modifiers in ways that are not fully captured by existing theory. Differences among obligate

sexuals, asexuals, facultative sexuals, and self-fertilizing lineages alter effective population size, recombination, and linkage, with complex consequences for both short- and long-term mutation-rate evolution.

Despite strong theoretical expectations, further work is needed to better understand the parameter space governing selection on mutation rates in asexual and selfing lineages. In addition, empirical support for these predictions remains limited. Much of the available evidence relies on indirect genomic proxies that conflate mutation rate with the effects of selection and demography, while direct estimates remain scarce, taxonomically restricted, and difficult to compare across studies. As a result, our ability to rigorously test theoretical predictions—particularly for intermediate and dynamic reproductive modes—remains severely constrained. A key challenge for the field is therefore to expand direct, genome-wide estimates of mutation rate across a broader diversity of taxa and reproductive strategies, ideally within standardized, replicated frameworks. Such data are essential for distinguishing the effects of reproductive mode from those of effective population size, life history, and genome architecture, and for rigorously testing and refining theoretical predictions of mutation-rate evolution.

7 Statements

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data Accessibility. Supplementary material is available online.

Declaration of AI use. We made limited use of AI-assisted technologies to improve the readability and grammar of our work.

Authors' Contributions.
J.L.: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration,
Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing;
S.S.: Conceptualization, Funding Acquisition, Validation, Writing – original draft, Writing – review & editing;
M.N.: Conceptualization, Funding Acquisition, Supervision, Validation, Writing – original draft, Writing – review & editing;
R.A.Z. Conceptualization, Funding Acquisition, Supervision, Validation, Writing – original draft, Writing – review & editing;
S.I.W.: Conceptualization, Funding Acquisition, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing;
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8 References

Almeida, Miguel Vasconcelos, Grégoire Vernaz, Audrey L. K. Putman, and Eric A. Miska. 2022. "Taming Transposable Elements in Vertebrates: From Epigenetic Silencing to Domestication." *Tr. Genet.* 38 (6): 529–53. https://doi.org/10.1016/j.tig.2022.02.009.

André, Jean-Baptiste, and Bernard Godelle. 2006. "The Evolution of Mutation Rate in Finite Asexual Populations." *Genetics* 172 (1): 611–26. https://doi.org/10.1534/genetics.105.046680.

Azevedo, Ricardo B. R., Rolf Lohaus, Suraj Srinivasan, Kristen K. Dang, and Christina L. Burch. 2006. "Sexual Reproduction Selects for Robustness and Negative Epistasis in Artificial Gene Networks." *Nature* 440 (7080): 87–90. https://doi.org/10.1038/nature04488.

Balloux, François, Laurent Lehmann, and Thierry de Meeûs. 2003. "The Population Genetics of Clonal and Partially Clonal Diploids." *Genetics* 164 (4): 1635–44. https://doi.org/10.1093/genetics/164.4.1635.

Bao, Kevin, Rutuja Gupte, Neil Braker, and Nathaniel P Sharp. 2026. "Contrasting Mutation Patterns in Haploid and Diploid Cells from Two Yeast Species." *Genetics* 232 (3): iyaf282. https://doi.org/10.1093/genetics/iyaf282.

Barrett, Spencer C. H., Robert I. Colautti, and Christopher G. Eckert. 2007. "Plant Reproductive Systems and Evolution During Biological Invasion." *Mol. Ecol.* 17 (1): 373–83. https://doi.org/10.1111/j.1365-294x.2007.03503.x.

Barrick, Jeffrey E, Dong Su Yu, Sung Ho Yoon, et al. 2009. "Genome Evolution and Adaptation in a Long-Term Experiment with *Escherichia Coli*." *Nature* 461 (7268): 1243–47. https://doi.org/10.1038/nature08480.

Bast, Jens, Darren J Parker, Zoé Dumas, et al. 2018. "Consequences of Asexuality in Natural Populations: Insights from Stick Insects." *Mol. Biol. Evol.* 35 (7): 1668–77. https://doi.org/10.1093/molbev/msy058.

Baur, Julian, and David Berger. 2020. "Experimental Evidence for Effects of Sexual Selection on Condition-Dependent Mutation Rates." *Nat. Ecol. Evol.* 4 (5): 737–44. https://doi.org/10.1038/s41559-020-1140-7.

Bell, Graham. 1982. *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. University of California Press.

Bergeron, Lucie A., Søren Besenbacher, Jiao Zheng, et al. 2023. "Evolution of the Germline Mutation Rate Across Vertebrates." *Nature* 615 (7951): 285–91. https://doi.org/10.1038/s41586-023-05752-y.

Bergeron, Lucie A, Søren Besenbacher, Tychele Turner, et al. 2022. "The Mutationathon Highlights the Importance of Reaching Standardization in Estimates of Pedigree-Based Germline Mutation Rates." *eLife* 11 (January): e73577. https://doi.org/10.7554/eLife.73577.

925 Bielas, Jason H., Keith R. Loeb, Brian P. Rubin, Lawrence D. True, and Lawrence A. Loeb. 2006.
926 “Human Cancers Express a Mutator Phenotype.” *Proc. Natl. Acad. Sci. U. S. A.* 103 (48):
927 18238–42. https://doi.org/10.1073/pnas.0607057103.

928 Bierzychudek, P. 1985. “Patterns in Plant Parthenogenesis.” *Experientia* 41 (10): 1255–64.
929 https://doi.org/10.1007/BF01952068.

930 Bull, J. J., R. Sanjuán, and C. O. Wilke. 2007. “Theory of Lethal Mutagenesis for Viruses.” *J.*
931 *Virol.* 81 (6): 2930–39. https://doi.org/10.1128/JVI.01624-06.

932 Chao, Lin, and Edward C. Cox. 1983. “Competition Between High and Low Mutating Strains of
933 *Escherichia Coli*.” *Evolution* 37 (1): 125–34. https://doi.org/10.2307/2408181.

934 Chao, L, C Vargas, B B Spear, and E C Cox. 1983. “Transposable Elements as Mutator Genes
935 in Evolution.” *Nature* 303 (5918): 633–35. https://doi.org/10.1038/303633a0.

936 Charlesworth, B. 1994. “The Effect of Background Selection Against Deleterious Mutations on
937 Weakly Selected, Linked Variants.” *Genet. Res.* 63 (3): 213–27.
938 https://doi.org/10.1017/S0016672300032365.

939 Charlesworth, B. 1990. “Mutation-Selection Balance and the Evolutionary Advantage of Sex
940 and Recombination.” *Genet. Res.* 55 (3): 199–221.
941 https://doi.org/10.1017/S0016672300025532.

942 Charlesworth, B., and C. H. Langley. 1986. “The Evolution of Self-Regulated Transposition of
943 Transposable Elements.” *Genetics* 112 (2): 359–83.
944 https://doi.org/10.1093/genetics/112.2.359.

945 Charlesworth, Brian. 2012. “The Effects of Deleterious Mutations on Evolution at Linked Sites.”
946 *Genetics* 190 (1): 5–22. https://doi.org/10.1534/genetics.111.134288.

947 Charlesworth, Brian, and Charles H. Langley. 1989. “The Population Genetics of *Drosophila*
948 Transposable Elements.” *Annu. Rev. Genet.* 23: 251–87.
949 https://doi.org/10.1146/annurev.ge.23.120189.001343.

950 Charlesworth, Deborah, and John H. Willis. 2009. “The Genetics of Inbreeding Depression.”
951 *Nat. Rev. Genet.* 10 (11): 783–96. https://doi.org/10.1038/nrg2664.

952 Charlesworth, Deborah, and Stephen I Wright. 2001. “Breeding Systems and Genome
953 Evolution.” *Curr. Opin. Genet. Dev.* 11 (6): 685–90. https://doi.org/10.1016/S0959-
954 437X(00)00254-9.

955 Chen, Hwei-yen, Therese Krieg, Brian Mautz, et al. 2023. “Germline Mutation Rate Is Elevated
956 in Young and Old Parents in *Caenorhabditis Remanei*.” *Evol. Lett.* 7 (6): 478–89.
957 https://doi.org/10.1093/evlett/qrad052.

958 Chen, Lian, and John J Wiens. 2021. “Multicellularity and Sex Helped Shape the Tree of Life.”
959 *Proc. R. Soc. B* 288 (1955): 20211265. https://doi.org/10.1098/rspb.2021.1265.

960 Colegrave, Nick. 2002. “Sex Releases the Speed Limit on Evolution.” *Nature* 420 (6916): 664–
961 66. https://doi.org/10.1038/nature01191.

962 Comeron, J M, A Williford, and R M Kliman. 2008. “The Hill-Robertson Effect: Evolutionary
963 Consequences of Weak Selection and Linkage in Finite Populations.” *Heredity* 100 (1): 19–31.
964 https://doi.org/10.1038/sj.hdy.6801059.

965 Cutter, A. D. 2006. “Nucleotide Polymorphism and Linkage Disequilibrium in Wild
966 Populations of the Partial Selfer *Caenorhabditis Elegans*.” *Genetics* 172 (1): 171–84.
967 https://doi.org/10.1534/genetics.105.048207.

968 Cutter, Asher D, Scott E Baird, and Deborah Charlesworth. 2006. “High Nucleotide
969 Polymorphism and Rapid Decay of Linkage Disequilibrium in Wild Populations of
970 *Caenorhabditis Remanei*.” *Genetics* 174 (2): 901–13.
971 https://doi.org/10.1534/genetics.106.061879.

972 Cutter, Asher D, M.-A. Félix, Antoine Barrière, and Deborah Charlesworth. 2006. “Patterns of
973 Nucleotide Polymorphism Distinguish Temperate and Tropical Wild Isolates of
974 *Caenorhabditis Briggsae*.” *Genetics* 173 (4): 2021–31.
975 https://doi.org/10.1534/genetics.106.058651.

976 Cutter, Asher D, James D Wasmuth, and Nicole L Washington. 2008. “Patterns of Molecular
977 Evolution in *Caenorhabditis* Preclude Ancient Origins of Selfing.” *Genetics* 178 (4): 2093–104.
978 https://doi.org/10.1534/genetics.107.085787.

979 Dawson, Kevin J. 1998. “Evolutionarily Stable Mutation Rates.” *J. Theor. Biol.* 194 (1): 143–57.
980 https://doi.org/10.1006/jtbi.1998.0752.

981 Decaestecker, Ellen, Luc De Meester, and Joachim Mergeay. 2009. “Cyclical Parthenogenesis
982 in *Daphnia*: Sexual Versus Asexual Reproduction.” In *Lost Sex: The Evolutionary Biology of*
983 *Parthenogenesis*, edited by Isa Schön, Koen Martens, and Peter Dijk. Springer.
984 https://doi.org/10.1007/978-90-481-2770-2_15.

985 Denver, Dee R., Larry J. Wilhelm, Dana K. Howe, Kristin Gafner, Peter C. Dolan, and Charles F.
986 Baer. 2012. “Variation in Base-Substitution Mutation in Experimental and Natural Lineages of
987 *Caenorhabditis* Nematodes.” *Genome Biol. Evol.* 4 (4): 513–22.
988 https://doi.org/10.1093/gbe/evs028.

989 Desai, Michael M., and Daniel S. Fisher. 2011. “The Balance Between Mutators and
990 Nonmutators in Asexual Populations.” *Genetics* 188 (4): 997–1014.
991 https://doi.org/10.1534/genetics.111.128116.

992 Dolgin, E. S., and B. Charlesworth. 2006. “The Fate of Transposable Elements in Asexual
993 Populations.” *Genetics* 174 (2): 817–27. https://doi.org/10.1534/genetics.106.060434.

994 Doolittle, W. F., T. B. L. Kirkwood, and M. A. H. Dempster. 1984. “Selfish DNAs with Self-
995 Restraint.” *Nature* 307 (5951): 501–2. https://doi.org/10.1038/307501b0.

996 Drake, John W., Elizabeth F. Allen, Susan A. Forsberg, Rosa-Maria Preparata, and Elaine O.
997 Greening. 1969. “Spontaneous Mutation: Genetic Control of Mutation Rates in Bacteriophage
998 T4.” *Nature* 221 (5186): 1128–32. https://doi.org/10.1038/2211128a0.

Dubin, Manu J, Pei Zhang, Dazhe Meng, et al. 2015. “DNA Methylation in *Arabidopsis* Has a Genetic Basis and Shows Evidence of Local Adaptation.” *eLife* 4 (May): e05255. https://doi.org/10.7554/eLife.05255.

Ewens, W. J. 2004. *Mathematical Population Genetics*. 2nd ed. Vol. 27. Springer.

Felsenstein, J. 1974. “The Evolutionary Advantage of Recombination.” *Genetics* 78 (2): 737–56. https://doi.org/10.1093/genetics/78.2.737.

Fijalkowska, Iwona J., Roel M. Schaaper, and Piotr Jonczyk. 2012. “DNA Replication Fidelity in *Escherichia Coli*: A Multi-DNA Polymerase Affair.” *FEMS Microbiol. Rev.* 36 (6): 1105–21. https://doi.org/10.1111/j.1574-6976.2012.00338.x.

Fisher, Ronald Aylmer. 1930. *The Genetical Theory of Natural Selection*. Clarendon Press.

Fitzsimmons, William J, Robert J Woods, John T McCrone, et al. 2018. “A Speed-Fidelity Trade-Off Determines the Mutation Rate and Virulence of an RNA Virus.” *PLoS Biol.* 16 (6): e2006459. https://doi.org/10.1371/journal.pbio.2006459.

Friedberg, Errol C, Graham C Walker, Wolfram Siede, Richard D. Wood, Roger A. Schultz, and Tom Ellenberger. 2006. *DNA Repair and Mutagenesis*. 2nd ed. ASM Press.

Furió, Victoria, Andrés Moya, and Rafael Sanjuán. 2005. “The Cost of Replication Fidelity in an RNA Virus.” *Proc. Natl. Acad. Sci. U. S. A.* 102 (29): 10233–37. https://doi.org/10.1073/pnas.0501062102.

Furió, Victoria, Andrés Moya, and Rafael Sanjuán. 2006. “The Cost of Replication Fidelity in Human Immunodeficiency Virus Type 1.” *Proc. R. Soc. B* 274 (1607): 225–30. https://doi.org/10.1098/rspb.2006.3732.

Gabriel, W., M. Lynch, and R. Bürger. 1993. “Muller’s Ratchet and Mutational Meltdowns.” *Evolution* 47 (6): 1744–57. https://doi.org/10.1111/j.1558-5646.1993.tb01266.x.

Gardner, A., and A. T. Kalinka. 2006. “Recombination and the Evolution of Mutational Robustness.” *J. Theor. Biol.* 241 (4): 707–15. https://doi.org/10.1016/j.jtbi.2006.01.011.

Gerrish, P J, and R E Lenski. 1998. “The Fate of Competing Beneficial Mutations in an Asexual Population.” *Genetica* 102-103: 127–44. https://doi.org/10.1023/A:1017067816551.

Gerrish, Philip J., Alexandre Colato, Alan S. Perelson, and Paul D. Sniegowski. 2007. “Complete Genetic Linkage Can Subvert Natural Selection.” *Proc. Natl. Acad. Sci. U. S. A.* 104 (15): 6266–71. https://doi.org/10.1073/pnas.0607280104.

Gerrish, Philip J., Alexandre Colato, and Paul D. Sniegowski. 2013. “Genomic Mutation Rates That Neutralize Adaptive Evolution and Natural Selection.” *J. R. Soc. Interface* 10 (85): 20130329. https://doi.org/10.1098/rsif.2013.0329.

Gervais, Camille, and Denis Roze. 2017. “Mutation Rate Evolution in Partially Selfing and Partially Asexual Organisms.” *Genetics* 207 (4): 1561–75. https://doi.org/10.1534/genetics.117.300346.

1035 Gifford, Danna R, Ernesto Berríos-Caro, Christine Joerres, et al. 2023. “Mutators Can Drive
1036 the Evolution of Multi-Resistance to Antibiotics.” *PLoS Genet.* 19 (6): e1010791.
1037 https://doi.org/10.1371/journal.pgen.1010791.

1038 Gillespie, J H. 1984. “Molecular Evolution over the Mutational Landscape.” *Evolution* 38:
1039 1116–29. https://doi.org/10.1111/j.1558-5646.1984.tb00380.x.

1040 Gioti, Anastasia, Jason E. Stajich, and Hanna Johannesson. 2013. “*Neurospora* and the Dead-
1041 End Hypothesis: Genomic Consequences of Selfing in the Model Genus.” *Evolution* 67 (12):
1042 3600–3616. https://doi.org/10.1111/evo.12206.

1043 Giraud, A, I Matic, O Tenaillon, et al. 2001. “Costs and Benefits of High Mutation Rates:
1044 Adaptive Evolution of Bacteria in the Mouse Gut.” *Science* 291 (5513): 2606–8.
1045 https://doi.org/10.1126/science.1056421.

1046 Glémin, Sylvain, Eric Bazin, and Deborah Charlesworth. 2006. “Impact of Mating Systems on
1047 Patterns of Sequence Polymorphism in Flowering Plants.” *Proc. R. Soc. B* 273 (1604): 3011–
1048 19. https://doi.org/10.1098/rspb.2006.3657.

1049 Glémin, Sylvain, Clémentine M. François, and Nicolas Galtier. 2019. “Genome Evolution in
1050 Outcrossing Vs. Selfing Vs. Asexual Species.” In *Evolutionary Genomics: Statistical and*
1051 *Computational Methods*, edited by Maria Anisimova. Methods in Molecular Biology. Springer
1052 New York. https://doi.org/10.1007/978-1-4939-9074-0_11.

1053 Green, Richard F., and David L. G. Noakes. 1995. “Is a Little Bit of Sex as Good as a Lot?” *J.*
1054 *Theor. Biol.* 174 (1): 87–96. https://doi.org/https://doi.org/10.1006/jtbi.1995.0081.

1055 Haigh, J. 1978. “The Accumulation of Deleterious Genes in a Population—Muller’s Ratchet.”
1056 *Theor. Popul. Biol.* 14: 251–67. https://doi.org/10.1016/0040-5809(78)90027-8.

1057 Hartfield, M. 2016. “Evolutionary Genetic Consequences of Facultative Sex and Outcrossing.”
1058 *J. Evol. Biol.* 29 (1): 5–22. https://doi.org/10.1111/jeb.12770.

1059 Herr, Alan J, Lindsey N Williams, and Bradley D Preston. 2011. “Antimutator Variants of DNA
1060 Polymerases.” *Crit. Rev. Biochem. Mol. Biol.* 46 (6): 548–70.
1061 https://doi.org/10.3109/10409238.2011.620941.

1062 Hersch-Green, E. I., H. Myburg, and M. T. J. Johnson. 2012. “Adaptive Molecular Evolution of a
1063 Defence Gene in Sexual but Not Functionally Asexual Evening Primroses.” *J. Evol. Biol.* 25 (8):
1064 1576–86. https://doi.org/10.1111/j.1420-9101.2012.02542.x.

1065 Hershberg, Ruth, and Dmitri A Petrov. 2008. “Selection on Codon Bias.” *Annu. Rev. Genet.* 42:
1066 287–99. https://doi.org/10.1146/annurev.genet.42.110807.091442.

1067 Hickey, Donal A. 1982. “Selfish DNA: A Sexually-Transmitted Nuclear Parasite.” *Genetics* 101
1068 (3-4): 519–31. https://doi.org/10.1093/genetics/101.3-4.519.

1069 Hill, W. G., and A. Robertson. 1966. “The Effect of Linkage on Limits to Artificial Selection.”
1070 *Genet. Res.* 8: 269–94. https://doi.org/10.1017/S0016672300010156.

Jablonski, D. 2008. “Species Selection: Theory and Data.” *Annu. Rev. Ecol. Evol. Syst.* 39: 501–24. https://doi.org/10.1146/annurev.ecolsys.39.110707.173510.

Jain, Kavita, and Apoorva Nagar. 2013. “Fixation of Mutators in Asexual Populations: The Role of Genetic Drift and Epistasis.” *Evolution* 67 (4): 1143–54. https://doi.org/10.1111/evo.12005.

Johnson, Toby. 1999a. “Beneficial Mutations, Hitchhiking and the Evolution of Mutation Rates in Sexual Populations.” *Genetics* 151 (4): 1621–31. https://doi.org/10.1093/genetics/151.4.1621.

Johnson, Toby. 1999b. “The Approach to Mutation–Selection Balance in an Infinite Asexual Population, and the Evolution of Mutation Rates.” *Proc. R. Soc. B* 266 (1436): 2389–97. https://doi.org/10.1098/rspb.1999.0936.

Jovelin, R, B C Ajie, and P C Phillips. 2003. “Molecular Evolution and Quantitative Variation for Chemosensory Behaviour in the Nematode Genus *Caenorhabditis*.” *Mol. Ecol.* 12 (5): 1325–37. https://doi.org/10.1046/j.1365-294X.2003.01805.x.

Keith, Nathan, Abraham E Tucker, Craig E Jackson, et al. 2016. “High Mutational Rates of Large-Scale Duplication and Deletion in *Daphnia Pulex*.” *Genome Res* 26 (1): 60–69. https://doi.org/10.1101/gr.191338.115.

Kent, Tyler V., Jasmina Uzunović, and Stephen I. Wright. 2017. “Coevolution Between Transposable Elements and Recombination.” *Phil. Trans. R. Soc. B* 372 (1736): 20160458. https://doi.org/10.1098/rstb.2016.0458.

Kimura, Motoo. 1967. “On the Evolutionary Adjustment of Spontaneous Mutation Rates.” *Genet. Res.* 9: 23–34. https://doi.org/10.1017/S0016672300010284.

Kimura, Motoo. 1983. *The Neutral Theory of Molecular Evolution*. Cambridge University Press.

Kimura, Motoo, and Tomoko Ohta. 1971a. “On the Rate of Molecular Evolution.” *J. Mol. Evol.* 1 (1): 1–17. https://doi.org/10.1007/BF01659390.

Kimura, Motoo, and Tomoko Ohta. 1971b. “Protein Polymorphism as a Phase of Molecular Evolution.” *Nature* 229 (5285): 467–69. https://doi.org/10.1038/229467a0.

Kokko, Hanna. 2020. “When Synchrony Makes the Best of Both Worlds Even Better: How Well Do We Really Understand Facultative Sex?” *Am. Nat.* 195 (2): 380–92. https://doi.org/10.1086/706812.

Kondrashov, A. S. 1988. “Deleterious Mutations and the Evolution of Sexual Reproduction.” *Nature* 336 (6198): 435–40. https://doi.org/10.1038/336435a0.

Kondrashov, A. S. 1997. “Evolutionary Genetics of Life Cycles.” *Annu. Rev. Ecol. Syst.* 28: 391–435. https://doi.org/10.1146/annurev.ecolsys.28.1.391.

Kondrashov, Alexey S. 1995. “Modifiers of Mutation-Selection Balance: General Approach and the Evolution of Mutation Rates.” *Genet. Res.* 66 (01): 53–69. https://doi.org/10.1017/S001667230003439X.

1107 Kong, Augustine, Michael L Frigge, Gisli Masson, et al. 2012. “Rate of *de Novo* Mutations and
1108 the Importance of Father’s Age to Disease Risk.” *Nature* 488 (7412): 471–75.
1109 https://doi.org/10.1038/nature11396.

1110 Krasovec, Marc, Rosalind E M Rickaby, and Dmitry A Filatov. 2020. “Evolution of Mutation
1111 Rate in Astronomically Large Phytoplankton Populations.” *Genome Biol. Evol.* 12 (7): 1051–59.
1112 https://doi.org/10.1093/gbe/evaa131.

1113 Kunz, Bernard A., Karthikeyan Ramachandran, and Edward J. Vonarx. 1998. “DNA Sequence
1114 Analysis of Spontaneous Mutagenesis in *Saccharomyces Cerevisiae*.” *Genetics* 148 (4):
1115 1491–505. https://doi.org/10.1093/genetics/148.4.1491.

1116 Lande, Russell. 1975. “The Maintenance of Genetic Variability by Mutation in a Polygenic
1117 Character with Linked Loci.” *Genet. Res.* 26 (3): 221–35.
1118 https://doi.org/10.1017/S0016672300016037.

1119 Leigh, Egbert Giles, Jr. 1970. “Natural Selection and Mutability.” *Am. Nat.* 104 (937): 301–5.
1120 https://doi.org/10.1086/282663.

1121 Leigh, Egbert Giles, Jr. 1973. “The Evolution of Mutation Rates.” *Genetics* 73 (Suppl.) (April):
1122 1–18.

1123 Lindsay, Sarah J., Raheleh Rahbari, Joanna Kaplanis, Thomas Keane, and Matthew E. Hurles.
1124 2019. “Similarities and Differences in Patterns of Germline Mutation Between Mice and
1125 Humans.” *Nat. Commun.* 10 (1): 4053. https://doi.org/10.1038/s41467-019-12023-w.

1126 Liu, Haoxuan, and Jianzhi Zhang. 2021. “The Rate and Molecular Spectrum of Mutation Are
1127 Selectively Maintained in Yeast.” *Nat. Commun.* 12 (1): 4044.
1128 https://doi.org/10.1038/s41467-021-24364-6.

1129 Loeb, Lawrence A., Clark F. Springgate, and Narayana Battula. 1974. “Errors in DNA
1130 Replication as a Basis of Malignant Changes.” *Cancer Res.* 34 (9): 2311–21.

1131 Loh, Ern, Jesse J. Salk, and Lawrence A. Loeb. 2010. “Optimization of DNA Polymerase
1132 Mutation Rates During Bacterial Evolution.” *Proc. Natl. Acad. Sci. U. S. A.* 107 (3): 1154–59.
1133 https://doi.org/10.1073/pnas.0912451107.

1134 Long, Hongan, Thomas G Doak, and Michael Lynch. 2018. “Limited Mutation-Rate Variation
1135 Within the *Paramecium Aurelia* Species Complex.” *G3* 8 (7): 2523–26.
1136 https://doi.org/10.1534/g3.118.200420.

1137 Long, Hongan, David J. Winter, Allan Y.-C. Chang, et al. 2016. “Low Base-Substitution
1138 Mutation Rate in the Germline Genome of the Ciliate *Tetrahymena Thermophila*.” *Genome*
1139 *Biol. Evol.* 8 (12): 3629–39. https://doi.org/10.1093/gbe/evw223.

1140 Lynch, Michael. 2008. “The Cellular, Developmental and Population-Genetic Determinants of
1141 Mutation-Rate Evolution.” *Genetics* 180 (2): 933–43.
1142 https://doi.org/10.1534/genetics.108.090456.

Lynch, Michael. 2010. “Evolution of the Mutation Rate.” *Tr. Genet.* 26 (8): 345–52. https://doi.org/10.1016/j.tig.2010.05.003.

Lynch, Michael. 2011. “The Lower Bound to the Evolution of Mutation Rates.” *Genome Biol. Evol.* 3 (January): 1107–18. https://doi.org/10.1093/gbe/evr066.

Lynch, Michael. 2016. “Mutation and Human Exceptionalism: Our Future Genetic Load.” *Genetics* 202 (3): 869–75. https://doi.org/10.1534/genetics.115.180471.

Lynch, Michael, Matthew S. Ackerman, Jean-Francois Gout, et al. 2016. “Genetic Drift, Selection and the Evolution of the Mutation Rate.” *Nat. Rev. Genet.* 17 (11): 704–14. https://doi.org/10.1038/nrg.2016.104.

Lynch, Michael, Farhan Ali, Tongtong Lin, Yaohai Wang, Jiahao Ni, and Hongan Long. 2023. “The Divergence of Mutation Rates and Spectra Across the Tree of Life.” *EMBO Rep.* 24 (10): e57561. https://doi.org/10.15252/embr.202357561.

Lynch, Michael, and Wilfried Gabriel. 1983. “Phenotypic Evolution and Parthenogenesis.” *Am. Nat.* 122 (6): 745–64. https://doi.org/10.1086/284169.

Manuel, Marc de, Molly Przeworski, Natanael Spisak, and Anastasia Stolyarova. 2026. “What Sets the Mutation Rate of a Cell Type in an Animal Species?” 10.1371/journal.pbio.3003799. *PLoS Biol.* 24 (6): e3003799. https://doi.org/10.1371/journal.pbio.3003799.

Mao, E. F., L. Lane, J. Lee, and J. H. Miller. 1997. “Proliferation of Mutators in a Cell Population.” *J. Bacteriol.* 179 (2): 417–22. https://doi.org/10.1128/jb.179.2.417-422.1997.

Maynard Smith, John, and John Haigh. 1974. “The Hitch-Hiking Effect of a Favourable Gene.” *Genet. Res.* 23: 23–35. https://doi.org/10.1017/S0016672308009579.

Melde, Robert H, Kevin Bao, and Nathaniel P Sharp. 2022. “Recent Insights into the Evolution of Mutation Rates in Yeast.” *Curr. Opin. Genet. Dev.* 76: 101953. https://doi.org/10.1016/j.gde.2022.101953.

Miller, Jeffrey H. 1996. “Spontaneous Mutators in Bacteria: Insights into Pathways of Mutagenesis and Repair.” *Annu. Rev. Microbiol.* 50 (1): 625–43. https://doi.org/10.1146/annurev.micro.50.1.625.

Misevic, D., C. Ofria, and R. E. Lenski. 2006. “Sexual Reproduction Reshapes the Genetic Architecture of Digital Organisms.” *Proc. R. Soc. B* 273 (1585): 457–64. https://doi.org/10.1098/rspb.2005.3338.

Møller, AP, and JJ Cuervo. 2003. “Sexual Selection, Germline Mutation Rate and Sperm Competition.” *BMC Evol. Biol.* 3 (1): 6. https://doi.org/10.1186/1471-2148-3-6.

Muller, H. J. 1932. “Some Genetic Aspects of Sex.” *Am. Nat.* 66 (703): 118–38. https://doi.org/10.1086/280418.

Muller, H. J. 1964. “The Relation of Recombination to Mutational Advance.” *Mutat. Res.* 1 (1): 2–9. https://doi.org/10.1016/0027-5107(64)90047-8.

1179 Neiman, M., T. F. Sharbel, and T. Schwander. 2014. “Genetic Causes of Transitions from
1180 Sexual Reproduction to Asexuality in Plants and Animals.” *J. Evol. Biol.* 27 (7): 1346–59.
1181 https://doi.org/10.1111/jeb.12357.

1182 Ness, Rob W., Andrew D. Morgan, Radhakrishnan B. Vasanthakrishnan, Nick Colegrave, and
1183 Peter D. Keightley. 2015. “Extensive de Novo Mutation Rate Variation Between Individuals and
1184 Across the Genome of *Chlamydomonas Reinhardtii*.” *Genome Res.* 25 (11): 1739–49.
1185 https://doi.org/10.1101/gr.191494.115.

1186 Nordborg, M, B Charlesworth, and D Charlesworth. 1996. “The Effect of Recombination on
1187 Background Selection.” *Genet. Res.* 67 (2): 159–74.
1188 https://doi.org/10.1017/S0016672300033619.

1189 Ollivier, M., T. Gabaldón, J. Poulain, et al. 2012. “Comparison of Gene Repertoires and
1190 Patterns of Evolutionary Rates in Eight Aphid Species That Differ by Reproductive Mode.”
1191 *Genome Biol. Evol.* 4 (2): 155–67. https://doi.org/10.1093/gbe/evr140.

1192 Olofsson, Peter, Logan Chipkin, Ryan C Daileda, and Ricardo B R Azevedo. 2023. “Mutational
1193 Meltdown in Asexual Populations Doomed to Extinction.” *J. Math. Biol.* 87 (6): 88.
1194 https://doi.org/10.1007/s00285-023-02019-y.

1195 Orive, M. E. 1993. “Effective Population Size in Organisms with Complex Life-Histories.”
1196 *Theor. Popul. Biol.* 44 (3): 316–40. https://doi.org/10.1006/tpbi.1993.1031.

1197 Paland, Susanne, and Michael Lynch. 2006. “Transitions to Asexuality Result in Excess Amino
1198 Acid Substitutions.” *Science* 311 (5763): 990–92. https://doi.org/10.1126/science.1118152.

1199 Payne, Bryan L, and David Alvarez-Ponce. 2018. “Higher Rates of Protein Evolution in the Self-
1200 Fertilizing Plant *Arabidopsis Thaliana* Than in the Out-Crossers *Arabidopsis Lyrata* and
1201 *Arabidopsis Halleri*.” *Genome Biol. Evol.* 10 (3): 895–900. https://doi.org/10.1093/gbe/evy053.

1202 Raynes, Yevgeniy, C. Scott Wylie, Paul D. Sniegowski, and Daniel M. Weinreich. 2018. “Sign of
1203 Selection on Mutation Rate Modifiers Depends on Population Size.” *Proc. Natl. Acad. Sci. U.*
1204 *S. A.* 115 (13): 3422–27. https://doi.org/10.1073/pnas.1715996115.

1205 Raynes, Y, and P D Sniegowski. 2014. “Experimental Evolution and the Dynamics of Genomic
1206 Mutation Rate Modifiers.” *Heredity* 113 (5): 375–80. https://doi.org/10.1038/hdy.2014.49.

1207 Roberts, Gilbert, and Marion Petrie. 2022. “Sexual Selection for Males with Beneficial
1208 Mutations.” *Sci. Rep.* 12 (1): 12613. https://doi.org/10.1038/s41598-022-16002-y.

1209 Sasaki, Joanna AND Reichardt-Gomez, Eriko AND Gunis. 2022. “Conditional GWAS of Non-
1210 CG Transposon Methylation in *Arabidopsis Thaliana* Reveals Major Polymorphisms in Five
1211 Genes.” *PLoS Genet.* 18 (9): 1–24. https://doi.org/10.1371/journal.pgen.1010345.

1212 Schaaper, Roel M. 1998. “Antimutator Mutants in Bacteriophage T4 and *Escherichia Coli*.”
1213 *Genetics* 148 (4): 1579–85. https://doi.org/10.1093/genetics/148.4.1579.

1214 Sharp, Nathaniel P., Linnea Sandell, Christopher G. James, and Sarah P. Otto. 2018. “The
1215 Genome-Wide Rate and Spectrum of Spontaneous Mutations Differ Between Haploid and

Diploid Yeast." *Proc. Natl. Acad. Sci. U. S. A.* 115 (22): E5046–55. https://doi.org/10.1073/pnas.1801040115.

Sloan, D. B., and V. G. Panjeti. 2010. "Evolutionary Feedbacks Between Reproductive Mode and Mutation Rate Exacerbate the Paradox of Sex." *Evolution* 64: 1129–35. https://doi.org/10.1111/j.1558-5646.2009.00869.x.

Sniegowski, Paul D., Philip J. Gerrish, Toby Johnson, and Aaron Shaver. 2000. "The Evolution of Mutation Rates: Separating Causes from Consequences." *BioEssays* 22 (12): 1057–66. https://doi.org/10.1002/1521-1878(200012)22:12<1057::AID-BIES3>3.0.CO;2-W.

Sturtevant, A. H. 1937. "Essays on Evolution. I. On the Effects of Selection on Mutation Rate." *Q. Rev. Biol.* 12 (4): 464–67. https://doi.org/10.1086/394543.

Sung, Way, Matthew S. Ackerman, Samuel F. Miller, Thomas G. Doak, and Michael Lynch. 2012. "Drift-Barrier Hypothesis and Mutation-Rate Evolution." *Proc. Natl. Acad. Sci. U. S. A.* 109 (45): 18488–92. https://doi.org/10.1073/pnas.1216223109.

Sung, Way, Abraham E. Tucker, Thomas G. Doak, Eunjin Choi, W. Kelley Thomas, and Michael Lynch. 2012. "Extraordinary Genome Stability in the Ciliate *Paramecium Tetraurelia*." *Proc. Natl. Acad. Sci. U. S. A.* 109 (47): 19339–44. https://doi.org/10.1073/pnas.1210663109.

Taddei, F., M. Radman, J. Maynard Smith, B. Toupance, P. H. Gouyon, and B. Godelle. 1997. "Role of Mutator Alleles in Adaptive Evolution." *Nature* 387 (6634): 700–702. https://doi.org/10.1038/42696.

Tenaillon, Olivier, Hervé Le Nagard, Bernard Godelle, and François Taddei. 2000. "Mutators and Sex in Bacteria: Conflict Between Adaptive Strategies." *Proc. Natl. Acad. Sci. U. S. A.* 97 (19): 10465–70. https://doi.org/10.1073/pnas.180063397.

Tenaillon, O, B Toupance, H Le Nagard, F Taddei, and B Godelle. 1999. "Mutators, Population Size, Adaptive Landscape and the Adaptation of Asexual Populations of Bacteria." *Genetics* 152 (2): 485–93. https://doi.org/10.1093/genetics/152.2.485.

Teterina, Anastasia A, John H Willis, Matt Lukac, Richard Jovelin, Asher D Cutter, and Patrick C Phillips. 2023. "Genomic Diversity Landscapes in Outcrossing and Selfing *Caenorhabditis* Nematodes." *PLoS Genet.* 19 (8): e1010879. https://doi.org/10.1371/journal.pgen.1010879.

Villegas, Laura I, Luca Ferretti, Thomas Wiehe, Ann-Marie Waldvogel, and Philipp H Schiffer. 2024. "Parthenogenomics: Insights on Mutation Rates and Nucleotide Diversity in Parthenogenetic *Panagrolaimus* Nematodes." *Ecol. Evol.* 14 (1): e10831. https://doi.org/10.1002/ece3.10831.

Wang, Yiguan, and Darren J Obbard. 2023. "Experimental Estimates of Germline Mutation Rate in Eukaryotes: A Phylogenetic Meta-Analysis." *Evol. Lett.* 7 (4): 216–26. https://doi.org/10.1093/evlett/qrad027.

Weinstein, Brooke, and Scott William Roy. 2026. "Controlling for Life-History Traits in Vertebrates Reveals That Effective Population Size Does Not Affect Mutation Rate or Genome

Size." *Proc. Natl. Acad. Sci. U. S. A.* 123 (6): e2519649123. https://doi.org/10.1073/pnas.2519649123.

Welch, David B. Mark, and Matthew S. Meselson. 2001. "Rates of Nucleotide Substitution in Sexual and Anciently Asexual Rotifers." *Proc. Natl. Acad. Sci. U. S. A.* 98 (12): 6720–24. https://doi.org/10.1073/pnas.111144598.

Whitlock, Alexander O. B., Kayla M. Peck, Ricardo B. R. Azevedo, and Christina L. Burch. 2016. "An Evolving Genetic Architecture Interacts with Hill–Robertson Interference to Determine the Benefit of Sex." *Genetics* 203 (2): 923–36. https://doi.org/10.1534/genetics.116.186916.

Wielgoss, Sébastien, Jeffrey E. Barrick, Olivier Tenaillon, et al. 2013. "Mutation Rate Dynamics in a Bacterial Population Reflect Tension Between Adaptation and Genetic Load." *Proc. Natl. Acad. Sci. U. S. A.* 110 (1): 222–27. https://doi.org/10.1073/pnas.1219574110.

Wiser, Michael J., Noah Ribeck, and Richard E. Lenski. 2013. "Long-Term Dynamics of Adaptation in Asexual Populations." *Science* 342 (6164): 1364–67. https://doi.org/10.1126/science.1243357.

Wright, Sewall. 1931. "Evolution in Mendelian Populations." *Genetics* 16 (2): 97–159. https://doi.org/10.1093/genetics/16.2.97.

Yang, Sihai, Long Wang, Ju Huang, et al. 2015. "Parent-Progeny Sequencing Indicates Higher Mutation Rates in Heterozygotes." *Nature* 523 (7561): 463–67. https://doi.org/10.1038/nature14649.

Yarbrough, Emily, and Christopher Chandler. 2024. "Patterns of Molecular Evolution in a Parthenogenic Terrestrial Isopod (*Trichoniscus Pusillus*)." *PeerJ* 12: e17780. https://doi.org/10.7717/peerj.17780.

Yin, Zhangyuan, Xu Liu, Aileen Ariosa, et al. 2019. "Psp2, a Novel Regulator of Autophagy That Promotes Autophagy-Related Protein Translation." *Cell Res.* 29 (12): 994–1008. https://doi.org/10.1038/s41422-019-0246-4.

Yonezawa, Katsuei. 1997. "Effective Population Size of Plant Species Propagating with a Mixed Sexual and Asexual Reproduction System." *Genet. Res.* 70 (3): 251–58. https://doi.org/10.1017/S0016672397002991.

Yonezawa, Katsuei, Takuro Ishii, and Tsukasa Nagamine. 2004. "The Effective Size of Mixed Sexually and Asexually Reproducing Populations." *Genetics* 166 (3): 1529–39. https://doi.org/10.1534/genetics.166.3.1529.

Zeyl, Clifford, Melissa Mizesko, and J. Arjan G. M. De Visser. 2001. "Mutational Meltdown in Laboratory Yeast Populations." *Evolution* 55 (5): 909–17. https://doi.org/10.1111/j.0014-3820.2001.tb00608.x.

Reproductive mode and the evolution of mutation rate: Electronic supplementary material

Method 1. Computing mean d_S for the *Capsella* species pair

Data sources

We retrieved annotation (GFF), coding DNA sequence (CDS), and protein FASTA datasets for *Arabidopsis thaliana*, *Capsella grandiflora*, and *C. orientalis* from publicly available resources. Specifically, we obtained *C. orientalis* data from ENA (PRJEB80551) (Kasianova et al. 2025), *C. grandiflora* from Phytozome (v1.1) (Slotte et al. 2013), and *A. thaliana* from the NCBI genome database (GCA_978657495.1).

Isoform filtering and sequence preprocessing

We filtered CDS and protein files to retain only the longest isoform per gene using the AGAT toolkit (agat_sp_keep_longest_isoform.pl) (Dainat 2026). We applied this approach to the GFF annotation file of each species to remove redundant transcript variants. From the filtered annotations, we extracted identifiers corresponding to retained coding sequences and used these curated identifier sets to filter both CDS and protein FASTA files with SeqKit v2.13.0 (Shen et al. 2016). This procedure ensured that only sequences corresponding to the longest isoforms were retained for downstream analyses.

Ortholog identification and sequence alignment

We inferred orthologous groups using OrthoFinder v3.1.4 (Emms et al. 2026). From the resulting orthogroups, we extracted single copy orthologs present in all three species (1:1:1 orthologs). We then generated species-specific lists of orthologous gene identifiers and extracted the corresponding protein and CDS sequences using seqkit.

We indexed all filtered CDS and protein files using SAMtools faidx v1.23.1 (Li et al. 2009), and performed pairwise and codon-aware alignments using a custom pipeline (Pairwise_align.sh). For each single-copy ortholog, this pipeline constructs individual protein and CDS datasets by extracting exactly one sequence per species. It then validates the presence of all required sequences prior to alignment and excludes ortholog groups with missing data. The pipeline finally aligns protein sequences for each ortholog independently using MAFFT v7.526 (Katoh et al. 2002; Katoh and Standley 2013). For each alignment, it ensures that exactly three sequences (one per species) are present. It finally generates codon-aware nucleotide alignments using PAL2NAL v14 (Suyama et al. 2006).

Phylogenetic reconstruction

We converted codon alignments into PHYLIP format using a custom Python script (paml_to_phylip.py). We standardized species names across all alignment files to consistent labels. We concatenated all codon alignments into a single file using AMAS concat and inferred a

species phylogeny with IQ-TREE v3.1.1 (Wong et al. 2026) under a codon substitution model with automatic model selection. This analysis yielded the following distances:

(*A. thaliana*: 0.4095855361, (*C. grandiflora*: 0.0442844570, *C. orientalis*: 0.0464908079))

Estimation of synonymous substitution rates

We estimated synonymous substitution rates (d_S) using codeml from the PAML package v4.10.10 (Yang 2007). We used the phylogenetic tree inferred by IQ-TREE, with a topology reflecting the close relationship between *C. grandiflora* and *C. orientalis* as shown above. For each codon alignment, we generated a control file from a template by specifying the alignment file, tree file, and output path. We ran codeml independently for each ortholog and stored the resulting output files. We used model = 1 in our codeml.ctl to allow each branch in the phylogenetic tree to have its own independent ω.

To minimize the effects of saturation, we filtered the data by removing d_S values below 0 and above 2. All analyses were performed in R version 4.5.2 (R Core Team 2024) and RStudio 2026.01.0 (Posit team 2025). We then calculated the mean d_S across the filtered values for each species and assessed statistical significance using a paired Wilcoxon signed-rank test.

Method 2. Invasion analyses

Genome

Individuals are diploid and have a single chromosome containing L fitness loci and 1 randomly located mutation-rate modifier locus. All loci are equally spaced.

Life-cycle

Stochastic, individual-based simulations were conducted within a Wright-Fisher framework (Ewens 2004). Individuals are diploid and undergo a mutation–selection–reproduction life cycle. Populations have a constant size N.

Mutation

Following reproduction, each offspring individual may acquire a new mutation at a fitness locus with probability U_d/L where U_d is the deleterious mutation rate. All mutations are irreversible and deleterious with the same effect, $s_d < 0$. The mutation-rate modifier locus does not mutate. An individual can acquire multiple mutations, but only one per locus.

Fitness

A genotype with i deleterious mutations in a homozygous state and j deleterious mutations in a heterozygous state has fitness:

$$w_{i,j} = (1 + 2s_d)^i (1 + s_d)^j \quad (1)$$

i.e., deleterious alleles acted additively within and multiplicatively among loci.

Mode of reproduction

Obligate asexuality

In obligate asexual populations, N individuals are sampled at random with replacement with probability proportional to their fitness and allowed to reproduce. Each offspring is an exact copy of the parent before mutation is allowed to operate. The parents are discarded after reproduction.

Obligate sexuality

In obligate asexuals, $2N$ individuals are chosen to reproduce at random with replacement with probability proportional to their fitness. We then create N pairs of individuals from this set at random without replacement (i.e., there is random mating). Each individual undergoes meiosis and generates one haploid gamete which fuses with a gamete from its paired individual. During meiosis, recombination events occur with probability r between adjacent loci. The parents are discarded after reproduction.

Facultative sexuality

In facultative sexuals, the entire population reproduces sexually every $1/\phi$ generations (i.e., synchronously), where ϕ is the probability of reproducing sexually; otherwise the population reproduces asexually.

Invasion simulations

Initially, all individuals are mutation-free at the fitness loci and have equal fitness, $w = 1$ (equation (1)). $N - 1$ individuals are homozygous for a wild type allele at the mutation-rate modifier locus and have a deleterious mutation rate U_d; one individual is heterozygous for an antimutator allele at the mutation-rate modifier locus and has a deleterious mutation rate of $U_d + \Delta U_d$ $(\Delta U_d < 0)$. A homozygote for the antimutator allele will have a deleterious mutation rate of $U_d + 2\Delta U_d$ (i.e., the antimutator allele is co-dominant). Thus, the following condition must be met: $-U_d < 2\Delta U_d < 0$.

Populations were allowed to evolve until the antimutator allele either fixed or was eliminated from the population. In obligate asexuals, fixation occurred in a heterozygous state, whereas in obligate or facultative sexuals fixation occurred in a homozygous state.

Parameters

In all simulations we used: population size, $N = 2000$ individuals; $L = 50$ fitness loci; wildtype deleterious mutation rate, $U_d = 0.02$ per generation; change in the deleterious mutation rate caused by an antimutator allele in a heterozygous state, $\Delta U_d = -0.005$; deleterious effect of a mutation in a heterozygous state, $s_d = -0.07$.

Software

Simulations were conducted using software written in Python 3.12 and NumPy 1.26 (Harris et al. 2020).

References

Dainat, J. 2026. “AGAT: Another Gff Analysis Toolkit to Handle Annotations in Any GTF/GFF Format (Version V1.7.0).” *Zenodo*, ahead of print. https://doi.org/10.5281/zenodo.3552717.

Emms, David M, Yi Liu, Laurence Belcher, Jonathan Holmes, and Steven Kelly. 2026. “OrthoFinder: Improved Phylogenetic Orthology Inference with Enhanced Accuracy and Scalability.” *Nat. Methods* 23 (7): 1327–33. https://doi.org/10.1038/s41592-026-03126-6.

Ewens, W. J. 2004. *Mathematical Population Genetics*. 2nd ed. Vol. 27. Springer.

Harris, Charles R., K. Jarrod Millman, Stéfan J. van der Walt, et al. 2020. “Array Programming with NumPy.” *Nature* 585 (7825): 357–62. https://doi.org/10.1038/s41586-020-2649-2.

Kasianova, Alexandra M, Vladislav D Mityukov, Dmitry A German, Artem S Kasianov, Aleksey A Penin, and Maria D Logacheva. 2025. “Chromosome-Scale Assembly of *Capsella Orientalis*, Maternal Progenitor of Cosmopolitan Allotetraploid *c. Bursa-Pastoris*.” *Genome Biol. Evol.* 17 (1): evaf009. https://doi.org/10.1093/gbe/evaf009.

Katoh, Kazutaka, Kazuharu Misawa, Kei-ichi Kuma, and Takashi Miyata. 2002. “MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform.” *Nucl. Acids Res.* 30 (14): 3059–66. https://doi.org/10.1093/nar/gkf436.

Katoh, Kazutaka, and Daron M Standley. 2013. “MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability.” *Mol. Biol. Evol.* 30 (4): 772–80. https://doi.org/10.1093/molbev/mst010.

Li, Heng, Bob Handsaker, Alec Wysoker, et al. 2009. “The Sequence Alignment/Map Format and SAMtools.” *Bioinformatics* 25 (16): 2078–79. https://doi.org/10.1093/bioinformatics/btp352.

Posit team. 2025. *RStudio: Integrated Development Environment for R*. Posit Software, PBC. http://www.posit.co/.

R Core Team. 2024. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/.

Shen, Wei, Shuai Le, Yan Li, and Fuquan Hu. 2016. “SeqKit: A Cross-Platform and Ultrafast Toolkit for FASTA/Q File Manipulation.” *PLoS ONE* 11 (10): e0163962. https://doi.org/10.1371/journal.pone.0163962.

Slotte, Tanja, Khaled M Hazzouri, J Arvid Ågren, et al. 2013. “The *Capsella Rubella* Genome and the Genomic Consequences of Rapid Mating System Evolution.” *Nat. Genet.* 45 (7): 831–35. https://doi.org/10.1038/ng.2669.

Suyama, Mikita, David Torrents, and Peer Bork. 2006. “PAL2NAL: Robust Conversion of Protein Sequence Alignments into the Corresponding Codon Alignments.” *Nucl. Acids Res.* 34 (July): W609–12. https://doi.org/10.1093/nar/gkl315.

Wong, Thomas K F, Nhan Ly-Trong, Huaiyan Ren, et al. 2026. “IQ-TREE 3: Phylogenomic Inference Software Using Complex Evolutionary Models.” *Mol. Biol. Evol.* 43 (5): msag117. https://doi.org/10.1093/molbev/msag117.

Yang, Z. 2007. “PAML 4: Phylogenetic Analysis by Maximum Likelihood.” *Mol. Biol. Evol.* 24 (8): 1586–91. https://doi.org/10.1093/molbev/msm088.