High levels of mitotic gene conversion are needed to effectively purge deleterious mutations in asexual organisms

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Abstract

Self-fertilisation and asexual reproduction are both hypothesised to lead to long-term extinction due to inefficient selection acting against deleterious mutations. Self-fertilisation can counter these effects through creating homozygous genotypes and purging deleterious mutations. Although complete asexuality lacks meiotic gene exchange, mitotic gene conversion is a mechanism for creating homozygous regions that could limit deleterious mutation accumulation in a similar manner to self-fertilisation. Here, we compare mutation accumulation in self-fertilising and facultative sexual populations subject to mitotic gene conversion, and quantify the relative efficacy of purging caused by the latter. We first show analytically that this purging is most effective with high levels of asexuality and gene conversion, and when deleterious mutations are recessive. We further show using simulations that, when mitotic gene conversion becomes sufficiently high in obligate asexuals, there is a sudden reduction in the mutation count and a jump in homozygosity, reflecting mutation purging. However, this mechanism is not necessarily as efficient at purging compared to high levels of self-fertilisation, and elevated rates of mitotic gene conversion seem to be needed compared to empirical estimates. Hence, while mitotic gene conversion can remove recessive deleterious mutations, self-fertilisation seems to be a more effective mechanism for doing so.

Teaser Text

Asexuality has been argued to be an evolutionary ‘dead end’, due to a lack of gene exchange causing inefficient selection acting against deleterious mutations. It has been proposed that asexuals can counter these negative effects through mitotic gene conversion, which exposes mutations to selection within individual lineages. Here, we theoretically investigate how effective this mechanism is. We compare results to those obtained when individuals reproduce by self-fertilisation, which has similar effects on exposing deleterious variants. While mitotic gene conversion can be effective in removing recessive deleterious mutations, high rates are required and it is not always as effective as selfing.

Introduction

Mutations are the ultimate source of genetic novelty in all organisms and are essential for biological evolution (Loewe and Hill 2010). However, the generation of variation is a double-edged sword; although favourable mutations may emerge that are crucial for long-term survival, it may also result in a burden of deleterious mutations, which are much more common than favourable ones (Eyre-Walker and Keightley 2007).

The reproductive system, which determines how offspring are generated in a population, has a profound impact on the prevalence of deleterious variation. Reproduction can either be with or without sex. Sex usually involves two phenomena (Hartfield and Keightley 2012): recombination (the exchange of genetic information between homologous DNA sequences, most commonly as meiotic crossing-over), and fusion of gametes (specialised cells with halved ploidy and DNA content). In the simplest case when asexuality is equivalent to clonal reproduction, the genotype of an offspring is identical to that of a parent, except for *de novo* mutations. Clonal reproduction encompasses diverse mechanisms, including fission or budding in unicellular eukaryotes, or budding (e.g., hydra, jellyfishes), fragmentation (e.g., corals) and whole-body fission (e.g., flatworms, starfish) in animals, or many forms of vegetative propagation in plants (De Meeûs et al. 2007). Nevertheless, most eukaryotic organisms undergo some variant of sexual reproduction, at least occasionally (Speijer et al. 2015). Facultatively sexual organisms reproduce via a mixture of sexual and asexual reproduction, so that genetic evolution is affected by the frequency of both recombination and clonal reproduction (Hartfield 2016).

Gametes from which offspring originate may also be derived from the same individual, leading to self-fertilisation or selfing. The opposite of selfing is panmictic outcrossing, with anyone in the population having an equal chance of being a mate of anyone else, with self-mating being rare or even prohibited (if, e.g., self-incompatibility mechanisms are present; Nettancourt 2001). Cloning and selfing are two types of uniparental reproduction, which forms a basis of their similarity (e.g., the lack of mate finding) and potential ecological advantages

(e.g., reproductive assurance; Baker 1955, 1967; Theologidis et al. 2014; Mráz and Mrázová 2021).

Despite these apparent similarities, cloning and selfing differ in the manner in which they affect selection acting against deleterious mutations. Muller's ratchet operates in asexual populations leading to stochastic mutation accumulation, due to their inability to generate offspring that have fewer deleterious alleles than those of their ancestors (Muller 1964; Haigh 1978). This mechanism is a form of selection interference, which refers to the effect of strong genetic linkage on the dynamics of variants under selection (Felsenstein 1974). In completely clonal populations all sites are in absolute genetic linkage, therefore the presence of multiple deleterious alleles selectively interferes with one another having a strong impact on mutation accumulation (Hill and Robertson 1966; Roze and Barton 2006; Hartfield and Keightley 2012). Recombination breaks this linkage and allows the dynamics of variants to respond to selection independently, i.e., more efficient removal of deleterious mutations and restoration of the most-fit genotype. Another important consequence due to the lack of recombination and gamete fusion in diploid asexuals is the divergence of homologous chromosomes found within one individual. The two genome copies are always transmitted together across the lineage, gradually and independently accumulating mutations leading to a high level of heterozygosity; a process known as 'allelic sequence divergence' or the Meselson effect (Mark Welch and Meselson 2000; Butlin 2002; Hartfield 2016).

In contrast to cloning, the main genetic consequence of selfing is genome homogenization. Under complete selfing, parents with heterozygous genotypes *Aa* have a 50% probability of losing heterozygosity, independent of the allele frequencies in the rest of the population. The probability that heterozygosity is retained decreases exponentially every generation, rapidly leading to overall genome homogenisation. This homogenisation also occurs, albeit at a lower rate, under partial selfing (Wright 1951). As a consequence, the effective recombination rate is reduced since recombination creates variability only when heterozygosity is present (Nordborg et al. 1996; Nordborg 2000). An additional consequence of increased homozygosity is the exposure of recessive deleterious alleles to selection, giving

rise to inbreeding depression (Charlesworth and Willis 2009). While this leads to an instant reduction in the fitness of selfers, the subsequent purging and removal of deleterious mutations from the population could eventually lead to elevated long-term fitness and persistence of the selfing population (Glémin 2003; Abu Awad and Billiard 2017). An outcome of selfing broadly shared with cloning is the removal of neutral variation through extensive linked selection acting against deleterious mutations, manifesting itself as a reduction in effective population size (Agrawal and Hartfield 2016).

Both reproductive systems have been called 'dead-end' strategies, meaning that species using them have an increased extinction probability. In asexual and selfing populations, this phenomenon has been often ascribed to mutational meltdown, i.e., the accumulation of deleterious mutations leading to extinction (Lynch et al. 1993, 1995; Olofsson et al. 2023), but other mechanisms have been proposed for the phylogenetically short-lived appearance of asexual species (Engelstädter 2008; Janko et al. 2008; Schwander and Crespi 2009). While the 'dead-end' hypothesis has also been invoked for selfing species (Wright et al. 2013), they can increase their fitness over time through purging deleterious mutations (Glémin 2003; Abu Awad and Billiard 2017), and this purging could also stop Muller's ratchet from operating (Charlesworth et al. 1993). Furthermore, there exist ancient asexuals that have seemingly existed for long periods of time in the absence of sexual reproduction, including species of bdelloid rotifers (Flot et al. 2013), oribatid mites (Brandt et al. 2021), ostracods (Tran Van et al. 2021), and trypanosomes (Weir et al. 2016). The persistence of these ancient asexuals begs the question of whether there are underexplored mechanisms at play that prevent these species from undergoing excessive accumulation of deleterious variants, similar to what occurs in self-fertilising species.

Although genome exchange is an intrinsic part of sexual reproduction, gene conversion is a mechanism of genome exchange that is also available to asexuals in the form of mitotic recombination (Lee et al. 2009). Gene conversion results in homozygous tracts, akin to the effect of selfing, and it has been shown to have an important impact on neutral diversity when sex is rare (Hartfield et al. 2016; Hartfield 2021). Mitotic gene conversion has been argued to

affect deleterious mutation accumulation in asexual species, including the bdelloid rotifer *Adenata vaga* (Flot et al. 2013; although see Vakhrusheva et al. 2020 who argue that these species show evidence of inter-individual gene exchange), the crustacean *Daphnia pulex* (Tucker et al. 2013) and the planarian worm *Schmidtea mediterranea* (Kershenbaum et al. 2024). Khakhlova and Bock (2006) also show experimental evidence of gene conversion eliminating deleterious mutations in plastids of tobacco plants, which are uniparentally inherited and are effectively asexual. Most of these studies argue that this gene conversion is beneficial for asexual species by removing deleterious mutations (an exception being Tucker et al. (2013) that argued that mitotic gene conversion reduces fitness by creating homozygous mutations). However, most of these arguments are verbal, and there exist little theoretical predictions to determine whether realistic levels of gene conversion can purge deleterious mutations, and hence maintain high fitnesses in asexual populations, in an analogous manner to self-fertilisation. One exception is a model in Flot et al. (2013), showing how gene conversion slows down Muller's ratchet in asexual populations.

The overall goal of this study is to investigate the efficacy of mitotic gene conversion in facultative sexual and asexual species in purging deleterious mutations, and compare its effects to that caused by self-fertilisation. We will use two complementary approaches: first, we develop single-locus analytical models to quantify the frequency of deleterious mutations under facultative sex and gene conversion, and compare results to equivalent models under self-fertilisation. We will then use multi-locus simulations to determine how both reproductive mechanisms influence the genome-wide prevalence of deleterious mutations.

Our principal questions are: (i) Under facultative sex, how does the frequency of deleterious variants (and hence also the population's mean fitness) scale with the prevalence of sex and gene conversion, both at individual loci and genome-wide? (ii) What values of sexual reproduction and gene conversion produce a level of mutation purging similar to a given level of self-fertilisation? (iii) Can realistic levels of mitotic gene conversion hence be as effective as self-fertilisation at purging deleterious mutations? The findings of this study will provide important quantification of a mechanism for maintaining the fitness of facultative

sexuals and asexuals that has been often discussed but seldom investigated theoretically. It also provides some insight as to which mode of uniparental reproduction might be favoured by evolution due to its effects on removing deleterious variation.

Single-Locus Mathematical Model

To gain some insight into how mitotic gene conversion affects the purging of deleterious mutations and how it compares to self-fertilisation, we investigate a single-locus model of deleterious allele frequencies in a deterministic diploid Wright-Fisher population subject to either facultative sex with sexual reproduction occurring at frequency σ (i.e., every generation, a fraction σ of offspring are generated sexually, and the remaining 1–σ are produced asexually), or self-fertilisation at rate *S* (using similar definitions as for facultative sex). We consider the frequency of a deleterious allele (denoted *A*) that arose from mutation from a wild-type allele *a* with probability μ; heterozygous mutant genotypes have fitness 1–*hs* and homozygotes 1–*s*, for *h* the dominance coefficient and *s* the selection coefficient. Mitotic gene conversion occurs with frequency γ, which converts heterozygotes to either wild-type or derived homozygote with equal frequency.

We can track the prevalence of deleterious mutations by creating numerical recursions of how the frequency is affected by selection, reproduction, mutation and gene conversion in non-overlapping generations (following the methods of Agrawal and Hartfield 2016, Hartfield 2021; see Supplementary *Mathematica* file for the full recursion equations). Although these recursions calculate the change in genotype frequencies over time, we can convert these genotype frequencies to allele frequencies; specifically, if the frequency of the mutant allele is p_A, then the frequency of the mutant homozygote is $p_A^2 + Fp_A(1 - p_A)$ (with similar equations for the wild-type homozygote), and the heterozygote has frequency $2\,p_A(1 - p_A)(1 - F)$. Here, *F* denotes the reduction in homozygosity compared to Hardy-Weinberg expectations (Wright 1951), and shows to what extent either gene conversion under facultative sex or self-

fertilisation affects genotype composition by reducing heterozygote frequencies. After making these transformations, we linearise the equations assuming $\mu, p_A \ll 1$ to obtain steady-state values of p_A and *F* (see Otto 2003 for a similar approach).

After these algebraic calculations, we obtain the general equation for the deleterious allele frequency:

$$\hat{p}_A = \frac{\mu}{s(F+h-Fh)} \quad (1)$$

This is a known form for mutation-selection balance under non-random mating (Ohta and Cockerham 1974; Glémin 2003). The inbreeding coefficient *F* in Equation 1 differs depending on the form of uniparental reproduction. Under selfing:

$$\hat{F}_{self} = \frac{((1-h\,s)\,S\,(1-\gamma) + 2\gamma)}{(2-(1-(2-h)\,s)\,S\,(1-\gamma))} \quad (2)$$

While under facultative sex:

$$\hat{F}_{asex} = \frac{\gamma}{\gamma+(1-\gamma)(s(1-\sigma)+\sigma)} \quad (3)$$

We can use heuristic arguments to compare *F* in each case to understand which reproductive mode has the greatest effect on homozygosity. In the absence of gene conversion and selection, so we only focus on the effects of selfing, then $\hat{F}_{self} \approx S/(2-S)$ in line with classic results on how selfing affects homozygosity (e.g., Nordborg and Donnelly 1997). Under facultative sex (Equation 3), positive $\hat{F}_{asex}$ is only formed when gene conversion is present; interestingly, dominance does not appear in Equation 3 and instead affects allele frequencies through the mutation-selection balance term (Equation 1). In the absence of selection and assuming that $\gamma << \sigma$ so the denominator approximates to σ, then $\hat{F}_{asex} \approx \frac{\gamma}{\sigma}$ (as also found by Hartfield 2021). By comparing these two approximations, we can show that the inbreeding values are equivalent if $S = 2/(1+\phi)$ for $\phi = \sigma/\gamma$ (Hartfield et al. 2016; Hartfield 2021). As sex is much more common than gene conversion under these assumptions (so $\phi >> 1$) then we see that gene conversion acting under frequent sex is only equivalent to a small selfing rate. Gene conversion becomes more effective once sex becomes rarer and hence is

more equivalent to elevated selfing rates, but note that this approximation for *S* will break down once gene conversion and sex act at the same rate.

Figure 1 illustrates these derived equations for different values of the rate of sex and mitotic gene conversion. If mutations are recessive (Figure 1a) then even small amounts of gene conversion can reduce the prevalence of deleterious mutations compared to outcrossing expectations, even for intermediate frequencies of sex. Conversely, the frequency of additive alleles are close to outcrossing expectations (Figure 1b). These results illustrate that mitotic gene conversion has the strongest effect on purging deleterious mutations if they are recessive, with the purging effect magnified as sex becomes rarer. Under self-fertilisation (Figures 1c, d), the same general results arise, but we note that only a small amount of selfing is needed to remove recessive deleterious mutations, reflecting the stronger effect of selfing on homozygosity. Mitotic gene conversion also only has a minor effect on allele frequencies (note that the contour lines are nearly vertical). Plots of $\hat{F}$ (Figure 2a, b) show that while mitotic gene conversion under facultative sex leads to elevated homozygosity, the increase is mostly modest unless sex is rare and deleterious mutations have a weaker selection effect. In contrast, under self-fertilisation then $\hat{F}$ values tend to be much higher (Figure 2c, d).

To test the accuracy of these approximate analytical solutions (Equations 1-3), we compared them to exact values obtained from numerical recursions over 50,000 generations, where the deleterious heterozygous had an initial frequency of 0.001 (Supplementary Figures S1 and S2, with code in Supplementary *Mathematica* file). We see that they match exact numerical recursions over a wide range of parameters. One caveat of these models is that they do not consider drift effects, and hence processes like Muller's ratchet (Muller 1964; Haigh 1978) that can arise when sex is rare.

Multi-Locus Simulation Methods

Simulation Outline

While the single-locus model provides insight into how rare sex and gene conversion affect deleterious mutation frequencies, it does not show how gene conversion affects longer genome regions (e.g., through longer gene-conversion events purging mutations at multiple loci, and its effects on the resulting disequilibrium between loci) and hence how effective it is in purging mutations across larger stretches of the genome. Hence, we next use simulations to compare how gene conversion leads to mutation removal in asexuals, compared to selfing species.

Simulations were written using SLiM v4.0.1 (Haller and Messer 2023), and were implemented in either the Wright-Fisher framework (where the population size is fixed and the probability of choosing parents are proportional to their fitness; hereafter 'WF model') or in the non-Wright-Fisher framework (where the population size can change depending on which parents reproduce or not; hereafter 'non-WF model'). Simulated populations consist of 5,000 diploid individuals, each with a genome size of L = 25 Mb that approximates the length of *Arabidopsis thaliana* chromosome 1 (The Arabidopsis Genome Initiative 2000). Individuals do not carry mutations at the start of the simulations. We let populations evolve for 60,000 generations, which is sufficient for the population variance to reach a steady-state (Supplementary Figure S3). After this time, 50 individuals are sampled and their genotypes were saved in a VCF file format for downstream analysis. The mutation rate was set to 4×10^{-9}, which is on the same order as that inferred from *Arabidopsis thaliana* (Ossowski et al. 2010). Per-base-position recombination rate was set to 4×10^{-8}, reflecting estimates obtained from crosses of *Arabidopsis thaliana* (Salome et al. 2012).

Simulated mutations were deleterious and mutation stacking is allowed by default. Two types of fitness models were investigated. They differ in whether selection and dominance coefficients were constant or drawn from a distribution (Table 1). In the first type, fixed values

of deleterious mutations and dominance coefficients were chosen. In the second type, the selection and dominance coefficients were drawn from a distribution based on Spigler et al. (2017) (see Table 1). Selection coefficient is modelled by a gamma distribution with shape parameter α and mean $\bar{s}$. The dominance coefficient of a mutation with selection coefficient *s* is then drawn from a uniform distribution bounded by 0 and e^{-ks} , where *k* is a constant (see Table 1 for exact values). Free parameters in the model, *k*, $\bar{s}$, and α, were defined so $\bar{s}$ and $\bar{h}$ match with parameters in our main simulations (*s* = 0.01, *h* = 0.2) and the shape parameter was based on Chen *et al.* (2017). The general rationale behind this model is to reflect empirical observations that strongly deleterious mutations tend to be more recessive (Simmons and Crow 1977; Agrawal and Whitlock 2011).

Implementing mitotic gene conversion in asexual reproduction

By default, recombination in SLiM (including gene conversion) only acts exclusively when meiosis is involved. Therefore, the reproduction cycle was manually modified through a reproduction call-back (i.e., a SLiM function for governing reproduction), so that mitotic gene conversion in asexuals can be simulated; this can be done only in non-WF model. For simplicity, the model applies only to a completely asexual population. The development of the code was facilitated by a post on the SLiM discussion board (Dussert and Haller 2019) and was extended based on the gene conversion model under SLiM Wright-Fisher simulations (Haller and Messer 2016).

When an offspring is generated, then the number of gene conversion events that acts within it is drawn from a Poisson distribution with a mean $L\gamma$ where γ denotes the rate of gene conversion initiating at a site. For each gene conversion event, the central position is first drawn uniformly through the chromosome. Then, the tract is extended to the left and right independently by drawing each length from a geometric distribution with parameter *p:*

$$p = \frac{-\lambda + \sqrt{\lambda}\sqrt{8+\lambda}}{2\lambda} \quad (2)$$

305

306 where λ is the total mean tract length we want to use in simulations. This parameterization is
307 used to account for the fact that gene conversion tracts with length zero are discarded. This
308 redefined value of *p* means that the mean tract length is λ after accounting for discarding
309 these tracts (see Supplementary *Mathematica* notebook for further information). Gene
310 conversion events that overlap with the chromosome ends are truncated so they stop at the
311 chromosome edges.

312 After the gene conversion start- and end-points are drawn, they are checked and
313 potentially modified as follows: tracts of length 0 are removed; gene conversion tracts may
314 have the same start or end positions, in which case, the start (or end) of one of them is shifted
315 to the right (left), so that gene conversion tracts overlap; or two gene conversion tracts overlap,
316 in which case two interior gene conversion positions are removed so that the resulting gene
317 conversion tract is a union of the two smaller ones. Since multiple gene conversion tracts can
318 overlap, this refinement process is repeated until all start and end positions are unique and
319 alternate after sorting.

320 Gene conversion can occur on either of the homologous chromosomes with equal
321 probability and results in the selected part of one chromosome being copied onto the same
322 position at the other chromosome. Note that for each offspring-production event, all gene
323 conversion tracts are in the same direction, i.e., material is transferred solely from one strand
324 to another during each reproduction event. Since the 'initiation' strand is randomly chosen for
325 each reproduction event, then this effect should not lead to a major bias in results. The mean
326 length of a gene conversion event is set to 4,000 base pairs; this value accounts for the fact
327 that empirical studies of mitotic gene conversion tends to find that gene conversion tracts are
328 bimodally distributed, with a combination of short (on the order of 1kb) and long tracts (on the
329 order of 10kb) (Yim et al. 2014; Chumki et al. 2016; Jia et al. 2021). Using a mean length of
330 4,000bp will thus enable a wide distribution of tract lengths to be drawn that cover both these
331 possibilities. We report simulation results in terms of the 'mean gene conversion rate per site',

332 which is equal to the initiation rate times the mean tract length. This value is approximately
333 equivalent to the gene conversion parameter γ in the single-locus model (Frisse et al. 2001).
334 To enable fair comparison between simulations, further changes to the non-WF model
335 were made so that it behaves as a standard Wright-Fisher model with a fixed population size
336 over time, based on section 16.15 in the SLiM Manual (Haller and Messer 2016). Briefly,
337 survival callback limits the age of an individual to a single generation, and reproduction
338 callback samples a constant number of parents with probability weighted by their fitness. By
339 comparing measurements of population fitness (Supplementary Figure S4) and mutation
340 count (Supplementary Figure S5) between the two simulations, we see that this new routine
341 yields similar behaviour under low gene conversion rates to the original WF model with
342 complete asexuality.
343 100 replicate simulations were run if *s*, *h* were fixed; 20 replicate simulations were run
344 for WF simulations (i.e., with partial selfing and cloning) where *s*, *h* are drawn from
345 distributions; and 10 replicate simulations were run for non-WF simulations (i.e., with
346 asexuality and gene conversion) where *s*, *h* are drawn from distributions. Fewer simulations
347 replicates were used in the latter two cases due to the much longer runtime of these
348 simulations.

349 Mutational Load Measurement

350 Deleterious mutation load was assessed based on two measurements to reflect those
351 used in empirical genomic studies (note these terms and measurements are also used by
352 Laenen et al. (2018) in an analysis of *Arabis alpina*). We calculate (i) the the number of
353 homozygous mutant genotypes (also called 'recessive load'), which is proportional to the
354 genetic load expressed by recessive mutations; and (ii) the total number of mutations present
355 in the population ('additive load'), which is proportional to the load caused by mutations with *h*
356 = 0.5 (Bertorelle et al. 2022). We also calculated the relative homozygosity for each simulation,
357 defined as 2*(number of homozygous mutations)/(total number of mutations). This

measurement can be interpreted as the proportion of deleterious mutations that are in homozygous state, across all sites and individuals. Simulation outputs were analysed using R (R Core Team 2024).

Measuring linkage and identity disequilibrium

Linkage disequilibrium was measured from simulations using similar methods to those in Hartfield et al. (2018) and Hartfield and Glémin (2024). Briefly, r^2 was measured using VCFtools (Danecek et al. 2011) after thinning SNPs so measurements were taken between sites that are 10kb apart and had a minor allele frequency of 5%. LD decay was plotted for each simulation replicate in bins of 0.5Mb up to 12.5Mb apart, while subsampling the results per bin so that they all contain the same number as in the lowest bin, but no more than 200 (in case of simulations with fixed fitness effects) or 100 (for simulations with variable fitness effects). Only replicates with the minimum number of points per bin larger or equal to 5 were retained.

Identity disequilibrium was measured using the g_2 statistic (David et al. 2007), as implemented in the 'inbreedR' package (Stoffel et al. 2016). This statistic measures the extent heterozygosity across a genome is correlated, relative to an expectation under independent sites. For all simulations, the output VCF file was converted to inbreedR format as described in the package's manual. The 'g2_snps' function was then used with 100 bootstraps for generating the mean g_2 estimate along with 95% confidence intervals.

Results

Comparing self-fertilisation and facultative sex

We first compared the mean fitness and mutation loads with different levels of self-fertilisation or asexual reproduction, to determine how mutation accumulation differs between

381 the two mating systems. These simulations were run using the WF model. All results in the
382 main text assume a fixed *s* and *h*; we will present results with varying *s*, *h* values at the end of
383 the results section.

384 Figure 3 shows how the mean fitness changes over time. Under self-fertilisation
385 (Figure 3a), while the mean fitness is fairly high overall, it is lowest when there is complete
386 outcrossing. It generally increases with the selfing rate, reaching a maximum for around 95%
387 self-fertilisation, but then drops for even higher rates. In contrast, the mean fitness drops
388 dramatically for highly asexual populations (Figure 3b).

389 Figure 4 (and Supplementary Figure S6) shows mutation counts under these
390 reproductive regimes. Selfing populations undergo mutation purging, as evidenced by a
391 decreasing number of mutations in the population (approximately linearly with the selfing rate)
392 and an increase in homozygous load reflecting higher inbreeding. In contrast, asexual
393 populations have approximately the same mutation counts (both total and homozygote) as
394 outcrossing populations, until the population becomes around 95% asexual. At this point, there
395 is a spike in homozygosity that is then reduced with even higher levels of asexuality, while the
396 total mutation count rapidly increases. The high mutation count reflects inefficient selection
397 acting once asexuality becomes prevalent, as reflected in the drop in mean fitness (Figure 3).
398 The spike in homozygosity likely reflects the fact that for high-but-incomplete asexuality,
399 mutation accumulation starts acting but there is still sufficient segregation to create
400 homozygous genotypes. In populations that become completely asexual (i.e., the level of
401 clonal reproduction approaches 1), there is a lack of genetic segregation so genotypes only
402 form as heterozygotes (the signature of 'allelic sequence divergence'). The relative
403 homozygosity (Supplementary Figure S7) also reflects these outcomes; it increases towards
404 1 with higher selfing, reflecting greater homozygosity due to inbreeding. In contrast, it stays
405 constant with increased asexuality up to 95% asexual reproduction, but then decreases with
406 higher rates. No apparent spike at 95% asexuality is observed using this measure.

Impact of mitotic gene conversion

Given this increased mutation count under low rates of sex, we next investigated to what extent mitotic gene conversion can reduce it. Here, we used the non-WF model with the new gene conversion routine implemented. Figure 5 plots the mean fitness for different per-base-pair initiation rates of gene conversion. For most values the mean fitness remains low, similar to results in the absence of gene conversion (Figure 3b). However, once mean gene conversion per site becomes sufficiently high (in this case, $4x10^{-4}$ and above) the mean fitness increases and starts reflecting that of outcrossing populations. These results imply that purging is indeed occurring, and this mechanism is validated when looking at mutation counts (Figure 6). Here, the mutation count drops while the homozygosity increases (as reflected with heightened relative homozygosity; Supplementary Figure S8), in line with purging reducing the total mutation prevalence and increasing the mean fitness.

Comparing mitotic gene conversion to self-fertilisation

We next compare the effects of mitotic gene conversion in asexuals to self-fertilisation, to determine to what extent the two effects are comparable. Figure 7 compares gene conversion in asexuals against a range of selfing rates. When looking at homozygosity, it seems that asexuality with the highest gene conversion rates ($4x10^{-2}$) yield the same homozygous counts as complete selfing, implying that the two are equivalent. However, the total mutation count is lower under any level of self-fertilisation, compared to the highest gene conversion rate used. Hence, it seems that while gene conversion in asexuals can purge mutations, it does not produce the same benefits in terms of reducing mutation load as observed under self-fertilisation.

Effects on Linkage and Identity Disequilibrium

As the level of uniparental reproduction increases, so does linkage disequilibrium across the genome (Supplementary Figures S9, S10). Linkage disequilibrium under self-fertilisation does not exceed 0.25 on average, while for asexual reproduction it jumps as the level of clonal reproduction exceeds 95% and stays at high values (r^2 of nearly 1) across the genome. These results reflect those previously obtained under investigations of LD under different reproductive modes (Hartfield et al. 2018; Hartfield and Glémin 2024). When gene conversion is included under clonal reproduction (Supplementary Figure S11), r^2 still stays constant over the length of genome investigated, but decreases from mean rates of $4x10^{-4}$ onwards and dropping below 0.25 and at a similar level to high selfing for the highest value investigated of $4x10^{-2}$.

Identity disequilibrium (ID), as measured by g_2, is mostly zero across simulations, only becoming non-zero for some high values of selfing or asexual reproduction. Mean ID is also zero in clonal populations even when gene conversion is present, although there is some variance around this value under very high gene conversion rates (Supplementary Figure S12).

Results where *s*, *h* are drawn from distributions

To test the generality of these results, we also investigate simulations where *s*, *h* are drawn from probability distributions, using the same mutation rate. In these cases the fitness variance also reaches a steady-state within 60,000 generations (Supplementary Figure S13). Mean fitness results are generally similar to the fixed *s*, *h* case, although if looking just at selfing populations, the highest fitness is realised for complete selfing (Supplementary Figure S14). Mutation accumulation in the absence of gene conversion acts slightly differently; while the highest mutation count occurs under high levels of sexual reproduction, the number of homozygous genotypes is reduced under both high levels of selfing and asexual reproduction (Supplementary Figures S15, S16). The relative homozygosity is higher than that for fixed *s*,

457 *h* cases under low rates of sex, but drastically drops once the rate of asexuality reaches 95%
458 and higher (Supplementary Figure S17). Gene conversion in asexuals restores population
459 fitness in a similar manner to the fixed *s, h* simulations (Supplementary Figure S18), with
460 slightly lower levels of gene conversion needed to purge deleterious mutations
461 (Supplementary Figure S19 – S21). LD and ID measurements are also qualitatively similar to
462 before (Supplementary Figure S22 – S25). Hence, it seems that the results found above do
463 not strongly depend on the fitness model used.

464 Discussion

465 Summary of results

466 In this study, we theoretically explored to what extent mitotic gene conversion in
467 facultative sexual species purge deleterious mutations. We additionally compared this process
468 to self-fertilisation, as the latter is known to purge deleterious mutations so we can compare
469 how effective the two are. Using a single-locus model (Figure 1), we first demonstrated that
470 mitotic gene conversion has the greatest effect in reducing mutation frequency when
471 asexuality is frequent, but also when mutations are recessive. We also show that, while the
472 process leads to elevated homozygosity (as measured by the inbreeding coefficient *F;* Figure
473 2), it will not be substantially elevated and match the effects of selfing unless populations are
474 fully asexual and the gene conversion rate is high. We subsequently used multi-locus
475 simulations to explore the effect of mitotic gene conversion on a genome-wide level. In the
476 absence of gene conversion (Figures 3, 4), highly-asexual species accumulate many
477 mutations as heterozygotes, leading to a drastic drop in fitness. When gene conversion is
478 present in fully asexual populations (Figure 5), then we do see a purging effect: once the gene
479 conversion rate exceeds a critical value (generally an mean rate of $4x10^{-4}$ per site) the total
480 mutation count drops and more mutations are present as homozygotes. These levels of gene
481 conversion also dramatically increase the fitness of asexual populations (Figure 5, Figure

S18). Despite these benefits of gene conversion, the total mutation count is lowest under high levels of self-fertilisation (Figure 7). Overall, both the single-locus and multi-locus results indicate that while high rates of gene conversion can purge deleterious mutations under asexual reproduction, these benefits do not always counter the costs of inefficient selection under clonality and self-fertilisation can act a stronger force at purging deleterious mutations. That said, lower levels of gene conversion (rate of 10^{-5} and above) can remove some deleterious mutations and increase the fitness of asexual organisms, and hence still be evolutionary favoured.

Comparing mutation accumulation in self-fertilising and asexual populations

One notable feature of the asexual-selfing comparison is the threshold behaviour of the mutation load. In the absence of gene conversion, neutral genetic diversity in asexual populations is similar to outcrossers unless the rate of sex is of the order $1/N$ (Bengtsson 2003; Ceplitis 2003; Hartfield et al. 2016; Hartfield 2021); background selection also incurs noticeable reductions in diversity in species with infrequent sex (Agrawal and Hartfield 2016). We see similar results with regards to the mutation count in Figure 4; it stays fairly stable under facultative sex unless populations become highly asexual. Conversely, under high selfing we generally see a drop in the mutation count and heightened homozygosity, in line with mutation purging. With very high levels of selfing, we see an elevation in both the homozygous mutation count and in the total mutation number. These increases likely arise due to an additional increase in mutation number due to genetic drift, as N_e can be dramatically reduced in selfing populations due to background selection effects (Kamran-Disfani and Agrawal 2014; Roze 2016). Note that this behaviour can change with the fitness model assumptions; under a model where s and h are drawn from distributions, we instead see a dramatic reduction in the

507 mutation count under high selfing, in line with a model where genetic variation is purged
508 (Charlesworth and Charlesworth 1995; Lande and Porcher 2015; Abu Awad and Roze 2018).
509

510 Effects of mitotic gene conversion, and comparison with 511 empirical estimates

512 Throughout this study, we compared purging via mitotic gene conversion in asexuals
513 to that via self-fertilisation. We showed that despite the benefits of mitotic gene conversion,
514 lower mutation counts were observed under high levels of self-fertilisation (see, e.g., Figure
515 7). These results indicate that self-fertilisation can be a more efficient means of purging
516 deleterious mutations, likely because it affects the whole genome and homozygous regions
517 can be formed more rapidly. In contrast, mitotic gene conversion as employed here only
518 affects a fraction of the genome every generation, so purging acts in a piecemeal manner.
519 This comparison seems to hold over different mutation models, selfing and gene conversion
520 rates; one caveat is that there could be a specific, untested combination of mutation and
521 recombination rates in selfers that might lead to different results.

522 Our simulation model shows that a mean mitotic gene conversion rate above $4x10^{-4}$
523 per site can start facilitating the purging of deleterious mutations. This is in line with previous
524 theoretical work showing that, once gene conversion becomes sufficiently high, then it can
525 substantially reduce genetic variation in asexual populations (Hartfield et al. 2016; Hartfield
526 2021). Although empirical estimates are sparse, they are usually much lower than this value,
527 ranging between 10^{-7} and 10^{-5} depending on the study organism (Mandegar and Otto 2007;
528 Xu et al. 2011; Flot et al. 2013; Sharp and Agrawal 2016). Potentially higher rates were
529 observed in rice; Jia et al. (2021) inferred a mitotic recombination rate of 10^{-6} with mean tract
530 lengths ranging between 30-50kb, leading to a per-locus rate on the order of 10^{-2}. Hence, it
531 seems that while some larger empirical estimates are sufficiently high to cause extensive
532 deleterious mutation purging, on the whole mitotic gene conversion is usually too weak a force

533 to lead to extensive purging in nature. However, note that the estimates of Jia et al. (2021)
534 consider all mitotic recombination events, both crossover and non-crossover events. Our
535 simulation procedure only considers non-crossover events, which converts more localised
536 genomic regions. Mitotic crossover events, in contrast, can convert much larger fractions of
537 the genome (Jia et al. 2021). Even though the net conversion rate per site seems the most
538 important parameter, longer conversion regions could have a stronger effect through affecting
539 multiple sites at once, in a similar manner to self-fertilisation. Other forms of asexual
540 reproduction that also create extensive homozygous regions, such as terminal-fusion
541 automixis (Mirzaghaderi and Hörandl 2016), could also affect enough of the genome to cause
542 large levels of mutation purging. Future research can determine whether these different factors
543 have a stronger effect on asexual fitness.

544 Another factor not considered in our study is the effect of small and/or changing
545 population sizes. In small populations, homozygotes can form more frequently due to genetic
546 drift, which can also lead to the purging of deleterious mutations. However, this process is only
547 effective at purging highly recessive mutations (Glémin 2003). Gene conversion and drift also
548 interact to affect the evolution of sex (Roze and Michod 2010). Given the large diploid
549 population size of 5,000 used in simulations, then drift should have a minimal influence on the
550 actual purging process under the multi-locus results, but could affect the baseline level of
551 deleterious mutations in the absence of gene conversion. It will be a rewarding avenue of
552 future research to determine how drift would affect these processes. Similarly, we also did not
553 consider demographic feedbacks that are induced during the purging period, where mutation
554 purging also causes a reduction in population size (Abu Awad and Billiard 2017). The
555 presence of these feedbacks could increase the extinction probability of populations during
556 gene-conversion induced purging. Another avenue for the potential further investigation is the
557 role of adaptive evolution and/or epistasis on the relative advantage of different reproduction
558 strategies, as they could impact the relative fitness of heterozygotes and homozygotes and
559 hence the outcomes of the reproductives modes studied here (Kondrashov and Kondrashov
560 2001; Abu Awad and Roze 2020).

An interesting case where gene conversion may be important under clonal reproduction is tumour evolution. Here, cells reproduce asexually by mitosis and can potentially accumulate deleterious mutations due to a lack of recombination (Ní Leathlobhair and Lenski 2022). Hence, the gene conversion mechanisms investigated here may prevent transmissible cancers from experiencing mutation meltdown (Ní Leathlobhair and Lenski 2022), and also activate linked mutations that disable tumour-suppressing genes and enable carcinogenesis (Takahashi and Innan 2022).

568 **Table 1. The summary of model parameters and values used for simulations**

Parameter	Values for simulations with f. e.*	
	constant	**non-constant**
Genome	Diploid, L = 25 Mbp	
Per-base mutation rate	4×10^{-9}	
Per-position recombination rate	4×10^{-8}	
Population size	5 000	
Sample size	50	
Selection	s = 0.01	s ~ Gamma($\bar{s}$, α) mean $\bar{s}$ = 0.01 shape α = 0.2
Dominance	h = 0.2	h ~ U(0, e^{-ks}) k = 91.63
Mean gene conversion tract length	λ = 4 000 bp	

569 * f. e. - fitness effects; selection and dominance coefficients

570

571 ***Alt text:*** *Table outlining parameter values used in computer simulations.*

572

573

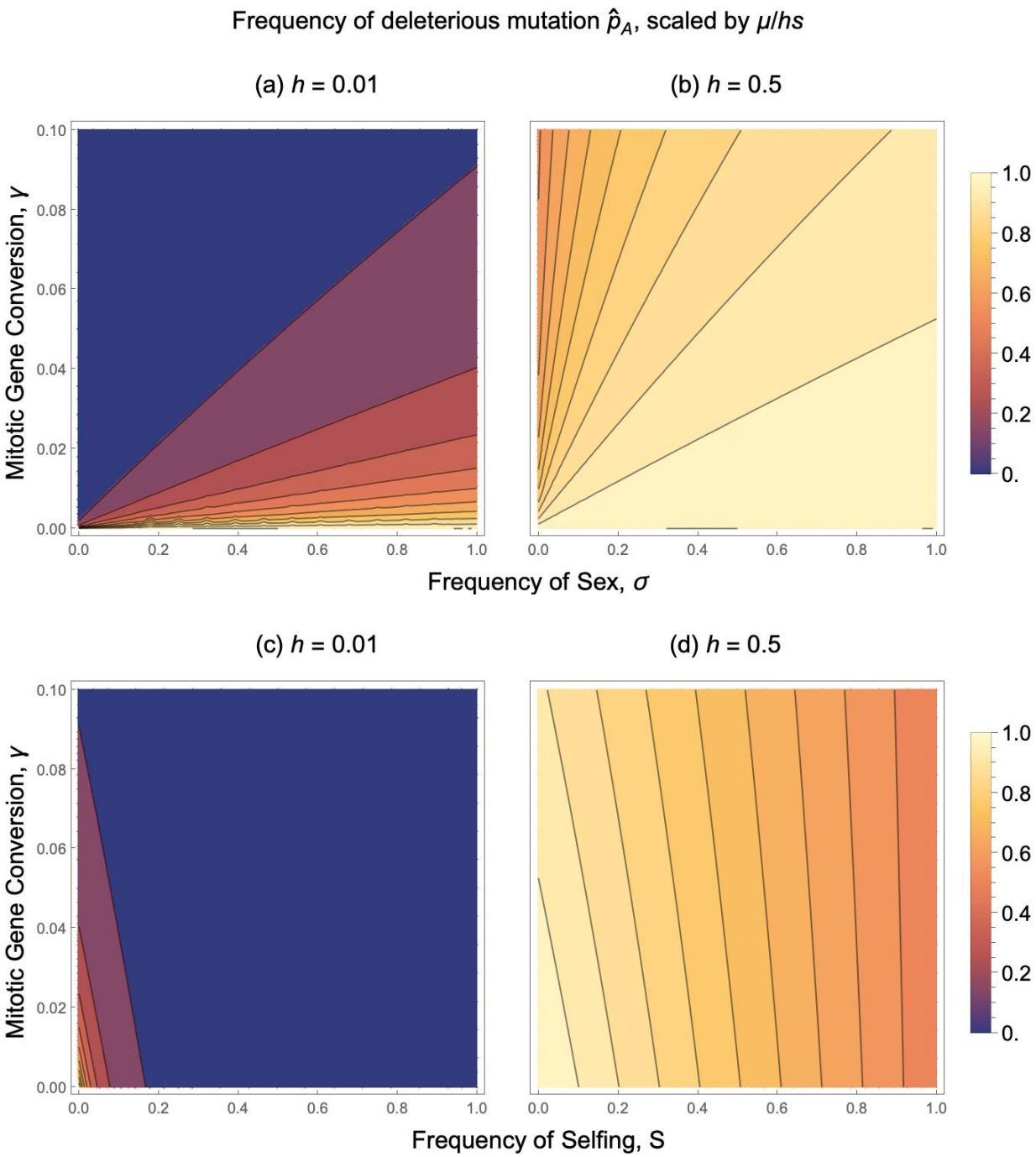

Figure 1: Plots of single-locus analytical results for the steady-state frequency of a deleterious mutation. *Results show Equation 1 for different dominance values, scaled to the expected result under outcrossing (μ/hs). Note that μ is not present in this scaled equation. Panels (a), (b) are for different frequencies of sex (with $\hat{F}$ given by Equation 3), while (c), (d) are for different frequencies of self-fertilisation (with $\hat{F}$ given by Equation 2).*

Alt text: *Contour plots showing deleterious mutation frequency with subfigures labelled a to d, illustrating effects of reproductive modes and mitotic gene conversion.*

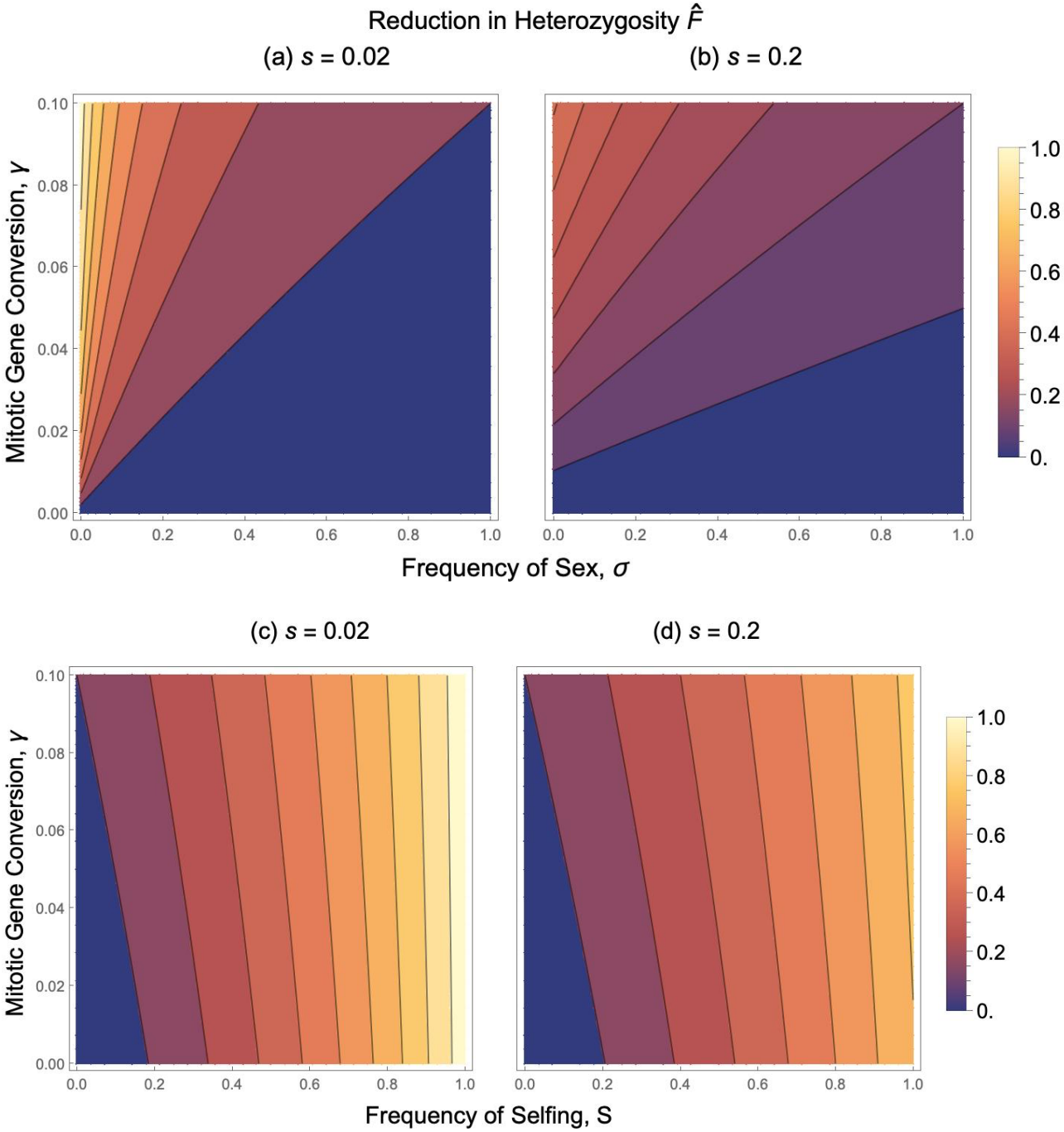

Figure 2: Plots of single-locus analytical results for the reduction in heterozygosity, $\hat{F}$. *Results show either Equation 2 under facultative sex (panels a, b), or Equation 3 under self-fertilisation (panels c, d).*

Alt text: *Contour plots showing reduction in heterozygosity with subfigures labelled a to d, illustrating effects of reproductive modes and mitotic gene conversion.*

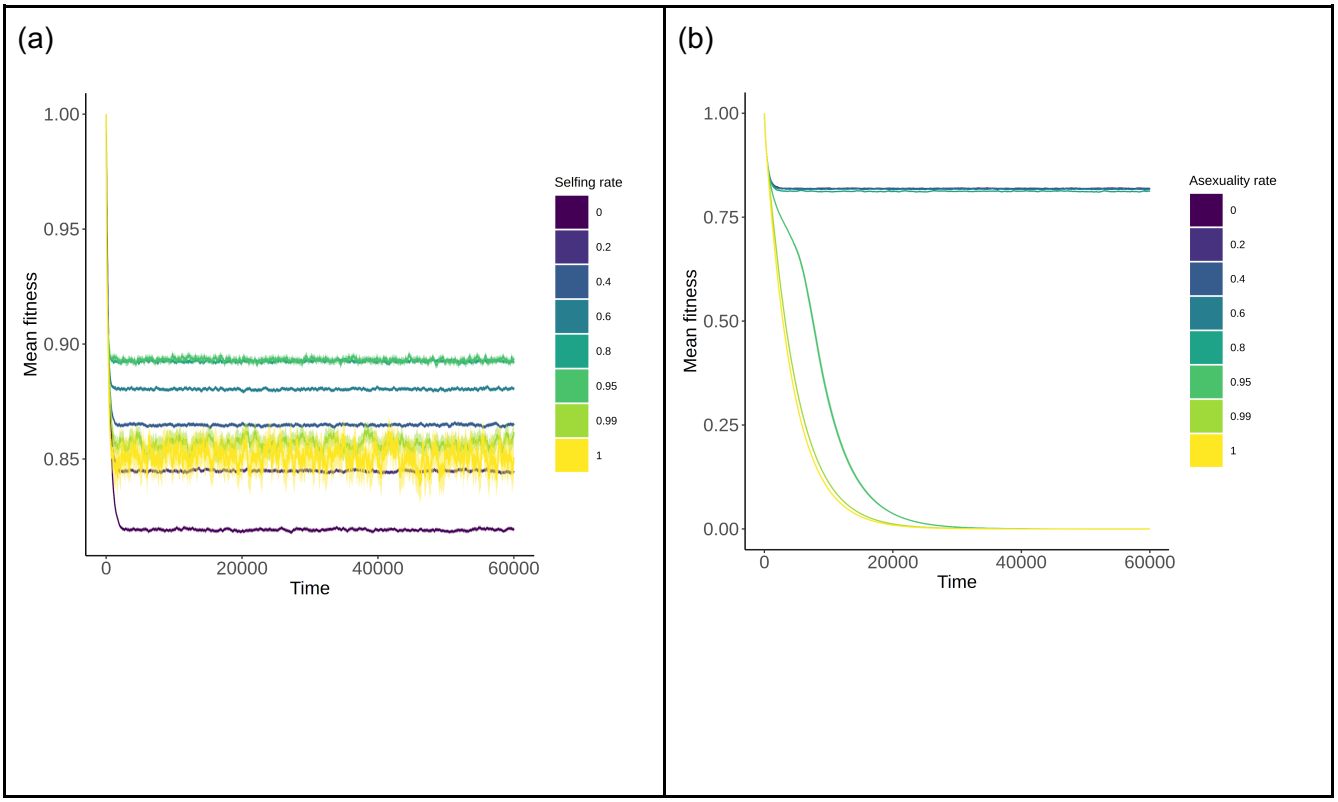

Figure 3: Mean fitness for different modes of uniparental reproduction. *Results are shown as a function of time, for (a) different rates of self-fertilisation or (b) asexual reproduction. Thick lines are mean values, while thinner shading around lines show 95% confidence intervals. Notice the change of scale on the y-axis between (a) and (b).*

Alt text: *Graphs showing the mean fitness over time for either self-fertilisation or partial asexual reproduction, with different colour lines showing different rates.*

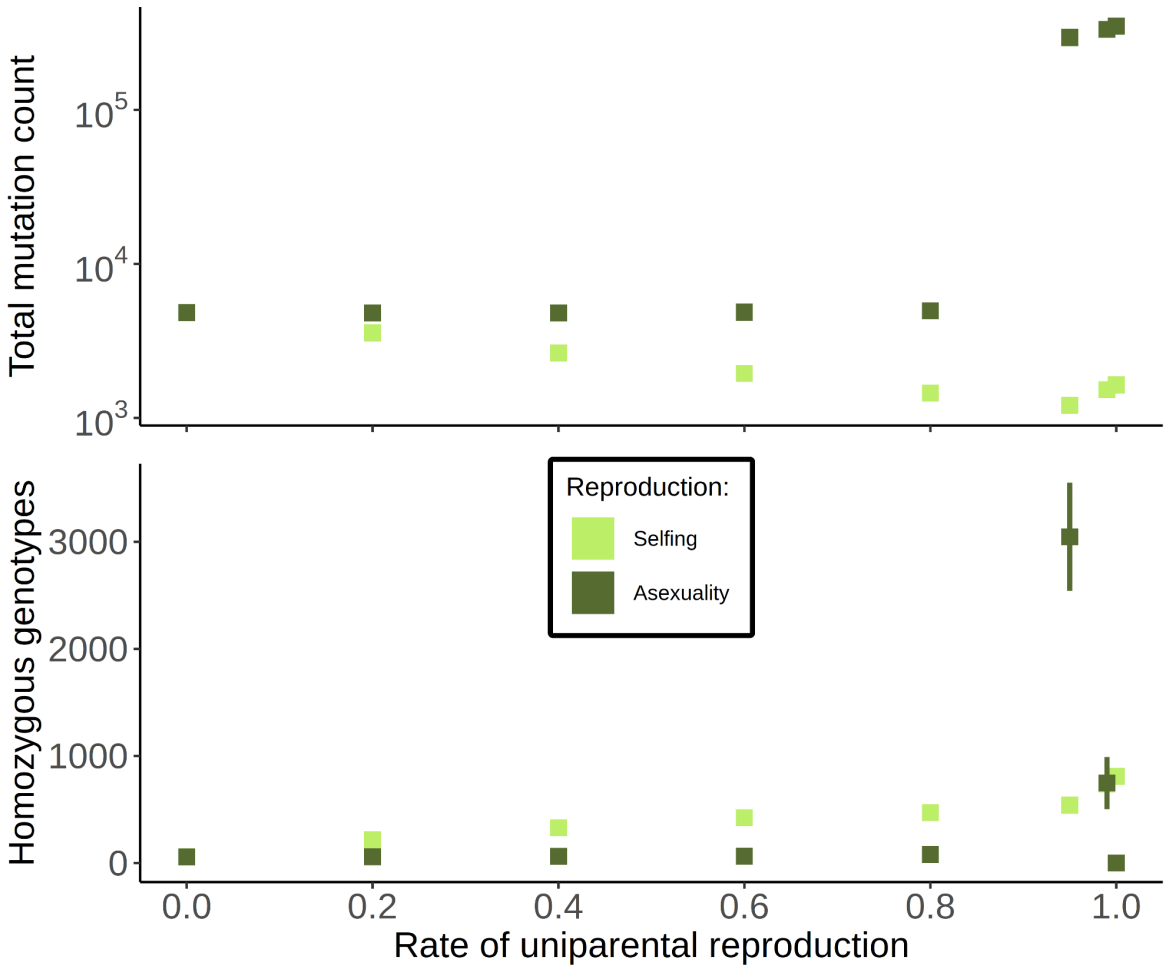

Figure 4: Deleterious mutation counts under different uniparental reproductive modes. *As a function of the rate of uniparental reproduction (either selfing or asexual reproduction), these plots show (top) the total mutation count present in the sample of 50 individuals, and (bottom) the number of homozygous genotypes present in the sample. Error bars represent 95% confidence intervals; if they are not shown then they lie within a point. See Supplementary Figure S6 for the total count on a linear scale.*

Alt text: *Graphs showing total mutation count and number of homozygote genotypes, with different points indicating different rates of either self-fertilisation or partial asexual reproduction.*

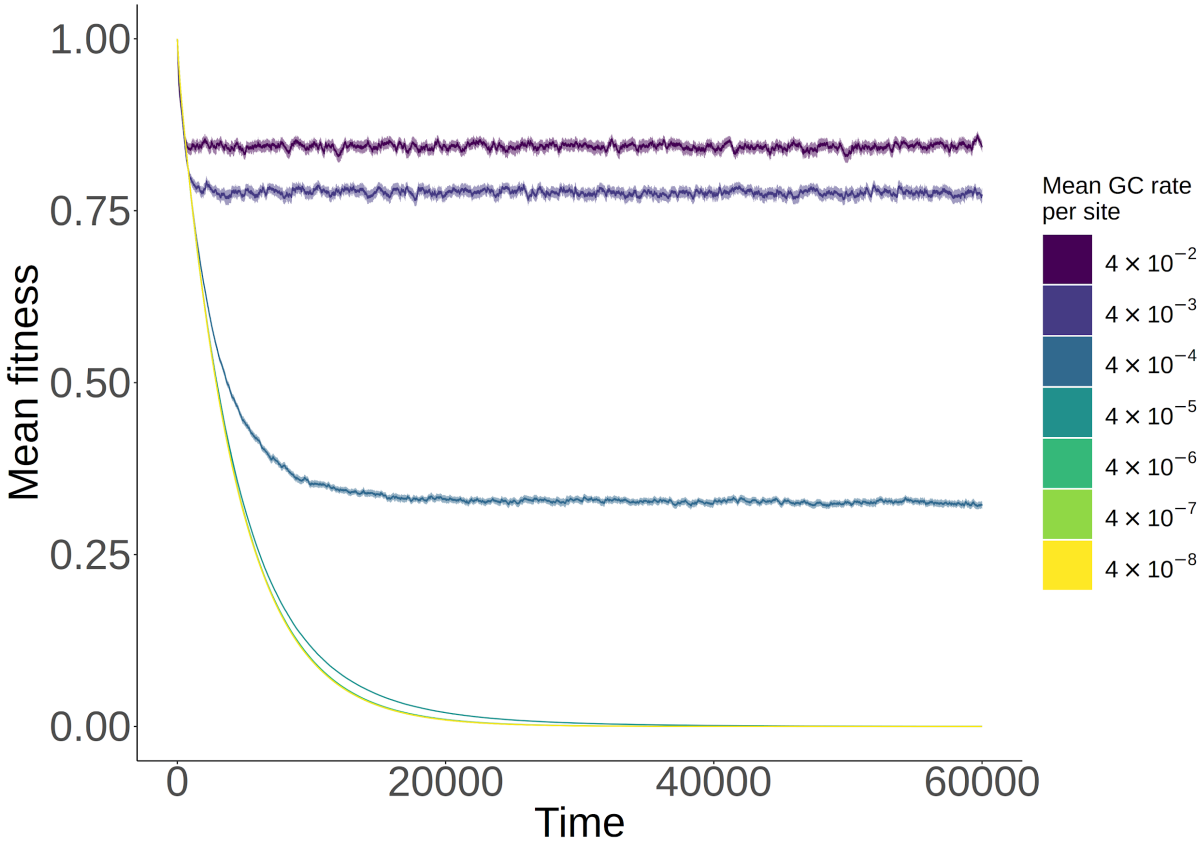

Figure 5: Mean fitness for obligately asexual populations when mitotic gene conversion is present at different rates. *Results are shown as a function of time. Thick lines are mean values, while thinner shading around lines show 95% confidence intervals.*

Alt text: *Graph showing the mean fitness over time for complete asexual reproduction, with different colour lines showing different rates of mitotic gene conversion.*

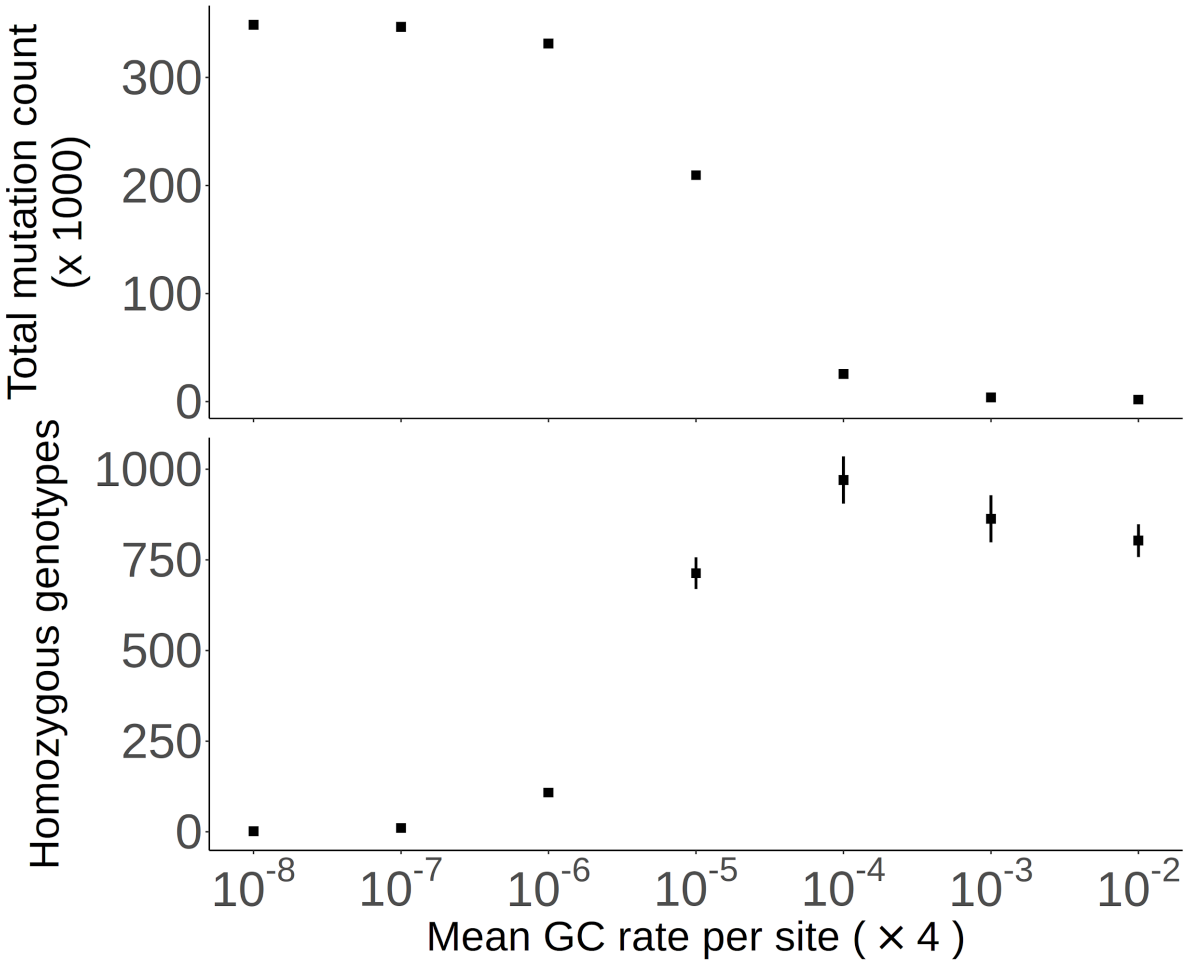

Figure 6: Deleterious mutation counts for obligate asexual populations with mitotic gene conversion present. *Results are shown as a function of the rate of gene conversion initiation at a single site. Plots show (top) the total mutation count present in the sample of 50 individuals, and (bottom) the number of homozygous genotypes present in the sample. Error bars represent 95% confidence intervals; if they are not shown then they lie within a point.*

Alt text: *Graph showing total mutation count and number of homozygote genotypes, with different points indicating different rates of gene conversion under complete asexual reproduction.*

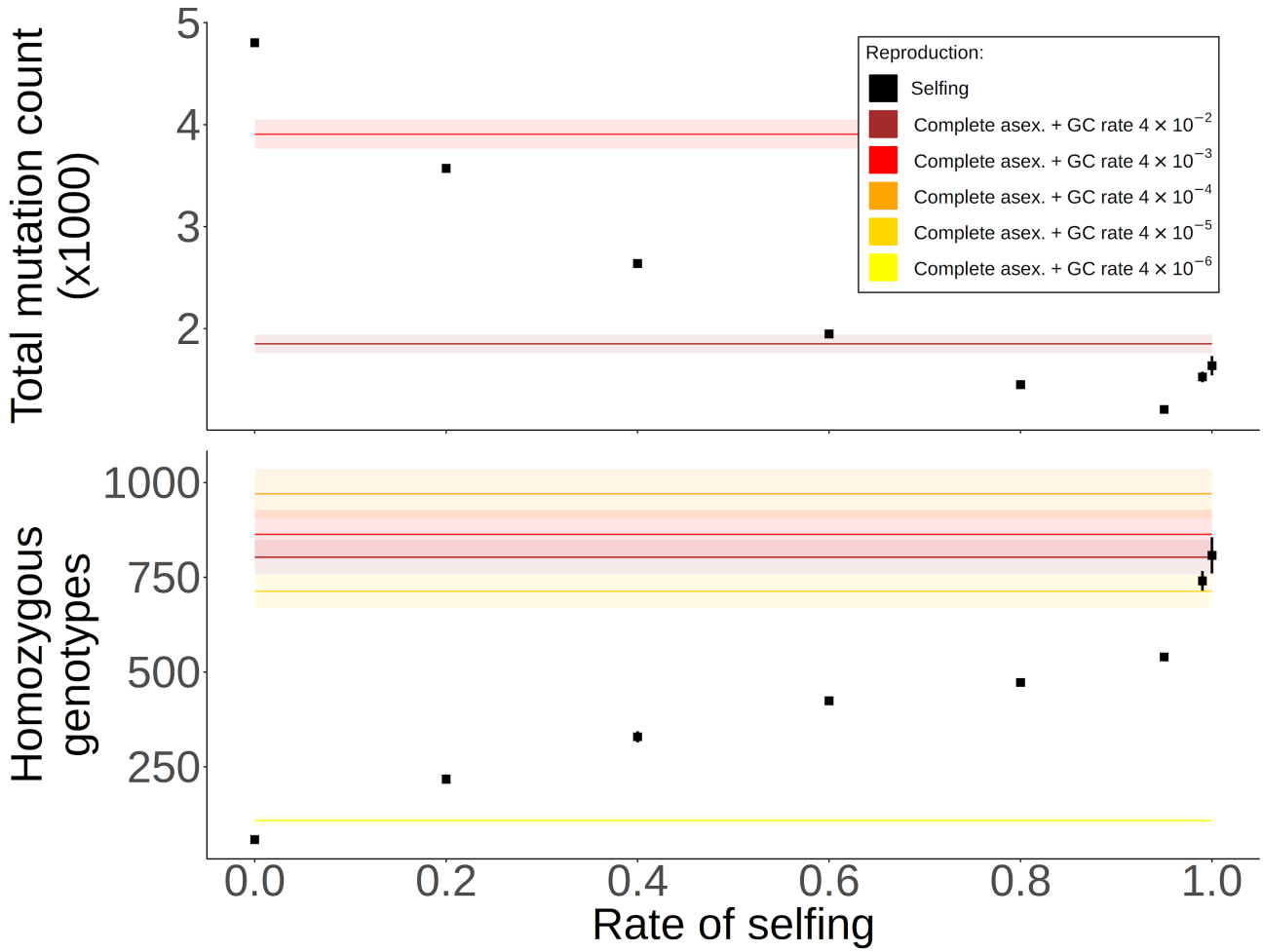

Figure 7: Comparing mutation counts under different levels of self-fertilisation to asexual populations subject to mitotic gene conversion***.*** *Plots show (top) the total mutation count present in the sample of 50 individuals, and (bottom) the number of homozygous genotypes present in the sample. Note that only* gene conversion *rates of* $4x10^{-2}$ *and* $4x10^{-3}$ *are shown in the top panel. The points show results under different levels of self-fertilisation; error bars represent 95% confidence intervals, and if they are not shown then they lie within a point. Coloured lines are results for asexuality with different levels of mitotic* gene conversion*, as indicated by the legend; shaded bands around lines indicate 95% confidence intervals.* ***Alt text:*** *Graph comparing total mutation count and number of homozygote genotypes, with points indicating different selfing rates, and horizontal lines are results for complete asexuality with different gene conversion rates.*

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