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POPULATION-LEVEL CONSEQUENCES OF INHERITABLE SOMATIC MUTATIONS AND THE EVOLUTION OF MUTATION RATES IN PLANTS

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ABSTRACT. Inbreeding depression, that is the decrease in fitness of inbred relative to outbred individuals, was shown to increase strongly as life expectancy increases in plants. Because plants are thought to not have a separated germline, it was proposed that this pattern could be generated by somatic mutations accumulating during growth, since larger and more long-lived species have more opportunities for mutations to accumulate. A key determinant of the role of somatic mutations is the rate at which they occur, which likely differs between species because mutation rates may evolve differently in species with contrasting life-histories. In this paper, I study the evolution of the mutation rates in plants, and consider the population-level consequences of inheritable somatic mutations given this evolution. I show that despite substantially lower somatic and meiotic mutation rates, more long-lived species still tend to accumulate larger amounts of deleterious mutations because of the increased number of opportunities they have to acquire mutations during growth, leading to higher levels of inbreeding depression in these species. However, the magnitude of this increase depends strongly on how mutagenic meiosis is relative to growth, to the point of being close to nonexistent in some situations.

1 Introduction

1 Plant growth is fueled by cell divisions occurring in meristems. Each shoot is produced by
2 an apical meristem and may bear axillary meristems, which are typically situated in the
3 axils of leaves and grow out to become the apical meristem of a new shoot upon activation
4 ([Burian et al., 2016](#)). As meristematic cells generate all the tissues constituting the shoot,
5 any mutation occurring in a meristematic cell will be borne by all the cells it gave rise to,
6 leading to genetic mosaicism within individual plants ([Schoen and Schultz, 2019](#)). Fur-
7 thermore, because meristems also give rise to reproductive tissues, mutations occurring
8 during growth before the differentiation of the germline, that is somatic mutations, may
9 be present in the gametes and hence be inherited (although how frequently the inheritance
10 of somatic mutations occurs is currently unknown, [Lanfear, 2018](#)). All else being equal, it
11 follows that the larger and the older a given plant grows, the more cell divisions it under-
12 goes and the more somatic mutations it should accumulate and transmit to its offspring,
13 potentially leading to a higher mutation load in more long-lived and larger species since
14 it is thought that most mutations are deleterious ([Eyre-Walker and Keightley, 2007](#)).

15 Inbreeding depression, that is the decrease in fitness of inbred relative to outbred
16 individuals ([Charlesworth and Charlesworth, 1987](#)), is thought to be mostly generated
17 by recessive deleterious mutations maintained at mutation-selection balance in popula-
18 tions ([Charlesworth and Willis, 2009](#)). Hence, [Scofield and Schultz \(2006\)](#) proposed that
19 somatic mutations accumulation could lead to higher inbreeding depression in larger and
20 more long-lived species. Consistent with this view, inbreeding depression was indeed shown
21 to increase strongly as life expectancy increases across plant species ([Duminil et al., 2009](#);
22 [Angeloni et al., 2011](#)). Furthermore, [Bobiwash et al. \(2013\)](#) showed that substantial in-

breeding depression was generated by somatic mutations in a study performed at the phenotypic level in old *Vaccinium angustifolium* clones. This is, however, the only empirical test of Scofield and Schultz (2006)’s idea. Besides, recent theoretical investigations have shown that variations in inbreeding depression can in principle be generated by differences in the fitness effect of mutations between species with contrasting life-histories (Lesaffre and Billiard, 2020), so that somatic mutations accumulation may not always be needed to explain variations in the magnitude of inbreeding depression across plant species. Moreover, theoretical investigations of the population-level consequences of somatic mutations accumulation are lacking, so that their role in the maintenance of high inbreeding depression in long-lived species remains poorly understood. Indeed, theoretical studies regarding somatic mutations in plants either focused on the case of favorable mutations, conferring resistance against herbivores (e.g. Antolin and Strobeck, 1985), or studied the fate of deleterious mutations subject to intra-organismal selection (Otto and Orive, 1995; Pineda-Krch and Lehtilä, 2002), but never considered the population-level consequences of recessive deleterious mutations (Schoen and Schultz, 2019). In summary, deleterious somatic mutations accumulation has been proposed as a mechanism to explain the rarity of selfing species among long-lived plants (Scofield and Schultz, 2006), consistent with empirical measures of inbreeding depression, but theoretical support for this idea remains scarce.

An important determinant of the consequences of somatic mutations accumulation is the rate at which said mutations accumulate during growth, that is the somatic mutation rate, which is defined here as the number of mutations occurring per unit of vegetative growth. This rate is likely influenced by evolutionary mechanisms similar to those affect-

ing mutation rates in general. For example, [Kimura \(1967\)](#) showed that mutation rates
 should be shaped by the opposition between the increase in the number of deleterious
 mutations borne by individuals with higher mutation rates on the one hand, which causes
 indirect selection against genetic variants increasing mutation rates to increase, and the
 direct fitness cost there is to increasing the fidelity of DNA replication on the other hand.
 Besides, [Lynch \(2011\)](#) proposed that selection to decrease the mutation rate should be-
 come weaker than genetic drift at some point in finite populations, thereby favoring the
 persistence of non-zero mutation rates. Nevertheless, the inheritability of somatic muta-
 tions in plants and their intrinsic link with growth and life expectancy likely contribute
 to shape the evolution of mutation rates in a specific manner which was never tackled
 theoretically. Great interest was however taken in empirically detecting somatic muta-
 tions and comparing mutations rates in a variety of plants species ranging from the very
 short-lived *Arabidopsis thaliana* to ancient, centuries old trees. In an analysis performed
 across many plant families, [Lanfear et al. \(2013\)](#) showed that taller species among pairs of
 sister species have significantly lower rates of molecular evolution, measured as the number
 of substitutions per site per 10^6 years. They argued that contrary to animals, this pattern
 is not a mere reflection of differences in generation time, which would reflect different rates
 of genome copying per unit of time, because somatic genome copying events contribute
 to the inheritable genetic variation in plants. Instead, they proposed that this pattern
 may be due to slower growth in taller species, which results in a lower number of mitosis
 (and therefore mutations) per unit of time. Consistent with this view, it was shown at the
 cellular level that axillary meristems cells are set aside early during the growth of a shoot
 ([Burian et al., 2016](#)), resulting in a number of cell divisions increasing linearly with the

number of branching events in trees although the number of terminal branches increases exponentially. Furthermore, multiple studies showed that somatic mutation rates tend to be considerably lower in taller, more long-lived species (Schmid-Siegert et al., 2017; Plomion et al., 2018; Hofmeister et al., 2019; Orr et al., 2020; Wang et al., 2019; Hanlon et al., 2019). For instance, Orr et al. (2020) found the somatic mutation rate per generation to be only ten times higher in *Eucalyptus melliodora* than in *Arabidopsis*, despite being > 100 times larger in size.

Thus, empirical evidence indicates that more long-lived species have acquired mechanisms to reduce the amount of mutations accumulated during growth on the one hand, but still present high levels of inbreeding depression on the other hand, which suggests that more long-lived species still accumulate more mutations despite above mentioned limiting mechanisms. The aim of the present study is to disentangle the relationship between these two observations. I first study the evolution of the mutation rate in plants, and then consider the number of mutations and the magnitude of inbreeding depression maintained at mutation-selection balance, given the evolutionarily stable mutation rate reached by the population. To do so, I extend the work of previous authors (Kimura, 1967; Gervais and Roze, 2017) to the case of a perennial population in which individuals grow as they age and accumulate mutations in doing so. I obtain analytical predictions which are then tested against the output of individual-centered simulations. I show that the evolutionarily stable mutation rate should decrease in plants as life expectancy increases, because deleterious mutations have more time to accumulate in more long-lived species. Furthermore, I show that despite substantially lower per year mutation rates, more long-lived species still tend to accumulate larger amounts of deleterious mutations because of higher per generation,

92 leading to higher levels of inbreeding depression in these species. However, the magnitude
93 of this increase depends strongly on how mutagenic meiosis is relative to growth.

2 Methods

94 **Model outline.** I consider a large population of hermaphroditic diploids. Individuals
95 survive between mating events with a constant probability S . Juveniles may only settle in
96 replacement of deceased individuals, so that population size is kept constant. Individuals
97 are assumed to be made of a trunk, which grows by one section between each flowering
98 event (FIG. 1). This growth model is neither intended to depict a particular kind of plants
99 nor to be a realistic model of plant growth. It was chosen because it is the simplest growth
100 model incorporating within-individual genetic mosaicism. Besides, as long as mutations
101 do not interfere with the growth process, as it is the case here (see below), more compli-
102 cated growth models would only alter the age distribution of sections within individuals,
103 which should not qualitatively alter the results presented in this study provided that older
104 individuals are still made of older sections on average.

105 Mutations at the selected loci occur both during meiosis and somatic growth. The
106 meiotic mutation rate of a given individual (u), which includes both mutations occurring
107 during meiosis and during the development of disposable reproductive parts, is determined
108 by its genotype at a single modifier locus. At this locus, I consider the fate of a rare mutant
109 (m) with a weak effect (ε) competing with a resident allele (M). This mutant allele is
110 codominant with the resident, so that an individual's meiotic mutation rate is given by
111 $u_{MM} = u_0$, $u_{Mm} = u_0 + \varepsilon$, or $u_{mm} = u_0 + 2\varepsilon$, depending on its genotype at the modifier.

112 Mutations occur due to the unrepaired misincorporation of nucleotides during DNA

113 replications, or due to DNA lesions occurring between replications which are not repaired
 114 in time before the next replication event (*i.e.* cell division), so that they end up being
 115 incorporated in the daughter cells' genome (Gao et al., 2016). Because there is, to my
 116 knowledge, no reason to expect DNA repair mechanisms to fundamentally differ between
 117 meiotic and somatic cell divisions, I hypothesized that meiotic and somatic mutation rates
 118 should evolve jointly to some extent. Importantly however, these two rates differ in at
 119 least two ways. First, they are not defined on the same scale. Indeed, while the somatic
 120 mutation rate is usually defined as a number of mutations per unit of growth, as it is the
 121 case in the present model, meiotic mutation rates are defined at the scale of a reproductive
 122 event. Thus, they may each cover a very different number of cell divisions, especially
 123 since recent empirical evidence has shown that the number of cell divisions separating
 124 axillary buds stem cells from those of the apical meristem they emerged from may be
 125 much lower than previously thought due to strong quiescence mechanisms (Burian et al.,
 126 2016). Second, meiotic cell divisions necessarily include recombination, causing additional
 127 double-strand DNA breaks and therefore giving the opportunity for more mutations to
 128 occur during meiosis than during mitosis (Magni and Von Borstel, 1962). Hence, the
 129 relationship between these two mutation rates is not straightforward, because different
 130 genetic events may happen and different numbers of cell divisions may occur over the course
 131 of a growth season and during a reproductive event. In the absence of a more mechanistic
 132 model, it is hard to give a biologically well-motivated shape to this relationship. Thus, in
 133 an effort to keep the model as simple as possible, I will assume that somatic mutations
 134 accumulate at rate γu per unit of growth (that is, per section, FIG. 1), where γ is a
 135 positive real number which allows one to tune the intensity of somatic mutation relative

136 to meiotic mutation. In other words, I assume there is a linear relationship between the
 137 two rates.

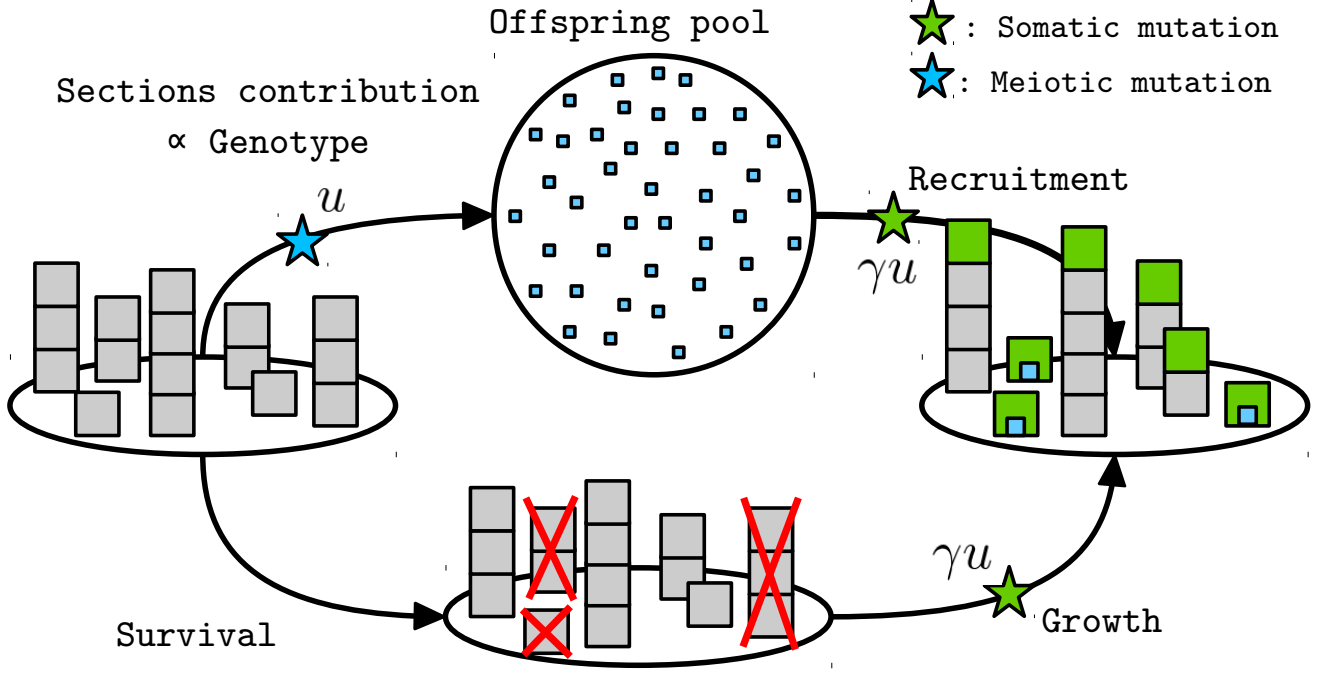


FIGURE 1: Life cycle of the modeled population. Small blue squares depict seeds. Green squares depict the sections grown during the last growing season. Juveniles go through one growing season before reproducing, and are therefore made of a single section as depicted by the green squares wrapping the small blue ones. Stars show the steps at which meiotic (blue) and somatic (green) mutation occurs. The rate at which mutation occurs is indicated beside each star.

138 I assume that any section can contribute to reproduction (FIG. 1). Self-fertilisation
 139 occurs at rate α , a fraction σ of which imperatively occurs within the same section. The
 140 remaining fraction $1 - \sigma$ can occur between sections within the individual. Introducing
 141 σ into the model enables one to study the effect of within versus between sections selfing
 142 more easily.

143 A section's fecundity is determined by its genotype at a very large number of biallelic
 144 loci acting multiplicatively. At these loci, allele 0 is an healthy allele, while allele 1 is a

mutated allele which diminishes the section's fecundity by a proportion s . In heterozygotes, allele 1 expresses proportionally to its dominance coefficient h . Following previous authors (Gervais and Roze, 2017), I also introduce a DNA replication fidelity cost function, f , which is an increasing function of the meiotic mutation rate u . Gervais and Roze (2017) considered a variety of cost functions and came to qualitatively similar conclusions in every case. Yet, most of their results were obtained using the cost function given in Equation (1),

$$f(u) = e^{-\frac{c}{u}}, \quad (1)$$

where c is the cost of replication fidelity, which is also used in this study. Thus, the fecundity of a section is given by

$$W = f(u) \times (1 - s)^{n_{\text{hom}}} (1 - sh)^{n_{\text{het}}}, \quad (2)$$

where n_{hom} and n_{het} are the number of mutations borne in the homozygous and heterozygous states, respectively.

Analytical methods. To study the model, I use the theoretical framework described in Kirkpatrick et al. (2002), which relies on indicator variables to describe individuals' multilocus genotypes. In the analytical work, the effect of the proportion of obligate within-section selfing (σ) is neglected since it is difficult to incorporate and will prove to have very little impact on the results. For the sake of brevity, derivations of the results presented in the following sections are detailed in Appendices I.1 and I.2 for results regarding the evolution of mutation rate and the mutation-selection equilibrium properties

163 of the population given the evolutionarily stable mutation rate, respectively.

164 **Individual-centered simulations.** Individual-centered simulations were run to test
165 the validity of analytical approximations. The simulation program was coded in C++11, is
166 available from GitHub and has been given a DOI using Zenodo (10.5281/zenodo.5166952).
167 In this program, individuals are represented by two chromosomes of length λ (expressed in
168 cM) with the modifier situated at the center and along which mutations can occur at any
169 position, so that infinitely many selected loci are effectively modeled (Roze and Michod,
170 2010).

171 **Modeled loci.** Following the work of Gervais and Roze (2017), it is assumed that
172 infinitely many alleles exist, coding for any value of $u \in [0, +\infty[$ exist at the modifier.
173 Mutations at the modifier occur at rate $u_m = 10^{-3}$, and the value coded by the new
174 allele is sampled from a Gaussian distribution centered on the former allele value with
175 standard deviation $\sigma_m = 10^{-2}$, which is truncated at zero to prevent the modifier from
176 going out of range. At selected loci, the number of mutations occurring on a chromosome
177 during a given mutation event is sampled from a Poisson distribution with mean u (γu for
178 somatic growth), and their position is sampled from a uniform distribution. Recombination
179 is modeled by exchanging segments between homologous chromosomes. The number of
180 crossing-overs is sampled in a Poisson distribution with mean λ and their positions are
181 sampled from a uniform distribution along chromosomes. Every time a mutation occurs,
182 the age of the section at which it occurred along the individual is stored, so that the
183 genotype of any section within an individual can be reconstructed at any time from the
184 individual genome. This method allows one to gain substantial computation time because

185 mutations are stored only once per individual instead of being copied once for each new
186 section.

187 **Sequence of events.** The population is kept of constant size, N . Between each
188 mating event, individuals have a constant survival probability S . If they survive, they
189 grow by one section, and mutations occur at rate γu in this section. If they die, they are
190 replaced by an offspring produced by the population. Any section within any individual
191 can be chosen as a parent, with a probability proportional to its fecundity (Equation
192 2). The offspring is produced by self-fertilisation with probability α , in which case the
193 chosen section mates with itself with probability σ , and with any section within the same
194 individual with probability $1 - \sigma$. When selfing occurs between sections, a second parental
195 section is selected within the individual. When the offspring is not produced by self-
196 fertilisation, which occurs at rate $1 - \alpha$, it is produced by random mating and a second
197 parent is selected from the whole population. Mutation occurs at rate u during meiosis.

198 **Measurements.** Once the equilibrium was reached, that is when both the muta-
199 tion rate and the average number of mutations per chromosome were at equilibrium, the
200 average number of mutations per chromosome in seeds, the average mutation rate and
201 inbreeding depression were measured. Although individuals are chimeric in the model, I
202 stuck with measuring inbreeding depression at the individual level to be in line with its
203 formal definition. To do so, I counted how many times each individual was chosen as
204 a parent before it died (*i.e.* I measured its lifetime reproductive success) and used this
205 quantity as a measure of lifetime fitness. Individuals were marked as being produced by
206 outcrossing (0), selfing within the same section (1), and selfing between sections within

207 the same individual (2), so that I was able to measure fitness differences between these
 208 various categories of individuals. Namely, I measured inbreeding depression, that is the
 209 decrease in fitness of selfed individuals relative to the outcrossed (δ_{01}), and autogamy de-
 210 pression (Schultz and Scofield, 2009; Bobiwash et al., 2013), that is the decrease in fitness
 211 of within-section selfed individuals relative to between-sections ones (δ_{12}). Ten replicates
 212 were run for each parameter set. Simulations were kept running for 10^6 and 2×10^5 re-
 213 productive seasons for life expectancies lower and higher than 200 reproductive seasons,
 214 respectively. Results were averaged over the last 10^5 reproductive cycles and 2×10^4 for
 215 life expectancies lower and higher than 200 reproductive seasons, respectively, and the
 216 95% confidence interval around the mean was also recorded.

3 Results

217 In what follows, life expectancy (E) will be used to discuss results instead of survival
 218 probability (S) for the sake of clarity and biological relevance. Given survival probability
 219 S , life expectancy can be computed as

$$E = \frac{1}{1 - S}. \quad (3)$$

3.1 Evolutionarily stable mutation rate

220 Let us first study the evolution of the mutation rate. It is shown in Appendix I.1 that the
 221 evolution of the mutation rate is the result of the opposition between the direct cost of
 222 DNA replication fidelity, which is higher when the mutation rate is lower, and the indirect
 223 selection caused by deleterious alleles which tend to be more frequently linked with modifier

alleles increasing the mutation rate (Equation A23). The resulting evolutionarily stable mutation rate is given by

$$u^* = \sqrt{-\frac{c}{\hat{s}_{ind}}}, \quad (4)$$

where \hat{s}_{ind} encapsulates the intensity of indirect selection acting on the modifier. Its expression is derived in Appendix I.1.5. FIGURE 2 shows the evolutionarily stable mutation rate as a function of life expectancy (top row), along with the intensity of indirect selection (bottom row), for cases where $\gamma = 1$, $\gamma = 0.1$ and $\gamma = 0.01$. I chose to focus on cases where $\gamma \leq 1$, that is on cases where more mutations are produced during meiosis (plus the production of disposable reproductive parts) than during the development of a new section, on the basis of three lines of evidence. First, direct observations of plant development at the cellular level indicate that cells destined to form axillary meristems undergo much fewer divisions than other cells from the moment they are produced in the apical meristem, which suggests that the number of cell divisions per branching event, and therefore the number of opportunities for mutations to accumulate, may be lower than previously thought (Burian et al., 2016). Second, estimates of somatic mutation rates per unit of growth tend to be low (Orr et al., 2020). Third, to my knowledge, the only experiment comparing the mutagenicity of meiosis and mitosis was performed by Magni and Von Borstel (1962) in yeast. They found meiosis to be 6 to 20 times more mutagenic than mitosis, which further suggests that γ may tend to be lower than 1. Besides, performing simulations with $\gamma > 1$ proved to be very challenging since the number of mutations accumulated in the population quickly became very high, causing simulations to run very slowly and consume a lot of resources.

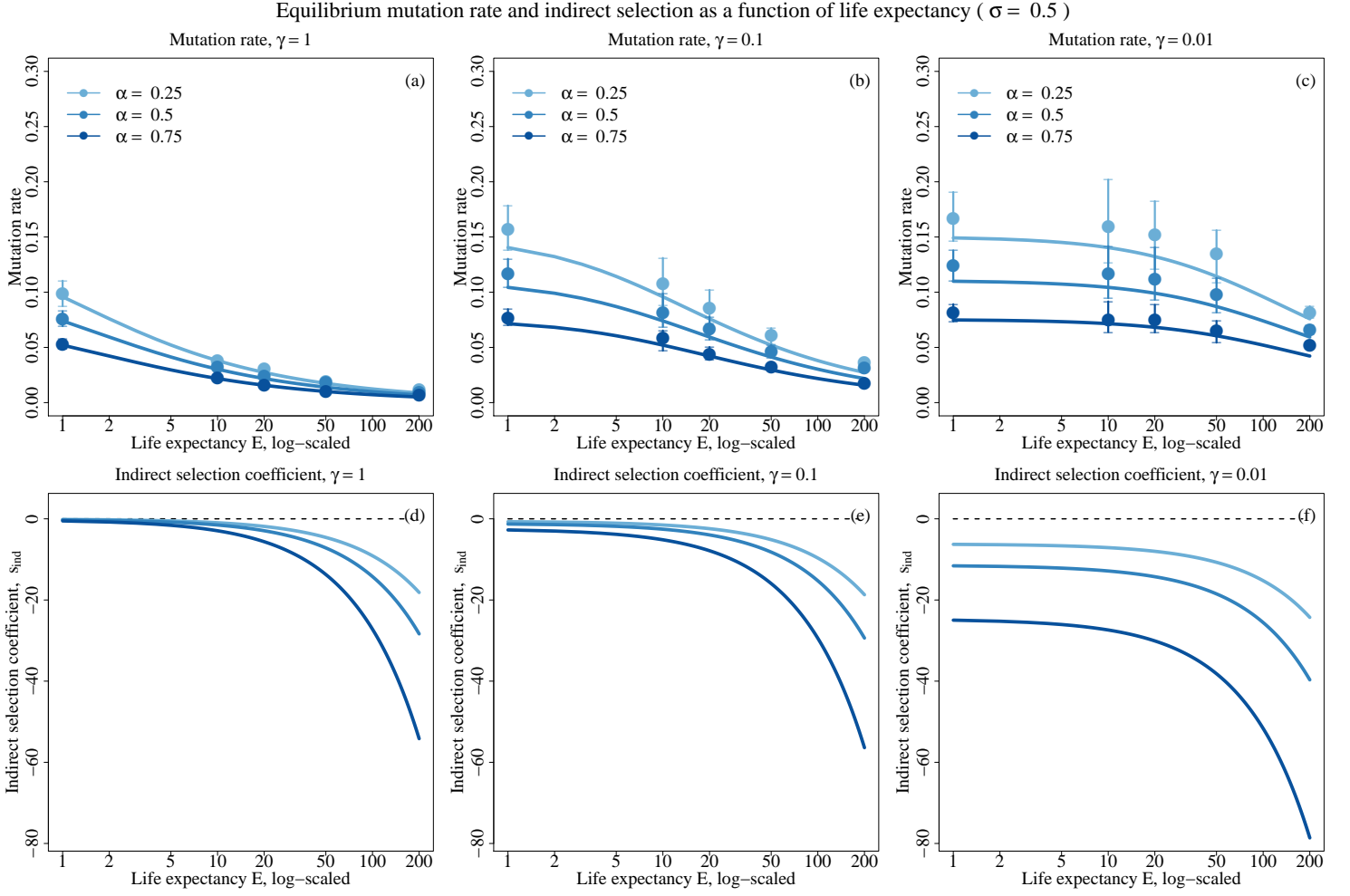


FIGURE 2: Evolutionarily stable mutation rate (top) and intensity of indirect selection (bottom) as a function of life expectancy (log-scaled) for various selfing rates (colors) and for $\gamma = 1$ (left), $\gamma = 0.1$ (middle) and $\gamma = 0.01$ (right). Other parameters values are $s = 0.05$, $h = 0.3$, $c = 0.0014$, $\lambda = 20$, and $\sigma = 0.5$. Dots depict simulation results and error bars depict the 95% confidence intervals. Lines depict analytical predictions.

245 The evolutionarily stable mutation rate decreases with life expectancy for all γ values
 246 (FIG. 2a-c). In both cases, this is due to the greater number of opportunities to accumulate
 247 deleterious mutations in more long-lived species because they go through more growth
 248 events, which in turn causes indirect selection to increase against alleles increasing the
 249 mutation rate because deleterious mutations become more numerous (FIG. 2d-f).

250 The mutation rate also decreases as the selfing rate (α) increases, which may seem

251 counter-intuitive since selfing tends to reduce the number of deleterious mutations seg-
252 regating in the population through purging [Roze \(2015\)](#). However, self-fertilisation also
253 causes genetic associations between selected loci and the modifier to increase, thereby in-
254 creasing indirect selection and resulting in a decrease of the evolutionarily stable mutation
255 rate when the selfing rate increases as shown by [Gervais and Roze \(2017\)](#). The results pre-
256 sented in FIG. 2 were obtained assuming half of selfing events occurred imperatively within
257 the same section ($\sigma = 0.5$). Cases with $\sigma = 0$ and $\sigma = 1$ were also investigated and yielded
258 very similar results, which are presented in FIG. S3 and S4, respectively, in Appendix II.
259 The very small effect of σ on the results is due to the relatively low evolutionarily stable
260 mutation rate, which causes few somatic mutations to occur during growth, and to the
261 fact that weak selection was assumed so that mutations have little effect on their bearer's
262 fitness.

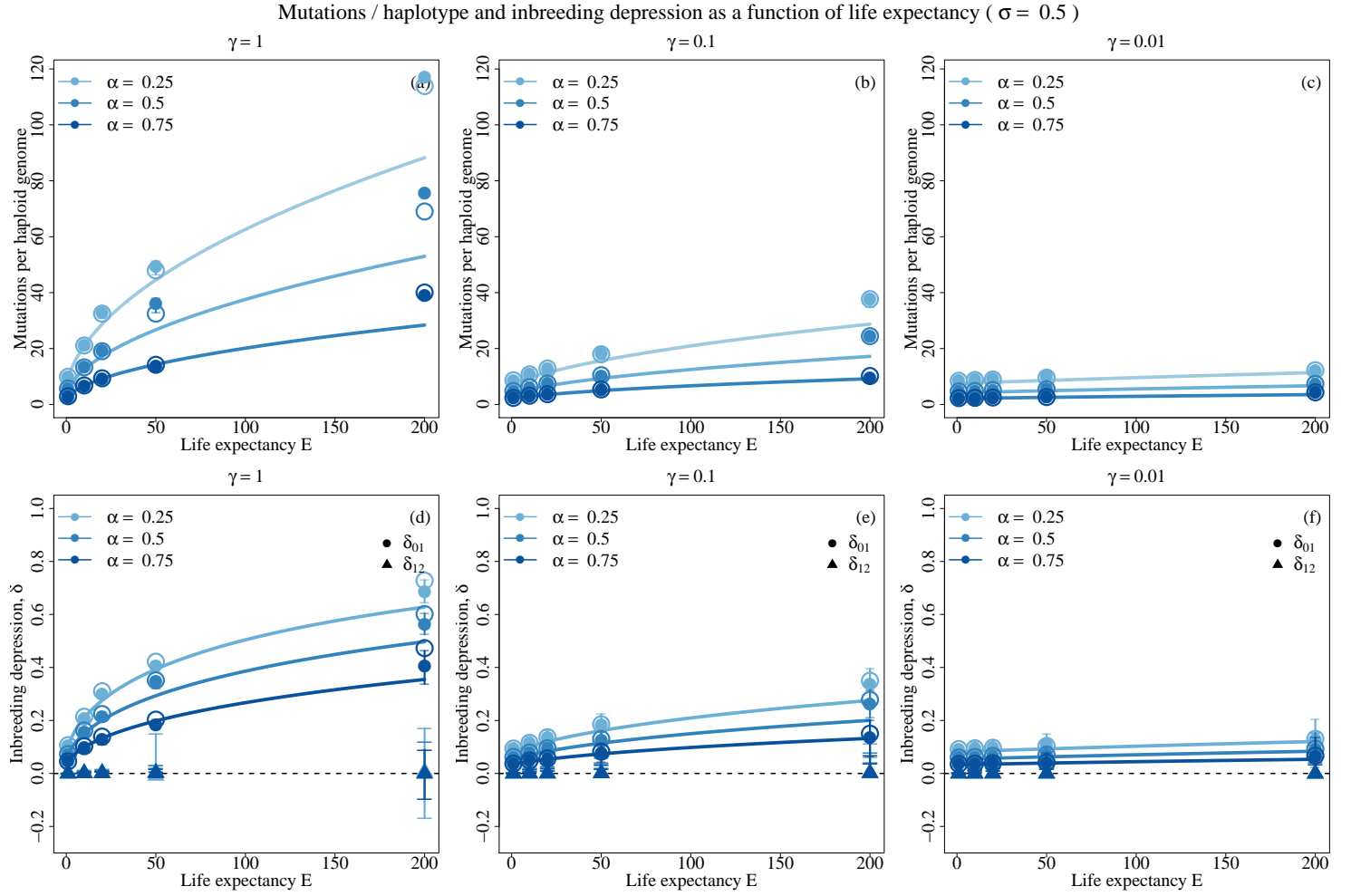


FIGURE 3: Average number of mutations per haploid genome (top) and inbreeding depression (bottom) as a function of life expectancy (log-scaled) for various selfing rates (colors) and $\gamma = 1$ (left), $\gamma = 0.1$ (middle) and $\gamma = 0.01$ (right). Other parameters values are $s = 0.05$, $h = 0.3$, $c = 0.0014$, $\lambda = 20$, and $\sigma = 0.5$. Filled dots depict simulation results and error bars depict the 95% confidence intervals. Lines depict analytical predictions. Open circles depict the value predicted by our analytical model when the equilibrium mutation rate from simulations is used instead of Equation 4. On the bottom row, dots indicate inbreeding depression (δ_{01}), while triangles indicate autogamy depression (δ_{12}).

3.2 Mutation-selection balance

263 Once the mutation rate has reached an equilibrium and the population is at mutation-
 264 selection balance, I show in Appendix I.2.1 that a leading order approximation of the

265 average number of mutations per haploid genome in juveniles (n) is given by

$$n \approx \frac{\hat{u}^*}{s[h + F(1 - h)]} - \gamma u^* \frac{S}{1 - S}, \quad (5)$$

266 where $u^* = \sqrt{-\frac{c}{\hat{s}_{ind}}}$, and $\hat{u}^* = \left(1 + \frac{\gamma}{1 - S}\right) u^*$ depicts the total mutation rate of the

267 population over the course of one timestep, including both meiotic and somatic mutations.

268 As for inbreeding depression calculated between outcrossed and selfed individuals (δ_{01}), it

269 is given by

$$\delta_{01} = 1 - \exp \left[-s(1 - 2h) \frac{1 + F}{2} \left(\frac{\hat{u}^*}{s[h + F(1 - h)]} - \gamma u^* \frac{S}{1 - S} \right) \right], \quad (6)$$

270 where $F = \frac{\alpha}{2 - \alpha}$, to leading order in s . Again, I do not consider the impact of the

271 proportion of selfing occurring within or between sections (σ) in the analytical model since

272 it is negligible. FIGURE 3 shows the number of mutations per haploid genome among

273 juveniles (n , top row), and inbreeding and autogamy depression (δ_{01} and δ_{12} , bottom

274 row) at mutation-selection balance. Deviations between analytical predictions (lines) and

275 simulations results (dots) are observed. They can be explained by the slight differences

276 between the predicted evolutionarily stable mutation rate and the equilibrium mutation

277 rate reached by simulations, which build up large differences in n when life expectancy

278 becomes high. Indeed, when the equilibrium mutation rate from the simulations is used

279 to predict n instead of Equation (4), the agreement between predictions (open circles) and

280 simulation results (dots) is restored.

281 The number of mutations maintained n increases with life expectancy in every case

282 due to the greater amount of opportunities for mutations to accumulate in more long-

283 lived species. Indeed, the denominator of the first term in Equation (5) shows that the
 284 intensity of selection is independent of life expectancy, while the total mutation rate \hat{u}^* is
 285 an increasing function of life expectancy in all investigated cases despite the fact that the
 286 equilibrium mutation rate *per mutagenic event* (u^*) decreases in more long-lived species
 287 (FIG. S1 in Appendix II shows the total mutation rate as a function of life expectancy in
 288 said cases). The increase of n with life expectancy becomes much lower when γ decreases
 289 to the point of being barely noticeable with $\gamma = 0.01$, despite the fact that the equilibrium
 290 meiotic mutation rate is slightly higher in that case. This result is generated by the joint
 291 effect of γ , which reduces the contribution of somatic mutations as it decreases, and of
 292 the evolution of mutation rate which is lower at the evolutionary equilibrium in more
 293 long-lived species (as an additional illustration, FIG. S2 in Appendix II compares the
 294 obtained \hat{u}^* with the one expected if the evolutionarily stable mutation rate for annuals,
 295 that is $E = 1$, is assumed for all life expectancies for various γ values). As a result of these
 296 effects, inbreeding depression gets lower as γ decreases and increases as life expectancy
 297 increases, but this increase becomes less and less marked for smaller γ values. Furthermore,
 298 consistent with the negligible effect σ had on the evolution of the mutation rate, almost
 299 no autogamy depression is generated (triangles in FIG. 2, bottom row).

4 Discussion

300 In this paper, I studied the evolution of the mutation rate when mutations accumulating
 301 during growth are assumed to be inheritable, and considered the consequences of such
 302 mutation accumulation for mutation load and inbreeding depression in species with varying
 303 degrees of perenniality.

4.1 Evolution of the mutation rate

I showed that the evolutionarily stable mutation rate decreases as life expectancy increases because of the greater number of opportunities to accumulate mutations during growth in more long-lived species, which makes indirect selection against alleles increasing the mutation rate stronger. However, although the mutation rate per mutagenic event (u), that is per growth season or per meiosis in the present model, decreased in more long-lived species, the total mutation rate (\hat{u}), that is the rate at which mutations entered the population through both somatic growth and meiosis, increased. Hence, results indicate that while we should expect more efficient mechanisms reducing the accumulation of deleterious mutations during growth to evolve in more long-lived species, so that their per unit of growth and per year mutation rate should be lower, their per generation mutation rates should still be higher. These predictions are in line with empirical evidence, which suggest that mutation rates per generation tend to be higher in more long-lived species although the mutation rates per unit of growth tend to be lower (Hofmeister et al., 2019; Hanlon et al., 2019; Orr et al., 2020).

I modeled the evolution of the mutation rate following the work of Kimura (1967), by assuming there is a direct fitness cost to DNA replication fidelity opposing the indirect selection generated by deleterious mutations linked to the modifier, so that the mutation rate was maintained greater than zero in response to a trade-off. An alternative mechanism, which is not mutually exclusive with the trade-off described above, was put forward by Lynch (2011). They proposed that selection should always act to reduce the mutation rate, down until it becomes so low that the selective advantage brought by any further reduction should be overwhelmed by genetic drift, thus maintaining non-zero mutation

326 rates because alleles further decreasing the mutation rate should at some point become
 327 effectively neutral, and thereby creating a lower bound for the evolution of the mutation
 328 rate (Lynch, 2011). This lower bound is inevitably influenced by effective population size,
 329 as it plays on the relative strength of selection and genetic drift. In the present model,
 330 I overlooked Lynch (2011)'s lower bound by assuming a large and fixed population size.
 331 Yet, effective population sizes are expected to be higher in more long-lived species in
 332 which generations overlap (Felsenstein, 1971; Charlesworth, 1980; Petit and Hampe, 2006;
 333 Duminil et al., 2009), which implies the lower bound described by Lynch (2011) should
 334 be met for lower mutation rates in said species. Hence, we should expect the decrease
 335 in the evolutionarily stable mutation rate described in this study to become sharper in
 336 conditions where Lynch (2011)'s lower bound is expected to matter for the evolution of
 337 the mutation rate.

4.2 Inbreeding depression

338 The larger total mutation rate in more long-lived species led to the maintenance of more
 339 mutations in the population at mutation-selection balance, and therefore to higher in-
 340 breeding depression in these species, consistent with results from meta-analyses which
 341 found inbreeding depression to increase in larger-statured, more long-lived species (Du-
 342 minil et al., 2009; Angeloni et al., 2011). Importantly however, the magnitude of the
 343 increase in the total mutation rate, and therefore in inbreeding depression with life ex-
 344 pectancy depended strongly on the relative mutagenicity of meiosis and growth, which
 345 was controlled by the γ parameter in this model. Indeed, while the increase in inbreeding
 346 depression was strong when γ was close to 1, that is when the same amount of mutation

347 was produced during meiosis and during growth between two flowering seasons, it became
 348 smaller as γ decreased, to the point of being barely noticeable for $\gamma = 0.01$. This was due
 349 to both the decrease of the evolutionarily stable total mutation rate (\hat{u}^*) and to the de-
 350 crease of γ , which made the contribution of somatic mutations to the mutation load more
 351 and more negligible compared with meiotic mutations. Hence, according to the results
 352 presented in this paper, for somatic mutations to be the main driver of the empirically
 353 observed increase in inbreeding depression in more long-lived species, roughly the same
 354 amount of mutations should be produced during growth between two flowering seasons
 355 and during reproduction.

4.3 Mating system evolution

356 Inbreeding depression is thought to be one of the main factors preventing the evolution of
 357 self-fertilisation (Lande and Schemske, 1985; Barrett and Harder, 2017). In Angiosperms,
 358 consistent with the observed increase in inbreeding depression in more long-lived species,
 359 there exists a strong correlation between mating systems and life-histories. Indeed, many
 360 self-fertilising species are annuals whereas most long-lived species are strictly outcrossing
 361 (Barrett and Harder, 1996; Munoz et al., 2016). Thus, somatic mutations accumulation
 362 was proposed as an explanation for this correlation (Schofield and Schultz, 2006). While the
 363 results presented in this study indicate that inbreeding depression increases with respect
 364 to life expectancy due to somatic mutations accumulation, particularly when γ is large,
 365 this increase is tempered by the decrease of the evolutionarily stable mutation rate with
 366 life expectancy. Furthermore, in agreement with results obtained by Gervais and Roze
 367 (2017), I showed that the evolutionarily stable mutation rate decreases as the selfing rate

368 increases because the modifier becomes more strongly associated with selected loci. These
 369 decreases of the mutation rate with respect to mating system and life expectancy, together
 370 with the purging effect of self-fertilisation (Roze, 2015), result in a substantial drop in the
 371 magnitude of inbreeding depression as the selfing rate increases in more long-lived species,
 372 potentially opening the way for the evolution of self-fertilisation. Hence, whether somatic
 373 mutations accumulation is sufficient to explain the correlation between life-history and
 374 mating system in Angiosperms when the mutation rate is allowed to evolve jointly with
 375 the mating system remains an open question.

4.4 Autogamy depression

376 In order to empirically estimate the contribution of somatic mutations accumulation to
 377 inbreeding depression using phenotypic data, a method was developed by Schultz and
 378 Scofield (2009). This method, called the autogamy depression test, relies on the compar-
 379 ison of the fitnesses of individuals produced by selfing within an inflorescence with those
 380 of individuals produced by selfing between distant inflorescences on the plant's crown
 381 (Schultz and Scofield, 2009; Bobiwash et al., 2013). In this paper, I performed such test
 382 by measuring autogamy depression (δ_{12}). Contrary to inbreeding depression, I found auto-
 383 gamy depression to be almost null in every case, even in situations where the contribution
 384 of somatic mutations accumulation to inbreeding depression was high. This result can be
 385 explained by the low evolutionarily stable mutation rates, and by the fact that we only
 386 considered mutations with a weak fitness effect. It suggests that the autogamy depression
 387 test should only be able to detect mutations with a large fitness effect in large individ-
 388 uals, where mutations have had time to accumulate. Thus, it implies that detecting no

389 autogamy depression in a given population cannot be taken as evidence of a negligible
390 contribution of somatic mutations accumulation to the population's mutation load.

4.5 Germline segregation and relative mutagenicity of growth and meiosis

391 The results presented above suggest that valuable insights into the evolutionary relevance
392 of somatic mutations and the evolution of the mutation rate in plants could be gained by
393 further investigating the γ parameter in this model, which depicts the relative mutagenicity
394 of meiosis and growth between two flowering seasons, and is likely influenced by at least
395 three important factors that were either overlooked or only partially accounted for in this
396 study.

4.5.1 Relative mutagenicity of meiosis and mitosis

397 First, it is necessarily influenced by how mutagenic meiotic divisions are in comparison with
398 mitotic divisions, about which little is known although one may expect meiotic divisions to
399 generate more mutations, as they generate many more double strand DNA breaks which
400 are required for recombination and are known to be particularly mutagenic events (Magni
401 and Von Borstel, 1962; Arbel-Eden and Simchen, 2019).

4.5.2 Number of cell divisions separating meristems

402 Second, it is influenced by the number of mitoses occurring between flowering buds. This
403 number depends on the growth habit of the considered species, because fast growing species
404 undergo more mitoses per unit of time than slow growing species, and because the rate
405 at which mitoses occur, and thus the growth rate, may interact with the evolution of the

406 mutation rate. For instance, investing in a higher fidelity of DNA replication may tend to
407 slow down individual growth.

408 The number of mitoses separating two meristems also depends on patterns of meris-
409 tematic stem cell divisions that were recently brought to light (Burian, 2021). Indeed,
410 although it has long been thought that the germline remains unsegregated up until a
411 meristem switches to the floral state in plants, Burian et al. (2016) showed that within
412 the apical meristem, the stem cells give rise to a specific cell lineage which will serve as
413 the axillary meristems' stem cells and spend most of their time in a quiescent, almost
414 non-dividing state, contrary to surrounding cell lineages which divide vigorously to effect
415 plant growth. Thanks to this mechanism, the number of mitoses separating two meristems
416 is greatly reduced and so is the number of cell divisions separating the seed from the ga-
417 metes, as the germline directly emerges from these stem cells. Hence, although it is clear
418 that the plant germline remains undifferentiated up until reproduction is triggered, it may
419 be considered segregated prior to differentiation, because the cells giving rise to it do not
420 suffer the same fate as surrounding somatic cell lineages, thus behaving as a functional
421 germline (Romberger et al., 1993; Burian, 2021). The exact timing of such segregation
422 during plant development is, however, not known (Lanfear, 2018). Therefore, it is impor-
423 tant to point out that the results presented in this study not only hold if the germline
424 segregates late in development, but that they would also hold if the germline was actually
425 segregated as early as the first embryonic cell division (as it is the case in animals) and
426 remained sheltered within meristems. Indeed, such segregated germline would still have
427 to go through a non-zero number of mitotic cell divisions to be passed from one meristem
428 to the next due to developmental constraints (Burian, 2021), so that the number of cell

divisions it goes through before reproduction would still be affected by individual growth and be higher in more long-lived, larger species. In summary, the validity of the results presented in this paper does not depend on the degree to which the germline is actually segregated in plants, but the existence of a functional germline as described above, irrespective of when germline segregation occurs, supports the idea that plants acquired physiological mechanisms favoring lower values of γ .

4.5.3 Intra-organismal selection

Finally, apart from mechanisms reducing the amount of mutations produced during growth, deleterious mutations may also be affected by intra-organismal selection, which may not only reduce the growth rate by eliminating mutated cells, but also efficiently purge deleterious mutations from the organism, so that little to no somatic mutation may be present in the gamete, which could make γ smaller among the mutations effectively transmitted to offspring. This could in turn affect the evolution of the mutation rate. Little is known, however, about the actual efficacy of intra-organismal selection in removing deleterious mutations since it was seldom investigated theoretical (Otto and Orive, 1995), and mostly empirically demonstrated to occur in the case of strongly beneficial mutations (e.g. Edwards et al., 1990; Simberloff and Leppanen, 2019).

The various elements discussed above show that γ is an emerging property of the interaction between a variety of physiological mechanisms rather than a fixed quantity, which advocates for the development of theoretical models treating it as such rather than as a fixed parameter, by incorporating growth, mutation and selection at the cellular level.

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