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 constrasting 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

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

Inbreeding depression, that is the decrease in fitness of inbred relative to outbred individuals (Charlesworth and Charlesworth, 1987), is thought to be mostly generated by recessive deleterious mutations maintained at mutation-selection balance in populations (Charlesworth and Willis, 2009). Hence, Scofield and Schultz (2006) proposed that somatic mutations accumulation could lead to higher inbreeding depression in larger and more long-lived species. Consistent with this view, inbreeding depression was indeed shown to increase strongly as life expectancy increases across plant species (Duminil et al., 2009; 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 contrastring 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 become weaker than genetic drift at some point in finite populations, thereby favoring the persistence of non-zero mutation rates. Nevertheless, the inheritability of somatic mutations 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 mutations 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 signficantly 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,

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.

2 Methods

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

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

Mutations occur due to the unrepaired misincorporation of nucleotides during DNA

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

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

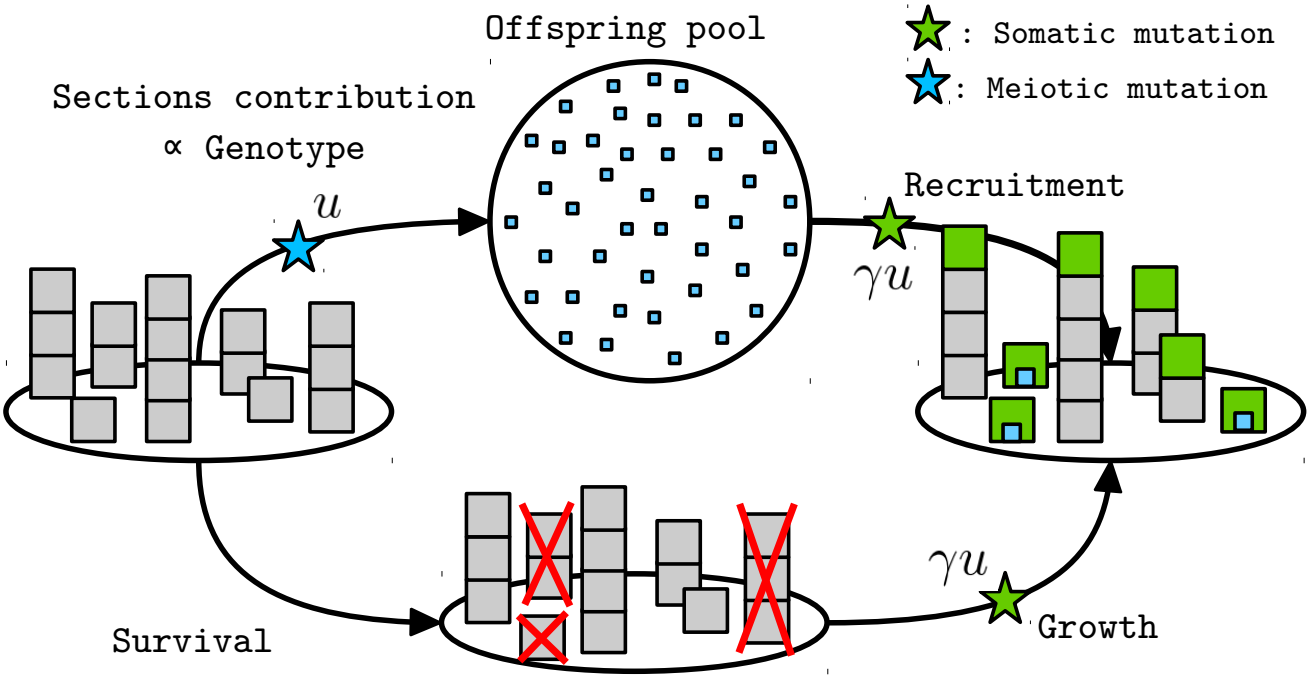

FIGURE 1: Life cycle of the modeled population. Small blue squares depict seeds. Green squares depict the sections grown during the last growing season. Juveniles go through one growing season before reproducing, and are therefore made of a single section as depicted by the green squares wrapping the 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.

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

A section's fecundity is determined by its genotype at a very large number of biallelic 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),

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

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

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

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

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

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

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

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

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

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

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

3 Results

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

$$E = \frac{1}{1 - S}. \tag{3}$$

3.1 Evolutionarily stable mutation rate

Let us first study the evolution of the mutation rate. It is shown in Appendix I.1 that the evolution of the mutation rate is the result of the opposition between the direct cost of DNA replication fidelity, which is higher when the mutation rate is lower, and the indirect 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

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

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 $\gamma \leqslant 1$, 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.

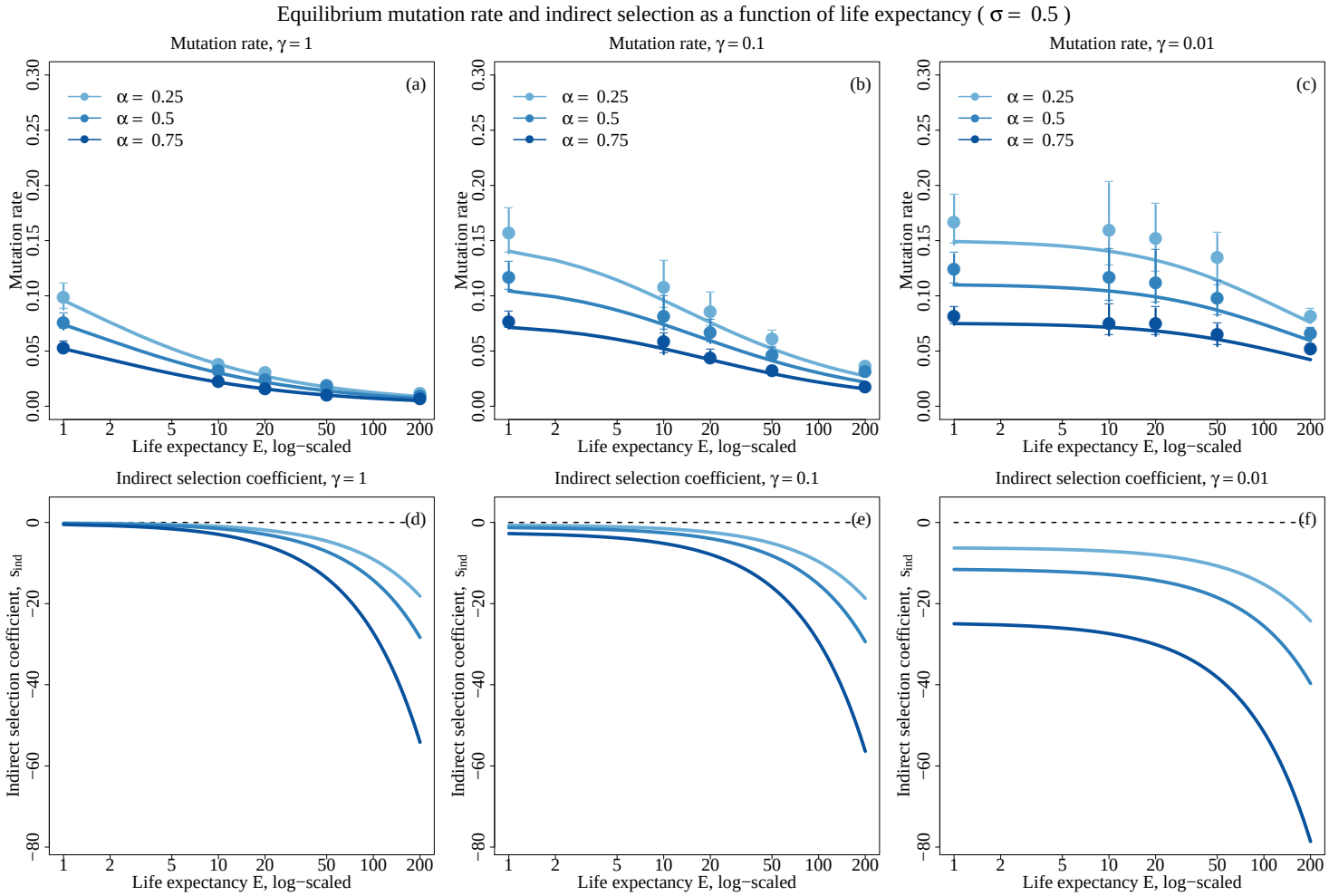

FIGURE 2: Evolutionarily stable mutation rate (top) and intensity of indirect selection (bottom) as a function of life expectancy (log-scaled) for various selfing rates (colors) and for $\gamma = 1$ (left), $\gamma = 0.1$ (middle) and $\gamma = 0.01$ (right). Other parameters values are $s = 0.05$, $h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

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

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

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

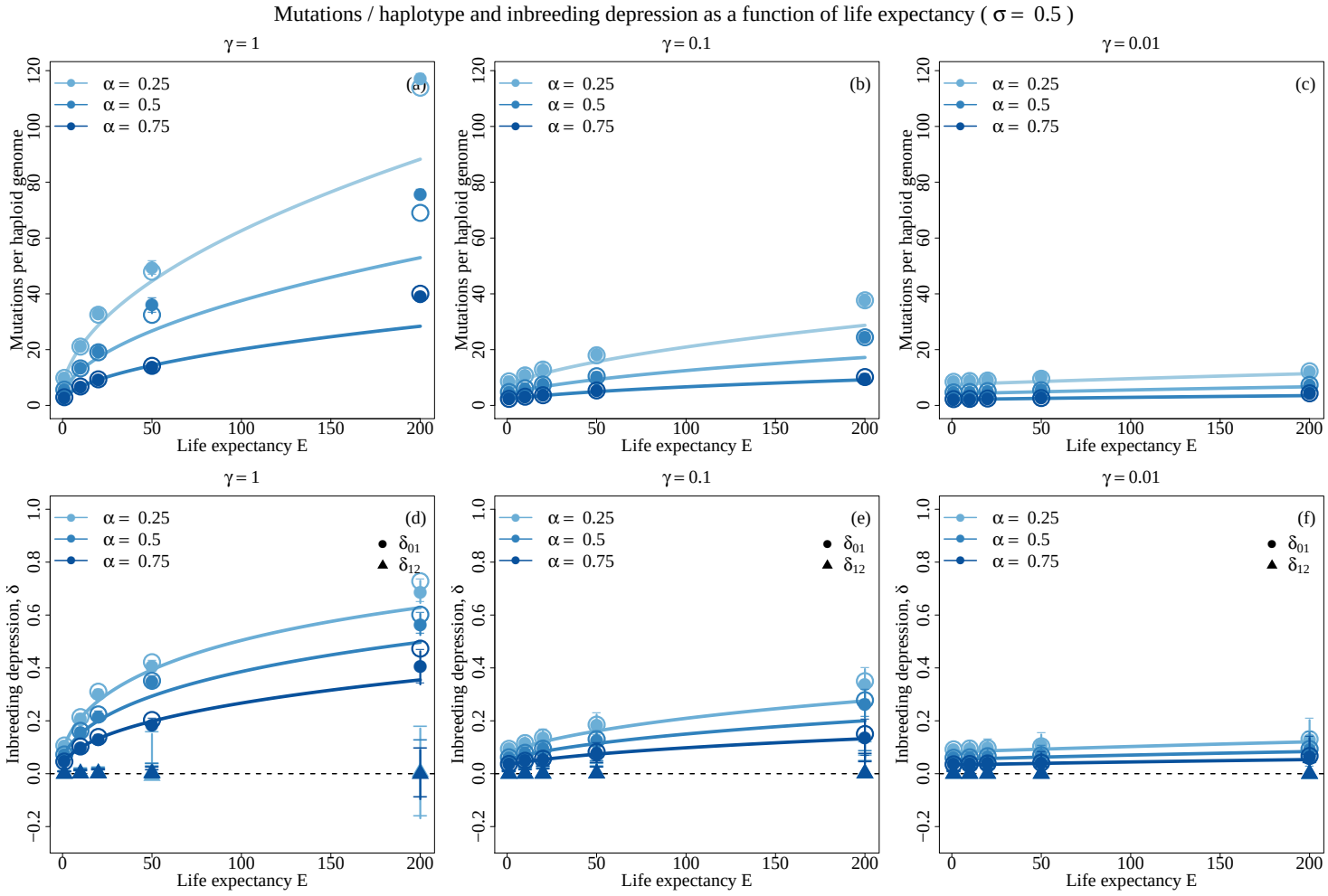

FIGURE 3: Average number of mutations per haploid genome (top) and inbreeding depression (bottom) as a function of life expectancy (log-scaled) for various selfing rates (colors) and $\gamma = 1$ (left), $\gamma = 0.1$ (middle) and $\gamma = 0.01$ (right). Other parameters values are $s = 0.05$, $h = 0.3$, $c = 0.0014$, $\lambda = 20$, 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

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

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

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

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 population over the course of one timestep, including both meiotic and somatic mutations. As for inbreeding depression calculated between outcrossed and selfed individuals (δ_{01}), it is given by

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

where $F = \frac{\alpha}{2-\alpha}$, to leading order in s. Again, I do not consider the impact of the proportion of selfing occurring within or between sections (σ) in the analytical model since it is negligible. FIGURE 3 shows the number of mutations per haploid genome among juveniles (n, top row), and inbreeding and autogamy depression (δ_{01} and δ_{12}, bottom row) at mutation-selection balance. Deviations between analytical predictions (lines) and simulations results (dots) are observed. They can be explained by the slight differences between the predicted evolutionarily stable mutation rate and the equilibrium mutation rate reached by simulations, which build up large differences in n when life expectancy becomes high. Indeed, when the equilibrium mutation rate from the simulations is used to predict n instead of Equation (4), the agreement between predictions (open circles) and simulation results (dots) is restored.

The number of mutations maintained n increases with life expectancy in every case due to the greater amount of opportunities for mutations to accumulate in more long-

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

4 Discussion

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

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

4.2 Inbreeding depression

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

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

4.3 Mating system evolution

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

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

4.4 Autogamy depression

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

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

4.5 Germline segregation and relative mutagenicity of growth and meiosis

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

4.5.1 Relative mutagenicity of meiosis and mitosis

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

4.5.2 Number of cell divisions separating meristems

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

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

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