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Abstract

The mutational meltdown, in which demographic and genetic processes mutually reinforce each other to accelerate the extinction of small populations, has been poorly quantified despite its paramount importance in conservation biology. Here, we present a model-based framework to study and quantify the mutational meltdown in a finite diploid population evolving continuously in time and subject to resource competition. We model slightly deleterious mutations affecting the population demographic parameters and we study how the rate of mutation fixation increases as the genetic load increases, a process that we investigate at two time scales: an ecological and a mutational scale. Unlike most previous studies we treat population size as a random process in continuous time. We show that, as deleterious mutations accumulate, the decrease in mean population size accelerates with time, relative to a null model with a constant mean fixation time. Studying the evolution of the mean fixation time at each new fixation allows us to quantify the mutational meltdown, which appears less severe than predicted by earlier theoretical work. We also emphasize the importance of measuring not only mean population size, but also demographic parameters, to assess extinction risks.

1 Introduction

Many evolutionary and ecological processes operating in natural populations influence, and are influenced by, population abundance or density (e.g. intraspecific and interspecific competition (Volterra, 1931; Lotka, 1932; Verhulst, 1844), reproduction (Clay and Shaw, 1981), trait evolution (Lande, 1976; Cherry, 1998), or fixation of deleterious mutations (Crow and Kimura, 1970, pp.418-430)). These abundance or density-dependent processes commonly affect population growth, leading to positive (as in the case of cooperation) or negative (as in the case of intraspecific competition) correlations between population growth and abundance/density. Allee effects refer to a positive relationship between population size and

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the population per capita deterministic growth rate (Stephens and Sutherland, 1999), at low density. Allee effects limit population viability because the growth rate of populations with an Allee effect becomes negative below a threshold abundance. This phenomenon alone can lead to extinction, but it may also be associated with gradual genetic deterioration, which reinforces the decline in population abundance and results in an extinction vortex (Gilpin and Soulé, 1986). The mutational meltdown (Lynch and Gabriel, 1990) is a particular form of extinction vortex in which demographic and genetic processes mutually reinforce each other. Spontaneous mildly deleterious mutations (Drake et al., 1981; Lynch et al., 1999; Haag-Liautard et al., 2007) can go to fixation and accumulate in small populations, leading to reduced growth rate and reduced population size, which in turn speeds up mutation accumulation (Lynch and Gabriel, 1990) and can precipitate population extinction.

The mutational meltdown has received much theoretical attention (Lynch et al., 1995; Theodorou et al., 2009; Robert, 2011) and is one of the main justifications for incorporating genetics into quantitative assessments of species or population viability (Traill et al., 2007), minimum viable population sizes (Shaffer, 1981; Gilpin and Soulé, 1986), and conservation status (Mace and Purvis, 2008). However, most existing theoretical treatments of the mutational meltdown remain qualitative, despite its paramount importance in conservation biology (Spielman et al., 2004). There is thus a need for more quantitative frameworks allowing to quantify the meltdown that results from the reciprocal interaction between demography and genetics, i.e. to gauge the actual acceleration of mutation accumulation, as compared with a null model assuming no effect of the current genetic load on the rate of future mutation accumulation, and to examine how demographic parameters affect the strength of the mutational meltdown. In this paper we present a new quantitative approach to analyzing mutational meltdown by computing the probability of fixation of slightly deleterious mutations in diploid populations with stochastic population dynamics. We are specifically interested in the reciprocal interaction between population size and fixation of small-effect deleterious mutations (Lande, 1994) in small populations. Unlike most previous studies (e.g. Lynch and Gabriel, 1990; Lande, 1994), we do not regard population size as a constant parameter, but instead build on the pioneering work of Champagnat et al. (2006) and treat population size as a random process in continuous time. The probability distribution of population size depends on individual demographic parameters, which are themselves determined by the genotypes of individuals at a large number of loci subject to recurrent deleterious mutation. Using this model of mutation accumulation in diploid populations with stochastic population dynamics, we assess how the demography-genetics (hereafter demo-genetic) feedback accelerates mutation accumulation in comparison to a null model assuming no effect of mutation accumulation on fixation time. We highlight that demographic parameters (birth and death rates) sometimes provide a more accurate picture of extinction risk than the more widely used average population size. For example, the sensitivity of the rate of fixation of mutations to changes in demographic parameters (which themselves dependent on the current fixation load) can be used to quantify the extent to which the overall demo-genetic feedback increases the risk of extinction. This allows us to identify situations where the demographic properties of populations might be associated with strong mutational meltdown.

2 The Model

We consider a population of hermaphroditic, randomly mating diploid organisms, following a logistic birth-and-death process. Mutation to slightly deleterious alleles follows an infinite site model, so that each new mutation occurs at a new locus at genomic rate $m_K = 2m/K$, where K is a scale parameter that goes to infinity to model rare mutations, a classical assumption in evolutionary genetic studies (e.g. Lande, 1994). We are interested in how the rate of fixation of deleterious mutations changes as mutations accumulate, and in the resulting change in demographic parameters, which we model at two time scales: an ecological and a mutational time scales. At the ecological time scale (i.e. in the limit when K goes to infinity without rescaling of time), a given mutation is lost or fixed before the next occurs. Individuals

are hence characterized by their genotype at the mutant locus, with two alleles (wild-type and deleterious mutant), and we examine the fate of a single deleterious allele evolving in a genetically homogeneous background (see section 2.1). In contrast, at the longer mutational time scale, new mutations are instantly fixed or lost and we examine the process of mutation accumulation and subsequent mutational meltdown in a population that is monomorphic at all times (see sections 2.2 and 3).

2.1 Evolution of a single biallelic locus at the ecological time scale

At the ecological time scale, we consider a single biallelic locus with a wild-type allele A and a mutant deleterious allele a. At this time scale, in the limit when K goes to infinity, as the mutation rate $m_K = 2m/K$ goes to 0, no other mutation occurs before the current mutation is fixed or lost. The population $Z_t := (i_t, j_t, k_t)$ is then defined at each time t by i_t, j_t, and k_t, the number of individuals with genotypes AA, Aa, and aa respectively. The process $(Z_t)_{t\geqslant 0}$ jumps from a point of $\mathbb{N}^3$ to one of its neighbors at a rate given by the birth and death rates of each genotype. We assume density-independent per capita fecundities b. In contrast, per capita death rates are density-dependent and combine intrinsic mortality (d) with mortality caused by intraspecific competition (rate c for any pair of individuals). In the absence of competition ($c = 0$), mean individual lifetime is thus $1/d$ and is decreased for $c > 0$. We assume that the deleterious allele a affects intrinsic death rates only, such that d is increased by δ and δ' in the heterozygote and homozygote genotypes, respectively. For $Z = (i, j, k)$ such that $N := i + j + k \geqslant 3$, the individual death rates of genotypes AA, Aa, and aa are thus respectively:

$$\begin{aligned} d_{AA}(Z) &:= d + c(N-1), \\ d^{\delta}_{Aa}(Z) &:= d + \delta + c(N-1), \\ d^{\delta'}_{aa}(Z) &:= d + \delta' + c(N-1). \end{aligned} \tag{1}$$

In principle, the selection parameters δ and δ' can be of either sign, but here we consider deleterious mutations only (i.e. $\delta' > 0$; the sign of δ is discussed later). Individuals are hermaphroditic and self-incompatible, all genotypes have identical fecundities b and we assume Mendelian reproduction, so that the total birth rates of genotypes AA, Aa and aa are:

$$\begin{aligned} b_{AA}(Z) &:= b\left[\frac{i(i-1)}{N-1} + \frac{ij}{N-1} + \frac{j(j-1)}{4(N-1)}\right], \\ b_{Aa}(Z) &:= b\left[\frac{ij}{N-1} + \frac{j(j-1)}{2(N-1)} + \frac{jk}{N-1} + \frac{2ik}{N-1}\right], \\ b_{aa}(Z) &:= b\left[\frac{k(k-1)}{N-1} + \frac{jk}{N-1} + \frac{j(j-1)}{4(N-1)}\right]. \end{aligned}$$

A key assumption of our model is that no death occurs when only two individuals are left in the population. This prevents extinction, but does not hamper our study of the accumulation of deleterious mutations and of the resulting decrease in mean population size. Without extinction, one of the two alleles eventually goes to fixation; we are interested in the probability $u^{\delta,\delta'}_{i,1,0}$ (which also depends on b, d, and c) that the mutant allele a goes to fixation given an initial population $(i, 1, 0)$, i.e. a population of $i+1$ AA individuals in which a single mutation occurred. More generally we denote by $u^{\delta,\delta'}_{i,j,k}$ the probability that allele a goes to fixation given an initial population (i, j, k). In the neutral case ($\delta = \delta' = 0$), probability theory tells us that $u^{0,0}_{i,j,k}$ is the initial frequency of allele a (see for example Crow and Kimura (1970), or Ewens (2004, p. 21)): $u^{0,0}_{i,j,k} = (j + 2k)/2N$, with $N = i + j + k$. In particular, $u^{0,0}_{i,1,0} = 1/2N = 1/2(i + 1)$. Note that this result does not hold if the population is allowed to go extinct: in this case, there is a non-zero probability that no allele goes to fixation. Assuming weak selection (slightly deleterious effects δ and δ'), we can approximate $u^{\delta,\delta'}_{i,j,k}$ by its Taylor expansion, i.e. we approximate the difference in the probability of

fixation between slightly deleterious alleles and neutral alleles by a linear function of δ and δ'. We prove that $(\delta, \delta') \mapsto u_{i,j,k}^{\delta,\delta'}$ is differentiable in $(0,0)$. Then we can write:

$$u_{i,j,k}^{\delta,\delta'} = \frac{j+2k}{2N} - \delta v_{i,j,k} - \delta' v'_{i,j,k} + o(|\delta| + |\delta'|),$$

where v and v' are also functions of demographic parameters b, d, and c. Note that the fixation probability, starting from state $(i,1,0)$, is then:

$$u_{i,1,0}^{\delta,\delta'} = \frac{1}{2N} - \delta v_{i,1,0} - \delta' v'_{i,1,0} + o(|\delta| + |\delta'|).$$

The ratio

$$\left| \frac{u_{i,1,0}^{\delta,\delta'} - u_{i,1,0}^{0,0}}{u_{i,1,0}^{0,0}} \right| = 2N \left| \delta v_{i,1,0} + \delta' v'_{i,1,0} \right| + o(|\delta| + |\delta'|) \tag{2}$$

is the deviation in the fixation probability between neutral alleles and deleterious mutations characterized by (1), and quantifies the strength of selection. To compute $v_{i,j,k}$ and $v'_{i,j,k}$ and study their dependence on the initial population (i,j,k) and on the population demographic parameters (b,d,c), we use the Kolmogorov-forward equation, which consists in decomposing $u_{i,j,k}$ according to the first event occurring in the population. Taking the first order in δ and δ' separately, we obtain that $(v_{i,j,k})_{i,j,k}$ verifies a second-order recurrence equation (see online appendix A). We find that

(i)

$$v_{i,j,k} = \frac{j(i-k)}{N} x_N + (i-k) \frac{N^2 - (i-k)^2}{N^2} y_N, \tag{3}$$

where $N = i+j+k$, and the sequence of vectors $(z_N)_{N \geqslant 2} := \begin{pmatrix} x_N \\ y_N \end{pmatrix}_{N \geqslant 2}$ is the unique bounded solution of a system of equations detailed in online appendix A.

(ii)

$$v'_{i,j,k} = \frac{kY}{N} x_N + j x'_N + Y(2N-Y) \left(\frac{y'_N}{N} - \frac{Y}{2N^2} y_N \right), \tag{4}$$

where $Y = 2i + j$ is the number of A alleles, the sequences (x_N) and (y_N) are the same sequences as in (3), and the sequence of vectors $(z'_N)_{N \geqslant 2} := \begin{pmatrix} x'_N \\ y'_N \end{pmatrix}_{N \geqslant 2}$ is the unique bounded solution of a system of equations detailed in online appendix A.

We now know that the fixation probability of a deleterious mutation, starting from $(i,1,0)$ is:

$$\begin{aligned} u_{i,1,0}^{\delta,\delta'} = \frac{1}{2N} \\ - \delta \left(\frac{N-1}{N} x_N + (N-1) \frac{2N-1}{N^2} y_N \right) \\ - \delta' \left(x'_N + (2N-1) \left(\frac{y'_N}{N} - \frac{2N-1}{2N^2} y_N \right) \right) \\ + o(|\delta| + |\delta'|) \end{aligned} \tag{5}$$

where $N = i+1$. Using stochastic calculus we also prove that $v'_{i,j,k} > 0$ for every (i,j,k) and that $v_{i,j,k}$ is of the sign of $i-k$. The biological interpretation of the effect of δ' is straightforward: if $\delta' > 0$ and $\delta = 0$, allele a has an overall detrimental effect, because aa individuals only are affected by δ'. The effect of δ is more intricate because it affects heterozygous individuals, with the same apparent effect on both alleles.

We prove that when a is initially the most frequent allele ($i < k$), allele a is favored when $\delta > 0$ (i.e. $v_{i,j,k} > 0$), relative to the neutral case ($\delta = 0$), all else being equal; the opposite is true when A is the most frequent allele initially ($i > k$). In particular, starting from a population $(i, 1, 0)$, allele a is deleterious if $\delta > 0$ and $\delta' = 0$, i.e. a typical case of underdominance. This result is consistent with Fisher (1922).

2.2 The mutational time scale

We move to the evolution of a population subject to recurrent deleterious mutations at multiple loci, which, under the assumption of rare mutations, requires a broader time scale. At the mutational scale, we thus scale time t by K, the parameter we used to model rare mutation (rate $m_K = 2m/K$). This allows emergence of new mutations and we prove, as in the haploid case (Champagnat and Lambert, 2007), that when K goes to infinity, new alleles go to fixation or disappear instantaneously, due to faster invasion than emergence of mutations. Hence in the limit, an initially monomorphic population stays monomorphic at every time t. We assume that all mutations have the same effect on intrinsic death rate as described earlier and that individual birth and competition rates b and c remain constant. We can therefore track consecutive fixations of deleterious mutations via the change in the intrinsic death rate D_t common to all N_t individuals in the population. At each time t, the population size N_t is a random variable following the stationary law of a logistic birth-and-death process with parameters b, D_t, and c. By solving a stationary system (see Grimmett and Stirzaker (2001, $p.260$) and online appendix B), we find the probability $p(N, b, d, c) := \mathbb{P}(N_t = N)$ that the population size at time t is equal to N given that D_t, the individual death rate at time t, is equal to d:

$$p(N, b, d, c) := \frac{\frac{1}{N}\prod_{k=2}^{N-1}\frac{b}{d+kc}}{\sum_{i=2}^{\infty}\frac{1}{i}\prod_{j=2}^{i-1}\frac{b}{d+jc}}. \tag{6}$$

Hence the probability distribution of the population size is directly controlled by its demographic parameters, which contrasts with previous approaches regarding population size as a constant parameter (e.g. Lande, 1994). A small population size therefore results from small values of the birth rate or large values of death rates. In this model, each new mutation gets either lost, with no effect on the death rate, or fixed instantaneously. In the latter case, the population death rate is increased by δ', since the population changes directly from being monomorphic with type AA to being monomorphic of type aa. $(D_t)_{t>0}$ is thus a jump process that jumps from a value d to $d + \delta'$ at rate $\tau(b, d, c, \delta, \delta')$ i.e. the rate of fixation of a deleterious mutation, which we compute later on. When a new deleterious mutation goes to fixation, the stationary law of the population size changes, due to an increase in the death rate of all individuals (from d to $d + \delta'$) causing a decrease in the mean population size. We can define and compute numerically the expected size of a population with demographic parameters b, d, and c:

$$\overline{N}(b, d, c) := \sum_{N=2}^{\infty} Np(N, b, d, c).$$

As the death rate D_t increases, we prove that the mean population size decreases gradually and approaches the minimum value of 2 (see figure 1 and table 1). Note that when all parameters are multiplied by a constant, the frequency of birth-and-death events is modified, but the distribution of the population size is unaffected (see equation (6)). In the following, we therefore keep the competition parameter constant and study the influence of other demographic parameters. Finally we examine the temporal dynamics of the mean population size by iterating the effect of mutation fixation on population size. In a population with initial demographic parameters b, d, and c, the mean time to fixation of a deleterious mutation is

$T(b,d,c,\delta,\delta') := 1/\tau(b,d,c,\delta,\delta')$; at time $t = T(b,d,c,\delta,\delta')$, we change the intrinsic death rate from d to $d+\delta'$ and the mean population size from $\overline{N}(b,d,c)$ to $\overline{N}(b,d+\delta',c)$. This is repeated through time to obtain the temporal dynamics of population size (see subsection 3.2).

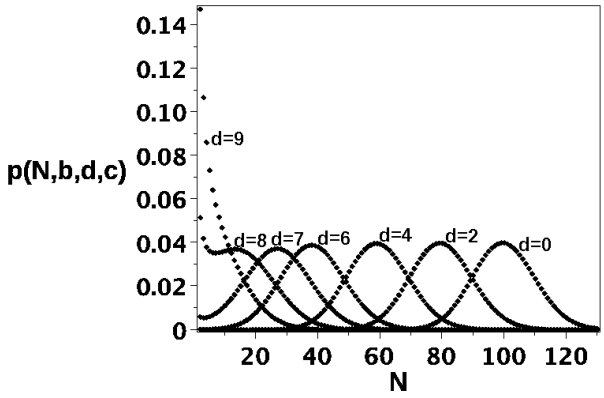


Figure 1: Distribution of the population size under different intrinsic death rates d. In this figure $b = 10$ and $c = 0.1$.

3 Computations and numerical results

3.1 Dynamics of a single deleterious allele at the ecological time scale

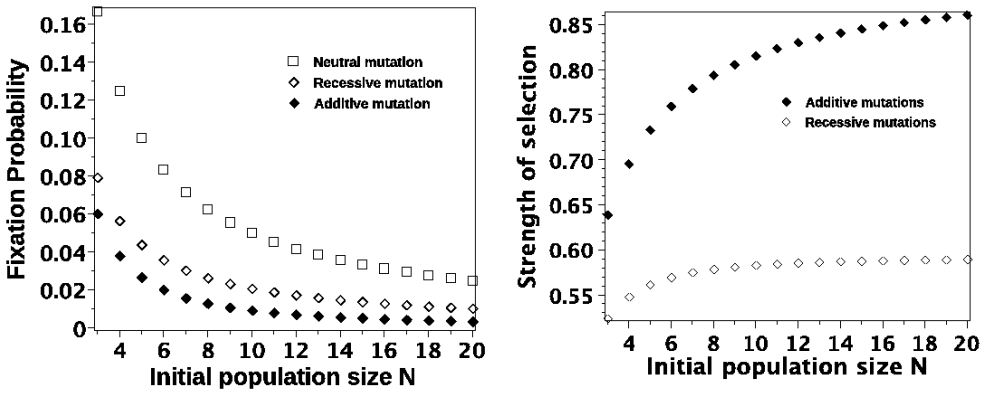
The probability of fixation of deleterious mutations, and its deviation from neutrality, depends on z_N and z'_N (equations (3) and (4)): although we cannot detail these formulae of as functions of N, b, d, and c much more, we can compute them numerically (see figure 9) to obtain fixation probabilities (figure $2A$). The probability of fixation of slightly deleterious mutations decreases as the population size increases (figure $2A$), but the strength of selection, i.e. the relative difference in fixation probability between a deleterious and a neutral mutation (equation (2)), is an increasing function of the initial population size (figure $2B$), which is consistent with classical results in evolutionary genetics (see Ohta, 1973, for example).

3.2 The mutational time scale

The genetic load accelerates the rate of fixation of deleterious mutations - Our eventual goal is first to prove the existence of a mutational meltdown and second to study how demographic parameters influence the strength of this meltdown. In a population of N individuals, the rate of fixation is $2mNu^{\delta,\delta'}_{N-1,1,0}$, which can be averaged over all population sizes N to obtain an overall rate of fixation of deleterious mutations $\tau(b,d,c,\delta,\delta')$, when the demographic parameters are b, d, and c:

$$\begin{aligned}\tau(b,d,c,\delta,\delta') :&= 2m\sum_{N=2}^{+\infty} N\, u^{\delta,\delta'}_{N-1,1,0}\, p(N,b,d,c)\\ &= m - 2m\sum_{N=2}^{+\infty} N\, p(N,b,d,c)\\ &\qquad\qquad \times (\delta\, v_{N-1,1,0} + \delta'\, v'_{N-1,1,0})\\ &\quad + o(|\delta| + |\delta'|)\end{aligned}$$

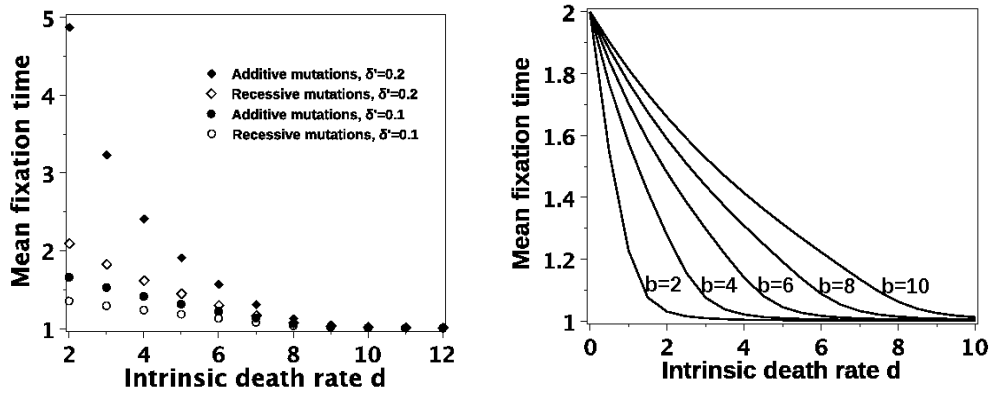
We use this formula to examine how the mean time T to fixation depends on population size, demographic parameters, and dominance relationship among alleles, which yields three important results. First, the



(A) (B)

Figure 2: Fixation probability (A) and strength of selection (B) of an additive ($\delta = \delta'/2$, closed diamonds) or recessive ($\delta = 0$, open diamonds) deleterious allele, as a function of the initial population size N. Squares in (A) represent the neutral fixation probability, i.e, $1/2N$. The strength of selection (B) is measured as the relative difference in fixation probability between a deleterious and a neutral mutation (equation (2)). Demographic parameters are $b = 10$, $d = 1$, $c = 0.1$, and $\delta' = 0.2$.

mean time to fixation is a decreasing function of the death rate d (figure $3A$), which suggests an increase of the rate of fixations of deleterious mutations, i.e. a mutational meltdown: fixation of deleterious mutations increases the intrinsic death rate, thereby causing faster fixation of new deleterious alleles, and so on. When the intrinsic death rate d becomes large (effectively infinite), the mean fixation time $T(b, d, c, \delta, \delta')$



(A) (B)

Figure 3: (A): Relationship between T, the mean time to fixation of a deleterious mutation, and the population intrinsic death rate d as a function of selection and dominance. Open symbols: recessive mutation ($\delta = 0$); closed symbols: additive mutation ($\delta = \delta'/2$); circles: $\delta' = 0.1$; diamonds: $\delta' = 0.2$. Other demographic parameters are $b = 10$, $c = 0.1$, and $m = 1$.(B): Relationship between the mean time to fixation of a deleterious mutation T and parameters b and d. Each curve corresponds to a fixed value of b. Other parameters are $\delta = 0.05$, $\delta' = 0.1$, $c = 0.1$ and $m = 1$.

converges the mean fixation time of a neutral mutation, ($1/m$, where m is the unscaled mutation rate), due to a small population size and dominant effects of drift over selection (see Ohta (1973) and Kimura (1979) for analog results in other models). As expected, the birth rate b has an opposite effect on the mean fixation time $T(b, d, c, \delta, \delta')$ (see figure 10 and figure $3B$), which is an increasing function of b due to higher population sizes, thus better elimination of deleterious alleles, at higher birth rates. Second, the time to fixation of a beneficial mutation with parameters $-\delta$ and $-\delta'$ is the symmetrical, with respect to the

neutral value $1/m$, of the time to fixation of a deleterious mutation with parameters δ and δ'. Hence, the mean time to fixation of a beneficial mutation is an increasing function of d, implying that the mutational meltdown may also be caused by lower fixation probabilities of beneficial mutations at higher intrinsic death rates. Third, and maybe most importantly, our results show no particular relationship between the mean population size and the mean time to fixation of deleterious mutations. In particular, unlike existing results with other models (see Crow and Kimura, 1970), the mean fixation time of deleterious mutations is not an increasing function of the mean population size $\overline{N}$. This can be seen for instance on figure $3B$: if we compare two populations with the following demographic parameters (Population 1: $b = 8$, $d = 1$, $c = 0.1$; Population 2: $b = 10$, $d = 2$, $c = 0.1$), population 1 has a smaller average population size ($\overline{N}(8, 1, 0.1) = 69$; $\overline{N}(10, 12, 0.1) = 79$), but a mean fixation time of deleterious mutations that is greater than that of population 2 ($T(8, 1, 0.1, 0.05, 0.1) = 1.771$; $T(10, 2, 0.1, 0.05, 0.1) = 1.6595$). This illustrates the interest of considering not only population size, but also demographic parameters when studying population extinction risks.

Population dynamics under a mutational meltdown - As deleterious mutations accumulate, the mean population size decreases more and more rapidly relative to a null model with a constant mean fixation time $T(b, D_0, c, \delta, \delta')$ (figure 4), which is caused by the acceleration of mutation fixations. As expected, the overall decrease in population size with additive mutations is slower than with recessive mutations, which go to fixation more rapidly. However we can hardly compare the influence of the acceleration of mutation fixations (mutational meltdown) in these two populations because they have different initial mean fixation times. Hence, the dynamics of population size across different mutation models are caused both by (1) different initial mean fixation times and (2) different accelerations of the fixations of deleterious mutations (the mutational meltdown per se). Similarly, although the acceleration in population decline may seem stronger for higher selection coefficient δ' (figure 4), this difference is also due to (1) differences in the accelerations of mutation fixations, (2) differences in the initial mean fixation times and (3) larger influence of mutation fixations on the mean population size for larger δ'. The extinction risk of populations subject to accumulation of deleterious mutations can thus be quantified first by the corresponding null model of extinction risk, providing a baseline for the rate of fixation of deleterious mutations, and second by the acceleration of mutation fixations, i.e. the mutational meltdown. From a conservation perspective, it is therefore crucial to quantify the part of the extinction risk that is due to the acceleration of mutation fixations, in order to define whether the meltdown can be neglected or not.

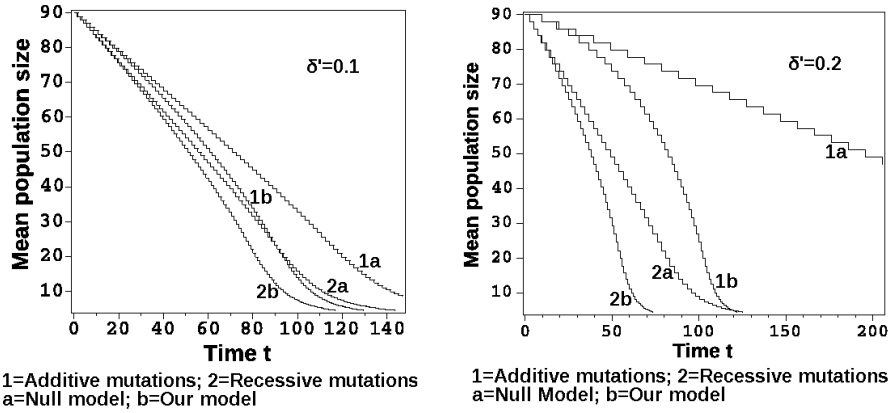
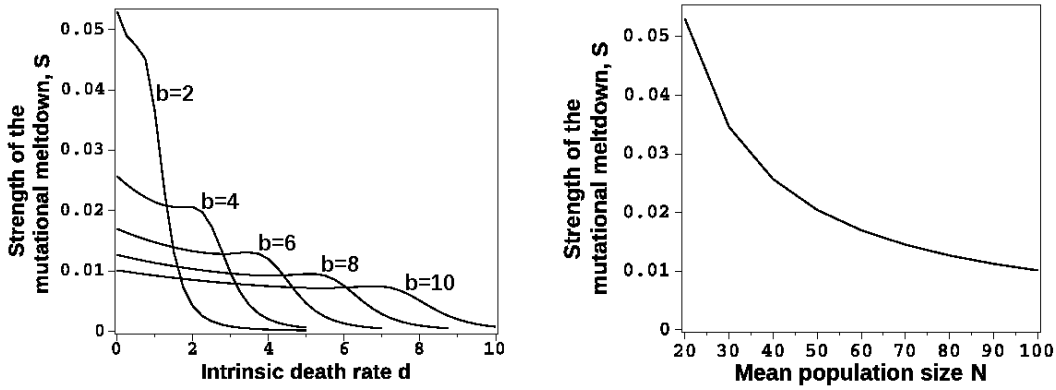


Figure 4: Temporal decrease in the mean population size. In this figure, $b = 10$, $c = 0.1$, $m = 1$, and the initial intrinsic death rate is 1. For (A), $\delta' = 0.1$ whereas for (B), $\delta' = 0.2$. We plot additive and recessive cases. For each case, we also plot the temporal dynamics of the mean population size in the corresponding null model.

Quantifying the extinction risk and the acceleration of fixations - We suggest that the extinction risk of a population is controlled by two quantities: first the time to fixation of the first deleterious mutation (see figure 3) which quantifies the risk of extinction in the null model, and second the positive real number:

$$S(b,d,c,\delta,\delta') := \frac{T(b,d,c,\delta,\delta') - T(b,d+\delta',c,\delta,\delta')}{T(b,d,c,\delta,\delta')},$$

which represents the acceleration of fixations between the first and the second mutation and quantifies the part of the extinction risk due to the mutational meltdown (see figure 5). S is a function of birth and death rates (figure $5A$) which can be used to define threshold values of the demographic parameters b and D_0 (initial intrinsic death rate) above or below which the mutational meltdown is small enough to be neglected. The strength of the mutational meltdown goes to 0 as the intrinsic death rate d goes to infinity: as mutations get fixed, the population goes more rapidly to extinction, due to a lower mean time to fixation of deleterious mutations, but the population tends to be less subject to mutational meltdown. Besides, when comparing populations with the same initial fixation time (figure $3B$), we show that the strength of the extinction vortex is a decreasing function of the mean population size (figure $5B$), so that a threshold mean population size, above which the mutational meltdown is small enough, can still be defined (see Lande, 1994).



(A) (B)

Figure 5: (A): Relationship between the strength of the mutational meltdown and demographic parameters b and d. Each curve corresponds to a fixed value of b. (B): Relationship between the strength of the mutational meltdown and the mean population size, when the initial fixation time is set to 2. Here, $d = 0$ and b was varied between 2 and 10 to obtain this curve (see figure $3B$). In both figures, $c = 0.1$, $\delta = 0.05$, $\delta' = 0.1$, and $m = 1$.

4 Comparison with other models of the mutational meltdown

Our approach is novel in that we combine for the first time in an analytical model two traditions: population genetics approaches, which frequently assume infinite (or at best finite, but constant) population sizes, with demographic approaches, which are often based on simplified genetic processes. Our results therefore reconcile these two approaches to generate novel results. Below we illustrate this by comparing our results to those obtained with classical population genetics models (the Wright-Fisher model in particular) and demo-genetic models in haploid populations.

4.1 Comparison with the Wright-Fisher model

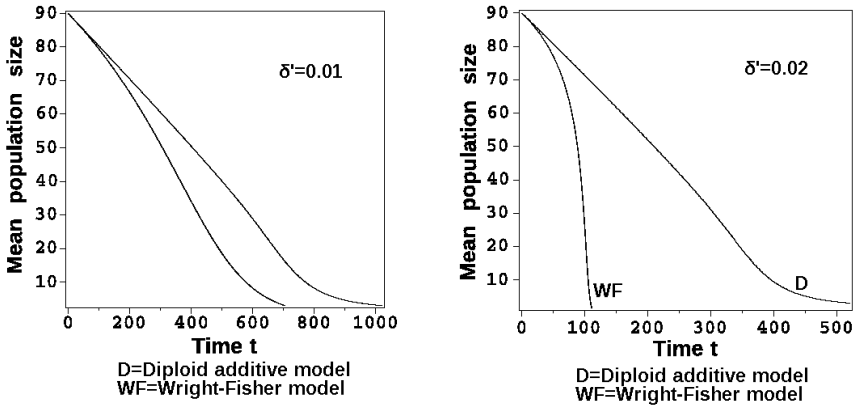
Crow and Kimura (see Crow and Kimura, 1970, p. 345) proved that the probability of fixation of a deleterious additive mutation in a population with large, constant size N is:

$$\frac{e^{2sN_e/N} - 1}{e^{4sN_e} - 1}, \tag{7}$$

where s is the selection coefficient of deleterious mutations and N_e is the effective population size, which is here a parameter of the model. We can use this formula to compare the temporal dynamics of the mean population size in the Wright-Fisher ($N_e = N$) vs. our model, which requires variable population size in the Wright-Fisher model. To obtain this, we use equation (4) of Clarke (1973) describing the change ΔN in population size N of a Wright-Fisher model as a function of the change in the mean fitness ΔW ($= -2s$ here): $\Delta N = N \times \Delta W$. We finally rescale time and fitness in the Wright-Fisher model so that the first mutation that goes to fixation has same mean fixation time and the same impact on the mean population size in both models. We show that the mutational meltdown is stronger in the Wright-Fisher model (figure 6), i.e. the mean population size collapses more rapidly. This can be understood examining the Taylor expansion of (7) when $N_e = N$:

$$\frac{1}{2N} - \frac{s}{2}\left[2 - \frac{1}{N}\right] + o(s)$$

which yields a strength of selection of $Ns[2 - 1/N]$, to be compared with equation (2) and figure $2B$ of our model: for large population sizes, the strength of selection is equivalent to $2Ns$ in the Wright-Fisher model whereas it is bounded in our model. As a result, the strength of selection and the time to fixation decrease more rapidly in the Wright-Fisher model as the population size decreases. This suggests that the mutational meltdown is overestimated with the Wright-Fisher model, assuming constant population size, compared to our model.



(A) (B)

Figure 6: Temporal dynamics of the mean population size with time, in our additive diploid model (D) and the Wright-Fisher model (WF). In these figures, $b = 10$, $c = 0.1$, $m = 1$ and the initial intrinsic death rate $d = 1$. For (A), $\delta' = 0.01$, whereas for (B), $\delta' = 0.02$.

4.2 Comparison with the haploid model of Champagnat and Lambert (2007)

In a more realistic demographic model, Champagnat and Lambert (2007) studied the fixation of small mutations in a haploid population. Comparing haploid vs. diploid populations is however not straightforward, particularly because the latter produce twice as many alleles in one birth and contain twice as much

genetic material for the same population size. We can nonetheless rely on the expectation that, under Hardy-Weinberg equilibrium and large population sizes, a diploid population under additive selection can be approximated by a haploid population. We choose to compare haploid vs. diploid models by considering populations with the same rate of fixation of neutral mutations, the same effect of mutations, and the same initial demographic parameters. Under these conditions, the temporal decrease in mean population sizes is in fact comparable in both models when deleterious mutations have additive effects in diploid organisms (figure 7). The only differences appear for really small populations, which is at least partly due to the fact that the minimum diploid population size is 2 whereas the minimum haploid population size is 1. Our model however allows us to examine non additive mutations, which cannot be studied in haploid models. This is particularly relevant in diploid organisms, in which most deleterious mutations have partly to fully recessive effects (García-Dorado et al., 2004). We show that the mutational meltdown is stronger with additive mutations than with recessive ones (figure 8): its strength increases with the effect of mutations in heterozygotes δ, when δ', the effect of mutations in homozygotes, is kept constant.

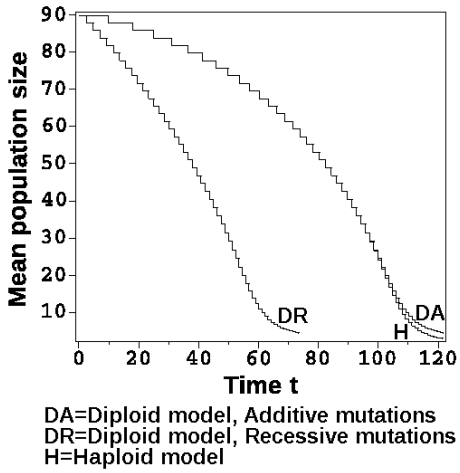


Figure 7: Temporal dynamics in the mean population size, in haploid vs. diploid populations. The lower curve (DR) is reproduced from the curve $2b$ of Fig. $4B$, i.e. the temporal dynamics of the mean population size in the recessive case, when $\delta = 0$. The upper curves are the change in the mean population size with time in a haploid population, derived from Champagnat and Lambert (2007) (H), and in a diploid population with additive mutations (DA, reproduced from the curve $1b$ of Fig. $4B$). Parameter values are $b = 10$, $c = 0.1$, $m = 1$, $\delta' = 0.2$ and the initial intrinsic death rate is $d = 1$.

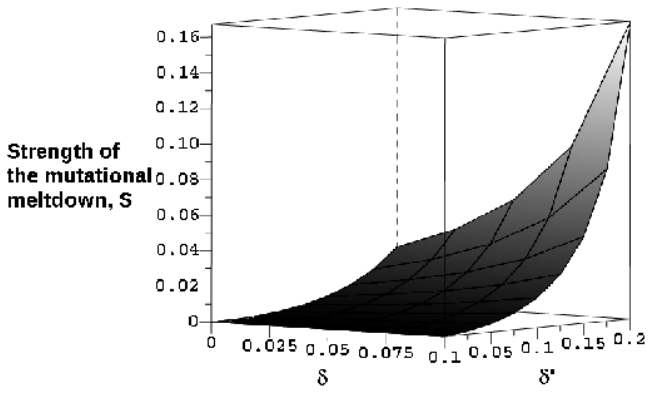


Figure 8: The strength of the mutational meltdown as a function of selection coefficients δ and δ'. In this figure, $b = 10$, $d = 1$, $c = 0.1$, and $m = 1$.

5 Discussion

In this paper, we provided a more quantitative framework for the study of mutational meltdown than previous theoretical treatments, and modeled the stochastic dynamics of population size in diploid organisms. As in several previous models, we demonstrated the existence of a mutational meltdown by showing that, as deleterious mutations accumulate, the population size decreases more and more rapidly relative to a null model with constant mean fixation time of deleterious mutations. We showed that this fixation time is a decreasing function of the intrinsic death rate, an increasing function of the fecundity, and converges towards the mean fixation time of neutral mutations as the intrinsic death rate goes to infinity. Our approach takes the study of mutational meltdown a step further by demonstrating that mean population size is not always the best indicator of extinction risk for populations subject to fixation of deleterious mutations. We also used our results to suggest a new quantification of the mutational meltdown (i.e. the acceleration of mutation fixations) and to define a threshold in the mean population size above which the mutational meltdown is small enough to be neglected. Our results finally suggest that the mutational meltdown per se may not be as severe as predicted by earlier population genetics models assuming constant population size or haploid models. In the following, we discuss the implications of our results for conservation and the limitations of our approach.

5.1 Quantifying the mutational meltdown

While the ultimate causes of most species extinctions are environmental (Brooks et al., 2002), various environmental constraints can have various secondary consequences on ecological and genetic processes. This implies that, depending on the situation faced by populations, the relative weights of non-genetic (e.g., ecological factors associated with highly variable and/or low average growth rate), genetic (e.g., inbreeding depression, accumulation of deleterious mutations) components and of their interaction (e.g., the mutational meltdown in the strict sense) may strongly vary. Here, we developed a framework that allows accounting for all three components. Although we focused on quantifying the interaction component (i.e., the demographically mediated effect of the load of fixed mutations on the rate of future mutation fixations), we also provided results on the "pure" genetic component (i.e., the rate of mutation fixation, independent from the load of fixed mutations) and revealed that the extinction risk for populations subject to recurrent deleterious mutation fixations can be quantified both with the initial mean fixation time of a deleterious mutation, and with the acceleration of mutation fixations; which quantifies the strength of the mutational meltdown. We also found out that the quantification of the extinction risk in terms of demographic parameters rather than population sizes can provide a more accurate vision of the population temporal dynamics. Indeed, populations with similar population sizes can have different initial times to fixation of deleterious mutations, therefore different risks of extinction. However, in populations with the same initial time to fixation, the strength of the mutational meltdown is a decreasing function of the initial mean population size, so that one can define a threshold population size above which the mutational meltdown can be neglected. Our results demonstrate that the magnitude of the mutational meltdown is highly dependent on the underlying demo-genetic model, but also on demographic and mutational parameters (e.g., level of dominance). Numerical applications indicate for instance that genetic models simpler than ours may strongly overestimate the magnitude of the mutational meltdown (see e.g. the Wright-Fisher model, figure 6). On the other hand, neglecting the mutational meltdown might lead to strong underestimation of the final/overall speed of mutation accumulation, and subsequently to overestimate the time to extinction (figure $4B$).

5.2 Limitations

One major limitation of our model is that a population cannot get extinct. If extinction can occur, the probability of fixation of neutral alleles is modified, particularly because the population can go extinct

before an allele gets fixed. This could be accounted for by computing the expected number of mutations that will get fixed before the population goes extinct, but this would require another time scale because the population would instantaneously go extinct at the mutational time scale of our present model. We also assumed no epistasis and free recombination of all loci (no linkage). Population genetic theory (Hill and Robertson, 1966; Felsenstein, 1974) and empirical results (Betancourt and Presgraves, 1990) suggest that the efficacy of natural selection is generally limited by linkage. The consideration of linkage is not expected to qualitatively modify our results regarding the occurrence of the mutational meltdown and the effects of demographic parameters on its magnitude, but it may engender a more detrimental effect of mutation accumulation in all cases. Similarly, synergistic or antagonistic epistasis is likely to influence the strength of selection. In particular, if deleterious mutations interact synergistically, they may be more efficiently removed by selection, which may result in a reduced load (Charlesworth, 1990). However, no clear pattern of epistasis (synergistic or antagonistic) is apparent from empirical studies (Elena and Lenski, 1997).

5.3 Implications and forthcoming works

Our results have critical implications in the field of conservation biology, in which the projected viability of endangered populations is generally derived from demographic models (based on specific demographic components, Beissinger and McCullough (2002)) and/or genetic models (based on population size, Franklin and Frankham (1998)). While mere juxtapositions of these two kinds of estimates might provide reasonable estimates of the extinction risk in some cases (Robert, 2011), our and other works (Lynch et al., 1995) indicate that the demo-genetic interaction may strongly affect both the dynamics of mutations (i.e., fixation rate) and population viability. However, proper consideration of this interaction requires the use of advanced concepts and sophisticated tools, which challenges its use as a standard/operational method in conservation. In this context, we hope that our framework will be useful in outlining a conceptual basis to differentiate situations in which the mutational meltdown has minor effects on the risk of extinction from those in which it cannot be neglected. Our choice of considering finite diploid populations of variable sizes and evolving continuously in time was motivated by the urgent need for theoretical models with strong practical implications in the field of biodiversity conservation. Most species of conservation concerns are diploid, iteroparous organisms (Seddon et al., 2005) and endangered populations generally exhibit high year-to-year variance in population size, either due to sampling or to process variation (Lande et al., 2003). Our overlapping generations framework allows not only to assume varying population sizes over long time scales but also to relax the assumption of constant population size within generations (as, e.g., in the modified Wright-Fisher model presented above). This is of particular interest to assess the viability of small populations of long-lived species (typically, bird and mammal species), in which population sizes may vary by orders of magnitude within a single generation, for both intrinsic (e.g. the mutational meltdown itself) or environmental reasons. Further, by incorporating explicit descriptions of birth and death processes, our framework also allows adding deterministic or stochastic variation in demographic parameters, which would allow us to model environmental stochasticity. An interesting perspective is to compare the weights of environmental stochasticity and deleterious mutation accumulation on the extinction risk of populations (Lande, 1993; Spielman et al., 2004).

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Online appendix A: Matrices

Taking then the first order in δ and δ' separately in the Kolmogorov-Forward equation (see Champagnat and Lambert, 2007), we obtain that $(v_{i,j,k})_{i,j,k}$ verifies the following second-order recurrence equation (with three indices, i, j, and k) and four initial conditions:

$$\begin{cases} (\Delta v)_{i,j,k} = \dfrac{j(i-k)}{2N(N-1)} \quad \forall (i,j,k) | N = i+j+k \geqslant 3 \\ v_{0,0,2} = v_{2,0,0} = 0 \\ v_{1,1,0} = \frac{1}{2} v_{2,1,0} + \frac{1}{2} v_{1,2,0} \\ v_{0,1,1} = \frac{1}{2} v_{0,1,2} + \frac{1}{2} v_{0,2,1} \\ v_{0,2,0} = \frac{1}{4} v_{1,2,0} + \frac{1}{4} v_{0,2,1} + \frac{1}{2} v_{0,3,0} \\ v_{1,0,1} = v_{1,1,1} \end{cases} \tag{8}$$

where

$$\begin{aligned} (\Delta v)_{i,j,k} = & \sum_{i=1}^{3} (bN + dN + cN(N-1)) v_{i,j,k} \\ & - [b_{AA}(Z) v_{i+1,m,k} + b_{Aa}(Z) v_{i,j+1,k} + b_{aa}(Z) v_{i,j,k+1} \\ & + (d + c(N-1))[i v_{i-1,j,k} + j v_{i,j-1,k} + k v_{i,j,k-1}] \end{aligned}$$

with $Z = (i,j,k)$. We find that v' satisfies similar equations. After some computations to find a sublinear solution (see Champagnat and Lambert, 2007) for this system of equations, we establish that v and v' can be written as follows:

(i)

$$v_{i,j,k} = \frac{j(i-k)}{N} x_N + (i-k) \frac{N^2 - (i-k)^2}{N^2} y_N,$$

where $N = i+j+k$ and the sequence of vectors $(z_N)_{N \geqslant 2} := \begin{pmatrix} x_N \\ y_N \end{pmatrix}_{N \geqslant 2}$ is the unique bounded solution of the following system of equations composed of a second-order recurrence equation and an initial condition:

$$\begin{cases} B_N z_{N+1} = C_N z_N + D_N z_{N-1} + f_N \quad \forall N \geqslant 4, \\ B_3 z_4 = \tilde{C}_3 z_3 + f_3 \end{cases}$$

where the matrices B_N, C_N, $\tilde{C}_3$, D_N and the vectors f_N can be computed by replacing v by Formula (3) in Equation (8) and are given further.

(ii)

$$v'_{i,j,k} = \frac{kY}{N} x_N + j x'_N + Y(2N - Y) \left(\frac{y'_N}{N} - \frac{Y}{2N^2} y_N \right),$$

where $Y = 2i + j$ is the number of A alleles, the sequences (x_N) and (y_N) are given in the first point and the sequence of vectors $(z'_N)_{N \geqslant 2} := \begin{pmatrix} x'_N \\ y'_N \end{pmatrix}_{N \geqslant 2}$ is the unique bounded solution of the following system of equations composed of a second-order recurrence equation and an initial condition:

$$\begin{cases} B'_N z'_{N+1} = C'_N z'_N + D'_N z'_{N-1} + f'_N \quad \forall N \geqslant 3, \\ \tilde{B'}_2 z'_3 = \tilde{C'}_2 z'_2 + \tilde{f'}_2. \end{cases}$$

The matrices B'_N, C'_N, $\tilde{C'}_2$, D'_N and the vectors f'_N are given further.

We find:

$$
\begin{aligned}
B_N &:= \frac{b}{2(N-1)(N+1)} \begin{pmatrix} 1 & \frac{2N^2+4N-3}{N+1} \\ 2N^2-3 & \frac{-3}{N+1} \end{pmatrix}, \\
C_N &:= (b+d+c(N-1)) \begin{pmatrix} 0 & \frac{1}{N} \\ 1 & 0 \end{pmatrix}, \\
\tilde{C}_3 &:= \begin{pmatrix} 0 & \frac{b+d+2c}{3} \\ b+\frac{d+2c}{3} & -(d+2c) \end{pmatrix}, \\
D_N &:= -\frac{d+c(N-1)}{N-1} \begin{pmatrix} 0 & \frac{N-3}{N-1} \\ N-2 & \frac{3}{N-1} \end{pmatrix}, \\
f_N &:= \begin{pmatrix} 0 \\ \frac{-1}{2N(N-1)} \end{pmatrix}, \\
B'_N &:= \frac{b}{N-1} \begin{pmatrix} 2N^2-2N-1 & \frac{-1}{N+1} \\ \frac{1}{2} & \frac{N^2+N-3/2}{N+1} \end{pmatrix}, \\
\tilde{B}'_2 &:= \begin{pmatrix} 1 & 9 \\ \frac{3}{2} & \frac{13}{2} \end{pmatrix}, \\
C'_N &:= (bN+dN+cN(N-1)) \begin{pmatrix} 2 & 0 \\ 0 & \frac{1}{N} \end{pmatrix}, \\
\tilde{C}'_2 &:= \begin{pmatrix} 0 & 4 \\ 1 & 3 \end{pmatrix} = C_3 - \begin{pmatrix} 0 & 0 \\ \frac{2}{3}(d+2c) & (d+2c) \end{pmatrix} \\
D'_N &:= -(d+c(N-1)) \begin{pmatrix} 2N-2 & \frac{2}{N-1} \\ 0 & \frac{N-2}{N-1} \end{pmatrix}, \\
f'_N &:= \begin{pmatrix} \frac{b}{N-1}(2N-1)\frac{y_{N+1}}{2(N+1)^2} \\ -(d+c(N-1))(4N+2)\frac{y_{N-1}}{2(N-1)^2} \\ \frac{b}{N-1}\left(2N^3+3N^2-4N-\frac{3}{2}\right)\frac{y_{N+1}}{2(N+1)^2} \\ -(bN+dN+cN(N-1))(2N-1)\frac{y_N}{2N^2} \\ +(d+c(N-1))(2N^2-7N+8)\frac{y_{N-1}}{2(N-1)^2} \end{pmatrix}, \\
\tilde{f}'_2 &:= \begin{pmatrix} x_2 - y_2 - x_3 + \frac{3}{2}y_3 \\ \frac{57}{56}y_3 - \frac{9}{8}y_2 \end{pmatrix}.
\end{aligned}
$$

Online appendix B: The population size

We find the formula of the stationary distribution of the population size by solving the stationary system (see Grimmett and Stirzaker, 2001, p. 260):

$$
\begin{cases}
b(N-1)p(N-1,b,d,c) \\
\quad +(d+cN)(N+1)p(N+1,b,d,c) \\
\qquad = N(b+d+c(N-1))p(N,b,d,c) \quad \forall N \geqslant 3 \\
\\
2bp(2,b,d,c) = 3(d+2c)p(3,b,d,c)
\end{cases}
$$

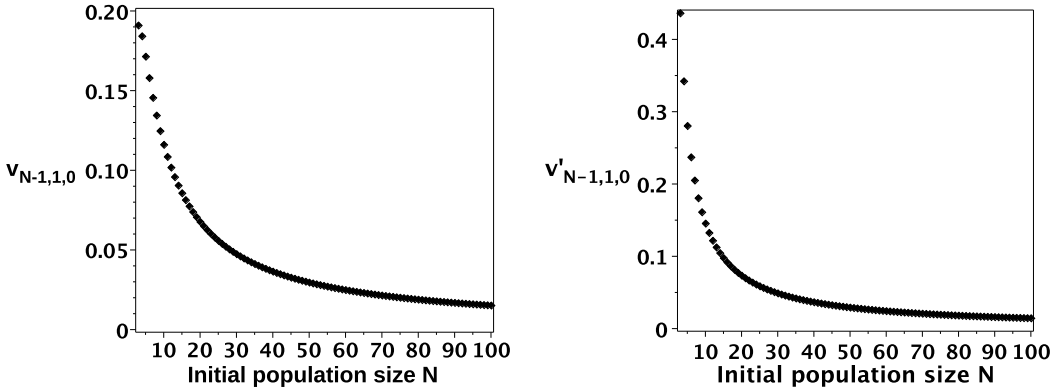
We obtain Table 1 for the decrease of the mean population size $\overline{N}$ when d increases.

d	$\overline{N}(b,d,c)$
0	100
2	80
4	59
6	38
8	16
10	6

Table 1: Correspondence between the death rate and the mean population size, for $b = 10$ and $c = 0.1$.

Online appendix C: v and v'

We obtain Figure 9 for the partial derivatives of the fixation probability, $v_{N-1,1,0}$ and $v'_{N-1,1,0}$, as functions of N.



(A) (B)

Figure 9: $v_{N-1,1,0}$ (A) and $v'_{N-1,1,0}$ (B) as functions of the initial population size N. The demographic parameters are $b = 10$, $d = 1$, $c = 0.1$.

Online appendix D: T as a function of b

We obtain figure 10 for the mean fixation time T as a function of b.

References

Beissinger, S., McCullough, D.E., 2002. Population viability analysis. University of Chicago Press, Chicago, U.S.A.

Betancourt, A., Presgraves, D., 1990. Linkage limits the power of natural selection in drosophila. Proceedings of the National Academy of Sciences of the United States of America 99, 13616–13620.

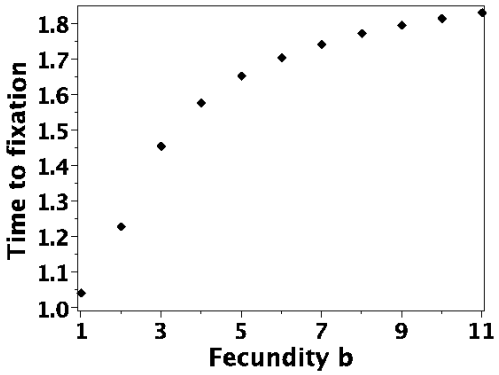


Figure 10: Relationship between the mean time to fixation of a deleterious mutation T and the fecundity b. In this figure, $d = 1$, $c = 0.1$, $\delta = 0.05$, $\delta' = 0.1$, and $m = 1$.

Brooks, T., Mittermeier, R., Mittermeier, C., Da Fonseca, G., Rylands, A., Konstant, W., Flick, P., Pilgrim, J., Oldfield, S., Magin, G., Hilton-Taylor, C., 2002. Habitat loss and extinction in the hotspots of biodiversity. Conservation Biology 16, 909–923.

Champagnat, N., Ferriere, R., Méléard, S., 2006. Unifying evolutionary dynamics: From individual stochastic processes to macroscopic models. Theoretical Population Biology 69, 297–321.

Champagnat, N., Lambert, A., 2007. Evolution of discrete populations and the canonical diffusion of adaptive dynamics. Annals of Applied Probability 17, 102–155.

Charlesworth, B., 1990. Mutation-selection balance and the evolutionary advantage of sex and recombination. Genetics Research 55, 199–221.

Cherry, J., 1998. Should we expect substitution rate to depend on population size? Genetics 150, 911–919.

Clarke, B., 1973. The effect of mutation on population size. Nature 242, 196–197.

Clay, K., Shaw, R., 1981. An experimental demonstration of density dependent reproduction in a natural population of diamorpha-smallii a rare annual. Oecologia 51, 1–6.

Crow, J., Kimura, M., 1970. An introduction to population genetics theory. Harper and Row. second edition.

Drake, J., Charlesworth, B., Charlesworth, D., Crow, J., 1981. Rates of spontaneous mutation. Genetics 148, 1667–1686.

Elena, S., Lenski, R., 1997. Test of synergistic interactions between deleterious mutations in bacteria. Nature 390, 395–398.

Ewens, W., 2004. An introduction to population genetics theory. 1. Theoretical Introduction. Springer. second edition.

Felsenstein, J., 1974. The evolutionary advantage of recombination. Genetics 78, 737–756.

Fisher, R., 1922. On the dominance ratio. Proceedings of the Royal Society of Edinburgh 52, 321–341.

Franklin, I., Frankham, R., 1998. How large must populations be to retain evolutionary potential? Animal Conservation 1, 69–70.

García-Dorado, A., Lopez-Fanjul, C., Caballero, A., 2004. Rates and effects of deleterious mutations and their evolutionary consequences, in: Evolution of Molecules and Ecosystems (Moya, A. and Fontdevila, E., eds). Oxford University Press, New York, pp. 20–32.

Gilpin, M., Soulé, M., 1986. Conservation Biology : The Science of Scarcity and Diversity. Sinauer associates.

Grimmett, G., Stirzaker, D., 2001. Probability and Random Processes. Oxford University Press. third edition.

Haag-Liautard, C., Dorris, M., Maside, X., Macaskill, S., Halligan, D., Charlesworth, B., Keightley, P., 2007. Direct estimation of per nucleotide and genomic deleterious mutation rates in drosophila. Nature 445, 82–85.

Hill, W., Robertson, A., 1966. The effect of linkage on limits to artificial selection. Genetics Research 8, 269–294.

Kimura, M., 1979. Model of effectively neutral mutations in which selective constraint is incorporated. Proceedings of the National Academy of Sciences of the United States of America 75, 1934–1937.

Lande, R., 1976. Natural selection and random genetic drift in phenotypic evolution. Evolution 30, 314–334.

Lande, R., 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. The American Naturalist 142, 911–927.

Lande, R., 1994. Risk of population extinction from fixation of new deleterious mutations. Evolution 48, 1460–1469.

Lande, R., Engen, S., Saether, B., 2003. Stochastic Population Dynamics in Ecology and Conservation. Oxford: Oxford University Press.

Lotka, A., 1932. The growth of mixed populations : two species competing for a common food supply. Journal of the Washington Academy of Sciences 22, 461–469.

Lynch, M., Blanchard, J., Houle, D., Kibota, T., Schultz, S., Larissa Vassilieva, J., 1999. Perspective : Spontaneous deleterious mutations. Evolution 53, 645–663.

Lynch, M., Conery, J., Burger, R., 1995. Mutation accumulation and the extinction of small populations. The American Naturalist 146, 489–518.

Lynch, M., Gabriel, W., 1990. Mutation load and the survival of small populations. Evolution 44, 1725–1737.

Mace, G., Purvis, A., 2008. Evolutionary biology and practical conservation : bridging a widening gap. Molecular Ecology 17, 9–19.

Ohta, T., 1973. Slightly deleterious mutant substitutions in evolution. Nature 246, 96–98.

Robert, A., 2011. Find the weakest link. a comparison between demographic, genetic and demo-genetic metapopulation extinction times. BMC Evolutionary Biology 11:260.

Seddon, P., Soorae, P., Launay, F., 2005. Taxonomic bias in reintroduction projects. Animal Conservation 8, 51–58.

Shaffer, M., 1981. Minimum population sizes for species conservation. Bioscience 31, 131–134.

Spielman, D., Brook, B., Frankham, R., 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences of the United States of America 101, 15261–15264.

Stephens, P., Sutherland, W., 1999. Consequences of the allee effect for behavior, ecology and conservation. Trends in Ecology and Evolution 14, 401–405.

Theodorou, T., Souan, H., Couvet, D., 2009. Metapopulation persistence in fragmented landscapes : significant interactions between genetic and demographic processes. Journam of Evolutionary Biology 22, 152–162.

Traill, L., Bradshaw, C., Brook, B., 2007. Minimum viable population size : a meta-analysis of 30 years of published estimates. Biological Conservation 139, 159–166.

Verhulst, P., 1844. Recherches mathématiques sur la loi d'accroissement de la population. Nouveaux Memoires de l'Académie Royale des Sciences et Belles-Lettres de Bruxelles 20, 1–52.

Volterra, V., 1931. Variations and fluctuations of the number of individuals in animal species living together, in: Animal Ecology. R.N. Chapman, ed., McGraw-Hill, New York, pp. 409–448.