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ORIGINAL PAPER

Prediction of the minimum effective size of a population viable in the long term

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Abstract
The establishment of the minimum size for a viable population (MVP) has been used as a guidance in conservation practice to determine the extinction risks of populations and species. A consensus MVP rule of 50/500 individuals has been attained, according to which a minimum effective population size of $N_e = 50$ is needed to avoid extinction due to inbreeding depression in the short term, and of $N_e = 500$ to survive in the long term. However, the large inbreeding loads (B) usually found in nature, as well as the consideration of selection affecting genetic diversity, have led to a suggestion that those numbers should be doubled (100/1000). Purging of deleterious mutations can also be a main factor affecting the suggested rules. In a previous simulation study, the reduction of B by the action of purging pointed towards an MVP intermediate between the two rules for short term survival. Here, we focused on the consequences of purging in the establishment of MVPs for long term survival. We performed computer simulations of populations under the action of purging, drift, new mutation, and environmental effects on fitness to investigate the extinction times and the loss of genetic diversity for a range of effective population sizes. Our results indicate that purging can reduce the MVP needed for a population to persist in the long term, with estimates close to $N_e = 500$ for species with moderately large reproductive rates. However, MVP values appear to be of at least $N_e = 1000$ when the species´ reproductive rates are low.

Keywords Effective population size · Inbreeding depression · Genetic purging · Inbreeding load · Extinction risk

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Introduction

In an ever-changing world where biodiversity is declining and human activity has an imposing presence, the indicators for population resilience are needed for the genetic management and conservation planning of endangered species. One of these indicators is the minimum size for a viable population (MVP) required to avoid a population from extinction in a given timeframe (Shaffer 1981; Traill et al. 2010). According to the International Union for Conservation of Nature, 28% of the assessed species are currently under risk of extinction (IUCN 2021). Integrating the multiple factors of diverse nature influencing the viability of a population, from environmental, demographic, and genetic stochasticity to deterministic factors such as habitat loss (Shaffer 1981; O'Grady et al. 2004; Brook et al. 2008; Frankham et al 2010), many researchers have focused on developing a general MVP value (see for example Reed et al. 2003b; Brook et al. 2006; Traill et al. 2007, 2010). The concept of MVP was introduced by Shaffer (1981), and despite the debate that has arisen in the past few years on the suitability of its use given its generality across species (and even populations) (Traill et al. 2010; Brook et al. 2011; Flather et al. 2011a, 2011b), it still has a great relevance in conservation practice, being a component, for instance, of the IUCN criteria to determine the threatened categories (IUCN 2012).

The MVP can be defined as the minimum size of a population capable of survival in a given timeframe in the face of the different adverse forces and uncertainties that affect its viability, the size below which the risk of extirpation or extinction is unacceptably high (Shaffer 1981). Different variants of MVP can be found in the literature depending on what minimum size is referred to, that is, the minimum carrying capacity, the minimum adult population size or, from a genetic point of view, the minimum effective population size (Nunney and Campbell 1993; Reed et al. 2003b), N_e, i.e. the size of an ideal population that would result in the same level of inbreeding or genetic drift as the real population (Wright 1938; Wang et al. 2016). In general, two distinctions about MVP can be made in terms of the time scale involved and the genetic forces that most influence the probability of persistence. One is the minimum population size in the short term, below which the viability of a population is seriously compromised by the decline of fitness due to inbreeding, i.e., inbreeding depression (Franklin 1980; Frankham et al. 2014; Caballero et al. 2017). The other is the minimum population size in the long term when, once the population has dealt with the initial inbreeding depression and survived, the loss of adaptive potential due to genetic drift compromises the ability of the population to face future environmental changes and, thus, the ability to persist in the long term (Franklin 1980; Frankham et al. 2014).

After a population suffers a reduction in size (whatever the original cause was and/or is), both genetic drift and inbreeding contribute to the decline of the population by reducing its fitness, which may reinforce the reduction in size (Gilpin and Soule 1986). First, deleterious mutations can become fixed due to the increased stochasticity in allele frequencies by drift (Lynch et al. 1993, 1995; Lande 1995). Second, inbreeding exposes in homozygosis recessive deleterious mutations that were previously hidden in heterozygous state when the population was large (the so-called inbreeding load, measured by the number of lethal equivalents; Morton et al. 1956), leading to a reduction of fitness (inbreeding depression). In the short term, both forces can lead the population to extinction (Lynch et al. 1993, 1995; Lande 1995; Frankham 2005; Wright et al. 2008). However, natural selection against the inbreeding load exposed in homozygosis, i.e. genetic purging (Hedrick and García-Dorado 2016), should also be considered when predicting the risk of extinction. As inbreeding

exposes recessive deleterious mutations, genetic purging removes or reduces the number of such mutations, thus ameliorating inbreeding depression (Hedrick and García-Dorado 2016). In terms of extinction risk, genetic purging may imply smaller MVPs in the short term than previously thought (García-Dorado 2015; Caballero et al. 2017), and perhaps also in the long term. Because purging reduces the inbreeding load, populations with a history of moderate size or occasional bottlenecks, and, therefore, with a history of past purging, would be less susceptible to future inbreeding depression than populations without past inbreeding (García-Dorado 2015). However, surviving inbreeding depression does not guarantee long-term persistence. Genetic diversity, and thus the adaptive potential of the population to face future environmental changes (Reed and Frankham 2003), is lost at a rate $1/(2N_e)$ per generation by drift in isolated populations (Wright 1969). With time, an equilibrium is reached where the loss of genetic diversity is compensated by new entries of variation through mutation (Franklin 1980). The establishment of MVPs in the long term relies on such equilibrium (Franklin 1980; Franklin and Frankham 1998).

In the past years, several rules of thumb have arisen to help the decision of which populations are of special conservation concern, and to help the planning and management of endangered species for short- or/and long-term survival. Franklin (1980) proposed the classic 50/500 recommendation for the minimum effective population size (N_e) required in the short and long term, respectively. The value $N_e = 50$ was derived from animal breeding programmes, where a rate of inbreeding of 1% per generation resulting from $N_e = 50$ is considered tolerable (Soulé 1980; Franklin 1980). The value $N_e = 500$ was derived from the expected equilibrium reached between the loss by drift and the gains by new mutation of additive genetic variance for a quantitative trait (Franklin 1980; Lande 1975). However, more recently Frankham et al. (2014) proposed that those values should be increased to at least 100/1000. They provided a more precise description of risk in the 'short term', suggesting a maximum of 10% decline in total fitness over five generations. On the one hand, considering this maximum decline in fitness, and given the high inbreeding load usually found in wild populations ($B \approx 6$ lethal equivalents; O'Grady et al. 2006), an effective size of at least 100 individuals would be needed to prevent extinction caused by inbreeding depression (Frankham et al. 2014). On the other hand, focusing on genetic diversity for fitness traits, rather than for peripheral traits, and including natural selection as a genetic force influencing genetic diversity (either removing or retaining it), Frankham et al. (2014) defended that an effective size of at least 1000 individuals would be needed to retain evolutionary potential.

Although Frankham et al. (2014) considered selection, the rules proposed by Frankham et al. (2014) and Franklin (1980) did not take into account the consequences of purging. Despite the effects of purging have often been considered weak or limited, to some extent supported by the apparent absence of purging (or weak purging) in several empirical analyses (Byers and Waller 1999; Reed et al. 2003a; Boakes et al. 2007), both theoretical and empirical results support that purging can be relevant under some particular situations (Swindell and Bouzat 2006; Ávila et al. 2010; Pekkala et al. 2012; López-Cortegano et al. 2016; Pérez-Pereira et al. 2021b). Note that purging can go unnoticed because it could be masked by other factors such as simultaneous adaptation or genetic management. In addition, proper estimates of B and N_e are not always available to allow for an informed study of population fitness and viability as a function of effective population size (García-Dorado 2015; López-Cortegano et al. 2016).

The study of MVPs is often addressed with population viability analysis (PVA), which incorporates both stochastic and deterministic factors based on population-specific parameters (Reed et al. 2003b). For instance, it is common to use the software VORTEX (Lacy

and Pollak 2021) in simulating different population sizes in order to determine the minimum size of a population capable of persistence given the level of stochasticity introduced in the model. Although the software includes the effects of inbreeding depression, at the moment the action of purging is restricted to full recessive lethals (Lacy and Pollak 2021). It ignores the purging occurring over an important fraction of the inbreeding load due to deleterious but not lethal alleles, and thus underestimates the role of purging in both the short and the long term (García-Dorado 2015). In a recent study, Caballero et al. (2017) performed computer simulations to investigate the effects of purging (against lethal and non-lethal alleles) on the short-term MVP. They found that, under the mutational models evaluated, a minimum effective size of $N_e \approx 70$ is sufficient to avoid extinction due to inbreeding depression, a value that is intermediate between the $N_e = 50$ rule by Franklin (1980) and the $N_e = 100$ rule by Frankham et al. (2014). However, the consequences of purging in relation to the MVP needed for a population to persist in the long term were not addressed. In the present study, we aim to investigate the MVP for the long term survival of a population by considering the consequences of purging through computer simulations, following a similar procedure as in Caballero et al. (2017) but for longer periods and with the incorporation of environmental effects on fitness.

Methodology

We carried out computer simulations of populations of various sizes to evaluate the extinction times under different mutational models. The simulation process consisted of two steps. First, we simulated large populations at the mutation-selection-drift equilibrium to create large base populations with a given inbreeding load B. Second, multiple lines of reduced sizes were derived from the large equilibrium populations and maintained over a large number of generations under the action of selection, drift, new mutation and environmental effects on fitness. The extinction time, genetic diversity, inbreeding load, and other properties of the lines over generations were recorded and analysed in relation to MVP.

Genomic model and mutational parameters

The genome consisted of 30,000 biallelic loci, of which 1000 were neutral and the remaining had deleterious effects on fitness (around 28,000 segregating deleterious loci). We assumed a genome size of $L = 20$ Morgans (Dumont and Payseur 2008). Deleterious mutations arose at a rate U per haploid genome and generation (with parameter values given below). They had selection coefficient s obtained from a gamma distribution with shape parameter β (mean selection coefficient $\bar{s}$), and dominance coefficient h obtained from a uniform distribution between zero and e^{-ks} (where k is a constant used to obtain the mean dominance coefficient $\bar{h}$; Caballero and Keightley 1994). An additional mutation rate U_L was included to add a class of lethal mutations ($s = 1$ and $h = 0.02$; Simmons and Crow 1977). For a single locus, the fitness values were 1, $1 - sh$ and $1 - s$ for the homozygous genotype of the wild allele, the heterozygous genotype, and the homozygous genotype of the mutant allele, respectively. The fitness of an individual was calculated multiplicatively across loci. We assumed soft selection, in the sense that selection acted on relative fitness obtained by dividing the fitness (as determined by the multiplicative model) of each individual at generation t by the mean fitness of the population at generation 0, as empirical estimates suggesting a high mutation rate (see below) seem to tolerate soft selection only

(Keightley 2012). The inbreeding load (B) was calculated as the sum for all loci of $s(1 - 2h)pq$ (Morton et al. 1956), where p and $q = 1 - p$ are the wild and mutant allele frequencies, respectively.

We adopted two mutational models, both being based on empirical evidences (Table 1). In Model I, the mutation rate U was obtained from Keightley (2012) for humans, the average selection coefficient $\bar{s}$ and shape parameter β from Boyko et al. (2008) for non-synonymous mutations in humans, and the mean dominance coefficient $\bar{h}$ from mutation accumulation experiments in *Drosophila*, *Caenorhabditis* and yeast (Caballero 2020). Model II corresponds to Model A from Caballero et al. (2017). Because the inbreeding load usually found in the wild is about $B \approx 6$ (O'Grady et al. 2006), we modified the original U of both models such that the mutation rate finally applied in simulations (U_{sim}) resulted in that approximate value of B in the base populations. Despite Models I and II assume low average fitness effects and the same mean dominance coefficient, they differ mainly in their distribution of s and h values (Fig. S1 in Supplemental Material). Model I is characterized by a larger proportion of small effect mutations and smaller dominance coefficients per locus, while Model II includes mutations of moderate effect and larger dominance coefficients, which can be more easily eliminated by purging. Both models included additionally the same rate of lethal mutations.

Simulation of populations

Ten (replicate) base populations simulated per model were maintained with random mating without selfing at a constant size of $N_b = 10{,}000$ individuals during 10,000 generations. From each base population, a sample of N individuals ranging from $N = 25$ to 1700, called a line hereafter, was obtained and maintained during 1000 generations to evaluate the risk of extinction and loss of genetic diversity measured as mean observed heterozygosity (H). For each scenario, 100 replicate lines were simulated per base population (i.e., a total of 1000 lines per scenario and model) and the results were averaged.

Individual fitness was assumed to be composed of two traits, fecundity (number of offspring produced, determined by 1/3 of the simulated loci) and viability (survival probability, determined by 2/3 of the simulated loci). The overall fitness (W) was the product of the fecundity (W_f) and viability (W_v) components. This resulted in an initial mean inbreeding load of $B_f \approx 2$ for fecundity and $B_v \approx 4$ for viability for both Models I and II, in agreement

Table 1 Mutational models

	U	U_{sim}	U_L	$\bar{s}$	β	$\bar{h}$	B (± sd)
Model I	1.1	0.34	0.015	0.02	0.2	0.2	5.967 ± 0.083
Model II	0.5	0.22	0.015	0.05	0.45	0.2	6.064 ± 0.097

U haploid deleterious mutation rate per generation, U_{sim} haploid deleterious mutation rate per generation used in the simulations to obtain the desired value of B, U_L haploid lethal mutation rate per generation, where the homozygous lethal selection coefficient is $s = 1$ and the dominance coefficient of lethal alleles is $h = 0.02$, $\bar{s}$ average homozygous selection coefficient for deleterious mutations, β shape parameter of the gamma distribution of homozygous selection coefficients, $\bar{h}$ average coefficient of dominance of deleterious mutations, B average and standard deviation of the inbreeding load of a large equilibrium population

with the empirical observations of O'Grady et al. (2006) for birds and mammals. The fecundity (W_f) and viability (W_v) of each individual was calculated multiplicatively across the involved loci. At each generation t, the relative fecundity or relative viability of individual i was obtained by dividing its genotypic value by the mean of the line at generation $t=0$ (i.e., $W_{f\,(t,\,i)}/\overline{W}_{f\,(0)}$ and $W_{v\,(t,\,i)}/\overline{W}_{v\,(0)}$). Under this multiplicative model of fitness, the probability of survival for a given inbreeding level F can be expressed as $S_F=e^{-(A\,+\,BF)}$ (Lynch and Walsh 1998), where A determines the probability of mortality in the panmictic population, which includes environmental causes of mortality, and BF accounts for the probability of mortality due to inbreeding. Thus, to account for a reduction in fitness due to environmental reasons, the values of W_f and W_v were subtracted an amount $A=0.3$, a value obtained as the average of empirical estimates for mammals and birds (Lynch and Walsh 1998).

Matings followed a polygamous system (an individual could mate multiple times with different individuals, but selfing was not allowed), where parents were chosen based on their fecundity and the number of offspring was proportional to the fecundity of both parents. The number of matings occurring in each generation was assumed to be half the number of breeding individuals. The number of offspring per couple was calculated as $K\times\sqrt{W_{f(mother)}\times W_{f(father)}}$, where K is the reproductive rate (note that this value is defined as twice the value of K considered by Caballero et al. 2017). The values of K ranged from 2 to 60, based on reproductive rates found in nature, especially for mammals (median 3), birds (median 6) and reptiles (median 14.4), measured as total number of eggs laid (mean clutch size × mean number of clutches) or young born (mean litter size × mean number of litters) per female per year (Fig. S2; Vance et al. 2003; Brook et al. 2006; Traill et al. 2007; Rytwinski and Fahrig 2011; Quesnelle et al. 2014). Although these empirical reproductive rates are not the same as the values of K used in the simulations, they can be considered approximations. The simulated K values represent the maximum number of offspring per mating pair, but individuals could mate multiple times in the same generation, which would be equivalent to one clutch or litter per year over multiple years in a single simulation generation, or to multiple clutches or litters in a single year in a single simulation generation. Each offspring was determined to survive if a random number taken from a uniform distribution in the range [0,1] was lower than its viability, W_v. From the alive offspring, a number of N individuals was randomly sampled as breeders for the next generation. However, the population size of the line could be reduced to less than N individuals if there were not enough alive offspring available, and the population could become extinct if the number of alive offspring was ≤ 1. Note that, here, the population size refers to the carrying capacity, which means that the number of individuals can be lower than N but not larger, which is a conservative assumption. We investigated the minimum value of K with which a population of a certain effective size N_e could persist in the long term with a 99% probability, where "long term" means a period of 40 generations (as suggested by several authors; Traill et al. 2007; Frankham et al. 2014) or the total length of the simulations, i.e., 1000 generations. The effective population size (N_e) of the lines was calculated from the achieved variance of family sizes (S_k^2) as $N_e=4N/(2+S_k^2)$ (Wright 1938). Under the simulated breeding system, the effective size was approximately equal to $0.6\times N$. Because N_e is the relevant parameter under discussion, all results from the study will refer to this parameter, rather than to N.

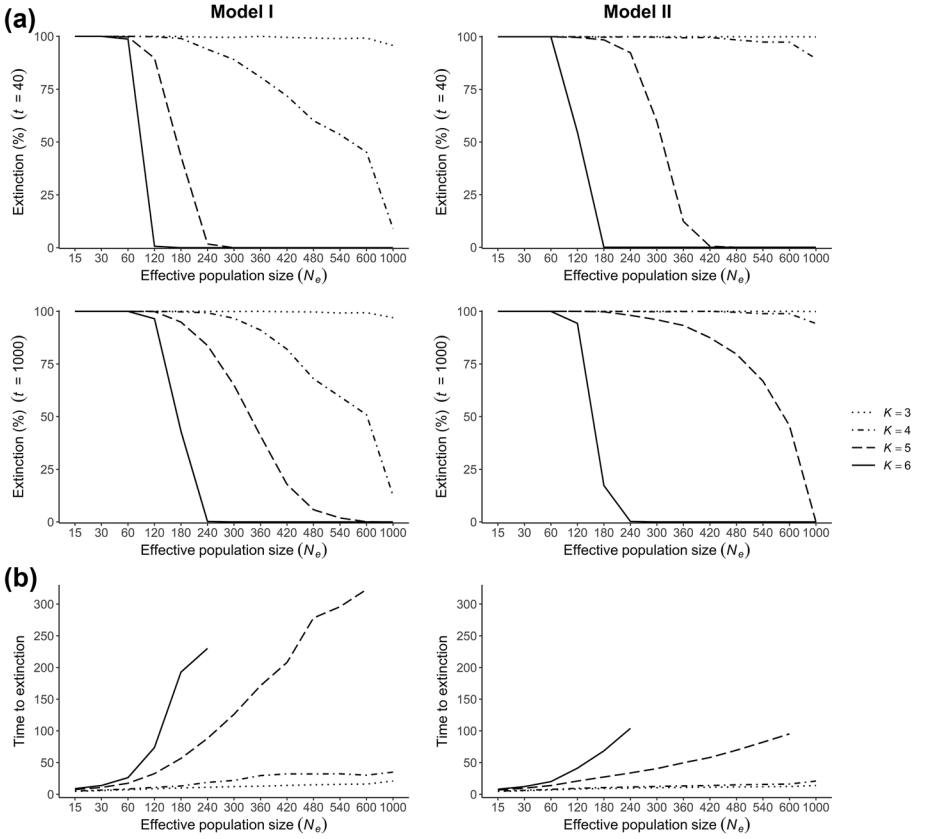

Fig. 1 **a** Probability of extinction, represented as percentage of extinct lines after 40 generations (first row) or 1000 generations (second row) for different effective population sizes (N_e) and reproductive rates (K) under two mutational models (see Table 1). **b** Mean times to extinction (in generations) for different effective population sizes (N_e) and reproductive rates (K)

Results

Probability of extinction

Extinctions were frequent under the two mutational models evaluated, particularly for lines with small effective population sizes and low reproductive rates. Model II incurred in general more extinctions than Model I (Fig. 1a). Times to extinction were considerably short on average (Fig. 1b), especially when reproductive rates were low ($K \leq 4$). In such cases, all populations became extinct in the first 25 generations approximately, regardless of N_e and the mutational model. Those generations were characterized by severe inbreeding depression and large reductions in effective size, to a greater extent under Model II, although under both models a recovery of fitness was observed after overcoming the phase of inbreeding depression, even exceeding the average fitness at generation 0, which can be attributed to purging (Fig. 2). For higher reproductive rates ($K = 5$ and 6), times to extinction were considerably shorter for Model II than Model I. With $K = 6$, no extinctions were observed for populations with $N_e > 240$ under both models.

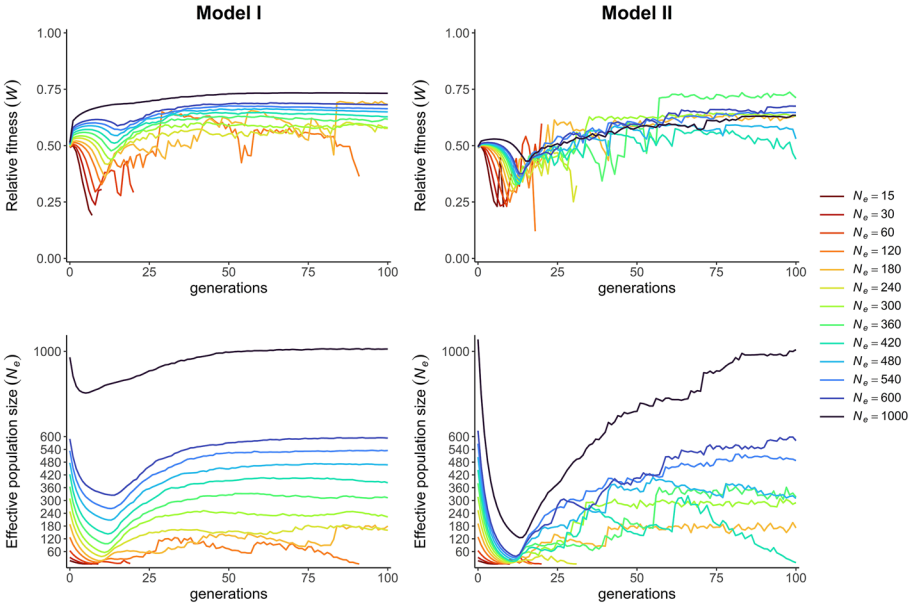

Fig. 2 Relative fitness (W) and realized effective population size (N_e) in the first 100 generations. Results correspond to a reproductive rate of $K=4$ under two mutational models (see Table 1)

To see more clearly the impact of the reproductive rate on extinction risk, we considered the minimum value of K with which a population of a certain effective size N_e could persist in the long term (40 or 1000 generations) with a 99% probability (Fig. 3). Results for Models I and II were in general similar, as well as for the two periods of time considered, suggesting that the critical time for extinction risk was the first generations during which inbreeding depression could be severe. Small populations ($N_e \leq 240$) only persisted in the long term if the reproductive rate was high. For example, for populations of effective size $N_e=60$, a reproductive rate of at least $K=10$ for both Model I and II was needed to persist 40 generations, and $K \approx 25$ for Model I or $K \approx 10$ for Model II to persist 1000 generations. However, larger K values than those explored in our simulations would be needed to ensure the persistence of populations with $N_e=15$, as no K value in our explored range was found that ensured the 99% persistence of such lines for 40 generations under Model I, and for 1000 generations under Model II. On the other hand, the minimum value of K seems to stabilize for population sizes above $N_e \approx 300$. This minimum value is around $K=5$, indicating that populations of sizes of that order with no extremely low reproductive rates could persist in the long term, at least under the simulated conditions. This result also indicates that, populations of species with reproductive rates below that value (i.e., $K<5$) would need effective sizes above $N_e=1000$ individuals to ensure long-term persistence.

Retention of genetic diversity

MVP results were in general consistent with those obtained for genetic diversity, measured as mean (observed) heterozygosity for neutral alleles, H, at generations $t=40$ and 1000

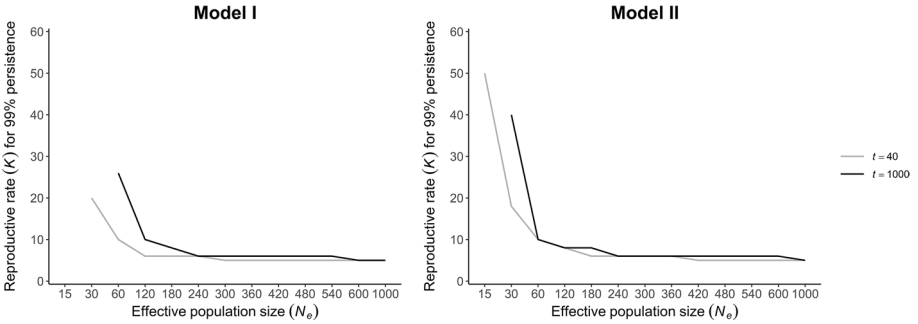

Fig. 3 Minimum reproductive rate (K) necessary for a population of effective size N_e to persist in the long term (40 or 1000 generations) with a 99% probability under two mutational models (see Table 1). The absence of a solid line for a particular N_e indicates that no K value was found that ensured the persistence of that line for the time t considered (evaluated up to a maximum of $K=60$)

(Fig. 4). For comparison, we also simulated populations in the absence of selection (i.e., neutral). Heterozygosity increased with population size, asymptoting to the initial H at generation 0 (indicated by a dashed blue line) for effective sizes $N_e > 500$ when measured at generation 40 under the two mutational models (first row in Fig. 4). Small differences were found between the simulations with and without selection. By generation 1000 (second row in Fig. 4), H was reduced substantially, to a larger extent in those simulations with

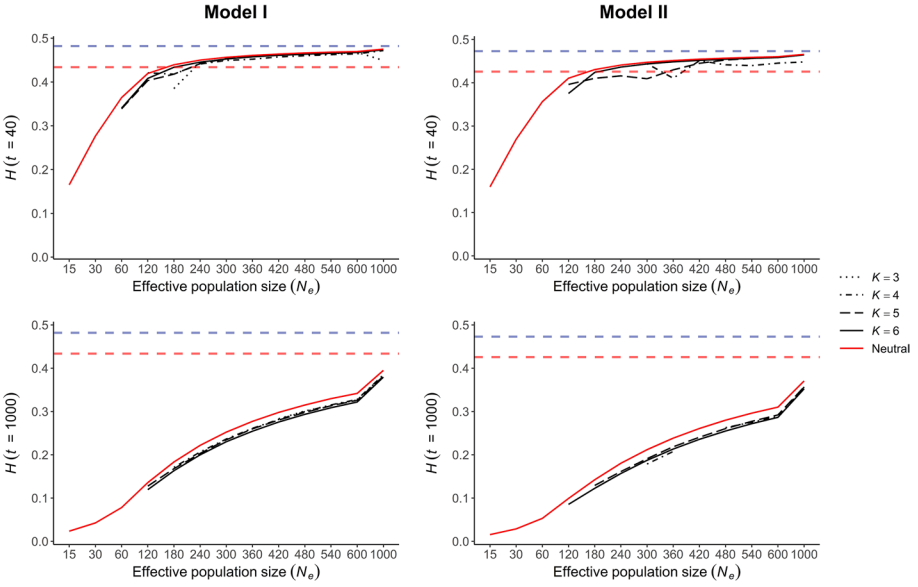

Fig. 4 Observed mean heterozygosity (H) for neutral alleles as a function of effective population size (N_e) of the lines at generations $t=40$ (first row) and $t=1000$ (second row) under two mutational models (see Table 1). Black lines indicate simulations with selection, the solid red line indicates simulations without selection (neutral) for comparative purposes, the horizontal dashed blue line indicates the initial mean heterozygosity at generation 0 (H_0), and the horizontal dashed red line indicates a 90% threshold in genetic diversity to be retained

selection, as expected, and larger effective sizes ($N_e > 1000$) would be needed to reach a stabilization in the curve of H as N_e increases.

In captive breeding programs, it is a common objective to retain 90% of within-population genetic diversity for 100 years (Frankham et al. 2010, 2014). Although we did not simulate time in years, this objective could be assessed at generation 40 (dashed red line in Fig. 4), a timeframe recommended to evaluate probability of extinction in the long term (Traill et al. 2007; Frankham et al. 2014). Under the particular conditions of our simulations, an effective size around $N_e \approx 240$ (Model I) or $N_e \approx 420$ (Model II) would be enough to reach this goal.

We also measured (observed) heterozygosity for deleterious alleles (Fig. 5), as their effect on fitness can be environment dependent in nature (i.e., deleterious in one environment but beneficial in others) and thus can also contribute to adaptation, especially those of mild fitness effect. Note that purging will have a direct impact on the heterozygosity for such alleles, so lower H values were expected in simulations with selection than in neutral simulations (where H is only reduced by drift). This can be observed at generation 1000 (second row in Fig. 5) and to a larger extent for larger populations, as expected. On the other hand, if we compare the two mutational models, lower heterozygosity was found under Model II. In terms of MVP, similar conclusions were reached as for the neutral alleles, the results suggesting that population sizes of the order $N_e \approx 240$ (Model I) or $N_e \approx 420$ (Model II) would be enough to attain the objective of retaining 90% of within-population genetic diversity for 40 generations (first row in Fig. 5). Thus, to be conservative, we can conclude that a minimum effective population size around $N_e \approx 400$ individuals would be needed for populations of species with a reproductive rate of at least $K = 5$ to persist in the long term with a 99% probability (Fig. 3) and to retain evolutionary potential

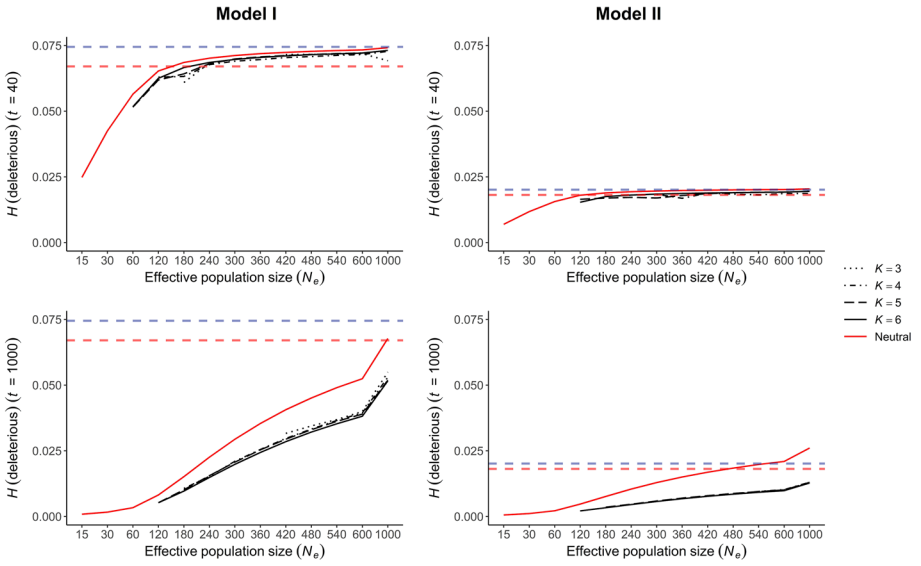

Fig. 5 Mean heterozygosity (H) for deleterious alleles as a function of effective population size (N_e) at generations $t = 40$ (first row) and $t = 1000$ (second row) under two mutational models (see Table 1). Black lines indicate simulations with selection, the solid red line indicates simulations without selection (neutral) for comparative purposes, the horizontal dashed blue line indicates the initial mean heterozygosity at generation 0 (H_0), and the horizontal dashed red line indicates a 90% threshold in genetic diversity to be retained

(Fig. 5). However, for populations of species with a reproductive rate $K<5$, effective sizes larger than 1000 would be needed to ensure long term persistence.

Discussion

We have evaluated the effects of purging on the minimum effective population size (MVP) required to avoid extinction in the long-term. By simulating populations under two sets of mutational parameters consistent with empirical data and under a simulation model that allowed the action of purging, we evaluated whether the MVP obtained under a purging model fits the classic rule of thumb $N_e=500$ (Franklin 1980) commonly applied in conservation, or, instead, the more recent modification to $N_e=1000$ suggested by Frankham et al. (2014). Our results suggest that the classic rule is fairly robust for a wide range of populations with moderately large reproductive rates ($K>5$), although species with low reproductive rates ($K<5$) will need MVPs closer to those suggested by Frankham et al. (2014), or even larger.

Results were somewhat more optimistic under the mutational Model I than under Model II, although similar conclusions were reached for both in terms of MVP. Although mean parameters (mainly selection coefficient $\bar{s}$ and dominance coefficient $\bar{h}$) did not differ substantially between the models, they did differ in their distribution, with more severely deleterious alleles under Model II. Thus, although the inbreeding load was adjusted by modifying the mutation rate to encompass an initial inbreeding load of about $B \approx 6$ lethal equivalents (on the order of that found in wild populations of mammals and birds; O'Grady et al. 2006), less intense inbreeding depression was observed under Model I than under Model II, with a consequent smaller reduction in population size in the first generations and, therefore, a lower risk of extinction and larger times to extinction for Model I. However, similar minimum N_e values for a viable population in the long term were obtained from the two models ($N_e \approx$ 300–400 for a reproductive rate $K>5$), probably as a consequence of purging. In fact, both models showed evidence of purging with a fitness rebound, relative to the mean fitness of the population at generation 0, once the populations survived the phase of inbreeding depression (see Fig. 2 as an example for a reproductive rate $K=4$, but an increase in fitness was also observed for other K values; not shown). Therefore, those lines that passed the phase of inbreeding depression would carry lower B values in the long term and, thus, would be more resilient to future inbreeding. This is consistent with the results of Yang et al. (2018), who found fewer loss-of-function variants in the critically endangered *Ostrya rehderiana* (with a long history of population decline) compared to its close relative *O. chinensis*. According to the authors, this could have helped the population to survive long periods of time at low effective population sizes despite the high genetic load and low levels of genetic diversity found in this species. We did not find large differences when evaluating extinction probability over a period of 40 *versus* 1000 generations (Fig. 3), as the first 40 generations were the main determinant of the persistence of a population over time, and therefore of the establishment of the MVP, at least under the constant environmental conditions considered in our simulations. Thus, using a standardized period of 40 generations to evaluate long-term MVPs, as suggested in previous studies (Traill et al. 2007; Frankham et al. 2014), seems reasonable.

As purging removes deleterious alleles, an increase in the frequency of wild-type homozygote genotypes would be expected and, therefore, a decrease in observed heterozygosity (H) for those loci. This is consistent with the higher reduction of H observed for

deleterious alleles compared to the model without purging (neutral model), particularly for large values of N_e (Fig. 5). It should be noted that purging is more effective for larger populations in which inbreeding progresses slowly, but the consequences become apparent later in time. This may have implications on the criteria to establish MVPs based on genetic diversity retention when deleterious alleles are included as part of genetic diversity contributing to evolutionary potential, particularly because the deleterious effects may be environment-dependent (Cheptou and Donohue 2011; Frankham et al. 2014). In our simulations, we used the objective of retaining 90% of genetic diversity in conservation programs as a guidance to establish the minimum N_e for maintaining adaptive potential in the long term (Frankham et al. 2010, 2014). When we measured H for deleterious alleles at generation $t=40$ (in accordance with the suggested time period to evaluate extinction probability), the conclusion was consistent with that reached for neutral genetic diversity and for extinction probability ($N_e \approx 400$), but differed when measured at generation $t=1000$, when the consequences of purging were apparent, and a larger MVP would be needed than that derived in the absence of purging.

Our results show that there is a strong dependence of the MVP on the reproductive rate of the species. Given the reproductive rates commonly found in nature (Vance et al. 2003; Brook et al. 2006; Traill et al. 2007; Rytwinski and Fahrig 2011; Quesnelle et al. 2014), the minimum $N_e=500$ could apply to a wide range of species. However, for many of these, particularly mammals, for which $K<5$ (Fig. S2), this would not be the case. The reproductive rates summarised in Fig. S2 were calculated as total number of eggs laid or young born per female per year. The median values of reproductive rates for mammals, birds and reptiles were 3, 6 and 14.4, respectively. To our knowledge, these estimates do not account for the probability of survival to reproductive age. In this sense, these estimates are comparable to the value of K used in simulations, as all the offspring expected for a given fecundity of the parents are produced, regardless of whether or not each of the offspring subsequently survives. However, these estimates are not fully comparable to our K values, as we evaluated offspring production per generation and assumed discrete generations, instead of offspring production per year for species with overlapping generations. In addition, mortality before reaching reproductive age could be very much higher than that simulated here. For instance, amphibians and reptiles may lay thousands of eggs, while only a few can hatch and survive to reproductive age. Thus, the values of K shown for these groups (as well as for insects) in Fig. S2 could be highly inflated in relation with the K used in our simulations, with the consequence that a larger fraction of populations would best fit the MVP suggested by Frankham et al. (2014), or even higher.

Brook et al. (2006) and Traill et al. (2007) have also reported a certain tendency of larger MVPs for lower reproductive rates, especially for mammals, birds, amphibians and insects (for which more data was available; see the supplementary material in the respective references). However, they found little correlation between variation in MVPs and life-history predictors of MVPs among taxa (including reproductive rate, body weight and generation length, among others), although life-history predictors did correlate with the IUCN threat status. Along with the large variation in MVP found within-species, empirical data suggest that the MVP of a particular species or population is rather environment-context specific (Flather et al. 2011a).

We are aware that our results could be considered too optimistic (small MVP), even if compared to the classic recommendation. Although our results indicate that purging is capable of reducing the MVP for long-term survival, in agreement with simulations of similar nature for short-term survival (Caballero et al. 2017) and theoretical expectations (García-Dorado 2015), our simulation design had some limitations. Environment effects

(included in our parameter $A = 0.3$) were assumed to be constant over time, which may not be realistic considering for example the gradual environmental changes associated with climate change. Deterministic factors, such as reductions in population size by overexploitation or human-driven habitat loss, or stochastic factors such as catastrophes (e.g., fires) have not been taken into account either. These factors could increase the risk of extinction and therefore the estimates of MVP. However, the absence of environmental stochasticity has made it possible to evaluate the consequences of purging with less noises that could obscure the conclusions drawn from this study. Thus, our results could be applied to populations in more or less stable environmental conditions where the deterministic threats (such as habitat loss or deterioration) have been removed or considerably reduced by conservation efforts. Our simulation results are probably applicable to a limited number of populations, so further studies encompassing the joint effects of purging and environmental stochasticity would be needed for a practical purpose.

Other factors such as adaptation to environmental changes and population regulation by density-dependence are also worthy of study given their impact on the risk of extinction (Vinton and Vasseur 2020). For instance, Brook et al. (2006) found smaller MVPs under three (negative) density-dependence models than under two density-independent models, the MVPs for the latter being around two orders of magnitude larger than those for the former. The possible explanation given by the authors is that density dependence implies an endogenous control of the population that gives more long-term stability and therefore less susceptibility to fluctuations by environmental changes. Thus, the assumption of density-dependence models could compensate the negative effects of possible environmental fluctuations, although more studies are needed in this regard. In our simulations, we have also assumed that the large ancestral population of size $N = 10{,}000$ individuals suffers a sudden drop down to the intended size of the line. This has a large impact on the inbreeding depression occurring in the line, as illustrated by the strong declines in effective size shown in Fig. 2. However, if the transit from the large population size to the reduced one would occur gradually, as it is perhaps more realistic, genetic purging would remove the deleterious load more efficiently, so that the eventual extinction risk of the final population would be probably lower. In this sense, our simulations are very conservative and lower MVPs could be expected in general if the load of the ancestral population is slowly removed before reaching a small population size.

Despite the fact that an important fraction of drivers of extinction risk has not been taken into account in our study, we observed a larger proportion of extinctions and shorter times to extinction than those found in the study performed by Kyriazis et al. (2020). These authors considered demographic stochasticity as a density-dependence function (Haller and Messer 2019), environmental stochasticity determining the carrying capacity each generation, and probability of random natural catastrophes. Their mutational parameters ($U \approx 0.2$, $\bar{s} \approx 0.02$, $\beta \approx 0.2$, $h = 0.25$ for alleles with $s < 0.02$ and $h = 0$ otherwise) could be compared with those of our Model I. For instance, with an ancestral carrying capacity of 10,000 individuals, Kyriazis et al. (2020) obtained a median value for times to extinction around 100 and 1500 generations for lines of carrying capacity $N = 25$ and 50 ($N_e \approx 17$ and 35 according to their ratio $N_e/N \approx 0.7$), respectively (see their Figs. 2 and S7). From our simulations, similar values of extinction times were reached only with reproductive rates of $K \approx 50$ or greater for $N_e = 15$–30. Thus, although our results are optimistic, we would expect MVP estimates to be even more so if evaluated under the conditions simulated by Kyriazis et al. (2020). One factor that could account at least for part of the differences in extinction rates observed in our study and theirs is the different reproduction model simulated. They considered overlapping generations and despite each mating resulted in only

one offspring, each individual could mate multiple times not only in the same generation but also in later generations if that individual was still alive. This system seems, therefore, to be equivalent to one of high reproductive rates in our simulation model. Another possible factor contributing to the different results of the two studies is that Kyriazis et al. (2020) simulations assumed population density-dependence, i.e., the probability of survival depended on individual fitness scaled by the ratio between the carrying capacity and the number of individuals.

We used a set of mutational parameters based on empirical data or other simulation studies. However, other sets of mutational parameters can be found in the recent literature, as those assumed by Kardos et al. (2021) and Pérez-Pereira et al. (2021a). The model considered by Kardos et al. (2021) was similar to Model II and, therefore, it should render results close to those found in the present study. However, a rather different set of parameters was assumed by Pérez-Pereira et al. (2021a). They assumed a rate of $U=0.2$ of mutations with relatively large effect (values of s obtained from a gamma distribution of shape parameter $\beta=0.33$, resulting in a mean of $\bar{s}=0.2$) and a mean dominance coefficient of $\bar{h}=0.283$. Because the effects of mutations in this model are substantially larger than those in Models I and II, it is expected that purging would be more efficient and the deduced values of MVP would be also lower. In fact, the simulation results considering this model, and including an additional mutation rate for lethals as in Models I and II (Supplemental File S1), suggest that the MVP for species with reproductive rate $K>5$ would be of the order of 200 individuals. Thus, the results from Models I and II seem more conservative than those with this alternative model. A final aspect to take into account is that we assumed that inbreeding depression was exclusively caused by partially recessive deleterious mutations and we ignored the possible contribution of overdominant mutations, which would not be removed by purging, and could change the estimates of MVP. However, most evidence indicates that the contribution of overdominance to inbreeding depression is minor compared to partial dominance (see, e.g., Hedrick 2012; Yang et al. 2017).

One issue of special relevance in the establishment of MVPs is the translation of effective population sizes (N_e) to actual number of individuals (N), and vice versa. Although N_e values provide more information about the genetic health of a population, the application of MVPs usually relies on N, which is more manageable on a conservation practical level (Frankham 2021). Until now, a ratio $N_e/N=0.1$ has been commonly used as a rule of thumb to convert between N_e and N values. The ratio was the average value found by Frankham (1995) in a study that included around 100 species of animals and plants, although recent analyses provided larger ratios (mean $N_e/N=0.35$ and median 0.3; Hoban et al. 2020). In any case, there is a huge variability of N_e/N ratios among taxa (and even within-species; Jamieson and Allendorf 2012), which makes it difficult to establish general rules, when defining both a standard N_e/N ratio and an MVP applicable to any taxon or population. In our simulations we obtained the values of N_e and presented the results as a function of them. Ideally, in conservation practice N_e/N ratios should be obtained by directly estimating N_e in the particular populations, which is commonly quantified from changes in allele frequencies due to drift from one generation to another (the variance effective size, N_{eV}) or from linkage disequilibrium between loci (N_{eLD}) (see Wang et al. 2016). In this regard, it is important to note that a minimum $N_e=500$ refers to the global effective size (i.e., metapopulation) rather than the local effective size (Jamieson and Allendorf 2012). It has been shown that estimates of local N_{eV} and N_{eLD} can underestimate both the local and global inbreeding effective size (N_{eI}, which measures the increase of inbreeding and is the estimate more relevant for the minimum N_e) in substructured populations (Hössjer et al. 2016; Ryman et al. 2019). Thus, when appropriate estimates of N_e cannot be obtained, or a ratio

N_e/N taxon-specific is not available, then the standard ratio $N_e/N = 0.1$ could be used as a conservative criterion (Hoban et al. 2020; Laikre et al. 2021).

To summarize, our simulation results suggest that purging may have an important role on the persistence of a population, providing MVPs for long-term survival that can be lower than those obtained by ignoring purging. We have obtained a (conservative) minimum N_e (~ 400) close to the classic rule of $N_e = 500$ when the reproductive rates of the species are not too small ($K > 5$). Given the available estimates of reproductive rates, our results suggest that the classic rule may be appropriate for a wide range of species, and thus supports its use on the IUCN Criteria to categorize threatened species, as well as its presence on the recent indicators suggested by Hoban et al. (2020) for the "post-2020" framework for biodiversity conservation by the Convention on Biological Diversity (CBD). However, caution should be taken for species with a low reproductive rate ($K < 5$), particularly mammals, where MVP values need to be larger than 1000, as suggested by Frankham et al. (2014). In any case, as stated by Hoban et al. (2020), MVPs are minimum guidelines for risk assessment, but values of N_e above the MVP do not imply that conservation measures are not required.

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Data availability Data and simulation programs are available at https://github.com/noeliaperezp/Long-term-MVP.

Declarations

Competing interests We declare no financial interests.

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