ON THE CONCEPT AND IMPLICATIONS OF GENETIC PURGING IN SMALL POPULATIONS

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Abstract

Genetic purging is increasingly considered a relevant factor in conservation genetics, as well as in evolutionary genetics. However, for a long time, it was de facto ignored when computing the expected evolution of population fitness under inbreeding (the inbreeding depression). More than a decade ago, I proposed a simple genetic analytical approximation to account for the consequences of genetic purging, the Inbreeding-Purging (IP) model. This note is intended to improve the insight on the meaning and consequences of purging using the frame of the IP model and its extension including continuous mutation and non-purging selection (the Full Model). I propose a more precise and conceptual definition of the inbreeding purged coefficient, which is a central parameter of the IP model. Then, using this definition, I provide a more straightforward deduction for the equation that predicts its evolution. I use the model to provide a unified discussion that clarifies the consequences of purging, including the pace and the efficiency of the process and the implications for the evolution of the deleterious burden, the inbreeding depression and the inbreeding load, which are relevant to evolutionary and conservation genetics and genomics.

Key words: Inbreeding depression; Inbreeding load; Deleterious burden; Population bottleneck; Shrunk populations; Small populations; Full Model; Conservation Genetics.

For decades, the depression of fitness expected from inbreeding was predicted using the classical equations derived by Morton, Crow, and Müller (1956), which are based on a neutral model that ignores natural selection to analyze the evolution of its very target, i.e., of fitness. Although it was often argued that evolutionary changes driven by selection occur slowly, the indiscriminate use of this model has to do with the lack of tractable equations that incorporate the effects of selection. However, selection can be greatly accelerated by inbreeding, which exposes, in homozygous by descent (IBD) individuals, deleterious recessive effects that were previously hidden in heterozygotes. This phenomenon, known as genetic purging, is relevant for many evolutionary genetic processes and is critical in conservation genetics, as the small population sizes of endangered species entails a continuous increase in expected inbreeding. More than a decade ago (García-Dorado 2012, GD2012 hereafter), I developed a simple theoretical approach (the Inbreeding-Purging or IP model) that allows to predict the joint consequences of inbreeding and purging by incorporating purging in the classical model developed by Morton et al. (1956). Here, I revisit the IP model (and its extension, the Full Model) to clarify some concepts and results. This contribution is not a revision of the state of art regarding genetic purging, so that I only quote a sample of the literature that I find useful or particularly illustrative for this purpose. First, I provide a more conceptual definition of the central parameter of the model, the inbreeding purged coefficient, and a more direct demonstration of the equation that predicts its evolution. Then, I discuss the implications of the model's predictions to the evolution of main genetic properties related to fitness in populations that undergo drastic reductions of their effective size.

The meaning of genetic purging under the IP model

Large populations can harbor many rare deleterious alleles that are at least partially recessive (we will use just "recessive" hereafter to include partial recessivity), accounting for a large hidden load, also known as inbreeding load B since Morton et al. (1956). Overdominant loci can also contribute some inbreeding load that cannot be eliminated by selection and will not be considered here. The Inbreeding-Purging (IP) model considers only the inbreeding load due to deleterious recessive alleles.

In the IP model, genetic purging is defined as the selective pressure against deleterious copies that is specifically prompted by inbreeding as it exposes their recessive deleterious effects in the homozygotes by descent. Genetic purging and the non-purging fraction of purifying selection are more formally defined in the next section.

Purging is prompted by inbreeding irrespectively of whether inbreeding is caused by the finite population size and/or by non-random mating. Glemin (2003) proposed the terms "purging by inbreeding" for purging due to non-random mating, and "purging by drift" for purging produced due to the finite population size. In the IP model, this notation is avoided because i) both non-random mating and reduced population size produce inbreeding (i.e., increased homozygosity by descent); ii) drift opposes natural selection (including purging) by reducing its efficiency, and only associates to purging as far as it results in increased inbreeding. In any case, Glemin uses a relatively different definition of purging that includes any increase in the intensity of natural selection that is prompted by inbreeding. Under this definition, and according to Glemin, inbreeding by non-random mating can cause increased selection in infinite populations even under additive gene action. This occurs because, under additivity, the fitness genetic variance is expected to increase with inbreeding due to the reduction of intermediate heterozygous genotypes. The additive variance in the neutral model is expected to increase by a factor (1+F) under additive gene action (Hill, 1996), although the actual increase should be smaller due to natural selection. Selection from this source is not considered purging in the IP model, where the term purging refers just to selection against the recessive component of the deleterious effects.

Although the IP model can predict purging under non panmictic mating, here I will concentrate on the consequences of purging due to inbreeding that arises as relatives mate by chance in a panmictic population after a drastic reduction of its effective size, as occurred in many endangered natural populations.

Of course, relatives can mate by chance in any finite population, even if its size has been stable for a long time. However, for a population whose size has been stable for so long that it can be considered at the mutation-selection-drift (MSD) equilibrium, the fitness decline expected from by-chance mating between relatives and from new deleterious mutations are continuously concealed by standard natural selection (i.e., non-purging selection, see below), except for the slow constant decline due to continuous fixation of deleterious alleles ascribed to drift (GD 2007). Therefore, the mean fitness of a panmictic populations that is roughly at the MSD balance does not experience inbreeding depression through generations. This population has, however, some hidden inbreeding load B that will produce inbreeding depression if inbreeding occurs. In fact, this hidden inbreeding load equals the rate of fitness depression with increased inbreeding expected in the absence of selection.

Definition and evolution of purged inbreeding

As explained in GD2012, the IP model predicts the joint consequences of inbreeding and purging using a purged inbreeding coefficient g. We will begin by defining this g coefficient in a more conceptual way than in GD2012, so as to obtain a more direct deduction of its evolution through generations.

Consider a panmictic population (selfing allowed) and a locus with a wild type (+) and a deleterious (m) allele, with fitnesses 1, 1-hs and 1-s for the three genotypes (++, +m, mm, respectively), where s is the selection coefficient against the homozygote and h is the degree of dominance. Thus, the deleterious effect expressed per single copy of m is hs when in heterozygosis and $s/2$ when in homozygosis. Therefore, we can split the potential per-allele deleterious effect into two parts: hs, that is expressed always, and an additional recessive component ($d=s/2-hs$) that is expressed only in homozygosis and that we denote purging coefficient. Thus, we could write the fitness of the three genotypes as

++	+m	mm
1	$1 - hs$	$1 - s = 1 - 2hs - 2d$

Note that the inbreeding load (Morton et al. 1956), responsible for inbreeding depression, is defined as

$$B = \sum_j 2d_j q_j (1 - q_j)\,, \qquad (1)$$

where the sum is over all the loci affecting fitness and q_j is the frequency of the deleterious allele in locus j.

Consider an ancestral large population (with effective size N_0), where a recessive deleterious allele segregates at low frequency (q_0). At generation 0, the population undergoes a bottleneck to a new stable size N, which causes a progressive increase in inbreeding as well as some purging selection.

We define purging, the extra selection induced by inbreeding, as the selection acting against d in the homozygotes by descent for the deleterious alleles that were present in the population at generations 0. Of course, the selection not induced by inbreeding continues to occur as in the original non-inbred population, although its efficiency is reduced due to increased drift. We denote standard selection (or non-purging selection) to this non-purging selective pressure, which occurs against the sh component and against the d component expressed in non-homozygous by descent genotypes (i.e., in the heterozygous and the hallozygous genotypes). Purifying selection includes both purging and standard non-purging selection. However, in the IP model, we are interested just on purging selection, but not in "standard selection" which is expected to operate very slowly.

Let the frequency of m at generation t be q_t, and the inbreeding coefficient be f_t. It can easily be shown (see appendix A in GD2012) that, considering just selection against the recessive component d in the homozygotes by descent at generation t -1, the expected frequency of m at generation t is approximately

$$q_t \approx q_{t\text{-}1} (1 - 2d f_{t\text{-}1}), \qquad (2)$$

where f_t is the classical Wright's (1922) inbreeding coefficient at generation t. Thus, the reduction in q by purging is proportional to the deleterious effect exposed by inbreeding (d) and to the probability of it being exposed in homozygotes by descent (f_t).

Note that we can interpret $q_t / q_{t\text{-}1} \approx (1 - 2d f_{t\text{-}1})$ as the probability that a t-1 gamete carrying a m_i copy produces a successful individual, relative to that of a randomly sampled gamete.

We define the purged inbreeding coefficient at generation t (g_t) as the probability that a random deleterious copy m_i present at t is in homozygosis by descent. In other words, g_t is the probability that, at generation t, given a randomly sampled deleterious copy m_i, a second copy X randomly chosen in the population is identical by descent (IBD) to m_i.

This can occur in two different ways:

i) The X copy derives from the same t-1 copy as m_i, and it has been transmitted to t despite purging. Since the probability of any copy at t-1 is 1/2N, from Equation 1, the probability of this is $(1/2N)(1-2d\,f_{t-1})$

ii) The X copy does not derive from the same t-1 copy as m_i (probability 1-1/2N), but from a different copy that was IBD to it (probability g_{t-1}), and that has been transmitted to generation t despite purging. Therefore, the probability of this is
$(1-1/2N)\,g_{t-1}\,(1-2d\,f_{t-1})$.

Therefore, the purged inbreeding coefficient at generation t can be expressed as a function of g_{t-1} as

$$g_t = [1/2N + (1-1/2N)\,g_{t-1}](1-2df_{t-1}) \qquad (3)$$

Equivalent expressions have been derived to predict the evolution of g_t from genealogical data (GD2012 for pedigrees with non-overlapping generations; and García-Dorado et al. 2016, allowing for overlapping generations).

There is no expression to directly compute g_t as a function of g_0. However, it can easily be shown (GD2012) that, as generations go on, g_t does not approach 1, but approaches the equilibrium value

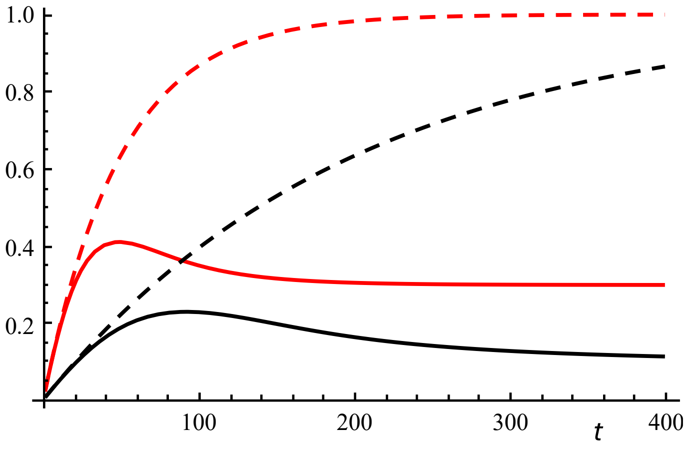
$$\hat{g} = \frac{1-2d}{1+2d(2N-1)} \quad , \qquad (4)$$

that can be very low for large d and/or N. These expressions give the evolution of g as a function of d for a single locus model or for any number of loci with identical d values. It should be noted that the above results and all the applications derived below give only approximate predictions. Quite extensive simulation has shown that the approximation is usually very good. However, it has been warned that this approach (as well as the Full Model one, see below) tends to overestimate the effects of purging for very small populations (for $Nd<1$).

Ignoring purging when predicting inbreeding depression is equivalent to assuming d=0 in equation 2, although not in Equation 1 when computing the inbreeding load B. This occurs because, in equation 2, d represents the purging coefficient, which could be denoted d_p and gives the deleterious effects concealed in homozygosis regarding the target of purging, *i.e.*, regarding the fitness trait that is expressed during the inbreeding-purging process. On the other hand, the d value used in the definition of B applies to fitness as expressed in any environment where inbreeding depression is evaluated (we could denote it d_B). It could be for example that a small population were maintained under very benign captivity conditions making purging inefficient ($d_p \approx 0$ in Equation 2) and then is introduced in the wild. The depression of fitness after that reintroduction can be dramatic, as it depends on g_t which will be roughly equal to f_t (as almost no purging occurred in captive conditions where $d_p \approx 0$), and on B computed using d_B , *i. e.* computed in terms of the effects of the deleterious alleles expressed in the wild. Here, for simplicity, I assume $d=d_p=d_B$.

Figure 1 gives the expected evolution of g_t and f_t through generations for two different effective population sizes. It is remarkable that, although the increase in g_t and in f_t is similar during a few initial generations, as f_t increases and triggers purging, g_t starts to increase more slowly than f_t, to later decline and approach its equilibrium value. That can be well below 1, particularly if the Nd product is large.

Figure 1: Evolution of the inbreeding purged coefficient and the classical inbreeding coefficient



Expected evolution under the IP model of the inbreeding purging coefficient g (solid lines) and the standard inbreeding coefficient f (dashed lines) through generations (t) after a large population ($N_0=10^6$) undergoes a stable reduction to a new effective size (N= 100 black line, or N=25 red line). Deleterious alleles have homozygous deleterious effect s=0.05 and dominance coefficient h=0.05, so that d=0.0225.

We need to note that the IP model predicts *g* as a function of *d* either for a model with a single locus or with any number of loci with the same *d* values. The IP model can however be applied to real situations where the different loci contributing inbreeding load *B* have different *d* values by using a single *d* value. This single *d* value should be interpreted as an effective purging coefficient, i.e., as the *d* value that produces the best possible predictions of the consequences of purging using the IP model, just as the effective population size allows to compute the best possible predictions of inbreeding using the ideal population model. Unfortunately, there is no analytical solution to compute the effective *d* value in terms of the *d* values of individual loci which, anyway, are usually unknown. However, the IP model has proved via simulation studies to produce very reasonable predictions using estimated effective *d* values under a wide set of conditions and distributions for the deleterious effects (García-Dorado 2012). Effective *d* values can be empirically estimated using the IP model, either from the evolution of average fitness for known effective population sizes or from pedigreed fitness data (Bersabé & García-Dorado 2013, García-Dorado et al. 2016, López-Cortegano et al. 2018). However, obtaining reliable estimates is very demanding in terms of experimental design and effort (López-Cortegano et al. 2016, 2021).

The consequences of inbreeding and purging on the deleterious burden

The more direct consequence of purging selection is the reduction of the burden of deleterious alleles per gamete. The per-gamete burden of segregating deleterious alleles was stable at the ancestral MSD equilibrium. After shrinkage, we will consider the burden of deleterious alleles per gamete, (D_t at generation *t*) computed as the addition of the frequency (q_t) over all the deleterious alleles that were segregating in the ancestral population as well as over all the deleterious mutations arisen during shrinkage, regardless whether they still segregate or became fixed by generation *t*.

For relatively short periods, the process can be explained using the simple IP approach that ignores both newly arisen deleterious mutations and standard non-purging selection. Then, the IP model predicts the evolution of the deleterious burden contributed by the alleles that were segregating in the ancestral non-inbred population (ancestral alleles). For a neutral locus, the expected frequency of any allele at any generation *t* is its ancestral frequency, and the probability that a given random copy is in homozygosis by descent is f_t. However, for a recessive deleterious allele m, the expected frequency at generation t is reduced by a factor q_0/q_t, due to genetic purging. Therefore, if we consider any single deleterious copy m_i at generation 0, the expected frequency of copies derived from it is also reduced by the same factor q_t/q_0. Thus, if we consider any given deleterious copy m_k at generation t that derives from m_i, the probability that a second random copy from generation t also derives from m_i should similarly have been reduced by q_t/q_0. In other words, the probability that, at generation t, any random deleterious copy m_k is in homozygosis by descent (g_t) has been reduced by a fraction q_t/q_0, compared to that of a neutral copy (f_t). This means that g_t can be interpreted as

$$g_t = f_t\, q_t/q_0 \qquad (5)$$

This allows the use of g_t to predict the consequences of purging on the average frequency of any deleterious allele and, therefore, on the deleterious burden D_t. For example, we can use g_t to predict the ratio of the expected deleterious frequency at t to the ancestral deleterious frequency as $q_t / q_0 \approx g_t / f_t$. Therefore, the deleterious burden expected at generation t can be predicted as

$$D_t = D_0\, g_t / f_t \qquad (6)$$

Similarly, we can predict the expected proportional reduction of the deleterious frequency (or of the deleterious burden D_t) due to purging, as

$$C_t = (q_t - q_0)/q_0 \;=\; (g_t - f_t) / f_t\,,$$

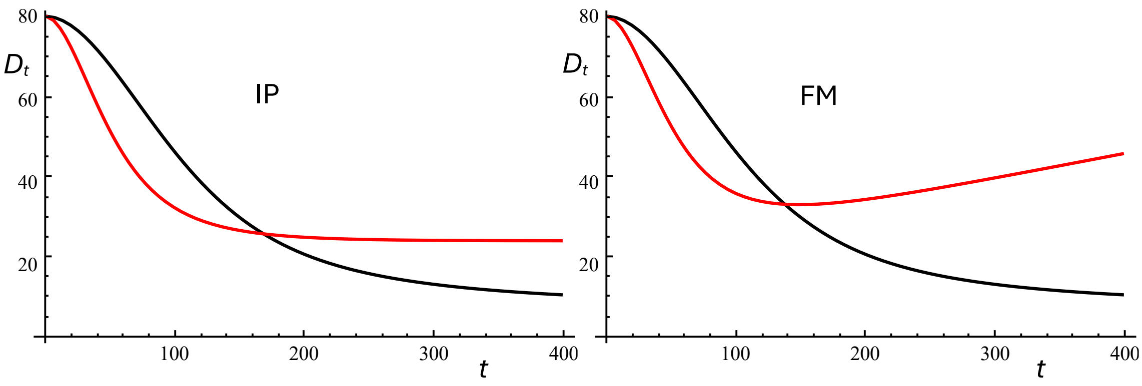
which represents the cumulative purging at generation t, and measures the overall efficiency of purging at generation t. Using (4), the overall cumulative purging asymptotically approaches

$$E = \;\hat{g} - 1, \qquad (7)$$

Thus, E measures the overall efficiency of purging expected in the long term.

Figure 2 gives the evolution for the expected deleterious burden when a very large population shrinks to a new effective size N for two different cases (N=25; N=100). The mutational parameter (used in this and other figures) have been chosen for illustrative purposes and are not representative of the properties of spontaneous mutation. We assume s=0.05, h=0.05 and we choose a rate of deleterious mutation per gamete and generation (λ=0.2) that accounts for a substantial initial inbreeding load (B=3.6) allowing for visually illustrative figures. Under the IP model (left panel), it can be seen that the expected deleterious burden monotonically declines due to purging and approaches an asymptotic value where all the expected purging has already occurred. When comparing shrunk populations of different sizes, the initial decline is quicker in the smaller population, but the final burden is larger.

Figure 2: Evolution of the deleterious burden after population shrinkage



Expected evolution of the deleterious burden D through generations (t) after a large population (N_0=10^6) undergoes a stable reduction to a new effective size (N= 100 black line, or N=25 red line). Deleterious mutations occur at a rate λ = 0.2 per gamete and generation, each with s=0.05 and h=0.05. The large ancestral population is assumed at the MSD balance with negligible probability of deleterious fixation, and its expected burden D_0 is computed using Equation 11 in DG2007. Left panel: after shrinkage, both new mutation and standard non-purging selection are ignored (IP model). Right panel: the deleterious burden is approximated considering inbreeding, purging, new mutation and standard non-purging selection (Full Model), as $D_t = D_0\, g_t/f_t + 2N\lambda U \sum_{i=0}^{t} f_t$, where U is the probability of fixation of new deleterious mutation at the MSD balance for the new effective sizes, respectively, and $\sum_{i=0}^{t} f_t$ can be computed as (t-2Nf_t) (see GD2007).

This leads to an apparent paradox: As larger sizes are considered for the shrunk population, the long-term efficiency of purging increases, but the short-term effect of purging declines, until the population is large enough that no burden decline is observed in the considered time scale. This shows that the role of purging is to accelerate the selective process at the cost of reducing its final efficiency. The effect of purging is therefore a matter of relative pace. A higher rate of decline (during some period) for the burden of ancestral alleles in smaller than in larger populations can be considered a footprint for genetic purging.

Note that the left panel (IP predictions) gives the evolution of just the burden for deleterious alleles that were segregating in the ancestral population (the ancestral-derived burden) and ignores new mutation and standard non-purging selection. For this ancestral derived burden, a larger decline in the smaller population should be ascribed to purging, but the existence of some decline after shrinkage is not, in itself, a proof of genetic purging, because standard non-purging selection is expected to cause some (even if very little) decline of the ancestral derived burden.

By contrast, the right panel of figure 2 includes the consequences of both standard non-purging selection and continuous new deleterious mutation after shrinkage, using the so-called Full Model (FM) in GD2012. Therefore, it includes both the deleterious mutations segregating in the ancestral population and the ones newly arising in the shrunk population, regardless of whether they still segregate or have reached fixation. In the absence of purging, this burden would be expected to continuously increase in finite populations, at a negligible rate at the ancestral population when $N_0s>>1$, but at an increased rate in the shrunk population where the input from new mutation remains constant but standard natural selection is less efficient due to drift. The decline of overall deleterious burden after population shrinkage (including both new deleterious mutations and the ancestral-derived burden) can therefore be considered a hallmark of genetic purging.

Predictions in the right panel of figure 2 were computed assuming that the ancestral population, at the MSD balance, was large enough that the probability of deleterious fixation was negligible and the overall deleterious burden was virtually stable (*i.e.*, in the ancestral population, natural selection removes all the deleterious burden introduced by mutation). The figure shows that, during some period after the effective population size shrinks to N, purging more than compensates for the loss of efficiency of non-purging selection to oppose genetic drift, leading to a net reduction of the overall deleterious burden that (despite new mutation) is initially more conspicuous in the smaller population. This reduction of the overall burden is only transient as, in the long term, natural selection is in general less efficient in smaller populations. Therefore, after an initial period where purging occurs more quickly in the smaller of both shrunk populations, the burden becomes smaller in the larger population where purging occurs more slowly. Furthermore, for the larger of both shrunk populations (where Ns>>1), the probability of deleterious fixation in the new MSD balance (and the corresponding rate of increase in burden) is close to zero, and the reduction in burden produced by purging becomes virtually stable during a very long period.

Therefore, the hallmark of purging caused by population shrinkage is a decline of the overall deleterious burden per individual, computed including all the deleterious copies irrespective of whether they correspond to alleles that still segregate or that are fixed and including both those that were segregating when shrinkage occurred and those that arise later due to new deleterious mutation. This decline in burden leads to a reduction that is transient but can persist for many generations. It occurs faster but is in the long term smaller in smaller populations. Thus, purging can be detected as a (transient) reduction of the burden of the shrunk population compared to that of the ancestral population, or as a (transient) smaller burden in the smaller of two (or more) populations with common origin.

When analyzing demographic contractions at the evolutionary time scale, where even the smaller considered population is relatively large, purging can imply a reduction in the deleterious burden during very long periods, even for deleterious alleles with very mild effect. This is supported by the molecular analysis of the burden of mutations for different classes of putatively deleterious effects in different species, as, for example, gorilla, ibex, lynx or tiger (Xue et al. 2015, Grossen et al. 2020, Khan et al. 2021, Kleinman et al. 2022).

In the next section, we will see that the short or medium-term effects of purging can be crucial to limit inbreeding depression below tolerable thresholds allowing for population survival, or to reduce the inbreeding load down to safe values for populations exposed to demographic challenges.

The consequences of inbreeding and purging on fitness inbreeding depression

The evolution of mean fitness under inbreeding has been predicted for decades using the theory that Morton et al. (1956), developed for the particular case of survival. That theory assumes that, although the frequency of the deleterious alleles responsible for the inbreeding load B of the ancestral population randomly drift, their expected value remains constant over generations; i.e., it ignores natural selection. Then, assuming fitness is multiplicative across loci, the fitness expected for a given inbreeding f is $W_f = W_0 \exp(-Bf)$. Therefore, t generations after population shrinkage, the expected fitness is

$$W_t = W_0 \exp(-B f_t), \qquad (8)$$

where f_t is the expected inbreeding at generation t, and fitness declines as inbreeding progresses at a rate that equals the inbreeding load B (Equation 1). Thus, the inbreeding load B (also denoted hidden load) equals the reduction of logarithmic fitness that would be expressed for fully inbred individuals (f_t =1) but that is hidden in non-inbred ones (f_t =0).

In general, loads defined in terms of logarithmic fitness are referred to by Morton et al. as “lethal equivalents”, where one lethal equivalent is the load that, if dispersed in many individuals, would cause on the average on death. This “lethal equivalent” concept was used by Morton et al. to measure both the load that is expressed in the non-inbred population (which is the load component they denote A, which also included non-genetic deaths) and the hidden genetic load (B). The rationale for using this “lethal equivalent” term to denote log-scaled fitness loads is that, due to the multiplicative nature of fitness, the fitness effects that have different deleterious alleles when they occur in different individuals, only add their total joint fitness effect when occurring in the same individual if fitness is measured in the logarithmic scale. For example, 1000 dominant alleles dispersed in 1000 individuals, each reducing survival by a 0.1% (*i.e.*, producing 0.1% probability of death), cause one expected death. In other words, adding their separate effects we obtain a total genetic damage of one genetic death or one lethal equivalent. However, if they concentrate on a single individual, they reduce its fitness by just a factor $(1\text{-}10^{-3})^{1000} \approx \exp(-1) = 0.368$. Since Morton et al., the term “lethal equivalent” has been widely used, as it may seem more intuitive than referring to a logarithm measure of fitness damage. However, at present it is more commonly used to measure B.

Although it has been widely recognized that, due to purging, the actual value of W_t can be larger than predicted by Equation 8, for many decades there were no simple equations allowing to include purging in the predictive model to obtain even approximate predictions using estimable parameters. Thus, it was usually considered that predictions provided by Equations 8 were reliable, in the confidence that purging should be effective just against deleterious alleles with severe effects.

Fortunately, the IP model provides a framework to predict the expected evolution of fitness taking into account both inbreeding and purging. At generation t, the expected decline in fitness ascribed to the increase in homozygosis by descent in locus i is approximately $W_0 - W_t = 2d_i\, q_{it}\, f_t$ (see appendix in GD2012). Therefore, assuming that, in the ancestral population, deleterious alleles segregate at low frequencies such that the locus i contributes an ancestral inbreeding load $B_i \approx 2d_i q_{i0}$, and using Equation 5, gives $W_t \approx W_0 - B_i\, g_t$. Accounting for all the deleterious alleles contributing inbreeding load for multiplicative fitness, we obtain

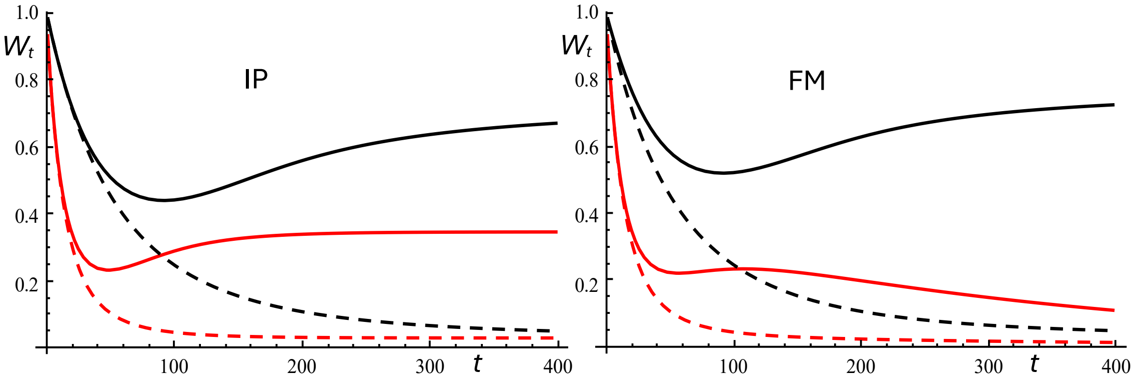
$$W_t \approx W_0 \exp(-Bg_t). \qquad (9)$$

Considering the evolution of g_t illustrated in Figure 1, expression 9 implies that, as inbreeding accumulates, purging tends to reduce the inbreeding depression compared to that predicted in the absence of purging by Equation 8, leading to some eventual (although partial) fitness recovery. Figure 3 (left panel) illustrates these IP predictions for the same deleterious mutation rate and effect and population sizes used in Figure 2.

Since purging leads to asymptotic g values smaller than one but larger than zero, the overall inbreeding depression is expected to be reduced due to purging, but inbreeding is always expected to lead to expected fitness values somewhat smaller than that of the original non-inbred population, even if the reduction can be very small in the long term when Nd>>1. This is so despite the reduction of the overall deleterious burden because, even during the period in which the inbred population has fewer deleterious copies, they are more often in homozygosis by descent.

Therefore, it is not that purging ascribed to shrinkage causes a net increase of fitness, but that it reduces the negative impact of inbreeding. Purging is never expected to fully conceal all the fitness depression caused by inbreeding, but it can reduce the pace of fitness decline and allow for some recovery, leading to a reduction of the fitness inbreeding depression that can be crucial to population survival. As in the case of the deleterious burden, the effects of purging on fitness take longer to be evident for larger populations than for smaller ones, but leads to higher asymptotic expected fitness.

Figure 3: Evolution of fitness after population shrinkage.



Expected evolution through generation of average fitness after a large population ($N_0=10^6$) undergoes a stable reduction to a new effective size (N= 100 black line, or N=25 red line). The large ancestral population is assumed to be at the MSD balance for deleterious mutations occurring at a rate $\lambda = 0.2$ mutations per gamete and generation, each with s=0.05 and h=0.05. Dashed lines give the prediction that considers inbreeding but ignores purging by using Equation 8. Solid lines in the left panel give the predictions that include inbreeding and purging using the IP model (Equation 9). Solid lines in the right panel give the prediction of the Full Model that accounts for inbreeding, purging, new mutation and standard selection (Equation 10). The expected inbreeding load at the MSD balance for both the ancestral and the shrunk effective population sizes (B and B*, respectively) is computed using Equation 13 in DG2007.

An interesting situation occurs when a shrunk population with size N undergoes some demographic recovery to a new larger effective size N' (where $N < N' < N_0$). After expansion, the previously accumulated inbreeding value (f_t) is retained (and continues to increase at a smaller rate), so that purging continues to occur against d in the corresponding IBD homozygous genotypes. This means that the per generation increase of g_t expected from random sampling ($1/2N'$) decays, the strength of purging ($2df_t$ in Equation 3) is not reduced. Therefore, g_t can experience more effective reduction and lead to an accelerated fitness recovery, as illustrated by predictions and simulations in GD2012. Nevertheless, this will only be possible as far as no substantial fixation has occurred before expansion, *i.e*, as far as the period of previous shrinkage is short enough to warrant not too large f values.

Ignoring standard selection and new deleterious mutations can lead to relevant bias in some occasions. On the one hand, if the shrunk population is very small, the risk of fixation of new deleterious mutation is large, and it can cause substantial fitness decline in the long term. On the other hand, when we consider increasing values for the effective size N of the shrunk population, the actual inbreeding depression expected when a given inbreeding f is attained, declines. The reason is that the per-generation fitness increase due to standard selection compensates a larger fraction of the inbreeding depression in larger populations (see Equation 14 in GD 2007). As a consequence, the actual inbreeding depression expected should approaches 0 as the size of the shrunk population considered approaches N_0, as no fitness decline is expected from inbreeding for a panmictic population that did not shrink (i.e., at the MSD balance for a stable size). To account for this, we need to take into account that, in fact, the population shifts from the ancestral population MSD balance for N_0, with inbreeding load B, to a new MSD balance corresponding to the smaller effective size N and, therefore with a smaller equilibrium value for the inbreeding load (B^*). During this shift, standard selection continuously compensates all the inbreeding depression that would occur at the new MSD balance, as well as all the decline ascribed to new deleterious mutations that are removed by standard selection. This gives the approximation for the fitness inbreeding depression given in GD2012's Full Model, according to which

$$W_t \approx W_0 \exp[-(B - B^*)g_t + M], \qquad (10)$$

Where M represents the fitness decline from new deleterious mutation, which is approximated using their fixation probability ($M = 2N\lambda Us$, where U is the fixation probability computed from diffusion theory; Kimura 1962). Using this approach, no inbreeding depression is predicted if the population does not shrink (*i.e.*, if N=N_0 so that B^*=B), as expected.

The right panel in Figure 3 shows the approximate prediction for the fitness decline under the Full Model. This approximate prediction assumes that the standard (non-purging) selection after the population shrunk, works as after the population attains the new Mutation-Selection-Drift balance (MSD), where the expected fitness will be stable through generations (except for the decline ascribed to the rate of fixation of deleterious mutations corresponding to the new shrunk stable size N ;GD2007). We see that, compared to the IP model, the inclusion of standard selection and new deleterious mutation in the Full Model implies some reduction in the rate at which fitness declines during purging, which is due to non-purging selection and is more conspicuous in the larger population with larger B^*. It also implies a long-term negative fitness slope due to deleterious fixation, more conspicuous in the smaller population, where Ns is smaller and close to 1.

However, after a dramatic reduction in population size, the IP model provides reasonable predictions in the short to medium term, which is fortunate since this is a particularly relevant situation in conservation genetics and since estimates for B^* and for the rate of fitness decline expected from new deleterious mutations are usually not available.

The consequences of inbreeding and purging on the evolution of the inbreeding load *B*

Starting from an inbreeding load B before population shrinkage and ignoring the consequences of natural selection, the inbreeding load is expected to decline in the shrunk population at the same rate that neutral gene diversity is lost by drift, i.e., by a factor $(1 - f_t)$:

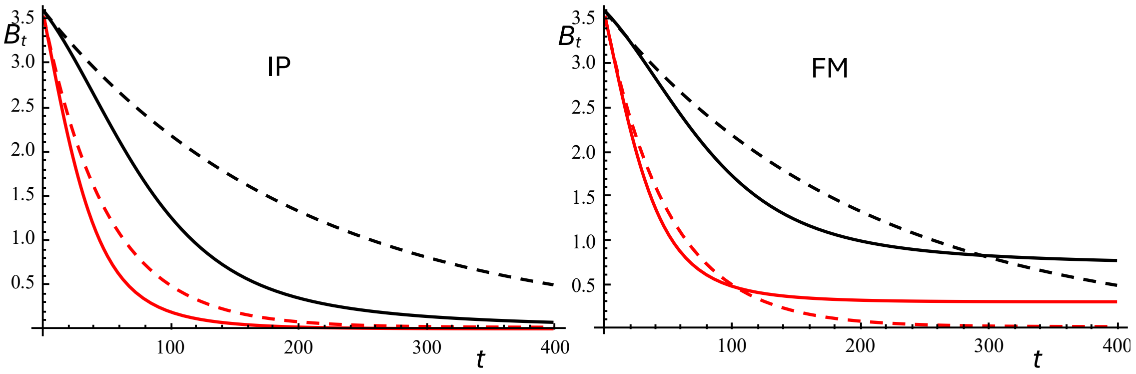
$$B_t = B\,(1 - f_t) \qquad (11)$$

However, purging accelerates this decline as it reduces the frequency of the deleterious alleles by a factor g_t/f_t. Therefore, after taking purging into account but ignoring new mutation and standard selection (IP model), B_t can be approximately predicted as

$$B_t = B\,(1\text{-}f_t)\,g_t/f_t. \qquad (12)$$

Figure 4 (left panel) shows the decline of the inbreeding load expected just by drift or considering purging (IP model). The inbreeding load is always exhausted in the long term, but the process is substantially accelerated when purging is considered. This acceleration is particularly conspicuous during an efficient purging process (see the first 150 generations for the larger population in the figure). Note that the exhaustion of B under purging contrasts with the evolution of the deleterious burden (Figure 2), which stabilizes at non-zero values because it includes the burden of fixed deleterious alleles.

Figure 4: Evolution of the inbreeding load after population shrinkage.



Expected evolution through generation of the inbreeding load after a large population ($N_0=10^6$) undergoes a stable reduction to a new effective size (N= 100 black line, or N=25 red line). The large ancestral population is assumed to be at the MSD balance for deleterious mutations occurring at a rate $\lambda = 0.2$ mutations per gamete and generation, each with $s=0.05$ and $h=0.05$. Dashed lines give the prediction that ignores purging by using Equation 11. Solid lines in the left panel give the predictions that include purging using the IP model (Equation 12). Solid lines in the right panel give the prediction of the Full Model that accounts for inbreeding, purging, new mutation and standard selection (Equation 13). The expected inbreeding load at the MSD balance for both the ancestral and the shrunk effective population sizes (B and B*, respectively) is computed using equation 13 in DG2007.

In practice, however, new mutation and standard selection continue to occur after population shrinks. The Full Model approach deals with the evolution of the inbreeding load by considering that the shrunk population shifts from the ancestral MSD balance, with inbreeding load B, to a new MSD balance with a smaller (but non-zero) stable inbreeding load B^*. Therefore, only a net $(B - B^*)$ inbreeding load is going to be lost due to inbreeding and purging in the transit to the new MSD balance. This shift can be approximately described by the expression

$$B_t = B^* + (B - B^*)\,(1\text{-}f_t)\; g_t / f_t. \qquad (13)$$

The right panel in Figure 4 illustrates the evolution of the inbreeding load after population shrinkage under the Full Model by using Equation 13. It shows that the IP model overestimates the rate of decline of the inbreeding load after population shrinkage and underestimates its asymptotic long-term expected value, particularly when the shrunk population is not too small. Even so, due to purging, shrunk populations that went through moderate shrinkage, can show substantial reduction of the inbreeding load and limited inbreeding depression (López-Cortegano et al. 2016, Pérez-Pereira et al. 2021). For example, our large population (*N*=100), has experienced a 37% fitness reduction by generation 200 (which represents a 42% of the reduction expected assuming no purging), but its inbreeding load has declined by a 91%. (right panels in Figures 3 and 4). Therefore, populations that went through moderate shrinkage in the past can, in some cases, be more resilient to future drastic bottlenecks (Pérez-Pereira et al. 2022).

General remarks

Putting together all the above predictions, they illustrate some important features of genetic purging in panmictic populations that are relevant in many contexts, as in the interpretation of the evolutionary process or the practice of conservation genetics. In what follows we briefly mention some of those implications. Of course, here we will let aside many other evolutionary and biological processes that need also be considered in practice.

For example, Our previous analysis implies that the observation of smaller deleterious burden in historically smaller populations compared to a larger population with a common origin, as those detected in genomic analysis for gorilla, ibex, lynx or tiger, shows that purging has played an evolutionary role. Despite this, larger panmictic populations are always expected to have larger fitness than smaller ones. On the other hand, the fitness difference can be irrelevant above some critical population size, so that it is very difficult to know how efficiently this process has limited fitness inbreeding depression through the evolutionary history of each population and whether the smaller populations will show a relevant fitness disadvantage at any time. Similarly, it is difficult to infer how far the smaller observed deleterious burden is associated with a smaller inbreeding load, responsible for smaller future inbreeding depression. Although insight can be gained using genomic inferences of putatively deleterious effects, fitness average and fitness inbreeding load can only be properly estimated from the experimental evaluation of fitness (García-Dorado & Hedrick 2023).

On the other hand, when planning the management of endangered populations, it should always be considered that the consequences of different *in situ* and *ex situ* strategies depend on the average fitness and the inbreeding load of the populations. Therefore, they are affected by previous purging processes, which depend on the corresponding demographic histories. In addition, management strategies interfere with future purging in different ways. For example, strategies controlling the number of offspring contributed by each parent interfere with purging opportunities and can have negative consequences for fertility traits if applied for long periods (GD2012), although they slow initial inbreeding depression and protect gene diversity and therefore adaptive potential. Similarly, minimizing the coancestry between mating individuals reduces immediate inbreeding depression at the cost of reduction purging opportunity, so that the convenience of this practice depends on the reproductive potential of the species and on the time scale in which the strategy is applied. Regarding genetic rescue, it can introduce inbreeding load from external sources in populations that might have undergo efficient purging in the past, but also produces hybrid vigor and introduces adaptive potential that could be critical under global environmental challenges (GD2012, García-Dorado 2015, Hedrick & García-Dorado 2016, Pérez-Pereira et al. 2021, Pérez-Pereira et al. 2025). The overall outcome of all these processes is difficult to predict. However, in general, purging needs to be taken into account in conservation management.

Here we have mainly been concerned with genetic purging in panmictic populations. Despite this I want to note that purging can also be boosted by intentionally mating related individuals (as with circular mating) to produce inbreeding. This strategy is expected to increase long term fitness and to reduce inbreeding load and resilience to future bottleneck episodes with little impact on genetic drift, but is usually discouraged in populations with limited reproductive potential, due to the risk derived from the initial inbreeding depression it produces (Caballero et al 2017).

Summarizing, purging in panmictic populations can be seen as an acceleration of the selective process at the cost of some loss of long-term efficiency. Regarding the deleterious burden, this long-term loss of efficiency arises from the larger rate of fixation of deleterious mutation. Therefore, If N is large enough to prevent deleterious fixation, the footprint of purging in genomic analysis can persist for a long evolutionary period, as in gorilla, ibex, lynx or tiger (Xue et al. 2015, Grossen et al. 2020, Khan et al. 2021, Kleinman et al. 2022). Regarding fitness, the reduction of the inbreeding depression during purging can allow for fitness levels above critical values for population survival. In parallel, although population shrinkage always increases to some extent the population´s extinction risk, it should be appreciated that populations that survived shrinkage are expected to have smaller fitness inbreeding load and can be more resilient to further bottlenecking. This is particularly to be expected after not too dramatic shrinkage, which can allow efficient purging and, therefore, a considerable reduction of the inbreeding load with little fitness decline.

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References

Bersabé, D., & García-Dorado, A. (2013). On the genetic parameter determining the efficiency of purging: an estimate for Drosophila egg-to-pupae viability. *Journal of evolutionary biology*, *26*(2), 375-385.

Caballero, A., Bravo, I., & Wang, J. (2017). Inbreeding load and purging: implications for the short-term survival and the conservation management of small populations. *Heredity*, *118*(2), 177-185.

García-Dorado, A. (2007). Shortcut predictions for fitness properties at the mutation–selection–drift balance and for its buildup after size reduction under different management strategies. *Genetics*, *176*(2), 983-997.

García-Dorado, A. (2012). Understanding and predicting the fitness decline of shrunk populations: inbreeding, purging, mutation, and standard selection. *Genetics*, *190*(4), 1461-1476.

García-Dorado, A. (2015). On the consequences of ignoring purging on genetic recommendations for minimum viable population rules. *Heredity*, *115*(3), 185.

García-Dorado, A., Wang, J., & López-Cortegano, E. (2016). Predictive model and software for inbreeding-purging analysis of pedigreed populations. *G3: Genes, Genomes, Genetics*, *6*(11), 3593-3601.

Garcia-Dorado, A., & Hedrick, P. (2023). Some hope and many concerns on the future of the vaquita. *Heredity*, *130*(4), 179-182.Glémin, S. (2003). How are deleterious mutations purged? Drift versus nonrandom mating. *Evolution*, *57*(12), 2678-2687.

Grossen, C., Guillaume, F., Keller, L. F., & Croll, D. (2020). Purging of highly deleterious mutations through severe bottlenecks in Alpine ibex. *Nature communications*, *11*(1), 1001.

Hedrick, P. W., & Garcia-Dorado, A. (2016). Understanding inbreeding depression, purging, and genetic rescue. *Trends in ecology & evolution*, *31*(12), 940-952.

Hill, W. G. (1996). Sewall Wright's``systems of Mating''. *Genetics*, *143*(4), 1499.

Khan, A., Patel, K., Shukla, H., Viswanathan, A., van der Valk, T., Borthakur, U., ... & Ramakrishnan, U. (2021). Genomic evidence for inbreeding depression and purging of deleterious genetic variation in Indian tigers. *Proceedings of the National Academy of Sciences*, *118*(49), e2023018118.

Kimura, M. (1962). On the probability of fixation of mutant genes in a population. *Genetics*, *47*(6), 713.

Kleinman-Ruiz, D., Lucena-Perez, M., Villanueva, B., Fernández, J., Saveljev, A. P., Ratkiewicz, M., ... & Godoy, J. A. (2022). Purging of deleterious burden in the endangered Iberian lynx. *Proceedings of the National Academy of Sciences*, *119*(11), e2110614119.

López-Cortegano, E., Vilas, A., Caballero, A., & García-Dorado, A. (2016). Estimation of genetic purging under competitive conditions. *Evolution*, *70*(8), 1856-1870.

López-Cortegano, E., Bersabé, D., Wang, J., & García-Dorado, A. (2018). Detection of genetic purging and predictive value of purging parameters estimated in pedigreed populations. *Heredity*, *121*(1), 38-51.

López-Cortegano, E., Moreno, E., & García-Dorado, A. (2021). Genetic purging in captive endangered ungulates with extremely low effective population sizes. *Heredity*, *127*(5), 433-442.

Morton, N. E., Crow, J. F., & Muller, H. J. (1956). An estimate of the mutational damage in man from data on consanguineous marriages. *Proceedings of the National Academy of Sciences*, *42*(11), 855-863.

Pérez-Pereira, N., Pouso, R., Rus, A., Vilas, A., López-Cortegano, E., García-Dorado, A., Quesada, H. & Caballero, A. (2021). Long-term exhaustion of the inbreeding load in Drosophila melanogaster. *Heredity*, *127*(4), 373-383.

Pérez-Pereira, N., Caballero, A., & García-Dorado, A. (2022). Reviewing the consequences of genetic purging on the success of rescue programs. *Conservation Genetics*, *23*(1), 1-17.

Pérez-Pereira, N., Kleinman-Ruiz, D., García-Dorado, A., Quesada, H., & Caballero, A. (2025). A Test of the Long-Term Efficiency of Genetic Rescue With Drosophila melanogaster. *Molecular Ecology*, e17690.

Wright, S. (1922). Coefficients of inbreeding and relationship. *The American Naturalist*, *56*(645), 330-338.

Xue, Y., Prado-Martinez, J., Sudmant, P. H., Narasimhan, V., Ayub, Q., Szpak, M., ... & Scally, A. (2015). Mountain gorilla genomes reveal the impact of long-term population decline and inbreeding. *Science*, *348*(6231), 242-245.