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NEWS AND COMMENTARY

Purging and MVP rules

On the consequences of ignoring purging on genetic recommendations for minimum viable population rules

A García-Dorado

In conservation practice, preliminary assessments of extinction risk as well as emergency decisions are often based on scarce information. Thus, a simple 50/500 rule of thumb has been applied for a long time as a guidance to determine when genetic threats become relevant to conservation, and to settle the genetic threshold to the minimum size for population viability (the so-called MVP). This rule, used, for example, in the elaboration of the International Union for the Conservation of Nature Red List criteria for threatened species, states that the effective population size (N_e) should be at least 50 to prevent the dramatic consequences from inbreeding depression in the short term, whereas a larger value ($N_e \geqslant 500$) would be needed to preserve adaptive potential in the long term (Franklin, 1980; Jamieson and Allendorf, 2012). As it is well known, these N_e values imply considerably larger censuses.

However, it has been recently proposed that these figures should be doubled (Frankham et al., 2014), a recommendation that could have important consequences on resource allocation but may be based on exceedingly simplifying assumptions (Franklin et al., 2014).

Frankham et al.'s proposal that N_e should be at least 100 to prevent extinction risk from inbreeding depression, was prompted by a bulk of recent estimates of the inbreeding load in the wild that are much larger than those previously obtained in captive conditions. Thus, a meta-analysis by O'Grady et al. (2006) on wild mammalian and avian species, corroborated by additional reports (Kruuk et al., 2002; Liberg et al., 2005; Walling et al.,

2011; Kennedy et al., 2014), concluded that the inbreeding load for overall fitness in the wild is on the average $B \approx 6$ haploid-recessive lethal equivalents, that is, about fourfold the estimate obtained in a meta analysis for captive conditions (Ralls et al., 1988) that had been widely used as a default value (Lacy, 1993). To derive this new $N_e = 100$ rule, Frankham et al. used the classical equation for fitness inbreeding depression

$$W_t = W_0 \text{Exp}[-B F_t], \qquad (1)$$

where W_t and F_t stand, respectively, for the average fitness and Wright's inbreeding coefficient at generation t, and the inbreeding load (B) is the rate of inbreeding depression. This expression assumes that the homozygosis for (partially) recessive deleterious alleles increases with inbreeding at the same rate as that for neutral alleles and, using $B = 6$, it predicts that $N_e = 50$ would cause the expected fitness to decline to 75% of its initial value in just five generations and to 0.2% in the long term.

However, as inbreeding promotes the expression of the recessive component of deleterious effects, it not only causes inbreeding depression but also leads to an increase of the efficiency of natural selection, known as genetic purging. Here I discuss the consequences of purging on Frankham et al.'s recommendation using the inbreeding–purging approach (García-Dorado, 2012), where the evolution of fitness is approximated by replacing F_t with a purged inbreeding coefficient ($g_t \leqslant F_t$) that determines the increase in homozygosis for the alleles that are being purged. This parameter can be computed as a function of N_e and of the intensity of purging, which is measured by a purging coefficient (d) that represents the magnitude of the deleterious effects concealed

in the heterozygous condition. For each particular deleterious allele, d depends both on the selection coefficient against homozygous (s) and on the degree of dominance (h) ($d = s(1-2h)/2$; note that, for any given d value, the intensity of purging does not depend of the underlying s and h coefficients). It has been shown that good approximations for fitness inbreeding depression can be obtained using an effective purging coefficient that applies to the overall inbreeding load (García-Dorado, 2012); however, it is convenient to separately consider the consequences of purging upon the inbreeding load ascribed to true recessive lethal alleles (B_L, with purging coefficient $d_L \approx 0.5$) from those ascribed to non-lethal alleles (B_{NL}, with effective purging coefficient d_{NL}). This gives

$$W_t = W_0 \text{Exp}\left[-B_L g_{Lt} - B_{NL} g_{NLt}\right]. \qquad (2)$$

In addition, the inbreeding load of the reduced population ascribed to deleterious alleles segregating in the original population can also be predicted as

$$B_t = \left(B_L g_{Lt} + B_{NL} g_{NLt}\right)(1 - F_t)/F_t, \qquad (3)$$

although the actual inbreeding load will be larger in the long term due to new deleterious mutation. For $d_L = d_{NL} = 0$, Equations (2) and (3) produce the corresponding classical neutral predictions.

To date, the only empirical estimate of the intensity of purging in the fraction of B not due to recessive lethal alleles, obtained in the lab for a partial measure of fitness in Drosophila (Bersabé and García-Dorado, 2013), suggests $0.02 < d_{NL} < 0.08$. However, it is reasonable to assume that, as B estimates in the wild are about four times those for captive and laboratory populations, d_{NL} in the wild should behave similarly ($0.08 < d_{NL} < 0.32$). To be conservative, I will illustrate the possible

A García-Dorado is at Departamento de Genética, Facultad de Biología, Universidad Complutense, Madrid, Spain
E-mail: augardo@ucm.es

consequences of purging in the wild considering $d_{NL} = 0.05$ or $d_{NL} = 0.15$.

Figure 1 (upper panels) gives the evolution of the fitness average of a previously large population after a reduction in size to $N_e = 25$, $N_e = 50$ and $N_e = 100$, as predicted by the classical neutral model or by the inbreeding–purging theory. As in O'Grady et al. (2006), I assume that the inbreeding load ($B = 6$) consists of a fraction $B_L = 2.5$ ascribed to recessive lethal alleles (purging coefficient $d_L = 0.5$) and another fraction $B_{NL} = 3.5$ due to non-lethal alleles undergoing purging ($d_{NL} = 0.05$ or $d_{NL} = 0.15$) or not ($d_{NL} = 0$). Figure 1 shows that: (i) the consequences of purging on fitness decline only become apparent after some inbreeding has accumulated, usually leading to a later fitness rebound; (ii) the larger the effective population size, the more generations are needed for purging consequences to become relevant, but this occurs at lower inbreeding levels and, therefore, after smaller fitness declines; (iii) the efficiency of purging in reducing fitness de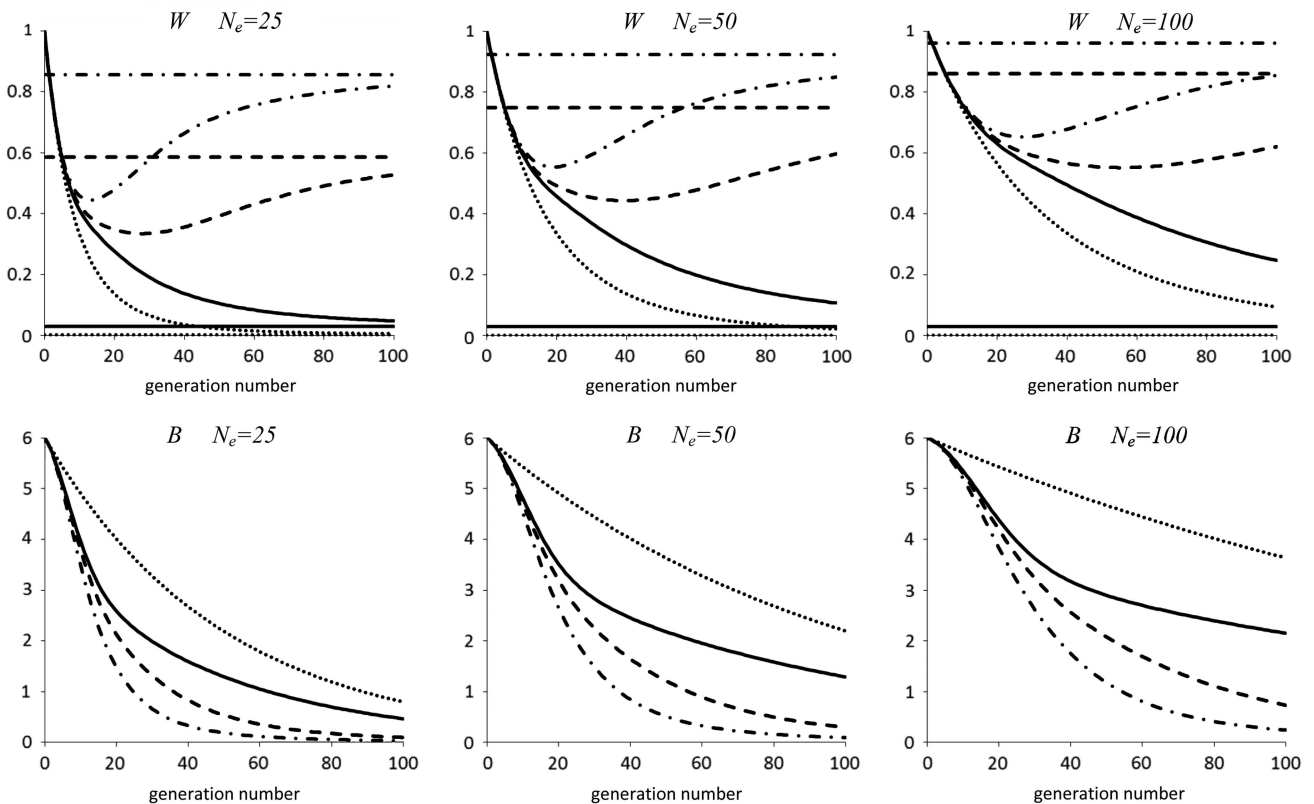pression is lower for the smaller populations, leading to reduced asymptotic fitness values. In fact, simulation results have shown that purging becomes inefficient for $N_e < 1/d$, due to genetic drift. As the appropriate N_e in this respect is the drift-effective population size, some inbreeding due to non-panmictic mating in moderate to large populations could produce situations particularly favorable for genetic purging, in agreement with theoretical and experimental results (Glémin, 2003; Ávila et al., 2010).

Figure 1 (lower panels) also gives an approximation for the evolution of the inbreeding load ascribed to deleterious alleles segregating in the original population, which drops much faster in the presence of purging than under drift alone ($d_L = d_{NL} = 0$). It clearly shows that the reduction of B caused by purging during the first generations can be mainly ascribed to lethal alleles, whereas that caused by less intense purge is delayed.

Frankham et al. (2014) argued that classical neutral predictions for the reduction of mean fitness are appropriate because purging has been shown to be modest. In fact, purging has not been detected in several experiments, but it has been observed in other instances, mainly under slow inbreeding (Crnokrak and Barrett, 2002; Leberg and Firmin, 2008). However, purging can pass undetected in experimentation, even for populations where it should be relevant in the medium term. The main reason is that, as shown by the inbreeding–purging predictions, either experimental inbreeding increases too fast to allow efficient purging or inbreeding is slow enough but the number of generations analyzed is too small (Hedrick, 1994; Frankham et al., 2001; Kennedy et al., 2014). Furthermore, experimental detection is often obscured by many factors, such as concurrent adaptation, genetic management or uncertainty regarding B or N_e values, and few experiments have addressed the evaluation of purging in the wild. Thus, the experimental support for the claim that purging is modest is, at least, controversial.

O'Grady et al. (2006) performed viability analyses assuming $B = 6$ for a range of mammal and avian species, concluding that the

Figure 1 Average fitness predicted for $B = 6$ using the inbreeding–purging approach (W, upper panels) together with the corresponding inbreeding load ascribed to deleterious alleles in the original population (B, lower panels), computed from Equations 2 and 3. Black Dotted lines: classical prediction ($d_L = 0$; $d_{NL} = 0$); magenta solid lines: purging acts only against recessive lethals ($B_L = 2.5$, with $d_L = 0.5$; $B_{NL} = 3.5$, with $d_{NL} = 0$), as assumed by O'Grady et al. (2006); blue dashed lines: purging acts against recessive lethals ($B_L = 2.5$, with $d_L = 0.5$) and against the remaining inbreeding load ($B_{NL} = 3.5$, with $d_{NL} = 0.05$); green dotted-dashed lines: purging acts against recessive lethals ($B_L = 2.5$, with $d_L = 0.5$) and against the remaining inbreeding load ($B_{NL} = 3.5$, with $d_{NL} = 0.15$). In all cases, the horizontal line represents the asymptotic fitness value. Plates, from left to right, are for $N_e = 25$, 50 and 100. A full color version of this figure is available at the Heredity journal online.

extinction rate increased dramatically in the medium term due to inbreeding depression. However, the VORTEX (Miller and Lacy, 1999) software used by O'Grady *et al.* only considers purging upon the fraction of inbreeding depression ascribed to truly lethal alleles ($B_L = 2.5$, extrapolated from *Drosophila*), but neglects purging upon the remaining inbreeding load ($B_{NL} = 3.5$). Figure 1 shows that, when B is large, considering purging only against recessive lethal alleles, as in O'Grady *et al.* (2006), accounts just for a small fraction of the medium-term consequences of overall purging, and for a negligible fraction of the long-term ones (solid lines). Thus, O'Grady *et al.* could have underrated the role of purging to a very relevant extent.

As the consequences of purging will often be negligible during the first generations, Frankham *et al.*'s warning regarding the immediate consequences of inbreeding depression should be taken into account. However, populations with a history of size decline or occasional bottlenecking can conceal smaller inbreeding load than the ancestral non-endangered populations, due to previous purging (Boakes *et al.*, 2006; Pujol *et al.*, 2009; Facon *et al.*, 2011). Certainly, the more drastic the bottlenecks, the larger should be the d value of the alleles that were efficiently purged, so that previous purging can lead to a smaller contribution of lethal alleles to overall B, and to smaller purging coefficients for non-lethal ones. Nevertheless, this reduction of purging intensity should be ascribed to the load that has already been purged, so that future inbreeding depression should be smaller than what would have been expected starting from the ancestral population. In any case, when predicting the short-term impact of inbreeding on fitness, it should be taken into account that many endangered populations may have inbreeding loads substantially smaller than the $B = 6$ value proposed by Frankham *et al.* (2014).

For the medium-long term, however, properly accounting for purging will produce much more optimistic prospects, as populations surviving the initial stages are likely to recover from most inbreeding depression. Thus, for many endangered populations, the critical issue is whether its reproductive potential is large enough so that the population can afford an important reduction of fitness for a few generations, before purging becomes capable of reversing the decline. If it is, the long-term survival will depend on the recovery of large numbers before adaptive potential is substantially eroded, rather than on inbreeding depression. Furthermore, after size recovery, purging upon previously accumulated inbreeding becomes more efficient, which can accelerate fitness rebound (see Figure 2 in García-Dorado, 2012). In addition, as purged populations are expected to conceal smaller inbreeding load, they should be more resilient against future episodes of drastic size reduction. Taking all things together, there is no reason to spare conservation efforts just because N_e is going to be small (even $N_e \approx 25$) in the near future.

In other words, the recommendation by Frankham *et al.* (2014) to increase N_e from 50 to 100 is based on the assumption that purging upon non-lethal deleterious alleles is irrelevant but, considering empirical evidences on the light of theoretical predictions, this assumption is not supported. As shown here, we need to account for purging upon the inbreeding load caused by both lethal and non-lethal deleterious alleles in order to determine the minimum effective size required to preserve populations that, even been actually endangered, can eventually recover from inbreeding depression. On the basis of present evidence, this effective size will often be closer to 50 than to 100.

CONFLICT OF INTEREST

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