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Review

Understanding Inbreeding Depression, Purging, and Genetic Rescue

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Inbreeding depression, the reduction of fitness caused by inbreeding, is a nearly universal phenomenon that depends on past mutation, selection, and genetic drift. Recent estimates suggest that its impact on individual fitness is even greater than previously thought. Genomic information is contributing to its detection and can enlighten important aspects of its genetic architecture. In natural populations, purging and genetic rescue mitigate fitness decline during inbreeding periods, and might be critical to population survival, thus, both mechanisms should be considered when assessing extinction risks. However, deliberate purging and genetic rescue involve considerable risk in the short and medium term, so that neither appears to be a panacea against high inbreeding depression.

What Is Inbreeding Depression?

Inbreeding depression (see Glossary), reduced fitness because of lower survival, mating, and/or reproduction in the progeny of related individuals compared to that of unrelated individuals, appears to be a nearly universal phenomenon and has been documented in many different organisms [1–7]. However, the amount of documented inbreeding depression varies for different species, for different populations, and for different individuals, and is difficult to analyze, particularly in the wild. Genomics is revolutionizing many aspects of evolutionary biology and is having a major impact on measuring and understanding inbreeding and inbreeding depression and their importance in evolution and conservation.

The extent of the variation in inbreeding depression also depends upon the evolutionary history of the species. In addition, the level of inbreeding depression for different components of fitness, such as survival, mating, or reproduction, can also vary, and it generally increases when the environment is more stressful or competitive [8–11]. Similarly, phenotypic traits correlated with fitness can be negatively influenced by inbreeding, and inbreeding depression can vary between males and females [12].

Inbreeding reduces fitness because part of the **genetic load** of populations, known as **inbreeding load**, is only expressed in homozygotes. This inbreeding load is a dynamic property influenced by a number of evolutionary factors in the past and present. It is thought to be fueled by continuous mutation to variants with a (partially) recessive deleterious effect on fitness, and eroded by both purifying selection and genetic drift. In addition, both the inbreeding load and the actual depression of fitness can be reduced by **purging** [13,14], that is, increased purifying selection facilitated by inbreeding; and by **genetic rescue** [15], that is, the introduction of beneficial variation from outside the population for genes with detrimental variation.

Trends

Inbreeding is being estimated using genomic approaches in many more species that do not have pedigrees.

Pedigree inbreeding is being used in combination with genomic estimation of relationships. This partnering provides insight to what the pedigree has missed, such as inbred or related founders and inbreeding before the pedigree was initiated.

Specific loci determining inbreeding depression are being found using various approaches, such as candidate loci, homozygosity mapping or genome wide association, particularly for variants with large effects.

Inbreeding depression, purging and genetic rescue are recognized as essential elements to be considered in conservation programs, and genomic monitoring of genetic rescue and purging are being used or considered to plan and monitor management.

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Multiple approaches indicate that inbreeding depression is caused by homozygosity of rare lethal, nearly recessive variants and partially recessive, detrimental variants [16]. From detailed examination of the source of inbreeding depression in *Drosophila melanogaster*, it appears that about half the inbreeding depression for viability is from almost recessive lethals and about half is from partially recessive detrimentals of small effect [17]. However, the population size of *D. melanogaster* is generally assumed to be quite large, and small populations can more likely purge lethal variants than low-effect detrimentals, so these proportions probably vary among species.

The other main hypothesis for the genetic basis of inbreeding depression is that it is caused by homozygosity of variants maintained by heterozygote advantage (overdominance). It is difficult to distinguish between these alternatives [18,19] because recessive detrimental variants might be in linkage disequilibrium, resulting in pseudo-overdominance [20]. However, while inbreeding depression is found in virtually all organisms, there are very few well-documented cases of heterozygote advantage [21]. Because of the strong evidence supporting the role of recessive detrimental variants causing inbreeding depression, we will not consider the overdominance hypothesis further.

Measuring Inbreeding

Before we discuss inbreeding depression, let us first discuss estimation of inbreeding, usually measured through Wright's **inbreeding coefficient *F*** [22]. Traditionally, the use of detailed pedigree data to estimate inbreeding, the probability of **identity by descent (IBD)** of the two alleles in a diploid individual from a common ancestor, has been thought to be the best approach [23,24]. However, reliable pedigrees are not available for many organisms and even when a pedigree is available, unknown relatedness and the limited depth and information of most pedigrees can result in an underestimation and/or inaccuracies of actual inbreeding levels. Further, pedigree inbreeding is the expectation of IBD for a given individual and substantial variation in actual IBD among progeny from a given type of mating is expected [25–29].

Genomic estimates of the level of inbreeding using thousands of SNPs have recently enabled a high degree of resolution in the estimation of the actual IBD proportion of individuals in many organisms without pedigrees (Box 1). As a result, significant inbreeding depression has been found more often when genomic measures of inbreeding were used than when pedigree measures were used [30–33].

General Approach To Estimating Inbreeding Depression

The classic approach for estimating inbreeding depression is to use data for a fitness-related trait from individuals with different levels of inbreeding. According to Morton *et al*. [34], assuming that the loci affecting fitness (survival) act independently and multiplicatively, w_F, the fitness of individuals with inbreeding F, becomes approximately

$$w_F = e^{-A-BF} = w_0 e^{-BF} \quad [1]$$

When there is no inbreeding, $w_0 = e^{-A}$, and the genetic portion of the reduction of w_0 is called the **mutation load** (Box 2).

This approach assumes that the average frequencies of the deleterious alleles remain constant during the inbreeding process, as for neutral alleles, and predicts that fitness will decline as inbreeding increases with a constant rate (B) that equals the inbreeding load, which is the load concealed in heterozygotes in the non-inbred population but expressed in homozygotes. B depends on the frequency of the deleterious alleles and the recessive component of their effects, and was originally interpreted as the number of recessive **lethal equivalents**, where a lethal equivalent is equal to 'a group of mutant genes of such number that, if dispersed in different

Glossary

Drift load: the steady decline in fitness due to random fixation of a fraction of the deleterious mutations that continuously occur in the population.
Genetic rescue: introduction of beneficial variation from outside the population at genes which are fixed for (or have a high frequency of) variants causing inbreeding depression in the population
Genetic load: the actual or potential reduction of population mean fitness due to genetic causes. Three main components of the genetic load can be considered: drift load, inbreeding load and mutation load.
Identity by descent (IBD): the situation where the two alleles are descended from a given allele in a common ancestor.
Inbreeding depression: the reduction of fitness because of lower survival, mating, and/or reproduction in the progeny of related individuals compared to the progeny of unrelated individuals.
Inbreeding load *B*: the genetic damage that is concealed in heterozygosis in the population and would be expressed in a complete homozygote. Under some simplifying assumptions (fitness multiplicative across unlinked non-epistatic loci) B equals the rate at which fitness declines with increasing inbreeding in the absence of selection (roughly, the % of reduction in fitness expected from each 0.01 increase in F).
Lethal equivalent: a group of mutant genes (or a single mutant gene) that if dispersed in different individuals, would cause on average one death
Inbreeding coefficient (*F*): The probability that an individual carries two IBD copies at a given neutral locus.
Minimum inbreeding: optimizing how breeding individuals should mate to minimize average inbreeding in the next generations. In the long term it induces some increase of mean inbreeding and some reduction of genetic diversity.
Minimum kinship: optimization of the number of breeding offspring contributed by each individual to minimize average coancestry in the next generation (self-coancestries included). It usually converges to the classical 'equal family contribution' strategy in a few generations.

Box 1. Measuring Inbreeding with Genomic Data

Genomic data can be used to estimate the true proportion of sites that are homozygous by descent in an individual (F_G hereafter) by reference to the current population under hypothetical random mating. For example, if the homozygosities of many SNPs are determined, then an estimate of inbreeding measuring the excess of observed homozygosity due to inbreeding is

$$F_H = \frac{O(Hom) - E(Hom)}{M - E(Hom)} \quad [I]$$

where $O(Hom)$ and $E(Hom)$ are the observed and expected Hardy–Weinberg numbers of homozygous loci in an individual and M is the number of loci examined [77,78]. This measure is on average negative when the parents are less related than expected by random mating, and has a distribution centered on 0 in a sample of non-inbred individuals [32]. It is analogous to the classic estimate of population inbreeding corresponding to the general expression

$$F_H = \frac{H_E - H_O}{H_E} \quad [II]$$

where H_E and H_O are the expected and observed frequencies of heterozygotes in the population, which measures inbreeding from non-random mating and/or population subdivision. This and other genomic measures related to multilocus heterozygosity generally are highly correlated [32,79].

If SNPs are mapped genomically, long homologous regions with many linked SNPs that are identical by descent at virtually all SNPs can be identified as identical by descent (IBD). These regions, usually several Mb long, are knowns as **runs of homozygosity (ROH)** [77]. The inbreeding coefficient F_{ROH} of an individual is estimated from these data as

$$F_{ROH} = \frac{\sum L_{ROH}}{\sum L_{AUT}} \quad [III]$$

where the numerator is the sum of the lengths of all the ROH of a given size or larger and the denominator is the sum of the overall length of autosomal genome covered by the SNPs utilized. F_{ROH} ranges from 0 to 1 and provides a direct estimate of the proportion of the genome in an individual that is IBD. It is therefore somewhat analogous to pedigree inbreeding but while IBD in pedigree inbreeding implies inheritance from common ancestor within the pedigree, IBD for ROH refers to common ancestors that are on average further back in time for shorter runs of homozygosity. Therefore, the distribution of the length of the runs of homozygosity contains information on the relatively recent demographic history of the population that can be useful to determine the impact of inbreeding and purging in the past [32].

Mutation load: the reduction in mean fitnesses when there is no inbreeding (Hardy–Weinberg proportions) due to lower fitness of homozygotes and/or heterozygotes for segregating deleterious mutations. It represents the expressed portion of the genetic load due to not fixed deleterious mutations.
Purging: increased purifying selection facilitated by inbreeding as it increases the homozygosity of partially recessive deleterious variants
Runs of homozygosity (ROH): regions of the genome that are identical by descent consisting of a given number of homozygous, adjacent SNPs over a given number of Mb. The longer the run, the closer is expected to be the most recent common ancestor

individuals, they would cause on average one death, for example, one lethal mutant, or two mutants each with 50 per cent probability of causing death, etc.' [34]. Thus, in the long term, inbreeding converts the inbreeding load B into the fixation load.

Most simply, the fitness averages for an outbred generation and an inbred generation with a known inbreeding level can be compared to estimate the effect of inbreeding [1]. Then, expression [1] can be solved to give B as

$$B = \frac{1}{F} \ln\left(\frac{w_0}{w_F}\right) \quad [2]$$

As perspective, when the proportion of reduction in fitness is the same as the increase in F, then B is slightly greater than 1. For example, if $F = 0.25$ and $w_F = 0.75$ (and $w_0 = 1$), then $B = 1.15$. The number of lethal equivalents per diploid zygote $2B$ is often used to describe the potential for inbreeding depression.

When fitness data from more levels of inbreeding are known, a maximum likelihood approach was initially used to estimate $2B$ [34]. Subsequently it was shown that the maximum likelihood approach had a low bias for a diverse range of simulated pedigrees and that the 95% confidence intervals were appropriate [35]. These parameter estimates can also be found using generalized linear modelling procedures available in standard statistical packages [36,37].

Estimates of Inbreeding Depression

Charles Darwin [3] experimentally documented the negative effects of inbreeding in plants. His interest in inbreeding depression was partly because he married his first cousin Emma Wedgewood and noticed the health problems of his 10 children, three of whom died before adulthood.

Box 2. Mutation Load

The population fitness can be reduced from its maximum because deleterious variants exist either as heterozygotes or homozygotes. When there is no inbreeding for a locus where the relative fitnesses of genotypes *AA*, *Aa*, and *aa* are 1, 1 – *hs*, and 1 – *s*, respectively, this reduction, called mutation load, is

$$L = 2hsq(1-q) + sq^2 \quad [I]$$

where *q* is the frequency of detrimental allele *a*. This mutation load accumulates over all segregating loci with detrimental effects.

Recently, mutation load has been estimated using genomic data and inferring fitness based on biological function scores that reflect the level of selection, where the more conserved phylogenetically mutant sites are assumed to be under stronger selection [80]. Using previously published selection coefficients [81], the estimated mutation load added over the whole genome in four human populations was very high [80], about 8 assuming $h = 0$ and about 15 assuming $h = 0.5$. Subsequently, another set of selection coefficients was used that were 20% to 28% less, and a mutation load of about 1.5 assuming $h = 0$ and about 3 assuming $h = 0.5$ was estimated in seven other human populations [82]. Most of the mutation load in both studies is from the class under intermediate selection ($s = 0.0045$ [81] or $s = 0.001$ [83]), while alleles in the next effect class ($s = 0.01$ and above [81] or 0.002 and above [83]) contribute little mutation load. The highest level of selection ($s = 0.002$ and above) in [83] constituted only about 1% of the deleterious mutant alleles per individual because of their low frequency.

Because both the frequency and the level of selection of most deleterious variants are very low, the high mutation load estimate apparently occurs because very many mutational variants were identified (between 13 400 and 23 700 mutants in the four populations in [81]. The mutation load measured in these studies is high but is the result of many loci, those causing most mutation load having very small deleterious effect. Since mildly deleterious effects are thought to be more or less additive, it is likely that most inbreeding depression is caused by variants with relatively large deleterious effect that cause almost no mutation load because they are at least partially recessive and segregate with low frequency. Nevertheless, the dominance for effects so small as those accounting for most mutation load has never been directly investigated. It also appears that approaches based on genomic evolution might not be able to identify variants with individually important effects, such as $s > 0.05$, including lethals, because there are few of them and they are individually rare. Thus either direct experimental approaches or population information, such as low allele sharing across populations and/or low frequency of mutants, might be necessary to identify variants responsible for the inbreeding load [80,83,84].

Recently, inbreeding depression in the Charles Darwin family has been documented by examining his pedigree and male parent inbreeding was significant for both family size and reproductive duration [38].

The number of lethal equivalents was originally estimated for several human populations and $2B$ was between three and five [34]. However, these estimates did 'omit abortions, early adult deaths, and cases of infecundity' with the conclusion that 'the value of *B* taken to include these cases is probably twice as great as we have given' [34].

Inbreeding depression was examined in captive ungulates and juvenile survival was found to be lower in inbred than in non-inbred progeny in 15 of 16 species [39]. Subsequently, a survey of pedigreed zoo populations found decreased juvenile survival in 36 of 40 mammal populations with a median of $2B = 3.14$ [7]. This inbreeding depression estimate was based only on juvenile survival and did not include other mortality or other fitness components, suggesting that inbreeding depression would likely be higher in natural populations [7]. A survey of the inbreeding depression in mammals and birds in wild populations, combined over fitness components, concluded that the number of lethal equivalents in a diploid ($2B$) was around 12 [6]. As an illustration of the impact of captive versus natural environments, the estimated number of lethal equivalents in the white-footed mouse in captivity was 0.5 while in a natural environment, it was 12.6 [40].

A number of high estimates of inbreeding depression have been found in recent years. For example, examination of inbreeding depression across life-history stages of the takahe, a flightless rail, found that the total number of lethal equivalents was $2B = 16.05$ [41] and this large overall number of lethal equivalents was the result of smaller values distributed over nearly all life stages. Captive inbred embryos and chicks in the 'Alalā, the Hawaiian crow, had much

reduced survival with $2B = 13.8$ [42] and in this case, nearly all the inbreeding was from a single pair of ancestors [20]. Other examples include fitness in the great tit ($2B = 6.4$ [43]), fitness and viability in collared flycatchers ($2B = 22.4$ and 14.9, respectively [44]), viability in a sparrow ($2B = 18$ [45]), and viability in red deer ($2B = 8.7$ [46]). Inbreeding depression from genomic data for early survival in a single individual of *Eucalyptus grandis* was $2B = 11.75$ [20]) (Box 3).

More studies on inbreeding depression in wild populations should be obtained in the near future due to the availability of molecular markers for many species. For example, inbreeding depression has been estimated for a wild population of red deer (*Cervus elaphus*) using over 30 000 SNPs to estimate inbreeding [31]. This study found inbreeding depression for many traits, and when inbreeding was 0.125, the predicted lifetime breeding success in females and males was only 18% and 5%, respectively, of that with no inbreeding. These data gave extremely high estimates of $2B = 27.4$ and 47.9, in females and males, respectively.

What are the factors that might influence these recent high estimated levels of inbreeding depression? Both the inclusion of more fitness components and measuring inbreeding

Box 3. Genomic Inbreeding Depression in Eucalyptus

Genomic data promises to provide other approaches to estimate and understand the level of inbreeding depression. For example, Hedrick *et al.* [20] used an approach related to that of Morton *et al.* [34] to estimate inbreeding depression for inbred versus outbred progeny in the Australian tree *Eucalyptus grandis*. For a group of 28 progeny produced by self-fertilization from a single parental tree, the whole genome was sequenced. Overall, 9560 genes that were heterozygous in the parent were examined in the progeny group. Given that there was no selection, the expectation is that 50% of these progeny would be homozygous (IBD) for these heterozygous loci.

However, only 34% of these genes were homozygous in this progeny group, a deficiency that was present on all 11 *E. grandis* chromosomes. Figure I gives the observed proportion of the three genotypes for 1019 genes along chromosome 1. Except for a short region on the far right end of the chromosome, the proportion of the two homozygotes is much less than 0.5 and averages around 30%, while the proportion of heterozygotes is much greater than 0.5 and averages 70%. Six of the 11 chromosomes have regions in which one homozygote is missing, indicating a potential lethal. These effects appear to be the result of very strong selection at many genes that cause high mortality when made homozygous by one generation of self-fertilization. It is likely that more than 100 genes, many with a substantial effect on viability, are contributing to this inbreeding depression. Overall, from this deficit of homozygotes, it was estimated that $w_F = 0.053$. Using expression [2] earlier, and assuming that $w_0 = 1$ and $F = 0.5$, then $2B \geq 11.75$ for viability in this one parental tree (note that this was a minimal estimate since there had been one generation of recombination, see [20]).

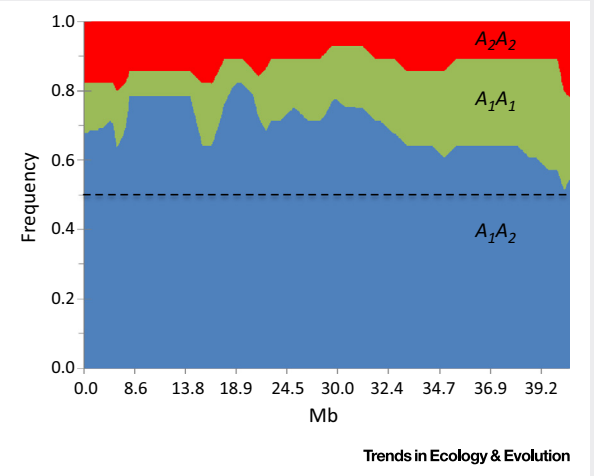

Figure I. Heterozygosity and Homozygosity across Chromosome 1. The proportions of heterozygotes (blue) and the two homozygotes (red and green) for 1019 single nucleotide polymorphisms (SNP) along chromosome 1 for 28 self-fertilized progeny from a single parent in *Eucalyptus grandis* [34]. The broken line gives the expected proportion of heterozygotes given no selection.

depression in natural or more realistic environments could result in higher inbreeding depression than the earlier estimates. In addition, for some species there might have been very high past effective population sizes, resulting in a high level of standing detrimental variation for recessive alleles and consequently high inbreeding depression, as discussed below.

Evolutionary Factors Influencing Inbreeding Depression

The inbreeding load is essentially the result of rare, nearly recessive lethals and partially recessive detrimentals [1]. An approximation for the equilibrium level of inbreeding load is given by assuming that detrimental mutation produces genetic variation, and purifying selection and genetic drift reduces it so that there is a mutation–selection–genetic drift balance [47].

In this approach, the rate of detrimental mutation per gamete per generation is U. The deleterious effect in the mutant homozygotes aa is s, and the proportion of this deleterious effect that is expressed in the heterozygotes Aa is h, so that, the fitnesses of genotypes AA, Aa, and aa are 1, $1-hs$, and $1-s$, respectively. The extent of genetic drift is determined by the effective population size N. Using these parameters, the equilibrium number of lethal equivalents B is approximately

$$B \approx \frac{sU(1-2h)}{1/(2N) + hs + s(1-2h)K} \quad [3]$$

where K is the proportion of deleterious copies that undergo selection in the homozygotes [47]. At equilibrium, the magnitude of B is basically a balance of the input from mutation in the numerator and the reduction from genetic drift and selection in the denominator.

As we mentioned above, the variants that reduce fitness with inbreeding have a distribution of effects but fall into two major groups [18], those with large effects (lethals or near lethals), almost completely recessive, and those with small or moderate effects (detrimentals) which are only partially recessive. For illustration here, we will examine some cases for these two categories of variants separately, lethals (Figure 1A) and detrimentals (Figure 1B).

Notice first that the number of lethal equivalents B increases linearly with U (Figure 1A). In addition, B is smaller in small populations for two reasons, that is, because genetic drift reduces genetic diversity at the loci responsible for B at a rate $1/(2N)$ and because of selection against the larger proportion K of alleles that are exposed as homozygotes. In general, B is a function of the level of dominance h because this determines the extent that heterozygotes are exposed to selection, and Figure 1B shows that even small h values can impose an important constraint on

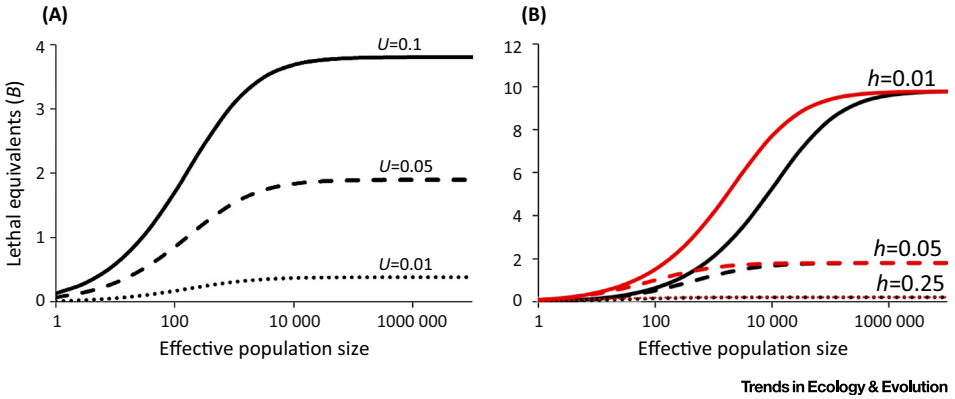

Figure 1. Inbreeding Load as a Function of Population Size. (A) The equilibrium inbreeding load for lethals ($s = 1$) with low dominance ($h = 0.025$) and different mutation rates U. (B) The equilibrium inbreeding load for detrimentals (black: $s = 0.1$; red: $s = 0.5$) with mutation rate $U = 0.1$, and different levels of dominance, both as a function of the effective population size.

B. For any given h value in large populations, B becomes independent of the level of selection against homozygotes s. This occurs because more deleterious alleles contribute more inbreeding load per copy, but have also lower frequency. From these considerations, at the equilibrium, lower inbreeding load would be predicted for small populations, while large populations are expected to experience more severe fitness reduction under inbreeding.

How Can High Inbreeding Depression Be Reduced?

In managed populations, minimizing inbreeding or mean kinship are the major approaches used to minimize inbreeding depression, primarily based on genealogical information but potentially based on detailed genomic data when available [27]. In addition, two other options have been advocated to reduce inbreeding depression in recent years, purging induced by deliberate inbreeding, and genetic rescue. Both options have advocates that suggest that these approaches can substantially reduce inbreeding depression but, as we discuss below, both options should be applied carefully and neither appears to provide a universal solution to the problem of high inbreeding depression.

Purging

Purging of inbreeding depression, that is, reduction of the frequency of detrimental variants that when homozygous result in inbreeding depression, can occur either naturally or by design [14,48,49]. Naturally, this occurs as inbreeding increases the frequency of homozygotes where recessive effects are exposed to selection. It has been recognized as particularly important for lethals and alleles with large recessive effects but it is also significant for partially recessive detrimentals with smaller effects, as long as the rate of inbreeding is not too fast [50]. The effectiveness of genetic purging depends on the architecture of the inbreeding load and on the rate of increase in the level of inbreeding. Many of the evolutionary genetic aspects of purging have been examined [13], including the impact of linkage [51].

The dynamics of purging can be shown by the evolution of fitness through inbreeding which, by generation t, can be approximated by

$$w_t = w_0 e^{-Bg}. \quad [4]$$

This expression is analogous to Equation 1, but the inbreeding coefficient F has been replaced with the coefficient of purged inbreeding g that is less than F as it is weighted by the reduction in frequencies of deleterious alleles caused by purging [13,52]. The evolution of g depends on inbreeding rates but also on a purging parameter d that represents the magnitude of the deleterious effect that is being exposed by inbreeding. More precisely, for single s and h values, d is the recessive component of the deleterious effects (i.e., the per-copy deleterious effect that is expressed in the homozygotes minus that expressed in the heterozygotes, $d = s/2 - hs$).

To illustrate the predictions from this approach, Figure 2 gives the expected change in fitness, starting from a large population with $B = 5$ (assuming that $s = 0.25$ and $h = 0.2$) after its effective size is reduced to either $N = 10$ or $N = 50$. First, notice the fitness drop in the early generations as detrimental genetic variation is exposed by inbreeding associated with genetic drift. Then, as fitness-lowering alleles are purged, the fitness rebounds to a higher level, somewhat below the original outbred value because of some fixation of detrimental alleles. In the smaller population, the initial reduction is greater and occurs more quickly, and the fitness rebound also occurs more quickly but the eventual fitness after purging is smaller than in the larger population. Inbreeding depression from recessive lethals is purged even more efficiently than in this example for detrimentals.

As purging reduces the frequency of the deleterious alleles, it also reduces the inbreeding load. Thus, after a reduction in population size, the inbreeding load will drop faster (and the rate of

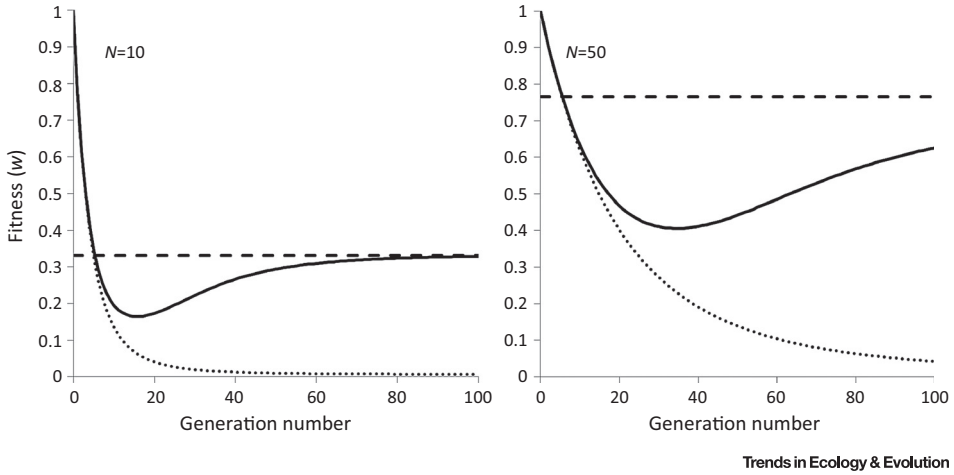

Figure 2. Effects of Purging on Fitness. The expected change in mean fitness starting from a large population after its effective population size is reduced to $N = 10$ or $N = 50$ ($B = 5$ due to detrimental alleles with $s = 0.25$ and $h = 0.2$). Unbroken lines: prediction from inbreeding with purging (Equation 4). Broken horizontal line: asymptotic value for the above prediction. Dotted lines: prediction when there is no purging (Equation 1).

fixation for deleterious alleles will be smaller) than expected just from genetic drift in the absence of purging.

Although purging has been observed in experimental laboratory populations, empirical evidence for purging in both captive and wild populations is not strong, partly because it is difficult to document inbreeding depression with substantial statistical power before and after purging. Purging has most often been detected under slow inbreeding [53–55], with strong purging being estimated under competitive conditions in *Drosophila* [10]. In captive populations, purging can also be difficult to detect because both inbreeding depression and purging seems to be lower under benign non-competitive conditions [54].

The efficiency of purging in the wild varies among populations. For example, detailed examination of the Stewart Island robin *Petroica australis rakiura* with a history of low population numbers producing relatively slow inbreeding, found no support for inbreeding depression, consistent with previous purging [56]. By contrast, for a population of the Chatham Island black robin *P. traversi* with a documented history of drastic bottlenecking, there remained a strong negative association of inbreeding and juvenile survival with $2B = 6.85$ (95% CI: 0.587, 13.11) [57].

Recently, the purging parameter has been estimated from the fitness decline of experimental populations of *Drosophila melanogaster*, and purging was found to be particularly important under competitive conditions [10,54]. Furthermore, a method and free software has been developed to estimate purging from fitness pedigreed data [37]. Therefore, estimates for d are likely to be increasingly available in the near future, allowing the prediction of the joint consequences of inbreeding and purging on the fitness evolution of endangered populations.

Purging can also be deliberately induced in conservation programs, potentially resulting in loss of detrimental variation and the avoidance of eventual decrease in fitness. This can be done by subdividing the population into isolated lines. However, increased genetic drift within the lines can lead to a reduction of the long-term efficiency of purging for mildly deleterious alleles [13,14]. Furthermore, drift also increases the probability of fixation for the deleterious mutations that continuously occur in the lines during the inbreeding process, particularly when effects are mild, causing an additional fitness decline [58,59], the so-called **drift load.** After subdivision, purging

can be further increased through between-lines selection [49] but this will increase drift at the metapopulation level, reducing overall genetic diversity and adaptive potential.

Purging in a managed population can also be implemented by artificially inducing some inbred matings (or identifying inbred individuals), which is not expected to cause increased drift [51,53]. This approach was suggested to have been applied successfully in Speke's gazelle [60] but subsequently the results were shown to be more likely ascribed to temporal husbandry improvement [61]. However, recent data from the Chatham Island black robin suggests that if a highly inbred chick inherits a proven genotype from a highly inbred parent, it has a fitness advantage [27,62].

Overall, purging has the potential to reduce the negative effects of inbreeding depression but it requires relatively slow inbreeding, and usually an early fitness decline occurs before the purging effect becomes apparent. Therefore, induced purging has potential dangers and might prove questionable as a deliberate strategy for the genetic health of a population. However, spontaneous purging is relevant to population survival, while genetic management strategies aimed to slow inbreeding and drift in captive populations hinder purging. Thus, **minimum kinship** slows inbreeding but, by making the number of offspring that an individual contributes to the breeding group independent of its fitness, it reduces the intensity of natural selection or even cancels it in the case of fecundity traits. Regarding **minimum inbreeding** (the choice of mates with minimum coancestry), purging is hampered for the sake of a reduction of immediate inbreeding, but subsequent inbreeding can be even larger than under no management. Thus, these strategies should not be intended as long-term conservation protocols. This implies that efforts should be made to warrant *in situ* conservation. *Ex-situ* conservation can be necessary for extremely endangered populations, but it should only be aimed as a provisional intervention, since the earlier management is relaxed and the population is reintroduced in its natural habitat, the better.

Genetic Rescue

When variants with a negative effect on fitness become fixed or reach a high frequency due to genetic drift in small populations, introduction of variation from outside the population can result in an increase in fitness, a phenomenon called genetic rescue [15,63]. In fact, genetic rescue has resulted in population recovery in Florida panthers [64], adders [65], bighorn sheep [66], prairie chickens [67], and wolves [68–70], and appears to have reduced inbreeding depression in a number of situations [71]. However, in at least one instance, genetic rescue eventually resulted in lower fitness and imminent extinction [68,72] (Box 4).

Although genetic rescue appears to be a possible 'silver bullet' for populations with low fitness, several cautionary aspects must be noted [73]. First, an appropriate source population might not be present in some cases, either because there are no other populations of the same species or because they are genetically different in some fundamental way. Second, swamping of locally adapted genetic variation [74] by genetic rescue might make the population more prone to extinction in the long run even though there could be a short term increase in numbers. Third, after a genetic rescue episode, even one with great success as in the Florida panther there is the potential for subsequent inbreeding and low effective population size [73] as seen in the Isle Royale wolves. Further, if immigrants come from a large source population and are very successful, then rare deleterious alleles might increase and result in an increase in inbreeding load.

Also, there might be diminishing returns from multiple genetic rescue episodes with significant effects initially but eventually resulting in the replacement of the population with ancestry from another population. However, genetic rescue can result in buying time for the population by increasing fitness and population size in the short term so that the basic threats to the species,

Box 4. Genetic Rescue in Wolves

Lowered fitness in a population potentially can be overcome by the natural or artificial introduction of individuals from outside the population, or genetic rescue. Because populations of many species have become small and isolated, genetic rescue might become of important significance in species management and recovery. However, the impact of genetic rescue over time is not clear and [73] advocated a long-term evaluation of its impact to determine the duration of the initial positive effect of genetic rescue and any possible negative effects.

Detailed genetic examination of the wolf population in the Isle Royale National Park on Lake Superior has provided insight into the process of genetic rescue. The Isle Royale wolf population was founded around 1950 by wolves from the mainland population most likely across an ice bridge. Until recently, the population has averaged around 25 animals in 3 to 4 packs. In 2008, it was discovered from genetic examination of scats that a male wolf migrated to Isle Royale in 1997 [65]. By 2008, based on analysis of the constructed pedigree, 59.4% of the genetic ancestry in the population descended from the migrant [72]. The great increase in his ancestry suggested that genetic rescue resulted in a large initial increase in population fitness. In fact, the benefit of genetic rescue was only temporary because the population has greatly declined in recent years and the population numbers of Isle Royale wolves in 2015 and 2016 were only 3, and 2 wolves, respectively. Figure I shows the three wolves observed in 2015. The adults are both father and daughter and half-sibs and the expected inbreeding coefficient of an offspring from them is 0.438. The pup appears to have an unusual tail and posture, is relatively small, and died within the first year, all indicators of potential inbreeding depression effects.

It is possible that this situation, in which natural genetic rescue now appears to be associated with imminent extinction, is not likely except in an extreme situation like the Isle Royale wolves. In this case, there was a single migrant whose genome swept through the relatively small extant population. It appears possible that although this migrant and his progeny had a fitness advantage, he also brought in new detrimental variation which by chance, or associated with the initial selective advantage of his offspring, increased in frequency.

Figure I. Wolves Observed in Isle Royale in 2015. The adult female is to the right and the adult male in the middle. The third animal, thought to be a pup, was not seen in 2016.

such as habitat loss or introduced species, could be addressed for successful recovery in long term [63].

Future of Inbreeding Depression Studies

Recently, the use of genomics rather than pedigrees to measure inbreeding has been advocated [27,29,31,75] and has the potential to revolutionize the understanding of inbreeding

depression. The evaluation of the impact of the different mechanisms, such as purging and genetic rescue, that might alleviate inbreeding depression needs also to be analyzed. Studying purging, however, requires detailed historical information on the rates of inbreeding, as provided by experiments with controlled effective sizes or by pedigree data. Nevertheless, data from mapped genomic diversity, as the distribution of ROH length, could contribute information in this respect.

Genomic studies can also potentially identify genes that result in inbreeding depression [32], for example, such loci might be lethals or morphological loci from other common or model species, or candidate loci. In addition, approaches such as those in determining mutation load, homozygosity mapping for recessive variants, and genome-wide association studies could be used to identify genes influencing inbreeding depression [32]. Further, ROH analysis could find genes that are lethal as homozygotes because when regions are missing as ROH, this would suggest presence of a lethal.

Several recent studies in the cheetah [76], Chillingham cattle [76], and island fox [77] have found very high genomic homozygosity that has accumulated over many generations. Neither the Chillingham cattle nor the island fox populations appear to have much lowered fitness and perhaps purging has occurred in their populations over time. The cheetah, which has a high frequency of deformed sperm, has high rates of putatively function-damaging, nonsynonymous mutations not found in other felids in a gene that influences sperm morphology.

Inbreeding depression is an important aspect of managing small captive populations. However, there have been successful breeding programs with small numbers of founders in the black-footed ferret, Przewalski's horses, California condor, and other species. In all these cases, the environment was initially made less stressful so that the extent of inbreeding depression was probably less. Perhaps as the numbers are increased, such species can tolerate inbreeding depression in a more natural environment, where more purging can take place.

Concluding Remarks

Recent evidences indicate that inbreeding can potentially cause drastic reductions in fitness, so that populations undergoing dramatic reductions in size are likely to require genetic management to prevent extinction in the short term. When an appropriate source population is available, one possibility to revert inbreeding depression and restore sustainable population size is genetic rescue, but the habitat should be simultaneously restored, as part of the hybrid vigor induced is only temporary and can later induce further inbreeding depression and swamp the identity of the population. Alternatively, sustainable population sizes can be recovered through *ex-situ* conservation, often using breeding strategies based on minimizing inbreeding and the loss of genetic diversity. However, this also slows purging, particularly for fecundity traits. Therefore, although deliberate purging is generally not advised due to the risk from short term fitness decline, choosing mates with minimum coancestry, is only advised for extremely endangered populations, and minimum kinship methods should not be considered as a long term conservation strategy. On the contrary, unmanaged breeding in competitive wild environments should be restored as soon as population numbers warrant slow inbreeding and spontaneous purging. Both genomics and appropriate analysis of pedigrees and inbreeding rate data are expected to improve our knowledge of the genetic architecture of inbreeding, contributing to optimizing management decision in each case (see Outstanding Questions).

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Outstanding Questions

What genes are involved in inbreeding depression and how can they be identified? What is the distribution of deleterious effects responsible for inbreeding depression and purging?

What is the past level of inbreeding in species with small population sizes today?

How can species survive demographic hazards given the high amount of inbreeding load often observed in the wild?

Using genomic monitoring, is it possible to determine how successful genetic rescue is going to be and why it succeeds or does not?

How much purging has already occurred in endangered populations and how this affects future inbreeding depression?

Will it be possible to estimate a predictive purging parameter from genomic information?

What does the association between fitness and genomic estimates of inbreeding tell us about the expected fitness of offspring with a given pedigree inbreeding?

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