The Crucial Role of Genome-Wide Genetic Variation in Conservation

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Abstract

The unprecedented rate of extinction calls for efficient use of genetics to help conserve biodiversity. Several recent genomic and simulation-based studies have argued that the field of conservation biology has placed too much focus on conserving genome-wide genetic variation, and that the field should instead focus on managing the subset of functional genetic variation that is thought to affect fitness. Here, we critically evaluate the feasibility and likely benefits of this approach in conservation. We find that population genetics theory and empirical results show that conserving genome-wide genetic variation is generally the best approach to prevent inbreeding depression and loss of adaptive potential from driving populations towards extinction. Focusing conservation efforts on presumably functional genetic variation will only be feasible occasionally, often misleading, and counterproductive when prioritized over genome-wide genetic variation. Given the increasing rate of habitat loss and other environmental changes, failure to recognize the detrimental effects of lost genome-wide genetic variation on long-term population viability will only worsen the biodiversity crisis.

Introduction

Decades of theoretical (1) and empirical (2, 3) research suggest that conserving genome-wide genetic variation improves population viability. Maintaining genetic variation, ***adaptive potential*** (see Glossary), and avoiding ***inbreeding depression*** are central motivations for maintaining large, connected natural populations. Principles of genetics and evolution have therefore played a large role in conservation biology since its inception (4, 5). The genomics revolution has inspired biologists to leverage genome analysis to advance conservation beyond what was possible with traditional genetics. Numerous studies have sequenced genomes of non-model organisms of conservation concern to understand population history, ***inbreeding*** depression, and the genetic basis of adaptation. A particularly exciting area of research has been to determine when and how functional genetic information can advance conservation.

Several recent studies suggest that too much emphasis has been placed on genome-wide genetic variation in conservation biology. For example, persistence of small populations for long periods of time despite low genetic variation, and the collapse of the Isle Royale wolf population after the infusion of genetic variation via immigration, have been interpreted as a challenge to the idea that genetic variation generally increases population viability (6-12). Additionally, a weak relationship between conservation status and genetic variation has been used to argue that genome-

wide (presumably neutral) genetic variation is of little importance to conservation (11). Several authors have thus advocated for an approach that focuses on functional genetic variation that is thought to directly affect fitness (including minimizing deleterious genetic variation) in place of the traditional emphasis on conserving genome-wide genetic variation (6-8, 11).

Here, we evaluate the theoretical and empirical basis of this challenge to the importance of genome-wide genetic variation and show that its premise is inconsistent with population genetic theory and empirical findings. While it is clear that functional genetic information can advance conservation, deemphasizing the maintenance of genome-wide genetic variation would increase the extinction risk of threatened populations.

1. Is genetic variation predictive of inbreeding and inbreeding depression?

Inbreeding depression is thought to be driven mainly by homozygous and ***identical-by-descent*** deleterious, partially recessive alleles (13), with lethal and small effect deleterious alleles contributing substantially (14). The constant input of new deleterious mutations (15-19) makes inbreeding depression a ubiquitous phenomenon that can push populations toward extinction (2, 20-23). One of the foundational predictions of theoretical population genetics is that the rate of loss of heterozygosity (***H***) per generation ($\Delta\bar{H}=1/2N_e$) is identical to the rate of increase in mean individual inbreeding (***F***), which is $\Delta\bar{F}=1/2N_e$ (24). $\bar{H}$ is therefore expected to be entirely predictive of $\bar{F}$ (24-29).

A more difficult, but crucial question is whether genome-wide genetic variation ($\boldsymbol{\pi}$) is predictive of inbreeding depression. Deleterious alleles are lost in small populations due to selection and genetic drift (30, 31), but they are also more often expressed in homozygotes in smaller populations due to inbreeding. Selective ***purging*** of large effect deleterious alleles following inbreeding combined with genetic drift may therefore result in low ***inbreeding load*** and little inbreeding depression in the most highly inbred populations with the lowest π. However, the presence of purging does not imply that high fitness is maintained in small populations with low π.

Population genetics theory predicts that larger populations will have higher neutral (24) and deleterious genetic variation (32, 33). This is illustrated in Figure 1, where simulated large populations have higher π (24) and higher inbreeding load (32-34) arising from segregating partially recessive deleterious alleles. These simulations assume empirically supported models of fitness and dominance (***h***) effects (*Supplementary Information* [SI]). Smaller populations have

lower π due to genetic drift, and fewer ***lethal equivalents*** due to genetic drift and purging. However, despite having fewer lethal equivalents, chronically smaller populations have lower mean fitness due to partially recessive deleterious alleles being expressed following inbreeding, and some reaching high frequency or fixation (i.e., high ***drift load***). Therefore, a negative relationship is expected between π and drift load for populations at mutation-drift-selection equilibrium.

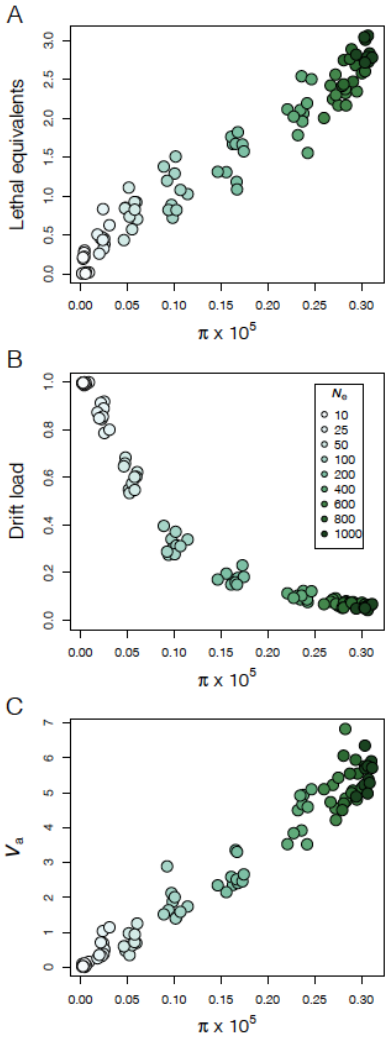

Figure 1. Relationship of nucleotide diversity (π) with the inbreeding load (lethal equivalents) (**A**), drift load (**B**), and additive genetic variance in a quantitative trait (V_a) (**C**). The data are from the 1,000th generation of 10 simulated populations with 9 different constant effective population sizes (N_e).

Equilibrium levels of π and drift load are not expected in populations with fluctuating population size or immigration rate. A common scenario with high conservation relevance is isolated populations that have experienced recent bottlenecks. The simulated data in Figure 2 shows that genome-wide π declines over time following a bottleneck, as expected from classical theory (24) (Figure 2A). This pattern is paralleled by lethal equivalents (Figure 2B) owing to the loss of deleterious alleles via genetic drift and purging of deleterious alleles expressed in homozygotes due to inbreeding (30, 31). However, the deleterious alleles remaining after a bottleneck often go to high frequency or fixation. This results in individuals being homozygous for increasingly more deleterious alleles (higher drift load, Figure 2C) as π declines inexorably during a sustained bottleneck, the same pattern expected for small populations at equilibrium (Figure 1). It is notable, though, that π, inbreeding load, and drift load can change at substantially different rates following a bottleneck. For example, drift load can become quite high before π declines substantially following a bottleneck (Figure 2A, 2C). However, small populations that already have low π are also expected to have low mean fitness due to ever-increasing drift load, which demonstrates that π is a good indicator of drift load and mean fitness. Occasional immigration can be sufficient to maintain high π and low drift load in small populations (Figure 2). This is one reason why maintaining connectivity is a priority in conservation biology, and why ***genetic rescue*** is an effective tool for managing small, isolated populations (30, 35, 36).

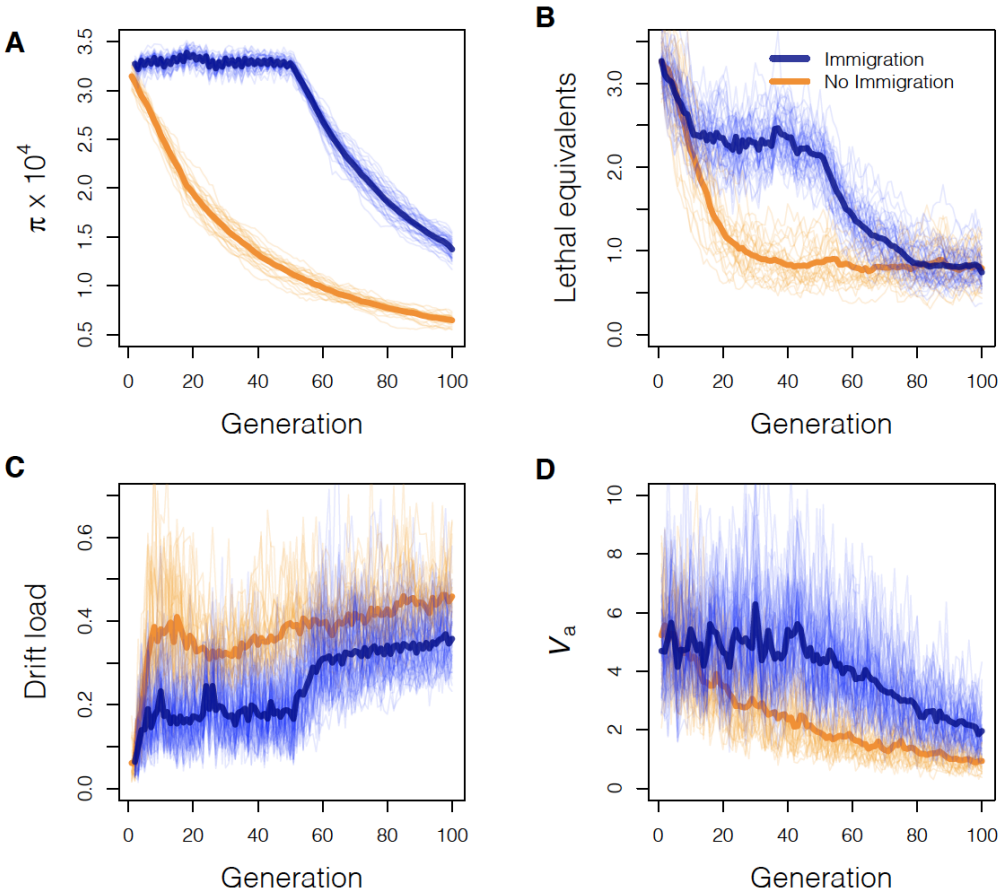

Figure 2. Genetic effects of bottlenecks with and without immigration. Nucleotide diversity (π) (**A**), number of lethal equivalents (**B**), drift load (**C**), and the additive genetic variance in a quantitative trait (V_a) (**D**) are shown for 100 generations after a simulated bottleneck in isolated populations (orange) and with 5 immigrants every 2 generations up to generation 50 (blue). Population size was held constant at N_e=1,000 for 1,000 generations before the bottleneck and then at N_e=25 starting at generation 0. The thin lines show the results from 25 replicates. The thick lines represent the mean across 25 replicates. Immigrants during the first 50 generations are from a population with N_e=500 that split from the receiving population the generation of the bottleneck. Details of the simulation model and parameters are provided in the SI.

Empirical data show that purging does not eliminate the extinction threat posed by inbreeding. Pedigree-based studies have yielded mixed results with regard to purging, with typically only a small portion of inbreeding depression being removed after sustained inbreeding in small populations (37-39). Analyses of 60 genomes from seven ibex species found that species which went through the most severe bottlenecks had more deleterious alleles (40). Alpine ibex,

which were once reduced to 100 individuals, had fewer highly deleterious alleles but more mildly deleterious alleles compared to Iberian ibex (bottleneck size 1,000 individuals). Empirical genetic data suggest small populations have higher drift load (40-42) which has resulted in lower population growth in populations with lower genetic variation (2, 3). In agreement with theoretical expectations outlined above, these data suggest that purging is insufficient to maintain high fitness in the face of strong genetic drift and inbreeding. Thus, the presence of genomic signatures of purging should not be taken as evidence for the absence of inbreeding depression, or for demographic stability of small populations.

The relationship between π and fitness is obviously complicated, particularly immediately after a bottleneck (Figure 2). Populations with the lowest π and highest inbreeding will also have the lowest inbreeding load on average due to reduced deleterious genetic variation via genetic drift and purging. However, these same genetically depauperate populations will typically have lower fitness than larger, genetically diverse populations on average due to ever-increasing drift load (Figures 1 & 2). The bottom line is that reduced fitness is generally expected in small, isolated, genetically depauperate populations due to inbreeding depression and the accumulation of drift load, and that maintaining genetic variation and population connectivity will increase long term viability.

2. Is genome-wide genetic variation predictive of adaptive potential?

The ability of populations to adapt to changing environmental conditions (***adaptive potential***) is fundamental for persisting through environmental change (43, 44). A core insight from theoretical genetics is that adaptation requires additive genetic variance (V_a) for the selected trait(s) (45). A lack of V_a can limit a population's response to selection and eventually lead to extinction (43, 44, 46). As with other types of genetic variation, V_a is affected by mutation at loci affecting the trait, selection, migration, and genetic drift (47). We therefore expect from first principles that larger populations will have higher π and higher V_a than small populations *on average* (Figure 1), and thus that π should be correlated with V_a. Despite strong theoretical support, determining the strength and importance of this relationship in real populations, especially those of conservation concern, has generated longstanding controversy (48).

Basic population genetic theory shows that population size and connectivity play major roles in determining V_a, and thus adaptive potential. Isolated populations below a certain size

should lose V_a due to genetic drift more rapidly than it is replenished via mutation (47). Additionally, recently bottlenecked populations that have lost π will eventually also lose V_a and evolutionary potential in the absence of immigration (Figure 2). However, while the eventual reduction in V_a in small populations is inevitable, the initial effects of a bottleneck on V_a can be complex. Recently bottlenecked populations may show decreases, stability, or even short-term increases in V_a due to the conversion of dominant or epistatic variance into V_a as allele frequencies change due to genetic drift (49-51). This potential conversion of nonadditive to additive variation in bottlenecked populations is highly stochastic across traits and populations, and is one of the processes that can cloud the relationship between molecular and quantitative trait variation (52). Nonetheless, the two important takeaways are: 1) although bottlenecks can complicate the prediction of declining V_a for any given trait in small populations, V_a will be reduced on average, especially for traits with primarily additive inheritance; and 2) eventually, the inexorable decline in π in very small populations means that all small populations will eventually lose V_a and their ability to adapt to environmental change. Adaptive potential in such populations will be severely limited unless V_a is replenished by new mutations or migration from differentiated populations (35) (Figure 2).

The hypothesis that small populations harbor less V_a has been tested empirically in both laboratory and field settings. Most experimental studies show declines in V_a and weaker responses to selection in small populations or following bottlenecks (53-55). On the other hand, field studies often find a weak association between V_a and genome-wide genetic variation when comparing across populations (48, 56); this weak relationship is likely due to a combination of factors, none of which refute the two takeaways described above.

As discussed above, empirical results suggest that V_a may initially increase after a bottleneck due to the conversion of epistatic and dominance variance to V_a (50, 57), and then decline after substantial inbreeding accumulates. Further, V_a is expected to vary among traits and populations depending on genetic architecture, mutation rate, and the mode and history of selection. In practice, most studies are unable to account for these factors and are generally only able to assess a few traits per species/population. Estimates of V_a for each trait are also typically based on a modest number of families. Although the number of traits, populations, and species studied has increased, determining the total V_a for fitness in a given population of conservation concern is not an attainable goal. Additionally, the vast majority of the best-characterized species

with respect to V_a in the wild (i.e., most of the species included in (48, 56) meta-analyses) are common. The species and populations in which the relationship between V_a and genetic variation is expected to be strongest, namely, declining species of conservation concern, tend to be most difficult to characterize.

Arguably the most important point is that the loss of genetic variation in small and/or bottlenecked populations is inevitable and will eventually lead to reduced V_a and reduce adaptive potential, regardless of short-term and stochastic outcomes. Isolated populations that remain small are unlikely to recover substantial V_a due to the slow rate of mutation and the counteracting loss of variation to genetic drift, and the lack of adaptive potential is problematic for long term viability (43, 44, 47).

3. What is the relationship between genome-wide genetic variation and population viability?

The central question regarding the role of genetic variation in conservation is whether populations with lower π are less likely to persist. Genetic effects on the persistence of a particular population are difficult to predict with certainty because there are many factors involved that are difficult to evaluate, including mating system and demographic history (32, 33), current and future environmental conditions (58), and the extent to which ***soft selection*** versus ***hard selection*** predominate (59, 60). Additionally, the highly stochastic demography of small populations, which is exacerbated by inbreeding depression (61), means that widely divergent outcomes can be expected across populations with the same environmental, demographic, and genetic starting conditions. However, theoretical empirical studies have yielded broadly applicable insights into the effects of genetic variation and inbreeding on population viability.

Population genetics theory predicts that small, isolated populations with low genetic variation are more likely to go extinct due to genetic effects than larger, more genetically diverse populations under empirically supported mutational assumptions (19, 22, 23, 62). *De novo* mutations following a bottleneck are expected to cause eventual extinction of very small, genetically depauperate populations via ***mutational meltdown*** (Figure S1) (19). The average time to extinction is shorter under the more realistic scenario where bottlenecked populations carry deleterious mutations at the outset (Figure 3). However, the extinction rate depends strongly on bottleneck duration, with longer restrictions conferring increased extinction due to both demographic stochasticity and the constant increase in drift load. Short-lived bottlenecks are one scenario where viability may sometimes be higher for historically smaller, less genetically diverse

populations that have fewer deleterious alleles at the outset of the bottleneck due to historical genetic drift and purging (Figures 1, 3A, 3B). However, this assumes inbreeding depression is the only genetic challenge operating, and simultaneous selection caused by environmental change may reverse this relationship. Longer bottlenecks in isolated populations are expected to result in very high extinction rates due to mutational meltdown regardless of the abundance of deleterious alleles at the outset (19) (Figure 3C).

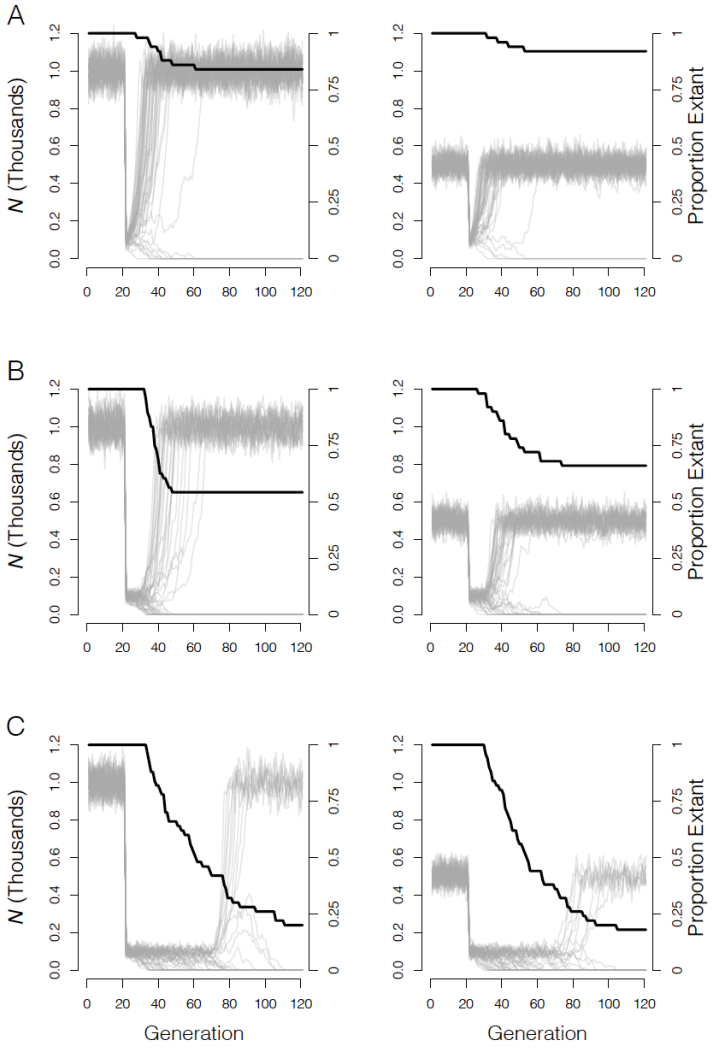

Figure 3. Population viability during bottlenecks from carrying capacity K=1,000 (left column) and K=500 (right column) to K=100. The bottlenecks were 2 (**A**), 10 (**B**), and 50 (**C**) generations in length. The black line shows the proportion of extant populations. Gray lines show population size for each of 50 replicate simulations in each scenario.

Empirical studies of population dynamics arguably provide the strongest evidence for the broad benefits of increased genetic variation for population viability. Numerous studies have almost universally found that populations with higher genetic variation have increased population growth and viability (63). For example, lower genetic variation was associated with reduced population growth in alpine ibex (3) and increased local extinction in Glanville fritillary butterflies (2). Inbred laboratory lines of animals, which quickly lose genetic variation, often become extinct substantially more rapidly than control lines (64, 65). Additionally, the infusion of genetic variation via natural (66) and facilitated immigration ('genetic rescue') nearly always increases population growth (35, 36, 67, 68) either by masking of deleterious recessive alleles, or by infusing adaptive genetic variation.

The collapse of the Isle Royale wolf population after a mainland male immigrated to the small population has been interpreted as a counter-example to the efficacy of genetic rescue (8). However, detailed documentation indicates that results from this unusual system are unsuitable as a general example of the likely demographic outcome of genetic rescue attempts (67, 69, 70). The immigration of only a single male into Isle Royale makes is unusual in the context of managed genetic rescue attempts which typically involve translocation of multiple individuals into a small population, e.g., (71-73). The single migrant male wolf dominated and increased reproduction, resulting in genetic rescue (an increase in population size following outbreeding). However, his extremely high reproduction resulted in very high inbreeding within two generations and the subsequent dramatic population decline (67, 69, 70). This male was likely just an opportunistic, successful migrant from the nearest population. It is unclear whether he carried an exceptional number of deleterious alleles that drove the subsequent decline, or if inbreeding following exceptionally high reproduction of any individual would have led to a similar demographic outcome.

Recovery of some populations from severe bottlenecks, and persistence of some populations despite small N_e and low genetic variation is often cited as a challenge to the idea that low genetic variation and inbreeding reduce population viability (6, 8, 9, 11, 74-77). Soulé (5) [p. 178] pointed out the fundamental flaw of this argument, which he referred to as the "fallacy of the accident" nearly 35 years ago: the only observable populations that have experienced bottlenecks are those that survived. The potentially numerous populations that went extinct under similar conditions are unobservable. Counting extant, genetically depauperate populations is therefore an unreliable metric of the extinction risk posed by lost genetic variation and inbreeding. Theoretical

population genetics and population ecology both predict that some populations will survive bottlenecks, and some lucky ones will persist for long periods at small population size. However, such cases are likely the rare exception, the lottery winners so-to-speak (5, 67).

The most immediate threats to small, genetically depauperate populations are demographic stochasticity and inbreeding depression. However, long term population persistence will in most cases require populations to adapt to environmental change (e.g., climate change, novel diseases, invasive species, etc.) (44, 78). Rapid adaptation to new conditions is possible, but requires sufficient genetic variation and relatively large population size (53, 79). All of the material above highlights the fundamental importance of maintaining large, connected, genetically diverse populations. Long term population viability requires having both manageable ***genetic load*** and adaptive potential associated with genome-wide genetic variation.

4. Simulation-based inferences of the effects of genetic variation and inbreeding on population viability

Simulation-based studies showed long ago that inbreeding depression can substantially increase extinction risk (23, 80). However, our increasing understanding of deleterious mutation parameters (e.g., deleterious mutation rates, and the distribution of fitness effects [DFE]) combined with the availability of sophisticated, user-friendly simulation software (81) will likely advance our understanding of inbreeding depression and purging within the field of conservation.

While there is much to learn about deleterious mutation parameters, a lot is known about the most important elements. First, deleterious mutations arise frequently (15, 16, 82-84), and large effect deleterious alleles appear to be a major driver of inbreeding depression (14, 85-87). For example, lethal alleles arose via mutation at a rate of ~3% per diploid genome in *Drosophila* (14). Inbreeding depression appeared to be largely due to highly deleterious alleles originating in a subset of pedigree founders in sheep and mice (86, 87). Lethal and other large effect deleterious alleles are frequently observed in small natural populations, humans, and model organisms (14, 83, 85, 88-90). The majority of humans and *Drosophila* likely carry one or more recessive lethal alleles (85, 89, 90). Deleterious mutations appeared at a rate of U=1.2 /diploid genome/generation in *Drosophila* (15) and U = 1.6 in hominids (16). Mutation accumulation studies show that the DFE for deleterious mutations is strongly bimodal, with most mutations having small to moderate effects (e.g. $|s|<0.25$) and a minority being lethal or semi-lethal (82).

Second, the degree of dominance (***h***) is strongly related to mutation effect size. Direct observation of dominance effects in yeast and *Drosophila* suggest that nearly neutral deleterious mutations are slightly recessive on average (h slightly less than 0.5), and highly deleterious mutations (e.g., $|s|>0.25$) are nearly fully recessive (h very near zero), with h declining exponentially as s increases in size (14, 91, 92). There is still much uncertainty regarding deleterious mutation parameters (see discussion below). However, the best available information suggests that reasonable values of U are >1, the DFE is strongly bimodal, and dominance declines substantially with increasing size of s. These findings guide the simulations presented above (details in SI).

Recently, results from genetically explicit simulations were used to argue that genome-wide genetic variation is of little importance to population viability, and that purging is likely to prevent extinction (8, 11, 74). However, these studies excluded large effect deleterious mutations (Figure S2) and assumed values of U that were between 2.6 and 92.3 times lower than the best estimate of U in *Drosophila* (Table 1). As a result, these models (8, 11, 74) produce substantially weaker inbreeding depression (<0.05 to approximately 1 lethal equivalent) than observed in real populations, where the median number of lethal equivalents for juvenile survival in captive mammals was 3.1 (93), and 12 for total fitness in wild mammals (23) (Figure S3). There is substantial uncertainty in deleterious mutation rates, and the DFE, particularly for non-model organisms. However, the discrepancy between the assumed mutation parameters and the resulting inbreeding depression in the aforementioned studies (8, 11, 74) and the best available empirical estimates (Table 1, Figure S3), yield results that underestimate the importance of genetic variation in conservation, and the efficacy of genetic rescue as a tool in conservation.

Table 1. Deleterious mutation rates used in previous simulation-based analyses of inbreeding depression and genetic rescue.

Study	Mutation target size	Mutation rate	Proportion deleterious	U*	$U_{Drosophila}/U$**
Teixeira & Huber (11)	1,000 exons	$1x10^{-5}$/exon/generation	0.66	0.013	**92.3**
Robinson et al. (74)	2,000 genes × 1,000bp	$1x10^{-8}$/bp/generation	0.7	0.028	**42.9**
Kyriazis et al. (8)	20,000 genes × 1,500 bp	$1x10^{-8}$/bp/generation	0.77	0.462	**2.6**

*U is calculated as 2 × mutation target size × mutation rate × proportion of mutations that are deleterious. **$U_{Drosophila}$ is the deleterious mutation rate per diploid genome, $U_{Drosophila}$ =1.2 (15).

5. Is the relationship between genetic variation and conservation status informative of the importance of genetic variation to population viability?

It has been suggested that a weak relationship between genetic variation and conservation status (e.g., IUCN Red List) means that genome-wide genetic variation is uninformative of extinction risk (11). However, this relationship is not universally expected, even though extinction risk is strongly affected by genome-wide genetic variation.

First, a lag is expected between reduced population size and the loss of genetic variation. Most threatened populations initially decline due to non-genetic factors (e.g., habitat loss, disease, climate change). Thus, multiple generations are required for a substantial reduction in genetic variation, even after severe bottlenecks (Figure 2A). Threatened populations that became small due to non-genetic factors may still have high genetic variation due to this lag. Second, failing to control for other factors that influence genetic variation (e.g., N_e, dispersal, generation time, and mutation rate (11)) can obscure the relationship between genetic variation and conservation status. In contrast, a study controlling for phylogeny (a proxy for the aforementioned confounding factors) showed a significant relationship between genetic variation and conservation status (94).

Differences among studies in the measures of genetic variation can further obscure true relationships between genetic variation and conservation status. Estimates of genetic variation for different species used in comparative studies vary widely in the number of sampled individuals and populations, and in the regions of the genome analyzed. Some studies estimate species-wide genetic diversity from a single individual (11, 95, 96) and compare different genetic data types across species (6, 96). Using single genomes to estimate species-wide genetic diversity is problematic because the individuals chosen may not be representative of the species as a whole (e.g., captive individuals (95)). Rather, multiple individuals and populations are necessary to accurately reflect a species' distribution of genetic variation (97, 98). Additionally, estimates of genetic diversity are affected by reference genome quality (99), mapping bias (100, 101), the methods used to measure genetic variation (e.g., whole genome sequencing, RNAseq, RADseq), and bioinformatics approaches (98, 99). Thus, sampling, genetic markers, and analyses should be standardized when measuring the relationship between genetic variation and conservation status.

Lastly, IUCN Red List status is an imperfect index of extinction risk because it is a subjective measure of population viability. The IUCN Red List is important for monitoring biodiversity, but the guidelines used to categorize threat levels within the Red List are subject to user interpretation, which can lead to inconsistent assessments (102-106). The imperfect

relationship between IUCN Red List status and extinction risk means that Red List status is an inappropriate surrogate for extinction risk in assessing the relationship between genome-wide diversity and extinction risk. Together these issues suggest that the weak relationship between genetic variation and conservation status has little bearing on the importance of genome-wide genetic variation for extinction risk.

6. What is the role of functional genetic variation in conservation?

The widespread availability of genomic data for non-model organisms has rapidly advanced our understanding of the genetic basis and evolution of fitness-related traits in natural populations, e.g., (107-111). This revolution has raised the question of how to effectively integrate functional genetic information into conservation practice (112-115). It has repeatedly been suggested that genetic assessment and management of threatened populations should be focused on variation at particular loci that affect particular fitness traits (11, 116-118). However, such gene-targeted conservation approaches are always difficult, and prone to failure for several reasons.

First, understanding the genetic basis of fitness remains extremely complicated and challenging (112, 114). While some important traits in natural populations are affected by loci with very large effects, most traits are determined by many small-effect loci (119-121). A comprehensive understanding of the genetic basis of such traits is out of reach for non-model organisms (122). To accurately understand the locus-specific effects on a trait and fitness requires information on dominance and pleiotropy, epistasis, genotype-by-environment interactions, and the amount of linkage disequilibrium with other loci influencing the trait or other fitness components (112). These factors are expected to vary among traits and to differ for the same trait among species and potentially among populations within a species, e.g., (107). Therefore, substantial effort is necessary to understand the conservation relevance of a particular genetic variant and predict whether the benefits of gene-targeted conservation actions outweigh potential detrimental effects (112, 114).

A classic example of the potential for undesirable outcomes of gene-targeted conservation management is the suggestion that genetic management of captive and wild populations should be designed around maintaining genetic variation at the major histocompatibility complex (MHC) (11, 116, 117, 123). The MHC has been studied in great detail in humans because of its importance in immunity, organ transplantation, and autoimmune disease, but its organization is poorly understood in most other vertebrates. Although there is strong evidence for its adaptive

importance, some variants have detrimental effects, and the adaptive effects of other variants appear to be environmentally dependent (124). Detailed examination of the fitness effects of MHC alleles and haplotypes is necessary to determine how much maintaining MHC variation enhances fitness.

Additionally, as highlighted multiple times over the last 35 years (112, 125-129), basing conservation management on a small subset of loci risks increasing the loss of genetic variation elsewhere in the genome. Such efforts would be counterproductive unless the gain in mean fitness associated with gene-targeted management is greater than the loss in fitness associated with lost genome-wide genetic variation (112). This highlights the challenges and pitfalls of gene-targeted conservation. When recommendations for maintaining genome-wide genetic variation versus particular adaptive variants are in conflict, a cost-benefit analysis of the two approaches should be performed and a composite solution identified (112). Recent cases where genomic analyses have revealed that large effect loci play a key role in traits of conservation importance, e.g., (107, 108, 110, 130) will be the first to empirically test the efficacy of gene-targeted conservation approaches.

Discussion

Genomic data should be used to challenge findings from population genetics theory and previous empirical data that form the basis for genetic management of small populations. Recent genomic studies provide useful fodder to determine how to effectively use genomic data to improve conservation in ways that were previously impossible. Examples are emerging of how understanding functional genetic variation could improve recommendations to conserve imperiled populations (107, 108, 110, 130), making genomic data more useful for conservation than ever before. However, genomic data have not discredited the decades worth of evidence that inbreeding depression, mutational meltdown, and loss of adaptive potential are major threats to conservation.

Identifying genetic variants that affect fitness traits undoubtedly advances understanding of the genetic basis of adaptation, and that is important in itself (131). However, placing conservation priority on a small, apparently adaptive portion of the genome ignores what may be the vast majority of variation elsewhere in the genome that will fuel adaptation to unpredictable future conditions (112, 114, 125, 126). This approach is reminiscent of the “adaptationist programme” that Gould & Lewontin (132) criticized >40 years ago for being overly enamored with adaptive explanations for interesting traits (‘spandrels’) without considering that they might have arisen by

447 accident, and that they are but one part of the whole, complex organism (114). Now, as then, we
448 should avoid the temptation to place undue priority on putatively adaptive loci ('molecular
449 spandrels' (133)) without first considering the rest of the genome. Our inability to predict future
450 changes in genotype-by-environment interactions should lead us to recognize the importance of
451 genome-wide genetic variation (including presently neutral variation), and more importantly, the
452 factors that make it possible – large livable habitats and natural patterns of connectivity among
453 them. Conserving genetic variation across the whole genome is almost certainly the most reliable
454 approach to conserve the genetic variation that matters.
455 We know of no convincing evidence that supports abandoning the focus on genome-wide
456 genetic variation in exchange for a focus on functional variation. The recent simulation studies that
457 have been used to discount the importance of genome-wide genetic variation in conservation (8,
458 11, 74) are based on assumptions that are inconsistent with the preponderance of empirical data on
459 the genetics of inbreeding depression and its effect on population viability (see above). Some
460 small populations may not suffer strong inbreeding depression, and some may not rebound
461 following the introduction of genetic variation. However, as pointed out in the formative years of
462 conservation biology, we must resist the temptation to dismiss the extinction risks associated with
463 lost genetic variation in small populations (5).
464 Although population genetics theory has done a remarkably good job of predicting patterns
465 now observable in genomic data, many questions remain unanswered that will improve the utility
466 of genomic data in conservation. For example, how prevalent is soft selection? The presence of
467 soft selection could help explain some of the instances where populations persist for long periods
468 despite inbreeding (59, 60). How much do U and the distribution of fitness effects for deleterious
469 mutations vary among taxa? U may be rather consistent within some taxonomic groups (e.g.,
470 mammals) where the number of genes is strongly conserved (134). Nevertheless, variation among
471 taxa in gene number, mutation rate, and the amount of intergenic DNA that is subject to
472 deleterious mutation is an important consideration for assessing the fitness effects of inbreeding.
473 Lastly, while it is clear that the distribution of mutation fitness effects is bimodal (82),
474 understanding the specific shape of this distribution, and how much this varies among taxa, is
475 important for our understanding of the extinction risks associated with small population size and
476 inbreeding.
477 Genomic data will undoubtedly continue to be used to revisit and refine insights gained
478 since genetics was first applied to conservation and to understand the extinction process (4, 5, 46,

135). So far, genomic data have reinforced earlier findings showing that genome-wide genetic variation is key to population viability. Given the increasing rate of habitat loss and fragmentation, failing to recognize and mitigate the effects of lost genome-wide genetic variation would only exacerbate the biodiversity crisis.

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Data availability

Materials to replicate the simulations are available at https://doi.org/10.5281/zenodo.5513957.

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Glossary:

Adaptive potential: The ability of a population to evolve adaptively in response to selection. Usually measured as narrow sense heritability (the proportion of phenotypic variance attributed to additive genetic effects).

Drift load: The reduction in mean fitness of a population due to homozygosity for deleterious alleles.

F: The individual inbreeding coefficient: the identical-by-descent fraction of an individual's genome.

Genetic load: The reduction in fitness due to all genetic effects arising from both segregating and fixed deleterious alleles.

***Genetic rescue*:** Increase in population growth or reduction in genetic load arising from the immigration of individuals with new alleles.

h: The dominance coefficient. A derived allele is recessive when h=0 (heterozygous genotypes have the same mean fitness as homozygous wildtypes), and dominant when h=1 (heterozygous genotypes have the same mean fitness as homozygous derived allele genotype), and additive when h=0.5 (heterozygous genotypes have fitness midway between the alternative homozygous genotypes).

H: Heterozygous fraction of an individual's genome.

***Hard selection*:** Where an individual's absolute fitness depends only on its phenotype or genotype and is independent of the phenotypes or genotypes of other individuals in the population.

Identical-by-descent: Two segments of DNA are identical-by-descent when they both descend from a single haploid genome in a recent ancestor.

Inbreeding: Mating between relatives.

Inbreeding depression: Reduced fitness of individuals whose parents are related.

Inbreeding load: A measure of the potential for inbreeding to reduce fitness, measured by the number of ***Lethal equivalents***, which is a set of alleles that would on average cause death when homozygous.

Mutational meltdown: Extinction of a population due to the synergistic interactions of population decline, genetic drift, and the accumulation of deleterious alleles.

π: Nucleotide diversity: expected proportion of nucleotide differences between randomly chosen pairs of haploid genomes in a population.

Purging: Increased selective elimination of deleterious, partially recessive alleles that are exposed to purifying selection via inbreeding.

Soft selection: Selection where an individual's fitness depends on its phenotype or genotype relative to others in the same population.

SUPPLEMENTARY INFORMATION

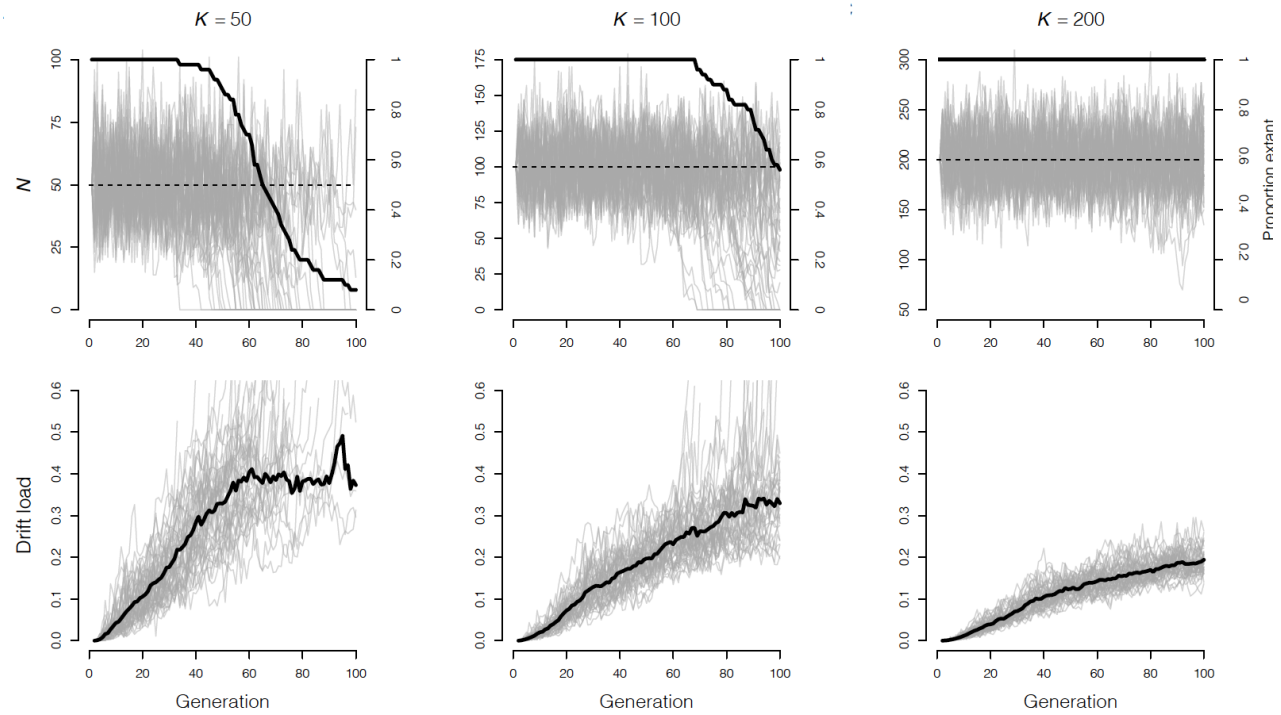

Figure S1. Mutational meltdown via *de novo* mutation in isolated populations. Panels in the top row show population size N (gray lines, left vertical axis), and the proportion of extant populations (thick black line, right vertical axis) for 50 replicate simulations of populations with carrying capacities (K) of 50 (**A**), 100 (**B**), and 200 (**C**). The bottom row shows the drift load for each simulation replicate (gray lines), and the mean across all non-extinct populations (thick black line). These simulations with hard selection have a ratio of effective population size (N_e) to N of approximately 0.25 on average (Figure S5).

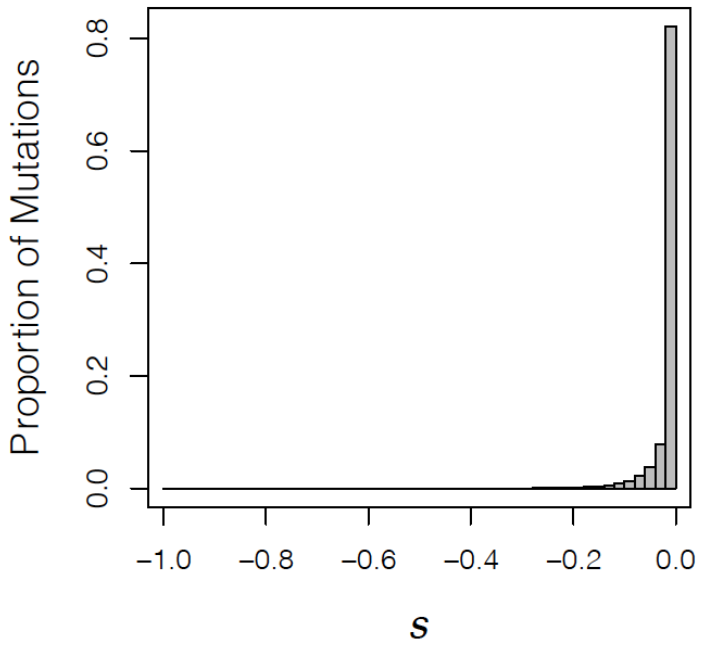

Figure S2. Gamma distribution (shape parameter = 0.186 and scale parameter = 0.071) of fitness effects (*s*) for deleterious mutation assumed in Teixeira and Huber (2021), Robinson et al. 2018, and Kyriazis et al. (2020). Highly deleterious mutations are effectively excluded here (compare to Figure S4 and the results reviewed in Eyre-Walker & Keightley (1)).

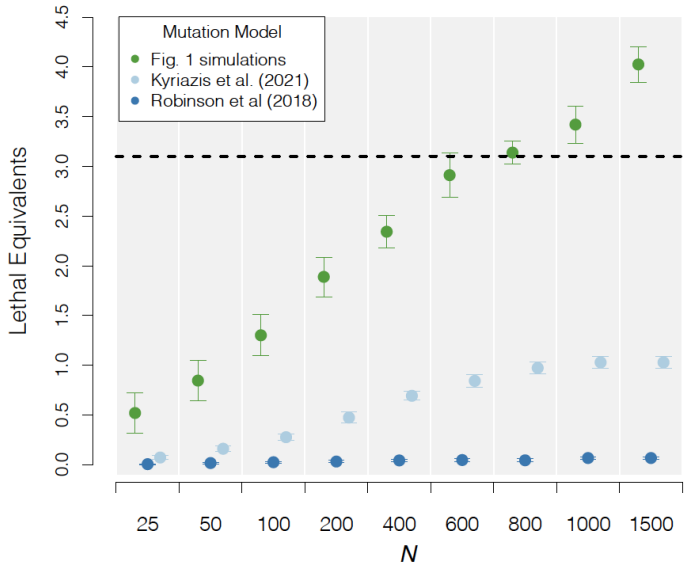

Figure S3. Number of lethal equivalents at approximate mutation-drift-selection equilibrium under the mutation models of Kyriazis et al. (2), Robinson et al. (3), and the simulation model in Figure 1 for constant population sizes ranging from $N_e = 25$ to $N_e = 1{,}500$. The error bars represent the standard deviation across 10 simulation replicates. The dashed line represents the median number of lethal equivalents for juvenile survival for captive mammals from Ralls et al. (4). Note that O'grady et al. (5) estimated an average of 12 lethal equivalents across all fitness components in wild mammals.

Simulations illustrating the relationship between genetic variation and fitness

We use individual-based simulations implemented in R (6-9) to illustrate the relationships among genetic variation, population size, additive genetic variance (V_a), inbreeding load, drift load, and population viability. These are intended to demonstrate patterns that arise directly from population genetics theory under empirically supported combinations of the key parameters. The simulated organism is a self-incompatible hermaphrodite, and has non-overlapping generations, and mean fecundity of 4 (6) when selection was hard (population size is temporally variable), and 2 when selection was soft (population size is temporally constant). Details on the implementation of hard versus soft selection are provided below. Partially recessive deleterious mutations, and mutations that affect the quantitative trait affect fitness by viability selection before breeding when population size is temporally variable (selection is hard), and during the reproduction phase when

population size is constant (selection is soft). The simulations in Figures 1 & 2 in the main text include both partially recessive mutations (as described below), and mutations that affect a quantitative trait (also described below). The simulations shown in Figures 3 (main text), S1, and S3 include partially recessive deleterious mutations, but do not incorporate selection on a quantitative trait.

Simulations with temporally variable population size (Figures 3 & S1) assume a ceiling model of density dependent fitness. Here, when population size is > carrying capacity (K), mean fitness is penalized so that the expected number of offspring forming the next generation is K.

Mutations affecting a quantitative trait under stabilizing selection

Our model for the inheritance of a quantitative trait is from Kardos & Luikart (6). The quantitative trait is assumed to have an optimal phenotype value of $\theta = 0$ (in arbitrary units), a per diploid genome per generation mutation rate of $U_q = 0.147$, with phenotypic effects (a) drawn from a uniform distribution ranging from -0.5 to 0.5, an environmental variance of $V_e = 4$. We assume a Gaussian fitness function:

$$W_{q,i}(z) = W_{\max} e^{-\frac{(z_i-\theta)^2}{2c^2}}, \qquad (1)$$

where $W_{q,i}(z)$ is the expected fitness of individual i with quantitative trait value z_i, c is the standard deviation of the fitness function [set to $c = 6$ as in (6)], z is the individual's phenotype value, and $W_{\max}$ is the expected fitness of an individual with phenotype of $z = \theta$ and no partially recessive deleterious mutations (set to $W_{\max} = 2.5$). $W_{\max}$ is equivalent to the intrinsic population growth rate for a perfectly adapted population with population size very near zero. Smaller values of $W_{\max}$ (e.g., $W_{\max} = 1.5$) resulted in nearly all large populations going to extinction before reaching mutation-drift-equilibrium for lethal equivalents when selection was hard (see below).

Deleterious mutations affecting fitness

Deleterious mutations act directly on individual fitness. We assume a deleterious mutation rate per diploid genome of $U = 1.2$, as observed in *Drosophila* (10), which is substantially lower than in hominids ($U = 1.6$) (11). The location of a mutation is assigned randomly across 38 chromosomes, the number of autosomes in *Canids* (12), each with a 50 cM genetic length. We assume a gamma

distribution of mutation fitness effects (s, the expected reduction in fitness for derived allele homozygotes relative to wild type homozygotes), with shape parameter = 0.5 and scale parameter = 0.1, augmented so that 5% of deleterious mutations are lethal (Figure S4). This distribution mimics the distribution of fitness effects observed in mutation accumulation experiments (1), and is consistent with known contribution of both lethal and small-effect, partially recessive mutations in model organisms, humans, and non-model organisms, e.g., (13-15). We assume an exponential model of the relationship between dominance (h) and s as $h = 0.5e^{-13s}$, which closely mimics experimental results in model organisms (16, 17), where mutations with s very near 0 are generally nearly additive ($h \approx 0.5$), and mutations with s near -1 (lethals) are essentially completely recessive ($h \approx 0$, Figure S4). Using the higher deleterious mutation rate of hominids would result in an even larger gap between the resulting fitness effects of inbreeding here compared to Teixeira & Huber (18), Robinson et al. (3), and Kyriazis et al. (2) (Figure S3).

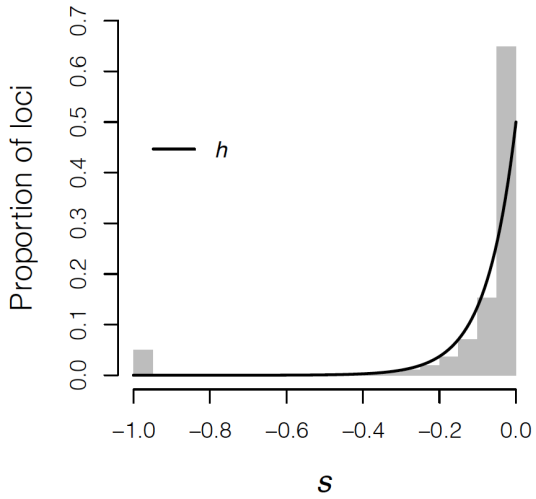

Figure S4. The distribution of selection coefficients (s) for deleterious mutations in our simulations. The black line shows the dominance coefficient h as a function of s.

The fitness reduction arising from partially recessive deleterious mutations for individual i is calculated as

$$\Delta W_i = \sum_{j=1}^{n} \eta_{i,j} \begin{cases} h_j s_j & \text{if } \eta_{i,j}=1 \\ s_j & \text{if } \eta_{i,j}=0 \text{ or } 2 \end{cases}, \qquad (2)$$

where $\eta_{i,j}$ is the count of the derived deleterious allele at the jth of the n loci where there has been a deleterious mutation in individual i. h_j and s_j are the dominance and selection coefficients, respectively, at locus j. Subtracting ΔW_i from $W_{q,i}(z)$ (eq. 1) yields the expected fitness of individual i given the fitness effects of the quantitative trait and partially recessive deleterious mutations.

Hard versus soft selection

Some of our simulations force population size to be constant (Figures 1, 2, S3) to simplify our analyses of the effects of population size on the parameters of interest. Constant population size implies that selection on the phenotype and arising from deleterious mutations was soft. Here, the mean fecundity is by definition 2, such that the population growth rate is exactly $\lambda = 1$, and the expected fitness of an individual with a particular genotype depends on the genotypes of others in the population (19). Selection in these cases is implemented during the reproduction phase.

Our other simulations allowed population size to fluctuate through time (Figures 3 and S1) to illustrate genetic effects on population viability. When population size is allowed to fluctuate through time, selection is hard, where an individual's fitness depends only on its genotype, and population fitness (population growth rate) depends on the collection of genotypes of all the individuals in the population (19). Here, selection is imposed via selection on juvenile survival before the breeding phase.

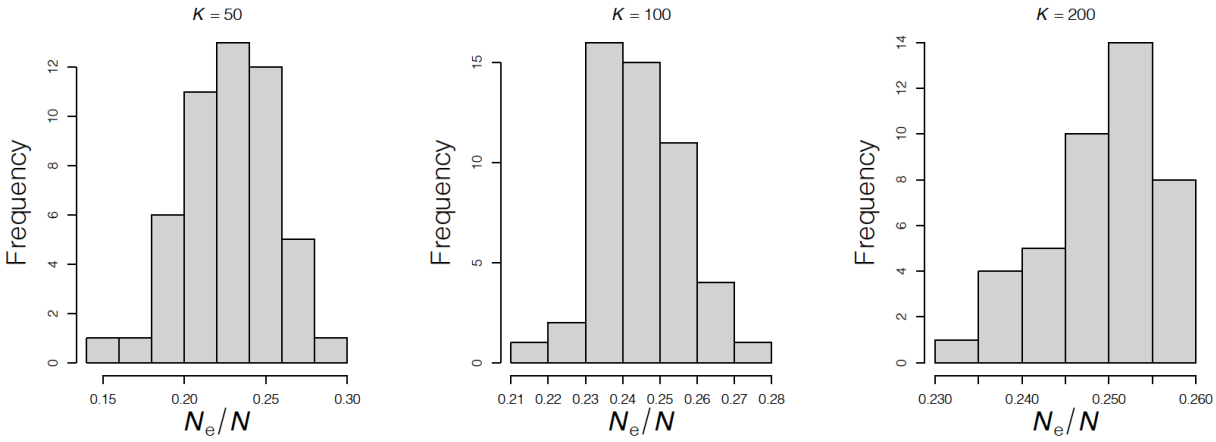

Figure S5. Distributions of the ratio of effective population size (N_e) to census population size (N) in simulations from Figure S1. N_e was calculated as $N_e = (1/\overline{\Delta F})/2$, where $\overline{\Delta F}$ is the mean per generation change in the pedigree inbreeding coefficient in the population over the first 50 generations of the simulation.

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