What can genome sequence data reveal about population viability?

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ABSTRACT

Biologists have long sought to understand the impacts of deleterious genetic variation on fitness and population viability. However, our understanding of these effects in the wild is incomplete, in part due to the rarity of sufficient genetic and demographic data needed to measure their impact. The genomics revolution is promising a potential solution by predicting the fitness effects of deleterious genetic variants (genetic load) bioinformatically from genome sequences alone, bypassing the need for costly demographic data. After a historical perspective on the theoretical and empirical basis of our understanding of the dynamics and fitness effects of deleterious genetic variation, we evaluate the potential for these new genomic measures of genetic load to predict population viability. We argue that current genomic analyses alone cannot reliably predict the effects of deleterious genetic variation on population growth, because these depend on demographic, ecological, and genetic parameters that need more than just genome sequence data to be measured. Thus, while purely genomic analyses of genetic load promise to improve our understanding of the composition of the genetic load, they are currently of little use for evaluating population viability. Demographic data and ecological context remain crucial to our understanding of the consequences of deleterious genetic variation for population fitness. However, when combined with such demographic and ecological data, genomic information can offer important insights into genetic variation and inbreeding that are crucial for conservation decision making.

1 | INTRODUCTION

The fitness effects of deleterious mutations have long been a central theme in evolutionary (Haldane, 1937; Wright, 1922; Wright, 1931) and conservation biology (Frankel & Soulé, 1981; Ralls & Ballou, 1982; Shaffer, 1981), and remain key to our growing understanding of the drivers of variation in individual fitness and population viability (Armstrong et al., 2021; Bozzuto, Biebach, Muff, Ives, & Keller, 2019; Huisman, Kruuk, Ellis, Clutton-Brock, & Pemberton, 2016; Kardos et al., 2023; Stoffel, Johnston, Pilkington, & Pemberton, 2021; Whiteley, Fitzpatrick, Funk, & Tallmon, 2015). Until recently, this body of work focused mainly on model organisms, captive populations, and a few intensively monitored wild populations where fitness can be measured directly (Bonnet et al., 2022). Therefore, predicted effects of deleterious mutations on the viability of natural populations are largely based on theory (Awad,

Gallina, Bonamy, & Billiard, 2014; Lande, 1994, 1998; Lynch, Conery, & Burger, 1995; Tanaka, 2000; Theodorou & Couvet, 2006; Wright, 1931), extrapolation from laboratory experiments (Bijlsma, Bundgaard, & Boerema, 2000; Frankham, 1995; Wright, 1922) and intensively-studied wild populations (Armstrong et al., 2021; Bozzuto et al., 2019; Dileo, Nair, Kardos, Husby, & Saastamoinen, 2024; Hedrick, Robinson, Peterson, & Vucetich, 2019; Kardos et al., 2023; Saccheri et al., 1998). The genomics revolution has inspired researchers to explore how genome sequence data can add to our understanding of the effects of deleterious genetic variation on the viability of wild populations where detailed demographic data are difficult to collect and rarely available (Bertorelle et al., 2022; van Oosterhout, 2020).

Here, we evaluate whether purely genomic analyses of deleterious genetic variation are likely to substantively advance our understanding of the effects of this genetic variation on population viability. Because current progress builds on past developments, we begin with an historical perspective on the theoretical and empirical basis of our understanding of the dynamics and fitness effects of deleterious genetic variation. We then discuss what genome sequence data alone, and genomics-informed simulation models, can reveal about the dynamics and fitness impact of deleterious genetic variation. We finish by arguing that, while intuitively appealing, purely genomic measures of genetic load combined with simulation models are currently insufficient to reliably predict population fitness. Field-based demographic studies remain key to our understanding of the influence of deleterious genetic variation on population viability.

2 | HISTORICAL PERSPECTIVE ON DELETERIOUS GENETIC VARIATION

Early studies of *Drosophila* (Morgan, 1915; Muller, 1930) revealed that most mutations were deleterious, and that the more detrimental mutations tended to be more recessive (Nei, 2013; Wright, 1922). These deleterious mutations generally reduced the fitness of individuals and the viability of populations in controlled experiments with inbred strains of maize (East & Jones, 1919; Shull, 1908), rats (King, 1918), guinea pigs (Wright, 1922), and livestock (McPhee, Russel, & Zeller, 1931). Strong concomitant selection could, however, counteract some of these detrimental effects (Castle, Carpenter, Clark, Mast, & Barrows, 1906; King, 1918), highlighting that both mutation and selection determine the dynamics of deleterious genetic variation.

Thus, by the late 1920s, there was ample evidence that all populations experienced a constant influx of deleterious mutations and ongoing selection against them. This motivated Fisher (1930) and Wright (1931) to explore theoretically how recurring mutations and selection would affect allele frequencies. While Fisher (1930) envisioned populations of 'many millions or thousands of millions' (p. 84), S. Wright (1931) had small livestock populations in mind and thus explored the effects of small population size. He concluded (p. 142) that deleterious mutations would cause two '*distinct degeneration processes*' in small and isolated populations: a rapid one involving inbreeding and a slow one involving the '*accumulation of injurious genes*'. Thus, Wright had already realized by 1931 that recurrent mutations would reduce fitness in different ways and that population size mediated these effects via genetic drift.

Predicting the magnitude of mutation-induced fitness reduction, however, remained elusive, in part because some of the crucial parameters – the strength of selection against a homozygous mutation (the selection coefficient, s) and the degree of dominance (h, the dominance coefficient reflecting the fitness of heterozygotes) – were difficult to measure empirically except in rare circumstances. Haldane (1937), and later Crow (1948) and Muller (1950) provided a partial breakthrough. Haldane (1937) showed that expected mean fitness was $\bar{W} = 1 - 2q(1-q)hs - q^2 s$, where q is the deleterious allele frequency and $1, 1-hs,$ and $1-s$ are the fitness of wildtype homozygotes, heterozygotes, and mutant homozygotes, respectively. This simplifies to $\bar{W} = 1 - 2qhs$ when we assume that q is <<1, which in turn assumes that genetic drift is sufficiently weak to allow selection to keep deleterious alleles at a low frequency. This further simplifies to $\bar{W} = 1 - 2\mu$ (where μ is the deleterious mutation rate per locus per generation) when deleterious alleles are removed by selection at the same rate as mutations produce them (i.e., under mutation-selection equilibrium). Muller (1950) derived similar formulae, which he applied to actual situations in *Drosophila* and humans, and he coined the term 'genetic load' for the reduction in average fitness at mutation-selection equilibrium. Thus, Haldane (1937) and Muller (1950) showed that, under restrictive assumptions, we only need to know the mutation rate to predict the effects of deleterious mutations on average fitness, whether the effects of an individual mutation (the size of s) are large or small (Crow, 1970).

Unfortunately, few real populations satisfy the assumptions of the models of Haldane (1937) and Muller (1950). First, the assumption that deleterious allele frequencies are always small ($q << 1$) does not apply to small populations where genetic drift is too strong for selection

to consistently prevent deleterious alleles from rising to high frequency. Secondly, the assumption of mutation-selection balance is violated in where population size changes through time. Such populations are often far from equilibrium, where predictions based on equilibrium assumptions no longer hold (Gravel, 2016; Spigler, Theodorou, & Chang, 2017). Finally, the deleterious mutation rate is difficult to estimate accurately except in rare circumstances. These limitations effectively restrict reliable application of the Haldane (1937) and Muller (1950) models to a small number of model organisms. As a consequence, there still aren't enough empirical data to test how well Haldane's and Muller's equations predict fitness (Agrawal & Whitlock, 2012).

Fortunately, Morton et al. (1956) discovered a way around some of these limitations by showing that the effects of deleterious mutations on mean fitness could be estimated via analysis of the reduction in fitness associated with increasing inbreeding. With data on individual fitness and inbreeding coefficients (F, the homozygous and identical-by-descent proportion of an individual's genome) (Wright, 1951) in hand, the cumulative effects of mutations could be estimated with a weighted linear regression relating the logarithm of fitness (e.g., survival probability, S) to the inbreeding coefficient F: $-\log(S) = A + BF$ (Morton et al., 1956; Nietlisbach, Muff, Reid, Whitlock, & Keller, 2019). In this model $A = \sum x + \sum q^2 s + 2\sum q(1-q)sh$, where x is the reduction in fitness due to an environmental factor, and $B = \sum qs - \sum q^2 s - 2\sum q(1-q)sh$. The summations are over all x's and all loci carrying deleterious alleles. Note that A, the y-intercept in the linear regression, is the expected reduction in fitness due to the summed effects of all environmental and genetic factors affecting fitness in the absence of inbreeding. The genetic part of A (the second and third terms) is equivalent to Haldane's model for genetic load. B, the slope in the regression model, is the expected reduction in fitness associated with complete inbreeding ($F = 1$), and is therefore commonly known as the inbreeding load (Charlesworth & Charlesworth, 1987).

The great advancement of the Morton et al. (1956) model was that the A and B parameters could be estimated empirically without identifying the underlying mutations or knowing the associated values of μ, s, or h. Morton et al. (1956) proposed $\sum qs$ as a useful measure of the total mutational damage per gamete. It is measured in units of lethal equivalents and corresponds to the reduction in fitness of a zygote formed by doubling the chromosomes of the gamete (thus F=1), and equals B plus the genetic part of A, with B representing the lower and

$B + A$ the upper bound of this quantity. Gravel (2016) showed that variants of $\sum qs$ are also useful measures of deleterious genetic effects in non-equilibrium situations. Because it is difficult to separate the genetic from the environmental component of A, it has become common practice to use B as a lower bound estimate of the total effects of mutations on fitness (Charlesworth & Charlesworth, 1987).

In the decades that followed, the Morton et al. (1956) approach has allowed estimation of the impact of deleterious mutations on fitness in numerous species and the exploration of many fundamental questions in evolutionary (Crow, 1993; Keller & Waller, 2002; Lewontin, 1974) and conservation biology (Ralls, Ballou, & Templeton, 1988; Ralls, Brugger, & Ballou, 1979). The main findings of these studies have been comprehensively summarized (Agrawal & Whitlock, 2012; Charlesworth & Charlesworth, 1987; Crnokrak & Roff, 1999; Crow, 1958, 1970; Crow, 1993; Hedrick & Kalinowski, 2000; Keller & Waller, 2002; Lewontin, 1974; Plough, 2016; Wallace, 1970, 1987). A central result that emerged was that the pattern of deleterious genetic variation was very different in large and small populations (Hedrick & García-Dorado, 2016; Kimura, Maruyama, & Crow, 1963; Willi et al., 2022), thus confirming Wright's (1931) insight that deleterious mutations affect fitness through different processes depending on population size. In large populations, there tends to be a large B due to numerous partially recessive deleterious alleles segregating at low frequencies. In small populations, on the other hand, B is reduced because inbreeding occurs more often and partially recessive detrimental alleles are therefore expressed more frequently in homozygous state. This exposes them to selection and small populations thus tend to be purged of part of the inbreeding load (Hedrick, 1994; Hedrick & García-Dorado, 2016; López-Cortegano, Moreno, & García-Dorado, 2021). Concurrently, however, mildly deleterious mutations can drift by chance to substantially higher frequencies or even fixation, enriching another type of genetic load known as 'drift load' (reduced fitness associated with the continual fixation of mildly deleterious alleles) (Whitlock, 2000). Because mildly deleterious alleles are far more common than the more severely deleterious mutations that are most readily purged (Crow, 1993), drift load can be orders of magnitude higher than inbreeding load in small populations (Kardos et al., 2021; Kimura et al., 1963). Many theoretical (Bataillon & Kirkpatrick, 2000; Charlesworth, 2018; Glémin, 2003; Kirkpatrick & Jarne, 2000; Lynch, Conery, & Burger, 1995) and empirical studies (Lohr & Haag, 2015; Mattila et al., 2012; Puurtinen, Knott, Suonpää, Ooik, & Kaitala, 2004; Willi,

Griffin, & van Buskirk, 2013) have since elaborated on these results and have explored the complex ways that demography impacts inbreeding and drift loads.

The range of species that contributed to these studies, however, was still restricted to study systems for which fitness data and information about inbreeding could be obtained. This is in part because data on fitness and *F* are difficult to collect in free-living organisms. Thus, the effects of deleterious genetic variation on individual fitness, and especially on population dynamics, have seldom been measured in wild populations. Additionally, the small size and difficulty of sampling many populations of conservation concern can severely limit the sample size available to measure the fitness effects of deleterious genetic variation. Therefore, the statistical power and precision are often quite low in studies of inbreeding depression in populations of conservation concern (Chapman, Nakagawa, Coltman, Slate, & Sheldon, 2009; Ford et al., 2018). The impact of deleterious mutations on population viability has therefore continued to be debated (Caro & Laurenson, 1994; Caughley, 1994; Creel, 2006; Hedrick, Lacy, Allendorf, & Soulé, 1996; Jamieson, 2007; Wootton & Pfister, 2015).

One of the major limitations – the difficulty of measuring *F* in the wild – has been partly solved by the rapidly increasing availability of genomic data (genotypic information at many thousands to millions of loci) beginning around 2007 (https://www.genome.gov/about-genomics/fact-sheets/Sequencing-Human-Genome-cost). Genomic measures of *F* are more precise than traditional pedigree- or genetic marker-based approaches (Ceballos, Joshi, Clark, Ramsay, & Wilson, 2018; Kardos, Luikart, & Allendorf, 2015; Keller, Visscher, & Goddard, 2011; Knief et al., 2015) and enable analyses of inbreeding depression in populations without extensive pedigrees. Several studies have since used genomic estimates of *F* along with demographic data to measure inbreeding depression in wild populations (Armstrong et al., 2021; Duntsch et al., 2023; Harrisson et al., 2019; Hoelzel et al., 2024; Hoffman et al., 2014; Huisman et al., 2016; Kardos et al., 2023; Niskanen et al., 2020; Stoffel et al., 2021) and some evaluated the effects of observed inbreeding depression on population dynamics (Armstrong et al., 2021; Kardos et al., 2023). Unfortunately, demographic analyses of inbreeding depression in the wild are still rare due to the high cost of genome sequencing and the difficulty of measuring fitness in the wild.

Purely genomic analyses of inbreeding and genetic load, and genomics-informed, evolutionary-demographic simulation models have been proposed as means to overcome these limitations (Bertorelle et al., 2022; Kyriazis, Robinson, & Lohmueller, 2023; Robinson, Kyriazis, Yuan, & Lohmueller, 2023; Robinson, 2023; van Oosterhout, 2020). Genome sequences are particularly appealing for the estimation of genetic load because they are thought to allow evaluation of the effects of deleterious genetic variation in a species' natural environment (Koufopanou, Lomas, Tsai, & Burt, 2015) without requiring the costly demographic data needed to directly measure fitness (Bertorelle et al., 2022). The premise of this approach is that we should be able to predict fitness of an individual or a population, based solely on a sample of genome sequences, if we can identify deleterious alleles, quantify their frequencies, and know enough about the associated values of h and the distribution of s (distribution of fitness effects, DFE). Being able to reliably predict fitness without having to directly measure demographic vital rates would provide a '*step-change*' in conservation (van Oosterhout, 2020) because it would enable evaluating the extinction risk of any population where genomic data are accessible.

Inspired by the potential for genomic data to reveal the fitness consequences of deleterious alleles, numerous studies have already used genome sequences to evaluate the dynamics and fitness effects of deleterious genetic variation in populations of conservation concern (Beichman et al., 2022; Bertorelle et al., 2022; Dussex et al., 2021; Grossen, Guillaume, Keller, & Croll, 2020; Hoffman et al., 2024; Kardos et al., 2023; Khan et al., 2021; Kyriazis, Wayne, & Lohmueller, 2021; Mathur & DeWoody, 2021; Mathur, Tomeček, Tarango-Arámbula, Perez, & DeWoody, 2023; Robinson, Brown, Kim, Lohmueller, & Wayne, 2018; Robinson et al., 2022; Smeds & Ellegren, 2023; Smeds, Huson, & Ellegren, 2024; Wilder et al., 2024; Xue et al., 2015). Additionally, several studies have used genomic estimates of parameters that determine genetic load (e.g., historical N_e, DFE, and the deleterious mutation rate) to parameterize evolutionary-demographic simulation models in order to predict effects of deleterious genetic variation on population dynamics and viability (Beichman et al., 2022; Dussex, 2024; Kyriazis, Beichman, et al., 2023; Kyriazis et al., 2021; Nigenda-Morales et al., 2023; Robinson et al., 2022; Wilder et al., 2024). For example, Robinson et al. (2022) inferred from simulations and genomic analyses of genetic load that the recovery of the highly endangered vaquita porpoise is unlikely to be limited by inbreeding depression. Moreover, genomic analyses of genetic load in ancient DNA from extinct species have been used to test

whether deleterious genetic variation might have contributed to the demise of populations that went extinct long ago (Dehasque et al., 2024; Rogers & Slatkin, 2017). Dehasque et al. (2024) concluded from temporal genomic analyses of genetic load in Wrangel Island wooly mammoths that deleterious genetic variation was unlikely to have contributed to the extinction of the population. The central objective of many such studies is to evaluate the impact of deleterious mutations on population dynamics and viability. It is therefore crucial to critically evaluate whether purely genomic analyses of genetic load and associated simulations are likely to be informative of population growth and viability.

How do deleterious mutations impact population viability in the wild? Theoretical (Lande, 1994; Lynch, Conery, & Burger, 1995; Wright, 1931) and experimental results (Bowman & Falconer, 1960; East & Jones, 1919; Frankham, 1995; Franklin, 1980; King, 1918; Lacy, Alaks, & Walsh, 1996; Leberg, 1990; McPhee et al., 1931; Meagher, Penn, & Potts, 2000; Shull, 1908; Soule, 1980; Wright, 1922) implied that inbreeding and drift load could limit population growth and increase the risk of extinction for inbred populations. Empirical results from wild populations have largely been consistent with this prediction. For example, wild populations with higher heterozygosity (lower inbreeding) have shown higher population growth (Bozzuto et al., 2019) and lower extinction probability (Saccheri et al., 1998) than those with lower genetic diversity. Inbreeding depression appears to have limited population growth in some (Armstrong et al., 2021; Kardos et al., 2023), but not all small natural populations (Johnson, Mills, Wehausen, Stephenson, & Luikart, 2011) where inbreeding depression has been measured. Masking the recessive fitness effects of deleterious alleles by outcrossing (i.e., 'genetic rescue', Box 1) nearly always reverses declines of small and isolated populations with low genetic variation and high inbreeding (Frankham, 2015; Whiteley et al., 2015), suggesting that some combination of fixed and segregating deleterious alleles had limited recovery of these populations. Maintenance of genetic variation and population connectivity and avoidance of inbreeding therefore remain crucial considerations in conservation (DeWoody, Harder, Mathur, & Willoughby, 2021; Frankham, 2005; Kardos et al., 2021; Soulé, 1987).

3 | ARE GENOMIC MEASURES OF GENETIC LOAD INFORMATIVE OF POPULATION VIABILITY?

3.1 | *Demography is the crux of extinction*

Predicting the impact of any factor on population viability requires understanding how strongly that factor influences population growth (Crouse, Crowder, & Caswell, 1987; Mills, 2012; Reed et al., 2002). Extinction is an inherently demographic process that requires demographic perspective and analyses to understand (Lande, 1988). The effects of deleterious genetic variation on population dynamics can be measured using demographic data by: (1) evaluating the relationship between population growth rate or extinction probability and population-based measures of inbreeding (Bozzuto et al., 2019; Saccheri et al., 1998), (2) measuring changes in population dynamics upon outcrossing (i.e., genetic rescue; Box 1) (Åkesson et al., 2016; Hogg, Forbes, Steele, & Luikart, 2006; Johnson et al., 2010; Madsen, Shine, Olsson, & Wittzell, 1999; Westemeier et al., 1998; Whiteley et al., 2015), or (3) estimating the relationship between vital rates (age- and sex-specific survival and reproduction) and *F* and then modelling the estimated effects on population growth using matrix models or individual-based simulations (Armstrong et al., 2021; Domingue & Teale, 2007; Johnson et al., 2011; Kardos et al., 2023). The fundamental advantage of these demographic approaches is that the fitness effects of deleterious genetic variation are measured directly.

Purely genomic studies of the demographic consequences of deleterious genetic variation essentially bypass the need for demographic data on wild populations (Beichman et al., 2022; Bertorelle et al., 2022; Kyriazis, Beichman, et al., 2023; Robinson et al., 2022). Predicting the demographic effects of deleterious genetic variants via genomic analysis is inherently a difficult task because the fitness effects and their interactions with extrinsic ecological factors cannot be measured directly from sequence data. A crucial question is whether genomic measures of genetic load can provide useful measures of population viability when the demographic effects are not measured. Below, we outline several reasons why current genomic measures of genetic load are unlikely to be informative of population dynamics and suggest future work to evaluate the efficacy of and improve these approaches.

3.2 | *Purely genomic methods are unlikely to reliably predict the impact of deleterious genetic variation on fitness*

First, genomic measures of genetic load do not themselves quantify effects of putatively deleterious alleles on fitness. Instead, methods to identify deleterious genetic variants (Adzhubei, Jordan, & Sunyaev, 2013; De Baets et al., 2012; McLaren et al., 2016; Wang, Li, & Hakonarson, 2010) usually classify putatively deleterious alleles by effects on protein structure (e.g., loss-of-function, missense, synonymous, intergenic, etc.) or degree of evolutionary constraint (Cooper et al., 2005). Mutations that appear to be more strongly conserved or to more substantially disrupt protein function are assumed to have larger fitness effects. On average, qualitative predictions of fitness effects of protein variants are likely to have some validity (Ralls, Sunnucks, Lacy, & Frankham, 2020). For example, putatively deleterious alleles had lower average frequencies than putatively neutral alleles in some studies e.g., (Grossen et al., 2020; Khan et al., 2021), suggesting that current methods are at least somewhat successful at identifying loci subjected to purifying selection. Additionally, 15% of manually curated loss-of-function mutations, for which none of the sequenced individuals were homozygous, turned out to be embryonic lethals in Belgian beef and New Zealand dairy cattle (Charlier et al., 2016). However, it remains unclear just how reliably genomic methods distinguish deleterious mutations from beneficial and neutral ones. Loss-of-function mutations are expected to be deleterious on average, yet they have shown a wide range of fitness effects (Karczewski et al., 2020), including beneficial rather than detrimental effects (Monroe et al., 2018; Xu & Guo, 2020). Additionally, predicted loss-of-function mutations are enriched for false positives compared to more benign mutations due to annotation errors and other technical artefacts (Karczewski et al., 2020), a problem that is likely exacerbated in species of conservation concern that lack high quality genome assemblies and annotations.

Furthermore, the relative contribution to genetic load of mutations in coding versus non-coding genomic regions remains unclear. While some genomic analyses of putatively deleterious genetic variation focus on coding portions of the genome, e.g., (Kardos et al., 2023), non-coding mutations must also be important because the great majority of trait-associated (Hindorff et al., 2009; Ibeagha-Awemu, Peters, Akwanji, Imumorin, & Zhao, 2016) and functionally constrained loci in vertebrates reside in non-coding regions (Lindblad-Toh et al., 2005; Meader, Ponting, & Lunter, 2010; Mouse Genome Sequencing Consortium, 2002; Rands, Meader, Ponting, &

Lunter, 2014; Rat Genome Sequencing Project Consortium, 2004). Similarly, it has proven difficult to empirically demonstrate substantial fitness consequences of mutations in ultra-conserved genomic elements (Snetkova, Pennacchio, Visel, & Dickel, 2022), a part of the genome that is thought to be under strong selection and of particular relevance for conservation (van Oosterhout, 2020). Thus, how well genomic approaches succeed at classifying mutations of different severity, and whether they can be translated into improved predictions of fitness and better conservation outcomes remains an open question (Speak et al., 2024).

A fundamental limitation of genomic methods that classify putatively deleterious alleles is that they do not explicitly measure either *s* or *h*, both of which are needed to translate the detection of putatively deleterious alleles into predictions of fitness. A potential solution is to use population genetic methods to estimate the DFE for deleterious alleles (Eyre-Walker & Keightley, 2007; Kim, Huber, & Lohmueller, 2017). This can be done by finding a distribution of *s* that is most consistent with observed levels of presumably neutral (synonymous) versus deleterious (nonsynonymous) genetic variation conditioned on an inferred demographic history (Kim et al., 2017; Robinson et al., 2022). However, population genetic methods are known to underestimate the number of strongly deleterious alleles because such alleles tend to have very low frequencies, resulting in downwardly biased estimates of the average *s* and proportion of deleterious alleles that are lethal or nearly so (Eyre-Walker & Keightley, 2007). Therefore, models parameterized with sequence-based estimates of the DFE are likely to lead to downwardly biased predictions of the cumulative fitness effects of deleterious alleles.

The utility of population genetic methods to estimate the DFE are also limited by being purely retrospective. The relevant population genetic patterns are the result of both recent and deep historical selection which are likely to differ from contemporary and future selection in rapidly changing environments. The cumulative fitness effects of deleterious genetic variation depend to varying degrees on environmental and ecological conditions (Dileo et al., 2024; Keller, 1998; Keller, Grant, Grant, & Petren, 2002; Meagher et al., 2000; Pemberton, Ellis, Pilkington, & Berenos, 2017) and are therefore expected to fluctuate through time and space. Genomic analyses of genetic load say nothing about the sensitivity of selection to environmental conditions. Applying biased measures of *s* and assuming that average past selection pressures hold in current and future environments is likely to result in a misleading understanding of the relevance of deleterious genetic variation to population dynamics.

An additional challenge of current genomic measures of genetic load is that they assume that loci affect fitness independently (Bertorelle et al., 2022) and in the same way under different environmental conditions. Experimental results from model organisms suggest that the fitness effects of *de novo* mutations can depend strongly on gene-by-gene-by-environment interactions. For example, particular mutations tended to confer increased fitness in *Plasmodium falciparum* and *Saccharomyces cerevisiae* genomic backgrounds that had low fitness in a particular environment, and to confer reduced fitness in environments where the genomic background had high fitness (Ardell, Martsul, Johnson, & Kryazhimskiy, 2024; Diaz-Colunga, Sanchez, & Ogbunugafor, 2023). Additionally, there is mounting evidence that the strong associations among loci that develop in small populations due to genetic drift (Ohta & Kimura, 1970) could lead to deleterious mutations being linked in repulsion and thus to the expression of pseudo-overdominance (Abu-Awad & Waller, 2023; Toczydlowski & Waller, 2023; Waller, 2021). Pseudo-overdominance, like overdominance at a single locus, leads to heterozygotes having higher fitness than either homozygote, which acts to oppose purging and maintain segregating deleterious genetic variation and inbreeding depression. For example, two closely linked deleterious recessive alleles that occur on different haplotypes can result in net heterozygous advantage and favor the maintenance of deleterious genetic variation at both loci (Waller, 2021). Empirical evidence from *Drosophila* (Latter, 1998) and simulations (Abu-Awad & Waller, 2023) suggests that pseudo-overdominance could partly explain the persistence of inbreeding depression in persistently small inbred populations (Toczydlowski & Waller, 2023). Genomic analyses of humans identified 22 genomic regions where pseudo-overdominance seems to maintain haplotypes with complimentary deleterious alleles (Gilbert, Pouyet, Excoffier, & Peischl, 2020). Thus, incorporating multi-locus perspective and gene-by-gene-by-environment effects will be necessary to improve the predictions of fitness effects of deleterious mutations and of the efficacy of purging.

While relative fitness is often all that matters if one wants to understand changes in allele frequencies, predicting genetic effects on population dynamics requires an understanding of effects on absolute fitness. Unfortunately, current genomic measures of genetic load contain no information about the expected absolute fitness of any individual. Translating genotypes at loci carrying putatively deleterious alleles into predictions of absolute fitness requires knowing the expected absolute fitness of either unloaded individuals or of individuals with known genetic

loads (e.g., level of inbreeding) in the same environment (Agrawal & Whitlock, 2012; Kardos et al., 2023), in addition to the mutation parameters discussed above. The ubiquity of mutation means that unloaded individuals do not exist, and demographic data and analyses are required to measure the relevant parameters (Morton et al., 1956). The unknown realized fitness effects of putatively deleterious alleles identified in sequence data combined with biases associated with population genetic methods to estimate the DFE mean that we currently have little understanding of how genomic measures of genetic load are related to individual fitness and population growth under contemporary ecological conditions.

3.3 | *Genomic measures of genetic load do not differentiate hard versus soft selection*

Another reason why genomic measures of genetic load alone are unlikely to be informative about population dynamics is that they do not reveal whether selection is hard vs. soft (Bell, Kovach, Robinson, Whiteley, & Reed, 2021; Wallace, 1975). Hard selection occurs when selective deaths or reproductive failures are additive (i.e., natural selection determines how many individuals survive or reproduce). Hard selection is therefore expected to affect population growth. On the other hand, under soft selection, selective deaths and reproductive failures are compensatory and thus determine *which*, not how many, individuals survive and reproduce, which has little or no direct effect on population growth rate. For example, under soft selection, individuals with lower genetic load are more likely to survive or reproduce than individuals with higher genetic load (Haldane, 1957) without a direct impact on population dynamics (Wallace, 1970).

Several lines of evidence suggest that hard selection is common in small populations (Frankham, 2015; Saccheri & Hanski, 2006). First, for evolution by natural selection to work, selection needs to be hard at least some of the time when populations compete. As (Crow, 1993) (p. 4) put it: "*Evolution by natural selection could hardly work at all if intra- and intergroup fitnesses were not positively correlated*". Second, classical laboratory experiments of inbreeding in guinea pigs and mice (Bowman & Falconer, 1960; Wright, 1922) showed that a significant proportion of inbred lines go extinct. Despite being carried out under benign laboratory conditions, selection in these experimental populations must have been at least partly hard, since it affected population size and increased extinction. Additionally, the near universal increase in population size following outcrossing in highly inbred and declining populations (Frankham, 2015; Whiteley et al., 2015) suggests that selection is often hard in small and declining

populations (Box 1). However, hard selection does not appear to be ubiquitous. Observations of strong inbreeding depression in populations that have persisted for a long time at small population size (Hoffman et al., 2014; Huisman et al., 2016; Stoffel et al., 2021) and of an uncoupling between individual fitness losses and population growth in response to climate change (Reed et al. 2013) suggest that soft selection is common in resource-limited natural populations.

The form of selection can change from hard to soft and *vice versa* through time with changing ecological conditions (Bell et al., 2021), and the presence of density dependence does not necessarily imply that selection is entirely soft (Agrawal & Whitlock, 2012). Thus, over time and spatial scales relevant for conservation, the hardness of selection may be quite variable depending on a number of factors, including environmental conditions (Bozzuto et al., 2019; Dileo et al., 2024). As a result, genetic load need not directly translate into effects on population size and contemporary extinction risk, as noted repeatedly for over 60 years (Agrawal & Whitlock, 2012; Brues, 1969; Clarke, 1973; Gravel, 2016; Haldane, 1957; Wallace, 1975, 1987, 1991). And as Kojima (1970) remarked, we should have ecological concepts ringing in our ears when thinking of genetic load and its consequences.

While it appears that selection must be at least somewhat hard on average, predicting exactly when selection will be hard and how hard it will be remains challenging (Keller, Biebach, & Hoeck, 2007). Such a predictive ability is crucial to be able to determine how strongly genetic load affects population viability. Purely genomic measures of genetic load are uninformative of the ecological details (Agrawal & Whitlock, 2012; John Burdon Sanderson Haldane, 1957) that determine the degree to which selection involving deleterious genetic variation is hard vs. soft. In the meantime, we are well advised to assume that selection will be partly hard in populations pushed beyond their natural conditions through anthropogenic environmental change (Saccheri & Hanski, 2006). Consequently, avoiding substantial loss of genetic variation and increase in inbreeding continues to be a crucial to conservation.

3.4 | *Purely genomic analyses do not reveal which vital rates are affected by deleterious alleles*

Genomic metrics of genetic load are unlikely to be informative of population dynamics because they contain no information on the affected vital rates (age- and sex-specific survival and reproduction). One of the foundational insights of population ecology is that different vital rates can have drastically different effects on population growth rate (Caswell, 2000; Cole, 1954; Crouse et al., 1987; Mills, 2012). For example, a given proportional reduction in adult survival often has a very different effect on population growth compared to the same reduction in juvenile survival (Box 2). Therefore, understanding the impact of deleterious genetic variation on population dynamics requires knowing which and to what extent different vital rates are affected (Box 2). The vital rates depressed by inbreeding appear to vary substantially across populations (Nietlisbach et al., 2019). For example, several studies of wild populations have found strong inbreeding depression for juvenile survival, and decreasing impacts of inbreeding on survival later in life e.g., (Armstrong et al., 2021; Huisman et al., 2016; Stoffel et al., 2021), while others have found that inbreeding affects fitness in later life stages (Johnson et al., 2011) or across the whole lifespan (Kardos et al., 2023). Some populations appear to experience substantial inbreeding depression for reproductive success in both sexes (Huisman et al., 2016; Niskanen et al., 2020), but others show sex-specific effects (Keller, 1998) or no detectable effects at all on breeding success (Kardos et al., 2023). It is likely that the vital rates depressed by deleterious genetic variation vary among populations and through time due to temporal and spatial variation in environmental conditions (e.g., intra- and inter-specific competition, climatic variation), and also depending on the DFE of segregating deleterious genetic variation (Husband & Schemske, 1996). Current genomic measures of genetic load provide little or no information on which vital rates are affected by deleterious genetic variants; they therefore do not capture the demographic details that determine the effects of deleterious genetic variation on population dynamics.

3.5 | *Purging and long-term persistence do not mean fitness effects of deleterious genetic variation are unimportant*

Evidence for long term small N_e and purging of deleterious alleles appears to be common in genomic studies of small populations e.g., (Dehasque et al., 2024; Grossen et al., 2020; Kardos et al., 2023; Khan et al., 2021; Kleinman-Ruiz et al., 2022; Mathur & DeWoody, 2021; Nigenda-Morales et al., 2023; Robinson et al., 2018; Robinson et al., 2022; Xue et al., 2015). Such results

have on occasion been interpreted as suggesting that purging has largely eliminated the threat imposed by deleterious genetic variation on population viability (Dehasque et al., 2024; Nigenda-Morales et al., 2023; Robinson et al., 2018; Robinson et al., 2022). We argue that this view is inconsistent with both empirical data and the central insight of population genetics outlined in the historical perspective above: small population size may lead to purging of strongly deleterious alleles and reduced inbreeding depression, but simultaneously leads to reduced fitness via the accumulation of high frequency and fixed mildly deleterious alleles (drift load) via genetic drift (Frankham, 2015; Hedrick & García-Dorado, 2016; Lande, 1995; Lynch, Conery, & Burger, 1995; Lynch, Conery, & Bürger, 1995; Whiteley et al., 2015). The frequent extinction of inbred lines and increase in fitness upon outcrossing in small, isolated populations further highlight that purging (which is expected in all small populations) does not reliably prevent fitness decline.

Furthermore, purging is unlikely to completely eliminate threats associated with inbreeding depression (Charlesworth & Willis, 2009; Toczydlowski & Waller, 2023). Demographic analyses frequently reveal inbreeding depression in populations with demographic history and genomic characteristics that are conducive to efficient purging. Substantial inbreeding depression occurs in haplodiploid species where purging should be especially efficient due to the expression of recessive, deleterious alleles in haploid males every generation (Henter, 2003). Additionally, inbreeding depression is commonly detected in populations with small historical N_e (i.e., in the tens or hundreds) for hundreds or thousands of generations e.g., (Kardos et al., 2023; Stoffel et al., 2021), and following a severe population bottleneck (Hoelzel et al., 2024). For example, the Southern Resident killer whales showed genomic signatures of both purging and small N_e recently (i.e., N_e in the tens for ~30 generations) and in deeper history (N_e in the 100's ~30-400 generations ago, and a deep historical N_e of ~5,000) (Foote et al., 2021; Kardos et al., 2023). Despite a history of quite small N_e, Southern Resident killer whales showed sufficiently strong inbreeding depression in survival to substantially reduce the population growth rate and viability (Kardos et al., 2023). Likewise, Soay sheep that have been isolated on small islands for thousands of years displayed substantial inbreeding depression for survival (Stoffel et al., 2021). We believe that genomic measures of genetic load and purging combined with analyses of historical N_e are crucial to our growing understanding of the long-term dynamics of different components of genetic load. However, current genomic metrics of genetic load are

by themselves insufficient to predict absolute fitness. Empirical demographic data and analyses are needed to directly evaluate the effects of deleterious genetic variation on absolute individual fitness and population viability (Allendorf, Ryman, & Kardos, 2023).

3.6 | *Relevance of simulation-based population viability analyses parameterized via genomic analysis*

Demographic simulations have played a crucial role in understanding the factors that affect population viability since the inception of conservation biology (Beier, Vaughan, Conroy, & Quigley, 2006; Beissinger & Westphal, 1998; Brook et al., 2000; Crouse et al., 1987; Reed et al., 2002; Shaffer, 1981, 1983). Stochastic simulations were crucial in showing that levels of inbreeding depression observed in model systems could influence extinction risk if they occur in wild populations (Brook, Tonkyn, O'Grady, & Frankham, 2002; Mills & Smouse, 1994; O'Grady et al., 2006). However, quantitative predictions of extinction risk based on simulation models are error-prone due to the limited amount and low quality of demographic data available in most study systems (Beissinger & Westphal, 1998). Recent advancements in genomics and simulation software are providing new opportunities, as well as substantial challenges.

Increasingly sophisticated and user-friendly simulation software (Guillaume & Rougemont, 2006; Haller & Messer, 2022) has enabled complex, genetically-explicit, individual-based, stochastic simulations of the effects of deleterious genetic variation and many other factors on population dynamics. Simulations can now accommodate nearly limitless combinations of historical N_e, deleterious mutation rate, DFE, life history, and genomic complexity of specific study populations or species (Dussex, 2024; Kardos et al., 2023; Kyriazis, Robinson, & Lohmueller, 2022; Kyriazis et al., 2021; Robinson, Kyriazis, Yuan, & Lohmueller, 2022; Robinson et al., 2022). Genomic estimates of the DFE, h, and U (the deleterious mutation rate per haploid genome) along with sequence-based estimates of historical demography are often used to parameterize individual-based simulations for population viability analysis (Beichman et al., 2022; Kyriazis, Beichman, et al., 2023; Kyriazis et al., 2024; Robinson et al., 2023; Robinson et al., 2022). This approach adds several parameters that are difficult to estimate to the traditional population viability analysis approaches that were already error-prone due to poorly parameterized demographic variables (Beissinger & Westphal, 1998). First, the inability to determine from genomic data which vital rates are affected by deleterious genetic variation

means that one usually has to assume which vital rates are affected by deleterious genetic variation. If the assumptions are wrong, wildly inaccurate predictions of population dynamics can result (Box 2), even if all of the other parameters are estimated accurately. Genomic estimates of the mutation parameters used to parameterize simulations are also highly error prone (as described above), which means that the modeled effects of deleterious genetic variation are likely to be far from the realized effects in real populations. Focusing population viability analyses on the wrong parts of the parameter space for mutation characteristics (Ralls et al., 2020), affected vital rates (Box 2), and importance of ecological factors (Beissinger & Westphal, 1998; Crouse et al., 1987; Lacy, 2000; Mills, 2012) can lead to misleading results that hinder conservation efforts. Without empirical measures of these effects, what may seem like reasonable assumptions for genetic effects on fitness are likely to result in erroneous predictions of imminent decline, demographic stability, or growth.

4 | CONCLUSIONS AND FUTURE DIRECTIONS

The limitations described above lead us to conclude that current genomic measures of genetic load are unlikely to materially improve our ability to measure population viability. This raises the question of how genomic measures of genetic load might still be useful for advancing conservation. One way molecular predictions of deleterious alleles could advance conservation is by improving our ability to predict *relative* fitness of individuals in a given environment. For example, translocating genetically variable individuals with relatively few putatively deleterious alleles into small inbred populations may result in more successful genetic rescue (increased future population growth) than translocation of individuals carrying more putatively deleterious alleles (Bertorelle et al., 2022; Khan et al., 2021; Christopher C Kyriazis et al., 2021; Whiteley et al., 2015). Captive breeding programs might maximize the fitness of offspring by selecting parents that share the fewest putatively deleterious alleles (Speak et al., 2024). Additionally, predictions of deleterious alleles might help to identify the loci underlying recessive phenotypes associated with reduced fitness (Bertorelle et al., 2022; Charlier et al., 2016; Dobrynin et al., 2015; Marty Kardos, Taylor, Ellegren, Luikart, & Allendorf, 2016), potentially enabling genomics-assisted selection against deleterious alleles in heavily managed captive (Moen et al., 2015) and wild populations (Ralls, Ballou, Rideout, & Frankham, 2000). In species of conservation concern where (semi-)lethal alleles are found to segregate and cause serious fitness

585 loss (Laikre, 1999; Ralls et al., 2000; Trask et al., 2016) such genomics-assisted selection may
586 offer ways to reduce the frequency of the disease-causing alleles without an associated severe
587 reduction in N_e. The likelihood of success of any of these conservation applications depends on
588 the extent to which predictions of deleterious genetic variation can be validated empirically and
589 improved in the future.
590 To advance conservation, genomic measures of genetic load would need to provide *more*
591 *information* about relative fitness than more traditional metrics such as genomic measures of *F*
592 and demographic history which have long been considered as important predictors of individual
593 fitness (Frankel & Soulé, 1981; Lukas F. Keller & Waller, 2002; Lynch, Conery, & Burger,
594 1995). This would require that predictions of deleterious alleles are generally accurate, and that
595 measures of genetic load based on predicted deleterious alleles are better predictors of fitness
596 than other genomic metrics (e.g., individual inbreeding). The accuracy of molecular predictions
597 could be tested by evaluating whether predicted highly deleterious alleles (e.g., mutations that
598 cause loss of gene function or occur in highly conserved genomic regions) coincide with loci
599 known to carry strongly deleterious alleles. For example, do molecular methods regularly predict
600 strongly deleterious alleles in genomic regions known to contain embryonic lethal or semi-lethal
601 alleles (Ralls et al., 2000; Trask et al., 2016), or in genomic regions where strongly deleterious
602 recessive fitness effects have been identified via association mapping (Stoffel et al., 2021)?
603 Additionally, whole-genome sequences combined with fitness data from long term studies of
604 wild populations (Clutton-Brock & Sheldon, 2010; J. M. Pemberton, Kruuk, & Clutton-Brock,
605 2022) could be used to test whether fitness is more strongly correlated with genomic measures of
606 genetic load than with genomic measures of *F* (Allendorf et al., 2023). Large sample sizes will
607 likely be required to obtain sufficient statistical power because genomic metrics of an
608 individual's genetic load (e.g., the number of homozygous, putatively deleterious alleles) and *F*
609 are expected to be highly correlated (M. Kardos et al., 2023). If this is generally true across
610 multiple study systems, then it would support to the idea that molecular measures of genetic load
611 can improve predictions of the relative fitness of individuals in a given population.
612 Genomic measures of genetic load are likely to benefit from improved genome
613 annotations and by accounting for potentially strong effects of structural genetic variants. The
614 quality of genome annotations in non-model species has not kept pace with the rapidly increasing
615 efficiency of genome-sequencing. For example, genes in non-model species are usually

identified by homology with known protein coding regions in model species, and by gene-predicting computational methods (Birney, Clamp, & Durbin, 2004; Kapustin, Souvorov, Tatusova, & Lipman, 2008; Kent, 2002). More accurate and contiguous reference genomes, and expanded use of long-read RNA sequencing of many different tissues in several individuals to identify transcribed genes, will likely improve the annotations of genes and identification of putatively deleterious alleles in non-model species (Kurylo, Guyomar, Foissac, & Djebali, 2023). Automated tools to discover putatively deleterious alleles will no doubt improve in the future, but deep manual curation will likely remain essential for some time to come. Such manual curation is widespread in livestock and human applications (Charlier et al., 2016; Singer-Berk et al., 2023) but less common in conservation applications. Most genomic analyses of deleterious genetic variation in wild populations have so far been limited to considering the effects of single nucleotide polymorphisms in coding and highly conserved genomic regions. Quantifying the contribution of structural genetic variants (e.g., inversions and insertion-deletions) might substantially improve future genomic estimates of genetic load (Fang & Edwards, 2024; Smeds et al., 2024).

Simulation models remain crucial for identifying the major drivers of population dynamics in threatened populations. However, these models are only as good as the data used to parameterize them (Beissinger & Westphal, 1998). The usefulness of purely genomic measures of genetic load to parameterize simulations is severely limited by their inability to reveal the strength, form, temporal variability, and environmental dependence of fitness effects, or which vital rates are involved. Some influential pre-genomics demographic simulation studies of inbreeding depression suffered from a similar limitation: the magnitude of inbreeding depression and affected vital rates were often extrapolated from other populations or even different species (Barry W Brook et al., 2002; L. Scott Mills & Smouse, 1994; O'Grady et al., 2006). Simulation-based assessments of population viability should be interpreted with extreme care in cases where demographic measures of these effects are unavailable. Models parameterized with output from purely genomic analyses of genetic load, or based on demographic data from other study populations are essentially what-if scenarios, and the results derived from such models should be interpreted accordingly as being speculative. Additionally, making assumptions about the strength of inbreeding depression and which vital rates are affected (Dussex, 2024; P. S. Miller, 2024; Williams et al., 2024) should especially be avoided when empirical estimates of these

effects are available in the same study populations (Åkesson et al., 2016; Bensch et al., 2006; M. Kardos et al., 2023; Liberg et al., 2005).

The issues outlined here highlight the continuing crucial role of field work to collect detailed, individual-level data on survival and reproduction. Such data, paired with high-quality genomic information will best advance our understanding of the demographic consequences of deleterious genetic variation. Unfortunately, such demographic data are rare for populations of conservation concern, and the availability of detailed demographic data does not ensure that the factors limiting recovery of threatened populations can be identified. For example, small sample sizes typical of the studies of threatened populations means that statistical power is often quite low to identify the environmental and genetic factors that influence fitness and population growth. Additionally, the factors limiting population growth can change through time, such that a conclusive finding regarding the demographic effects of deleterious genetic variation over one period of time may not hold in the future.

We argue that in light of the theoretical and empirical insights on genetic load of the last hundred years, data on population trend, environmental conditions, and genetic variation (e.g., genomic measures of heterozygosity and inbreeding) are the most important pieces of information regarding whether deleterious genetic variation is likely impacting population dynamics. Specifically, inbreeding depression and the accumulation of drift load should be leading hypotheses for the lack of recovery of populations with low genetic variation in environments that appear to be sufficient to support population growth. This view is supported by the strong evidence that infusion of genetic variation via translocation nearly universally increases population growth in such situations (Frankham, 2015; Whiteley et al., 2015) (Box 1). Interpreting purely genomic estimates of genetic load without considering the ecological and genetic complexities outlined above is likely to result in spurious inferences about the factors that drive population dynamics in threatened populations, which can mislead conservation decision making.

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Box 1: Genetic rescue

Genetic rescue is a reduction in extinction probability of small, inbred populations caused by gene flow (Bell et al., 2019). It is best quantified as an increase in population growth by more than can be attributed to the demographic contribution of immigrants (Ingvarsson, 2001). The primary mechanism of genetic rescue is typically assumed to be a reduction in genetic load due to the masking of deleterious alleles, but it can also be caused by the introduction of additive genetic variation on which selection can act, thereby reducing maladaptation in small populations with strong genetic drift (Bell et al., 2019; Whiteley et al., 2015). Genetic rescue can be mediated by people, or it can occur naturally, e.g., when an organism disperses from one population to another of its own accord. Genetic rescue is related to, but distinct from, other similar terms. Evolutionary rescue is an adaptation-dependent reversal of population decline due to maladaptation to novel environmental conditions (Carlson, Cunningham, & Westley, 2014; Gonzalez, Ronce, Ferriere, & Hochberg, 2013). Assisted gene flow is the managed movement of individuals or gametes between populations within a species' range to facilitate adaptation to changing environments (Aitken & Whitlock, 2013).

Increasing evidence demonstrates that genetic rescue works in the vast majority of cases. Some of the best examples of genetic rescue come from conservation management efforts to increase population growth rates of small, imperiled populations. The introduction of 20 male adders (*Vipera berus*) to an isolated population in Sweden suffering from severe inbreeding depression resulted in a dramatic demographic recovery (Madsen et al., 1999). Other examples of favorable population-level fitness responses to human-mediated immigration include Florida panthers (*Puma concolor coryi*) (Johnson et al., 2010) and Rocky Mountain bighorn sheep (*Ovis canadensis*) (Hogg et al., 2006; J. M. Miller, Poissant, Hogg, & Coltman, 2012). Controlled experiments in copepods (*Tigriopus californicus*) (Hwang, Northrup, Alexander, Vo, & Edmands, 2011), plants (*Rutidosis leptorrhynchoides*) (Pickup, Field, Rowell, & Young, 2013), flour beetles (*Tribolium castaneum*) (Hufbauer et al., 2015), Trinidadian guppies (*Poecilia reticulata*) (Fitzpatrick et al., 2020), and many other species also show positive effects of immigration on absolute fitness. In a literature review of studies that have rigorously tested for absolute fitness effects (on population size or growth rate) of migration across generations, the vast majority (14/18; 78%) showed either positive (n = 10) or a mix of positive and no absolute fitness effects (n = 4) (Whiteley et al., 2015).

The observation that genetic rescue attempts usually successfully increase population size or growth rates suggests that many target populations are small due at least in part to deleterious genetic effects. It also suggests that selection against inbred individuals is not entirely soft, as discussed in the main text.

Box 2: Demographic impacts of inbreeding depression involving different vital rates

Selection involving different vital rates can have very different effects on population growth rate depending on life history strategy. To demonstrate this effect, we measured the effects of reducing stage-specific annual survival using information from age-specific life tables (Tables S1-S6) for six species representing a wide range of life histories: great tit (*Parus major*) (Bouwhuis, Charmantier, Verhulst, & Sheldon, 2010; Bouwhuis, Choquet, Sheldon, & Verhulst, 2012), Dall sheep (*Ovis dalli*) (Simmons, Bayer, & Sinkey, 1984), killer whale (*Orcinus orca*) (Olesiuk, Bigg, & Ellis, 1990), grizzly bear (*Ursus arctos horribilis*) (Harris, White, Schwartz, & Haroldson, 2007; Schwartz, Haroldson, & White, 2006), hoop pine (*Araucaria cunninghami*), and the copepod *Mesochra lilljeborgi* (Waples, Luikart, Faulkner, & Tallmon, 2013). For each species, we calculated the expected finite rate of population growth (λ) (Supplementary Methods) from the unaltered lifetables. To measure the sensitivity of λ to variation in survival at different life stages, we calculated λ after reducing the annual survival rate of either juveniles or adults by half. Figure 1 shows that λ was most strongly impacted by juvenile survival in the great tit, killer whale, and copepod. However, λ for Dall sheep, grizzly bear, and hoop pine was most strongly impacted by adult survival. These results show that knowing which life stage is most affected by selection is crucial for determining the effects of selection on population growth (Crouse et al., 1987).

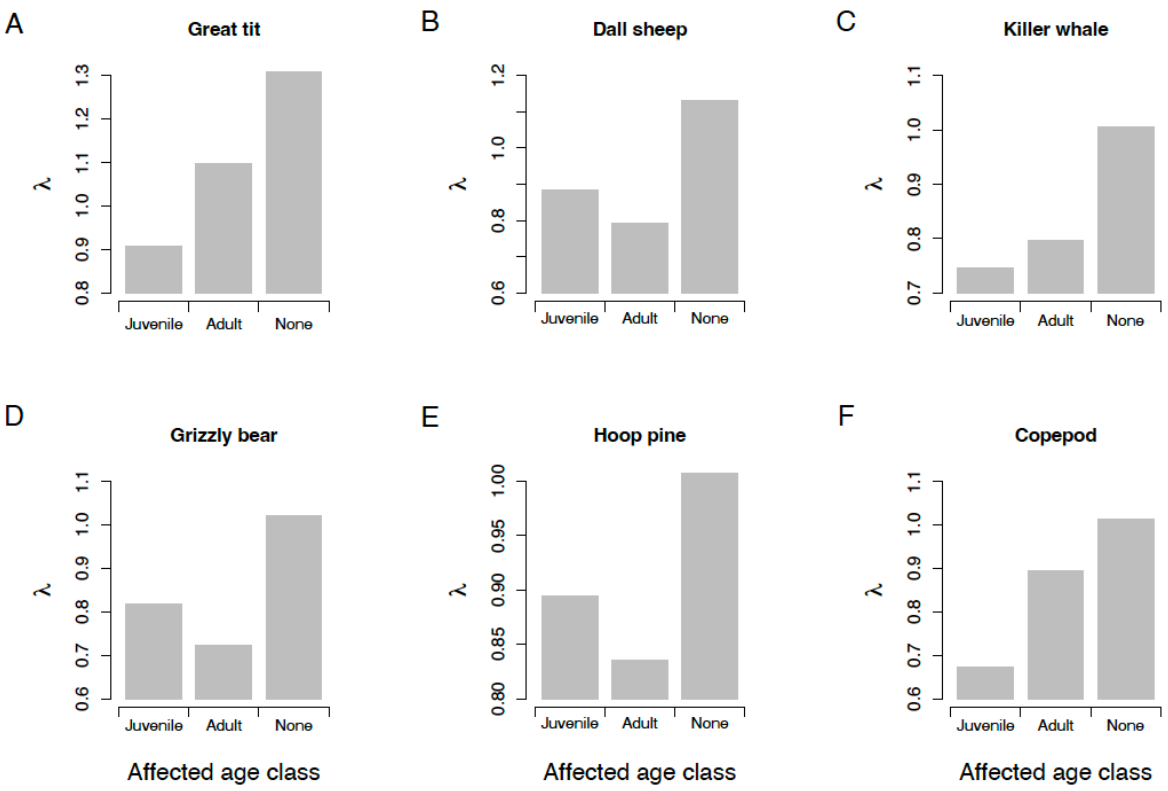

Figure 1. Effects of stage-specific survival on population growth rate (λ) in great tit, Dall sheep, killer whale, grizzly bear, hoop pine, and copepod.

What are the implications of these differences in the sensitivity of the population growth rate to variation in survival at different life stages? And what does it mean for the impact of inbreeding depression on population viability? To answer this question, we applied the individual-based simulation model of Kardos et al. (2023) to two species with very different life histories: the great tit (short lifespan, high fecundity) and Dall sheep (long lifespan, low fecundity) (Figure 2).

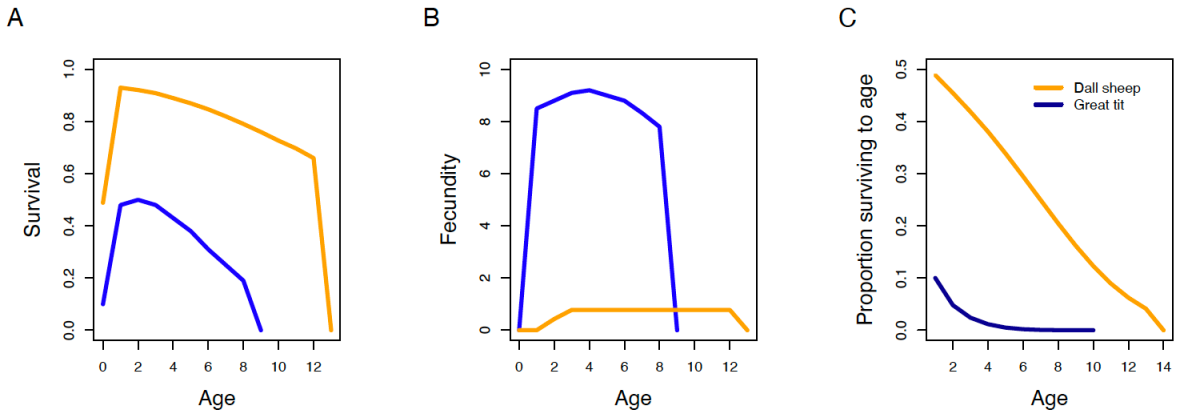

Figure 2. Age-specific survival, fecundity, and proportion of individuals surviving to age in great tit and Dall sheep.

For each species we modeled a single closed population with carrying capacity $K = 100$ and initial population size of 50 individuals sampled from a stable age distribution (Supplementary Methods) in year 0. We modeled inbreeding depression for annual juvenile survival, adult survival, or for both juvenile and adult annual survival. We assumed an inbreeding load of $B = 3$ for great tit, and $B = 1$ for Dall sheep given the much lower λ for Dall sheep compared to great tit (Figure 1). Simulation details are in the Supplementary Materials. We projected each simulated population forward through time for 50 years or until extinction, and repeated this 300 times for each species and combination of affected vital rates. The results are shown in Figure 3. Consistent with the sensitivity analysis (Figure 1), great tit population growth was more strongly reduced by inbreeding depression on juvenile than adult survival. Populations with inbreeding depression affecting only adult survival grew initially (on average) while those with inbreeding depression for juvenile survival typically declined rapidly (Figure 3). Also consistent with the sensitivity analysis, Dall sheep populations were most affected by inbreeding depression for adult survival: those with inbreeding depression for juvenile survival declined slowly on average over 50 years, but the same strength of inbreeding depression for adult survival resulted in population decline and more than 50% of simulated population going extinct by year 35. Additionally, the strongest impact of inbreeding depression on population growth was observed in both species when it affected both juvenile and adult survival. These simulation results demonstrate that assuming inbreeding depression acts on a particular vital

rate(s) without a strong empirical justification can result in wildly misleading predictions of the relative and absolute impact of deleterious genetic variation on population growth and viability.

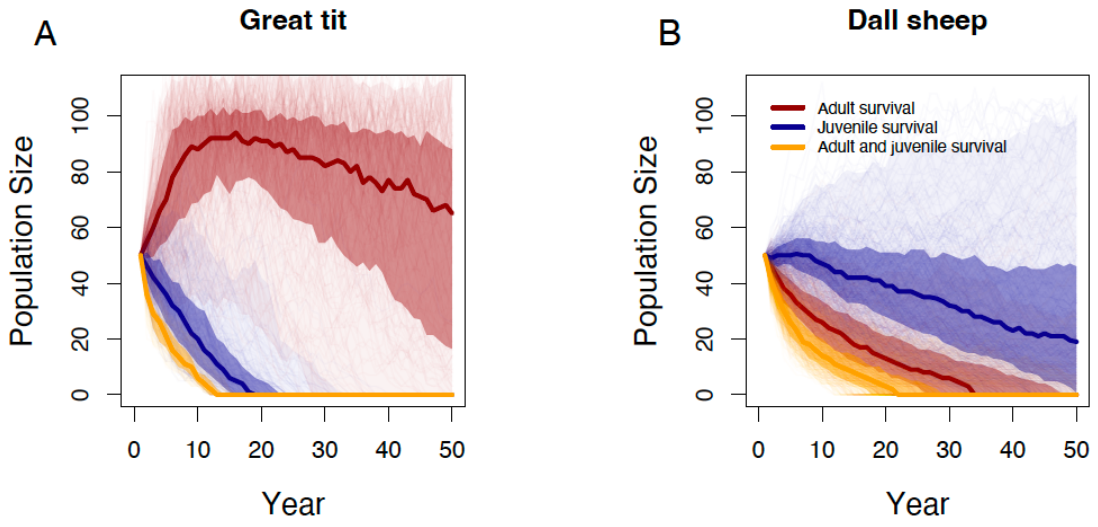

Figure 3. Effects of inbreeding depression for different vital rates on great tit (A) and Dall sheep (B) population growth. Population size is shown through time for simulations with inbreeding depression (3 lethal equivalents in great tit and 1 lethal equivalent in Dall sheep) affecting juvenile (blue), adult (red), and both juvenile and adult annual survival (orange). Results are shown for 300 replicate simulations for each species and combination of vital rates.