SYNTHESIS

Using Computational Simulations to Model Deleterious Variation and Genetic Load in Natural Populations

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ABSTRACT: Deleterious genetic variation is abundant in wild populations, and understanding the ecological and conservation implications of such variation is an area of active research. Genomic methods are increasingly used to quantify the impacts of deleterious variation in natural populations; however, these approaches remain limited by an inability to accurately predict the selective and dominance effects of mutations. Computational simulations of deleterious variation offer a complementary tool that can help overcome these limitations, although such approaches have yet to be widely employed. In this perspective article, we aim to encourage ecological and conservation genomics researchers to adopt greater use of computational simulations to aid in deepening our understanding of deleterious variation in natural populations. We first provide an overview of the components of a simulation of deleterious variation, describing the key parameters involved in such models. Next, we discuss several approaches for validating simulation models. Finally, we compare and validate several recently proposed deleterious mutation models, demonstrating that models based on estimates of selection parameters from experimental systems are biased toward highly deleterious mutations. We describe a new model that is supported by multiple orthogonal lines of evidence and provide example scripts for implementing this model (https://github.com/ckyriazis/simulations_review).

Keywords: computational simulations, deleterious mutations, distribution of fitness effects, genetic load, inbreeding depression, population genetics.

Introduction

New mutations are constantly entering a population, some fraction of which are deleterious to organismal fitness (Eyre-Walker and Keightley 2007; Keightley 2012). The burden of deleterious variation carried by a population is referred to as its "genetic load," defined as the reduction in fitness due to segregating and fixed deleterious mutations (Muller 1950; Agrawal and Whitlock 2012; Hedrick and Garcia-Dorado 2016). Genomic methods are now commonly used to characterize deleterious variation in wild populations (Kardos et al. 2016; Díez-del-Molino et al. 2018; Bertorelle et al. 2022), although the best approaches for leveraging such datasets to estimate genetic load remains an active area of research. In particular, empirical measures of putatively deleterious variation have seen increased use in conservation genomics studies (Bertorelle et al. 2022); however, these measures remain relatively crude and are often challenging to interpret (Cooper and Shendure 2011; She and Jarosz 2018; Huber et al. 2020; Sandell and Sharp 2022).

In light of the limitations of empirical measures of deleterious variation and genetic load, the aim of this review is to encourage more ecological and conservation genomics researchers to employ computational genetic simulations. To that end, we first provide an overview of simulations of deleterious genetic variation, discussing the key parameters involved and how such methods can be used to model genetic load. Next, we discuss several approaches for validating these models, either by comparing predicted and observed patterns of genetic variation or by comparing the predicted inbreeding load to an estimate for a given species. Using this validation approach for three recently proposed deleterious variation models, we then demonstrate that models based on experimental estimates of selection parameters are biased toward highly deleterious mutations. Finally, we propose a new model that is supported by several orthogonal sources of evidence and conclude with a discussion of future directions in the field. Our hope is that this

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review will provide useful information for researchers aiming to incorporate simulation-based approaches into genomic studies of load, enabling more comprehensive assessments of the ecological and conservation relevance of deleterious genetic variation.

Defining Genetic Load

Understanding the implications of genetic load for organismal fitness and population viability is a topic of longstanding interest in population and conservation genetics (Haldane 1937; Muller 1950; Morton et al. 1956; Kimura et al. 1963; Agrawal and Whitlock 2012; Henn et al. 2015; Hedrick and Garcia-Dorado 2016). Several definitions of genetic load have been put forth in the literature recently, often with the aim of partitioning genetic load into "realized" and "potential" load (e.g., Mathur and DeWoody 2021; Bertorelle et al. 2022). Here, we adhere to the definition of genetic load as the realized reduction in mean fitness in a population due to segregating and fixed deleterious mutations (note that "mutation load" typically refers only to mutations segregating under mutation-selection balance; Muller 1950; Agrawal and Whitlock 2012). The genetic load of a population at a single locus i (L_i) is given by

$$L_i = 2hsq(1 - q) + sq^2, \tag{1}$$

where s is the selection coefficient of a mutation, h is the dominance coefficient, and q is the mutation frequency (Agrawal and Whitlock 2012). Here, we parameterize fitness assuming that homozygous mutant individuals have a fitness of $1 - s$ and heterozygous individuals have fitness $1 - hs$, such that s is positive for deleterious mutations. In equation (1), the effect of deleterious mutations found as heterozygotes is captured by the $2hsq(1 - q)$ term, and the effect of homozygous deleterious mutations is captured by the sq^2 term. For fixed mutations ($q = 1.0$), the genetic load is therefore equal to s. Genetic load at a single locus can be related to mean population fitness at locus i ($\bar{W}_i$) as $\bar{W}_i = 1 - L_i$. When assuming that fitness is multiplicative across sites (i.e., ignoring epistasis and linkage disequilibrium), the mean genome-wide genetic load of a population can be obtained by multiplying $\bar{W}_i$ across sites, such that $L_{\text{total}} = 1 - \prod \bar{W}_i$. Thus, the units of genetic load are in terms of multiplicative fitness scaled from 0 to 1.

Another important quantity for understanding the impacts of deleterious variation is the inbreeding load, which quantifies the rate at which fitness is lost under increasing levels of inbreeding (Morton et al. 1956; Hedrick and Garcia-Dorado 2016). Unlike the genetic load, the inbreeding load is measured in terms of lethal equivalents, which represent a summed quantity of s for recessive deleterious mutations that are masked as heterozygotes. For a randomly mating population, the inbreeding load at a single locus i (B_i) is given by (Morton et al. 1956)

$$B_i = sq - sq^2 - 2hsq(1 - q) = sq - L_i. \tag{2}$$

This equation demonstrates that the inbreeding load at a single locus is determined by the frequency and fitness effect of a mutation (sq) minus the effects that are expressed as homozygotes (sq^2) and heterozygotes ($2hsq(1 - q)$). To calculate the total inbreeding load across a diploid genome ($2B$), this quantity can be summed across sites with deleterious mutations and multiplied by 2 to account for diploidy, such that $2B = 2\sum B_i$.

These fundamental principles demonstrate that an essential component of estimating the genetic load and inbreeding load (hereafter referred to together as "load") using genetic variation data is knowing s and h for individual mutations. However, although some progress has been made in predicting whether a mutation is likely to be neutral or deleterious (e.g., Cooper et al. 2005; Kumar et al. 2009; Choi et al. 2012; Cingolani et al. 2012; Kircher et al. 2014), accurately predicting h and s for individual mutations in genomic sequencing data remains a major challenge, even in humans and model organisms (Cooper and Shendure 2011; She and Jarosz 2018; Huber et al. 2020; Sandell and Sharp 2022). For example, a recent simulation study demonstrated that genomic evolutionary rate profiling (GERP; Cooper et al. 2005), a popular method for predicting the deleterious effect of mutations based on evolutionary conservation, cannot reliably distinguish weakly deleterious mutations from strongly deleterious mutations (Huber et al. 2020), although the method is commonly used for this purpose (e.g., Henn et al. 2016; Marsden et al. 2016; Van Der Valk et al. 2019; Dussex et al. 2021). Similarly, experimental studies in yeast have found that methods such as SIFT (Kumar et al. 2009) and PROVEAN (Choi et al. 2012) are poor predictors of the fitness effect of a mutation (She and Jarosz 2018; Sandell and Sharp 2022) that provide only crude proxies of s. Moreover, these methods do not provide any information on dominance, an essential component of quantifying load. These limitations are unlikely to be fully overcome, particularly for nonmodel organisms, implying that methods for quantifying load based on sequence data will remain somewhat crude approximations for the foreseeable future.

Overview of Simulation-Based Approaches

Computational simulations using evolutionary models provide an alternate way of quantifying load that alleviates

many of the limitations discussed above. Simulations are widely used in population genetics (e.g., Marjoram and Donnelly 1994; Akey et al. 2004; Ramachandran et al. 2005; Fu et al. 2014; Harris and Nielsen 2016; Henn et al. 2016; Uricchio et al. 2016; Adrion et al. 2020*a*) yet remain underused in ecological and conservation genomics. Historically, this may be due to a relative lack of simulation tools capable of modeling ecologically realistic scenarios and an often steep learning curve for using simulation software that may be poorly documented (Hoban et al. 2012). However, many of these challenges have been addressed by the forward-in-time genetic simulation program SLiM (Haller and Messer 2016, 2019, 2023), which offers a flexible array of models incorporating realistic ecological dynamics as well as comprehensive documentation and a graphical user interface. Other similar programs include Nemo (Guillaume and Rougemont 2006; Cotto et al. 2020) and SimBit (Matthey-Doret 2021), both of which have been applied in a conservation genetics context (Grossen et al. 2020; Grummer et al. 2022). Finally, another important recent advance in simulation software in population genetics is the growth of simulation resources through the PopSim consortium (Adrion et al. 2020*a*; Lauterbur et al. 2023), including a library of demographic models, recombination maps, and other useful simulation parameters for a growing number of species.

Simulations are broadly useful in evolutionary genetics because they can serve the critical function of revealing which evolutionary scenarios are consistent with observed patterns of genetic variation. All studies of genetic variation in natural populations suffer from the limitation that they observe a single outcome of a stochastic evolutionary process, where underlying mechanisms are largely unobservable. Simulations allow researchers to model this evolutionary process and determine which mechanisms (e.g., genetic drift, gene flow, selection, migration) are needed to explain observed patterns of variation in a dataset. Moreover, the process of using simulations can be extremely valuable for developing intuition on how various evolutionary forces interact to influence patterns of genetic variation, improving the ability of researchers to design evolutionary genetics studies and interpret their results.

For studies aiming to assess the ecological and conservation relevance of deleterious variation, simulations can be especially useful for quantifying load, which can be directly tabulated from the simulation output (see sec. 1 of the supplemental PDF). Simulations can therefore be used to complement empirical measures of load, providing a framework in which to interpret observed patterns and verify that they are expected under a plausible evolutionary model. Moreover, simulations can go beyond empirical measures by projecting load under various future scenarios, illuminating how actions in the present day may impact load decades or centuries from now. Finally, modern simulation tools, such as the ecologically realistic models supported by SLiM (Haller and Messer 2019, 2023), also offer the potential to conduct an analysis of future extinction risk while incorporating genome-scale genetic variation (e.g., Robinson et al. 2022), analogous to the population viability analysis approaches that have long been employed in conservation genetics (e.g., Lacy 1993, 2019; Beissinger and Westphal 1998; Brook et al. 2000).

In summary, simulation-based approaches have much to offer for genomic studies of deleterious variation in wild populations, yet their application remains relatively limited. In table 1, we have summarized existing studies that employ simulations along with genomic analyses to investigate load in organisms ranging from Alpine ibex to Chinese crocodile lizards. We suggest that future research should incorporate similar approaches to those implemented in these studies to provide a more thorough investigation of load in wild populations.

What Are the Components of a Simulation of Deleterious Genetic Variation?

Modeling deleterious genetic variation in a simulation framework at a minimum requires specifying a population history, mutation rate, recombination rate, deleterious mutation target size, and distribution of selection and dominance coefficients (table 2). The extent to which these parameters need to be tailored to a focal organism will vary depending on the researcher's objectives. Many studies may focus on using simulations primarily to explore qualitative dynamics of deleterious variation under various demographic and genetic scenarios. For example, one may be interested in asking: what are the qualitative effects of a bottleneck on genetic load under two extreme scenarios where deleterious mutations are either additive or fully recessive? For these studies, tailoring the simulation parameters to the focal organism may not be crucial, so long as the chosen parameters are reasonable.

For studies aiming to make more quantitative statements about genetic load or project future population trends, tailoring simulation parameters to the focal organism may be more critical. For example, demographic history can vary widely between populations and has a large influence on deleterious genetic variation. Thus, having a reliable demographic model is a crucial factor in modeling load. Fortunately, historical demographic parameters can be readily inferred from genomic datasets, although estimating recent demography (i.e., during the last tens or hundreds of generations) remains challenging (Beichman et al. 2018; Nadachowska-Brzyska et al. 2022). Importantly, computational simulation models need not assume that populations are at mutation-selection-drift equilibrium. However, it is typical to run a burn-in for forward-in-time simulations

Table 1: Recent studies combining simulations with empirical genomic data to explore impacts of small population size on deleterious variation in nonhuman species

Study	Organism	Simulation software	DFEs	Question addressed with simulations
Beichman et al. 2023	Sea otter	SLiM	Kim et al. 2017[a]	How has the fur trade bottleneck impacted genetic load in the sea otter?
Dussex et al. 2021	Kākāpō	SLiM	Mean $s = .024$[b]	Has purging occurred in the Stewart island kākāpō population?
Grossen et al. 2020	Alpine ibex	nemo	Mean $s = .01$[b]	How has deleterious variation been impacted by a recent human-mediated bottleneck?
Kyriazis et al. 2023*a*	North American moose	SLiM	Kim et al. 2017[a]	How have bottlenecks influenced purging and genetic load in North American moose?
Marsden et al. 2016	Domestic dog	PReFerSim	Boyko et al. 2008	How has the domestication bottleneck shaped deleterious variation in dogs?
Robinson et al. 2018	Channel island fox	SLiM	Kim et al. 2017	How has deleterious variation been impacted by small population size in island foxes?
Robinson et al. 2019	Gray wolf	SLiM	Kim et al. 2017	How does the large North American wolf population size influence recessive deleterious variation?
Robinson et al. 2022	Vaquita	SLiM	Estimated by authors[a]	Are vaquitas doomed to extinction by inbreeding depression?
Stoffel et al. 2021	Soay sheep	SLiM	Eyre-Walker et al. 2006[c]	Are short runs of homozygosity purged of deleterious variation?
Takou et al. 2021	*Arabodopsis lyrata*	PReFerSim	Estimated by authors	Do range-edge populations have elevated genetic load?
Xie et al. 2022	Chinese crocodile lizard	SLiM	Kim et al. 2017	Have population declines resulted in purging?

[a] Sensitivity analysis conducted with additional distribution of fitness effects (DFE).
[b] Mean for gamma distribution, not based on explicit analysis.
[c] The DFE uses shape parameter from Eyre-Walker et al. (2006) and mean s of 0.01, 0.03, and 0.05.

to reach equilibrium levels of genetic diversity in an ancestral population before modeling subsequent population size changes, when the population is no longer at equilibrium. This ability to model nonequilibrium dynamics is a key advantage of simulation approaches, as most natural populations are likely not at equilibrium, although many classic analytical results in population genetics assume equilibrium conditions (Brandvain and Wright 2016).

The mutation rate is another essential component influencing levels of deleterious variation in a population,

Table 2: Evolutionary forces relevant to modeling load and how these forces impact load

Evolutionary force	Impact on genetic load	Impact on inbreeding load
Population size (N)	Decrease with increasing N	Increase with increasing N
Mutation rate (μ)	Increase with increasing μ	Increase with increasing μ
Deleterious mutation target size (G)	Increase with increasing G	Increase with increasing G
Distribution of fitness effects (s)	Depends on N[a]	Increase with increasing mean s
Dominance distribution (h)	Increase as h increases from 0 to .5	Decrease as h increases from 0 to .5
Recombination rate (r)	Decrease with increasing r	Decrease with increasing r

[a] Note that under classical models of mutation load, due to mutations segregating under mutation-selection balance, s does not impact load. However, this result does not hold when considering fixed mutations and finite population size. See Kimura (1963) for a detailed analysis of the effects of s, h, and N on genetic load.

although high-quality mutation rate estimates (i.e., based on a large number of sequenced trios) do not exist for the vast majority of species (although see Bergeron et al. 2023). However, mutation rates can also be estimated from substitution rates between species, an approach that is now widely feasible given the abundance of whole-genome sequencing data (Lynch et al. 2016). Recombination rates can also influence load, as negative selection against deleterious alleles may be impeded in regions of low recombination (Charlesworth 2012; Berdan et al. 2021; Sianta et al. 2023). To model these dynamics for a species of interest, a growing number of approaches exist for estimating recombination rates from genomic datasets from as little as one diploid individual (e.g., Barroso et al. 2019; Adrion et al. 2020*b*). Tailoring the genome structure (i.e., the length and number of genes and the extent of noncoding deleterious variation, which together determine the deleterious mutation target size) of a simulation to a specific organism can also be an important component of a simulation, particularly for studies aiming to model population dynamics due to the total impact of deleterious mutations. To aid in this, a growing number of annotated reference genomes are now available, which can provide useful information on genome structure, particularly for protein-coding regions of the genome (Paez et al. 2022).

Finally, the joint distributions of selection and dominance coefficients are essential components of modeling deleterious variation and load. These distributions determine the effect that new mutations exert on fitness, as well as the corresponding dominance coefficient of a mutation. Although there is broad agreement that strongly deleterious mutations tend to be highly recessive, the parameters of the distribution of dominance coefficients remain especially poorly known (Simmons and Crow 1977; Caballero and Keightley 1994; García-Dorado and Caballero 2000; Agrawal and Whitlock 2011; Huber et al. 2018). Much more is known about the distribution of selection coefficients for new mutations, often termed "the distribution of fitness effects" (DFE), although most studies remain focused on humans and model organisms such as *Drosophila* (Eyre-Walker and Keightley 2007; Huber et al. 2017; Kim et al. 2017; fig. 1). Given the importance of the DFE for simulations of deleterious variation as well as recent debate over DFE parameters (Kardos et al. 2021; Pérez-Pereira et al. 2021; Garcia-Dorado and Hedrick 2022; Pérez-Pereira et al. 2022; Kyriazis et al. 2023*b*), below we provide a more detailed review of this topic.

What Is the DFE and How Is It Estimated?

The DFE is a probability distribution that quantifies the selective effect (s) of new mutations entering the population, that is, what fraction of new mutations are adaptive, neutral, weakly deleterious, or strongly deleterious. Here, we focus our discussion on the deleterious portion of the DFE, given that adaptive mutations do not contribute to load. Importantly, the DFE is not an estimate of s for segregating variation and therefore does not directly quantify load (see sec. 1 of the supplemental PDF; figs. S1, S2), a misconception that has recently spread in the literature (e.g., Jones et al. 2020; Kutschera et al. 2020). Instead, the fate of a mutation after it enters a population and whether it will segregate and potentially reach fixation will be influenced by selection as well as the stochastic effects of genetic drift and linkage. Thus, quantifying segregating variation and load using the DFE requires modeling these effects under a given demographic model (for an example, see sec. 1 of the supplemental PDF; figs. S1, S2).

Historically, the DFE was estimated primarily using experimental mutation accumulation approaches (Mukai 1964; Simmons and Crow 1977; Eyre-Walker and Keightley 2007; Halligan and Keightley 2009). However, these approaches are limited to detecting the small fraction of deleterious mutations that have large enough effects to be observed in a laboratory setting (although typically excluding lethals; Davies et al. 1999; Eyre-Walker and Keightley 2007; Halligan and Keightley 2009; see sec. 2 of the supplemental PDF; table S1). These limitations motivated the development of sequence-based approaches for estimating the DFE over the past two decades (Eyre-Walker and Keightley 2007). Sequence-based methods estimate the DFE on the basis of differences in the synonymous (assumed to be neutral) and nonsynonymous (assumed to be primarily neutral and deleterious) site frequency spectra (SFS), a summary of allele frequencies in a sample (Eyre-Walker et al. 2006; Eyre-Walker and Keightley 2007; Boyko et al. 2008; Kim et al. 2017; Tataru et al. 2017; see sec. 2 of the supplemental PDF). Specifically, these methods typically use the synonymous SFS to account for neutral demographic effects and, conditioning on inferred demographic or nuisance parameters, then estimate the parameters of the distribution of s for new nonsynonymous mutations (most commonly, the mean and shape parameters of a gamma distribution). Thus, although these approaches have much greater power for estimating the weakly deleterious portion of the DFE, existing sequence-based DFEs are generally limited to nonsynonymous single-nucleotide variants (although see Torgerson et al. 2009). Finally, one important limitation of sequence-based approaches is that they typically assume that all mutations have additive effects on fitness, given that information on the distribution of dominance coefficients is very limited (although see Huber et al. 2018). Consequently, sequence-based DFE approaches may not be well powered for estimating the relatively small portion of the DFE that is highly recessive and strongly deleterious, including recessive lethals (Wade

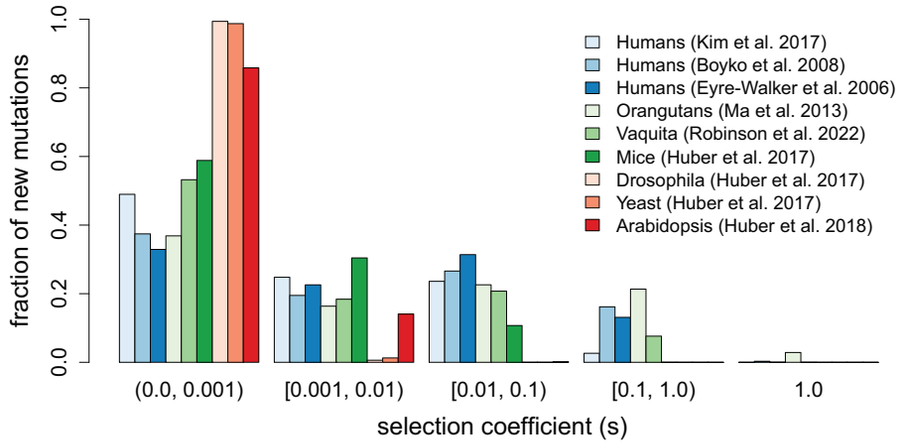

Figure 1: Representative estimates of the distribution of fitness effects from sequence-based approaches. Distributions are plotted in discrete bins of weakly deleterious ($0 < s < 0.001$), moderately deleterious ($0.001 \leq s < 0.01$), strongly deleterious ($0.01 \leq s < 0.1$), semilethal ($0.1 \leq s < 0.99$), and lethal ($s \geq 0.99$) mutations. Distribution of fitness effects (DFE) estimated for humans are colored in shades of blue, DFEs for nonhuman mammals are colored in shades of green, and nonmammalian DFEs are colored in shades of red. Note the higher fraction of weakly deleterious mutations in nonmammalian DFEs.

et al. 2023). See section 2 of the supplemental PDF for a detailed discussion of the nuances and limitations of sequence-based versus experimental methods to infer the DFE.

A growing number of studies have used sequence-based methods to estimate the DFE for nonsynonymous mutations in various taxa, including humans, nonhuman primates, mice, *Arabidopsis*, *Drosophila*, and the highly endangered vaquita porpoise (Eyre-Walker et al. 2006; Boyko et al. 2008; Ma et al. 2013; Chen et al. 2017; Huber et al. 2017, 2018; Kim et al. 2017; Tataru et al. 2017; Castellano et al. 2019; Robinson et al. 2022; fig. 1). In general, these studies estimate a relatively high proportion of weakly deleterious mutations (here defined as $s < 1e{-}3$). These sequence-based estimates are also in agreement with a broad literature in population genetics and functional genomics suggesting that the majority of nonsynonymous mutations have relatively minimal effects on fitness (Cassa et al. 2017; Agarwal and Przeworski 2021; Kruglyak et al. 2022; Agarwal et al. 2023). Another finding from sequence-based DFE studies is that the fraction of new mutations that are weakly deleterious appears to vary among major taxonomic groups. For example, studies in mammals generally estimate ~50% of mutations as weakly deleterious, whereas studies in *Arabidopsis*, *Drosophila*, and yeast suggest that >80% of new nonsynonymous mutations are weakly deleterious (fig. 1). Comparative analyses of the DFE have suggested that such differences may be related to species complexity (Huber et al. 2017) as well as life history traits, such as selfing (Arunkumar et al. 2015; Chen et al. 2017).

How Can We Validate Simulation Models?

As can be seen from the above discussion, there are many different parameters in a model of deleterious variation, and the estimation of each of these parameters often comes with some uncertainty. Moreover, such parameter estimates do not exist for the vast majority of species, which may result in researchers employing parameter estimates from other (ideally, closely related) species. Given these many potential sources of uncertainty, an important step in formulating a simulation model is ensuring that it is reasonable with some form of validation (Lotterhos et al. 2022).

One approach for validating a simulation model is to test whether patterns of genetic variation generated under the model agree with patterns observed in an empirical genomic dataset. Various summary statistics can be useful for this exercise. Examination of the simulated and observed SFS can be particularly informative, as the SFS captures all allele frequency information in a dataset and is therefore a rich source of information for model validation (Schraiber and Akey 2015; Ragsdale et al. 2018). Comparing simulated versus observed heterozygosity, which is itself a summary of the SFS, can also be informative as a simple test of whether a model can predict basic aspects of an observed dataset. Additionally, in populations where inbreeding is present, comparing the observed and simulated distribution of runs of homozygosity can provide valuable information on whether the demographic parameters of a model are reasonable (for an example, see Kyriazis et al. 2023*a*). Finally, examining patterns of linkage disequilibrium may also be useful, as patterns of linkage disequilibrium

represent a valuable source of information that is not contained in the SFS (Schraiber and Akey 2015; Ragsdale et al. 2018).

Such comparisons between simulated and empirical patterns of genetic variation can give some reassurance that a model is reasonable. However, for models that aim to examine ecological population dynamics due to genome-wide effects of deleterious variation, it may be important to also assess whether a model agrees with more direct, field-based estimates of fitness. For most species, this can pose a major challenge, as the long-term observational data that are typically required for measuring fitness remain rare (Sheldon et al. 2022). Moreover, another major hurdle is that comparing model-based predictions of genetic load to field-based estimates is not straightforward, as an empirical estimate of genetic load requires the existence of a "mutant-free" reference genotype where deleterious mutations are absent (Agrawal and Whitlock 2012; Robinson et al. 2023). Given that all organisms are burdened to some degree by deleterious mutations, this problem makes quantifying genetic load empirically a near-impossible task (Agrawal and Whitlock 2012).

A more feasible approach for comparing the effects of deleterious mutations between a model and empirical measurements of fitness is to examine the predicted inbreeding load. As described above, the inbreeding load quantifies the rate at which fitness is lost in a population as levels of inbreeding increase (Morton et al. 1956; Keller and Waller 2002; Hedrick and Garcia-Dorado 2016). This quantity can be measured empirically in systems where estimates of fitness and the inbreeding coefficient exist at an individual level (note that these individual-level data provide a population-level estimate of the inbreeding load; for a detailed evaluation of common methodological approaches for measuring the inbreeding load, see Nietlisbach et al. 2019). Thus, for species where such empirical estimates of the inbreeding load exist, it may be possible to compare these empirical estimates to those derived from a simulation model as a means to further validate model parameters.

However, there are several potential hurdles in comparing observed and predicted inbreeding load for model validation. First, empirical estimates of the inbreeding load do not exist for the vast majority of populations or species; thus, it is unlikely that an estimate is available for model validation in a given population of interest. Second, even in cases where empirical estimates of the inbreeding load do exist, often they are not reliable (Nietlisbach et al. 2019). Accurately estimating the inbreeding load is not a trivial task: it requires large sample sizes; accurate estimates of the inbreeding coefficient, ideally from genomic data; high variance in inbreeding in a population; and a reliable proxy for fitness (Kalinowski and Hedrick 1999; Nietlisbach et al. 2019). Relatively few studies exist that combine all of these elements, leading to wide variance in available estimates (for further discussion, see sec. 3 of the supplemental PDF).

Given these challenges, another approach may be to compare model-based predictions of the inbreeding load to empirical estimates that are averaged across populations or species. For instance, one widely cited estimate of the median diploid inbreeding load ($2B$) for juvenile survival in mammals is $2B = 3.1$, derived from an analysis of 40 captive mammalian populations (Ralls et al. 1988). More recently, an analysis of the "total" inbreeding load in wild vertebrates proposed a much higher $2B = 12$, based on data from 12 primarily bird species (O'Grady et al. 2006). However, this estimate was shown to be upwardly biased and unreliable because of issues with the statistical methods and underlying datasets (Nietlisbach et al. 2019; see sec. 3 of the supplemental PDF). Given these issues, Nietlisbach et al. (2019) conducted a reanalysis and found a median inbreeding load for survival to sexual maturity in wild vertebrates of $2B = 4.5$. This value may serve as a useful point of comparison for validating model-based predictions. However, we emphasize that the inbreeding load of a given population is expected to vary on the basis of its genomic and demographic characteristics; thus, model-based predictions may often be higher or lower than this estimate. Additional reliable estimates of the inbreeding load in wild populations will be valuable for further validating models in the future.

Validating Recent Deleterious Mutation Models

A great deal of recent debate has been concerned with the parameterization of models of deleterious variation (Kardos et al. 2021; Pérez-Pereira et al. 2021, 2022; Garcia-Dorado and Hedrick 2022; Kyriazis et al. 2023*b*). Much of this debate has occurred in response to the model employed by Kyriazis et al. (2021), a model that aimed to reflect deleterious mutation parameters in canids. Specifically, this model employed a genome structure consisting of 30 Mb of coding sequence across 38 different chromosomes as informed by the structure of the dog genome (Lindblad-Toh et al. 2005), yielding an overall rate of deleterious mutation of $U = 0.42$ per individual (for details, see sec. 4 of the supplemental PDF). Since estimates of selection and dominance parameters do not exist for canids, this model also employed a sequence-based DFE estimated from humans by Kim et al. (2017) and a dominance distribution that was proposed for humans by Henn et al. (2016; fig. 2; table 3; for details, see sec. 4 of the supplemental PDF).

These parameters were criticized by Kardos et al. (2021) and Pérez-Pereira et al. (2022), both of whom argued that the Kim et al. (2017) DFE did not reflect the high proportion of strongly deleterious variation inferred from experimental studies. Additionally, these authors also criticized

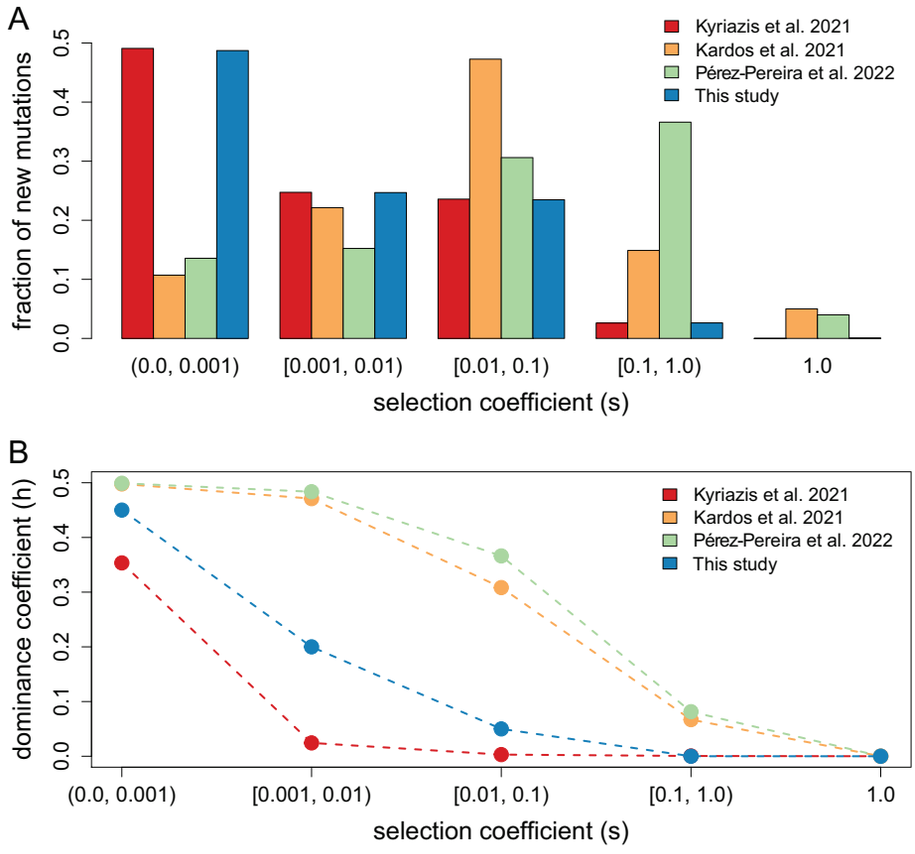

Figure 2: Comparison of distribution of fitness effects (DFE) and dominance models employed by Kyriazis et al. (2021), Kardos et al. (2021), and Pérez-Pereira et al. (2022), as well as the model described in this study. *A*, DFEs of new deleterious mutations for each model. *B*, Distribution of dominance coefficients for each respective model. Note that the dominance distribution from Pérez-Pereira et al. (2022) assumes a distribution of h for each value of s (see sec. 4 of the supplemental PDF). See table 3 for more details on these models.

the dominance distribution based on Henn et al. (2016) as being too recessive (fig. 2). Kardos et al. (2021) and Pérez-Pereira et al. (2022) instead proposed DFEs with a much greater proportion of highly deleterious alleles (here defined as $s > 0.01$) and dominance distributions that are far less recessive, loosely based on experimental results (fig. 2; table 3; for details, see sec. 4 of the supplemental PDF). These DFEs suggest that a large majority of new mutations are highly deleterious (~67% for Kardos et al. 2021; ~71% for Pérez-Pereira et al. 2022; fig. 2), in stark contrast to all existing sequence-based estimates for nonsynonymous mutations in diverse plant and animal taxa (fig. 1). Finally, these authors also propose varying deleterious mutation rates compared with Kyriazis et al. (2021), with Kardos et al. (2021) assuming $U = 1.2$ and Pérez-Pereira et al. (2022) assuming $U = 0.4$ (table 3).

It is not surprising that the sequence-based DFE parameters estimated by Kim et al. (2017) and employed by

Table 3: Comparison of distribution of fitness effects (DFE), dominance, and mutation rate parameters

Model	Mean s	Shape	Lethals (%)	Mean h	U
Kyriazis et al. 2021	.0131	.186	0	.18	.42
Kardos et al. 2021	.05	.1	5	.31	1.2
Pérez-Pereira et al. 2022	.2	.33	0	.28	.4
This study	.0131	.186	.3	.28	.63

Note: Note that the mean s and shape are for parameterizing a gamma distribution, and "lethals" denotes an additional proportion of recessive lethals augmented to the gamma distribution. Mean h denotes the mean dominance coefficient for new mutations under each DFE (note that this value is impacted by the assumed DFE, as DFEs with more strongly deleterious mutations will tend to have a more recessive mean h when keeping the distribution of h constant). U represents the diploid genomic deleterious mutation rate from each of these models.

Kyriazis et al. (2021) do not agree with experimental estimates of the DFE. As noted above, experimental estimates are well known to be biased toward highly deleterious variation, as the impacts of more weakly deleterious alleles may go undetected in an experimental setting (Davies et al. 1999; Eyre-Walker and Keightley 2007). To quantitatively demonstrate the implications of such extreme selection parameters, we compared these three proposed models in terms of whether the predicted nonsynonymous SFS from these models agrees with the observed SFS from a large sample of human genomes from the 1000G dataset (Auton et al. 2015; for details, see sec. 5 of the supplemental PDF). As described above, the SFS is a useful tool for model validation, as it summarizes all allele frequency information in a sample. Given that Kardos et al. (2021) and Pérez-Pereira et al. (2022) claim that their models are applicable to mammals, their models should predict patterns of genetic variation that are consistent with those seen in humans.

When running SLiM simulations under a human demographic model and outputting the simulated SFS (see sec. 5 of the supplemental PDF; fig. S3), we find that the predicted patterns of genetic variation from the Kardos et al. (2021) and Pérez-Pereira et al. (2022) models are not consistent with those observed in humans. Specifically, the nonsynonymous SFS from these models is greatly shifted toward rare variation, with a large majority of variants predicted to be singletons (variants with a count of one in the sample; fig. 3; table S2). For instance, the Kardos et al. (2021) model yields ~76% of variants as singletons, and the Pérez-Pereira et al. (2022) model yields ~71% as singletons (table S2). However, only ~57% of variants are singletons in the 1000G dataset (table S2). This surplus of rare variation is due to the very strong predicted effects of negative selection under these models, which results in deleterious alleles being held at extremely low frequency. By contrast, the Kyriazis et al. (2021) model makes predictions that are in good agreement with observed patterns in humans, with ~54% of variants predicted to be singletons (fig. 3; table S2; for further discussion, see sec. 5 of the supplemental PDF). Overall, these results further confirm previous findings that experimentally derived selection parameters are biased toward highly deleterious variation. Additionally, although our comparison here focuses on humans, the similarity between sequence-based human DFE estimates and those from other mammalian species (fig. 1) suggests that similar results would be obtained when comparing model predictions to patterns of genomic variation in other taxa.

As another approach for validating these models, we compared the predicted inbreeding load from each model to empirical estimates of the inbreeding load in humans. Several estimates of the inbreeding load are available in humans, including an estimate of $2B = \sim 4$ from Morton et al. (1956) and $2B = 1.4$ from Bittles and Neel (1994). Additionally, Gao et al. (2015) estimated ~0.6 recessive lethal mutations per human, which represents the fraction of the inbreeding load that is due to lethal mutations. To assess which models are consistent with these estimates in humans, we again ran simulations under a human demographic model (fig. S4). For each model, we assumed the genomic deleterious mutation rate proposed by each article (table 3). However, we also present results using the same human mutational parameters for all models

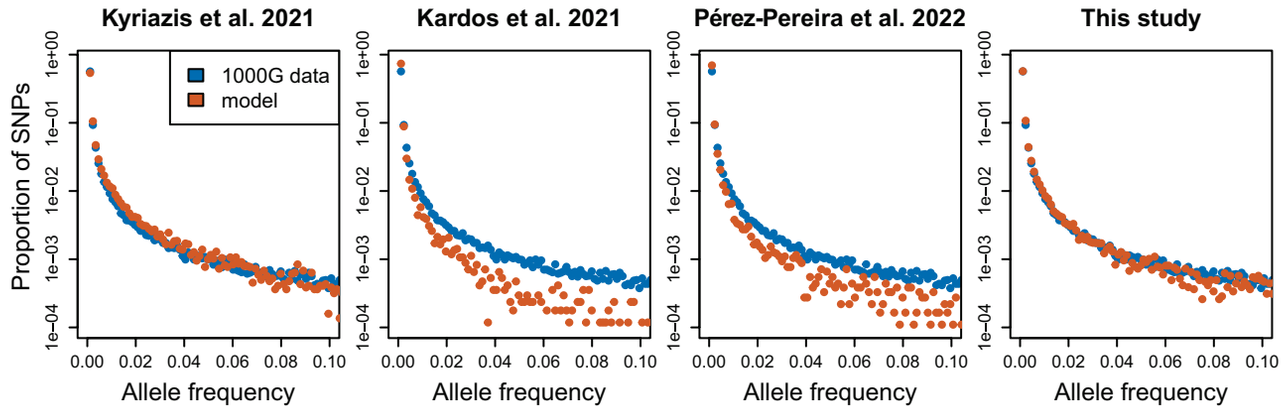

Figure 3: Predicted proportional nonsynonymous site frequency spectra (SFS) from various distribution of fitness effects and dominance models compared with SFS from 1000G data. Note that the predicted SFS from the Kyriazis et al. (2021) model and the model proposed in this study fit the 1000G data well, whereas the predicted SFS from the Pérez-Pereira et al. (2022) and Kardos et al. (2021) models are greatly shifted toward rare alleles due to the overabundance of strongly deleterious variation in these models. See figure S3 for plots of simulated versus empirical synonymous SFS and table S2 for proportion of singletons and common variants predicted by each model. SNP = single-nucleotide polymorphism.

($U = 0.63$; see sec. 5 of the supplemental PDF) to facilitate more direct comparison of how these DFE and dominance parameters affect the predicted inbreeding load. Note that although Kardos et al. (2021) and Pérez-Pereira et al. (2022) conducted a similar analysis of comparing the predicted inbreeding load from their models to an empirical estimate, their analysis assumed small equilibrium effective population sizes that do not reflect those observed in natural populations (see sec. 6 of the supplemental PDF; figs. S5, S6). By contrast, our analysis employs a demographic model that was estimated for humans by Kim et al. (2017) that includes complex nonequilibrium dynamics (see sec. 5 of the supplemental PDF), as informed by observed patterns of synonymous variation in humans (fig. S3).

Here, we find that all models greatly overshoot empirical estimates of the inbreeding load in humans. Specifically, the Kardos et al. (2021) and Pérez-Pereira et al. (2022) models predict very high inbreeding loads of $2B = 38.0$ and $2B = 20.4$, respectively, while the Kyriazis et al. (2021) model predicts an inbreeding load of $2B = 7.6$ (figs. 4, S4). Moreover, the Kardos et al. (2021) and Pérez-Pereira et al. (2022) models also predict a high number of recessive lethal mutations (30.4 and 7.8 mutations per human, respectively), whereas the Kyriazis et al. (2021) model predicts no such lethal mutations (fig. 4). Thus, none of these models are consistent with empirical inbreeding load estimates in humans, although overpredictions are especially notable for the Kardos et al. (2021) and Pérez-Pereira et al. (2022) models. Importantly, the predictions from the Kardos et al. (2021) and Pérez-Pereira et al. (2022) models are also not consistent with results from nonhuman species, where inbreeding loads are typically on the order of ~5 (Nietlisbach et al. 2019) and recessive lethal counts are consistently on the order of ~1.5 (Simmons and Crow 1977; McCune et al. 2002). Finally, note that results are qualitatively similar when assuming the same genomic deleterious mutation rate for all models ($U = 0.63$; fig. S7), suggesting that much of the overprediction of the inbreeding load for the Kardos et al. (2021) and Pérez-Pereira et al. (2022) models is due to the assumed DFE and dominance parameters.

Given the shortcomings of these existing models, we propose a new model based on an analysis of the DFE in humans under a nonadditive model of dominance (M. I. A.

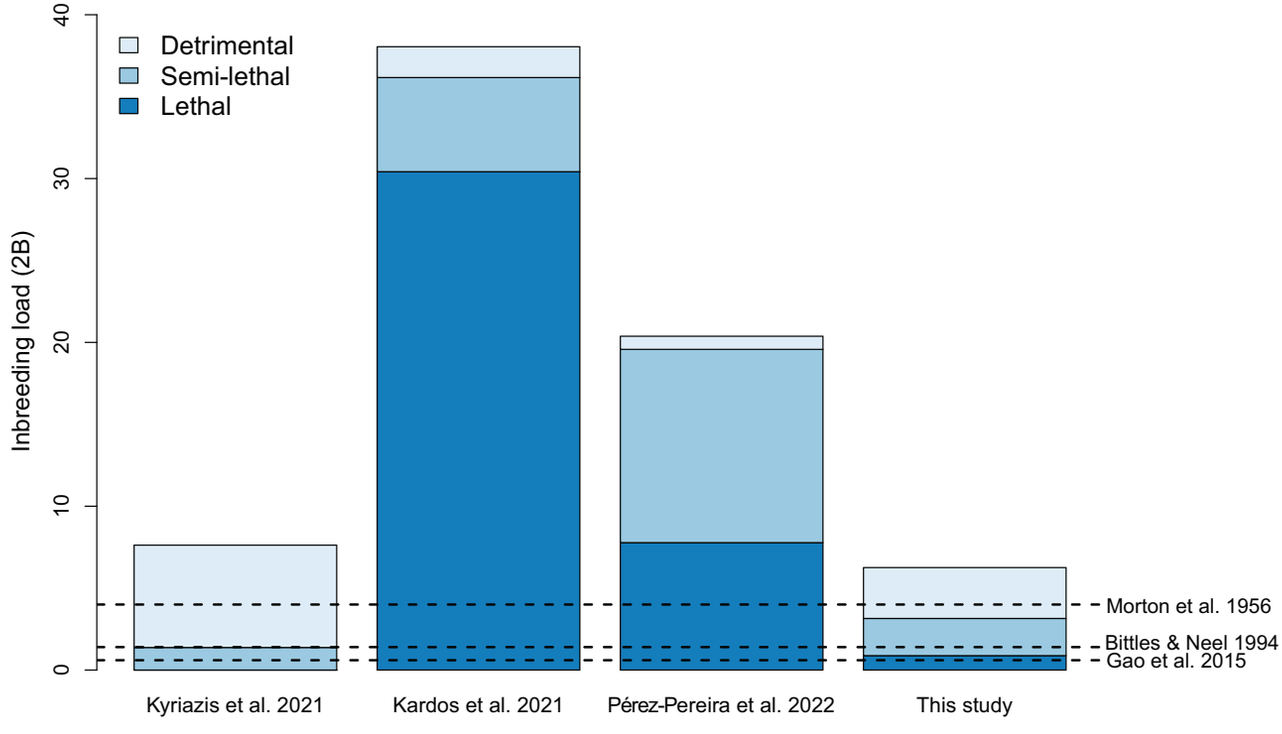

Figure 4: Predicted inbreeding load for different deleterious variation models under a human demographic model. Colors depict the contribution of inbreeding load from each class of deleterious mutations, with the total height of each bar representing the total predicted inbreeding load ($2B$). Detrimentals are here defined as mutations with $s < 0.1$, semilethals as mutations with $0.1 \leq s < 0.99$, and lethals as mutations with $s \geq 0.99$. Dashed lines show the estimated number of lethals per diploid human from Gao et al. (2015) and inbreeding load estimates for humans from Bittles and Neel (1994) and Morton et al. (1956).

Cavassim, C. C. Kyriazis, and K. E. Lohmueller, unpublished manuscript) as well as an estimate of the recessive lethal portion of the DFE (Wade et al. 2023). In brief, this new model assumes a somewhat less recessive dominance distribution compared with that assumed by Kyriazis et al. (2021) and is augmented with a small proportion (0.3%) of recessive lethal mutations (fig. 2; for details, see sec. 5 of the supplemental PDF). Indeed, simulation results under this model are in much better agreement with empirical estimates. Specifically, this new model predicts an inbreeding load of $2B = 6.3$, including ~0.9 recessive lethals per diploid (fig. 4). Although the total predicted inbreeding load slightly exceeds empirical estimates in humans, this result is expected given that these empirical estimates are based only on juvenile survival and may therefore be underestimates of the full inbreeding load (Morton et al. 1956; Bittles and Neel 1994). Importantly, this model also predicts patterns of genetic variation that closely align with those observed in humans (fig. 3; table S2; for further discussion, see sec. 5 of the supplemental PDF).

Overall, this analysis demonstrates that sequence-based DFE estimates can explain empirical estimates of fitness when making slight adjustments to account for their shortcomings in estimating the proportion of recessive lethal mutations (Wade et al. 2023). Thus, sequence-based DFEs remain preferable for modeling deleterious variation because they account for the impacts of both weakly and strongly deleterious variation. To facilitate use of this new model in future simulation studies, we have provided an example SLiM script available on GitHub (https://github.com/ckyriazis/simulations_review).

Conclusions and Remaining Questions

In summary, computational simulation tools represent a valuable resource for studying deleterious variation in natural populations that has been largely untapped by the ecological and conservation genomics community. As genomic datasets for wild species continue to grow, the necessity of employing such simulation tools to help interpret patterns in these data will also increase. Although parameterizing a computational simulation model may seem like a daunting task for a first-time user, we hope that this perspective article will provide a useful starting point for many of the key components.

Several outstanding questions remain to be addressed, which have implications for our fundamental understanding of deleterious variation and inbreeding depression as well as for our ability to better parameterize simulation models. These include the following.

How can we better estimate dominance parameters? Few estimates of the distribution of dominance parameters are available, and those that do exist sometimes conflict with one another (Simmons and Crow 1977; Caballero and Keightley 1994; Agrawal and Whitlock 2011; Huber et al. 2018). As dominance is an essential component of inbreeding depression, obtaining a better understanding of dominance will be essential to improving our ability to model the effects of inbreeding depression in natural populations.

How much does the DFE differ across taxa? Most available estimates of the DFE are for mammals, with few existing estimates in other animal or plant taxa (fig. 1). Obtaining a better understanding of the DFE across diverse taxa will help determine whether it is justified to use mammalian DFEs, such as the human DFE presented in this article (fig. 2), in simulations for other vertebrate taxa where DFE estimates are not available.

How much does balancing selection contribute to inbreeding depression? Although the focus of this review is deleterious variation, studies in *Drosophila* suggest that inbreeding depression cannot be fully explained by deleterious alleles under mutation selection balance and that other factors, such as balancing selection, may play a role (Charlesworth and Charlesworth 1999; Charlesworth 2015). These studies notably contrast with our finding that deleterious variation models in humans are sufficient to explain empirical estimates of the inbreeding load (fig. 4). Additional research is needed to better understand the reasons for these conflicting findings.

How can we better parameterize the effects of other sources of deleterious variation, including noncoding variation and structural variation? Although our discussion in this article is focused on deleterious mutations arising at nonsynonymous sites in coding regions, there are several other sources of deleterious variation, including variants in noncoding regions and structural variants. Although some evidence suggests that noncoding deleterious mutations tend to be primarily weakly deleterious (Torgerson et al. 2009; Murphy et al. 2022; Dukler et al. 2022), there have been few attempts to quantify the selective effects of structural variants (although see Abel et al. 2020). Quantifying the selective effects of these types of mutations will provide critical information for better parameterizing models of deleterious variation.

What is the role of epistasis? Epistasis, or interactions between variants at different loci, is a factor that is typically ignored by models of deleterious variation and genetic load (Agrawal and Whitlock 2012). In many cases, this assumption seems justified, as there remains little convincing evidence for epistasis playing a major role in shaping the genomic landscape of deleterious variation (Carr and Dudash 2003; Agrawal and Whitlock 2012; Garcia and Lohmueller 2021; Sandler et al. 2021). Nevertheless, more research is needed to evaluate the potential impact of epistasis and understand its influence on deleterious variation and load.

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Statement of Authorship

C.C.K., J.A.R., and K.E.L. conceived the study. C.C.K. conducted analyses and wrote the manuscript with input from all authors.

Data and Code Availability

All simulation and plotting scripts are available at https://github.com/ckyriazis/simulations_review and have been archived in a Zenodo repository (https://doi.org/10.5281/zenodo.7925628; Kyriazis 2023*c*).

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"It is, moreover, a successful application of the principle of evolution, the theory forming the warp and woof of the work, and thus according with nature, while a wholesome and reverent tone pervades the pages." Figured: "The pioneers of the army of milk givers." From the review of Buckley's *Winners in Life's Race* (*The American Naturalist*, 1884, 18:47–50).