



Genetic load: genomic estimates and applications in non-model animals

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Abstract | Genetic variation, which is generated by mutation, recombination and gene flow, can reduce the mean fitness of a population, both now and in the future. This ‘genetic load’ has been estimated in a wide range of animal taxa using various approaches. Advances in genome sequencing and computational techniques now enable us to estimate the genetic load in populations and individuals without direct fitness estimates. Here, we review the classic and contemporary literature of genetic load. We describe approaches to quantify the genetic load in whole-genome sequence data based on evolutionary conservation and annotations. We show that splitting the load into its two components — the realized load (or expressed load) and the masked load (or inbreeding load) — can improve our understanding of the population genetics of deleterious mutations.

Fitness

A measure of the capability of an individual or genotype to survive and reproduce.

Deleterious mutations

Poorly adapted genetic variants that reduce fitness relative to the wild-type variant, thereby contributing to the genetic load. The mutations that remain deleterious across alternative environments are called ‘unconditionally deleterious’.

The evolutionary forces of mutation, recombination and gene flow introduce novel genetic variants into populations that form the substrate for natural selection and genetic drift, thereby driving evolutionary change. However, assuming that a population is adapted to its current environment, random changes to its genetic variation are more likely to be deleterious than beneficial¹. Even for a novel beneficial variant, substitution of its ancestral variant creates a burden on the population, which is the cost of natural selection². The resulting reduction in individual and mean population fitness is the genetic load, which could be considered the price paid by a species for its capacity for further evolution³.

Various ‘load’ definitions have been proposed to describe different processes that can lead to a reduction in fitness (BOX 1). The term genetic load was introduced in 1950 (REF.⁴). With a focus on the phenotypic effect, it is defined as “the proportion by which the population fitness is decreased in comparison with an optimum genotype”⁵. This definition emphasizes the loss of fitness in the present population. However, it ignores any potential future loss in fitness caused by partially recessive deleterious mutations whose effects are not fully expressed. The ‘total load’⁶ includes those masked mutations; for the purpose of this Review, the term ‘genetic load’ refers to the ‘total load’.

In the field of conservation genomics, understanding the total genetic load is important to assess its impact on the health and viability of endangered populations, both now and in the future. According to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, 30,449 out of 65,364 (46.6%) species

of animals, fungi and plants are in decline⁷. During population decline, the composition of the genetic load changes, with many previously masked mutations becoming expressed. Hence, understanding the genetic load, as well as its composition, helps to guide future conservation efforts.

Empirical studies on the relationship between inbreeding and fitness estimates have improved our understanding of genetic loads in populations that have undergone demographic changes^{8–12}. Such studies are relevant for evolutionary biology and conservation, but assessing individuals’ fitness is challenging in wild populations, especially in threatened species. Genomes contain valuable information about an individual’s ancestry and health, including its genetic load. Recent studies have been able to analyse genetic load dynamics using whole-genome data even in the absence of fitness data (Supplementary Table 1).

Here, we review the classic population genetic definition of the genetic load¹³. We discuss the unit the load is measured in, that is, the lethal equivalents, and show how components of the load can be defined at population and individual levels. We then describe current approaches that estimate the load using whole-genome sequence data without direct fitness estimates. Furthermore, we summarize what we have learnt from empirical studies that have estimated the load using whole genomes in wild animal populations, which have mainly focused on vertebrates (Supplementary Table 1). Finally, we discuss promising research areas and potential future developments in the theoretical and applied study of the genetic load. We focus on sexually reproducing outcrossing

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<https://doi.org/10.1038/s41576-022-00448-x>

Inbreeding depression

The reduction of fitness due to breeding between relatives.

Effective population size

(N_e). The number of individuals in an idealized population that shows the same amount of genetic drift (that is, random fluctuation of allele frequencies and loss of gene diversity) as the actual population.

Bottlenecks

(Also known as population bottlenecks). Sharp reductions in the effective population size (N_e) over one or multiple generations.

Genetic rescue

Artificial (re)introduction of new (or rare) genetic variants into a population with the aims of reducing inbreeding depression, increasing genetic variation and population viability.

diploid animals, although some of the concepts we cover are also relevant to plants and microorganisms, depending on their evolutionary dynamics. Also, we consider only (unconditionally) deleterious mutations, and not the genetic load that can be introduced by novel, better-adapted mutations that replace original variants.

The genetic load components

From a population genetics perspective, the genetic load can be defined as a statistic that summarizes the selection and dominance coefficients of deleterious mutations as a function of their frequencies in a population^{13,14} (Eqs (1–3)) (BOX 2). The genetic load can also be expressed as a function of the genotype of these mutations in an individual genome (Eqs (5–7)) (BOX 2). Furthermore, assuming that the fitness effects of mutations act independently and multiplicatively across loci, the impact of the genetic load on fitness is approximated by Eqs (8) and (9) (BOX 3). Some confusion has arisen in the literature because the fitness effects of mutations are generally calculated multiplicatively, particularly for traits that affect survival probability. However, the value of the load (that is, the lethal equivalents) is calculated by summing the selection coefficients (BOX 2).

In diploid organisms, the genetic load can be partitioned into the realized load (also known as the expressed load)⁶ and the masked load (also known as the inbreeding load^{15,16} or potential load^{17,18}). The realized load reduces the fitness in the current generation, whereas the masked load quantifies the potential fitness loss due to (partially) recessive deleterious mutations that may become expressed in future generations depending on the population's demography (for example, inbreeding, population decline or subdivision). The genetic load is the sum of the realized load and the masked load. This terminology emphasizes the effect of the load rather than processes that give rise to the load (BOX 1). Put simply, the genetic load is binary: the deleterious fitness effects of a mutation can be either expressed (in the realized load) or not expressed (in the masked load). Furthermore, the mean fitness of a population subject to the negative effects of deleterious mutations is approximately equal to the negative exponent of the realized load (BOX 3).

The unit of the load components is the lethal equivalent, which was originally defined in terms of additive fitness effects as “a group of mutant genes of such number that, if dispersed in different individuals, they would cause on the average one death, e.g., one lethal mutant, or two mutants each with 50 per cent probability of causing death, etc.”¹³. However, under a model of multiplicative fitness effects, a more accurate definition of a lethal equivalent is a group of mutant alleles with a summed selection coefficient equal to one. Assuming that the fitness effects of mutations are multiplicative across loci, the survival probability of an individual with a realized load of one lethal equivalent is approximately equal to e^{-1} (~36.8%), and with two lethal equivalents equal to e^{-2} (~13.5%). This can also be understood as the zero term in a Poisson distribution whose mean is in lethal equivalents, that is, the proportion of individuals that carry zero lethal equivalents¹⁹.

This second definition of lethal equivalents assumes that each mutation reduces fitness by a given probability, which might be more appropriate for studies on the survival rate, such as in the analysis of inbreeding depression or the extinction risk in conservation genomics. Accordingly, individuals with a realized load of more than one lethal equivalent can still survive. This definition makes the explicit assumption that (semi-) lethal mutations have low frequencies in the population and, hence, that homozygous lethals are rare. If (semi-) lethal mutations are common, fitness is not equal to the negative exponent of the realized load. For example, death is certain for an individual that expresses a lethal mutation (and not e^{-1} ~36.8%) (Supplementary Information 1,2).

Depending on the age of a population, the genetic load has a non-linear relationship with the effective population size (N_e) (FIG. 1). Small populations that have persisted for long periods of time are expected to possess the highest genetic load because many slightly deleterious mutations have become fixed, which elevates the realized load. By contrast, small populations tend to possess a low masked load. Hence, they are not expected to show much inbreeding depression¹⁷, as mating between close relatives does not substantially increase the number of homozygous deleterious loci. The masked load increases with population size, making large populations more prone to inbreeding depression during population decline (FIG. 1). Although bottlenecks purge some highly deleterious mutations, thereby reducing the genetic load^{20–22}, they also convert the masked load into the realized load. Furthermore, prolonged bottlenecks eventually result in a persistent realized load due to the fixation of deleterious mutations²³ (FIG. 2). Consequently, even after demographic recovery, migration or genetic rescue might be required to replace fixed deleterious variants and restore fitness¹⁸.

From genomes to load estimates

In the absence of direct fitness information, genetic load estimates can be obtained from whole-genome sequencing data in two steps. First, the deleterious effects of mutations are predicted. Second, the deleterious scores of these mutations are summarized to produce a load

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Box 1 | Definitions of different types of load

Different types of load have been introduced in the literature (reviewed elsewhere^{6,160}). Some definitions emphasize the processes giving rise to the load, whereas others were introduced to describe effects on genetic variation, fitness or population persistence. Although these definitions are historically interesting and conceptually insightful, the differences can be confusing. In addition, these loads are often of limited practical relevance when analysing whole-genome sequence data of wild organisms because they lack the analytical framework to study them quantitatively.

Mutation (or mutational) load^{4,115,161,162}

The reduction of fitness due to (recurrent) deleterious mutations or the reduction of fitness at the mutation–selection equilibrium.

Drift load^{159,163,164}

The reduction of fitness due to the increase in frequency (up to fixation, the fixation load; during range expansion, the expansion load) of deleterious mutations due to random genetic drift.

Evolution load (also referred to as evolutionary, transitory, lag or substitution load)^{165,166}

The reduction of fitness due to deleterious (suboptimal, maladapted) mutations during the spread of superior adaptive variants.

Inbreeding load^{15,17}

The reduction of fitness due to deleterious recessive mutations unmasked by inbreeding. The inbreeding load is the same as the masked load or potential load¹⁷.

Segregation (or segregating) load, sometimes used as a synonym of balanced load^{6,167}

The reduction of fitness due to segregating deleterious mutations; the balanced load is the reduction of fitness due to mutations that are deleterious in both homozygous genotypes, but not in the heterozygote.

Migration load and hybrid load^{168–170}

The reduction of fitness due to deleterious (maladapted) mutations introgressed from a different population or species after migration or hybridization.

Recombination load^{171,172}

The reduction of fitness due to the break up of favourable combinations of alleles at different loci due to recombination.

Other types of load

Other types of load have been described, including the ecological load¹⁷³, heterogeneous environment load⁶, environmental load¹⁷⁴, incompatibility load^{5,175}, meiotic drive load^{176,177}, pleiotropic load¹⁷⁸, gametic load¹⁷⁹, non-local load¹⁸⁰, sheltered load^{181–183} and gender load¹⁸⁴.

index, or a set of indices, which can be considered as proxies and used to study the load components.

Predicting deleterious mutations. Various methods have been developed to predict the fitness consequences of mutations that can be broadly grouped into two categories (TABLE 1): methods analysing the evolutionary conservation across a multispecies alignment; and approaches estimating the expected effects based on the type of substitution and its known effects in model species. A third, less widely used, source of information is the level of gene expression of the mutated gene.

Evolutionary conservation approaches can be applied to whole-genome sequences without annotations. A multiple sequence alignment is built to assess the level of conservation at individual nucleotide or genomic regions across lineages. Point mutations, insertion and deletions (indels) and structural variants can all contribute to the genetic load²⁴, but most predictors focus on point mutations (TABLE 1; Supplementary Table 1). A score quantifying the harmful effects of each variant is then assigned under the assumption that the

level of conservation in the alignment reflects its functional importance^{25–28}. The accuracy of the prediction increases with the number and the phylogenetic distance of the species in the alignment, as long as anchor species at intermediate evolutionary distances are also included^{26,29}. Large alignments can be challenging given that there is considerable turnover of constrained sites across phylogenies³⁰, and because they are computationally intensive. Lineage-specific substitutions should be considered with great care, as they may represent lineage-specific adaptations or may reflect changes in selection pressures, rather than unconditionally deleterious mutations³⁰.

One of the most widely used methods based on evolutionary conservation, especially in wild animals, is genomic evolutionary rate profiling (GERP)^{26,31}. A GERP score is a continuous variable estimating the average number of substitutions that would have accumulated under neutrality but have been removed (‘rejected’) by purifying selection. High scores reflect many rejected mutations and indicate that substitutions are only rarely tolerated during evolution^{26,31}. Sites with high GERP scores are assumed to experience strong negative selection, although turnover of sites and changes in selection pressures across the phylogeny set limitations on the use of comparative genomic approaches³⁰.

An alternative approach to evolutionary conservation methods consists of the direct inference of possible mutation effects using information from biochemical studies or functional annotations of the site or region where the mutation occurs^{32,33}. For example, the chemical properties of amino acids can be used to assign numerical scores to any of the possible changes³⁴. Also, substitutions in a coding region can be categorized as either synonymous (more likely to be selectively neutral) or non-synonymous (possibly deleterious). This type of information can be translated into a categorical variable such as low, moderate or high according to their predicted nearly neutral, mildly deleterious or highly harmful type of change. Additional information based on protein structure, experimentally known effects of the mutation or more detailed genome annotations can provide further data on the effects of variants such as missense, frameshift, stop-gain and loss of function, as done, for example, by SnpEff^{32,35}. A derived mutation can erroneously be predicted as deleterious when it has been identified using one or a few outgroups, or when multiple mutations occur at the same site. Such errors can be avoided by integrating the probability of multiple mutations at the same site based on the overall mutational spectrum³⁶ in the target species or population and applying corrections based on the DNA sequence context³⁷.

Predictions of the negative impact of a mutation become more precise and reliable as the quality of the genome annotation increases, and if evolutionary conservation is included as one of the annotation variables (for example, combining conservation scores and mutation type with local DNA structure, GC content, distance to splice sites and gene expression)^{33,38–40} (TABLE 1). Some of these ensemble approaches, such as ANNOVAR³³, PolyPhen³⁹, SIFT^{38,41} and VEP⁴⁰, directly

Anchor species

A species that is added to the genome alignment between two evolutionarily distantly related taxa to help connect them and facilitate the identification of conserved elements.

Mutational spectrum

The rate of different types of DNA mutations in different sequence contexts.

DNA sequence context

Nucleotide composition around the focal mutation.

target functional effects of regions that are already known from existing databases^{33,38–41}, whereas others, such as combined annotation-dependent depletion (CADD)⁴² or GWAVA⁴³, move beyond the available

information and assign a deleteriousness score to all potential mutations genome-wide, instead of relying on only the established ones^{42–44}. These methods provide reliable evidence of the fitness effect of variants, but are

Box 2 | Genetic load components

A population perspective

A population's genetic load at the gametic level is the sum of the selection coefficient s_i (the fitness reduction due to deleterious mutation i) of mutations at L loci, multiplied by their frequencies q_i (REF.¹³):

$$\text{Genetic load(population)} = \sum_{i=1}^L q_i s_i \quad (1)$$

The genetic load is independent of genotype frequencies and, hence, theoretically is not affected by inbreeding, random genetic drift or recombination. Only mutation and natural selection cause a directional change by increasing and decreasing the genetic load, respectively. By contrast, the portion of the genetic load that is realized (that is, whose fitness effects are expressed) does depend on genotype frequencies, as shown in Eq. (2). The first term expresses the homozygous effects, and the second term captures the effects of loci with heterozygous mutations, where h_i is the dominance coefficient of mutation i :

$$\text{Realized load(population)} = \sum_{i=1}^L q_i^2 s_i + 2 \sum_{i=1}^L q_i [1 - q_i] h_i s_i \quad (2)$$

The remaining part of the genetic load is not expressed, and thus stays hidden from selection. Crow⁶ referred to this as the inbreeding load, but we prefer to call this the masked load considering that inbreeding tends to reduce this load by unmasking it. This terminology also avoids the confusion between a condition (that is, the fitness effects of mutations that have remained masked) and a process (inbreeding):

$$\text{Masked load(population)} = \sum_{i=1}^L q_i s_i - \sum_{i=1}^L q_i^2 s_i - 2 \sum_{i=1}^L q_i [1 - q_i] h_i s_i \quad (3)$$

Inbreeding converts the masked load into the realized load, by increasing the frequency of homozygotes by $(1-F)q_i^2 + Fq_i$ and by reducing the frequency of heterozygotes by $2(1-F)q_i(1-q_i)$, where F is the inbreeding coefficient¹⁸⁵. Substituting these genotype frequencies increases the realized load whilst reducing the masked load by the same amount:

$$\text{Masked load(inbred population)} = \sum_{i=1}^L q_i s_i - \left\{ \sum_{i=1}^L (1-F)q_i^2 s_i + Fq_i s_i \right\} - \left\{ 2(1-F) \sum_{i=1}^L q_i [1 - q_i] h_i s_i \right\} \quad (4)$$

Equations (1–4) are visualized in Supplementary Fig. 6.

An individual perspective

The different types of loads can also be calculated for individuals, which may be of practical use when individual genomes have been sequenced. The individual genetic load represents the potential fitness burden of mutations in the individual's genome, affecting its fitness and the fitness of its descendants. It is equal to the sum of the selection coefficients (s_i) across all loci i that are homozygous for the mutant allele, plus half the selection coefficients (s_j) of all heterozygous mutant loci j (REF.⁶⁹):

$$\text{Genetic load(individual)} = \sum_{i=1}^{L(\text{hom})} s_i + \sum_{j=1}^{L(\text{het})} 0.5 s_j \quad (5)$$

The factor 0.5 associated with the heterozygous loci in Eq. (5) expresses the probability that a given deleterious mutation at a heterozygous locus is passed on to an offspring (not the dominance coefficient).

The loss in fitness due to an individual's genetic load is captured by its realized load, and is equal to the sum of all selection coefficients of all homozygous mutant loci plus the sum of the product of the dominance coefficient (h_j) and selection coefficient of all heterozygous loci:

$$\text{Realized load(individual)} = \sum_{i=1}^{L(\text{hom})} s_i + \sum_{j=1}^{L(\text{het})} h_j s_j \quad (6)$$

Similar to Eq. (3), also at the individual level, part of the genetic load at the heterozygous sites is not expressed, and this constitutes an individual's masked load:

$$\text{Masked load(individual)} = \sum_{j=1}^{L(\text{het})} (0.5 - h_j) s_j \quad (7)$$

This equation shows that additive mutations ($h=0.5$) do not contribute to the masked load, and, indeed, these mutations do not contribute to inbreeding depression. This expression is also known as the purging coefficient, $d=s(0.5-h)$, defined elsewhere¹⁸⁶.

Under Hardy–Weinberg genotype frequencies, the values of the loads calculated using Eqs (5–7) and averaged over multiple individuals approximate to the population's values computed using Eqs (1–3), respectively. Furthermore, Eqs (5–7) can be approximated by using average s and h coefficients instead of their sums (Supplementary Information 4).

Box 3 | Load components under different conditions and the effect on fitness

Theoretically, a population could possess a large genetic load and still have a high fitness. For example, the genetic load of F1 hybrids of parents from two genetically diverged populations is mainly present as a masked load, and hence the deleterious fitness effects of these mutations are not completely expressed (compare with heterosis)¹⁸⁷. The fitness of individuals is only affected by the realized load, and for fitness-related traits such as viability and survival it can be calculated by multiplying the fitness effect across all homozygous and heterozygous loci L :

$$\text{Individual fitness} = \prod_{i=1}^{L(\text{hom})} (1 - s_i) \prod_{j=1}^{L(\text{het})} (1 - h_j s_j) \quad (8)$$

where s_i and s_j are the selection coefficients of mutations in homozygous loci i and heterozygous loci j , respectively, and h_j is the dominance coefficient. The mean population fitness can be approximated by taking the negative exponent of the realized load:

$$\begin{aligned} \text{Mean population fitness} &\approx (1 - \{\bar{s}\bar{q}^2 + 2\bar{q}(1 - \bar{q})\bar{h}\bar{s}\})^L \approx e^{-\{\bar{s}\bar{q}^2 + 2\bar{q}(1 - \bar{q})\bar{h}\bar{s}\} \cdot L} \\ &\approx e^{-\text{RealizedLoad}} \end{aligned} \quad (9)$$

where \bar{s} and \bar{h} are the mean selection and dominance coefficient averaged across loci, \bar{q} is the mean frequency of deleterious mutations and L is the number of loci carrying deleterious mutations. Both Eqs (8) and (9) ignore other (non-genetic) causes for fitness loss (compare with the intercept of the regression line in REF.⁵), and they assume that the fitness effects are independent and act multiplicatively across loci. Furthermore, this equation assumes Hardy–Weinberg genotype frequencies and that (semi-)lethal mutations are rare (Supplementary Information 1,2).

mostly available for model organisms and require specific genomic alignments and annotations. When focusing on ultraconserved elements⁴⁵, scores obtained from the genome of a model animal can potentially be lifted over to a closely related non-model species.

Gene expression data can also be used to predict the potential fitness impact of specific variants. This approach takes advantage of the negative correlation observed between gene expression and protein polymorphism: highly expressed genes are usually associated with highly conserved coding sequences^{46–49}. Mutations in highly expressed genes should therefore be prioritized in genetic load estimates. However, this approach relies on an additional correlation step between the data and the prediction and requires known gene expression levels²². Furthermore, gene expression data cannot be applied to single nucleotides, limiting their current application to filtering out highly downregulated genes from genetic load analyses²².

Translating deleteriousness scores into genetic load proxies. The predicted effects of variants have been used to produce genetic load proxies for single individuals or populations, and several approaches have been proposed (FIG. 3; Supplementary Table 1). If numerical scores are available (for example, GERP or CADD scores), these can be summed or averaged across the genome of each individual^{50–53}. These values can be further averaged across individuals to obtain quantities related to the population's genetic load, which can be standardized using neutral variation^{22,54,55}. When variants can only be classified into categories according to the type of substitution or the intensity of their harmful effect, load indices based on the number or ratios of the observed variants in each category can be computed^{56–62}. These indices

can also be computed separately for homozygous and heterozygous loci^{56–58,63–66}, or they can be calculated for deleterious variants that occur at high frequency or are fixed in different populations^{56,67}. Furthermore, indices suitable for comparing different populations or samples with different ages (for example, ancient versus modern) have been developed^{22,66,68}.

Unfortunately, given the various approaches used to translate deleteriousness scores into genetic load proxies, there is no agreed gold standard that enables comparison of the load components across studies (Supplementary Table 1). Even in humans, the load estimates differ markedly depending on how they are calculated^{69–72}.

Empirical genetic load estimates

Until recently, the approaches described above were applied mainly to model and domesticated species, where genomic data are abundant and the crucial validation of the predicted damage of each variant is frequently possible through functional studies. However, their use in wild non-model species is rapidly increasing (Supplementary Table 1).

Genetic load estimates in model and domesticated species.

Model organisms, such as *Drosophila melanogaster*, have substantially contributed to our understanding of how genetic load manifests in genomes^{73–76}. Experimental validations were used to assess the fitness effects of specific mutations⁷⁶, and often populations containing known harmful mutations were used to shed light on the synergistic epistatic effects and the role of mutations in inbreeding depression^{77,78}.

These genomic studies have started to reveal important insights into the impact of deleterious mutations both in coding and non-coding regions, which have been validated experimentally in some cases. Causative non-coding variants in human diseases are often predicted through scores based on diverse genomic features derived from gene model annotations, evolutionary constraints and functional predictions, such as GERP, GWA and CADD^{31,42,43,79} (TABLE 1; Supplementary Table 1). These multi-source informed predictions have recently been extended to non-human organisms, such as mouse⁴⁴, chicken⁸⁰ and pig⁸¹. The challenge now lies in connecting genome-wide bioinformatics predictions and experimental validation of fitness effects. Meta-analyses in livestock show consistent fitness reduction when genomics-derived inbreeding measurements increase⁸². For example, a 1% increment in inbreeding results in an average decrease of 1.3% for a given trait value^{83,84}. Furthermore, genetic load seems to decrease with haplotype age, suggesting purging of deleterious variants within the breeding population^{82,84,85}. Potentially recessive lethal variants can be identified by screening pedigrees for missing homozygous haplotypes^{86,87}, and causal mutations can be validated by combining functional annotations with carrier × carrier mating. Such variants can greatly compromise population fertility, and lethal recessive haplotypes likely constitute >10% of the genetic causes of stillbirths in purebred pigs^{88,89}.

Besides phenotypic validations of predicted harmful mutations in living organisms, novel developments in

Ultraconserved elements
Highly conserved genomic
regions with (near) identical
nucleotide sequences in
evolutionarily distant taxa.

organoids, single-cell analyses and CRISPR–Cas genome editing greatly enhance our understanding of the functional impact of mutations (reviewed elsewhere^{90,91}). Such studies help to bridge the gap between sequence-derived predictions and fitness-related estimates of genetic load, and their insights may eventually be used to alleviate load in target populations⁹².

Genetic load estimates in wild animals. Recent empirical insights into the genetic load have been obtained using whole-genome data without fitness data in wild animals^{22,51,63,64,93} (Supplementary Table 1). Empirical studies in natural populations show that large ancestral

populations accumulate substantial masked load^{10,51}. During population decline, part of this load is lost by random genetic drift, and part is purged by selection as recessive deleterious mutations with large fitness effects become exposed by inbreeding^{53,56,94}. However, a proportion of the masked load is converted into the realized load, resulting in inbreeding depression¹⁸. Both the type of demographic contraction and the distribution of the fitness effects of mutations play important roles in determining these processes^{10,21}. Harmful variants can become fixed during extreme bottlenecks, but those with larger fitness effects are likely to be purged during long-term declines^{56,67,95–99}. Persistently small,

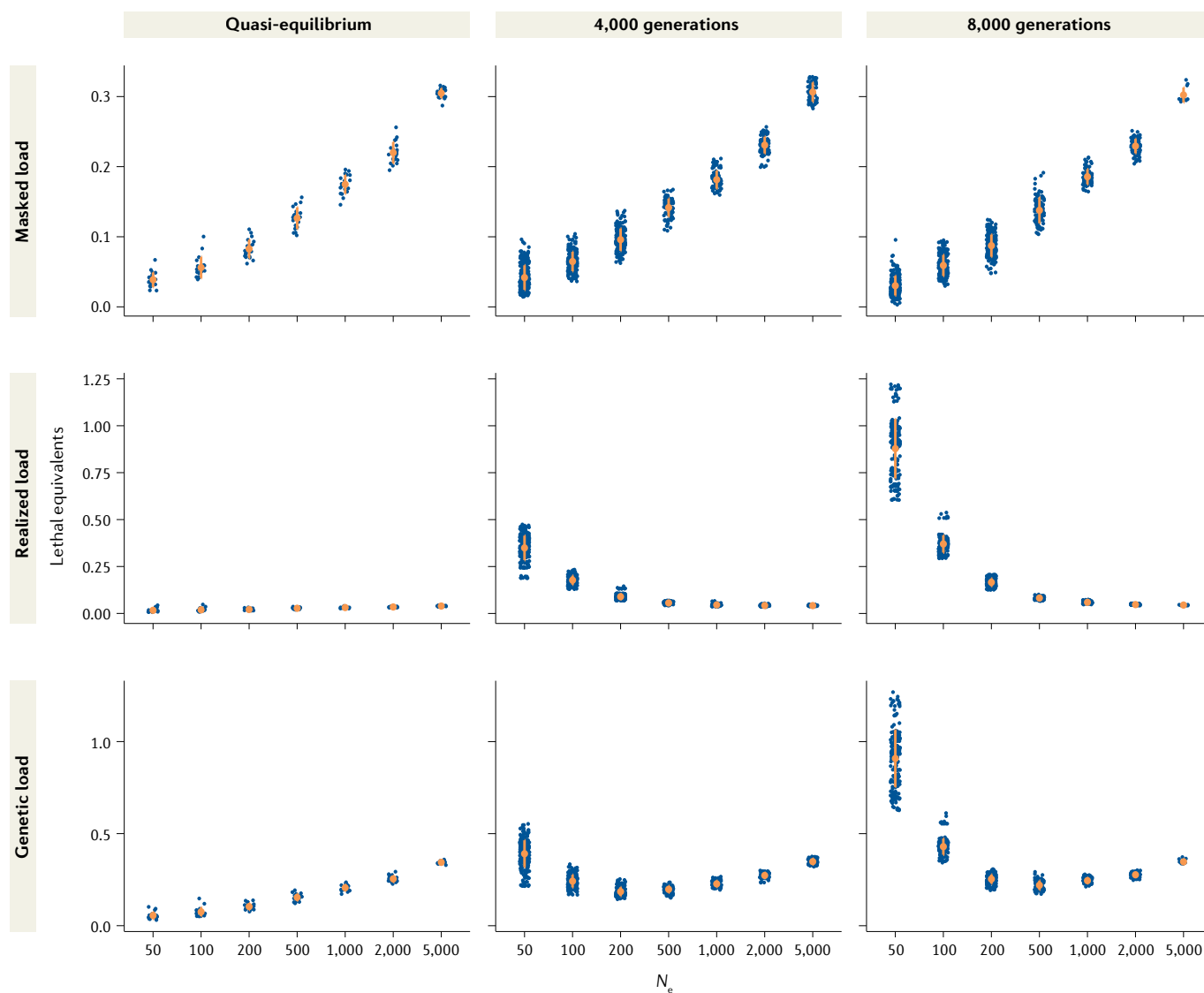


Fig. 1 | Effects of N_e on genetic load partition. Individual-based forward simulations in SLiM¹⁵⁷ of genetic load expressed in lethal equivalents, simulating a gamma distribution of selection (s) and dominance (h) coefficients that reflect empirical observations (Supplementary Information S13). Simulations with constant effective population size (N_e) (yellow dots and bars represent mean and standard deviation across replicates, blue dots indicate individual replicates). Columns refer to populations at quasi-equilibrium (after a burn-in of $2N_e$ generations), and 4,000 and 8,000 generations later, respectively. Note x axis shows N_e rather than generation time, and lethal equivalents plotted on y axis are

quasi-equilibrium values of the load reached after $t = 2N_e$ generations. Masked load increases with N_e and reaches equilibrium after circa $2N_e$ generations (top). By contrast, realized load continues to increase, particularly in small populations (middle). Strong genetic drift across many loci overwhelms the purifying effects of selection in populations with small N_e , resulting in gradual fixation of slightly deleterious mutations¹⁵⁸. Such mutational meltdown generates a steady decline in fitness²⁰ and could even lead to extinction¹⁵⁹. The genetic load (bottom) is the sum of the masked and the realized load, and its behaviour does not reveal relevant dynamics of the components.

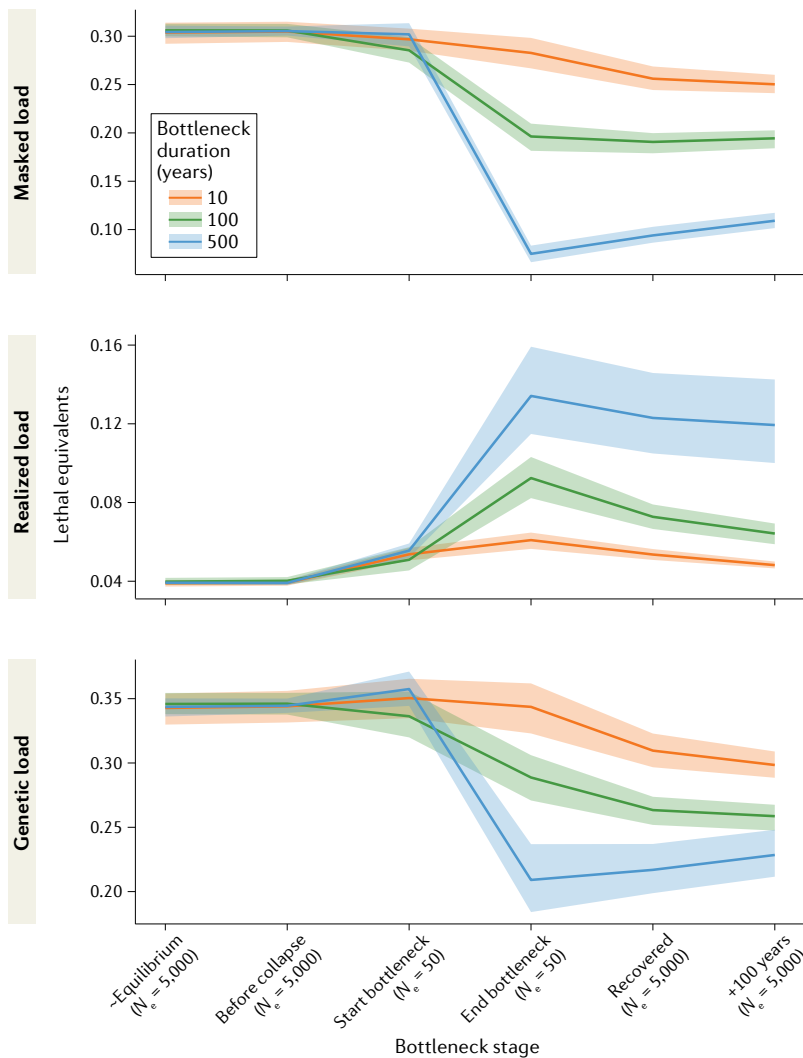


Fig. 2 | Effects of demographic bottlenecks on genetic load partition. Individual-based forward simulations in SLiM¹⁵⁷ of genetic load expressed in lethal equivalents, simulating a gamma distribution of selection (s) and dominance (h) coefficients that reflect empirical observations (Supplementary Information 3). Effects of demographic bottlenecks of different duration ($t = 10, 100$ or 500 years) on genetic load dynamics (average generation 2.6 years). Six stages are shown: quasi-equilibrium at twice the effective population size ($2N_e$) to reach quasi-equilibrium, followed by another 100 years at full N_e , 100 years of exponential collapse, a bottleneck stage ($N_e = 50$) of different duration, 100 years of exponential recovery and another 100 years after recovery. During a bottleneck, inbreeding and drift increase homozygosity, converting masked load into realized load. This load conversion has two main consequences. Firstly, purifying selection purges some (mostly highly) deleterious mutations, thereby reducing genetic load^{20–22}. Secondly, many (mostly mildly) deleterious mutations escape purifying selection, resulting in overall increase of realized load^{20–22}. After demographic recovery, purifying selection reduces realized load. However, prolonged bottleneck results in persistent realized load due to fixation of deleterious mutations. These simulations illustrate the dynamics of load conversion and do not assess fitness or extinction risk¹²⁹. Fitness and population viability of post-bottleneck populations are affected also by factors not simulated in this model, such as overall loss of adaptive variation, compensatory adaptive mutations, correlation between population density and fitness (allele effect), environmental change and loci under balancing selection¹¹¹.

Runs of homozygosity
Long and contiguous regions of a diploid genome where all nucleotide sites are homozygous.

isolated populations are expected to exhibit increased load accumulation due to strong genetic drift^{18,59,62,64}. In this case, highly harmful mutations might be effectively purged when exposed to selection under most

conditions, but mildly deleterious variants can accumulate during demographic collapse^{22,100} (FIG. 2).

The patterns observed in wild animals highlight that the genetic architecture and degree of dominance also determine the fate of deleterious mutations^{21,101}. Accumulation of genetic load has been linked to an increased homozygosity of strongly deleterious recessive alleles^{51,63,93,102} or a rise in frequency of harmful mutations^{53,59}, or both^{58,100}. Within genomes, non-coding regions, chromosomal rearrangements, regions with runs of homozygosity and introgressed loci carrying disadvantageous alleles showed an enrichment of genetic load^{53,59,103,104}.

Remarkably, there is little correlation between the IUCN Red List status of species and their estimated genetic load^{105,106}. Whereas some endangered species have a higher genetic load than their more abundant counterparts^{55,57,58,63,64,67,93,96,98,102}, others seem to have a low load^{51,107}. This poor concordance could be partly explained by the inconsistency amongst studies in estimating and reporting the genetic load, hindering effective conservation management. In addition, on its own, the genetic load is not a particularly informative statistic to assess the genetic health of a population. For example, the number of lethal equivalents calculated using Eq. (1) is not (immediately) affected by inbreeding or genetic drift, as it takes time to purge deleterious mutations¹⁰⁸. Moreover, mildly deleterious mutations are expected to accumulate slowly in populations with a long-term small N_e , counteracting the purifying effects of selection (FIG. 1). Once fixed in a population, these mutations no longer contribute to inbreeding depression¹⁰. Therefore, more informative than merely reporting the total genetic load is to delineate this statistic into its components, that is, the masked load and the realized load. These components capture the current loss in fitness of the population, as well as the predicted future fitness loss caused by deleterious mutations that may lower the population's long-term viability¹⁷. Such information is crucial for correct management decisions, including genetic rescue, assisted gene flow, population supplementation and reintroduction programmes^{10,63,64,109,110}. Conservation geneticists tend to prioritize maximizing genetic diversity rather than minimizing the genetic load¹¹¹, but with better data and understanding of the genetic load, a more balanced approach is likely to improve conservation outcomes¹¹².

Future directions

Towards a standardized use of genomic data to predict load components. Previous studies quantified the genetic load by counting the scores of derived homozygotes twice, and the scores of heterozygotes once^{65,69,97,113} (BOX 2). With estimates of the dominance coefficients, the relative realized load and relative fitness of individuals could be calculated (BOXES 2,3). However, these would be rough approximations, and more accurate estimates of the masked load and the realized load require the identification (and, possibly, the validation with empirical fitness data) of the relationship between deleteriousness scores and selection coefficients, and improved estimates of the dominance coefficients. Such data may

become available — for example, as specific distributions for different bins of selection coefficients^{114–118} — with the whole-genome sequencing of thousands of species^{119–121}. The improved alignments and annotations of large data sets of whole genomes^{42,79,120,122,123} and the integration of phylogenomic and population genomics approaches¹²⁴ will help to rapidly advance the field. Genomic sequences generated for individuals with fitness data across their entire life history, from deceased embryos to healthy adults, could be very useful for validating the genomic measures of realized load. Fitness data of threatened vertebrates and samples collected post-mortem in zoos could provide a largely overlooked, valuable resource for such studies¹²⁵.

The genotype–fitness relationship. The relationship between genome sequence-derived estimates of the load and their fitness consequences is mostly untested and usually relies on previously identified disease-causing mutations in model organisms and humans¹²⁶. Whereas functional studies on specific mutations predicted to be deleterious are important, most potentially harmful mutations will not be experimentally validated (or even explored) in most species. A gap has emerged between quantitative genetic studies and sequence-oriented studies in genetic load investigations. The former focus on direct fitness effects and are often limited to model organisms, and the latter are based on comparative data sets of many species but lack direct fitness estimates. We therefore need improved tools to bioinformatically identify deleterious mutations, such as EVE (evolutionary model of variant effect)¹²⁷, which predicts the pathogenicity of protein variants by modelling the distribution of sequence variation across species, and VIVID, which integrates evolutionary conservation and functional analyses of variants with 3D protein models¹²⁸. In addition, more field-based studies are needed that correlate phenotypic fitness values to the genomic predictions of the load components of individuals in their natural environment. Our understanding of the relationship between load scores, selection and dominance coefficients and fitness effects can be further improved by simulations^{23,85,109,129}.

How ancient DNA data can contribute to genetic load investigations. Empirically, the effects of demographic events, such as bottlenecks, population fragmentation and population size decline, can best be studied using temporal genomic samples (that is, ancient and historical museum-preserved DNA). Time-series genomic data have been successfully used to show how the accumulation of genetic load increases in response to early domestication bottlenecks (the ‘cost of domestication’)^{130,131} and to more recent artificial breeding practices^{50,132}. Comparisons between contemporary and historical samples from museum collections have also shown increases in the number of deleterious mutations associated with higher inbreeding after demographic bottlenecks^{96,98}. Theoretically, only the realized load is expected to increase after a rapid population decline, and, hence, it would be interesting to re-examine these data and calculate the separate load components.

Ancient DNA data from extinct populations and species can yield information on the genetic load dynamics before extinction¹³³. They can also help to test the predicted correlation between increased genetic load and extinction probability¹³⁴, and further our understanding of the genomic signature of population decline and extinction¹³⁵. The assembly of reference genomes from museum samples or from ancient samples represents an important development to avoid the bias introduced when mapping ancient data to evolutionarily distant modern genomes^{136,137}. However, the calling of genotypes from ancient DNA data remains challenging^{138–140}, and may require additional laboratory and computational efforts to confirm the presence of deleterious mutations.

Practical applications in conservation biology. Both ex situ and in situ conservation of endangered species are increasingly implementing genome-level information to more accurately maintain high genetic diversity, maximize the representation of different subpopulations, minimize kinship and inbreeding, assess genetic introgression and preserve local adaptation^{22,63,141}. Future conservation actions could benefit from including proxies of genetic load components, for example, to guide captive breeding¹¹, genetic rescue^{111,129} and reintroduction

Table 1 | Main approaches to predict deleteriousness from genomic data

Approach	Principle	Pros	Cons	Examples
Evolutionary conservation	Mutations at sites with a reduced number of substitutions compared with neutral expectations in multiple alignments are likely harmful	Annotation-free; allows direct comparisons between many species	Alignments among distant species could imply errors and missing regions; computationally intensive	GERP ³¹ , PhyloP ²⁸
Basic annotation, physicochemical properties	Well-known functional effects of mutations in coding regions across species are used to classify a mutation	Alignment-free; simple to use and robust in classifying major classes of deleteriousness (for example, non-synonymous, stop codons, loss of function)	Requires basic annotation; focused on coding regions; numerical scores are not always predicted	SnpEff ³² , Grantham scores ³⁴
Extended annotation	Diverse information (for example, experimental evidence, physicochemical properties and evolutionary conservation) on the predicted harmful effects of variants is weighted and integrated into one metric	Exploits multiple information types	Still largely limited to humans, model and domesticated organisms where multiple data sources are available	PolyPhen ³⁹ , SIFT ³⁸ , ANNOVAR ³³ , VEP ⁴⁰ , CADD ⁴² , GWAVA ⁴³

These approaches are designed principally to quantify the load due to SNPs. Other types of polymorphisms (for example, copy number variation, short tandem repeats, transposable elements) are not necessarily captured with these methods. CADD, combined annotation-dependent depletion; GERP, genomic evolutionary rate profiling.

Scores related to deleteriousness		
Categorical basic (e.g. synonymous/non-synonymous substitution)	Categorical, deleteriousness (e.g. high or moderate effect)	Numerical (e.g. GERP score)
Proxies of the genetic load based on the deleteriousness scores (Most of the indices below can be separately computed at homozygous or heterozygous sites, within different classes of allelic frequencies and within or outside runs of homozygosity)		
Number or proportions of: <ul style="list-style-type: none"> non-synonymous sites derived alleles Ratio between: <ul style="list-style-type: none"> variation at non-synonymous (or zero-fold degenerate) sites and variation at synonymous (or fourfold degenerate) sites number of private mutations in different populations 	Number or proportions of: <ul style="list-style-type: none"> fixed deleterious alleles segregating deleterious alleles Ratio between: <ul style="list-style-type: none"> number of high-damage sites and number of low-damage sites number of sites with deleterious mutations and number of synonymous variants number of private deleterious mutations in different populations number of deleterious alleles in homozygotes versus heterozygotes genotypes SFS only at deleterious alleles	Sum of scores <ul style="list-style-type: none"> Averages across: <ul style="list-style-type: none"> all derived alleles only homozygous loci only highly conserved sites only coding sites

Fig. 3 | Genetic load proxies used with whole genomes in wild animals. ‘Categorical, basic’ refers to very simple annotations of a variant. ‘Categorical, deleteriousness’ refers to partitions where some direct and supported class of damage produced by a variant can be identified. ‘Numerical’ refers to scores with a continuous value for the impact of the substitution. For each category, lower section maps different statistics frequently used to summarize scores in populations or individuals and obtain metrics related to the genetic load. When homozygous or heterozygous genotypes for a derived allele are identified, the ratio between realized load and masked load can be calculated, thereby providing information about the population’s vulnerability to (future) inbreeding. Additional details and references available in the main text and Supplementary Table 1. GERP, genomic evolutionary rate profiling; SFS, site frequency spectrum.

simulating the historic population size and demographic trajectories. Such data can be derived from analyses that use whole-genome sequence data to estimate the time to the most recent common ancestor^{142,143}, and methods to infer N_e from the spectrum of linkage disequilibrium between pairs of loci¹⁴⁴. Furthermore, individual-based models can be used to assess the future conservation needs of species¹⁴⁵.

The classical genetic conservation paradigm that globally high diversity equals a healthy population is currently being debated, and it is increasingly clear that we need to better understand the consequences of the different components (neutral, beneficial or deleterious) of diversity^{10,23,146–148}. Genome-wide diversity correlates positively with higher (current) fitness^{149–151}, and higher diversity is thought to increase the (future) adaptive potential of wild populations¹⁵². Similarly, highly diverse populations typically carry an elevated masked load. For example, computer simulations suggest that high diversity in source populations used for genetic rescue could introduce deleterious variation into the recipient population, ultimately resulting in more inbreeding depression and a greater extinction risk¹²⁹. Such advice goes against decades of conservation wisdom^{147,148,153} and has made the application of genomic simulations in conservation a much-discussed issue¹¹¹. Ultimately, the specific effects of different types of genetic variation on fitness are complex and will depend on the ancestral population size (large versus small)^{17,66}, recent demographic dynamics (for example, severity and duration of bottlenecks)⁹⁹, the expected amount of environmental change^{154,155} and management interventions such as assisted gene flow and genetic rescue^{109,156}. We hope that an improved framework to estimate the genetic load and its components will help to deepen these conversations within the conservation genetic community.

Published online 8 February 2022

programmes¹⁰⁹. We foresee that individual-based modelling approaches will become increasingly important, for example, to assess the genetic load that has accumulated in the ancestral population. This could be inferred by

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Acknowledgements

The authors thank D. Charlesworth and A. Caballero for helpful comments on a previous version of the manuscript. C.v.O. was supported by the Royal Society International Collaborations Award (ICAR1\201194) and the Earth and Life Systems Alliance (ELSA). G.B. and F.R. were supported by the University of Ferrara (Italy). G.B., F.R., A.I. and E.T. were funded by the MIUR PRIN 2017 grant 201794ZXTL to G.B. M.B. was financially supported by the Dutch NWO Veni grant n. 016.Veni.181.050. H.E.M. was funded by an EMBO long-term fellowship (grant 1111-2018) and the European

Union's Horizon 2020 research and innovation programme under a Marie Skłodowska-Curie grant (840519). C.B. is funded by the Wellcome grant WT207492.

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G.B., F.R., C.B., H.E.M. and C.v.O. researched the literature. G.B., F.R., E.T., H.E.M. and C.v.O. substantially contributed to discussions of the content. G.B., F.R., M.B., E.T., H.E.M. and C.v.O. wrote the article. All authors reviewed and/or edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Genetics and the authors thank Katerina Guschanski and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Supplementary information

The online version contains supplementary material available at <https://doi.org/10.1038/s41576-022-00448-x>.

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