Mutation Load: The Fitness of Individuals in Populations Where Deleterious Alleles Are Abundant

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Abstract

Many multicellular eukaryotes have reasonably high per-generation mutation rates. Consequently, most populations harbor an abundance of segregating deleterious alleles. These alleles, most of which are of small effect individually, collectively can reduce substantially the fitness of individuals relative to what it would be otherwise; this is mutation load. Mutation load can be lessened by any factor that causes more mutations to be removed per selective death, such as inbreeding, synergistic epistasis, population structure, or harsh environments. The ecological effects of load are not clear-cut because some conditions (such as selection early in life, sexual selection, reproductive compensation, and intraspecific competition) reduce the effects of load on population size and persistence, but other conditions (such as interspecific competition and load on resource use efficiency) can cause small amounts of load to have strong effects on the population, even extinction. We suggest a series of studies to improve our understanding of the effects of mutation load.

INTRODUCTION

New mutations enter a population each generation. Of those that affect fitness, most are deleterious (Keightley & Lynch 2003). Excluding those with severe (lethal) effects, a deleterious allele can persist for multiple generations before it is eradicated by selection. Meanwhile, other mutations arise, so that deleterious alleles are always present.

Humans are no exception to this phenomenon. The average human carries 250 to 300 loss-of-function mutations (1000 Genomes Project Consortium 2010). Inferences from molecular population genetics data indicate that the average person also carries another several hundred less severely deleterious amino acid variants (Eyre-Walker et al. 2006, Charlesworth & Charlesworth 2010). Based on the amount of sequence constraint across the genome (Eöry et al. 2010), it is reasonable to speculate that the number of deleterious alleles carried at noncoding sites at least matches, and is likely several times greater than, the number at nonsynonymous sites. It is thus reasonable to say that the average human carries well over a thousand deleterious mutations.

The genomics era has greatly improved our ability to assess the abundance of deleterious alleles segregating in populations, but the question remains whether these deleterious alleles matter much or at all. This depends on what it means to "matter." Deleterious alleles are not the stuff of adaptation, so they are sometimes regarded as an uninteresting and unimportant part of the evolutionary process. However, some have argued that deleterious mutations provided the selection pressures that led to a variety of major adaptive phenomena, including diploidy (Otto & Goldstein 1992), recombination and sex (Kondrashov 1988, Keightley & Otto 2006), outcrossing (Lande & Schemske 1985, Charlesworth et al. 1990), secondary sexual traits (Rowe & Houle 1996, Houle & Kondrashov 2002), and various aspects of genomic complexity (Lynch & Conery 2003, Lynch 2007).

However, the simplest and most direct way that one might expect deleterious mutations to matter is by causing a reduction in fitness. The loss of fitness resulting from deleterious alleles maintained by mutation-selection balance is known as mutation load.

WHAT IS MUTATION LOAD?

H.J. Muller (1950) wrote a paper famously titled "Our Load of Mutations," in which he argued that deleterious alleles make a substantial contribution to human mortality and disease and, more generally, discussed the reduction in fitness due to recurrent mutation. According to Crow (1993), the term mutation load arose from Muller's title and has been a part of the evolutionary genetics lexicon ever since. Mutation load is sometimes used loosely to describe a variety of consequences of segregating deleterious alleles including the incidence of genetic disorders and the magnitudes of inbreeding depression and standing genetic variance in fitness resulting from mutational input. However, here we use it in its formal sense to refer to the reduction in fitness due to the presence of deleterious mutations segregating at mutation-selection balance.

This concept traces back to Haldane's (1937) classic theoretical paper, in which he quantified the expected "loss of fitness" resulting from mutation-selection balance. For a simple one-locus model with fitnesses of $W_{AA} = 1$, $W_{Aa} = 1 - hs$, and $W_{aa} = 1 - s$, the equilibrium frequency of the deleterious allele is $q \approx \mu/hs$ (assuming $hs \gg \mu$), where μ is the mutation rate for $A \rightarrow a$. Mean fitness is $\bar{W} = 1 - 2pqhs - q^2 s$, which is closely approximated by $\bar{W} \approx 1 - 2qhs$ when the deleterious allele is rare ($q \ll 1$) and simplifies to $\bar{W} \approx 1 - 2\mu$ at mutation-selection balance. Mutation load is the extent to which individuals are less fit than they would be otherwise because of mutation. Using $\bar{W}_{NoMut} = 1$ to represent the mean fitness in the absence of mutation, mutation load is defined by $L \equiv (\bar{W}_{NoMut} - \bar{W})/\bar{W}_{NoMut}$, which in the one-locus case is $L = 2\mu$.

Haldane extrapolated his single-locus results across the genome by making two simplifying assumptions: no epistasis and no linkage disequilibrium. Under these assumptions, total mean fitness is equal to the product of mean fitnesses with respect to each locus,

$$\bar{W} = \prod \bar{W}_i = \prod (1 - 2\mu_i) \approx \prod e^{-2\mu_i} = e^{-U},$$

where $\bar{W}_i$ is the mean fitness with respect to locus i, and μ_i is the mutation rate for that locus. $U = \Sigma(2\mu_i)$ is the genome-wide rate of deleterious mutation, where the 2 arises because in diploid organisms each individual has two copies of each gene, each with a chance of a mutation. From the equation above, the genome-wide mutation load is $L = 1 - e^{-U}$.

Haldane's prediction for mean fitness $\bar{W} = e^{-U}$ is rather disturbing once we consider realistic values for U. For example, if there is only a single deleterious mutation per genome per generation on average ($U = 1$)—close to the estimate for *Drosophila* (Haag-Liautard et al. 2007)—then mean fitness is less than 40% of what it would be in the absence of mutation. With a recent estimate for humans of $U = 2.2$ (Keightley 2012), Haldane's result predicts an onerous load of 89%. Of course, Haldane's analysis makes a number of assumptions, and below we discuss some of the subsequent development of the theory. Nonetheless, his prediction provides a baseline for thinking about the potential magnitude of mutation load. Was he close to being right? If so, what do such large loads mean?

Before discussing these questions, we return to the one-locus model to clarify some key terms. In this model, the fitness of each genotype is defined relative to the best type. Thus, mean fitness $\bar{W}$, as used in the context of mutation load, refers to the average fitness of individuals relative to the best type; it does not refer to their absolute fitness. Critically, mutation load refers to the reduction in fitness of individuals, not populations, and the relationship between the two can be complex.

In Haldane's model, the extent to which a mutation reduces the mean fitness (i.e., the hs in $\bar{W} \approx 1 - 2qhs$) is exactly the same as the selection pressure that determines the equilibrium allele frequency (i.e., the hs in $q \approx \mu/hs$) so that these two effects of selection cancel each other out, making L independent of s. This has likely contributed to the appeal of mutation load among theoretical population geneticists as it implies that we need to know nothing about the ecology—or even strength—of selection. However, this sentiment is misleading. Haldane's model and most other mutation load models describe population genetics, not demography or ecology. Consequently, we cannot easily link $\bar{W}$ (or L) to any measure of population performance unless we have a specific ecological framework for doing so.

The biggest limitation to empirically measuring mutation load sensu stricto is identifying a mutation-free reference genotype. In fact, such an individual is unlikely to exist if the mutation rate is reasonably high and if most mutations are only weakly deleterious. Without the reference genotype, mutation load cannot be measured. So is the subject worthy of study at all? There are at least three reasons why it is. First, it is interesting to know how much less fit the average individual is than it could be if there were no segregating deleterious alleles. Even if we cannot directly quantify this loss of fitness empirically, we can use theory to inform ourselves about what relevant parameters to measure (e.g., mutation rate, epistasis, inbreeding) so we can estimate the load based on the theoretical models. Second, although we cannot measure absolute load, we should be able to compare the loads under different circumstances (relative load) to test the importance of various factors predicted by theory to influence load. Third, we can study the ecological consequences of load in models and by empirical manipulation of the load.

THE GENOME DELETERIOUS MUTATION RATE, *U*

The most crucial parameter to predicting the mutation load is the genomic deleterious mutation rate, *U*. Several recent reviews provide summaries of estimates of *U* as well as descriptions of the methods by which *U* is estimated and their associated caveats (Baer et al. 2007, Halligan & Keightley 2009). Consequently, we limit our discussion to a few major points. Microbes (with the exception of viruses) tend to have very small mutation rates ($U < 0.01$, Drake et al. 1998), so mutation load is unlikely to be important in these taxa. However, for multicellular plants and animals, recent mutation rate estimates are often above 0.1 and sometimes greater than 1 (Baer et al. 2007, Halligan & Keightley 2009), i.e., well into the range where Haldane would predict a substantial load. For example, recent estimates in some well-studied taxa include: *Caenorhabditis elegans*, $U \approx 0.25$ to 2.5; *Drosophila melanogaster*, $U \approx 1.2$ to 1.4; humans, $U \approx 2.2$ (Denver et al. 2004, 2009; Haag-Liautard et al. 2007; in each case the higher estimates come from Keightley 2012).

The measures of *U* reported above were determined by combining direct measures of the per-nucleotide mutation rate μ, the total genome size *G*, and the fraction of nucleotides constrained by selection *c*; i.e., $U = \mu Gc$ (Kondrashov & Crow 1993). Selective constraint is estimated using between-species comparisons from the proportion of variants of a particular type, for example nonsynonymous amino acid changes that are missing relative to variants presumed to be neutral, such as synonymous changes. Classically, *U* was inferred from the rates of phenotypic changes in fitness of genetic lines maintained without selection; such estimates of *U* are typically much smaller. However, phenotypically based methods underestimate *U* because they are insensitive to mutations that have weak effects on fitness that are difficult to measure in the lab (Halligan & Keightley 2009, Keightley & Halligan 2009). Most mutations have weak effects (Davies et al. 1999, Estes et al. 2004, Bégin & Schoen 2006, Keightley & Eyre-Walker 2007), but they should not be neglected; the per-locus load is independent of *s* as long as the allele is not nearly neutral. The estimates based on $U = \mu Gc$ are not perfect but are likely to be of the correct order of magnitude.

Considerable uncertainty in *U* persists for several reasons including (*a*) ongoing refinements in measuring constraint, especially for noncoding regions, (*b*) measurement error in μ, and (*c*) variation among genotypes in mutation rates. For example, in a single *Drosophila* study (Haag-Liautard et al. 2007), estimates of *U* from three genotypes were 0.66, 0.94, and 2.56. If the species-wide average *U* is closer to the lowest of these estimates, then Haldane's equation gives a load of 48%, whereas with the higher estimate a load of 92% is predicted.

Understanding the intraspecific variation in *U* (Baer et al. 2005, Haag-Liautard et al. 2007, Conrad et al. 2011) is not only necessary to refine our estimates of the species-wide average but is also interesting in its own right. Recent evidence suggests that an individual's mutation rate may be correlated to its fitness (Goho & Bell 2000, Agrawal & Wang 2008, Sharp & Agrawal 2012). This relationship results in a positive feedback loop whereby individuals of low genetic quality transmit mutations at an elevated rate, creating offspring that are of even worse genetic quality. This feedback loop causes average mutation rate to evolve and differentially affects the equilibrium load as well as the extinction risk of populations with different reproductive modes (Agrawal 2002, Shaw & Baer 2011).

FACTORS AFFECTING RELATIVE FITNESS

Rule of Thumb

The calculation of load, as based on Haldane's model, is relatively simple when there is random mating, no linkage disequilibrium, and no gene interaction. When, as in most realistic scenarios,

one or more of these assumptions is violated, the realized load depends on details of selection and reproduction. Fortunately, there are some general principles for understanding how various factors affect load. Extending the earlier work of King (1966), Kondrashov & Crow (1988) provided perhaps the clearest perspective.

At equilibrium, the number of mutations entering the population, U per offspring, must equal the number removed by selection. This selection is manifested through a reduction in relative fitness of loaded genotypes. The "lost" relative fitness, which occurs in the form of death or a partial or complete failure to reproduce, represents the load. From the general model of Kondrashov & Crow, it can be shown that the load is $L = U/(\bar{z} - \bar{y})$, where $\bar{y}$ and $\bar{z}$ are the mean numbers of mutations carried by the winners and losers of selection, respectively. It is easiest to think about this in the special case when selection only acts on survivorship; in that case, the "winners" are the survivors who have the opportunity to reproduce and the "losers" are the individuals who die through selection. More generally, we can calculate $\bar{y}$, the mean number of mutations in winners, by averaging over all individuals weighted by their offspring number, and for losers, $\bar{z}$ can be calculated by weighting each individual by its lost fecundity, i.e., the difference between its fecundity and that possible for an unloaded genotype. If an individual loses all of its fitness due to selection, this is counted as one full "selective death." The difference in the number of mutations between these two groupings of individuals $\bar{z} - \bar{y}$ represents the number of mutations eliminated per selective death. Because the probability of selective death (or, in more general terms, the amount of fitness loss per capita) is the load, L, and the number of mutations removed per selective death is $\bar{z} - \bar{y}$, the average number of mutations eliminated by selection per capita is $(\bar{z} - \bar{y})L$. At equilibrium, the selective loss of deleterious alleles per capita must be balanced by mutation, U. Rearranging, this gives $L = U/(\bar{z} - \bar{y})$. The number of mutations eliminated per selective event (relative to U) can be thought of as the efficiency of selection. When many mutations are eliminated in each selective event, relatively few individuals need to lose fitness in order to balance out the influx of new mutations across the entire population. Thus, any factor that increases the number of mutations eliminated per selective death helps to reduce load. Remember, though, that selective death is just inaccurate shorthand; alleles can be selectively removed through reduced fecundity or lowered mating success, and dying zygotes and offspring are often replaced by healthier siblings. These selective events count as selective deaths without creating visible carnage in the population.

The utility of $L = U/(\bar{z} - \bar{y})$ can be illustrated by considering a simple case. In the single-locus diploid model described above ($U = 2\mu$) with some expression in the heterozygote, a selective loser carries one deleterious allele, whereas selective winners have none, so we obtain $L = 2\mu/(1 - 0) = 2\mu$. In the case of a totally recessive mutation, the deleterious allele remains rare, so selective winners still have, on average, close to zero mutations. However, selective losers carry two deleterious alleles, so $L = 2\mu/(2 - 0) = \mu$, which is the same result obtained by other means (Haldane 1937). In the case of totally recessive mutations, selection is twice as efficient; each selective death removes twice as many mutations, so the load is only half as large as when mutations have heterozygous effects.

Epistasis

Just as interactions between alleles at the same locus (i.e., dominance) can affect the efficiency of selection, so can epistasis (interlocus interaction). When genes interact such that having multiple deleterious alleles together is worse than expected based on their individual effects, i.e., "synergistic" (or negative) epistasis, then selection is more efficient and the load is reduced (Kimura & Maruyama 1966, King 1966, Kondrashov 1982, Charlesworth 1990). Perhaps the simplest case is

one in which individuals carrying less than a threshold number of mutations T have high fitness but those with more than T mutations have low fitness. Immediately following selection, the average number of mutations is somewhat less than T. Through recombination, a substantial frequency of offspring is produced carrying more than T mutations. Consequently, the losers of selection can carry, on average, quite a few more deleterious mutations than the winners; multiple mutations are eliminated with each selective death, substantially reducing the load.

This type of threshold selection is one rather extreme form of epistasis. Others have modeled fitness as a quadratic function of mutation number, implying negative pairwise gene interactions (Charlesworth 1990, Howard & Lively 1998). Such models also show a reduction in load but the effect is less dramatic than in the threshold case. Consistent with these results, Hansen & Wagner (2001) studied a somewhat more general model of epistasis and found that higher-order gene interactions potentially have a stronger influence on the load, especially when mutation rates are high, as the effect of k^{th} order epistasis is proportional to U^k.

The empirical evidence for epistasis is mixed. Studies examining the fitness effects from combinations of small numbers of specific mutations have found a mix of both positive and negative interactions with no strong epistasis on average (vesicular stomatitis virus, Sanjuán et al. 2004; *Escherichia coli*, Elena & Lenski 1997; yeast, Jasnos & Korona 2007; *Aspergillus*, de Visser et al. 1997b; *D. melanogaster*, Whitlock & Bourguet 2000, Wang et al. 2009). The observation that epistasis is highly variable among gene-combinations and with a mean close to zero is consistent with a landscape model of mutational effects (Martin et al. 2007). However, there appears to be an interesting, but somewhat tenuous, pattern across taxa in which multicellular eukaryotes (which have higher U) tend to have more evidence of synergistic epistasis than unicellular organisms (Sanjuán & Elena 2006, Agrawal & Whitlock 2010).

There are several caveats that apply to the data sets mentioned above. First, the experimental procedures bias most studies, to varying degrees, against observing interactions with strong negative epistasis (e.g., synthetic lethals). Second, it is possible that synergistic epistasis only becomes prevalent under particular environmental conditions (King 1967, Kondrashov 1988, Peck & Waxman 2000, Peters & Keightley 2000, Kishony & Leibler 2003). However, studies measuring epistasis in different environments have found no evidence that epistasis becomes more synergistic under stress (Jasnos et al. 2008, Wang et al. 2009). Third, the studies above examine interactions among small numbers of mutations, thereby missing out on higher-order interactions. Yet, studies that presumably examine the effects of larger numbers of (unidentified) mutations also tend to show no epistasis or only weak synergism (Mukai 1969, de Visser et al. 1997a, West et al. 1998, Peters & Keightley 2000). Fourth, most studies examine epistasis among random combinations of mutations. There is no reason to expect any directional epistasis among random mutations (Martin et al. 2007, Agrawal & Whitlock 2010), but there are reasons to expect epistasis among genes within functional pathways (Szathmáry 1993, Keightley 1996, Segrè et al. 2005, Sanjuán & Nebot 2008). Rice (1998) argued that pathway epistasis is ignored by existing empirical studies but could contribute to reducing load. He considered a model in which the genome was divided into 100 pathways with synergistic epistasis occurring among genes within pathways but no epistasis among pathways. Even though epistasis would be observed in only 1% of random mutant pairs (and likely to pass undetected in an experiment), Rice found that total load was greatly reduced.

Asexual Reproduction

When selection is multiplicative, there is no difference in mutation load between asexual and sexual populations (assuming random mating for the latter and ignoring drift). However, epistasis changes the load of sexual populations but does not affect asexual populations (Kimura & Maruyama

1966, Kondrashov & Crow 1988). This difference arises because of the important role of sex and recombination, which reduces linkage disequilibria.

Modifying an approach used by Rice (1998), we can express the load as $L = 1 + \beta\sigma^2/U$, where β is the regression of mutation number on fitness relative to the best type (which will be negative) and σ^2 is the variance in mutation number, which depends on allele frequency as well as linkage disequilibria. Considering various forms of epistasis, Rice (1998) found that, in asexual populations, the disequilibria always evolve in such a way as to compensate for changes made to the fitness function (i.e., so that the product $\beta\sigma^2$ remains constant). With recombination, the disequilibria remain relatively closer to zero regardless of how selection changes, resulting in "mismatches" between selection and the genetic variance, which further result in lower or higher loads, depending on the sign of epistasis. The contrast between how the loads of sexual and asexual populations are affected by epistasis illustrates the potential importance of disequilibria in determining load.

Nonrandom Mating

In many if not most sexual species, mating is not completely random. For example, theory suggests that positive assortative mating should be a common outcome whenever male-male competition or female mate choice is costly (Fawcett & Johnstone 2003). This type of mating expands the variance in number of deleterious alleles per offspring, allowing for a greater difference in mutation number between selection's winners and losers. Rice (1998) found that even weak positive assortative mating for fitness could substantially reduce the load. Correlations in body size between mates are reasonably common in nature but explicit tests of nonrandom mating with respect to fitness have been rare (reviewed in Sharp & Agrawal 2009). Moreover, the relevant correlation is the genetic correlation in fitness between mates, which is likely weaker than any observed phenotypic correlation. More information on genetic correlations is required to assess by how much positive assortative mating reduces load in nature.

Inbreeding is another common form of nonrandom mating. In many taxa, matings occur between relatives more often than expected by chance, often simply due to geography. This creates an excess of homozygotes. Returning to the one-locus model, the losers of selection are more often homozygotes than if mating were truly random, increasing the efficiency of selective deaths, because in inbred individuals two alleles are removed per selective death rather than one. This effect is magnified if deleterious alleles are at least partially recessive because then selection falls disproportionately on the homozygotes, further increasing $\bar{z}$, by a fraction proportional to the inbreeding coefficient or more. This homozygosity effect has been noted by several researchers (Crow & Kimura 1970, Whitlock 2002, Glémin et al. 2003, Roze & Rousset 2004) and can cause a substantial reduction in genome-wide load for reasonably low levels of inbreeding (Agrawal & Chasnov 2001), provided deleterious alleles are partially recessive. The available evidence supports partial recessivity (Simmons & Crow 1977, Phadnis & Fry 2005, Agrawal & Whitlock 2011, Manna et al. 2012) though much of the data comes from a small number of taxa (mostly *D. melanogaster* and *Saccharomyces cerevisiae*) and good estimates of h for typical small-effect genes are lacking.

Spatial Effects

In addition to causing inbreeding, spatial structure may also lead to local competition, which counters the homozygosity effect described above (Whitlock 2002, Glémin et al. 2003, Roze & Rousset 2004). Mutant individuals can be sheltered from selection if they tend to compete for resources against their mutant relatives. This lessens the difference in mutational burden

between the losers and winners of natural selection ($\bar{z} - \bar{y}$), thereby increasing the mutation load. The importance of the "local competition effect" depends strongly on the ecological details of population regulation and the nature of selection on individual genes (Holsinger & Pacala 1990, Agrawal 2010, Laffafian et al. 2010). The local competition effect can be particularly strong if some juveniles die before reaching reproductive maturity but consume limited local resources, making resources unavailable to others. However, load is only increased if surviving mutants can take advantage of the resources made available by the death of their mutant relatives.

The discussion above assumes that selection is the same across space but this need not be the case. Consider mutations that are deleterious in some environments but neutral in others. Kawecki and colleagues (Kawecki 1995, Kawecki et al. 1997) showed that the buildup of such alleles in habitats where they are neutral can hinder populations from adapting to habitats where these alleles would be selectively eliminated. This result arises from an interaction between ecological and evolutionary effects. Moreover, if the demographic contribution from rarely used habitats is low, then selection within such habitats becomes ineffective, allowing the accumulation of alleles with deleterious effects specific to these habitats. If these types of deleterious alleles prevent establishment in marginal habitats, then this would represent a major unseen effect of load.

Roze (2012) argued that spatial variation in the average strength of selection would reduce the load. Variation in selection intensity among demes creates positive linkage disequilibrium, increasing the efficiency of selection. Moreover, the nonlinear relationship between average mutation number and average fitness means that demes receiving good genotypes benefit more than demes receiving bad ones suffer. These effects occur only when some environments are more selective across the genome than others. Though the change in selection between environments is often variable among genes, several experiments (Kishony & Leibler 2003, Jasnos et al. 2008, Wang et al. 2009) have documented reasonably consistent changes (i.e., selection on the majority of genes is stronger in some environments than in others), although this does not necessarily correlate with the quality of the environment. The reasons why certain environments are more selective remain elusive (Martin & Lenormand 2006, Agrawal & Whitlock 2010).

Temporal Effects

Just as selection can vary over space, it can also vary over time. We are unaware of any formal models of load when selection varies over time. The simplest case can be considered as follows. Imagine that every t generations there is an extreme weather event. There are some loci that, if in a mutated state, are lethal during these events but are neutral otherwise. Immediately following one of these events, the population is free of such alleles. Assuming the mutation rate to such loci is U, then by the next event the frequency of individuals carrying one or more of these alleles is $1 - e^{-Ut}$. Calculating the geometric mean fitness over one complete cycle (i.e., $t - 1$ generations without selection and one generation with selection), we obtain $\bar{W} = \sqrt[t]{e^{-Ut}} = e^{-U}$, which is Haldane's classic result. Of course, this average belies the temporal variation in mean fitness, which is maximal ($\bar{W} = 1$) between extreme weather events but quite low during those events ($\bar{W} = e^{-Ut}$). Moreover, temporal variation may be more important in selective contexts beyond the simple scenario described above. For example, if there is synergistic epistasis on small-effect mutations during extreme weather events, then the period between events allows for more rounds of recombination, increasing the efficiency of selection when it next occurs. If such temporal variation in selection is common, point estimates of load could be very misleading.

Genetic Drift

Genetic drift can also affect the amount of mutation load experienced by a population. First, drift can cause the fixation of deleterious alleles, which in itself causes a reduction in fitness called

drift load or finite population load (Crow 1970). This type of load has been considered by some researchers as part of mutation load (Kimura et al. 1963), but not by others (Crow 1970). When drift is strong relative to selection, deleterious alleles can reach fixation provided that the rate of reverse mutation is not too high. The overall genetic load in small populations can be much larger than predicted by deterministic mutation load theory (Kimura et al. 1963, Bataillon & Kirkpatrick 2000, Glémin 2003, Haag & Roze 2007), and such load can potentially even contribute to the extinction of small populations (Lynch et al. 1995). Contrary to intuition, intermediate levels of drift can lead to a net reduction in load from partially recessive alleles because of purging, but this only occurs under very limited combinations of N, h, and s (Kimura et al. 1963, Glémin 2003). Even when drift is strong, the load attributable to segregating mutations (rather than total genetic load) can be reduced simply because the deleterious alleles are fixed rather than segregating.

For alleles that are not nearly neutral, the expected mutation load in a finite population will be similar to the deterministic prediction. However, this is not true at the level of a single locus at any specific time point. In a small population, there may be no deleterious alleles present at any given time, whereas at other loci the frequency of deleterious mutations may be higher than expected by deterministic mutation-selection balance predictions. As a result, it can be very difficult to infer the strength of selection against a particular deleterious mutation in a finite population. However, it is possible to infer distributions of selection coefficients for categories of mutations (e.g., nonsynonymous sites) from population-level sequence data (Eyre-Walker et al. 2006, Keightley & Eyre-Walker 2007, Boyko et al. 2008).

WHEN DOES LOAD HAVE ECOLOGICAL CONSEQUENCES?

It is clear that deleterious mutations can affect the relative fitness of individuals, making them less able to compete against individuals with fewer low-fitness alleles. However, it is not as obvious that such relative declines in fitness affect the absolute mean fitness of the population. Some have implied that a population cannot persist with a heavy mutation load (Kondrashov & Crow 1993, Kondrashov 1995, Nachman & Crowell 2000, Reed & Aquadro 2006). Numerous others (Haldane 1957, Turner & Williamson 1968, Wallace 1968, Mather 1969, Wallace 1970) have argued that mutation load is largely irrelevant to ecology because of density-dependent processes. Wallace (1968, 1975) used the terms soft selection to refer to cases where selective deaths would otherwise be replaced by nonselective (ecological) deaths and hard selection to refer to cases where they would not. Because of the perceived ubiquity of density dependence, many believe selection is typically soft and, therefore, load is ecologically unimportant (i.e., if selection were not removing individuals because of bad genotypes, then more individuals would die because of competition for limited resources). However, the ecological importance of load has remained controversial for several decades, partly because the debate has typically occurred without considering explicit ecological models (but see Clarke 1973a,b).

In fact, ecological processes are unlikely to completely mask the effects of genetic load. The effect of variation in fitness components on ecological success has been studied often (e.g., MacArthur 1972; Schoener 1973, 1976; Abrams 2003; Abrams et al. 2003; Schreiber & Rudolf 2008). These studies have explicitly looked at the effects of resource acquisition rates, fecundity, and survival on equilibrium population size. These models usually find that reductions in fitness components caused a decrease in population size. The key to understanding the effects of load on ecology is intuitive: Load affects a species population size if individuals that die selective deaths remove resources that would otherwise be available to other members of the same species. If an individual dies before consuming any resources and if the resources freed up are consumed by the same species, then load has little effect on population size. However, if a dying individual has

consumed resources without reproduction or if the resources freed up are consumed by another species or otherwise lost, load affects population size.

To frame our discussion, we have made explicit calculations about the ecological effects of load (**Supplemental Text 1**). We use a MacArthur (1972) type model of population growth rather than the logistic model (as used by Clarke 1973a,b) because the parameters of the latter (especially "carrying capacity") can be difficult to interpret in terms of individual-level traits (Matessi & Gatto 1984) and can lead to misleading conclusions.

First consider the case where the consumer population is at low density so that its consumption of resources is limited not by a scarcity of resources but by an upper limit of the consumption rate, C_{max}. The conversion efficiency of resources to offspring is b and the death rate is d. In that case, the proportional change in consumer population size is $bC_{max} - d$. This is equivalent to the intrinsic growth rate of the consumer population. Any decline in consumption rate or conversion efficiency, or increase in death rate, leads to a drop in the ecological performance of the consumer species in this situation. Thus, mutation load in any of these parameters would affect a population's growth rate at low density.

At the other extreme, when the consumption of resources is at equilibrium with the resource input, we have to consider the effect of the consumption on the amount of resources. We take MacArthur's (1972) simplest model for the replenishment of the resources; resource R becomes available at rate I and is depleted only by consumption by this consumer, which happens with acquisition rate a. Therefore the dynamics of the resources are given by $\partial R/\partial t = I - aNR$, where N is the population size of the consumer, and the dynamics of the consumer species are given by $\partial N/\partial t = (baR - d)N$. At equilibrium (i.e., the carrying capacity), N will be

$$\hat{N} = \frac{bI}{d}.$$

$\hat{N}$ is higher with greater conversion efficiency and lower with lower death rate, but it is unaffected by the resource capture rate, a. This is because if an individual captures resources but uses them inefficiently or dies, it eliminates some reproductive potential of the species. If it does not capture resources well, then those resources are still available for other individuals of the species to use. In this simple model, load can have either zero effect on N (in the case of acquisition ability) or cause a proportional reduction in N (for load on birth rate, b, or generation time, $1/d$), depending on what traits are affected.

Real organisms pass through different life stages where they feel the selective effects of different mutant alleles and are subjected to different types of ecological regulation. The timing of selection can greatly affect load. For example, Wallace (1991) suggested that load would not reduce a population's productivity if it occurred early in life, e.g., at the zygote stage. Presumably the logic behind this claim is that dying zygotes have used fewer resources than dying adults, and therefore their competitive effect on conspecifics would be reduced.

To investigate this issue further, we expanded our model to include the possibility of early selective deaths. Imagine L of the population dies due to deleterious alleles each generation and does so before reaching full adulthood. If individuals that die selective deaths consume only a fraction β of the resources that a healthy adult would consume, then it can be shown (**Supplemental Text 1**) that the equilibrium population size is

$$\hat{N} = \frac{bI}{d}\frac{(1-L)}{(1-L(1-\beta))}.$$

Selective deaths of adults after consumption but before reproduction reduce the size of the species in proportion to load. However, if the selective deaths occur early in life, such that β is small, the demographic consequences of load can be greatly reduced, at least at the adult stage.

In situations like the example above, load can reduce the equilibrium abundance of juveniles but have little effect on the abundance of adults if density regulation occurs after selective juvenile mortality and prior to the censusing of adults. Thus, the answer to how greatly load affects abundance depends on what life stage is being censused.

Interspecific Competition

As far as we are aware, all previous explicit discussions of the ecological effects of load focus on the effects of intraspecific competition. However, when organisms compete interspecifically for resources, the ecological effects of load can be much more drastic, as shown in **Supplemental Text 1**. Load on most vital rates can greatly reduce equilibrium population size in the presence of a close competitor. If the two species are sufficiently similar (such that their acquisition rates of various resources are similar), a small increase in load can cause extinction of one species in the presence of a competitor (**Figure 1**).

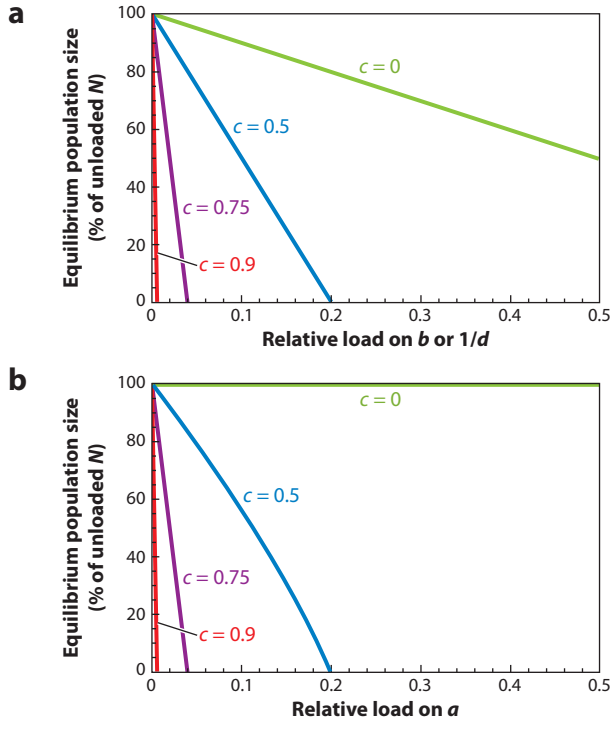

Figure 1

When species compete strongly for resources, a small amount of load in one species can cause a disproportionately large effect in its equilibrium population size. In some cases, relatively small levels of load can cause species extinction. This graph plots the results from the calculations in **Supplemental Text 1** for load in either (*a*) fecundity or longevity or (*b*) acquisition rate. It assumes that two consumer species are identical to each other except that one has a relatively larger load (as plotted on the x-axis) and that the two species are competing for two resources, each of which is better used by one of the species. The effects of load on population size and persistence depend on how ecologically similar the two species are, as measured by c, the relative resource acquisition rate of a species on its suboptimal resource, compared to its preferred resource. (These calculations assume that each species acquires one of the resources better than the other by the same ratio as for the unloaded genotypes.) When this c ratio is high, very small levels of load can cause extinction. Other parameters used for these calculations for the default unloaded genotypes are: $b = 0.02$, $d = 0.1$, $a = 1$, $I_1 = I_2 = 500$.

In the one-species model, the effects of load on the equilibrium abundance of adults could be masked by ecological compensation if load were expressed as juvenile mortality. This type of masking of load is less likely to occur in species limited by interspecific competition. In a two-species, two-stage model with two resources available (see **Supplemental Text 1**), early deaths free up resources, but those resources are available to both species. As a result, the dynamics of the two-species model continue to show similar effects of load regardless of when in the life cycle that load occurs.

Abrams (2003), with a more complicated model of consumer competition including population dynamics of the resource species and type 2 functional responses, similarly showed that the ecological effects of changing vital rates can be large, whether through mutation or other mechanisms. Counterintuitively, a decrease in consumption rates can, in some cases, cause an increase of the population density of that species, if the consumer species previously were overexploiting the resource. The ecological effects of changing life-history parameters can be complicated to predict and are somewhat model dependent. One of our main conclusions is that we need to be ecologically explicit before we can claim to know the effects of load on population size or persistence.

However, four points are clear. First, density-dependent effects do not necessarily compensate for load, i.e., the mere existence of density dependence does not preclude severe demographic consequences of mutation load (see **Supplemental Figures 1** and **2**). Second, load affecting different life-history traits (e.g., resource capture rate versus conversion efficiency) may or may not differ in the ecological consequences, depending on the circumstances. Third, the demographic consequences of load can differ between life stages (e.g., juveniles versus adults). Fourth, in populations limited by strong interspecific competition, the demographic consequences of load can be particularly strong and could result in competitive exclusion.

To evaluate the importance of load in mediating interspecific competition, we first need to ask how likely it is that relative load varies between species. Point estimates for the rates of evolution at synonymous sites between two closely related species of *Drosophila* differ by ~10% (Cutter 2008). A similar difference occurs between related species of *Caenorhabditis* (Cutter 2008). Because neutral evolution is a function of mutation rate per unit time, such differences could reflect differences in either mutation rate per generation or in generation time. With these caveats, though, estimates of rates of mutational decline indicate that related *Caenorhabditis* species (or even strains within species) may vary substantially in mutation load (Baer et al. 2005, Phillips et al. 2009).

The Effects of Correlations among Fitness Components

Of the models we have described, load on fecundity or longevity always has some effect on equilibrium population size, provided that this size is determined by intra- or interspecific competition. With intraspecific competition only, load on resource acquisition ability does not affect population size (assuming no resource decay). However, there is still selection on acquisition rate, even with pure intraspecific competition, meaning that selection should still be effective on this trait even though it does not affect population size. This raises the possibility that selection on acquisition ability could, through pleiotropy, reduce the load on other vital rates. If new mutations that deleteriously affect fecundity or longevity also reduce acquisition ability, then selection on the latter can also make selection indirectly more effective on the former. As a result, the equilibrium frequency of alleles decreasing fecundity or longevity becomes decreased, and load on those vital rates also becomes reduced. Therefore, with intraspecific competition and positive correlations between the effects of alleles on acquisition rate and fecundity or longevity, the effect of total load on equilibrium population size can be reduced by selection on acquisition ability. A similar effect is seen with sexual selection on males when the effects on male mating success are positively

correlated with the effects on female productivity (Whitlock & Agrawal 2009). Sexual selection is a form of intraspecific competition (by males for access to females), so it is not strange that it should behave similarly to the intraspecific competition case presented here.

HOW TO STUDY LOAD EMPIRICALLY

Given that deleterious mutation rates in many multicellular organisms are on the order of $U = 1$ or more (Baer et al. 2007, Halligan & Keightley 2009, Keightley 2012), then there should be substantial mutation loads in these taxa. But are individuals really as much less fit as predicted by Haldane? If not, then why not? Despite the topic being of continued theoretical investigation for more than 70 years, there is very little evidence that Haldane's theory is even approximately correct, even though the predicted effects should be large.

The problem of identifying a mutant-free reference genotype makes measuring the absolute mutation load a near impossible task. However, major tenets of mutation load theory should be testable by comparing individuals from populations that have evolved under different mutation rates. In a number of systems, mutation rate can be manipulated in replicate populations. According to Haldane, if two populations evolved with deleterious mutation rates of U and $U + \Delta U$, then the average fitness of individuals of the second population relative to those of the first should be $e^{-\Delta U}$ (i.e., increasing the mutation rate by $\Delta U = 1$ should result in a ~60% reduction in fitness). By combining manipulations of mutation rate with other types of treatments, experimental evolution could be used to test the roles of sexual selection or population structure in load or whether load affects the outcome of interspecific competition.

However, there are several difficulties in employing this approach. First, the populations should be well adapted initially so that there is little scope for beneficial mutations. (Alternatively, experimental mutagenesis may be stopped after some time followed by a test of whether selection is able to improve fitness by purging the load.) Second, to test the theory quantitatively, it is necessary to estimate the mutation rate in each treatment. Modern advances in sequencing allow direct estimates of the total mutation rate to be obtained more easily. However, discerning the fraction of mutations that are deleterious in the lab is difficult (Davies et al. 1999, Bégin & Schoen 2006). Classic mutation accumulation studies with fitness measures may need to be combined with direct estimates of μ to determine the deleterious mutation rate under each treatment. Third, it can take a long time to approach mutation-selection balance. For example, if the mutation rate is increased by $\Delta U = 1$ deleterious mutation per genome per generation, then it would take ~120 generations for fitness to decline 80% of its expected amount if $s = 0.01$ (and longer if s or ΔU is smaller). However, by obtaining a time series of data, one could infer the equilibrium load after tens or hundreds of generations even if equilibrium is not reached. Finally, adaptation to the experimental mutagenesis procedure may introduce unwanted differences between populations (Nothel 1987). In principle, this can be tested by comparing the sensitivity to mutagenesis in control populations with that in populations that have evolved with it.

Despite these difficulties, experimental evolution offers the most direct way to test mutation load theory. However, few studies have employed this approach (Sankaranarayanan 1964, 1965; Tobari & Murata 1970a,b; Springman et al. 2010), and all suffer from poor replication or lack of proper controls (see also Anderson et al. 2004). Perhaps the best study was conducted by Bruce Wallace. He maintained populations of *D. melanogaster* with and without chronic exposure to gamma radiation for ~120–150 generations (Wallace 1956, 1959, 1991). Though he collected an impressive amount of data over a four-year period, he had several treatments and none of them were replicated, so it is difficult to draw strong conclusions. Taken at face value, the observed fitness reductions were 5–30% (depending on which fitness measure and which reference are

used). Unfortunately, it is impossible to assess whether these numbers accord with Haldane's expectation because there is no quantitative estimate of how much radiation elevated the mutation rate (ΔU). Though such studies are challenging, there are clearly a number of fundamental issues that remain to be tested using experimental evolution.

Although direct testing of load remains the primary challenge, there are other avenues requiring further exploration. Mutation rate is the most important parameter affecting load, and sequencing technologies are making this easier to measure. We can now begin more detailed studies of the variation in mutation rate both within species as well as between closely related (and potentially competing) species. Predicting ecological consequences of load will also require an understanding of how different types of ecologically relevant traits, including male reproductive success (Mack et al. 2000, Whitlock & Agrawal 2009, Mallet et al. 2011), are affected by mutation. The extent of pleiotropy between different traits is a key issue but will be challenging to resolve for typical mutations of small effect. Finally, the nature of selection with respect to both gene interaction (epistasis, dominance) and environmental effects (whether selection changes predictably across the genome) will continue to be an issue of importance.

RELEVANCE OF LOAD IN BASIC AND APPLIED PROBLEMS

By its formal definition, mutation load refers to the reduction in the average fitness of individuals due to mutation. As such, it is relevant to those situations where we are interested in the absolute fitness of individuals relative to what it might be otherwise. One obvious area of concern is human health, as highlighted by Muller's classic 1950 paper, as well as by several prominent papers since (Crow 1997, Lynch 2010). To some extent, mutation load is not a treatable problem because it is so diffuse (i.e., many thousands of rare deleterious alleles of small effect affecting fitness in a variety of ways). Nonetheless, Muller (1950) and Crow (1997) warned against escalating the problem by increasing the mutation rate, either through increased exposure to mutagens in our environment or by shifting reproduction to later ages when mutation rates are higher. They also pointed out that the relaxation of selection by medical intervention allows for the accumulation of more mutations. In the long term, the realized mutation load is only reduced so long as medical technology continues to outpace the higher frequency of deleterious alleles.

Of course, mutation load is also relevant to the health of other organisms with high genomic mutation rates. Mutation load likely affects equilibrium population densities and could play a role in determining the outcome of interspecific competition. As such, mutation load may be subject to species-level selection. Selection among species is the stock-in-trade of community ecology, but it has had a more mixed reception in evolutionary biology (Whitlock 1996). Nonetheless, this idea is at the root of theoretical models using load-based explanations to provide an advantage of sexual populations over obligately asexual populations (Kondrashov 1982, Charlesworth 1990, Agrawal 2001, Agrawal & Chasnov 2001, Siller 2001) but is equally applicable to ecological competition between related sexual taxa. For example, Kawecki (1994) suggested that the evolution of ecological specialization could be enhanced by the greater genetic load of generalists (see also Fry 1996, Holt 1996, Whitlock 1996). Mutation load can also be a conservation concern because load (*a*) lowers initial vital rates and (*b*) is the source of the segregating deleterious alleles that can drift to fixation (i.e., the conversion of mutation load to drift load), potentially leading to mutational meltdowns (Lynch et al. 1995).

Recently, the possibility of using mutation load as a means of treating bacterial and viral infection has been studied (Loeb et al. 1999, Eigen 2002, Anderson et al. 2004, Bull et al. 2007, Bull & Wilke 2008, Martin & Gandon 2010). In lethal mutagenesis, drugs target pathogen DNA or RNA replication systems, increasing the mutation rate and driving up the load. If the load becomes

sufficiently high, pathogens can be driven to lower densities or to extinction. Both theoretical (Eigen 2002, Bull et al. 2007, Bull & Wilke 2008, Martin & Gandon 2010) and empirical (Loeb et al. 1999, Crotty et al. 2001, Anderson et al. 2004) studies support the potential utility of this idea. As with other disease treatments, the evolution of resistance is possible, though it may be selected less directly than in response to typical treatments (Freistadt et al. 2004, Martin & Gandon 2010).

If we use the term mutation load more liberally to refer to the consequences of mutation-selection balance, then mutation load is relevant to a whole host of other issues, including inbreeding depression and the evolution of outcrossing (Lande & Schemske 1985, Charlesworth et al. 1990), the maintenance of genetic variance in fitness (Charlesworth & Hughes 1999), the evolution of sexually selected male traits and female preference (Rowe & Houle 1996, Houle & Kondrashov 2002), and rates of adaptation under the influence of background selection (Charlesworth 2012).

CONCLUSIONS: THE SUSTAINABILITY OF LOAD

Given current estimates of U, classical theory would predict large loads for many multicellular eukaryotes. Haldane's theory applied with a recent estimate of $U = 2.2$ (Keightley 2012) would predict the average person to be ~90% less fit than a mutant-free competitor (i.e., $L \approx 0.9$). Some have argued (e.g., Kondrashov 1988, Crow 1997, Nachman & Crowell 2000) that the high load predicted by Haldane's calculation is incompatible with the continued persistence of a species such as ours, which has a relatively low reproductive capacity. The conclusion, some researchers claim, is that mutations must act nonmultiplicatively to reconcile the high genomic mutation rate with the continued success of the human population. Although we agree that the increased efficiency of selection under some models of epistasis and inbreeding can play an important role in determining the exact value of load, we also argue that there is no reason to say that the load predicted by Haldane's equation is not sustainable by humans.

First, remember that the low reproductive capacity of humans is for typical (loaded) individuals, not unloaded ones. It is a mistake to ask whether our current population could withstand a further 90% reduction in fitness because that would be applying the load twice. More importantly, we have emphasized that load has no direct relationship to population abundance or persistence. Instead, mutation load refers to the reduction in fitness of individuals, not populations, relative to a mutation-free reference genotype. To ask whether a given amount of load is plausible, we can ask what mutation load predicts the fitness of an unloaded individual to be.

Some have skeptically asked, "Is it reasonable that an unloaded genotype placed into the current population would produce $1/(1 - L) \approx 10$ times as many offspring as the average?" It is difficult to envision what a mutant-free hominid would be capable of given that it is unlikely that a hominid with fewer than 1,000 deleterious alleles has ever existed (see Introduction, above). Even limiting ourselves to data, it is clear that human reproductive capacity is greater than what happens on average. For example, to sustain a population, each female needs to produce on average only two adult offspring, whereas the world record is 67 (Glenday 2010), which is greater than predicted for the mean of an unloaded genotype, and males have the capacity for even greater reproductive success. (Remember, load can affect fitness through any fitness component, so the fitness deficit may be expressed through lowered fecundity and lower mating success, as well as through mortality.) Nevertheless, physiological constraints on reproductive capacity may limit the fitness of an ideal genotype so that it is only slightly better than a typical genotype when both are given unlimited resources. However, such constraints do not set an upper limit on load.

The question posed at the beginning of the previous paragraph is not quite correct because it focuses on putting an unloaded individual into a loaded population rather than the reverse. The load as calculated under Haldane's assumption more accurately reflects how much less fit the

average individual would be than its neighbors if it were placed into a population consisting only of mutant-free genotypes. In competition for limited resources (e.g., food, shelter, mates) against a population of unloaded individuals, a typical, heavily loaded individual may do very poorly, being only one-tenth (or less) as fit. In other words, a high maximal absolute fitness of the ideal genotype is not needed to explain a high load. Rather, we need only that the ideal genotype be much more fit than the typical individual when in competition, which can result from the low absolute fitness of the latter rather than the high fitness of the former. In principle, a load of any magnitude is compatible with species persistence because heavily loaded individuals can have high absolute fitness when competing against one another, even though each would have negligible fitness if forced to compete in a population of unloaded individuals.

The idea that species cannot persist with high loads, independent of other assumptions, is incorrect. However, load can reduce population sizes (even with density-dependent regulation) and possibly cause extinction, but the magnitude of these effects depends heavily on other assumptions. As we argue above, we know that populations can persist with their current values of load, but this does not imply that populations with even greater loads could persist in the face of competition with other, possibly less loaded, species.

Because deleterious mutations happen, individuals must be less fit than they could be. In principle, we could infer how fit individuals might be if we knew how load worked, but we do not. We do not even know whether mutation rate affects fitness in the manner Haldane predicted 70 years ago. If it does not, then why? Regardless, large loads have the potential to have ecological consequences. Is load (or relative loads) an important aspect of ecology? These are some of the simplest, yet most pressing, questions regarding mutation load that have remained unanswered for decades. Hopefully, they will not remain so for decades more.

DISCLOSURE STATEMENT

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LITERATURE CITED

1000 Genomes Project Consortium. 2010. A map of human genome variation from population-scale sequencing. *Nature* 467:1061–73

Abrams PA. 2003. Effects of altered resource consumption rates by one consumer species on a competitor. *Ecol. Lett.* 6:550–55

Abrams PA, Brassil CE, Holt RD. 2003. Dynamics and responses to mortality rates of competing predators undergoing predator-prey cycles. *Theor. Popul. Biol.* 64:163–76

Agrawal AF. 2001. Sexual selection and the maintenance of sexual reproduction. *Nature* 411:692–95

Agrawal AF. 2002. Genetic loads under fitness-dependent mutation rates. *J. Evol. Biol.* 15:1004–10

Agrawal AF. 2010. Ecological determinants of mutation load and inbreeding depression in subdivided populations. *Am. Nat.* 176:111–22

Agrawal AF, Chasnov JR. 2001. Recessive mutations and the maintenance of sex in structured populations. *Genetics* 158:913–17

Agrawal AF, Wang AD. 2008. Increased transmission of mutations by low-condition females: evidence for condition-dependent DNA repair. *PLoS Biol.* 6:e30
Agrawal AF, Whitlock MC. 2010. Environmental duress and epistasis: How does stress affect the strength of selection on new mutations? *Trends Ecol. Evol.* 25:450–58
Agrawal AF, Whitlock MC. 2011. Inferences about the distribution of dominance drawn from yeast gene knockout data. *Genetics* 187:553–66
Anderson JP, Daifuku R, Loeb LA. 2004. Viral error catastrophe by mutagenic nucleosides. *Annu. Rev. Microbiol.* 58:183–205
Baer CF, Miyamoto MM, Denver DR. 2007. Mutation rate variation in multicellular eukaryotes: causes and consequences. *Nat. Rev. Genet.* 8:619–31
Baer CF, Shaw F, Steding C, Baumgartner M, Hawkins A, et al. 2005. Comparative evolutionary genetics of spontaneous mutations affecting fitness in rhabditid nematodes. *Proc. Natl. Acad. Sci. USA* 102:5785–90
Bataillon T, Kirkpatrick M. 2000. Inbreeding depression due to mildly deleterious mutations in finite populations: Size does matter. *Genet. Res.* 75:75–81
Bégin M, Schoen DJ. 2006. Low impact of germline transposition on the rate of mildly deleterious mutation in *Caenorhabditis elegans*. *Genetics* 174:2129–36
Boyko AR, Williamson SH, Indap AR, Degenhardt JD, Hernandez RD, et al. 2008. Assessing the evolutionary impact of amino acid mutations in the human genome. *PLoS Genet.* 4:e1000083
Bull JJ, Sanjuán R, Wilke CO. 2007. Theory of lethal mutagenesis for viruses. *J. Virol.* 81:2930–39
Bull JJ, Wilke CO. 2008. Lethal mutagenesis of bacteria. *Genetics* 180:1061–70
Charlesworth B. 1990. Mutation-selection balance and the evolutionary advantage of sex and recombination. *Genet. Res.* 55:199–221
Charlesworth B. 2012. The effects of deleterious mutations on evolution at linked sites. *Genetics* 190:5–22
Charlesworth B, Charlesworth D. 2010. *Elements of Evolutionary Genetics*. Greenwood Village, CO: Roberts and Co.
Charlesworth B, Hughes KA. 1999. The maintenance of genetic variation in life-history traits. In *Evolutionary Genetics: From Molecules to Morphology*, ed. RS Singh, CB Krimbas, pp. 369–92. Cambridge, UK: Cambridge Univ. Press
Charlesworth D, Morgan MT, Charlesworth B. 1990. Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution* 44:1469–89
Clarke B. 1973a. Effect of mutation on population size. *Nature* 242:196–97
Clarke B. 1973b. Mutation and population size. *Heredity* 31:367–79
Conrad DF, Keebler JEM, DePristo MA, Lindsay SJ, Zhang YJ, et al. 2011. Variation in genome-wide mutation rates within and between human families. *Nat. Genet.* 43:712–14
Crotty S, Cameron CE, Andino R. 2001. RNA virus error catastrophe: direct molecular test by using ribavirin. *Proc. Natl. Acad. Sci. USA* 98:6895–900
Crow JF. 1970. Genetic loads and the cost of natural selection. In *Mathematical Topics in Population Genetics*, ed. K-I Kojima, pp. 128–77. Berlin: Springer-Verlag
Crow JF. 1993. Mutation, mean fitness, and genetic load. In *Oxford Surveys in Evolutionary Biology*, ed. D Futuyma, J Antonovics, pp. 3–42. Oxford, UK: Oxford Univ. Press
Crow JF. 1997. The high spontaneous mutation rate: Is it a health risk? *Proc. Natl. Acad. Sci. USA* 94:8380–86
Crow JF, Kimura M. 1970. *An Introduction to Population Genetics Theory*. Minneapolis: Burgess Publ.
Cutter AD. 2008. Divergence times in *Caenorhabditis* and *Drosophila* inferred from direct estimates of the neutral mutation rate. *Mol. Biol. Evol.* 25:778–86
Davies EK, Peters AD, Keightley PD. 1999. High frequency of cryptic deleterious mutations in *Caenorhabditis elegans*. *Science* 285:1748–51
de Visser JAGM, Hoekstra RF, van den Ende H. 1997a. An experimental test for synergistic epistasis and its application in *Chlamydomonas*. *Genetics* 145:815–19
de Visser JAGM, Hoekstra RF, van den Ende H. 1997b. Test of interaction between genetic markers that affect fitness in *Aspergillus niger*. *Evolution* 51:1499–505
Denver DR, Dolan PC, Wilhelm LJ, Sung W, Lucas-Lledo JI, et al. 2009. A genome-wide view of *Caenorhabditis elegans* base-substitution mutation processes. *Proc. Natl. Acad. Sci. USA* 106:16310–14

Denver DR, Morris K, Lynch M, Thomas WK. 2004. High mutation rate and predominance of insertions in the *Caenorhabditis elegans* nuclear genome. *Nature* 430:679–82

Drake JW, Charlesworth B, Charlesworth D, Crow JF. 1998. Rates of spontaneous mutation. *Genetics* 148:1667–86

Eigen M. 2002. Error catastrophe and antiviral strategy. *Proc. Natl. Acad. Sci. USA* 99:13374–76

Elena SF, Lenski RE. 1997. Test of synergistic interactions among deleterious mutations in bacteria. *Nature* 390:395–98

Eöry L, Halligan DL, Keightley PD. 2010. Distributions of selectively constrained sites and deleterious mutation rates in the hominid and murid genomes. *Mol. Biol. Evol.* 27:177–92

Estes S, Phillips PC, Denver DR, Thomas WK, Lynch M. 2004. Mutation accumulation in populations of varying size: the distribution of mutational effects for fitness correlates in *Caenorhabditis elegans*. *Genetics* 166:1269–79

Eyre-Walker A, Woolfit M, Phelps T. 2006. The distribution of fitness effects of new deleterious amino acid mutations in humans. *Genetics* 173:891–900

Fawcett TW, Johnstone RA. 2003. Mate choice in the face of costly competition. *Behav. Ecol.* 14:771–79

Freistadt MS, Meades GD, Cameron CE. 2004. Lethal mutagens: broad-spectrum antivirals with limited potential for development of resistance? *Drug Resist. Updat.* 7:19–24

Fry JD. 1996. The evolution of host specialization: Are trade-offs overrated? *Am. Nat.* 148:S84–107

Glémin S. 2003. How are deleterious mutations purged? Drift versus nonrandom mating. *Evolution* 57:2678–87

Glémin S, Ronfort J, Bataillon T. 2003. Patterns of inbreeding depression and architecture of the load in subdivided populations. *Genetics* 165:2193–212

Glenday C, ed. 2010. *Guinness World Records 2011*. New York: Bantam Books

Goho S, Bell G. 2000. Mild environmental stress elicits mutations affecting fitness in *Chlamydomonas*. *Proc. R. Soc. B* 267:123–29

Haag CR, Roze D. 2007. Genetic load in sexual and asexual diploids: segregation, dominance and genetic drift. *Genetics* 176:1663–78

Haag-Liautard C, Dorris M, Maside X, Macaskill S, Halligan DL, et al. 2007. Direct estimation of per nucleotide and genomic deleterious mutation rates in *Drosophila*. *Nature* 445:82–85

Haldane JBS. 1937. The effect of variation on fitness. *Am. Nat.* 71:337–49

Haldane JBS. 1957. The cost of natural selection. *J. Genet.* 55:511–24

Halligan DL, Keightley PD. 2009. Spontaneous mutation accumulation studies in evolutionary genetics. *Annu. Rev. Ecol. Evol. Syst.* 40:151–72

Hansen TF, Wagner GP. 2001. Epistasis and the mutation load: a measurement-theoretical approach. *Genetics* 158:477–85

Holsinger KE, Pacala SW. 1990. Multiple-niche polymorphisms in plant populations. *Am. Nat.* 135:301–9

Holt RD. 1996. Adaptive evolution in source-sink environments: direct and indirect effects of density-dependence on niche evolution. *Oikos* 75:182–92

Houle D, Kondrashov AS. 2002. Coevolution of costly mate choice and condition-dependent display of good genes. *Proc. R. Soc. B* 269:97–104

Howard RS, Lively CM. 1998. The maintenance of sex by parasitism and mutation accumulation under epistatic fitness functions. *Evolution* 52:604–10

Jasnos L, Korona R. 2007. Epistatic buffering of fitness loss in yeast double deletion strains. *Nat. Genet.* 39:550–54

Jasnos L, Tomala K, Paczesniak D, Korona R. 2008. Interactions between stressful environment and gene deletions alleviate the expected average loss of fitness in yeast. *Genetics* 178:2105–11

Kawecki TJ. 1994. Accumulation of deleterious mutations and the evolutionary cost of being a generalist. *Am. Nat.* 144:833–38

Kawecki TJ. 1995. Demography of source-sink populations and the evolution of ecological niches. *Evol. Ecol.* 9:38–44

Kawecki TJ, Barton NH, Fry JD. 1997. Mutational collapse of fitness in marginal habitats and the evolution of ecological specialisation. *J. Evol. Biol.* 10:407–29

Keightley PD. 1996. Metabolic models of selection response. *J. Theor. Biol.* 182:311–16

Keightley PD. 2012. Rates and fitness consequences of new mutations in humans. *Genetics* 190:295–304
Keightley PD, Eyre-Walker A. 2007. Joint inference of the distribution of fitness effects of deleterious mutations and population demography based on nucleotide polymorphism frequencies. *Genetics* 177:2251–61
Keightley PD, Halligan DL. 2009. Analysis and implications of mutational variation. *Genetica* 136:359–69
Keightley PD, Lynch M. 2003. Toward a realistic model of mutations affecting fitness. *Evolution* 57:683–85
Keightley PD, Otto SP. 2006. Interference among deleterious mutations favours sex and recombination in finite populations. *Nature* 443:89–92
Kimura M, Maruyama T. 1966. The mutational load with epistatic interactions in fitness. *Genetics* 54:1337–51
Kimura M, Maruyama T, Crow JF. 1963. Mutation load in small populations. *Genetics* 48:1303–12
King JL. 1966. The gene interaction component of genetic load. *Genetics* 53:403–13
King JL. 1967. Continuously distributed factors affecting fitness. *Genetics* 55:483–92
Kishony R, Leibler S. 2003. Environmental stresses can alleviate the average deleterious effect of mutations. *J. Biol.* 2:14
Kondrashov AS. 1982. Selection against harmful mutations in large sexual and asexual populations. *Genet. Res.* 40:325–32
Kondrashov AS. 1988. Deleterious mutations and the evolution of sexual reproduction. *Nature* 336:435–40
Kondrashov AS. 1995. Contamination of the genome by very slightly deleterious mutations: Why have we not died 100 times over? *J. Theor. Biol.* 175:583–94
Kondrashov AS, Crow JF. 1988. King's formula for the mutation load with epistasis. *Genetics* 120:853–56
Kondrashov AS, Crow JF. 1993. A molecular approach to estimating the human deleterious mutation rate. *Hum. Mutat.* 2:229–34
Laffafian A, King JD, Agrawal AF. 2010. Variation in the strength and softness of selection on deleterious mutations. *Evolution* 64:3232–41
Lande R, Schemske DW. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39:24–40
Loeb LA, Essigmann JM, Kazazi F, Zhang J, Rose KD, Mullins JI. 1999. Lethal mutagenesis of HIV with mutagenic nucleoside analogs. *Proc. Natl. Acad. Sci. USA* 96:1492–97
Lynch M. 2007. *The Origins of Genome Architecture*. Sunderland, MA: Sinauer Assoc.
Lynch M. 2010. Rate, molecular spectrum, and consequences of human mutation. *Proc. Natl. Acad. Sci. USA* 107:961–68
Lynch M, Conery JS. 2003. The origins of genome complexity. *Science* 302:1401–4
Lynch M, Conery J, Bürger R. 1995. Mutation accumulation and the extinction of small populations. *Am. Nat.* 146:489–518
MacArthur RH. 1972. *Geographical Ecology*. New York: Harper and Row
Mack PD, Lester VK, Promislow DEL. 2000. Age-specific effects of novel mutations in *Drosophila melanogaster*. II. Fecundity and male mating ability. *Genetica* 110:31–41
Mallet MA, Bouchard JM, Kimber CM, Chippindale AK. 2011. Experimental mutation-accumulation on the X chromosome of *Drosophila melanogaster* reveals stronger selection on males than females. *BMC Evol. Biol.* 11:156
Manna F, Martin G, Lenormand T. 2012. Fitness landscapes: an alternative theory for the dominance of a mutation. *Genetics* 189:923–37
Martin G, Elena SF, Lenormand T. 2007. Distributions of epistasis in microbes fit predictions from a fitness landscape model. *Nat. Genet.* 39:555–60
Martin G, Gandon S. 2010. Lethal mutagenesis and evolutionary epidemiology. *Philos. Trans. R. Soc. B* 365:1953–63
Martin G, Lenormand T. 2006. The fitness effect of mutations across environments: a survey in light of fitness landscape models. *Evolution* 60:2413–27
Matessi C, Gatto M. 1984. Does *K*-selection imply prudent predation? *Theor. Popul. Biol.* 25:347–63
Mather K. 1969. Selection through competition. *Heredity* 24:529–49
Mukai T. 1969. Genetic structure of natural populations of *Drosophila melanogaster*. VII. Synergistic interaction of spontaneous mutant polygenes controlling viability. *Genetics* 61:749–61
Muller HJ. 1950. Our load of mutations. *Am. J. Hum. Genet.* 2:111–76

Nachman MW, Crowell SL. 2000. Estimate of the mutation rate per nucleotide in humans. *Genetics* 156:297–304

Nothel H. 1987. Adaptation of *Drosophila melanogaster* populations to high mutation pressure: evolutionary adjustment of mutation rates. *Proc. Natl. Acad. Sci. USA* 84:1045–49

Otto SP, Goldstein DB. 1992. Recombination and the evolution of diploidy. *Genetics* 131:745–51

Peck JR, Waxman D. 2000. Mutation and sex in a competitive world. *Nature* 406:399–404

Peters AD, Keightley PD. 2000. A test for epistasis among induced mutations in *Caenorhabditis elegans*. *Genetics* 156:1635–47

Phadnis N, Fry JD. 2005. Widespread correlations between dominance and homozygous effects of mutations: implications for theories of dominance. *Genetics* 171:385–92

Phillips N, Salomon M, Custer A, Ostrow D, Baer CF. 2009. Spontaneous mutational and standing genetic (co)variation at dinucleotide microsatellites in *Caenorhabditis briggsae* and *Caenorhabditis elegans*. *Mol. Biol. Evol.* 26:659–69

Reed FA, Aquadro CF. 2006. Mutation, selection and the future of human evolution. *Trends Genet.* 22:479–84

Rice WR. 1998. Requisite mutational load, pathway epistasis and deterministic mutation accumulation in sexual versus asexual populations. *Genetica* 102–103:71–81

Rowe L, Houle D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. B* 263:1415–21

Roze D. 2012. Spatial heterogeneity in the strength of selection against deleterious alleles may strongly reduce the mutation load. *Heredity* 109:137–45

Roze D, Rousset FO. 2004. Joint effects of self-fertilization and population structure on mutation load, inbreeding depression and heterosis. *Genetics* 167:1001–15

Sanjuán R, Elena SF. 2006. Epistasis correlates to genomic complexity. *Proc. Natl. Acad. Sci. USA* 103:14402–5

Sanjuán R, Moya A, Elena SF. 2004. The contribution of epistasis to the architecture of fitness in an RNA virus. *Proc. Natl. Acad. Sci. USA* 101:15376–79

Sanjuán R, Nebot MR. 2008. A network model for the correlation between epistasis and genomic complexity. *PLoS ONE* 3:e2663

Sankaranarayanan K. 1964. Genetic loads in irradiated experimental populations of *Drosophila melanogaster*. *Genetics* 50:131–50

Sankaranarayanan K. 1965. Further data on the genetic loads in irradiated experimental populations of *Drosophila melanogaster*. *Genetics* 52:153–64

Schoener TW. 1973. Population growth regulated by intraspecific competition for energy or time: some simple representations. *Theor. Popul. Biol.* 4:56–84

Schoener TW. 1976. Alternatives to Lotka-Volterra competition: models of intermediate complexity. *Theor. Popul. Biol.* 10:309–33

Schreiber S, Rudolf VHW. 2008. Crossing habitat boundaries: coupling dynamics of ecosystems through complex life cycles. *Ecol. Lett.* 11:576–87

Segrè D, DeLuna A, Church GM, Kishony R. 2005. Modular epistasis in yeast metabolism. *Nat. Genet.* 37:77–83

Sharp NP, Agrawal AF. 2009. Sexual selection and the random union of gametes: testing for a correlation in fitness between mates in *Drosophila melanogaster*. *Am. Nat.* 174:613–22

Sharp NP, Agrawal AF. 2012. Evidence for elevated mutation rates in low-quality genotypes. *Proc. Natl. Acad. Sci. USA* 109(16):6142–46

Shaw FH, Baer CF. 2011. Fitness-dependent mutation rates in finite populations. *J. Evol. Biol.* 24:1677–84

Siller S. 2001. Sexual selection and the maintenance of sex. *Nature* 411:689–92

Simmons MJ, Crow JF. 1977. Mutations affecting fitness in *Drosophila* populations. *Annu. Rev. Genet.* 11:49–78

Springman R, Keller T, Molineux IJ, Bull JJ. 2010. Evolution at a high imposed mutation rate: adaptation obscures the load in phage T7. *Genetics* 184:221–32

Szathmáry E. 1993. Do deleterious mutations act synergistically? Metabolic control theory provides a partial answer. *Genetics* 133:127–32

Tobari I, Murata M. 1970a. Changes of genetic loads in experimental populations of *Drosophila melanogaster* with radiation histories. *Jpn. J. Genet.* 45:387–97

Tobari I, Murata M. 1970b. Effects of X-rays on genetic loads in a cage population of *Drosophila melanogaster*. *Genetics* 65:107–19
Turner JRG, Williamson MH. 1968. Population size, natural selection and genetic load. *Nature* 218:700
Wallace B. 1956. Studies on irradiated populations of *Drosophila melanogaster*. *J. Genet.* 54:280–93
Wallace B. 1959. Studies of the relative fitnesses of experimental populations of *Drosophila melanogaster*. *Am. Nat.* 93:295–314
Wallace B. 1968. Polymorphism, population size, and genetic load. In *Population Biology and Evolution*, ed. RC Lewontin, pp. 87–108. Syracuse, NY: Syracuse Univ. Press
Wallace B. 1970. *Genetic Load. Its Biological and Conceptual Aspects*. Englewood Cliffs, NJ: Prentice-Hall
Wallace B. 1975. Hard and soft selection revisited. *Evolution* 29:465–73
Wallace B. 1991. *Fifty Years of Genetic Load: An Odyssey*. Ithaca, NY: Cornell Univ. Press
Wang AD, Sharp NP, Spencer CC, Tedman-Aucoin K, Agrawal AF. 2009. Selection, epistasis, and parent-of-origin effects on deleterious mutations across environments in *Drosophila melanogaster*. *Am. Nat.* 174:863–74
West SA, Peters AD, Barton NH. 1998. Testing for epistasis between deleterious mutations. *Genetics* 149:435–44
Whitlock MC. 1996. The Red Queen beats the Jack-of-All-Trades: the limitations on the evolution of phenotypic plasticity and niche breadth. *Am. Nat.* 148:S65–77
Whitlock MC. 2002. Selection, load, and inbreeding depression in a large metapopulation. *Genetics* 160:1191–202
Whitlock MC, Agrawal AF. 2009. Purging the genome with sexual selection: reducing mutational load through selection on males. *Evolution* 63:569–82
Whitlock MC, Bourguet D. 2000. Factors affecting the genetic load in *Drosophila*: synergistic epistasis and correlations among fitness components. *Evolution* 54:1654–60

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