Substitution load revisited: reproductive excess, the proportion of deaths that are selective, and adaptation's speed limit

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ABSTRACT

Haldane's Dilemma refers to the concern that the need for many "selective deaths" to complete a substitution (i.e. selective sweep) creates a speed limit to adaptation. However, discussion of this concern has been marked by confusion over which features of adaptation produce speed limits, what those limits are, and the consequences of violating speed limits. The term "substitution load" has been particularly unhelpful in this regard. Here we distinguish different historical lines of reasoning that lead to speed limits. We identify one line of reasoning, focused on finite reproductive excess, that has not yet been fully addressed. We develop this into a more general theoretical model that can apply to populations with any life history, even those for which a generation or even an individual are not well defined. As proof of principle, we apply this model to a dataset measuring survival of 517 different genotypes of *Arabidopsis thaliana* grown in eight different environmental conditions. These data suggest highly permissive limits to the speed of adaptation in all environmental conditions. This is because a much higher proportion of deaths contribute to adaptation than were anticipated during historical discussions of speed limits.

Keywords: cost of selection, adaptation rate, genetic load, fitness component, biological individual

LAY SUMMARY

Neutral theory was predicated on theoretical arguments that adaptation is subject to a speed limit. We resolve confusions regarding historical speed limit arguments, which depend on differences in fitness, not variance (differences in fitness squared). We generalize the underlying concepts of selective deaths and reproductive excess to populations with any life cycle, even those for which an "individual" and hence generation and fitness, are poorly defined. We apply the revised theory to Arabidopsis data, demonstrating the potential for future related experiments.

INTRODUCTION

During an adaptive sweep, new alleles need to be substituted for old alleles across an entire population. This means that all individuals with the old alleles need to leave no descendants, and individuals with new alleles must produce enough offspring to replenish the population. These requirements put a limit on the speed at which sweeps can happen, which could be prohibitive if many sweeps need to occur quickly. Haldane (1957) used this reasoning to propose a rough estimate of the maximum speed at which sweeps could accumulate. This speed limit, later known as Haldane's dilemma (Van Valen 1963) motivated the development of Kimura's (Kimura 1968) neutral theory. However, the underlying logic has been challenged on multiple counts (Ewens 1970; Felsenstein 1971; Kern and Hahn 2018; Maynard Smith 1968). In these discussions, conceptually distinct approaches to quantifying the issue are often described using identical terms, which apart from being confusing, leaves unresolved the critical question: what is the upper limit on the speed of adaptation, and does it matter for natural populations?

Here we first synthesize the historical literature, drawing out several key quantities (see Results). First, a population has a "reproductive excess", meaning how many individuals at a given life history stage are in excess of the minimum number required to avoid a decline in population size over the next life cycle (see Glossary for definitions of the terms used here). The second key quantity is the "selective deaths"

(including foregone fertility) required for selection to effect a change in allele frequency. These can be quantified as the degree to which the mean individual survives and reproduces worse than the best genotype present. The "cost of selection" is the number of selective deaths required to achieve a given adaptation rate. Haldane (1957) assumed, without evidence, that at most 10% of deaths were selective, an assumption repeated by all subsequent work.

Haldane (1957) confused the cost of selection, which is related to differences between the mean individual and the best genotype present, with the lag load, which compares to an ideal genotype that is unlikely to exist (Ewens 1970). However, Nei (1971) and Felsenstein (1971) derived a near-identical speed limit to Haldane's, without this flaw, by examining the requirement for selective deaths within a model in which reproductive excess is finite.

Explicit consideration of reproductive excess means abandoning relative fitness models, which consider only one life history stage with adult population size N, with an implied infinite number of juveniles (Bertram and Masel 2019). Models of finite reproductive excess need at least two life history stages: adults and juveniles, where the latter shows reproductive excess relative to the former. Selective deaths are then relative not to a single adult population size N as in Haldane's (1957) model, but to a denominator describing the population size at the appropriate life history stage (Kimura and Crow 1969).

An emphasis on life history transitions rather than generations is a strength rather than a weakness of the selective deaths view. One of the many flaws of the concept of "fitness" (Van Valen 1989) is the difficulty of defining a "generation" for many species, especially colonial species for which an "individual" is not well defined (Wilson and Barker 2021). Consider for example the budding yeast *Saccharomyces cerevisiae.* Is each mitotic division a generation? Or each life cycle spanning from meiosis to meiosis, with variable number of mitoses in between? Or the span between outcrossing events, with variable occurrences of selfing as well as mitoses in between? Or is a generation best defined ecologically with respect to dispersal between resources that allow growth? Problems defining a generation arise for a broad range of species (albeit not humans, nor many other animal species), but are

resolved when population dynamics are viewed as a series of life history transitions. The "generation" that matters in this view is not the concept of one complete life cycle, but rather the "generation" of reproductive excess, in contrast to other life history transitions that involve survival rather than reproduction.

After synthesizing the literature, here we reformulate and generalize Nei's (1971) and Felsenstein's (1971) ideas to selection on both fecundity and survival, to life cycles with selection at more than one stage, and to life cycles with a variable number of stages. We clarify the concepts of reproductive excess and selective deaths, and use our general theory to pose two empirically accessible questions. First, how much reproductive excess does a genotype or population produce, beyond what is needed to avoid decline? Second, what fraction of deaths are selective (and how does this compare to the 10% guess made by Haldane)? Posing questions in this form allows us to make the first empirical estimates with which to ground Haldane's approach. We use data from Exposito-Alonso et al. (2019), who counted or estimated every plant grown and seed produced of *A. thaliana* cultivars from 517 different genotypes in one season, under 8 distinct environmental conditions. These data are not representative of natural conditions, but they suffice to illustrate how such an analysis can be done. Ours is the first direct application of Haldane's selective death arguments to empirical data, representing proof of principle.

METHODS

Environmental conditions

We re-analyze the data of Exposito-Alonso et al. (2019), who used a 2 × 2 × 2 design for environmental conditions, with the three treatments being climate, water availability, and adult density (see github for raw data and analysis code). We treat these as eight separate populations. For climate, plants were grown in outdoor field stations in either Tübingen, Germany (near the center of the species range of *A. thaliana*) or in Madrid, Spain (at the southern edge of the range). Plants were all artificially watered. The high-water treatment matched soil moisture levels near the station in Germany, and the low-water treatment

matched soil moisture levels near the station in Spain. To generate high adult density, thirty seeds of the same genotype were planted per pot. For low density, several seeds (~10) were planted per pot, enough to ensure that at least one seed would germinate, but few enough that the seeds were unlikely to inhibit each other pre-germination. To avoid any competition between adult plants in the low density treatment, only one seedling, chosen at random, was retained after germination, and the rest were plugged out and discarded. We refer to each treatment with a three-letter abbreviation: M or T for Madrid or Tübingen, L or H for low or high water, and I or P for a single individual plant or a population of thirty plants per pot. For example, the treatment with thirty seeds per pot grown in Madrid with high water is abbreviated as MHP.

Within each of the eight populations, seeds from 517 fully homozygous plant genotypes (taken from a parental generation grown under controlled conditions to control for parental effects) were grown in pots that included only plants of that genotype. The number of replicate pots per genotype per population was occasionally as few as one due to experimental losses, but mostly ranged between five to seven replicates. Our interest is in differences among genotypes, not among replicates. We therefore calculate key quantities of interest for each genotype-environment combination by averaging across replicates.

Selective deaths

The experiment can be mapped reasonably easily onto theoretical treatments. In each environmental treatment, a starting population of seeds grows into adult plants, experiencing both selective deaths and non-selective deaths as they proceed from seeds to seedlings to adults. Plants which survive to become adults then produce k seeds on average, some of which would normally constitute the next generation, although the experiment concludes at the end of season. The experiment does not capture the life history stage of seed dispersal to fertile ground, to complete the life cycle that began with seeds planted in a pot.

Juvenile deaths must be treated differently for the low- and high-density treatments. In the low-density treatment, where exactly one seedling is retained after germination, we do not have access to data on

selective seed deaths, and so consider only seedling selective deaths. In the high-density treatment, any of the thirty seeds that fail to survive to the end of the experiment are counted as deaths, whether due to seed death before germination or to subsequent seedling death; our selective death calculations do not differentiate between these two life history transitions. This means that across the two life history transitions at which juvenile plants can die (as planted seeds before germination and as seedlings), only one set of juvenile deaths is recorded in each density treatment, but they are not comparable. They are combined seed and seedling deaths in the high-density case, and seedling deaths alone in the low-density case. Histograms are shown in Supplementary Figures 1-2.

In each treatment, we score the observed juvenile death rate of the highest performing genotype as the baseline extrinsic mortality for all genotypes (i.e. as non-selective deaths). Conceptually (ignoring a correction for extreme value bias that is treated in the Supplement), for each life history transition in each environmental condition we have:

$$\text{Selective deaths in the population} = \sum_i n_i(d_i - d_{best})$$

where n_i is the starting population of genotype i at that life history transition, d_i is the genotype's average death rate during that life history transition, and d_{best} is the average death rate of the genotype with the lowest death rate for that life history transition in that environment.

Test for genetic variance in fecundity

Selective "deaths" can also be defined for unrealized fecundity. We did not analyze this here, because of lack of evidence for significant genetic differences in fecundity. In support of this, we performed an ANOVA test on fecundity in each environmental condition. We only have information on fecundity as an aggregate per replicate pot (rather than per individual plant in the high-density condition), so we compare among-genotype variance to among-replicate variance. Note, however, that we remove replicates that had no adults surviving to reproductive maturity, as well as genotypes with only a single replicate pot with

surviving adults (and therefore no way of estimating among-replicate variance). All surviving adults produced at least some seeds. We Box-Cox transformed the data for each pot with surviving adults in each environmental condition (see Supplementary Figure 3 for post-transform histograms) before performing the ANOVA.

Proportion of juvenile deaths selective

Because *A. thaliana* is an annual plant, all juveniles will die by the end of the season, whether as selective deaths during the experiment, non-selective deaths during the experiment, or non-selective deaths after the end of the experiment. From this we obtain, for each environmental condition:

$$\text{Fraction of juvenile deaths selective} = \frac{Selective\ deaths\ of\ juveniles}{Starting\ population}$$

In the high-density populations, the starting population is 30 seeds per pot. In the low-density populations, the starting population is 1 seedling per pot.

Pairwise genotype comparisons

For every possible pair of genotypes, we repeat the analysis above to estimate selective deaths and the proportion of deaths which are selective, using the better genotype of the pair as the 'best' genotype in the calculation of selective deaths. With only two genotypes, we do not adjust for extreme value bias. Using whole-genome information, we calculated the total number of SNP differences between each pair (Hamming distance, number of allele differences out of 1,353,386 biallelic SNPs) using PLINK v1.9.

RESULTS

Synthetic historical review

Haldane made two somewhat different arguments in his seminal 1957 paper, muddying the waters from the beginning. In the first argument, he defined "selective deaths" as the subset of deaths $s(1-p)N$ that

contribute to a change in the allele frequency p, where s is the selection coefficient. This was on the basis that the $(1-p)N$ individuals that lack a beneficial mutation experience s more deaths than they would if they had the mutation, and those extra deaths are required for selection to have its effects. Note that reduced fecundity is mathematically equivalent to increased mortality, and selective "deaths" can thus result from losing potential offspring, not just literal deaths.

Haldane defined the "cost of selection" as the number of selective deaths occurring during a substitution (i.e. a selective sweep from low allele frequency to fixation). He calculated this cost as the integral of $s(1-p)N$ over the course of a sweep from allele frequency $p = p_0$ to close to 1 (Figure 1A). In a haploid population of constant size N, one sweep requires $N \times D$ selective deaths, where $D = -\ln(p_0)$+O(s). For appropriately small s and p_0 (Haldane suggests $s < \frac{1}{3}$ and $p_0 = 10^{-4}$), the first term dominates, making D nearly independent of the selection coefficient. For alternative assumptions about ploidy, dominance, and degree of inbreeding, D is a different function of p_0, but s remains unimportant unless close to 1 (Haldane 1957). In a representative case of $p_0 = 10^{-4}$ at a diploid autosomal locus with no dominance, $D = 18.4$. Haldane conservatively estimated that 20-30N selective deaths are likely to be typical for a sweep.

Haldane's second argument about adaptation rate limitations relied on load calculations. Load is a reduction of a population's fitness relative to a reference optimal genotype (Figure 1B). Haldane considered x loci independently undergoing sweeps, such that the current allele frequency at the i^{th} locus reduces population fitness by a factor of $1 - d_i$ relative to its post-sweep value. The fitness of the population is then lower than that of a hypothetical perfect population by a factor of $\prod_{i=1}^{x}(1-d_i) \approx e^{-\sum_{i=1}^{x} d_i}$. Haldane claimed that this load relative to an ideal genotype implies that the fraction of deaths that are selective is $\sum d_i$. This was incorrect; the better reference point is the best genotype actually present in the population. With $30N$ selective deaths required to complete each independent selective

sweep, and N deaths available per generation, Haldane obtained an average spacing between fixation events $n \geq \frac{30}{\sum d_i}$ generations.

Haldane's fitness reduction relative to an ideal genotype was later named lag load (Maynard Smith 1976), inspired by lagging adaptation to a changing environment, where new mutations are required to keep up (Bertram, Gomez, and Masel 2017). (This is distinct from "evolutionary rescue" (Bell 2017) from imminent population decline, without asking whether a similar disastrous scenario will promptly recur.) Lag load can be defined even in a static environment, where innovative new adaptive alleles reveal the possibility of an even better optimal genotype. The size of a lag load is not important *per se*; what matters is that it is stable rather than growing. While speed limits do not threaten the persistence of a species adapting in a constant environment, real species do face rapidly changing environments (biotic and abiotic) that can threaten population persistence. I.e., for population persistence, the speed of adaptation must keep up with the speed of environmental change.

Haldane argued *a priori* that species could probably only sustain about 10% selective deaths (which he incorrectly equated with 10% lag load) for any serious length of time. From this assumption, he derived a speed limit of around one sweep every 300 generations, later called "Haldane's dilemma" (Van Valen 1963). All subsequent authors have continued to assume a 10% figure.

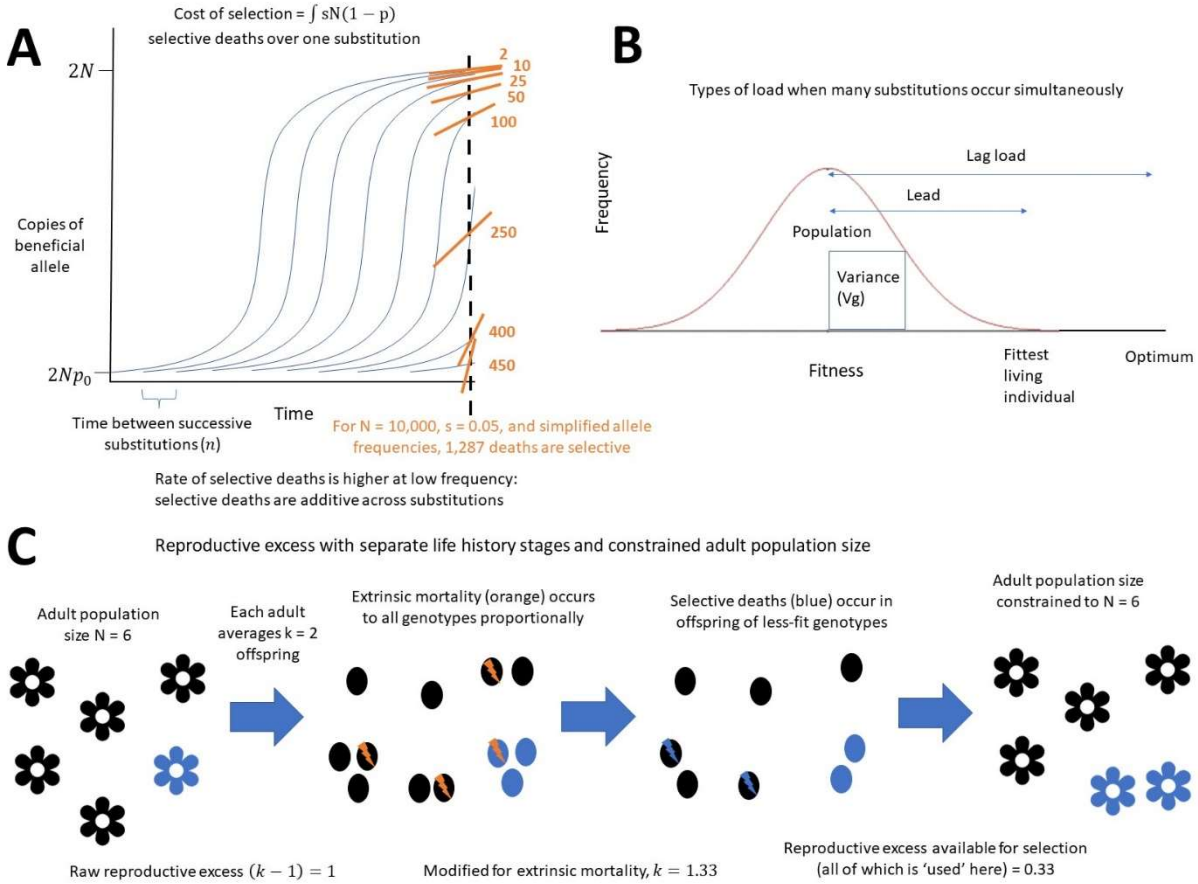
The fact that there are so many amino acid substitutions, each requiring a sweep, was the original evidence supporting neutral theory (Kimura 1968). Kimura and Ohta (1971) plugged in estimates of the actual rate of substitution in mammalian lineages as n in Haldane's equation $L = e^{-30/n}$, which produced what they considered to be an excessively large lag load. Although still a lag load argument, their argument was subtly different from Haldane's, arguing that a high lag load implies that typical individuals would need to have a biologically implausible fraction of their offspring die (Kimura and Ohta 1971).

Ewens (1970) pointed out that Haldane's and Kimura's load arguments improperly use the lag load (comparison to an ideal genotype) to calculate selective deaths, where they should have mean fitness

relative to the most fit individual present. In a population with many sweeps occurring at once, the likelihood that even a single individual has the ideal combination of alleles is vanishingly small (Figure 1B). More recent travelling wave theories have rediscovered the importance of this relative type of load, and named it the "lead" (Desai and Fisher 2007).

Prior to modern travelling wave theory, approximations for the lead were derived from variance in fitness (Ewens 1970; Kimura 1969). In the case of many independent sweeps at once, variance in fitness (after normalizing mean population fitness as 1) is approximately s/n, where s is the selection coefficient of an adaptive allele and n is the number of generations between fixation events (Ewens 1970). The fittest genotype likely to be present can be estimated using the statistics of extreme values. E.g., for a population of size 10^6, the most extreme fitness value likely to appear is around 4.9 standard deviations above the mean (Ewens 1970). Using Haldane's 10% as an estimate of the lead (instead of the lag load) yields $4.9\sqrt{s/n} = 0.1$. For $s = 0.01$, n is around 20, much less than Haldane's estimate of 300, and n is lower still for lower s. In other words, Ewens (1970) found that many simultaneous sweeps do not imply an implausibly large lead, and the corresponding speed limit of $n \approx 20$ is not an obstruction with respect to observed rates of amino acid divergence. Similar arguments have been applied to deleterious mutation load (Galeota-Sprung, Sniegowski, and Ewens 2020).

Although Ewens' argument revolved around lead, which is a difference between fitnesses, his approach continued the traditional emphasis of evolutionary genetics on variance in fitness, which describes the mean square of differences (Crow 1958; Ewens 2004; Fisher 1930). Modern traveling wave theory instead derives the lead directly from s, N, and the beneficial mutation rate U, and obtains the variance in fitness variance only downstream from that (Desai and Fisher 2007), rather than relying on our ability to directly measure fitness and its variance as an input to the calculation.



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238 **Figure 1.** Three different types of arguments have been used to argue for limits to the speed of adaptation.
239 A) The cost of selection is the number of selective deaths that must occur over time to complete a single
240 sweep (each sweep shown as a logistic curve). The cost of selection at one timepoint is the sum of the
241 costs for each current sweep, illustrated as the slopes of the orange lines, each calculated as the subset of
242 deaths $s(1-p)N$ that contribute to a change in the allele frequency p. B) Load arguments calculate the
243 reduction in mean fitness of a population from what it could be. C) Finite reproductive excess imposes an
244 upper limit on how many selective deaths per generation a population can sustain, which sets an upper
245 bound to how fast substitutions can occur.

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Maynard Smith (1976) made a quite different argument against speed limits, claiming that the reason Haldane's dilemma is not a problem is pervasive synergistic epistasis. Synergistic epistasis increases differences in fitness above those expected from differences in the numbers of beneficial mutations, thereby making each selective death more likely to count towards a larger number of sweeps at once. A persistent source of confusion has been that in his model of truncation selection, Maynard Smith also made the shift from Haldane's absolute fitness to a more standard population genetic relative fitness, and hence from lag load to lead. The fact that Haldane's dilemma did not arise in Maynard Smith's model might therefore be due to reasons put forth by Ewens, rather than due to epistasis.

Although Ewens' lead-based approach negates arguments that convert lag load into a speed limit (i.e. Haldane's second argument), it doesn't address Haldane's first line of argument: the cost of natural selection in terms of selective deaths. Confusion between these two disparate lines of argument was exacerbated by the fact that different papers use the term "substitutional load", which we avoid here, to mean very different things. 'Substitutional load' has been used to refer to what we here call the lag load (Kimura and Ohta 1971), the cost of selection (Kimura 1968), the lead (Maynard Smith 1976), the number of offspring that the most fit genotype must produce (Ewens 2004), the sum of lag load across all generations involved in a substitution (Kimura 1960; Nei 1971), and even more broadly to refer to variance rather than load-based arguments when made in the context of similar questions (Ewens 1970). This confusion in terminology has obscured the consequences of formulating Haldane's dilemma in different ways.

Nei (1971) and Felsenstein (1971) made a key advance that was perhaps not fully appreciated amidst the confusion. The relative fitness models which dominate population genetics (e.g. Wright-Fisher and Moran) implicitly assume inexhaustible reproductive excess (Bertram and Masel 2019). This can be seen easily in simulations using a rejection sampling method – when fitness is low, an absurd number of zygotes might be generated and discarded prior to filling the N slots. However, real populations have a finite reproductive excess, e.g. human females do not easily have more than 20 infants. This constrains

272 members of the next generation to come from the options contained within that finite set of potential
273 offspring. This concept has been applied to lethal mutagenesis strategies for anti-viral drugs (Bull,
274 Sanjuán, and Wilke 2007).

275 Nei (1971) and Felsenstein (1971) each modelled independently evolving sites in a haploid population
276 with adult population size N. Each adult has fecundity k, i.e. produces k offspring prior to juvenile deaths
277 (Figure 1C, first arrow). In their deterministic models, k is exact, but the theory readily generalizes to
278 interpreting k as an expectation. The raw reproductive excess is thus $(k-1)N$, with $k > 1$. Some
279 reproductive excess is lost to non-selective mortality, set not by an extrinsic rate, but derived from
280 population size regulation after selective mortality (Figure 1C, far right). Extrinsic mortality occurring
281 prior to selective mortality, and hence at a fixed rate, can be folded into a lower value of k. While the
282 number of selective deaths available for adaptation is then still denoted $(k-1)N$, this no longer
283 represents raw reproductive excess (Figure 1C, second arrow). Haldane's estimate of $k = 1.1$ (resulting in
284 a maximum of 10% selective deaths), which Nei (1971) and Felsenstein (1971) retain, includes the fact
285 that extrinsic mortality substantially reduces the fecundity available to be 'used' for selective deaths.

286 The population then undergoes sweeps, all with the same initial frequency p_0 and selection coefficient s
287 applying to survival rather than fecundity. Each sweep follows the same trajectory with a mean delay of n
288 generations between sweeps (Figure 1A). Given independent sites, the cost of selection is summed across
289 loci at any given point in time (e.g. slopes of orange lines in Figure 1A); Haldane's integral is a method of
290 calculating the expectation of this sum. Comparing this cost to the reproductive excess of the population
291 produced the novel result that the minimum spacing n is $-\ln(p_0)/\ln(k)$ (Felsenstein 1971; Nei 1971).
292 For Haldane's estimates of $p_0 = 10^{-4}$ and $k = 1.1$, this yields $n = 97$ generations between selective
293 sweeps. This can be compared to Haldane's original spacing of $-\ln(p_0)/\ln\left(\frac{W_{max}}{\bar{W}}\right) = 92$ for a
294 denominator (somewhat oddly described by Haldane as a selection intensity) $= \ln\left(\frac{W_{max}}{\bar{W}}\right) = 0.1$.

This new limit based on the finite nature of reproductive excess is much slower than the speeds predicted by lead-based arguments, but is similar in magnitude to Haldane's original result. Importantly, this speed limit calculation is not subject to the same criticisms as Haldane's original argument. Where Haldane compared the mean fitness of the population to the mean fitness of a hypothetical population, Nei's (1971) and Felsenstein's (1971) approach compares the available reproductive excess to the reproductive excess required to effect changes in allele frequencies. Even if no individual exists who possesses the beneficial allele at every segregating site, each sweep still requires a certain fraction of deaths to contribute to its selection. It is the finite nature of reproductive excess that directly produces this limit on the rate of adaptation.

Felsenstein's (1971) and Nei's (1971) formulations of Haldane's dilemma define the amount of reproductive excess that is available for selective deaths as $(k-1)N$ after controlling for extrinsic mortality, where N is the population size prior to the generation of reproductive excess. But the value of k is an effective value that can conceal much, the "-1" assumes that perfect density regulation demands no excess individuals above N, and the N refers always to the same adult life history stage. This "effective" reproductive excess parameter is better conceived of as a measure of the proportion of deaths that are selective than as a true reproductive excess, and indeed they used Haldane's estimate for 10% deaths being selective to set $k = 1.1$. Just because a species like *A. thaliana* has high fecundity (high raw reproductive excess), this tells us nothing about the proportion of deaths that are selective.

Theory

Previous theoretical treatments by Nei (1971) and Felsenstein (1971) assume that all genotypes have the same fecundity (k), i.e. that there is no selection on fecundity, only on the single life history transition representing survival. They also assume that extrinsic mortality has a density-dependent component such that the combination of selective and non-selective mortality is perfectly balanced with fecundity. These are obviously not realistic assumptions. Next, we extend the theory in a variety of ways.

Selective deaths during generative life history transitions

Previous theoretical treatments by Nei (1971) and Felsenstein (1971) emphasize literal deaths. We can also describe differences in fecundity as selective 'deaths'. This is because mathematically, foregone fecundity is equivalent to deaths that take place immediately after fecundity, and can be treated as:

$$\text{Selective } '\text{deaths}' \text{ during differential fecundity} = N_i(b_{best} - b_i)$$

where N_i is the number of reproductive mature adults, b_i is the fecundity of genotype i, and b_{best} is the fecundity of the genotype with the highest fecundity in that environment.

Reproductive excess within a fixed life cycle

Next we generalize from just one life history transition experiencing selection, to multiple that occur in a consistent order. We consider a life history transition j that starts with population size N_j and ends at population size $N_{j+1} = k_j N_j$: We now define

$$\text{Reproductive excess after transition } j = k_j N_j - N_{min,j+1}$$

where $N_{min,j+1}$ is the minimum population size at the end of transition j that is required in order for the population to achieve size of N_j at the beginning of transition j in the next life history cycle. Note that $k_j > 1$ indicates fecundity while $k_j \leq 1$ indicates survival.

To produce selective deaths, $k_{i,j}$ must depend on genotype i. To capture density regulation, $k_{i,j}$ for at least some values of j must depend on population size N_j. The values $k_{i,j}$ can also be functions of the genotype frequencies and/or an absolute measure of time. Two life history transitions (survival and fecundity) is the minimum, but each of these can be broken up into multiple transitions. For example, survival ($k < 1$) can be broken into components representing survival at different ages, or a selective component depending only on genotype vs. a density-dependent extrinsic mortality component depending only on N_j vs. an extrinsic mortality component occurring at a constant rate.

Reproductive excess can be calculated either with respect to the best genotype present (i.e. the one most likely to become fixed), or with respect to the population mean, by using different values of k_j and $N_{min,j+1}$. Reproductive excess with respect to the population mean is needed to avoid population decline in the next generation, while reproductive excess with respect to the best genotype describes the ability to avoid population decline that would continue even after the best genotype has swept to fixation. The best choice depends on the particulars of the population in question. For example, studying balancing selection calls for the population mean, while studying evolutionary rescue calls for the best genotype.

To calculate reproductive excess with respect to the population mean, we solve for $N_{min,j+1}$ in:

$$N_j = N_{min,j+1} \sum_i f_i \prod_{x \neq j} k_{i,x},$$

where f_i is the frequency of genotype i at the beginning of the transition. With respect to the best genotype, we instead solve for $N_{min,j+1}$ in:

$$N_j = N_{min,j+1} \prod_{x \neq j} k_{best,x}.$$

Nei and Felsenstein as a special case

Nei (1971) and Felsenstein (1971) treated reproductive excess in the conceptually simple case of only two alternating life history transitions: births and deaths. Deaths included *only* selective deaths, while the "effective" fecundity transition was non-selective. They handled non-selective deaths by collapsing them into the value of $k_{fecundity\_effective}$, either before selection on survival (in which case non-selective deaths reduce $k_{fecundity\_effective}$ in the current generation), or after selection on survival (in such a way as to exactly balance out any available deaths that were "unused" by selection in the current generation, by reducing $k_{fecundity\_effective}$ in the subsequent generation). The product $k_{fecundity\_effective} \times k_{selective\_mortality}$ was thus constrained to not exceed 1, via a fudge factor in the former. They solved for equality to 1 in order to calculate the maximum amount of selective deaths. When this equality is

363 satisfied, $k_{fecundity\_effective}$ can be interpreted as the product of actual fecundity and non-selective
364 survival. Fig. 1C interprets this scheme in a temporal manner, proceeding first through non-selective
365 fecundity to produce raw reproductive excess, then the non-density-dependent component of extrinsic
366 mortality, then selective deaths, and finally density-dependent extrinsic mortality to cap the population
367 size at *N* adults. They score reproductive excess as subject to the first but not the second form of reduction
368 down to "effective" fecundity, minus the *N* individuals needed to replace the population.

369 *Reproductive excess beyond a fixed life cycle*

370 Not all organisms proceed through the exact same sequence of life history transitions every time, e.g. with
371 budding yeast experiencing a variable number of mitoses in between each meiosis, and a variable number
372 of selfing events between each outcrossing. In this case we cannot take the product of an exact series of
373 transitions. Instead, we privilege the life history transition that produces the most severe bottleneck,
374 assuming that the population will spring back to vibrancy after. We define a minimum number of
375 individuals N_{bot} who need to make it through to the other side of the bottleneck, and define

376 $$\text{Reproductive excess at transition } j = k_j N_j - \text{min. needed to ensure } N_{bot} \text{ after bottleneck}$$

377 We now need to take the expectation over all possible series of life history transitions, and solve for
378 $N_{min,j+1}$ in

379 $$N_{bot} = \mathrm{E}\left(N_{min,j+1} \prod_{x}^{\substack{life\ histo\ \ stages \\ between\ j\ and\ bot}} k_{best,x} \right).$$

380 The precise value of N_{bot} will be informed by the ecology of the species in question. It may be small,
381 such as when just a modest number of new hosts, each colonized by just one infectious microorganism, is
382 sufficient to ensure the population's future. The appropriate value of N_{bot} is the smallest population size
383 that reliably escapes extinction.

Comparison to fitness

Values of k in our framework are equivalent to fitness components, with respect to absolute rather than relative fitness. Haldane obtained selective deaths from $sN(1-p)$ over a time step of a complete generation, where s is the selection coefficient with respect to relative fitness. We have shown how selective deaths can be derived directly from the underlying population dynamic model, without requiring either generation or relative fitness to be defined first.

Antagonistic pleiotropy is treated quite differently in a selective deaths framework than for fitness components. Per-generation fitness is the product of fitness components, such that when a genotype that benefits fitness in one life history transition bears an antagonistically pleiotropic cost at another, the costs and benefits at least partially cancel out. In contrast, selective deaths accrue across life history transitions – each selective death absorbs reproductive excess, and there is no reason for them to cancel out. Similarly, there is no cancelling out across generations, e.g. seasonally fluctuating selection must incur many selective deaths in order to effect the large allele frequency fluctuations that have been observed around the long-term mean (Kelly 2022; Machado et al. 2021; Rudman et al. 2022). This high demand for selective deaths also applies, given life history trade-offs, to unobserved effects that more quickly cancel out between successive life history transitions.

In the simple case of just births at rate b and deaths at rate d, classic population genetic per-generation fitness corresponds to the effective reproduction number b/d, while the Malthusian parameter gives an alternative formulation of fitness as $b-d$. A Malthusian approach is generally preferred when dealing with complications of age- or stage-structured populations. Our approach extends an effective reproduction number framing to these more complex scenarios, while avoiding dependence on the definition of one "generation". While the Malthusian approach is sufficient for many purposes, something closer to an effective reproduction number approach is required to capture the finite nature of reproductive excess and the corresponding limits to selective deaths and hence adaptation. We note that

often the question being asked is simply what will invade, in which case either approach can be used (Lehmann et al. 2016; Metz, Geritz, and Nisbet 1992; Roff 2008).

Comparison to travelling wave models

It is instructive to calculate the required reproductive excess and the proportion of deaths selective in the asexual relative fitness model treated by Desai and Fisher (2007), whose Eq. 39 solves for the lead qs as a function of s, U, and N. They define the lead qs in Malthusian fitness terms, but we approximate it here in terms of per-generation fitness. With an approximately constant population size, the mean genotype has absolute fitness ~ 1, and the best genotype present (the nose) has absolute fitness $\sim 1 + qs$. The lead qs can thus be conceived of as the minimum reproductive excess, with respect to the optimal genotype, that is required in order to avoid limits to adaptation. This aligns closely with the parameter $k - 1$ of Nei (1971) and Felsenstein (1971). The actual reproductive excess in Desai and Fisher's (2007) model is infinite, as for all models that assume a constant population size and treat only relative fitness.

We next calculate the proportion of deaths that are selective. The entire reproductive excess of the best genotype present, $RE \geq 1 + qs$, represents non-selective deaths (or foregone fecundity) among its offspring. Other genotypes all experience the same rate of non-selective deaths. The per-capita odds that the next death hits a specific average individual rather than a specific nose individual are $1 : 1/(1 + qs)$. An average parent therefore expects $RE(1 + qs)$ offspring deaths during the time in which it expects the RE non-selective offspring deaths that represent a generation, making the proportion of deaths that are selective equal to $1 - 1/(1 + qs)$.

For a substantial range of parameters, especially with rapid adaptation with large s, U, and N, both the minimum required reproductive excess, and the proportion of juvenile deaths that are selective, exceed the previously assumed value of 0.1 (Figure 2). This application to the model of Desai and Fisher (2007) helps clarify the distinction between these two related properties, which were confounded into a single value of k by Nei (1971) and Felsenstein (1971). In this particular model, with its explicit adults and

implicit juveniles under selection, they are both functions of the lead qs, but this need not continue to be so simple when more complex life histories are considered.

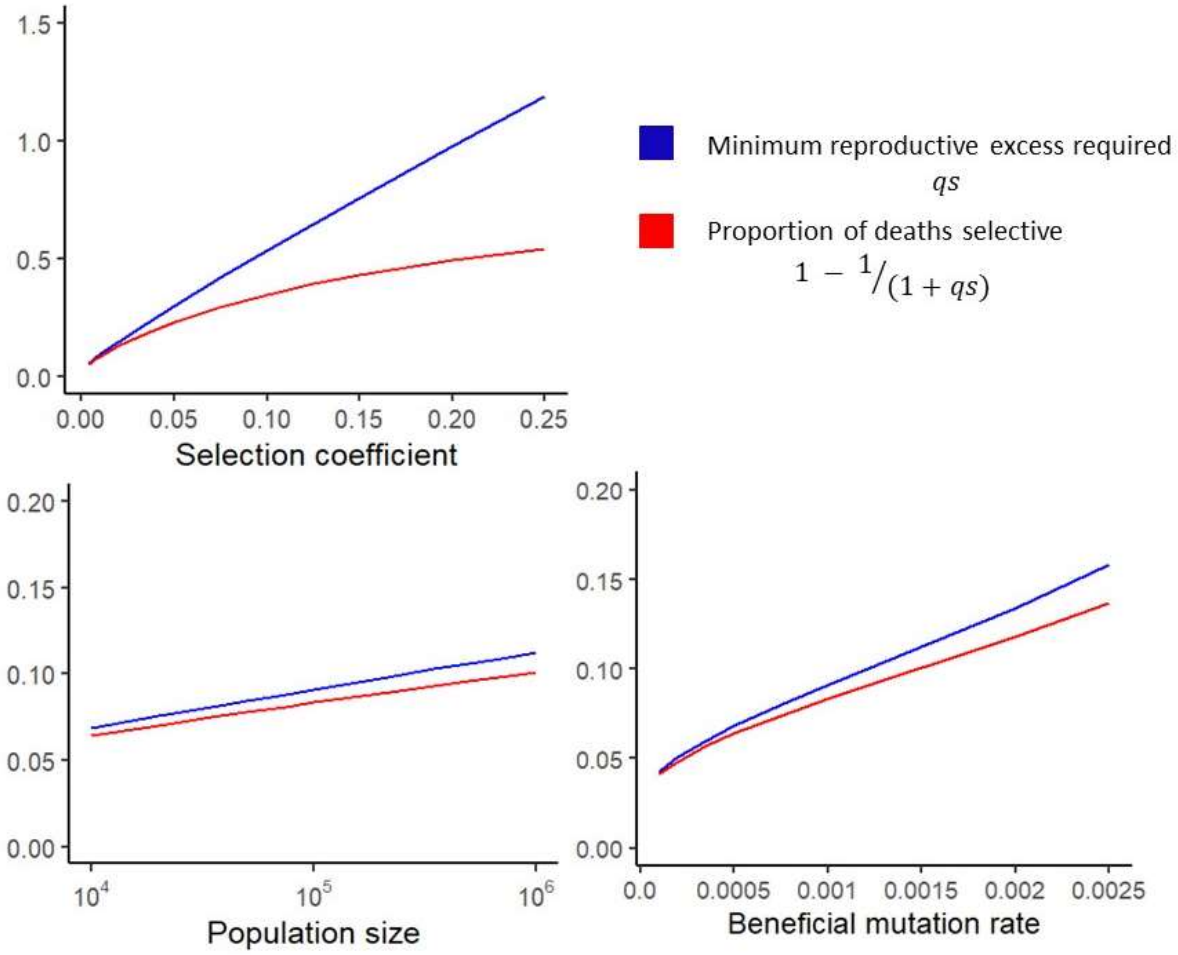


Figure 2. As the adaptation rate goes up with increasing U, s, and N, following the multiple mutations regime of Desai and Fisher (2007), so do the proportion of deaths selective and the minimum reproductive excess required to sustain that rate of adaptation. Parameter ranges are truncated to avoid the regime $\frac{s}{U} < 3$, for which the assumptions of the Desai and Fisher's (2007) model break down. The minimum required reproductive excess qs and the corresponding proportion of selective deaths $1 - 1/(1+qs)$ were calculated by numerically solving Equation 39 for q in Desai and Fisher (2007).

Application of theory to Arabidopsis *data*

The experiment analyzed here has a fixed life cycle of four life history transitions (adults producing seeds, seeds successfully dispersing to suitable habitat, seeds surviving to be seedlings, seedlings surviving to be adults). Matching this, we define reproductive excess (RE) four different ways, as illustrated in Figure 3. Given the presence of many poorly adapted genotypes in the experiment, we perform each calculation with respect to the best genotype (denoted by the prime symbol $'$), yielding:

$$\mathrm{RE(seed\ survival)} = k'_{seed\_survival} N_{seeds} - \frac{N_{seeds}}{k'_{seedling\_survival} k'_{fecundity} k'_{dispersal}}$$

$$\mathrm{RE(seedling\ survival)} = k'_{seedling\_survival} N_{seedlings} - \frac{N_{seedlings}}{k'_{fecundity} k'_{dispersal} k'_{seed\_survival}}$$

$$\mathrm{RE(fecundity)} = k'_{fecundity} N_{adults} - \frac{N_{adults}}{k'_{dispersal} k'_{seed\_survival} k'_{seedling\_survival}}$$

$$\mathrm{RE(dispersal)} = k'_{dispersal} N_{seeds\_produced} - \frac{N_{seeds\_produced}}{k'_{seed\_survival} k'_{seedling\_survival} k'_{fecundity}}$$

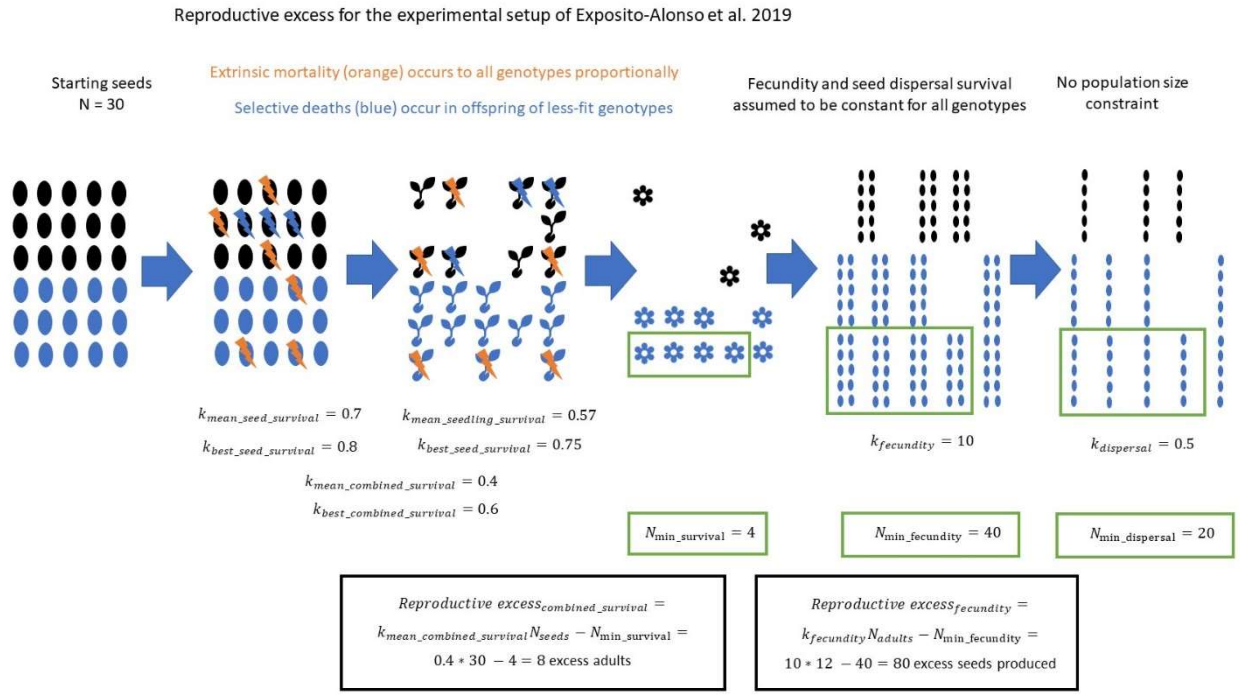
In the high density environmental conditions, we use a single survival transition to cover both seeds and seedlings. Under low density conditions, where one seedling was chosen at random from the product of 10 planted seeds, we use $k_{seed\_survival} = 0.1$ for all genotypes. We treat all genotypes as having the same $k_{fecundity}$ (due to lack of evidence for genetic variation – see Experimental Results below). Our experiment provides no information about values of $k_{dispersal}$, so we consider a range from 0.01 to 0.1, equal across genotypes. Because we lack data on the number of seeds after dispersal, we do not calculate a reproductive excess for this transition. This means that we calculate only two reproductive excesses for each environmental condition, one for survival and one for fecundity, although the calculations are different between low- and high-density conditions. These reproductive excesses are given by:

462 $$\text{RE}(\text{fecundity}, \text{low density}) = k_{fecundity} N_{adults} - \frac{N_{adults}}{0.1 k_{dispersal} k'_{seedling_{survival}}} \quad (1)$$

463 $$\text{RE}(\text{survival}, \text{low density}) = k'_{seedling\_survival} N_{seedlings} - \frac{N_{seedlings}}{0.1 k_{dispersal} k_{\,fecundity}} \quad (2)$$

464 $$\text{RE}(\text{fecundity}, \text{high density}) = k_{fecundity} N_{adults} - \frac{N_{adults}}{k_{dispersal} k'_{survival}} \quad (3)$$

465 $$\text{RE}(\text{survival}, \text{high density}) = k'_{survival} N_{seeds} - \frac{N_{seeds}}{k_{dispersal} k_{fecundity}} \quad (4)$$



466

467 **Figure 3**. Worked example of reproductive excess for the life history transitions of *A. thaliana* in the
468 experimental setup from Exposito-Alonso *et al* (2019). We found no evidence for between-genotype
469 differences in fecundity and the experimental setup provides no information about seed dispersal, so we
470 show no selective deaths during these transitions. Specific values of k are chosen for illustrative purposes.

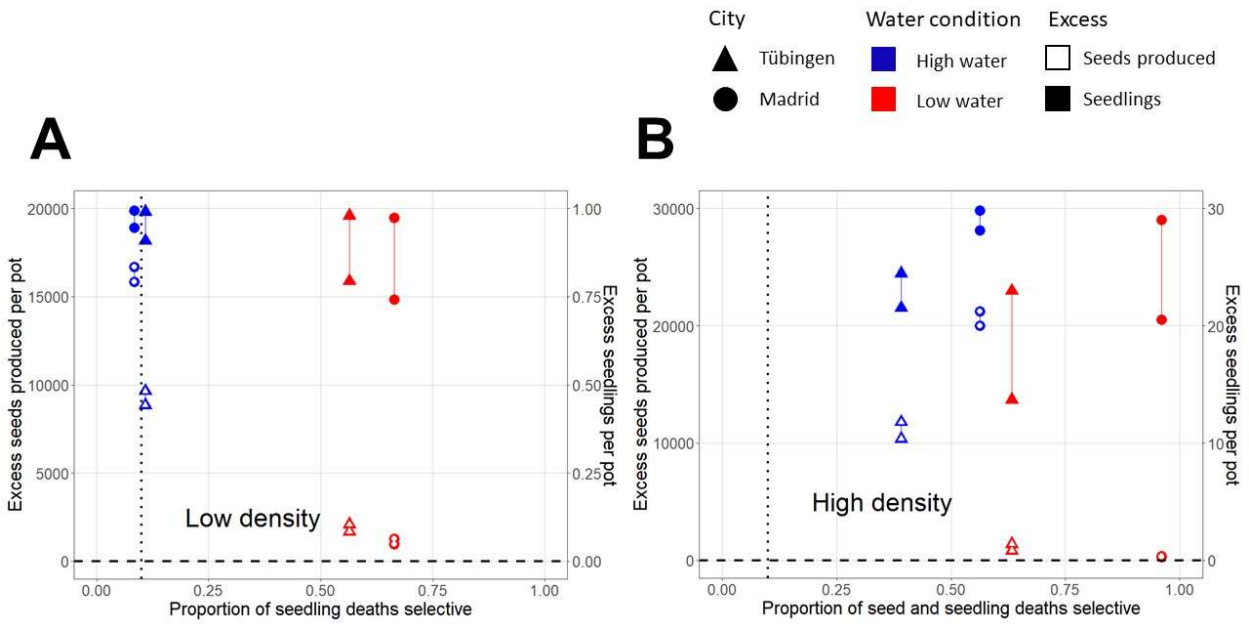
471

472 **Experimental**

Our empirical findings are restricted to selective deaths relating to juvenile survival. We did not analyze selective 'deaths' attributable to differences in fecundity, because an ANOVA on fecundity within and among genotypes showed no statistical support for any difference in mean fecundity among genotypes in six of the eight experimental conditions. Even in the two conditions with statistical significance, among-genotypic variance was three times smaller than among-replicate variance. A more sensitive MCMCglmm model with Poisson errors and controlling for replicate found non-zero heritability in 6/8 environments, but still below 10%, which we consider low enough to neglect.

The proportion of *A. thaliana* deaths that are selective substantially exceeds Haldane's 10% estimate in six out of eight experimental conditions, and is close to it in the other two (Figure 4 y-axis, Supplementary Table 2). In Madrid with low water and high density, as many as 95% of deaths are selective.

A priori, we expect high water and low density to be more benign, which might increase reproductive excess and/or reduce the proportion of deaths selective. While we cannot compare seed deaths at low density to seed plus seedling deaths at high density, these predictions are confirmed for high vs. low water: strongly in the case of selective deaths and excess seeds, and weakly with respect to excess seedlings (Figure 4, Supplementary Table 2). Estimated reproductive excess is fairly insensitive to the 10-fold range we consider for the proportion of seeds that successfully disperse to suitable habitats (vertical line length in Figure 4).



491

492 **Figure 4.** The proportion of deaths that are selective generally exceeds Haldane's 10% estimate (dotted
493 vertical line), with ample reproductive excess (above zero, shown as dashed horizontal line) especially
494 under high-water conditions. Selective deaths shown on the x-axis apply either to seeds plus seedlings (A)
495 or to seedlings alone (B). Reproductive excess in seeds produced vs. seedlings surviving is shown with
496 open vs. closed symbols, corresponding to values shown on the left and right y-axes, respectively.
497 Reproductive excess is with respect to what the genotype with the highest survival produced above what
498 would be required to replace the starting population of a pot. Note that reproductive excess of seedlings
499 cannot exceed one per pot under low density conditions, and 30 per pot under high density conditions.
500 Reproductive excess of seeds produced was calculated using equation 1 for low-density conditions and
501 equation 3 for high-density conditions. Reproductive excess of surviving seedlings was calculated using
502 equations 2 and 4. All reproductive excesses are shown as a vertical range, with the lower bound
503 calculated using $k_{dispersal} = 0.01$ and the upper bound calculated using $k_{dispersal} = 0.1$. The
504 proportions of deaths that are selective are adjusted for extreme value bias as shown in Supplementary
505 Table 1. Values can be found in Supplementary Table 2.

506

We expected *a priori* that harsher environmental conditions would have higher extrinsic mortality. However, this wasn't the case. We can estimate extrinsic mortality as the death rate of the best genotype, after correcting for extreme value bias (see columns 3 and 4 of Supplementary Table 1). Most environmental conditions saw a highest-fitness genotype with perfect survival, or close to it. We saw the most extrinsic mortality in the THP environmental condition, which is not one of the harsher conditions. Nei (1971) and Felsenstein (1971) implicitly assume high extrinsic mortality via their choice of value for reproductive excess; we explicitly account for extrinsic mortality during mortality transitions. Extrinsic mortality might of course be much higher in natural conditions, lowering the proportion of deaths below the high values observed here.

The artificially high genetic diversity in our experiment might inflate the proportion of deaths that are selective. If this were the case, then we expect that competition between more similar genotypes should lead to a smaller estimate for this proportion. We tested this prediction by repeating our analysis on every pair of genotypes, as though they were the only two genotypes in the experiment, and looking for a correlation between genetic distance and proportion of deaths selective. Note that we use genetic distance, much of it presumably neutral, as a proxy for genetic differences related to adaptation. Although some statistically significant correlations were observed in some environmental conditions, the direction of correlation was evenly split between negative and positive (as seen in Table 1), and the highest R^2 value observed in any environmental condition was $0.096^2 = 0.0092$, for seedling deaths in the MLI environment, which we deem biologically insignificant. This is reassuring with respect to the artificially high genetic diversity in our experiment.

Life history stage	Seedling deaths		Combined seed and seedling deaths	
Environmental condition	Spearman's rho	p-value	Spearman's rho	p-value
MLI	0.096	2.2e-16		
MHI	0.0004	0.88		
TLI	-0.0024	0.39		
THI	-0.0056	0.042		
MLP			0.055	2.2e-16
MHP			0.011	4.9e-5
TLP			-0.0081	0.0031
THP			-0.0085	0.002

Table 1: Genetic distance between a pair of genotypes does not consistently correlate with proportion of deaths selective. Visualization of each of the eight relationships is available in Supplementary Figures 4-5.

DISCUSSION

The prevailing consensus is that Haldane's Dilemma poses no real limitation to the speed of adaptation, despite persistent confusion as to the reason. Here we began by clarifying the primary issue with load-based arguments: correcting Haldane's comparison to the best theoretically possible genotype, to instead compare to the best genotype actually present, enables far more rapid adaptation. However, the historical consensus that adaptation is not significantly limited largely overlooks a different and crucial type of limitation pointed out by Nei (1971) and Felsenstein (1971), one that depends not on relative load but on the finite nature of reproductive excess. If we accepted Haldane's previously unchallenged 10% guess as an estimate for the reproductive excess that is available for selective deaths to take place among, or as an estimate of the proportion of deaths that are selective, then the rate of adaptation would be significantly

542 limited. We clarified and extended theoretical arguments regarding selective deaths drawn from finite
543 reproductive excess, along with a proof-of-concept application to an experimental dataset. Our extension
544 applies flexibly to different life histories, including those for which a "generation" is poorly defined, e.g.
545 colonial organisms.

546 Our experimental results suggest a possible resolution to Haldane's concern that the rate of adaptation
547 might be substantively limited. Our illustration of the model of Desai and Fisher (2007) confirms that the
548 finite nature of reproductive excess would indeed limit the speed of adaptation if only 10% of deaths were
549 available for selection. But the smallest proportion of selective deaths we observed across 8
550 environmental conditions was 8.5%, while in the most adverse environmental conditions, 95% of deaths
551 were selective. Relaxing this auxiliary assumption about a critical parameter value resolves Haldane's
552 concerns.

553 The data we use for our proof of concept measured selective deaths under artificial conditions. An
554 obvious concern with our setup is that with genotypes representing Europe-wide diversity of *A. thaliana*,
555 exaggerated differences between the best-adapted and worst-adapted genotypes would inflate estimates of
556 the proportion of deaths that were selective. These concerns are partially mitigated by our unexpected
557 finding that the genetic distance between genotypes is not consistently related to the proportion of deaths
558 which would be selective in a competition between genotypes. However, two similar genotypes in our
559 experiment represent more genetic distance than might be present within a typical natural population, and
560 even closely related genotypes might differ in important fitness-associated traits. Future work under more
561 natural conditions (e.g. with higher extrinsic mortality) and in different species (e.g. less fecund) remains
562 necessary to reach the conclusion that the proportion of deaths that are selective is typically high. Our
563 framework is flexible enough to be customized for any species, using whichever life history transitions
564 best describe that species' life history.

565 Empirical demonstration of the concepts, even with serious caveats about the generalizability of the
566 empirical example, makes the concepts more concrete. This is especially important because disparate

usage of the term 'substitutional load' in the literature, as well as the variety of underlying lines of reasoning involved, has made this topic unnecessarily opaque. One aspect of our current work is simply to clarify the variety of lines of reasoning that produce limits on the rate of adaptation. Our more specific theoretical and empirical analyses then develop a line of reasoning about reproductive excess and selective deaths that was not previously resolved. The attention of creation science to this matter (Remine 2005, 2006) highlights the importance of resolving it.

This approach, building on Haldane (1957), Nei (1971), and Felsenstein (1971), is not identical to standard modes of reasoning in genetics. In particular, quantitative genetics approaches follow Fisher (1930) to focus on variances — sums of differences squared — while selective deaths and reproductive excess are, like load, both differences, with no square operation. We hesitate to call this aspect of our approach 'novel', because it is clearly quite old, but it nevertheless comes across as novel with respect to aspects of contemporary evolutionary genetics.

Interestingly, the concept of relative load was later reinvented as the "lead", as part of calculations that derived the actual speed of adaptation v (rather than limits to it) from the beneficial mutation rate U, the population size N, and the per-mutation selection coefficient s (Desai and Fisher 2007). One reason this solution was not available to Haldane was that population genetics had not yet begun to treat origination processes (McCandlish and Stoltzfus 2014). Instead of treating a steady input of beneficial new mutations, Haldane considered a scenario in which environmental change activates beneficial variants within standing genetic variation. Indeed, a variant's initial frequency $-\ln(p_0)$ is the primary factor in determining the maximum speed of adaptation. Some adaptation comes not from activation of standing genetic variation, but from *de novo* mutations each appearing at initial frequency $1/N$ or $1/2N$. A lead-based approach was used for the latter to derive the rate of beneficial sweeps in asexuals as $\frac{2s \ln[Ns]}{\ln^2[s/U]}$ for the simple case of constant s (Desai and Fisher 2007), for parameter ranges in which the previously derived rate UNs does not apply. Here we relaxed the assumption of infinite reproductive excess made by

591 this relative fitness model, and calculated the minimal reproductive excess required for the model to hold.
592 We also reveal the model's implied fraction of deaths (or foregone fecundity) that are selective. Both
593 quantities are functions of the lead.

594 Another approach, starting with Kimura (1961), is to use the framework of information theory to set
595 bounds on the speed of adaptation. Natural selection increases the information stored in genomes (Adami
596 2012). Kimura calculates the amount of information acquired per sweep in terms of p_0 and then relates
597 this to the cost of selection using Haldane's equation that $D = -\ln(p_0)$ (Kimura 1961). More recent
598 approaches treat the bounds placed on the accumulation of information in much more detail, while
599 treating either the Malthusian parameter (McGee et al. 2022) or classic discrete time relative fitness
600 (Hledík, Barton, and Tkačik 2022). Both these approaches define an information "cost", but this is not
601 equal to our cost in terms of selective deaths.

602 The historical significance of Haldane's arguments about limitations to adaptation is that they were
603 convincingly used to support neutral theory. This was framed as a dilemma because data on the rates of
604 amino acid divergence among species seemed to exceed Haldane's speed limit. The development of
605 neutral theory resolved this apparent dilemma by suggesting that most amino acid substitutions are
606 neutral and do not count against the speed limit. However, the basis for this historical argument is now on
607 troubled ground, because recent literature argues that the fraction of substitutions explained by adaptation
608 can be high (Galtier 2016; Murga-Moreno et al. 2023; Sella et al. 2009; Uricchio, Petrov, and Enard
609 2019), and that on shorter timescales, as much as 37% of allele frequency change is attributable to
610 adaptation (Buffalo and Coop 2020). For example, recent experiments have shown rapid, pervasive
611 seasonal adaptation in *Drosophila* (Bertram 2021; Kelly 2022; Machado et al. 2021). There are other
612 possible resolutions — e.g. some estimates include substitutions of neutral alleles via hitchhiking.
613 Nevertheless, it is curious that the empirical collapse of historical arguments for neutral theory has not led
614 to a re-evaluation of related arguments by Haldane. Here we revise Haldane's arguments for the modern

615 era, finding that Haldane's revised arguments are compatible with empirical evidence for abundant
616 adaptation while still posing upper limits that might matter in some contexts.

617 We note, however, that Haldane (1957) used a one-locus model — linkage disequilibrium will typically
618 make the conversion of selective deaths to adaptation less efficient than implied by the assumption of
619 independence used both in his calculations, and in the subsequent calculations of Nei (1971) and
620 Felsenstein (1971). Here we also considered the other extreme, in the form of the asexual model of Desai
621 and Fisher (2007). The focus of the current work is to calculate the required reproductive excess and the
622 proportion of deaths that are selective; more work is required to quantify how this is converted into
623 adaptation in a broader range of models of linkage disequilibrium and epistasis, as well as life histories
624 and antagonistic pleiotropy.

625 Excitingly, unlike most approaches in evolutionary genetics, the approach we describe does not require
626 the quantity 'fitness', which is deceptively difficult to define in a manner that can be generalized to all
627 circumstances (Ariew and Lewontin 2004; Bertram and Masel 2019; Doebeli, Ispolatov, and Simon 2017;
628 Van Valen 1989). Standard quantitative definitions of either relative or absolute 'fitness' require a clear
629 definition of a 'generation' over which change is assessed, which in turn requires a clear definition of an
630 'individual' whose life cycle a generation captures (Wilson and Barker 2021). While "lineage fitness"
631 solves a number of problems (Akçay and Van Cleve 2016; Graves and Weinreich 2017; King and Masel
632 2007), it does so at the cost of defining the fittest genotype to be that which tends to eventually prevail,
633 sacrificing much of the quantitative benefit of 'fitness'. Our generalized selective deaths approach is
634 derived from selection, but does not require 'fitness' to be defined. Rather, we measure selective deaths
635 from pairwise differences in fecundity and survival between each genotype and the best genotype present,
636 during each life history transition. Reproductive excess corresponding to that life history transition
637 indicates how stringent selection can be, without triggering a decline in population size. This approach
638 applies at each life history transition and can therefore be generalized to species with complex life
639 histories, where it becomes difficult to define a 'generation' and therefore fitness.

GLOSSARY

Absolute fitness of an adult: expected number of individual offspring surviving to adulthood (defined here for hermaphrodite species — half this value, if reproducing sexually with males).

Absolute fitness of a juvenile: expected number of individual offspring (or half this value, if reproducing sexually with males). Failure to survive to adulthood (reproductive maturity) implies zero offspring.

Adult: Reproductively mature individual. More than one adult life history stage may be defined.

Cost of selection: The number of selective deaths that must occur over time to accomplish defined evolutionary change, e.g. to complete a single selective sweep.

Generation: A set of life history transitions that ends the first time it returns, with new individuals, to the same life history stage (e.g. adult or juvenile) where it began.

Individual: An organism that meets a loosely-defined set of criteria (Wilson and Barker 2021), including a shared genome, and the degree of integration of parts. Whether e.g. a group of microbes is a closely connected ecological community vs. an individual organism may be a matter of biological judgment.

Juvenile: Individuals that are not yet reproductively mature. More than one juvenile life history stage may be defined, e.g. before vs. after dispersal.

Lag load: The difference in fitness between a theoretical best genotype that might not be present in the population and the average genotype present.

Lead: The difference in fitness between the best genotype present in the population and the average genotype present.

Life history transition: Survival (i.e. persistence of an individual), reproduction (i.e. generation of new individuals) and/or organismal growth from one life history stage to the next

Load: A difference in fitness between an actual genotype or population and a reference. See "lag load" and "lead" as concrete examples.

Relative fitness: expected relative genetic contribution to the next generation

Reproductive excess: The degree to which a hypothetical population concludes a life history transition with a larger population than the minimum required to complete a life history cycle without the population shrinking in size.

Selective deaths: The subset of deaths (or foregone fertility) that contributes to selective changes in allele frequency. This can be quantified as how many deaths each genotype experiences that would not have been experienced if that genotype were replaced by the best genotype.

DATA AVAILABILITY

All analysis was performed in R, and our code is available on GitHub:

www.github.com/josephmatheson/selective_deaths.

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AUTHOR CONTRIBUTIONS

Conception of the paper by Joanna Masel and Moises Exposito-Alonso. Design and interpretation by Joseph Matheson and Joanna Masel. Data and details of experimental methodology provided by Moises Exposito-Alonso. Data analysis mostly by Joseph Matheson, with the MCMCglmm model analyzed by

Moises Exposito-Alonso. Manuscript drafted by Joseph Matheson and substantively revised by all authors.

CONFLICT OF INTEREST

The authors declare no conflicting interests.

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SUPPLEMENTARY INFORMATION

Contents

Supplementary Methods

Correction for extreme value bias

The estimated best genotype is subject to extreme value bias, leading to overestimation of the number of selective deaths. I.e., the best genotype observed is likely not only to be a superior genotype, but also to have outperformed its own expected death rate by chance. The more uncertainty in estimated genotypic survival rates, relative to true genetic variance, the worse the extreme value bias problem. Here we attempt a rough estimate of the magnitude of extreme value bias using the observed noise among replicates and among genotypes. We then subtract a conservative estimate of bias from our best observed genotype, and redo our calculations of selective deaths.

We perform 10,000 simulations per environmental treatment. In each simulation, we assume that the observed 517 genotypic death rates are the 'true' values for each genotype (thus slightly overestimating genetic variance within the population). We then resample the number of surviving seeds 'observed' for each replicate of that genotype using a binomial distribution and calculate the resulting observed death rate.

In each simulated dataset, we took the best observed genotype and recorded the difference between its observed value and its actual genotypic value, then averaged these differences across the 10,000 simulations to obtain estimated bias. We then adjusted our estimate of the number of selective deaths to incorporate this estimated bias:

$$\text{Total adjusted selective deaths} = \sum_{i=1}^{517} n_i(d_i - (d_{best} + bias)).$$

Adjustments to selective deaths are shown in Supplementary Table 1. Across all environmental treatments, adjusting for extreme value bias leads to negligible change in the estimate of the proportion of deaths which are selective.

Supplementary Tables

Environmental condition	Among-genotype variance	Observed maximum survival rate	Estimated bias	Unadjusted proportion of deaths selective	Adjusted proportion of deaths selective
MLI	0.0618	1	0	0.664	0.664
MHI	0.0144	1	0	0.0847	0.0847
TLI	0.0337	1	0	0.564	0.564
THI	0.0139	1	0	0.109	0.109
MLP	0.0113	1	0	0.96	0.96
MHP	0.0433	1	0	0.564	0.564
TLP	0.023	0.947	0.00418	0.633	0.629
THP	0.0195	0.871	0.0195	0.391	0.372

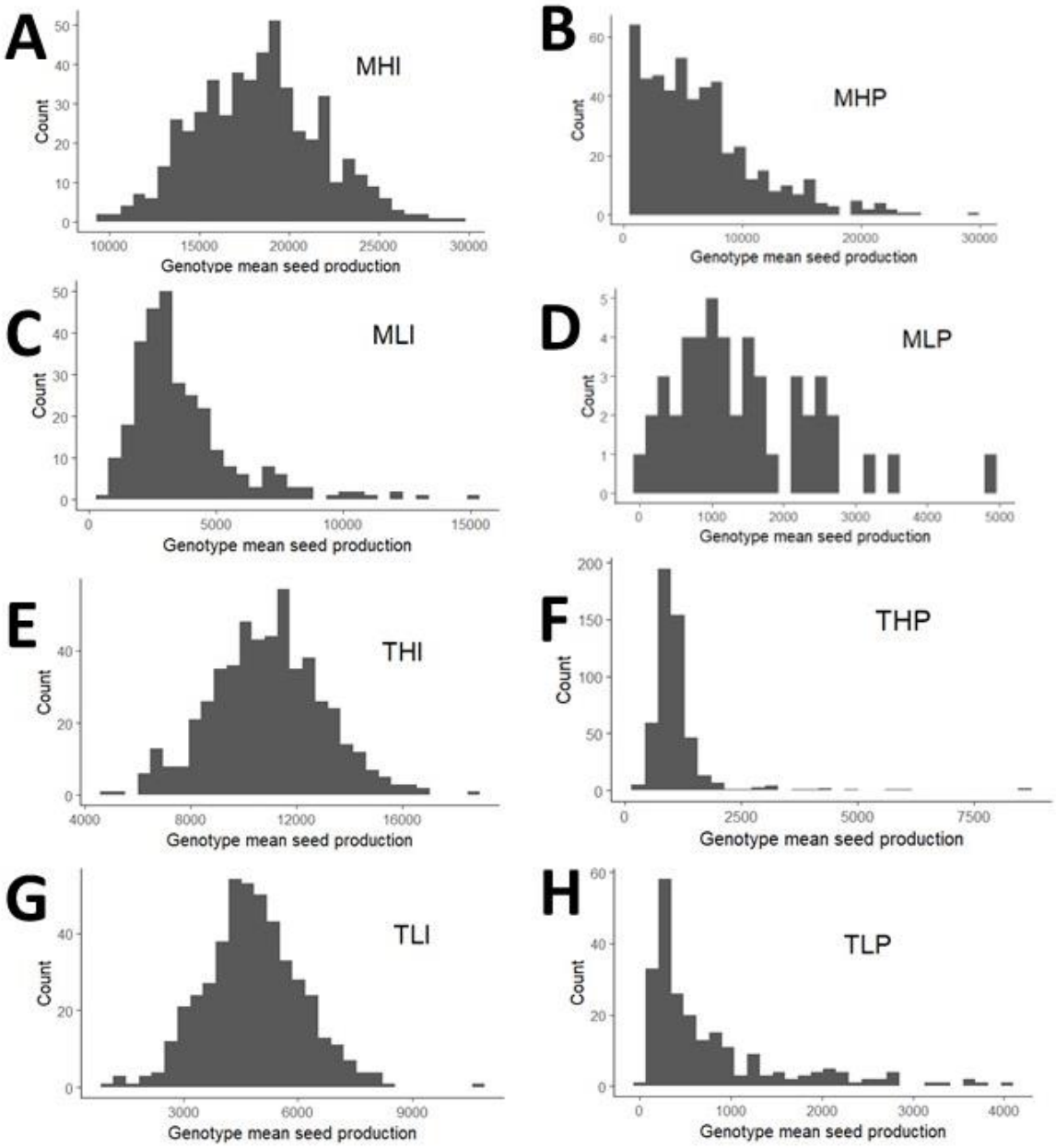
Supplementary Table 1: Adjusting for extreme value bias has little effect on the estimated proportion of selective deaths.

In six out of eight environmental conditions, at least one genotype had all plants survive until the end of the experiment. This genotype then has an observed death rate of zero and an observed between-replicate variance of zero, and thus our approach will not find any bias. Determining bias in this case would require attempting to fit and then resample from a true distribution of genotypic values that includes death rates near but not equal to zero. We did not pursue this avenue, because resampling genotypes would add a new source of variance, and we need only correct for the extreme value bias pertaining to the genotypes actually studied. Given the small degree of bias observed in the two environmental conditions in which no genotype experienced perfect survival, we consider our approach sufficient to conclude that extreme value bias has little quantitative effect on our results.

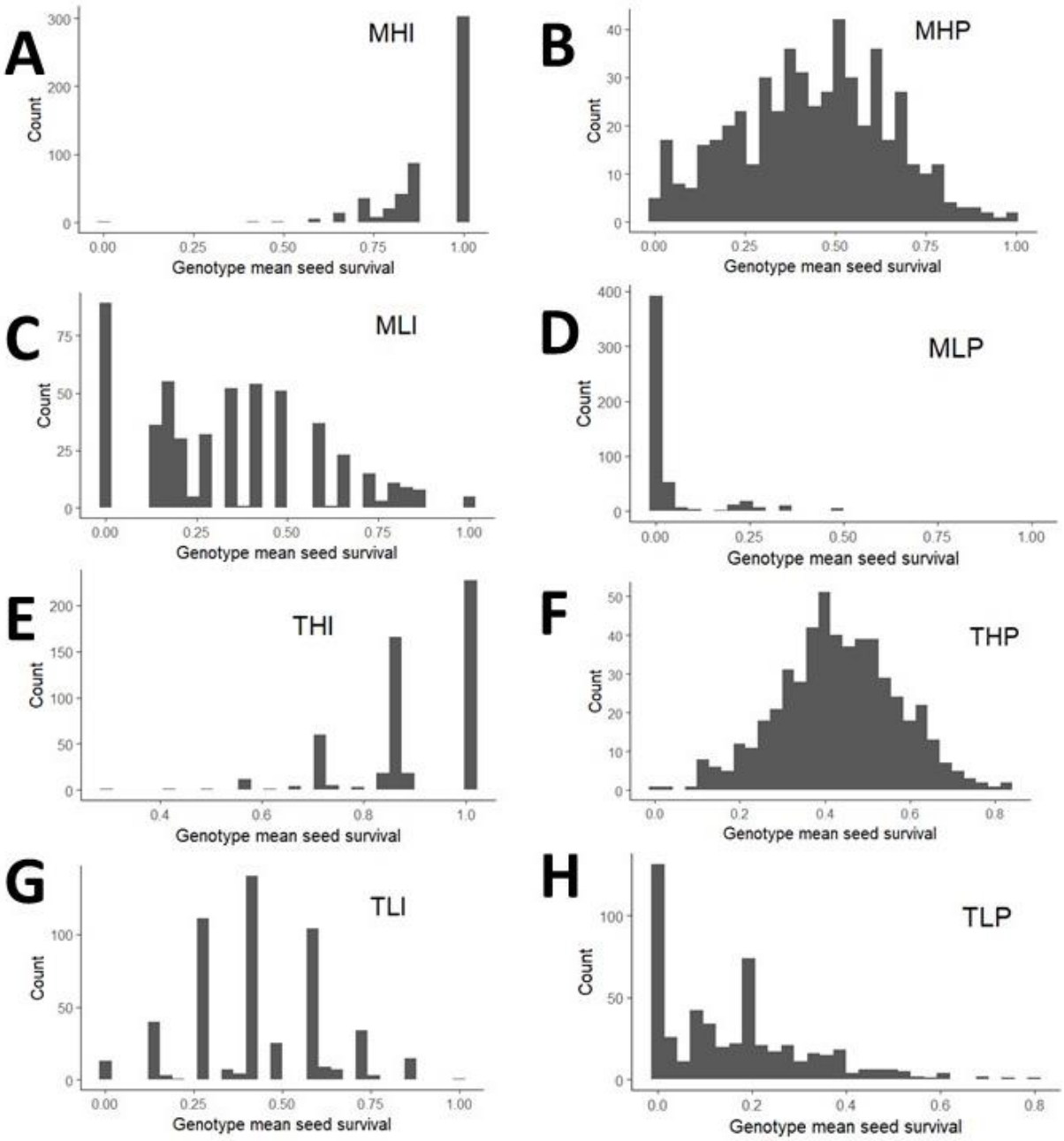
	Proportion of seedling deaths selective	Proportion of combined seed and seedling deaths selective	Excess seedlings per pot after seedling survival transition	Excess seedlings per pot after seed and seedling survival transition	Excess seeds produced per pot after fecundity transition
MLI	0.664		0.742-0.974		967-1,269
MHI	0.0847		0.945-0.995		15,856-16,680
TLI	0.564		0.794-0.979		1,683-2,075
THI	0.109		0.908-0.991		8,848-9,651
MLP		0.960		20.51-29.05	257-364
MHP		0.564		28.16-29.82	20,029-21,207
TLP		0.633		13.71-22.97	833-1,396
THP		0.391		21.52-24.47	10,374-11,796

Supplementary Table 2**.** Proportion of deaths that are selective are above Haldane's 10% estimate for most environmental conditions. Reproductive excess after the survival and fecundity life history transitions are high. Note that reproductive excess per pot for low-density conditions cannot exceed one, and excess per pot for high-density conditions cannot exceed 30. For reproductive excess, lower bounds are calculated using $k_{dispersal} = 0.01$ and higher bounds are calculated using $k_{dispersal} = 0.1$. Reproductive excess values for fecundity are rounded to the nearest integer; approximate methods were used to estimate seed number.

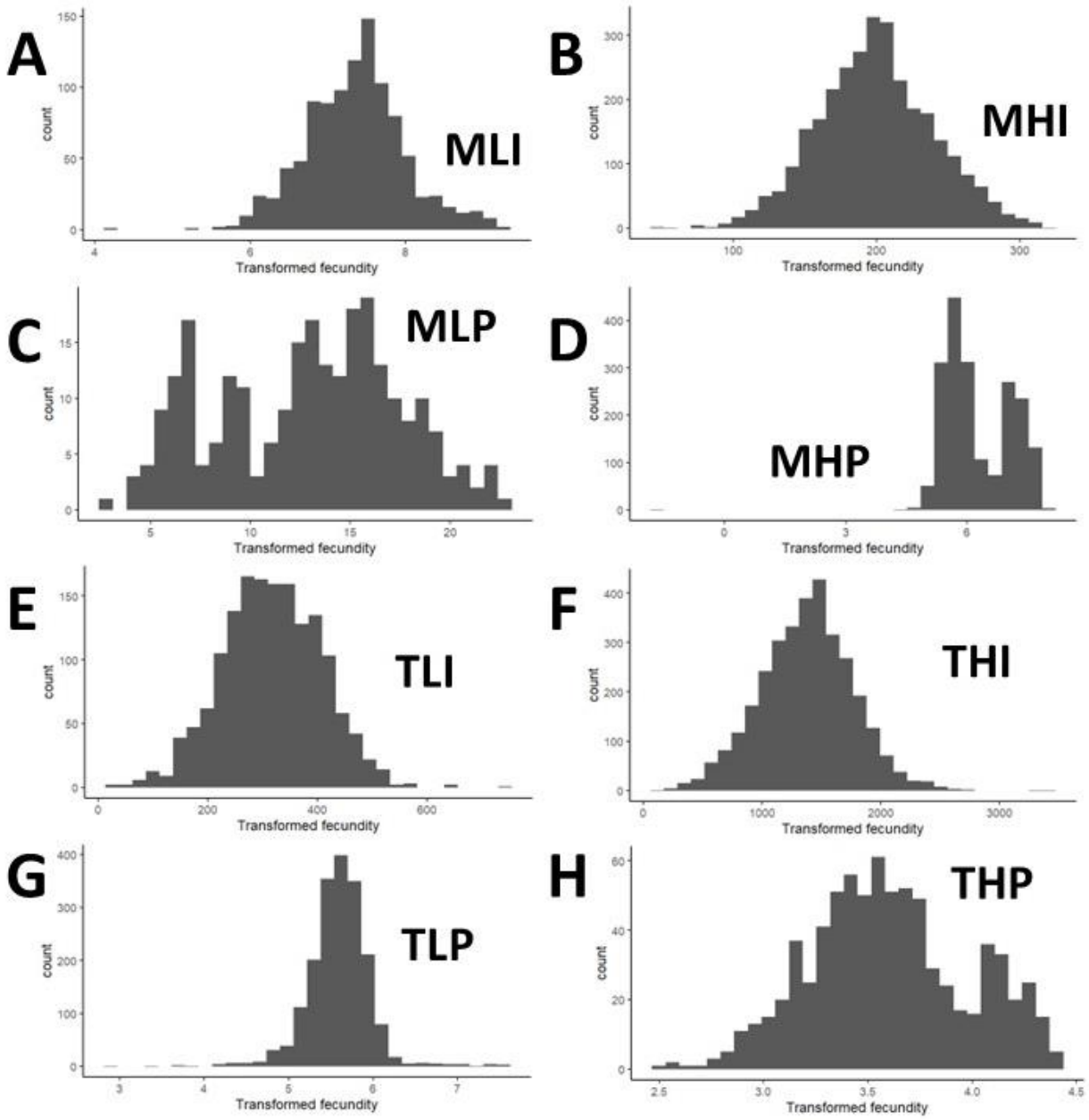
Supplementary Figures



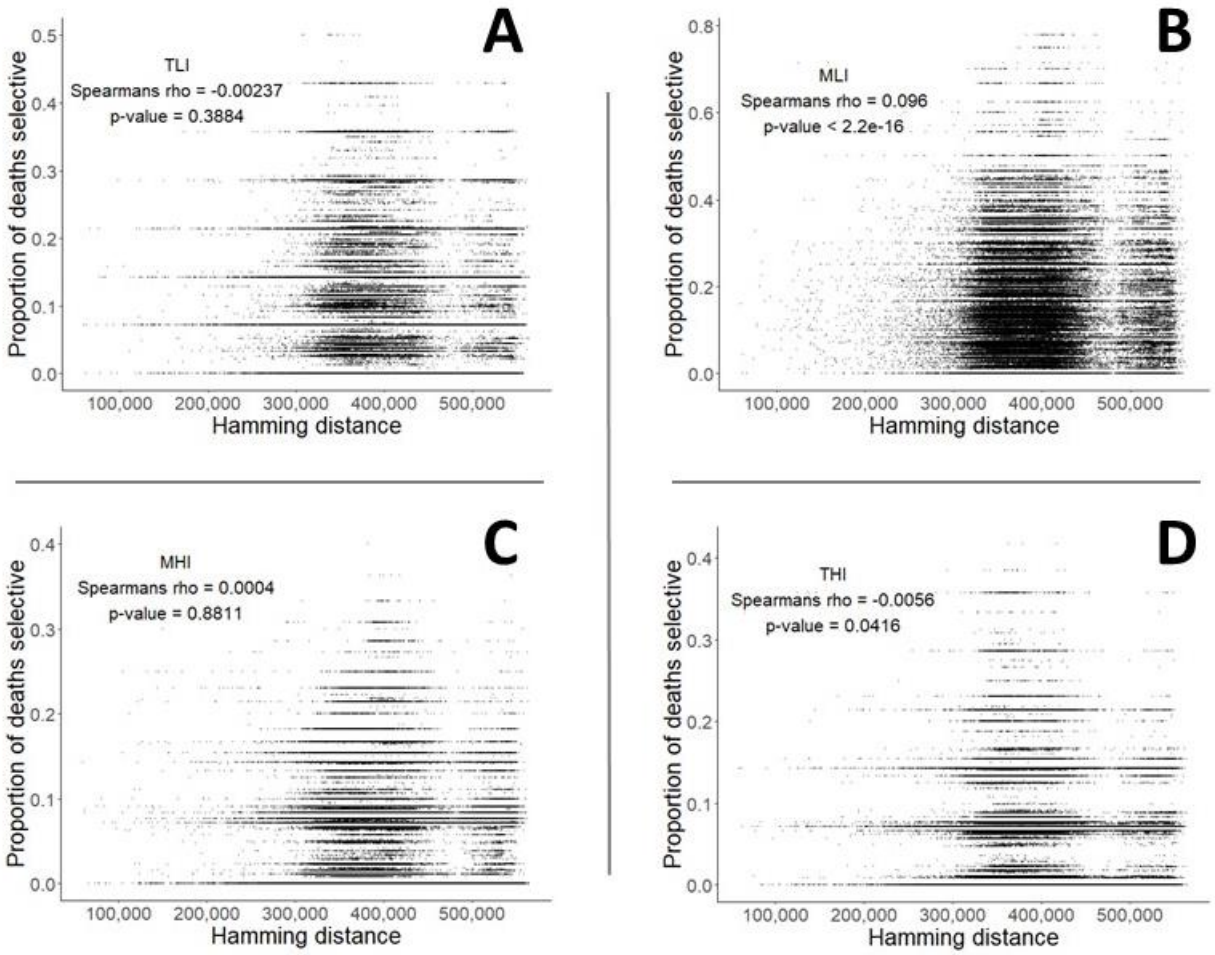
Supplementary Figure 1**.** Histograms of genotype mean seed production for every genotype with at least one surviving adult in each of the eight environmental conditions.



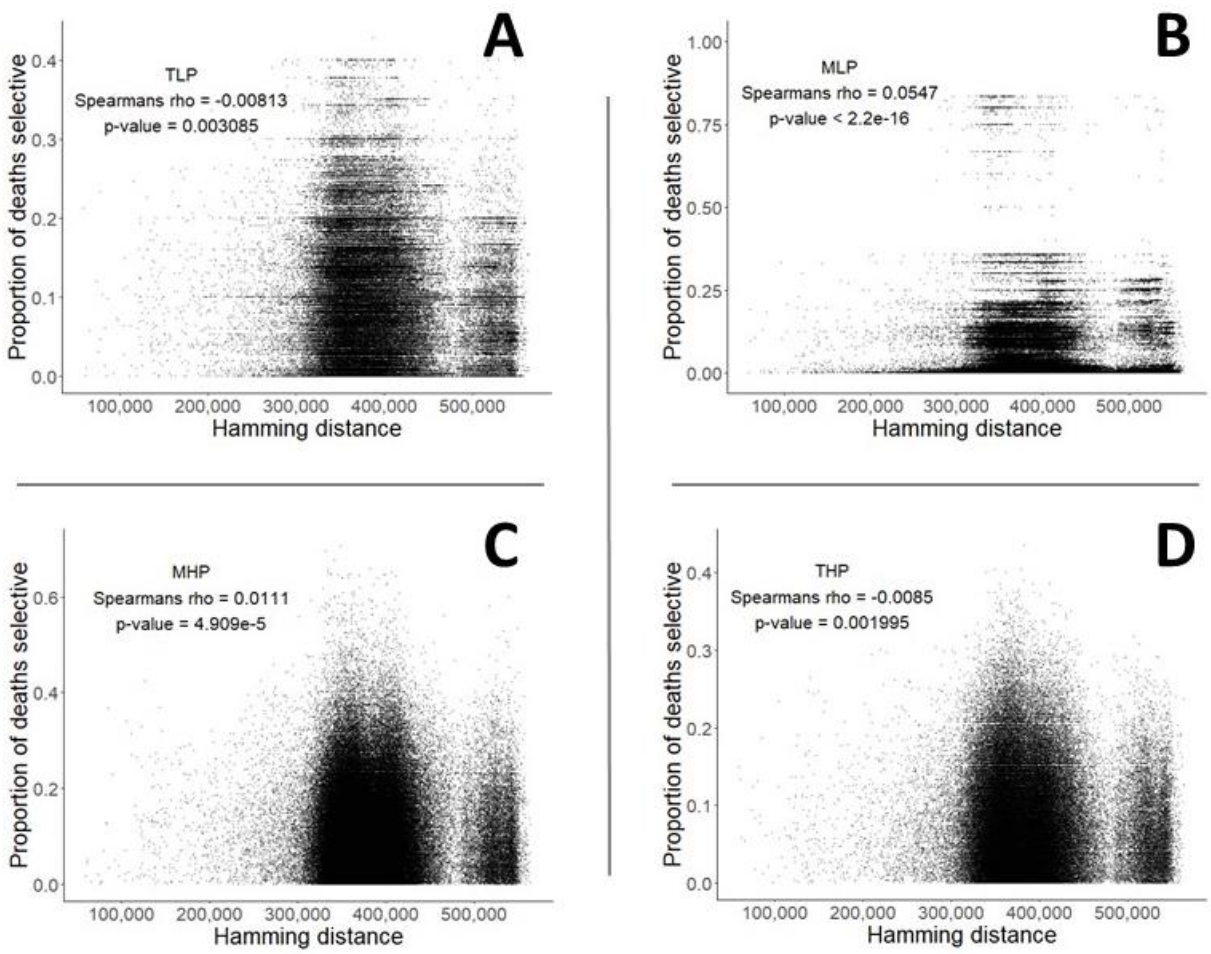
Supplementary Figure 2. Histograms of genotype mean seed survival for every genotype in each of the eight environmental conditions.



Supplementary Figure 3**.** Histograms of Box-Cox transformed fecundity values for every pot with surviving adults in each of the eight environmental conditions. Lambda values for the Box-Cox transformations are (A) -0.02, (B) 0.46, (C) 0.18, (D) -0.06, (E) 0.63, (F) 0.75, (G) -0.18, (H) -0.06



Supplementary Figure 4**.** Hamming distance does not substantially predict the proportion of deaths selective during the life history stage of combined seed and seedling survival at low density.



Supplementary Figure 5**.** Hamming distance does not substantially predict the proportion of deaths selective during the life history stage of seedling survival at high density.