Substitution load revisited:
a high proportion of deaths can be selective

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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, especially with respect to the term "substitution load". Here we distinguish different historical lines of reasoning, and identify one, focused on finite reproductive excess and the proportion of deaths that are "selective" (i.e. causally contribute to adaptive allele frequency changes), 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. The actual speed of adaptive evolution is coupled to the proportion of deaths that are selective. The degree to which reproductive excess enables a high proportion of selective deaths depends on the details of when selection takes place relative to density regulation, and there is therefore no general expression for a speed limit. As proof of principle, we estimate both reproductive excess, and the proportion of deaths that are selective, from a dataset measuring survival of 517 different genotypes of *Arabidopsis thaliana* grown in eight different environmental conditions. These data suggest that a much higher proportion of deaths contribute to adaptation, in all environmental conditions, than the 10% cap that was anticipated as substantially restricting adaptation 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 must be substituted for old alleles across an entire population. This means that individuals with the old alleles must leave no descendants that carry them, and individuals with new alleles must produce enough offspring to replenish the population. These requirements limit the number of sweeps that can happen within a given time. Haldane (1957) used this reasoning to propose a rough estimate of the maximum speed of adaptation. This speed limit, later known as Haldane's dilemma (Van Valen 1963), motivated the development of Kimura's (Kimura 1968) neutral theory. However, its underlying logic has been challenged on multiple counts (Ewens 1970; Felsenstein 1971; Kern and Hahn 2018; Maynard Smith 1968).

Here we first synthesize the historical literature, drawing out two key quantities (see Results). First, a population has a "reproductive excess", meaning how many individuals could be produced at a given life history stage, in excess of requirements, if all individuals had the best genotype present (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 finite reproductive excess limited the budget for selective deaths, such that at most 10% of deaths were selective, an assumption repeated in 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). Once multiple life history stages are considered, assumptions regarding when and how density regulation. operates must be made explicit (Haldane 1956; Nicholson 1933).

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 (Smith et al. 2024). The "generation" that matters in this view is not 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 the best genotype have? 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.

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 an increase in the allele frequency p, where s is the selection coefficient. These deaths are considered selective because the $(1-p)N$ individuals that lack a beneficial mutation expect s more deaths than they would if they had the mutation, and those extra deaths are required for selection to have

its effects. Selective "deaths" can include absence of potential offspring, not just literal deaths, because reduced fecundity is mathematically equivalent to increased mortality.

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 needed for a sweep.

Haldane's second argument 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 ith 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 conversion was incorrect; the better reference point to calculate the fraction of deaths that are selective 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). Lag load can be defined even in a static environment, where innovative new adaptive alleles reveal the possibility of an even better optimal genotype. Large lag load is not a problem *per se*; what matters is that it is stable rather than growing. While speed limits cannot threaten the persistence of a species adapting in a constant environment, real species face rapidly changing environments (biotic and abiotic) that can threaten population persistence. I.e., for a population to persist, adaptation must keep up with the speed of environmental change.

Haldane argued *a priori* that species could 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 $\frac{30}{\sum d_i} = \frac{30}{0.1}$ =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. 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 used 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 fitness difference between the average genotype and the best genotype currently present and named it the "lead" (Desai and Fisher 2007). Prior to this, approximations for the lead were derived from variance in fitness (Ewens 1970; Kimura 1969). With 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. around 4.9 standard deviations above the mean for a population of size 10^6 (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.

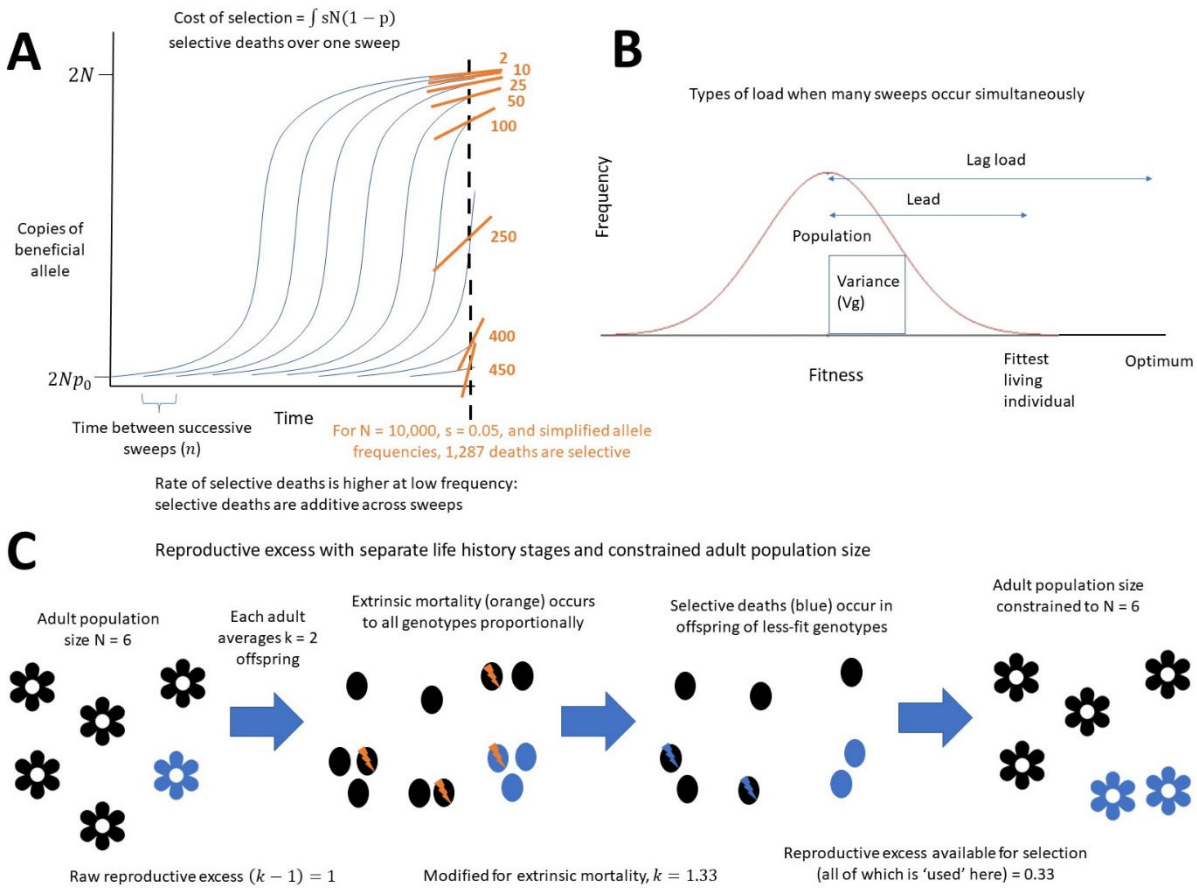

Figure 1. Three different types of arguments have been used to argue for limits to the speed of adaptation. A) The cost of selection is the cumulative number of selective deaths that must occur to complete a single sweep (each sweep shown as a logistic curve). The cost of selection at one timepoint is the sum of the costs for each current sweep, illustrated as the slopes of the orange lines, each calculated as the subset of deaths $s(1-p)N$ that contribute to selection on allele frequency p. B) Load is a difference between fitnesses: lag load is the difference between the mean fitness and a hypothetical optimal genotype, while the lead is the difference between the mean fitness and the best currently living genotype. C) Finite reproductive excess imposes an upper limit on how many selective deaths per generation are possible, which sets an upper bound to how fast substitutions can occur.

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), this does not negate Haldane's first line of argument that 20-30N selective deaths are needed for a sweep. Nei (1971) and Felsenstein (1971) made a key advance along this first line of argument, which was perhaps not fully appreciated amidst the confusion.

The cost of selective deaths must be paid out of a finite budget of reproductive excess. 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 when simulating selection on zygote viability using rejection sampling– 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 give birth to more than 20 infants. This constrains members of the next generation to options within a finite set of potential offspring. This concept has been applied to lethal mutagenesis strategies for anti-viral drugs (Bull, Sanjuán, and Wilke 2007).

Nei (1971) and Felsenstein (1971) each modelled independently evolving sites in a haploid population with adult population size N. Each adult has fecundity k, i.e. produces k offspring prior to juvenile deaths (Figure 1C, first arrow). In their deterministic models, k is exact, but the theory readily generalizes to interpreting k as an expectation. The raw reproductive excess is thus $(k-1)N$, with $k > 1$. Extrinsic mortality occurring prior to selective mortality, and hence at a fixed rate, can be folded into a lower value

of k (Figure 1C, second arrow). Haldane's estimate of $k = 1.1$ (resulting in a maximum of 10% selective deaths), which Nei (1971) and Felsenstein (1971) retain, includes the fact that extrinsic mortality substantially reduces the fecundity available to be 'used' for selective deaths. Population size regulation after selective mortality (Figure 1C, far right) will on average consume the remaining reproductive excess that was not used by selection.

The population undergoes sweeps, all with the same initial frequency p_0 and selection coefficient s applying to survival rather than fecundity. Each sweep follows the same trajectory with a mean delay of n generations between sweeps (Figure 1A). Given independent sites, the cost of selection is summed across loci at any given point in time (e.g. slopes of orange lines in Figure 1A); Haldane's integral is a method of calculating the expectation of this sum. Comparing this cost to the reproductive excess of the population produced the novel result that the minimum spacing n is $-{\ln(p_0)}/{\ln(k)}$ (Felsenstein 1971; Nei 1971). For Haldane's estimates of $p_0 = 10^{-4}$ and $k = 1.1$, this yields $n = 97$ generations between selective 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 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 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 $(k-1)N$. 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 N refers only to the 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

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

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 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.

For example, consider a microbial population in batch culture that alternates between exponential growth, stationary phase, and dilution life history transitions. For stationary phase, k is close to 1 but kN is relatively large because N is large. Many cells will be lost in the subsequent 1/100 dilution, but then exponential growth will typically yield N cells from any starting density, so long as seeding is successful. $N_{min,} > 100$ is thus required to recover N live cells at the beginning of the next stationary phase. In contrast, for the dilution life history transition, $k \sim 0.01$ and N_{min} in its aftermath may not be much larger than 1.

Reproductive excess for transition j provides the maximum budget for how many selective deaths could occur during transition j, without upsetting population dynamics by overwhelming the compensatory capacity of density regulation during other transitions. The calculation of $N_{min,j+1}$ includes density regulation at all life history transitions except j, while density regulation during transition j is held constant in its representation by $k_j N_j$. Reproductive excesses for different life history transitions cannot be summed or otherwise combined.

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 into 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 (Bell 2017) 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:

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

Speed limits in the general model

Nei (1971) and Felsenstein (1971) had only one value of reproductive excess $RE = (k-1)N$, and one (adult) population size *N*, from which they derived a speed limit of $-\ln\left(\frac{RE}{N}+1\right) \Big/ \ln\left(p_0\right)$ substitutions per generation. When selective deaths occur at only one life history transition j, this calculation applies with RE_j and N_j.

However, because each selective death reduces k for the transition at which it occurs, it reduces reproductive excess at all other transitions, in a manner that depends on assumptions about when and how density regulation occurs. E.g., the selective death of an adult tree creates a canopy opening into which saplings may grow, in addition to reducing the number of seeds available to grow into it. Both these density-regulation effects dampen the degree to which selective deaths at one life history transition reduce reproductive excess at other transitions. Density regulation makes the reproductive excess "budgets" for the selective death "costs" partially, but only partially, independent across life history transitions. Reproductive excess calculations invoke N_{min} as a counterfactual – how many losses at the focal life history transition could density regulation compensate for, holding all else constant? There is no simple counterfactual for perturbing selective deaths at multiple transitions at once, even when each invokes distinct, non-pleiotropic mutations. Life history trade-offs make matters worse, because the same allele might cause selective deaths at multiple transitions, even when the allele frequency is not changing. We thus reach the negative result that there is no general formula for a speed limit when selection occurs during more than one life history transition.

Nei and Felsenstein as a special case

Nei (1971) and Felsenstein (1971) treated reproductive excess given only two alternating life history transitions: births and deaths. Deaths included *only* selective deaths, while the "effective" fecundity transition was non-selective. Non-selective deaths were collapsed into $k_{fecundity_effective}$, either before selection on survival (reducing $k_{fecundity_effective}$ in the current generation), or after selection on survival (balancing out deaths that were "unused" by selection in the current generation, by reducing $k_{fecundity_effective}$ in the subsequent generation). When $k_{fecundity_effective} \times k_{selective_mortality}$ is set to 1 to calculate the maximum amount of selective deaths, $k_{fecundity_effective}$ can be interpreted as the product of actual fecundity and non-selective survival. Fig. 1C interprets this scheme in a temporal manner, proceeding first through non-selective fecundity to produce raw reproductive excess, then the non-density-dependent component of extrinsic mortality, then selective deaths, and finally density-

dependent extrinsic mortality to cap the population size at *N* adults. They score reproductive excess as subject to the first but not the second form of reduction down to "effective" fecundity, minus the *N* individuals needed to replace the population.

Reproductive excess beyond a fixed life cycle

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

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

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

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

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

Comparison to fitness

Haldane obtained selective deaths from $sN(1-p)$, where *s* is the selection coefficient with respect to per-generation relative fitness. Our k values are equivalent to absolute fitness components per life history

transition. We have shown how selective deaths can be derived from the underlying population dynamic model, without requiring either generation or relative fitness to be defined first.

In the simple case of an absolute fitness version of the Wright-Fisher model, adults of genotype i produce k_i offspring, then die. Selective deaths per capita $k_{best} - k_{mean}$ is equal to the difference in absolute fitness, and reproductive excess per capita is $k_{mean} - \frac{1}{k_{best}}$.

However, these simple relationships break down once selection occurs at multiple life history stages. 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, costs and benefits cancel out. In contrast, selective deaths never cancel out; indeed, incomplete density compensation causes selective deaths to accrue across life history transitions. 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).

Discrete time models such as Wright-Fisher typically emphasize the effective reproductive number rather than the Malthusian parameter, i.e. equivalent to b/d rather than $b - d$ in a continuous time model with births at rate b and deaths at rate d. Malthusian approaches are generally used to handle the complications arising from multiple life history transitions (Charlesworth 1994). Our approach enables an effective reproduction number framing in these more complex scenarios, while still avoiding the need to define one "generation". This enables treatment of the finite nature of reproductive excess.

Comparison to travelling wave models

Desai and Fisher's (2007) Eq. 39 solves for the fitness difference between the highest-fitness and the average asexual genotypes (the 'lead'), as a function of U, N, and relative fitness s, In a population of approximately constant size, the mean genotype has absolute fitness ~ 1, and the best genotype present

(the 'nose') has absolute fitness $\sim 1 + qs$. The minimum reproductive excess implicitly required to maintain constant population size is equal to the lead qs. 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 be so simple when more complex life histories are considered. We note that the adaptation speed has a one-to-one relationship with the proportion of deaths that are selective, whereas reproductive excess imposes a speed limit without having a direct effect on speed.

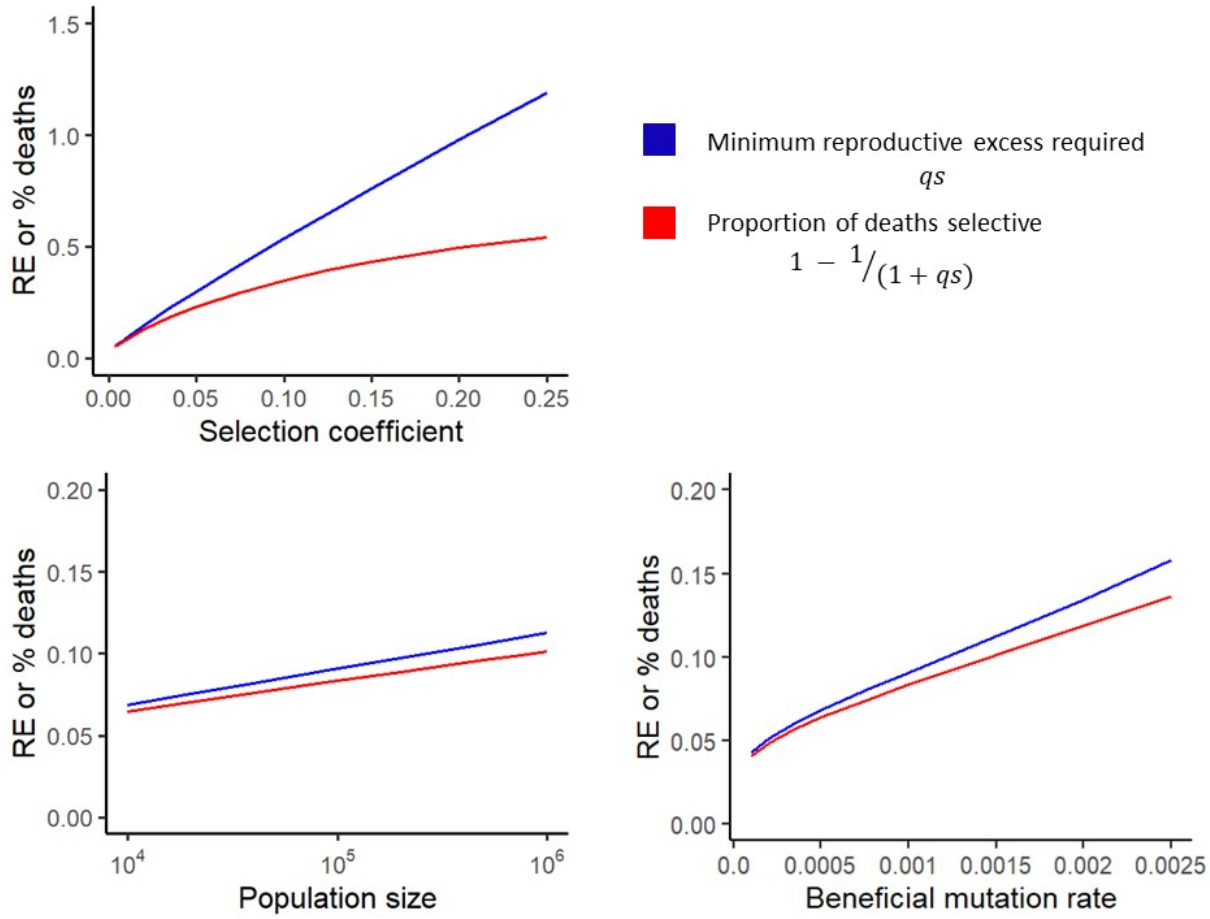

Figure 2. As the adaptation rate goes up with increasing U, s, and N, following the multiple mutations regime of Desai and Fisher (2007), so do the proportion of deaths selective and the minimum reproductive excess required to sustain that rate of adaptation. Parameter ranges are truncated to avoid the 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).

Experimental proof of concept

We measure reproductive excess and the proportion of deaths that are selective for the first time in an empirical system using data from Exposito-Alonso et al. (2019) (Figure 3). Briefly (see Supplementary

Information for details), 517 homozygous lines of *Arabidopsis thaliana* were grown in 2 × 2 × 2 environments of varying location, water availability, and adult density. For high density, thirty seeds of the same genotype were planted per pot, and selective deaths are for the transition from seed to adult. For low density, ~10 seeds were planted per pot, and only one seedling, chosen at random, was retained after germination; selective deaths are for the transition from seedling to adult. Fitness differences in fecundity were not significant (see Supplementary Results).

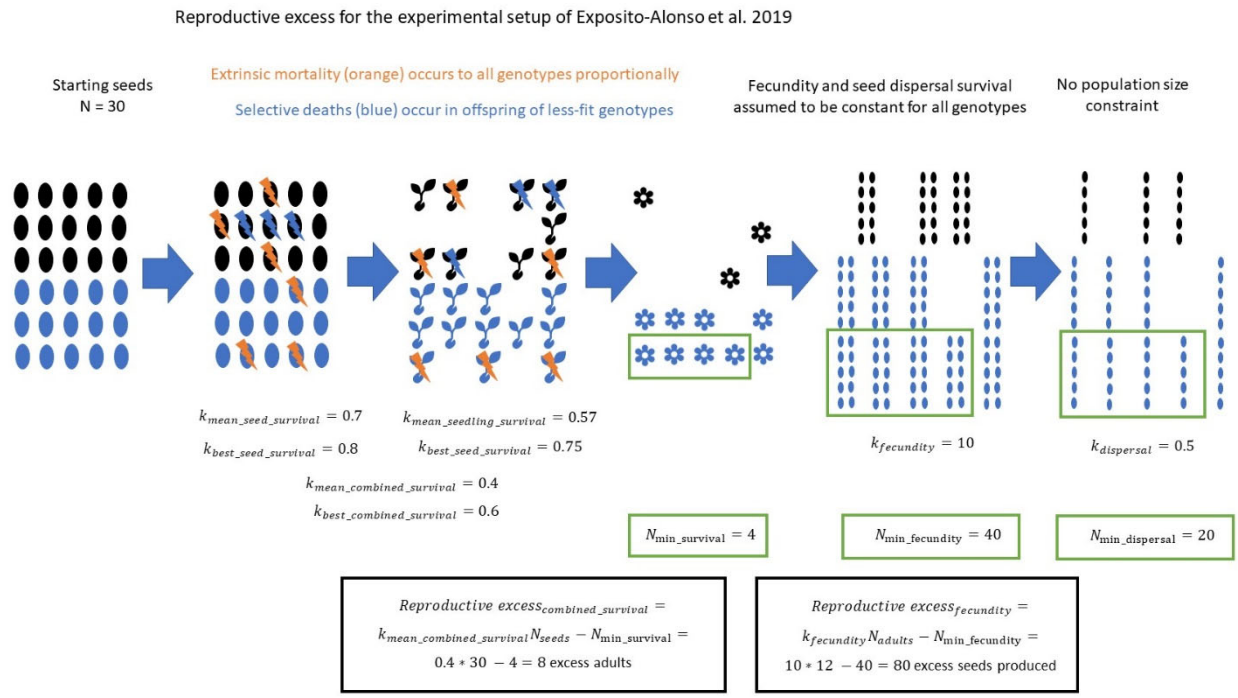

Figure 3. Worked example of reproductive excess for the life history transitions of *A. thaliana* in the experimental setup from Exposito-Alonso *et al* (2019). We found no evidence for between-genotype differences in fecundity and the experimental setup provides no information about seed dispersal,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

The proportion of deaths that are selective exceeds 10% (Figure 4 x-axis, Supplementary Table 2). In Madrid with low water and high density, as many as 95% of deaths were selective.

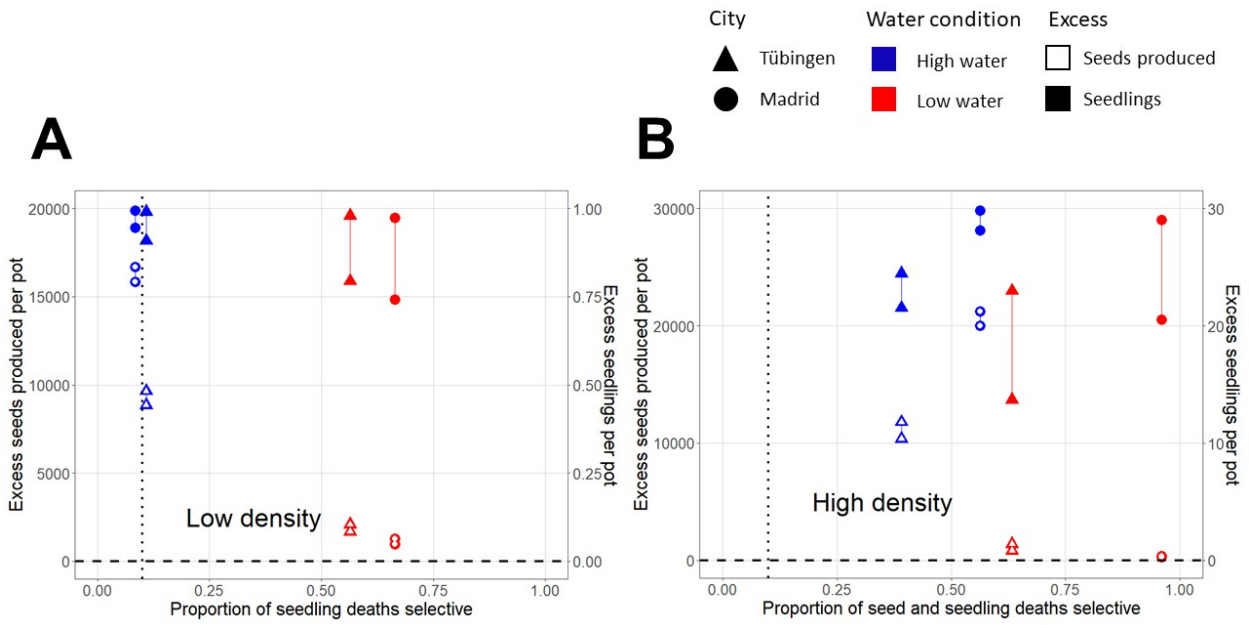

Figure 4. <u>The proportion of deaths that are selective generally exceeds Haldane's 10% estimate (dotted vertical line), with ample reproductive excess (above zero, shown as dashed horizontal line) especially under high-water conditions.</u> Selective deaths shown on the x-axis apply either to seeds plus seedlings (A) or to seedlings alone (B). Reproductive excess is calculated (see Supplementary Information) for two life history transitions: seeds produced and seedlings surviving to adulthood. These are shown with open and closed symbols, respectively, and correspond to values shown on the left and right y-axes, respectively. Note that reproductive excess of seedlings cannot exceed one per pot under low density conditions, and 30 per pot under high density conditions. Reproductive excesses are shown as a vertical range representing high and low values of seed survival during dispersal, a life history transition for which we lacked empirical data, and therefore considered a 10-fold plausible range. The proportions of deaths that are selective are adjusted for extreme value bias as shown in Supplementary Table 1. Values can be found in Supplementary Table 2.

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 seedling 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).

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 speed limit arguments; with appropriate comparisons to the best genotype currently present, rapid adaptation is possible. 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. Given an assumed 10% limit on the fraction of deaths that could be selective, and allowing selection at just one life history transition, the rate of adaptation is significantly limited (Felsenstein 1971; Nei 1971). We clarified and extended theoretical arguments regarding selective deaths drawn from finite reproductive excess, to apply even to life histories for which a "generation" is poorly defined, e.g. colonial organisms. Speed limits depend on when and how density regulation occurs, and our proof-of-principle empirical analysis suggests that the fraction of selective deaths may not be limited to 10%; these results might explain why adaptation is not significantly limited.

Nei (1971) and Felsenstein (1971) collapsed linkage disequilibrium, density regulation, antagonistic pleiotropy, etc. into a single 'effective' reproductive excess, and then solved for a hypothetical equilibrium where all such reproductive excess was converted to selective deaths. They thus demonstrated that finite reproductive excess imposed a speed limit, without referring to lag load. Our more complete treatment emphasizes firstly that reproductive excess varies by life history stage and secondly that the degree to which selective deaths during one life history transition reduces reproductive excess at other transitions depends on how and when density regulation occurs. Taken together, these theoretical results argue against the usefulness of singular estimates of 'speed limits' to adaptation in general, although they do not preclude the possibility that some populations in some cases will have limits on their rate of adaptation.

We apply the concepts of reproductive excess and selective deaths to empirical data for the first time. This proof-of-principle application was sufficient to suggest a novel possibility: Haldane's 10% limit to selective deaths may be too low. The smallest proportion of selective deaths we observed across 8 environmental conditions was 8.5%, while in the most adverse environmental conditions, 95% of deaths were selective. Relaxing this auxiliary assumption about a critical parameter value resolves Haldane's concerns. Future work under more natural conditions (e.g. with higher extrinsic mortality) and in different species (e.g. less fecund) remains necessary to reach the conclusion that the proportion of deaths that are selective is typically high. Our theoretical framework is flexible enough to be customized for any species, using whichever life history transitions best describe that species' life history. Uses might include understanding the limits to adaptation given high fecundity (Hutchings 2000).

Empirical demonstration of the concepts makes the concepts more concrete. One contribution 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.

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.

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 Haldane's 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 this relative fitness model, and calculated the minimal

reproductive excess required for the model to hold. We also reveal the model's implied fraction of deaths (or foregone fecundity) that are selective. Both quantities are functions of the lead.

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

The historical significance of Haldane's arguments about limitations to adaptation is that they were convincingly used to support neutral theory. This was framed as a dilemma because data on the rates of amino acid divergence among species seemed to exceed Haldane's speed limit. The development of neutral theory resolved this apparent dilemma by suggesting that most amino acid substitutions are neutral and do not count against the speed limit. However, the basis for this historical argument is now on troubled ground, because recent literature argues that the fraction of substitutions explained by adaptation can be high (Galtier 2016; Murga-Moreno et al. 2023; Sella et al. 2009; Uricchio, Petrov, and Enard 2019). On shorter timescales, recent experiments have shown rapid seasonal adaptation in *Drosophila* (Bertram 2021; Kelly 2022; Machado et al. 2021). There are other possible resolutions — e.g. some estimates include substitutions of neutral alleles via hitchhiking. Nevertheless, it is curious that the empirical collapse of historical arguments for neutral theory has not led to a re-evaluation of related arguments by Haldane. Here we revise Haldane's arguments for the modern era, finding them compatible with empirical evidence for abundant adaptation, while still posing upper limits that might matter in some contexts.

We note, however, that Haldane (1957) used a one-locus model — linkage disequilibrium will typically make the conversion of selective deaths to adaptation less efficient than implied by the assumption of independence used both in his calculations, and in the subsequent calculations of Nei (1971) and Felsenstein (1971). Here we considered both this one-locus extreme, and the other extreme in the form of the asexual model of Desai and Fisher (2007).

Excitingly, unlike most approaches in evolutionary genetics, the approach we describe does not require the quantity 'fitness', which is deceptively difficult to define in a manner that can be generalized to all circumstances (Ariew and Lewontin 2004; Bertram and Masel 2019; Doebeli, Ispolatov, and Simon 2017; Smith et al. 2024; Van Valen 1989). Standard quantitative definitions of either relative or absolute 'fitness' require a clear definition of a 'generation' over which change is assessed, which in turn requires a clear definition of an 'individual' whose life cycle a generation captures (Wilson and Barker 2021). Our generalized selective deaths approach is derived from selection, but does not require 'fitness' to be defined. Rather, we measure selective deaths from pairwise differences in fecundity and survival between each genotype and the best genotype present, during each life history transition. Reproductive excess corresponding to that life history transition indicates how stringent selection can be, without triggering a decline in population size. This approach applies at each life history transition and can therefore be generalized to species with complex life 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 of the optimal genotype would conclude 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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