Can Purifying Natural Selection Preserve Biological Information?

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Abstract

Most deleterious mutations have very slight effects on total fitness, and it has become clear that below a certain fitness effect threshold, such low-impact mutations fail to respond to natural selection. The existence of such a selection threshold suggests that many low-impact deleterious mutations should accumulate continuously, resulting in relentless erosion of genetic information. In this paper, we use numerical simulation to examine this problem of selection threshold.

The objective of this research was to investigate the effect of various biological factors individually and jointly on mutation accumulation in a model human population. For this purpose, we used a recently-developed, biologically-realistic numerical simulation program, Mendel's Accountant. This program introduces new mutations into the population every generation and tracks each mutation through the processes of recombination, gamete formation, mating, and transmission to the new offspring. This method tracks which individuals survive to reproduce after selection, and records the transmission of each surviving mutation every generation. This allows a detailed mechanistic accounting of each mutation that enters and leaves the population over the course of many generations. We term this type of analysis *genetic accounting.*

Across all reasonable parameters settings, we observed that high impact mutations were selected away with very high efficiency, while very low impact mutations accumulated just as if there was no selection operating. There was always a large transitional zone, wherein mutations with intermediate fitness effects accumulated continuously, but at a lower rate than would occur in the absence of selection. To characterize the accumulation of mutations of different fitness effect we developed a new statistic, selection threshold (ST_d), which is an empirically determined value for a given population. A population's selection threshold is defined as that fitness effect wherein deleterious mutations are accumulating at exactly half the rate expected in the absence of selection. This threshold is mid-way between entirely selectable, and entirely unselectable, mutation effects.

Our investigations reveal that under a very wide range of parameter values, selection thresholds for deleterious mutations are surprisingly high. Our analyses of the selection threshold problem indicate that given even modest levels of noise affecting either the genotype-phenotype relationship or the genotypic fitness-survival-reproduction relationship, accumulation of low-impact mutations continually degrades fitness, and this degradation is far more serious than has been previously acknowledged. Simulations based on recently published values for mutation rate and effect-distribution in

humans show a steady decline in fitness that is not even halted by extremely intense selection pressure (12 offspring per female, 10 selectively removed). Indeed, we find that under most realistic circumstances, the large majority of harmful mutations are essentially unaffected by natural selection and continue to accumulate unhindered. This finding has major theoretical implications and raises the question, "What mechanism can preserve the many low-impact nucleotide positions that constitute most of the information within a genome?"

Key words: deleterious mutation, genetic deterioration, mutation accumulation, near-neutral, population genetics, selection threshold, simulation

Introduction

More than forty years ago, Muller [1] concluded that there exists a class of low-impact mutations that are beyond the reach of natural selection. Kimura greatly expanded upon this theme, using mathematical modeling to study the problem [2]. Although Kimura initially described such mutations as 'neutral', Ohta [3–6] argued that such mutations should more accurately be termed 'nearly neutral', and Kimura later agreed [7, 8]. Kondrashov realized that very low impact mutations are not only inherently unselectable, but they also create a profound evolutionary paradox [9]. Later, Lynch *et al*. [10, 11] and Higgins and Lynch [12] provided evidence that accumulation of low-impact mutations plays an important role in the extinction process. Recently, Loewe [13] showed that accumulation of nearly neutral mutations is a theoretical problem even for haploid genomes as small as that of human mitochondria. His analysis suggests that accumulation of nearly-neutral mutations within the mitochondria alone could potentially lead to human extinction. Given that nearly-neutral mutations have such profound biological implications, it would seem important to understand better the primary factors that control the accumulation of low-impact deleterious mutations.

A useful way to conceptualize selection's ability to influence the accumulation of low-impact mutations is in terms of signal versus noise. 'Signal' corresponds to the level of influence a mutation has on its own transmission. 'Noise', by contrast, corresponds to various types of interference that reduce the correlation between a mutation's effect on functional fitness and its probability of transmission. When the signal is weak and the noise is sufficiently strong, the signal is obscured and selection breaks down. At that point the correlation between the mutation's effect on functional fitness and the likelihood of that mutation's transmission becomes too small for selection to affect the frequency of that mutation in the population in any significant way.

Kimura [7] was the first to attempt to quantify the threshold for selection breakdown. His calculations focused only on the influence of one source of 'noise' on the rate of mutation fixation, i.e., that of gametic sampling. Kimura found that the

strength of this confounding effect on selection varies inversely with the effective population size, N_e. In small populations, a relatively small number of gametes are extracted to produce the next generation. This restricted gametic sampling results in sampling error that leads to random fluctuations in each allele's frequency within the population. These random fluctuations represent a type of noise that interferes with selection. It is well known that this type of genetic drift is strong in small populations and can override all but the strongest selection pressures. However, in larger populations the gametic sampling error is smaller, and thus the resulting random fluctuations in allele frequency are smaller. Therefore, selection for low-impact mutations can be more effective in larger populations. Restricting his analysis to this single source of noise, Kimura developed his now well-known approximation of the magnitude of the selection coefficient needed to overcome drift, expressed as $s = 1/(2N_e)$. This expression implies a direct relationship between the selection threshold and the effective population size N_e [7]. Most subsequent studies of nearly-neutral mutations and their accumulation have utilized this estimate for the point at which selection breaks down and genetic drift becomes predominant [9–13].

It is obvious, however, that there are other sources of biological noise besides gametic sampling. All of these other sources of noise should reduce the correlation between the magnitude of the effect (d_i) of a specific mutation on the functional fitness of an individual and the influence of that mutation on the individual's reproductive success. Lynch [14], for example, notes that small population size, large nucleotide distances between crossovers, and high mutation rates all synergistically reduce the efficiency of natural selection. To study some of these biological factors and to quantify how they affect the selection threshold beyond their predicted direct effect on the selection coefficient, *s,* we adopt a numerical simulation strategy using the program Mendel's Accountant (Mendel) [15, 16, http://www.MendelsAccountant.info]. This numerical approach affords us much flexibility to explore the biological complexity of the mutation-selection process, as it actually occurs in nature. Numerous other studies have explored mutation accumulation via simulation [17–19], including the consequences of a non-uniform distribution of mutational effects. We extend those explorations by including environmental variance, a range of different mutation rates, and various forms of selection (truncation, partial truncation, and standard probability selection).

The earliest reference to the idea of a selection threshold seems to be from Muller [1]. He stated, "*There comes a level of advantage, however, that is too small to be effectively seized upon by selection, its voice being lost in the noise, so to speak. This level would necessarily differ greatly under different circumstances (genetic, ecological, etc.), but this is a subject that has as yet been subject to little analysis… although deserving of it.*" Muller's recognition that there are deleterious

mutations that are practically invisible to the selection process contributed to his overall concern about genetic deterioration. It also contributed to his concern about the problem of linkage-mediated deterioration in fitness ("Muller's ratchet"). The goal of this paper is to explore the biological circumstances (to which Muller alluded) that can make a large fraction of deleterious mutations immune to selection. Our results reveal that even modest degrees of either environmental variance or randomness in the selection process (probability selection) cause selection breakdown for most deleterious mutations, and this problem is compounded by high mutation rates.

Results

Conditions allowing perfect purifying selection

Several experiments were first conducted to discover the region of parameter space in which there is zero near-neutral mutation accumulation. We found that complete elimination of near-neutrals requires that all sources of noise be reduced to either extremely low levels or zero. As a general rule, this requires zero environmental variation (heritability = 1), perfect truncation selection, sufficiently high selection intensity, and sufficiently low mutation rates to maintain near-zero genetic variance. Only when these conditions were satisfied was selection effective enough to preclude accumulation of nearly neutral mutations. Under these special circumstances, low-impact mutations were eliminated just as if they were fully lethal. This was because under these conditions, selection becomes a matter of simply choosing between mutant versus non-mutant individuals. We obtained this result, for example, for the case of zero environmental variance, perfect truncation selection, a mutation rate of one mutation per individual per generation, and the default reproduction rate of six offspring per female (allowing for selection to eliminate 2/3 of all offspring, maintaining a constant population size). In this case, the Poisson distribution defining the number of new mutations assigned to each offspring yielded enough individuals with no mutations (37% on average) so that truncation selection against all mutations still allowed maintenance of the designated population size. This guaranteed elimination of all individuals with even a single mutation, regardless of how small the mutation's effect. As in all other experiments reported here, replicate experiments with different random number seeds produced no meaningful differences in outcome. Therefore for this and all following analyses, we will only report results from single representative runs.

Effects of high mutation rate and mutation-mutation interference

We next conducted a series of similar experiments, but with mutation rates of 5, 10, 20, and 40 per diploid genome per generation. For mutation rates greater than one new mutation per individual, a type of biological noise arises associated with selection interference among mutations. Results are summarized in Figure 1, which plots the mutation fitness effect versus mutation accumulation relative to the neutral expectation. While high-impact mutations had zero accumulation, extremely low-impact mutations displayed accumulation fractions approaching 1.0. The transition zone between these two extremes is characterized by an *S*-shaped curve. We define the selection threshold for deleterious mutations (ST_d) as the midpoint of this transition zone. More specifically, ST_d is the value of mutational fitness effect for which the accumulation fraction is 0.5, indicating that half as many mutations have accumulated as would be expected under complete neutrality (i.e., no selection). This can be visualized in Figures 1, 2, 3, and 4 as the intersection of the horizontal line corresponding to 0.5 on the y-axis and the curve that plots the fraction of mutational retention.

As shown in Figure 1, mutation rates greater than one per offspring resulted in accumulation of low-impact alleles. Increasing the mutation rate resulted in the accumulation of alleles with increasingly large fitness effects. In other words, higher mutation rates lead to progressively higher ST_d values. This means that increasing numbers of alleles that would otherwise have been selectable (to the left of the threshold) became unselectable (to the right of the threshold). With a mutation rate of 10, almost half of all deleterious mutations were retained, with a nearly constant accumulation rate of 4.5 mutations per individual per generation. The mean population fitness declined continuously, reflecting this accumulation of deleterious mutations, but the decline was very slow because the accumulating alleles had very small fitness effects. Figure 1 illustrates that an increased mutation rate, and consequent selection interference among alleles, led to ST_d values increasing from 6.8×10^{-9} for a mutation rate of 5; to 7.4×10^{-8} for a mutation rate of 10; to 5.2×10^{-7} for a mutation rate of 20; to 3.2×10^{-6} for a mutation rate of 40. At the highest mutation rate, 75% of the mutations were below the selection threshold, and hence were effectively unselectable.

Effects of environmental variance

We conducted a series of similar experiments, but instead of increasing mutation rate, we kept the rate at one per offspring and introduced environmental variance, quantified in terms of fitness heritability (i.e., genotypic variance/phenotypic

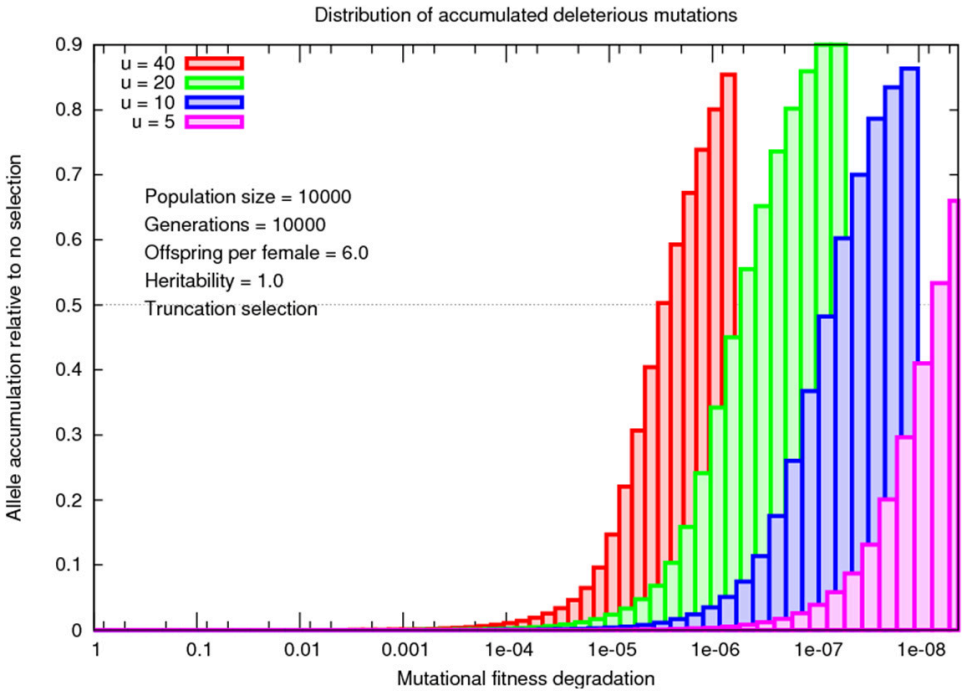

Fig. 1. Fractional retention of mutations as a function of fitness effect for various mutation rates. In these experiments, fitness heritability is 1.0 (i.e., there is no environmental noise), and truncation selection is chosen (i.e., there is no randomness in the selection process). Results for average mutation rates of 5, 10, 20, and 40 new mutations per offspring are displayed. Mutational fitness effect is shown using a log scale along the x-axis, with lethal mutations assigned the value of 1.0. Mutations of small effect are entirely unselectable, and have a fractional retention of 100% (y-axis value of 1.0), while mutations of large effect are eliminated entirely by selection and have a fractional retention of zero. The selection threshold (ST_d) is defined as that fitness effect class which has a fractional retention value of 0.5 (indicated by the dotted line). Note that selection breakdown becomes progressively worse as mutation rate increases. For a mutation rate of 1 per offspring on average, all mutations are selectively eliminated, so mutation accumulation is 0. With an average of 1 new mutation distributed in a Poisson manner and with four of every six offspring selectively eliminated, truncation selection is able to exclude every offspring that has one or more mutations. Because of the very large number of mutations accumulated in these experiments, given computer memory limitations, mutations with extremely small effects were not all tracked in detail, although their effects were fully accounted for. For this reason, the right edge of the distributions end at different fitness effect values.

variance ratio). To illustrate our findings we present three cases with fitness heritabilities of 0.4, 0.04, and 0.004 (Figure 2).

As can be observed in Figure 2, modest levels of environmental variance led to substantial ST_d levels. Heritability of fitness in nature has often been found to be very low, and such a fitness heritability value ($h^2 = 0.004$) yielded a high ST_d (2.6×10^{-5} after 10,000 generations). Given this level of environmental variance,

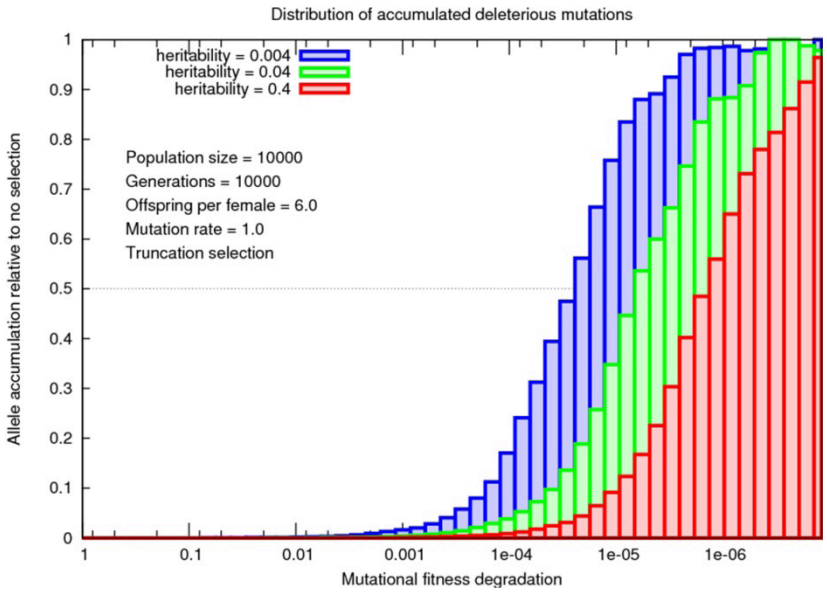

Fig. 2. Fractional retention of mutations as a function of fitness effect for various fitness heritabilities. In these experiments, the mutation rate is 1.0 per offspring on average, and truncation selection was applied (i.e., there was no randomness in the selection process). Results for fitness heritabilities of 0.4, 0.04, and 0.004 are displayed. Note that selection breakdown became progressively worse as heritability decreased (i.e., environmental variance increased). The selection threshold value for the lowest heritability value is 2.6×10^{-5}.

the average mutation count per individual increased at nearly a constant rate of 0.86 mutations per individual per generation. This means that 86% of all the newly arising mutations were below the selection threshold and were essentially unselectable, in spite of very intense selection pressure.

Effects of varying degrees of randomness within the selection process

In another series of experiments we examined the manner in which some randomness in the selection process itself (e.g., partial or complete probability selection) influences ST_d (Figure 3).

Figure 3 summarizes two experiments in which the only source of noise was a specified degree of randomness inherent to the selection process. These experiments were similar to the case that displayed zero mutation accumulation (that is, a mutation rate of one per offspring and zero environmental variance). However, instead of truncation selection, we applied two other forms of selection, i.e., probability selection and what we refer to as partial truncation (quasi-truncation)

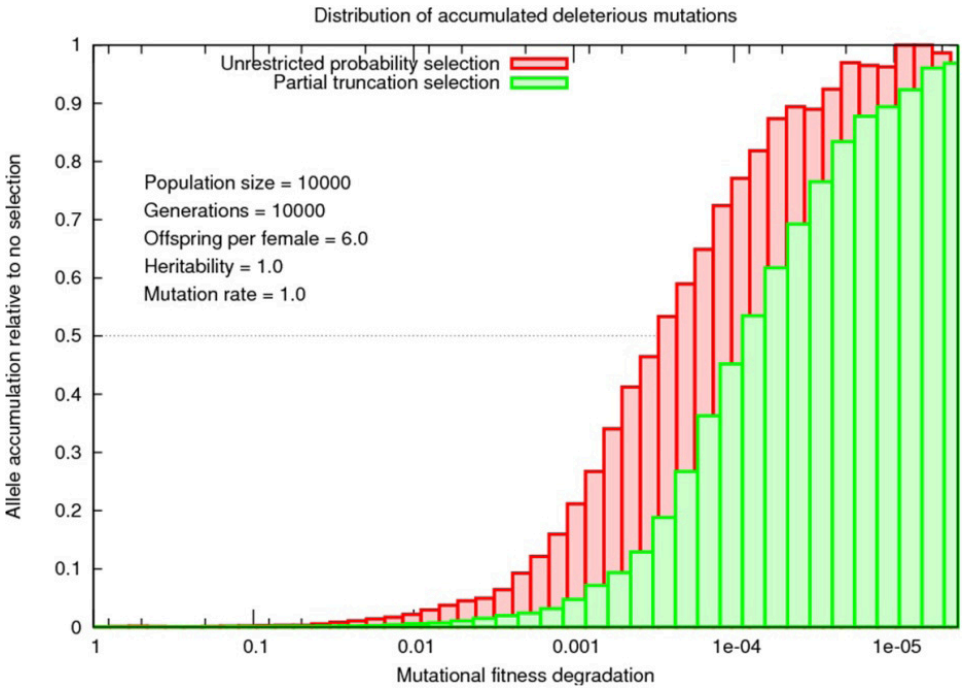

Fig. 3. Fractional retention of mutations as a function of fitness effect for various types of selection. In these experiments, the mutation rate was 1.0 per offspring and the fitness heritability was 1.0. Results are shown for three selection modes: truncation, partial truncation, and probability selection. Under truncation selection with this low mutation rate, all mutations are eliminated so that the fraction of mutations retained is zero for all fitness effect values (all bars in histogram have zero value). This occurs because, with new mutations distributed in a Poisson manner and with four of every six offspring selectively eliminated, truncation selection is able to exclude every offspring with one or more mutations. Note that selection breakdown becomes progressively worse as the level of randomness in the selection process increases. The transition from full truncation selection to partial truncation to probability selection results in increasing selection threshold (ST) values. The ST_d for probability selection is 3×10^{-4}.

selection. Under probability selection, the probability of an individual's reproduction is directly proportional to that individual's phenotypic fitness, such that even individuals with relatively low phenotypic fitness still have some likelihood of reproducing. It is generally understood that probability selection corresponds most closely to what occurs under natural circumstances. Probability selection contrasts strongly with truncation selection wherein there is no element of randomness. Under truncation selection, all individuals above a specific phenotypic value have a 100% probability of reproduction, while all individuals below that value have zero probability of reproduction. Such full truncation selection is almost never realized, even under the highly controlled conditions of artificial plant or animal

breeding. The selection method we refer to as partial truncation (sometimes also referred to as "broken-line" selection) is intermediate between truncation selection and probability selection.

Figure 3 shows that probability selection led to a profound increase in ST_d (3.0×10^{-4}). The mean mutation count per individual over 10,000 generations increased at the nearly constant rate of 0.93 per generation. This means that 93% of all mutations were essentially unselectable. Mean fitness declined by a total of 9%. The noise introduced by the random aspects of probability selection resulted in a much higher ST_d than any other single source of noise we examined. Even with partial truncation selection, the ST_d was high (8.4×10^{-5}), resulting in 91% of all mutations being unselectable. Even a very moderate degree of randomness in the selection process makes a large fraction of all mutations unselectable.

Effects of minimal levels of noise from multiple sources

Here we present an experiment that combines minimal levels of noise from multiple sources. The purpose of this experiment was to estimate the lower limit for ST_d values in typical mammalian populations. We chose what we felt were "best case" parameter settings, but it should be clear that the settings used are biologically unrealistic in that there should be much more noise in most natural circumstances. The parameter choices were: (a) partial truncation selection; (b) a mutation rate of 5.0; and (c) a fitness heritability of 0.4. Results from this experiment are shown in Figures 4–7.

Figure 4 shows that multiple sources of noise, even at minimal levels, result in a very appreciable ST_d value (7.6×10^{-5}). In this instance 90% of all mutations were below the selection threshold and were hence effectively unselectable. Some mutations accumulated which had fitness effects as large as 0.001. Selection breakdown was essentially complete below 0.00001.

The higher mutation rate of this experiment resulted in a higher mean mutation count and a much more severe reduction in fitness (Figures 5–7).

Figure 5 shows the distribution of mutant allele accumulation in greater detail, using a linear scale for the x-axis and focusing on just low-impact alleles. Moving from left to right, a smooth transition is evident from fully-selectable alleles to partially-selectable alleles, and finally to alleles that are entirely unselectable.

Figure 6 shows that the rate of mutation accumulation was remarkably constant at 4.5 mutations per individual per generation over 10,000 generations, even with intense selection pressure. Given the mutation rate of 5.0, only 10% of deleterious mutations were successfully eliminated by selection. We consistently observed a very constant rate of mutation accumulation, even when experiments were

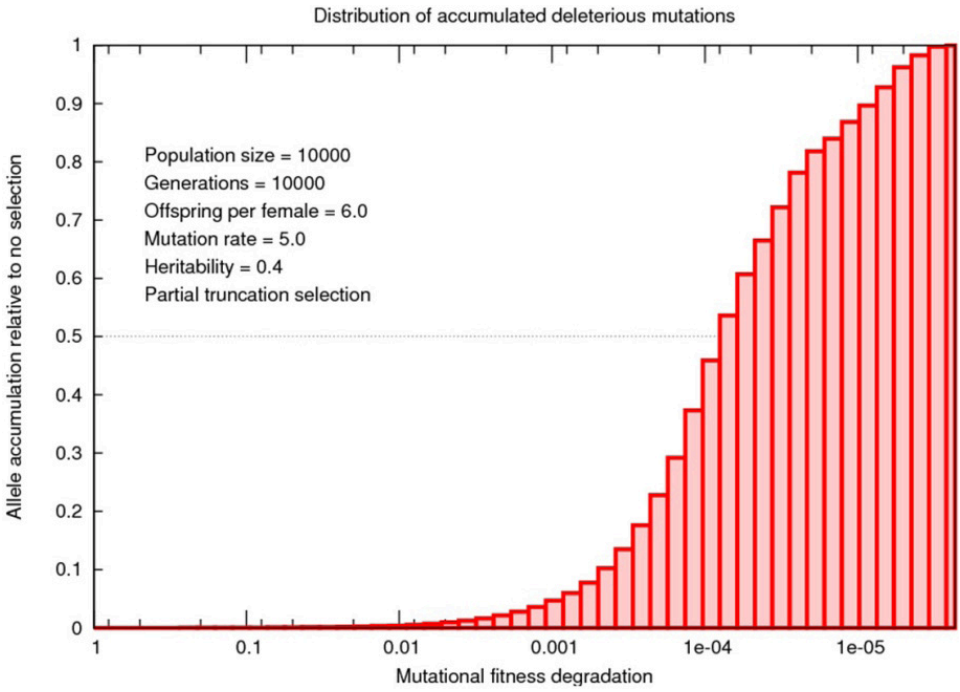

Fig. 4. Fractional retention of mutations as a function of fitness effect, with multiple sources of noise. This case used a mutation rate of 5.0 new mutations per offspring on average, a fitness heritability of 0.4, and partial truncation selection. Note that even with these modest levels of noise, ST_d was appreciable (7.6×10^{-5}).

extended to the point of extinction or to the point of computer memory overflow (due to large numbers of accumulated mutations being tracked for every individual).

Figure 7 shows that, under biologically relevant conditions, the population's mean fitness declined continuously as mutation count per individual increased. In this particular case, fitness declined by 16% during the first 10,000 generations. When this experiment was extended to the limits of computer memory, fitness declined to near extinction in 40,831 generations, with an average accumulation of 174,890 mutations per individual. The rate of fitness decline was essentially linear after generation 10,000.

Effects of larger population size, more time, and more recombination

Figure 8 shows the effects of population size on ST_d over time, using partial truncation selection with the same settings as for the case displayed in Figs. 4–7. Here,

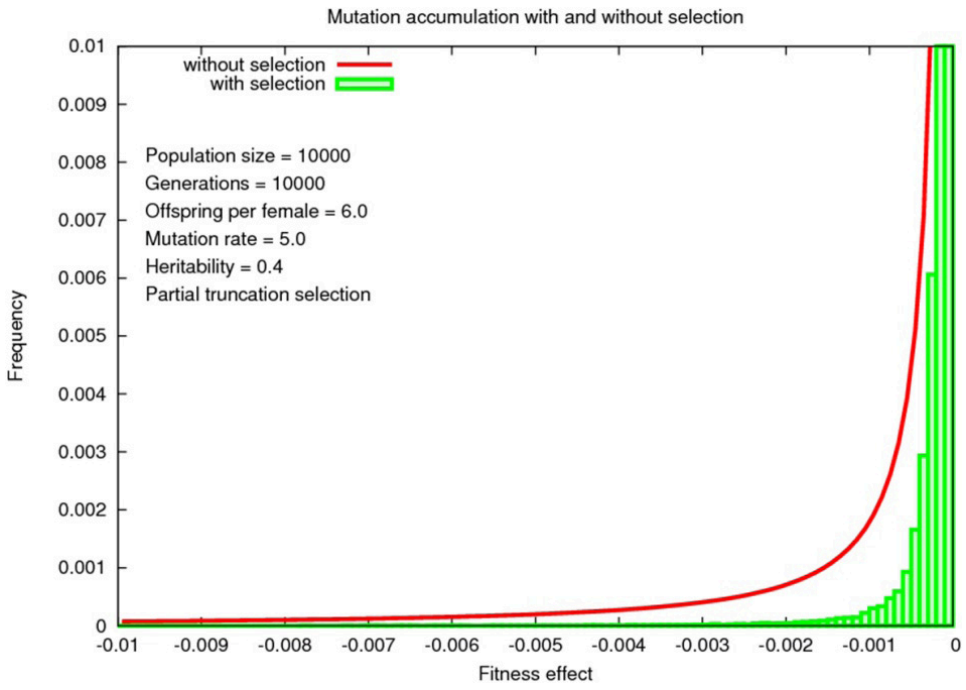

Fig. 5. Mutation distribution as a function of fitness effect, shown on a linear scale. The case is the same as shown in Fig 4. The curved line is the theoretical distribution with no selection. The histogram is the actual mutation distribution given intense selection. Note that only a small portion of the vertical and horizontal scales is displayed.

as in all our other simulations, when starting with zero genetic variance (as might occur after a severe bottleneck), ST_d values initially start very high but decline rapidly. This is due to the accumulation of segregating alleles in the population as time increases, such that selection has more to act upon and so becomes more effective. As the amount of genetic variance approaches an equilibrium, the decline in ST_d levels off. As this happens the initially drastic decline in ST_d reaches a plateau. As can be seen in Figure 8, for a population size of 100, the ST_d declined noticeably until generation 2000 and became relatively stable after roughly 4000 generations. For a population of 1,000, the ST_d value became relatively stable after roughly 6000 generations. For a population of 10,000, the ST_d value was still falling after 10,000 generations, meaning the population had not yet reached an equilibrium for selection efficiency (i.e., a constant value for ST_d).

When this experiment was extended, we saw that for the population size of 10,000, there was no significant decline in ST_d after roughly 150,000 generations. Larger populations clearly took longer to reach selection equilibrium, but given enough time (assuming that selection could consistently favor the same alleles

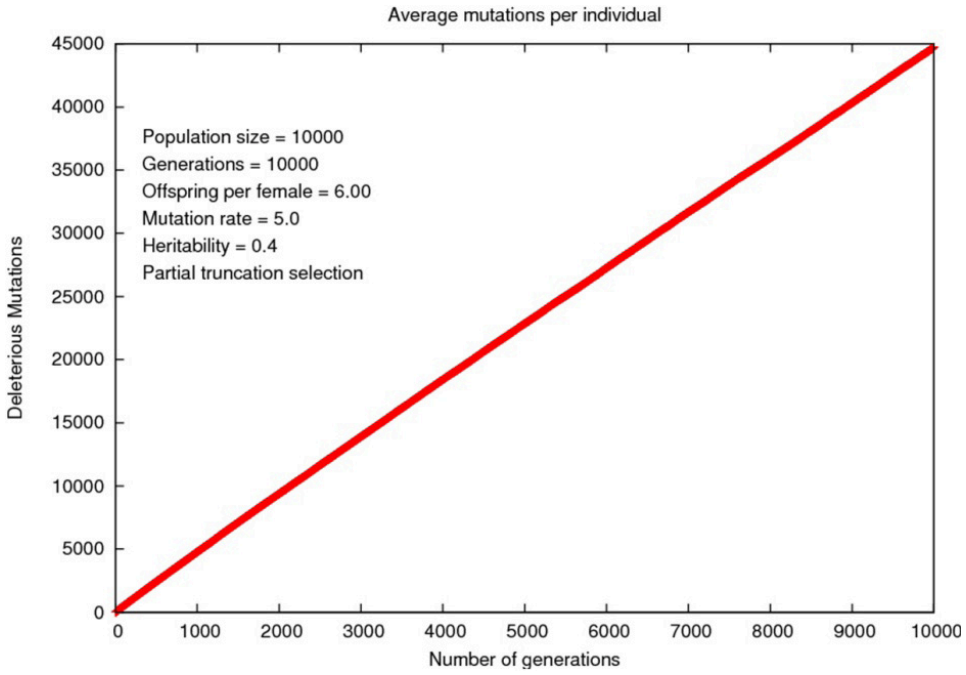

Fig. 6. Mean mutation count per individual as a function of generation number. The case is the same as shown in Figs. 4 and 5. With no selection, the mean mutation count would have been 50,000 after 10,000 generations, compared to the 45,000 actually accumulated.

throughout this many generations), reach markedly lower final ST_d values. In the time frame of this experiment, increasing the population size from 1,000 to 10,000 slowed fitness decline only modestly (average fitness of 0.84 vs. 0.79 at generation 10,000). This result may seem surprising in light of the conventional wisdom that selection effectiveness is directly proportional to population size. However, increasing population size from 1,000 to 10,000 reduced the ST_d at generation 10,000 by only a small amount on an absolute scale (1.5×10^{-4} to 7.2×10^{-5}), and thus did not greatly slow the decline of fitness.

Figure 9 shows the effect of population size on percent retention after 10,000 generations. Within this limited amount of time, there was only a trivial advantage in having population sizes greater than 5,000. With a population size of 5,000, the rate of mutation accumulation was 89.38%. Doubling the population size to 10,000 resulted in 89.05% accumulation, and doubling the population size again to 20,000 resulted in no further improvement (89.05% accumulation). It is clear that the advantage of larger population size beyond 1000 is only realized in deep time, which seems to imply the need for some type of very long-term selection equilibrium, which may be conceptually problematic.

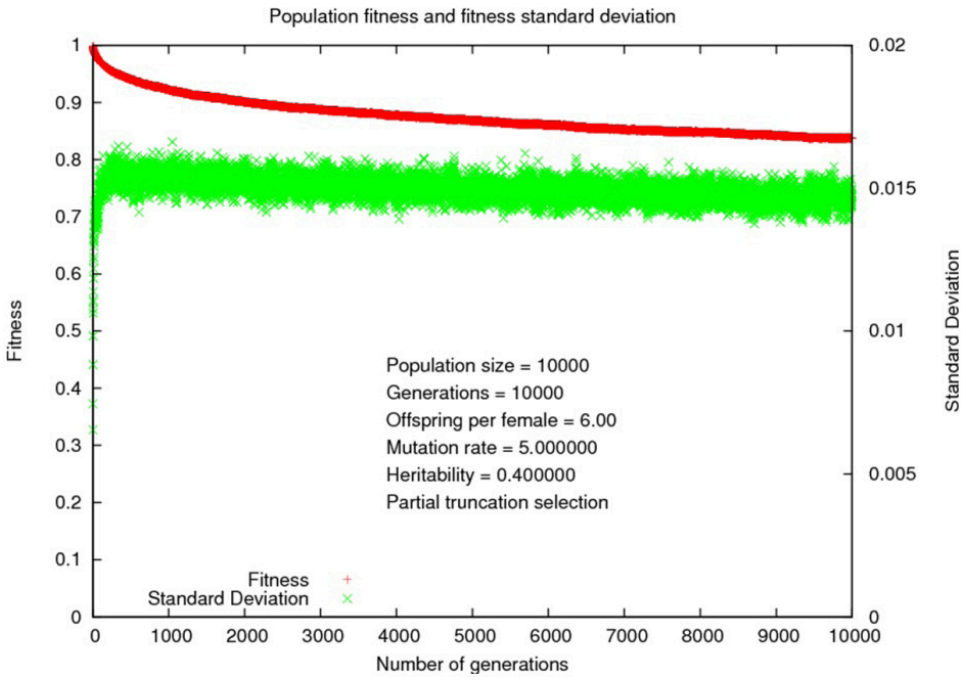

Fig. 7. Mean population fitness (red) and fitness standard deviation (green), as a function of generation number. The case is the same as shown in Figs. 4, 5, and 6, with a mutation rate of 5.0 new mutations per offspring on average, a fitness heritability of 0.4, and partial truncation selection. The accumulating mutations reduced mean fitness by 16% after 10,000 generations.

In a related series of experiments (data not shown), we found that having fewer than 500 linkage blocks resulted in much more severe mutation accumulation due to selection interference between mutations and due to Muller's ratchet. However, increasing the number of linkage blocks beyond 1,000 had very little additional benefit, apparently because mutations in proximal linkage blocks separated only rarely (two randomly placed crossovers per chromosome per generation), even though proximal mutations were technically in different linkage blocks.

Experiments using the latest estimate of human mutation rate and fitness effect distribution

For mutation accumulation simulations to have relevance to the biological world, the mutation rate and the distribution of mutational fitness effects must be reasonably realistic. The experiments summarized in Figure 1–9 used the most conservative parameters settings possible, representing best case scenarios for halting mutation accumulation. However, all these experiments employed Mendel's default

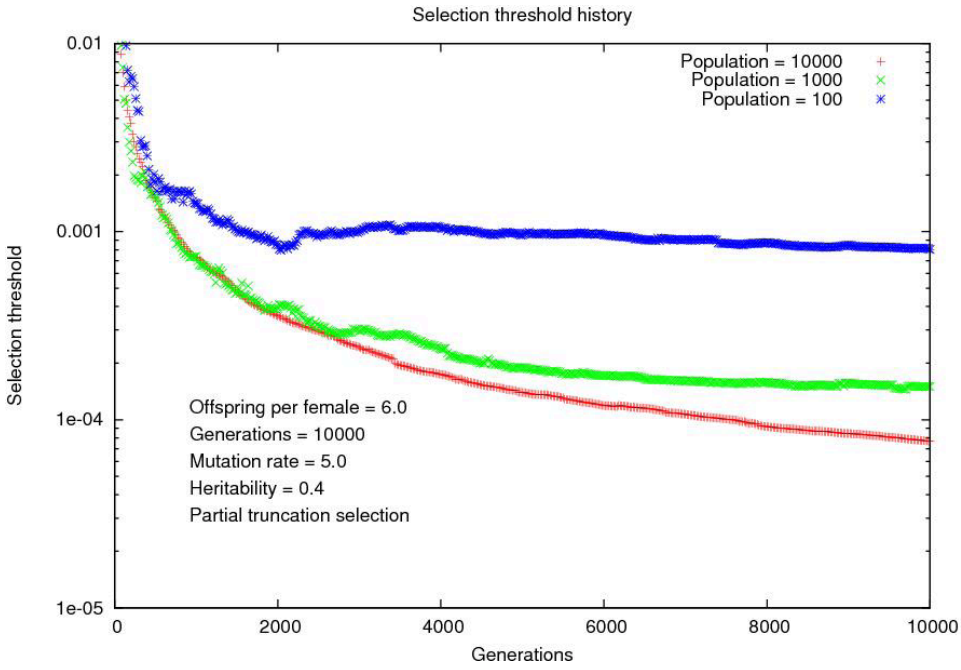

Fig. 8. Selection threshold (ST_d) as a function of generation number for three population sizes. Population sizes of 100, 1000, and 10,000 were used. Except for population size, parameters for these three cases were identical to those for the case shown in Figures 4–7. ST_d values for each population size were initially very high and decreased rapidly. For population sizes of 100 and 1000, there was little or no decrease in ST_d values after 2,000 to 4,000 generations. For the population of 10,000, ST_d values stabilized much later.

setting for mutation fitness effect distribution — and some might challenge this distribution. Therefore we report two Mendel experiments using the most recently published estimate of the human mutation fitness effect distribution (24), which required shifting the fitness effect distribution toward higher-impact mutations. The sum of different types of mutations discussed by Lynch (24) is approximately 8 –10 per individual that are apparently under at least weak selection, implying some level of deleterious effect. More specifically, Lynch estimated that each newborn human inherits an average of approximately 0.86 deleterious mutations that cause amino-acid changes in polypeptides, plus an additional 2 to 3 deleterious mutations of substantial effect (averaging 10^{-2} or stronger), including major deletions, gene duplications, and splice-site mutations. This means that there is an average of at least 3 distinctly deleterious mutations per newborn — a very conservative estimate that we chose to use in these experiments. Lynch reported various other types of mutations whose effects are almost certainly deleterious, but possibly weak, so these were not considered in these experiments. The default distribution of fitness

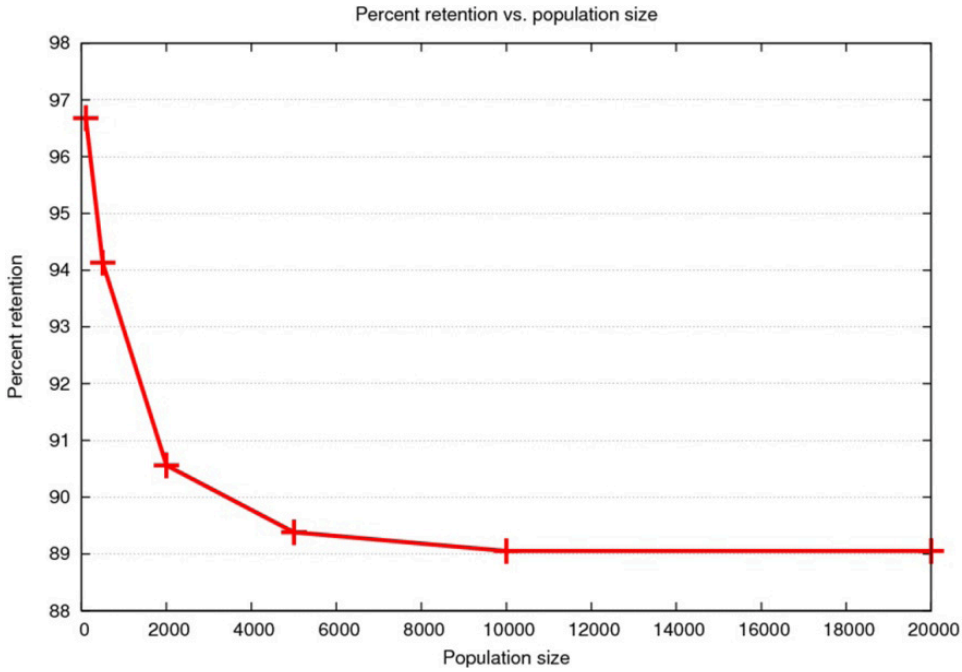

Fig. 9. Percent retention of deleterious mutations as a function of population size within 10,000 generations. The parameters for these experiments were the same as for figure 8, but with population sizes ranging from 100 to 20,000. Within the time frame of 10,000 generations, increasing population size beyond 5,000 resulted in no meaningful improvement in selection efficacy.

effects in Mendel's Accountant was adjusted to match Lynch's estimate of 27% of effects stronger than 10^{-2}, with the minimum fitness effect being adjusted upward to 10^{-6} by setting the genome size at 10^6, thus excluding from consideration the several other mutations per newborn, the effects of which might be less than 10^{-6} per mutation. The resulting distribution of fitness effects had a much higher mean fitness effect than the Mendel default distribution, and is a reasonable approximation of Lynch's distribution (ignoring all very low-impact mutations).

We ran two Mendel experiments using this new fitness effect distribution, employing a mutation rate of just 3 new deleterious mutations per newborn. The first experiment employed both partial truncation selection and a very high fitness heritability (0.4), as with the previous experiments. The second experiment used all the same parameters, except that it employed probability selection — which is much more realistic. Figure 10 shows the fitness history of these experiments. The result of using the Lynch fitness effect distribution was much faster degeneration than when using Mendel's default settings. The initial rate of fitness decline was approximately 5% per generation (data not shown), agreeing well with the fitness decline surmised by Lynch. However, over deeper time, as genetic variation for fitness built up, selection

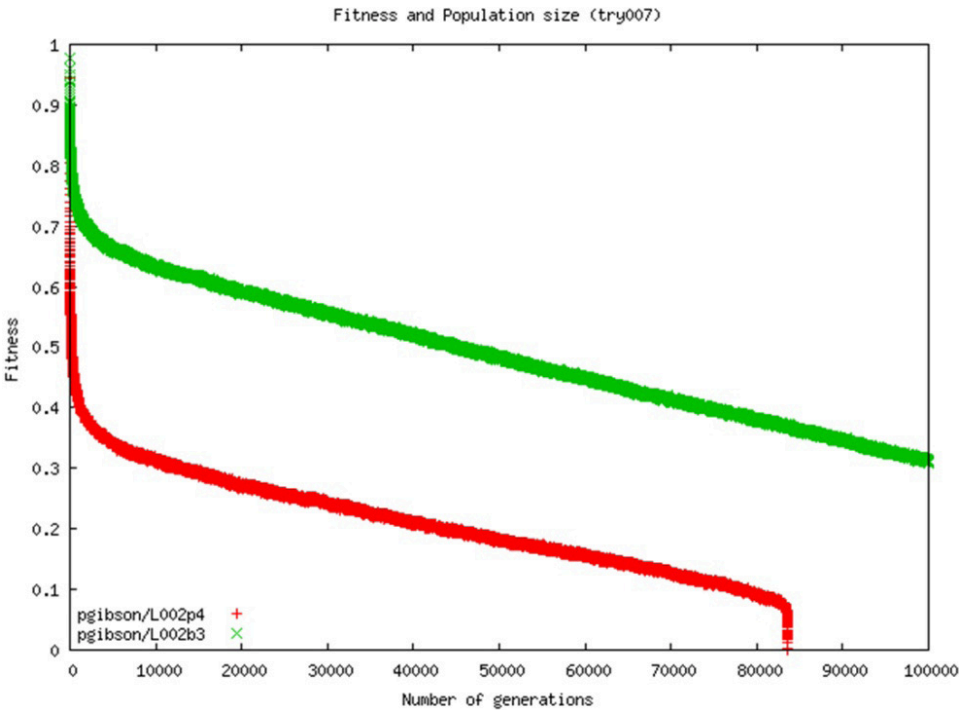

Fig. 10. Fitness history using the latest estimate of human mutation rate and fitness effect distribution, comparing partial truncation selection versus probability selection. The upper line (green) resulted from a run using partial truncation selection. The lower line (red) resulted from an identical run, but employing probability selection. In both cases, a fitness effect distribution was employed based upon Lynch [24], strongly skewed toward higher impact mutations. In both cases, the mutation rate was just 3, again in agreement with Lynch [24]. Population size was 10,000, fitness heritability was 0.4.

could act upon a wider range of variation and thus became more effective, slowing the decline, but not stopping it. The rate of fitness decline over time became extremely linear, with partial truncation selection resulting in a mean fitness of 0.31 after 100,000 generations. Using probability selection, the rate of fitness decline also became extremely linear over time, resulting in extinction at generation 83,647.

Discussion

General Implications

This study shows that, under conditions relevant to many mammalian populations, the large majority of deleterious mutations should escape purifying selection.

Given a specific population and specific circumstances, there must be a certain point where selection against low-impact mutations breaks down. Numerical simulation allows us to empirically determine this selection threshold, ST_d, for any particular set of conditions. We expand on previous work by showing that the value of ST_d is not a simple function of population size, but is affected by numerous variables. To our knowledge, the methodology used here (i.e., numerical simulation based on genetic accounting) provides the most biologically relevant treatment of the problem of germline mutation accumulation to date. The theoretical and practical implications of these results should be of wide interest.

For a typical mammalian model population (e.g. 10,000 individuals, genome size of 3 billion), our estimate for the lower limit of ST_d is in the range of 10^{-4} to 10^{-5}. Thus even with minimal levels of biological noise interfering with the phenotypic expression of the genotype, those deleterious mutations which reduce fitness by less than 10^{-4} to 10^{-5} will largely escape purifying selection and will accumulate linearly. We show that three important sources of noise which substantially increase the value of the selection threshold in large populations are: (1) selection interference between mutations; (2) environmental variance; and (3) any significant degree of probability selection (in contrast to truncation selection, which never occurs in nature). Our experiments show that depending on these variables, ST_d values for mammalian species may be as high as 10^{-3} or higher. Given Mendel's default fitness effect distribution, ST_d values in the range of 10^{-3} to 10^{-5} results in 82–97% of all deleterious mutations becoming effectively unselectable.

Our simulations indicate that the on-going accumulation of low-impact mutations results in continuous fitness loss. Consistent with the findings of others, our analyses reveal that the greatest contributor to this fitness loss is not the entirely unselectable mutations (having negligible fitness effects even in large numbers), but rather the accumulation of mutations with effects near the selection threshold. We observe that mutations in this zone accumulate more slowly than if there was no selection, yet still accumulate continuously and in large numbers. This transition zone between mutations that are entirely selectable and entirely un-selectable is often at least two orders of magnitude wide and typically encompasses fitness effects on the order of 0.001 to 0.00001. Accumulating alleles within this transition zone are primarily responsible for the reduction in fitness.

In view of the expected accumulation of low-impact mutations, it is important to estimate accurately the lower limit of effects that respond effectively to selection. Over the past several decades it has been tacitly assumed that population size is the primary determinant of this lower limit. This important assumption, explicit in Kimura's famous formula, $s = 1/(2Ne)$ [7], has been used by most investigators for defining the threshold for selection breakdown. However, our extensive

investigations have indicated that mutation rate, environmental variance, selection mode, and time are all important variables that affect ST_d in addition to population size. In populations of 1000 or more, these other variables are often more important than population size. We consistently observed that, regardless of the mode of selection, increasing population size beyond 1,000 provided only modest gains in selection efficiency in the time frame of thousands of generations. The advantages of population sizes beyond 10,000 were only realized after tens of thousands of generations, and even that depended on the very questionable assumption that all selection coefficients could remain constant. It is clear that selection breakdown is not a simple function of population size. In other words, Kimura's famous formula represents an over-simplification of biological reality and the failure to consider other sources of noise can therefore lead to serious error and serious under-estimation of the selection threshold problem. This is especially true when mutation rates are above 1 per individual per generation (resulting in substantial selection interference between mutations), or when the effect of truncation or quasi-truncation selection is considered instead of simple probability selection. Although future studies should explore the behavior of larger populations in much deeper time (as greater computational power becomes available), the present results strongly suggest that population sizes larger than 10,000 will have a minimal effect on the effect on ST_d values.

The inability of natural selection to effectively remove large numbers of low-impact mutations has major implications regarding the long-term maintenance of the genetic integrity of populations. A substantial but unknown fraction of the many mutations in each eukaryotic individual must be deleterious. Yet this study indicates that most such deleterious mutations are too subtle to respond to natural selection. How can this be? Unless some entirely unknown mechanism is operating, it appears that net genetic deterioration is an inevitable aspect of the mutation/selection process, given known mutation rates and fitness effects. It is widely supposed that within any viable population, natural selection must be able to act effectively on deleterious mutations at millions of loci simultaneously, even though most such mutations have vanishingly small fitness effects and their selection is compromised by multiple levels of interfering biological noise. The results of the current study involving biologically realistic numerical simulation clearly show that selection simply cannot do this. This simple reality seems to be widely understood by leading population geneticists (e.g., see references [1–13]), yet it appears to be generally regarded as a matter of small significance judging by the lack of much serious investigation into factors influencing mutation accumulation. However, if natural selection cannot reasonably be expected to halt degeneration of genomic information, then there must be a profound problem with the present formulation of neo-Darwinian theory. We suggest this is a matter of great significance and should interest all serious scholars.

Robustness of Findings

The primary findings of this study are that the selection threshold problem is real and that it is more serious than generally recognized. These findings are very robust. Our basic conclusions do not depend on a narrow range of parameter settings; rather the same picture emerges under all reasonable biological settings, indicating that the basic phenomenon is fundamental. Our most realistic simulations (see Figures 7 and 10) still employed extremely conservative parameter settings, based upon the premise that most mutations are entirely neutral, the premise of partial truncation selection, and the premise of a very high fitness heritability. We do not believe any of these assumptions are reasonable--they were applied only to define the lower range of the deleterious selection threshold for a model human population. Simulations with what we consider to be more realistic parameter settings have indicated an even more serious erosion of genetic information than is presented here.

We suggest that, unlike many phenomena in the realm of physics, the biology of population dynamics is too complex to be reliably reduced to a small set of equations. The primary deficiency we observe in prior mutation accumulation studies is the extreme simplification that has been required both in mathematical formulations and in numerical simulations. Common simplifying restrictions include assuming that all mutation effects are equal and that environmental variance is zero; usually also assuming perfect probability selection or perfect truncation selection. These simplifications may be why previous analytical models have not fully illuminated the phenomenon of mutation accumulation. Such extreme simplification is no longer required. Today's rapidly expanding computational resources and much more sophisticated numerical simulations provide the capacity for comprehensive numerical simulations that can address population genetic systems in their entirety, simultaneously considering all the major variables that affect mutation accumulation.

Mendel's Accountant was programmed to be a comprehensive numerical simulation, reflecting biological reality as closely as possible for all the primary variables known to influence selection effectiveness [14, 15]. Mendel empirically and mechanistically tracks the basic biological processes of mutation, meiosis, crossover, gamete formation, mating, zygote formation, and selection. During the course of thousands of generations, millions of individuals are simulated, and hundreds of millions of mutations are tracked individually and continuously — an approach we call *genetic accounting*. This approach allows us to observe empirically how different biological factors interact as they influence selection efficiency, requiring far fewer prior assumptions and far less abstraction than the conventional algebraic analysis. We have repeatedly seen that, given parameter settings that correspond

to the standard simplifying assumptions, Mendel supports the expectations of classic population genetic theory. However, in simulations that more realistically reflect the complexity of living populations (i.e., multiple sources of noise), Mendel's Accountant illuminates some fundamental problems in standard theory that were previously clouded by unrealistic simplifications. Mendel's Accountant thus marks a significant step forward in our ability to understand the problem of mutation accumulation, building upon the foundational work of Kimura and Ohta.

We have found these results to be highly reproducible. Replicated runs employing alternate random number seeds produce essentially identical results, creating only trivial variations. Other researchers can replicate the experiments reported here by downloading the Mendel's Accountant program along with its user manual at www.mendelsaccountant.info and by using the parameter settings listed in Appendix 1 for those parameters not presented in the specific experiments above.

Readers may ask whether we explored enough parameter space to enable us to reach the overall conclusions we claim. While the results of any given numerical experiment will, of course, depend on the specific parameter choices of the investigator, yet for each parameter, we included values that encompassed a range that was wider than seemed biologically reasonable, and explored an extensive number of combinations of the various parameters. These investigations revealed that a high selection threshold and continuous, nearly linear mutation accumulation are universal across all reasonable portions of parameter space. The results of these investigations cannot be summarized in any single paper, although our previous publications summarize many of our results [15, 16]. These extensive investigations have indicated that mutation rate, environmental variance, selection mode, and time are important variables that affect ST_d — in addition to population size. In populations of 1000 or more these factors are often more important than population size. For this reason we focused this paper on those specific variables, exploring the full range of their potential effects. In so doing we consistently find that the majority of deleterious mutations are not selectable, except within small and extremely unrealistic slivers of parameter space (e.g., the combination of less than 1 mutation per individual, no environmental variance, and full truncation selection). In this light, our conclusion that most deleterious mutations are beyond the reach of natural selection appears to be robust.

Potential Effects of Other Factors

Some will question the Mendel default settings for fitness effect distribution. We have tested other distributions and have not found them to produce fundamentally different results. In particular, in this paper we used Mendel to examine the latest

estimate of the human mutation rate and the human fitness effect distribution, as recently reported by Lynch [24]. We observed that using the Lynch-based parameter settings, we saw much more rapid fitness decline than when using the Mendel default settings (Figure 10). Shifting the fitness effect distribution toward significantly higher impact mutations makes the fitness decline problem much worse. Lynch's estimate of a rate of only 3 to 4 mutations/person/generation with distinctly negative consequences (non-synonymous coding sites plus other high impact mutations) is very dependent on the assumption that outside of the 1.5% of the genome that directly codes for protein, most of the genome is functionally inert. However, the findings of ENCODE [25] and others [26] now suggest that most of the genome is transcribed and much more than 1.5% of the genome has sequence-dependent function. This information suggests that a much more realistic mutation rate estimate would be well above 5 non-neutral mutations per generation, since more than 5% of the genome appears to have sequence-dependent function. A non-neutral mutation rate higher than Lynch's estimate is also supported by a recent reviews of mutations associated with human disease [27, 37], which cite many instances in which single-nucleotide substitutions in various types of non-coding regions are implicated in debilitating human diseases, as are synonymous mutations in both coding and non-coding regions. The normal Mendel default value of 10 new mutations per individual seems more realistic, and in our view is still too conservative.

It has been speculated by Lynch [24] and others that greater fecundity and more difficult living conditions in the past resulted in enhanced natural selection which may have been powerful enough to stop deleterious mutation accumulation. In order to test that hypothesis, simulations were conducted with 12 offspring per female, no random death, and a mutation rate of 3. These settings result in ten of every twelve offspring being selectively removed. This very extreme form of selection slowed mutation accumulation and the rate of fitness decline, but did not stop it. After 10,000 generations, fitness declined to 0.22 with probability selection, and 0.57 with partial truncation. In both cases, mutations of non-trivial effect were still accumulating and fitness was still declining when the runs ended.

Do recessive or dominant mutations give different results? We have done many experiments (data not shown) which indicate, as expected, that using an all-recessive mutation model (rather than co-dominant ones, as in this study) results in a slower rate of fitness decline, but also results in the accumulation of higher numbers of mutations, more fixation, and higher ST_d values. Thus, mutation accumulation is ultimately more damaging to the population when all mutations are recessive than when they are co-dominant.

Given the problem of the continuous accumulation of deleterious mutations, it is important to consider the role beneficial mutations might play in alleviating this problem. For the sake of simplicity and clarity, this study does not address beneficial mutations, but we focus on this topic in a companion paper [28]. In that paper we show there is a selection threshold for beneficial mutations very similar in magnitude to the one for deleterious mutations. We find that, while beneficial mutations can offset some of the damage from accumulating deleterious mutations, beneficial mutations that are substantial enough to respond to selection tend to strongly interfere with the selective removal of deleterious mutations. This is due both to selection interference and to the physical linkage of beneficial and deleterious mutations (which tends to makes both less selectable).

It has been postulated that a special form of selection, based essentially on mutation count (rather than fitness), might be a possible solution to the near-neutral paradox [29], and it has been suggested that such a situation might arise due to synergistic epistasis. In companion papers we deal with the special case of selection based upon mutation count [30] and the mechanism of synergistic epistasis [31]. Our results clearly show neither of these mechanisms can substantially slow mutation accumulation under real-world conditions.

Conclusion

In conclusion, numerical simulation shows that realistic levels of biological noise result in a high selection threshold. This results in the ongoing accumulation of low-impact deleterious mutations, with deleterious mutation count per individual increasing linearly over time. Even in very long experiments (more than 100,000 generations), slightly deleterious alleles accumulate steadily, causing eventual extinction. These findings provide independent validation of previous analytical and simulation studies [2–13]. Previous concerns about the problem of accumulation of nearly neutral mutations are strongly supported by our analysis. Indeed, when numerical simulations incorporate realistic levels of biological noise, our analyses indicate that the problem is much more severe than has been acknowledged, and that the large majority of deleterious mutations become invisible to the selection process. Even apart from numerical simulation, it would seem readily obvious that the following factors should interfere with selection effectiveness and thereby increase the threshold for selection: (a) large functional genome size; (b) high mutation rate; (c) significant environmental variance; (d) randomness in the selection process; (e) extensive linkage; and (f) small or fragmented populations. These factors are characteristic of all higher life forms [14] and should therefore be included in any future analyses. Our numerical simulation program

incorporates all these factors, and suggests that the threshold for selection breakdown should be very substantial for most eukaryotic species. As stated by Keightley and Eyre-Walker "How humans and related species evade the effects of mutation load on an evolutionary time scale is also an open question" [32]. It is unclear what factors could realistically stop the decline of fitness due to mutation accumulation, although studies of the effects of bottlenecks, sub-populations, and other possible factors are underway using Mendel's Accountant. This issue deserves much more serious investigation, and Mendel's Accountant provides a biologically realistic simulation approach for such investigations.

Materials and Methods

We have applied Mendel's Accountant to simulate biological reality as closely as possible. Mendel introduces new mutations into the population every generation and tracks each mutation through the processes of recombination, gamete formation, mating, and transmission to the new offspring. This method tracks which individuals survive to reproduce after selection and records the transmission of each surviving mutation every generation. This allows a detailed mechanistic accounting of each mutation that enters and leaves the population over the course of many generations. We term this type of analysis *genetic accounting*, as reflected in the name of the program, Mendel's Accountant [15, 16]. Its inner workings are described in great detail elsewhere [15]. It meticulously records and tracks huge numbers of discrete genetic events over time. This discrete approach contrasts with the traditional approach that has been used by population geneticists for the past nine decades who have sought to represent the processes solely in terms of analytical equations and then to solve these equations. Like any accounting program, its primary limitations are the appropriateness of the input data, in this case a set of parameters that characterizes the particular biological circumstance the user wants to investigate, and the computer processing speed and memory.

Although Mendel is designed with the ability to model a broad spectrum of haploid and diploid organisms, for the sake of simplicity we have limited our consideration in this paper to sexual diploid organisms with large genomes. We use parameters appropriate for human populations because more is generally known about the relevant values in humans than in other complex eukaryotes. We start with a genetically-uniform population, approximating the relative genetic uniformity that follows a significant population bottleneck, and we initially assign each individual a fitness of 1. Across the experiments reported here, we keep all input parameters constant, except for the following: (1) mutation rate; (2) environmental

variance; (3) selection mode; (4) population size; (5) number of linkage blocks; and (6) number of generations.

Mendel's calculations use a mutation's *effect on functional fitness (fitness effect)*, rather than its *selection coefficient,* in order to disentangle the genetic impact of a mutation on biological function from the selection process itself. In much of the population genetic literature, the selection coefficient and the influence of a given mutation on genetic fitness (fitness effect) have been equated by definition, which is true only when probability selection is combined with the multiplicative model of mutational effects and no other confounding factors occur. However, with other forms of selection and with the inclusion of other factors, a complex relationship emerges between a mutation's impact on functional fitness, its predicted selection coefficient, and its actual selectability [33, 34]. This actual selectability determines the change in allele frequencies, which by definition corresponds to the actual selection coefficient. *Functional fitness* is a concept integrating every element that influences survival and reproduction. We believe that the term functional fitness is both easily understood and conceptually useful. Our investigations show that numerous factors confound the correlation between a mutation's effect on functional fitness and its actual selectability.

Mendel outputs a new statistic we term *deleterious selection threshold* (ST_d), which marks the center of the transition zone in fitness effect between mostly selectable and mostly unselectable deleterious mutations. ST_d can be defined as the mutational fitness effect value at which the number of mutant alleles in the population is 50% of the number expected if there were no selection. The computed ST_d value lies at the mid-point of the transition zone separating large-effect, selectable mutations (that display nearly zero accumulation) and small-effect unselectable mutations (that display nearly 100% accumulation). This statistic provides, at any desired generation, a simple empirical basis for comparing selection effectiveness among cases involving different biological parameters. In this paper we restrict our discussion to only a few of the factors that influence this threshold, namely, mutation rate, environmental variation, selection scheme, population size, and degree of linkage.

The mutation rates we employ are based upon an estimate of approximately 100 new human mutations per person per generation [20, 21]. We adjust this estimate based on the fraction of the human genome assumed to be functional. We consider a minimal estimate of the functional genome to be 1% (yielding a functional mutation rate of 1), and a very conservative estimate to be 5% (yielding a functional mutation rate of 5). In light of increasing evidence of extensive genomic functionality [26, 27], we also examine functional mutation rates of 10, 20, or 40 new mutations per individual per generation, corresponding to a 10%, 20%, and 40% functional genome, respectively. By discounting the mutation rate based upon the

size of the functional genome, we are postulating a very conservative mutation rate because we effectively remove from consideration all non-functional DNA. This eliminates from consideration any absolutely neutral mutations. In this paper, for clarity and brevity, only detrimental mutations are considered, although the fate and impact of beneficial mutations are reported in a companion paper by Sanford *et al.* [28].

In Mendel, mutations follow an "infinite sites" model, and a Poisson distribution describes the random number of new mutations assigned to each individual. The distribution of mutational effects is a Weibull-type distribution [22] of the form $d = exp(ax^{\gamma})$, where d is the effect of a homozygous pair of mutant alleles, a is the inverse of the functional genome size, x is a uniformly distributed random number between 0 and 1, and γ is determined by the frequency of high-impact mutations and their user-defined cut-off value. All these parameters, as well as degree of dominance and numerous other variables, can be specified by the Mendel user.

While there is room for debate regarding the exact shape of the mutation effect distribution curve, its general shape is considered by most scientists to be exponential, with high impact mutations rare and very low impact mutations strongly predominant. There should be a fairly smooth distribution curve going from the rare semi-lethal to the typical low-impact, non-neutral mutation, and this curve should be approximately exponential in character. If this were not true and higher-impact mutations were more common, humans would quickly become extinct, given that we have such a high mutation rate and have already accumulated very large numbers of deleterious mutations.

The Weibull-type distribution, widely used in engineering for modeling degradation processes [22], readily accommodates the wide range of effects that we want to consider (eight or more orders of magnitude). This function is similar to a gamma distribution but allows a wider range of fitness effects. The use of this distribution is based on the evidence that even synonymous mutations and mutations in non-coding regions often have at least a very slightly deleterious effect [35, 36]. Indeed, two recent papers [23, 36] contend that the two-parameter Weibull distribution fits biological reality very well. Because of the basic similarity of exponential distributions, there is little reason that alternative exponential-type distributions should give substantially different results. An obvious consequence of the strong skewing of the mutational effects towards very small values in these exponential distributions is that a high proportion of the mutations are unselectable. In experiments where the distribution was shifted to yield more high-impact mutations, the proportion of mutations eliminated by selection was somewhat higher. However, fitness loss was even more rapid than when the distribution was more strongly skewed toward low-impact values, *because the mean*

effect on fitness from the mutations that did accumulate was higher. Thus, except at very low mutation rates in conditions that allow for perfect purifying selection, shifting the mutation distribution toward higher-impact mutations actually intensifies the problem of continuous mutation accumulation and ever-increasing genetic load.

The nature of genetic information requires that, as the functional genome size increases, the fractional information content of each individual nucleotide must be less and less. For example, in genomes with one hundred million functional nucleotides, a typical individual nucleotide change must have an extremely small impact on total information content, perhaps on the order of one part in one hundred million. While the impact of an individual mutation on fitness could be larger or smaller than the inverse of the functional genome size, it would seem reasonable that most non-neutral mutations would have at least that great an effect in view of the interdependent nature of many biological functions. Therefore, it seems reasonable to use the inverse of the functional genome size as the minimum fitness effect to be considered for non-neutral mutations.

For these experiments, we set $a = 3 \times 10^{-9}$ (reflecting the inverse of 3×10^{8} bp, a conservative estimate of the *functional* genome size in humans), thus setting the lower limit of the mutational effect for homozygous mutations in the model. Thus, the magnitude of homozygous mutational effects ranges from -1 (lethal) to -3×10^{-9}. For the cases described in this study, we set the value of γ by specifying high-impact mutations as those with a homozygous fitness effect of at least 0.1, with a frequency of 0.001, reflecting an estimate that one in a thousand mutations in humans reduces fitness by ten percent. This parameterization generates almost no lethal mutations and very few nearly lethal mutations. As discussed earlier, using distributions that give greater frequencies of lethal and semi-lethal mutations had little effect on mutation accumulation, and resulted in more rapid fitness decline.

Our experience has taught us that if the curve is too steep it does not correspond to reality, since in such a distribution, most mutations are very nearly neutral such that accumulation of large numbers of these mutations has almost no effect on fitness, even in the absence of selection. Likewise if the curve is too shallow it also results in an unrealistic scenario in which most mutations have substantial deleterious effects, such that mutation accumulation leads to very rapid extinction, even with intense selection. Our default mutation distribution was reached by considering: (1) the empirical data that is available concerning fitness effects for low-impact mutations in complex organisms, (2) general understanding of the effect of mutations on biological function, and (3) simulations that tested a range of distribution characteristics. It is our view that this default distribution is biologically reasonable. Moreover, we observe that moderate shifting of the distribution in either direction does not change the result that most deleterious mutations are unselectable.

To avoid potential confounding effects of variable degrees of dominance, we have defined the mutational fitness effect of all mutations in terms of their homozygous state. For simplicity, the present study treated all mutations as co-dominant. However, Mendel offers the flexibility to specify the fractions of recessive and dominant mutations and also their levels of heterozygous expression.

We consider four cases of environmental variance: zero environmental variance (heritability of 1.0); slight variance (heritability of 0.4); moderate variance (heritability of 0.04); and high variance (heritability of 0.004). While a heritability value of 0.04 would be very small for a simple phenotypic trait such as height, it is about 10-fold higher than what is commonly estimated for total fitness heritability [8]. Indeed, heritability of fitness is often found to be too small to measure. Selection is always based on each individual's phenotypic fitness, which reflects not only the genotype but also random environmental effects. A given heritability is achieved in Mendel by adding a random number to each individual's genotypic fitness to yield its phenotypic fitness value. These numbers are drawn from a zero-mean normal distribution of random numbers with a variance determined by the specified heritability.

We consider three types of selection: a) perfect truncation selection (approximating the sort of artificial selection applied in plant and animal breeding); b) standard probability selection (in which the probability of survival and reproduction is proportional to phenotypic fitness); and c) partial truncation (an intermediate type of selection, also called broken-line selection). A level of partial truncation was selected that gives results midway between strict probability and strict truncation selection (partial truncation input parameter = 0.5).

Parameters that were fixed for most of the evaluations in this study included: (a) six offspring per female (which implies that, averaged over the population, four out of six offspring are selected away based on phenotypic fitness); (b) Weibull-type distribution of homozygous mutation effects (mean value of -5.4×10^{-4}, median value of -1.4×10^{-7}, and 0.1% of the mutations with effects exceeding 0.1 in magnitude); (c) no beneficial mutations; (d) all mutations co-dominant; (e) mutation effects combine additively; (f) no random death; (g) no fertility decline associated with fitness decline; (h) a diploid sexual species; and (i) dynamic recombination within 23 sets of chromosomes, with two random crossovers per chromosome every generation. Unless specified otherwise, the number of linkage blocks across a haploid set of 23 chromosomes was 989 (43 per chromosome) and the population size was maintained at 10,000 reproducing individuals (30,000 offspring in each generation).

Addendum *— These numerical simulation studies have been theoretical in nature, based upon biologically realistic numerical simulations. A new study of actual*

mutation accumulation with the H1N1 Influenza virus now provides strong empirical validation of our findings. See: Carter R.C. & Sanford, J.C. (2012). A new look at an old virus: patterns of mutation accumulation in the human H1N1 influenza virus since 1918. Theoretical Biology and Medical Modeling 9:42doi:10.1186/1742-4682-9-42. That study analyses actual mutation accumulation within the H1N1 Influenza viral genome since 1918. During the entire history of human H1N1, mutations accumulated in a perfectly linear fashion — exactly as seen in all our theoretical studies. In the course of 90 years, almost 15% of the viral genome mutated, with mutation count increasing at a very constant rate. During this time, viral fitness, as reflected by associated human mortality rates, declined continuously and systematically from 1918 all the way to the apparent extinction of the human H1N1 strain in 2009.

We also append another significant new citation appearing since the finalization of this chapter. See: Sanford, J. & Nelson, C. (2012). The Next Step in Understanding Population Dynamics: Comprehensive Numerical Simulation, Studies in Population Genetics, in: M. Carmen Fusté (Ed.), ISBN: 978-953-51-0588-6, InTech, Available from: http://www.intechopen.com/books/studies-in-population-genetics/the-next-step-in-understanding-population-dynamics-comprehensive-numerical-simulation.

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Appendix 1: *Key parameter settings and their basis*

Mutation rate = 5 (unless otherwise specified). Although the human mutation rate is known to be in the range of 100 new mutations per person per generation [20, 21, 24], we use the extremely conservative number of just 5 as the default. This presumes that at least 95% of the human genome is perfectly inert "junk", which is contrary to the mounting evidence indicating a substantial fraction of the human genome has function [25, 26]). More realistic mutation rates only make the selection threshold problem worse.

Population size = 10,000 (unless otherwise specified). This default population size would be realistic for an isolated tribe, and is the most commonly used figure in human evolutionary scenarios, but obviously does not apply to modern populations. However, in our simulations, we observe that increasing population size beyond 1,000 results in only modest and rapidly diminishing benefits in terms of selection efficiency and reduced ST_d.

Generations = 10,000 (unless otherwise specified). Sufficient to approach selection equilibrium for population sizes of 100 to 5,000.

Fraction of beneficial mutations = 0.0. While beneficials are desirable in themselves, they confound selection against deleterious mutations, tending to make the ST_d problem worse. The effect of beneficial mutations on ST_d are dealt with in a companion paper.

Selection mode = partial truncation (unless otherwise specified). It is generally understood that probability selection best characterizes selection in nature and that strict truncation selection is never observed in nature. Our partial truncation treatment is extremely conservative, being halfway between probability selection and truncation selection.

Offspring per female = 6. In Mendel's default mode, all surplus progeny are selected away. Since two offspring per female are needed for population continuity, this setting causes two thirds of all progeny to be selected away (intense selection).

Chromosomes = 23 sets; Linkage blocks = 989 (unless otherwise stated). In most experiments we use 989 linkage blocks, evenly distributed across chromosomes. We have determined empirically that additional linkage blocks have little benefit in terms of improved selection efficiency and reduced ST_d, but require more computer memory and decrease the problem size possible. The program models two randomly positioned crossovers per chromosome pair per generation.

Distribution of mutation effects = Weibull distribution, wherein 0.1% of all mutations reduce fitness by 10% or more. This results in a mean mutation effect which

reduces fitness by roughly 0.1%. Altering the shape of the distribution to be either steeper or less steep does not significantly affect the ST_d phenomenon.

Dominant versus recessive = co-dominance. Although Mendel allows mutations to be partially dominant, for simplicity we make all mutations in this paper co-dominant. We have observed that this parameter has only a very minor impact on ST_d.

Heritability = 0.4 (unless otherwise specified). This is a very generous heritability, since it is widely recognized that under natural conditions fitness heritabilities are typically too small to measure and are easily an order of magnitude lower than our default setting. Low heritability reflects high environmental variance.

Population sub-structure = none. Mendel allows modeling of tribal population sub-structure with specified migration rates between tribes, but here we only model a simple population with fully random mating.

Mutation effects combination method = additive. Mendel also allows use of the multiplicative model, but we feel the additive model is more realistic. Use of the multiplicative model does not significantly affect the ST_d phenomenon.

To reproduce these results: all other settings can be set to the normal Mendel default settings (Version 1.2.1).