Selection Threshold Severely Constrains Capture of Beneficial Mutations

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Abstract

Background. In a companion paper, careful numerical simulation was used to demonstrate that there is a quantifiable *selection threshold,* below which low-impact deleterious mutations escape purifying selection and, therefore, accumulate without limit. In that study we developed the statistic, ST_d, which is the mid-point of the transition zone between selectable and un-selectable deleterious mutations. We showed that under most natural circumstances, ST_d values are surprisingly high, such that the large majority of all deleterious mutations are un-selectable. Does a similar selection threshold exist for beneficial mutations?

Methods. As in our companion paper we here employ what we describe as *genetic accounting* to quantify the selection threshold (ST_b) for beneficial mutations, and we study how various biological factors combine to determine its value.

Results. In all experiments that employ biologically reasonable parameters, we observe high ST_b values and a general failure of selection to preferentially amplify the large majority of beneficial mutations. High-impact beneficial mutations strongly interfere with selection for or against all low-impact mutations.

Conclusions. A selection threshold exists for beneficial mutations similar in magnitude to the selection threshold for deleterious ones, but the dynamics of that threshold are different. Our results suggest that for higher eukaryotes, minimal values for ST_b are in the range of 10^{-4} to 10^{-3}. It appears very likely that most functional nucleotides in a large genome have fractional contributions to fitness much smaller than this. This means that, given our current understanding of how natural selection operates, we cannot explain the origin of the typical functional nucleotide.

Key words: beneficial mutation, genetic degeneration, mutation accumulation, near-neutral, population genetics, selection threshold, simulation

Introduction

Muller [1] first argued that at a certain point, low-impact mutations should become outside the reach of natural selection. Muller's primary concern was the accumulation of deleterious mutations. Later, Kimura used rigorous mathematical analysis to validate this idea [2]. While Kimura initially described such mutations as 'neutral', Ohta [3–6] argued that such mutations should more accurately be termed 'nearly neutral', and Kimura eventually acknowledged this [7, 8]. Again, their focus was on deleterious mutations. Kondrashov described how low-impact mutations which are essentially un-selectable create a profound evolutionary paradox [9], because deleterious mutations should accumulate continuously, causing continuous fitness decline. Lynch *et al*. [10,11] and Higgins and Lynch [12] showed that accumulation of low-impact deleterious mutations should be a key factor in the extinction process. More recently, Loewe [13] demonstrated that the accumulation of nearly neutral deleterious mutations in just the human mitochondrial chromosome could theoretically eventually lead to extinction.

In a companion paper [14], numerical simulation was used to clearly show that the problem of continuously accumulating low-impact deleterious mutations is indeed a very real problem. We showed that under any given biological circumstance there is a definitive "selection threshold" for mutational fitness effect, and mutations with a fitness effect below this threshold accumulate largely unhindered by the selection process. We further showed that, under realistic conditions, this selection threshold is surprisingly high, in the range of 10^{-4} to 10^{-3}. Those findings indicate that most deleterious mutations should be un-selectable, confirming "Kondrashov's Paradox" [9] and reinforcing long-standing concerns about genetic load [1–13].

One widely-cited mechanism which might counteract the accumulation of slightly deleterious mutations is the concept of "compensating mutations", as first proposed by Ohta [3] and later expanded by others [15,16]. Ohta proposed that for each accumulating deleterious mutation, there is somewhere else in the genome a beneficial mutation that has a more or less equal but opposite compensating effect on fitness. This could not possibly be happening independent of selection, because we know that deleterious mutations strongly outnumber beneficial mutations [17–26]. Therefore the hypothesis of compensating mutations would only be conceivable if there could be effective selection for "equal but opposite" beneficial mutations. This appears problematic because the deleterious mutations are accumulating precisely because their fitness effects are too small to be selectable. Logically one might suspect that beneficial mutations with fitness effect values of similar amplitude would be equally un-selectable. This raises important questions. Is there a selection threshold for beneficial mutations? Under biologically realistic

circumstances, how large might such a selection threshold be? What are the biological implications of such a threshold?

Kimura [7] attempted to quantify the threshold for selection breakdown. His calculations focused on deleterious mutations and considered the influence of only one source of biological 'noise' on the rate of mutation fixation, that of gametic sampling. It is obvious, however, that there are other sources of biological noise besides gametic sampling. Except under strict probability selection (for which transmission of a gamete to the next generation is in strict proportion to the relative fitness of the parent), each of these other sources of noise should influence the selection threshold. Lynch [27], for example, notes that small population size, large nucleotide numbers between crossovers, and high mutation levels all synergistically reduce the efficiency of natural selection. To study some of these biological factors and to quantify how they affect the selection threshold, we have implemented a numerical simulation strategy using a program named Mendel's Accountant [28, 29]. Mendel's Accountant (Mendel) is freely available at http://www.MendelsAccountant.info. This numerical approach enables us to explore the biological complexity of the mutation-selection process as it actually occurs in nature in a way not before possible.

As early as 1964, Muller called for more research aimed at better understanding the selection threshold problem [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.*" The companion paper [14] does the very analysis which Muller felt was needed for deleterious mutations. The goal of this second paper is to describe the parallel analysis relative to the factors that affect the selectability of beneficial mutations.

Results

Conditions allowing optimal selection for beneficial mutations

To better understand the selection threshold phenomenon, we employed the same methodology described in our companion paper [14], conducting numerical simulation experiments using the genetic accounting program called "Mendel's Accountant". The details of how Mendel's Accountant works and how we conducted our experiments are given in the methods section at the end of this paper.

We first conducted experiments to see if there were any parameter settings that allowed selection to amplify beneficial allele frequencies across the full range of

mutational fitness effects. We found that even under idealized selection conditions and zero biological noise, perfect selection for low-impact beneficial mutations never occurs. In this regard, beneficial mutations have a distinctly worse selection threshold problem than do deleterious mutations, because given the same biological parameters that allow all deleterious mutations to be selected away, a large fraction of beneficial mutations remain immune to selective amplification. Even with high selection intensity, minimal selection interference, zero environmental variation, and perfect truncation selection, we observe a significant ST_b, as seen in Figure 1.

Figure 1 displays the rate of accumulation of beneficial mutations as a function of mutational fitness effect, relative to the case of zero selection. Mutational fitness

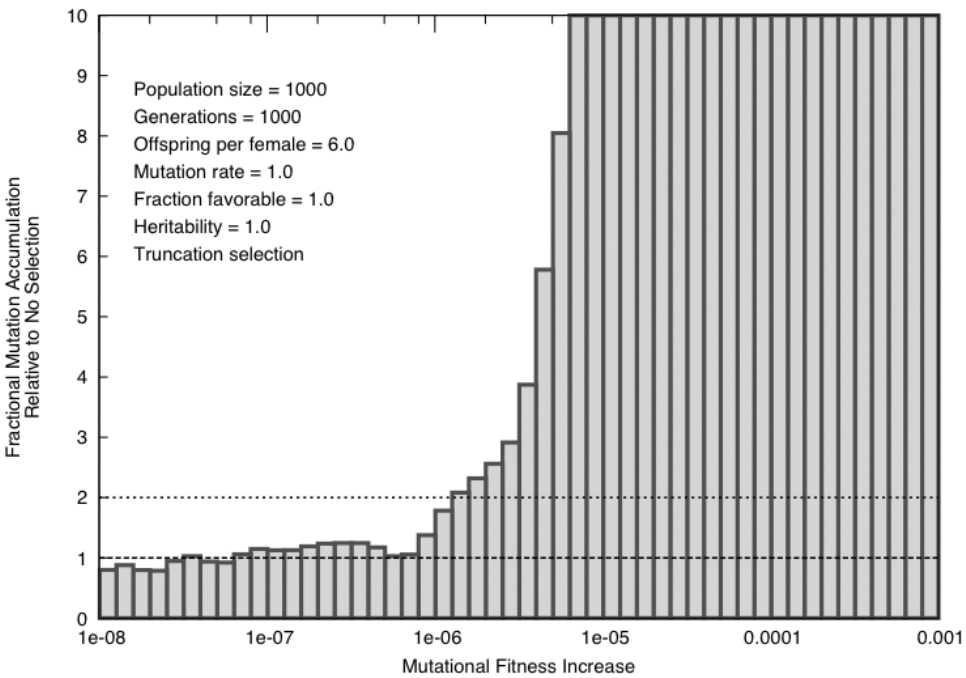

Fig. 1. Accumulation of beneficial mutations as affected by degree of benefit — optimal selection case. This experiment employed extremely unrealistic parameters chosen for maximal selection efficiency (low mutation rate, no deleterious mutations, 67% of all progeny were selected away every generation using truncation selection, with zero environmental variance). Beneficial mutation effects on fitness ranged from 3×10^{-8} to 1.0×10^{-3} (x axis). The height of the bins (y axis) reflects the relative rate of accumulation, compared to that expected when there is no selection. Bins at or near 1.0 are not responding to selection (see lower dotted line). Bins at or near 2.0 (see upper dotted line) are accumulating twice as fast as expected when there is no selection (we define this as the beneficial selection threshold — ST_b). Bins above 2.0 can be seen to be accumulating at increasingly rapid rates. Mutational effects falling in the first two orders of magnitude of mutational effect failed to respond to selection. Note that the vertical scale is clipped at a value of 10.

effect, shown on the x-axis using a logarithmic scale, ranges from a minimum non-neutral mutational value up to a maximal fitness effect. We define the minimal non-neutral mutation value as the reciprocal of the functional genome size (in this case we are considering a human population, and are assuming only 10% of the genome is functional). Each bin represents a fitness effect interval, and the height of the bin reflects the accumulation ratio of that class of mutations relative to the case of no selection. A height of 1.0, therefore, corresponds to the level of accumulation that occurs when selection is entirely ineffective (i.e., a mutation's frequency is influenced only by genetic drift). We define the beneficial selection threshold ST_b as the fitness effect value for which the distribution has the value 2.0. (i.e. the first fitness effect interval which displays twice the accumulation ratio expected in the absence of selection). This is in contrast to the deleterious selection threshold, ST_d, which is defined as the fitness effect where mutation accumulation is half of what is expected in the absence of selection. The beneficial selection threshold value can be seen visually in Figure 1 as the intersection point of the upper dotted line with the mutation distribution (at 1.34×10^{-6}). To the right of this selection threshold value, the heights of all bins increase rapidly because selection is highly effective in amplifying beneficial mutation frequency in this region.

Figure 1 reveals that, even under these idealized selection conditions, there is a fitness effect interval spanning more than two orders of magnitude, in which selection was exerting no meaningful influence on mutational frequency. This "zone of no selection" included all mutations from the smallest effect (3×10^{-8}), up to a value of just over 10^{-6} ($ST_b = 1.34\times10^{-6}$). This basic result was highly reproducible across multiple independent replicates that employed different random number seeds (data not shown). This method of representing the accumulating mutations is very useful, yet fails to convey the actual number of mutations in each bin, because the bin height represents merely a ratio of the actual mutation count versus the mutation count expected in the case of zero selection. It is important to realize that the mutation distribution is approximately exponential, so that the bins on the far left (i.e., low-impact mutations) contain the vast majority of beneficial mutations, while the bins on the right (i.e., high-impact mutations), even when filled, represent very few mutations. Even in this idealized selection experiment, given this mutation effect distribution, we actually observed that 92.7% of all beneficial mutations lay below the selection threshold. There will be occasional high-impact beneficial mutations that arise beyond the range of mutation effects of this experiment (above .001), but they will be so rare as to have very little effect on the fraction of mutations which are not selectable. As we will see, higher-impact beneficial mutations actually make the selection threshold problem worse, and need to be considered separately.

Effect of environmental variance

In the preceding experiment, parameters were chosen to maximize selection efficiency without any regard for biological realism. Two of the most unrealistic aspects of that experiment were the use of truncation selection and the assumption of zero environmental variation. To explore the influence of environmental variation, we conducted a series of experiments using identical parameters, except that we increased the level of environmental variance, quantified in terms of fitness heritability (the ratio of genotypic variance to total phenotypic variance). Figure 2 shows three cases, with fitness heritabilities (h^2) of 0.4, 0.04, and 0.004. Resulting ST_b values were 1.69×10^{-6}, 6.29×10^{-6}, and 1.4×10^{-5}, respectively. As can be observed, higher levels of environmental variance led to higher ST_b levels and a larger no-selection zone. The lowest fitness heritability value we used ($h^2 = 0.004$) is from Kimura [8], and is in keeping with the enormous impact environmental variance has on total phenotypic fitness under natural conditions. That particular heritability value yielded an ST_b approximately one order of magnitude higher

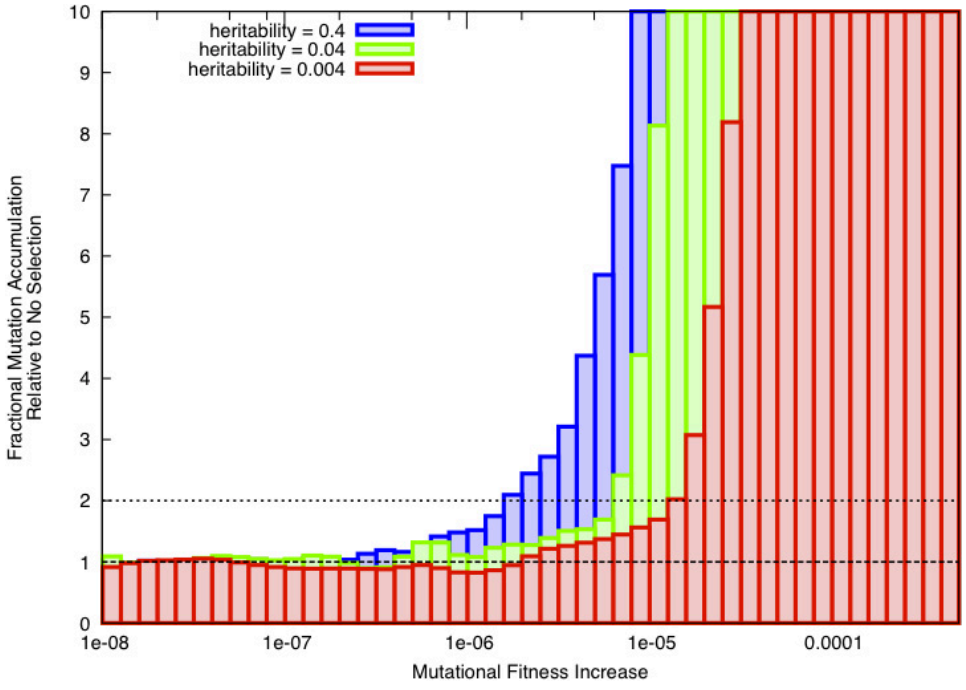

Fig. 2. Accumulation of beneficial mutations as affected by degree of benefit — introducing increasingly realistic levels of environmental variance. This figure combines the results of three experiments which employed the same unrealistic parameters as Figure 1, but simply introduced varying degrees of environmental variance (as reflected by heritability values less than 1.0). Heritability values (h^2) are shown in the figure. As can be seen, adding realistic levels of environmental variance increased the ST_b value by an order of magnitude.

than the zero environmental variance case (Figure 1), and we observed that in that instance 98.8% of the beneficial mutations fell below the selection threshold.

Introduction of probability into the selection process

In another series of experiments, we examined how more realistic modes of selection impact the beneficial selection threshold. Figure 3 contrasts our first experiment which employed truncation selection to more realistic cases employing partial truncation and probability selection. Figure 3 compares the results from the case shown in Figure 1 (red) with identical runs, but with partial truncation (green) or probability selection (blue).

It is well known that probability selection corresponds most closely to what occurs in nature. Under probability selection, the probability of an individual's reproduction is directly proportional to that individual's phenotypic fitness. Under this type of selection, even individuals with relatively low phenotypic fitness still have some likelihood of reproducing. Probability selection contrasts strongly with truncation selection, for which all individuals above a specific phenotypic fitness value have a 100% probability of reproduction, while all individuals below that value have zero probability of reproduction. Full truncation selection is an idealized version of artificial (conscious) selection, as employed by plant or animal breeders — it never happens in nature. The selection method we refer to as partial truncation (sometimes also referred to as "broken-line" selection) is intermediate between full truncation selection and probability selection. In this experiment we have employed a form of partial truncation representing an exact 50/50 blending of classical probability selection and full truncation selection.

Figure 3 shows that introducing even a modest degree of probability selection (partial truncation) results in markedly higher ST_b values. The ST_b value for partial truncation selection in this otherwise idealized selection experiment (2.54×10^{-4}) was more than two orders of magnitude larger than for pure truncation selection (1.68×10^{-6}). Full probability selection, which is commonly recognized as the actual mode of selection happening in nature, led to a complete breakdown of selection over the entire range of mutational effects considered in this experiment (the maximal beneficial fitness effect being 0.001). This indicates that the ST_b must have been greater than 0.001. We have consistently observed that the noise associated with the random aspects of probability selection leads to a greater increase in selection thresholds than any other source of noise we have examined. The only exception to this is in the case of extremely beneficial mutations, as will be described below. It is clear that even moderate levels of randomness in the selection process (i.e., a limited degree of "survival of the luckiest"), causes the vast majority of beneficial mutations to become un-selectable.

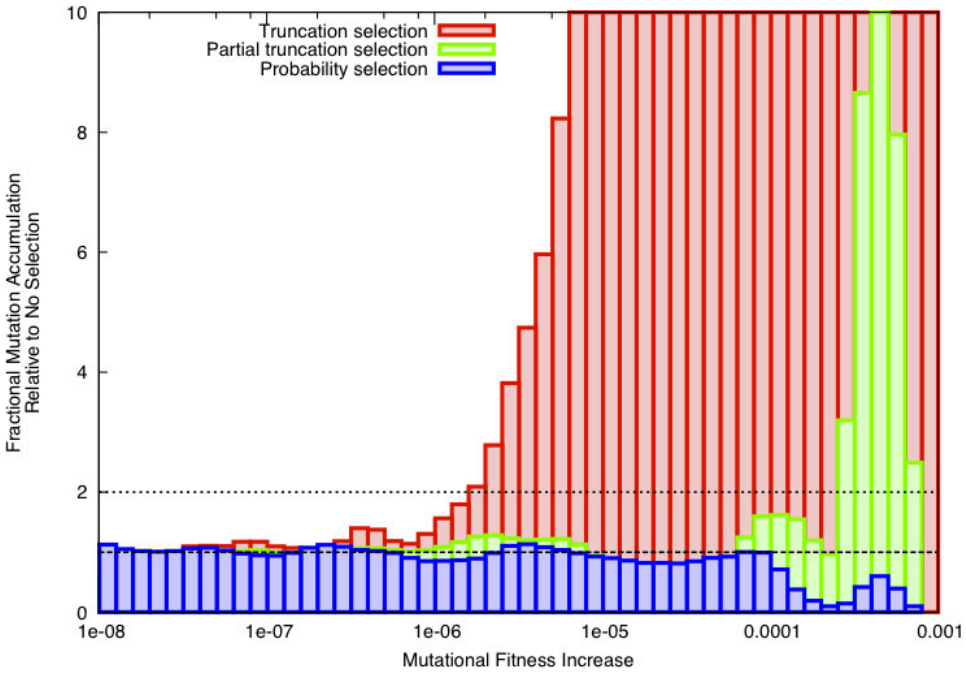

Fig. 3. Accumulation of beneficial mutations as affected by degree of benefit, employing three different modes of selection. Parameters are the same as in Figure 1, except that increasingly realistic forms of selection are introduced. Red: full truncation selection. Green: partial truncation selection (0.5). Blue: probability selection. As can be seen, introduction of probabilistic selection increased ST_b by roughly three orders of magnitude. The blue and green distributions become sparse on the right side of the figure because given an exponential distribution of mutational effects, alleles in this range were very rare apart from selective amplification.

Effect of high mutation rate and consequent selection interference among beneficial mutations

We next conducted a series of experiments, still using truncation selection and zero environmental variance, but with higher beneficial mutation rates, ranging from 5 to 40. As mutations accumulate, there arises a type of biological noise associated with *selection interference* among the mutations. Figure 4 summarized a series of experiments that reveal that increasing the rate of beneficial mutations lead to higher selection thresholds. This means that as mutation rate increases, more and more of the alleles that otherwise would be selectable escape selection. Increased mutation rate and the consequent selection interference among alleles resulted in ST_b values increasing from 1.68×10^{-6} for a mutation rate of 5; up to 5.84×10^{-6} for a mutation rate of 10; up to 1.00×10^{-5} for a mutation rate of 20; up to 1.46×10^{-5}

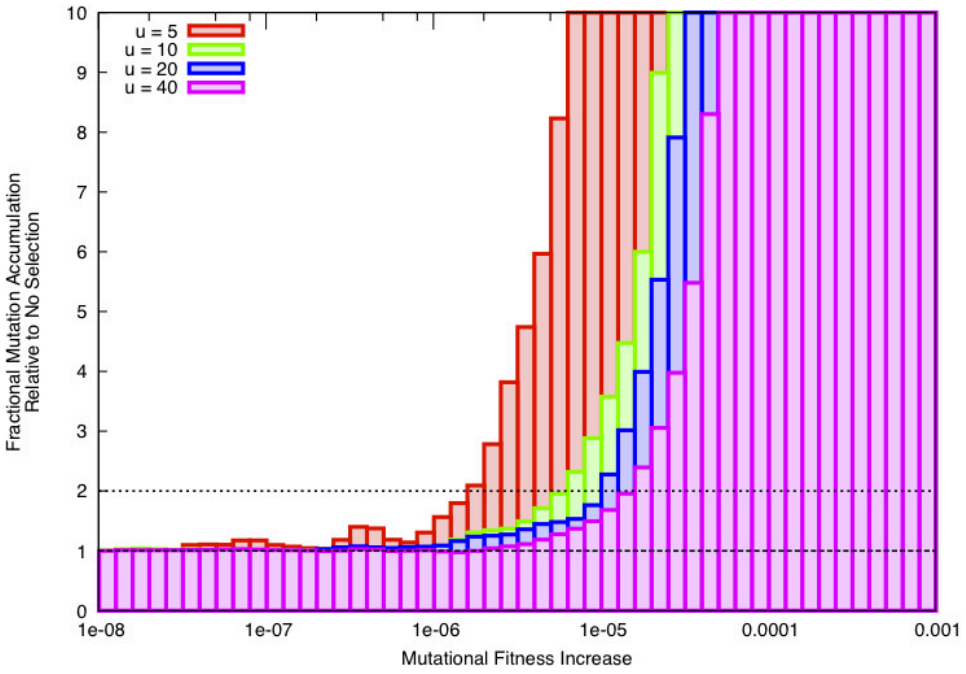

Fig. 4. Accumulation of beneficial mutations as affected by degree of benefit, employing four different mutation rates. Parameters are the same as in Figure 1, except that increasingly higher mutation rates (u) are introduced. As can be seen, higher mutation rates cause substantially higher ST_b values, due to selection interference.

for a mutation rate of 40. This last ST_b value for a mutation rate of 40 indicates that 98.8% of the beneficial mutations were below the selection threshold.

Effect of extremely beneficial mutations

Until this point, we have employed a ceiling value of 0.001 for beneficial mutational fitness effects. The rationale for this choice is given in the discussion section and was employed because very high-impact mutations need to be handled separately. We therefore conducted experiments with higher maximal fitness effect values, up to 1.0. When homozygous, a single beneficial mutation with a fitness effect of 1.0 will double the fitness of any individual, relative to the initial fitness value. We find that the inclusion of mutations with fitness values of 0.1 or greater have such a profound effect on the behavior of the whole population that we refer to them as "extremely beneficial" mutations. As can be seen in Figure 5, when we repeated the experiment illustrated in Figure 1, but merely extended the upper range of beneficial mutational effects up to 1.0, the result was a very dramatic

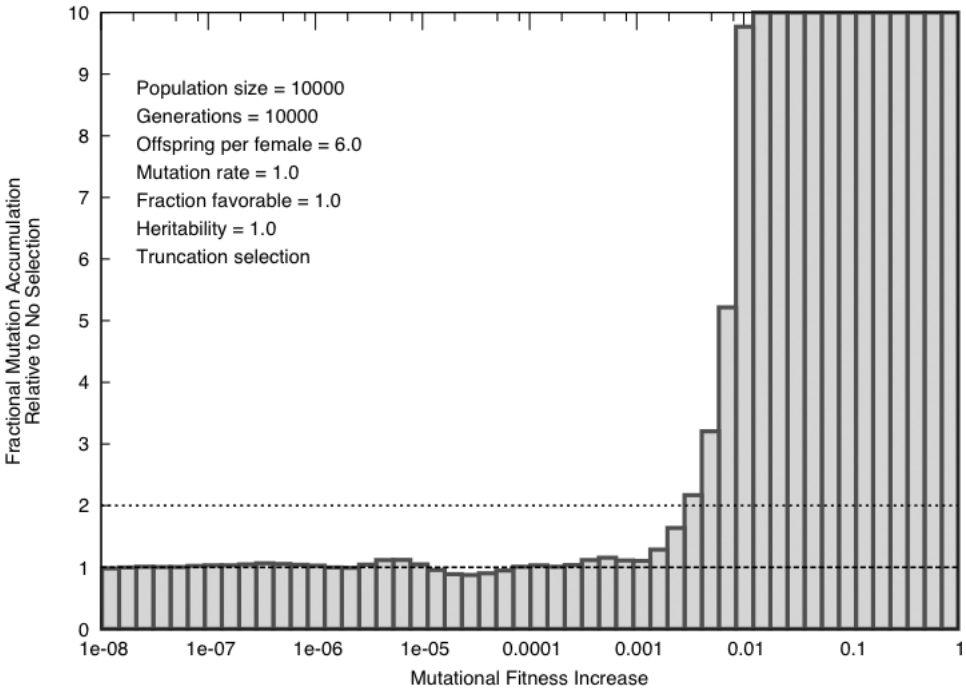

Fig. 5. Accumulation of beneficial mutations as affected by degree of benefit, when extremely beneficial mutations are allowed. Parameters are the same as in Figure 1, except that the maximal mutational fitness effect has been increased from .001 to 1.0. Allowing extremely beneficial mutations causes intense selection interference and raises the selection threshold more than 3 orders of magnitude.

increase in the ST_b value (2.96×10^{-3}). This was the single factor in our studies that by itself most dramatically increased the beneficial selection threshold.

The ST_b value seen in Figure 5 indicates that 98.0% of the beneficial mutations were below the selection threshold. This ST_b value is more than three orders of magnitude greater than what is seen in Figure 1 and is comparable to the increase we see when we switch from truncation selection to probability selection. Ironically, the effects of very high-impact beneficial mutations overshadow low-impact beneficial mutations so profoundly that it results in selection breakdown for all beneficial mutations with fitness effects less than approximately 0.001. This is true even when all other factors are chosen to minimize the selection threshold, including full truncation selection and zero environmental variance. These very high-impact beneficial mutations are in a sense "too selectable". The very rare alleles that are represented on the far right of Figure 5 dominate the selection process and exhaust almost all the selection potential available. This represents the most dramatic form of selection interference we have seen in over six years of experimentation with genetic accounting methodology.

Effect of adding deleterious mutations

The experiments described above show that increasing beneficial mutation rates leads to increased selection interference, and that introduction of extremely beneficial mutations leads to an especially profound type of selection interference. However, all experiments described thus far have involved only beneficial mutations. We know that, in reality, the majority of mutations are deleterious. To what extent do beneficial and deleterious mutations affect each other's relative selectability? To address this question we conducted an experiment similar to that of Figure 1 with truncation selection, zero environmental variance, and just one new beneficial mutation per offspring. In addition to the average of one beneficial mutation per offspring, we also added an average of one deleterious mutation per offspring. This experiment yielded a selection threshold for deleterious mutations of 2.30×10^{-6}, as shown in Figure 6. By contrast, the parallel case (as described in our companion paper [14]), with one new deleterious mutation per offspring but

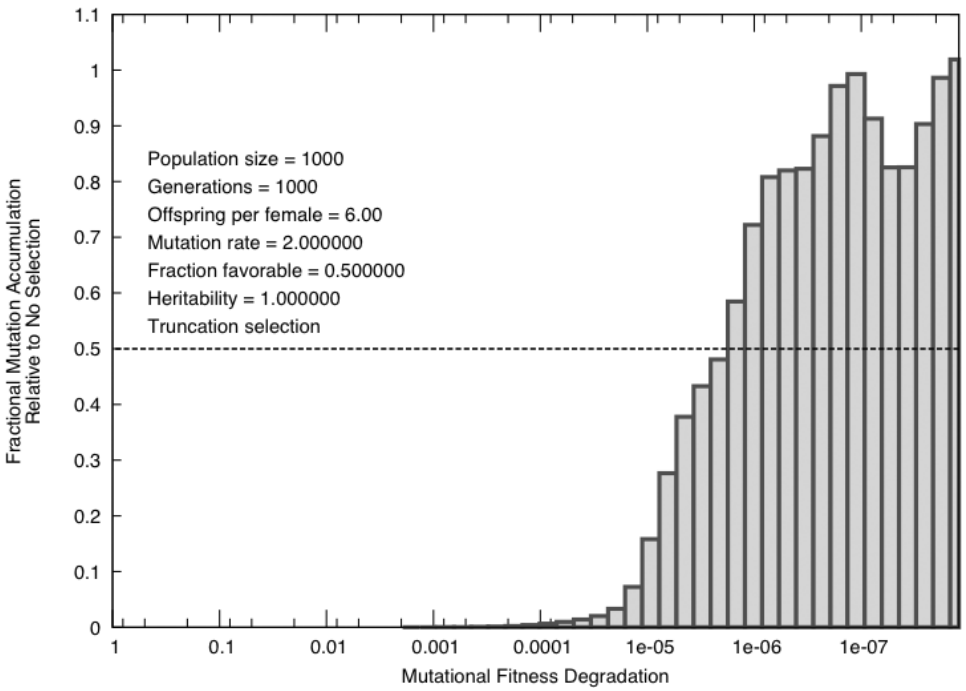

Fig. 6. Accumulation of deleterious mutations as affected by degree of harmfulness, given equal rates of deleterious and beneficial mutations. Parameters are the same as in Figure 1, except that an equal rate of deleterious mutation was added. Selection interference due to the accumulating beneficial mutations causes very significant accumulation of deleterious mutations under conditions where none would have accumulated otherwise (see companion paper [14]).

zero new beneficial mutations per offspring, gave the result of *zero* deleterious mutations accumulated.

The beneficial mutations clearly caused very serious selection interference in terms of the selectability of the deleterious mutations. However, the converse was not true. The accumulation of deleterious mutations only had a very modest effect on the accumulation of beneficial mutations. This can be seen by comparing Figure 7 ($ST_b = 2.00\times10^{-6}$) with Figure 1 ($ST_b = 1.34\times10^{-6}$). This asymmetrical aspect of selection interference between deleterious and beneficial mutations reflects a fundamental difference in dynamics between purifying selection versus positive selection. Purifying selection very effectively eliminates high-impact deleterious mutations, such that the remaining deleterious mutations are all low-impact, have a highly diffuse genetic effect, and constitute a minor source of noise relative to the selectability of the beneficial mutations. However, positive selection amplifies only the very high-impact beneficial mutations, which then very effectively "highjack" almost all the selection potential of the population, severely diminishing the effectiveness of purifying selection.

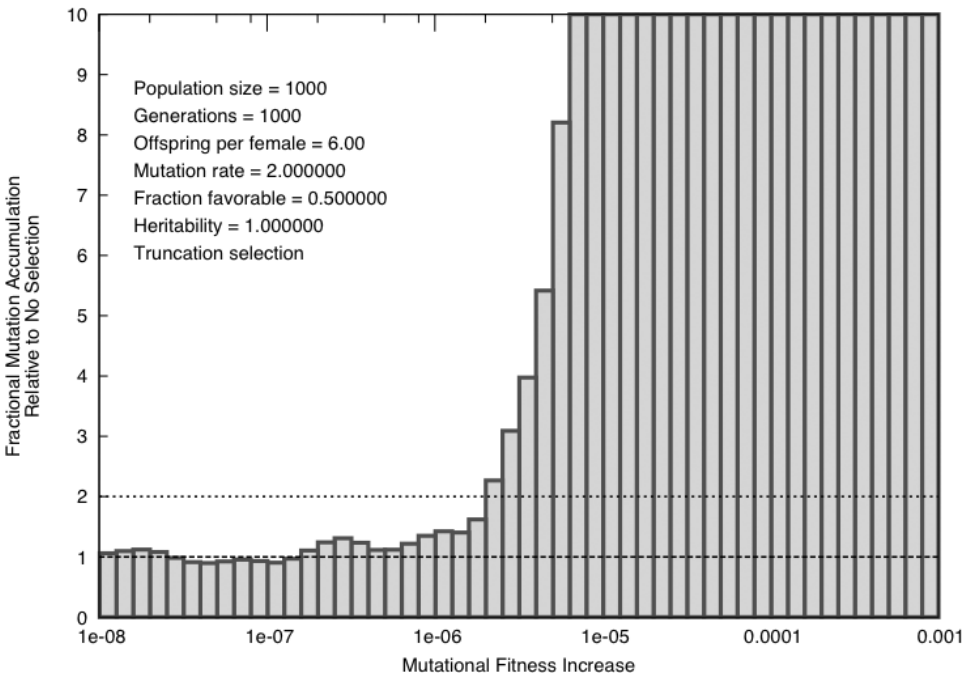

Fig. 7. Accumulation of beneficial mutations as affected by degree of benefit, given equal rates of deleterious and beneficial mutation. Parameters are the same as in Figure 1, except that an equal rate of deleterious mutation was included. When deleterious mutations are included, they have minimal effect on the selection threshold of beneficial mutations (contrast with Figure 1, where there were no deleterious mutations).

Effect of multiple sources of noise, at minimal levels

Here we present an experiment that combines minimal levels of noise from all the primary factors affecting selection threshold. The key parameter settings were as follows: a very conservative mutation rate (5.0), a very conservative level of environmental variance ($h^2=0.4$), an intermediate value for the maximal beneficial effect (0.1), and an extremely generous selection mode (50% truncation). We also added a minimal number of deleterious mutations (50% of mutations being harmful). We chose these highly unrealistic settings so that we might approximate a lower limit on the beneficial selection threshold that might be expected for a typical mammalian population. Results from this experiment are shown in Figures 8 and 9.

As seen in Figure 8, given multiple sources of biological noise at minimal levels (including interfering beneficial mutations), deleterious mutations accumulated massively, resulting in a ST_d value of 2.34×10^{-3} (97.7% of deleterious mutations were below the selection threshold). Likewise, these minimal levels of biological

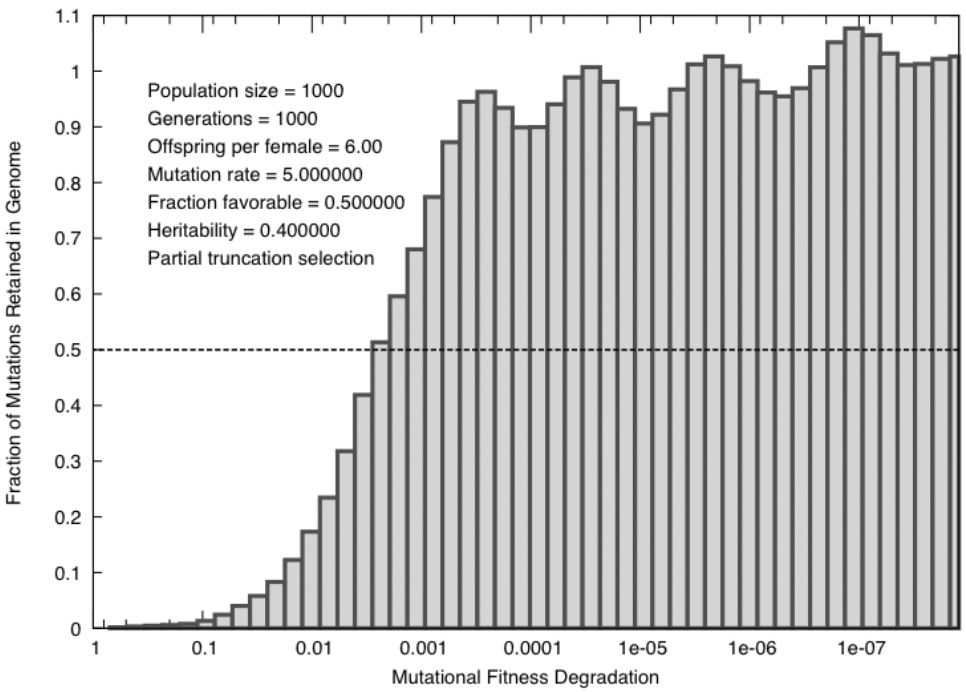

Fig. 8. Accumulation of deleterious mutations as affected by degree of harmfulness, with multiple sources of noise at low levels. Critical parameters: mutation rate = 5, fraction beneficial = 0.5, maximum beneficial effect = 0.1, fitness heritability = 0.4, partial truncation = 0.5. Multiple sources of noise, even at minimal values, cause very high ST_d values.

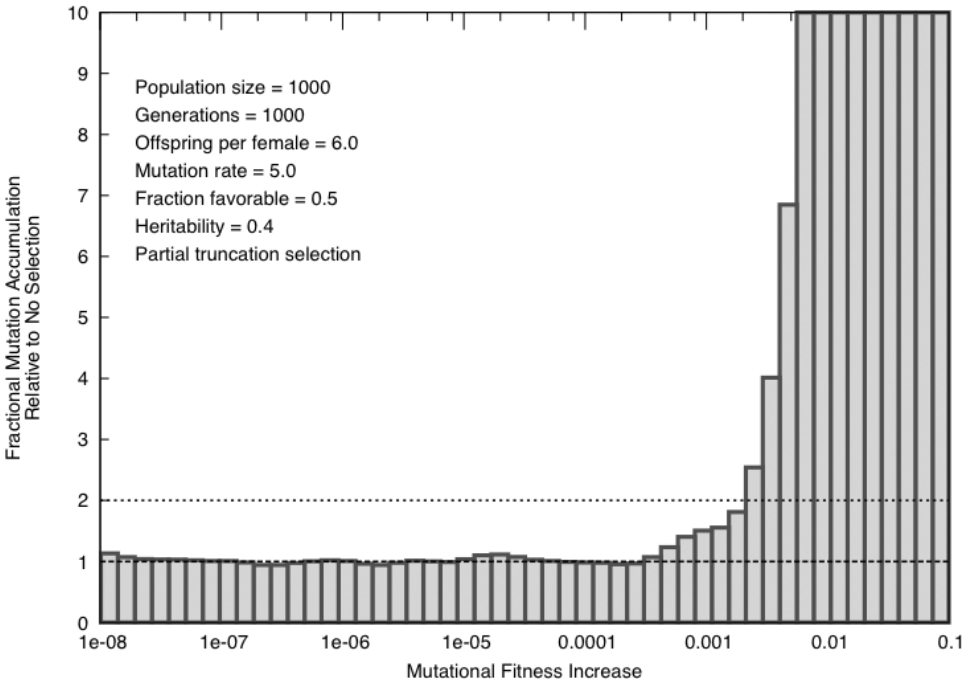

Fig. 9. Accumulation of beneficial mutations as affected by degree of benefit, with multiple sources of noise at low levels. Parameters as in Figure 8. Multiple sources of noise, even at minimal values, cause very high ST_b values.

noise combined with interfering deleterious mutations resulted in the failure to amplify almost all beneficial mutations (Figure 9), resulting in a ST_b value of 1.96×10^{-3} (99.4% of all beneficial mutations were below the selection threshold).

Modest levels of noise with a larger population

Here we present an experiment that combines larger population size with levels of noise which are more realistic but still very modest. The key parameter settings were as follows: mutation rate (10); environmental variance ($h^2 = 0.04$); beneficial mutations (10%), and a more realistic selection mode (partial truncation, but with 10% truncation and 90% probability selection). All prior experiments necessarily employed a modest population size of 1000, because the parameters settings were so extremely unrealistic that they resulted in massive amplification of certain beneficial mutations, which would then exhaust available RAM resources (16 GB). In this experiment, using more realistic parameters, we were able to employ a larger population size of 10,000. These more realistic settings

were chosen in order to approximate a more realistic lower limit for the beneficial selection threshold, as might be expected for a typical mammalian population. Results from this experiment are shown in Figures 10 and 11.

As seen in Figure 10, given a mixture of deleterious and beneficial mutations, combined with multiple sources of biological noise at modest levels, and with a larger population size, deleterious mutations again accumulated at very high rates, resulting in the highest ST_d value of this study, which was 4.96×10^{-3} (98.5% of all deleterious mutations were below the selection threshold).

Likewise, given a mixture of deleterious and beneficial mutations, combined with multiple sources of biological noise at modest levels, and with a larger population size, there was a failure to amplify the vast majority of beneficial mutations (Figure 11), resulting in the highest ST_b value of this study, which was 3.16×10^{-3} (99.6% of beneficial mutations were below the selection threshold).

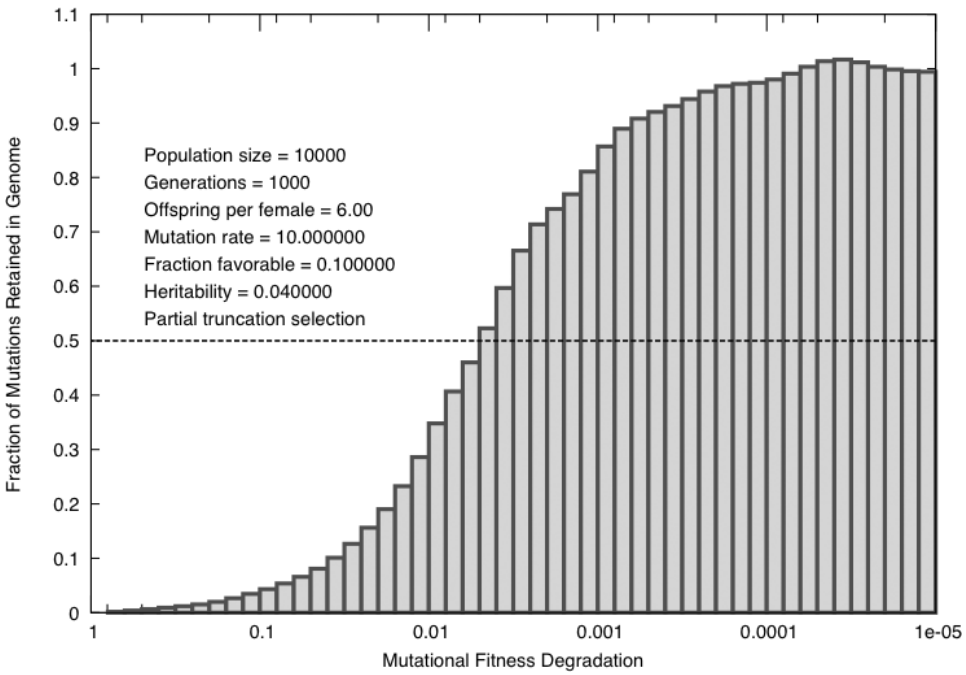

Fig. 10. Distribution of accumulating deleterious mutations, with multiple sources of noise at modest levels, larger population. Critical parameters: population size = 10000, generations = 1000, mutation rate = 10, fraction beneficial = 0.1, maximum beneficial effect = 0.1, fitness heritability = 0.04, partial truncation = 0.1. Multiple sources of noise, even at modest levels, and even with larger population size, cause very high ST_b values. Note: due to memory limits, in this experiment we used a tracking limit of 1.0×10^{-5}, and so could not plot the lowest three orders of low-impact mutations which would have been on far right.

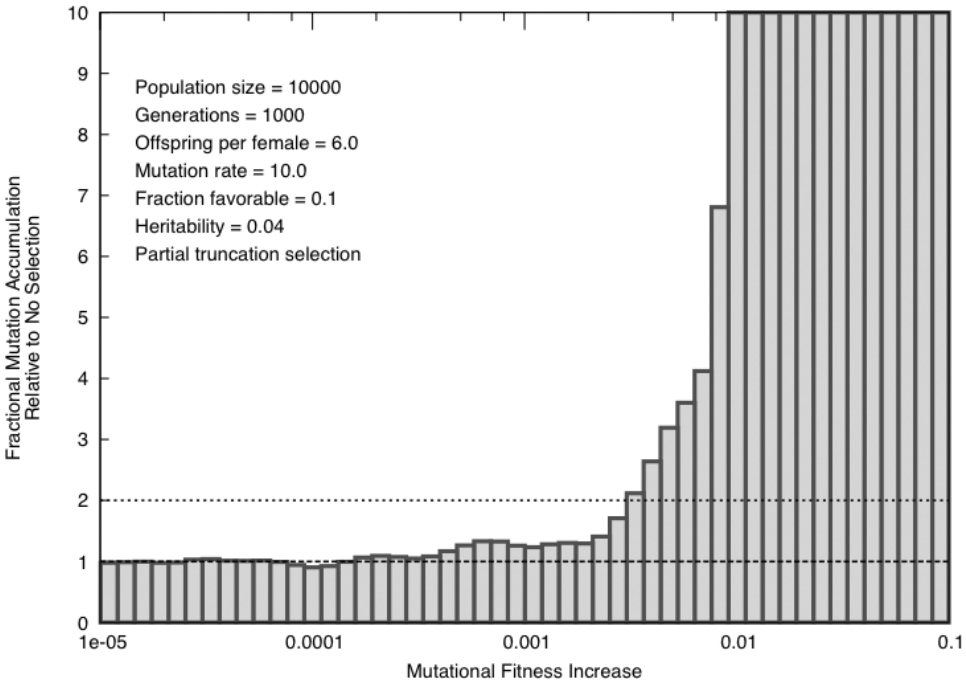

Fig. 11. Distribution of accumulating beneficial mutations, with multiple sources of noise at modest levels, larger population. Critical parameters: population size = 10000, generations = 1000, mutation rate = 10, fraction beneficial = 0.1, maximum beneficial effect = 0.1, fitness heritability = 0.04, partial truncation = 0.1. Multiple sources of noise, even at modest levels, and even with larger population size, cause very high ST_b values. Note: due to memory limits, in this experiment we used a tracking limit of 1.0×10^{-5}, and so could not plot the lowest three orders of low-impact mutations which would have been on far left.

The effect of time on ST_d and ST_b values

Here we present examples of how ST values can change over time. In all of our experiments where we begin with zero genetic variance, we see that ST values are initially exceptionally high, but rapidly decline as the population moves toward selection equilibrium, at which point ST values stabilize.

Figure 12 gives an example of this, where both beneficial and deleterious mutations are accumulating (population size = 1000, mutation rate = 5, fraction beneficial = 0.5, maximum benefit = 0.1, heritability = 0.4, partial truncation = 0.5). After 2000 generations, it can be seen that the beneficial mutations begin to approach selection equilibrium more rapidly than the deleterious mutations. After 5000 generations, ST values are very stable. In this experiment, ST_b stabilized at a slightly higher value than ST_d.

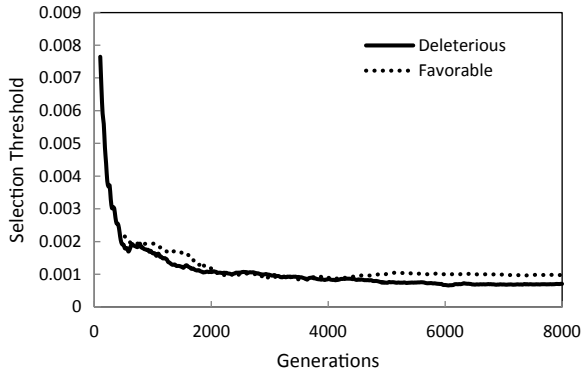

Fig. 12. Deleterious and beneficial selection thresholds plotted over time, with multiple sources of noise. It takes many generations to reach selection equilibrium. The beneficial and deleterious selection thresholds equilibrate at very nearly the same levels. Beneficial selection threshold cannot be plotted until about generation 500 (until then, there are too few beneficial mutations to produce meaningful data).

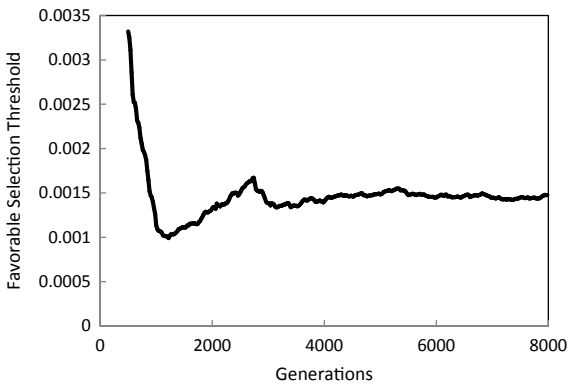

Fig. 13. Beneficial selection threshold plotted over time for a larger population, when extremely beneficial mutations are allowed. Critical parameters: population size = 10000, generations = 8000, mutation rate = 1, fraction beneficial = 1.0, maximum beneficial effect = 1.0, fitness heritability = 1.0, full truncation. Plotting started at generation 500.

Figure 13 shows how ST_b changes over time in the special case where there is a larger population (10,000), but extremely beneficial mutations are allowed (beneficial fitness effects up to 1.0), and all other parameters are optimized for selection efficiency (population size = 10,000; mutation rate = 1; fraction beneficial = 1,; heritability = 1; full truncation). Runs which include high-impact beneficial mutations tend to become limited by computer memory, because of the rapid amplification of those beneficial mutations. For that reason, longer-term experiments such as this require that all unnecessary tracking be suspended. Even with

this accommodation, memory overflowed in this experiment after 8,000 generations. Adding extremely beneficial mutations, even under ideal conditions, greatly increases initial and ending ST_b values. ST_b values reach a minimum after roughly 1000 generations (about 2×10^{-3}) and then gradually increased due to growing selection interference as high-impact mutations increased.

Discussion

This analysis leaves no doubt that there must be a very significant selection threshold for beneficial mutations in higher organisms. This threshold is not a simple function of population size, but is affected by numerous factors. The reality of such a threshold has profound theoretical and practical implications. Our results show that the beneficial selection threshold for higher eukaryotes should be so large under realistic biological circumstances that nearly all beneficial mutations must be below that threshold. This constitutes a mystery. If the vast majority of beneficial mutations lie below the selection threshold and thus are not acted upon by selection, how can we explain the origin of low-impact functional nucleotides? Most functional nucleotides within a large genome must each make only an extremely small fractional contribution to total fitness, and therefore certainly must lie below the selection thresholds we are seeing. Simple logic therefore suggests that most functional nucleotides in large genomes could not have arisen via selection, at least not as natural selection is presently understood to operate.

There is substantial room for discussion regarding which parameter choices would be most appropriate for a given species and which choices might be most representative of a given natural circumstance. However, if we use extremely conservative estimates for all the relevant parameter choices that affect selection threshold, we should be able to estimate reasonably well the *lower limits* for mammalian ST_b values. The experiment summarized in Figures 10 and 11 does just this, yielding a ST_b value of approximately 3×10^{-3}. We have found that whenever we combine multiple sources of noise, even when using our most conservative parameter settings, we see ST_b values in this range. Therefore, we suggest that 10^{-3} is a reasonable approximation of the beneficial selection threshold for a typical mammalian population.

Even given extremely unrealistic selection parameters which confer the smallest possible selection threshold (Figure 1), we show that the large majority of beneficial mutations still lie below that threshold. When we introduce greater and greater levels of biological realism into our experiments, the selection threshold problem becomes progressively more severe (Figures 2–11). For example, our experiments show that when there are higher rates of mutations, or when there are

just both classes of mutations (both beneficial and deleterious), this can cause strong selection interference, which further increases selection threshold values (Figures 4, 5, 6). This is seen when we increased beneficial mutations rates beyond one new mutation per offspring (Figure 4), or when we simultaneously allow both deleterious and beneficial mutations (Figure 6). We see this most dramatically (Figure 5), when we introduce very high-impact beneficial mutations, which strongly interfere with selection for all other mutations. The problem of selection interference has been casually recognized in several earlier papers [20, 26, 30], but no attempt has been made to quantify its effect under realistic circumstances, and the problem has largely been dismissed. Our studies suggest that selection interference is extremely important, and cannot be properly understood except by using biologically realistic genetic accounting programs such as Mendel's Accountant. This approach appears to bring the greatest clarity to the problem of selection interference and provides an excellent research tool for those who wish to study the problem further.

In a large genome (e.g., 10^8 functional nucleotides), non-neutral mutations must typically have very tiny fitness effects, with a lower limit of perhaps $\pm 10^{-8}$. Given that both deleterious and beneficial mutations have selection threshold magnitudes in the range of 10^{-3} or higher, it becomes clear that there exists a "zone of no-selection" which covers several orders of magnitude in fitness effect on either side of zero. We have previously shown that, when considering deleterious mutations by themselves, the large majority must fall within this "no selection zone" [14]. We here show that when high rates of beneficial mutations are included in the analysis, the selection breakdown for deleterious mutations becomes still worse (Figures 6, 8, 10). More importantly, we show that beneficial mutations themselves consistently have a very high selection threshold under all reasonable conditions (Figures 3, 5, 7, 9, 11). We show that given reasonable parameter settings, more than 99% all beneficial mutations are consistently un-selectable, leaving only a very small number of outlying high-impact beneficial mutations subject to selection. These findings raise a number of questions.

Can low-impact beneficial mutations contribute to genome building?

Building genomes without the use of low-impact nucleotides is very problematic. Since the time of Darwin it has been commonly thought that evolution must occur through an endless series of miniscule improvements (i.e. one nucleotide at a time). In light of our findings, this does not appear feasible. If beneficial mutations with fitness effects of less than 0.1% are not selectable, then evolution must only

advance via larger and more discrete steps. For example, if fitness typically advances in increments of 1–10%, then only 10 to 100 mutational steps would be needed to double biological functionality. But the typical functional nucleotide in a large genome is generally assumed to carry a selection coefficient orders of magnitude smaller than this. How did such low-impact functional nucleotides arise? It is widely recognized that we each carry tens of thousands of deleterious mutations, yet we remain fairly robust, indicating that the damaged functional nucleotide sites in our genome must generally have each been conferring very tiny contributions to fitness. If selection cannot preserve such functional nucleotides, how could selection have put them in their place to begin with?

Can high-impact beneficial mutations explain the origin of the genome?

A very high-impact beneficial mutation (an extremely beneficial mutation), can obviously contribute to genome building, but only in a very limited sense. Indeed, we observe that, given high rates of high-impact beneficial mutations, net fitness can increase rapidly, even while a much larger number of deleterious mutations are continuously accumulating at a steady rate. Under these conditions we can see huge leaps in fitness scores, yet this improvement is entirely dependent upon only a handful of isolated, unlinked, non-complementary mutations. Under these conditions, selection can at best eliminate the worst deleterious mutations, while amplifying only the highest-impact beneficial mutations.

In terms of numerical scores within a simulation experiment, just a few extremely beneficial mutations can more than compensate for large numbers of low-impact deleterious mutations. But this leads to increasing "fitness" only in a narrow and artificial sense. In the broader sense, the whole genome is still degenerating, because, while a few nucleotide sites are being improved, large numbers are being degraded. This type of trade-off is not sustainable, as it results in a shrinking functional genome size. More and more nucleotide sites are losing their specificity, and hence their functionality. Taken to the extreme, this would eventually yield a biological absurdity — a functional genome consisting of a handful of high-impact nucleotide sites that somehow code for all of the organism's functionality.

Extremely beneficial mutations undoubtedly play an important role in adaptation to specific environmental circumstances, as in the case of microbial resistance to antibiotics, or in the case of human resistance to malaria. However, beyond this type of dramatic adaptation to some lethal external factor, extremely beneficial mutations seem to have very limited explanatory power in terms of genome building.

To the extent that extremely beneficial mutations are undergoing selection, our experiments show that they cause a sharp increase in both ST_d and ST_b values. This is a serious problem, because it means extremely beneficial mutations are hijacking most of the "selection power" inherent in the surplus population, and thus are contributing to selection breakdown for the vast majority of both deleterious and other beneficial mutations. Another way of expressing this is that the organism is being improved relative to only a few highly specific traits, but otherwise is "rusting out" in innumerable other ways. The actual fitness gain in such cases is generally no more than a transient response to a fluctuating environmental condition and so is fundamentally superficial, yet the cost is a continuously growing genetic load involving systematic, long-term, and irreversible decay of innumerable and essential internal functions.

Natural selection must explain more than just a few high-impact nucleotide sites. It needs to also explain all the low-impact nucleotide sites surrounding any given high-impact nucleotide site — because these create the proper context which gives the high-impact nucleotide its functionality. Because extremely beneficial mutations must be extraordinarily rare, there is a statistical necessity for extremely beneficial mutations to arise singly, unlinked, and with functional independence, and this profoundly limits their utility. They are self-limiting in that they can only accomplish the types of things that a single typographical error might achieve. Naturally, a single nucleotide change can readily destroy a function or interfere with some key interaction. But a single nucleotide change generally is not expected to create, *de novo*, any new complex functionality. If only high-impact nucleotide positions are selectable, where do the many low-impact nucleotides come from which create the context for the rare high-impact nucleotide?

Can equal-but-opposite compensating mutations stop degeneration?

One implication of high selection thresholds for beneficial mutations is that Ohta's hypothesis of compensating mutations [3,15,16] does not appear viable. A multitude of low-impact deleterious mutations cannot be systematically compensated by selection for equal-but-opposite beneficial mutations at other sites in the genome. Our analysis indicates that selection thresholds for beneficial mutations are comparable in amplitude to those for deleterious mutations, so equal-but-opposite beneficial mutations must be equally un-selectable, rendering such a stabilizing mechanism inoperative.

Can high-impact compensating beneficial mutations stop degeneration?

A single high-impact beneficial mutation can, in a limited sense, compensate for many low-impact deleterious mutations. If there were enough high-impact beneficial mutations, this might appear to solve the problem of genetic degeneration. We have conducted extensive analyses of this question using Mendel and find that stopping genetic degeneration is feasible only when the rate of high-impact beneficial mutations is sufficiently high.

A major unknown for any genetic simulation is the exact frequency of beneficial mutation. Beneficial mutations are generally considered much too rare to allow empirical determination of their exact rate. In this paper we used extremely high fractions of beneficial mutation (10–100%), not because we consider such high numbers to be realistic, but because it was necessary in order to obtain definitive estimates for ST_b. We needed to generate a relatively large number of beneficial mutations to define the selection thresholds in a reproducible manner. When we use rates of beneficial frequencies that are consistent with estimates of other investigators [19, 20] and that seem reasonable to us (e.g., less than one in 10,000), beneficial mutations have essentially no effect. In all our experiments where deleterious mutations outnumber beneficial mutations by 3–6 orders of magnitude, the beneficial mutations exert essentially no effect on fitness change over time (except rare and anomalous mutations which are extremely beneficial and create a short-term spike in fitness).

Might beneficial mutations be common?

Is it possible that the rate of beneficial mutations might actually be extremely high, such that random drift and just a little selection might fill the genome with functional nucleotides? This does not seem reasonable because it would imply that practically any sequence is equally functional and that functional sequence information requires little specificity. However, most biologists understand that functional information is very specific, and thus beneficial mutations must be very rare. Indeed, beneficial mutation rates have often been estimated to be in the range of only one in a million [19, 20]. A large majority of geneticists acknowledge the scarcity of beneficial mutations, and complain of the difficulty in studying them due to their scarcity [17–26]. However, a few scientists have argued that beneficial mutations might be extremely common, even approaching 50% of all non-neutral mutations [31, 32]. If applied to the written information within a given assembly manual, this concept would suggest that 50% of all typographical errors in a set of

instructions will result in an improved product. This is obviously not reasonable, as it implies that almost any letter sequence will specify the same instruction. These issues are dealt with in more depth in another companion paper [33].

It is sometimes argued that genetic information must actually be quite non-specific, because many random changes have been thought to be perfectly neutral. This common misconception arose in part because of the casual use of the term "neutral mutation" to describe any low-impact mutation that escapes selection. However, on a functional level, the perfect neutrality of any mutation is neither testable nor logical. Every mutation should logically have some biological effect, no matter how small. Significantly, synonymous mutations, the long-standing paragon of neutral mutation, can no longer be assumed to be neutral. Synonymous mutations can be non-neutral because synonymous codon substitutions can profoundly affect RNA stability, protein translation rate, and even protein folding [34]. In a parallel development, the long-held paradigm of "junk DNA" is increasingly being challenged [35], undermining the other primary rationale for assuming that most mutations are perfectly neutral.

To address the issue of neutral mutation, Mendel's Accountant allows the mutation rate to be discounted by whatever fraction the user feels is a reasonable estimate of the rate of neutral mutation. For example, for the experiment summarized in Figures 10 and 11, we used a mutation rate of just 10, even though the actual human mutation rate is known to be in the range of 60–100. This reflects the premise that 90% of the genome is perfectly inert, and so 90% of all mutations are neutral, which we feel is extremely over-generous. We have earnestly sought to circumvent the confusion associated with the concept of neutral mutation by only considering mutations within the "functional genome" (as opposed to any junk DNA sequences). By focusing only on the functional genome, we feel we can focus just on those mutations within "functional sequences". To be functional, sequences must be specific, and so random changes within such sequences should very rarely increase their functionality.

For many reasons, unambiguously beneficial mutations must be very rare, and beneficial mutations above the selection threshold must be extraordinarily rare [33]. Invoking high rates of extremely beneficial mutations does not seem to offer a realistic solution to the selection threshold problem.

Possible criticisms

A possible criticism of this study might be that no one really knows the exact distribution of beneficial mutations. Therefore, some might claim that the Weibull distribution we used in these studies may be distorting our conclusions about

selection threshold for beneficial mutations. However, our results do not depend on the precise shape of the distribution curve. As long as the distribution is approximately exponential, we get similar results and reach the same basic conclusions. There is essentially unanimous consent that the beneficial mutation distribution must be approximately exponential [17,23,24,26,36–43], with high-impact mutations being very rare and very low-impact mutations being the vast majority. Indeed various papers [38, 42, 44], contend that the Weibull distribution fits biological reality as well or better than the other variations on the basic exponential theme.

A second possible criticism of this study might be that our thesis is contradicted by a large volume of scientific literature that uses DNA sequence comparisons to infer historical positive selection events for great numbers of putative beneficial mutations. To the extent that theory and actual observations conflict, there arises a scientific paradox which demands a reexamination of either the standing theory, or the observed data, or both. We naturally acknowledge the operation of selection for beneficial mutations in the past, but argue that such selection is severely constrained by the reality of selection threshold, as this study and common sense both demand. Natural selection, as presently understood, simply cannot do what so many are attributing to it — at least relative to low-impact mutations. It is noteworthy that a significant part of this body of literature that claims proof of positive selection in the past (based upon observed sequence variability in the present), may suffer from systematic error and is now being challenged [45–47]. Authors arguing for ubiquitous positive selection in the past, based solely upon sequence data, need to explain why their observed sequence variations might not be explained just as readily using alternative mechanisms such as differential mutational rates or ordinary statistical fluctuations. At the same time, they rightfully should point to the findings of this study and include in their discussion the theoretical problems inherent in selecting simultaneously for a multitude of very low-impact mutations with both positive and negative effects.

A third possible criticism of this study might be that our results are unique to our program and that this program was specifically designed to give these results. Yet in truth we went to great lengths to design Mendel to best reflect biological reality, and it is in fact clear that Mendel's Accountant is the most biologically-realistic forward-time population genetics numerical simulation yet developed. Furthermore, apart from specific details, our observations are in good agreement with what sound population genetics and logic would predict, and our work reflects an expansion, not a reversal, of previous studies [1–29]. Moreover, in another paper in these proceedings [48], and also in a separate paper [49], it is shown that the digital genetics simulation program known as 'Avida' produces very similar results regarding selection threshold and selection breakdown as we report here — when Avida is run using realistic fitness effects.

In fact, Avida shows selection thresholds substantially worse than what we report here [48, 49].

Concluding comments

Our findings raise a very interesting theoretical problem — in a large genome, how do the millions of low-impact (yet functional) nucleotides arise? It is universally agreed that selection works very well for high-impact mutations. However, unless some new and as yet undiscovered process is operating in nature, there should be selection breakdown for the great majority of mutations that have small impact on fitness. We have now shown that this applies equally to both beneficial and deleterious mutations, and we have shown that selection interference is especially important when there are high-impact beneficial mutations. We conclude that only a very small fraction of all non-neutral mutations are selectable within large genomes. Our results reinforce and extend the findings of earlier studies [1–13], which in general employed many simplifying assumptions and rarely included more than a single source of biological noise. We show that selection breakdown is not just a simple function of population size, but is seriously impacted by other factors, especially selection interference. We are convinced that our formulation and methodology (i.e., genetic accounting) provide the most biologically-realistic analysis of selection breakdown to date.

Methods

For both the companion paper [14] and this paper, our basic approach has been to develop and employ the computer program Mendel's Accountant (henceforth "Mendel" for short) to simulate genetic change over time. Mendel's numerical approach introduces a discrete set of new mutations into the population every generation and then tracks each mutation through the processes of mating, recombination, gamete formation, and transmission to the new offspring in all successive generations. Our 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 [28,29]. Its inner workings are described in great detail elsewhere [28]. Mendel is designed to mimic Mendelian heredity as we currently understand it. It acts as a meticulous accounting program to record and track huge numbers of

discrete genetic events over time. This discrete approach contrasts sharply with the traditional approach that has been used by population geneticists for the past nine decades that has sought to represent the processes solely in terms of analytical equations and then to solve these equations by hand. Like any accounting program, Mendel's primarily limitation is the requirement that the inputs' parameter values be clearly and honestly stated, so they properly characterizes the particular biological circumstance the user wants to investigate.

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. 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. In the experiments reported here, we keep all parameters constant, except for the following: 1) mutation rate, 2) environmental variance, 3) fraction of beneficial mutations, 4) selection mode, 5) population size, and 6) number of generations.

Mendel's calculations use a mutation's *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 [50, 51]. 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 selectability.

In Mendel, a Poisson distribution describes the random number of new mutations assigned to each individual. Mutations obey an "infinite sites" model, and the distribution of mutational effects is a Weibull-type distribution [52], of the form $d = exp(ax^{\gamma})$. Here 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 defining cut-off value. All these parameters, as well as degree of dominance and numerous other variables, can be specified by the Mendel user. The Weibull-type distribution, widely used in engineering for modeling degradation processes [52], 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 effect.

In regard to the parameters needed to characterize the Weibull distribution, for deleterious mutations we use $a = 3\times10^{-9}$ (reflecting the inverse of 3×10^{8} bp, a conservative estimate of the functional genome size in humans), which serves as the lower limit of the mutational effect for homozygous mutations in the model. Thus, the magnitude of homozygous deleterious mutational effects ranges from −1 (lethal) to -3×10^{-9}. With the Weibull-type distribution, mutations of small effect are much more frequent than those with large effect. To set the value of γ for the cases described in this study, we specify as high-impact mutations those with a homozygous deleterious fitness effect of at least 0.1 and fix their frequency at 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. Lethals have little effect on mutation accumulation, and thus are ignored in this analysis.

In this paper, when we specify the distribution of mutations, we must also include the beneficial mutations. Apart from their relative abundance, which is a user input, Mendel generates the distribution for deleterious and beneficial mutations in a very similar manner, such that they have the same basic shape to their distribution, except for their range. We take minimum magnitude for deleterious and beneficial mutations to be the same (one divided by the functional genome size). However, while the largest negative effect for deleterious mutations is always −1.0 (there can always be a few entirely lethal mutations), the maximum value Mendel allows for beneficial mutations is user-specified. While we believe a limiting value for beneficial effects in higher organisms should be on the order of a percent or less, we evaluate ST_b with values as large as +1.0. The distribution for beneficial mutation effects has the form $d = d_0 exp(ax^{\gamma})$, where d_0 is the limiting beneficial effect, a is the reciprocal of the product of the functional genome size and d_0, and γ is determined by the same parameters as deleterious mutations except that the cutoff value for "high-impact" mutations is scaled by the factor d_0.

Mendel outputs a statistic that we term *selection threshold* (ST), which marks the center of the transition zone in fitness effect between selectable and un-selectable mutations. For deleterious mutations, ST_d is defined as the mutational fitness effect value at which the number of mutant alleles in the population is exactly half 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 essentially zero accumulation) and small-effect un-selectable mutations (that display essentially 100% accumulation). This statistic provides, at any desired generation, a simple empirical basis for comparing selection effectiveness among cases involving different biological parameters.

For beneficial mutations, a similar statistic, ST_b, can be defined as the mutational fitness effect value at which the number of mutant alleles in the population is exactly twice that of the number expected if there were no selection. This provides a very useful benchmark for tracking at what point selection for low-impact mutations breaks down, and has a basic symmetry with the deleterious selection threshold. The computed ST_b value lies at a critical point where beneficial mutation effects start to be strongly amplified. This marks the transition zone separating large-effect, extremely selectable mutations (which display greatly accelerated accumulation rates) and very small-effect un-selectable mutations that display accumulation rates consistent with random drift.

Our choice for mutation rate is informed by recent estimates that tend to fall in the range of 100 new human mutations per person per generation [52, 53]. 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 very conservative estimates to be 5% and 10% (yielding functional mutation rates of 5 and 10). In light of increasing evidence of extensive genomic functionality [35], we also examine functional mutation rates of 20 or 40 new mutations per individual per generation, corresponding to a 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 also eliminates from consideration all mutations which are absolutely neutral.

In regard to environmental variance, we consider four cases: zero environmental variance (fitness heritability of 1.0), small variance (fitness heritability of 0.4), moderate variance (fitness heritability of 0.04), and large variance (fitness 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 still about 10-fold higher than what is commonly estimated for total fitness heritability [8]. Indeed, heritability of overall fitness is often found to be too small to measure. Selection is always based on each individual's phenotypic fitness, which reflects the genotype fitness plus a random environmental effect. In Mendel, a given heritability is achieved 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 just the right variance to produce the desired heritability.

We consider three relative frequencies of deleterious versus beneficial mutation: a) deleterious mutations are entirely absent; b) the deleterious mutation rate equals the beneficial mutation rate; and c) the deleterious mutations are 9-fold

more common than the beneficial mutations. We consider three types of selection: a) perfect phenotypic 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 for most cases that gives results midway between strict probability and strict truncation selection (partial truncation input parameter = 0.5), but in more realistic cases we use partial truncation with 10% truncation selection and 90% probability selection (partial truncation input parameter = 0.1).

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); b) Weibull-type distribution of homozygous mutation effects (0.1% of the mutations with effects larger in magnitude than 0.1 for deleterious mutations and 0.1 times the limiting value for beneficial mutations); c) all mutations co-dominant; d) mutation effects combine additively; e) no random death; f) no fertility decline associated with fitness decline; g) a diploid sexual species; and h) 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 1,000 reproducing individuals (3,000 offspring in each generation).

Addendum —

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References

1. Muller HJ (1964) The relation of recombination to mutational advance. Mutat Res 1:2–9.
2. Kimura M (1968) Evolutionary rate at the molecular level. Nature 217:624–626.
3. Ohta T (1973) Slightly deleterious mutant substitutions in evolution. Nature 246:96–98.
4. Ohta T (1974) Mutational pressure as the main cause of molecular evolution and polymorphism. Nature 252:351–354.
5. Ohta T (1992) The nearly neutral theory of molecular evolution. Ann Rev Ecol Syst 23:263–286.
6. Ohta T (2002) Near-neutrality in evolution of genes and gene regulation. Proc Natl Acad Sci USA 99:16134–16137.
7. Kimura M (1979) Model of effectively neutral mutations in which selective constraint is incorporated. Proc Natl Acad Sci USA 76:3440–3444.
8. Kimura M (1983) Neutral Theory of Molecular Evolution. Cambridge Univ. Press, New York.
9. Kondrashov AS (1995) Contamination of the genome by very slightly deleterious mutations: why have we not died 100 times over? J Theor Biol 175:583–594.
10. Lynch M, Conery J, Burger R (1995) Mutation accumulation and the extinction of small populations. Am Nat 146:489–518.
11. Lynch M, Conery J, Burger R (1995) Mutational meltdown in sexual populations. Evolution 49(6):1067–1080.
12. Higgins K, Lynch M (2001) Metapopulation extinction caused by mutation accumulation. Proc Natl Acad Sci 98:2928–2933.
13. Loewe L (2006) Quantifying the genomic decay paradox due to Muller's ratchet in human mitochondrial DNA. Genet Res 87:133–159.
14. Gibson P, Baumgardner J, Brewer W, Sanford J (2013) Can purifying natural selection preserve biological information? In: Marks II RJ, Behe MJ, Dembski WA, Gordon B, Sanford JC (eds) Biological Information — New Perspectives. World Scientific, Singapore, pp. 232–263.
15. Hirsch A, Fraser H (2001) Protein dispensability and rate of evolution. Nature 411:1046–1049.
16. Poon A, Otto S (2000) Compensating for our load of mutations: freezing the meltdown of small populations. Evolution 54: 1467–1479.
17. Kibota T, Lynch M (1996) Estimate of the genomic mutation rate deleterious to overall fitness in E. coli. Nature 381:694–696.
18. Charlesworth B, Charlesworth D (1998) Some evolutionary consequences of deleterious mutations. Genetica 103:3–19.

19. Elena SF, Ekunwe L, Hajela N, Oden SA, Lenski RE (1998) Distribution of fitness effects caused by random insertion mutations in *Escherichia coli*. Genetica 102/103:349–358.
20. Gerrish P, Lenski R N (1998) The fate of competing beneficial mutations in an asexual population. Genetica 102/103:127–144.
21. Crow J (2000) The origins, patterns and implications of human spontaneous mutation. Nature Reviews 1:40–47.
22. Bataillon T (2000) Estimation of spontaneous genome-wide mutation rate parameters: whither beneficial mutations? Heredity 84:497–501.
23. Imhof M, Schlotterer C (2001) Fitness effects of advantageous mutations in evolving Escherichia coli populations. Proc Natl Acad Sci USA 98:1113–1117.
24. Orr H (2003) The distribution of fitness effects among beneficial mutations. Genetics 163: 1519–1526.
25. Keightley P, Lynch M (2003) Toward a realistic model of mutations affecting fitness. Evolution 57:683–685.
26. Barrett RDH, M'Gonigle LKM, Otto SP (2006) The distribution of beneficial mutation effects under strong selection. Genetics 174:2071–2079.
27. Lynch M (2006) The origins of eukaryotic gene structure. Mol Biol Evol 23(2):450–468.
28. Sanford J, Baumgardner J, Gibson P, Brewer W, ReMine W (2007) Mendel's Accountant: a biologically realistic forward-time population genetics program. Scalable Computing, Practice and Experience [http://www.scpe.org] 8(2):147–165.
29. Sanford J, Baumgardner J, Gibson P, Brewer W, ReMine W (2007) Using computer simulation to understand mutation accumulation dynamics and genetic load. In 7th International Conference on Computational Science, Beijing, China, May 27–30, 2007 Proceedings, Part II, LNCS 4488. Edited by Shi Y, van Albada GD, Dongarra J, Sloot PMA, Berlin/Heidelberg: Springer-Verlag:386–392.
30. Keightley P, Otto S (2006) Interference among deleterious mutations favors sex and recombination in finite populations. Nature 443:89–92.
31. Joseph S, Hall D (2004) Spontaneous mutations in diploid Saccharomyces cerevisiae: More beneficial than expected. Genetics 168:1817–1825.
32. Dickinson W (2008) Synergistic fitness interactions and a high frequency of beneficial changes among mutations accumulated under relaxed selection in Saccharomyces cerevisiae. Genetics 178:1572–1578.
33. Montañez G, Marks II RJ, Fernandez J, Sanford JC (2013) Multiple overlapping genetic codes profoundly reduce the probability of beneficial mutation. In: Marks II RJ, Behe MJ, Dembski WA, Gordon B, Sanford JC (eds) Biological Information — New Perspectives. World Scientific, Singapore, pp. 139–167.
34. Kudla G., Murray AW, Tollervey D, Plotkin JB (2009) Coding-Sequence Determinants of Gene Expression in Escherichia coli. *Science* 324:5924.

35. The Encode Project Consortium (2007): Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 447:799–816.
36. Piganeau G, Eyre-Walker A (2003) Estimating the distribution of fitness effects from DNA sequencing data: implications for the molecular clock. Proc Natl Acad Sci USA 100:10335–10340.
37. Estes S, Phillips PC, Denver DR, Thomas WK, Lynch M *et al*. (2004) Mutation accumulation in populations of varying size: The distribution of mutational effects for fitness correlates in *Caenorhabditis elegans*. Genetics 166:1269–1279.
38. Sanjuan R, Moya A, Elena S (2004) The distribution of fitness effects caused by single nucleotide substitutions in an RNA virus. Proc Natl Acad Sci 101:8396–8401.
39. Keightley P, Charlesworth B (2005) Genetic instability of C elegans comes naturally. Trends in Genetics 21:67–70.
40. Eyre-Walker A, Woolfit M, Phelps, T (2006) The distribution of deleterious amino acid mutations in humans. Genetics 173:891–900.
41. Kassen R, Bataillon T (2006) Distribution of fitness effects among beneficial mutations before selection in experimental populations of bacteria. Nature Genetics 38:484–488.
42. Martin G, Lenormand T (2008) The distribution of beneficial and fixed mutation effects close to an optimum. Genetics 179:907–916.
43. Lynch M, Sung W, Morris K, Coffey N, Landry CR, Dopman EB, Dickinson WJ, Okamoto K, Kulkarni S, Harti DL, Thomas WK (2008) A genome-wide view of the spectrum of spontaneous mutations in yeast. Proc Natl Acad Sci USA 105:9272–9277.
44. Peris JB, Davis P, Cuevas JM, Nebot MR, Sanjuán R (2010) Distribution of fitness effects caused by single-nucleotide substitutions in bacteriophage f1. Genetics 185:603–609.
45. Hughes A (2007) Looking for Darwin in all the wrong places: the misguided quest for positive selection at the nucleotide level. Heredity 99:364–373.
46. Hughes A (2008) The origin of adaptive phenotypes. Proc Natl Acad Sci USA 105:13193–13194.
47. Kryukov G, Schmidt S, Sunyaev S (2005) Small fitness effect of mutations in highly conserved non-coding regions. Human Molecular Genetics 14:2221–2229.
48. Nelson C, Sanford J (2013) Computational evolution experiments reveal a net loss of genetic information despite selection. In: Marks II RJ, Behe MJ, Dembski WA, Gordon B, Sanford JC (eds) Biological Information — New Perspectives. World Scientific, Singapore, pp. 338–368.
49. Nelson C, Sanford J (2011) The effect of low impact mutations in digital organisms. Theoretical Biology and Medical Modeling 8:9.
50. Milkman R (1977) Selection differentials and selection coefficients. Genetics 88:391–403.

51. Kimura M, Crow JF (1978) Effect of overall phenotypic selection on genetic change at individual loci. Proc Natl Acad Sci USA 75(12):6168–6171.
52. Weibull W (1951) A statistical distribution function of wide applicability. J Appl Mech-Trans ASME 18(3):293–297.
53. Kondrashov AS (2002) Direct Estimate of human per nucleotide mutation rates at 20 loci causing Mendelian diseases. Hum Mutat 21:12–27.
54. Nachman MW, Crowell SL (2000) Estimate of the mutation rate per nucleotide in humans. Genetics 156:297–304.
55. Lynch M (2010) Rate, spectrum, and consequences of human mutation. Proc Natl Acad Sci USA 107:961–968.

Appendix I: *Key parameter settings and their justification:*

Mutation rate = 1, 5, or 10 (unless otherwise specified). Although the human mutation rate is known to be roughly 100 new mutations per person per generation [53–55], we typically use the extremely conservative maximal value of 10. This presumes that at least 90% 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 [35]. More realistic mutation rates only make the selection threshold problem worse.

Population size = 1,000 (unless otherwise specified). This default population size would be realistic for an isolated tribe or set of tribes. Population sizes larger than 1,000 do not significantly decrease ST values or change the percent of mutations which are un-selectable [14], but when we allow extremely beneficial mutations in larger populations, their rapid multiplication leads to overflow of memory.

Generations = 1000 (unless otherwise specified). We find that this is sufficient for ST_b to largely stabilize for the population sizes we have been studying.

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 and represents extremely intense selection.

Distribution of mutation effects = Weibull distribution, wherein 0.1% of all mutations reduce fitness by 10% or more. Altering the shape of the distribution to be either steeper or less steep, does not significantly affect the ST phenomenon.

Dominant versus recessive = co-dominance. Although Mendel allows some mutations to be partially or fully dominant, while others are partially or fully recessive,

for simplicity we make all mutations in this paper co-dominant. We have observed that this parameter has only a minor impact on ST values.

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

To reproduce these results: all other settings can be set to the normal Mendel default settings (Version 1.4.3).