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USING NUMERICAL SIMULATION TO BETTER UNDERSTAND FIXATION RATES, AND ESTABLISHMENT OF A NEW PRINCIPLE: HALDANE'S RATCHET

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ABSTRACT

In 1957, Haldane first described a fundamental problem with evolutionary theory. This problem eventually became widely known as "Haldane's Dilemma". The essence of this problem is that even given a steady supply of beneficial mutations plus deep time, the rate that such mutations reach *fixation* is too slow to achieve meaningful evolution. After more than 50 years, this fundamental problem remains unresolved. ReMine has gone far beyond Haldane's original mathematical analysis, and has developed "cost theory analysis" which strongly supports Haldane's thesis. Here we examine this long-standing problem using an entirely different approach. We employ advanced numerical simulation of the mutation/selection process to empirically measure the fixation rates of beneficial, neutral, and deleterious mutations. We do this employing both realistic and optimized population parameters. In our numerical simulations, each new mutation is tracked through time until it is either lost due to drift or becomes fixed in the population.

We first confirm that our numerical simulations correctly tallying the fixation of neutral mutations. We show that neutral mutations go to fixation just as predicted by conventional theory (i.e., over deep time the fixation rate approached the gametic mutation rate). We also show that the reason the vast majority of neutral mutant alleles fail to go to fixation, is because they lost due to drift, and this rate of loss rapidly approached 100% as population size is increased.

We then show that given realistic distributions of mutation fitness affects, the vast majority of all mutations (including deleterious and beneficial mutations), are similarly lost due to random drift. In terms of fixations, deleterious mutations went to fixation only slightly slower, while beneficial mutations went to fixation only slightly faster, than neutral mutations.

We then perform large-scale experiments to examine the feasibility of the ape-to-man scenario over a six million year period. We analyze neutral and beneficial fixations separately (realistic

rates of deleterious mutations could not be studied in deep time due to extinction). Using realistic parameter settings we only observe a few hundred selection-induced beneficial fixations after 300,000 generations (6 million years). Even when using highly optimal parameter settings (i.e., favorable for fixation of beneficials), we only see a few thousand selection-induced fixations. This is significant because the ape-to-man scenario requires tens of millions of selective nucleotide substitutions in the human lineage.

Our empirically-determined rates of beneficial fixation are in general agreement with the fixation rate estimates derived by Haldane and ReMine using their mathematical analyses. We have therefore independently demonstrated that the findings of Haldane and ReMine are for the most part correct, and that the fundamental evolutionary problem historically known as “Haldane’s Dilemma” is very real.

Previous analyses have focused exclusively on beneficial mutations. When deleterious mutations were included in our simulations, using a realistic ratio of beneficial to deleterious mutation rate, deleterious fixations vastly outnumbered beneficial fixations. Because of this, the net effect of mutation fixation should clearly create a ratchet-type mechanism which should cause continuous loss of information and decline in the size of the functional genome. We name this phenomenon “Haldane’s Ratchet”.

INTRODUCTION

Genome building requires the systematic fixation of large numbers of newly-arising beneficial mutations. Each new mutation in a population arises as an extremely rare, single-copy allele. It is initially vastly outnumbered by the natural “wild type” alleles at that locus. For this reason, even when there is strong natural selection, any newly arising beneficial mutation has an overwhelming probability of being lost due to random genetic drift. To contribute to the genome-building process, the mutant allele must survive random loss and increase in frequency until it drives the wild type allele to extinction. Only when this fixation happens can the beneficial mutation be considered an evolutionary advance, representing a single “click” upward in the evolutionary ratchet. In this way, beneficial fixations represent a type of scorecard in terms of evolutionary advance.

However it is widely recognized that not all fixations are beneficial. Random drift can also cause either neutral or deleterious mutations to go to fixation. When a deleterious mutation goes to fixation, it represents irreversible genetic damage. In this way, deleterious fixations can also be seen as scorecards in the evolutionary process, but in this case each “click” in the ratchet is downward, rather than upward. Therefore, a most realistic measure of the direction and rate of evolutionary change is the relative accumulation of beneficial versus deleterious fixations.

J.B.S. Haldane was one of the first geneticists to understand the evolutionary implications of fixation events and their rates. He first introduced the problem of the "slowness of evolution" with his controversial work on *The Cost of Natural Selection* (Haldane, 1957). The key to Haldane's realization was that there is a very high biological "cost" to selecting away all the wild-type alleles (which represent the vast majority of the population). Using some very loosely formulated mathematics, he showed that even given a very favorable evolutionary scenario, roughly 300 generations were required to fix a single beneficial mutation. Such a low rate of fixation makes major evolutionary advance essentially impossible, even given deep time. This problem has for many decades been referred to as "Haldane's Dilemma". At Haldane's rate of fixation (on average, 300 generations per fixation), and presuming a divergence of man from a chimp-like ancestor roughly 6 million years ago (about 300,000 generations), according to Haldane only about 1,000 beneficial mutations could have become fixed in the human lineage. At such an incredibly slow rate of fixation, it is hard to imagine how the mutation-selection process could have transformed an ape to a man. The actual functional difference between the chimp and human genome is not a matter of just a few thousand nucleotides. Minimally, tens of millions nucleotide substitutions are required (Britten, 2002).

Several decades after Haldane, it became clear that even very similar organisms differed from each other at many millions of genetic sites. Kimura recognized the validity of Haldane's dilemma, and so he concluded that mutation/selection could not even begin to explain all these differences – not even given deep time. It was on this basis that Kimura formulated his now famous "neutral theory of evolution", claiming that most genetic differences separating taxa are non-beneficial, and only arise due to random mutation and random genetic drift (Kimura; 1968, 1983). This raises an obvious question: where does the new information come from that allows evolutionary advance? Adding still another layer to the problem, Ohta (1973) recognized neutral mutations should more accurately be defined as "nearly neutral." She realized there is no such thing as truly neutral mutations. Even the slightest nucleotide change should have some effect on fitness. Kimura eventually agreed with Ohta, causing him to redefine his "neutral mutations" as "effectively neutral mutations" (beyond the reach of selection). This view is supported by contemporary population geneticists such as Eyre-Walker & Keightley (2007):

> *… it seems unlikely that any mutation is truly neutral in the sense that it has no effect on fitness. All mutations must have some effect, even if that effect is vanishingly small. However, there is a class of mutations that we can term effectively neutral … As such, the definition of neutrality is operational rather than functional; it depends on whether natural selection is effective on the mutation in the population or the genomic context in which it segregates, not solely on the effect of the mutation on fitness.*

If most mutations are very slightly deleterious, and therefore un-selectable, there should be continuous genetic damage accumulating in all higher genomes (Kondrashov, 1995). With this

ever-increasing “genetic load” due to the accumulation of low-impact deleterious mutations, it is highly questionable that fixation of a few rare beneficial mutations could ever compensate for this type of comprehensive erosion of genetic information. Instead of helping to resolve Haldane’s dilemma, Kimura and Ohta’s work revealed the dilemma was even more profound than Haldane could have understood.

Numerous authors have tried to explain away Haldane’s dilemma, but have only produced highly convoluted and conflicting arguments (Van Valen, 1963; Maynard Smith, 1968; Crow, 1968; Felsenstein, 1970; Morgan, 1970). For example, in 1990, Phelps proposed a hypothetical means to ‘speed up’ the rate of evolution through what he called “rank selection”, a highly unrealistic form of truncation selection (Phelps, 1997). There has still not been a satisfactory or generally-recognized resolution of the problem of Haldane’s dilemma.

In the last decade, ReMine has worked diligently to bring clarity to the problem of Haldane’s Dilemma through a much more rigorous development of “cost theory”, which goes far beyond Haldane’s work. ReMine has brought much-needed clarification of the cost of substitution (ReMine 2005, 2006). ReMine’s work strongly confirms the reality of Haldane’s dilemma. The main limitation to ReMine’s mathematical approach (and similar mathematical approaches as employed by Haldane, Kimura, Phelps, etc.) is that biological populations are extremely complex, and mathematical models use highly simplified formulas to try to understand this complexity. Because of this, the validity of these mathematical models is always debatable, because there will always be alternative ways to try and reduce a complex biological system into a simplified formula. This yields an apparent impasse; the mathematicians cannot agree, and the typical biologist has no basis on which to assess the mathematical claims being made. This is a primary reason why, even after 50 years of debate, the problem of Haldane’s Dilemma is still so poorly understood.

It is the goal of this paper to employ a non-mathematical approach to bring greater clarity to the topic of Haldane’s Dilemma. For the first time, we can accurately track the fixation process using what we call *comprehensive numerical simulation* (Sanford & Nelson, 2012). We do this using the genetic accounting program called *Mendel’s Accountant* (Sanford *et al.*, 2007). The power of *Mendel’s Accountant* is found in its ability to simultaneously and comprehensively simulate all the major known factors that affect fixation rates as would occur in real populations.

METHODS AND RESULTS

We utilized the program *Mendel’s Accountant* (Mendel) to simulate the fixation process. This program has been described in detail elsewhere (Sanford *et al.*, 2007; Baumgardner *et al.*, 2008; Sanford and Nelson, 2012; Gibson *et al.*, 2013; Sanford *et al.*, 2013). This is the most advanced and biologically-realistic forward-time population genetics program to date. Mendel is the first population genetics program which is capable of *comprehensive* numerical simulation of the

mutation/selection process (Sanford and Nelson, 2012), meaning that it simulates all the major variables that affect the outcome of the mutation/selection process.

Except where noted, each experiment employed probability selection, where the probability of an individual's reproduction is directly proportional to the individual's phenotypic fitness. In this way, 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 and contrasts strongly with truncation selection. Truncation selection is highly unrealistic and never happens even in artificial breeding experiments, let alone in nature.

Before using Mendel to evaluate the validity of Haldane's Dilemma, it was first necessary to confirm that Mendel can reliably and accurately track fixation rates. We ran four separate experiments modeling different sizes of relatively small human populations (Figure 1). Each of the experiments had the following fixed parameters: ploidy = diploid; reproduction = sexual; mating = random; linkage = dynamic recombination; new mutations per individual = 1 (all neutral); fitness heritability = 0.2; offspring per female = 2; generations = 13,500. The only difference between each of the four experiments was population size.

We observed that the rate of fixation of neutral mutations eventually approached the gametic rate of neutral mutation, as predicted by classical theory. However, the observed fixation rate only approached the theoretical rate after a very long "waiting time", which we define as "waiting time to fixation rate equilibrium". It was observed that the time to fixation rate equilibrium increased dramatically as population size increased. For instance, Figure 1 shows that the population size of 100 reached equilibrium roughly 10 times faster than a population size of 1,000. Likewise, using a human population size consistent with human evolutionary theory (10,000), the waiting time to fixation rate equilibrium for neutral mutations was far more than 100,000 generations (more than 2 million years). After 13,500 generations (about 270,000 years), the population of 10,000 individuals was not even approaching fixation rate equilibrium.

In agreement with classic theory, the rate of neutral allele loss was close to 99.5% for a population size of 100, and quickly approached 100% loss as population size increased. Our results indicate that for those rare neutral alleles that were not lost to drift, the average time to fixation was approximately $4N_e$ – again in general agreement with classical theory. These results show that Mendel is correctly simulating neutral fixations and its output is consistent with known genetic theory.

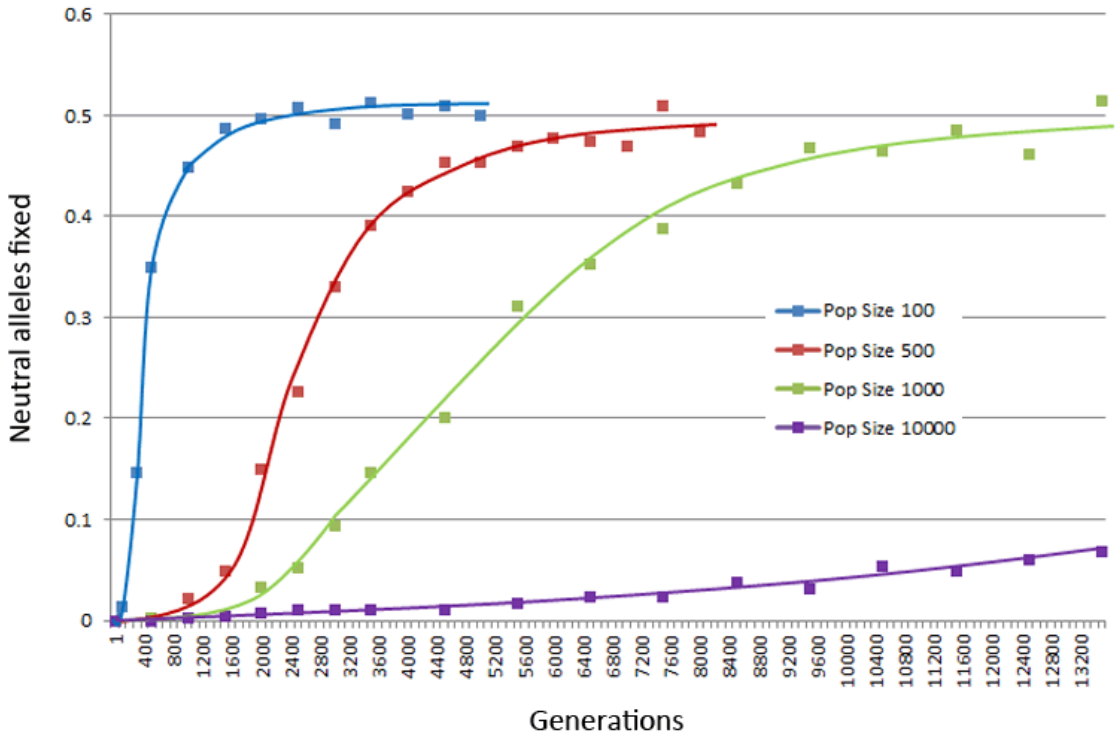

Figure 1. Rate of neutral fixation is primarily dependent upon mutation rate, in agreement with classic theory, but there is a very long waiting time before fixation rate equilibrium is reached, and this waiting time is profoundly influenced by population size. Numerical simulation using Mendel's Accountant reveals that in deep time neutral fixation rate approaches the gametic neutral mutation rate (0.5). However, the larger the population size, the more generations are required for fixation rate equilibrium to be reached. Given a realistic population size (10,000 or greater), fixation rate does not even begin to approach the mutation rate, even after 13,500 generations.

In our next Mendel experiment (Figure 2) we compared the fixation rates of beneficial and deleterious mutations to the neutral fixation rate in order to determine the effect of selection on fixation rates. We used the same parameters as Figure 1 except that the population size was 1,000 and the mutation rate was set to 3 per individual per generation. One third of the mutations were neutral, one third were beneficial, and one third were deleterious. For the non-neutral mutations, the distribution of the beneficial and deleterious mutation effects was a Weibull distribution (a type of exponential distribution, Sanford *et al.*, 2007). The range of deleterious mutations was 3.3×10^{-9} – 1.0. The range of beneficial mutations was 3.3×10^{-9} – 0.01. These parameters reflect a realistic distribution of mutation effects wherein the large majority of mutations are nearly neutral, and where beneficial mutations have a much lower mean effect than deleterious mutations (as reflected by the upper limits of mutation effects). These parameters allowed Mendel to empirically determine the rate at which these three classes of mutations, when given realistic distributions, should go to fixation when subjected to natural selection.

Neutral mutations went to fixation at the expected rate (approaching 0.5 fixations per generation as fixation equilibrium was approached). The beneficial mutations went to fixation slightly faster than did neutrals, but this difference was not substantial (2.7% faster). Likewise, the deleterious mutations went to fixation slightly slower than did neutrals, but again the difference was not substantial (2.6% slower). The difference between the rate of neutral fixation and the rate of beneficial fixation we define as the "rate of selective fixation". The rate of selective fixation was very modest. This indicates that, while selection affects fixation rate, the effect of selection on fixation rate is surprisingly weak under realistic conditions. The waiting time to selection equilibrium was similar for all three classes of mutation. Of all the beneficial mutations that arose in this population, 99.78% were lost from the population due to genetic drift.

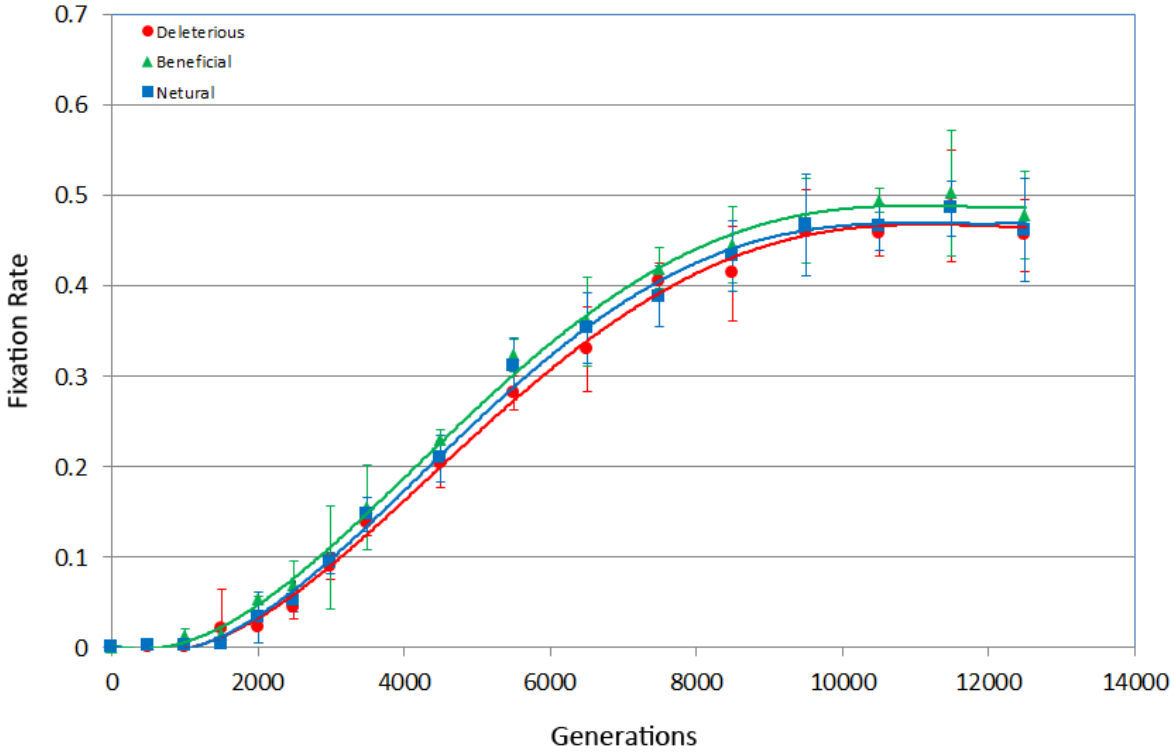

Figure 2. Rate of fixation for neutral, beneficial, and deleterious mutations, based on a population size of 1,000. Each curve was the average of 5 replicate experiments. Time to fixation rate equilibrium was similar for all three classes of mutations. The fixation rate for beneficial mutations was slightly higher due to selective amplification (2.7%). Likewise, deleterious approached equilibrium at a rate slightly lower than the expected for neutrals, due to a small amount of purifying selection (2.6%). Mendel's Accountant reveals that the vast majority of beneficial and deleterious fixations arose due to genetic drift.

We next conducted a similar but slightly longer term experiment, where mutations in each class were occurring at more realistic rates (Figure 3). The parameters utilized in Mendel were otherwise identical to our previous experiments, including a population size of 1,000. The inputs unique to this experiment include 20,000 generations and a total non-neutral mutation rate of 10

per individual. We assumed the ratio between beneficial versus deleterious mutations was 1:1000 (on average, 0.01 beneficials versus 9.99 deleterious mutations per individual per generation). We excluded neutral mutations from this analysis, given the understanding that there is no such thing as a perfectly neutral mutation (being more accurately defined as "nearly-neutral)." Based on these more realistic mutation settings, our results showed that at the end of the run, there were only 10 beneficial fixations, while there were 6,775 deleterious fixations (creating a fixation ratio of 1:677, compared to the 1:1000 ratio of the newly arising mutations). Even with strong selection, deleterious fixations far outnumbered the beneficial fixations.

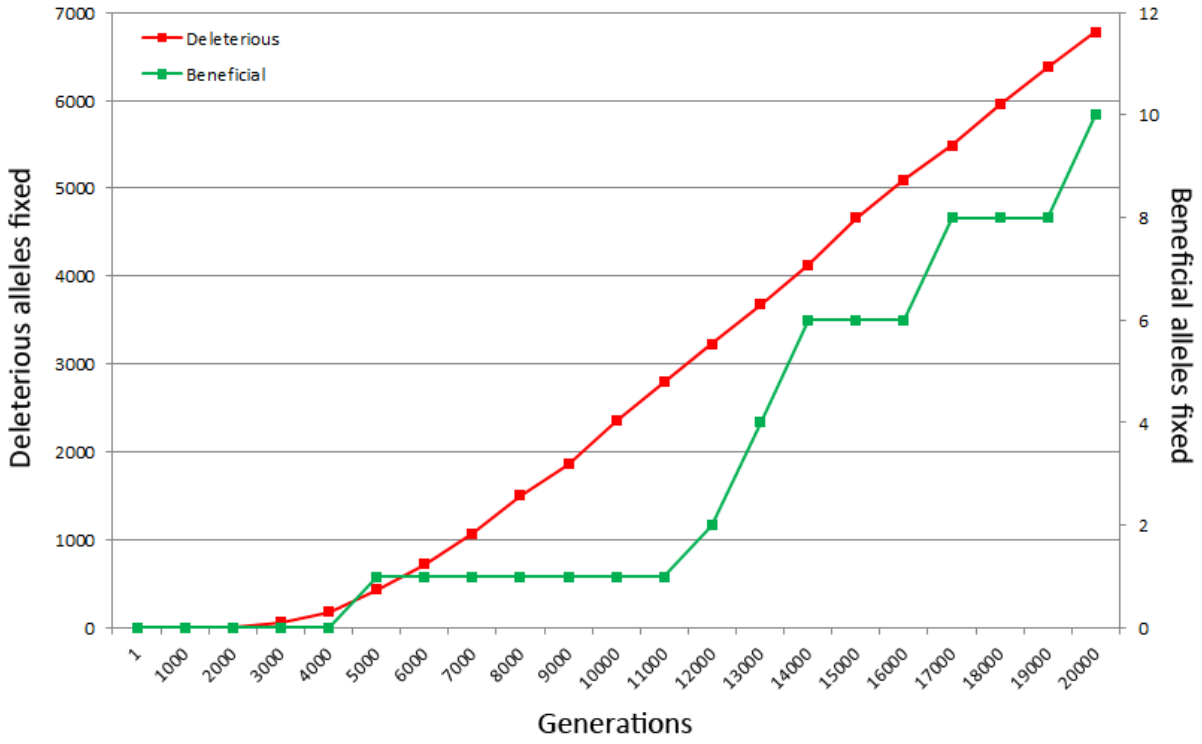

Figure 3. Fixation of beneficial versus deleterious mutations, in an experiment where deleterious mutations were 1000-fold more common than beneficial mutations. Deleterious fixations vastly outnumbered beneficial fixations, even in the face of strong selection (50% selective elimination of all progeny). At the end of the experiment there had been 6,775 deleterious fixations, and only 10 beneficial mutations. Note that the scale on the right is for number of beneficial fixations, while the scale on the left is for the number of deleterious fixations.

We next conducted a series of much larger experiments, simulating very deep time (300,000 generations). This is the postulated time since the divergence of chimpanzee and man, assuming a generation time of 20 years (roughly six million years). One set of experiments was conducted with a population size of 1,000, and another set was done with a population size of 10,000.

The series of small population experiments (Table 1, rows 1-3, designated qrtsyg, qrtqaq, and qrtimc) all employed the same parameters as in Figures 2 and 3, with a population size of 1,000, and ran for 300,000 generations. Beneficial and neutral mutations fixations were simulated separately, as simultaneous simulation of both would have caused overflow of computer memory (given 128 gigabytes RAM). Deleterious mutation accumulation could not be simulated through such deep time, as it always either caused overflow of memory or premature termination due to extinction. As before, the non-deleterious mutation rate was 0.01 per individual per generation.

Table 1. A summary of "deep-time" simulations. Shown for each simulation: Run ID; key parameter differences; mean mutation count per individual; total fixations; selective fixations; percent allele loss due to drift; and the selection threshold at end of experiment.

Run ID	Key Parameter Difference	Mutations/ Individual (av.)	Total Fixation	Selective Fixation	% Loss	Selection Threshold
qrtsyg	1000 pop size/all neutral	2870	1413	0	99.95	-----
qrtqaq	1000 pop size/default distr.	3048	1492	79	99.94	6.9×10^{-4}
qrtimc	1000 pop size/extended distr.	3413	1684	271	99.94	4.2×10^{-3}
qrtmoi	10,000 pop size/all neutral	2997	1234	0	99.99	-----
qrtsvs	10,000 pop size/default distr.	3469	1456	222	99.99	$\sim 6.8 \times 10^{-5}$
qrtniw	10,000 pop size/extended distr.	4190	1824	590	99.99	$\sim 2 \times 10^{-3}$
qrtoju	optimized selection settings*	5977	2710	1476	99.98	$\sim 2 \times 10^{-3}$

*larger population (10,000); increased lower limit of beneficial effect (1.0×10^{-6}); increased upper limit of beneficial effect (1.0); plus partial truncation selection (0.5).

~ using certain settings, tracking of selection thresholds broke down after 200,000 generations.

The first, small, deep-time population simulation was a preliminary run with only neutral mutations. After 300,000 generations, the total number of neutral fixations was 1,413 (Table 1, first row, designated qrtsyg). The difference between the number of neutral fixations in this run, and the number of beneficial fixations observed in the next two runs (where both selection and drift are operating), allows us to calculate the number of beneficial fixations arising specifically due to positive selection.

A second, small, deep-time population simulation (Table 1, second row, designated qrtqaq), employed Mendel's default range of beneficial mutation effects (3.3×10^{-9} – 0.01). The total number of beneficial fixations was 1,492 after 300,000 generations. When we subtract the number of fixations which would have gone to fixation if the mutations had been neutral (1,492), we see that only 79 fixations arose specifically due to selection. The selection threshold for beneficial mutations was 6.9×10^{-4}.

A third, small, deep-time population experiment (Table 1, third row, designated qrtimc) employed the same parameters, except that the upper range of beneficial mutation effects was 100-fold higher (up to a fitness effect of 1.0, wherein a single mutation quite unrealistically doubles fitness). The total number of beneficial fixations was 1,684 after 300,000 generations. When we subtract the number of neutral fixations which would have gone to fixation (1,413), we see that only 271 fixations arose specifically due to selection. The reason the rate of fixation did not increase more sharply was due to a dramatically higher selection threshold of 4.2×10^{-3}. This higher selection threshold was due to "selection interference", wherein the higher-impact beneficials interfered with the selection for the lower-impact beneficial mutations (Sanford *et al.*, 2012).

A second series of deep-time simulations (Table 1, rows 4-6, designated qrtmoi, qrtsvs, and qrtniw) employed the same parameters as above, but with a larger population size of 10,000. As before, the mutation rate was 0.01 per individual per generation.

The first, large, deep-time population simulation (Table 1, fourth row, designated qrtmoi) was again a preliminary run with only neutral mutations. After 300,000 generations, the total number of neutral fixations was 1,234. We observed that 99.99% of all neutral alleles were lost due to drift.

A second, larger population simulation in deep time (Table 1, fifth row, designated qrtsvs) again employed the default range of beneficial mutation effects (3.3×10^{-9} – 0.01). The total number of beneficial fixations was 1,456 after 300,000 generations. When we subtract the number of fixations which would have gone to fixation if the mutations were neutral (1,234), we see that only 222 fixations arose specifically due to selection. The selection threshold for beneficial mutations was 6.8×10^{-5}.

A third, large, deep-time population experiment (Table 1, sixth row, designated qrtniw) employed the same parameters, except that the upper range of beneficial mutation effects was 100-fold higher (up to a fitness effect of 1.0, as above). The total number of beneficial fixations was 1,824 after 300,000 generations. Subtracting the number of neutral fixations which would have gone to fixation (1,234) reveals that only 590 fixations arose specifically due to selection. As in the previous example with artificially large beneficial fitness effects, there was a breakdown in selection due to "selection interference". The selection for higher impact

beneficials interfered with the selection for the lower–impact beneficial mutations (Sanford *et al.*, 2013).

A fourth, large, deep-time population experiment was added (Table 1, seventh row, designated qrtoju) which employed highly optimized parameters reflecting a best-case scenario clearly transcending biological reality. The lower limit of beneficial effects was raised by three orders of magnitude, up to 3.3×10^{-6} (reflecting a functional genome size of only 300,000). The upper limit for beneficial effects was set at 1.0 (one mutation doubles fitness). Instead of natural probability selection, partial (50%) truncation selection was employed (Mendel can model 100% probability selection or 100% truncation selection, or any intermediate degree of selection). Even given these highly unrealistic optimized conditions, the total number of beneficial fixations was only 2,710 after 300,000 generations. Of these only 1,476 (2,710 – 1,234) were selectively fixed. The selection threshold was roughly 1.5×10^{-3}. During this same time, we would expect more than 100,000 deleterious mutations to have been fixed (extrapolated from Figure 3).

In all the experiments involving only beneficial mutations, fitness obviously had to increase. However, when the upper limit of beneficial effects was 0.01 (already large compared to expected biological reality), total fitness increase was always trivial. When the upper limit of beneficial effect was 1.0 (maximally one mutation causes fitness doubling), fitness increase was very significant, and in the optimized run fitness went from 1.0 up to 110 (Table 1). However, this dramatic increase in fitness was primarily due to a very limited number of very high-impact beneficial mutations; many of which still had not gone to fixation.

The percent loss of alleles due to random drift was always extremely high, as would be expected. Improving the parameters affecting selection efficiency did almost nothing to reduce this loss, and larger populations consistently made the percent loss proportionately greater (Table 1).

DISCUSSION

Comprehensive numerical simulation strongly affirms the general conclusions of Haldane (1957) and ReMine (2005, 2006) regarding the problem generally known as *Haldane's Dilemma*. Given realistic biological conditions, the rate of fixation of beneficial mutations is much too slow to allow significant forward evolution. These new results from numerical simulation, combined with the mathematical results from ReMine and Haldane, represent three independent demonstrations of the same problem. All three use different methodology, but they all clearly demonstrate the same basic reality. The slow fixation problem historically referred to as Haldane's Dilemma is real. Comprehensive numerical simulation brings much-needed clarity to this subject, which has not been possible using only mathematical analysis.

As can be seen in Figure 1, Mendel simulates neutral mutation fixations accurately, such that the fixation rate approaches the gametic mutation rate (half the mutation rate per individual per generation). However, there is a long waiting time before a population reaches equilibrium in terms of the optimal fixation rate. This waiting time is extremely long for larger populations, and

during this waiting time, the fixation rate is dramatically lower than predicted by theory. In large populations (millions or billions of individuals), it is not clear that such equilibrium can realistically be reached even in deep time, especially since there will necessarily be semi-isolated sub-populations. Furthermore, the vector or direction of selection cannot be expected to be constant through such deep time, causing selective reversals and largely precluding establishment of fixation rate equilibrium.

As can be seen in Figure 2, given a realistic range of mutational fitness effects, beneficial and deleterious mutations largely behave like neutral mutations; they are primarily driven to fixation by random genetic drift. Only the fixations of the highest-impact beneficial or highest-impact deleterious mutations are influenced by natural selection.

As can be seen in Figure 3, when there is a realistic ratio of good to bad mutations (1:1000), the rate of bad fixations vastly exceeds the rate of good fixations (by over 600-fold). This is consistent with the work of Gibson *et al.* (2013). If deleterious mutations are systematically going to fixation much more frequently than beneficial mutations in every generation, this guarantees systematic degeneration of the genome. Since fixations are essentially irreversible, this establishes a downward ratchet mechanism, which we refer to as “Haldane’s Ratchet”.

As can be seem in Table 1, deep time does not resolve Haldane’s Dilemma, but actually make the problem worse. All the experiments summarized in Table 1 involved very deep time (300,000 generations). In terms of human evolution, this represents roughly 6 million years (assuming a generation time of 20 years). This approximates the reputed time since the human and chimpanzee lineages diverged. Even given a very generous and continuous supply of beneficial mutations, in six millions years only a few thousands beneficial fixations could have occurred. Realistically, only a few hundred of these would have been actually due to selection. Surprisingly, the total number of beneficial fixations remained roughly the same (1,000-2,000 fixations), even given different population sizes and different mutation fitness effect distributions (Table 1). Even given highly optimized selection parameter settings (including partial truncation selection), the number of beneficial fixations was only 2,710, and of those only 1,476 were due to selection (Table 1). It is interesting to note that increased population size improved the selective fixation rate only slightly (because the larger population size reduced the population’s selection threshold).

While increasing the upper range of beneficial mutation effects greatly increased mean fitness gain (deleterious mutations being ignored), this had very small impact on rate of fixation. This is due to *selection interference*, wherein the high-impact beneficial mutations interfere with the selective fixation of the lower impact mutations which otherwise would have been selectable (Sanford *et al.*, 2013; Nelson and Sanford, 2011). The strength of selection interference due to high-impact beneficial mutations can be very striking, and has not been adequately characterized by population geneticists before, due to the lack of comprehensive numerical simulation tools prior to this time.

There are three basic reasons why beneficial mutations go to fixation so slowly. *Most importantly, beneficial mutations are very rare* (Montanez *et al.*, 2013). This is obviously true, both in terms of observation and from theoretical considerations. The functional genome (ignoring any possible "junk DNA") represents life's *specifications*. Specifications are by their very nature *specific*. Random changes in very exact specifications must systematically reduce that specificity – i.e., the probability of improving what is being specified becomes vanishingly small. In this paper, all our simulations that involved beneficial mutations employed a rate of beneficial mutation of one per 100 individuals per generation. This is a very generous rate. We also assume a 1:1000 ratio of good versus bad mutations. Since this ratio is often assumed to be closer to 1:1,000,000, we are clearly being over-generous. Some argue that mutation accumulation experiments (i.e., Lenski *et al*., 1994), provide evidence for extremely high beneficial to deleterious ratios. However, these claims reflect lack of understanding of what such experiments really measure. Mutation accumulation experiments only document gross changes in performance, usually measured in a single dimension of fitness (trait). Such studies are entirely blind to the vast majority of mutations actually accumulating in the study population. The recent and profound discovery that there are multiple over-lapping codes in higher genomes must profoundly reduce the likelihood of beneficial mutations still further, and must make *unambiguously* beneficial mutations almost unimaginably rare (Montanez *et al*., 2013). Given that beneficial mutations arise very rarely, they can only very rarely be fixed.

The second reason beneficial fixations are so slow to accumulate is that the vast majority of all new mutations, including beneficials, are lost due to random drift while they are still very rare alleles. This phenomenon is well understood, and is dramatically demonstrated in this paper. In all the experiments conducted in this study, we consistently see that even for beneficial mutations, over 99.9% are lost due to random genetic drift. This is true because the probability of fixation of any allele is directly proportional to its frequency in the population at any given time, and all new mutations are, by definition, at the lowest frequency possible (1/(2n)). Thus, drift usually happens very quickly, before selection has a chance to "grab hold of" the beneficials. This problem becomes increasingly worse as population size increases (Table 1).

The third reason beneficial fixations are so slow to accumulate is that most beneficial mutations must have a very tiny effect on fitness. In man, each beneficial mutation changes only one out of 3 billion letters in the genomic instruction manual, and reflects a very miniscule change in the genome's total information content. This single letter change is a tiny drop within an ocean of phenotypic variation within the population. The population's phenotypic variation is partly due to millions of other segregating mutations in the population, and is partly due to countless differences in each individual's specific environmental circumstances. The bottom line is that the vast majority of beneficial mutations will have a fitness effect below the population's selection threshold (Sanford *et al*., 2013). This makes such mutations invisible to natural selection. These nearly neutral mutations will drift toward fixation at essentially the same rate as perfectly neutral mutations. This is clearly seen in Figure 2 and Table 1. Given the same conditions, the rate of

beneficial fixation is consistently only slightly higher than the rate of neutral fixation. This makes it clear that most of the fixations observed for beneficial mutations only resulted from random genetic drift, and would have gone to fixation even without selection. Therefore, we must subtract the number of neutral fixations from the total number of beneficial mutations that went to fixation, to see how many beneficials went to fixation due to selection. What we see is that only a few hundred fixations result from selection, even after 300,000 generations using realistic settings (Table 1). This is lower than the fixation rates which either Haldane or ReMine predicted using their mathematical formulations, but their analysis did not include consideration of the selection threshold problem.

The ape-to-man scenario requires the fixation of tens of millions of mutations within each lineage. Most such mutations would necessarily have been nearly-neutral in their effect, but none can be assumed to have been perfectly neutral. It is widely agreed that many such fixations would have been slightly deleterious. Yet to enable a net increase in fitness (i.e., allowing increased intelligence in the human lineage, etc.), and even to simply avoid extinction due to accumulating deleterious mutations, the large majority of these tens of millions of fixations would have had to have been beneficial. The scenario clearly demands over ten million beneficial fixations. Yet the fundamental problem of Haldane's Dilemma only permits the selective fixation of hundreds, or at best, thousands of beneficial mutations in that six million year time period. The ape-to-man scenario falls short of the needed beneficial fixations by a factor of at least three orders of magnitude.

CONCLUSIONS

In light of our investigations, significant genome-building via the mutation/selection process appears essentially impossible. Except in the case of population bottlenecks where fixations are independent of selection, fixations must occur at an extremely slow rate, and only over very deep time. The amount of time required for fixation increases rapidly as population size increases, and also as the population breaks up into geographically isolated sub-populations. Every time there is a change in the environment, the direction of the selection vector can change, resetting the waiting time to selection equilibrium. This means that the examples given in this paper are extremely generous (since we assume a relatively small population size, no sub-population structure, and a constant direction of the selection vector).

The actual genomic difference between chimpanzee and man is still contested, but is minimally 5% (150 million nucleotides, equal to 75 million nucleotide changes in each lineage), and appears to be very much higher (Tompkins, 2012). Given our results, selective fixations could only explain a few hundred of those genetic differences, constituting a very trivial amount of information. However, these few hundred fixations could never arise in something as simple as a coherent text string, because each would arise and act independently and they would be randomly scattered throughout the genome. The type of trivial genetic modification associated with a few hundred beneficial fixations could not even explain the origin of a new sub-species

within the presumed ape-like common ancestor. Yet while these few beneficial mutations were being fixed, at least 100,000 low-impact deleterious fixations would have accumulated. Deleterious fixations would have caused extinction very early in the timeline.

It appears that genomes must degenerate unless there is some unknown stabilizing force far more potent than the mutation/selection process. Given what is now known, regardless of the specific scenario, deleterious fixations should vastly outnumber beneficial fixations, creating a net loss of information every generation. Because fixations are essentially irreversible events, this creates a downward "ratchet". In recognition of Haldane's pioneering work in this area, we refer to this phenomenon as "Haldane's Ratchet". Ironically, Haldane never even considered deleterious fixations. This type of irreversible genetic degeneration is remarkably consistent with the Biblical view of the history of life.

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