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Using Numerical Simulation to Test the Validity of Neo-Darwinian Theory

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Abstract

Evolutionary genetic theory has a series of apparent "fatal flaws" which are well known to population geneticists, but which have not been effectively communicated to other scientists or the public. These fatal flaws have been recognized by leaders in the field for many decades—based upon logic and mathematical formulations. However population geneticists have generally been very reluctant to openly acknowledge these theoretical problems, and a cloud of confusion has come to surround each issue.

Numerical simulation provides a definitive tool for empirically testing the reality of these fatal flaws and can resolve the confusion. The program *Mendel's Accountant* (Mendel) was developed for this purpose, and it is the first biologically-realistic forward-time population genetics numerical simulation program. This new program is a powerful research and teaching tool. When any reasonable set of biological parameters are used, Mendel provides overwhelming empirical evidence that all of the "fatal flaws" inherent in evolutionary genetic theory are real. This leaves evolutionary genetic theory effectively *falsified*—with a degree of certainty which should satisfy any reasonable and open-minded person.

Keywords

Mutation, Natural selection, Darwinian evolution, Genetic load, Genetic entropy

Introduction

The concept of biological evolution existed long before Charles Darwin. What Darwin added was what seemed to be a credible naturalistic mechanism which might drive the evolutionary process. He proposed a mechanistic force which might cause evolution to actually happen spontaneously, and therefore "naturally". Darwin's mechanism was simply the idea of spontaneous variation (mutation) plus differential reproduction (natural selection). Since the time of Darwin, evolutionary theory has been elaborated into a very sophisticated system of thoughts, theories, and equations. However, the simple concept of mutation/selection remains at the very heart of all these elaborate thought systems.

The book *Genetic Entropy and the Mystery of the Genome* (Sanford, 2005) uses logic and some simple calculations to make it clear that there are very fundamental problems with using the mutation/selection mechanism to explain evolution. A series of compelling arguments are used in that book to show that in the long run mutation/selection can not produce a net gain in information. Those same arguments are used to show that selection can not even stop the gradual but certain degradation of genetic information (traditionally referred to as "genetic load", but better termed "genetic entropy"). Taking all these arguments at face value, evolutionary theory appears to be demonstrably false. Historically, each of the arguments summarized in the book *Genetic Entropy,* have been begrudgingly acknowledged within the population genetics literature. However such acknowledgement has not been communicated to the broader scientific community or to the general public. The fact that the textbook version of evolutionary genetic theory appears to be fundamentally dysfunctional appears to constitute a "trade secret" among genetic theorists.

There is now an empirical method which can be used to objectively, empirically, and conclusively test the viability of evolutionary genetic theory. This new methodology is called *numerical simulation.* The key scientific operation which is needed to test evolutionary theory does not involve complex mathematical formulations, but simply involves *tracking and*

counting mutations within populations. It is essentially an advanced accounting problem. Good and bad mutations are entering real populations continuously. Some of these are adding up, while others are being subtracted away (either by selection or by random drift). The key mathematical operations needed here are just addition and subtraction. Computer-aided numerical simulation allows a researcher to mechanistically track every single mutation within a virtual population, from the time each mutation enters the population until its allele frequency goes to either zero or 100%. Each mutation can be realistically processed in a biologically accurate and explicit manner, such that its transmission to the next generation is based upon: (a) Mendelian segregation; (b) stochastic variation; and (c) the mutation's affect on its own probability of transmission to the next generation (its "fitness affect"). Because the neo-Darwinian process is strictly mechanistic, numerical simulation can precisely and rigorously model this process.

If one knows how many good and bad mutations enter a population each generation, and if one knows which individuals within that population reproduce and pass on their mutations, numerical simulation allows us to count precisely how fast the good and the bad mutations are accumulating. One can see exactly what is happening in terms of transmission and selection. It ceases being a matter of philosophy or abstract reasoning, but simply becomes a matter of straight-forward mechanics and arithmetic.

The analysis of the mutation/selection process by numerical simulation is much like accounting. It is concrete and objective, even as accounting is concrete and objective. In a business, one starts with certain assets (resources) and liabilities (debts). Net worth is simply assets minus debts. Every day there are transactions. There are incoming revenues (additions) and outgoing expenses (subtractions). Debts are paid off and new debts are incurred. At the end of the year the accountant will tally net worth, and determine if there was net profit or net loss. The bottom line is not so much where a given dollar went, the central issue is always "was there a net profit or a net loss? This is neither abstract nor philosophical. It is a question with a concrete and verifiable answer—something for which the IRS can hold us legally accountable.

In the same way, at any given point in time, a real living population has a certain "net worth." This is the *fitness* of the species, which is the total "biological functionality" of the species. Species' fitness derives from the total *genetic information* stored up in the species' genome. Every generation, new mutations arise within every individual's genome. The good mutations are like income—they add to the species' net worth or fitness. The bad mutations are like expenses—they subtract from net worth. If there are more bad mutations than good mutations, there must obviously be a decline in net worth. This means that there is a net loss of information in the genome, and a corresponding decline in fitness. In this case the species has lost some of its biological functionality. Such loss of biological functionality will be physically manifested in measurable characteristics such as shorter life span, reduced intelligence, or lower fertility. If the bad mutations are much more numerous than the good, and they continue to accumulate faster than the good mutations, there will a net loss of information every generation, and a continuous net reduction in fitness. It then becomes only a matter of time until such a species' goes extinct. This is just like a business which every year has expenses which exceed income. Deficit spending can only go so long before there is a serious problem. The accumulation of good and bad mutations is a simple matter of arithmetic.

In business there are ways to cut losses and protect assets. So, given a viable business opportunity, net losses can sometimes be converted into net gains by proper management. The same is true in biology. Natural selection can be seen as the business manager of a species—always trying to reduce the deficit spending. How does the manager, natural selection, do this? This manager has only one mechanism available—that is to prevent a certain number of individuals within the population from reproducing. That is the one and only mechanism whereby natural selection can slow down genetic decline. If an individual is selected out of the population, then a specific set of mutations, both good and bad, are subtracted from the accountant's ledger. If we know which specific individuals are selected away, and exactly which mutations they were carrying, we can tally exactly how many mutations remain within the population. So even after we introduce the selection process, we are still talking about simply tracking mutations and basic arithmetic.

Using paper and pencil is no longer a viable method for modern accounting—nor is it practical for tracking mutation accumulation in populations. In real populations there are many individuals, and each individual has many mutations. Each mutation has a specific fitness value and a specific chromosomal location. In just a few hundred generations, one must typically track the transmission of many millions of individual mutations. These mutations get shuffled as they are being passed from parent to offspring. Some are selected away and some accumulate. This constitutes a huge accounting task—something ideally suited to advanced computer programming. An honest computer accounting program is all that is needed for allowing an explicit/empirical/experimental approach to understanding mutation accumulation. It was for

this reason that a team of geneticists and computer scientists developed the program *Mendel's Accountant* (Sanford, Baumgardner, Gibson, Brewer, & Remine 2007a, 2007b; Baumgardner, Sanford, Brewer, Gibson, & Remine, 2008). This program has been extensively validated in terms of its fidelity in modeling the neo-Darwinian process (see Sanford, et al., 2007a). The primary underlying assumption of this program is simply the neo-Darwinian mechanism itself, as it is taught in all textbooks. Therefore, if Mendel fails to demonstrate evolution, the fault is not in the program (which faithfully models neo-Darwinian theory), but is in the theory itself.

Mendel's Accountant is essentially a very advanced genetic spreadsheet, useful for studying the outcome of the mutation/selection process. The program allows us to correctly tally accumulating mutations, just as they would accumulate in nature. *Mendel's Accountant* serves as a powerful teaching tool because it can very graphically reveal how the mutation/selection process really works. It does this in a way that any open-minded person should be able to understand and accept. It is also a powerful research tool, which allows us to empirically find answers to otherwise unmanageably complex genetic questions.

Specifying Realistic Input Parameters

Before running Mendel, one must input honest data. A spreadsheet can only produce honest results if honest data is entered into it. Just as a spreadsheet does not have its own "built-in" input data (income, expenses, etc.), Mendel's input data is not built into it. The user is responsible for inputting honest data.

These input decisions require a certain familiarity with the literature surrounding the organism under study (genome size, mutation rate, reproductive rate). If users so desire, they can apply dishonest input data within Mendel, just like they can put false numbers onto a financial spreadsheet. But in that same sense, they should then be held accountable for how and why they did this, and they must be prepared to defend what they have done. Just as users of financial spreadsheets are ultimately accountable to stockholders and the IRS, users of genetic spreadsheets should be accountable to the rest of the scientific community and to the public. The most important data points that must be entered honestly are: (a) the mutation rate; (b) the fraction of mutations which are beneficial; (c) the mutation distribution; and (d) the selection efficiency. The user manual for Mendel's Accountant (www.mendelsaccountant.info) describes in detail how to input all the relevant data for different biological situations in the most honest way possible. Mendel's specific results depend on the specific input data used. However the *general* patterns which Mendel reveals are surprisingly consistent—as long as the input data which is used is even remotely realistic biologically. These general output patterns are revealed in the example given below. In this particular example Mendel's human default parameters (see the user manual at www.mendelsaccountant.info) are used, except for the following exceptions: (a) the frequency of beneficial mutations is increased 10,000-fold so that the ratio of deleterious to beneficial is 9:1; (b) for simplicity, all mutations are made co-dominant.

Although we use here the default mutation rate for Mendel (which is presently set at ten new mutations per individual per generation), there is growing evidence that this should be set about one order of magnitude higher. We presently use a mutation rate of only ten just to be generous to evolutionary theory, allowing for the notion that 90% of the genome might be irrelevant "junk DNA." If this example employed the accepted human mutation rate (>100), the degeneration described below would be much more severe and extinction would be rapid. The default selection pressure used in this example (six children per female, four of which are selected away every generation), represents extremely intense selection.

Mutations Accumulate in a Strictly Linear Manner

The most striking aspect of Mendel's output is that *mutation count per individual* always increases in a strictly linear manner. Without any selection, if each person gets ten new mutations every generation, then obviously the mutation count per person will increase by ten every generation. So after 100 generations, everyone should have, on average, 1,000 mutations. Mendel's first figure always plots the mutation count per individual over time. If we set Mendel to create ten mutations per person, and we set selection intensity at zero (that is, allowing exactly two offspring/female), and we run Mendel for 100 generations, then Figure 1a always shows an average of 1,000 mutations per individual (not shown here).

To produce this simple result Mendel employed a very elaborate and cyclic process. Mendel had to generate one million different mutations in this particular run (as this case specified a population of 1,000 individuals). Every one of these mutations was individually tracked. Each mutation had its own distinctive mutational effect on fitness and a specified degree of dominance. Each mutation was assigned to a specific linkage block within a specific chromosome which was initially within a specific individual. All this information was tracked for each mutation. Mendel paired and inter-mated individuals, recombined linkage blocks, and transmitted gametes to create the next generation of individuals. All the while, Mendel was tallying and reporting mutation counts. The computational aspects of this program

are described in detail elsewhere in this conference (Baumgardner et al., 2008). In this paper we will just focus on what the Mendel program can show us about biology.

One can do countless Mendel runs, changing any parameters (such as population size, etc)—but if there is no selection, Figure 1a will always show a straight line whose angle upward is solely determined by the mutation rate. There is a linear increase of average mutation count per individual. When there are beneficial mutations (green) as well as deleterious mutations (red), both the red and green lines will go up in a strictly linear manner. This will be true after 100 generations, and also after 100,000 generations.

What happens if we add selection to the equation? Selection will preferentially eliminate some of the least fit individuals, and so will preferentially eliminate some of the more deleterious mutations associated with those individuals. At the same time selection will preferentially favor the reproduction of some of the most fit individuals, and thus will affect the transmission of some of the beneficial mutations. So the pattern of mutation accumulation will be altered. Will mutations still accumulate in a linear fashion? Mendel allows us to explicitly answer this question.

When we use any type of realistic input parameters, Mendel reveals absolutely linear accumulation of bad mutations, even when strong selection is applied (see Figure 1a, red line). This is extremely significant, because conventional wisdom among evolutionary theorists would hope that mutation count would eventually level off (Crow, 1997). Mendel only reveals such a non-linear accumulation of mutations in one extremely artificial "special case"—which does not appear to apply to any real-world population (this special case makes all mutation of equal effect). Apart from this exception, the only aspect of deleterious mutations accumulation which is changed by selection is the precise slope of the straight line—regardless of the biological input parameters used. Strong selection will only cause a slightly shallower slope for the accumulation of bad mutations. The basic nature of deleterious mutation accumulation, either with or without selection, is essentially the same, and only differs by a very small degree.

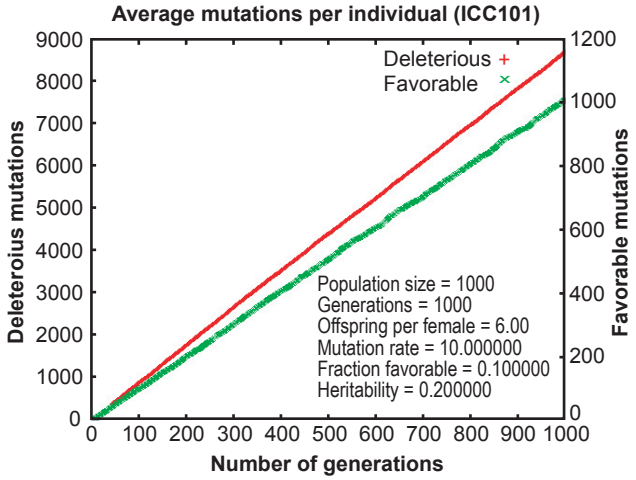

Figure 1a. Even when there is intense selection, mutation count per individual increases linearly—with the slope being for the most part a simple function of the mutation rate.This applies for both deleterious mutations (red line), and beneficial mutations (green line). The scale for deleterious mutations is shown on left, the scale for beneficial mutations is shown on right. If there had been no selection, the red line would reach slightly higher (9,000 mutations per individual, rather than 8,730). The green line would reach very slightly lower (1000 per individual, rather than 1011). In this example the ratio of deleterious to beneficial mutations was artificially set extremely low (9:1), yet after selection the deleterious mutations are still accumulating almost 9 times faster than the beneficials.

When we add beneficial mutations to this picture, we see a similar effect. Selection only causes a slightly steeper slope, but the line is still perfectly linear for the beneficial mutations (Figure 1a, green line). The reason these lines remain straight and do not change their slope significantly when selection is added, is because most mutations are *nearly-neutral* in effect (see Kimura, 1983; Kondrashov, 1995; Sanford, 2005), and are consequently immune to selection. Only the worst (or best) mutations can be selected, and all the rest continue to accumulate in a linear fashion.

When we increase the mutation rate up to the actual rate that is known for humans (more than 100 per person), effective selection breaks down almost completely—due to extensive *selection interference*. At this point, there is no longer any significant difference in mutation counts per individual—with or without selection. When mutation rates are very high the slopes of the mutation count plots are nearly identical—with or without selection.

What do these direct empirical observations (Figure 1a) tell us about genetic theory? These simple observations reveal profound truths about selection and mutation accumulation. The most important single observation is that even with strong selection, mutation accumulation is strictly linear. This means that there is absolutely nothing that can be done to stop the problem of genetic entropy. As long as the mutation rate remains constant, any large genome will accumulate deleterious mutations at a very steady rate. Since bad mutations outnumber good mutations by many orders of magnitude, even after selection, the bad mutations will still be accumulating much faster than good mutations. Selection only slows this accumulation very slightly—it does not even begin to stop it. Typically we see that selection eliminates substantially less than 10% of the deleterious mutations (data not shown). All the rest simply build up within

the genome at a steady rate. As we will soon see, even when there are some rare beneficial mutations which are also accumulating, these only have a trivial effect on the subsequent fitness decline.

It is true that selection can eliminate the very worst mutations and multiply the very best ones—and this goes a long way toward reducing the rate of degeneration. However, the main point here is that information-bearing nucleotide positions are continuously being degraded in a strictly linear fashion, and so the size of the "functional genome" is continuously shrinking in a linear fashion. Even if the fitness effects of a few major beneficial mutations could somehow fully counteract the effects of many minor deleterious mutations, the genome would still be degenerating in terms of total number of functional nucleotides. This must obviously lead to eventual extinction.

Beneficial mutations are clearly very rare. Because of this, in the absence of selection the slope for beneficial mutations is essentially zero, compared to the slope of deleterious mutations. Because Figure 1a is self-scaling and the beneficial mutations have their own scale on the right side of the figure, this fact is easily missed, unless one keeps careful track of the two different scales (bad scaling on left, good scaling on right). Even when strong selection is applied, there is only a slight change of the slopes for good and bad mutations. Only if good mutations were arising at almost the same rate as bad mutations could selection have any chance of reversing their relative abundance. Numerous biological variables can be changed, but these only modulate the rate of degeneration. The bottom line is that the accumulation rates for good versus bad mutations are profoundly different, and selection can only very marginally change their relative rate of accumulation.

Mendel empirically demonstrates the reality of linear accumulation of deleterious mutations, with or without selection. This unambiguously demonstrates that genetic entropy is real, validating the historical concept of genetic load (Wallace, 1987). When using any realistic input parameter values and then "turning the crank"—we always see genetic entropy in operation. This is fundamentally true—even as the second law of thermodynamics is true. Using the Mendel program, this simple reality is verified experimentally. It is a matter of arithmetic and straightforward accounting. This first output figure of Mendel, all by itself, effectively disproves the neo-Darwinian theory.

Fitness Declines Continuously Over Time

What happens to biological fitness as mutations accumulate? If mutations are accumulating linearly and are overwhelmingly deleterious, it should be obvious that biological fitness will also decline. Mendel's second output figure consistently shows this (see Figure 1b). When mutations are combined additively, all populations can be seen to consistently decline at a nearly constant rate, except for a brief initial period when the population is "equilibrating" and decline is especially rapid.

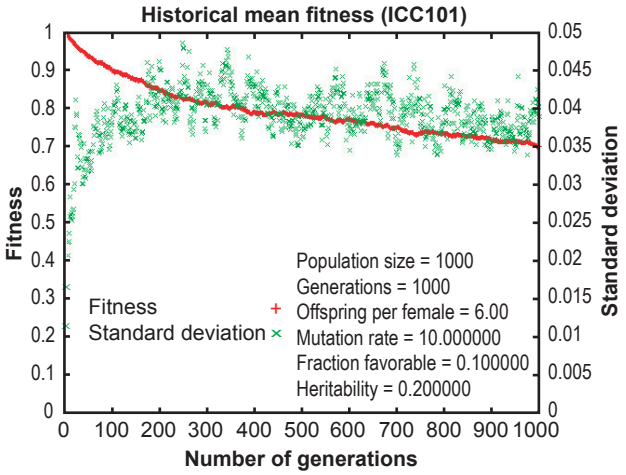

Figure 1b. Fitness (red) declines as deleterious mutations accumulate (fitness scale on left).Without selection this same run reaches a fitness of 0.0 (extinction) in 882 generations. With intense selection the fitness decline is much more gradual, because the worst mutations are eliminated very effectively. Fitness decline is initially quite rapid until there is enough genetic variation for selection to act upon (green plots fitness standard deviation—scale shown on right). After about 200 generations the population reaches near-equilibrium in terms of selection efficiency and begins a nearly linear phase of degeneration which continues until extinction is approached (not shown). When fitness goes below 0.2 and extinction approaches, the rate of degeneration again accelerates due to the phenomenon of mutational meltdown (not shown).

The "equilibrating period" is an artifact of our starting conditions. In a new Mendel run, we unrealistically start with a population which has zero mutations and no natural variation (although this can be avoided by re-starting a Mendel run from an older, equilibrated population). During the equilibration phase the rate of degeneration is especially rapid because selection does not have enough genetic variation to act upon. But over time a population reaches an equilibrated level of variation, as seen by the leveling off of the standard deviation for fitness (plotted in green in Figure 1b). After this happens the rate of decline becomes nearly constant. Naturally, such constancy is dependent upon a stable biological situation. If mutation rate, or population size, or fertility, or numerous other variables are changed—the rate of decline will also change. What is particularly striking is that no input parameter can be manipulated, in a biologically reasonable manner,

such that the decline is actually halted or reversed. This can be extensively demonstrated experimentally using Mendel.

Can this second Mendel output figure be used to predict the time to extinction? It can—but only on a very crude level. The time to extinction depends on the rate (slope) of the fitness decline. This rate of decline is primarily affected by two things: (1) the mutation rate; and (2) the average mutation-effect. While the mutation rate is something which can be empirically measured, the average mutation effect cannot be measured directly. Most mutations are nearly-neutral, and so have an effect on fitness which is too subtle to measure. However, a certain fraction of all mutations (the "major" mutations) have a phenotypic effect which is very readily apparent. If we understand this upper range of the mutation distribution (the frequency of major mutations), then we can then approximate the rest of the distribution. This can be done because we know the over-all mutation rate and we know the genome size.

The genome size tells us the approximate value for the smallest indivisible unit of information which can be changed within that genome. We know that the basic *genomic unit of information* is the nucleotide. Since the human genome size contains 3 billion nucleotides, the typical deleterious mutation (the loss of one functional nucleotide) will reduce information by a factor of roughly 0.0000000003. So the most frequent class of mutation effects (the mode of the distribution), will be roughly at this point in the distribution. Given knowledge of both ends of the mutation distribution, we can then fairly accurately fill in the middle of the distribution (it is essentially an exponential curve).

Once given the mutation rate and the mutation distribution, we can make a theoretical estimation of the minimum time to extinction—assuming there is no selection. We can simply continue the run until fitness becomes zero. But we know that this theoretical time to extinction is just a minimal estimate. This is because we know there is always some selection happening in nature. At the very least, there are always a few lethal and near-lethal mutations which will automatically eliminate themselves from the population. Since such mutations cause a disproportionate amount of fitness decline, their elimination very significantly slows degeneration. The actual effectiveness of selection will determine the difference between the potential versus the real time to extinction.

The effectiveness of selection will be affected by such things as selection intensity (what fraction of the individuals are prevented from mating), biological noise, and population size. When realistic estimates are plugged in for these parameters, Mendel can give us a very rough approximation of actual time to extinction. Given current understanding, the greatest source of uncertainty is the mutation distribution. For example, we are still uncertain about exactly how many human mutations have a "major effect". If less than one in a thousand (.001) mutations are "major", then the shape of the distribution curve is very steep and almost all mutations are nearly-neutral, and the rate of decline will be very slow. In these cases, Mendel shows a very gradual decline which is largely unaffected by the presence or absence of selection. This is because the degeneration is almost entirely due to the accumulation of near-neutrals. On the other hand, if over one in a hundred (.01) mutations are "major," then the rate of degeneration becomes very fast, and effective selection becomes critical for preventing very rapid extinction. In our estimation, the actual rate of major mutations in man is somewhere between these two values. We have noted that as the population approaches extinction, selection becomes more effective because some individuals have a fitness of zero—being eliminated with 100% certainty (partial truncation selection). This can delay the extinction event.

There is another especially important variable which affects the time to extinction, and counteracts the effect of the near-extinction partial truncation selection. As a population degenerates, there is one biological variable which can not be kept constant—fertility. It is well known that declining genetic fitness causes declining fertility. But any reduction in fertility will accelerate the rate of fitness decline—creating an accelerating downward cycle. Declining fertility reduces population surplus, which in turn reduces selection intensity (simply because there are fewer surplus individuals that can be "selected away"). This in turn causes still more rapid mutation accumulation. This causes faster fitness decline, and this reduces fertility still further. The vicious cycle begins to accelerate—causing what is called *mutational meltdown*. The slope of the fitness decline curve becomes steeper and steeper when this happens, causing actual time to extinction to become shorter. So we need to qualify our statement that fitness decline is linear. It is linear only when the population is in the "steady-state decline phase." Fitness decline should actually be non-linear (faster) in the very early and very late phases of population decline.

The Problem of the "Near-Neutral Zone"

The disastrous accumulation of mutations, and the corresponding decline in fitness, is largely due to the problem of nearly-neutral mutations. The problem of near neutral mutations has been known for a long time. Muller first mentions it when describing Muller's ratchet (1964), and it was extensively developed conceptually and mathematically by

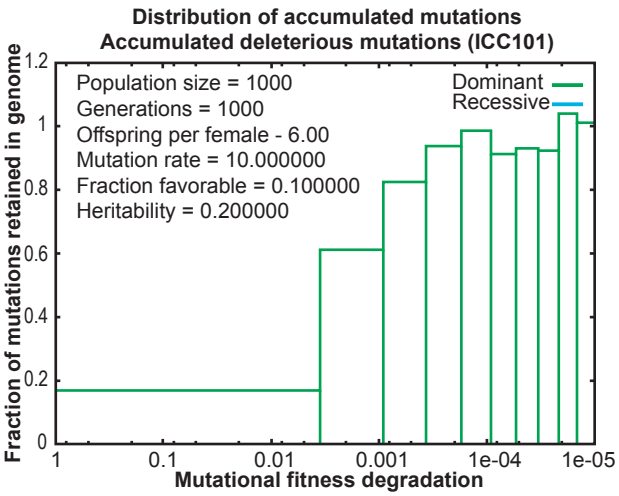

Figure 2. Which mutations are accumulating? This plot shows the full range of deleterious mutations, plotted on a log scale, with mutational effects ranging from lethal (–1.0) to nearly neutral (–0.00001). The "extremely near-neutral mutations" are not plotted here (ranging from –0.00001 to zero). Bin widths are scaled such that if there is no selection, each bin will come up to 1.0 on the left scale. This scale reflects the fraction of each mutation class which is accumulating. The high-impact mutations are largely selected away (first bin on left). Minor mutations are accumulating at intermediate levels (next two bins), and all the "nearly-neutral mutations" are accumulating freely (all other bins to right). The "extremely near neutral mutations" (off chart to right) are obviously also accumulating freely (not shown).

Kimura (1979, 1983). Kondrashov expanded upon this problem further (Kondrashov, 1995). Sanford (2005), describes the actual distribution of mutation-effects in depth. It is very clear that most mutations in large genomes must be nearly-neutral (see also Sanford et al., 2007a).

The logic behind this problem is very compelling. Since the human genome has 3 billion nucleotides, each nucleotide contains on average, about .0000000003 of the total genomic information. The information of the genome should most typically increase or decrease by this amount—this is the fundamental *genomic unit* or *mutational unit*. So the loss of a typical functional nucleotide (a single deleterious point mutation) should decrease biological fitness by about this amount. But normally we can only measure biological effects that increase or decrease fitness by about 10% (0.1). So most mutations are a million fold more subtle than what we can actually measure. This means there is no practical way we could detect or artificially select against such mutations. Mother Nature (natural selection) has exactly the same problem. While it is widely believed that she has more time to select for these more subtle mutations, in nature much more biological noise is interfering with such selection. Therefore, most mutations should be inherently un-selectable. Only those mutations which have unusually large effects should be selectable. All population geneticists should know this, and it is a huge problem for genetic theory (Kondrashov, 1995). Unfortunately most biologists (that is, molecular biologists) are still ignorant of this, and certainly the public has not been informed of this. The *Mendel* program, for the first time, lets us empirically demonstrate the reality of near-neutral mutations, and Mendel can be used to reveal the actual shape of the "near-neutral zone." *Mendel* can also be used to demonstrate how the shape of this zone changes depending on biological parameters, and reveals how this in turn affects the rate of degeneration. Figures 2 and 3 of Mendel graphically reveal the near-neutral zone for deleterious mutations.

Figure 2 uses a bar diagram to show which types of mutations are accumulating, and which are not. Mutations are placed into bins depending upon how strong an effect each mutation has on fitness. The log scale on the bottom is designed so that when there is no selection, all bins will be filled to approximately the same height (apart from sampling variation). In Figure 2 we can see that the mutations with the largest effects (those in the bin furthest from zero), are hardly accumulating at all. These are the major mutations, and selection removes them very effectively. However, the mutations with the smallest fitness effect are all freely accumulating (the bins nearest zero). These are the nearly-neutral mutations. Any honest mutation accounting program will show that near-neutral mutations exist, and that the "no-selection" zone is an objective reality. The bins in the middle of the Figure 2 represent a transition zone. These middle bins are filling up, but more slowly that the bins on the far right. These bins are not truly "un-selectable" because selection is still partially acting upon them. They are actually "minor mutations" which are accumulating primarily because of *selection interference*. These minor mutations are the most damaging to a population, because they have substantial fitness effects yet are still accumulating at a very significant rate.

What is not very easy to see in Figure 2 is that the near-neutrals represent the vast majority of all mutations (90–99%). This is unclear because the bins were scaled to be the same height, in order to more precisely locate the borders of the *near-neutral zone*. But in actuality, the number of mutations per bin increases exponentially as the fitness effect gets smaller. Almost all mutations are in those few bins nearest zero (off scale).

What about the beneficial mutations? At realistic rates, beneficial mutations accumulate in such small numbers that there are not enough data points to plot their distribution accurately. When the beneficial

mutations are greatly exaggerated we can get enough data to plot. What we see is the mirror image of what happens with deleterious mutations. The bins with the least affect accumulate mutations just as if there was no selection. Only the highest-impact beneficial mutations are seen to actually respond to selection. We can actually see at what point this happens, because only the very highest-impact bin accumulates mutations faster than would be expected if there was no selection. Therefore, even for beneficial mutations, near-neutrals are a verifiable reality, and this other half of the *near-neutral zone* is just as real and measurable as for deleterious mutations. In fact, Mendel clearly demonstrates that overwhelmingly, almost all beneficial mutations are entirely un-selectable.

In summary, Figure 2 consistently shows us that when selection is applied, the worst mutations are selected away, the nearly-neutral mutations accumulate unhindered, and minor mutations accumulate at intermediate rates. Therefore, we have demonstrated, through empirical experimentation, that the near neutral problem is very real! Strangely, there are many molecular biologists who feel near-neutrals could not possibly be real, because they are *totally convinced* that all of the functional nucleotide

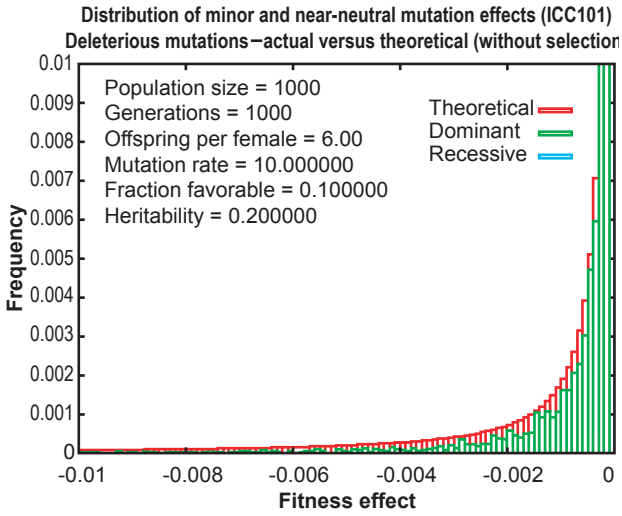

Figure 3. Which mutations are accumulating? This figure shows in greater detail the nature of Kimura's "no-selection zone." Only mutational effects ranging from .01 to zero are plotted, using a natural scale. The red bins represent the natural distribution of mutations when there is no selection.The green bins represent the distribution of the actual mutations which are accumulating. Essentially all mutations stronger than .01 are selected away (off scale). The three bins nearest zero also go off scale (on top), but are "nearly neutral" and are accumulating freely. All the other bins are "minor mutations" and are accumulating more and more freely as they approach the near-neutral zone on the right.

sites which they study must have arisen by natural selection. But this is only a *belief* on their part—a belief which can now be conclusively shown to be wrong.

The exact place where selection begins to break down depends on numerous biological variables (Baumgardner et al., 2008) and Mendel can be used experimentally to determine where this point is, given a specific set of biological parameters. To briefly summarize observations from hundreds of Mendel runs, when using realistic parameters, we consistently see that the "no-selection zone" begins around +/−.001 for both good and bad mutations. This means that selection is only really effective for mutations which change fitness by at least one part in a thousand. The vast majority of all mutations are much more subtle than this, so it should not be surprising that most mutations are un-selectable. In fact, a typical mutation should be many orders of magnitude smaller than this selection limit, and such mutations must be entirely immune to selection. While the exact point at which mutations begin to accumulate depends on specific circumstances, it is very clear that most mutations are un-selectable.

Figure 3 takes a more careful look at the near-neutral zone, using a natural linear scale. This figure shows the theoretical distribution of deleterious mutations (in red), and superposes over this, the mutations which are actually accumulating (green). If there is no selection, the accumulating mutations can be seen to match very closely the theoretical distribution. When selection is applied, we can see that the worst mutations are eliminated (Figure 3). But the majority of all mutations will accumulate exactly the same as if there was no selection. Figure 3, with its natural linear scale, more clearly shows us that the "no-selection zone" does not have a clear border, but actually has a very wide "transition zone". Within this transition zone purifying selection is only partially effective and becomes progressively weaker as the mutational effect decreases.

While the no-selection zone is always present, it can be greatly reduced whenever mutation rate is low, or where selection is extremely intense, or where noise is minimal. Therefore in microbial systems the near-neutral problem should be greatly reduced, and most mutations should be effectively eliminated. Some extremely small genomes may be extremely resistant to genetic entropy.

Figures 2 and 3 both conclusively demonstrate that "Kimura's no-selection zone" is very real, and they show that that this "no-selection zone" encompasses most bad mutations and essentially all good mutations. Kimura's no-selection zone appears to generally cover mutations ranging from −.001 to +.001. This is one of the primary reasons why mutation count always goes

up linearly (Figure 1a), and why fitness consistently goes down linearly (Figure 1b).

The Problem of Linkage

The problem of linkage was first described by Muller (1964). Since that time linkage has been recognized as a serious problem for evolutionary theory, and thus many theorists have tried to make the problem "go away". The problem is that our genome is made up of large linkage blocks which do not recombine, and which are on average 30,000 nucleotides long (about the size of a typical gene). So mutations accumulate within *clusters that never break apart.* But one of the essential things that selection **must** accomplish if forward evolution is to be feasible, is to *separate* the good mutations from the bad mutations. Given that most mutations are bad, it should be obvious that any rare good mutation will always be linked to many bad mutations within its linkage cluster. This is one reason why selection for beneficials is so extremely ineffective (see Figure 1a). This is illustrated more clearly in Figure 4. When realistic rates and distributions of beneficial mutations are employed, all beneficial linkage blocks gradually disappear. Rare beneficial effects are systematically cancelled out by the rapidly accumulating bad mutations which are physically linked to them (Figure 4). This means that almost every single one of the "building blocks" of the genome (there are about 100,000 linkage groups in man) are systematically degenerating. This has been independently been confirmed by another recent study (Loewe, 2006). The problem of linkage sheds light on the evolutionary relevance of horizontal gene transfer. Since all genomic building blocks (linkages) must degenerate, the transfer of such building blocks between species does not create new information—any incoming (or outgoing) linkage blocks will be degenerating just as fast as the rest of the genome.

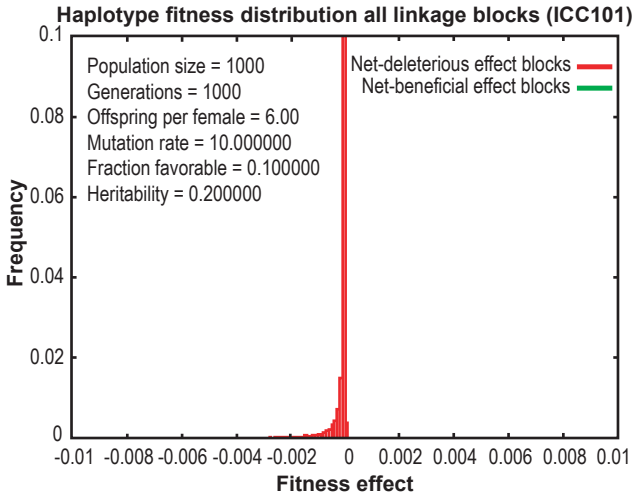

Figure 4. The distribution of linkage block effects. Mutations occur is clusters within linkage blocks within chromosomes. These mutational clusters (haplotypes) are physically linked and do not undergo recombination. So all the mutations within a cluster act like a single mutation, and are inherited like a single gene. The mutational effect of each linkage cluster is simply the net mutational effects of all the mutations in that linkage cluster. Mutation cluster fitness effects are plotted above. All linkage blocks with a net deleterious effect are to the left of zero and are shown in red. All linkage blocks with a net beneficial effect are to the right of zero and are shown in green (there are none in this example). Even though 1 in 9 mutations were made beneficial in this experiment, there are essentially no linkage blocks with a net beneficial effect. This is because each beneficial mutation is linked to an average of nine deleterious mutations—which consistently override the beneficial effect.

Allele Frequency and Fixation

The mutations that can not be selected away within a population will continuously accumulate. A small number will increase in frequency up to the point of fixation (such that every individual will be homozygous for such mutations). However, the vast majority of mutant alleles will drift out of the population, including most of the beneficials. Mendel's Figure 5 shows allele frequencies at the end of a run, and the fraction of alleles that have become fixed.

In all Mendel runs, every new mutation first enters the population as a singe copy. These extremely rare alleles will either be quickly lost, or will drift further into the population—becoming more frequent. Except in very small populations, this drifting process is extremely slow. A disproportionate fraction of all mutant alleles will remain very rare (piling up on the left, with frequencies of less than 1%). The more common alleles that have drifted into the population become distributed quite uniformly—establishing an essentially level distribution across all frequencies ranging from 5% to 99%. These "common alleles" represent what is essentially a "conveyor belt of mutant alleles"—which gradually moves to the right. When alleles get to the far right, they become fixed when they have an allele frequency of 100%. Mendel shows that this conveyor belt moves *extremely* slowly except in very small populations. This applies equally to beneficial mutations—confirming the essence of "Haldane's Dilemma" (Haldane, 1957).

Mendel shows that after a very long initial period of equilibration, mutations finally start being fixed (initially all allele frequencies are low, so for a long time zero fixations can happen). When the point is finally reached where fixations begin to happen, mutations start to be fixed each generation at a steady rate. This rate of fixation is almost exactly as fast as the new mutations are arising within the population (at the other end of the conveyor belt). Mendel's Figure

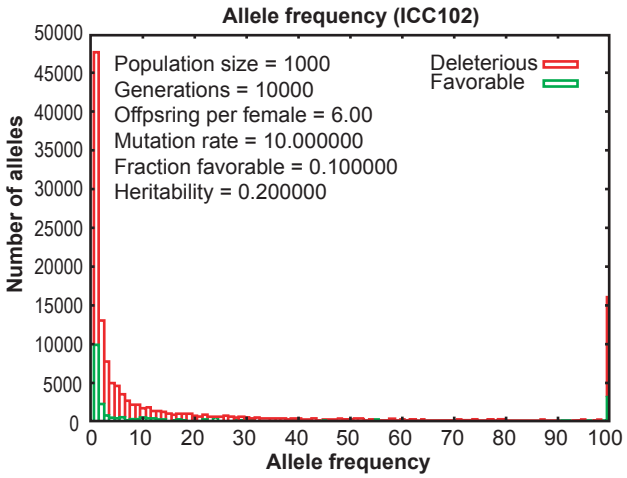

Figure 5. After one thousand generations no beneficial or deleterious alleles had been fixed. For this reason the run was extended to 10,000 generations (shown above). At this point 16,116 deleterious mutations had been fixed, and 3,193 beneficial alleles had been fixed. If the rate of beneficials had not been artificially enhanced in this run by roughly 10,000 fold (being set at one in nine), zero beneficials would have been fixed, even in 10,000 generations. Deleterious fixations represent irreversible damage to the genome and are the exact antithesis of evolution.

5 consistently shows three important things: (1) the beneficial alleles remain a trivial part of the entire picture; (2) even the very rare selectable beneficial mutations migrate to the right at glacial speeds; (3) fixations are overwhelmingly deleterious, even with intense selection.

The fixation of beneficial mutations is the yardstick of evolution. Fixation of deleterious mutations is the precise antithesis of evolution. Figure 5 (like Figures 1a, Figure 1b, and Figure 4), conclusively demonstrates that mutation/selection does not result in evolution, but rather results in degeneration. Genetic entropy is demonstrably real, and is an integral part of genomic change over time.

Conclusion

At its most fundamental level, evolutionary genetic theory must be about tracking mutations and allele frequencies. It boils down to a very large accounting problem. To objectively test evolutionary genetic theory the thing that has been lacking has been a practical mechanism for tracking each mutation, through large populations, over many generations, in a biologically realistic manner. This has now become possible for the first time, using the numerical simulation program called *Mendel's Accountant*. This program is a powerful teaching and research tool. It reveals that all of the traditional theoretical problems that have been raised about evolutionary genetic theory are in fact very real and are empirically verifiable in a scientifically rigorous manner. As a consequence, evolutionary genetic theory now has no theoretical support—it is an indefensible scientific model. Rigorous analysis of evolutionary genetic theory consistently indicates that the entire enterprise is actually bankrupt. In this light, if science is to actually be self-correcting, geneticists must "come clean" and acknowledge the historical error, and must now embrace honest genetic accounting procedures.

While numerical simulations can not honestly be used to support evolutionary theory, a surprisingly wide range of very reasonable biological input parameters give rise to Mendel output compatible the biblical account of a recent creation (not shown). Biologically reasonable Mendel input parameters produce output consistent with: (a) rapid local adaptation followed by phenotypic stabilization; (b) a spike in genetic variation followed by continuously declining diversity; (c) rapid genetic degeneration tapering into a more gradual but continuous genetic decline; and (d) many extinction events.

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