Can Synergistic Epistasis Halt Mutation Accumulation? Results from Numerical Simulation

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Abstract

The process of deleterious mutation accumulation is influenced by numerous biological factors, including the way in which the accumulating mutations interact with one another. The phenomenon of negative mutation-to-mutation interactions is known as synergistic epistasis (SE). It is widely believed that SE should enhance selective elimination of mutations and thereby diminish the problem of genetic degeneration. We apply numerical simulation to test this commonly expressed assertion.

We find that under biologically realistic conditions, synergistic epistasis exerts little to no discernible influence on mutation accumulation and genetic degeneration. When the synergistic effect is greatly exaggerated, mutation accumulation is not significantly affected, but genetic degeneration accelerates markedly. As the synergistic effect is exaggerated still more, degeneration becomes catastrophic and leads to rapid extinction. Even when conditions are optimized to enhance the SE effect, selection efficiency against deleterious mutation accumulation is not appreciably influenced.

We also evaluated SE using parameters that result in extreme and artificially high selection efficiency (truncation selection and perfect genotypic fitness heritability). Even under these conditions, synergistic epistasis causes accelerated degeneration and only minor reductions in the rate of mutation accumulation.

When we included the effect of linkage within chromosomal segments in our SE analyses, it made degeneration still worse and even interfered with mutation elimination. Our results therefore strongly suggest that commonly held perceptions concerning the role of synergistic epistasis in halting mutation accumulation are not correct.

Key words: mutation accumulation, synergistic epistasis, mutational meltdown, numerical simulation, Mendel's Accountant

Introduction

There is a significant body of literature indicating that direct selection against deleterious mutations is insufficient to halt mutation accumulation [1–5]. This has

recently been validated using biologically realistic numerical simulations [6–9]. A primary reason for this result is that most deleterious mutations have extremely small effects on fitness and thus are invisible to selection [10–15].

Some have argued that this fundamental issue might be resolved if selection is not ultimately based directly upon the biological effects of individual mutations acting in isolation of one another, but instead is based largely upon interactions between mutations, interactions that act to compound the biological effects of the individual mutations. Such effect-enhancing interaction between deleterious mutations has been termed synergistic epistasis (SE). It is widely claimed that SE acts to slow deleterious mutation accumulation and thereby helps prevent genetic degeneration and mutational meltdown [16–29]. We will refer to this concept as the SE hypothesis.

The logic behind this hypothesis is somewhat counterintuitive. The reasoning is that, while the number of mutations per individual increases in roughly a linear manner, the number of potential mutation-mutation interactions increases in a non-linear fashion. The number of pair-wise interactions increases as the square of the mutation count, for example. Hence, if SE effects are significant, then at a certain point individuals who carry the most mutations might conceivably begin to display a significant reduction in fitness relative to the rest of the population. This, in turn, might increase selection against high mutation count individuals and thereby eliminate a larger total number of mutations from the population than would occur otherwise. Eventually, this intensifying selection against high mutation count individuals, if sufficiently strong, might stabilize the mutation count and thereby halt further genetic degeneration. This SE hypothesis is counterintuitive, because in most circumstances increasing the negative effects of deleterious mutations on fitness only serves to increase the rate of fitness decline and hasten mutational meltdown and extinction. For the SE hypothesis to be viable, the selection against high mutation count individuals must be sufficiently strong so that at some point it is able to counter the associated increased rate of fitness decline.

The circumstances under which selection, apart from any SE effects, can come to be based primarily upon mutation count, rather than the additive or multiplicative fitness effects of the individual's mutations, has been discussed by several investigators [17-20]. In a companion paper [9], we apply numerical simulation to test the efficacy of selection based upon mutation-count entirely apart from SE effects. In this paper we apply numerical simulation in a similar manner to evaluate whether or not SE has the ability to halt mutation accumulation.

Interactions among mutations within a genome are diverse in their impact. Any two mutations may act independently of each other (that is, have no interaction, which leads to the standard additive model), act multiplicatively (the multiplicative model), diminish each other's effect (antagonistic epistasis), or compound

each other's effect (synergistic epistasis). Undoubtedly, all of these types of interactions operate in any sizeable genome. Therefore it is not reasonable to assume all mutation-mutation interactions in any genome are exclusively of a single type. Nevertheless, non-interaction should be the norm, with the other types of interaction being the exceptions. The only rationale for modeling a 100% multiplicative model or a model with SE contributions from 100% of the deleterious mutation interactions is to try to understand in which direction the exceptional interactions tend to pull the overall behavior away from the norm of additivity.

It is noteworthy that the main exceptions to the general rule of additivity pull in opposite directions. Both antagonistic epistasis and the multiplicative model drive population fitness in the direction opposite to that of synergistic epistasis. That is to say, as mutation count increases, both the antagonistic epistasis model and the multiplicative model cause fitness decline to slow down, while SE causes fitness to decline faster and faster. So when combined, the other types of interactions should cancel out the effects of the SE interactions in whole or in part, leaving what should closely approximate an additive model. Therefore, in a complex genome it would seem most realistic to assume the additive model, with interactions constituting a low level of "genetic noise" (which we would normally just refer to simply as "epistasis" or "general epistasis").

We therefore conclude that a genetic model in which all mutations interact in a synergistic manner is an artificial model, one that does not represent any real biological population. Moreover, such a model contradicts an extensive body of population genetics literature, which for nearly 90 years has been built on the assumption that most mutational effects combine either additively or multiplicatively (the latter effectively counteracting any generalized SE effect). The idea of genome-wide generic SE interaction is virtually never invoked, except as special pleading as a theoretical mechanism to halt mutation accumulation and degeneration. The present study uses numerical simulation to show that even if there were widespread and generic SE, it still could not halt mutation accumulation. Instead, what is seen is that as SE effects become stronger, there is more and more genetic degeneration, just as logic and common sense would suggest.

Methods

The program Mendel's Accountant [6], hereafter referred to as Mendel, is applied to study the effects of SE on mutation accumulation and genetic degeneration. This software uses realistic genetic accounting to study mutation accumulation [7–9].

There is enormous biological complexity inherent in the mutation/selection process when it is considered at the level of the whole genome and the whole

population. It is not reasonable to assume that such complexity can be effectively captured by any tractable set of analytic equations. However, thanks to the computational capabilities now available, complex systems of this type can now be routinely analyzed using numerical simulation. Mendel, developed over the past five years, is a genetic accounting program which can actually do this. This software models and tracks a complete biological system, from individual mutations, to mutation-mutation interactions, to linkage blocks, to chromosomes, to genotypes, to phenotypes, to mating/recombination events, to sub-populations, to whole populations. Using Mendel, all the appropriate parameters are accounted for and are specified by the program user, and the computational processing is faithful to our understanding of how genetic systems operate.

The basic process underlying this numerical simulation is as follows. Mendel creates a population with specified biological characteristics. The individuals in this population are allowed to create gametes, mate, and generate offspring for a new generation. Each offspring inherits the mutations in the gametes from its two parents, including possible new mutations that arose in the germ line of the parents during their lifetime. Each new mutation has its own fitness effect and its own genome location involving a specific linkage block. Mendel then calculates the genotypic fitness of each offspring based upon the net effect of all the mutations it carries. Random environmental noise is next added to obtain a value for phenotypic fitness. Selection is then applied, based on phenotypic fitness, to determine which of the offspring will mate and reproduce to create the next generation. Although Mendel readily treats beneficial mutations, for the sake of clarity in this paper we include deleterious mutations only. We use Mendel's human default parameters, as might reflect a small human population, except as indicated. Apart from these exceptions, the default parameters in all our experiments are as follows: ploidy = diploid; reproduction = sexual; mating = random; linkage = dynamic; new mutations per individual = 10; beneficials = none; offspring per female = 4 (resulting in 50% selective elimination); population size = 1000; generations = 2000; haploid genome size = 3 billion; rate of high impact mutations (fitness impact of 0.1 or higher) = .001; gene expression = complete co-dominance; fitness heritability = 1.0; fertility decline with fitness decline = none; selection type = probability.

Modeling general epistasis

Mutational interactions are, by their very nature, unique and specific, so it is somewhat problematic to account for interactions in a generic manner. However, there is one generic aspect of nucleotide interactions which we can easily describe

and model, namely, the phenomenon of general epistasis. General epistasis reflects the net effect of all types of mutation-mutation interaction. When there is genetic diversity within a sexual population and the segregating nucleotides recombine with each other every generation, many specific interactions in the parent are destroyed, and many new interactions are created in the progeny. These changing interactions generation to generation result in what is called epistasis. The overall effect of such epistasis is a type of non-heritable variation (noise), resulting in lower heritability and reduced selection efficiency. So the dominant effect of the ever-changing nucleotide interactions within the genome is generic epistasis, which hinders selection efficiency to a modest degree. In numerical simulations, the phenomenon of general epistasis can very reasonably be modeled simply by decreasing the genotypic heritability parameter by an appropriate amount.

Modeling additive interactions

While generic epistatic interaction as described above is significant, by far the most common relationship between any two given nucleotides should be *non-interaction* (or vanishingly small interaction). Like any two misspellings in a long text, any two nucleotides in a large genome will have a vanishingly small chance of having any meaningful direct interaction. When two letters are changed in a text, they generally need to be in the same word, or at least in the same sentence, to have any reasonable likelihood of interaction (wherein one affects the meaning of the other). In the same way, any two mutations are unlikely to interact significantly unless they are in the same gene, or at least in the same pathway. The vast majority of mutations should not significantly interact with one another.

The *non-interaction* of most mutations is the theoretical basis for the conventional additive model for combining the effects of mutations within an individual. The additive model assumes that as mutations accumulate, each new mutation affects fitness independently of the others. Under this model if an individual in a population has an initial fitness of 1.0, and we introduce two independent harmful mutations, with each reducing fitness by an increment of 0.1, the resulting fitness will be 0.8. If we then introduce a good mutation that increases fitness by an increment of 0.1, the new fitness will be 0.9. The mutational effects of all the mutations in a given individual are simply added. The additive model is commonly employed in population genetics because in a large genome it is only reasonable to assume that non-interaction is the rule and interaction is the exception. Mendel employs the additive model of mutation effect combination as its default.

Modeling multiplicative interactions

The most common alternative to the additive model is the multiplicative model. Under this model, as mutations accumulate, their mutational effects combine multiplicatively. This means that as deleterious mutations accumulate, they have less and less effect relative to the original fitness, while as beneficial mutations accumulate they have greater and greater effect. To draw an analogy, deleterious mutations act similarly to inflation eroding the value of a bank account, while beneficial mutations act as earned interest which is being compounded. This type of interaction is only reasonable where mutations act in a sequential manner, with one interaction building upon the effect of another, in series. This might plausibly occur when multiple mutations affect the same biochemical pathway. While some specific sets of mutations will doubtless interact multiplicatively, it is not reasonable to assume that all mutations would or could interact in this way. It is also not reasonable to use the multiplicative model as the primary method of combining mutational effects, because a purely multiplicative model can never reach a fitness of zero (i.e., extinction). In fact, under the strict multiplicative model, a small genome might have every nucleotide become mutated, with the genotype still retaining a positive fitness.

In the big picture, on the level the whole genome, the additive model should most generally be true, with the multiplicative model being applicable only to a limited number of special interactions. In other words, multiplicative interactions should only represent deviations from the norm of additive interaction. Mendel has been designed to allow any blend of additive and multiplicative interaction, ranging from 100% additive to 100% multiplicative. In our opinion, a fraction of 0.99 additive and 0.01 multiplicative interactions is reasonable, but this choice is left to the Mendel user.

By allowing any fraction of additive and multiplicative general interaction, and by adjusting heritability downward to allow for general epistatic noise, Mendel allows for the modeling of the primary mutation-mutation interactions.

Modeling synergistic epistasis

Mendel has also been designed, however, to handle the special type of reinforcing interaction between mutations known as synergistic epistasis. Like multiplicative interaction, SE interaction must be viewed as a deviation from the general rule of non-interaction (i.e., the additive model). SE interaction implies that as deleterious mutations accumulate, each additional mutation has a greater and greater effect on fitness. This is the exact antithesis of multiplicative interaction, wherein each

deleterious mutation has less and less effect on fitness. Both multiplicative and SE interactions represent deviations from the additive model, but they pull in opposite directions. To the extent that multiplicative and SE interactions occur at a similar frequency, they should largely cancel each other. Viewing the genome as a whole, if 90% of all mutations combine additively, and 5% combine multiplicatively and 5% combine via SE, the result should be that the two types of deviation mostly cancel, yielding results nearly equivalent to a purely additive model. For most genetic simulations, a realistic and practical choice is simply to use the standard additive model.

Because SE has often been invoked as a hypothetical mechanism which might be able to halt mutation accumulation, we have included it as an option in Mendel. In doing so, we have endeavored to treat SE in as biologically realistic a manner as possible. Our implementation, however, involves a few assumptions which we shall now review.

First, we assume a reference genotype. From an evolutionary perspective all nucleotides have arisen by mutation, so viewed from that perspective, all nucleotides are "mutant". However, to treat SE in the normal sense of that term logically requires a reference genotype relative to which "mutations" may unambiguously be defined. The approach employed in Mendel is to assume a population with zero initial genetic variation, as might be approximated by a population after a severe bottleneck at a specific point in time. All subsequent mutations causing deviation from that starting genotype are tracked individually and contribute to the distinct set of mutations and hence to the mutation count of each member of the population in subsequent generations. This assumption of a reference genotype is inherent to Mendel's underlying formulation and does not apply in any special way to the treatment of SE. Note that when there is just one mutation in a genome, all the interactions involving that mutation are with non-mutant nucleotides, so 100% of that mutation's fitness effect is due to its interactions with non-mutant sites. Thus all solitary mutations have a non-epistatic effect on fitness that arises entirely from its interactions with non-mutant nucleotide sites.

As additional mutations accumulate, however, there are more and more potential mutation-mutation interactions. As the mutation count increases, the deleterious SE contribution to fitness increases at an accelerating rate, accelerating because the number of possible pair-wise interactions increases in proportion to the square of the number of mutations. A second assumption is that we restrict our SE treatment to these pair-wise interactions, that is, to interactions between pairs of individual mutations. A third is that we assume the strength of the SE effect on fitness is directly proportional to the non-epistatic fitness effects of each of the mutations in the pair. This means that if a mutation's effect on the non-mutant genome is small, then the SE contribution from its interactions with other mutations likewise is small.

We further assume that, in regard to SE interactions, it is proper to distinguish between linked mutation pairs, that is, those which reside within the same linkage block on a chromosome and those pairs which reside in separate linkage blocks. Linked mutations are inherited together. Not only are the non-epistatic fitness effects of all such mutations inherited together, but the SE effects of all their mutual interactions are as well. By contrast, genetic recombination progressively tends to scramble mutations that are not linked together. Hence, the SE contribution from non-linked mutations has a transient component. The SE effects arising from the non-linked interactions which change from one generation to the next act like a type of noise that interferes with the selection process. Therefore, realistic modeling of SE requires that linked and non-linked SE effects be treated separately. We therefore partition the SE effects on fitness into two parts, one involving interactions between deleterious mutations occurring in the same linkage block (linked interactions) and the other part involving interactions of deleterious mutations on different linkage blocks (non-linked interactions). SE effects from linked interactions are inherited, while part of those from non-linked interactions are transient and act, in effect, as a type of noise as far as the selection process is concerned.

Another major difference between linked and non-linked SE interactions is the relative magnitude of their effects. Intuitively, the strongest SE interactions should be within the same linkage block, even as two misspellings in an encyclopedia are likely to interact more strongly if they occur within the same chapter or paragraph or sentence. Two mutations are most likely to interact if they occur within the same protein-coding sequence or at least the same genic region. Therefore, the treatment in Mendel includes separate scaling factors for each of these two categories of SE effects. Normally, the scaling factor for linked interactions should be much larger (perhaps by a factor of 1000) than the one for non-linked interactions.

Since linked SE interactions are inherited perfectly, they must always make the degeneration problem worse. This is because the SE contributions act to reinforce the negative non-epistatic fitness effects of the mutations on each linkage block and, in effect, make the non-epistatic effects even more negative.

Let us now consider how Mendel actually treats the linked SE interactions. We assume the amplitude of the linked SE effect of each pair-wise interaction to be proportional to the product of non-epistatic fitness effects of the paired mutations. If a mutation's effect on the non-mutant genome is small, the SE contribution from its interactions with other mutations is likewise small. If we denote the number of mutations in a given linkage block by m, the number of pair-wise interactions each mutation has with the other mutations is $m\text{-}1$, and the total number of unique

pair-wise interactions in the linkage block is $m(m-1)/2$. Mendel stores the fitness f (relative to unity, when no mutations are present) of each linkage block as well as the number m of mutations it carries.

Mendel computes the SE contribution to fitness whenever a new mutation is added to the linkage block. This contribution is proportional to the non-epistatic effect of the new mutation times the sum of the non-epistatic effects of each of the individual mutations already present on the block. When these SE contributions are accumulated, each of the $m(m-1)/2$ unique pair-wise interactions is accounted for. These contributions are scaled by a user-specified factor α. We also assume co-dominance for these SE interactions, which implies each haploid occurrence of a mutation gives 50% expression of the mutation's total non-epistatic value. This reduces the SE effect by a factor of 0.25. We note that, because mutations within a given linkage block are passed intact from one generation to the next, the SE effects arising from linked mutations are also passed intact from parent to offspring. Therefore, as we have already noted, the net result of including SE relative to linked deleterious mutations is always to increase the magnitude of their negative effect on fitness.

Mendel treats the non-linked SE interactions in a similar manner. Let M be the total number of mutations in the genome of a given member of the population and n be the number of equal-sized linkage blocks. The total number of unique pair-wise interactions between mutations is $M(M-1)/2$, the mean number of mutations per linkage block is M/n, and the approximate number of linked interactions is $n(M/n)[(M/n)-1]/2 = M(M-n)/2n$. With this approximation, the number of non-linked interactions becomes $(1-1/n)M^2/2$ and the ratio of the number of non-linked interactions to linked ones is $n-1/(1-n/M)$. With n typically 1000 or greater, as M becomes much greater than n, this ratio approaches n. In other words, as the total number of mutations becomes large relative to n, the number of non-linked mutations approaches n times the number of linked mutations.

Let us denote by F the overall genotypic fitness, apart from any SE effects, of a given member of the population. We assume the amplitude of the non-linked SE effect of each pair-wise interaction to be proportional to the product of non-epistatic fitness effects of the two mutations in each pair. The total non-linked SE fitness contribution is then nearly proportional to the sum of the non-epistatic fitness effects of all the individual mutations, $(1-F)$, but scaled to account for the portion of the mutations which are linked using the factor $(1-1/n)$, times the mean non-epistatic fitness effect of these mutations, $(1-F)/M$, times the number of unique pair-wise interactions, $(1-1/n)M/2$, that each non-linked mutation has with the others. This estimate has included the contributions from the self-interaction of each of the mutations, contributions that should not be included and which

Mendel omits. However, when the total number of mutations is large, the sum of these contributions is relatively small, in which case the estimate is reasonable accurate. We again assume co-dominance, which implies each haploid occurrence of a mutation gives 50% expression of the mutation's non-epistatic value. This reduces the overall contribution by a factor of 0.25. We scale this non-linked SE contribution with a user-specified input parameter β. As already mentioned, one expects that interaction between mutations within the same linkage block will, on average, have much greater SE effects than mutations which are more distant within the genome. Hence, a value for β much less than α is usually appropriate. The resulting approximate expression for the non-linked SE contribution to individual fitness is therefore $0.125\beta(1\text{-}F)^2(1-1/n)^2$. Mendel corrects this by subtracting away the sum of the self-interaction contributions.

We note that the negative SE contribution to fitness from all the non-linked interactions is proportional to $(1\text{-}F)^2$. Since the number of linkage blocks is typically 1000 or greater, the factor $(1-1/n)^2$ can usually be approximated as unity. The SE contribution from non-linked interactions is larger for individuals in the population with lower fitness and smaller for individuals with higher fitness. It therefore tends to accentuate the spread in fitness across the population and thus to enhance selection efficiency. Since fitness F tends to be dominated by the relatively few mutations in the high-impact tail of the fitness effect distribution, F is largely insensitive to mutation count. This non-linked SE contribution is therefore insensitive as well. Since the mean mutation fitness effect is directly proportional to $(1\text{-}F)$, the overall impact of this SE contribution from non-linked interactions is to increase the mean negative mutational fitness effect, just as is the case for the SE contribution from the linked interactions. Therefore, the net effect of SE for both linked and non-linked interactions should be a higher rate of fitness decline with time. There is nothing from a theoretical standpoint to suggest otherwise.

Finally in this section, let us estimate what a biologically reasonable value might be for the non-linked scaling factor β. The total SE fitness contribution in Mendel for non-linked mutations, assuming no linkage at all, is approximated by the expression $0.125\beta(1\text{-}F)2$, where F is the individual genotypic fitness. A plausibly hard upper bound on the magnitude of β might be the value that drives F to zero when, without SE, the fitness F of a given individual is 0.5. In this case, $\beta = 0.5/(0.125\times 0.5^2) = 16$. This means that, if the accumulated mutations in a given individual reduce the fitness of a given individual to 0.5 without SE, then with SE and β =16, the fitness of this individual drops to zero. In our view, a biologically realistic value for the non-linked scaling factor β should therefore be no larger than 1.0 and more plausibly on the order of 0.1 or less.

Results

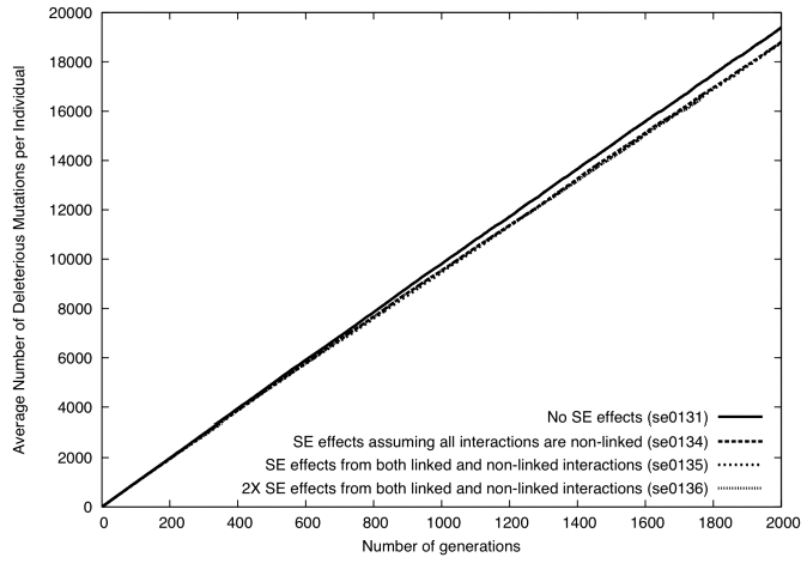
Preliminaries

We ran a number of experiments with Mendel's Accountant to ascertain a reasonable value for the linked scaling factor α relative to the non-linked factor β. We found that choosing α some 2000 times larger than β gave comparable SE contributions from linked relative to non-linked mutations. We considered cases with just under 2000 total linkage blocks for the diploid genome, or about 1000 for the haploid genome. This implies much larger linkage blocks and more linked mutations that observations would suggest for most organisms. Therefore, α should almost certainly be chosen larger than 2000 relative to β when the number of linkage blocks is increased if one wants the SE contribution from linked mutations to be comparable to that from linked mutations.

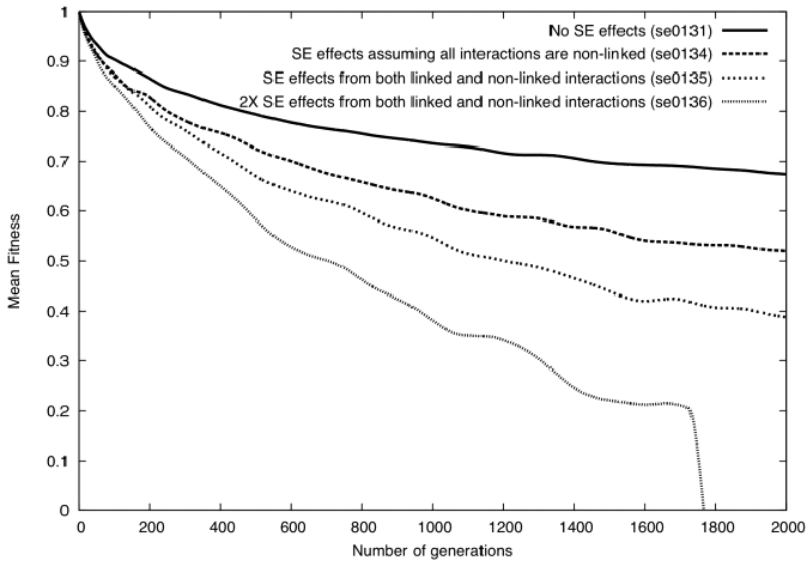
Large SE effects and modest selection pressure

We begin our exploration of the SE effects on fitness with SE scaling factors α and β that are large but with the selection pressure, controlled by fertility, relatively low. For a low level of selection pressure we chose a fertility of 1.1, which for a constant population size, implies that only 10% of the offspring in each generation do not reproduce. For SE scaling parameters we chose 10 for the non-linked mutation pairs and 2×10^4 for the linked mutation pairs, or 2000 times the non-linked scaling factor β. These parameter choices are about 100 times the maximum values we consider to be biologically realistic. What we found was that the effects on fitness after 2000 generations were too small to quantify, even though mean fitness due to normal mutation accumulation had decreased by 33%. Typically, we found that the mean number of accumulated mutations after 2000 generations was about 0.7% smaller with this level of SE relative to no SE. Despite the small effect on fitness, these values of 10 for β and 2×10^4 for α are likely still far higher than is realistic for most natural populations. Nevertheless, these experiments prompted us to explore what larger values for α and β were might reveal concerning SE behavior.

Let us consider cases with the same low selection intensity but with $\beta = 300$ and $\alpha = 6\times10^5$, both 30 times larger than before. Figure 1 displays the mutation accumulation and the population fitness histories for the following four cases: (1) no SE effects, (2) SE effects from non-linked interactions only, (3) SE effects from both linked and non-linked interactions, and (4) SE effects from both linked and non-linked interactions, but with both scaling factors doubled.



(A)



(B)

Fig. 1. Mutation accumulation (A) and the population fitness histories (B) for modest selection pressure and extreme SE interactions for four cases: (1) no SE effects, (2) SE effects from non-linked interactions only, (3) SE effects from both linked and non-linked interactions, and (4) SE effects from both linked and non-linked interactions, but with both scaling factors twice as large as in case (3). All cases apply probability selection, perfect genotypic heritability, and a fertility of 1.1, which implies 10% of the offspring in each generation do not reproduce in the next. The scaling factor for non-linked SE interactions in cases (2) and (3) is 3×10^2 and for linked interactions in case (3) is 6×10^5

Several features of these numerical experiments are readily apparent. First, the effects on mutation accumulation are relatively small given the large values of the SE scaling factors. With the mean mutation rate of 10 new mutations per offspring, if there were no selection, the average number of mutations per individual would be 20,000. The actual numbers of accumulated mutations per individual after 2000 generations for the first three cases are 19405, 18807, and 18763, respectively. The average number of accumulated mutations for case (3) is only 642 (3%) fewer than the case with no SE included, despite the large SE scaling factors. Also noteworthy is the fact that case (4) undergoes mutational meltdown at generation 1766 due to the strong deleterious SE effect on fitness.

These experiments show that it is possible, at least numerically, to make the SE effect sufficiently strong to drive a population to extinction. However, the scaling factors required for this to take place within 2000 generations are extreme.

Extreme SE effects and moderate selection pressure

In our next set of experiments we increase the selection pressure to a moderately high level. Instead of a fertility of 1.1, we choose a fertility of 2.0. This means that twice as many offspring are produced in each generation than are allowed to reproduce in the succeeding generation. That is, the selection process excludes half the offspring in each generation from reproducing in the next. For SE scaling factors we use 10^5 for non-linked interactions and 2×10^8 for linked interactions, and then examine a case with both scaling factors increased. Figure 2 displays the mutation accumulation and the population fitness histories for the following cases: (1) no SE effects, (2) SE effects assuming all interactions are non-linked, (3) SE effects from both linked and non-linked interactions, and (4) SE effects from both linked and non-linked interactions, but with scaling factors five times larger. The mean numbers of accumulated mutations after 2000 generations for the first three cases are 19570, 16510, and 16110, respectively. Cases (4) underwent mutational meltdown in generation 1960. We note that even with the SE effects exaggerated to this degree there is no hint that mutation accumulation can be halted, or even slowed to any significant degree, before mutational meltdown takes place.

Extremely exaggerated SE effects and extreme selection pressure

For this final set of cases we retain the fertility of 2.0, but instead of probability selection, we apply truncation selection. Truncation selection is artificial in that there is no randomness in the selection process. With a fertility of 2.0, each offspring

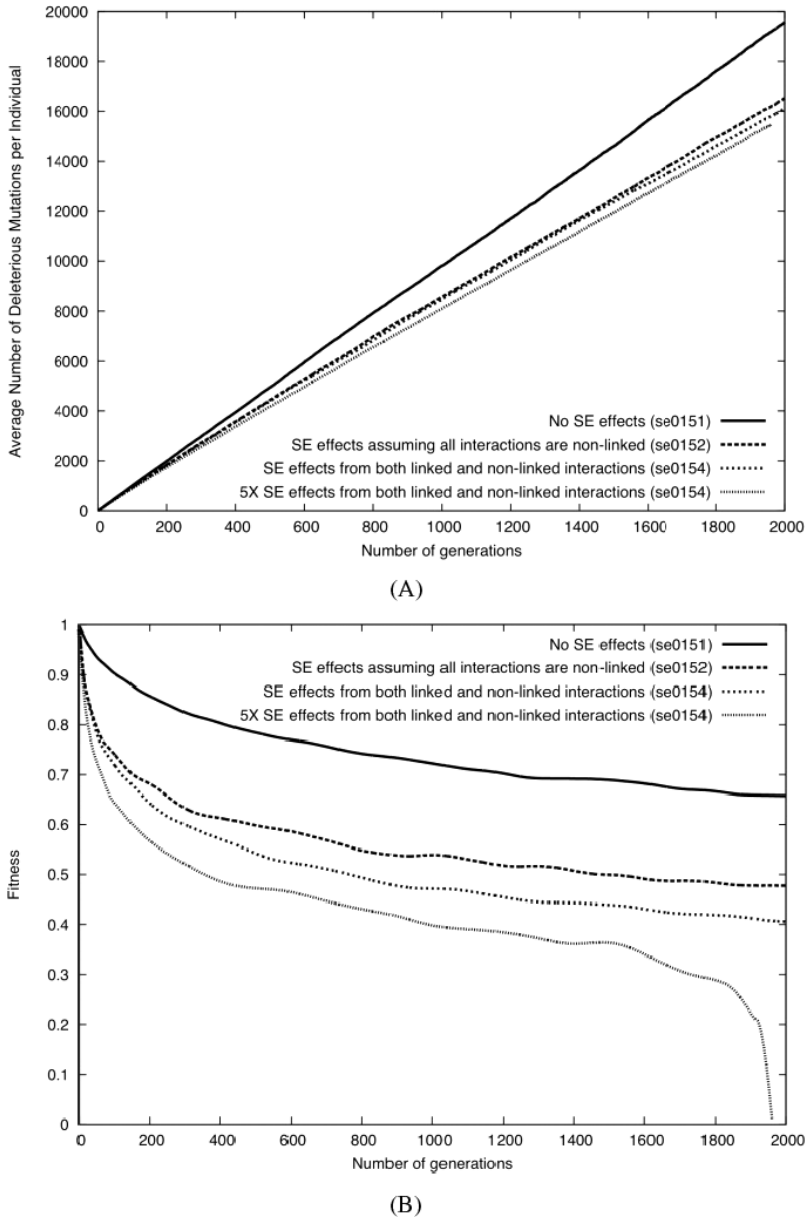


Fig. 2. Mutation accumulation (A) and the population fitness histories (B) for moderate selection pressure and extremely exaggerated SE interactions for cases: (1) no SE effects, (2) SE effects assuming all interactions are non-linked, (3) SE effects from both linked and non-linked interactions, and (4) SE effects from both linked and non-linked interactions, but with scaling factors 5 times larger. All cases apply probability selection, perfect genotypic heritability, and a fertility of 2.0, which implies half the offspring in each generation do not reproduce in the next. The scaling factor is 10^5 for non-linked SE interactions in cases (2) and (3) and 2×10^8 for linked interactions in case (3).

with fitness below the median value is selected away and does not reproduce in the succeeding generation, while each offspring with fitness above the median value does survive to reproduce. For SE scaling factors we use 5×10^5 for non-linked interactions and 10^9 for linked interactions, the same values that gave meltdown in the previous set of experiments. We also include a non-linked case with a scaling factor three times as large. Figure 3 displays the mutation accumulation and the population fitness histories for the following four cases: (1) no SE effects, (2) SE effects assuming all interactions are non-linked, (3) SE effects from both linked and non-linked interactions, and (4) SE effects assuming all interactions are non-linked with a scaling factor of 1.5×10^6. It is noteworthy that with truncation selection, fewer mutations accumulate for case (1) with no SE effects than for cases (2), (3), and (4) which include significant SE effects. The mean numbers of accumulated mutations after 2000 generations are 14388, 15480, 14510, and 14700 for these cases, respectively. In other words, instead of reducing mutation accumulation, SE actually *increases* the rate of mutation accumulation slightly in these experiments. This is almost certainly because SE increases the fitness variance considerably which makes the selection process less efficient. Also to be observed is that case (4) is in the process of mutational meltdown at generation 2000. These cases show persuasively that even with SE greatly exaggerated and selection efficiency also greatly exaggerated, SE fails to halt, or even slow, the accumulation of deleterious mutations.

Discussion

The importance of genic interactions

Like the letters in a text, nucleotides have meaning only within the context of other nucleotides, which is to say that nucleotides interact extensively. Such interaction between symbolic characters is the underlying basis for all language and all information systems. Functional genetic information is the basis of life and results from extensive networks of extremely specific, consistently positive, nucleotide-nucleotide interactions. Most mutations are deleterious because most represent disruptions of these networks of highly optimized sets of positive nucleotide-nucleotide interactions.

A given mutation's net biological effect arises from all of its actual interactions with other nucleotides within the genome. Each new mutation may have several or perhaps several dozen very specific significant interactions. A beneficial mutation is beneficial because it involves more positive total interactive effects than negative interactive effects. Most mutations are deleterious because, again, they disrupt

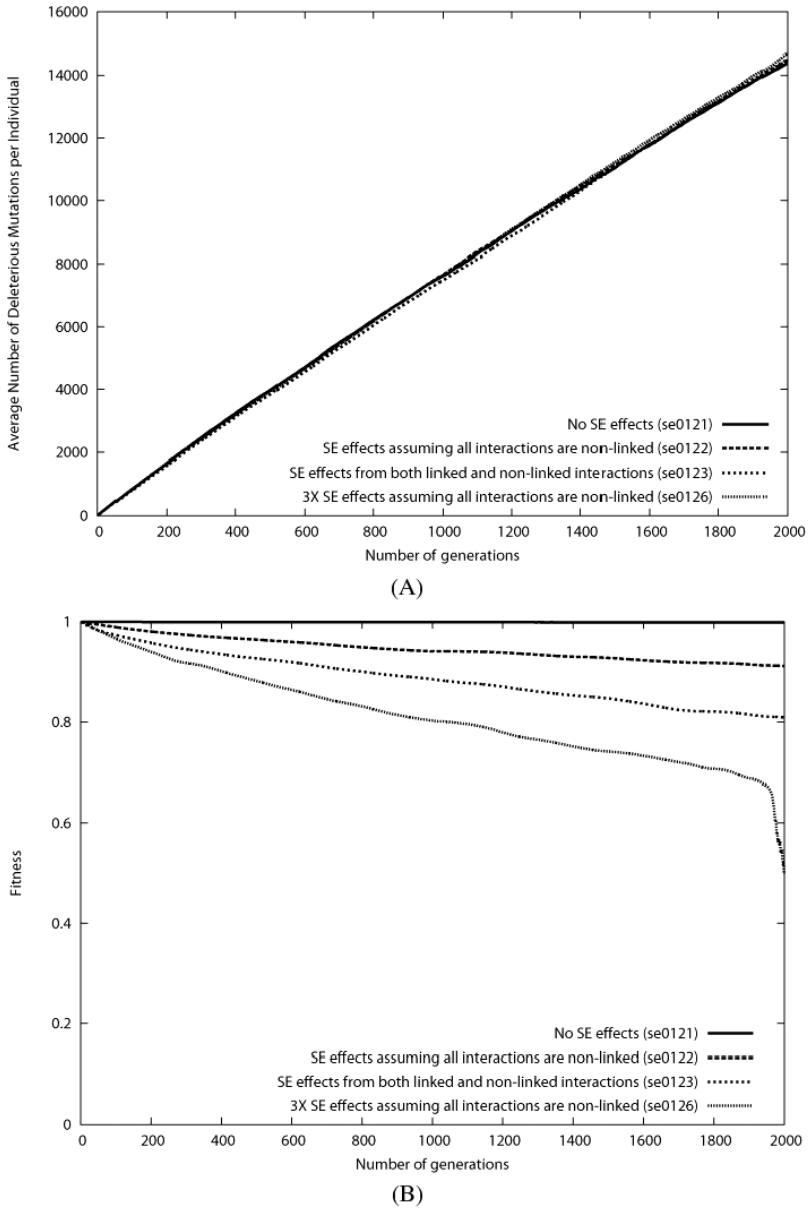


Fig. 3. Mutation accumulation (A) and the population fitness histories (B) for extremely exaggerated selection pressure and extremely exaggerated SE interactions for four cases: (1) no SE effects, (2) SE effects assuming all interactions are non-linked, (3) SE effects from both linked and non-linked interactions, and (4) SE effects assuming all interactions are non-linked, but with a scaling factor three times as large. All cases apply truncation selection, perfect genotypic heritability, and a fertility of 2.0, which implies 50% of the offspring in each generation do not reproduce in the next. The scaling factor for non-linked SE interactions for cases (2) and (3) is 5×10^5, for linked interactions in case (3) is 1×10^9, and for non-linked interactions in case (4) is 1.5×10^6.

or degrade existing highly specific positive nucleotide interactions that represent functional genetic specifications.

It is impossible to model all the possible interactions between nucleotides in a large genome. For example, the haploid genome of man has roughly 3 billion nucleotides. The number of potential pair-wise nucleotide interactions therefore is roughly 5×10^{18}. This is still an underestimate, because we are diploid and heterozygous at millions of sites, making the potential number of interactions even larger. Like widely spaced pairs of letters in a large book, the vast majority of nucleotide-nucleotide interactions surely have negligible effects. When there is a meaningful interaction, the biological effect can range from strongly negative to strongly positive. However, the vast majority of interactions that are not entirely neutral are surely still extremely subtle and nearly-neutral. We note that nearly-neutral interactions are beyond measurement, are not suited to empirical analysis, and therefore can be modeled only in a generic way.

The significance of SE

The primary reason that SE is of interest today is because it has been invoked as a mechanism that might possibly be able to halt mutation accumulation. This SE hypothesis, as we refer to it, has been embraced and advocated by several population geneticists, but it has never been demonstrated to work. In fact, the hypothesis is notably counterintuitive. In a non-selective setting, SE logically must accelerate genetic degeneration and lessen the time to extinction. This is because as deleterious mutations accumulate, SE guarantees that, on average, each new mutation must have a greater and greater deleterious effect.

However, it has been argued that, within a strongly selective setting, mutation accumulation might be halted if the SE effects were acute enough to activate what we refer to as the mutation-count mechanism (MCM). This mechanism requires selection to be strongly directed against those individuals within a population that have a higher mutation count than average. This conceivably might allow elimination of more mutations at less selective cost (that is, fewer individuals need be selected away). In a companion paper, we show that the MCM mechanism can operate only under certain highly artificial circumstances [9]. This special mechanism appears to be feasible only in sexually reproducing populations in which the range of mutational fitness effect variation is extremely narrow, the environmental variance is small, and truncation selection prevails. Arguably, these conditions never occur together in the natural world.

However, the MCM still might conceivably be activated, it has been argued, if extensive, strong, generic, non-linked SE interactions occur. Under such

circumstances, fitness reduction from SE interactions might increase at an ever accelerating rate (while mutation count is increasing at a more or less constant rate), such that a *mutation-count threshold* arises. Above such a threshold, additional mutations might result in catastrophic fitness loss, triggering very strong truncation selection. If the SE effect were strong enough, mutation count might conceivably overwhelm the factors which otherwise would dominate (such as mutation rate, the mutation effect values themselves and their distribution, and environmental variance). At such a mutation-count threshold, truncation selection based primarily on mutation count might then potentially halt mutation accumulation and stop mutational degeneration completely. At the point of such a threshold, a newly arising small-effect mutation might have the same impact as a nearly lethal mutation (because both affect mutation count the same), even though in realty they might differ in their biological effects by orders of magnitude.

Is the SE mechanism described above even technically feasible? This study was designed to answer that question. If the SE effects are not actually strong enough to create the required level of truncation selection based on mutation count, then the very SE interactions conjectured to save the genome will instead more rapidly destroy it.

Testing the limits of SE

To probe the limits of how well the SE mechanism might conceivably work, we performed numerical experiments granting the SE hypothesis every possible advantage: 1) we allowed *all* mutation-mutation interactions to be SE interactions; 2) we included no interactions that were multiplicative or involved antagonistic or general epistasis; 3) we neglected the effects of linkage entirely; 4) we applied perfect truncation selection and perfect heritability; and 5) we allowed SE effects to assume extreme values, far beyond what is biologically realistic. Cases (2) and (4) of Figure 3 incorporate all of these generous concessions.

Are these concessions reasonable? No. It is not reasonable, for example, to make all mutations interact synergistically, because the vast majority of mutations should not interact with each other at all. In the big picture, non-interaction should be the norm, and simple additivity should describe how most mutations combine. Moreover, interactions that behave in a multiplicative manner as well as antagonistic epistatic interactions contribute to fitness in a manner opposite to that of SE. Further, it is not reasonable to neglect mutational linkage. Almost all SE interactions should be between mutation pairs that are tightly linked. Zero linkage is therefore a major concession benefiting the SE hypothesis. We make

this concession simply because mutational linkage clearly neutralizes the mutation count mechanism [9]. When two mutations are linked, not only are the mutations inherited together but their SE effects are as well, and this results inexorably in accelerated fitness decline. Moreover, it is not reasonable to assume zero environmental noise (a heritability of 1) or to employ strict truncation selection. We make all these concessions only because in another paper we have already shown that the MCM is largely negated by low fitness heritability and probability selection [9].

Finally, although there should be a rational limit for how large each specific SE penalty should be relative to the basal, non-epistatic mutational fitness effect (as measured for a given mutation in an otherwise non-mutant genome), we allowed the amplitudes of the SE effects to become extreme. We showed earlier that the total SE fitness contribution in Mendel for non-linked mutations, assuming no linkage at all, is approximated by the expression $0.125\beta(1\text{-}F)2$, where F is the genotypic fitness. We applied this formula to show that, if the accumulated mutations in a given individual reduce its fitness to 0.5 without SE, then with SE and a value for β of 16, the fitness of this individual drops to zero. We argued that a biologically realistic value for β should plausibly be on the order of 0.1 or less. In our numerical experiments we see a discernible SE effect only when we use unrealistically exaggerated non-linked SE scaling (300 and 600 in Figure 1, 10^5 and 5×10^5 in Figure 2, and 5×10^5 and 1.5×10^6 in Figure 3). In these experiments the scaling factor values for the SE contribution were orders of magnitude beyond a plausible upper limit. This represents a major concession to the SE model, yet, instead of activating a strong MCM, the large scaling values led consistently to accelerated genetic decline.

Cases (2) and (4) of Figure 3 incorporate all of these features that strongly favor the SE hypothesis. What we observe is that even with all these highly unrealistic concessions, the mutation count per individual *actually increases slightly*, rather than decreases, relative to the case of no SE. Even with exaggerated selection efficiency, both forms of SE cause starkly accelerated fitness decline relative to the default case of mutation non-interaction. We found that in order to see any noteworthy SE effect at all, the SE scaling factors must be larger than anything that seems biologically reasonable. Even when we do this, we do not observe the effects which are so widely ascribed to the SE mechanism (halting of mutation accumulation and stabilization of fitness). Instead we see the opposite. If SE has any effect at all, it consistently makes genetic degeneration worse. The larger the SE effect, the more rapid is the degeneration. This agrees with the logical expectation of what should happen when there is the on-going accumulation of increasingly severe mutational damage.

Modeling SE realistically

To model SE realistically, the net SE effect must be only a slight deviation from the standard additive model, most SE interactions must arise from mutations within the same linkage block, individual SE effects must have reasonable limits, there must be small fitness heritability, and selection must be characterized primarily by the probability model. These constraints all reflect biological reality as we understand it. Modeling SE in accord with any one of these five constraints *negates* the SE hypothesis. When we model SE under what we believe are the most realistic conditions, we consistently see no meaningful SE effect on either mutation accumulation or fitness decline. We feel this reflects biological reality; that is, generic SE effects are necessarily small, are strongly overshadowed by much more significant biological phenomena, and do not affect mutation accumulation in any significant way.

Pros and cons of the SE hypothesis

It might be argued that logically there should always be some selection against high mutation count individuals, so this should help slow mutation accumulation. In particular, the SE mechanism should create an increased penalty against the high mutation count individuals, strengthening the potential MCM. The problem with this line of reasoning is that, while higher mutation count will have some correlation with lower fitness, this correlation under natural conditions will be extremely weak. The major reason for this weak correlation is the large variation in the magnitude of mutation fitness effects. Some mutations have substantial effects, but most have small to vanishingly small effects. Individuals in a population with random mating should all have approximately the same number of mutations, due to averaging. Moreover, most mutations are nearly neutral. The primary reason some individuals display reduced fitness relative to the others is due to only a few substantial mutations and not because of some small difference in total mutation count. Realistic numerical simulation consistently confirms that this is true [this paper and 7–9].

Cases of genuine SE genetic interactions are well documented. Most involve the interactions of relatively large-impact mutations, usually within the same gene or same pathway and affecting a single trait. These specific examples of SE should not be interpreted to imply, however, that SE effects arise from interactions from *every* pair of mutations throughout the genome. Naturally, high impact mutations can be expected to produce a few strong and measurable interactions, some of which will be synergistic. The interactions among such mutations, as

well as the mutations themselves, are then highly selectable. For a simple trait whose character is determined by only few genes, each gene is highly significant relative to that trait. In a sense the "genome" for that trait is small, which makes every mutation in that limited system potentially significant. Because the "genome" is small, the likelihood that two mutations within it will display an SE interaction is larger that it would be otherwise. However, in a large functional genome with billions of nucleotides, which encode for thousands of traits, the likelihood that mutations in distant parts of the genome will have significant mutual SE interaction is tiny.

Experimental evidence of generic genome-wide SE in living populations has been inconclusive [30, 31]. The inferred absolute amplitudes of generic SE effects are small. These studies on the extent of generic SE in natural populations in no way support a conclusion that the SE mechanism acts to slow genomic degeneration. Our own analyses consistently show that regardless of the extent of generic SE in a genome, SE consistently accelerates degeneration and does almost nothing to slow mutation accumulation.

The SE hypothesis is that SE interactions cause truncation selection at a critical threshold, such that any further mutation (even the lowest impact mutation) acts essentially as if it were lethal. If SE stabilizes genomes and stops genomic degeneration in this way, then constant and intense selection must operate just below that threshold, such that any additional mutations will be severely detrimental. This means that the population stabilizes just a few mutations short of disaster (mutational meltdown). Another way of saying this is that the population is stabilized against mutational meltdown/extinction by maintaining itself on the verge of extinction. Ironically, in this state of extreme selective tension, an improvement in environmental conditions (e.g., good weather, fewer predators) could result in significantly relaxed selection, which could lead to mutation accumulation beyond the threshold, which could then lead to extinction in the more favorable environment. This seems more than counterintuitive. It is, in reality, entirely unreasonable. How could any population remain balanced on such a knife edge for millions, or even thousands, of generations?

Numerous mutation accumulation experiments have been performed involving a laboratory population of plants or animals placed in a state of relaxed selection for many generations. Such experiments cannot truly eliminate selection (there is always selection for embryo viability and fertility), but selection can be greatly reduced. Usually, the observed fitness decline is slow and gradual [32], consistent with very limited levels of SE. In the few cases where degeneration was more accelerated [27], it can readily be attributed to a few major interactions between a few high impact mutations (major mutations are naturally expected to have major interactions).

Genetic bottlenecks, often invoked in evolutionary scenarios, result in greatly reduced selection (because genetic drift overrides selection when population size is small). This also ought to result in mutation accumulation past the critical SE threshold, causing mutational meltdown and rapid extinction. Since such SE-induced meltdown is generally not thought to occur, this also seems to argue against the SE hypothesis.

Therefore, many lines of evidence, based upon both logic and biological data, argue strongly against the SE hypothesis. These evidences have now been validated by the numerical simulations carried out in this study. Our findings are consistent with the findings of Butcher [19], but apply to sexual as well as asexual species. While any one of these individual lines of evidence by itself might be insufficient to discredit the SE hypothesis, taken together they constitute an overwhelming case against the SE hypothesis, strong enough in our view to constitute falsification.

A very recent paper by Crow [33], forcefully argues against any significant role for epistasis in affecting selection efficiency. This would seem highly significant because the same author has for decades been a leading proponent for theoretical mechanisms that might resolve the mutational degeneration paradox, including the MCM and SE hypotheses. Crow now states, "My main objective here is to show that the breeders' practice of ignoring epistasis in quantitative selection is fully justified…In general, the smaller the effects, the more nearly additive they are. Experimental evidence for this is abundant…Multiple factors with individually small effects acting in a near-additive manner seem to be the rule… although there may be large dominance and epistatic components, selection acts only on the additive variance…For these reasons, one would expect that epistatic variance would have only a small effect on predicting the progress of selection…Any attempt to include epistatic terms in prediction formulae is likely to do more harm than good."

In summary, there appears to be neither theoretical nor observational support for the idea that a generic SE mechanism exists in nature capable of halting mutation accumulation or of stabilizing natural populations against mutational meltdown. Given that the SE hypothesis has so many glaring problems, one might ask how it ever became widely accepted. The SE hypothesis seems to have been proposed solely as a possible means for dealing with one of the as yet unsolved difficulties for the classic neo-Darwinian model. It appears to have become widely accepted only because no alternative mechanism could be identified that might conceivably stop deleterious mutation accumulation. We suggest that until a more credible mechanism can be discovered for halting deleterious mutation accumulation, the genetic degeneration problem should most honestly be described simply as a paradox that is yet to be explained.

Conclusions

1. Theoretical considerations show that SE should not be able to stop mutation accumulation. It has already been shown in our companion paper that with any realistic distribution of mutational fitness effects, the mutation count mechanism (MCM) does not operate and is of no avail in stopping deleterious mutation accumulation [9]. There is no theoretical basis for thinking that SE could stop mutation accumulation, even if it could activate the MCM effect. In this paper we show that for both linked and non-linked mutations, SE simply serves to *amplify* the fitness effect differences among mutations whenever the SE effect is directly related to the base, non-epistatic effect. In the case of linked mutations, the SE effects, like the linked mutations themselves, are inherited generation to generation, and therefore act simply as enhancements to the basal, non-epistatic mutational fitness effects. We show that the same is true of the non-linked SE interactions. Because of these enhancements to the basal mutation fitness effects, in both cases SE therefore logically can only serve to accelerate fitness decline and hasten mutational meltdown.
2. Consistent with simple logic, this paper's careful numerical simulations suggest that SE does nothing to halt mutation accumulation. In fact, even numerical experiments using truncation selection and perfect genotypic heritability show SE slightly *enhances* mutation accumulation. To the extent that SE has any noteworthy effect at all, it consistently accelerates degeneration. When realistic levels of linkage are included, this degeneration is accelerated even more.
3. If somehow these first two conclusions were not valid and the SE hypothesis were actually true, all species should mutate right up to the brink of their mutation-count threshold. Biological observations, however, do not support any type of mutation count threshold. In nature, if the SE hypothesis were true, any relaxation of selection pressure (a more favorable environment or a bottleneck episode) would be expected to cause rapid extinction. Likewise, lab mutation accumulation experiments, wherein selection is artificially relaxed, would be expected to result in rapid and catastrophic fitness meltdown. Neither result has ever been observed.
4. The SE hypothesis seems to have been proposed solely as a possible means for dealing with one of the as yet unsolved difficulties for the classic neo-Darwinian model. It appears to have become widely accepted only because no alternative mechanism has yet been identified that might conceivably stop deleterious mutation accumulation. The genetic degeneration problem remains unresolved.

Addendum – *Since the finalization of this chapter, a significant new paper has been published. See: Sanford, J. & Nelson, C. (2012). The Next Step in Understanding Population Dynamics: Comprehensive Numerical Simulation, Studies in Population Genetics, in: M. Carmen Fusté (Ed.), ISBN: 978-953-51-0588-6, InTech, Available from: http://www.intechopen.com/books/studies-in-population-genetics/the-next-step-in-understanding-population-dynamics-comprehensive-numerical-simulation .*

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