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# INDIRECT SELECTION ON THE MUTATIONAL LANDSCAPE:

## AN EVOLVED ROLE FOR THE MUTABILITY OF REPETITIVE DNA?

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## ABSTRACT

Computer simulations reveal an abstract **mutational landscape**, analogous to the familiar "adaptive landscape", in which long-term survivorship is a function of mutability. On a mutational landscape, the average fitness of a population over many generations depends both on the prevailing mutation rate and on the typical range of fitness effects that individual mutations may have. When these mutability parameters are themselves heritable and permitted to vary by mutation, they can evolve in predictable ways. **Indirect selection** occurs when alleles governing mutability parameters are eliminated or retained in a population through consistent association with the deleterious or beneficial traits which result from their action. Even if selection never acts directly on mutational mechanisms, indirect selection can nevertheless move a population uphill on the mutational landscape. Under conditions of persistently suboptimal fitness when there is a recurring opportunity for advantageous mutational variation, mutability can evolve toward a peak on the mutational landscape

where mutations are frequent and each mutation has only a small quantitative impact on fitness. However, indirect selection is effective only when the parameters which govern mutability are hereditary and closely linked to the resulting mutations.

Certain patterns of repetitive DNA, particularly the simple sequence repeats (microsatellites and minisatellites), have properties which correspond closely to those that are favored by indirect selection in these simulations. These properties, which assure an abundant supply of quantitative genetic variation, suggest that simple sequence repeats may have evolved by indirect selection to function as mutationally adjustable regulators of gene activity. Thus the presence and phylogenetic distribution of genetically variable microsatellite sequences may indicate the location of regulatory loci with significance for recent or ongoing evolutionary change in quantitative traits.

## INTRODUCTION

Conventional evolutionary theory presumes that "mutations are accidents and accidents will happen" (Sturtevant 1937). However, research over the last three decades has revealed many complex mutational mechanisms and thereby stimulated a growing appreciation that not all mutations are properly characterized as accidental (Arber 1995). Indeed, "the most successful genomes may be those that have evolved to be able to change quickly and substantially" (Pennisi 1998). Mechanisms which govern the rate and pattern of mutation may be selected indirectly if they are inherited along with favored alleles, but the consequences of indirect selection have not yet been intensively investigated.

An evolved role for repetitive, microsatellite DNA in mutational modification of gene regulation is suggested by several properties of these simple sequence repeats. Simple sequence repeats are widespread in eukaryotic genomes. They are often integrated into functional gene complexes, where

the number of tandemly repeated units can exert a quantitative effect on gene activity (Kashi, King and Soller 1997). The most common mode of mutation in simple sequence repeats is the addition or subtraction of a single repeat unit. These step-wise mutations typically have only a small impact on fitness. Such mutations are both frequent and fully reversible; they can supply abundant quantitative variation with minimal risk of severely deleterious effect, prompting the characterization of simple sequence repeats as "evolutionary tuning knobs" (King, Soller and Kashi 1997) or "agents of evolution" (Moxon and Wills 1999). This mode of mutation is intrinsic to repetitive DNA. Mutation rate and typical mutation size are both based on sequence features such as motif length and purity of motif repetition. Since these parameters are both heritable and site-specific, they are therefore subject to evolution by indirect selection (King and Soller 1999).

In this paper, computer simulations reveal how mutability can affect fitness and evolve by indirect selection. In these simulations, indirect selection in a fluctuating environment can favor mutability parameters which are similar to those of simple sequence repeats.

## METHODS

Genetic changes in an evolving population are simulated using a simple Mendelian model of population genetics with mutation. Basically, alleles at one locus govern one quantitative trait. Fitness, or probability of genotype survival, is based on this single trait. The alleles at this trait-determining locus are also subject to mutation. Two other loci determine the parameters of mutation, specifically the rate of mutation and the range of allele values that can result from individual mutations. More detailed features of the model are listed below.

The simulations are carried out by a computer program written in QuickBasic 4.5. All stochastic events, including mutation of individual alleles, independent assortment of alleles into gametes, fusion of gametes to form new genotypes, and survival of individual organisms, are based on QuickBasic's random number generator.

**Population model:** The simulated population consists of diploid, sexually reproducing, hermaphroditic, randomly mating organisms with non-overlapping generations. Each organism is modelled as a discrete individual genotype, which may or may not survive to contribute gametes to the subsequent generation. At each generation, gametes from surviving parents come together at random to produce 1000 offspring which are then subjected to selection before becoming parents themselves.

**Genetics:** Each genotype consists of two alleles at each of three polymorphic loci. The genotype at one of these three loci determines the value for a single, quantitative trait. Each allele at this single, trait-determining locus has a quantitative value; the two allele values present in each genotype are summed to determine the value of the associated trait. Alleles at the other two loci govern mutability parameters; their only effect occurs during the gamete stage when they determine the rate and the range of mutation (see below) at the single associated trait-determining allele. The two mutability loci may be tightly linked to the single trait locus (i.e., with no recombination), or these three loci may assort independently (as if located on three separate chromosomes).

**Selection and environment:** Selection is hard, eliminating individuals and thus reducing the size of the standing population. Fitness for each genotype is simply the probability of survival for organisms having the trait value associated with that genotype. The environment determines a single, optimal

trait value for which survival probability is 1.00; all other trait values confer a lower survival probability. The survival probability for a particular trait value decreases as a function of the difference between this value and the optimal trait value. [At a difference of three units of trait value, fitness will be reduced by about one-half.]

The environment (i.e., the optimal trait value and hence the selection coefficient for each genotype) is itself a function of time, measured in generations. The environment can remain constant or can fluctuate from generation to generation. Environmental fluctuations may be regular (sinusoidal) or random. For regular fluctuations, the period may be short or long and the amplitude may be large or small.

Alleles at the two mutability loci play no role whatsoever in the survival of a genotype, in the production of gametes, or in the successful fusion of gametes to form new individuals.

**Mutation:** Mutations occur during the haploid gamete stage and produce a random change in allele value. Two mutability loci determine respectively the rate and the range of mutation at the single trait-determining locus; thus these mutability parameters are both hereditary. The *mutation rate* for the trait locus is the *per-generation probability* that a mutation will occur in any particular trait-determining allele. The *mutation range* for the trait locus is the *maximum change* in allele value that can result from a single mutation.

Very large mutations are always severely deleterious; even when mutations have only small effect on the trait locus, at least half will be nominally deleterious by shifting the allele away from the current optimum. The mutability loci may themselves have fixed (monomorphic) allele values, or these alleles

may be polymorphic and subject to infrequent random changes. [The probability that such changes will yield specific values for mutated mutability parameters is uniformly distributed on log scale.]

Each experiment simulates the evolution of a single population over many generations. In every experiment, allele values for the trait-determining locus are free to mutate. Alleles for the mutability loci can either be preset to a single fixed value or else can be free to mutate with a probability of $10^{-3}$ per allele per generation. Environmental conditions, whether constant or fluctuating, are established at the start of each experiment.

To map the **mutational landscape** associated with particular environmental conditions, an array of experiments is conducted under those conditions. In each experiment of the array, the parameters of mutation rate and mutation range are preset at values which are varied systematically from experiment to experiment. The frequencies of the trait-determining alleles then evolve in the chosen environment, while survivorship of the population is averaged over many generations. Average survivorship for the entire array of experiments is plotted as a function of the preset mutation rates and mutation ranges, as illustrated in **Figure 1**. Such plots have been constructed for experiments representing a variety of environments, including uniform, unvarying conditions as well as rapid, moderate, slow and random fluctuations at high and low amplitudes.

**Indirect selection** is demonstrated by experiments in which the mutability alleles (i.e., alleles at the two loci that govern the parameters of mutation rate and mutation range) are not preset to fixed values but are free to mutate. A population is initialized with uniform values for these mutability alleles and then allowed to evolve over several thousand generations. At the end of the experiment, the predominant mutability alleles are recorded. Such experiments have been conducted several times for

a variety of environmental conditions, both with tight linkage among loci and with independent assortment among loci, as illustrated in **Figure 2**.

In all experiments, it is important to note that survival of an individual organism is determined *only* by the value of a single quantitative trait. Mutability loci play *no* direct role in determining the survival or reproductive success of individual genotypes. Alleles at these loci are selected only indirectly, when they occur in genotypes whose survival probability is based on alleles at the single trait-determining locus.

## RESULTS

**Alleles at the trait-determining locus evolve by direct selection:** In each experiment, the frequencies of trait-determining alleles evolve from generation to generation. In each generation, those genotypes are favored whose associated trait values most closely approximate the optimal trait value for that generation. Overall survivorship in each generation depends on the range of genotypes produced by extant alleles at the trait-determining locus.

Under constant environmental conditions one particular allele will increase in frequency. This allele constitutes the homozygous genotype whose associated trait value is optimal for the constant environment. Unsurprisingly, as this most-fit allele approaches fixation, survivorship approaches the maximal value of one hundred percent. Any other alleles, including newly arising mutants, will then necessarily produce genotypes which depart from the optimal trait value and will therefore be selected against. The genetic load associated with any mutability will thus cause some reduction below maximal survivorship.

However, if the environment is changing, the genotypes produced by previously-selected alleles become less fit with each passing generation so overall survivorship declines. If mutant alleles that can form fitter genotypes do arise, their frequencies will increase, as will survivorship, at least until further environmental change reduces adaptation once again. Under such circumstances, the gene pool continually evolves by mutation and selection as the population continually adapts to new conditions. How closely the predominant genotypes track the environmental optimum depends not only on the rate of environmental change and the strength of selection but also on the diversity of available alleles. This genetic variation depends in turn on the rate and the range of mutation.

**Survivorship depends on mutation rate:** Average survivorship over many generations depends on the rate at which mutations alter individual trait-determining alleles. As noted above, in a constant environment direct selection eventually brings a single allele close to fixation. From that point on, average survivorship will be greater when mutation rate is lower. However, the situation becomes more interesting with a changing environment. Selection in a slowly changing environment eventually exhausts preexisting variation. As change continues, survivorship declines with each subsequent generation until new mutations provide access to more-fit genotypes. Although survivorship can be very high when available alleles produce optimally-fit genotypes, survivorship can decline precipitously as conditions change. The occurrence of advantageous mutations permits high fitness to be regained, at least for limited periods. However, if such mutations arise too infrequently (much less than $10^{-3}$ mutations per generation) the average survivorship over many generations is significantly depressed. In contrast, a moderately high mutation rate (in these simulations, greater than $10^{-2}$ mutations per generation) provides an abundant supply of variation for adaptive response to changing

conditions. Such a high mutation rate steadily depresses survivorship through the occurrence of deleterious mutations. But survivorship can nevertheless remain quite stable and reasonably high while the environment is experiencing substantial fluctuations. Frequent deleterious mutations do increase the genetic load. However, average survivorship with a moderately high mutation rate may nevertheless exceed that attained with a very low mutation rate.

**Survivorship depends on mutation range:** Average survivorship over many generations also depends on the range of mutation. If mutation range is large (i.e., if a given mutation can cause a change in allele value that may range from very small to very large), then most mutant alleles will be severely deleterious regardless of environmental conditions. Survivorship will be correspondingly depressed in proportion to mutation rate. However, if mutation range is small (i.e., if all mutations cause only small changes in allele value), then the occurrence of many mutant alleles will have a less severe impact on survivorship. Furthermore, if the environment is changing so that previously favored alleles can no longer constitute genotypes associated with high survival probability, then any mutation which produces only a small change in allele value stands an approximately even chance of being beneficial. Therefore, a moderately low range for mutational effect interacts with a moderately high mutation rate to confer average survivorship which is both high and stable.

**The Mutational Landscape:** If a particular rate of mutation and a particular range of mutation are fixed at the start of an experiment, the resulting average survivorship over many generations will be a function of these two parameters. The results of many such experiments, with systematic pairing of

different values for rate and range mutation, can be presented as a three-dimensional surface to illustrate how average survivorship depends on mutation rate and mutation range.

**Figure 1** shows two examples of such plots, which may be conceptualized as "mutational landscapes". Higher points on each landscape indicate regions of higher average survivorship. In all of the tested conditions involving a fluctuating environment (i.e., rapid, moderate, slow, and random; mild and severe), the mutational landscape is highest where the mutation rate is moderately *high* and the mutation range is moderately *low*. Very low survivorship along the right edge of the mutational landscape reflects the genetic load associated with an exceedingly high rate of severely deleterious mutation. The depressing effect of high mutation rates, especially when most mutations are deleterious, as well as the steep increase in survivorship as mutation rate decreases, matches conventional expectations. More interesting is the decline in survivorship when mutation rate decreases too far. Reduced survivorship at very low mutation rates reflects the scarcity of alleles capable of constituting highly fit genotypes, so that there is inadequate variation for repeated adaptation to environmental changes. The decline in survivorship with very low mutation rates may be either precipitous (as illustrated in **1a**) or gentle (**1b**), depending on the severity of environmental fluctuations. In the extreme case of a constant environment, survivorship does not decline but asymptotically approaches one hundred percent as mutation rate declines toward zero. But in any fluctuating environment, survivorship at extremely low mutation rates remains significantly and consistently depressed relative to the peak of the landscape.

The basic result illustrated in **Figure 1** is quite robust. Although the precise shape of the mutational landscape varies with the frequency and amplitude of environmental fluctuations (including random fluctuations), plots of the mutational landscape indicate that average survivorship is always

highest for a moderately high mutation rate (more than $10^{-3}$ mutations per generation), regardless of mutation range. Even when the vast majority of mutations are severely deleterious (i.e., when mutation range is large), average survivorship is still highest with a moderately high mutation rate. Similarly, at least for moderately high mutation rates, average survivorship is always highest for moderate low values of mutation range. This simply means that frequent mutations can maximize the efficiency of ongoing adaptation to changing conditions, while small mutation size can minimize the associated cost. Stated even more succinctly, *mutability is advantageous in a changing environment.*

**Indirect Selection:** If values for mutation rate and mutation range are not preset and unchangeable but are themselves permitted to vary, the frequencies of the alleles which determine these parameters will evolve over time. In a constant environment, unsurprisingly, mutation rate evolves to the lowest available value. At that point, mutation range becomes irrrelevant and evolves by drift. However, as with the mutational landscape described above, a changing environment produces more interesting effects. In **Figure 2** the predominant allele values for mutation rate and mutation range are plotted on the mutational landscape, for several experiments in which these two mutability loci were permitted to vary and evolve for several thousand generations. When the mutability loci are free to assort independently (**open circles**), predominant allele values may remain near the initial allele values (e.g., **2a**) or move toward lower mutation rates, with associated lower levels of average survivorship (**2b**). Indeed, if the amplitude of environmental fluctations is relatively large, then with unlinked loci extinction of the population is likely (**crosses** in **2a**). However, when these two loci are tightly linked to the locus which determines trait value (**solid circles**), the parameters which they govern converge consistently on values near the peak of the mutational landscape (both **2a** and **2b**). This result is

typical, regardless of the detailed shape of the mutational landscape. *Indirect selection can effectively increase the predominant mutation rate within a population, but only when mutability loci are tightly linked to the trait-determining locus.*

## DISCUSSION

With either a constant or a changing environment, any mutability alleles that are repeatedly associated with less-fit genotypes will be slowly eliminated from the population. However, the converse is also true. Any mutability alleles that are repeatedly associated with successful genotypes will be retained within the population. In either case, selection acts *directly* only on the phenotypic traits produced by genotypes. But the process by which alleles governing mutability parameters are eliminated from or retained within a population, through consistent association with the deleterious or beneficial traits, may accurately be characterized as *indirect* selection. In light of the prevailing view that "natural selection of mutation rates has only one possible direction, that of reducing the frequency of mutation to zero" (George Williams, 1966), one might wonder whether indirect selection could find a favorable balance between the costs and the benefits of mutation. The simulations reported here demonstrate that as long as mutability alleles are tightly linked to any resultant mutant alleles, *the process of **indirect selection** can indeed be quite effective at leading a population toward a peak of the **mutational landscape** where average fitness over many generations is maximized.*

Unsurprisingly, indirect selection in a constant environment will favor mutability alleles which minimize the cost of deleterious alleles by minimizing the mutation rate in the population. However, in a fluctuating environment indirect selection will minimize this cost by favoring both a moderately high mutation rate and a low mutation range. Note that indirect selection for mutability operates even

when the immediate survival of individual genotypes is unaffected by mutability parameters. In other words, although mutability alleles may be invisible to direct selection, over the long run favorable mutability alleles can be selected indirectly but nonetheless effectively by linkage to the alleles that do directly promote immediate survival. However, these simulations also demonstrate that linkage is critical. *Indirect selection can promote maximal long-term fitness, at the peak of the mutational landscape, only when mutability alleles are tightly linked to the resulting mutants.*

Significantly, selection *against* mutability alleles is far less effective than selection *in favor of* such alleles. This is because direct selection can only eliminate deleterious alleles one at a time as they arise but can sweep a single beneficial mutation to fixation. Suppose, for example, that two mutability alleles occurred at similar frequencies within a large population, one yielding a relatively high mutation rate of $10^{-2}$ mutations per generation and other yielding a much lower mutation rate of $10^{-8}$. If *every* mutation at a tightly linked locus were lethal (with a fitness of zero), one percent of the high-rate alleles would be eliminated in each generation (in effect, the high-rate allele would have a fitness of approximately 0.99 relative to the low-rate allele). Many generations would pass before that allele could be selectively eliminated from the population, and many mutations would arise from the action of the high-rate allele. In contrast, if *any* beneficial mutation arose during those many generations, even only one, direct selection favoring that beneficial mutant could promptly fix the high-rate allele in the population. This curious asymmetry assures that alleles promoting mutability can endure through repeated spells of adverse selection, as long as the beneficial effects of mutability do have some occasional opportunity to prevail.

Although the basic idea of a changeable environment is not implausible, its idealized simulation by regular sinusoidal fluctuations certainly is unrealistic. However, this convention was adopted

primarily to demonstrate most clearly the process of indirect selection. The basic shape of the mutational landscape appears to be similar regardless of the frequency or amplitude of regular fluctuations, and also for at least those few patterns of random fluctuation that have been tested. Furthermore, *any* circumstance which creates some advantage for newly arising genetic variation may reasonably be expected to sustain indirect selection favoring mutability. Such circumstances encompass any persistent change in the physical or biotic environment, including such special cases as competitive escalation or evolving symbiosis. Appropriately constrained mutability may also be advantageous in heterogenous environments, especially in conjunction with small populations where beneficial variation may repeatedly be lost to drift. Even in the presence of relatively constant external conditions, complex traits governed by functionally integrated sets of genes could evolve indefinitely through recursive, reciprocal coadaptation, and thereby drive indirect selection for appropriately constrained mutability in each of the affected loci.

Although the genetic model for these simulations is based on simple Mendelian principles, several key features may appear quite unconventional. These include not only the mere existence of heritable alleles governing mutation rate and mutation range, but also the tight linkage between these mutability alleles and their associated trait-determining loci. However, all of these features are characteristic of at least some real genes, particularly genes incorporating the simple sequence repeats of microsatellite DNA. Repetitive DNA can "slip" during replication, so that new alleles may include more or fewer copies of the repeating motif. The size of a single-step mutation depends on the length of the repeating motif. How readily such mutations occur depends on the number of individual base pair substitutions that modify the repeating motif, as well as on the length of the repeating motif itself (reviewed in King and Soller 1999). Both the mutation rate and the typical mutation size that are associated with this

mechanism are thus consequences of the repeating sequence itself. They are therefore both site-specific and heritable, so they are susceptible to heritable, site-specific variation. Furthermore, when such a sequence is integrated into a functional gene complex, the number of repetitions can exert a quantitative influence on an associated trait (Kashi, King and Soller 1997). Quite plausibly, then, alleles with different effects on phenotype can share similar mutability characteristics, while alleles with different mutability characteristics can share similar effects on phenotype (King and Soller 1999).

Not only can the three distinct parameters of mutation rate, mutation range, and quantitative phenotypic effect all be heritable, in principle they can all vary independently at a single locus. A single repetitive DNA locus can encode phenotype by the number of repeats; it can encode typical mutation size by the length of the repeating motif; and it can encode mutation rate by the purity of repetition. And since all three parameters depend on the same locus, all three are absolutely linked together. Inheritance of an individual simple sequence repeat allele will involve not only inheritance of that allele's particular effect on gene expression but also its particular rate and typical size of mutation, the same parameters by which that individual allele itself originated. These are exactly the properties which are required, in the simulations reported here, for effective indirect selection on the mutational landscape.

This would be a remarkable set of properties for any gene, but it is not necessarily unique to simple sequence repeats. As long as the mutability of a gene is related to that gene's specific base pair sequence, the mutability parameters will be site-specific and heritable and therefore susceptible to indirect selection. This should be the case whether mutability is intrinsic to the DNA itself (as it apparently is for simple sequence repeats) or governed by enzymes which recognize specific binding sites. As reported by Trifonov (1989), even within protein coding genes there is room for additional

functional properties to be encoded, for example by variation in the third base position of synonymous codons, without altering the specific gene product. *A single gene may have multiple "meanings", including both phenotypic function and also mutability. These meanings may be independently encoded, independently variable, and independently selected.*

## CONCLUSION

Under the conditions of these simulations, indirect selection is an effective agency for shaping mutability. The mutability parameters which are indirectly selected in these simulations are similar to those which characterize the simple sequence repeats of microsatellite DNA. Indirect selection may also be an effective evolutionary process under natural conditions. Site specific modes of mutation may be shaped by selection acting indirectly, through the long-term success that is provided by a reliable supply of low-cost genetic variation. The peculiar characteristics of simple sequence repeats may indeed reflect an indirectly-selected function of mutability, such that genes regulated by these elements are evolutionarily adjustable. Such a "tuning knob" function for simple sequence repeats (King, Soller and Kashi 1997) supports and adds a new level of quantitative interpretation to E.H. Davidson's proposal that "the evolutionary plasticity of repeat sequence location in the genome could be regarded as a source of possibilities for new ontogenetic regulatory patterns" (Davidson 1982). The presence and phylogenetic distribution of simple sequence repeats may indicate the location of loci whose evolutionary adjustment is adaptively significant. Furthermore, the concept of indirect selection may find far more general application, as the basis for understanding a variety of genomic and developmental patterns which confer evolutionary adaptability.

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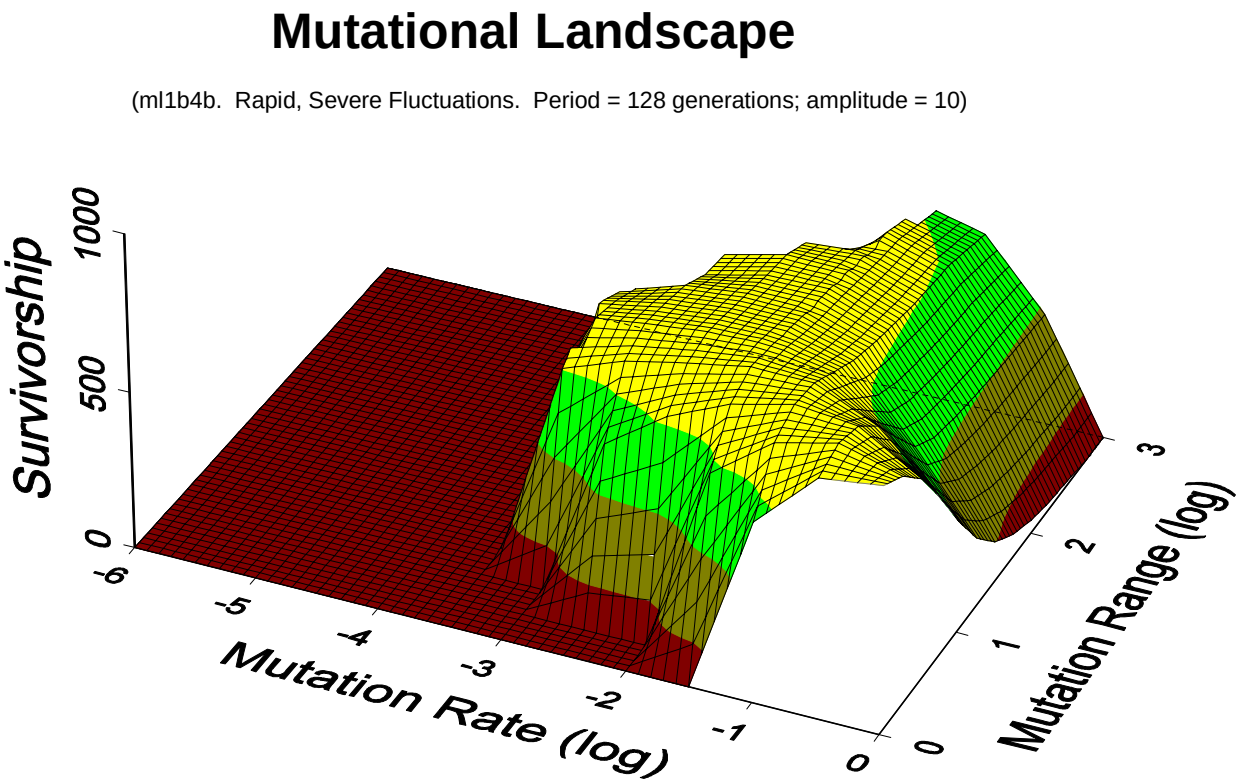


**Figure 1a.** Three dimensional plot of survivorship (averaged over 1536 generations) as a function of mutation rate and mutation range. 169 simulations were conducted, at intervals of 0.5 on the log scale

for mutation rate and 0.25 on the log scale for mutation range. Between these points, the surface was smoothed by linear interpolation. For each of the simulations, the environment fluctuated sinusoidally with a period of 128 generations and an amplitude of plus or minus 10 arbitrary units. The same arbitrary units were used to measure the impact of mutation on fitness. (A mutation range of 2 on the log scale means that any individual mutation could add or subtract any value up to 100 units to the original value of the mutating allele.) See text for further explanation.

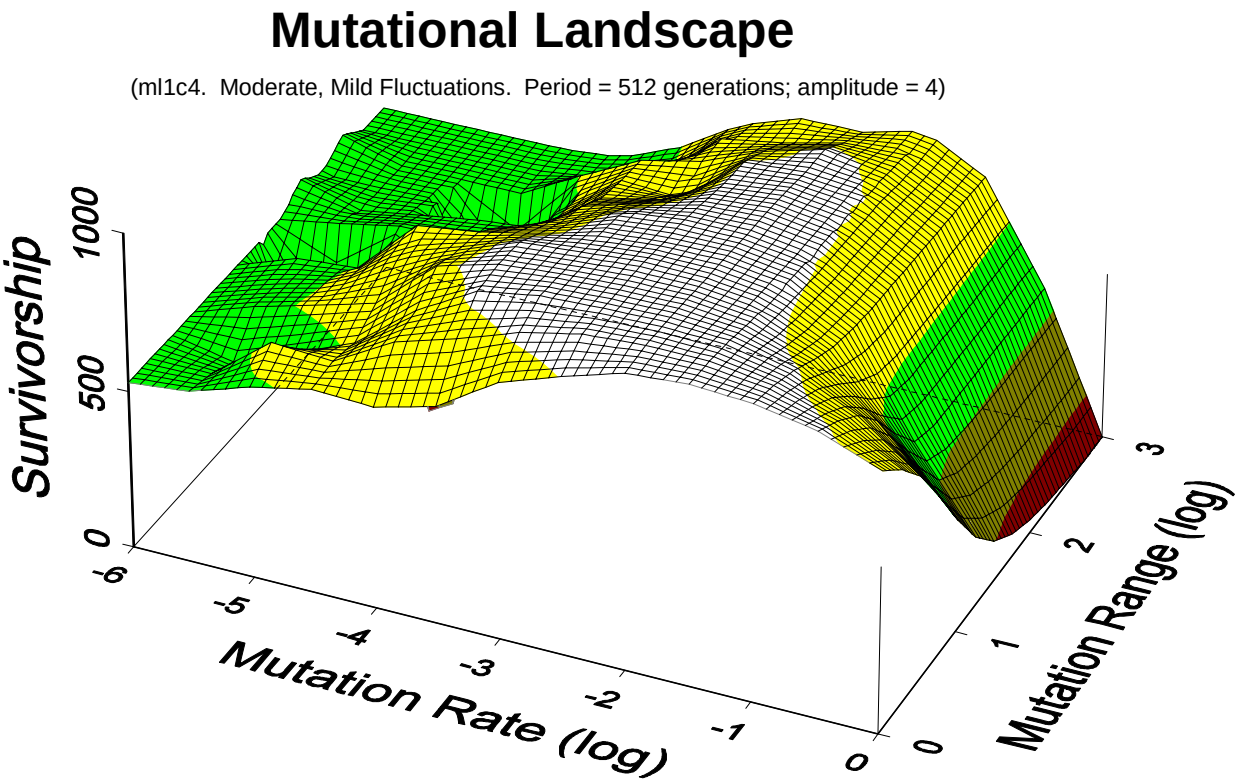


**Figure 1b.** Three dimensional plot of survivorship (averaged over 1536 generations) as a function of mutation rate and mutation range. 169 simulations were conducted, at intervals of 0.5 on the log scale for mutation rate and 0.25 on the log scale for mutation range. Between these points, the surface was smoothed by linear interpolation. For each of the simulations, the environment fluctuated sinusoidally with a period of 512 generations and an amplitude of plus or minus 4 arbitrary units. The same arbitrary units were used to measure the impact of mutation on fitness. (A mutation range of 2 on the log scale means that any individual mutation could add or subtract any value up to 100 units to the original value of the mutating allele.) See text for further explanation.

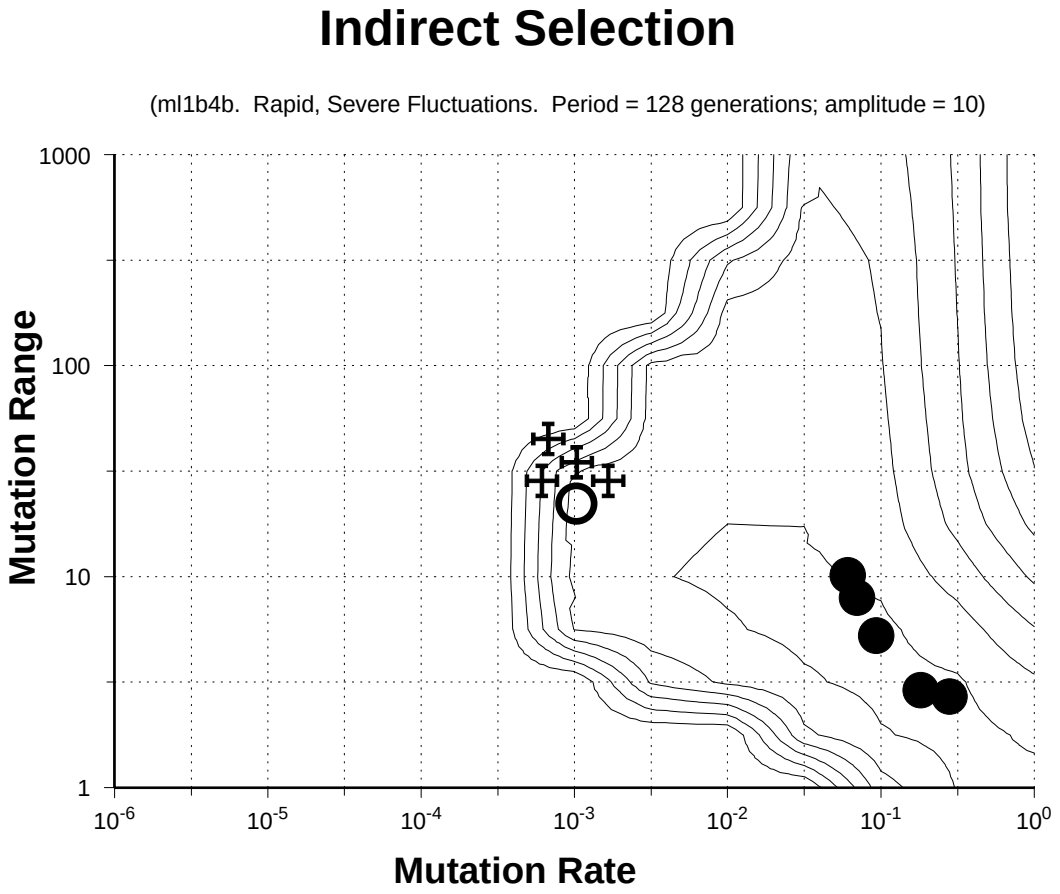


**Figure 2a.** Each circle indicates the parameters of mutation rate and mutation range which predominated at the end of 8192 generations during which these parameters were free to vary and evolve. **Solid circles** indicate experiments in which mutability parameters were **linked** to trait-determining loci. These results cluster near the peak of the corresponding mutational landscape (shown by contour lines). **Open circles** and **crosses** indicate experiments in which mutability parameters were **not linked** to trait-determining loci; the crosses mark experiments which were ended prematurely by extinction of the population. (The crosses are clustered around the values at which each experiment was initialized, suggesting that extinction occurred before the mutability parameters could evolve away from initial values. The single open circle demonstrates that extinction is not inevitable for initialization at these values.) For each experiment, the environment fluctuated sinusoidally with a period of 128 generations and an amplitude of plus or minus 10 arbitrary units, as in Figure 1a. See text for further explanation.

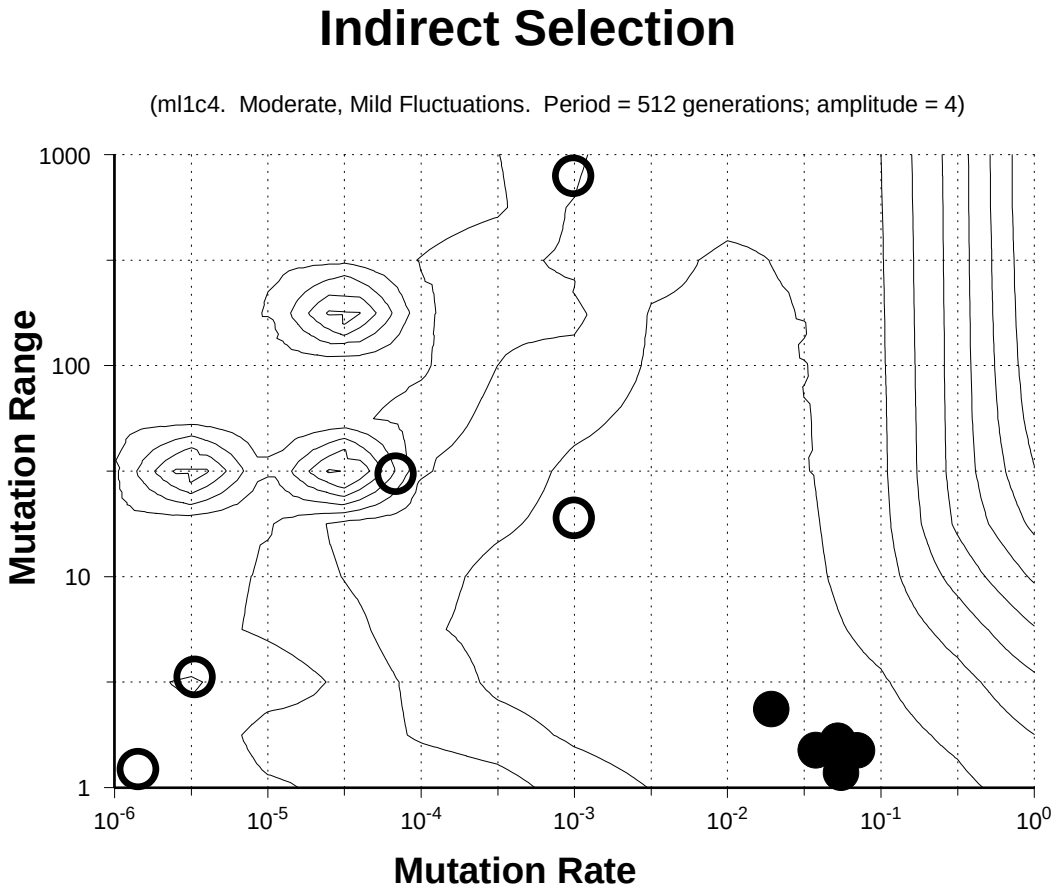


**Figure 2b.** Each circle indicates the parameters of mutation rate and mutation range which predominated at the end of 8192 generations during which these parameters were free to vary and evolve. **Solid circles** indicate experiments in which mutability parameters were **linked** to trait-determining loci. These results cluster near the peak of the corresponding mutational landscape (shown by contour lines). **Open circles** indicate experiments in which mutability parameters were **not linked** to trait-determining loci. For each experiment, the environment fluctuated sinusoidally with a period of 512 generations and an amplitude of plus or minus 4 arbitrary units, as in Figure 1b. See text for further explanation.