Random with Respect to Fitness or External Selection? An Important but Often Overlooked Distinction

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Abstract

Mutations are often described as being "random with respect to fitness." Here we show that the experiments used to establish randomness with respect to fitness are only capable of showing that mutations are random with with respect to current external selection. Current debates about whether or not mutations are directed may be at least partially resolved by making use of this distinction. Additionally, this distinction has important mathematical, experimental, and inferential implications.

1 Introduction

Mutations are often described as being "random with respect to fitness," both in the standard literature and in textbooks describing mutation and evolution (for recent examples see Sober (2003), Bromham (2016), Roy (2016), and Tollefsbol (2022)). Others prefer terminology such as "undirected" instead of "random" but the meaning is essentially the same (Eagle, 2005). While this is often repeated, the standard evidence on which it is based is insufficient to make the claim. The present paper will show that, at most, the experiments used to show that mutations are random with respect to *fitness* would be more accurately described by saying that they show that mutations are random with respect to *selection* (or, even more specifically, current external selective pressure). This distinction is extremely important for understanding, communicating, and investigating the process of evolution at a molecular level.

2 Distinguishing Internal And External Selection

While there are many definitions of fitness, fitness is essentially the quantitative representation of an organism's ability to survive and reproduce successfully in a particular environment (Orr, 2009). However, as pointed out by Lewontin (2003), the causal picture of selection is not always as simplistic as might be assumed. Organisms can modify their environments, which then modifies the fitness landscape associated with their genome (Laland et al., 2016). More impor-

tantly for the present analysis, selection is not solely dependent on the external environment. That is, part of the selective context includes the organism's own internal organization.

We can then think about two different aspects of selection—internal selection and external selection. External selection is related to how much the organism is "fit" to the environment. Internal selection is related to how much the organism is fit to the organism's own internal ability to achieve homeostasis and basic reproductive functions. For instance, if a zygote for some reason lacked all DNA polymerase genes, it would be subject to internal selection. The organism would not be able to survive in any environment.[1] Recent studies have shown that internal selective effects are important for many genes. For instance, studies of essential genes show that there are many genes which cannot undergo deletion mutations without disrupting the internal environment of the cell or the development of the organism (Glass et al., 2006; Bartha et al., 2018).

Thus, an organism's fitness consists both of its internal consistency and functioning as well as the relationship between that organism's functioning and the external environment. Therefore, decreases in fitness can happen due to either internal or external selection pressures.

This distinction between internal and external selection can be considered either intuitionally or inductively. The intuitional definition is that internal selection relates to the cohesiveness of the fundamental anatomy of the organism. Many biologists have pointed out that organisms exhibit deep patterns of cohesiveness which are required to be maintained at moderate evolutionary timescales (Montévil and Mossio, 2015). The requirement to maintain this cohesiveness would be considered internal selection by the present conceptions. Mutations that affect the operations of the organism that interact with its environment, or are more incidental to the core biology of the creature, would be considered being constrained by external selection.

[1]Even viruses, which do lack DNA polymerase genes, can do so *because they have other biophysics which allow for it.* Even for a virus, there are configurations which are not fit in any environment.

The inductive definition would be to take a mutation and ask if there exists a reasonably-encounterable environment in which the mutation would not be detrimental.[2] If the mutation is detrimental in every reasonably-encounterable environment, then it can be considered to be subject to internal selection. If there are reasonably-encounterable environments where the mutation is beneficial, then it can be considered to be constrained by external selection instead.[3]

3 Mutation Experiments Before Watson and Crick

The Luria-Delbrück (fluctuation test) and Lederberg (replica plating) experiments performed in the 1940s and early 1950s are often said to demonstrate that the tested mutations were random with respect to fitness (Luria and Delbrück, 1943; J. Lederberg and E. M. Lederberg, 1952). Briefly, these experiments can be used to determine whether or not mutations arise in *response* to selection pressures, or if the mutations arose prior (and therefore independently of) those pressures. It is important to note that both of these experiments were performed prior to Francis Crick's sequence hypothesis (Crick, 1958) which paved the way for the modern understanding of how DNA operates as the carrier of genetic information. When the mechanism of encoding genetic traits was unknown, it could be plausibly stated that these experiments showed that mutations were random with respect to fitness generally. After all, the actual range of potential mutations were unknown at the time since it was unknown what the possibility space even looked like.

After the confirmation of the sequence hypothesis,

[2]By "reasonably-encounterable" I only mean to exclude conditions that one would only be able to achieve in a carefully controlled laboratory environment and not in the wild.

[3]There is utility in also considering edge cases as well, such as where a subsystem of an organism may have internal selective constraints, but that subsystem is not a critical subsystem of the organism, and, thus, losing the subsystem could be neutral or even advantageous in some environments. These are useful distinctions, but for the purpose of the present discussion, we will consider these to be under the broad category of being subject to external selection.

however, the possibility space of mutations became knowable, as well as the potential fitness impacts of those potential changes. Unfortunately, the results and terminology from the prior decades were carried over relatively unaltered in the face of these newer developments. The fact that Lederberg and Luria-Delbrück couldn't possibly know the distribution of fitness effects over the total *potential* mutation space did not prevent biologists of that time from importing their results into the DNA revolution.

What Luria-Delbrück and Lederberg showed is that, at least for the mutations they were analyzing, the mutations occurred at random with respect to *external selection*. Both of these experiments work by examining the rate of arrival of mutations which allow for adaptation and examining whether selection alters this arrival rate. These experiments have shown that, for a great number of known mutations, selection does not alter the arrival rate of mutations.

However, are the mutations that do arrive more or less likely to be consistent with internal selective pressures compared to other mutations? Neither experiment is able to answer this question. To answer this question properly, one would need to analyze the biochemical effects both of mutations that do and do not occur, and analyze the relative rates of those effects in those categories.

4 An Abstract Look at Mutation Space

To better understand the possibilities, let us imagine an organism with an extremely limited genome with a small, finite number of genetic possibilities available. Let us say that the organism can have one of the following genetic states: A, B, C, D, E, F, or G, and it mutates freely between these in a uniform random distribution. Let us then propose three environments: X, Y, and Z. Now, let's say that,

- in environment X, A performs the best,
- in environment Y, B performs the best,
- in environment Z, C performs the best,
- D is detrimental in all environments (because it has lower internal fitness),
- E is lethal in all environments (because it is not fit to any environment),
- F is lethal in all environments (because of problems of internal fitness), and
- G is lethal in all environments (also because of problems of internal fitness).

If a mutation is random with respect to *external selection*, then the mutation space is unaltered by external selective pressures in the environment. This means that being in environment X won't cause a mutation to configuration A to be any more likely than it was in environment Y or Z. The mutation space, whatever it is, stands unaltered in the face of selection pressures.

However, if a mutation is random with respect to *fitness*, then the mutation space is completely unaltered by *any* selective concerns. Let us now say that, rather than the mutations being a uniform random distribution across all seven possibilities, mutations in this organisms are structured in a way such that configuration F, though a genetic possibility, never occurs (or occurs extremely rarely). While this still leaves degradative and even lethal mutations within the potential mutational spectrum, it decidedly shifts the distribution of mutations in *favor* of fitness. This is actually true whether or not the original distribution of mutations was uniformally random or not, but considering the uniform random case makes the fitness effect of excluding mutations from the distribution more obvious.

Experiments such as Lederberg and Luria-Delbrück would be able to detect if environment X caused a mutation to A to become more likely. However, if a mutation to F is never seen because the mutations are biased in favor of internal fitness, neither experiment would detect such a phenomena. They can both only work with mutations that are detectable—they cannot draw conclusions on mutations that do not occur. However, such a question can at least in principle be examined in the genomics age (Bartlett, 2020). Experiments can be setup which

compare outcomes from mutations that are known to be random (because the experiment created them to be so) and mutations that occur naturally, and their effects compared. We can use the comparison between the fitness effects of naturally-occurring mutations and known-random mutations to check if the hypothesis that mutations are random with respect to fitness is correct.

When thinking about real genomes, it is true that there are definitely biochemical reasons for the range of mutations to be biased towards certain mutations more than others (i.e., the distribution is not a uniform random distribution). Random with respect to fitness does not require that each site be equally mutable. However, an arbitrary (even if non-random) biasing of which bases were more likely to mutate would have no intrinsic reason to favor function over non-function (Eagle, 2005). Therefore, the mere fact that mutations have a biochemical bias does not give any reason to suppose that those mutations would favor function over non-function. In fact, given the expansive size of degenerative mutations in the abstract space of genetic configurations (Soskine and Tawfik, 2010), there is no inherent reason to think an arbitrary biasing of mutations would be in favor of function over non-function. Having such a favoring would be something that required explanation—in other words, it could not be said to be due to mutations which were *random* with respect to fitness.

5 Relationship to the Modern Synthesis

Previous studies have tended to either attempt to merge newer studies into the paradigm of the modern synthesis (Merlin, 2010) or present these newer understandings as a thorough undermining of it (Jablonka and Lamb, 2005). Both of these approaches miss the mark because they both fail to distinguish the specific nuances of what previous experiments showed compared to what we are finding now. A third approach is that of evo-devo, which will be covered in Section 6.

Merlin (2010), for instance, wants to show that most of the new findings fall into the same categories defined under the modern synthesis. This is done by essentially broadening the definition of random mutation beyond what the modern synthesis specifies. Merlin significantly broadens the concept of random mutation to mean only that the changes are not exclusively advantageous. Merlin stated, "All mutations are "evolutionary chance" mutations since they are not genetic changes specifically produced in an (exclusively) advantageous manner in response to a given environmental challenge." Merlin qualifies the term "exclusive" by stating that, in this context, exclusive means "if and only if it is part of a local increase of the mutation rate and the physicochemical processes causing it clearly makes the probability of a beneficial mutation higher than the probability of other deleterious or neutral mutations in the same environment." Requiring a process to *exclusively* produce advantageous results (even in this qualified sense) to be considered directed is quite a high bar—one in which many end-directed biological processes could not meet.[4]

This is contradicted by many in the modern synthesis. For instance, Simpson (1960) says that mutations "arrive, however, by chance, and their effects are random in the sense that the cause of a mutation has no evident relationship to the nature of the result and the effects are unoriented with respect to usefulness or adaptation in the organism." Mayr (1961) similarly said that mutations are the result of errors of replication, and that the "occcurrence of a given mutation is in no way related to the evolutionary needs of the particular organism or of the population to which it belongs." If a mutation is an *error* in replication, and it is *in no way related* to the evolutionary needs of the organism, then this is in favor of the idea of them being random with respect to fitness, not solely external selection. Appendix C of Stoltzfus (2021) yields a whole host of citations from

[4] As an example, even teleonomical processes such as predators hunting prey do not exclusively yield their goals, even by Merlin's qualified definition. Vermeij (1982) noted that many predatory species have capture rates less than 25%, yet none would qualify these as undirected. While there is some amount of chance in the process, the mere existence of stochastic variations is not what the authors of the modern synthesis had in mind when they developed the concept of random mutations.

the primary authors of the modern synthesis about the randomness of mutation which almost uniformly follow in a similar vein.

This is a much more specific cause-effect relationship than Merlin's grouping allows for, essentially excluding any sort of governing process behind mutations whatsoever. Merlin essentially took the fact that the mutational process doesn't exclusively produce beneficial mutations to mean that the developers of the modern synthesis would be fine with the expansion of the definition. It is unlikely, given their statements, that this is the case. Such a set of causes certainly wasn't predicted or expected by them.

The more problematic aspect, however, is that by rewriting the modern synthesis in this way prevents investigation of the importance of the distinctions of processes that are occurring. Specifically, here, we are looking at the distinction between mutations being random with respect to fitness as a whole and being random with respect to external selection. Later sections of this paper will describe the mathematical (Section 7), experimental (Sections 8 and 9), and inferential (Section 10) importance of this distinction. Simply classifying such processes as being the same as the ones described by the modern synthesis simply because they do not produce exclusively beneficial mutations only continues to drive the misunderstandings that are perpetuated in the literature.

Likewise, it is also important not to overemphasize the directedness of mutation. Replica plating and the fluctuation test still show that, at least for the most well-studied mutations, mutations are random with respect to external selective forces. Knowing the limits of these experiments is not equivalent to throwing them out. This is why making such a distinction is important. Making better distinctions allows us to preserve what was previously discovered while simultaneously preparing for future investigations.

6 Relationship to Evo-Devo

Another interesting approach to the question of the fitness effects of mutations is evolutionary developmental biology, or evo-devo. Essentially, evo-devo says that the mutations are random but the developmental processes canalize the resulting phenotypes. This is reflected in statements by proponents from the beginning of the subject through the present. Wagner and Altenberg (1996) stated, "for adaptation to occur, these systems must possess 'evolvability,' i.e., the ability of random mutations to sometimes produce improvement." In other words, the presumption of the randomness of mutations developed by the modern synthesis is maintained, but evo-devo aims to show that the organism's evolved genotype-phenotype map makes it more likely that the genotypically random mutations produce outcomes that are phenotypically beneficial. More recently, Rosa and Villegas (2022) stated, "the probabilities invoked in evo-devo do not concern the distribution of mutations, but rather the distribution of resulting phenotypes". As noted by Salazar-Ciudad and Cano-Fernández (2023), the process of development means that the phenotype mutation space is not isotropic—in other words, development limits the phenotypic possibility space that random mutations can induce.

The question of how the developmental pathways influence the genotype/environment to phenotype mapping is an interesting question, but orthogonal to the one we are presently concerned with. Evo-devo is primarily concerned with the production of phenotypes from genotypes, and how organisms harness the development process and other tools to make a higher percentage of the mutational spectra beneficial.

Evo-devo is correct to point out the highly complex relationship between changes in genotype and the resulting phenotype due to developmental processes. However, in the present paper, our primary consideration is whether or not the phenotype of the organism feeds back to help narrow the choices of which *genotypes* get produced, rather than following the modern synthesis in its presumption that the mutational process is blind concerning which particular mutations occur.

A slightly different question is sometimes asked as well. Beatty (2010) (as well as others) have noted the fact that the present state of the organism influences future probabilities, such that the past evolution influences future evolution. That is, different genomes are different distances from each other, and taking one path makes you genetically closer to one path in-

stead of another. Previous mutations will influence the reach of the genome to other mutations (for a given mutation rate and population size) as well as affect the genotype/phenotype map for those mutations. Thus, what is selected in the present generation is greatly influenced by history. Again, while this is an important consideration, what is presently being considered is the possibility that the distribution of actual mutations that occur in the present generation are in any way correlated with the fitness effects of those mutations on the organism as a whole (including internal fitness).

For instance, let's say that an organism has genotype A. In the next generation, an insertion sequence is inserted at a specific position to yield genotype B. Then, in the third generation, a single-nucleotide polymorphism (SNP) occurs within the insertion sequence, leading to genotype C. It is clear that the history of the organism is influencing the likelihood of future mutations. That is, from genotype A, going directly to genotype C is incredibly unlikely (since it has both the results of the insertion sequence and the subsequent SNP). However, once the insertion sequence has been inserted in generation B, the genotype in generation C becomes much more likely. However, recognizing the sequence dependence of these mutations is orthogonal to the question of whether the mutational spectra that occurs within genotype B is random with respect to the overall fitness of genotype B, or if instead there are mutations that are preferred or avoided based on their likelihood of impacting fitness.

While the evo-devo world has helped immensely to show that the way that organismal genotypes and genotype-phenotype maps evolve is not as straightforward as previously assumed, the questions that have been tackled so far by evo-devo are largely orthogonal to our present discussion.

7 The Fitness Effect of Avoiding Lethals

Section 4 examined, in general terms, how merely excluding even a subset of lethal mutations from the set of possibilities skews the distribution towards benefit. This specific case of environmentally-independent lethal avoidance can be readily analyzed mathematically. Since such lethals have a fitness of zero in any environment, this makes analyzing their removal more straightforward.

The expected resulting fitness value W of an organism after mutation is simply the average of each particular possibility (W_i) multiplied by its probability of occurring (p_i).

$$E[W]_{\text{inc}} = \sum_{i \in n} p_i W_i. \tag{1}$$

Since, for any i, $W_i \geq 0$ and $p_i \geq 0$, excluding *any* W_x where $W_x = 0$ will improve the expected fitness value (assuming they aren't all zero).

We can determine the effect of excluding a particular lethal on expected post-mutational fitness by renormalizing. Since all p_i add up to one,

$$E[W]_{\text{exc}} = \left(\sum_{i \in n, i \neq x} p_i W_i \right) \frac{1}{1 - p_x}. \tag{2}$$

However, since the fitness value being removed is a zero, the summation in Equation 1 is equivalent to the summation in Equation 2. Therefore, we can rewrite Equation 2 as

$$E[W]_{\text{exc}} = E[W]_{\text{inc}} \frac{1}{1 - p_x}. \tag{3}$$

Note that Equation 3 doesn't depend on any particular prior distribution of mutations (i.e., it is true whether or not the mutations are distributed uniformly throughout the genome). Since $0 < p_x < 1$, then $E[W]_{\text{exc}} > E[W]_{\text{inc}}$.

This equation can also be adapted to situations where certain lethals are *reduced* instead of eliminated. Conceptually, this can be thought of as splitting the mutation into two separate alternatives and having one of them eliminated. For instance, if the occurrence p_x is merely reduced by 30%, then one simply needs to replace p_x with $0.3\,p_x$ in the equation.

Additionally, excluding non-lethal but detrimental mutations can also be analyzed mathematically. If we

define a "detrimental" mutation as one which is below the expected fitness value of the organism which would otherwise include them ($W_x < E[W]_{\text{inc}}$), then an avoidance strategy that strictly avoids these mutations would have the effect of increasing fitness. If $W_x < E[W]_{\text{inc}}$ and $p_x > 0$, then

$$\begin{aligned} -p_x W_x &> -p_x E[W]_{\text{inc}} \\ E[W]_{\text{inc}} - p_x W_x &> E[W]_{\text{inc}} - p_x E[W]_{\text{inc}} \\ E[W]_{\text{inc}} - p_x W_x &> (1-p_x) E[W]_{\text{inc}} \\ \frac{E[W]_{\text{inc}} - p_x W_x}{1-p_x} &> E[W]_{\text{inc}} \\ E[W]_{\text{exc}} &> E[W]_{\text{inc}}. \end{aligned} \quad (4)$$

Note that these results assume strict avoidance measures. In other words, the only result of excluding lethals or detrimental mutations is that they don't occur. It is theoretically possible to have a mutational mechanism which happens to avoid certain lethals, but also alters the probabilities of other mutations as well. Nonetheless, the present model at least yields a starting point for understanding how avoidance mechanisms impact the expected fitness of an organism.

8 Lethal Avoidance in Experimental Biology

As shown, the avoidance of lethals shifts the abstract fitness of mutations towards benefit. This is true even if only some classes of lethals are avoided. Many experiments have shown that organisms do have strategies of lethal avoidance. Moxon et al. (1994) suggests a distinction between "housekeeping genes" and "contingency genes," where the former are genes whose products focus on the internal or environment-invariant needs of the organism, and the latter are genes whose products focus on the environment-specific needs of the organism. Their results suggest that the mutation rate in housekeeping genes in bacteria are often lower. This suggests the possibility that the organism's mutational mechanism is avoiding certain lethals while not removing the possibilities of other mutations.

More recently, a similar phenomena has been found in multicellular plants. Studies of de novo mutations in *Arabidopsis thaliana* show that mutation frequency is reduced by two thirds in essential genes (Monroe et al., 2022). More specifically, they find that "genes subject to stronger purifying selection have a lower mutation rate."

The results of Equation 3 suggest that such mutational strategies, even if they were random with respect to external selection forces, are not random with respect to fitness in general.

9 Cyclical Mutations for Fitness Enhancement

Another way of biasing mutations in favor of fitness even when random with respect to external selection is through cyclical mutational mechanisms. A cyclical mutational mechanism is one in which reversions to a previous configuration is significantly more likely than for an arbitrary mutation. A simple example of a cyclical mutation would be a site-specific, reversible DNA inversion. These are commonly found as means of rapid adaptations in bacteria (Putte and Goosen, 1992; Henderson et al., 1999; Cui et al., 2012).

A more complex example of cyclical mutations has been discovered for *Neisseria*. Neisseria has an expressed pilin gene, *pilE*, as well as numerous silent pseudogenes, $pilS_i$. The mutational mechanism by which *pilE* varies is through one-way recombination events with the *pilS* genes (Cahoon and Seifert, 2011). In other words, there is a finite number of existing variations of the pilin gene which are regularly cycled through. Thus, rather than having to navigate the (very large) abstract mutation space, the actual mutational mechanism simply *cycles* through a standing set of variations through recombination. This recombination system is one-way (into the expression locus), thus preserving the variability.

While the abstract study of the benefit of this system has not been explored, it biologically makes sense that these alternatives are more likely to have a high fitness compared to other arbitrary sequences. Sequence space, in general terms, is extremely sparse

in terms of which sequences contain function. The sequences that are present in *Neisseria* for this function are all able to serve successfully in the expression locus in some environment. Therefore, even if the recombination to a particular *pilS* sequence is random with respect to the external selective environment, it is likely that these mutational alternatives are not random with respect to fitness as a whole.

10 *In Vitro* vs *In Vivo* Mutation Space

If the mutation rates of negative or lethal outcomes may be suppressed, then studies of the effects of mutations *in vitro* cannot be used as a proxy for the distribution of the effects of mutations *in vivo*, since *in vitro* studies assume a random distribution of mutations. If that assumption is unfounded, then so are the results of such studies. As an example, Soskine and Tawfik (2010) analyzes the outcomes of arbitrary *in vitro* mutations of a β-lactamase protein, and measures the fitness effects for the mutations. While there is no reason to doubt the fitness values they measured, if such studies are done in the absence of studies about the distribution of such mutations as they occur *in vivo*, then the distribution of fitness effects found cannot be equated to the expected distribution of fitness effects of real mutations *in vivo*, despite the fact that they are used that way today.

An example of this is Graur (2017), which uses the *in vitro* distribution data as a proxy for the distribution of *in vivo* mutations, and uses that as the basis of estimating parameters for population dynamics in humans. However, if there are mutational mechanisms which bias the fitness of mutations, even if it remains random with respect to external selection, then the *in vitro* data cannot properly serve as a proxy for *in vivo* outcomes.

This is not a lone instance, as Serohijos and Shakhnovich (2014) has suggested using such proxies generally as a basis for the integration between biophysics and evolutionary population genetics. Essentially, in this model, biophysical simulations of proteins resulting from random mutations are used as a means of inferring the distribution of fitness effects. Again, if mutations are only random with respect to external selection, and not random with respect to fitness in general, then this biophysics approach cannot be properly used as a proxy for the distribution of fitness effects of mutations in populations.

11 Conclusion

What we have established is that the *concepts* of "random with respect to fitness" and "random with respect to external selection" are distinct concepts, and that the Luria-Delbrück and Lederberg experiments are only capable of determining whether or not a mutation is random with respect to external selection, not fitness as a whole. Recognizing this distinction is crucial to understanding the results of modern genetics studies, which often shows that organisms have mutational mechanisms which are decidedly biased in favor of fitness irrespective of whether or not they are biased in favor of selection.

Current genetic studies show that organisms can do this by suppressing bad alternatives, such as the lowering of the mutation rate in genes subject to purifying selection in *Arabidopsis thaliana*, or by promoting good solutions, such as providing specific mutational alternatives in *Neisseria*. In either case, the effects of such mutations *in vivo* are decidedly more fit than abstract mutations *in vitro*.

Making this distinction may help clarify many of the challenges associated with recent questions of directed mutation. Many of those who are in favor of the concept of directed mutation point to increased mutation rates where mutations are likely to be beneficial (Jablonka and Lamb, 2005). Those not in favor of the concept point out that very few if any of these have been shown to be the result of selective pressures (Merlin, 2010). Making the critical distinction between "random with respect to fitness" and "random with respect to external selection" may help avoid misunderstandings in a number of cases.

Even more importantly, this distinction is important for knowing the limitations of using biophysical data about potential mutational effects as proxies for actual mutations occurring *in vivo*. If mutations are

only random with respect to external selection, and not fitness as a whole, then such inferences are unjustified.

12 Statements and Declarations

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