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Reflections

Two sides of the same coin: A population genetics perspective on lethal mutagenesis and mutational meltdown

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Abstract

The extinction of RNA virus populations upon application of a mutagenic drug is frequently referred to as evidence for the existence of an error threshold, above which the population cannot sustain the mutational load. To explain the extinction process after reaching this threshold, models of lethal mutagenesis have been proposed, in which extinction is described as a deterministic (and thus population size-independent) process. As a separate body of literature, the population genetics community has developed models of mutational meltdown, which focus on the stochastic (and thus population-size dependent) processes governing extinction. However, recent extensions of both models have blurred these boundaries. Here, we first clarify definitions in terms of assumptions, expectations, and relevant parameter spaces, and then assess similarities and differences. As concepts from both fields converge, we argue for a unified theoretical framework that is focused on the evolutionary processes at play, rather than dispute over terminology.

Key words: lethal mutagenesis; mutational meltdown; Hill–Robertson interference; Muller's Ratchet.

1. Introduction

Fisher (1930) argued that an intermediate mutation rate is likely optimal for populations to survive, as it ensures a constant input of beneficial mutations providing the 'fuel' for adaptation, while limiting the impact of accumulating deleterious mutations. Subsequently, the effects of high population mutation rates and the related risk of population extinction have been explored thoroughly in the theoretical literature, spanning the fields of both population genetics and virology.

This literature has spawned a number of concepts describing the extinction of populations owing to the excessive accumulation of deleterious mutations. From population genetics, the mutational meltdown model (Lynch and Gabriel 1990) has been proposed, invoking previously developed evolutionary processes including Muller's ratchet (Muller 1964; Felsenstein 1974) and Hill–Robertson interference (Hill and Robertson 1966; McVean and Charlesworth 2000). Relatedly, from the study of virus evolution, the model of lethal mutagenesis (Bull, Sanjuán, and Wilke 2007; Wylie and Shakhnovich 2012) has emerged.

Despite the different fields in which these ideas were developed, there is a considerable amount of parallelism between these notions. Though mutational meltdown is generally discussed within the context of 'small' population sizes in which stochastic effects play an important role, and lethal mutagenesis is generally discussed within the context of 'large' population sizes driven by deterministic factors—recent extensions of

the latter model have relaxed this assumption to incorporate stochastic effects (Wylie and Shakhnovich 2012). Hence, the extent to which these models are overlapping descriptions of related processes or events, or are even subsets of one another, is unclear. This has resulted in an inherent confusion when invoking these models to describe biological observations.

We here seek firstly to clearly define and review these models in terms of parameter requirements, predicted effects, and biological relevance, and then compare them side-by-side in light of their similarities and differences. Fundamentally, we propose that the notion of lethal mutagenesis largely describes the deterministic subset of the dynamics of mutational meltdown, and thus call for a more integrated focus on the underlying processes driving extinction.

2. Lethal mutagenesis

Originally introduced and coined to describe a therapeutic strategy for curing vaccine-lacking viral diseases, the concept of lethal mutagenesis refers to the drug-induced increase in viral mutation rate (achieved through incorporating non-complementary nucleotides or nucleotide analogs during the DNA/RNA replication process), which reduces population mean fitness (and increases mutation load) and in turn leads to a decline in viral population size and eventual extinction (Loeb et al. 1999; Anderson, Daifuku, and Loeb 2004; Bull, Sanjuán, and Wilke 2007). Although the concept itself has been applied to both RNA and DNA viruses—including human immunodeficiency virus (HIV), influenza A virus (IAV) and hepatitis B virus (HBV) (see Anderson, Daifuku, and Loeb 2004)—results were initially discussed in light of Eigen's error catastrophe model (Eigen 1971) owing to the lack of a separate, formal theoretical framework of lethal mutagenesis.

In an attempt to synthesize existing empirical and theoretical work, Bull, Sanjuán, and Wilke (2007) proposed a general theory of lethal mutagenesis, which, as the authors noted, "offered [nothing] ... specifically original", but is "rather [an] ... application of simple models and the interpretation of those results in the context of empirical methods ... that made this [theory] original". This model is composed of three basic features: First, a genotype-to-fitness map $\omega(g)$ characterizes how the number of (deleterious) mutations affects the genotype's fitness; second, a mutation-rate-to-fitness map addresses how the population mean fitness at mutation-selection balance changes with the rate of mutations U (Haldane 1932; Bürger 1998); finally, a demographic model that links the two above components with an ecological component $R_{max}(e)$ that quantifies the (environmentally dependent) maximal absolute population growth rate (sometimes also called the maximal fecundity). Therefore, the population *absolute* mean fitness $\bar{W}$ is given by

$$\bar{W} = \mathbf{R}_{max}(\boldsymbol{e})\bar{w}(\boldsymbol{\omega}(\boldsymbol{g}), \mathbf{U}).$$

Depending on the choice of the underlying demographic model, the number of viral particles in the next (discrete) generation N_{t+1} is then simply a function of the current population size N_t and the absolute mean fitness (e.g., a simple exponential growth model of the form $N_{t+1} = N_t\bar{W}$ or a more complex density-dependent model; see Gabriel and Bürger 1992). However, independent of the choice of demographic model, the population will eventually become extinct if the mean absolute fitness $\bar{W} < 1$, such that the population can no longer maintain itself. This can happen either because the mean *relative* fitness $\bar{w}$ drops below a critical value (i.e., mutation load becomes too high), or because $R_{max}(e)$ drops below unity as a consequence of a change in the environment (e.g., due to the application of a novel drug treatment).

In this general formulation, viral populations are assumed to be large initially such that genetic drift is not affecting mutation-selection balance, recombination is thought to be absent, and beneficial mutations are disregarded (see below for a discussion of recent relaxations of these model assumptions). This simplified model allows the calculation of critical mutation rates U_c, beyond which absolute mean fitness drops below unity and the population will become extinct. In particular, under a multiplicative genotype-to-fitness map $\omega(i) = (1-s)^i$, where the fitness of a genotype is reduced by a constant s per deleterious mutation, the mean relative fitness is $\bar{w} = e^{-U}$ (Kimura and Maruyama 1966). Thus, the population would go extinct if $U > U_c = \mathrm{Log}[R_{max}(e)]$. Notably, due to environmental dependence of $R_{max}(e)$, there is no universal critical mutation rate across viral populations—not even for a single species.

As shown by the above back-of-the envelope calculation, lethal mutagenesis is independent of population size—a result made explicit in the original model in noting that it is fundamentally a deterministic process that will operate even in very large populations (Bull, Sanjuán, and Wilke 2007). Importantly, although the outcome of lethal mutagenesis is deterministic, population dynamics, extinction times, and individual trajectories of mean absolute fitness are not. Thus, demography is nonetheless important here (Nowak and May 2000), where finite population sizes will always induce an additional (drift) load that is not accounted for in these models, but which can have a strong effect on population dynamics owing to the fixation of deleterious mutations further decreasing population mean fitness.

3. Mutational meltdown

Muller's ratchet (Muller 1964; Felsenstein 1974) describes the stepwise loss of the fittest class of individuals in a population and the associated reduction in absolute fitness due to the accumulation of deleterious alleles, or drift load (Whitlock and Bourguet 2000). In a finite population, Muller's ratchet eventually results in the extinction of the population if it is not opposed by compensatory or beneficial mutations. Lynch and Gabriel (1990) were the first to combine the study of population dynamics (i.e., size changes and absolute growth rates) and population genetics (i.e., allele frequency distributions and relative fitnesses) in order to describe this extinction process, which they termed "mutational meltdown". In a series of papers, the authors proposed and analyzed various related models and discussed the properties and implications of mutational meltdown in both asexual and sexual populations (Lynch and Gabriel 1990; Gabriel, Lynch, and Bürger 1993; Lynch et al. 1993; Lynch, Conery, and Burger 1995a, b; Gabriel and Bürger 1994).

This process is fundamentally described with respect to the accumulation of mutations over time. The mean number of mutations, $\bar{n}(t)$, depends on the carrying capacity K, the absolute growth rate R, the deleterious effect of a mutation s, and the (deleterious) mutation rate μ, and can be expressed as

$$\bar{n}(\mathbf{t}+1) \cong \bar{n}(\mathbf{t}) + \mu - \mathbf{s}\left[\mu + \boldsymbol{\sigma}_{\boldsymbol{n}}^2(\mathbf{t})\left(1 - \frac{1}{\mathbf{K}}\right)\right],$$

where $\sigma_n^2(t)$ describes the variance in n over time, which is generated by mutation and reduced by selection. The dynamics of mutation accumulation, beginning with an isogenic population,

can then be split into three phases (see Fig. 1 of Lynch et al. 1993): first, mutations accumulate rapidly, until mutation-selection-balance is reached. This is followed by a slower constant-rate accumulation of mutations at constant population size (i.e., when the population is at its carrying capacity). Once the mean viability drops below 1/R, carrying capacity cannot be maintained and population size starts to decline, thus increasing the susceptibility to further accumulate deleterious mutations (which in turn again reduces mean viability), ultimately resulting in rapid population extinction.

One important difference in the dynamics of the meltdown model is the dependence on the order of events in the life cycle (i.e., whether selection acts before or after population size regulation). In the first case, the carrying capacity K can be maintained over a long period, resulting in a constant population size and linear accumulation of mutations, followed by a rapid extinction phase (Lynch and Gabriel 1990; Lynch et al. 1993). In the second case, each click of the ratchet (i.e., loss of the least-loaded class of individuals) results in fewer offspring; thus the population size declines gradually and the speed of the ratchet increases over time (Gabriel, Lynch, and Bürger 1993). Independent of the type of model, the conclusions from these papers were that extinction times of a few hundreds of generations are expected for small populations, and that the process is slowed by roughly an order of magnitude in sexual populations (Lynch, Conery, and Burger 1995b). A simple rule determines the beginning of the final meltdown phase, which was subsequently used in Lande's treatment of the same problem in a quantitative genetics framework (Lande 1994, 1998), and in models of lethal mutagenesis (Bull, Sanjuán, and Wilke 2007; and see below): the population is doomed to extinction when the mean viability decreases below the reciprocal of the absolute growth rate (i.e., the number of offspring an individual can produce; Lynch et al. 1993). An interesting finding is that an intermediate magnitude of the deleterious selection coefficient minimizes the time to extinction through mutational meltdown; this is of particular interest given the recently accumulating empirical evidence for a generally bimodal distribution of fitness effects (DFEs) of new mutations (e.g., Eyre-Walker and Keightley 2007; Hietpas et al. 2011, 2013; Bank et al. 2014).

Beyond the "extinction threshold", other elements of lethal mutagenesis were indeed first discussed in the seminal papers on mutational meltdown (Lynch and Gabriel 1990; Lynch et al. 1993). First, Lynch et al. (1993) describe the conditions under which mutational meltdown is driven by genetic drift versus by mutational pressure, and conclude that "when the mutation rate is on the order of 1 per individual per generation [...] the [least-loaded] class will be lost due to mutation pressure alone". Second, the authors demonstrate that the extinction time is only relatively weakly (logarithmically) dependent on the population size. Finally, Lynch et al. (1993) compare the mean fitness reached after the first phase with that of an infinite-population under mutation-selection-balance (i.e., the starting point for lethal mutagenesis; Kimura and Maruyama 1966), and observe that it is indeed only slightly larger in the case of a finite population. Thus, though generally associated with small-population size effects, mutational meltdown has been discussed both with regards to high-mutation-rate regimes and large population sizes (Lynch and Gabriel 1990).

4. Comparing and interpreting the models

"Everything should be made as simple as possible, but not simpler."
Albert Einstein

The notion of lethal mutagenesis arose to provide a comprehensive framework to describe the deterministic (and thus population size-independent) processes that lead to population extinction via the crossing of a distinct error threshold. In contrast, the notion of mutational meltdown was fundamentally concerned with the stochastic nature of this extinction process, invoking classical population genetic models describing small population size dynamics. However, as discussed, the model of mutational meltdown has also been examined with regards to large population sizes. Further, recent extensions of the model of lethal mutagenesis have begun to consider stochastic effects. For example, Wylie and Shakhnovich (2012) studied the role of population size and mutation rate on extinction times, finding, as expected, that small populations may go extinct very quickly, whereas large populations survive almost indefinitely.

Thus, in some ways the model of lethal mutagenesis has converged with that of mutational meltdown, certainly in terms of appreciating the importance of stochastic processes in driving extinction events. Indeed, the genetic processes underlying population extinction are governed by the *effective* population size, N_e (Wright 1931; Crow 1954; Charlesworth 2009). Estimates of N_e/N taken from over 100 species (excluding viruses), demonstrated that census population size is on average an order of magnitude larger owing to fluctuating population sizes, unequal sex-ratios, and/or variance in reproductive success (Frankham 1995). In particular, the latter has been argued to significantly affect viral populations (Neher and Hallatschek 2013; Irwin et al. 2016). The ladder-like genealogy of the influenza A virus hemagglutinin segment, for example, suggests that only a few viruses seed the entire next generation (Grenfell et al. 2004). Along the same lines, estimates of effective population size in HIV range from 10^3 to 10^6, but generally show an extraordinarily low N_e/N ratio (Pennings, Kryazhimskiy, and Wakeley 2014).

Another similarity common to both models is the need to incorporate the potential effects of beneficial and compensatory mutations in modifying the rate of fitness decline and time to extinction (see Manrubia, Domingo, and Lázaro 2010; Bull et al. 2013). Specifically, small increases in mutation rate may improve the ability of populations to respond to novel environmental challenges, and there indeed exist examples of selection for hypermutator strains in bacteria under particular stressors (e.g., Sniegowski, Gerrish, and Lenski 1997; Gerrish, Colato, and Sniegowski 2013). However, owing to the effects of Hill–Robertson interference, as well as the far greater input of newly arising deleterious relative to beneficial mutations, this concern fundamentally suggests a need to quantify the *extent* to which mutation rates must be increased in order to ultimately result in population extinction.

Recent empirical studies attempting to test the genetic models underlying lethal mutagenesis have largely failed to match its (qualitative) predictions (Springman et al. 2010; Bull et al. 2013). This is presumably owing to the evolutionary mechanisms neglected by the (original) theory such as adaptive evolution (i.e., beneficial/compensatory mutations), interactions between mutations (i.e., epistasis) and non-constant mutational effect sizes (i.e., the DFEs). While Antoneli et al. (2013) recently derived a generalization of the lethal mutagenesis extinction criterion that allows for a small fraction of (fixed effect) beneficial mutations, epistasis and the DFE are inherently connected to the genotype-to-fitness and/or the genotype-to-phenotype map. Two alternatives to the frequently used multiplicative fitness model of Kimura and Maruyama (1966) have been proposed: First, in biophysics-based fitness landscape approaches,

the DFE is derived from the mutational effects on protein folding and its thermodynamic properties (e.g., Chen and Shakhnovich 2009; Stich, Lázaro, and Manrubia 2010; Wylie and Shakhnovich 2011, 2012). While these approaches may indeed account for an important class of mutational fitness effects in viruses (Wylie and Shakhnovich 2011), they have been criticized for directly equating fitness with protein stability—thus neglecting ecological aspects underlying fitness (Martin and Gandon 2010). As an alternative, phenotypic landscape models naturally accommodate epistasis, variation in mutational effects, and compensatory mutations (e.g., Fisher 1930 for Geometric Model; for recent empirical support see Martin Lenormand 2006; Cooper, Ostrowski, and Travisano 2007; Hietpas et al. 2013; Achaz et al. 2014; Tenaillon 2014). Under these models mutations, instead of directly affecting fitness, change n (unknown) quantitative traits (e.g., cell-to-cell transmission rate or levels of drug tolerance) which are considered to be under (Gaussian) stabilizing selection centered around an optimum. However, despite the conceptual differences between these two approaches, the resulting shapes of the DFEs are surprisingly similar (compare Fig. 3 in Wylie and Shakhnovich 2011 with Fig. 1 in Martin and Lenormand 2006), perhaps emphasizing that variable mutational effects must be accounted for (Bull et al. 2013).

The best evidence for the empirical observation of population extinction driven by increased mutation rates comes from the experimental evolution literature in which these stochastic effects are prominent by design—for example, in yeast where population sizes were artificially kept small (Zeyl, Mizesko, and De Visser 2001). Investigation in this area is particularly active in the study of RNA viruses, where the impact of mutagenic agents administered either alone or combined with antiviral inhibitors has been widely assessed (e.g., Loeb et al. 1999; Crotty, Cameron, and Andino 2001; Pariente et al. 2001; Airaksinen et al. 2003; Grande-Pérez et al. 2005; Bank et al. 2016). In other words, this literature has focused on experiments directly modulating either effective population size or mutation rate. It is additionally of note that several empirical papers claiming to study mutational meltdown do not observe extinction of their study population (e.g., Rowe and Beebee 2003; Shoubridge and Wai 2008; Allen et al. 2009; Willi 2013; Woodruff 2013), which may partly be owing to a confusion of terminology: the *process* of Muller's ratchet and the *event* of mutational meltdown are sometimes used interchangeably.

In order to avoid future confusion, we propose here that focusing on the action and interaction of the underlying evolutionary processes will likely be more informative for quantifying evolutionary dynamics and developing clinically relevant treatment strategies, will be more effective than quibbling about the proper (largely semantic) nomenclature surrounding the extinction event itself. Namely, both extinction models discussed here are fundamentally concerned with the notion of mutation-selection-(drift) balance (e.g., Haldane 1937; Kimura and Crow 1964; Bürger 1989) and its induced mutational load (also see the helpful theoretical results of Hermisson et al. 2002 that relate this equilibrium behavior to changing mutation rates). Further, Muller's ratchet and Hill–Robertson interference are fundamentally the processes driving the loss of fitness and eventual extinction. Though these processes have been well characterized in the population genetics literature, further progress must be made to extend these results to account for particular features of virus biology; for example by considering the large variance in reproductive success, the population structure induced by compartmentalization, the interplay of fluctuating population sizes and changing environmental pressures, and the effects of tissue-specific drug permeability.

Conflict of interest: None declared.

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