doi:10.1111/evo.14107

Imposed mutational meltdown as an antiviral strategy

Jeffrey D. Jensen,[1,2,3] Ryan A. Stikeleather,[4] Timothy F. Kowalik,[5] and Michael Lynch[1,4,6]

[1]*School of Life Sciences, Arizona State University, Tempe, Arizona 85281*

[2]*Center for Evolution & Medicine, Arizona State University, Tempe, Arizona 85281*

[3]*E-mail: jeffrey.d.jensen@asu.edu*

[4]*Biodesign Center for Mechanisms of Evolution, Arizona State University, Tempe, Arizona 85281*

[5]*Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, Massachusetts 01655*

[6]*E-mail: mlynch11@asu.edu*

Received August 14, 2020
Accepted October 10, 2020

Following widespread infections of the most recent coronavirus known to infect humans, SARS-CoV-2, attention has turned to potential therapeutic options. With no drug or vaccine yet approved, one focal point of research is to evaluate the potential value of repurposing existing antiviral treatments, with the logical strategy being to identify at least a short-term intervention to prevent within-patient progression, while long-term vaccine strategies unfold. Here, we offer an evolutionary/population-genetic perspective on one approach that may overwhelm the capacity for pathogen defense (i.e., adaptation) – induced mutational meltdown – providing an overview of key concepts, review of previous theoretical and experimental work of relevance, and guidance for future research. Applied with appropriate care, including target specificity, induced mutational meltdown may provide a general, rapidly implemented approach for the within-patient eradication of a wide range of pathogens or other undesirable microorganisms.

KEY WORDS: **Antivirals, lethal mutagenesis, mutational meltdown, population genetics, SARS-CoV-2.**

Both the avoidance and facilitation of extinction are key issues in applied evolutionary biology, the first being in the domain of conservation genetics, and the second being the goal of pathogen eradication. Over the past few decades, the notion that an excessive input of deleterious mutations can drive population extinction has been explored in numerous contexts. Speaking generally, mutational meltdown (Lynch and Gabriel 1990; Lynch et al. 1993) invokes stochastic effects, and was initially focused on small populations of endangered species. The dynamics of mutation accumulation may be viewed in three phases (Fig. 1). First, starting from a newly founded (or bottlenecked) population, mutations accumulate relatively rapidly. Second, as a quasi-mutation-selection-drift balance is reached, a slower steady-state rate of mutation accumulation occurs, provided that the mutation load is low enough to allow for sustained population size. Finally, once mean viability drops to the point that the average individual cannot replace itself, the population size begins to decline, sentencing the population to a relatively rapid downward spiral towards extinction.

The driver of this final mutational-meltdown phase is the accelerated reduction in the efficacy of natural selection that ensues once population size starts to decline. Given that the vast majority of fitness-impacting mutations are deleterious rather than beneficial (Crow 1993; Lynch et al. 1999; Bank et al. 2014a; and see reviews of Bank et al. 2014b; Eyre-Walker and Keightley 2007), declining population size facilitates the further fixation of deleterious variants, which in turn further reduces the efficacy of selection, and so on – a compounding effect that may eventually lead to extinction.

This model has been investigated in the context of both sexual and asexual populations. In the complete absence of recombination (e.g., obligately asexual organisms), extinction by mutational meltdown may be nearly unavoidable, with the time to extinction being relatively predictable given enough knowledge

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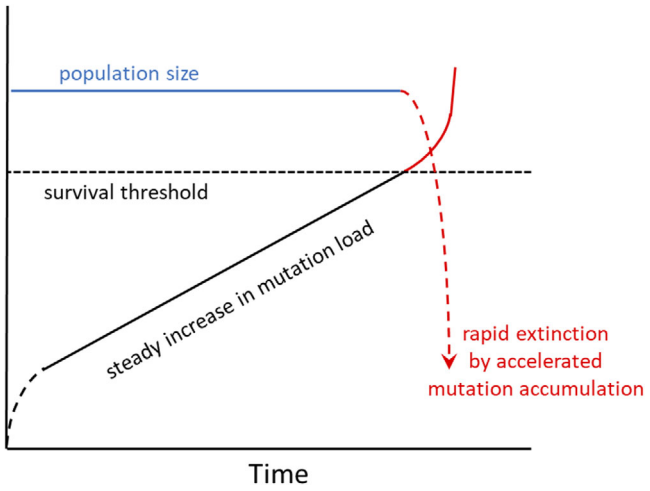

Figure 1. **An idealized schematic of mutational meltdown. Starting with a genetically homogeneous base population, deleterious mutations accumulate relatively rapidly for a short period (phase 1, dashed line), until a point is reached at which the rate of input of mutations is balanced by the rate of selective removal. In this second steady-state phase (solid black line), although mutation and selection pressures remain relatively balanced, there is a progressive increase in the average mutation load owing to the stochastic processes outlined in Figure 2. The final and rapid meltdown phase (solid red line) is initiated once the mean mutation load is high enough that the population is incapable of numerical replacement. The blue line represents the viral population size, which remains constant until the mutation load reaches the survival threshold, at which point there is a rapid decline toward extinction.**

about mutation rates and effects (Lynch and Gabriel 1990; Lynch et al. 1993). Compensatory and/or back mutations can serve to slow the process (Wagner and Gabriel 1990; Poon and Otto 2000; Goyal et al. 2012), as can recombination, provided genome-wide mutation rates are in the realm of that typically observed in eukaryotes (Gabriel et al. 1993; Lynch et al. 1995). However, sufficiently high mutation rates can overwhelm the ability of natural selection to remove mutation load, even in enormous populations with recombination, leading to nearly deterministic extinction (e.g., Lynch 2020). Thus, given that many pathogens, viruses in particular, have very high mutation rates, they may commonly reside close enough to threshold levels of mutation load that moderate increases in error rates will ensure extinction.

A Consideration of Viral Mutation Rates

It has long been thought that viral mutation rates are roughly inversely correlated with genome size (Gago et al. 2009), with the usual interpretation being that viruses with small genomes are condemned to such states owing to the nature of their replication machinery. For this reason, many assume that RNA-based viral genomes are smaller than DNA genomes owing to intrinsically error-prone replicative RNA polymerases (Drake and Holland 1999; Elena and Sanjuán 2005). However, recent observations suggest that the direction of causality may be reversed. That is, rather than genomes being small because their mode of genome replication is error-prone, when genomes are small, the selection pressure for replication fidelity (per nucleotide site) is reduced, owing to the fact that selection operates on the genome-wide deleterious mutation rate (Kimura 1967; Lynch 2008).

Based on this central point, the drift-barrier hypothesis (Lynch 2011, 2012; Lynch et al. 2016) provides a general explanation for the 1000-fold range in mutation-rate variation across the tree of cellular life. It also explains why the subset of polymerases dedicated to just the repair of small regions of damaged DNA have elevated error rates, and why secondary lines of defense such as mismatch repair are much more error-prone than the earlier polymerization step. These ideas readily extend to viruses, with observations on coronaviruses (CoVs) providing especially strong support. The order Nidovirales, which includes CoVs, have genome sizes that are considerably larger than those of many other RNA viruses, with approximately two-thirds of the genome allocated to replication-related functions. Like other single-stranded RNA viruses, CoVs replicate by use of an RNA-dependent RNA polymerase (RdRp), but unlike other such viruses, they have a secondary proof-reading subunit. Deactivation of the proofreader leads to ~20-fold reduction in replication fidelity (Smith et al. 2013; Graepel et al. 2017), which means that the proofreader removes ~95% of errors at the polymerization stage.

Laboratory evolution experiments show that murine hepatitis virus (MHV) with a deactivated proofreader (created with two alanine substitutions at key catalytic sites) reverts to its wild-type mutation rate in <200 cell passages (Graepel et al. 2017, Graepel et al. 2019). Remarkably, however, the compensatory mutations are never back mutations at the altered proofreading positions, nor anywhere else in the proofreading domain. Rather, they are scattered over the three other subunits contributing to replication (including the polymerase domain). These results are fully concordant with the theory of evolutionary layering in surveillance mechanisms – because selection operates on the total error rate, an added layer of defense results in relaxation of selection on the component parts (Lynch 2012). This eventually results in a decline of the total system performance to the level preceding the addition of the second layer, with neither component at its peak performance (Frank 2007). Consistent with this expectation, the polymerization step of CoV replication alone appears to have a high error rate compared to that in RNA viruses that do not encode a proofreader (Ferron et al. 2018).

Many studies indicate that viruses are quite capable of evolving lower mutation rates (e.g., Pfeiffer and Kirkegaard 2003; Coffey et al. 2011; Sadeghipour and McMinn 2013; Meng and Kwang 2014), indicating that they are by no means up against a biophysical barrier (although, see Fitzsimmons et al. 2018), but are instead stalled by some other evolutionary effect. The drift-barrier hypothesis accounts for such behavior by postulating that, as with cellular life, viral mutation rates are relentlessly pushed to lower levels by natural selection, the power of which becomes compromised once any further improvement in replication fidelity conferred by an anti-mutator is lower than the power of genetic drift. Despite their enormous absolute population census sizes, owing to their mode of transmission, viruses typically appear to have extremely low effective population sizes, N_e (on the order of hundreds to thousands; Hughes 2009; Miyashita and Kishino 2010; Renzette et al. 2013), with the power of drift being $\sim 1/N_e$. Theory predicts that the selective advantage of an anti-mutator is equal to the reduction in the genome-wide deleterious mutation rate (U_D). This means that if $N_e = 1000$, once U_D has evolved down to 0.001, even the perfect anti-mutator that completely eliminated error production cannot be promoted by selection. If the total genomic mutation rate in coronaviruses is on the order of $\sim$0.1 (e.g., Eckerle et al. 2010), and given that few anti-mutators have a $>10\%$ improvement in fidelity, it is likely that given their genome size, coronaviruses cannot evolve much lower mutation rates. However, recombination is also a significant factor here, as discussed below.

Although these arguments are inconsistent with the conventional wisdom that viruses are actively selected to have high mutation rates so as to deal with environmental challenges, it should be noted that: (1) this usual view is not supported by any direct empirical evidence; (2) it has been extraordinarily difficult to show how optimizing mutation rates can be maintained by selection, especially with recombining genomes; and (3) every observation on coronavirus replication fidelity to date is consistent with the drift-barrier hypothesis.

A Primer on Mutational Meltdown

Given these natural constraints on mutation-rate evolution, we next consider the potential effects of an artificial modification of the underlying rate (or on the proofreader itself). At least two processes contribute to the stochastic dynamics driving the accumulation of deleterious mutations under the meltdown model. First, Muller's ratchet describes the decline of fitness owing to the stochastic loss of the most-fit genotype (Fig. 2A; Muller 1964; Felsenstein 1974), a phenomenon generally considered to be substantially exacerbated in the absence of recombination. In short, owing to the recurrent input of deleterious mutations and their slow removal by selection, the members of a population will vary in terms of the number of carried deleterious mutations, with the form of the distribution being roughly Poisson. Because very few individuals reside in the most-fit class, it is highly vulnerable to stochastic loss. Provided that reversion- and/or compensatory mutations are rare, once the fittest class is lost, the new "least-loaded" class is less fit than the previous version. These successive losses are referred to as clicks of the ratchet. While Haigh (1978) hypothesized that the expected number of individuals in the least-loaded class is likely to be the most important determinant of the speed of the ratchet, the rate of fitness loss has since been demonstrated to depend more specifically on the effective population size, mutation rate, and the magnitude of deleterious selection coefficients (Stephan et al. 1993; Gordo and Charlesworth 2000). Although the full mathematical details of the process remain to be worked out, the general properties of Muller's ratchet are widely appreciated.

What may be less appreciated is the nature of the driving force underlying the ratchet. The stochastic loss of the fittest class is generally viewed as a consequence of genetic drift in finite populations – with a typically very small number of individuals residing in the most-fit class, there is a high chance that, just by sampling, no progeny from this class will contribute to the next generation. However, the ratchet can also be driven by mutation pressure alone, particularly when mutation rates are high – this happens when all surviving offspring from the most-fit class have acquired at least one additional deleterious mutation. The transition between drift-driven versus mutation-driven stochastic loss occurs when the deleterious-mutation rate is on the order of 1 per individual per generation (Lynch et al. 1993). In this case, even for very large population sizes and free recombination, mutation pressure can overwhelm selection (Lynch and Gabriel 1990), driving population extinction at rates only weakly dependent on population size (Lynch 2020).

Second, while high population mutation rates will naturally also increase the input of beneficial mutations, the far greater input of deleterious variants implies that any adaptive change will likely be linked to (and eventually overwhelmed by) other fitness-reducing variants (Fig. 2B). Similarly, while compensatory/back mutations may represent an avenue towards rescue (Wagner and Gabriel 1990; Poon and Otto 2000; Goyal et al. 2012), this will also be compromised by a high genome-wide mutation rate. These linkage effects, termed weak-selection Hill-Robertson interference, also decrease the probability of fixation of beneficial variants, particularly in low-recombination rate environments (Hill and Robertson 1966; McVean and Charlesworth 2000).

THE RELATIONSHIP OF MUTATIONAL MELTDOWN TO LETHAL MUTAGENESIS

Formally related to the concept of mutational meltdown, but more traditionally discussed in the context of viral therapeutic

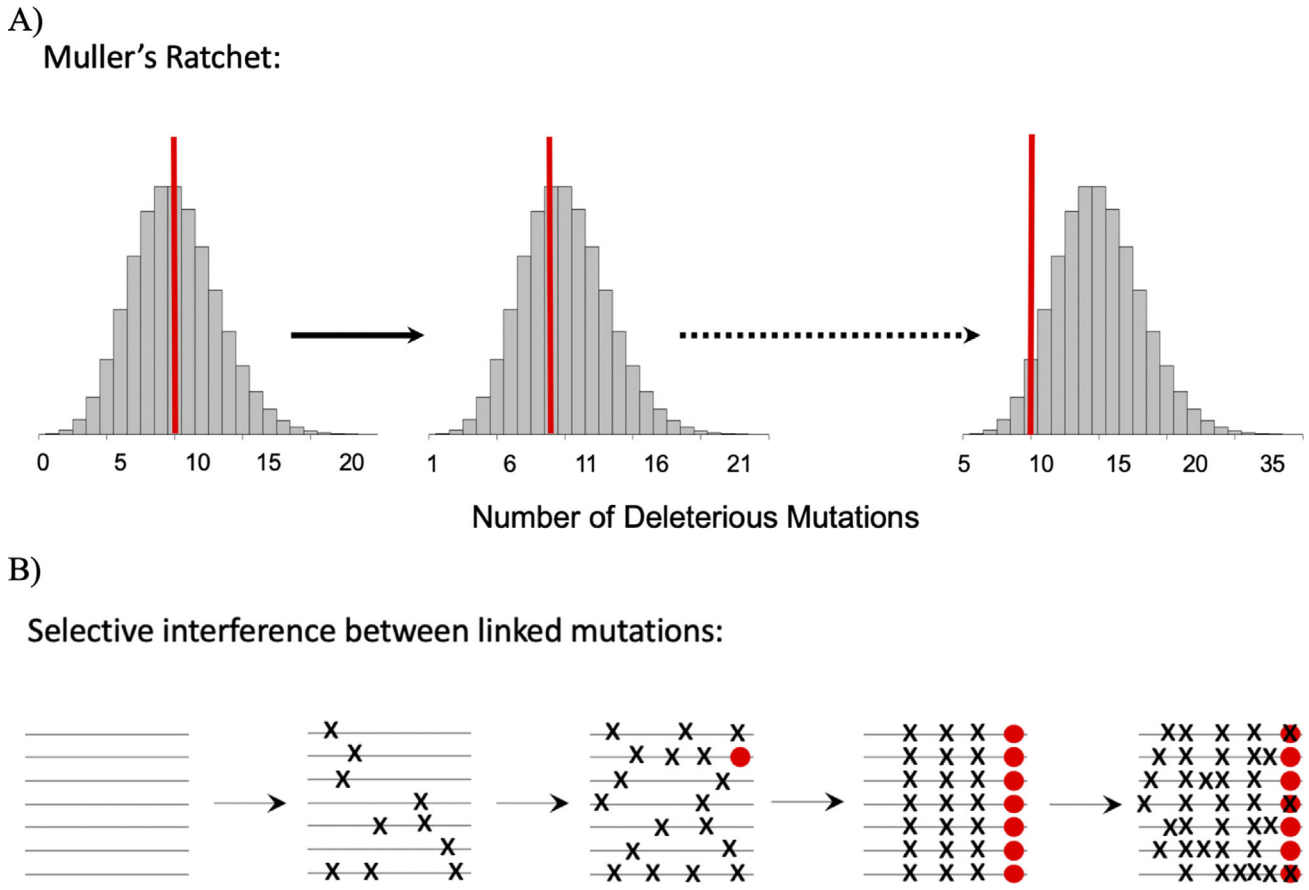

Figure 2. **(A) At approximate selection-mutation-drift balance, an asexual population has an approximately constant steady-state distribution close to Poisson in form, but with the mean number of deleterious mutations progressively increasing, as the least-loaded class (typically containing a very small number of individuals) is stochastically lost (and not recovered). For reference, the red line denotes the mean number of deleterious mutations per individual at time 0. (B) The progressive buildup of deleterious mutations (indicated with an x) over time, shown with a sample of seven genomes. In the earliest episode, the population has progressed to the point that no individual carries less than one deleterious mutation (the ratchet has clicked once). In the next episode, all individuals carry at least two deleterious mutations, but one has acquired a beneficial mutation (red dot) conferring a net selective advantage that sweeps this chromosomal type to fixation, dragging along three deleterious mutations and transiently removing all variation from the population. Finally, more deleterious mutations accumulate on this previously beneficial background, obliterating the prior selective advantage. Should one of the previously fixed deleterious mutation have increased the mutation rate, this final episode will have also incurred a higher rate of accumulation of mutations.**

strategies, the concept of lethal mutagenesis has been proposed as a route to eventual extinction via increased mutational load (Loeb et al. 1999; Anderson et al. 2004). The literature here has focused on large-population size/deterministic expectations (Bull et al. 2007; Wylie and Shakhnovich 2012), and was initially framed around the notion of error catastrophe (Eigen 1971). As such, it does not explicitly invoke the same stochastic processes described above, but otherwise the principles are the same as for the meltdown model: (1) a genotype-fitness map characterizes the relationship between the number of deleterious mutations and fitness; (2) an evolved balance between mutational input and selective removal defines population mean fitness at equilibrium; and (3) a demographic model links these two with the notion of R_{max}, which quantifies the maximum absolute population growth rate. As with mutational meltdown, extinction is inevitable when the mutational load becomes large enough to drop the population growth rate below the level necessary for maintenance. While the outcome of lethal mutagenesis is viewed as deterministic, the demography is nonetheless important (Nowak and May 2000), and finite population sizes (as in any real population) require the consideration of stochastic effects.

As previously discussed (Matuszewski et al. 2017; Fabreti et al. 2019; Jensen and Lynch 2020), subsequent extensions of these models have begun to blur the simple stochastic/deterministic boundaries described above. For example, Wylie and Shakhnovich (2012), working ostensibly under the

lethal mutagenesis model, described the relationship of faster extinction times under smaller population sizes. Although the concept of lethal mutagenesis is still sometimes discussed as it is distinct from mutational meltdown, it is clear that stochastic effects are important, with a full understanding of mutation-driven extinction requiring a joint accounting of the processes of mutation, selection, and genetic drift (Haldane 1937; Kimura and Crow 1964; Burger 1989). Given the greater generality of the model, we will hereafter refer to mutation-driven extinction as mutational meltdown.

Experimental Insights into Mutational Meltdown

Empirical evidence examining mutational meltdown largely comes from experimental-evolution studies, in which population sizes and/or mutation rates are artificially modulated. For example, Zeyl et al. (2001) established 12 replicate populations from two yeast strains differing in mutation rate by roughly two orders of magnitude. Modulating effective population size by a series of bottlenecks, they observed extinctions in high-mutation-rate populations, with dynamics being linked to the mutational-meltdown model. This model has also recently been examined in the context of genetic load in cancer cell populations (Persi et al. 2018; Zhang et al. 2019). Speaking more to our specific purpose here, multiple investigations have focused on RNA viruses. For example, Loeb et al. (1999) investigated increased mutation rates in HIV populations using a pro-mutagenic nucleoside analog, observing a loss of viral replicative potential in seven of nine experiments after one to two dozen serial passages (with no loss observed in 28 control cultures). Using ribavirin as a mutagen and working with poliovirus, Crotty et al. (2001) observed that a roughly 10-fold increase in mutagenesis in the presence of the drug resulted in a nearly complete loss of viral genome infectivity. Airaksinen et al. (2003), also working with ribavirin, but applied to foot-and-mouth disease virus, reported the ability of mutagenesis-based treatment to eliminate the virus in infected cells. The utility of ribavirin has been reported for multiple other viruses (Crotty et al. 2000; Lanford et al. 2001; Severson et al. 2003). Most pertinent, Sheahan et al. (2020) recently demonstrated that the ribonucleoside analog EEID-1931 has a mutagenic effect on SARS-CoV-2 passaged in cell culture. Significantly, working in a mouse model, they further observed a positive correlation between increased viral mutation rates (assessed by the frequency of observed mutations) and the degree of therapeutic efficacy.

Relatedly, recent studies using the drug favipiravir have shown an ability to inhibit the RNA-dependent RNA polymerase (RdRp) of multiple RNA viruses including Ebola, yellow fever, chikungunya, enterovirus, and norovirus (and see Baranovich et al. 2013; Furuta et al. 2013). Utilizing experimental passaging of influenza A virus (IAV) at different concentrations of favipiravir, Bank et al. (2016) observed a strong fit to predicted meltdown dynamics – a linear rate of mutational accumulation in time until a transition point was reached, followed by a sharp increase in mutation load and population collapse (and see Lumby et al. 2020). Interestingly, under low favipiravir concentrations, evidence for adaptation to the drug was observed – population growth rates initially declined, but began to recover in later passages. A number of mutations were identified as potential candidates driving adaptive selective sweeps related to this shift. One cluster of mutations in the polymerase subunit PA was associated with the subcellular localization of viral RdRp components, and two of these RdRp mutations conferred resistance to favipiravir in IAV-infected cells (Goldhill et al. 2018). Nonetheless, under high concentrations of favipiravir, the Bank *et al*. study observed no mutational escape, consistent with the idea that even in the presence of newly arising beneficial resistance mutations, the linked deleterious load under strong mutation pressure can be simply too great to enable significant net adaptation (Pénnison et al. 2017), again emphasizing the potential advantage of such a genome-wide target size.

In an extension of this work in IAV, Ormond et al. (2017) examined the effects of favipiravir combined with oseltamivir, a frequently used drug that acts as a competitive inhibitor of the viral surface neuraminidase (NA) glycoprotein, which is responsible for binding host-cell sialic acid to enable the release of viral progeny (Moscona 2005). Using drugs in combination is an established clinical strategy (Mitchison 2012), and this particular combination had been observed to offer synergistic benefits *in vivo* in mouse (Smee et al. 2013). Ormond *et al*. observed the same dynamics as the Bank *et al*. study in populations treated with favipiravir alone, and observed rapid drug-resistance evolution in populations treated with oseltamivir alone (also consistent with previous studies, Renzette et al. 2014; Foll et al. 2014). However, when present in combination, extinction actually proceeded more quickly, with the results again suggesting that the hitchhiking of deleterious mutations (augmented by favipiravir) along with the oseltamivir-resistance mutations accelerated the development of the fixed deleterious load and the resultant population decline (Fig. 2b).

Needed Studies and Open Questions

Importantly, as opposed to targeting a specific genomic function, the input of deleterious mutations resulting from mutation-inducing drugs is a genome-wide effect, thus increasing the rate of mutational degradation of all functional regions. A significant limitation of therapeutics targeting specific genomic regions is that they provide consistent targets for counter-adaptation in the

form of resistance mutations. For example, as noted above, the influenza drug oseltamivir acts as a competitive inhibitor by binding to a hydrophobic pocket in the viral surface protein, an action that can be thwarted by mutations near the binding site (Collins et al. 2008). Although the high fitness cost to the virus of the most common oseltamivir-resistant mutation, NA H274Y (or H275Y, depending on the NA type), was initially thought to make the development of resistance unlikely (Ives et al. 2002), resistance spread rapidly in the 2007/8 influenza season (Moscona 2009; and see Bloom et al. 2010). Thus, the enhancement of genome-wide mutagenesis may provide a substantial advantage for future treatment strategies.

Important theoretical and experimental extensions are required to move this field of research forward in ways that apply to a broader range of viruses as well as to other pathogens (bacteria, fungi, and protists). Pénnison et al. (2017) have made a number of important steps in this regard. Specifically, they considered the joint input of beneficial and deleterious mutations, characterizing the resulting trade-off in fixation probabilities as mutation rates rise, and demonstrating that these linkage effects may greatly reduce the probability of fixation of beneficial mutations relative to the unlinked expectation (Haldane 1927; Barton 1995; Bachtrog and Gordo 2004; Charlesworth 2013). They concluded that the critical mutation rate necessary for mutational meltdown is defined by the point at which no beneficial mutation has a sufficient selective advantage to offset the subsequent arrival of linked deleterious effects (what they term "the effects of lineage contamination"). A number of unresolved issues remain, however, as the full form of the frequency distribution of fitness effects (DFE), which defines the critical point, is difficult to accurately quantify in natural populations (although improved inference approaches are actively being developed, see Johri et al. 2020). Accounting for variable selection coefficients will be a necessary component of future models, as earlier work often made the unrealistic assumption of fixed deleterious effects, leaving the influence of interference effects under realistic DFEs largely unexplored.

In addition, strongly skewed progeny distributions inherent to viral reproduction violate many assumptions underlying common population-genetic models (i.e., a single 'individual' may produce progeny far in excess of simple random replacement), requiring a so-called multiple-merger coalescent framework for analysis (e.g., Irwin et al. 2016; Matuszewski et al. 2018; Sackman et al. 2019). When a single virion can produce large numbers of new virions, this will alter expected within-host mutational dynamics in synergistic ways, as a new mutation associated with a large skew event may rapidly increase in frequency stochastically. Relatedly, as initial work on meltdown focused on small population sizes, the often extremely large viral population sizes underlying infections require further consideration. Promisingly, despite huge variation in census sizes observed across organisms,

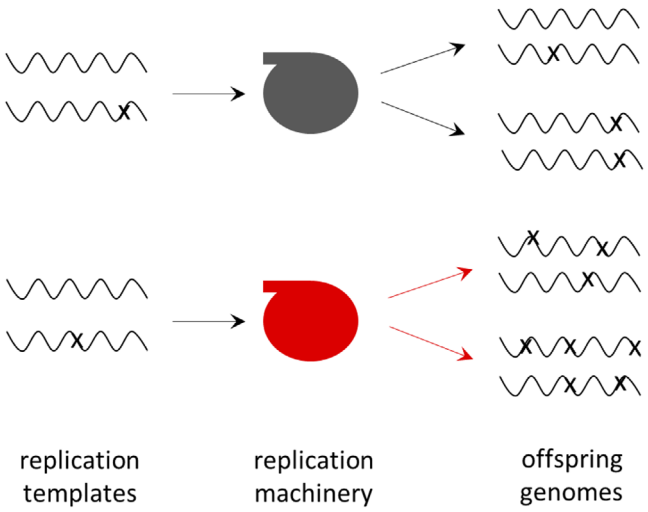

Figure 3. An idealized schematic of mutation accumulation among viral genomes within an individual host cell. On the left are four viral genome templates (e.g., negative-strand RNAs), two of which carry novel mutations (marked by an x) that have arisen within the host cell. In the center, the shapes represent nonmutant (grey) and mutant (red) replication complexes residing within the host cell; the latter may have arisen from transcriptional errors or be results of prior genomic mutations within the original host-cell colonists. To the right is a subset of viral progeny genomes, with the variant replicase generating an elevated number of mutations. Although a number of details are omitted, the figure illustrates the molecular population-genetic aspects of RNA virus replication that need to be evaluated in future applications of mutational-meltdown theory.

genetic effective population sizes appear remarkably constrained (Neher 2013; Lynch 2020; Lynch and Trickovic 2020), likely because of the strong interference effects noted above, which cause populations to behave as though they are much smaller than absolute numbers would imply.

Furthermore, despite the enormous number of virions that can reside within an infected individual, the peculiar replication dynamics and genome structure of RNA viruses may substantially boost their vulnerability to mutation accumulation beyond the expectations for current mutational-meltdown theory (Fig. 3). First, approximately two-thirds of the SARS-CoV-2 genome encodes for proteins associated with genome replication. This suggests that a substantial fraction of genomic mutations (and transcription errors) will directly influence replication fidelity, bringing in an element of synergism not accounted for in the existing theory but expectedly accelerating the rate of mutation accumulation.

Second, as multiple sets of replication proteins and viral genomes can reside within viral replication compartments in single host cells, cross-engagement can occur in a sort of "public-goods" scenario (Lynch and Gabriel 1990). That is, unlike the

situation in cellular organisms where there is a closed one-to-one relationship between individual genomes and their products, here a mutant polymerase can engage with a nonmutant template or vice versa (from either a prior replication or from a coinfection), further magnifying the rate of mutation proliferation (although see the discussion pertaining to defective interfering particles below). Support for this idea derives from observations, more than 60 years ago, showing that influenza virus incurs an elevated rate of fitness loss when the multiplicity of infection, and hence molecular crosstalk, is maintained at a high level (von Magnus 1954).

Perhaps most importantly, the roughly 30-kb SARS-CoV-2 genome exhibits evidence for recombination breakpoints across the genome (Wu et al. 2020), consistent with earlier observations on homologous recombination during co-infection of coronaviruses (Lai 1990; Lai and Cavanagh 1997; Graham and Baric 2010). The ability of recombination to facilitate purging of deleterious variants by freeing beneficial mutations from deleterious mutation-laden backgrounds, and the mutation rate necessary to overwhelm this effect, requires further study. Given estimated viral polymerase error rates (e.g., Drake and Holland 1999; Zhao et al. 2004; Sanjuan et al. 2010), SARS-CoV-2 and other RNA viruses may already reside near a mutational tipping point, even with recombination, as discussed above. Assuming viral mutation rates are as high as previously suggested, recombination may not be able to free beneficial alleles faster than the buildup of an induced linked deleterious load if the rate were to be artificially increased (and see Santiago and Caballero 2020). Furthermore, for complex viral adaptations involving multiple mutational changes, recombination may even accelerate meltdown in high mutation-rate contexts when the rate of recombination between the relevant sites is greater than the selective advantage afforded by the complex adaptation (Lynch 2010; Weissman et al. 2010).

Related to the point made above on the indiscriminate use of damaged public goods, it has long been known that various forms of nonhomologous recombination cause viral populations to make defective interfering particles, including truncated genomes, which hijack the replication machinery, thereby reducing the transmissibility of intact genomes (Huang 1973; Poirier et al. 2015). In effect, this leads to a sort of molecular parasitism that progressively drives down mean population fitness even in the absence of point-mutation-driven clicks of the ratchet. The matter is of relevance here because in addition to elevating the point mutation rate (Baranovich et al. 2013; Arias et al. 2014), drugs such as favipiravir may also cause premature chain termination (see discussion of Abdelnabi et al. 2017). The degree to which these two types of genomic alterations jointly influence mutational meltdown, in presumably nonadditive ways, also requires further attention.

Importantly, any practical application of mutational meltdown will need to consider the molecular genetics of the target species. The structure of the SARS-CoV-2 replicative RdRp protein, nsp12, has been solved (Kirchdoerfer and Ward 2019), but other cofactors are essential for processive replication of the large 30-kb genome (Subissi et al. 2014). Unique to large (26 to 32 kb) RNA viral genomes is a subunit, nsp14, which harbors a proofreader domain (Gorbalenya et al. 2006; Eckerle et al. 2010; Sevajol et al. 2014). Homologs of nsp14 are not found among smaller (13 to 16 kb) RNA viral genomes, suggesting that proofreading is likely a key to avoiding mutational meltdown in larger genomes (Gorbalenya et al. 2006). Inactivation of the putative proofreading domain in SARS-CoV nsp14 increases mutation rates ~20-fold (Eckerle et al. 2010; Smith et al. 2013). Moreover, inactivation of proofreading combined with treatment using RNA viral mutagens synergistically decreases infectivity and viral titers, while increasing mutation rates in both MHV and SARS-CoV (Smith et al. 2013). Thus, drugs targeting nsp14 may represent a particularly valuable avenue in future design, perhaps even more so in combination with other viral RNA mutagens. While evolutionary layering might suggest that the polymerase could compensate for such a loss, it is an open question as to whether the necessary virus-generation time-scale would be of relevance for any given patient infection (e.g., perhaps rendering it of differing applicability for acute vs. chronic infections).

Newly Emerging Clinical Results

Wang et al. (2020) recently evaluated the antiviral efficiency of remdesivir against a clinical isolate of SARS-CoV-2, finding that it is modestly effective in vitro (and see Li and De Clercq 2020; Beigel et al. 2020), with similarly encouraging results recently reported from infected rhesus macaques (Williamson et al. 2020). Importantly, SARS-CoV was previously found to be more sensitive to remdesivir in the absence of nsp14 proofreading (Agostini et al. 2018). As of Summer 2020, initial published results have been reported from small-scale clinical trials of multiple drugs in Shenzhen (Dong et al. 2020); based on a few dozen patients with controls, the preliminary data suggest faster viral clearance with favipiravir compared with other tested treatments. The fact that nucleoside analogues, such as remdesivir and favipiravir, which are incorporated into replicating RNA genomes, can be removed by RdRp proofreading (D'Abramo et al. 2004), serves to further highlight the importance of this domain and its likely role in future developments (and see Agostini et al. 2018). However, greater clarification on the mechanism of any given therapeutic - whether it directly causes point-mutation accumulation or chain termination, for example - will be of importance.

Finally, although existing theoretical, experimental, and clinical results are promising with respect to the utility of

mutational meltdown as a within-patient eradication strategy, a number of practical questions remain to be addressed. For example, an understanding of the optimal drug concentration necessary to induce meltdown is of particular significance. A minor increase in the mutation rate may not only be insufficient to achieve the desired meltdown result but may be dangerous in the sense of enabling the spread of adaptive/resistance mutations, without generating a sufficiently deleterious linked-mutational load. This, of course, is a general concern with any non-lethal anti-microbial. In addition, the general time-scale of patient-level mutation-driven extinction remains as a key question. While previous calculations in RNA viruses appear promising in this regard (e.g., Lynch et al. 1993), a full examination of the relevant underlying parameters in SARS-CoV-2, including improved estimates of both mutation and recombination rates, will be essential. In this regard, long-term mutation-accumulation experiments across CoVs, followed by whole-genome sequencing, will be of great value. Nonetheless, the discussed evidence encouragingly suggests that mutation-driven extinction may successfully be accelerated by pressuring viral populations with drugs in combination, or by simultaneously targeting the proof-reading mechanism; and candidate drugs already in existence have been partially evaluated for patient safety (e.g., Nagata et al. 2014).

What is reasonably clear is that the emergence of new pandemics will remain a constant threat, and thus the development of a therapeutic first-response generally applicable across viruses, such as drug-induced mutational meltdown, is a research area that merits further study - an area that the field of population genetics can greatly inform.

ACKNOWLEDGMENTS

The authors thank Matt Dean, Jim Bull, and an anonymous reviewer for helpful comments and suggestions that improved the manuscript. This work was funded by National Institutes of Health grant R01-GM135899 to J.D.J., National Institutes of Health grant R35-GM122566-01 and US Department of Army MURI award W911NF-14-1-0411 to M.L., and subawards from the US Department of Defense award W81XWH-15-1-0317 and National Institutes of Health grant P01AI129859 to T.F.K.

LITERATURE CITED

Abdelnabi, R., A. T. S. Morais, P. Leyssen, I. Imbert, S. Beaucourt, H. Blanc, M. Froeyen, M. Vignuzzi, B. Canard, J. Neyts, et al. 2017. Understanding the mechanism of the broad-spectrum antiviral activity of favipiravir (T-705): Key role of the F1 motif of the viral polymerase. J. Virol 91:e00487–17.

Agostini, M. L., E. L. Andres, A. C. Sims, R. L. Graham, T. P. Sheahan, X. Lu, E. C. Smith, J. B. Case, J. Y. Feng, R. Jordan, et al. 2018. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. MBio 9:1–15.

Airaksinen, A., N. Pariente, L. Menendez-Arias, and E. Domingo. 2003. Curing of foot-and-mouth disease virus from persistently infected cells by ribavirin involves enhanced mutagenesis. Virology 311:339–349.

Anderson, J. P., R. Daifuku, and L. A. Loeb. 2004. Viral error catastrophe by mutagenic nucleosides. Ann. Rev. Microbiol. 58:183–205.

Arias, A., L. Thorne, and I. Goodfellow. 2014. Favipiravir elicits antiviral mutagenesis during virus replication in vivo. eLife 3:e03679.

Bachtrog, D., and I. Gordo. 2004. Adaptive evolution of asexual populations under Muller's ratchet. Evolution 58:1403–1413.

Bank, C., R. T. Hietpas, A. Wong, D. N. A. Bolon, and J. D. Jensen. 2014a. A Bayesian MCMC approach to assess the complete distribution of fitness effects of new mutations: uncovering the potential for adaptive walks in challenging environments. Genetics 196:841–852.

Bank, C., G. B. Ewing, A. Ferrer-Admetlla, M. Foll, and J. D. Jensen. 2014b. Thinking too positive? Revisiting current methods in population genetic selection inference. Trends Genet. 30:540–546.

Bank, C., N. Renzette, P. Liu, S. Matuszewski, H. Shim, M. Foll, D. N. Bolon, K. Zeldovich, T. F. Kowalik, R. W. Finberg, et al. 2016. An experimental evaluation of drug induced mutational meltdown as an antiviral treatment strategy. Evolution 70:2470–2484.

Baranovich, T., S. S. Wong, J. Armstrong, H. J. Marjuki, R. J. Webby, R. G. Webster, and E. A. Govorkova. 2013. T-705 (favipiravir) induces lethal mutagenesis in influenza A H1N1 viruses in vitro. J. Virol 87:3741–3751.

Barton, N. H. 1995. Linkage and the limits to natural selection. Genetics 140:821–841.

Beigel, J. H. K. M. Tomashek, L. Dodd, A. Mehta, B. Zingman, A. Kalil, E. Hohmann, H. Chu, A. Luetkemeyer, S. Kline, D. Lopez de Castilla, et al. 2020. Remdesivir for the treatment of Covid-19 - preliminary report. N. Engl. J. Med. in press.

Bloom, J. D., L. I. Gong, and D. Baltimore. 2010. Permissive secondary mutations enable the evolution of influenza oseltamivir resistance. Science 328:1272–1275.

Bull, J. J., R. Sanjuan, and C. O. Wilke. 2007. Theory of lethal mutagenesis for viruses. J. Virol 81:2930–2939.

Burger, R. 1989. Linkage and the maintenance of heritable variation by mutation-selection balance. Genetics 121:175–184.

Charlesworth, B. 2013. Background selection 20 years on. J. Hered 104:161–171.

Coffey, L. L., Y. Beeharry, A. V. Bordería, H. Blanc, and M. Vignuzzi. 2011. Arbovirus high fidelity variant loses fitness in mosquitoes and mice. Proc. Natl. Acad. Sci. USA 108:16038–16043.

Collins, P. J., L. F. Haire, Y. P. Lin, J. Liu, R. J. Russell, P. A. Walker, J. J. Skehel, S. R. Martin, A. J. Hay, and S. J. Gamblin. 2008. Crystal structures of oseltamivir-resistant influenza virus neuraminidase mutants. Nature 453:1258–1261.

Crotty, S., D. Maag, J. J. Arnold, W. Zhong, J. Y. Lau, Z. Hong, R. Andino, and C. E. Cameron. 2000. The broad-spectrum antiviral ribonucleoside ribavirin is an RNA virus mutagen. Nature Med. 6:1375-1379.

Crotty, S., C. E. Cameron, and R. Andino. 2001. RNA virus error catastrophe: direct molecular test by using ribavirin. Proc. Natl. Acad. Sci. USA 98:6895–6900.

Crow, J. F. 1993. Mutation, mean fitness, and genetic load. Oxf. Surv. Evol. Biol. 9:3–42.

D'Abramo, C. M., L. Cellai, and M. Götte. 2004. Excision of incorporated nucleotide analogue chain-terminators can diminish their inhibitory effects on viral RNA-dependent RNA polymerases. J. Mol. Biol. 337:1–14.

Dong, L., S. Hu, and J. Gao. 2020. Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discov. Ther. 14:58–60.

Drake, J. W., and J. J. Holland. 1999. Mutation rates among RNA viruses. Proc. Natl. Acad. Sci. USA 96:13910–13913.

Eckerle, L. D., M. M. Becker, R. A. Halpin, K. Li, E. Venter, X. Lu, S. Scherbakova, R. L. Graham, R. S. Baric, T. B. Stockwell, et al. 2010.

Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication is revealed by complete genome sequencing. PLoS Pathog. 6:e1000896.

Eigen, M. 1971. Self-organization of matter and the evolution of biological macromolecules. Naturwissenschaften 58:465–523.

Elena, S. F., and R. Sanjuán. 2005. Adaptive value of high mutation rates of RNA viruses: separating causes from consequences. J. Virol. 79:11555–11558.

Eyre-Walker, A., and P. D. Keightley. 2007. The distribution of fitness effects of new mutations. Nat. Rev. Genet. 8:610–618.

Fabreti, L. G., D. Castro, B. Gorzoni, L. M. R. Janini, and F. Antoneli. 2019. Stochastic modeling and simulation of viral evolution. Bull. Math. Biol. 81:1031–1069.

Felsenstein, J. 1974. The evolutionary advantage of recombination. Genetics 78:737–756.

Ferron, F., L. Subissi, A. T. Silveira De Morais, N. T. T. Le, M. Sevajol, L. Gluais, E. Decroly, C. Vonrhein, G. Bricogne, B. Canard, et al. 2018. Structural and molecular basis of mismatch correction and ribavirin excision from coronavirus RNA. Proc. Natl. Acad. Sci. USA 115:E162–E171.

Fitzsimmons, W. J., R. J. Woods, J. T. McCrone, A. Woodman, J. J. Arnold, M. Yennawar, R. Evans, C. E. Cameron, and A. S. Lauring. 2018. A speed-fidelity trade-off determines the mutation rate and virulence of an RNA virus. PLoS Biol. 16:e2006459.

Foll, M., Y. P. Poh, N. Renzette, A. Ferrer-Admetlla, C. Bank, H. Shim, A. S. Malaspinas, G. Ewing, P. Liu, D. Wegmann, et al. 2014. Influenza virus drug resistance: a time-sampled population genetics perspective. PLoS Genet. 10:e1004185.

Frank, S. A. 2007. Maladaptation and the paradox of robustness in evolution. PLoS One 2:e1021.

Furuta, Y., B. B. Gowen, K. Takahashi, K. Shiraki, D. F. Smee, and D. L. Barnard. 2013. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antiviral Res 100:446–454.

Gabriel, W., M. Lynch, and R. Burger. 1993. Muller's ratchet and mutational meltdowns. Evolution 47:1744–1757.

Gago, S., S. F. Elena, R. Flores, and R. Sanjuán. 2009. Extremely high mutation rate of a hammerhead viroid. Science 323:1308.

Goldhill, D. H., A. J. W. te Velthuis, R. A. Fletcher, P. Langat, M. Zambon, A. Lackenby, and W. S. Barclay. 2018. The mechanism of resistance to favipiravir in influenza. Proc. Natl. Acad. Sci. USA 115:11613–11618.

Gorbalenya, A. E., L. Enjuanes, J. Ziebuhr, and E. J. Snijder. 2006. Nidovirales: Evolving the largest RNA virus genome. Virus Res. 117:17–37.

Gordo, I., and B. Charlesworth. 2000. On the speed of Muller's ratchet. Genetics 156:2137–2140.

Goyal, S., D. J. Balick, E. R. Jerison, and R. A. Neher. 2012. Dynamic mutation-selection balance as an evolutionary attractor. Genetics 191:1309–1319.

Graepel, K. W., X. Lu, J. B. Case, N. R. Sexton, E. C. Smith, and M. R. Denison. 2017. Proofreading-deficient coronaviruses adapt for increased fitness over long-term passage without reversion of exoribonuclease-inactivating mutations. mBio 8:e01503–17.

Graepel, K. W., M. L. Agostini, X. Lu, N. R. Sexton, and M. R. Denison. 2019. Fitness barriers limit reversion of a proofreading-deficient coronavirus. J. Virol. 93:e00711–19.

Graham, R. L., and R. S. Baric. 2010. Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. J. Virol 84:3134–3146.

Haigh, J. 1978. The accumulation of deleterious genes in a population — Muller's ratchet. Theor. Popul. Biol. 14:251–267.

Haldane, J. B. S. 1927. A mathematical theory of natural and artificial selection. Part V. Selection and mutation. Proc. Camb. Philos. Soc. 23:838–844.

Haldane, J. B. S. 1937. The effect of variation in fitness. Am. Nat. 71:337–349.

Hill, W. G., and A. Robertson. 1966. The effect of linkage on limits to artificial selection. Genet. Res. 8:269–294.

Huang, A. S. 1973. Defective interfering viruses. Annu. Rev. Microbiol. 27:101–117.

Hughes, A. L. 2009. Small effective population sizes and rare nonsynonymous variants in potyviruses. Virology 393:127–134.

Irwin, K. K., S. Laurent, S. Matuszewski, S. Vuilleumier, L. Ormond, H. Shim, C. Bank, and J. D. Jensen. 2016. On the importance of skewed offspring distributions and background selection in virus population genetics. Heredity 117:393–399.

Ives, J. A., J. A. Carr, D. B. Mendel, C. Y. Tai, R. Lambkin, L. Kelly, J. S. Oxford, F. G. Hayden, and N. A. Roberts. 2002. The H274Y mutation in the influenza A/H1N1 neuraminidase active site following oseltamivir phosphate treatment leave virus severely compromised both in vitro and in vivo. Antiviral Res. 55:307–317.

Jensen, J. D., and M. Lynch. 2020. Considering mutational meltdown as a potential SARS-CoV-2 treatment strategy. Heredity 124:619–620.

Johri, P., B. Charlesworth, and J. D. Jensen. 2020. Towards an evolutionarily appropriate null model: jointly inferring demography and purifying selection. Genetics 215:173–192.

Kimura, M., and J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. Genetics 49:725–738.

Kimura, M. 1967. On the evolutionary adjustment of spontaneous mutation rates. Genet. Res. 9:23–34.

Kirchdoerfer, R. N., and A. B. Ward. 2019. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. Nat. Commun. 10: 2342.

Lai, M. M. 1990. Coronavirus: organization, replication, and expression of genome. Annu. Rev. Microbiol. 44:303–333.

Lai, M. M., and D. Cavanagh. 1997. Molecular biology of coronaviruses. Adv. Virus Res. 48:1–100.

Lanford, R. E., D. Chavez, B. Guerra, J. Y. Lau, Z. Hong, K. M. Brasky, and B. Beames. 2001. Ribavirin induces error-prone replication of GB virus B in primary tamarin hepatocytes. J. Virol. 75:8074–8081.

Li, G., and E. De Clercq. 2020. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). Nat. Rev. Drug Discov. 19:149–150.

Loeb, L. A., J. M. Essigmann, F. Kazazi, J. Zhang, K. D. Rose, and J. Mullins. 1999. Lethal mutagenesis of HIV with mutagenic nucleoside analogs. Proc. Natl. Acad. Sci. USA 96:1492–1497.

Lumby, C. K., L. Zhao, M. Oporto, T. Best, H. Tutill, D. Shah, P. Veys, R. Williams, A. Worth, J. Illingworth, et al. 2020. Favipiravir and Zanamivir cleared infection with Influenza B in a severely immunocompromised child. Clin. Infect. Dis. in press.

Lynch, M. 2008. The cellular, developmental, and population-genetic determinants of mutation-rate evolution. Genetics 180:933–943.

———. 2010. Scaling expectations for the time to establishment of complex adaptations. Proc. Natl. Acad. Sci. USA 107:16577–16582.

———. 2011. The lower bound to the evolution of mutation rates. Genome Biol. Evol. 3:1107–1118.

———. 2012. Evolutionary layering and the limits to cellular perfection. Proc. Natl. Acad. Sci. USA 109:18851–18856.

Lynch, M., and W. Gabriel. 1990. Mutation load and the survival of small populations. Evolution 44:1725–1737.

Lynch, M., R. Burger, D. Butcher, and W. Gabriel. 1993. The mutational meltdown in asexual populations. J. Hered. 84:339–344.

Lynch, M., J. Conery, and R. Burger. 1995. Mutational meltdown in sexual populations. Evolution 49:1067–1080.

Lynch, M., J. Blanchard, D. Houle, T. Kibota, S. Schultz, L. Vassilieva, and J. Willis. 1999. Spontaneous deleterious mutations. Evolution 53:645–663.

Lynch, M., M. Ackerman, J.-F. Gout, H. Long, W. Sung, W. K. Thomas, and P. L. Foster. 2016. Genetic drift, selection, and evolution of the mutation rate. Nature Rev. Genetics 17:704–714.

Lynch, M., and B. Trickovic. 2020. A theoretical framework for evolutionary cell biology. J. Mol. Biol. in press.

Matuszewski, S., L. Ormond, C. Bank, and J. D. Jensen. 2017. Two sides of the same coin: a population genetics perspective on lethal mutagenesis and mutational meltdown. Virus Evol. 3:1–5.

Matuszewski, S., M. E. Hildebrandt, G. Achaz, and J. D. Jensen. 2018. Coalescent processes with skewed offspring distributions and nonequilibrium demography. Genetics 208:323–338.

McVean, G. A., and B. Charlesworth. 2000. The effects of Hill–Robertson interference between weakly selected mutations on patterns of molecular evolution and variation. Genetics 155:929–944.

Meng, T., and J. Kwang. 2014. Attenuation of human enterovirus 71 high-replication-fidelity variants in AG129 mice. J. Virol. 88:5803–5815.

Mitchison, D. A. 2012. Prevention of drug resistance by combined drug treatment of tuberculosis. Handb. Exp. Pharmacol. 211:87–98.

Miyashita, S., and H. Kishino. 2010. Estimation of the size of genetic bottlenecks in cell-to-cell movement of soil-borne wheat mosaic virus and the possible role of the bottlenecks in speeding up selection of variations in trans-acting genes or elements. J. Virol 84:1828–1837.

Moscona, A. 2005. Oseltamivir resistance–disabling our influenza defenses. N. Engl. J. Med. 353:2633–2636.

Moscona, A. 2009. Global transmission of oseltamivir-resistant influenza. N. Engl. J. Med. 360:953–956.

Muller, H. J. 1964. The relation of recombination to mutational advance. Mutat. Res. 106:2–9.

Nagata, T., A. K. Lefor, M. Hasegawa, and M. Ishii. 2014. Favipiravir: a new medication for the Ebola virus disease pandemic. Dis. Med. Pub. Health Prep. 9:79–81.

Neher, R. A. 2013. Genetic draft, selective interference, and population genetics of rapid adaptation. Ann. Rev. Ecol. Evol. Syst. 44:195–215.

Nowak, M., and R. May. 2000. Virus dynamics: mathematical principles of immunology and virology. Oxford Univ. Press, Oxford, U.K.

Ormond, L., P. Liu, S. Matuszewski, N. Renzette, C. Bank, K. Zeldovich, D. Bolon, T. Kowalik, R. Finberg, J. D. Jensen, et al. 2017. The combined effect of oseltamivir and favipiravir on influenza A virus evolution. Genome Biol. Evol 9:1913–1924.

Pénnison, S., T. Singh, P. Sniegowski, and P. Gerrish. 2017. Dynamics and fate of beneficial mutations under lineage contamination by linked deleterious mutations. Genetics 205:1305–1318.

Persi, E., Y. I. Wolf, D. M. Leiserson, E. V. Koonin, and E. Ruppin. 2018. Criticality in tumor evolution and clinical outcome. Proc. Natl. Acad. Sci. USA 115:E11101–10.

Pfeiffer, J. K., and K. Kirkegaard. 2003. A single mutation in poliovirus RNA-dependent RNA polymerase confers resistance to mutagenic nucleotide analogs via increased fidelity. Proc. Nat.l Acad. Sci. USA 100:7289–7294.

Poirier, E. Z., B. C. Mounce, K. Rozen-Gagnon, P. J. Hooikaas, K. A. Stapleford, G. Moratorio, and M. Vignuzzi. 2015. Low-fidelity polymerases of Alphaviruses recombine at higher rates to overproduce defective interfering particles. J. Virol. 90:2446–2454.

Poon, A., and S. P. Otto. 2000. Compensating for our load of mutations: freezing the meltdown of small populations. Evolution 54:1467–1479.

Renzette, N.bson., B. Bhattacharjee, D. Fisher, M. R. Schleiss, J. D. Jensen, and T. F. Kowalik. 2013. Rapid intrahost evolution of human cytomegalovirus is shaped by demography and positive selection. PLOS Genet. 9:e1003735.

Renzette, N., D. R. Caffrey, K. B. Zeldovich, P. Liu, G. R. Gallagher, D. Aiello, A. J. Porter, E. A. Kurt-Jones, D. N. Bolon, Y. P. Poh, et al. 2014. Evolution of the influenza A virus genome during development of oseltamivir resistance in vitro. J. Virol. 88:272–281.

Sackman, A., R. B. Harris, and J. D. Jensen. 2019. Inferring demography and selection in organisms characterized by skewed offspring distributions. Genetics 211:1019–1028.

Sadeghipour, S., and P. C. McMinn. 2013. A study of the virulence in mice of high copying fidelity variants of human enterovirus 71. Virus Res 176:265–272.

Sanjuan, R., M. R. Nebot, N. Chirico, L. M. Mansky, and R. Belshaw. 2010. Viral mutation rates. J. Virol. 84:9733–9748.

Santiago, E., and A. Caballero. 2020. The value of targeting recombination as a strategy against coronavirus diseases. Heredity in press.

Sevajol, M., L. Subissi, E. Decroly, B. Canard, and I. Imbert. 2014. Insights into RNA synthesis, capping, and proofreading mechanisms of SARS-coronavirus. Virus Res. 194:90–99.

Severson, W. E., C. S. Schmalijohn, A. Javadian, and C. B. Jonsson. 2003. Ribavirin causes error catastrophe during Hantaan virus replication. J. Virol. 77:481–488.

Sheahan, T., A. Sims, S. Zhou, R. Graham, A. Pruijssers, M. Agostini, S. Leist, A. Schafer, K. Dinnon III, L. Stevens, et al. 2020. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci. Transl. Med. 12:eabb5883.

Smee, D. F., E. B. Tarbet, Y. Furuta, J. D. Morrey, and D. L. Barnard. 2013. Synergistic combinations of favipiravir and oseltamivir against wild-type pandemic and oseltamivir-resistant influenza A virus infections in mice. Future Virol. 8:1085–1094.

Smith, E. C., H. Blanc, M. C. Surdel, M. Vugnuzzi, and M. R. Denison. 2013. Coronaviruses lacking exoribonuclease activity are susceptible to lethal mutagenesis: evidence of proofreading and potential therapeutics. PLoS Pathog. 9:e1003565.

Subissi, L., C. C. Posthuma, A. Collet, J. C. Zevenhoven-Dobbe, A. E. Gorbalenya, E. Decroly, E. J. Snijder, B. Canard, and I. Imbert. 2014. One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities. Proc. Natl. Acad. Sci. USA 111:E3900–E3909.

Stephan, W., L. Chao, and J. Smale. 1993. The advance of Muller's ratchet in a haploid asexual population: approximate solution based on diffusion theory. Genet. Res. 61:225–232.

von Magnus, P. 1954. Incomplete forms of influenza virus. Adv. Virus Res. 2:59–79.

Wagner, G. P., and W. Gabriel. 1990. Quantitative variation in finite parthenogenetic populations: what stops Muller's Ratchet in the absence of recombination? Evolution 44:715–731.

Wang, M., R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, W. Zhong, and G. Xiao. 2020. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 30:269–271.

Weissman, D. B., M. W. Feldman, and D. S. Fisher. 2010. The rate of fitness-valley crossing in sexual populations. Genetics 186:1389–1410.

Williamson, B. N., F. Feldmann, B. Schwarz, K. Meade-White, D. P. Porter, J. Schulz, N. van Doremalen, I. Leighton, C. Kwe Yinda, L. Perez-Perez, et al. 2020. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. Nature 585:273–276.

Wu, F., S. Zhao, B. Yu, Y. M. Chen, W. Wang, Z. G. Song, Y. Hu, Z. W. Tao, J. H. Tian, Y. Y. Pei, et al. 2020. A new coronavirus associated with human respiratory disease in China. Nature 579:265–269.

Wylie, C. S., and E. I. Shakhnovich. 2012. Mutation induced extinction in finite populations: lethal mutagenesis and lethal isolation. PLoS Comput. Biol. 8:e1002609.

Zeyl, C., M. Mizesko, and J. A. de Visser. 2001. Mutational meltdown in laboratory yeast populations. Evolution 55:909–917.

Zhang, Y., Y. Li, T. Li, X. Shen, T. Zhu, Y. Tao, X. Li, D. Wang, Q. Ma, Z. Hu, et al. 2019. Genetic load and potential mutational meltdown in cancer cell populations. Mol. Biol. Evol. 36:541–552.

Zhao, Z., H. Li, X. Wu, Y. Zhong, K. Zhang, Y. P. Zhang, E. Boerwinkle, and Y. X. Fu. 2004. Moderate mutation rate in the SARS coronavirus genome and its implications. BMC Evol. Biol. 4:21.

Associate Editor: M. D. Dean
Handling Editor: T. Chapman