Fitness changes in a constant environment

Some useful notes (hopefully)

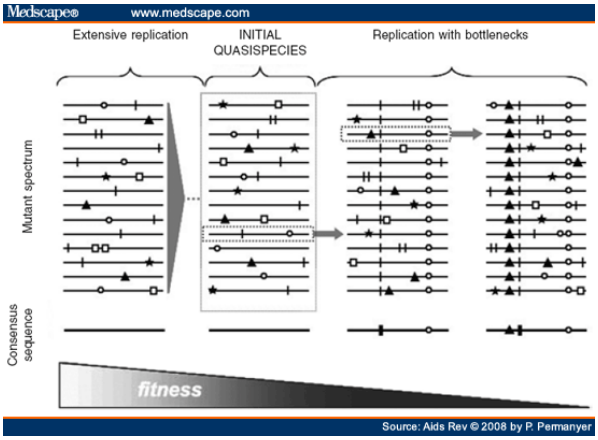
***** High mutation rates do not necessarily result in rapid evolution. Something must drive mutation to increase or decrease frequency.

***** To do experimental evolution with viruses one uses a "wild type" that usually has a history of replication under particular conditions that is sufficiently long to have promoted good adaptation, but not to take the population to the top of a fitness peak.

* To avoid DIP interference we do passages at low MOI. A *passage* represents two (liquid regime) or three (plaque passages) rounds of replication. During each round of replication there are two copy events: from the genome to the antigenome and from the antigenome to the genome. Thus, each round of replications consists of 2 generations. A liquid regime includes 4 generations and plaque-to-plaque passages 6 generations. In each generation the selective forces vary.

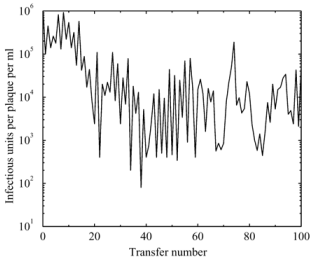
Evolution under drift: Muller's ratchet

To study evolution under random drift we use an experimental design in which selection is minimized by using small populations during infection during transmission and carrying out for many generations. The most dramatic case is that in which the population size is limited to one individual and we do that by picking an individual plaque. Note that selection cannot be completely ignored and the sample will always be biased because variants of low virulence or extremely low fitness will never be sampled.



Using plaque-to-plaque passages and following many replicas of the experiment Chao demonstrated overall fitness loss in phage phi-6. Because the mutation(s) in any given genome are randomly distributed and randomly picked, it is not possible to predict the outcome of a single replica. This experiment has been repeated with many viruses and consistently produced similar results.

FMDV under Muller's ratchet



In long-term experiments different viral species may show different behaviors. While sometimes Muller's ratchet results in extinction in others fitness stabilizes and the population can be maintained for long periods of time because of extensive epistasis and the relative frequency of compensatory mutations. Escarmis has done the most detailed work analyzing the dynamics on evolution under extreme genetic bottlenecks in FMDV.

Muller's ratchet operation depends on the initial fitness of the population so the size of the bottleneck required for fitness loss increases with fitness. This is because more fit populations tend to

have higher deleterious mutation rates. Muller's ratchet does not stop during exposure to new environmental conditions.

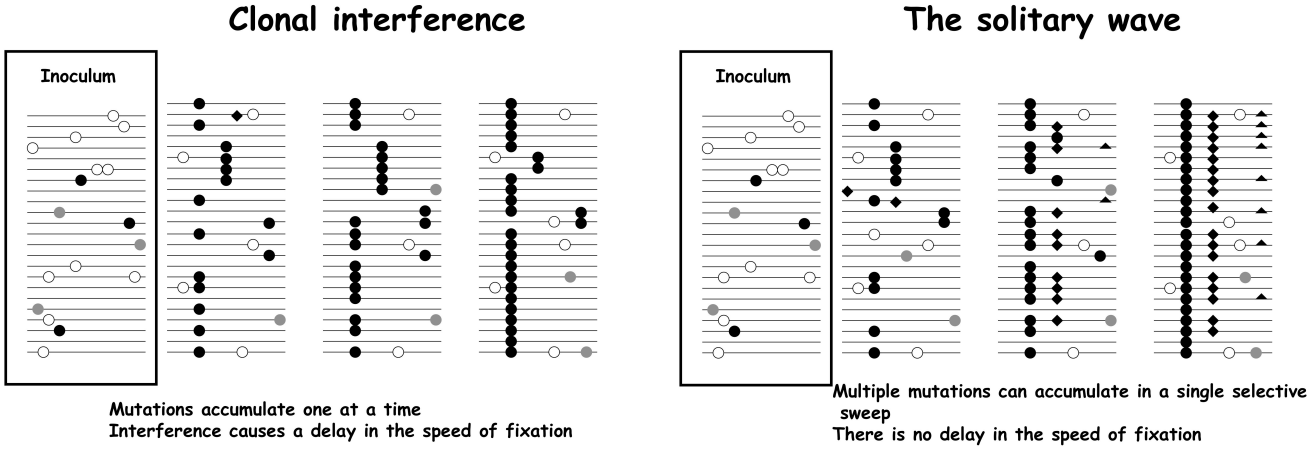
Sex does help with recovery from Muller's ratchet, but it is complicated

How do you stop Muller's ratchet? By regenerating mutation-free genomes by recombination. Chao continued his studies with phage phi-6 and allowed evolution of populations with or without sex. This is not a trivial experiment because of two problems: superfinfection exclusion and complementation. Superinfection exclusion occurs when a virus induces a change in the cell that inhibits infection by other viruses and its consequence is that increasing amount of virus in the inoculum may not necessarily result in higher levels of coinfection. Complementation provides the defective mutant the wt function in trans so it weakens selection. On last thing to consider is the generation of defective genomes (cheaters) that can accumulate.

Mutation can also mediate the recovery of Muller's ratchet.

Fitness changes under positive selection

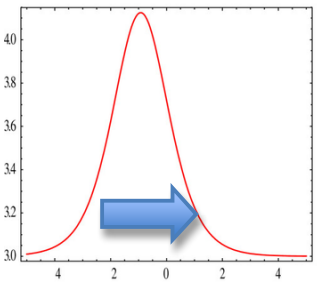
When the population size is sufficiently high and selection is operational viral populations are expected to increase in fitness. There are a number of models that describe/predict the behavior of populations under selection, including clonal interference and solitary wave.

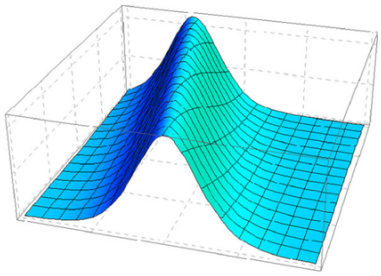


Phil Gerrish and Rich Lenski developed clonal interference theory (Gerrish P. J., Lenski R. E. 1998 The fate of competing beneficial mutations in an asexual population. *Genetica* **102/103**, 127–144. (doi:10.1023/A:1017067816551)). The basis of clonal interference is that when mutation rates are low beneficial variation is limiting and the speed of adaptation is limited by the waiting time between beneficial mutations. Clonal interference has other predictions that other models shared, such as the delay in the speed of fixation in very large populations due to competition among genomes carrying different beneficial mutations (clonal interference effects).

In contrast solitary wave applies at high mutation rates and the delay caused by the waiting times does not apply. Tsimring's work (RNA Virus Evolution via a Fitness-Space Model. Lev S. Tsimring, Herbert Levine, and David A. Kessler. Phys. Rev. Lett. **76**, 4440 – Published 3 June 1996) provided the basis for solitary wave, which was developed by Rouzine, Wakeley and collaborators (Rouzine, Igor M., John Wakeley, and John M. Coffin. 2003. "The solitary wave of asexual evolution." Proc. Natl. Acad. Sci., USA 100 (2): 587-592.).

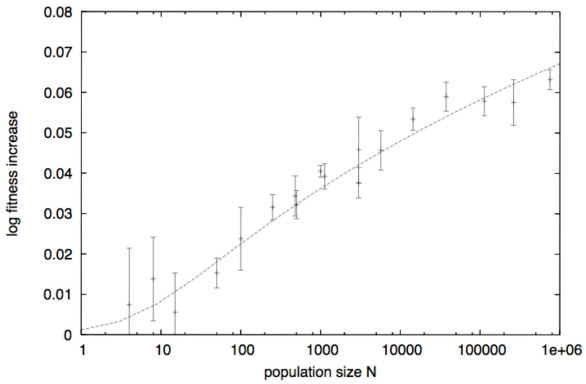
The initial modeling efforts were an attempt to explain the observation that the increase in VSV fitness during large-population passages was exponential and monophasic (for wt-like strains) or biphasic (for deleterious mutants, typically coming from repeated bottlenecking). The initial faster phase in the later corresponds to the collapse of the distribution toward the highest fitness class. The travel of the distribution corresponds to the selection of new beneficial mutations. Adaptation in FMDV is also exponential, but phi-6 increases fitness linearly.



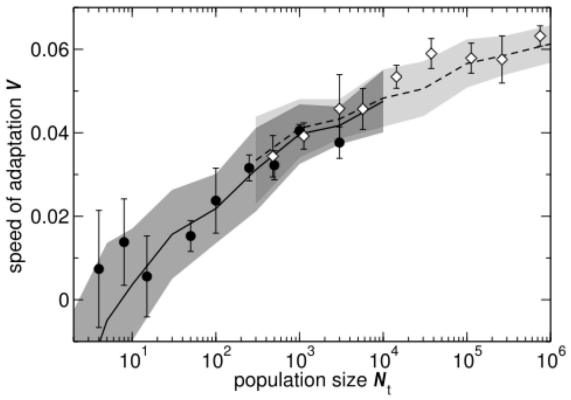


The next figure shows the fit of the experimental data obtained for VSV to clonal interference (left) and solitary wave (right). Each data point represents a replica of an experiment in which the virus underwent selection for 20 passages at different transmission population sizes (Nt) but a constant MOI and periodic determinations were done to measure fitness changes. These changes were used to calculate the speed of adaptation for each Nt.

Fit to clonal interference



Fit to the solitary wave modified

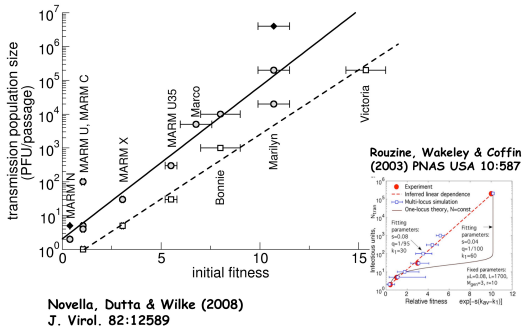


Dutta et al. (2008) J. Virol. 82: 4354

The main difference between the two fits is the use of biologically-relevant parameters in the case of solitary wave, while for clonal interference the fit required unrealistic values of mutation rates and other parameters. Note that we can observe clonal interference effects at large Nt, but clonal interference theory is not a good descriptor of viral behavior because beneficial variation is not limiting. This is in contrast to the results from Lenski, Gerrish and others using bacteria, where indeed the model explains well the observed behavior and its consistent with limits to adaptation placed by the low mutation rates.

At a given population size fitness increases for as long as beneficial variation and/or combinations of mutants can be consistently sampled during transmission. The higher the fitness the more difficult it becomes to find additional beneficial variation and fitness tends to stabilize, although the variance can be

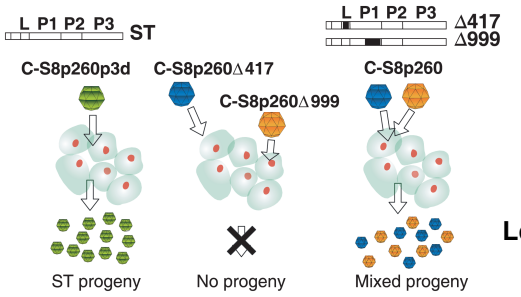
Fit to 17 years of VSV evolution



Novella, Dutta & Wilke (2008) J. Virol. 82:12589

very high. Adaptation can continue if the Nt is increased.

Consistent with of solitary wave theory, sequencing the evolved strains at different times during their evolution showed that all except 1 of the secondary (beneficial) mutations identified arose in genomes that were still at very low frequency.



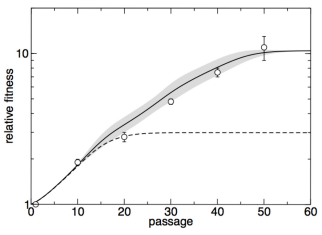
As we have already seen, evolution of populations at high MOI under constant conditions results in different dynamics and selects for cheaters/defectors/DIPs. Once aspect that is particularly interesting is the potential evolution of segmentation or multipartite viruses.

Left figure: Multipartite FMDV

Some aspects of sequence evolution

The types of mutations accumulated during prevailing negative selection has not been tested experimentally, but populations under positive selection of drift have dN/dS close to one. This value in adapting populations is driven by the positive selection of synonymous mutations, which is illustrated by the identification of their parallel evolution. Sequencing of molecular clones from several viruses, including poliovirus and VSV suggest that the polymerase error results in excess transitions in the order of 10-fold. Departures from this value may help with the identification of positive selection. Parallel evolution of all types of mutations is common under selection. Not surprisingly the types of mutations accumulated during the operation of Muller's ratchet is different with insertions/deletions and infrequent types of infrequent more commonly found.

Initial adaptation is mediated by preexisting variation



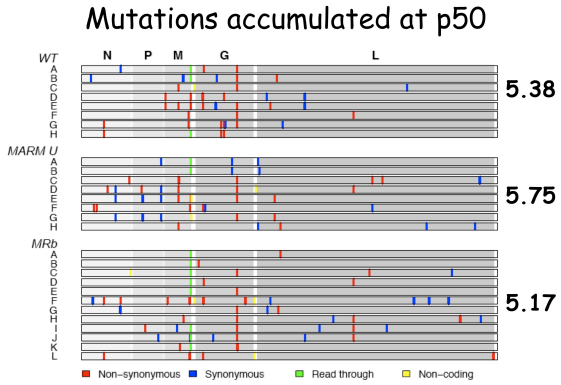
Adaptive mutations increase their frequency very slowly, even when they have high selection coefficient. While this could have been expected it often comes at a surprise and some researcher interpret the stability of the consensus sequence as a reflection of a phenotypically stable population. It is not uncommon to see several-fold differences in fitness before the first change in the consensus is identified, particularly if the computer does the identification.

While new mutations are important in the long run, the initial steps of adaptation are mediated by preexisting variation.

The evolution of adaptability

We have seen that fitness and robustness increase during selection and decrease during drift. The same tendency is observed in the evolution of adaptability, so relaxed selection allows adaptability loss. Thus, repeated bottleneck results in populations that have lost adaptability. In the majority of the replicas fitness can still increase fairly quickly once conditions favor selection over drift, but in some instances the phenotype can be fairly dramatic and there is a long period of stasis before fitness starts to increase.

Low adaptability can be the result of lower beneficial mutation rate or of beneficial mutations having a smaller effect. Low overall mutations rates are unlikely because many of the mutants that have adaptability defects have no non-synonymous mutations in the polymerase. Furthermore, the number of mutations accumulated once fitness is recovered (after 50 passages) does not differ in variants with or without adaptability defects. Thus that change the beneficial mutation rate is the result of a decrease in the positive value of mutations, not the number of beneficial mutations that can be generated.



The genetic basis of differences in adaptability can be remarkably limited: as little as 2-3 mutations can produce an obvious phenotype. This observation is consistent with extensive epistasis throughout the genome.

Figure Credits

Muller's ratchet experiment: Resistance to extinction of low fitness virus subjected to plaque-to-plaque transfers: Diversification by mutation clustering. Escarmis, C (Escarmis, C); Gomez-Mariano, G (Gomez-Mariano, G); Davila, M (Davila, M); Lazaro, E (Lazaro, E); Domingo, E (Domingo, E). JOURNAL OF MOLECULAR BIOLOGY. Volume: 315 Issue: 4 Pages: 647-661. DOI: 10.1006/jmbi.2001.5259

Solitary wave: https://www.researchgate.net/figure/264629762_fig1_Figure-1-Bell-shaped-2D-and-3D-plot-of-the-solitary-wave-The-graphs-have-been-plotted [accessed Feb 1, 2016]

Multipartite FMDV: Viral Genome Segmentation Can Result from a Trade-Off between Genetic Content and Particle Stabilit. Samuel Ojosnegros, Juan García-Arriaza, Cristina Escarmís, Susanna C. Manrubia, Celia Perales, Armando Arias, Mauricio García Mateu, Esteban Domingo. PLoS Genetics DOI: 10.1371/journal.pgen.1001344.