Studying Population Genetic Processes in Viruses: From Drug-Resistance Evolution to Patient Infection Dynamics

Jeffrey D Jensen, Arizona State University, Tempe, AZ, United States

Glossary

Background Selection Reduction in genetic diversity due to purifying selection against deleterious mutations at linked sites.

Distribution of Fitness Effects (DFE) The statistical distribution of selection coefficients of newly arising mutations, as compared to a reference genotype.

Mutational Meltdown The accumulation of stochastic effects which may result in population decline and ultimately extinction.

Selective Sweep The result of a beneficial mutation being driven to high frequency by positive selection, together with linked variation.

Site Frequency Spectrum (SFS) Summary of the frequencies of observed segregating mutations in a population.

In this article, I briefly summarize the population genetic environment in which within-patient viral populations are evolving – discussing the roles of genetic drift as modulated by the infection history of the patient, selection acting on newly arising deleterious and beneficial variants and the related linked selection effects on the genome, and the underlying mutation and recombination/reassortment rates as well as replication behavior. I conclude with a consideration of how an improved understanding of these processes specifically, and evolutionary genomics in general, can inform therapeutic strategies in the future.

The Population Dynamics of Infection

The natural starting point in any evolutionary analysis is a consideration of the effects of genetic drift. This stochastic evolutionary force is generally described in terms of the effective population size (N_e) – that is, the idealized size of the population which would experience the observed amount of genetic drift. This is opposed to the census population size (N), that is, the current number of individuals in the population. There are multiple reasons why N_e may be strongly reduced relative to N, including changes in census population sizes over time as well as natural selection itself, as will be described throughout this article. While viral population census sizes are known to often be exceptionally large, it is important to appreciate that viral effective population sizes are much more constrained, owing both to underlying modes of transmission and infection dynamics as described below – with N_e having been estimated to only be on the order of hundreds to thousands (Hughes, 2009; Miyashita and Kishino, 2010; Renzette *et al.*, 2013). This is significant with regards to viral population genetics as it suggests an upper limit on the efficiency of natural selection, given that this efficacy is dictated by N_e. It relatedly serves as a reminder of the pervasively important role of genetic drift in governing evolutionary outcomes, and the generally unjustified adaptation-centric view of virus genome evolution still forwarded in certain sections of the virology community.

Thus, before treating the topic of selection, one must firstly consider the neutral population dynamics of the organism in question. In the case of a viral population sampled from a patient, these dynamics likely include a strong population bottleneck (*i.e.*, a temporary reduction in population size) associated with the initial infection – these bottlenecks may be quite severe, with some primary infections being established by a very small number of infecting units. After this initial infection, the viral population will often next enter a phase of rapid population growth. This combination of bottlenecking and growth will leave signatures in the genomic patterns of variation, which in turn may be used to estimate the timing of infection, the severity of the bottleneck, the rate of within-patient population growth, and indeed the relative difference between N_e and N. In certain viruses that compartmentalize, these dynamics might also include population sub-structuring, in which isolated or semi-isolated viral populations are established in different compartments (*e.g.*, in different organs) within a single host. These compartments may or may not be connected by gene flow, via the plasma for example.

Multiple approaches have been developed within the field of population genetics to infer these demographic models and their underlying parameters. It is perhaps most helpful to introduce such methodology within the context of a specific application. For this purpose, I will examine recent work in human cytomegalovirus (HCMV), a member of the Herpesviridae family of dsDNA viruses. HCMV is characterized by a particularly large genome ($\sim$235,000 base pairs), and is nearly ubiquitous in human populations, with a seroprevalence of 30%–90% in the US and $>$90% in adults outside of the developed world (Kenneson and Cannon, 2007; Boeckh and Geballe, 2011). Though asymptomatic in most individuals, HCMV can lead to severe symptoms in immunocompromised patients and neonates, and is the most common cause of birth defects resulting from an infectious agent (Hassan and Connell, 2007; Manicklal *et al.*, 2013).

A large literature has accumulated demonstrating that HCMV has high levels of genetic variation within a host (Spector *et al.*, 1984; Drew *et al.*, 1984; Haberland *et al.*, 1999; Meyer-König *et al.*, 1998; Faure-Della Corte *et al.*, 2010), despite encoding a DNA polymerase with proofreading activity (Nishiyama *et al.*, 1983), with several recent high-throughput whole-genome studies revealing the full extent of this variation (Renzette *et al.*, 2011, 2013, 2014, 2015, 2016, 2017; Hage *et al.*, 2017; Pokalyuk *et al.*, 2017). Utilizing such whole-genome data sampled from the urine and plasma compartments of five congenitally infected infants, Renzette *et al.* (2013) found that the sub-structuring effect of compartmentalization was particularly strong – as assessed by F_{ST}, a measure of differentiation between populations – with these two compartments from a single patient being as diverged as samples from two unrelated patients. Utilizing the observed frequencies of genome-wide segregating sites in these congenitally infected patient samples – the site frequency spectrum (SFS) – they additionally inferred the demographic model characterizing each infection. Specifically, Renzette *et al.* inferred a population bottleneck associated with the initial infection (*i.e.*, the movement of the virus from the maternal compartment to the fetal plasma compartment), and a second bottleneck associated with the subsequent infection of the kidney compartment (*i.e.*, as assessed by the urine sample). The severity of the bottleneck characterizing the initial infection was not as strong as earlier results had suggested, with dozens or even hundreds of virions characterizing fetal infection. By estimating the age of the bottleneck, this work also provided the first inference of the timing of fetal infection.

In follow-up work with longitudinal sampling of the plasma, urine, and saliva compartments, Pokalyuk *et al.* (2017) also found evidence for subsequent admixture with maternal HCMV populations after the initial infection and compartmentalization, suggesting re-infection post-birth via, for example, breast milk (Numazaki, 1997; Enders *et al.*, 2011). While these mixed infections can certainly be a significant player in governing levels and patterns of genomic variation as described by Pokalyuk *et al.*, there has been an unfortunate tendency of some authors to only focus on this admixture process while neglecting the variety of other selective and demographic processes which also determine levels of variation (*e.g.*, Cudini *et al.*, 2019; though see Jensen and Kowalik, 2020).

Given that no current method exists to prevent maternal-fetal transmission, or to reduce the severity of fetal infection (Britt, 2017), such a characterization of population dynamics may be crucial to future therapeutic strategies. For example, clinically-imposing a more severe population bottleneck during pregnancy may reduce HCMV variability within the fetus, limiting the pool of variation on which natural selection may subsequently act, thereby potentially improving treatment outcomes. Immunotherapy advances have produced therapeutics capable of reducing the rate of maternal transmission in other viruses, including hepatitis B and HIV, and may thus represent a promising route for HCMV as well (Tseng and Kao, 2017; Voronin *et al.*, 2017).

Direct Selection and Linked Selection Effects

With regards to the direct action of natural selection in viral populations, positive selection related to antiviral drug resistance or immune evasion has received particular attention for obvious reasons (see review of Irwin *et al.*, 2016a). This search for adaptive loci is generally based around identifying the patterns of selective sweeps (Maynard Smith and Haigh, 1974) in viral genomes, owing to the genetic hitchhiking effects induced from the rapid rise in frequency of the beneficial mutation towards fixation. However, though it receives comparatively less attention in the virology literature, it is well understood that the vast majority of new fitness-impacting mutations in any organism are deleterious rather than beneficial (*e.g.*, Crow, 1993; Lynch *et al.*, 1999; Bank *et al.*, 2014b; and see reviews of Eyre-Walker and Keightley, 2007; Bank *et al.*, 2014a). The selective removal of these harmful variants by purifying selection will serve to further reduce the effective population size to an extent largely dictated by the rate of recombination and the strength of selection (Charlesworth *et al.*, 1993; Charlesworth, 2013). In addition, genomic linkage to this frequent input of deleterious mutations will reduce the probability of fixation of other linked sites – including reducing the likelihood of adaptation (Hill and Robertson, 1966; and see Pénisson *et al.*, 2017).

Importantly, just as the increase in frequency of a beneficial mutation can lead to the genetic hitchhiking of linked variation towards fixation in the genome (leading to a selective sweep effect), so too can the removal of the much larger input of deleterious mutations lead to the genetic hitchhiking of linked neutral variants towards loss. This effect – the loss and reduction in frequency owing to linkage with deleterious variants – is known as background selection (Charlesworth *et al.*, 1993). This effect can be of major consequence in viral populations given their coding-dense genomes (in which most new mutations are expected to be deleterious), particularly in the absence of recombination. Though this is yet to be thoroughly studied in viral populations, initial work has found a much more dominant role for background selection relative to selective sweeps in shaping within-patient genomic variation (*e.g.*, Renzette *et al.*, 2016).

Quantifying the relative input of deleterious, neutral, and beneficial mutations, means understanding the shape of the distribution of fitness effects (DFE). There are three general types of methods for performing such inference. The first involves the artificial creation of a mutation, one at a time or in combination, in order to measure the resulting fitness effect on an otherwise wildtype background under lab-controlled environmental conditions (*e.g.*, Fowler *et al.*, 2010; Hietpas *et al.*, 2011, 2012; Bank *et al.*, 2014b). The second approach, mutation-accumulation, maintains a population in a laboratory and allows mutations to naturally occur and accumulate, and fitness is generally quantified and related to this accumulation at multiple time points (*e.g.*, Foll *et al.*, 2014; Ferrer-Admetlla *et al.*, 2016; Lynch *et al.*, 2016; Long *et al.*, 2018). Aside from these two experimental approaches, a third approach is based on the direct sampling of natural population data (*e.g.*, from a patient infection, in the case of viral populations) – using either single time point (*e.g.*, Keightley and Eyre-Walker, 2007; Schneider *et al.*, 2011; Tataru *et al.*, 2017; Johri *et al.*, 2020) or multiple time point data (*e.g.*, Malaspinas *et al.*, 2012; Mathieson and McVean, 2013; Foll *et al.*, 2015;

Sackman *et al.*, 2019). These approaches generally rely on fitting a population history using patterns of variation at neutral sites, and then using that history to quantify the DFE at putatively selected sites.

One particularly important aside here of great relevance to the virology community, is the danger of basing such selection analyses on consensus sequences (*i.e.*, the representation of each patient's viral sample by a single sequence, which represents the most common allele at each site in a given within-patient viral population). While common practice, this tremendous loss of information (*e.g.*, all rare alleles – which represent the vast majority of variation – are neglected), combined with the associated ascertainment issue of only viewing high frequency mutations, can wreak havoc on evolutionary inference. For example, Renzette *et al.* (2017) demonstrated that earlier work based on consensus sequences had incorrectly arrived at the conclusion that most sampled segregating variation in HCMV was strongly selected owing to this consensus ascertainment – whereas when full within-patient population-level data was considered, it was clear that most segregating variation was in fact neutral or nearly neutral, consistent with general expectations (Kimura, 1968; Ohta, 1973; and see Jensen *et al.*, 2019; Morales-Arce *et al.*, 2020b).

Finally, it is important in this regard to appreciate that the correct evolutionary null model must necessarily account for the population infection dynamics discussed in the first section, together with the purifying and background selection effects discussed above. As viral populations are characterized by changing population sizes owing to infection bottlenecks and subsequent growth, and as the input of deleterious mutations is a constant process, an accounting for these processes is necessary to establish a baseline expectation for levels and patterns of variation, on top of which positively selected outliers may be identified. A neglect of these neutral dynamics can lead to serious mis-inference. For example, Feder *et al.* (2016) examined consensus HIV-1 population sequence data from 6717 drug-treated patients sequenced over a 24-year span. They found, as expected, that the change in variation observed in the viral population was correlated with the degree of treatment effectiveness. They used this pattern to argue that less effective treatments may be associated with so-called soft selective sweeps (*i.e.*, positive selection on a previously neutrally segregating genomic variant, or on multiple simultaneously occurring and identical genomic variants), whereas more effective treatments may be associated with hard selective sweeps (*i.e.*, positive selection on a *de novo* beneficial mutation). Perplexingly, they assumed identical strengths of positive selection, and identical within-patient demographic histories, between patients receiving effective and ineffective treatments. Revisiting this data, Harris *et al.* (2018) demonstrated that if one accounts for these variable fitness effects, and the inherent reality that more effective treatments reduced viral population sizes more than less effective treatments, these data observations can be explained entirely without invoking soft selective sweeps. Hence, it is always crucial to first account for the underlying neutral dynamics.

Other Key Aspects of the Population Genetic Environment

Apart from the effects of genetic drift and direct and linked selection described above, multiple other evolutionary processes are necessary to consider in order to develop a more holistic view of within-host viral population dynamics. For example, while the DFE describes the fitness effects of mutations, the rate of input of mutation is itself an important evolutionary parameter, as is the rate of recombination/reassortment, as that dictates the extent to which these newly arising mutations will be linked (and thus experience Hill-Robertson interference). Inference about mutation and recombination is similarly possible from with-patient polymorphism data. Returning to the HCMV example, Renzette *et al.* (2015) calculated genome-wide rates of mutation and recombination in 500 base pair windows across the genome for 48 longitudinal samples from 18 patients, inferring an average mutation rate of 2.0×10^{-7} new mutations per site per replication, similar to rates observed from murine cytomegalovirus (Drake and Hwang, 2005; Sanjuan *et al.*, 2010; and see review of Sackman *et al.*, 2018).

Importantly, viral mutation rates have generally been thought to be inversely correlated with genome size (Gago *et al.*, 2009), with the general interpretation being, for example, that RNA-based viral genomes are smaller than DNA genomes owing to intrinsically error-prone polymerases (Drake and Holland, 1999; Elena and Sanjuán, 2005). However, it appears more likely that, given that selection operates on the total genome-wide deleterious mutation rate (Kimura, 1967; Lynch, 2008), the selection pressure for replication fidelity (per site) is simply reduced in smaller genomes. This interpretation, as described in the 'drift-barrier hypothesis', has been widely supported across the tree of life (Lynch, 2011, 2012). In other words, genome-wide viral mutation rates are pushed to the lowest levels possible by natural selection, thus predicting the widely-observed correlation between effective population size and mutation rate, as well as between genome size and mutation rate. While some in the virology community retain the notion that viruses may be selected to maintain high mutation rates to better deal with fitness challenges, there is simply neither empirical nor theoretical support for the validity of such a view.

Finally, as much of population genetic inference is based on the Wright-Fisher model and Kingman coalescent - in which there is an assumption of a Poisson-shaped progeny distribution in which the variance equals the mean and is small relative to the population size – there is a growing appreciation that violations of this assumption must be accounted for when studying virus populations, which are highly variable in this regard. As such, virus genealogies are better characterized by more generalized Moran and multiple-merger coalescent models (see Donnelly and Kurtz, 1999; Pitman, 1999; Sagitov, 1999; Schweinsberg, 2000; Eldon and Wakeley, 2008; Matuszewski *et al.*, 2018; and see reviews of Tellier and Lemaire, 2014; Irwin *et al.*, 2016b). Specifically, violations of this progeny distribution assumption may elevate linkage disequilibrium even in the presence of frequent recombination (Eldon and Wakeley, 2008; Birkner *et al.*, 2013), and skew estimates of F_{ST} (Eldon and Wakeley, 2009). As such, these model violations may be mistaken for either population size change or positive selection if not accounted for (Matuszewski *et al.*, 2018; Sackman *et al.*, 2019).

Recent theoretical and statistical developments have provided some inroads into estimating, and thus accounting for, progeny skew from within-host pathogen populations. This work has demonstrated an ability to co-estimate the degree of progeny skew together with population size change, mutation rate, and positive selection from within-patient polymorphism data (Eldon *et al.*, 2015; Matuszewski *et al.*, 2018; Sackman *et al.*, 2019; Morales-Arce *et al.*, 2020a), providing a more appropriate null model for the study of pathogen evolution.

An Outlook on Evolutionarily Informed Treatment Strategies

The ability of the above described inference approaches to study drug-resistance mechanisms, and thus quantify the likelihood of adaptively evading a given therapeutic strategy within an experimental evolution setting, has been well-justified in the literature. Namely, identifying positively selected mutations in experimental viral populations challenged with possible drug-treatments individually or in combination, may elucidate potential routes to resistance, and highlight drugs requiring more complex (and lower probability) mutational evasion routes. For example, the commonly used influenza drug oseltamivir acts as a competitive inhibitor by binding to a hydrophobic pocked in the viral surface protein – an action which can be effectively blocked by mutations near the binding site (Collins *et al.*, 2008). Initially, the high fitness cost of the most common oseltamivir-resistant mutation, NA H275Y, was hypothesized to make the development of resistance unlikely (Ives *et al.*, 2002) – though resistance spread rapidly in the 2007–8 flu season owing to the limited number of required mutational steps (Moscona, 2009; Bloom *et al.*, 2010), an important drawback that became clearer in subsequent experimental evolution studies (*e.g.*, Foll *et al.*, 2014).

Other strategies with wider genomic effects may thus prove more fruitful – ideally ones requiring at least multiple mutation steps in order to confer resistance. Over the past decades, the notion that an excessive input of mutations can drive population extinction has been explored both theoretically and empirically, beginning with the foundational work of Lynch and Gabriel (1990) and Lynch *et al.* (1993). This possibility owes to the fact that the vast majority of fitness-impacting mutations are deleterious, or said another way, there are many more ways to disrupt rather than improve genomic function. This so-called mutational meltdown is achieved once the mean viability of the population drops to the point that the average individual cannot replace itself, at which time the population begins to decline resulting in a snowball effect leading to ultimate extinction. The cause of this final meltdown-phase is in fact the accelerated reduction in the efficacy of natural selection that results from the decline in N_e – that is, the declining population size allows for the further fixation of deleterious mutations via genetic drift, which in turn further reduces the efficacy of selection, allowing for further deleterious fixations, and so on.

Given that many viruses are naturally characterized by high mutation rates, and thus may commonly reside near a threshold of mutational load, a therapeutic increase in mutation rates may indeed induce this phase in which the input of new mutation overwhelms the ability of natural selection to remove this deleterious load. While such a scenario is often referred to as lethal mutagenesis in the virology literature (Bull *et al.*, 2007), the mutational meltdown framework is more general and critically incorporates the additional effects of genetic drift inherent to all populations (see Matuszewski *et al.*, 2017). The ability of an increased mutation rate to induce such extinction in a patient viral population has been relatively well explored and justified in RNA viruses (Loeb *et al.*, 1999; Crotty *et al.*, 2000; Lanford *et al.*, 2001; Crotty *et al.*, 2001; Severson *et al.*, 2003; Airaksinen *et al.*, 2003), using a variety of drugs including ribavirin and, more recently, favipiravir (Furuta *et al.*, 2013; Baranovich *et al.*, 2013; Bank *et al.*, 2016; Ormond *et al.*, 2017). Pertinent to the 2020 pandemic, Sheahan *et al.* (2020) recently demonstrated that the ribonucleoside analog EEID-1931 has a mutagenic effect in SARS-CoV-2 passaged in cell culture and, working in a mouse model, found a positive correlation between increased viral mutation rates and the degree of therapeutic efficacy. This work lends support to other recent calls to better investigate the potential therapeutic strategy of mutational meltdown in the context of CoV-2 (Jensen and Lynch, 2020; Santiago and Caballero, 2020; Jensen *et al.*, 2020).

While this represents a potentially promising and generalized treatment strategy for existing (and future unknown) pathogens, a number of important open questions remain in this regard. These will necessitate further theory work related to the necessary mutation rate turn-up required to induce this effect with realistic DFEs, as well as a more complete exploration of the interplay with underlying recombination rates (when applicable); as well as a more cohesive experimental effort to accurately quantify natural viral mutation rates via mutation-accumulation studies, in order to establish baseline values. What is clear however is that new viral pathogens will continue to emerge, a comprehensive understanding of their evolutionary trajectories will be critical for designing effective clinical treatments and interventions, and hence that a better union of the fields of population genetics and medicine will prove to be of great value.

See also: Antiretroviral Therapy – Nucleoside/Nucleotide and Non-Nucleoside Reverse Transcriptase Inhibitors. Antiviral Classification. HIV Integrase Inhibitors and Entry Inhibitors. Management of Adenovirus Infections (*Adenoviridae*). Management of Hepatitis A and E Virus Infection. Management of Herpes Simplex Virus Infections (*Herpesviridae*). Management of Influenza Virus Infections (*Orthomyxoviridae*). Management of Patients With Chronic Hepatitis B (*Hepadnaviridae*) and Chronic Hepatitis D Infection (*Deltavirus*). Management of Respiratory Syncytial Virus Infections (*Pneumoviridae*). Management of Varicella-Zoster Virus Infections (*Herpesviridae*). Protease Inhibitors. Treatment and Prevention of Herpesvirus Infections in the Immunocompromised Host

References

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

Bank, C., Ewing, G.B., Ferrer-Admettla, A., Foll, M., Jensen, J.D., 2014a. Thinking too positive? Revisiting current methods of population genetic selection inference. Trends in Genetics 30, 540–546.

Bank, C., Hietpas, R.T., Wong, A., Bolon, D.N., Jensen, J.D., 2014b. 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., Renzette, N., Liu, P., *et al*., 2016. An experimental evaluation of drug-induced mutational meltdown as an antiviral treatment strategy. Evolution 70, 2470–2484.

Baranovich, T., Wong, S., Armstrong, J., *et al*., 2013. T-705 (favipiravir) induces lethal mutagenesis in influenza A H1N1 viruses in vitro. Journal of Virology 87, 3741–3751.

Birkner, M., Blath, J., Eldon, B., 2013. An ancestral recombination graph for diploid populations with skewed offspring distribution. Genetics 193, 255–290.

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

Boeckh, M., Geballe, A.P., 2011. Cytomegalovirus: pathogen, paradigm, and puzzle. Journal of Clinical Investigation 121, 1673–1680.

Britt, W.J., 2017. Congenital human cytomegalovirus infection and the enigma of maternal immunity. Journal of Virology 91, e02392.

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

Charlesworth, B., Morgan, M., Charlesworth, D., 1993. The effect of deleterious mutations on neutral molecular variation. Genetics 134, 1289–1303.

Charlesworth, B., 2013. Background selection 20 years on. Journal of Heredity 104, 161–171.

Collins, P.J., Haire, L., Lin, Y., *et al*., 2008. Crystal structures of oseltamivir-resistant influenza virus neuraminidase mutants. Nature 453, 1258–1261.

Crotty, S., Cameron, C., Andino, R., 2001. RNA virus error catastrophe: direct molecular test by using ribavirin. Proceedings of the National Academy of Sciences of the United States of America 98, 6895–6900.

Crotty, S., Maag, D., Arnold, J., *et al*., 2000. The broad-spectrum antiviral ribonucleoside ribavirin is an RNA virus mutagen. Nature Medicine 6, 1375–1379.

Crow, J.F., 1993. Mutation, mean fitness, and genetic load. Oxford Series in Evolutionary Biology 9, 3–42.

Cudini, J., Roy, S., Houldcroft, C., *et al*., 2019. Human cytomegalovirus haplotype reconstruction reveals high diversity due to superinfection and evidence of within-host recombination. Proceedings of the National Academy of Sciences of the United States of America 116, 5693–5698.

Donnelly, P., Kurtz, T.G., 1999. Particle representations for measure-valued population models. Annals of Probability 27, 166–205.

Drake, J.W., Holland, J.J., 1999. Mutation rates among RNA viruses. Proceedings of the National Academy of Sciences of the United States of America 96, 13910–13913.

Drake, J.W., Hwang, C.B.C., 2005. On the mutation rate of herpes simplex virus type 1. Genetics 170, 969–970.

Drew, W.L., Sweet, E.S., Miner, R.C., Mocarski, E.S., 1984. Multiple infections by cytomegalovirus in patients with acquired immunodeficiency syndrome: documentation by Southern blot hybridization. Journal of Infectious Diseases 150, 952–953.

Eldon, B., Birkner, M., Blath, J., Freund, F., 2015. Can the site-frequency spectrum distinguish exponential population growth from multiple-merger coalescents? Genetics 199, 841–856.

Eldon, B., Wakeley, J., 2008. Linkage disequilibrium under skewed offspring distribution among individuals in a population. Genetics 178, 1517–1532.

Eldon, B., Wakeley, J., 2009. Coalescence times and Fst under a skewed offspring distribution among individuals in a population. Genetics 181, 615–629.

Elena, S.F., Sanjuán, R., 2005. Adaptive value of high mutation rates of RNA viruses: Separating causes from consequences. Journal of Virology 79, 11555–11558.

Enders, G., Daiminger, A., Bäder, U., Exler, S., Enders, M., 2011. Intrauterine transmission and clinical outcome of 248 pregnancies with primary cytomegalovirus infection in relation to gestational age. Journal of Clinical Virology 52, 244–246.

Eyre-Walker, A., Keightley, P.D., 2007. The distribution of fitness effects of new mutations. Nature Reviews Genetics 8, 610–618.

Faure-Della Corte, M., Samot, J., Garrigue, I., *et al*., 2010. Variability and recombination of clinical human cytomegalovirus strains from transplantation recipients. Journal of Clinical Virology 47, 161–169.

Feder, A.F., Rhee, S.-Y., Holmes, S.P., *et al*., 2016. More effective drugs lead to harder selective sweeps in the evolution of drug resistance in HIV-1. eLife 2016, 5.

Ferrer-Admetlla, A., Leuenberger, C., Jensen, J.D., Wegmann, D., 2016. An approximate Markov model for the Wright-Fisher diffusion and its application to time series data. Genetics 203, 831–846.

Foll, M., Poh, Y.-P., Renzette, N., *et al*., 2014. Influenza virus drug resistance: A time-sampled population genetics perspective. PLOS Genetics 10, e1004185.

Foll, M., Shim, H., Jensen, J.D., 2015. A Wright-Fisher ABC-based approach for inferring per-site effective population sizes and selection coefficients from time-sampled data. Molecular Ecology Resources 15, 87–98.

Fowler, D.M., Araya, C.L., Fleishman, S.J., *et al*., 2010. High-resolution mapping of protein sequence-function relationships. Nature Methods 7, 741–746.

Furuta, Y., Gowen, B., Takahashi, K., *et al*., 2013. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antiviral Research 100, 446–454.

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

Haberland, M., Meyer-Konig, U., Hufert, F.T., 1999. Variation within the glycoprotein B gene of human cytomegalovirus is due to homologous recombination. Journal of General Virology 80 (Pt 6), 1495–1500.

Hage, E., Wilkie, G.S., Linnenweber-Held, S., *et al*., 2017. Characterization of human cytomegalovirus genome diversity in immunocompromised hosts by whole-genome sequencing directly from clinical specimens. Journal of Infectious Diseases 215, 1673–1683.

Harris, R.B., Sackman, A., Jensen, J.D., 2018. On the unfounded enthusiasm for soft selective sweeps II: examining recent evidence from humans, flies, and viruses. PLOS Genetics 14, e1007859.

Hassan, J., Connell, J., 2007. Translational mini-review series on infectious disease: Congenital cytomegalovirus infection: 50 Years on. Clinical and Experimental Immunology 149, 205–210.

Hietpas, R., Jensen, J.D., Bolon, D.N.A., 2011. Experimental illumination of a fitness landscape. Proceedings of the National Academy of Sciences of the United States of America 108, 7896–7901.

Hietpas, R., Roscoe, B., Jiang, L., Bolon, D.N.A., 2012. Fitness analyses of all possible point mutations for regions of genes in yeast. Nature Protocols 7, 1382–1396.

Hill, W.G., Robertson, A., 1966. The effect of linkage on limits to artificial selection. Genetics Research 8, 269–294.

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

Irwin, K.K., Laurent, S., Matuszewski, S., *et al*., 2016b. On the importance of skewed offspring distributions and background selection in virus population genetics. Heredity 117, 393–399.

Irwin, K.K., Renzette, N., Kowalik, T., Jensen, J.D., 2016a. Antiviral drug resistance as an adaptive process. Virus Evolution 2, 1–10.

Ives, J.A., Carr, J., Mendel, D., *et al*., 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 Research. 55, 307–317.

Jensen, J.D., Kowalik, T.F., 2020. A consideration of within-host human cytomegalovirus genetic variation. Proceedings of the National Academy of Sciences of the United States of America 117, 816–817.

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

Jensen, J.D., Payseur, B., Stephan, W., *et al*., 2019. The importance of the Neutral Theory in 1968 and 50 years on: a response to Kern & Hahn 2018. Evolution 73, 111–114.

Jensen, J.D., Stikeleather, R., Kowalik, T., Lynch, M., 2020. Imposed mutational meltdown as an antiviral strategy. Evolution 74, 2549–2559. doi:10.1111/evo.14107.

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

Keightley, P.D., Eyre-Walker, A., 2007. Joint inference of the distribution of fitness effects of deleterious mutations and population demography based on nucleotide polymorphism frequencies. Genetics 177, 2251–2261.

Kenneson, A., Cannon, M.J., 2007. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Reviews in Medical Virology 17, 253–276.
Kimura, M., 1967. On the evolutionary adjustment of spontaneous mutation rates. Genetics Research 9, 23–34.
Kimura, M., 1968. Evolutionary rate at the molecular level. Nature 217, 624–626.
Lanford, R.E., Chavez, D., Guerra, B., *et al.*, 2001. Ribavirin induces error-prone replication of GB virus B in primary tamarin hepatocytes. J. Virol. 75, 8074–8081.
Loeb, L.A., Essigmann, J., Kazazi, F., *et al.*, 1999. Lethal mutagenesis of HIV with mutagenic nucleoside analogs. Proceedings of the National Academy of Sciences of the United States of America 96, 1492–1497.
Long, H., Sung, W., Kucukyildirim, S., *et al.*, 2018. Evolutionary determinants of genome-wide nucleotide composition. Nature Ecology and Evolution 2, 237–240.
Lynch, M., Ackerman, M.S., Gout, J.-F., *et al.*, 2016. Genetic drift, selection and the evolution of the mutation rate. Nature Reviews Genetics 17, 704–714.
Lynch, M., Blanchard, J., Houle, D., *et al.*, 1999. Spontaneous deleterious mutation. Evolution 53, 645–663.
Lynch, M., Burger, R., Butcher, D., Gabriel, W., 1993. The mutational meltdown in asexual populations. Journal of Heredity 84, 339–344.
Lynch, M., Gabriel, W., 1990. Mutation load and the survival of small populations. Evolution 44, 1725–1737.
Lynch, M., 2008. The cellular, developmental, and population-genetic determinants of mutation-rate evolution. Genetics 180, 933–943.
Lynch, M., 2011. The lower bound to the evolution of mutation rates. Genome Biology and Evolution 3, 1107–1118.
Lynch, M., 2012. Evolutionary layering and the limits to cellular perfection. Proceedings of the National Academy of Sciences of the United States of America 109, 18851–18856.
Malaspinas, A.-S., Malaspinas, O., Evans, S.N., Slatkin, M., 2012. Estimating allele age and selection coefficients from time-serial data. Genetics 192, 599–607.
Manicklal, S., Emery, V.C., Lazzarotto, T., Boppana, S.B., Gupta, R.K., 2013. The "Silent" global burden of congenital cytomegalovirus. Clinical Microbiology Reviews 26, 86–102.
Mathieson, I., McVean, G., 2013. Estimating selection coefficients in spatially structured populations from time series data of allele frequencies. Genetics 193, 973–984.
Matuszewski, S., Hildebrandt, M.E., Achaz, G., Jensen, J.D., 2018. Coalescent processes with skewed offspring distributions and non-equilibrium demography. Genetics 208, 323–338.
Matuszewski, S., Ormond, L., Bank, C., Jensen, J.D., 2017. Two sides of the same coin: a population genetics perspective on lethal mutagenesis and mutational meltdown. Virus Evolution 3, 1–5.
Maynard Smith, J., Haigh, J., 1974. The hitch-hiking effect of a favourable gene. Genetics Research 23, 23.
Meyer-König, U., Ebert, K., Schrage, B., Pollak, S., Hufert, F., 1998. Simultaneous infection of healthy people with multiple human cytomegalovirus strain. Lancet 352, 1280.
Miyashita, S., Kishino, H., 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. Journal of Virology 84, 1828–1837.
Morales-Arce, A.Y., Harris, R.B., Stone, A.C., Jensen, J.D., 2020a. Evaluating the contributions of purifying selection and progeny-skew in dictating within-host *Mycobacterium tuberculosis* evolution. Evolution 74, 992–1001.
Morales-Arce, A.Y., Sabin, S.J., Stone, A.C., Jensen, J.D., 2020b. The population genomics of within-host *Mycobacterium tuberculosis*. Heredity 126, 1–9. doi:10.1038/s41437-020-00377-7.
Moscona, A., 2009. Global transmission of oseltamivir-resistant influenza. New England Journal of Medicine 360, 953–956.
Nishiyama, Y., Maeno, K., Yoshida, S., 1983. Characterization of human cytomegalovirus-induced DNA polymerase and the associated 3′-to-5′, exonuclease. Virology 124, 221–231.
Numazaki, K., 1997. Human cytomegalovirus infection of breast milk. FEMS Immunology and Medical Microbiology 18, 91–98.
Ohta, T., 1973. Slightly deleterious mutant substitutions in evolution. Nature 246, 96–98.
Ormond, L., Liu, P., Matuszewski, S., *et al.*, 2017. The combined effect of oseltamivir and favipiravir on influenza A virus evolution. Genome Biology and Evolution 9, 1913–1924.
Pénisson, S., Singh, T., Sniegowski, P., Gerrish, P., 2017. Dynamics and fate of beneficial mutations under lineage contamination by linked deleterious mutations. Genetics 205, 1305–1318.
Pitman, J., 1999. Coalescents with multiple collisions. Journal of Applied Probability 27, 1870–1902.
Pokalyuk, C., Renzette, N., Irwin, K.K., *et al.*, 2017. Characterizing human cytomegalovirus reinfection in congenitally infected infants: an evolutionary perspective. Molecular Ecology 26, 1980–1990.
Renzette, N., Bhattacharjee, B., Jensen, J.D., Gibson, L., Kowalik, T.F., 2011. Extensive genome-wide variability of human cytomegalovirus in congenitally infected infants. PLOS Pathogens 7, e1001344.
Renzette, N., Gibson, L., Bhattacharjee, B., *et al.*, 2013. Rapid intrahost evolution of human cytomegalovirus is shaped by demography and positive selection. PLOS Genetics 9, e1003735.
Renzette, N., Gibson, L., Jensen, J.D., Kowalik, T.F., 2014. Human cytomegalovirus intrahost evolution – A new avenue for understanding and controlling herpesvirus infections. Current Opinion in Virology 8, 109–115.
Renzette, N., Kowalik, T.F., Jensen, J.D., 2016. On the relative roles of background selection and genetic hitchhiking in shaping human cytomegalovirus genetic diversity. Molecular Ecology 25, 403–413.
Renzette, N., Pfeifer, S.P., Matuszewski, S., Kowalik, T.F., Jensen, J.D., 2017. On the analysis of intrahost and interhost viral populations: Human cytomegalovirus as a case study of pitfalls and expectations. Journal of Virology 91, e01976-16.
Renzette, N., Pokalyuk, C., Gibson, L., *et al.*, 2015. Limits and patterns of cytomegalovirus genomic diversity in humans. Proceedings of the National Academy of Sciences of the United States of America 112, E4120–E4128.
Sackman, A., Harris, R.B., Jensen, J.D., 2019. Inferring demography and selection in organisms characterized by skewed offspring distributions. Genetics 211, 1019–1028.
Sackman, A.M., Pfeifer, S.P., Kowalik, T.F., Jensen, J.D., 2018. On the demographic and selective forces shaping patterns of human cytomegalovirus variation within hosts. Pathogens 7, 16.
Sagitov, S., 1999. The general coalescent with asynchronous mergers of ancestral lines. Journal of Applied Probability 36, 1116–1125.
Sanjuan, R., Nebot, M.R., Chirico, N., Mansky, L.M., Belshaw, R., 2010. Viral mutation rates. Journal of Virology 84, 9733–9748.
Santiago, E., Caballero, A., 2020. The value of targeting recombination as a strategy against coronavirus diseases. Heredity 125, 169–172. doi:10.1038/s41437-020-0337-5.
Schneider, A., Charlesworth, B., Eyre-Walker, A., Keightley, P.D., 2011. A method for inferring the rate of occurrence and fitness effects of advantageous mutations. Genetics 189, 1427–1437.
Schweinsberg, J., 2000. Coalescents with simultaneous multiple collisions. Electronic Journal of Probability 5, 1–50.
Severson, W.E., Schmalijohn, C., Javadian, A., Jonsson, C., 2003. Ribavirin causes error catastrophe during Hantaan virus replication. Journal of Virology 77, 481–488.
Sheahan, T., Sims, A., Zhou, S., *et al.*, 2020. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Science Translational Medicine 12, eabb5883.
Spector, S.A., Hirata, K.K., Neuman, T.R., 1984. Identification of multiple cytomegalovirus strains in homosexual men with acquired immunodeficiency syndrome. Journal of Infectious Diseases 150, 953–956.
Tataru, P., Mollion, M., Glémin, S., Bataillon, T., 2017. Inference of distribution of fitness effects and proportion of adaptive substitutions from polymorphism data. Genetics 207, 1103–1119.
Tellier, A., Lemaire, C., 2014. Coalescence 2.0: A multiple branching of recent theoretical developments and their applications. Molecular Ecology 23, 2637–2652.
Tseng, T.C., Kao, J.H., 2017. Elimination of hepatitis B: Is it a mission possible? BMC Medicine 15, 1–5.
Voronin, Y., Jani, I., Graham, B.S., *et al.*, 2017. Recent progress in immune-based interventions to prevent HIV-1 transmission to children. Journal of the International AIDS Society 20, e25038.

Further Reading

Charlesworth, B., Charlesworth, D., 2010. Elements of Evolutionary Genetics. USA: Roberts and Company Publishers.
Kimura, M., 1983. The Neutral Theory of Molecular Evolution. UK: Cambridge University Press.
Provine, W.B., 2001. The Origins of Theoretical Population Genetics. USA: University of Chicago Press.
Wakeley, J., 2009. Coalescent Theory: An Introduction. USA: Roberts and Company Publishers.
Walsh, B., Lynch, M., 2018. Evolution and Selection of Quantitative Traits. UK: Oxford University Press.