The Puzzle of HIV Neutral and Selective Evolution

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Abstract

HIV is one of the fastest evolving organisms known. It evolves about 1 million times faster than its host, humans. Because HIV establishes chronic infections, with continuous evolution, its divergence within a single infected human surpasses the divergence of the entire humanoid history. Yet, it is still the same virus, infecting the same cell types and using the same replication machinery year after year. Hence, one would think that most mutations that HIV accumulates are neutral. But the picture is more complicated than that. HIV evolution is also a clear example of strong positive selection, that is, mutants have a survival advantage. How do these facts come together?

Key words: **neutral evolution, selective evolution, HIV.**

The neutral theory of molecular evolution (Kimura 1983) has had a profound impact on evolutionary theory and the methods we use to study molecular evolution in general. Although selection clearly has had a large impact on how lifeforms have evolved (Darwin 1859), many features have also arisen due to various stochastic events such as catastrophic population size reductions and "lucky" survival in new or momentarily open niches. Interestingly, HIV captures many of these aspects.

The origin of the human immunodeficiency virus (HIV) is complicated; it has crossed the host species barrier several times from several primate hosts into humans (Sharp et al. 1994; Sharp and Hahn 2011). There are two types of HIV, HIV-1, and HIV-2, with different origins in chimpanzees, gorillas, and sooty mangabeys. In addition, simian immunodeficiency viruses (SIVs), as the virus is called when it is not in humans, have also jumped host many times such that some other primate hosts besides humans also are infected by more than one type of primate lentivirus (PLV, the collective name of HIVs and SIVs). The diversity among all these PLVs is very large, with pairwise distances up to 40–50%, yet cross species jumps are still possible. The pandemic version of HIV-1 was likely introduced into humans as recently as in the late 1800s or the early 1900s (Korber et al. 2000; Worobey et al. 2008), although many previous, but globally unsuccessful, introductions may have occurred in the more distant past. This raises the question of how so genetically different viruses can jump into and infect so many different primate hosts.

HIV-1 is also extremely diverse within humans, both on the between human hosts level and within a single infected human. For instance, the genetic distance between HIV-1 variants reaches 25% globally between so called subtypes (Leitner 1997) (major pandemic lineages forming distinct phylogenetic clades without significantly detectable recombination between them). Within a single host, HIV-1 can reach diversity levels of 5–10% that have diverged 10% from the founding variant(s) only years after infection (Shankarappa et al. 1999; Immonen et al. 2015). Compared with humans, who differ only 0.1% from each other after 2.5 My of evolution, HIV-1 indeed displays extreme levels of genetic variation. The extreme within-host variation explains the global variation, as all variation, of course, emerges while the virus replicates within a host. Thus, many mutants in an HIV-1 population that exist in a single host survive and are subsequently transmitted. As HIV-1 within-host populations are vast, in the order of 10^6–10^{10}, quickly raising to appreciable (and detectable) levels would be unlikely unless selection was involved. Indeed, natural evolution of HIV-1 within-host populations is driven by the interaction of the virus and the host's immune response. In particular, the surface proteins of HIV-1 (encoded by the *env* gene) are under heavy attack by human antibodies which quickly adapt to neutralize the most prevalent HIV-1 variants (Richman et al. 2003; Wei et al. 2003; Bunnik et al. 2008). Thus, HIV-1 escape mutants will have an advantage and rise to high levels. HIV-1 mutations occur at a very high rate, where all single point mutations occur daily, creating a diverse pool from where escape mutants may be drawn (Coffin 1995). HIV-1 lacks proof reading, causing mutations in nearly every replication round (Mansky and Temin 1995). The lack of proof reading has several advantages, 1) it allows fast replication as proof reading takes time and requires additional repair mechanisms, 2) it generates mostly defective forms of HIV-1 (Aldovini and Young 1990; Rusert et al. 2004), which still need to be killed by the immune system, and among which replication competent HIV-1 can hide, and 3) it generates the genetic variation that facilitates immune escape. The antibody pressure on *env* explains why it is the most diverse gene on the HIV-1 genome. Although all genes are under some selective pressure mediated by the humoral (antibodies) or cellular (CTL) immune systems, additional pressure on HIV-1 is exerted by antiviral treatment using synthetic drugs. Many current anti-HIV drugs target HIV specific proteins such as the reverse transcriptase and the proteinase, both vital for replication. This targeted pressure also selects mutants from the diverse, low frequency pool that

Perspective

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HIV-1 constantly regenerates. Drug induced pressure selects for specific mutations regardless of genetic background, which have been carefully mapped for each antiviral drug, whereas immune pressure is different in every human. When strong pressure is exerted on certain sites, other sites with little or no benefit may tag along and rise to high levels through hitchhiking (Gillespie 2000; Neher and Leitner 2010; Zanini and Neher 2013; Pennings et al. 2014).

The population size and replication rate are important factors that contribute to the probability of how HIV-1 drug resistance develops (Bonhoeffer et al. 1997; Nowak et al. 1997; Nijhuis et al. 1998; Duffy et al. 2008; Alexander and Bonhoeffer 2012). Recent work studying population size dynamics and genetic diversity as a function of drug pressure revealed that the stronger the drug pressure was, the more diversification followed the drug-induced population bottleneck (Fun et al. 2018). Thus, more severe drug-induced extinction was followed by more detectable diversification. When large extinctions occurred, previously occupied niches opened up for new virus variants. Many new mutations can then be accepted and as they do no compete for resources until the population regains a size limited by the carrying capacity, or many of the preexisting low-frequency variants have a chance to rise to high relative frequencies. Hence, strong selection against present variants may drastically reduce population size, where stochastic effects may determine which variants get a chance to survive. Indeed, while specific drug mutations become fixed, the path to those drug resistance mutations differs in different patients (Fun et al. 2018).

Although the census HIV-1 within-host population size is vast, the effective population size (N_e) has typically been estimated to be much smaller. Many early estimates were in the order of 10^3–10^4 (Brown 1997; Nijhuis et al. 1998; Seo et al. 2002), but some suggested N_e in the order of 10^6 (Coffin 1995; Rouzine and Coffin 1999), approaching the census size. N_e is important because drift has more impact in smaller populations while selection is more important in large populations in explaining how genetic variants evolve in a population. Hence, while N_e is an important parameter explaining population dynamics and the rise of escape mutations, the estimation of the effective population size itself is based on assumptions about neutrality and linkage disequilibrium. This has caused debate on whether the evolution of HIV-1 within a host is mostly driven by drift or selection. The lower estimates of N_e were typically based on the assumption of neutrality. However, it has been pointed out that estimates based on genetic diversity at neutral sites cannot be applied to diversity at sites under selection, for example, sites under drug selection (Kouyos et al. 2006). The large N_e of 10^6, on the other hand, was estimated under the assumption of positive selection, but instead ignored the effects of recombination, another complicating factor in HIV-1 evolution. In this context, drift in combination with selection can generate a state of negative linkage disequilibrium, known as the Hill–Robertson effect (Hill and Robertson 1966). This would favor recombination as an evolutionary mechanism as it can reduce the time to fixation. HIV-1 recombination is indeed a major mechanism for evolution (Zhuang et al. 2002; Zhang et al. 2010), with a rate on par with the substitution rate (Neher and Leitner 2010).

Because transient decreases of N_e significantly enhance the importance of drift over selection, bottleneck effects during HIV-1 evolution are of particular importance. As already mentioned, significant bottlenecks can occur during antiviral treatment. The perhaps less severe, but much more frequent bottlenecks due to immune surveillance also reduce the effective population size (Frost et al. 2001). Classic population genetics predicts that the effective population size is given by the harmonic mean of the census size, thus trending towards a small size. Sites under neutral evolution will therefore be more affected by bottlenecks than selected sites because the diversity at neutral sites is larger and would take longer time to regenerate. Intriguingly, the most severe bottleneck during HIV-1 evolution occurs during transmission, linking within-host evolution to between-host evolution and the global scale of HIV-1 evolution.

Transmission causes a severe bottleneck, allowing only a small number of viruses to establish a new population in a new host. Because HIV-1 replication in the donor host mostly generated junk HIV-1, even transmission of many virus particles would only constitute a small number of viable viruses. Many studies have tried to estimate the number of viruses that establish infection, and while the results vary and depend on experimental methodology and mode of transmission, it is clear that a very severe bottleneck takes place, permitting only a single or sometimes tens of viruses to establish a new infection (McNearney et al. 1992; Wolfs et al. 1992; Zhang et al. 1993; Keele et al. 2008; Salazar-Gonzalez et al. 2009; Rieder et al. 2011).

The rapid evolutionary rate observed at the within-host level appears not to be sustained at the epidemiological level, however, decreasing by an order of magnitude (Alizon and Fraser 2013). Although several theories have been proposed to explain the rate differences (Herbeck et al. 2006; Maljkovic Berry et al. 2007; Maljkovic Berry et al. 2009; Pybus and Rambaut 2009; Lythgoe and Fraser 2012; Alizon and Fraser 2013), some authors have suggested that the evolutionary rate discrepancy could result from a disproportionate fraction of transmissions involving latent virus that is genetically closer to the founder than the average virus in the plasma population (Lythgoe and Fraser 2012; Vrancken et al. 2014). Interestingly, it has been shown that the evolutionary rate on the global level is inversely correlated with the rate of spread (Berry et al. 2007), that is, the faster HIV-1 spreads, the slower it evolves on the between-host level. This difference is explained by the fact that early infection involves nearly neutral evolution as the immune system is not fully active, whereas later in the infection it is. Thus, if HIV-1 is transmitted during the acute infection phase, then HIV-1 evolves at the slower neural rate also on the between-host scale, whereas if transmissions occur during the chronic phase then the between-host rate would be more similar to the high within-host rate seen later, and during most of, HIV-1 within-host evolutionary time. Such differences have been shown to exist between fast and slow epidemics (Berry et al. 2007). If HIV-1 was selectively transmitted, for example, preferentially

selecting for some feature that makes it more transmissible, no such correlation would be detectable. Furthermore, it has been shown that the between-host diversity is shaped by the interplay of the transmission rate and the within-host selection rate (involving both escape from, and reversions of, host-specific CTL mediated mutations), which together can maintain high between-host diversity (Poon et al. 2007). Of course, there may be both neutral and selective mechanisms involved in transmission, such that regardless of genetic background (composed of neutral sites) a few sites that make transmission or early outgrowth possible (selected sites) determine infection probability. HIV-1 evolution is thus described by both neutral and selective evolution. Depending on what the goal with a particular analysis is, one may carefully choose models and methods based on either neutral or selective assumptions. Sometimes it may be necessary to filter sites into separate categories and treat the sets differently.

One powerful set of methods includes phylodynamic methods to infer how HIV (and other pathogens) spread among humans (and other hosts). Based on the observation that a between host HIV-1 phylogeny is related to the transmission history between the hosts (Leitner et al. 1996), recent advances using within-host coalescent models have connected within-host evolution to between-host evolution (Ypma et al. 2013; Jombart et al. 2014; Romero-Severson et al. 2014; Kenah et al. 2016; Didelot et al. 2017; Romero-Severson et al. 2017). These models assume neutral evolution as well as neutral transmission. These models have shown that different phylogenetic patterns may reveal epidemiological relationships, that is, who-infected-whom and when (Romero-Severson et al. 2016), and that taking within-host evolution into account when investigating global level epidemics has profound impact on reconstructing transmission networks (Giardina et al. 2017) and incidence trends (Volz et al. 2017).

The development of new sequencing technologies has already had an impact on HIV-1 research, and undoubtedly will continue to reveal new insights into HIV-1 evolution. Many of the open issues outlined here will be investigated, previous results based on more limited data will be challenged, and new questions will arise. The interplay between neutral and selective mechanisms will be further illuminated, necessitating both neutral and selective models to interpret the fascinating evolution of HIV-1.

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