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Genetic bottlenecks in intraspecies virus transmission

John T McCrone[1] and Adam S Lauring[1,2]

Ultimately, viral evolution is a consequence of mutations that arise within and spread between infected hosts. The transmission bottleneck determines how much of the viral diversity generated in one host passes to another during transmission. It therefore plays a vital role in linking within-host processes to larger evolutionary trends. Although many studies suggest that transmission severely restricts the amount of genetic diversity that passes between individuals, there are important exceptions to this rule. In many cases, the factors that determine the size of the transmission bottleneck are only beginning to be understood. Here, we review how transmission bottlenecks are measured, how they arise, and their consequences for viral evolution.

Addresses
[1] Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI 48109, USA
[2] Division of Infectious Diseases, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, USA

Corresponding author: Lauring, Adam S (alauring@med.umich.edu)

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Many viral pathogens exist as diverse populations within infected hosts. The diversity present in this 'mutant swarm' provides the raw material on which selection can act. Although populations within a host may reach as high as 10^{14} virions [1], viruses are frequently subject to bottleneck events as they spread within and between hosts [2]. These bottlenecks drastically reduce the size of the population and, consequently, its genetic diversity. Because the population that develops after a genetic bottleneck is derived from a small sample of the ancestral population, this process can dramatically alter the relative frequency of mutations in the population.

The stringency of the transmission bottleneck plays an important role in linking within-host processes to a pathogen's larger evolutionary dynamics. Stringent, or tight, transmission bottlenecks limit the diversity of the founding population in the recipient and alter the mutational composition of the population in the recipient relative to that in the donor (Figure 1, top). However, if the transmission bottleneck is loose, transmission does not significantly impact variant frequencies and the composition of the founding population in the recipient more closely matches that present in the donor at the time of transmission (Figure 1, bottom).

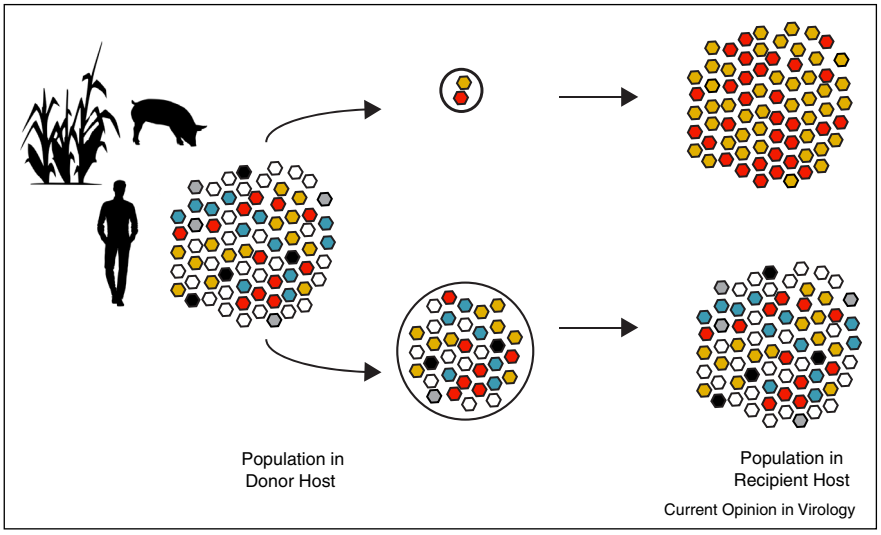
Although transmission bottlenecks play an important role in viral evolution, relatively little is known about their size and determinants. Many quantitative studies suggest that bottlenecks are tight [3,4]; however, there are exceptions and even conflicting reports for viruses with similar transmission pathways. Importantly, the factors that determine the stringency of the transmission bottleneck are poorly understood. Here, we briefly review how transmission bottlenecks are measured, how they arise, and their impact on viral evolution across biological scales. For a more comprehensive review of bottlenecks, including those found at the within-host and cellular scale, we direct the reader to reference [3].

Measuring transmission bottlenecks

Transmission bottlenecks are measured by their effect on viral diversity. In experimental systems, within-host diversity can be approximated using a defined population of viruses that are tagged with genetic markers. If the markers are selectively neutral, the number of distinct markers that pass from donor to recipient reflects the sampling event of the bottleneck as opposed to selection within either host (Figure 2a). This technique has been used to qualitatively estimate a stringent bottleneck for aphid transmission of cucumber mosaic virus (an average of 3 of 12 markers were transmitted) [5] and aerosol transmission of influenza in ferrets and guinea pigs (2–5 of 100 sequence tags were transmitted) [6••]. In a particularly elegant experiment, Moury and colleagues artificially inoculated aphid vectors with mixtures of 2 Potato Y virus mutants prior to feeding the aphids on pepper plants [7•]. By modeling the number of plants exposed to only one of the mutants, Moury *et al.* found that aphid transmission imposes a bottleneck of 0.5–3.2 virions on Potato Y virus.

Because natural systems do not offer the opportunity for a barcoding approach, early studies characterized the transmission bottleneck qualitatively based on the degree of shared diversity found within transmission pairs (Figure 2b). Clonal sequencing of influenza virus isolates from swine and equine transmission chains found

Figure 1



The effect of transmission bottlenecks on viral diversity. In a variety of hosts (e.g. humans, pigs, plant shown here), stringent bottlenecks (top) limit the size and diversity of a population and drastically alter their composition. The large populations that pass through loose bottlenecks (bottom) allow for transmission of rare variants. As a result the diversity of the population in the recipient approximates that of the donor.

transmission pairs shared minority variants [8–10]. Studies of aphid, mechanical, and vertical transmission of Zucchini Yellow Mosaic Virus found similar results [11,12]. These studies suggest that transmission bottlenecks are sometimes sufficiently loose to allow for the transmission of low-frequency mutations.

More quantitative approaches can also be employed to estimate the transmission bottleneck from shared diversity data. In these models, the transmission process is assumed to be a random sampling of the donor population and individual variants are assumed to be transmitted independently of one another. The probability that a variant is transmitted is derived from a binomial distribution and is positively correlated with its frequency in the donor and the size of the bottleneck. More complexity can be incorporated into these models to tease apart the relative impact of within-host and between-host processes (see Ref. [13••] for a thorough discussion and comparison of common models). One such model has been used to estimate a loose bottleneck of roughly 200 genomes in a recent study of human transmission of influenza virus [13••,14]. This estimate is much larger than that provided by the barcode experiments previously discussed. The large discrepancy in these studies highlights the need for a more complete understanding of the viral, host, and environmental factors that determine transmission bottleneck sizes.

When only one member of a transmission pair is available, the diversity present in the infected host can be used to estimate the number of genotypes in the founding population. Coalescent theory works backward in time, tracing the evolutionary history of the current population back to common ancestors [15]. Coalescent models based on the current diversity, the viral evolutionary rate, and the estimated time of infection can be used to determine how many genotypes were present in the founding population (Figure 2c). Phylogenetic analysis of HIV evolution suggests that most infections derive from small founding populations of only one genotype [16,17]. A similar approach has been used to estimate a stringent transmission bottleneck for HCV [18•,19–21].

Determinants of bottleneck size

Most transmission studies suggest tight bottlenecks and small founding populations (see tables in [3,4]). However, as mentioned above, these estimates can vary significantly depending on the virus, host, route of transmission, and experimental design. Understanding the factors that determine the size of the transmission bottleneck is vital to interpreting the effect transmission has on viral evolution. Work in Tobacco etch virus (TEV) suggests that the size of the bottleneck is dose dependent, with higher exposure doses corresponding to larger founding populations [22]. Evidence from mixed infections of influenza virus in a guinea pig model is consistent with a dose dependence model [23]. Further support comes from experimental infections with tagged influenza clones in ferret and guinea pig models, which indicate that the more limiting exposure dose of aerosol transmission imposes a significantly more stringent bottleneck than contact transmission [6••]. Additionally, coinfection by other pathogens, which can limit innate defenses and modulate the immune response, has been correlated with loose bottlenecks in HIV and HCV [24–26]. Taken