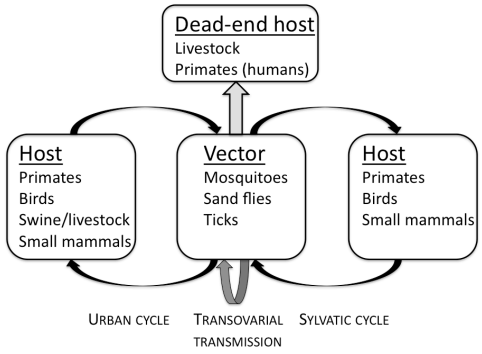
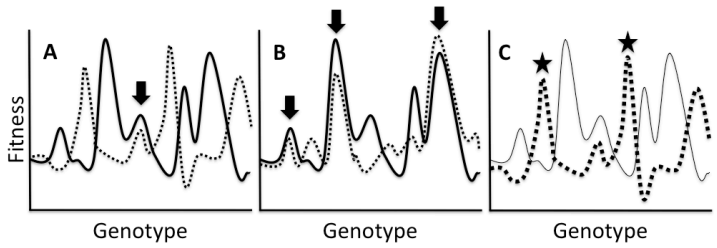
Fitness evolution during host alternation

One of the more interesting questions of evolutionary biology is what are the mechanisms leading to radiation and subsequent speciation. Niche differentiation provides a mechanism to promote coexistence and, potentially, radiation as populations adapt to specific environments. This process is one of specialization and contrasts with the selection of populations that are capable of using doing reasonably well across environments: the generalists. Assuming tradeoffs both strategies have a cost.



RNA viruses in general, and arthropod-borne viruses (arboviruses), are considered master generalists because of their ability to do well in multiple environmental conditions. Arboviruses have life cycles that include at least a step of replication in a vertebrate host and a step of replication in an arthropod vector. These cycles can be more complicated and the virus can spillover to hosts that cannot support viral replication sufficiently to continue the transmission cycle (dead-end hosts).

The cost of specialization can be understood in terms of overlapping peaks in the fitness landscape provided by vector and host and provided a theoretical explanation for the relatively slow speed of evolution found in natural populations of arboviruses: if there are few overlapping fitness peaks (high cost of adaptation to one environment) selection will trap the populations in those overlapping regions.



Note that we will be talking about two types of costs/tradeoffs: (1) the specific cost that adaptation to EA had in EB and (2) the broad cost of generalization and specialization.

Experimental tests

Several groups have been studying the evolution of arboviruses from different families under changing conditions that reproduce alternation between vectors and hosts. In cell culture when viruses adapted to one environment (EA) are tested for fitness in another environment (EB) the results are unpredictable (see lecture 2: trade offs/costs). Very often increases in EA correlate with increases in EB, although seldom there is an increase in EB as large as the increase observed during adaptation in EB (e.g. there is a cost). Other potential result is that adaptation in EA produces no change in EB. Finally, sometimes adaptation to EA results in fitness loss in EB (e.g. there is a tradeoff). The correlations between cell lines can be asymmetric and results in one direction do no necessarily predict the correlations in the other direction. For instance, if adaptation in EA results in adaptation in EB, adaptation in EB may have any possible result in terms of adaptation to EB. Studies with different mammalian cell types produced similar results.

Trade offs in phage may be relatively common and caused by antagonistic pleiotropy. In most studies with mammalian viruses, and regardless of the existence of tradeoffs or other costs, alternation results in fitness increases in both host and vector without evidence of cost to generalization. It seems that despite what we (humans) think, as far as the viruses go vectors and hosts can look like fairly similar environments.

For studies in cell culture the only exception to the rule was observed in VSV during adaptation to persistent infection of sand fly cells, a more realistic set up. Here the dynamics were asymmetric with trade offs found only during adaptation to persistence in sand fly. In that case fitness can decrease to spectacularly in the mammalian cells. The cycling resulted in trajectories of fitness evolution identical to that observed during adaptation to persistence, and alternation alleviates the defect only partially. The main conclusion is that the environment provided by persistence is the main determinant of VSV evolution. This result is consistent with studies in vivo suggesting that the role of the mammals in the replication cycle is minimal.

The cell culture studies were followed by several in vivo studies using live vectors and animal models. There were few surprises and sometimes there were tradeoffs to replicating in one of the environments, but sometimes there weren't. Regardless, during alternation there were two results, either the virus adapted concomitantly to both host and vector or one of the environment played no role in viral evolution. Taken together the results suggest that the best explanation to viral stability in nature is that these pathogens are already at the top of fitness peaks in the relevant environments and alternation does not promote evolutionary stability.

Fitness evolution was not a good predictor of virulence evolution. While adaptation to sand fly persistence does result in loss of virulence in the mammalian cells, the kinetics of virulence changes do not correlate well with the kinetics of fitness changes.

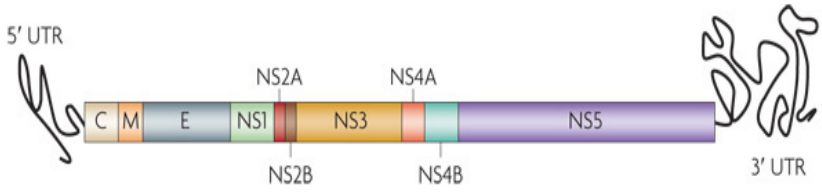
Genomic evolution

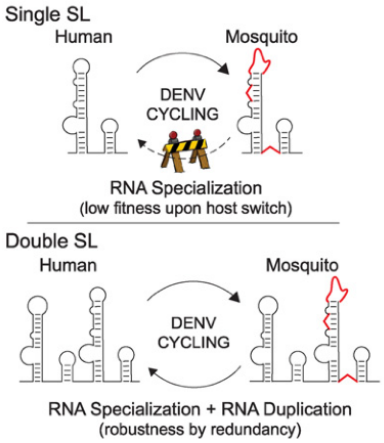
There are no data regarding fitness of natural virus populations and the speed of evolution is estimated from sequencing data. To understand the basis of adaptation or evolutionary stasis the experiments so far described included the determination of (full) genomic sequences.

Once more, despite predominant positive selection and minimal drift the typical dN/dS of evolving populations is close to 1.0. In evolving VSV the number of mutations correlated better with the extent of fitness gains than with the complexity of the environment. The results of alternation were always similar to those of adaptation to the vector alone. In other viral systems the results varied, but alternations produced the fewest mutations only in one case (TBEV) in which each regime was represented by a single replica. The vector-passaged and alternated regimes not only resulted in the same number of mutations, but there was a high degree of parallel evolution. In the absence of trade off the same mutations are present in single-cell passages and alternating passages.

The trade off observed in VSV during alternation of acute (lytic) mammalian infection and persistent vector infection mapped to the termini, in which both types of regimes selected for relatively large insertions in the promoters. Many host-range determinants have been studies in viruses, but we know less about trade off determinants (in part because they are a lot less frequent than we thought). However, poliovirus adapted to grow in cell culture becomes attenuated in vivo and the basis of the tradeoff map in the IRES element of the 5' terminus. Note that in the case of poliovirus fitness and virulence correlate. In experiments with Dengue it is possible to observe trade

offs, but alternation results in fitness increase in host and vector. The trade off maps to a secondary structure at the 3' terminus of the genome, which require different nucleotides for optimal function in host and vector. The simultaneous adaptation to both environments is mediated by duplication of the structure followed by the accumulations of vector-specific mutations in one copy. In vivo the results are a bit different and the mosquitoes determine the speed of evolution, both in terms of fitness and mutation accumulation.

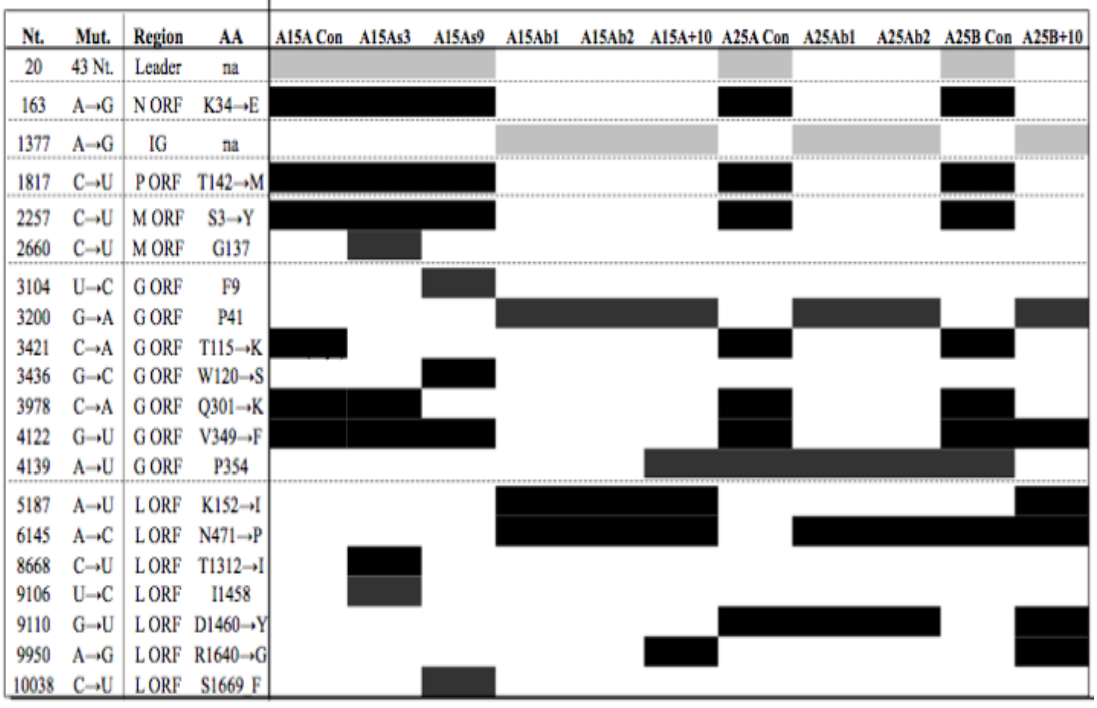




Antagonistic pleiotropy explains all cases of trade off analyzed (except for one paper that had some problems with the loss of genetic markers).

Adaptability changes during host alternation

The long-terms effects of alternation have received less attention. After adaptation of VSV to persistence recovery of fitness if very fast and it is mediated by mammalian-adapted variants that are maintained in the population even in the absence of periodic mammalian replication. Alternation did not slow down the speed of adaptation.



In contrast other results suggest that alternation may sometimes result in loss in adaptability in VSV when the virus fitness is measured at extreme temperatures. The problem with these studies is that the loss of the genetic marker in some lines makes it impossible to determine whether they are specialists or generalists, and based on alternation alone the result is not as clean. These results may be meaningful, though. Studies done with Chikingunya did demonstrate convincingly that virus adapted to alternation improved in both host and vector, but population diversity decreased significantly and the adaptation to new environments (antibody, mutagen) was limited compared to the serially-passaged counterparts.

Figure Credits

Dengue Genome: Guzman, M. G. et al. Dengue: A continuing global threat. Nature Reviews Microbiology **8,** S7–S16 (2010).

Dengue evolution: Villordo SM, Filomatori CV, Sánchez-Vargas I, Blair CD, Gamarnik AV (2015) Dengue Virus RNA Structure Specialization Facilitates Host Adaptation. PLoS Pathog 11(1): e1004604. doi:10.1371/journal.ppat.1004604