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Viral host-adaptation: insights from evolution experiments with phages

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Abstract

Phages, viral parasites of bacteria, share fundamental features of pathogenic animal and plant viruses and represent a highly tractable empirical model system to understand viral evolution and in particular viral host-adaptation. Phage adaptation to a particular host genotype often results in improved fitness by way of parallel evolution whereby independent lineages hit upon identical adaptive solutions. By contrast, phage adaptation to an evolving host population leads to the evolution of increasing host-range over time and correlated phenotypic and genetic divergence between populations. Phage host-range expansion frequently occurs by a process of stepwise evolution of multiple mutations, and host-shifts are often constrained by mutational availability, pleiotropic costs or ecological conditions.

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Introduction

Evolutionary studies of phages, viral parasites of bacteria, can reveal general principles of viral biology because phages share many of the fundamental features of pathogenic animal and plant viruses [1]. Large populations of phages and their bacterial hosts can be easily propagated in controlled laboratory environments where short generation times favour rapid evolutionary rates. Moreover, lytic phages in particular (i.e. those that must kill their host cell to reproduce), have simple life histories and relatively small, well-understood genomes allowing reliable mapping of genotype to phenotype and the formulation of a detailed knowledge of the pathways to adaptation. Importantly, phages are themselves of ecological and economic importance, for example, in structuring natural bacterial communities [2] and for their undesirable effects on bioprocessing of food and waste products by bacteria [3]. As a result of these factors, phages have emerged as a key model system for the study of viral evolution and in particular viral host-adaptation [1]. To date a wide range of phages and their associated bacterial hosts have been employed in experimental evolution studies encompassing a broad sweep of phylogenetic diversity and different forms of genomic organisation [1,4]. In this article we summarise recent advances in our understanding of viral host-adaptation arising from experimental evolution of phages. We highlight the differences in the pattern and process of phage evolution depending upon whether adaptation is against a fixed or a coevolving host, and outline the genetic and ecological factors that shape the evolution of phage host range.

Experimental evolution

As with all viruses, phage replication depends on intimate interactions with a number of host cell components, from receptors that mediate entry, to transcription and translation

machinery that produce virus particles, to cell wall components that are disrupted to lyse the host. Evolution can rapidly tune the kinetics of these reactions, and the huge population sizes that phage can reach, combined with the frequency of mutation and their generally compact genomes, ensures that there is abundant variation on which selection can efficiently act [5]. When repeatedly exposed to a given host genotype, phages tend to evolve higher growth rates [6-11] and/or an increased phage-imposed reduction in host growth [8,12] when compared with their ancestors (Figure 1). Many genes, including those involved in capsid structure [11,13], tail fibres [14] and viral replication [13], have been identified as undergoing changes during adaptation to a host, although many studies have been conducted with confounding factors (such as increased temperature, or growth in a chemostat) making it difficult to identify the genetic targets specific to host adaptation *per se*. A principal feature of phage host-adaptation is that evolution can be highly parallel, with fixation of the same mutations occurring in independently evolving populations of phage. Indeed, 'replaying the tape' of evolution often has repeatable consequences [13], and 'rewinding the tape' by reversing the selective pressures (i.e. adapting back to the host of the ancestral phage) sees the appearance of mutations reverting the phage genome towards the ancestral state [6,15,16]. Where adaptation does not necessarily require many amino acid changes [7,17] and there are limits to the number of beneficial mutations available due to the small size and high levels of pleiotropy in a virus genome [18], parallel evolution might be expected [19,20]. (Pleiotropy refers to the case where a single gene affects multiple functions.) Interestingly, parallel 'silent' mutations (those that do not affect the amino acid sequence of translated virus products) have been frequently observed, indicating that selection can be strong on features other than translated gene products, such as nucleic acid secondary structure or translation efficiency [6,7,11].

The implication of parallel adaptation is that the mutations involved have consistent beneficial effects on fitness, as phages optimise their interactions with host processes in the context of their own genetics and ecology. However, these fitness effects can be highly specific, with different adaptive paths open to different genotypes. Individual mutations may not act independently, but rather be dependant on the pre-existing genetic background (i.e. epistasis) [7,10]. When three related species of phage were adapted to a host, parallel evolution within each species was high, but the different species did not acquire similar mutations at homologous sites as they adapted [21]. Epistasis, coupled with an incomplete understanding of the precise structures and processes involved in the phage lifecycle, mean that whilst qualitative phenotypic trends occurring as a phage adapts to its host tend to be predictable, anticipating the exact effects of selection in shaping phenotype or genotype has proved somewhat difficult. For example, adaptation to altered host receptor lipopolysaccharide resulted in changes to the ϕX174 virus capsid as predicted, but these changes were located internally, not in the receptor binding site, and seemed to play a more general role in enhancing capsid stability than receptor specificity [11]. Likewise, selection under conditions favouring an altered lysis time yielded phages with phenotypes qualitatively in line with predictions but significantly divergent from the values predicted by a theoretical model [22,23]. Nevertheless, the fact that phage fitness tends to plateau under consistent selection [6,7,10] points to a dynamic in which evolution on a fixed host is selecting for an optimal phenotype in the context of the experimental conditions, towards which the population converges.

Evolving optimum fitness

Several obstacles may prevent viruses from attaining this optimum. Some traits may

require many simultaneous mutations to evolve, or are constrained by the physical properties of the genes and proteins involved, and thus are unlikely to appear in small populations or over short periods of time. Population bottlenecks, caused by events such as transmission, dispersal, and population dynamics often occur during virus evolution [24-26] and have the potential to shape the course of evolution by preventing the appearance and spread of beneficial mutations and facilitating fixation of deleterious ones [15,27]. Similarly, neutral and slightly deleterious mutations can hitchhike to fixation, if they fortuitously occur alongside a beneficial mutation in an expanding clone. The high mutation rate and small genome size, particularly of RNA viruses, can potentially overcome these problems by replenishing population diversity, allowing even a clonal virus to overcome the genetic restrictions of a bottleneck and efficiently explore the local fitness landscape [28]. A balance must be struck, however, because an elevated rate of mutation can inhibit optimal fitness by consistently mutating viruses away from a fitness peak [29], which in combination with frequent bottlenecks can lead to lethal mutagenesis and extinction [29].

Viruses within a population can also compete with one another. Clonal interference, whereby the rate of adaptation is inhibited by competition between different beneficial mutations, can be a major impediment to adaptation [30] although at high mutation rates and population size its effects can be somewhat alleviated by mutational supply [31]. Where supply of hosts is limited, multiplicity of infection (MOI) is high, and virus clones can co-infect the same host. Co-infection can allow for recombination, potentially increasing the rate of adaptation (although this effect can be small if co-infecting phages are closely related [31]. More importantly perhaps, co-infecting virus clones must compete for host resources. Under such conditions, there may not be a universal optimum genotype. In some cases, virus clones can parasitize the genes of co-infecting

viruses, resulting in a 'prisoner's dilemma' where the fitness of a particular genotype is dependent on the frequency of competitors [32]. Phages can even evolve the means to detect co-infection, and modulate their infection strategy accordingly. Phage ϕ2 evolved under high MOI killed *Pseudomonas fluorescens* host cells more rapidly than those evolved under low MOI, but only when assay MOI was high [33]. Such adaptive phenotypic plasticity raises the possibility that life-history plasticity is a trait amenable to selection in viruses. The tensions resulting from these competing interests mean that the dynamics of these relationships are rapid and unpredictable, and virus evolution can continue even in long-term cultures [13].

Experimental coevolution

In contrast to the optimisation of viral fitness achievable during experimental evolution, scenarios where bacteria too are allowed to evolve - i.e. experimental *co*evolution [34] - may preclude net gains in viral fitness due to the potential for continual reciprocal adaptation and counter-adaptation inherent to antagonistic coevolution. Indeed, empirical studies, across a range of bacteria-phage systems, reveal that rapid, persistent antagonistic coevolution is a common outcome of co-propagation of bacteria and phage [35-39] (challenging the view that bacteria-phage coevolution is universally constrained [40]). These studies suggest that bacteria-phage coevolution typically takes the form of an arms-race of repeated cycles of evolution of bacterial-resistance followed by evolution of phage to infect these resistant bacteria, which can last for several hundreds of bacterial generations [35,37,41]. This process is driven by predominantly directional selection favouring the evolution of broader virus host-range and, concomitantly, broader bacterial resistance range through time, but no corresponding increase in viral growth rate on the ancestral host [8] (Figure 1). As such, for a given population, phages from

later in the experiment can typically infect bacterial genotypes from the past, even though these bacteria will tend to be resistant to their own contemporaneous phages [37,39,42].

Recurrent selective sweeps of new infectivity mutations leads to the accumulation of multiple infectivity mutations per phage genome, and a tendency for host-range and the number of substitutions to be positively correlated [37,42]. Indeed, this stepwise accretion of mutations appears to be crucial to effective host-range expansion [12,17]. Continual reciprocal selection for innovation tends to accelerate viral evolution in coevolving populations compared to viral populations adapting to a fixed host environment [14,38], in some cases as much as doubling the nonsynonymous substitution rate [14]. Moreover, coevolution leads to greater between-population divergence of viral genomes than does selection against a fixed host [14]. Thus, while common loci are targeted by selection across replicate coevolving populations, the specific sites of mutations and/or the combinations of co-occurring mutations vary, giving rise to phenotypic divergence in viral host-range between populations [14] (Figure 1). In some phages, host-range mutations have been observed in genes known to encode infectivity determinants such as proteins for host-binding (e.g. tail fibre protein in phage ϕ2 [14,42], host recognition protein J in phage lamda [17], whereas, in other phages, host-range mutations have been observed in genes of unknown function, suggesting novel mechanisms of bacteria-phage interaction [37].

Host-range expansion & host-shifts

The expansion or shift of viral host range is one of the most pertinent features of viral evolution. Many recent examples of emerging human viral diseases, such as HIV, bird/swine flu and SARS are the result of expanding host range [1]. However, co-culture

studies have demonstrated that host range shifts are generally highly constrained by both genetic and ecological factors.

While coevolution leads to the stepwise build-up of broad host-range, larger shifts onto more distantly related hosts presents a greater challenge for viruses. Attempts to experimentally evolve phages to infect novel hosts often fail, even when the new host is of the same species [12,43]. Where larger numbers of mutations are required to infect a novel host, especially where those mutations act synergistically, the likelihood of evolving infectivity in one step is greatly reduced. For example, when exposed to resistant *P. fluorescens* strains (derived from coevolution with phage ϕ2), the ancestral phage genotype could evolve to infect hosts in one step, but only when relatively few mutations were required [12]. Interestingly, sequencing of host range mutants has shown that host range evolution is almost always associated with mutations in specific host attachment proteins (tail-fibre proteins in ϕ2 [12] and T7 [44], and attachment protein P3 in ϕ6 [43,45], suggesting a relatively limited array of virus genes on which selection can act. Access to the native host, either through migration [46] or in a mixed host environment [47,48], can greatly increase the potential for host range shifts. Susceptible hosts support a 'source' population for host range mutants able to infect novel hosts with poor efficiency ('sink' populations). Source populations provide the means to maintain population size and thus mutation supply, increasing the opportunity for beneficial mutations to arise.

Where host range mutations do arise they often result in antagonistic pleiotropic effects, reducing fitness in the native host [8,43,45], or even causing a loss of infectivity [9]. Evolution of host range is therefore likely to be subject to a trade-off in the benefits of host range expansion vs. specialisation. Phage selection experiments have demonstrated the role of several key ecological variables that shape the outcome of host

range evolution. Firstly, availability of susceptible hosts during adaptation to a novel host can hinder, as well as facilitate, the evolution of range shifts [48]. When evolved in varying ratios of susceptible:novel hosts, phage ϕ2 only evolved to infect a novel host when the frequency of susceptible hosts was between 0.1 – 1% relative to the novel host [48]. Phage genotypes able to grow on both the native and novel hosts were shown to have a significantly lower growth rate on each host population compared with host specialists. Thus, in the presence of greater frequencies of susceptible hosts, even where novel hosts remain in the majority, the pleiotropic cost of expanded host range results in selection against generalists. Furthermore, where host range expansions did occur, the vast majority of host range mutants evolved to specialise on the novel host alone, resulting in a corresponding increase in fitness on the new host. A similar pattern of adaptation was observed in the ϕ6 phage of *Pseudomonas syringae* [9], suggesting that even where host range shifts are favoured, antagonistic pleiotropy will favour divergent evolution of host specialists rather than the stable existence of generalists.

Secondly, host range shifts are also dependent on the intrinsic quality of the novel host resource. Under optimal foraging theory, where there is a disparity in the profitability of different hosts, specialism on the more profitable host will be favoured. For example, populations of a generalist T7 phage able to infect two *E. coli* host strains, one strain of which was less profitable that the other, evolved to infect only the more profitable host [44]. Crucially, selection for optimal foraging is not dependant on a cost of generalism. Indeed, in this study avoidance of the poor quality host was associated with a drop in infectivity on the preferred host [44].

Thirdly, Bono et al. have shown that intraspecific competition for resources can be a major driving force behind the evolution of generalism. Increasing the MOI of ϕ6 phages in *P. syringae* populations lead to an increase in the probability of generalist emergence

[49]. Furthermore, the authors find that the impact of intraspecific competition was positively associated with the quality of the novel host, driving rapid host range expansions where competition is strong and host quality is relatively equal, and slow rates of host range evolution where novel hosts are considerably less profitable than the native host [49].

Concluding remarks

Experimental studies of phage evolution have yielded substantial advances in our understanding of viral host-adaptation. Several clear trends emerge: First, viral adaptation to a given host genotype often results in parallel evolution and the convergence of independent lineages upon a shared adaptive solution via the same genetic targets, although this parallelism does not appear to be conserved when comparing evolution of different phage species. Second, reciprocal evolution of the bacterial host tends to accelerate phage evolution through continual selection for increased infectivity and leads to greater between-population divergence. Third, phage host-range expansion occurs during coevolution by stepwise evolution involving multiple mutations, and large host-shifts appear to be constrained by mutational accessibility, pleiotropic costs and ecological factors.

It is interesting to consider the extent to which these evolutionary patterns translate to viruses of eukaryotes. First, there are clear and important biological differences between prokaryotic and eukaryotic hosts, for example, multicellular eukaryotes tend to live in much smaller populations than bacteria, reproduce more slowly and only a small fraction of host cells are germline. These differences are likely to generate contrasting selection and demand different adaptive solutions. Nevertheless, several aspects of studies on viruses and their eukaryotic hosts appear consistent with the general patterns outlined

249 above. For example, as with phages, adaptation to a particular host favoured parallel
250 adaptations in experimental evolution of influenza virus A [50] and tobacco etch
251 potyvirus (TEV) [51]. Antagonistic pleiotropy and multiple steps to host range expansion
252 have been recorded in experimental TEV evolution [51] and in the history of feline
253 panleukopenia virus evolution [52]. Although rapid coevolution between viruses and their
254 eukaryotic hosts is constrained at the organismal level by the relatively slow eukaryote
255 rate of replication (and hence coevolution), coevolutionary dynamics are apparent in the
256 interaction between virus serotypes and the adaptive immunity and immune memory of
257 vertebrates. As with bacteria-phage coevolution, this can lead to rapid evolution and
258 between-population divergence, as has been observed in the case of HIV-antibody
259 coevolution within a patient [53] and the 'antigenic drift' of foot-and-mouth disease virus
260 [54] and influenza virus [55] between host populations.

261 Numerous questions remain for future research. Existing studies have largely focused
262 on phage adaptation at the interface of virus-host attachment, yet the recent discovery of
263 CRISPR-based immunity suggests huge potential for dynamic intracellular responses by
264 bacteria to act as a driver of phage evolution (e.g. see [56,57]). Very few studies have
265 addressed the extent to which the evolutionary characteristics of phages are
266 phylogenetically conserved (although see [21]) suggesting exciting potential for
267 'comparative' experimental evolution. In particular, it is unclear why some bacteria-
268 phage interactions undergo extensive, prolonged arms race coevolution whereas others
269 apparently do not (e.g., [58,59]). While host-adaptation by lytic phages has been well
270 studied, temperate phages have not been extensively studied in an experimental
271 evolution context (although see [60]). Finally, natural phage communities are highly
272 diverse, thus it is important to understand how the evolution of phages in isolation scales
273 up when embedded in more complex, species rich phage communities.

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An elegant study of optimal life history evolution in a temperate phage

Figure Legend

Figure 1. Contrasting trajectories of viral adaptation in terms of growth rate and host-range phenotypes for viruses evolving against a fixed host genotype (E - i.e. ‘evolving’)

or a coevolving host population (C – i.e. ‘coevolving’). Arrows show the trajectory of evolution from the ancestral phenotype (A – i.e. ‘ancestral’) and while trajectories are parallel for evolving viral lineages, they are divergent for coevolving viral lineages (compare ‘E’ and ‘C’ trajectories). Adapted from data presented in ref. 8.