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Hall, James Pj, Harrison, Ellie and Brockhurst, Michael A orcid.org/0000-0003-0362-820X
(2013) *Viral host-adaptation : insights from evolution experiments with phages*. *Current Opinion in Virology*. pp. 572-577. ISSN 1879-6265

<https://doi.org/10.1016/j.coviro.2013.07.001>

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1 **Viral host-adaptation: insights from evolution experiments with phages**

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11

12 **Abstract**

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

23 *Published in Current Opinion in Virology 3:572-7 doi: 10.1016/j.coviro.2013.07.001*

24 **Introduction**

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

45

46 **Experimental evolution**

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

49 machinery that produce virus particles, to cell wall components that are disrupted to lyse
50 the host. Evolution can rapidly tune the kinetics of these reactions, and the huge
51 population sizes that phage can reach, combined with the frequency of mutation and
52 their generally compact genomes, ensures that there is abundant variation on which
53 selection can efficiently act [5]. When repeatedly exposed to a given host genotype,
54 phages tend to evolve higher growth rates [6-11] and/or an increased phage-imposed
55 reduction in host growth [8,12] when compared with their ancestors (Figure 1). Many
56 genes, including those involved in capsid structure [11,13], tail fibres [14] and viral
57 replication [13], have been identified as undergoing changes during adaptation to a host,
58 although many studies have been conducted with confounding factors (such as
59 increased temperature, or growth in a chemostat) making it difficult to identify the genetic
60 targets specific to host adaptation *per se*. A principal feature of phage host-adaptation is
61 that evolution can be highly parallel, with fixation of the same mutations occurring in
62 independently evolving populations of phage. Indeed, ‘replaying the tape’ of evolution
63 often has repeatable consequences [13], and ‘rewinding the tape’ by reversing the
64 selective pressures (i.e. adapting back to the host of the ancestral phage) sees the
65 appearance of mutations reverting the phage genome towards the ancestral state
66 [6,15,16]. Where adaptation does not necessarily require many amino acid changes
67 [7,17] and there are limits to the number of beneficial mutations available due to the
68 small size and high levels of pleiotropy in a virus genome [18], parallel evolution might
69 be expected [19,20]. (Pleiotropy refers to the case where a single gene affects multiple
70 functions.) Interestingly, parallel ‘silent’ mutations (those that do not affect the amino
71 acid sequence of translated virus products) have been frequently observed, indicating
72 that selection can be strong on features other than translated gene products, such as
73 nucleic acid secondary structure or translation efficiency [6,7,11].

74 The implication of parallel adaptation is that the mutations involved have consistent
75 beneficial effects on fitness, as phages optimise their interactions with host processes in
76 the context of their own genetics and ecology. However, these fitness effects can be
77 highly specific, with different adaptive paths open to different genotypes. Individual
78 mutations may not act independently, but rather be dependant on the pre-existing
79 genetic background (i.e. epistasis) [7,10]. When three related species of phage were
80 adapted to a host, parallel evolution within each species was high, but the different
81 species did not acquire similar mutations at homologous sites as they adapted [21].
82 Epistasis, coupled with an incomplete understanding of the precise structures and
83 processes involved in the phage lifecycle, mean that whilst qualitative phenotypic trends
84 occurring as a phage adapts to its host tend to be predictable, anticipating the exact
85 effects of selection in shaping phenotype or genotype has proved somewhat difficult. For
86 example, adaptation to altered host receptor lipopolysaccharide resulted in changes to
87 the ϕ X174 virus capsid as predicted, but these changes were located internally, not in
88 the receptor binding site, and seemed to play a more general role in enhancing capsid
89 stability than receptor specificity [11]. Likewise, selection under conditions favouring an
90 altered lysis time yielded phages with phenotypes qualitatively in line with predictions but
91 significantly divergent from the values predicted by a theoretical model [22,23].
92 Nevertheless, the fact that phage fitness tends to plateau under consistent selection
93 [6,7,10] points to a dynamic in which evolution on a fixed host is selecting for an optimal
94 phenotype in the context of the experimental conditions, towards which the population
95 converges.

96

97 **Evolving optimum fitness**

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

99 require many simultaneous mutations to evolve, or are constrained by the physical
100 properties of the genes and proteins involved, and thus are unlikely to appear in small
101 populations or over short periods of time. Population bottlenecks, caused by events such
102 as transmission, dispersal, and population dynamics often occur during virus evolution
103 [24-26] and have the potential to shape the course of evolution by preventing the
104 appearance and spread of beneficial mutations and facilitating fixation of deleterious
105 ones [15,27]. Similarly, neutral and slightly deleterious mutations can hitchhike to fixation,
106 if they fortuitously occur alongside a beneficial mutation in an expanding clone. The high
107 mutation rate and small genome size, particularly of RNA viruses, can potentially
108 overcome these problems by replenishing population diversity, allowing even a clonal
109 virus to overcome the genetic restrictions of a bottleneck and efficiently explore the local
110 fitness landscape [28]. A balance must be struck, however, because an elevated rate of
111 mutation can inhibit optimal fitness by consistently mutating viruses away from a fitness
112 peak [29], which in combination with frequent bottlenecks can lead to lethal mutagenesis
113 and extinction [29].

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

124 viruses, resulting in a 'prisoner's dilemma' where the fitness of a particular genotype is
125 dependent on the frequency of competitors [32]. Phages can even evolve the means to
126 detect co-infection, and modulate their infection strategy accordingly. Phage ϕ 2 evolved
127 under high MOI killed *Pseudomonas fluorescens* host cells more rapidly than those
128 evolved under low MOI, but only when assay MOI was high [33]. Such adaptive
129 phenotypic plasticity raises the possibility that life-history plasticity is a trait amenable to
130 selection in viruses. The tensions resulting from these competing interests mean that the
131 dynamics of these relationships are rapid and unpredictable, and virus evolution can
132 continue even in long-term cultures [13].

133

134 **Experimental coevolution**

135 In contrast to the optimisation of viral fitness achievable during experimental evolution,
136 scenarios where bacteria too are allowed to evolve - i.e. experimental coevolution [34] -
137 may preclude net gains in viral fitness due to the potential for continual reciprocal
138 adaptation and counter-adaptation inherent to antagonistic coevolution. Indeed,
139 empirical studies, across a range of bacteria-phage systems, reveal that rapid, persistent
140 antagonistic coevolution is a common outcome of co-propagation of bacteria and phage
141 [35-39] (challenging the view that bacteria-phage coevolution is universally constrained
142 [40]). These studies suggest that bacteria-phage coevolution typically takes the form of
143 an arms-race of repeated cycles of evolution of bacterial-resistance followed by
144 evolution of phage to infect these resistant bacteria, which can last for several hundreds
145 of bacterial generations [35,37,41]. This process is driven by predominantly directional
146 selection favouring the evolution of broader virus host-range and, concomitantly, broader
147 bacterial resistance range through time, but no corresponding increase in viral growth
148 rate on the ancestral host [8] (Figure 1). As such, for a given population, phages from

149 later in the experiment can typically infect bacterial genotypes from the past, even
150 though these bacteria will tend to be resistant to their own contemporaneous phages
151 [37,39,42].

152 Recurrent selective sweeps of new infectivity mutations leads to the accumulation of
153 multiple infectivity mutations per phage genome, and a tendency for host-range and the
154 number of substitutions to be positively correlated [37,42]. Indeed, this stepwise
155 accretion of mutations appears to be crucial to effective host-range expansion [12,17].
156 Continual reciprocal selection for innovation tends to accelerate viral evolution in
157 coevolving populations compared to viral populations adapting to a fixed host
158 environment [14,38], in some cases as much as doubling the nonsynonymous
159 substitution rate [14]. Moreover, coevolution leads to greater between-population
160 divergence of viral genomes than does selection against a fixed host [14]. Thus, while
161 common loci are targeted by selection across replicate coevolving populations, the
162 specific sites of mutations and/or the combinations of co-occurring mutations vary, giving
163 rise to phenotypic divergence in viral host-range between populations [14] (Figure 1). In
164 some phages, host-range mutations have been observed in genes known to encode
165 infectivity determinants such as proteins for host-binding (e.g. tail fibre protein in phage
166 ϕ 2 [14,42], host recognition protein J in phage lamda [17], whereas, in other phages,
167 host-range mutations have been observed in genes of unknown function, suggesting
168 novel mechanisms of bacteria-phage interaction [37].

169

170 **Host-range expansion & host-shifts**

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

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

176 While coevolution leads to the stepwise build-up of broad host-range, larger shifts onto
177 more distantly related hosts presents a greater challenge for viruses. Attempts to
178 experimentally evolve phages to infect novel hosts often fail, even when the new host is
179 of the same species [12,43]. Where larger numbers of mutations are required to infect a
180 novel host, especially where those mutations act synergistically, the likelihood of
181 evolving infectivity in one step is greatly reduced. For example, when exposed to
182 resistant *P. fluorescens* strains (derived from coevolution with phage $\phi 2$), the ancestral
183 phage genotype could evolve to infect hosts in one step, but only when relatively few
184 mutations were required [12]. Interestingly, sequencing of host range mutants has
185 shown that host range evolution is almost always associated with mutations in specific
186 host attachment proteins (tail-fibre proteins in $\phi 2$ [12] and T7 [44], and attachment
187 protein P3 in $\phi 6$ [43,45], suggesting a relatively limited array of virus genes on which
188 selection can act. Access to the native host, either through migration [46] or in a mixed
189 host environment [47,48], can greatly increase the potential for host range shifts.
190 Susceptible hosts support a 'source' population for host range mutants able to infect
191 novel hosts with poor efficiency ('sink' populations). Source populations provide the
192 means to maintain population size and thus mutation supply, increasing the opportunity
193 for beneficial mutations to arise.

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

199 range evolution. Firstly, availability of susceptible hosts during adaptation to a novel
200 host can hinder, as well as facilitate, the evolution of range shifts [48]. When evolved in
201 varying ratios of susceptible: novel hosts, phage $\phi 2$ only evolved to infect a novel host
202 when the frequency of susceptible hosts was between 0.1 – 1% relative to the novel host
203 [48]. Phage genotypes able to grow on both the native and novel hosts were shown to
204 have a significantly lower growth rate on each host population compared with host
205 specialists. Thus, in the presence of greater frequencies of susceptible hosts, even
206 where novel hosts remain in the majority, the pleiotropic cost of expanded host range
207 results in selection against generalists. Furthermore, where host range expansions did
208 occur, the vast majority of host range mutants evolved to specialise on the novel host
209 alone, resulting in a corresponding increase in fitness on the new host. A similar pattern
210 of adaptation was observed in the $\phi 6$ phage of *Pseudomonas syringae* [9], suggesting
211 that even where host range shifts are favoured, antagonistic pleiotropy will favour
212 divergent evolution of host specialists rather than the stable existence of generalists.

213 Secondly, host range shifts are also dependent on the intrinsic quality of the novel host
214 resource. Under optimal foraging theory, where there is a disparity in the profitability of
215 different hosts, specialism on the more profitable host will be favoured. For example,
216 populations of a generalist T7 phage able to infect two *E. coli* host strains, one strain of
217 which was less profitable than the other, evolved to infect only the more profitable host
218 [44]. Crucially, selection for optimal foraging is not dependant on a cost of generalism.
219 Indeed, in this study avoidance of the poor quality host was associated with a drop in
220 infectivity on the preferred host [44].

221 Thirdly, Bono et al. have shown that intraspecific competition for resources can be a
222 major driving force behind the evolution of generalism. Increasing the MOI of $\phi 6$ phages
223 in *P. syringae* populations lead to an increase in the probability of generalist emergence

224 [49]. Furthermore, the authors find that the impact of intraspecific competition was
225 positively associated with the quality of the novel host, driving rapid host range
226 expansions where competition is strong and host quality is relatively equal, and slow
227 rates of host range evolution where novel hosts are considerably less profitable than the
228 native host [49].

229

230 **Concluding remarks**

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

242 It is interesting to consider the extent to which these evolutionary patterns translate to
243 viruses of eukaryotes. First, there are clear and important biological differences between
244 prokaryotic and eukaryotic hosts, for example, multicellular eukaryotes tend to live in
245 much smaller populations than bacteria, reproduce more slowly and only a small fraction
246 of host cells are germline. These differences are likely to generate contrasting selection
247 and demand different adaptive solutions. Nevertheless, several aspects of studies on
248 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.

274

275 **Acknowledgements**

276 Our work is supported by grants from the European Research Council (COEVOCON
277 311490) and the Natural Environment Research Council (NE/H005080/1) to M.A.B.

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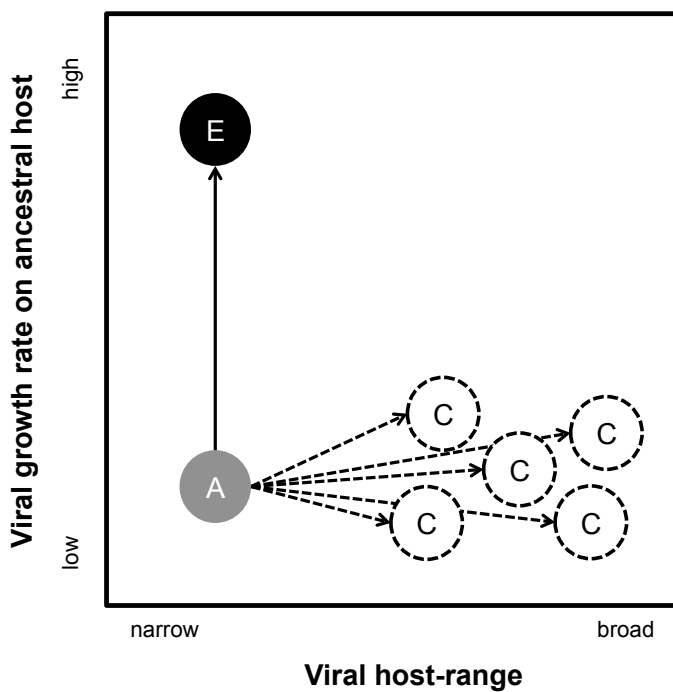
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513 **Figure Legend**

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

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



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