Viruses can suffer ‘life-history’ trade-offs that prevent simultaneous improvement in fitness traits, such as improved intrahost reproduction at the expense of reduced extrahost survival. Here we examine reproduction-survival trade-offs and other trait compromises, highlighting that experimental evolution can reveal trade-offs and their associated mechanisms. Whereas ‘curse of the pharaoh’ (high virulence with extreme stability) may generally apply for viruses of eukaryotes, we suggest phages are instead likely to suffer virulence/stability trade-offs. We examine how survival/reproduction trade-offs in viruses are affected by environmental stressors, proteins governing viral host range, and organization of the virus genome. Future studies incorporating comparative biology, experimental evolution, and structural biology, could thoroughly determine how viral trade-offs evolve, and whether they transiently or permanently constrain virus adaptation.

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This review will highlight how experimental evolution can be used to study life history trade-offs in viruses with a focus on trade-offs involving extracellular survival. In particular, we will stress the importance of comparative and mechanistic approaches to studying the generalities of life history trade-offs in viruses.

Types of life history trade-offs in viruses
Viruses are selected to maximize their overall fitness but the lifecycle comprises life history parameters (fitness components) that could be subject to conflicting trade-offs (Figure 1). The three main categories of virus trade-offs are intracellular, extracellular/intracellular and inter-environmental. Intracellular trade-offs can occur among traits within a cell, especially those relating to timing of cell lysis. Extracellular/intracellular trade-offs involve maximizing survival (particle stability) outside of the host versus reproduction within the host. Interevironmental trade-offs are trade-offs between fitness in different abiotic or biotic environments (hosts). This final category of trade-off is not extensively covered in this review but see [13–16,17**]. Also, we do not discuss trade-offs that explicitly involve host immune function, such as trade-offs between replication fidelity and immune escape [18].

Intracellular trade-offs — lysis timing
Virus fitness combines reproduction within the host, and virus transmission between hosts; for this reason, viruses of bacteria should be selected to optimize the time at
Figure 1

A depiction of generalized life history trade-offs that may occur in viruses. **Intracellular trade-offs:** (a) upon initial infection of the cell, certain bacteriophages can ‘choose’ between lysogeny (vertical transmission across host generations) versus lysis (destruction of the cell to promote horizontal transmission to a new host individual). (b) A trade-off in lytic phage can occur where increased burst size (progeny produced per cell) can lead to extended lysis time (increased transmission time to new cells), and vice versa. **Intracellular/Extracellular trade-offs:** (c) A wide variety of viruses should face possible trade-offs between rapid intracellular reproduction at the expense of reduced extracellular survival, especially when exposed to environmental stressors; for example, faster reproduction results in lower survival under elevated temperature. **Interenvironmental trade-offs** (d) Viral trade-offs may involve fitness differences between viruses across hosts (biotic environments) and (e) fitness differences between viruses on a single host across abiotic environments.

which they lyse (destroy) the host cell [19]. For temperate viruses, the ‘choice’ is to remain lysogenic in the current host cell (vertical transmission) versus lyse the host (horizontal transmission). The molecular mechanisms underpinning lysogeny are well characterized for phage λ [20] and robust theory explains its ‘decision’ [21,22]. The decision for λ to choose lysogeny over lysis depends on viral genome concentration within the host cell, such that host cell size combines with multiplicity of infection (MOI; the number of viruses co-infecting the cell) to determine the fate of the host cell [23]. The threshold for the switch to lysis from lysogeny, due to DNA damage, is variable among wild lambdoid phages and evolvable in the lab [24]. A related phage showed mechanistic differences, which affected the threshold for when to forgo lysogeny [25]. Presumably, the ecological basis for choosing lysogeny is that infection at high MOI signals rarity of susceptible hosts and overabundance of infectious viruses in the local environment [26]. Understanding the mechanism of genome concentration revealed the unpredicted contribution of host cell size in governing the switch but whether this effect is generalizable to other virus families would require a comparative approach, to test if genome concentration generally determines lysogeny versus lysis [4,23].

Strictly lytic viruses should also evolve optimal lysis timing [19]. Here, the trade-off is between increased burst size versus shortened burst time, and has a simple underlying mechanism: time. Viral particles inside the cell are assumed to be produced linearly through time from a point following infection; thus, delaying lysis should promote reproduction, whereas accelerating lysis reduces production but allows particles to more rapidly establish new infections [19,27]. Optimal lysis timing will depend on the rate of viral production, and the number of available hosts in the environment [27]. Studies with phage identified key proteins involved in lysis timing [28–30], and demonstrated that strains with intermediate lysis times experienced the greatest fitness [19]. Experimental evolution studies have manipulated host density as an environmental factor, to show that lysis timing is an evolvable trait; phage populations evolve faster lysis under high susceptible host density and slower lysis when hosts are scarce [31,32]. Most phages control lysis via holins [28], proteins that form holes in the cell membrane, and both altered expression and amino-acid substitutions in holin genes can affect evolution of lysis timing [30,31]. Some evolution studies showed that despite mutations in holins, phage populations did not evolve theoretically predicted optimum lysis times [32,33*]. The exact reasons are unresolved, but possible explanations include genetic constraints in pleiotropic virus genes, and insufficient time for spontaneous mutations of large effect to appear and fix in experimental populations [33*]. Also, if the assumption of linear intracellular particle production...
is incorrect, the trade-off and the predicted optimum lysis time would differ from model predictions, causing a mismatch to the data [32]. Nevertheless, the common mechanism for lysis and the wide variety of phages in which optimal lysis timing has been demonstrated suggest that this trade-off is generalizable for strictly lytic viruses.

**Extracellular versus intracellular trade-offs**
A trade-off between virus traits for intracellular reproduction versus extracellular survival would essentially constitute the classic life history trade-off between reproduction and survival. However, unlike the resolved mechanisms governing intracellular trade-offs, no obvious mechanisms explain reproduction/survival trade-offs in viruses. Furthermore, there is no a priori reason to expect such trade-offs in viruses, because the intracellular and extracellular environments strongly differ and survival and reproduction could be modular in viruses. However, strong evidence for reproduction/survival trade-offs in viruses comes from an elegant comparative study among phages that infect *Escherichia coli* [2]. The study measured replication and mortality rates at 37 °C for a large collection of distinct coliphages, and showed that faster intracellular reproduction correlated with higher extracellular mortality (lower survival). The remainder of this review covers whether this trade-off is evident in experiments where viruses evolve in stressful environments that degrade particles.

**Trade-offs and environmental stress**
Brief (5 min) heat shocks have provided a straightforward stressor that can be manipulated in the lab to cause virus mortality [34,35**,36] but other stressors have included lowered pH [37,38], UV irradiation [39], and exposure to urea [40] or salt [41] (Table 1). Whilst some experiments showed high degrees of convergence among virus populations evolved to withstand a stressor [42], others found a wide range of adaptive mutations between related phages [43]. Strikingly, experiments evolving unrelated phage to withstand heat shock have found that mutations in many different proteins can lead to increased extracellular survival. In some cases, phages adapted to heat shock by strengthening the structural capsid proteins [44,45**] whereas other phages increased survival via mutations to internal proteins such as viral lytic enzymes [35**,40]. In addition, viruses adapted to withstand a particular stressor (e.g. urea) have often not shown an advantage when exposed to a different stressor (e.g. temperature) [40,45**]. Overall, the empirical evidence indicates various ways for viruses to withstand environmental stressors and improve their extra-cellular survival, implying that a generalized mechanism for reproduction/survival trade-offs in viruses is unlikely.

Our work allowed populations of cystovirus phage Φ6 to evolve under periodic extracellular heat shocks that caused high mortality, and we demonstrated that the improved survival led to reduced growth on host bacteria at normal temperatures [35**]. To examine the underlying mechanism for the trade-off, we identified a single adaptive mutation in the viral lytic enzyme and determined how the amino acid substitution changed this protein’s structure. The mutation filled a hydrophobic pocket in the enzyme, stabilizing the protein under higher temperatures, and explaining the increased viral survival. Potentially, the survival/reproduction trade-off in the virus could have been mediated by a stability/activity trade-off in the lytic enzyme. However, we found no measurable differences in activity between the ancestor and mutant enzymes at normal temperature, suggesting that the trade-off was not mediated solely by changes in enzyme activity. Presumably, increased enzyme stability had a pleiotropic effect that changed protein–protein interactions but the exact details for this trade-off remain unclear. Also, the trade-off may be specific to phage Φ6, and not generalizable to the related Reoviridae family that infects animal hosts or other families of viruses.

The comparative trade-off shown in coliphages [2] suggests that experimental evolution of increased survival in phage should significantly decrease reproduction and vice versa. However, some studies that have explicitly

<table>
<thead>
<tr>
<th>Virus</th>
<th>Selective pressure</th>
<th>Trade-off</th>
<th>Cost to adaptation</th>
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</thead>
<tbody>
<tr>
<td>Phage Φ6 [35**]</td>
<td>Heat shock</td>
<td>Yes</td>
<td>Lowered reproduction</td>
</tr>
<tr>
<td>Phage T7 [40]</td>
<td>Urea</td>
<td>Yes</td>
<td>Lowered reproduction</td>
</tr>
<tr>
<td>Phage Qb [60]</td>
<td>Increased fecundity</td>
<td>Yes</td>
<td>Reduced thermostability</td>
</tr>
<tr>
<td>Phage Qb [47]</td>
<td>Heat shock</td>
<td>No</td>
<td>No cost</td>
</tr>
<tr>
<td>Phage ΦX174 [45**]</td>
<td>Heat shock; low pH</td>
<td>No</td>
<td>No cost</td>
</tr>
<tr>
<td>West Nile virus [87]</td>
<td>Low pH</td>
<td>Yes</td>
<td>Lowered fitness at normal pH</td>
</tr>
<tr>
<td>Foot and mouth disease virus [38]</td>
<td>Low pH</td>
<td>Yes</td>
<td>Smaller plaque size</td>
</tr>
<tr>
<td>Vesicular stomatitis virus [46]</td>
<td>Prolonged transmission</td>
<td>Yes</td>
<td>Lowered fecundity</td>
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looked for reproduction/survival trade-offs in phage have found that trade-offs on initial adaptive mutations are small [40,46] or absent [45*,47]. Without understanding the mechanism behind the trade-off, it is difficult to postulate reasons for how the trade-off may be avoided. It is possible that viruses are under fewer constraints in a lab environment, or are far from a fitness peak such that pleiotropic mutations can increase multiple fitness traits. Data for phage \( \Phi X 174 \) deviates from the line of best fit in the De Paepe and Taddei study, which may explain why this phage improved both reproduction and survival when evolved under periodic heat shock [2]. To the best of our knowledge, no published virus evolution experiments have examined how trade-offs evolve over long periods of times, such as 100 or more passages in the laboratory. Without mechanistic knowledge, it is impossible to predict whether compensatory mutations can overcome trade-offs in viruses in a similar manner to how bacteria overcome the fitness costs of antibiotic resistance.

The curse of the pharaoh

The examples of trade-offs in eukaryotic viruses in Table 1 come from evolution studies performed in tissue-cell culture. However, studies comparing virus virulence (damage to the host caused by intrahost growth) and survival in whole animal infections have found either no trade-off [17**], or a positive correlation between virulence and survival [48], the apparent opposite of the reproduction/survival trade-off seen in coliphages. The ‘curse of the pharaoh’ theory refers to the mysterious death of Lord Carnavon after entering the tomb of the Egyptian pharaoh Tutankhamen; this idea poses that pathogens which are extremely long-lived in the environment, should evolve high virulence [49-51]. The virulence/transmission trade-off states that rapid host mortality reduces the likelihood of host to host transmission. Therefore, only viruses that can rely on environmental transmission due to high extracellular survival can evolve high virulence. The predictions of this theory were confirmed for a sample of human respiratory viruses [48 but see 52]. How can the curse of the pharaoh be reconciled with the survival/reproduction trade-off seen in phages? Virulence for eukaryotic viruses as measured by human mortality is quite different from the reproductive rate of phage in bacteria or that of viruses in cell culture. Viruses that are directly transmitted from host to host without environmental exposure are subject to a virulence/transmission trade-off: fast reproduction that kills the host (high virulence) will reduce the probability of transmission. By contrast, for lytic phage, there is no transmission advantage to lower virulence as the only way to successfully infect new cells is through lysis. The reproduction/survival trade-off has likely not been observed in pathogenic viruses due to the more complex environment of a whole organism and epidemiological constraints.

Host range and survival

No virus should be infinitely durable, as every virus must ‘break’ to enter a cell and initiate reproduction. This implies a potential trade-off between viral entry and extracellular survival. Keen [6*] showed that for a sample of coliphages, there was a negative correlation between reproductive rate and host range, as measured by the ability to lyse members of an \( E. coli \) reference strain collection. The mechanism could be due to the energetic cost of producing multiple tail fibers [6*] or, more intriguingly, could represent a constraint in the entry apparatus. Viruses with a greater host range may have more flexible attachment proteins and be at greater risk for misfolding or other environmental damage to the entry apparatus. There have been multiple experimental studies expanding host range so this theory should be relatively easy to test [14,53,54]. Additional circumstantial evidence for this trade-off comes from a study showing Vesicular Stomatitis Virus evolved on multiple hosts grew relatively poorly in an extreme thermal environment compared to viruses that evolutionarily specialized on a single host [55].

Genome organization

A general mechanism that has been proposed to cause reproduction/survival trade-offs in viruses is that of capsid strength and packaging density of the genome [2]. Viruses have a narrow limit in which they can package their genetic material: too much genetic material and the genome will not fit into the capsid, but too little and there may not be enough pressure to successfully eject the genome to initiate infection upon cell binding [56]. Viruses with stronger capsids or less genetic material will be better able to survive environmental stress but may have slower reproduction due to increased expenditure on the capsid or slower ejection of the genetic material [56]. Recent work with Foot and Mouth Disease Virus (FMDV) has found that passing FMDV at high MOI in cell culture led to the evolution of complementary deletion mutants in a process akin to segmentation of the genome [57,58,59**]. These viruses had higher capsid stability and were significantly better at reproducing than wildtype virus at high MOI [59**]. These experiments show the importance of capsid stability as a mechanism for increasing virus survival but also that with radical genome reorganization, the trade-off can be overcome as FMDV improved in both reproduction and survival [59**].

Concluding remarks

Experimental evolution has shown the first steps viruses take to alter their position along trade-offs but many questions remain unanswered: What are the mechanisms of trade-offs? How general are the mechanisms across viruses? How do trade-offs evolve over longer periods? Whilst mechanistic details are known for intracellular trade-offs, the mechanisms that generally
underlie reproduction/survival trade-offs in viruses remain unclear. Although capsid strength is one possibility, the diversity of genes fixing adaptive mutations across experiments suggests multiple mechanisms may be at work. Without mechanistic details, it is difficult to extrapolate results from studies in a single virus and generalize them to other viruses. The lack of longer-term studies of trade-offs means that it is unknown how viruses respond to continued selective pressure and whether compensatory mutations can affect the shape of trade-offs. Structural, comparative and evolutionary approaches will all be needed to determine the constraints and mechanisms of life history evolution, suggesting that interdisciplinary approaches are warranted. Finally, the curse of the pharaoh shows that in more complex natural systems other trade-offs affecting life history can be of great importance. Despite comparative studies, the evolution of life history trade-offs are rarely studied in natural environments so their importance to viral evolution in nature remains to be seen, and should be the focus of future work.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

This paper evolves two related phage to have altered lysis times. The phages did not fully match the theoretical predictions perhaps due to genetic constraint.


The first paper to use structural data in an attempt to elucidate the mechanism of a life-history trade-off in phase 4F.


This paper tests for a reproductive trade-off following evolution to both heat shock and acidic conditions. They did not find a reproductive cost to increased survival. Uses molecular dynamics in an attempt to show the mechanism causing adaptation.


This paper explores how point mutations can lead to segmentation of the genome in Foot and Mouth Disease Virus. This segmentation led to an increase in both viral fitness and stability.