The evolution of life history trade-offs in viruses
Daniel H Goldhill and Paul E Turner

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.

Addresses
Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520, USA

Corresponding author: Turner, Paul E (paul.turner@yale.edu)

Introduction: why study life history trade-offs in viruses?

Life history trade-offs reflect fundamental constraints in the ability for evolving populations to simultaneously maximize traits such as the inability to evolve both increased reproduction and extended survival [1]. Interestingly, all biological populations seem vulnerable to life history trade-offs, including viruses [2–4]. However, the mechanisms underlying life history trade-offs in biological populations often remain elusive [5], and it is unknown how trade-offs can evolve in either the short or the long term.

Viruses are especially powerful and useful systems for elucidating trade-offs [4,6•]. Most viruses have relatively small and compact genomes, which should increase the likelihood of trade-offs due to expected pleiotropy (single genes coding for multiple proteins) and multifunctional proteins that play different roles during the viral lifecycle [7]. Viruses tend to have short generation times, large population sizes and ease of culture, allowing efficient experimental evolution studies that examine life history trade-offs [7]. Whole-genome sequencing easily permits identification of mutations changing life history traits, in genome comparisons between ancestral and evolved viruses. Last, viral protein changes can reveal fundamental constraints and biophysical mechanisms of life history trade-offs.

In addition, because viruses are exceedingly common on earth and affect other organisms in myriad ways [8], studying their life history trade-offs illuminates processes of global significance. For example, virus life history trade-offs could impact virus effects on: nutrient cycling in oceans and other ecosystems [9]; viral diversity [4]; epidemiological dynamics and emergence of virus pathogens in hosts such as humans [10,11]; and successful engineering of viruses for vaccines, and in industrial applications [12].

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.
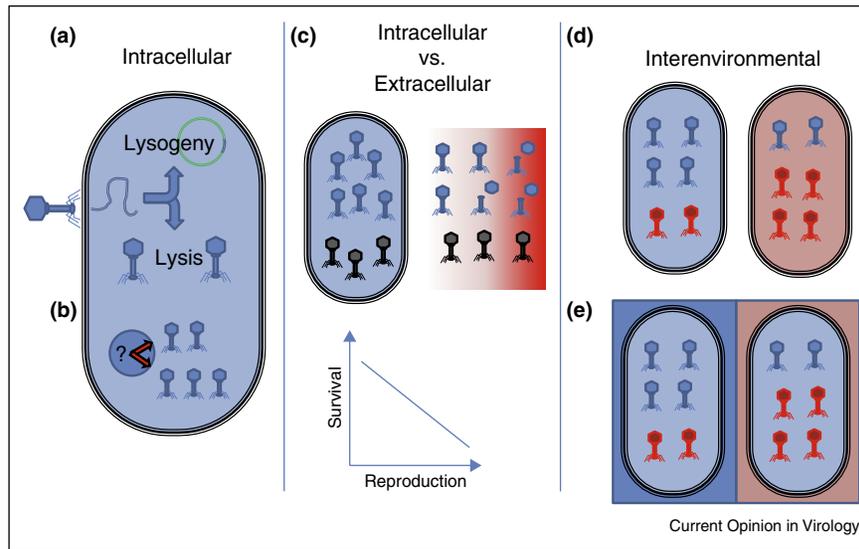
Types of life history trade-offs in viruses

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. Interenvironmental 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 forego 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 1

Examples of experimental evolution studies that tested for virus life-history trade-offs.

Virus	Selective pressure	Trade-off	Cost to adaptation
Phage Φ6 [35••]	Heat shock	Yes	Lowered reproduction
Phage T7 [40]	Urea	Yes	Lowered reproduction
Phage Qβ [60]	Increased fecundity	Yes	Reduced thermostability
Phage Qβ [47]	Heat shock	No	No cost
Phage ΦX174 [45•]	Heat shock; low pH	No	No cost
West Nile virus [37]	Low pH	Yes	Lowered fitness at normal pH
Foot and mouth disease virus [38]	Low pH	Yes	Smaller plaque size
Vesicular stomatitis virus [46]	Prolonged transmission	Yes	Lowered fecundity

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 ΦX174 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 passaging 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.

Acknowledgements

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Stearns SC: *The Evolution of Life Histories*. Oxford: Oxford University Press; 1992, .

2. De Paepe M, Taddei F: **Viruses' life history: towards a mechanistic basis of a trade-off between survival and reproduction among phages**. *PLoS Biol* 2006, **4**:e193.

3. Abedon ST: *Chapter 1 — Phage Evolution and Ecology*. Elsevier Inc.; 2009.

4. Gudelj I, Weitz JS, Ferenci T, Claire Horner-Devine M, Marx CJ, Meyer JR, Forde SE: **An integrative approach to understanding microbial diversity: from intracellular mechanisms to community structure**. *Ecol Lett* 2010, **13**:1073-1084.

5. Flatt T, Heyland A (Eds): *Mechanisms of Life History Evolution*. Oxford University Press; 2011.

6. Keen EC: **Tradeoffs in bacteriophage life histories**.
• *Bacteriophage* 2014, **4**:e28365.
Compares a range of coliphage to demonstrate a trade-off between host range and extra-cellular survival.

7. Elena SF, Lenski RE: **Microbial genetics: evolution experiments with microorganisms: the dynamics and genetic bases of adaptation**. *Nat Rev Genet* 2003, **4**:457-469.

8. Wasik BR, Turner PE: **On the biological success of viruses**. *Annu Rev Microbiol* 2013, **67**:519-541.

9. Suttle CA: **Viruses in the sea**. *Nature* 2005, **437**:356-361.

10. Rohani P, Breban R, Stallknecht DE, Drake JM: **Environmental transmission of low pathogenicity avian influenza viruses and its implications for pathogen invasion**. *Proc Natl Acad Sci USA* 2009, **106**:10365-10369.

11. Handel A, Brown J, Stallknecht D, Rohani P: **A multi-scale analysis of influenza A virus fitness trade-offs due to temperature-dependent virus persistence**. *PLoS Comput Biol* 2013, **9**:e1002989.

12. Luongo C, Winter CC, Collins PL, Buchholz UJ: **Increased genetic and phenotypic stability of a promising live-attenuated respiratory syncytial virus vaccine candidate by reverse genetics**. *J Virol* 2012, **86**:10792-10804.

13. Bull J, Badgett M, Wichman H: **Big-benefit mutations in a bacteriophage inhibited with heat**. *Mol Biol Evol* 2000, **17**:942-950.

14. Duffy S, Turner PE, Burch CL: **Pleiotropic costs of niche expansion in the RNA bacteriophage Φ6**. *Genetics* 2006, **172**:751-757.

15. Knies JL, Kingsolver JG, Burch CL: **Hotter is better and broader: thermal sensitivity of fitness in a population of bacteriophages**. *Am Nat* 2009, **173**:419-430.

16. Hall JP, Harrison E, Brockhurst MA: **Viral host-adaptation: insights from evolution experiments with phages**. *Curr Opin Virol* 2013, **3**:572-577.

17. Handel A, Lebarbenchon C, Stallknecht D, Rohani P: **Trade-offs**
•• **between and within scales: environmental persistence and within-host fitness of avian influenza viruses**. *Proc Roy Soc B* 2014, **281**:20133051.
Demonstrates that there is no link between extra-cellular survival and within-host viral load for influenza A viruses. They did find a trade-off between persistence of viruses in different thermal environments.

18. Coffey LL, Beeharry Y, Borderia AV, Blanc H, Vignuzzi M: **Arbovirus high fidelity variant loses fitness in mosquitoes and mice**. *Proc Natl Acad Sci* 2011, **108**:16038-16043.

19. Wang I-N: **Lysis timing and bacteriophage fitness**. *Genetics* 2006, **172**:17-26.

20. Ptashne MA: *Genetic Switch: Phage Lambda Revisited*. Third Edition. Cold Spring Harbor Laboratory Press; 2004.

21. Weitz JS, Mileyko Y, Joh RI, Voit EO: **Collective decision making in bacterial viruses**. *Biophys J* 2008, **95**:2673-2680.

22. Joh RI, Weitz JS: **To lyse or not to lyse: transient-mediated stochastic fate determination in cells infected by bacteriophages**. *PLoS Comput Biol* 2011, **7**:e1002006.

23. Zeng L, Skinner SO, Zong C, Sippy J, Feiss M, Golding I: **Decision making at a subcellular level determines the outcome of bacteriophage infection**. *Cell* 2010, **141**:682-691.

24. Refardt D, Rainey PB: **Tuning a genetic switch: experimental evolution and natural variation of prophage induction**. *Evolution* 2009, **64**:1086-1097.

25. Bullwinkle TJ, Koudelka GB: **The lysis-lysogeny decision of bacteriophage 933W: a 933W repressor-mediated long-distance loop has no role in regulating 933W PRM activity**. *J Bacteriol* 2011, **193**:3313-3323.

26. Stewart FM, Levin BR: **The population biology of bacterial viruses: why be temperate**. *Theor Popul Biol* 1984, **26**:93-117.

27. Wang I-N, Dykhuizen DE, Slobodkin LB: **The evolution of phage lysis timing**. *Evol Ecol* 1996, **10**:545-558.

28. Wang I-N, Smith DL, Young R: **Holins: the protein clocks of bacteriophage infections**. *Annu Rev Microbiol* 2000, **54**:799-825.

29. Catalão MJ, Gil F, Moniz-Pereira J, São-José C, Pimentel M: **Diversity in bacterial lysis systems: bacteriophages show the way**. *FEMS Microbiol Rev* 2012, **37**:554-571.

30. Young R: **Phage lysis: three steps, three choices, one outcome**. *J Microbiol* 2014, **52**:243-258.

31. Abedon ST, Hyman P, Thomas C: **Experimental examination of bacteriophage latent-period evolution as a response to bacterial availability**. *Appl Environ Microb* 2003, **69**:7499-7506.

32. Heineman R, Bull J: **Testing optimality with experimental evolution: lysis time in a bacteriophage**. *Evolution* 2007, **61**:1695-1709.

33. Chantranupong L, Heineman RH: **A common, non-optimal
• phenotypic endpoint in experimental adaptations of
bacteriophage lysis time**. *BMC Evol Biol* 2012, **12**:37.
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.

34. McBride R, Ogbunugafor C, Turner P: **Robustness promotes
evolvability of thermotolerance in an RNA virus**. *BMC Evol Biol*
2008, **8**:231.

35. Dessau M, Goldhill D, McBride RC, Turner PE, Modis Y: **Selective
•• pressure causes an RNA virus to trade reproductive fitness for
increased structural and thermal stability of a viral enzyme**.
PLoS Genet 2012, **8**:e1003102.
The first paper to use structural data in an attempt to elucidate the
mechanism of a life-history trade-off in phage Φ6.

36. Goldhill D, Lee A, Williams ES, Turner PE: **Evolvability and
robustness in populations of RNA virus Φ6**. *Front Microbiol*
2014, **5**:35.

37. Martin-Acebes MA, Saiz JC: **A West Nile virus mutant with
increased resistance to acid-induced inactivation**. *J Gen Virol*
2011, **92**:831-840.

38. Vazquez-Calvo A, Caridi F, Sobrino F, Martin-Acebes MA, Sandri-
Goldin RM: **An increase in acid resistance of foot-and-mouth
disease virus capsid is mediated by a tyrosine replacement of
the VP2 histidine previously associated with VP0 cleavage**. *J
Virol* 2014, **88**:3039-3042.

39. Ogbunugafor CB, McBride RC, Turner PE: **Predicting virus
evolution: the relationship between genetic robustness and
evolvability of thermotolerance**. *Cold Spring Harb Symp Quant
Biol* 2009, **74**:109-118.

40. Heineman RH, Brown SP: **Experimental evolution of a
bacteriophage virus reveals the trajectory of adaptation
across a fecundity/longevity trade-off**. *Plos One* 2012,
7:e46322.

41. Costafreda MI, Perez-Rodriguez FJ, D'Andrea L, Guix S, Ribes E,
Bosch A, Pinto RM: **Hepatitis A virus adaptation to cellular shut-
off is driven by dynamic adjustments of codon usage and
results in the selection of populations with altered capsids**. *J
Virol* 2014, **88**:5029-5241.

42. Rokyta DR, Abdo Z, Wichman HA: **The genetics of adaptation for
eight microvirid bacteriophages**. *J Mol Evol* 2009, **69**:229-239.

43. Bollback JP, Huelsenbeck JP: **Parallel genetic evolution within
and between bacteriophage species of varying degrees of
divergence**. *Genetics* 2008, **181**:225-234.

44. Lee KH, Miller CR, Nagel AC, Wichman HA, Joyce P, Ytreberg FM:
**First-step mutations for adaptation at elevated temperature
increase capsid stability in a virus**. *Plos One* 2011, **6**:e25640.

45. McGee L, Aitchison E, Caudle SB, Morrison A, Zheng L, Yang W,
• Rokyta DR: **Payoffs, not tradeoffs, in the adaptation to
ostensibly conflicting selective pressures in a ssDNA virus**.
2014, in press.
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.

46. Wasik B, Bhushan A, Ogbunugafor CB, Turner PE: **Delayed
transmission selects for increased survival of vesicular
stomatitis virus**. 2014, in press.

47. Domingo-Calap P, Pereira-Gómez M, Sanjuán R: **Selection for
thermostability can lead to the emergence of mutational
robustness in an RNA virus**. *J Evol Biol* 2010, **23**:2453-2460.

48. Walther BA, Ewald PW: **Pathogen survival in the external
environment and the evolution of virulence**. *Biol Rev* 2004,
79:849-869.

49. Bonhoeffer S, Lenski RE, Ebert D: **The curse of the pharaoh: the
evolution of virulence in pathogens with long living
propagules**. *Proc Roy Soc B* 1996, **263**:715-721.

50. Gandon S: **The curse of the pharoah hypothesis**. *Proc Roy Soc
B* 1998, **265**:1545-1552.

51. Roche B, Drake JM, Rohani P: **The curse of the pharaoh
revisited: evolutionary bi-stability in environmentally
transmitted pathogens**. *Ecol Lett* 2011, **14**:569-575.

52. Kastenmayer RJ, Maruri-Avidal L, Americo JL, Earl PL,
Weisberg AS, Moss B: **Elimination of A-type inclusion formation
enhances cowpox virus replication in mice: Implications for
orthopoxvirus evolution**. *Virology* 2014, **452–453**:59-66.

53. Meyer JR, Dobias DT, Weitz JS, Barrick JE, Quick RT, Lenski RE:
**Repeatability and contingency in the evolution of a key
innovation in phage lambda**. *Science* 2012, **335**:428-432.

54. Bono LM, Gensel CL, Pfennig DW, Burch CL: **Competition and
the origins of novelty: experimental evolution of niche-width
expansion in a virus**. *Biol Lett* 2012 http://dx.doi.org/10.1098/
rsbl.2012.0616.

55. Alto BW, Turner PE: **Consequences of host adaptation for
performance of vesicular stomatitis virus in novel thermal
environments**. *Evol Ecol* 2009, **24**:299-315.

56. Nurmemmedov E, Castelnovo M, Catalano CE, Evilevitch A:
**Biophysics of viral infectivity: matching genome length with
capsid size**. *Quart Rev Biophys* 2008, **40**:327-356.

57. Garcia-Arriaza J, Manrubia SC, Toja M, Domingo E, Escarmis C:
**Evolutionary transition toward defective RNAs that are
infectious by complementation**. *J Virol* 2004, **78**:11678-11685.

58. Ojosnegros S, García-Arriaza J, Escarmís C, Manrubia SC,
Perales C, Arias A, Mateu MG, Domingo E: **Viral genome
segmentation can result from a trade-off between genetic
content and particle stability**. *PLoS Genet* 2011, **7**:e1001344.

59. Moreno E, Ojosnegros S, García-Arriaza J, Escarmís C,
•• Domingo E, Perales C: **Exploration of sequence space as the
basis of viral RNA genome segmentation**. *Proc Natl Acad Sci
USA* 2014, **111**:6678-6683.
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.

60. Garcia-Villada L, Drake JW: **Experimental selection reveals a
trade-off between fecundity and lifespan in the coliphage Qβ**.
Open Biol 2013, **3** 130043–130043.