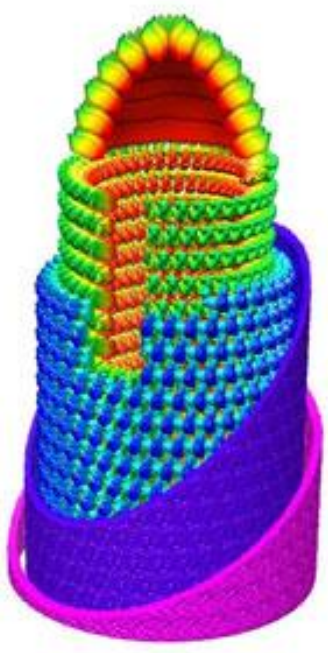
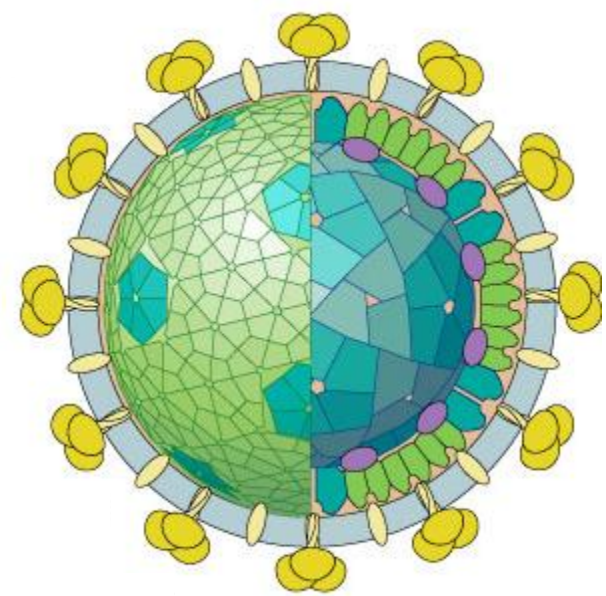
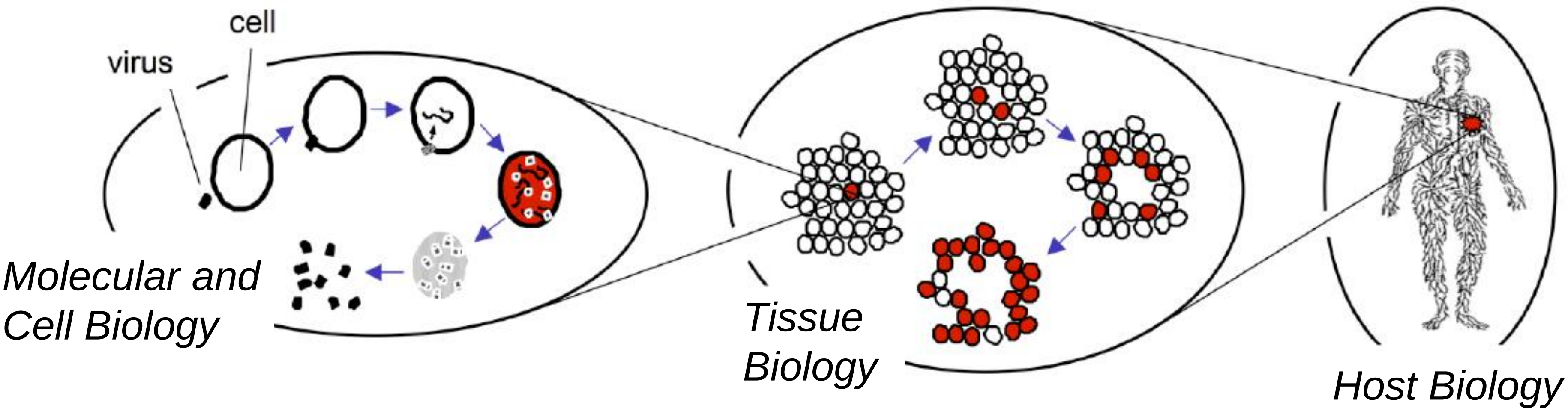
RNA Virus Adaptation (Or Not) To Environmental Change



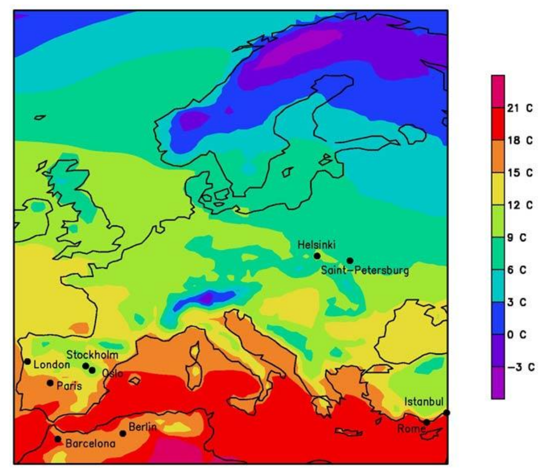


Paul E. Turner
Department of Ecology & Evolutionary Biology, Yale University
Microbiology Graduate Program, Yale School of Medicine

Viruses Often Encounter Environmental Change



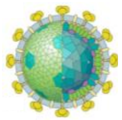
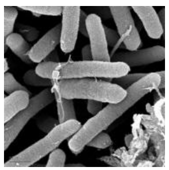
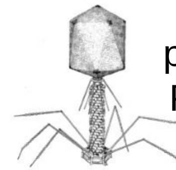
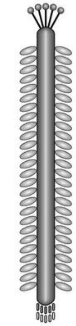
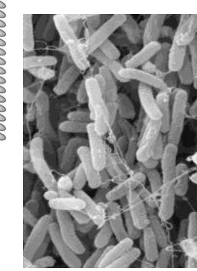
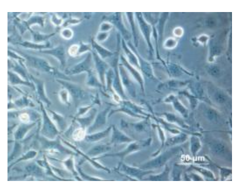
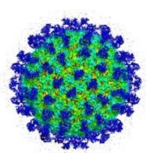
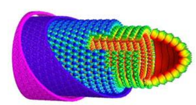
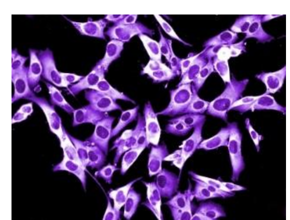
Vector Biology



Ecosystems

Wasik and Turner 2013 *Ann Rev Microbiol*
Goldhill and Turner 2014 *Curr Opin Virol*

Current Study Systems

	dsRNA	ssRNA(+)	ssRNA(-)	dsDNA	ssDNA
Phages	 phage ϕ6  			 phage PP01 	 phage M13 
Viruses of eukaryotes		 Sindbis virus   Dengue virus 	 Vesicular stomatitis virus 		

Outline

- Phage experiments
- Experimental evolution of segmented RNA phage $\phi6$
  - Prisoner's dilemma
  - Evolution of survival versus reproduction
- Phage therapy

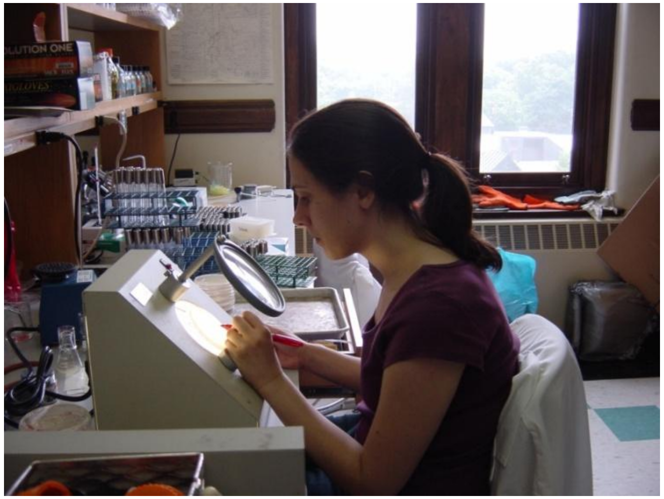
Phage Experiments

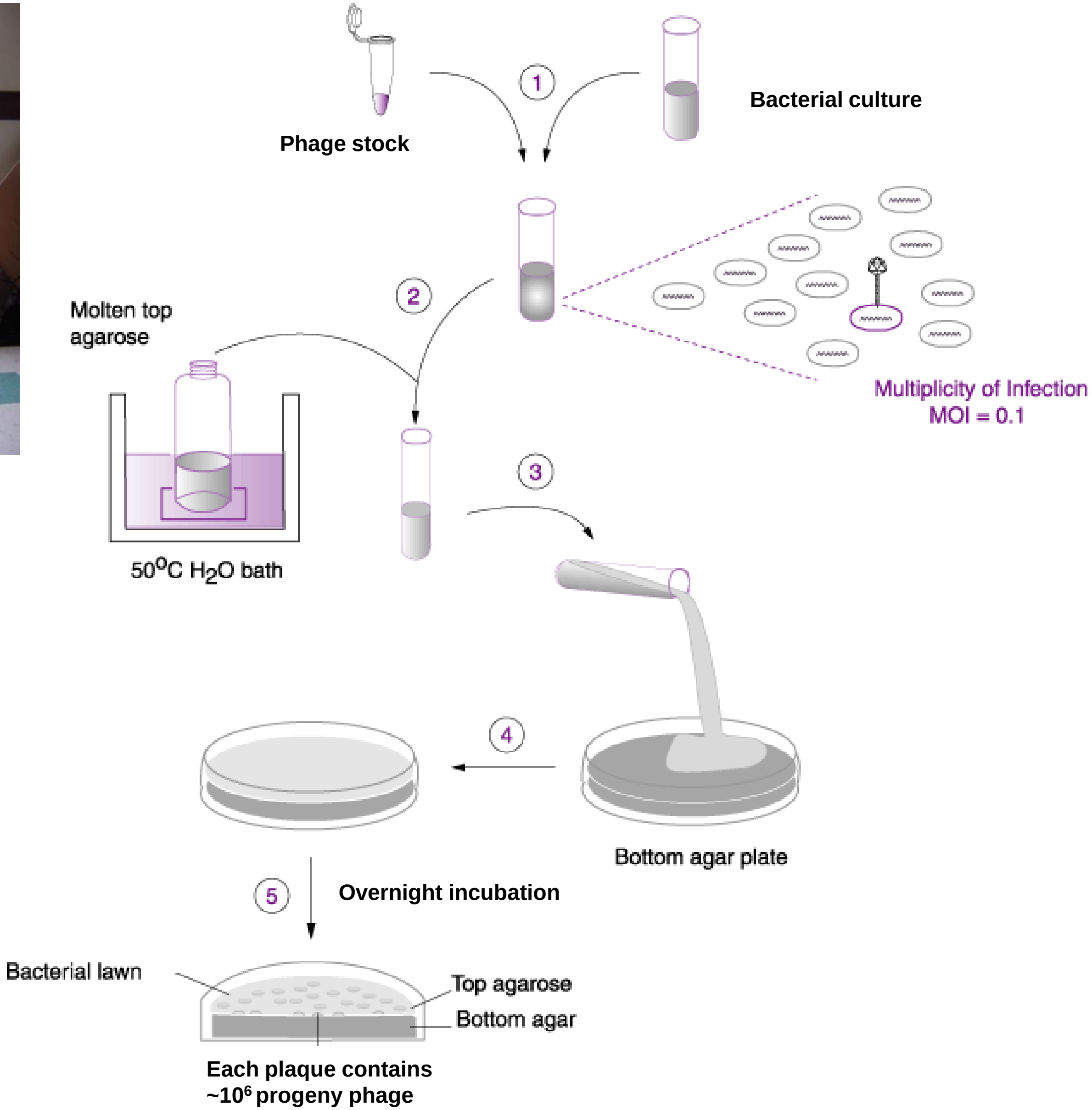
Power of experimental evolution using RNA phages

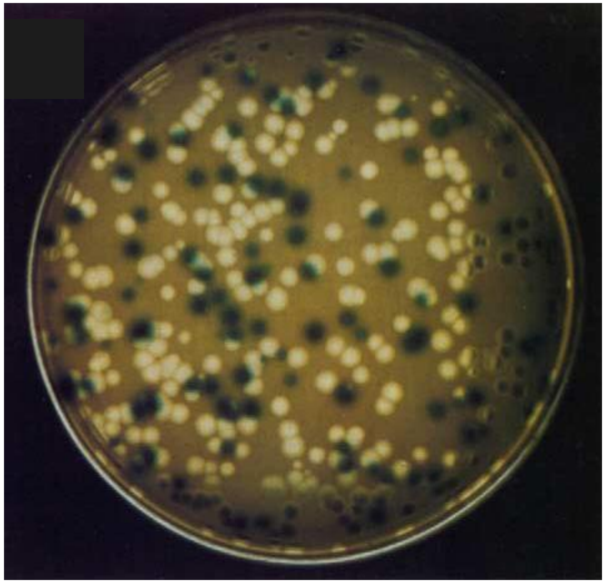
- High mutation rates
- Large population sizes
- Short generation times
- Small genome sizes
- *Indefinite freezer storage (fossil record)*

Rapid evolution

Traditional visualization of phage growth (fitness)

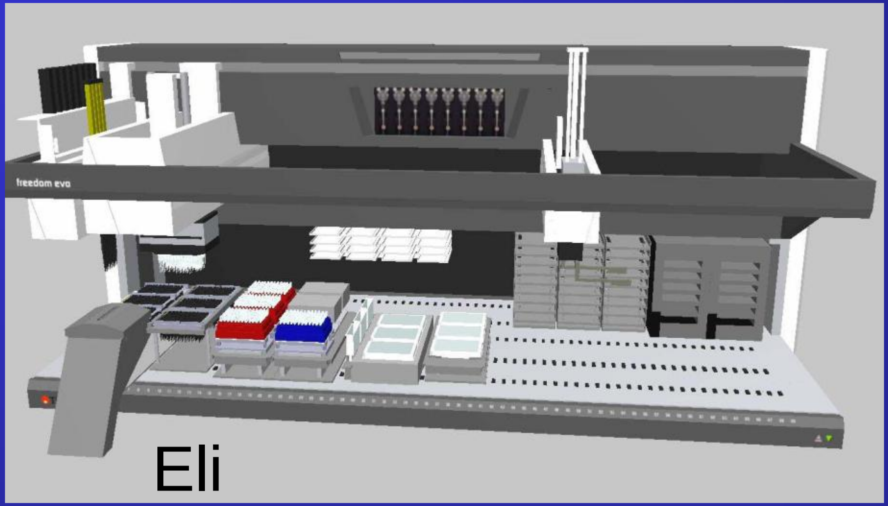






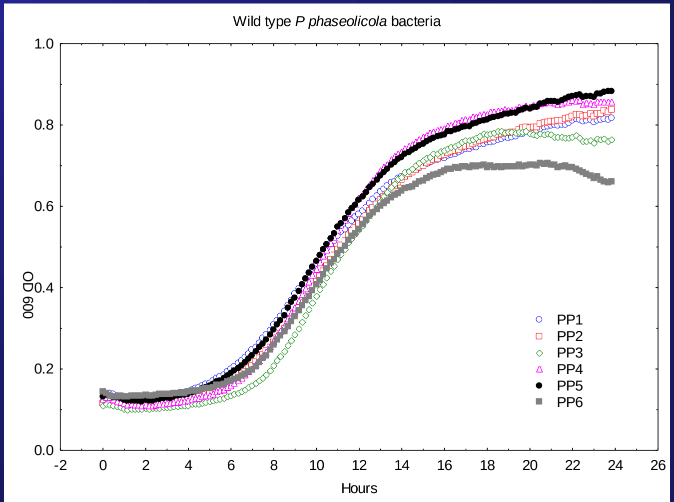
phage plaques

Novel methods for studying phage fitness

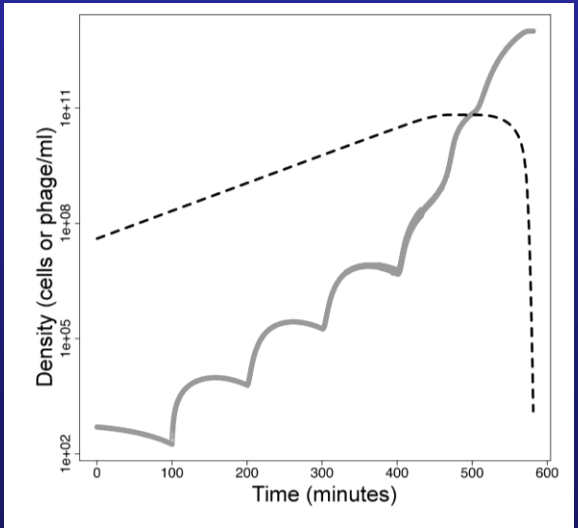


Liquid-handling robot

Microplate reader



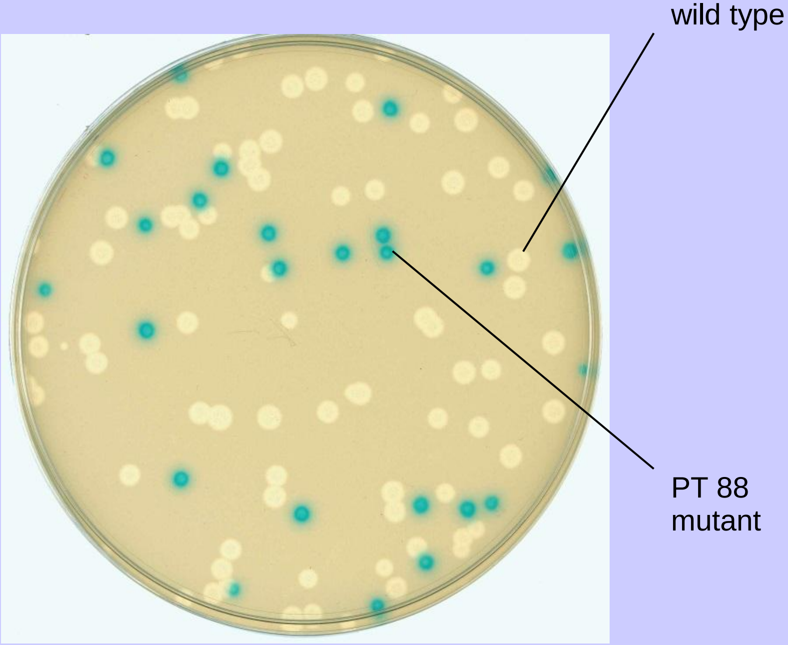
Bacterial growth curves

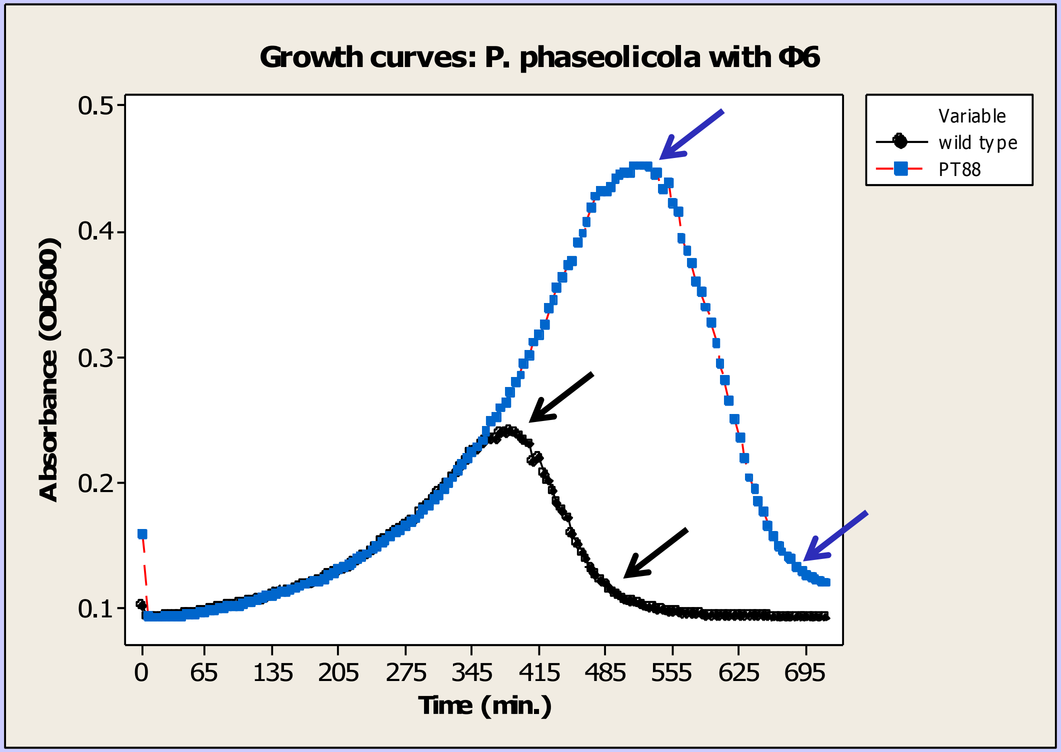


Inferred phage growth

Novel methods for measuring phage fitness

Phage too small to count directly.
Measure phage fitness by tracking host?



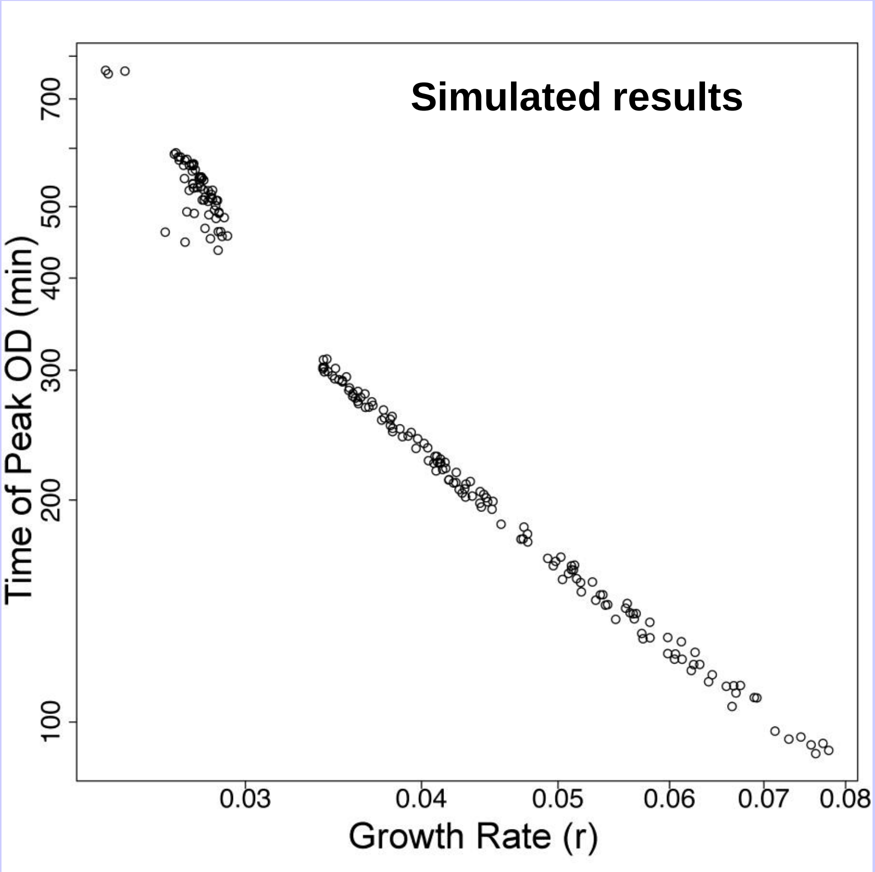


Traditional method:
Grow two strains on one plate and count plaques.

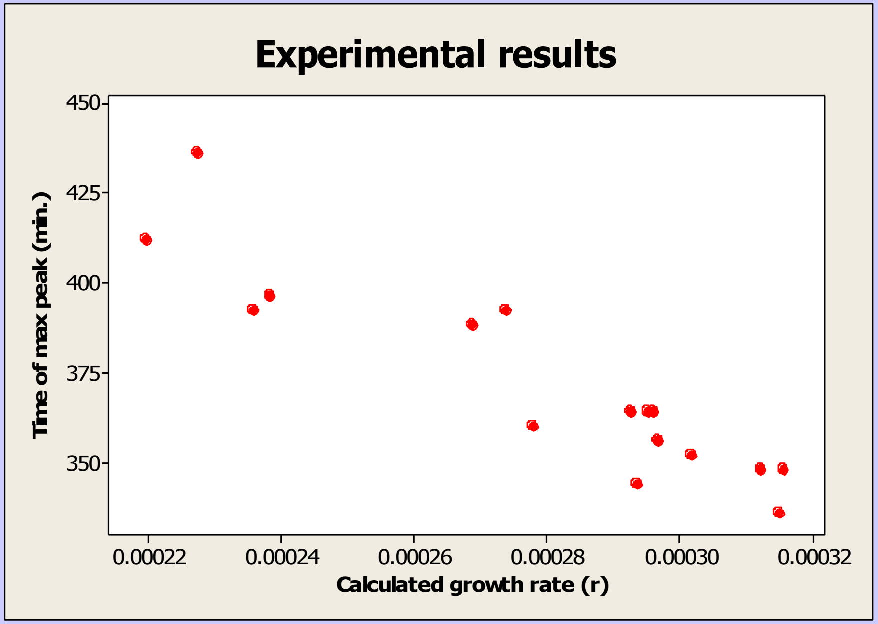
Problems: Time consuming,
Small sample size

New method:
Measure growth curves of infected hosts in liquid.
Strong host growth (time of max OD; longer extinction time) means lower phage fitness

High throughput measures of phage fitness



Simulations show that peak time correlates with growth rate

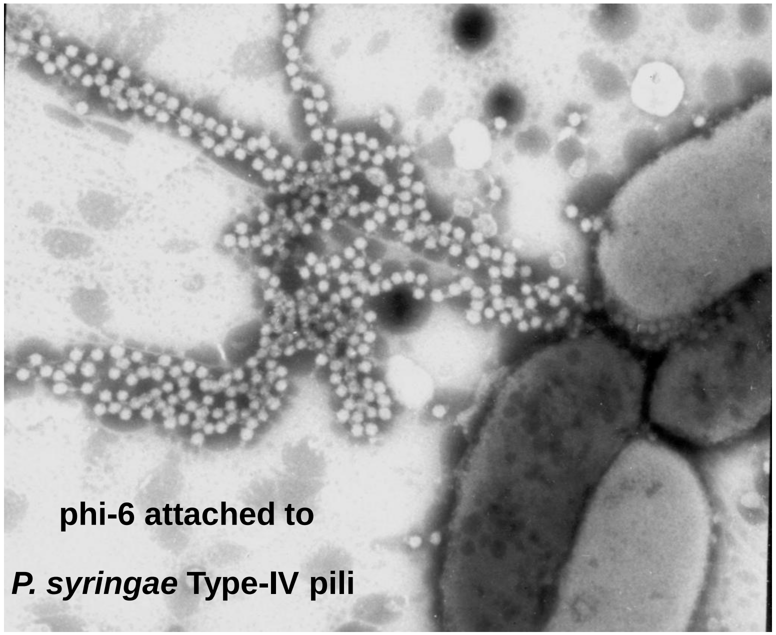


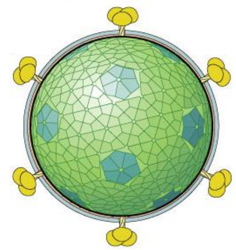
Empirical results show similar correlation

Turner et al. 2012 *J Microbiol Meth*

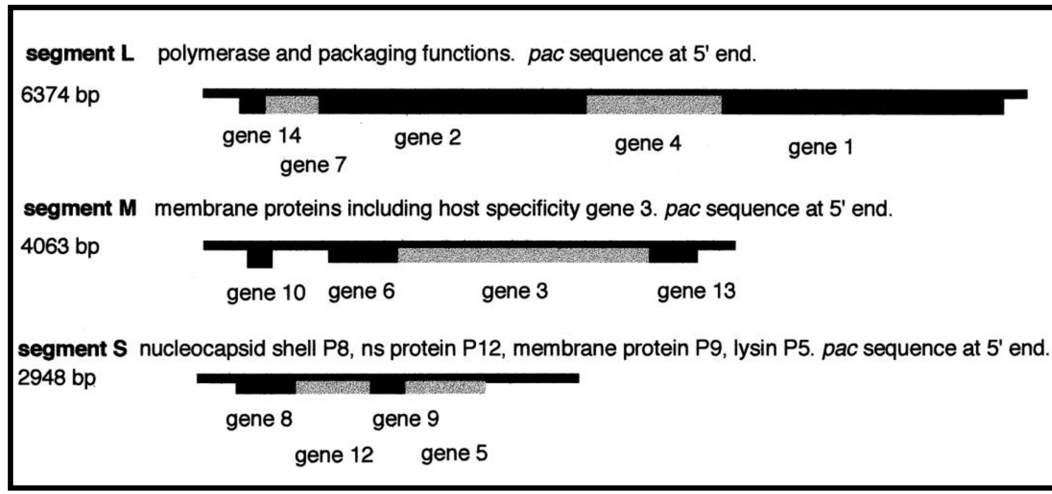
Experimental Evolution of Phage $\phi 6$

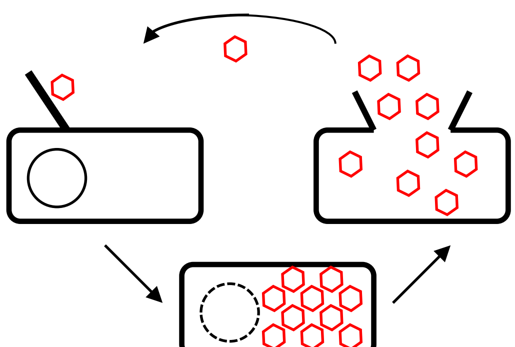
Model: segmented RNA phage phi-6





- 13kb dsRNA Cystovirus
- lipid envelope
- lytic infection



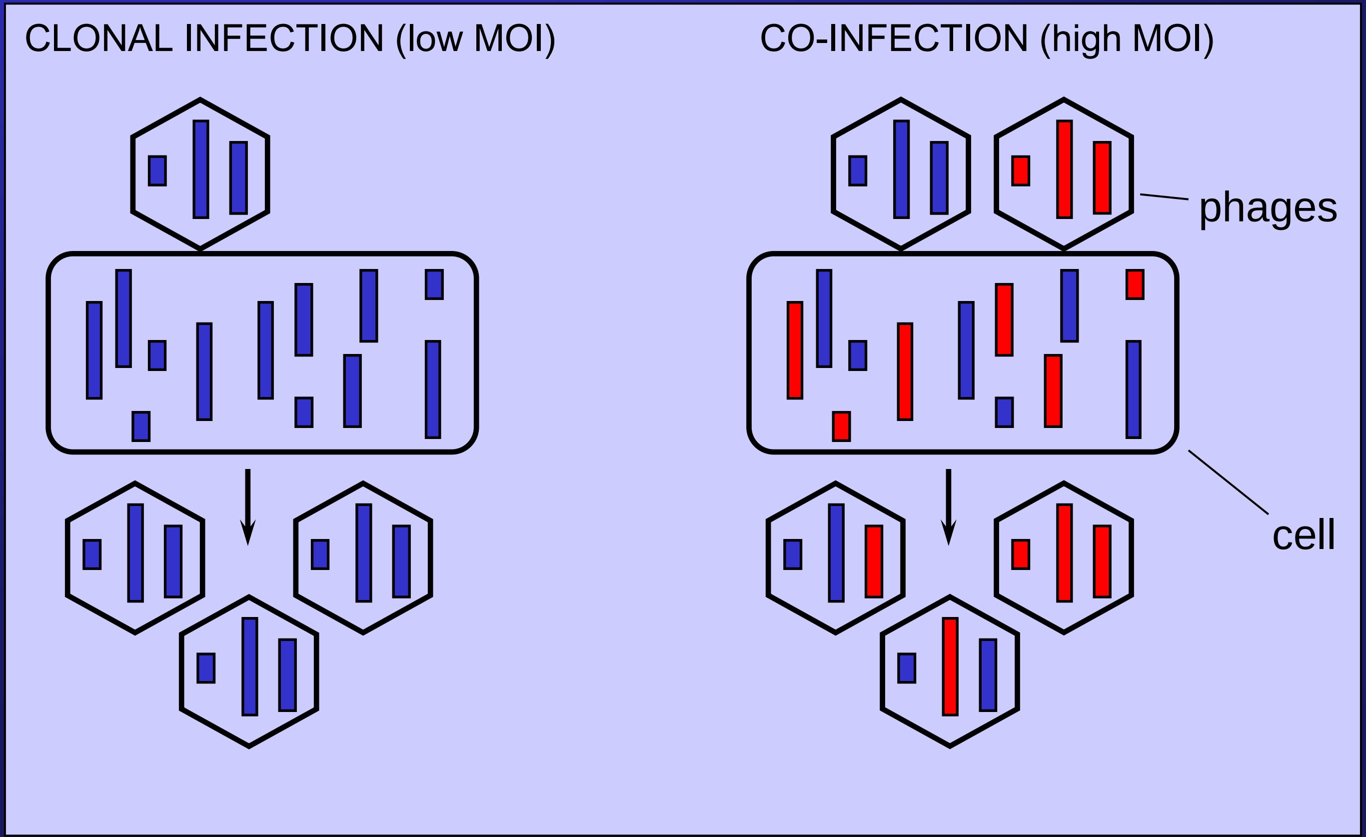


Turner & Chao 1999 *Nature*
Turner et al. 1999 *J Virology*
Montville et al. 2005 *PLoS Biology*
McBride et al. 2008 *BMC Evol Biol*

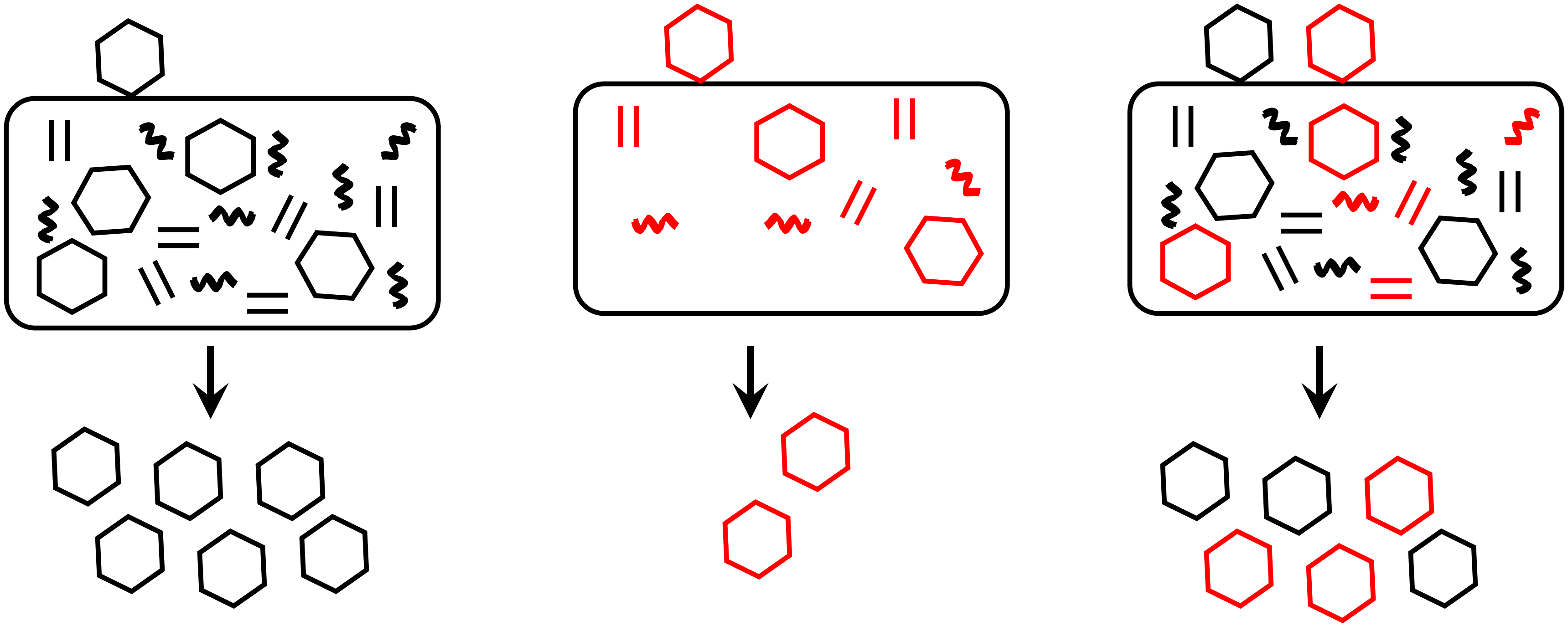
Does co-infection accelerate adaptation in an RNA virus?

Phage populations evolved at low vs. high MOI on *P. syringae* for 250 generations

Co-infecting viruses became LESS fit than ancestor, but only at low MOI
(Turner and Chao 1998 *Genetics*)



Evolution of ‘cheating’ is possible in viruses



Hypothetical wildtype

Hypothetical cheater

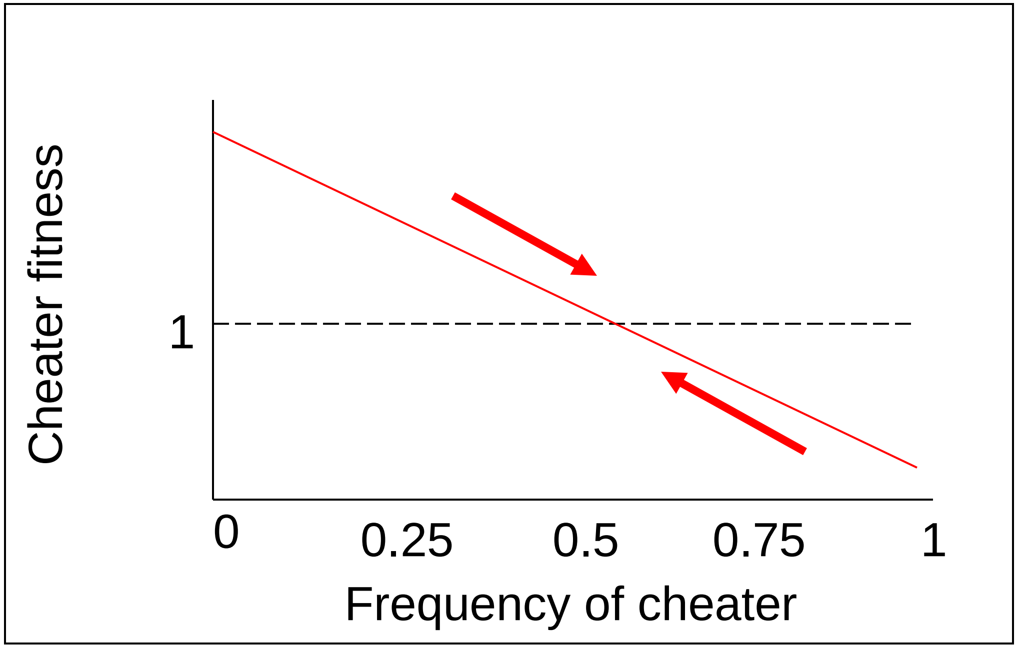
Cheater advantaged following co-infection

Payoff Matrix from evolutionary game theory

	Cooperate	Cheat
Cooperate	1	$1 - s_1$
Cheat	$1 + s_2$	$1 - c$

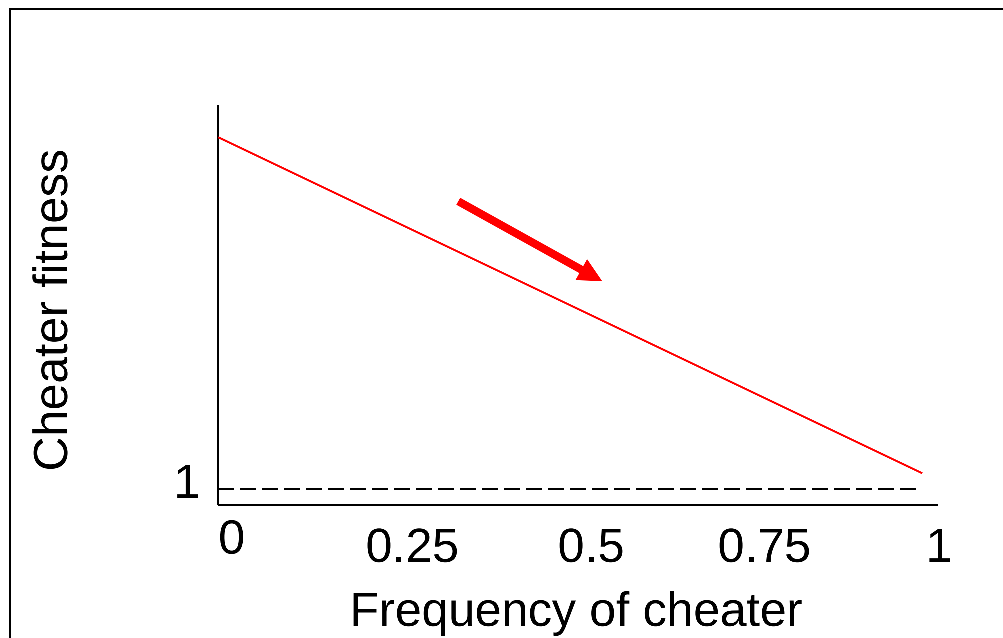
$(1\text{-}c) < (1\text{-}s_1)$ Mixed equilibrium (mixed ESS)

	Cooperate	Defect
Cooperate	1	$1 - s_1$
Defect	$1 + s_2$	$1 - c$



$(1-c) > (1-s_1)$ Prisoner's dilemma (cheating as pure ESS)

	Cooperate	Defect
Cooperate	1	$1 - s_1$
Defect	$1 + s_2$	$1 - c$



	Cooperate	Defect
Cooperate	1	$1 - s_1$
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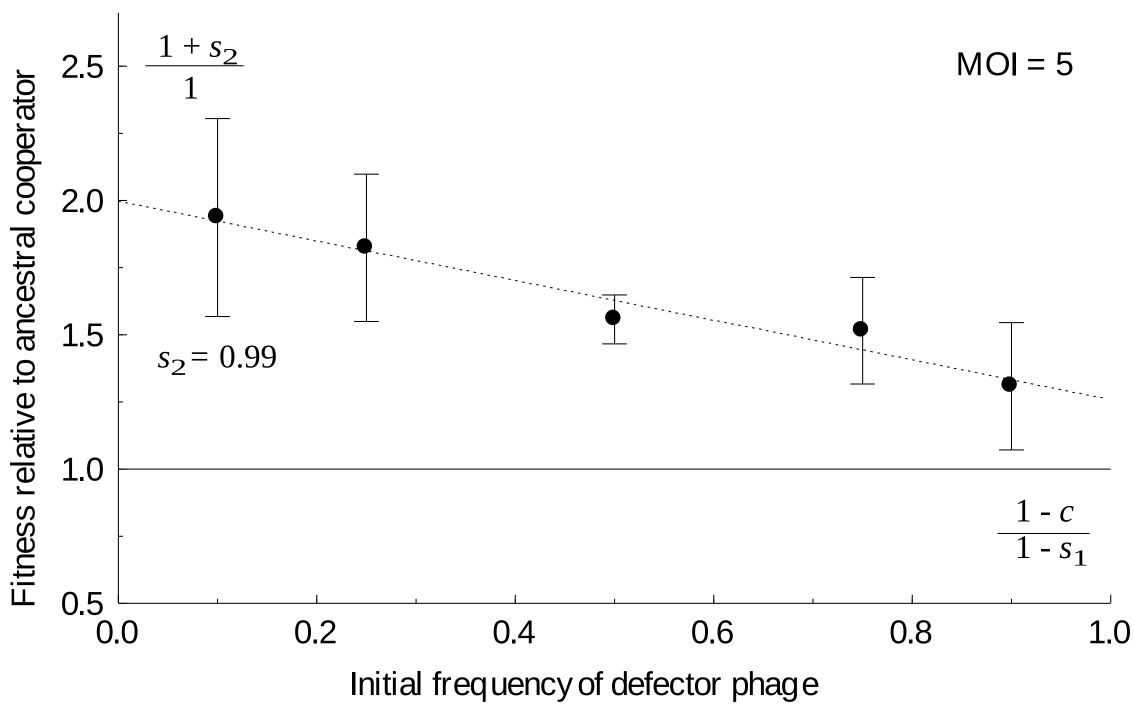


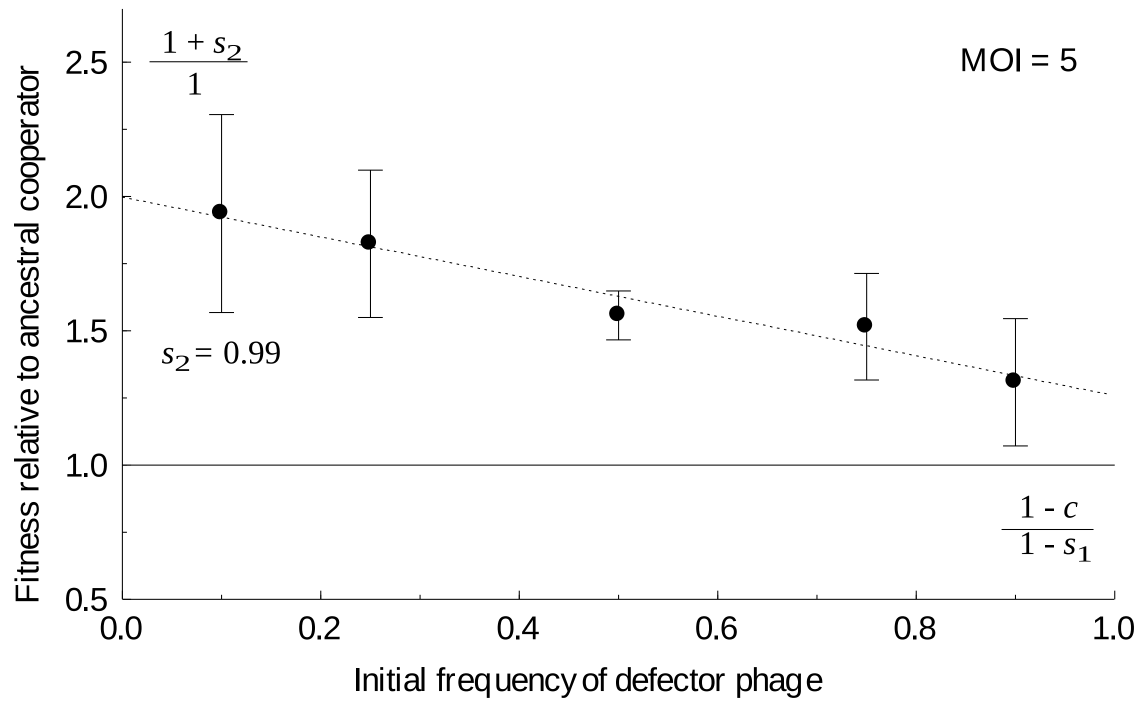
Turner and Chao 1999 *Nature*

	Cooperate	Defect
Cooperate	1	1 - s_1
Defect	1 + s_2	1 - c

	Ancestor	Evolved
Ancestor	1	0.65
Evolved	1.99	0.83

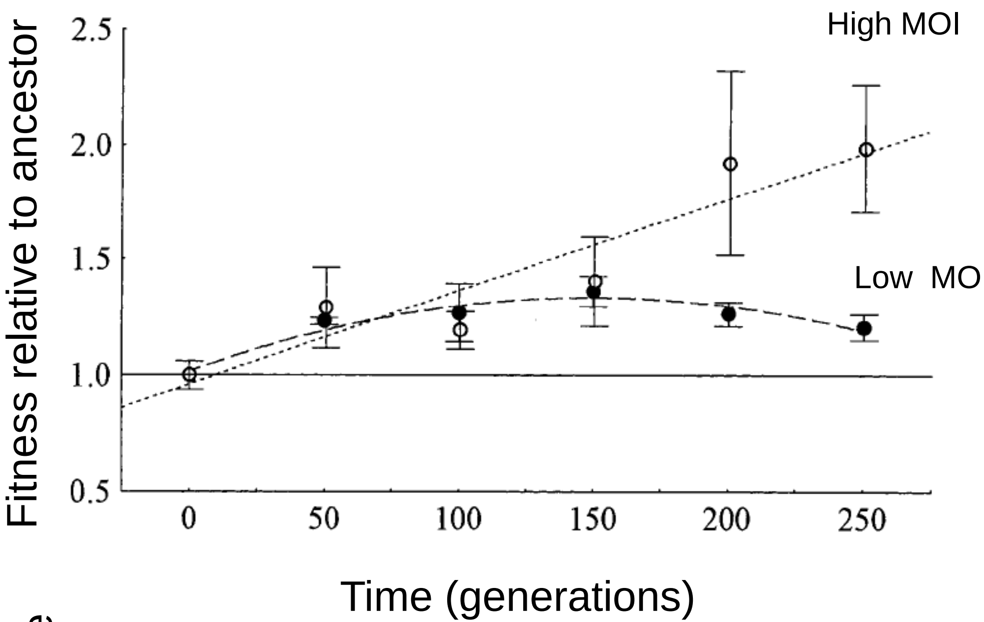
Prisoner's dilemma:

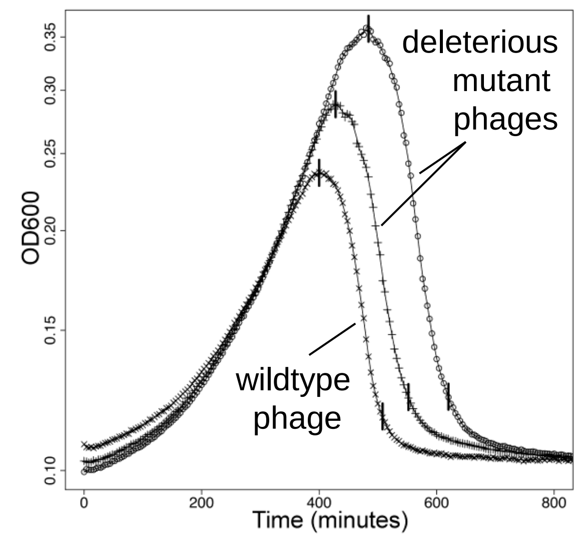
$(1\text{-}c) > (1\text{-}s_1)$



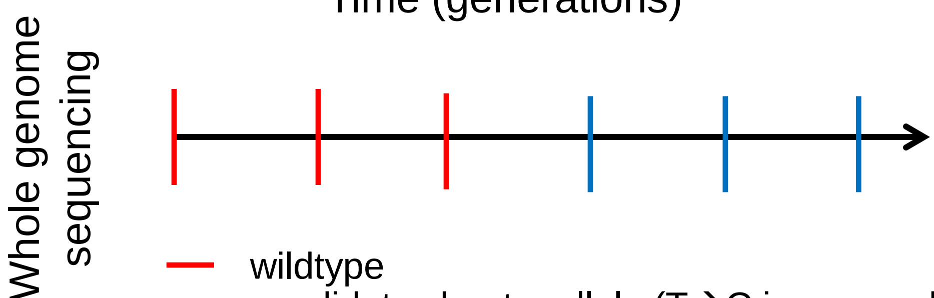
Turner and Chao 1999 *Nature*

Using host growth curves to study evolved cheater phage

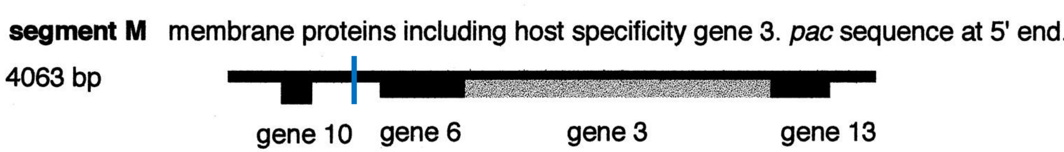




Does mutated M segment alone, cause fitness cost at low MOI?

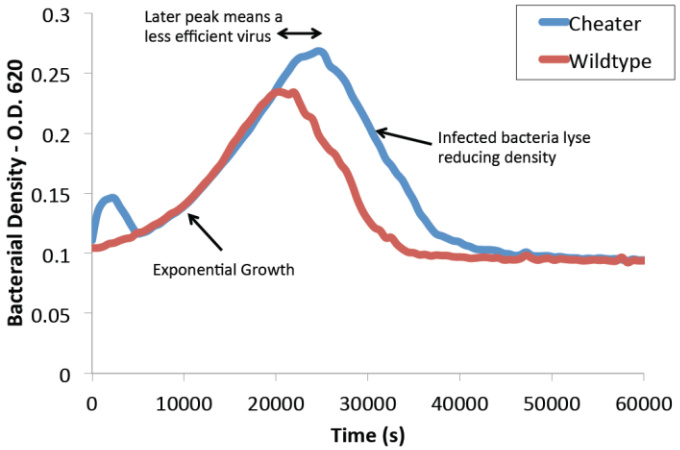


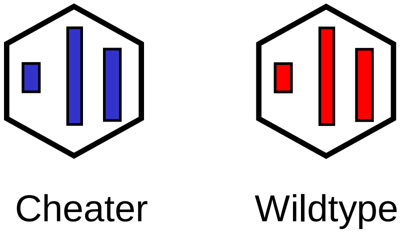
wildtype
candidate cheater allele (T→C in non-coding region on M segment)



Evidence that a cheater RNA segment can evolve in phi-6

Low MOI
(clonal infection)
environment





Arnold, Goldhill et al. (*unpublished*)

Evidence that a cheater RNA segment can evolve in phi-6

Low MOI
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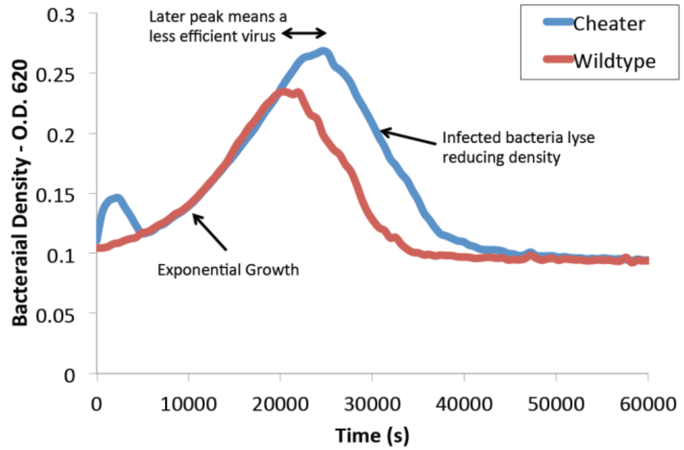
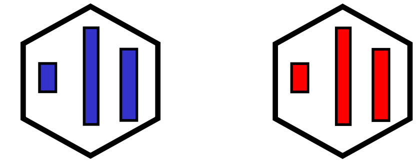
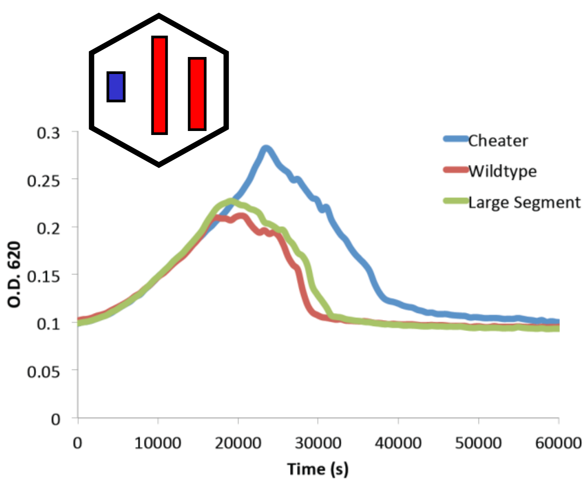
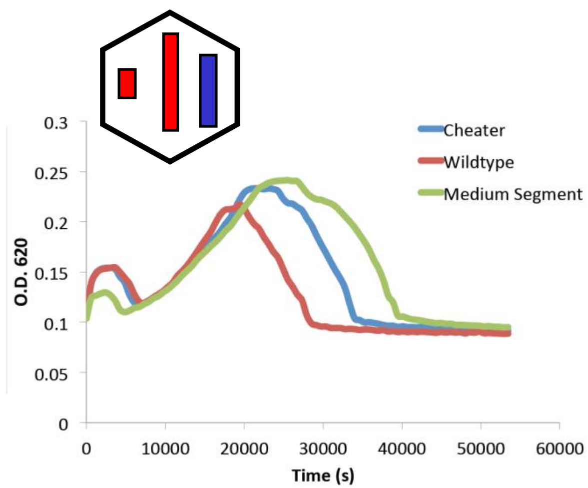
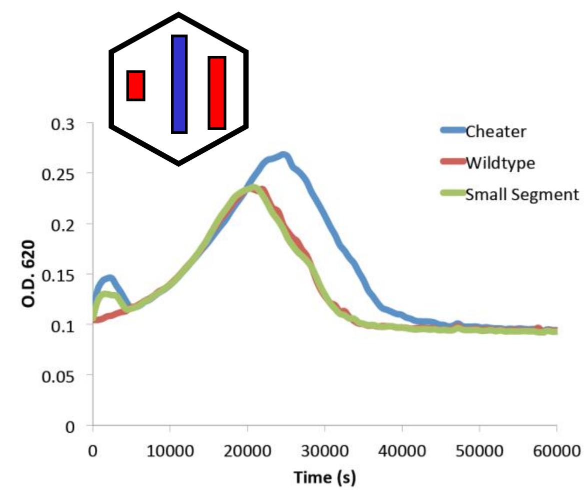




Cheater Wildtype

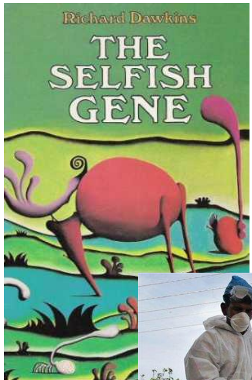


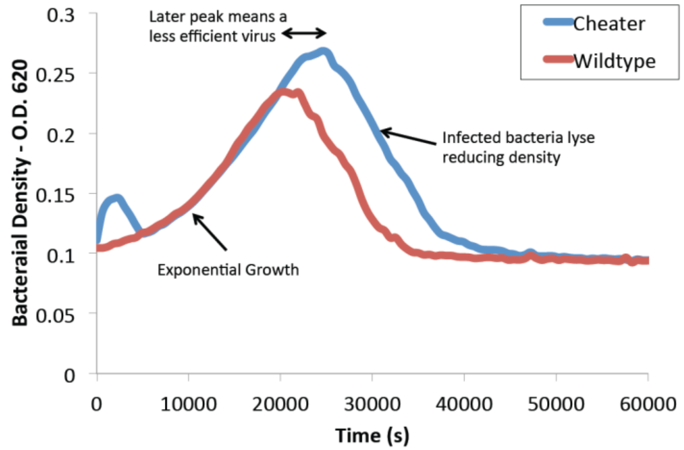


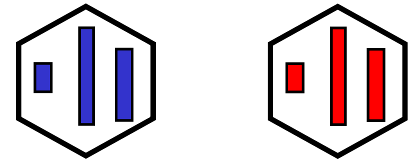


Arnold, Goldhill et al. (*unpublished*)

Evidence that a cheater RNA segment can evolve in phi-6

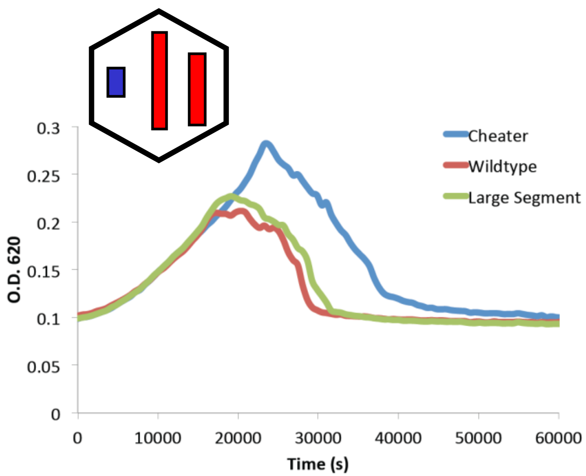


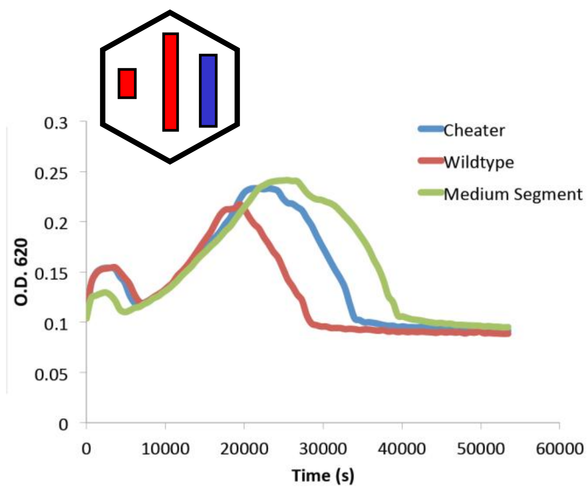


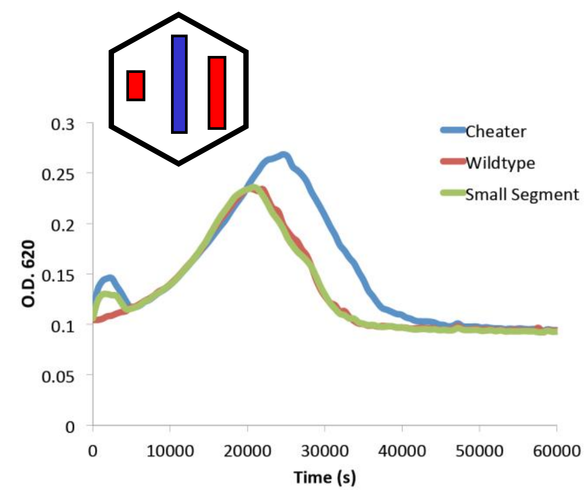


Cheater

Wildtype

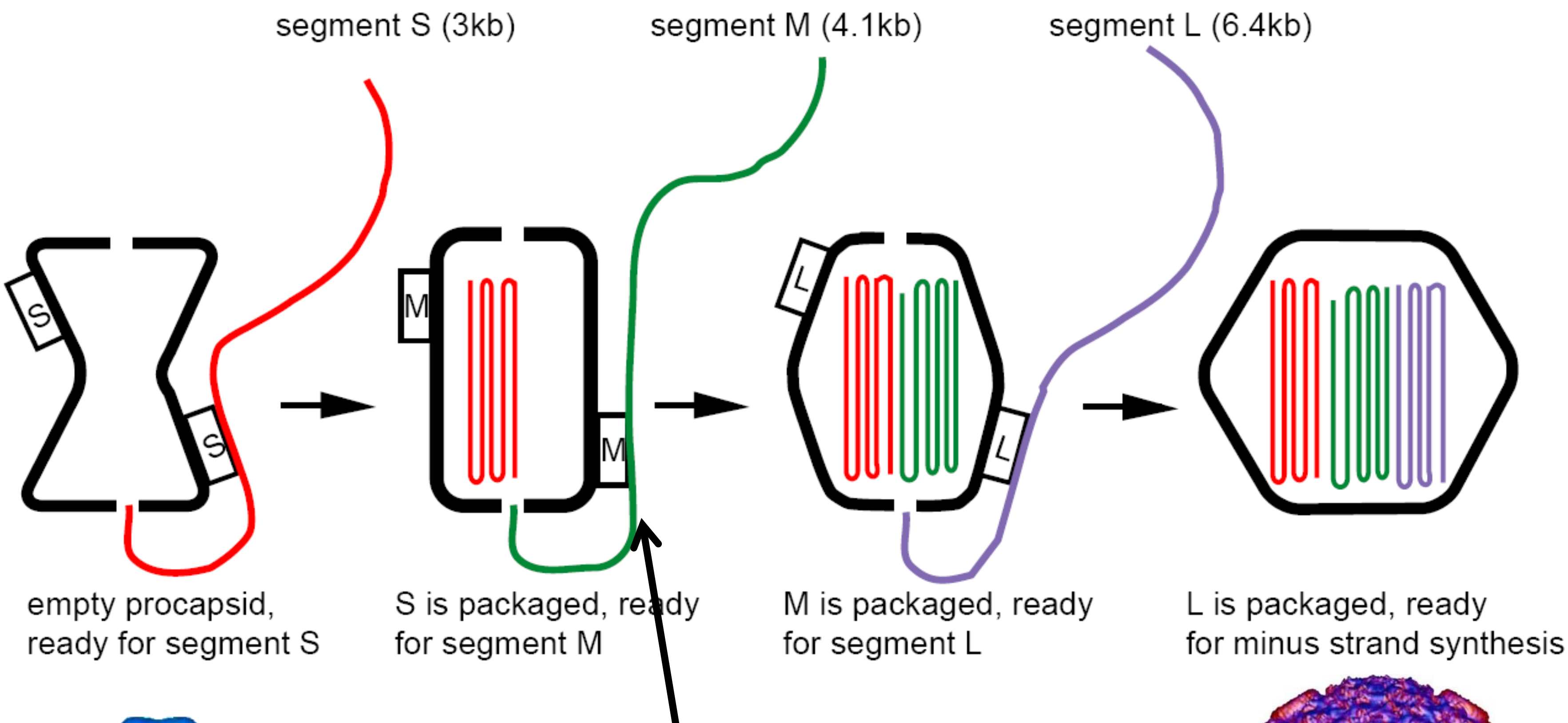


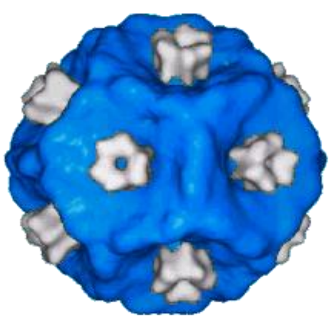




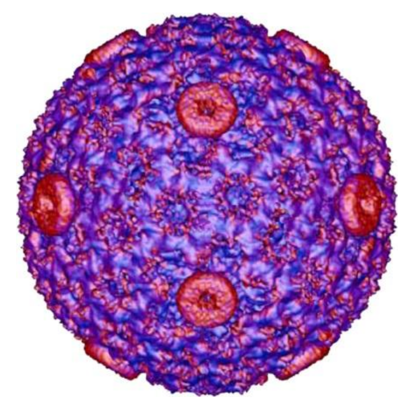
Arnold, Goldhill et al. (*unpublished*)

What is the cheating mechanism?



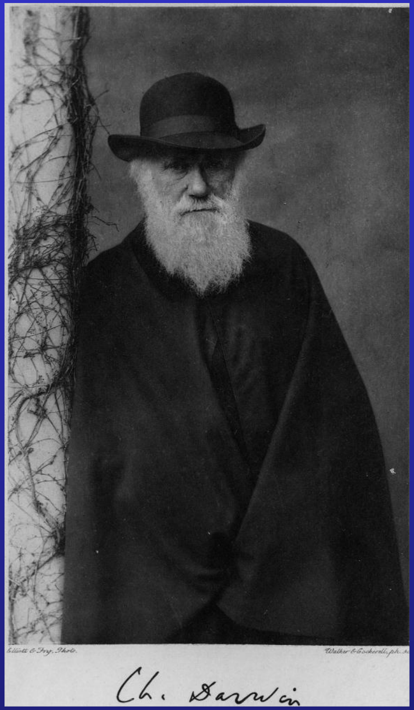


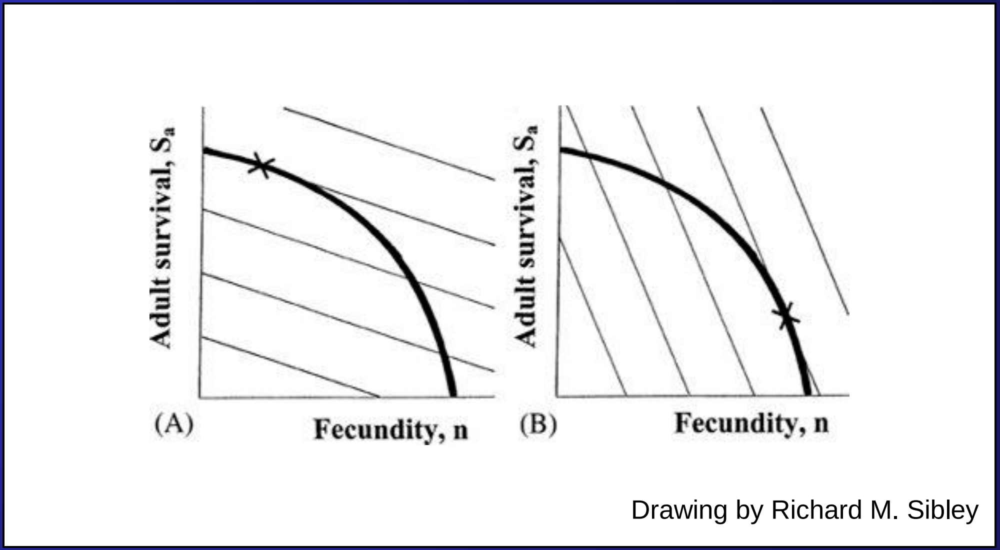
Greater packaging affinity of mutated M segment is likely cheater mechanism



Mindich 2004 *Virus Research*, Turner & Chao 2003 *Am Nat*

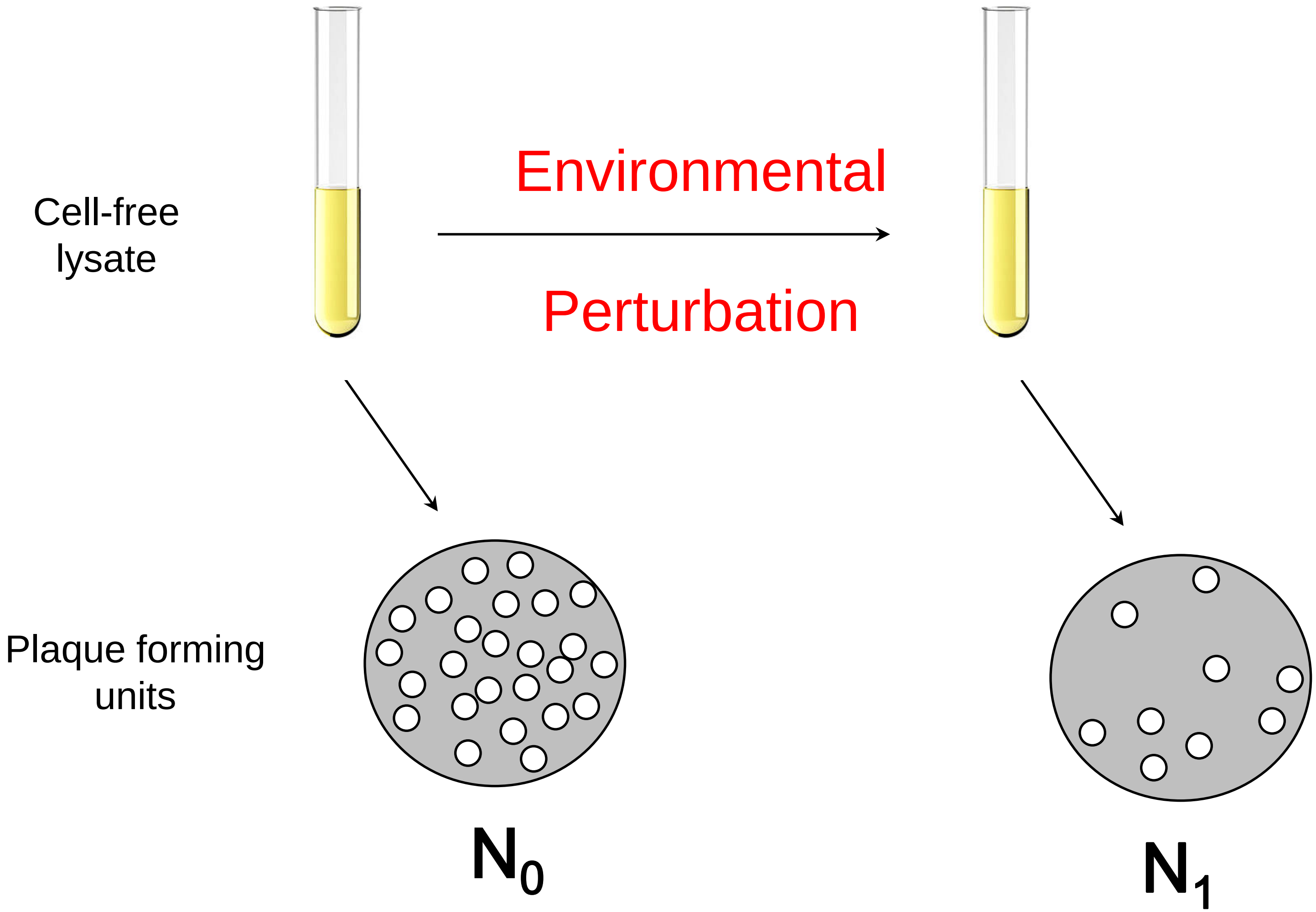
Natural selection can be driven by differential reproduction <u>and</u> survival





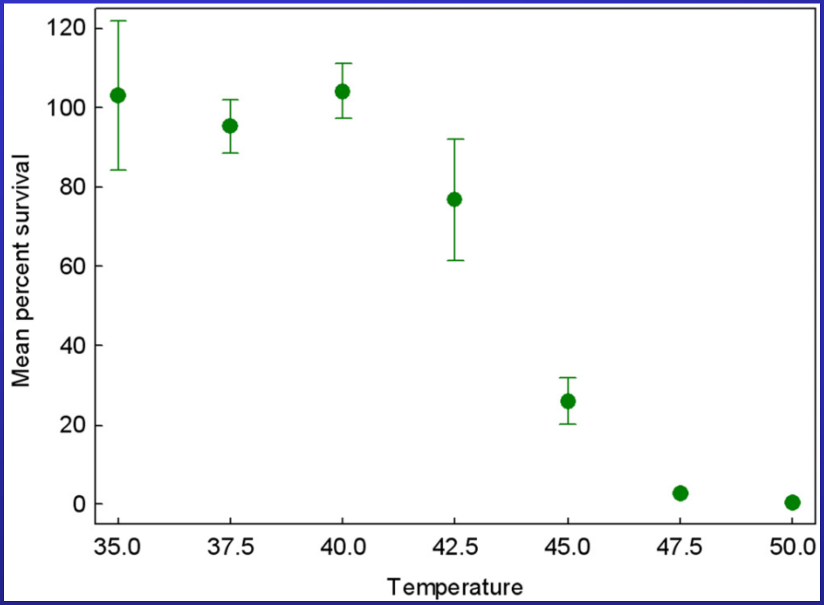
Life-history theory predicts that survival and reproduction cannot be simultaneously maximized.

Phage Survival Assay



$$\% \text{ Survivors} = (N_1/N_0) * 100$$

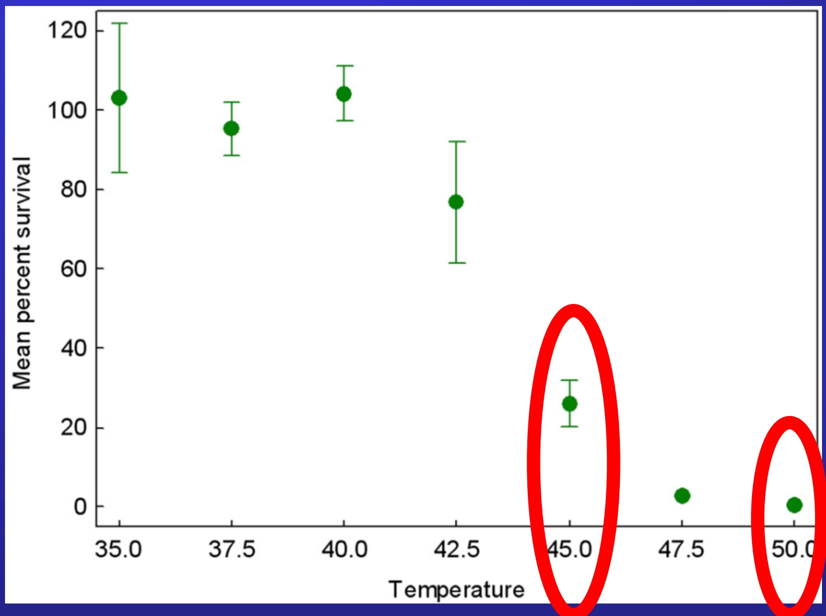
Phi-6 reaction norm following 5-min heat-shock



Typical lab environment: 25^{o}C

McBride et al. 2008, *BMC Evol Biol*

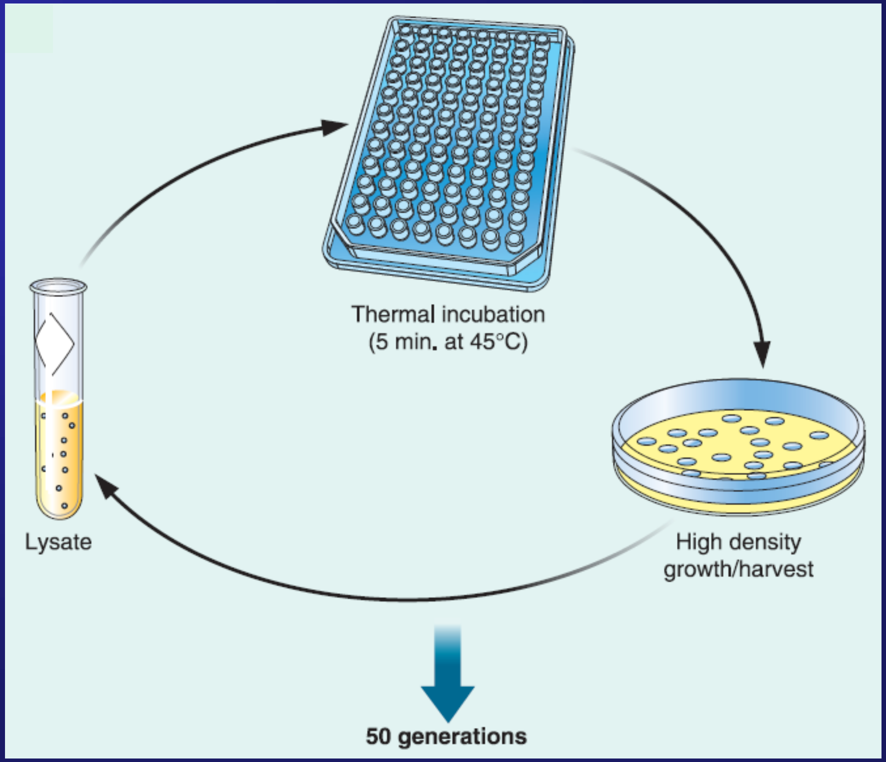
Can phage survival adaptively improve?



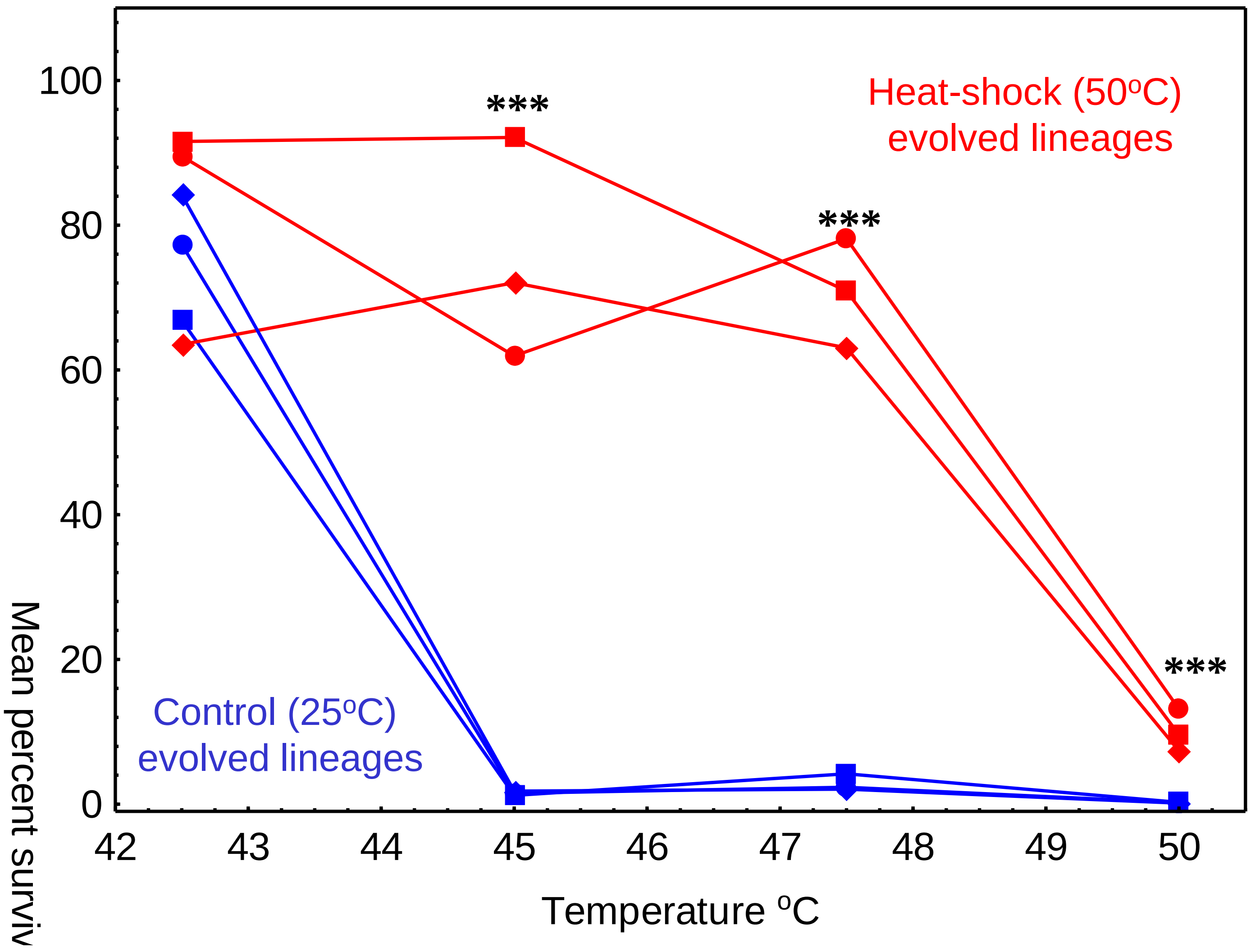
Daniel Goldhill

Rob McBride
(Sapphire Energy)

Brandon
Ogbunugafor
(Harvard)



Results: phenotypic adaptation (thermotolerance)



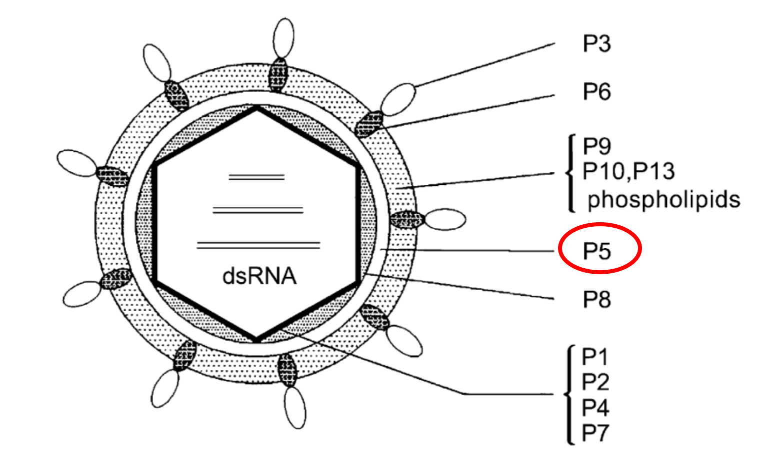
Dessau, Goldhill et al. 2012 *PLoS Genetics*

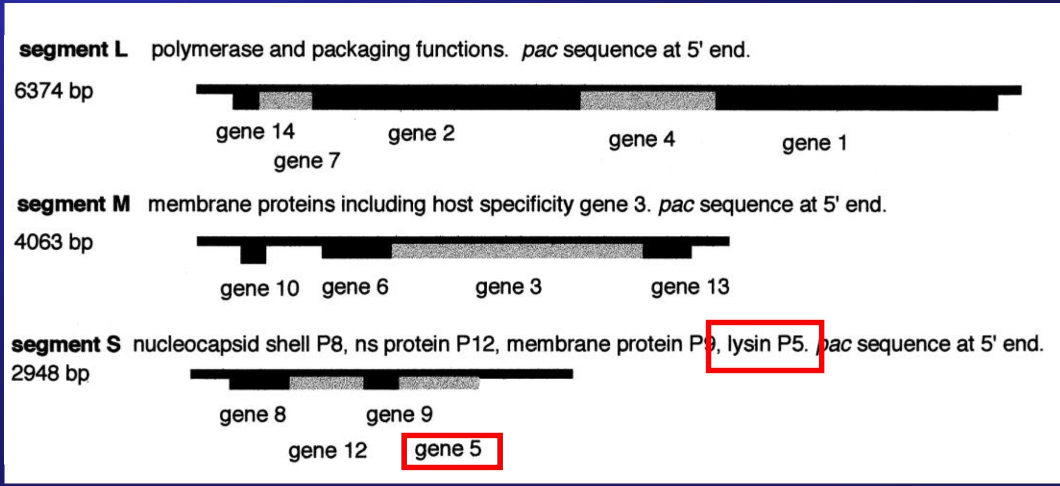
How does thermotolerance evolve in phi-6?

3 thermotolerance evolution studies

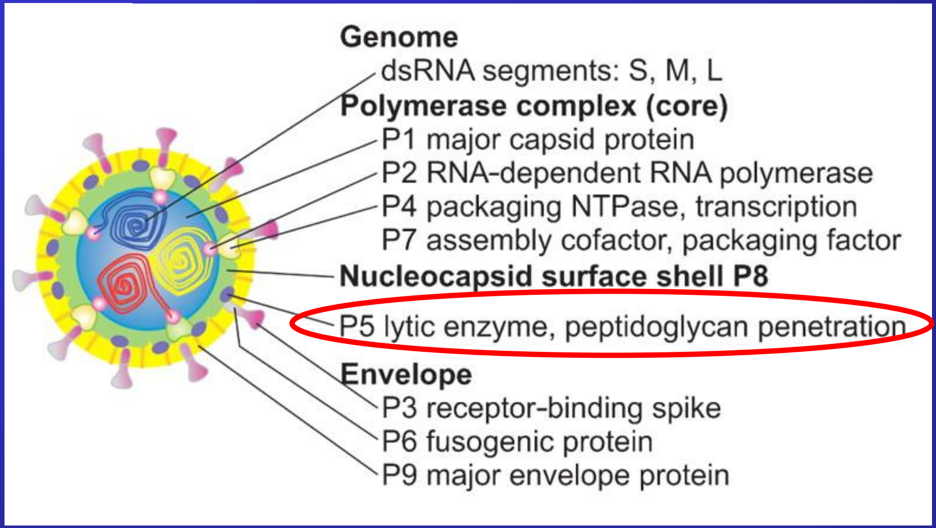
- Robust vs. Brittle clones evolved at 45°C
  (McBride et al. 2008)
- Robust vs. Brittle populations evolved at 45°C
  (Goldhill et al. submitted)
- Wild type clones evolved at 50°C
  (Dessau, Goldhill et al. 2012)

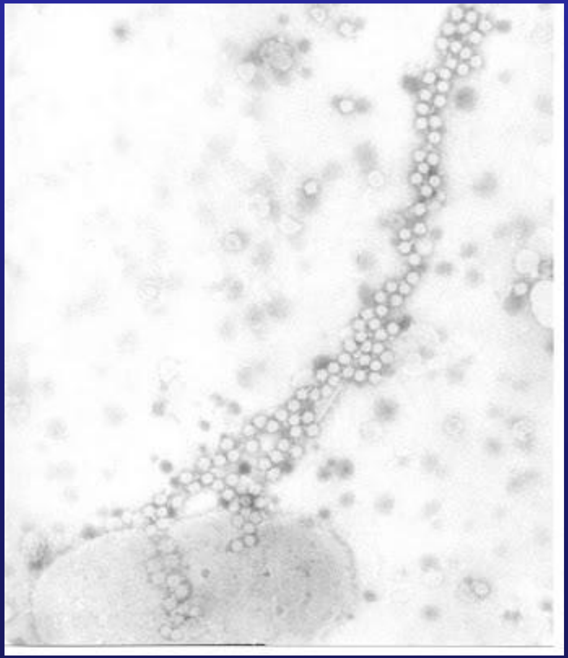
S segment:
P5 lysin gene mutation
G2238U transversion V→F

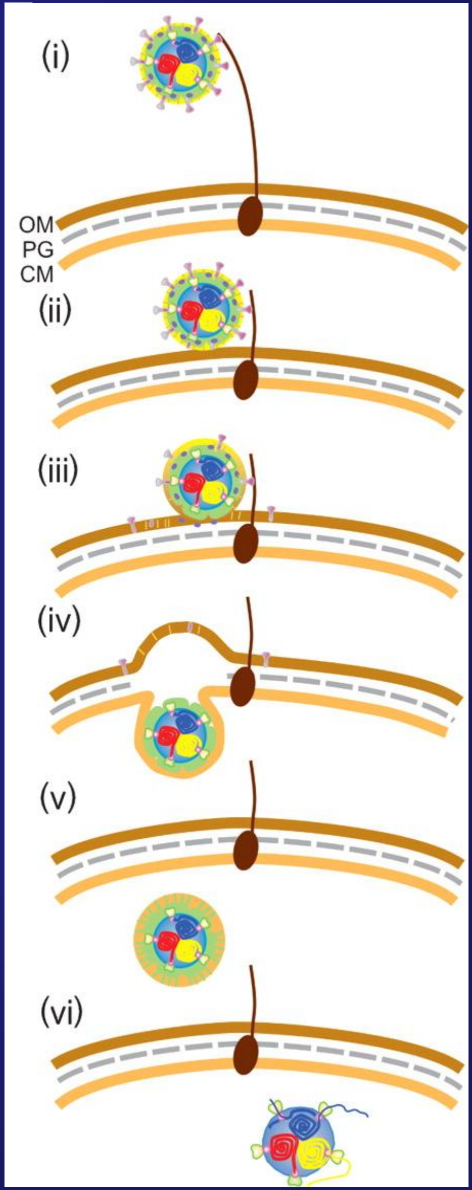




How does thermotolerance evolve in phi-6?



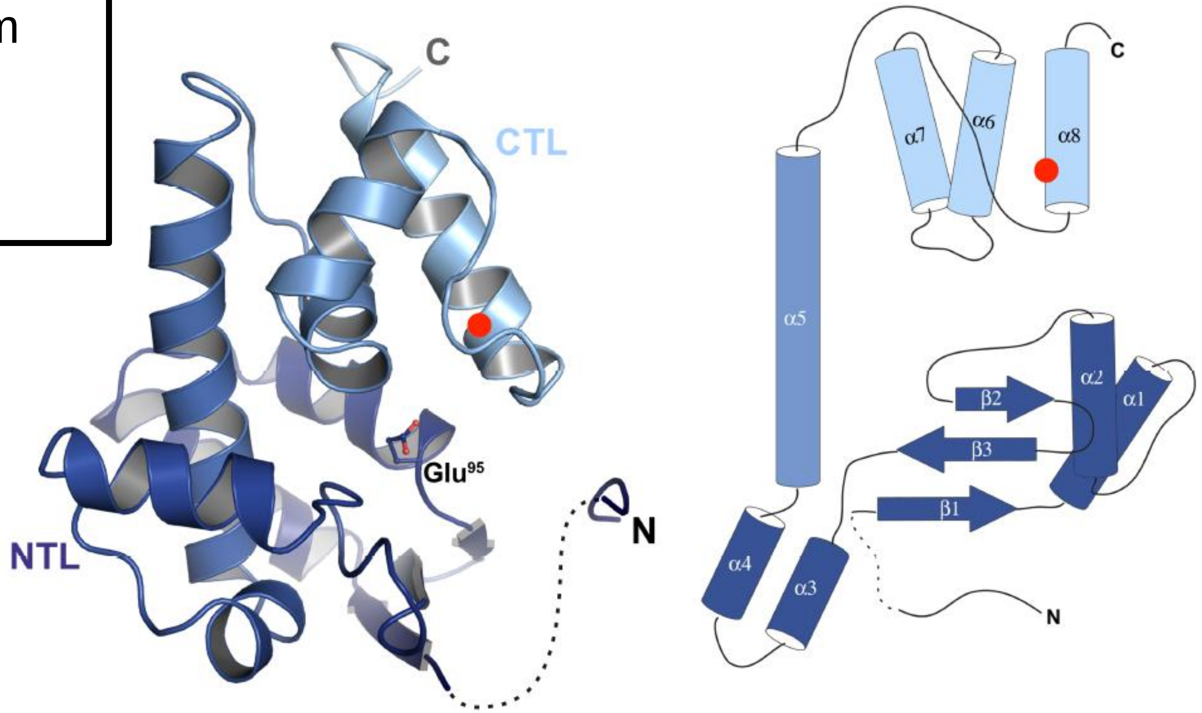




Cvirkaite-Krupovic et al 2010 *J Gen Virol*

Comparing wild-type and 'thermotolerant' P5 lysin enzymes

- Structure
  - X-ray crystallography
- Stability
  - Circular Dichroism
- Activity
  - Enzyme assay



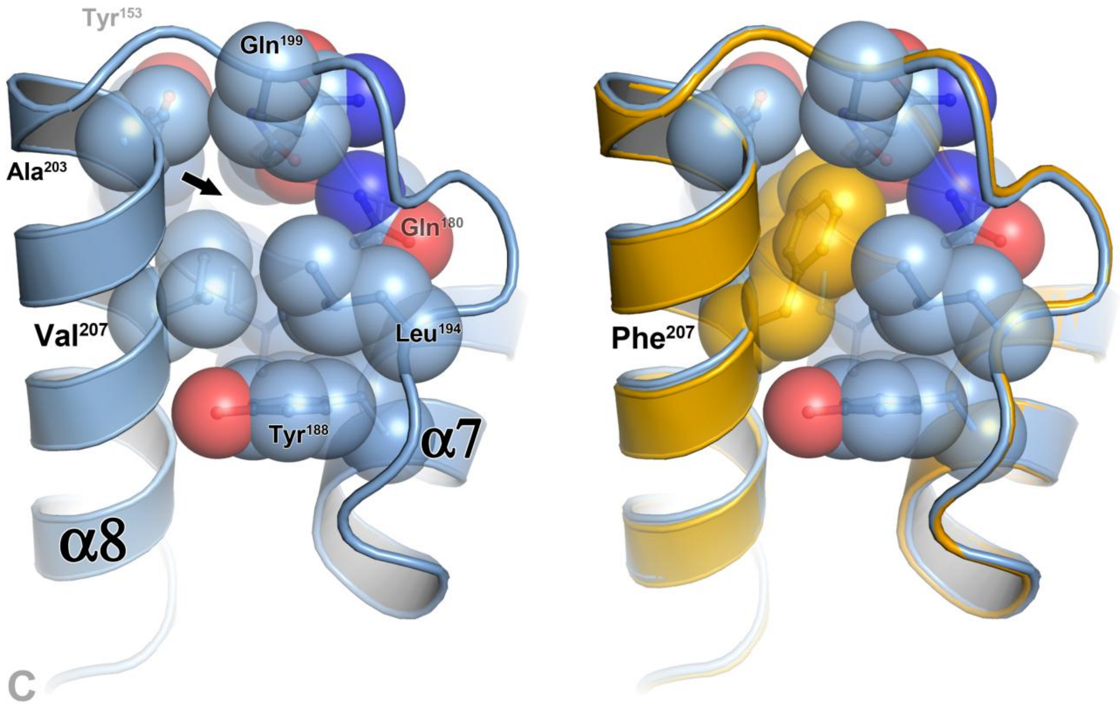
Structure of P5 protein
and region affected by mutation

Dessau, Goldhill et al. 2012 *PLoS Genetics*

Comparing wild-type and 'thermotolerant' P5 lysin enzymes

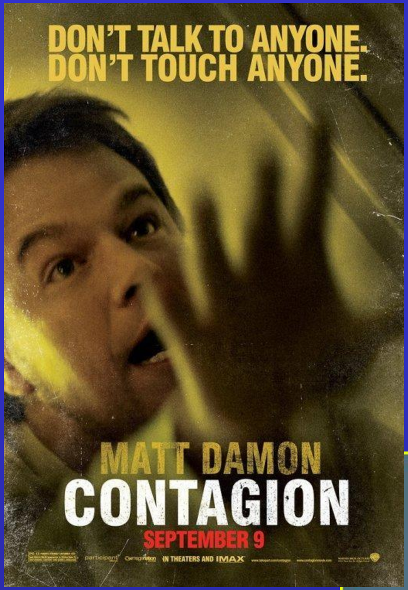
- Structure
  - X-ray crystallography
- Stability
  - Circular Dichroism
- Activity
  - Enzyme assay

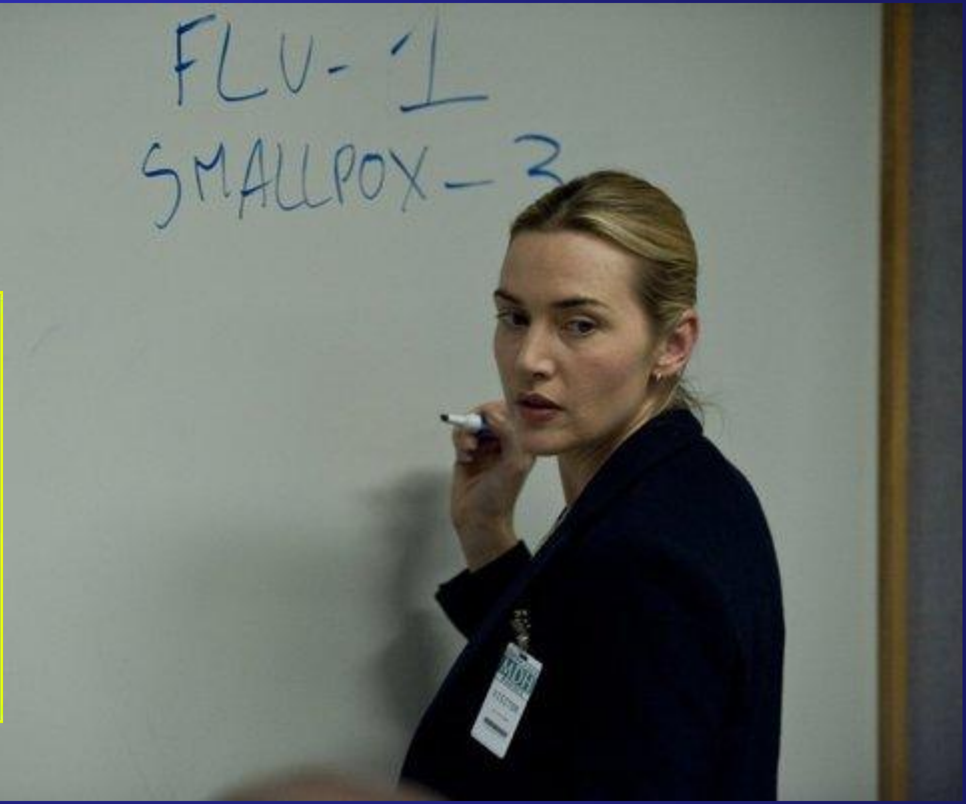
Phenylalanine fills a hydrophobic pocket stabilizing the protein



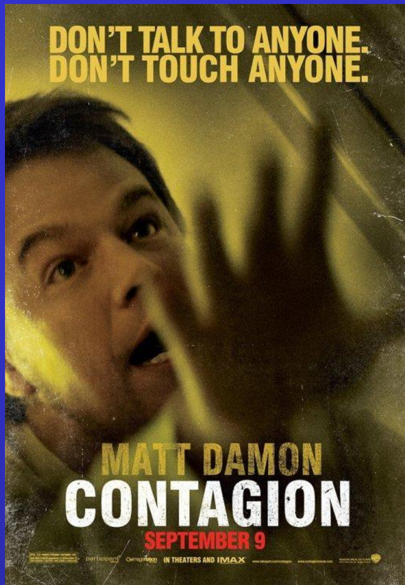
Dessau, Goldhill et al. 2012 *PLoS Genetics*

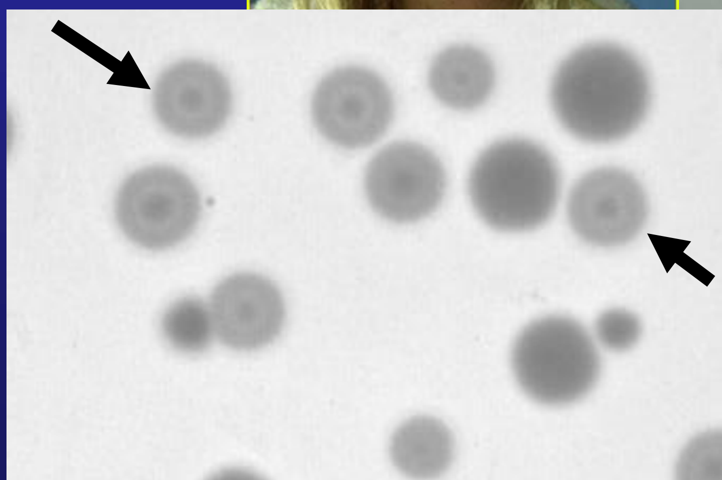
Is thermotolerance costly?





Is thermotolerance costly?

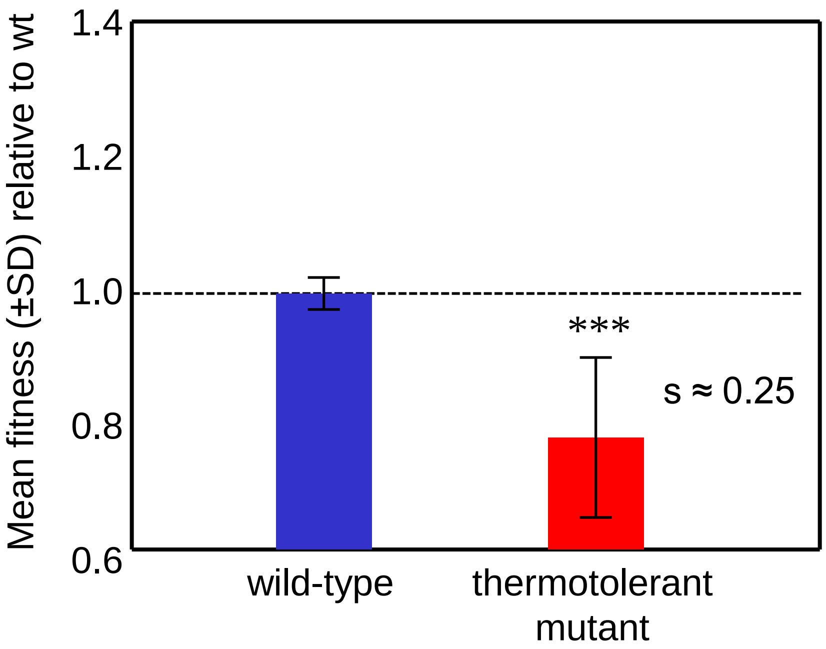




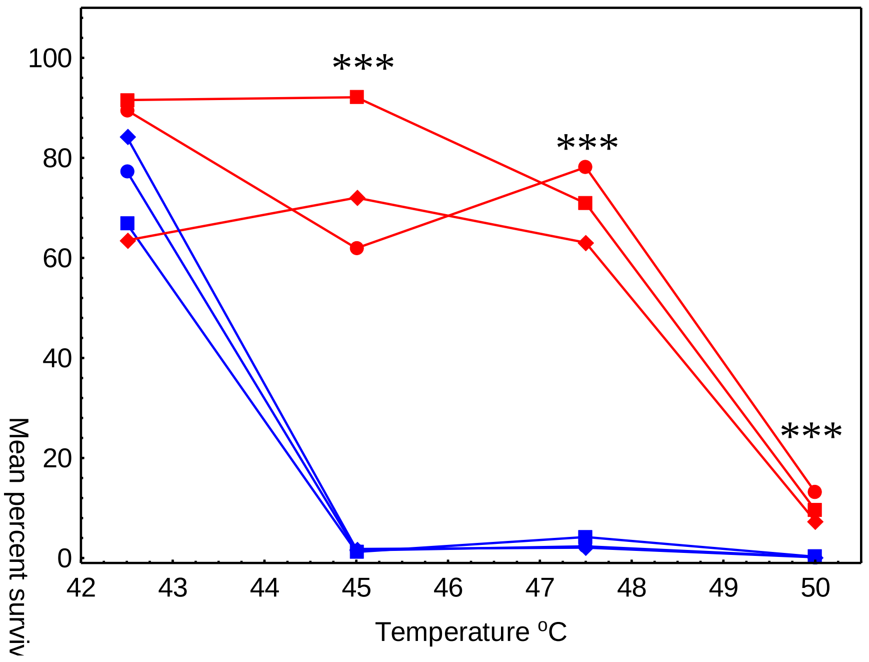
Thermotolerant mutants form
'bull's-eye' plaques at 25^{o}C

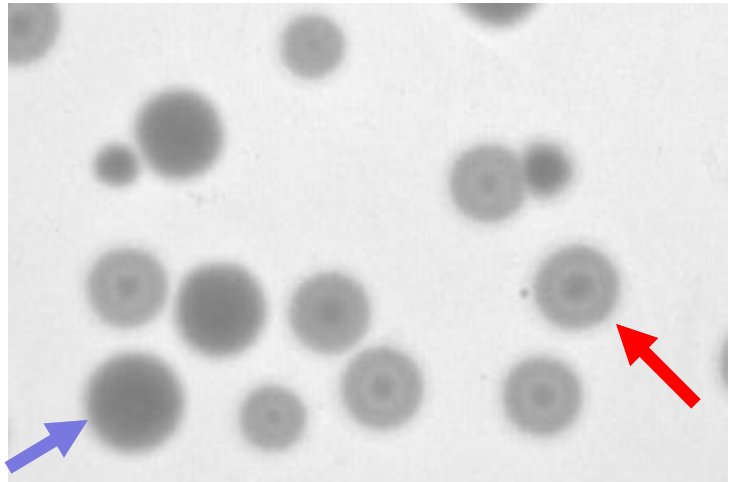
Antagonistically pleiotropic allele

Reproduction at 25°C



Survival at 45 – 50°C





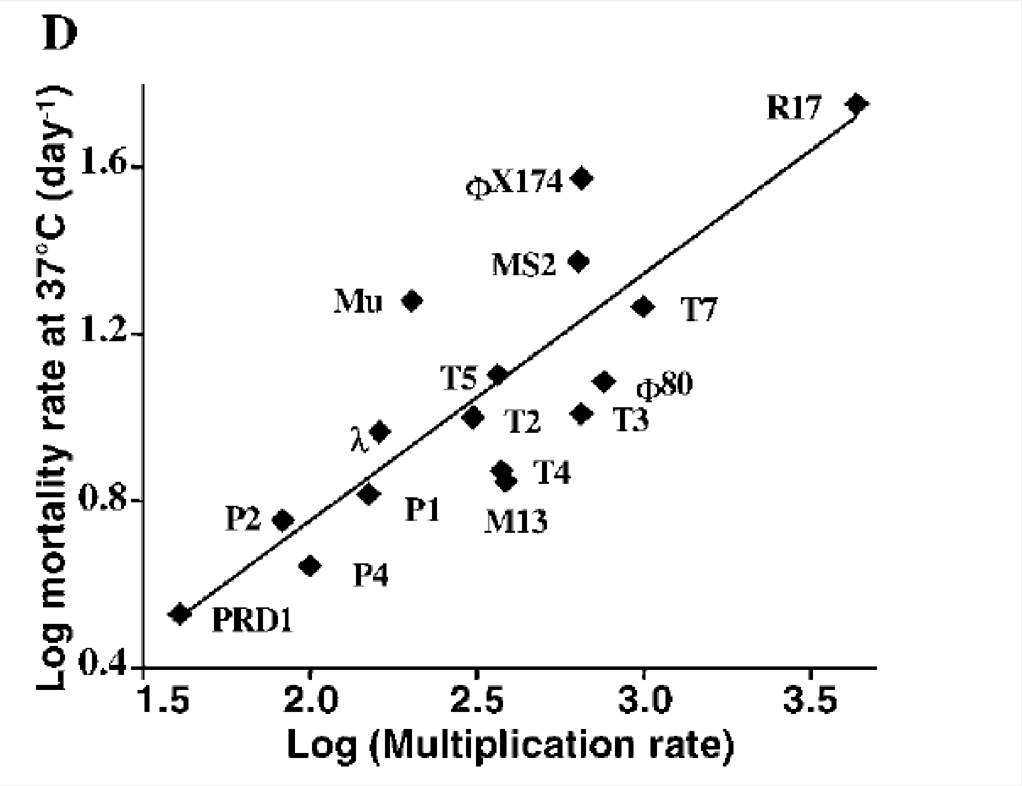
V207F causes 'life-history' tradeoff between survival and reproduction

Dessau, Goldhill et al. 2012 *PLoS Genetics*

Viruses' Life History: Towards a Mechanistic Basis of a Trade-Off between Survival and Reproduction among Phages

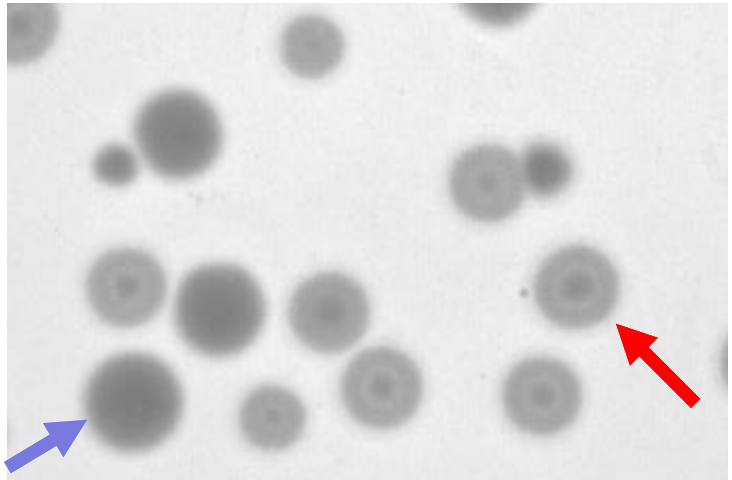
Marianne De Paepe, François Taddei*

Laboratoire de Genetique Moleculaire, Evolutive et Medicale, University of Paris 5, INSERM, Paris, France

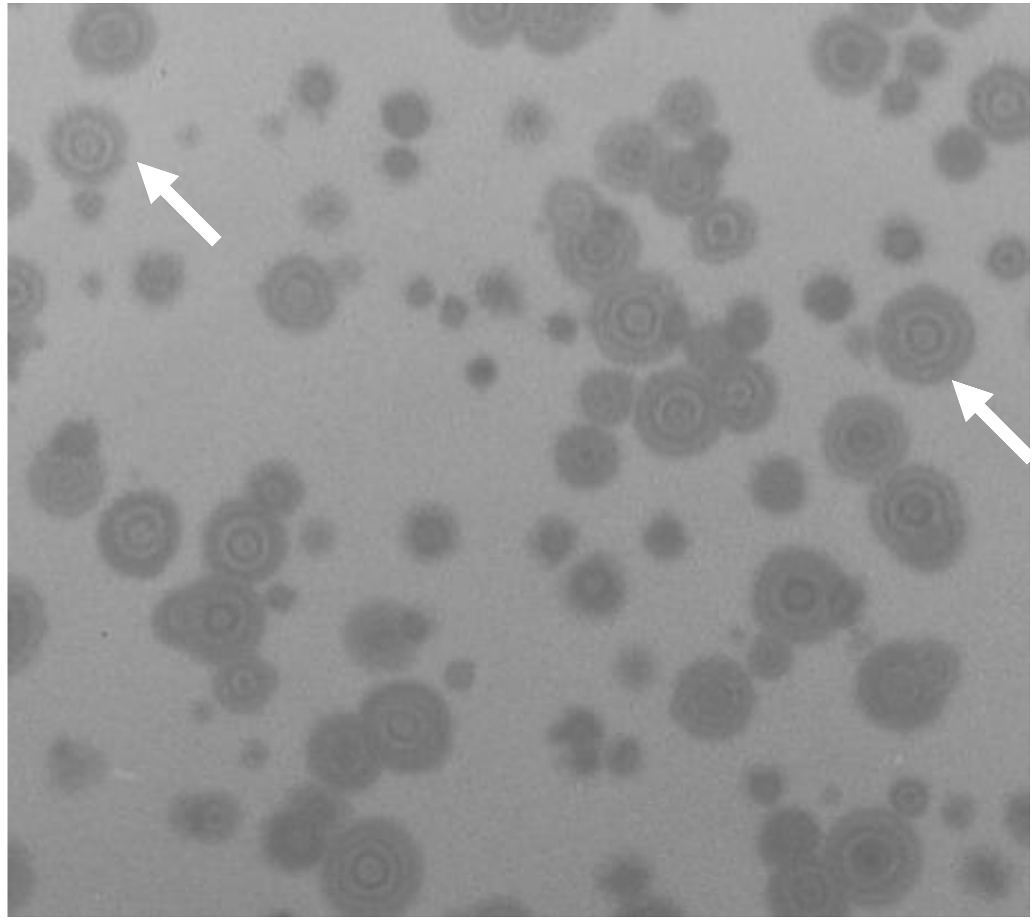


de Paepe and Taddei 2006 (PLoS Biol)

What causes bull's-eye plaque phenotypes?



V207F trades off reproduction for survival



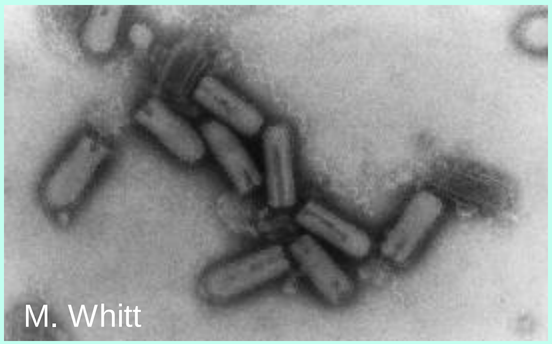
Do cystovirus populations harbor variability for life-history strategies? (phage phi-12 plaques)

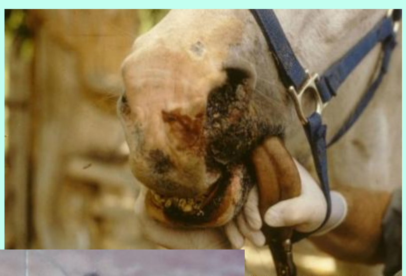
What causes bull's-eye plaque phenotypes?

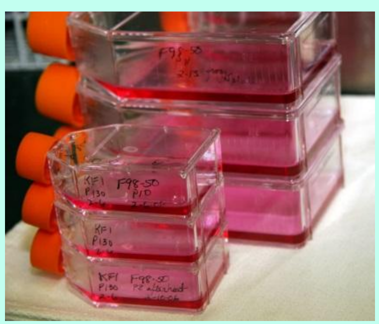
[Time lapse video of bull's-eye plaque formation]

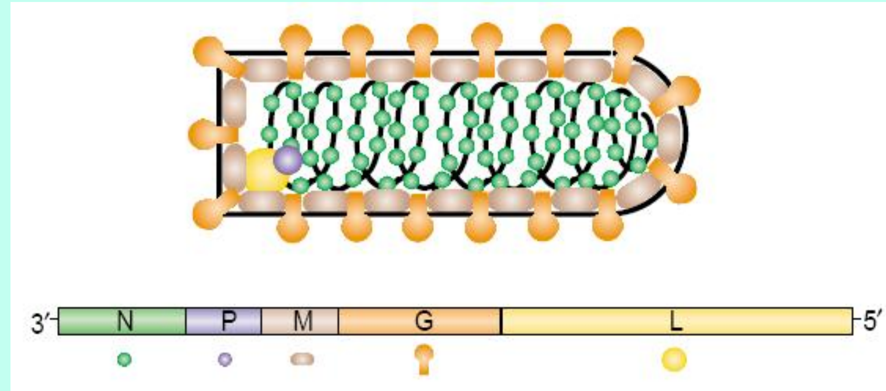
Vesicular Stomatitis Virus

- (-)ssRNA virus
- ~11 kb genome
- Arbovirus

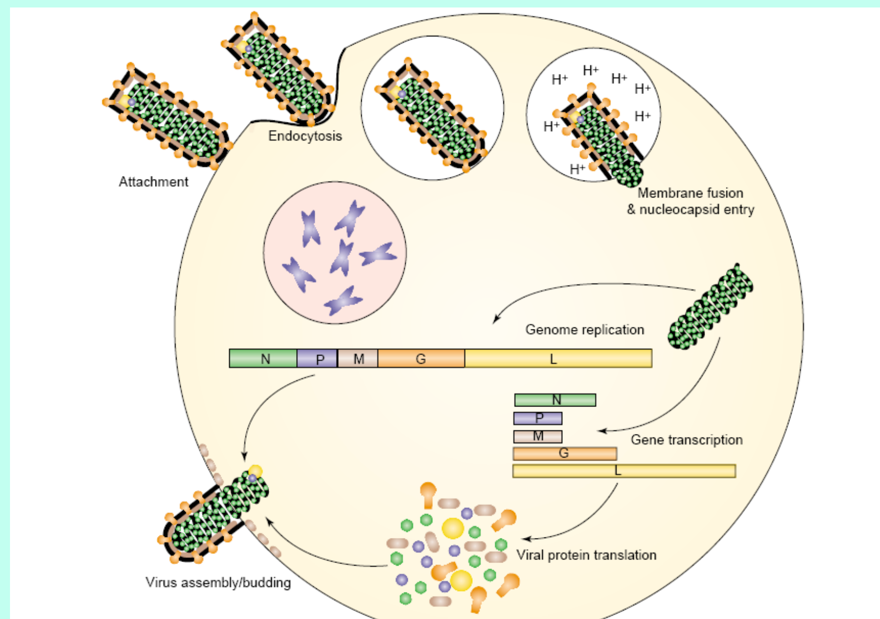

M. Whitt





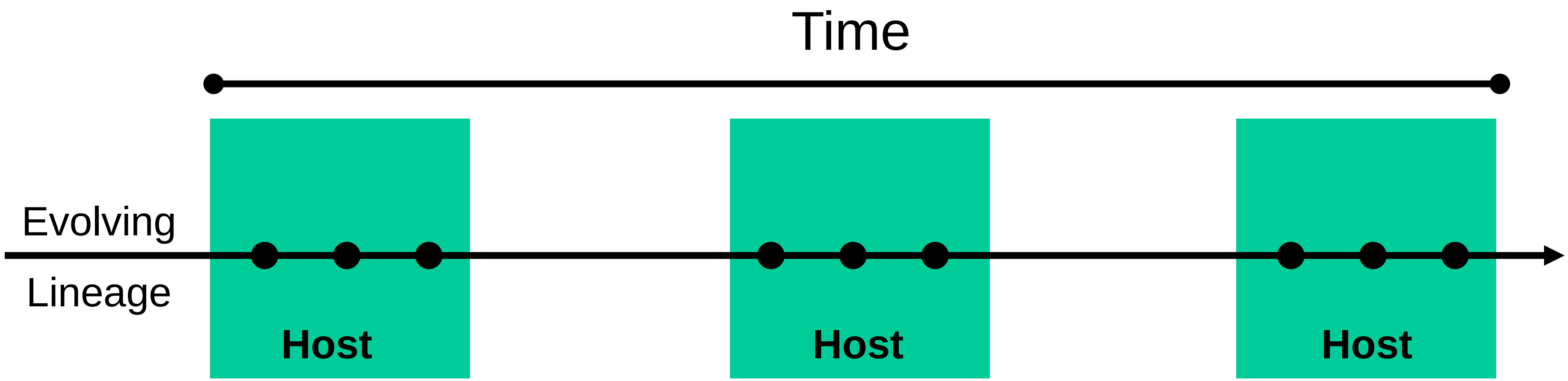


Lichty et al 2004

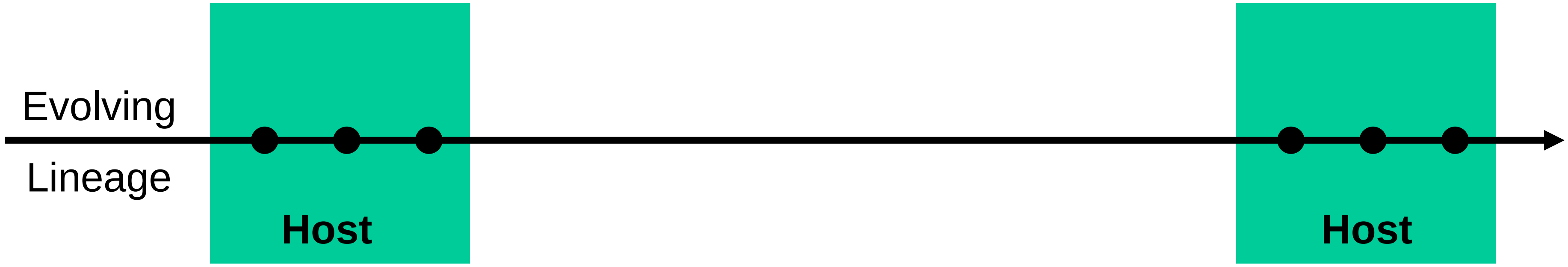


Lichty et al 2004

How should transmission time select for survival?



Ordinary transmission time
Strong selection for reproduction
Weak selection for survival



Prolonged transmission

Tradeoff?

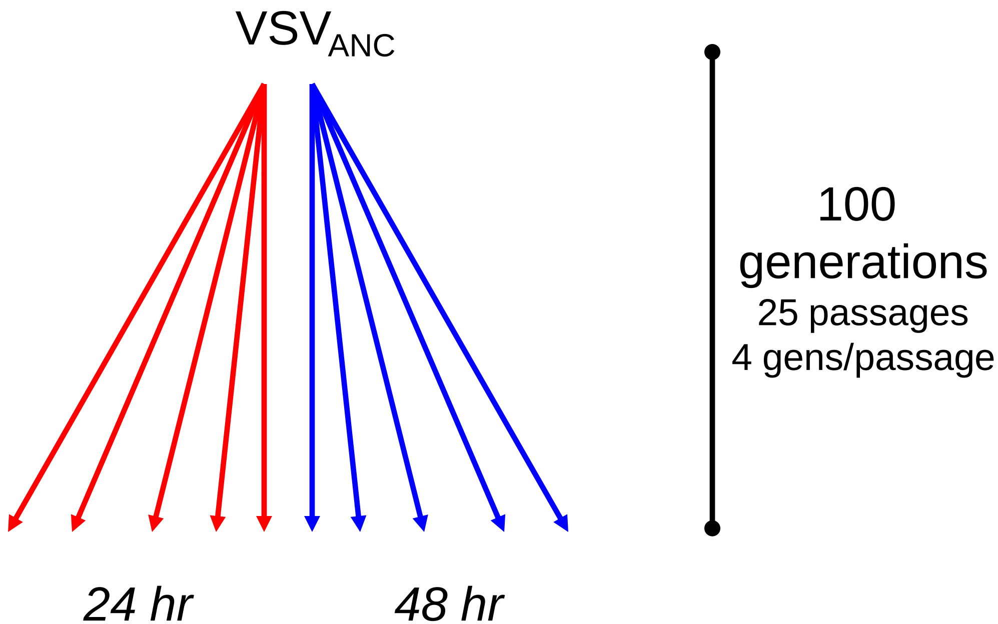
Strong selection for reproduction
Strong selection for survival

Bonhoeffer et al. 1996
Caraco & Wang 2008

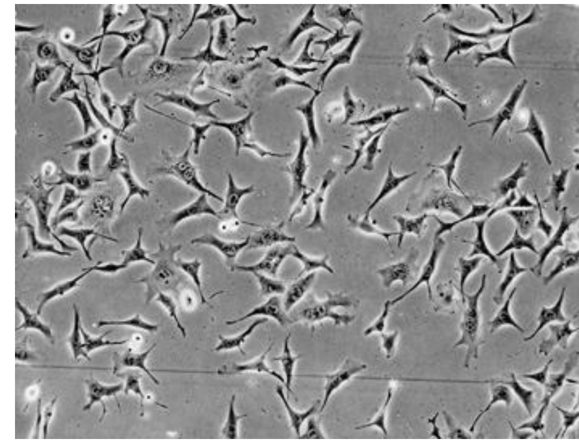
Transmission challenge:

24 hr vs. 48 hr

Experimental design:



baby hamster kidney
(BHK) cells

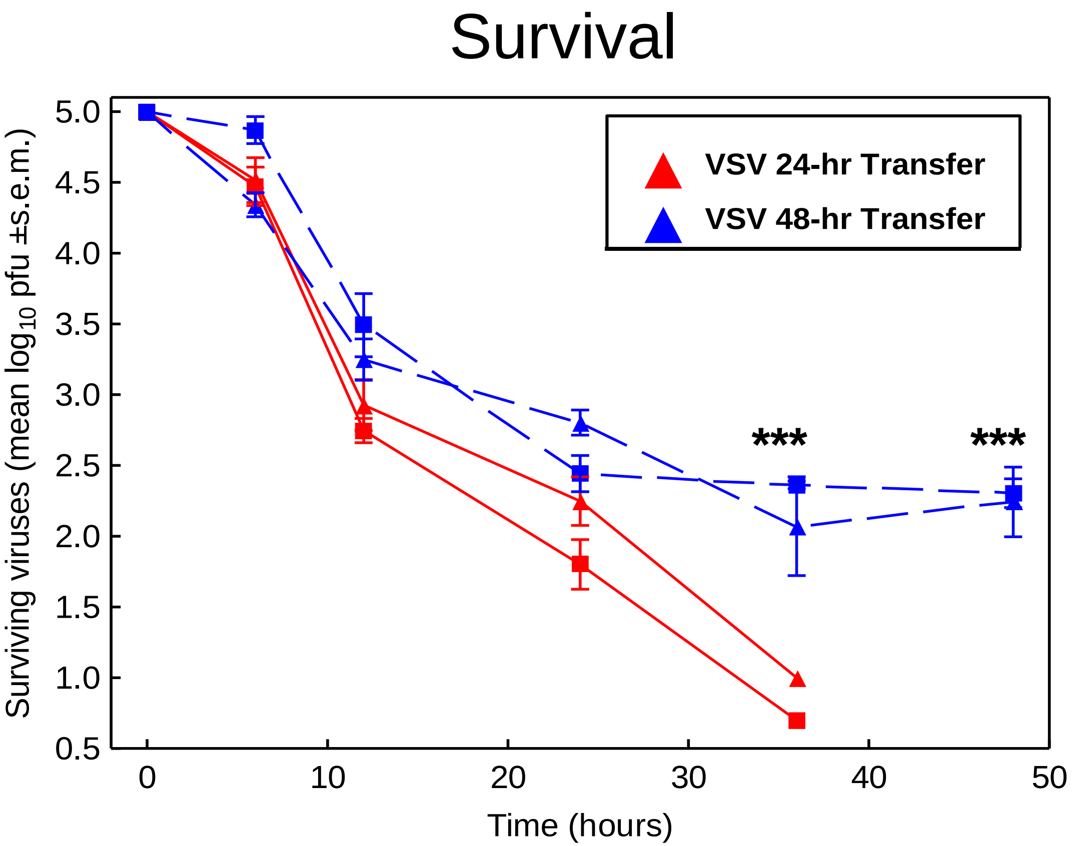


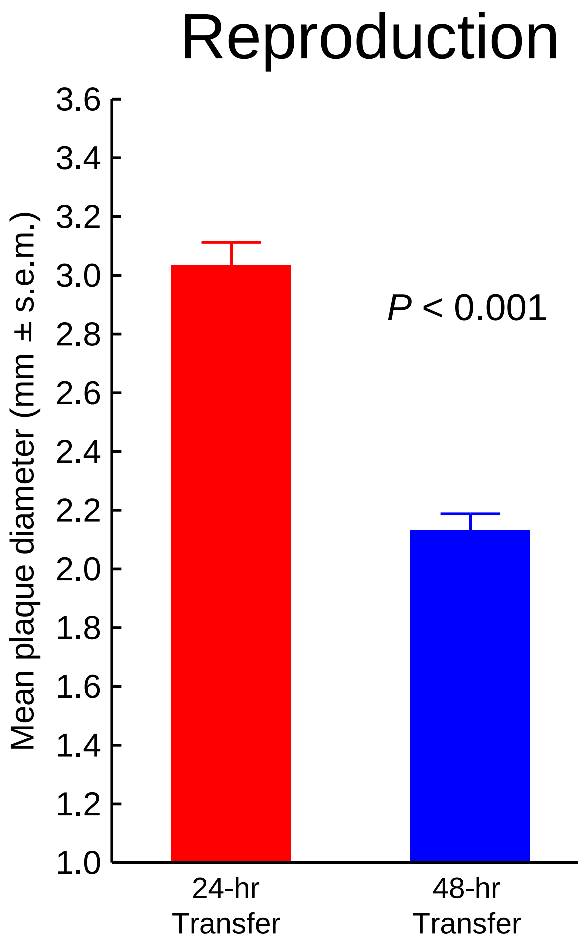
Brian Wasik
(Cornell U)

Ambika Bhushan
(Harvard Med)

Wasik, Bhushan et al. *Evolution* (in press)
(see also: Elena 2001 *Infect Genet Evol*)

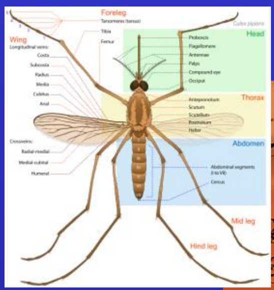
Survival trades-off with reproduction in VSV evolution





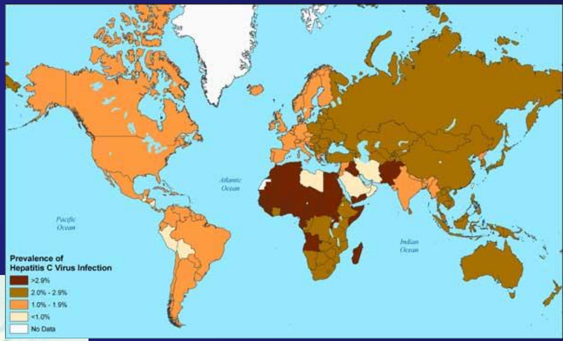
Wasik, Bhushan et al. *Evolution* (in press)

Effects of temperature on survival of Dengue virus and of Sindbis virus



Effects of transmission time on survival of Rotavirus and of Hepatitis C Virus

(Ogbunugafor, Hartl)



Conclusions / Future Work

- Phages play by same evolutionary rules as other systems.
- Classic life-history trade-off between survival and reproduction (inability to maximize both).
- Can compensatory mutations overcome this constraint?
  - Long-term (140 day) experimental evolution suggests YES.
  - BUT, <u>contingent</u> on first thermotolerance mutation that fixes in population – otherwise population is fated to <u>extinction</u>.
- Phage therapy efforts may benefit from evolutionary thinking

ACKNOWLEDGMENTS

Current lab members

Stacy Arnold
Ben Chan
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Daniel Goldhill
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Lynette Perez
Matthew Ribeiro
Qainat Shah
Smita Shukla
Mark Sistrom
Beth Williams

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John Dennehy (CUNY Queens)
Siobain Duffy (Rutgers U)
Jeremy Draghi (British Columbia)
Remy Froissart (Montpellier)
David Kysela (Indiana U)
Rob McBride (Sapphire Energy)
Rebecca Montville (NY Pub Sch)
Nadya Morales (BASF)
Brandon Ogbunugafor (Harvard)
Kara O’Keefe (San Fran Pub Hlth)
Kendra Pesko (U New Mexico)
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Teeming horde of undergrads too numerous to list

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John Wertz (Coli Stock Center)

Project High Hopes

NSF

NATIONAL INSTITUTES OF HEALTH