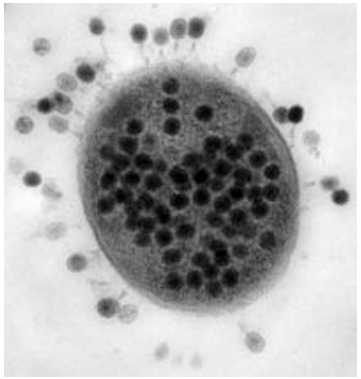
Using Phage to Select for Evolution of Reduced Virulence in Pathogenic Bacteria



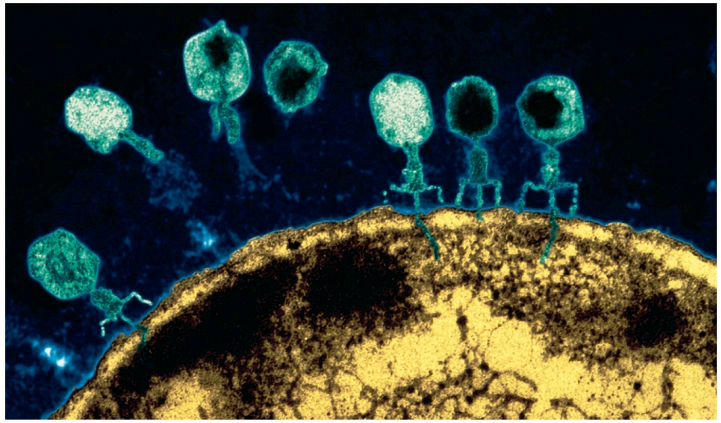
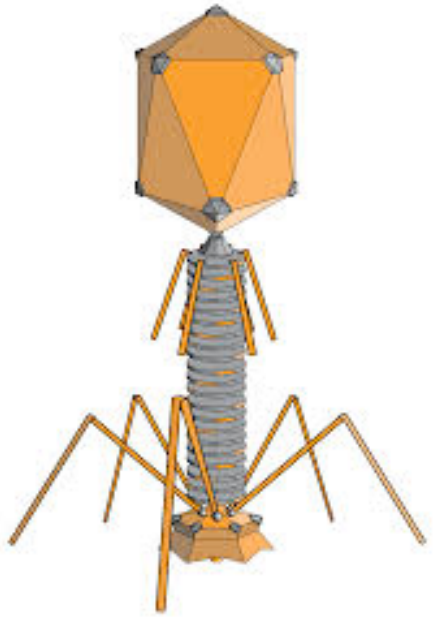


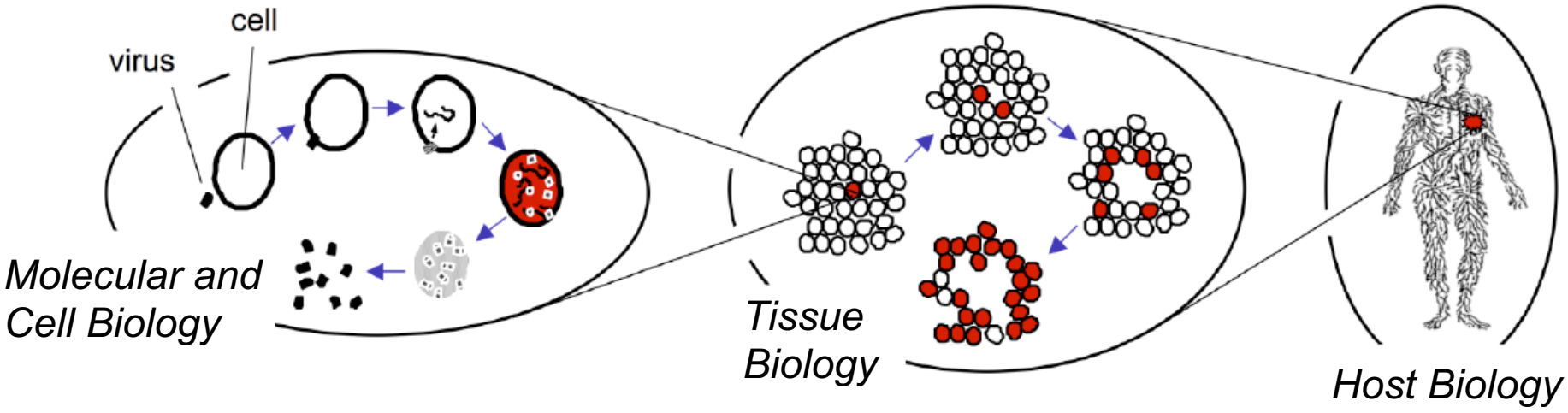
Figure 5-24
Introduction to Genetic Analysis, Tenth Edition
© 2012 W. H. Freeman and Company



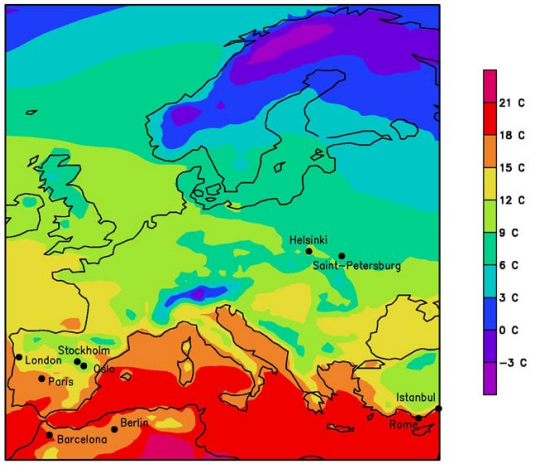
Paul E. Turner

Interim Dean of Science, Professor of Ecology & Evolutionary Biology, Yale University;
Microbiology Faculty, Yale School of Medicine

Virus genetics, genomics and evolution under environmental change



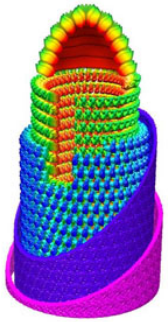
Vector Biology



Ecosystems

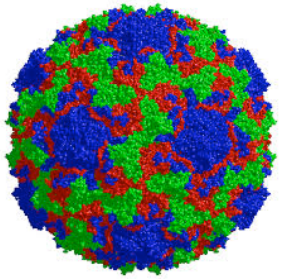
see Wasik & Turner 2013. *Annual Review of Microbiology*

Current virus study systems

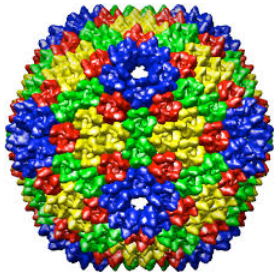


vesicular stomatitis virus

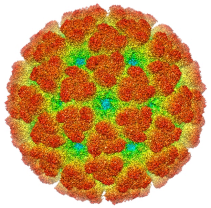
(-)ssRNA



rhinovirus

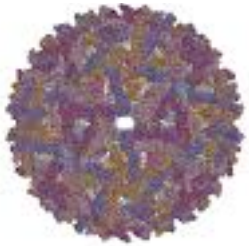


dengue virus



chikungunya virus

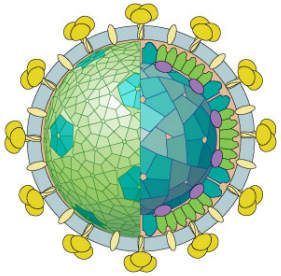
(+)ssRNA



Sindbis virus

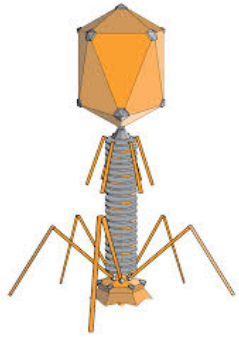
phage M13

ssDNA



phage phi-6

dsRNA



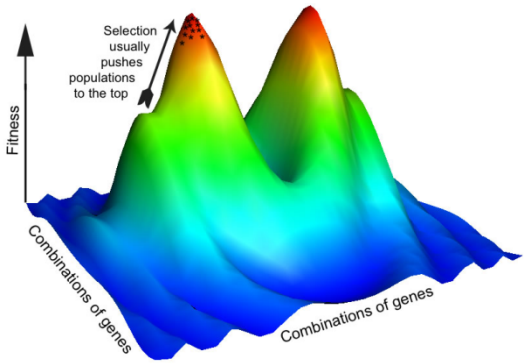
phage T2

dsDNA

Breadth of Research Topics

Basic — *Applied*

Tempo and Mode of Evolution & Adaptation



Ecology & Evolution of Infectious Disease

Improved and Novel Applications Using Viruses

Breadth of Research Topics

Basic — *Applied*

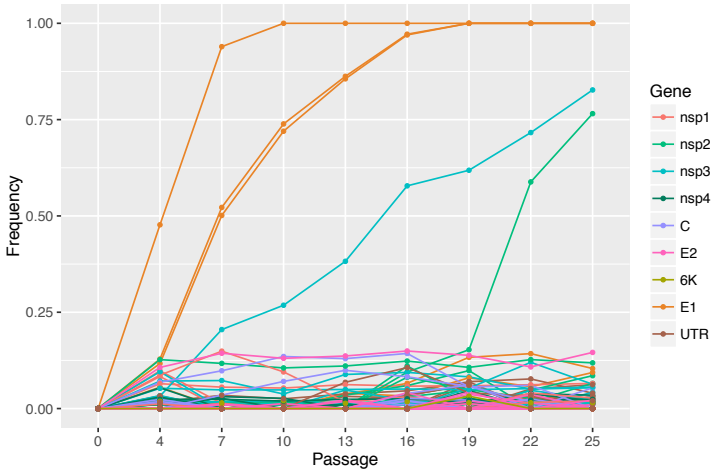
Tempo and Mode of Evolution & Adaptation

	Co-operate	Defect
Co-operate	1	1 - s_1
Defect	1 + s_2	1 - c

	ϕ6	ϕH2
ϕ6	1	0.65
ϕH2	1.99	0.83

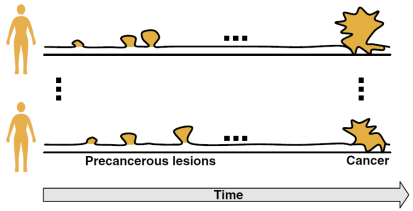
Turner and Chao 1999, *Nature*

Ecology & Evolution of Infectious Disease



Morley & Turner 2017, *Evolution*

Improved and Novel Applications Using Viruses

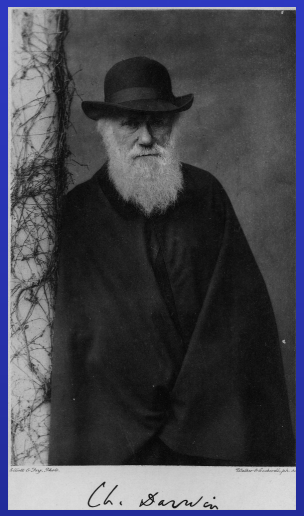


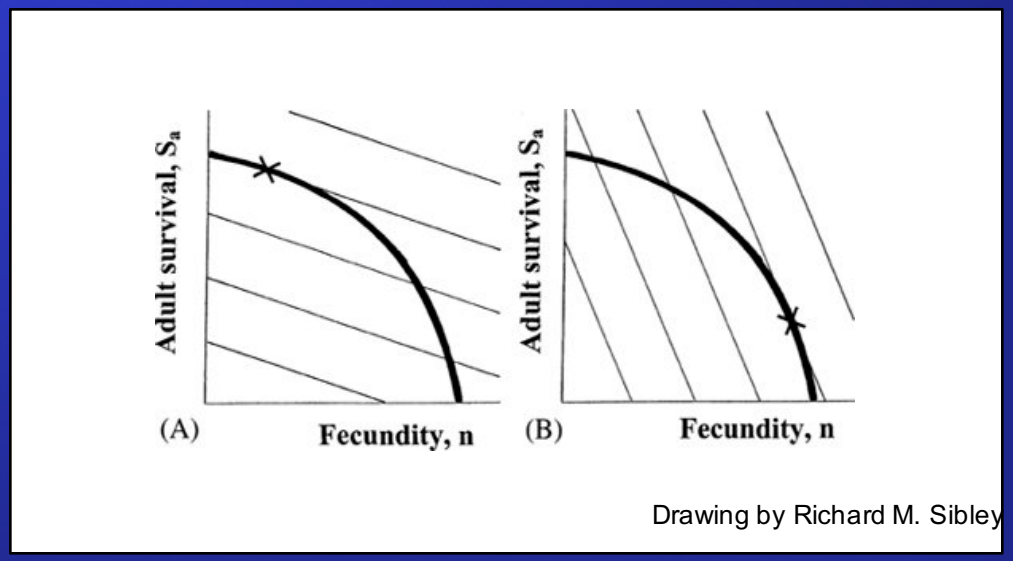
Sprouffske et al 2012

Turner et al., *unpubl.*

Why are there so many species on Earth?

- Evolution involves *compromises*.
- Natural selection can produce Trade-offs: improvement in one trait at the expense of performance in another trait.





Drawing by Richard M. Sibley

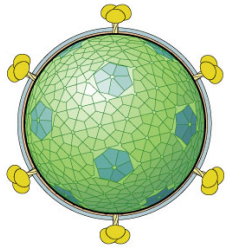
Steve Stearns (Yale)

- Life-history Theory: in general, traits cannot be simultaneously maximized.
- Often true for survival vs. reproduction – the cornerstones of evolution by natural selection.

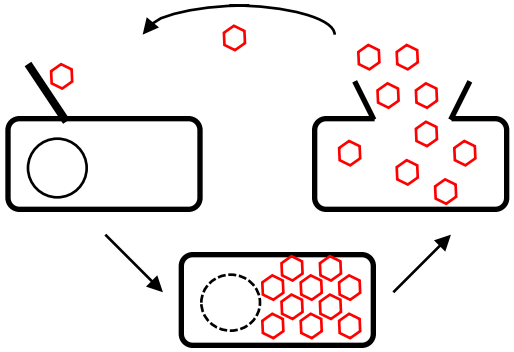
Goldhill and Turner (2014) *Current Opinion in Virology*
Wasik and Turner (2013) *Annual Review of Microbiology*

Life-history trade-off: survival vs. reproduction

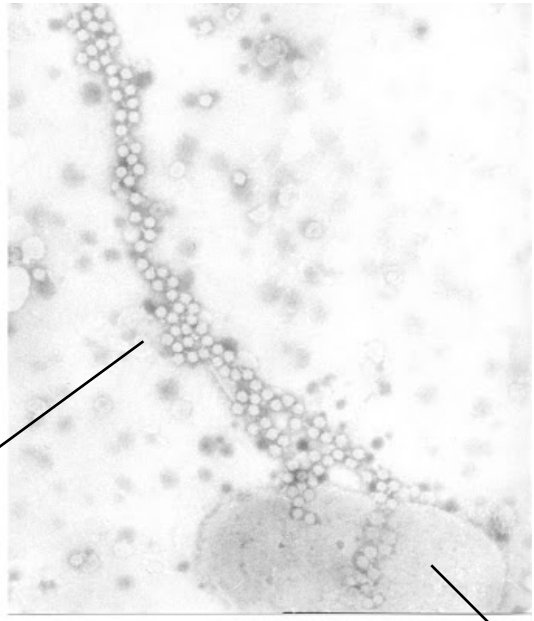
Example: phage phi-6 infection of *Pseudomonads*



- infects *Pseudomonas syringae* pathovars
- 13kb dsRNA segmented genome
- lytic infection cycle



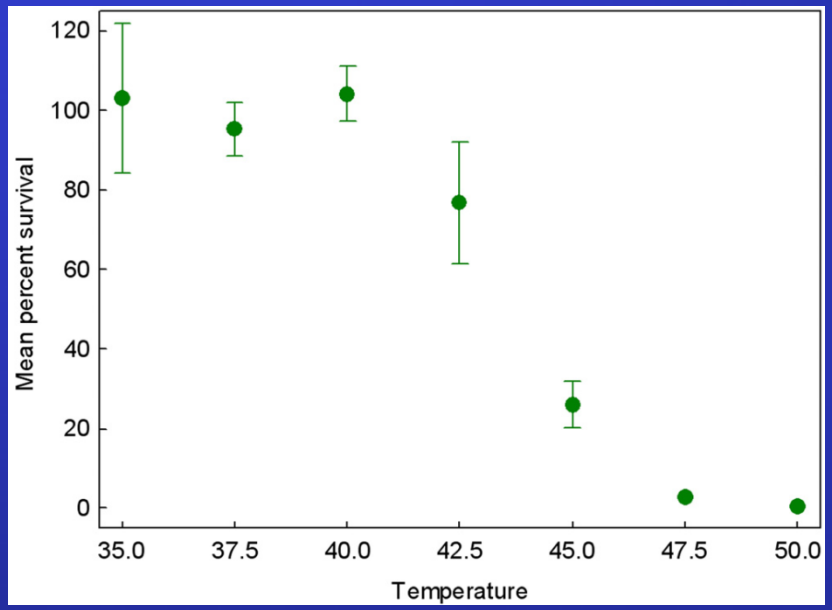
P. syringae bacteria attached to leaf surface



phage particles attached to host type-IV pilus

P. syringae host cell

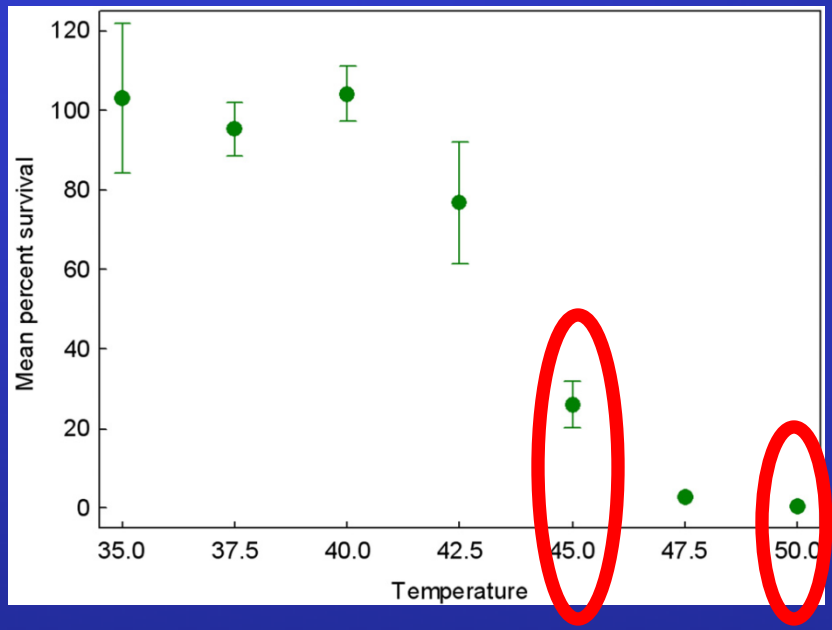
Reaction norm for wt phi-6 following 5-min heat-shock

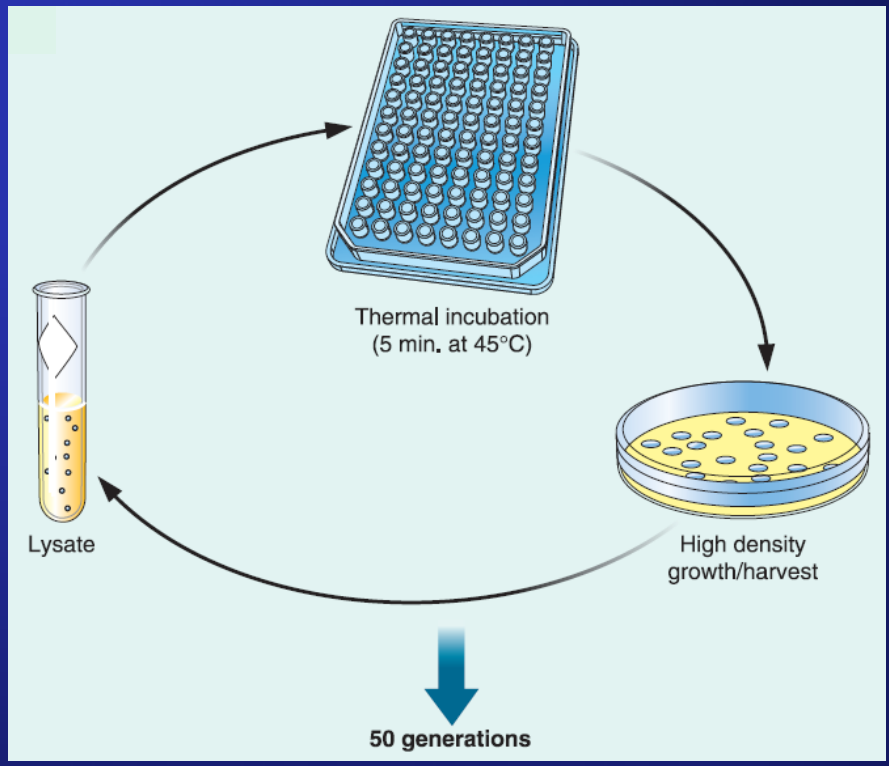


Typical lab environment: 25^{o}C

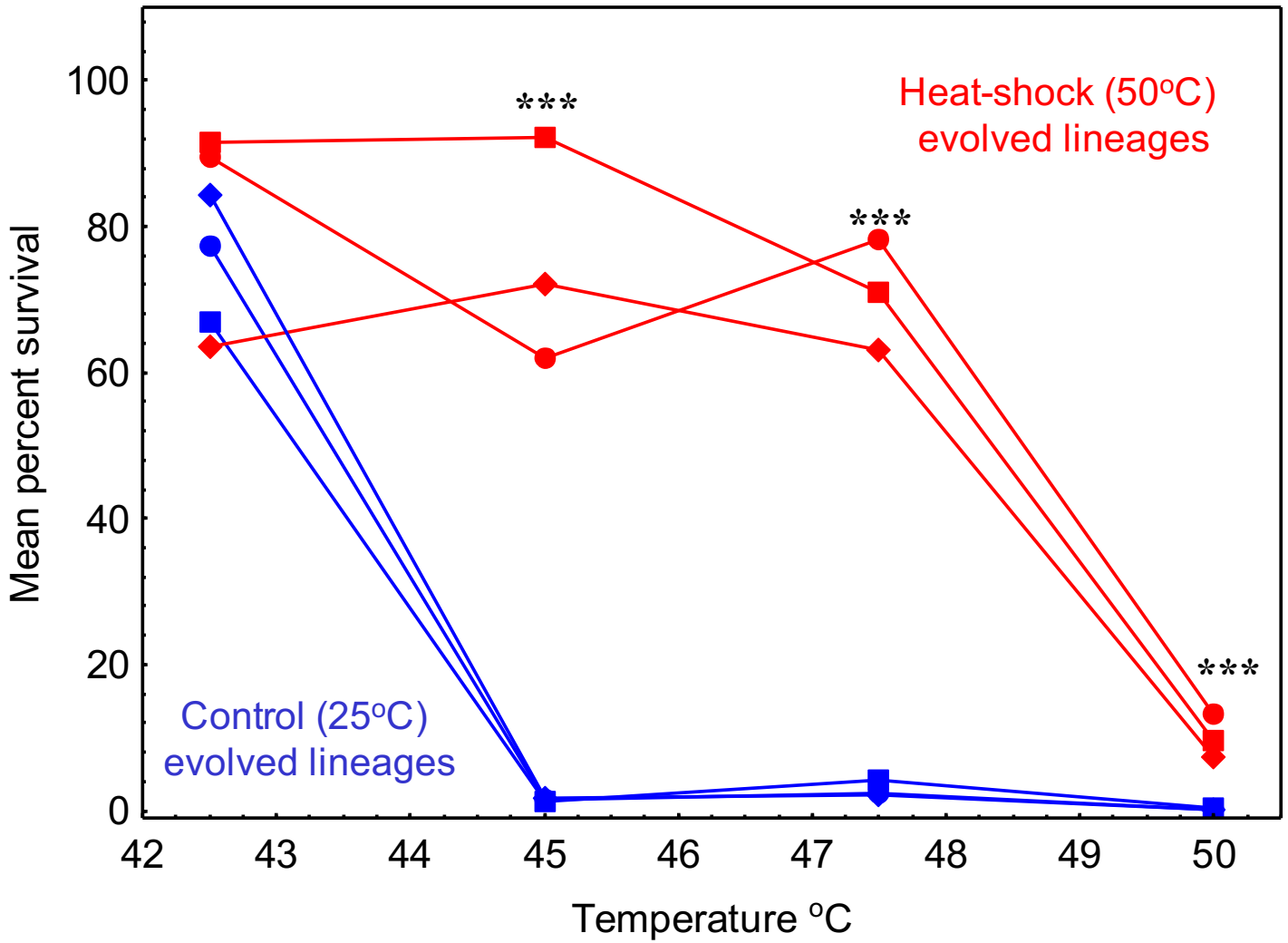
McBride et al. 2008, *BMC Evol Biol*

Can thermotolerance evolve in dsRNA phages?





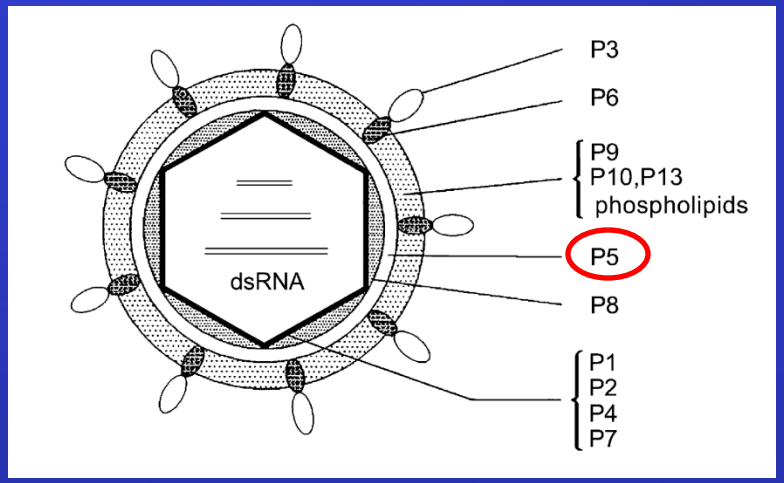
Results: Heat shock (thermotolerance) selection improves environmental robustness



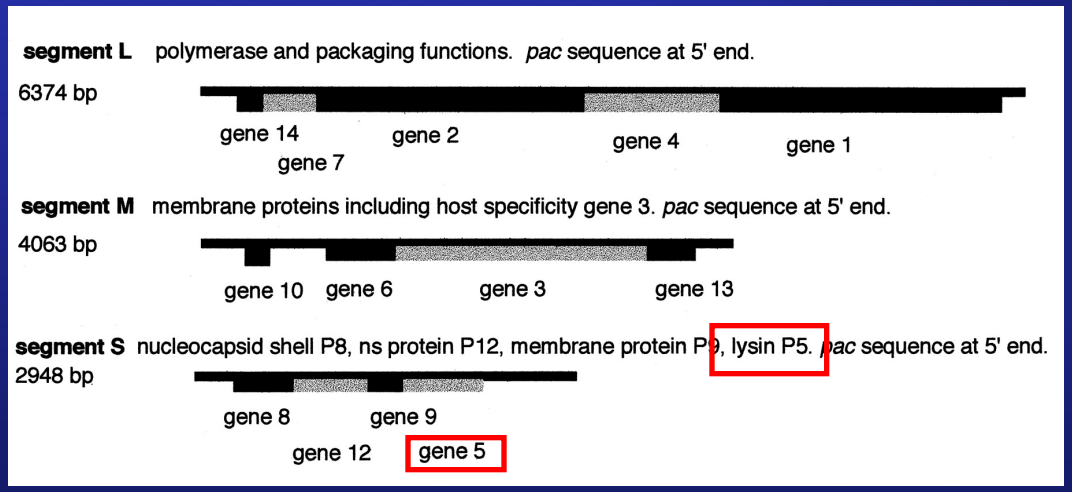
Dessau, Goldhill et al. 2012 *PLoS Genetics*

See also:
McBride et al. 2008
Goldhill et al. 2014

How does thermotolerance evolve in phi-6?



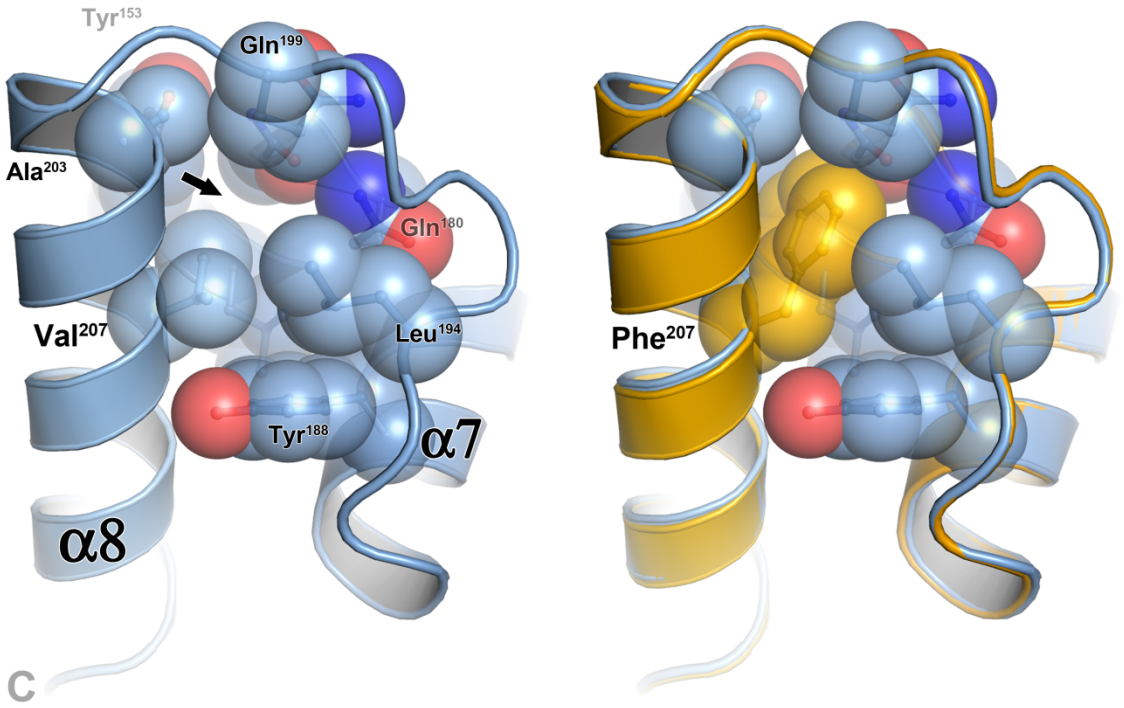
S segment:
P5 lysin gene mutation
V207F (G2238U transversion)



V207F in P5 lysin enzyme causes thermotolerance

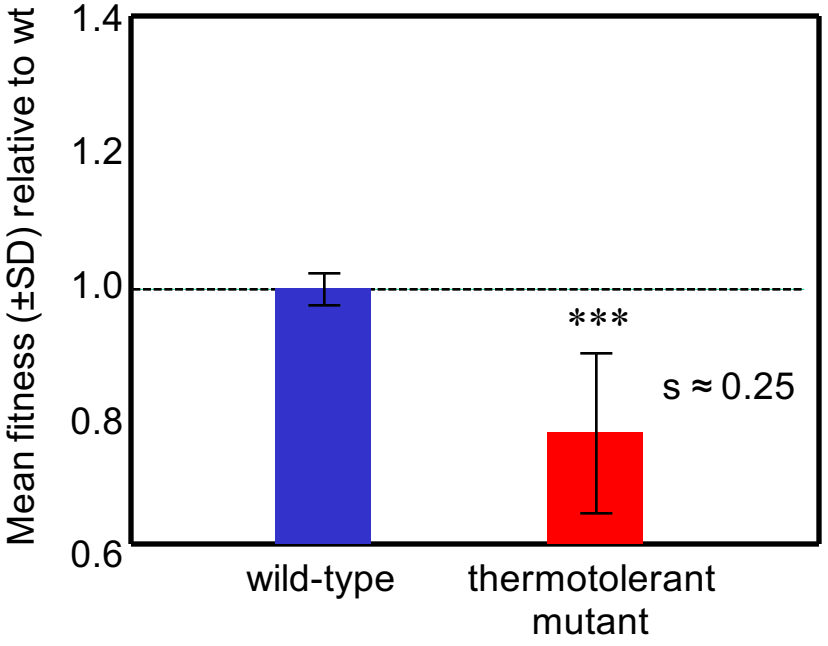
- Structure
  - X-ray crystallography
- Stability
  - Circular Dichroism
- Activity
  - Enzyme assay

Phenylalanine fills a hydrophobic pocket stabilizing the protein

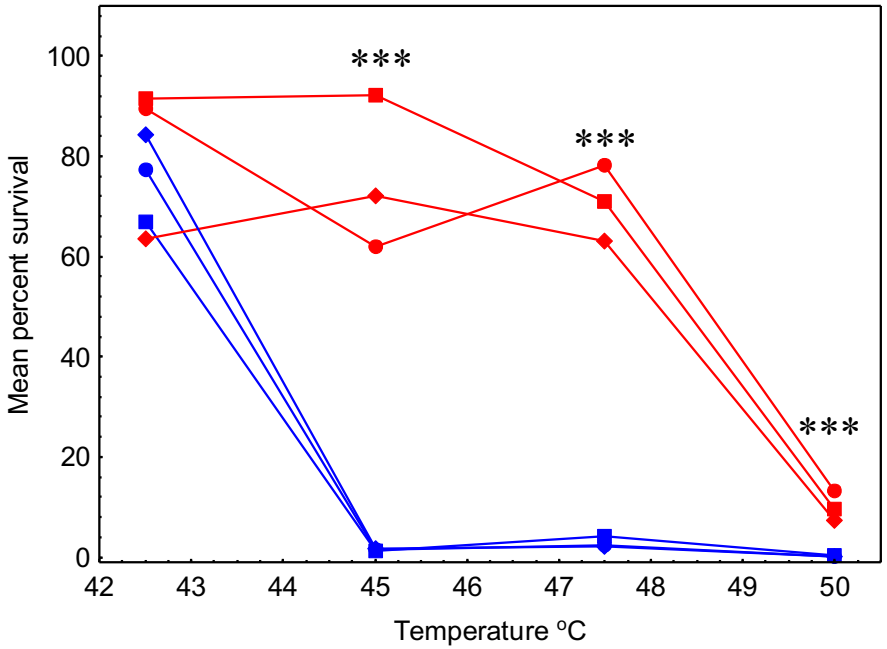


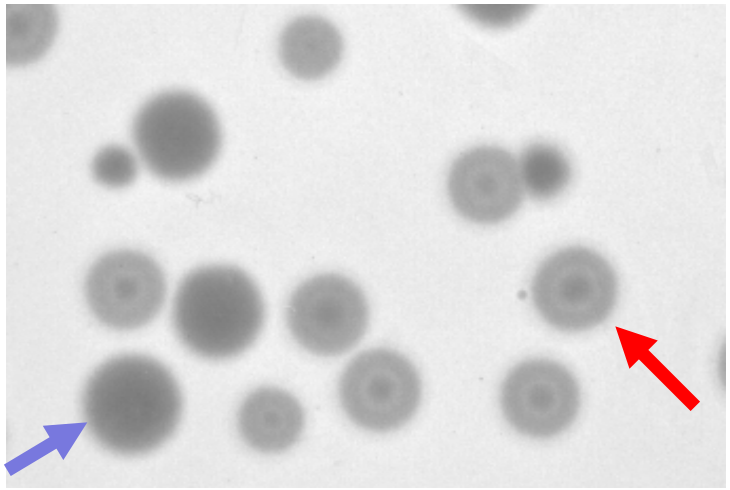
Dessau, Goldhill et al. 2012 *PLoS Genetics*

Reproduction at 25°C



Survival at 45 – 50°C





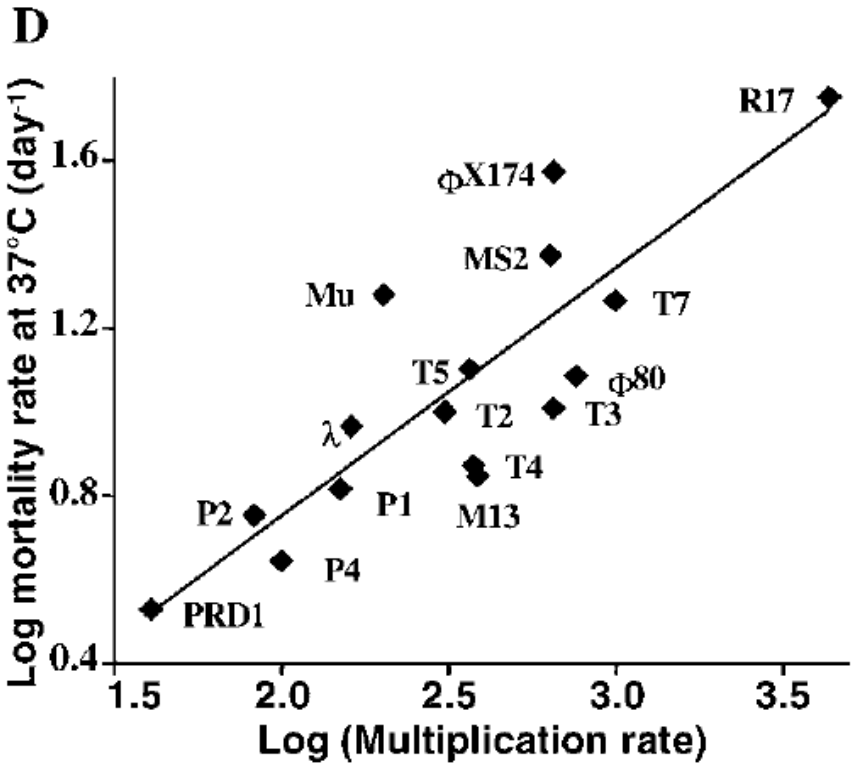
V207F causes 'life-history' tradeoff between survival and reproduction

Dessau, Goldhill et al. 2012 *PLoS Genetics*

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

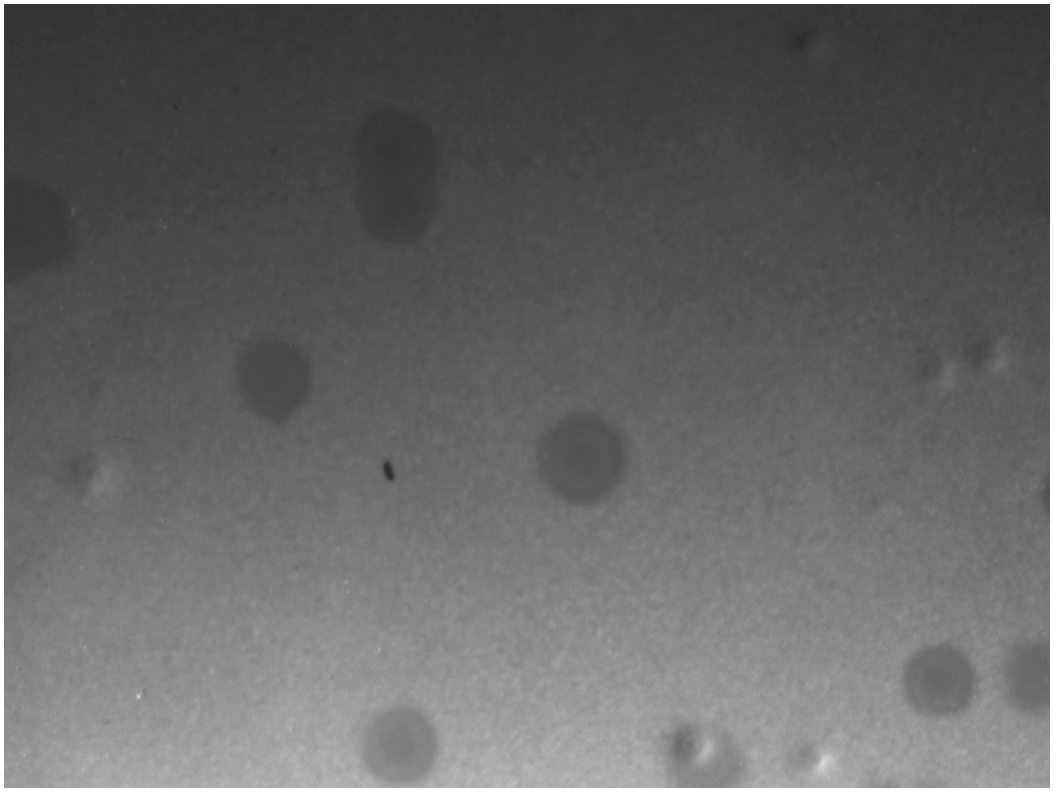
Marianne De Paepe, François Taddei*

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



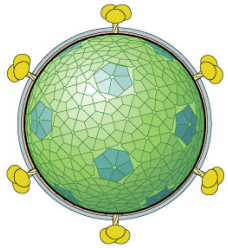
de Paepe and Taddei 2006 (PLoS Biol)

Time lapse video of bull's-eye plaque formation

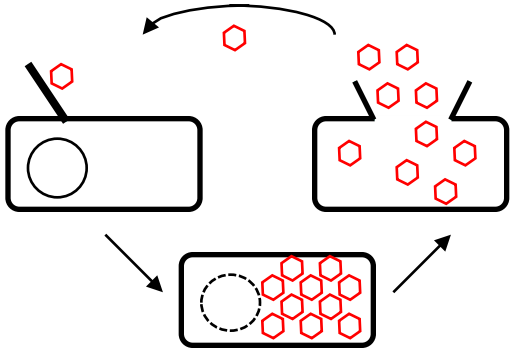


Can phage therapy exploit evolutionary trade-offs?

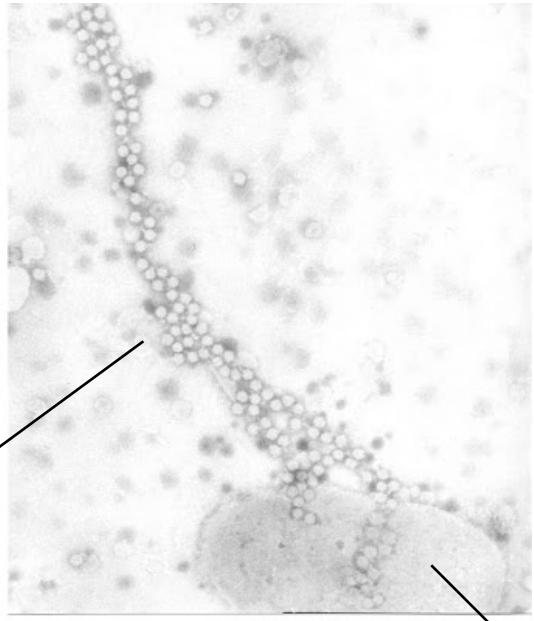
Example: phage phi-6 infection of *Pseudomonads*



- infects *Pseudomonas syringae* pathovars
- 13kb dsRNA segmented genome
- lytic infection cycle



P. syringae bacteria attached to leaf surface

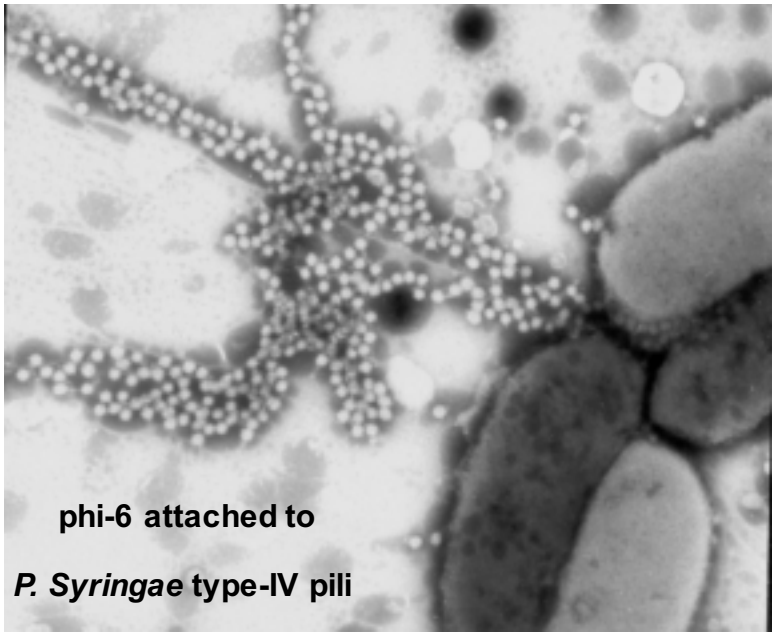


phage particles attached to host type-IV pilus

P. syringae host cell

Resistance to phage phi-6 reduces *P. phaseolicola* virulence

P. syringae pv. *phaseolicola* can evolve resistance to phi6 by eliminating type-IV pili



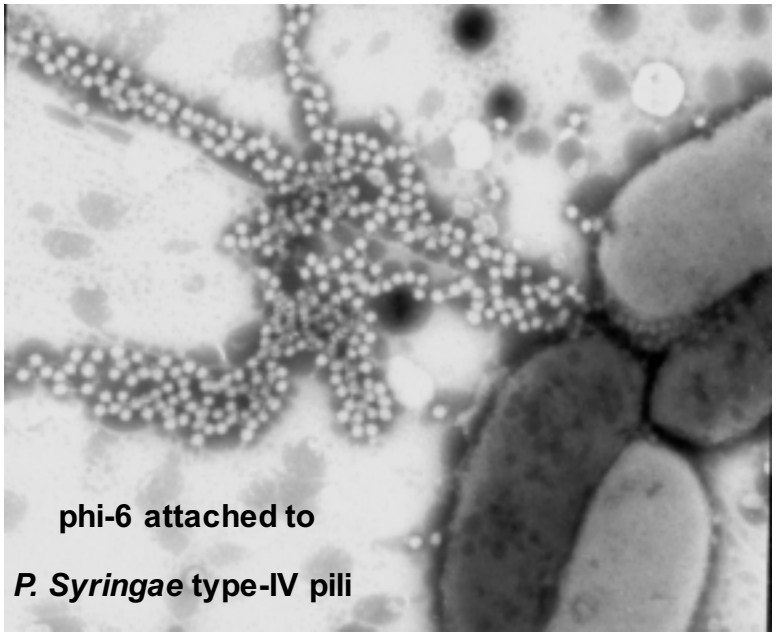
Halo blight disease

Pilus loss reduces conditional virulence
(cannot traverse leaves, but still infectious)
Evolutionary Trade-off

Romantschuk, *Ann Rev Pytopathol* (1992)
see also: Dennehy et al. *Ecol Lett* (2007)
Sistrom et al. *PLoS ONE* (2015)

Resistance to phage phi-6 reduces *P. phaseolicola* virulence

P. syringae pv. *phaseolicola* can evolve resistance to phi6 by eliminating type-IV pili



Halo blight disease

Pilus loss reduces conditional virulence
(cannot traverse leaves, but still infectious)
Evolutionary Trade-off

Indicates phage selection can LOWER bacterial pathogenicity

Romantschuk, *Ann Rev Pytopathol* (1992)
see also: Dennehy et al. *Ecol Lett* (2007)
Sistrom et al. *PLoS ONE* (2015)

Evolution of Antibiotic Resistance

- First line antibiotics are becoming less useful as prevalence of multi-drug resistant (MDR) bacteria increases

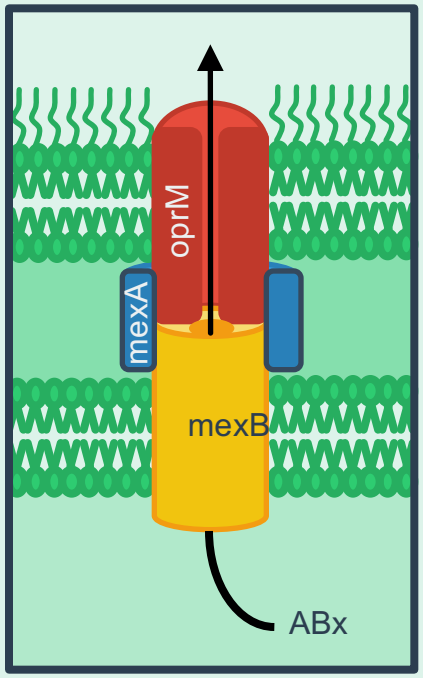
- MDR *Pseudomonas aeruginosa* particularly worrisome for: cystic fibrosis, severe burn, and immune compromised patients

Pseudomonas aeruginosa

Evolution of Antibiotic Resistance

- First line antibiotics are becoming less useful as prevalence of multi-drug resistant (MDR) bacteria increases

- MDR *Pseudomonas aeruginosa* particularly worrisome for: cystic fibrosis, severe burn, and immune compromised patients



Efflux pumps are transport proteins that help some bacteria to efficiently remove a wide variety of drugs from the cell

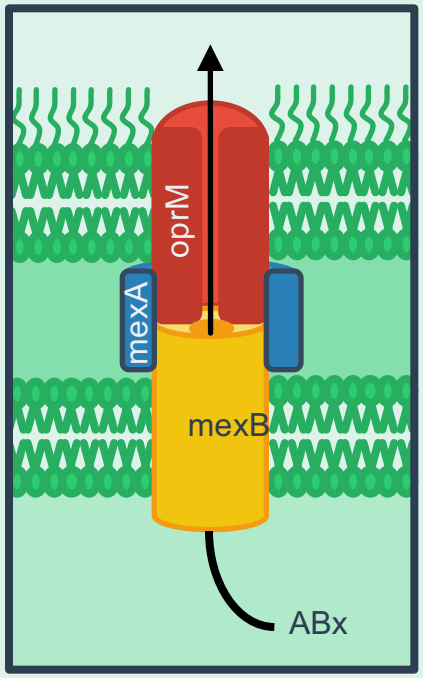
Also function in host colonization, evasion of host immunity, and biofilm formation

Pseudomonas aeruginosa

Evolution of Antibiotic Resistance

- First line antibiotics are becoming less useful as prevalence of multi-drug resistant (MDR) bacteria increases

- MDR *Pseudomonas aeruginosa* particularly worrisome for: cystic fibrosis, severe burn, and immune compromised patients



Efflux pumps

- Typically chromosome encoded; genetically conserved
- Generally found in Gram negative bacteria
- Major determinant of aminoglycoside, macrolide, and tetracycline resistance in *P. aeruginosa*

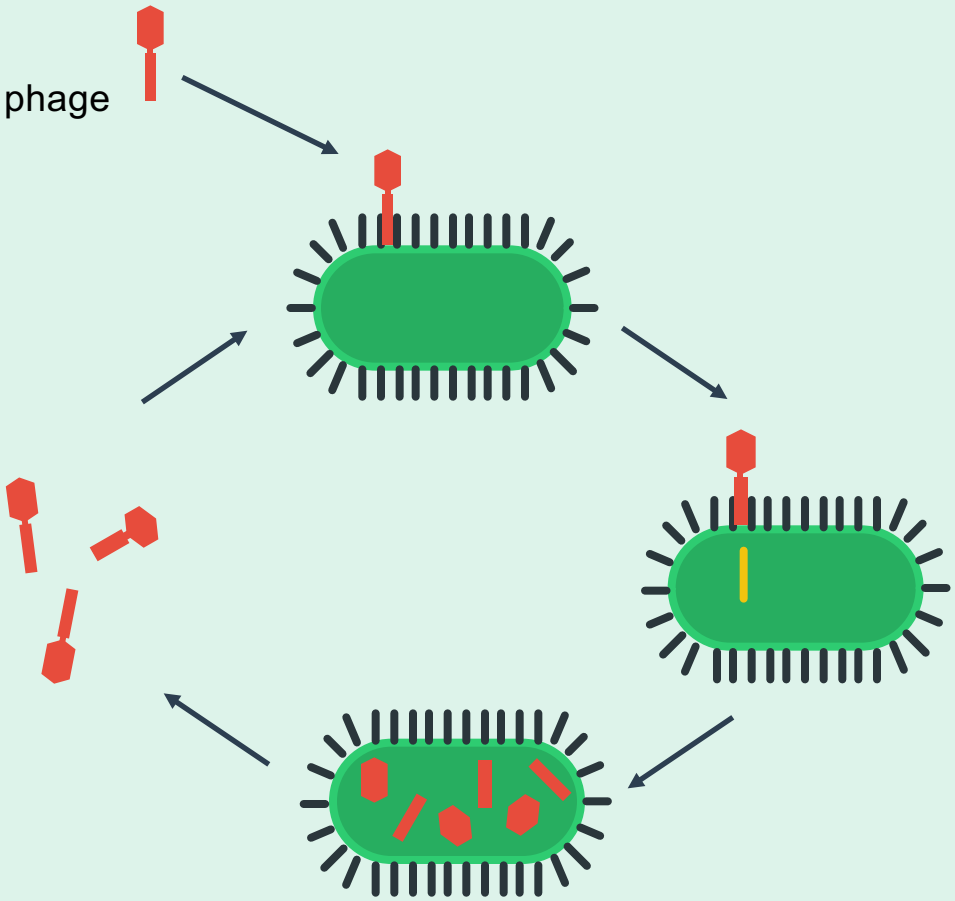
Phage Therapy

- While Western medicine invested in antibiotics Russians and others developed phages as ‘drugs’.

Russian soldiers in WW II

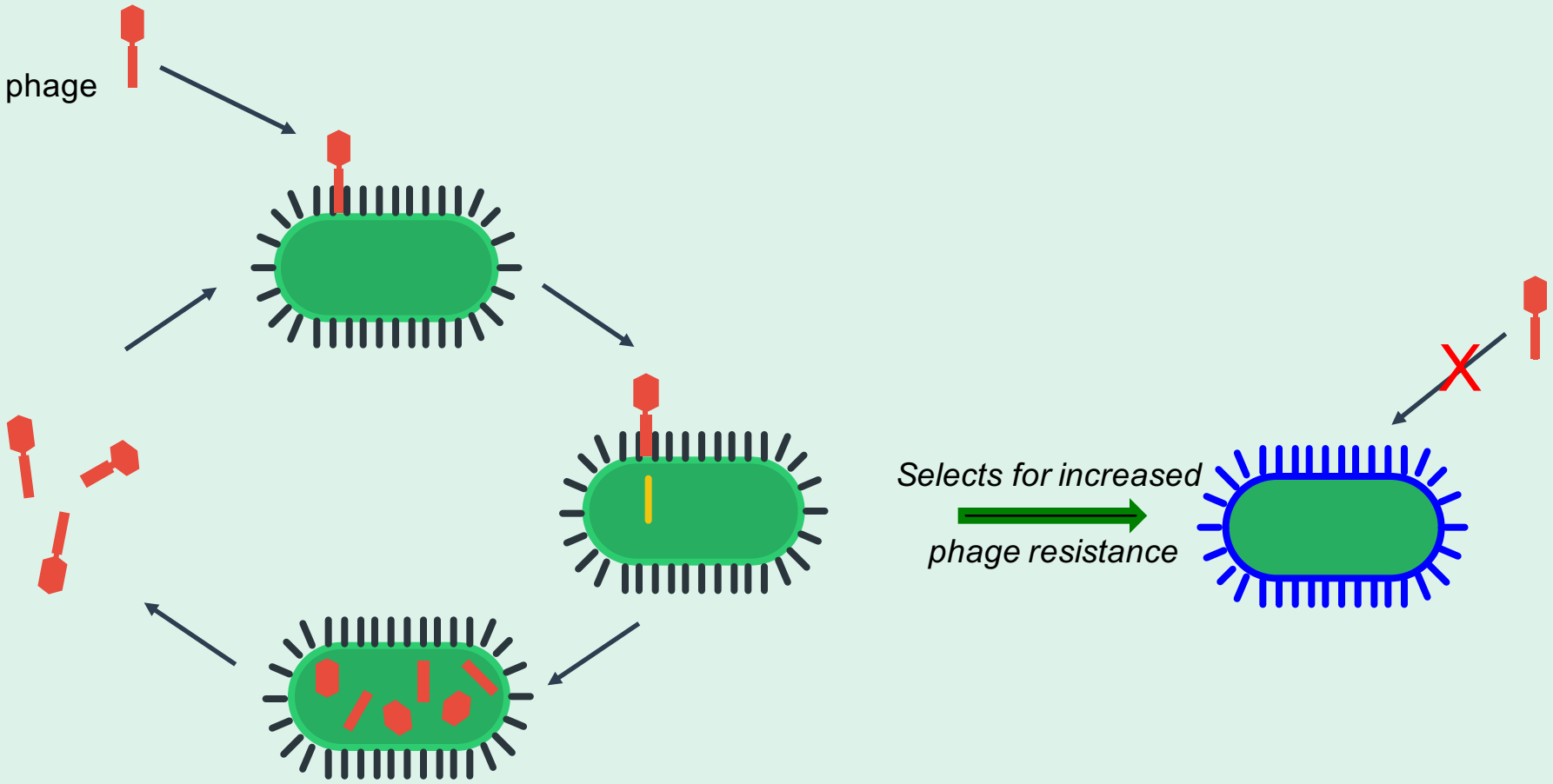
Cholera rehydration

Forcing a Genetic Trade-off to Improve Antimicrobial Therapy



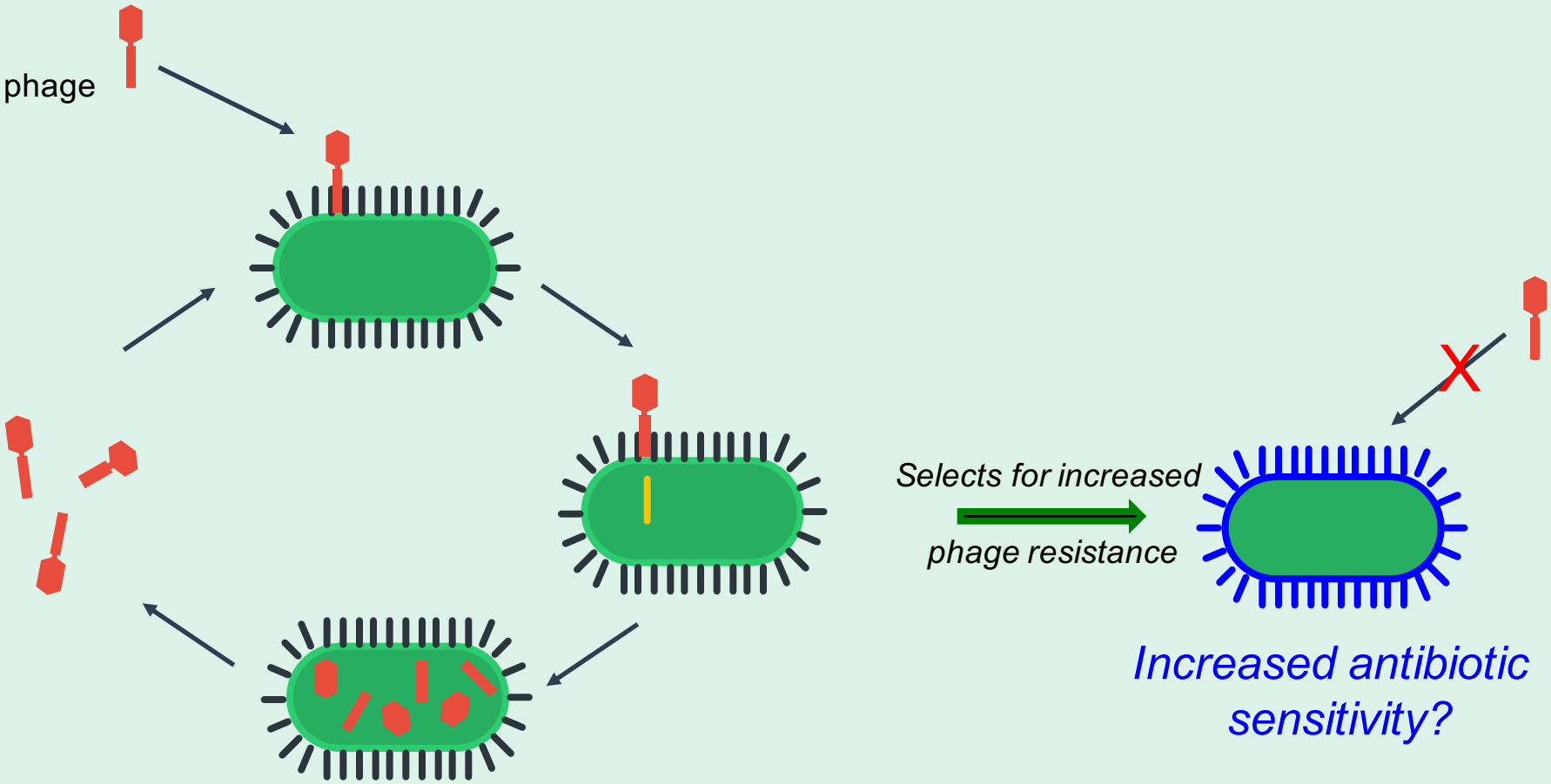
Infection cycle
of lytic bacteriophage

Forcing a Genetic Trade-off to Improve Antimicrobial Therapy



Infection cycle
of lytic bacteriophage

Forcing a Genetic Trade-off to Improve Antimicrobial Therapy



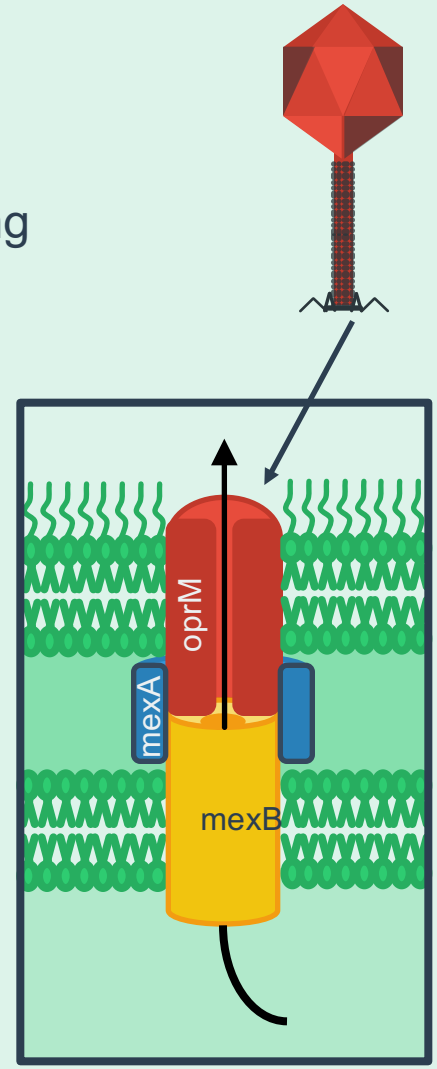
A genetic trade-off between phage resistance and antibiotic sensitivity would:

- Improve antimicrobial therapy
- Extend the lifetime of our current antibiotic arsenal

Forcing a Genetic Trade-off to Improve Antimicrobial Therapy

Phage OMKO1 (family Myoviridae)

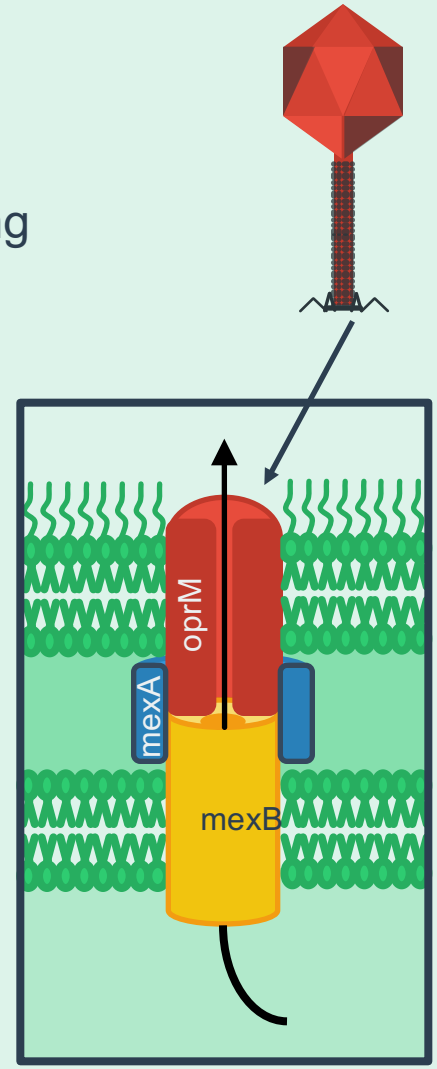
- Lytic phage that binds to oprM on cell surface (confirmed using mutant knockout library)
- Discovered, sequenced (242kb genome), and characterized at Yale University in 2016



Forcing a Genetic Trade-off to Improve Antimicrobial Therapy

Phage OMKO1 (family Myoviridae)

- Lytic phage that binds to oprM on cell surface (confirmed using mutant knockout library)
- Discovered, sequenced (242kb genome), and characterized at Yale University in 2016



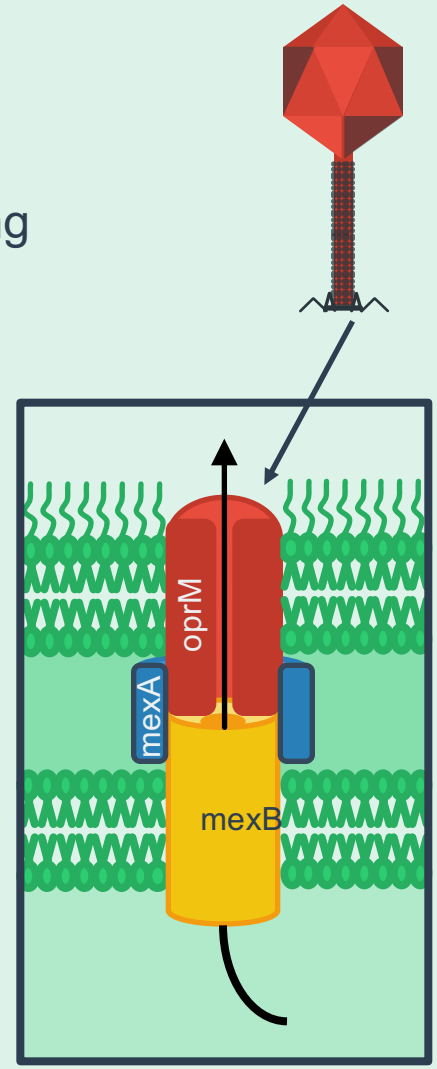
Phage OMKO1 forces a genetic trade-off:

- Phage sensitive bacteria can efflux antibiotics but are killed by phage OMKO1
- Phage resistant mutants have impaired ability to efflux antibiotics

Forcing a Genetic Trade-off to Improve Antimicrobial Therapy

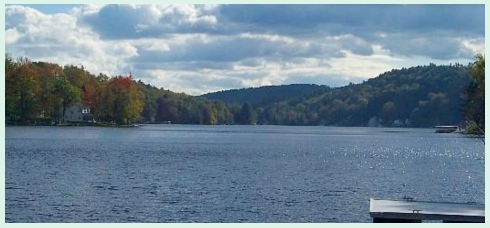
Phage OMKO1 (family Myoviridae)

- Lytic phage that binds to oprM on cell surface (confirmed using mutant knockout library)
- Discovered, sequenced (242kb genome), and characterized at Yale University in 2016



Phage OMKO1 forces a genetic trade-off:

- Phage sensitive bacteria can efflux antibiotics but are killed by phage OMKO1
- Phage resistant mutants have impaired ability to efflux antibiotics



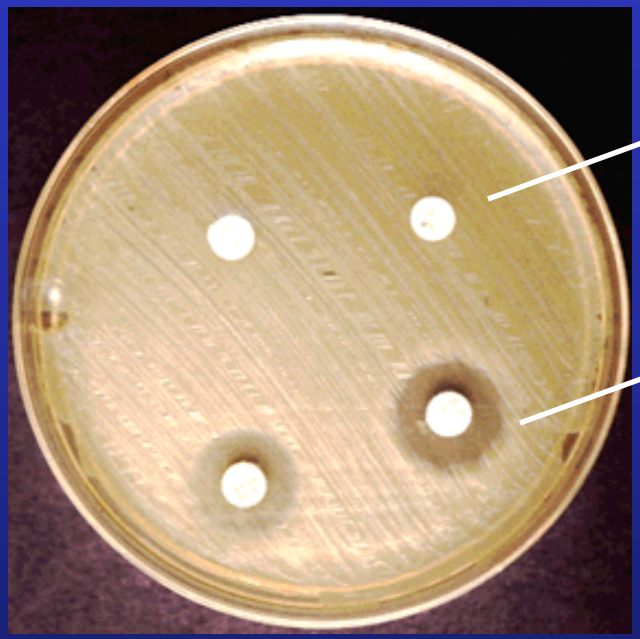
Lake in Connecticut

Using Evolution-thinking to Improve Phage Therapy

- Evolution of *P. aeruginosa* resistance to phage OMKO1 causes *sensitivity* to certain drugs (desired trade-off):

Phage OMKO1

Lytic virus (family *Myoviridae*)



Growth of MDR strain in presence of drug (no zone of inhibited growth)

Growth of phage-resistant MDR strain; kill zone measures minimum inhibitory concentration (MIC)

Chan et al. 2016, *Scientific Reports*

Forcing a Genetic Trade-off to Improve Antimicrobial Therapy in Clinical Isolates

Efflux provides resistance

Antibiotic	Class	Isolate MIC (mg/L)	Phage Resistant Isolate MIC (mg/L)	Fold-increased Drug Sensitivity
Tetracycline	Tetracycline	92.1	7.15	12.88
Erythromycin	Macrolide	265.5	21.75	12.21

Chan et al. 2016, *Scientific Reports*

Forcing a Genetic Trade-off to Improve Antimicrobial Therapy in Clinical Isolates

	Antibiotic	Class	Isolate MIC (mg/L)	Phage Resistant Isolate MIC (mg/L)	Fold-increased Drug Sensitivity
Efflux provides resistance	Tetracycline	Tetracycline	92.1	7.15	12.88
	Erythromycin	Macrolide	265.5	21.75	12.21
Efflux may provide resistance	Gentamicin*	Aminoglycoside	2.41	1.13	2.13
	Tobramycin*	Aminoglycoside	3.63	1.12	3.24
	Ciprofloxacin*	Fluoroquinolone	3.1	0.77	4.03
	Ceftazidime	Cephalosporin	1.12	0.45	2.49

* > 1 isolate showed reversal from clinical resistance to susceptibility (EUCAST 2015 breakpoints)

Chan et al. 2016, *Scientific Reports*

Forcing a Genetic Trade-off to Improve Antimicrobial Therapy in Clinical Isolates

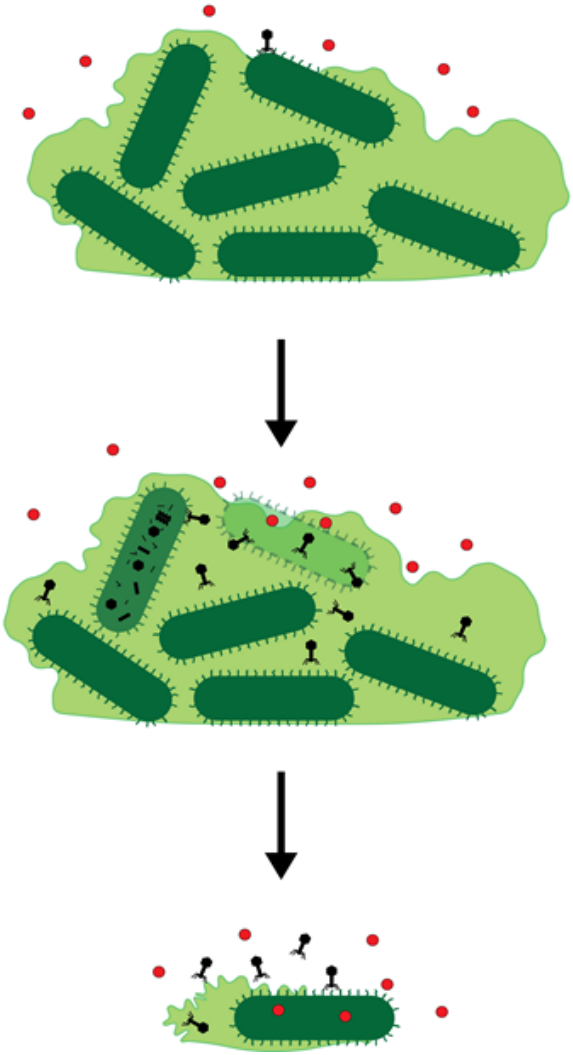
	Antibiotic	Class	Isolate MIC (mg/L)	Phage Resistant Isolate MIC (mg/L)	Fold-increased Drug Sensitivity
Efflux provides resistance	Tetracycline	Tetracycline	92.1	7.15	12.88
	Erythromycin	Macrolide	265.5	21.75	12.21
Efflux may provide resistance	Gentamicin*	Aminoglycoside	2.41	1.13	2.13
	Tobramycin*	Aminoglycoside	3.63	1.12	3.24
	Ciprofloxacin*	Fluoroquinolone	3.1	0.77	4.03
	Ceftazidime	Cephalosporin	1.12	0.45	2.49
Efflux does not provide resistance	Ampicillin	Penicillin	>256	>256	0

ONLY drugs exported by Multi-drug efflux (Mex) pump system were affected.

* > 1 isolate showed reversal from clinical resistance to susceptibility (EUCAST 2015 breakpoints)

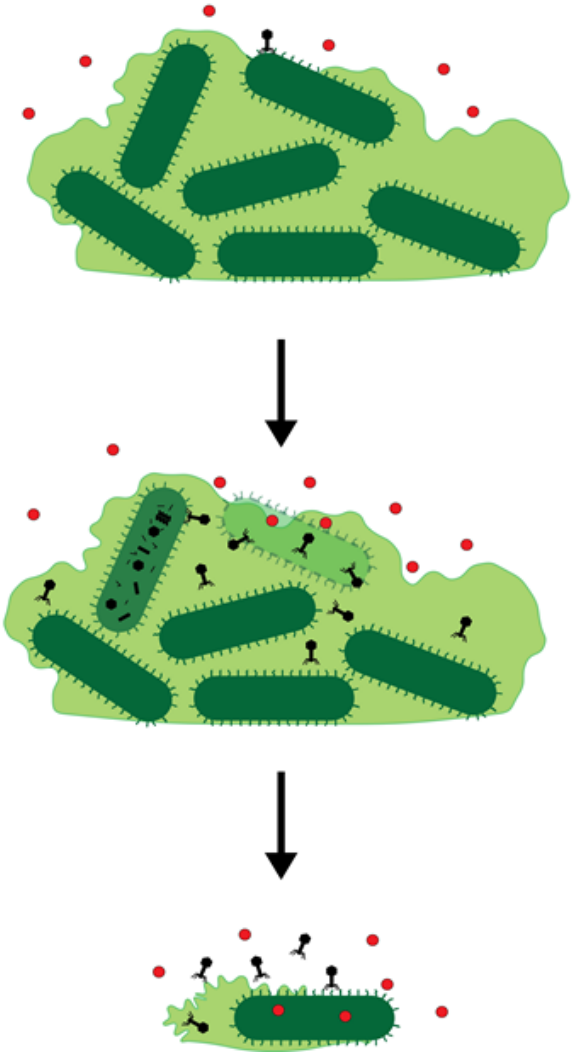
Chan et al. 2016, *Scientific Reports*

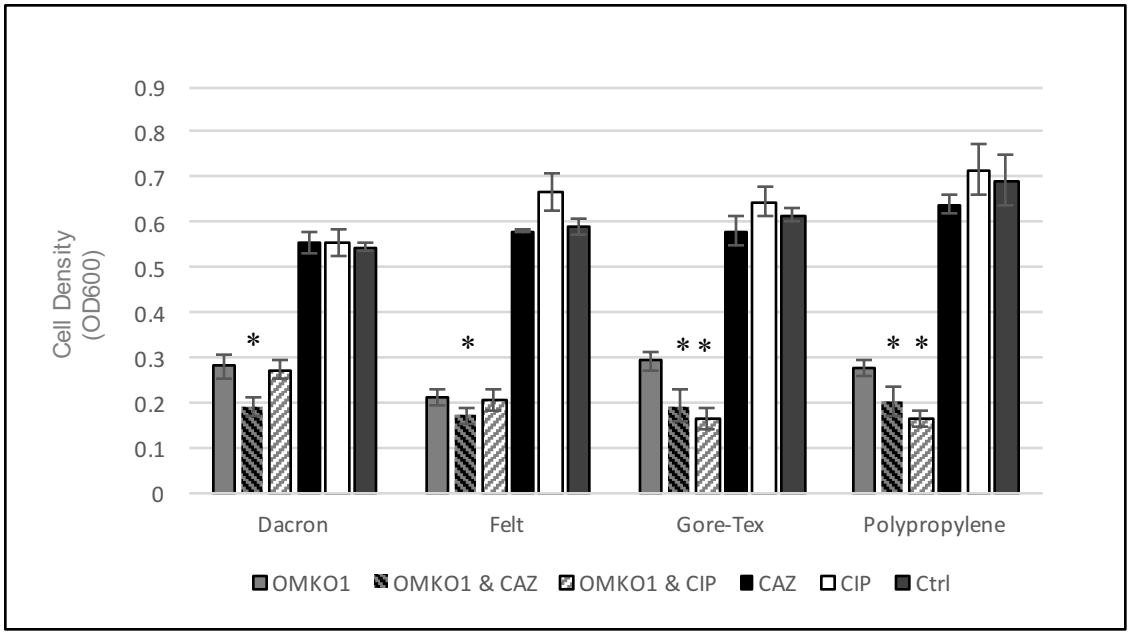
Phage OMKO1 + Antibiotic Improves Biofilm Breakdown



Chan et al. (submitted)

Phage OMKO1 + Antibiotic Improves Biofilm Breakdown





CAZ = ceftazidime
CIP = ciprofloxacin

Chan et al. (submitted)

Future Work and Applications

Trade-off observed for:

- Laboratory Model strains (PA01, PA14)
- Clinical isolates of multiple sources (otitis, diabetic foot ulcer, osteomyelitis, contaminated prosthesis)
- Environmental isolates (bacteria sampled from estuaries, and from human homes)

Objective: Examine impact of phage OMKO1 on a larger set of isolates (e.g., clinical isolate repository)

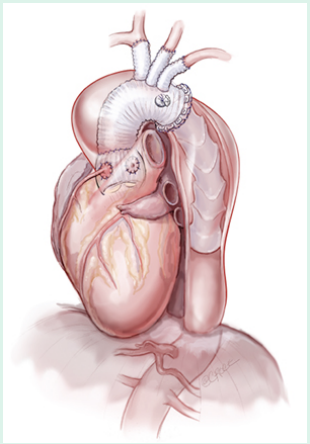
Future Work and Applications

Trade-off observed for:

- Laboratory Model strains (PA01, PA14)
- Clinical isolates of multiple sources (otitis, diabetic foot ulcer, osteomyelitis, contaminated prosthesis)
- Environmental isolates (bacteria sampled from estuaries, and from human homes)

Objective: Examine impact of phage OMKO1 on a larger set of isolates (e.g., clinical isolate repository)

Objective: With FDA approval, use phage to treat chronically infected human volunteers.
Example: Successful treatment of MDR *P. aeruginosa* biofilm infection associated with aortic arch replacement (Chan et al. submitted).



aortic arch replacement

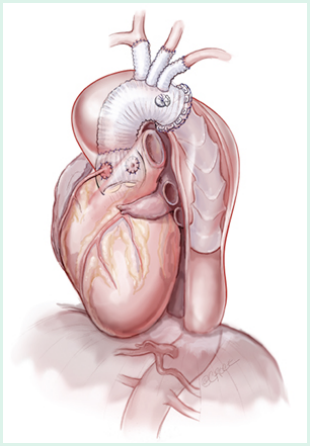
Future Work and Applications

Trade-off observed for:

- Laboratory Model strains (PA01, PA14)
- Clinical isolates of multiple sources (otitis, diabetic foot ulcer, osteomyelitis, contaminated prosthesis)
- Environmental isolates (bacteria sampled from estuaries, and from human homes)

Objective: Examine impact of phage OMKO1 on a larger set of isolates (e.g., clinical isolate repository)

Objective: With FDA approval, use phage to treat chronically infected human volunteers.
Example: Successful treatment of MDR *P. aeruginosa* biofilm infection associated with aortic arch replacement (Chan et al. submitted).



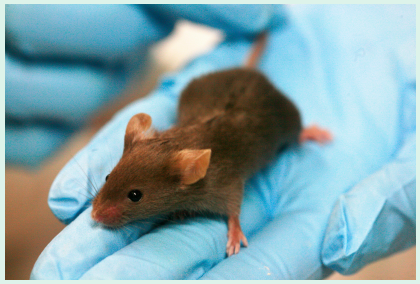
<u>See media for more info:</u>
Public Radio International's "Science Friday" (June 3, 2016)

"The People's Pharmacy" (Show 1052 – The Challenge of Antibiotic Resistant Superbugs)

Carl Zimmer – STAT online news (7 December 2016)

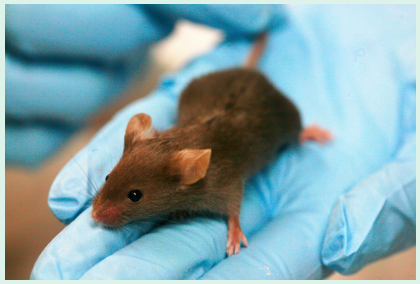
Future Work and Applications

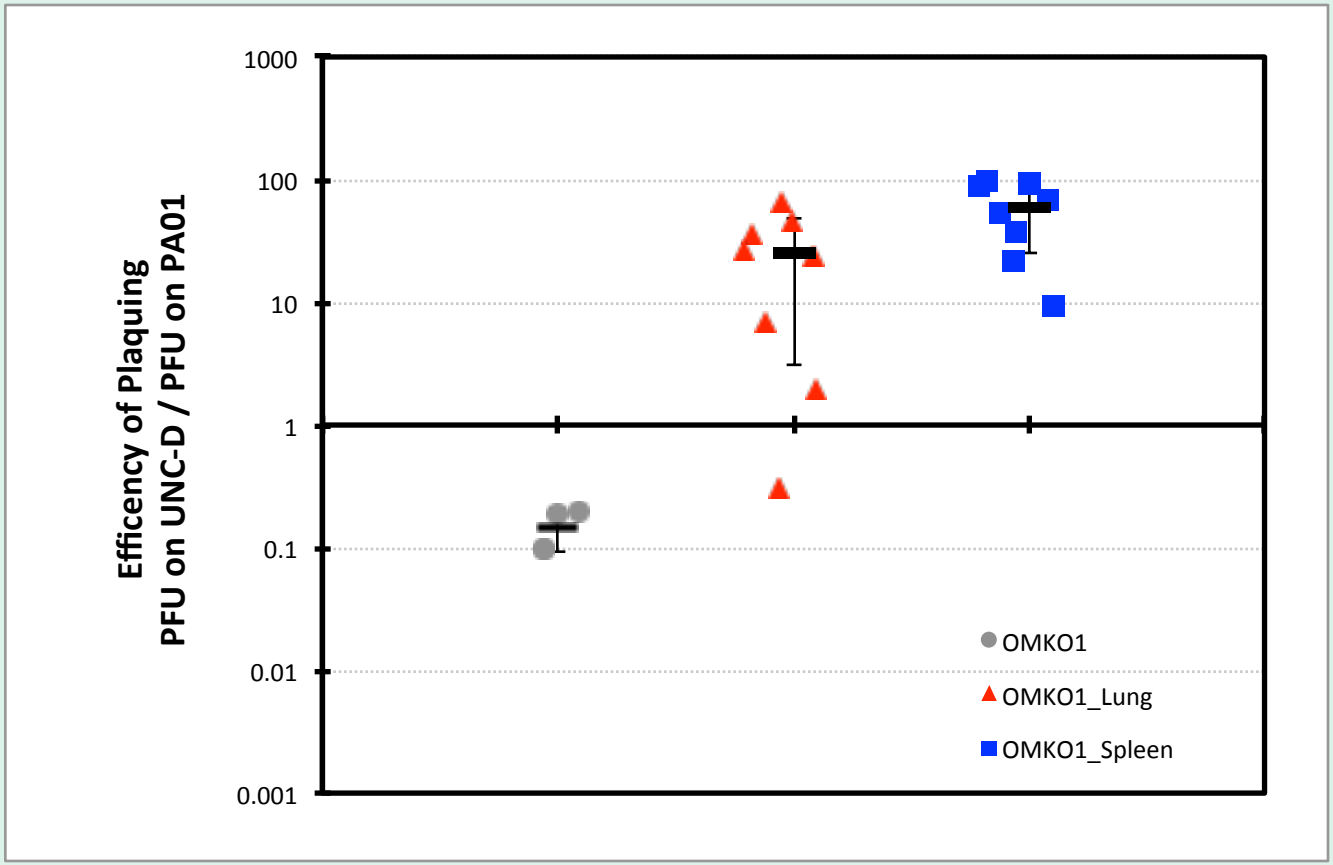
Objective: Test safety/efficacy in mouse model for lung pneumonia in immuno-compromised patients;
NIH Preclinical Services Award (2017)



Future Work and Applications

Objective: Test safety/efficacy in mouse model for lung pneumonia in immuno-compromised patients;
NIH Preclinical Services Award (2017)



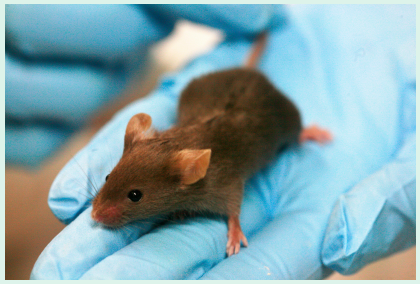


UNC-D = pathogenic strain in acute pneumonia model
PA01 = lab strain

Kortright et al. (unpublished)

Future Work and Applications

Objective: Test safety/efficacy in mouse model for lung pneumonia in immuno-compromised patients; **NIH Preclinical Services Award (2017)**



Objective: Continue our clinical application of OMKO1 (acquired IND in 2016 for compassionate use); Establish safety and efficacy of OMKO1 in a clinical trial

Target diseases for clinical trial(s):

- Hospital acquired pneumonia
- Cystic fibrosis associated pulmonary infections
- Catheter-associated UTIs.
- Burns

Chan et al. 2016, *Future Microbiology*

Future Work and Applications

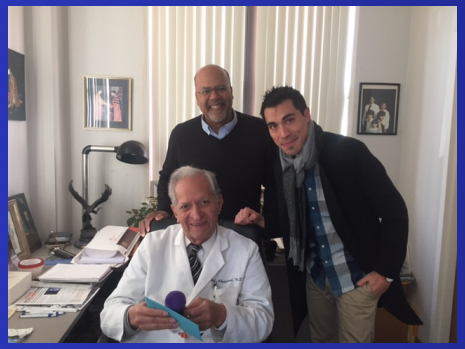
Phage binding in other medically important pathogens:

Shigella flexneri (OmpA; OmpC)
Escherichia coli (TolC)
Klebsiella pneumoniae
Salmonella typhi
Vibrio cholerae (?)

Develop phages for biocontrol
in agricultural systems

Chan et al. 2016, *Future Microbiology*

ACKNOWLEDGMENTS



Ben Chan, PhD
Research Scientist

Deepak Narayan, M.D.
Prof of Surgery, Yale School of Medicine
Chief of Plastic Surgery, West Haven VA

John Wertz, PhD
Director, Coli Genetic Stock Center at Yale

<u>Current lab members</u>
Stacy Arnold
Lisa Bono
Kevin Brown
Alita Burmeister
Ben Chan
Rachel Done
Andrea Gloria-Soria
Kaitlyn Kortright
Kenichi Okamoto

BEACON

Project
High Hopes

NSF

NATIONAL INSTITUTES OF HEALTH