Excursions into the Population and Evolutionary Dynamics of Bacteriophage

Bruce R. Levin
Department of Biology
Emory University
Atlanta, GA 30307
blevin@emory.edu

Awesome Eco-Evolutionary Dynamics in Nature and the Lab Encounter

Kavli Institute, Santa Barbara - July 26, 2017

EcLF

NATIONAL INSTITUTES OF HEALTH

Google Hangouts

Interruptions, with comments, questions, opinions or whatever are most welcome, but please speak loudly

“Bacteriophage are the most abundant organisms on earth”

Some 10^{31} phage about 10Xs that of bacteria

But we know shit about phage in natural communities of bacteria (microbiomes to be more trendy)

- Do phage regulate the densities of bacterial populations?
- What is the contribution of phage to determining the species and strain composition of bacterial communities?
- What is the contribution of phage to horizontal gene transfer and the adaptation of bacteria to their environment?
- **What are the conditions under which phage will be maintained in bacterial populations – their existence conditions?**
- **Under what conditions will temperate and lytic modes of phage replication evolve and be maintained.**

Outline

Part 1

Population and Evolutionary dynamics of bacteriophage - the issues and the questions

Lytic phage – communities dominated by bacteria

- Leaky resistance hypothesis
- Leaky resistance evidence

Temperate phage

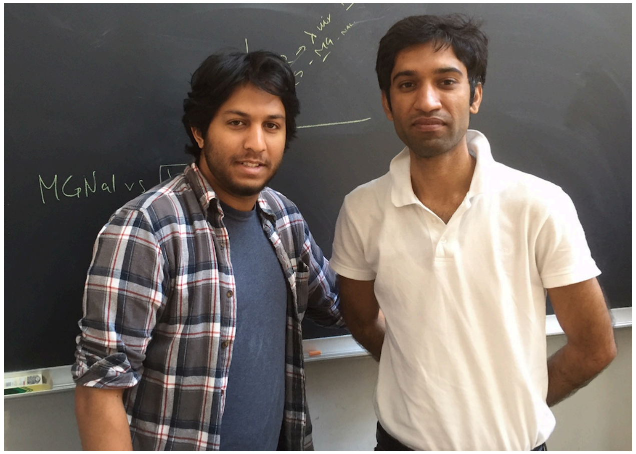
- Establishment and maintenance

Temperate and lytic phage together

Part 2 (If time permits)

Phage and the epidemiology of Cholera

The Population and Evolutionary Dynamics of Bacteriophage: The Virtues of Virulence and Temperance

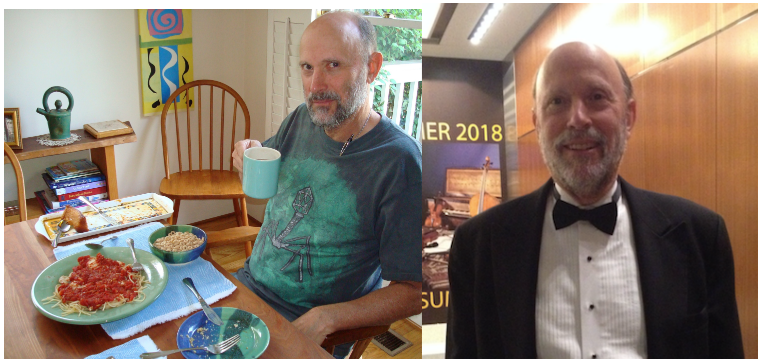


Nilang Shah Waqas Chaudhry
Emory University, Atlanta

Maros Pleska
IST, Vienna

Ingrid McCall

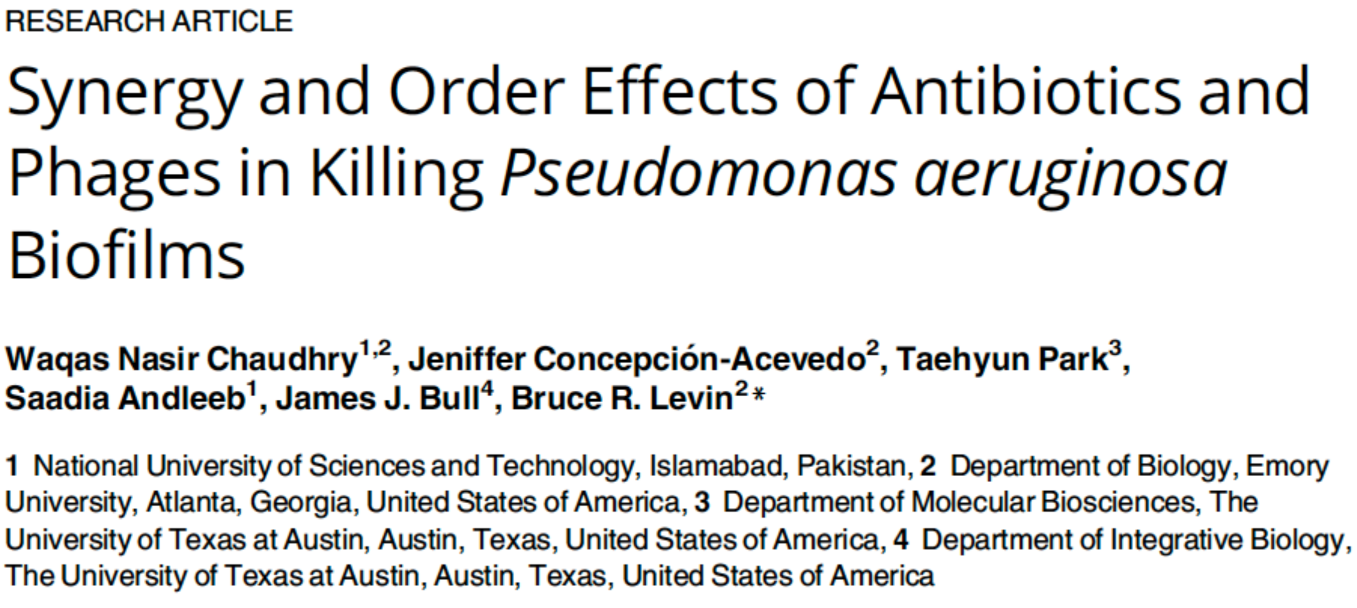
Howie Weiss
Georgia Tech



Jim (Jumbo) Bull
University of Texas

Therapeutic Phage from Tbilisi, GA

Advertisement



RESEARCH ARTICLE

Synergy and Order Effects of Antibiotics and Phages in Killing *Pseudomonas aeruginosa* Biofilms

Waqas Nasir Chaudhry[1,2], **Jeniffer Concepción-Acevedo**[2], **Taehyun Park**[3], **Saadia Andleeb**[1], **James J. Bull**[4], **Bruce R. Levin**[2]*

1 National University of Sciences and Technology, Islamabad, Pakistan, **2** Department of Biology, Emory University, Atlanta, Georgia, United States of America, **3** Department of Molecular Biosciences, The University of Texas at Austin, Austin, Texas, United States of America, **4** Department of Integrative Biology, The University of Texas at Austin, Austin, Texas, United States of America

* blevin@emory.edu

PLoS One DOI:10:1731/journal.pone
0168615. January 11, 2017

Waqas Chaudhry

Antecedents

"It was a long time ago but at the time it felt like the present".

Frank M. Stewart (1918-2011)

Necessary condition for co-existence

$P \leq N \leq P+R$

P- Phage

N –Bacteria

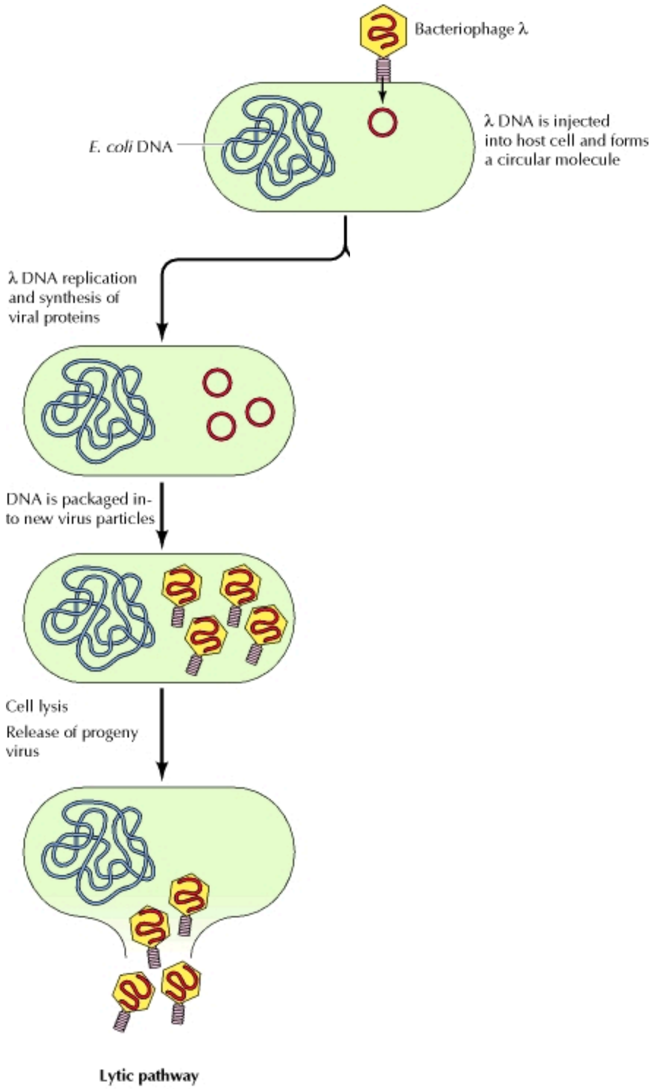
R - Resources

Campbell, A., *Conditions for the existence of bacteriophage.* Evolution, 1961. **15**: p. 153-165.

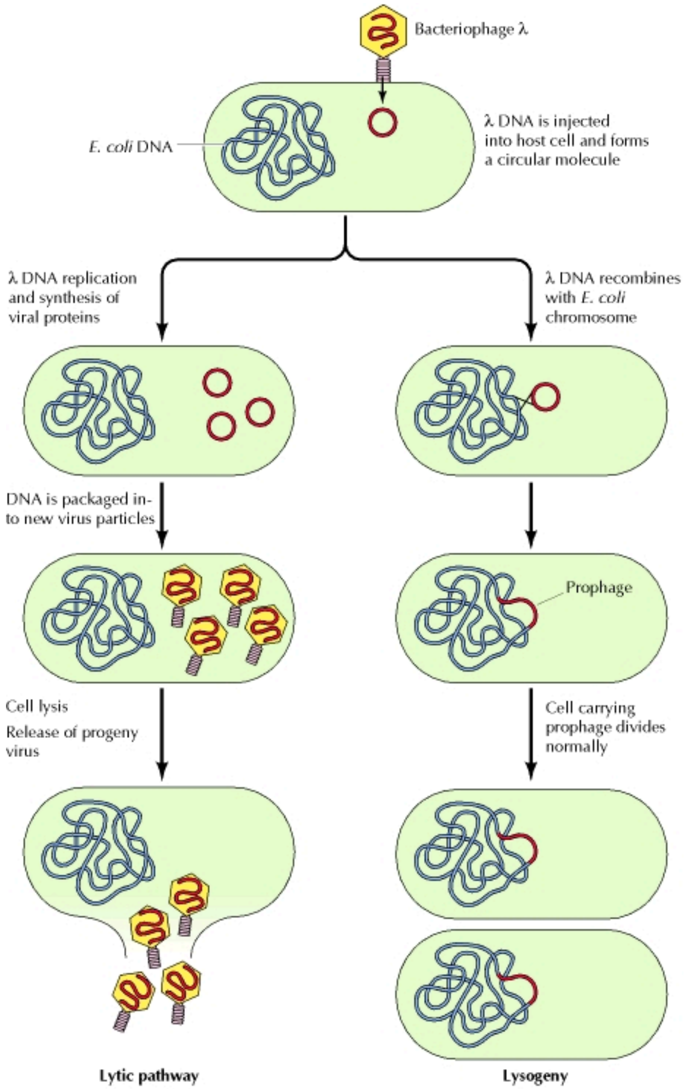
Levin, B. R., F. M. Stewart, and L. Chao. 1977. Resource - limited growth, competition , and predation: a model and experimental studies with bacteria and bacteriophage. American Naturalist 977:3-24.

Stewart, F. M., and B. R. Levin. 1984. The population biology of bacterial viruses: Why be temperate? Theor. Pop. Biol. 26:93-117.

Lytic Phage


Bacteriophage λ
E. coli DNA
λ DNA is injected into host cell and forms a circular molecule
λ DNA replication and synthesis of viral proteins
DNA is packaged into new virus particles
Cell lysis
Release of progeny virus
Lytic pathway

Temperate Phage



Virtues of Temperance (lysogeny)

- Horizontally (infectiously) transmitted
- Vertically Transmitted (can become established and maintained at any density)
- Can directly or indirectly increase the fitness of their host bacteria and thereby themselves.

The Virulent Caveat -

Lytic Mutants can replicate on lysogens - Would they not take over?

The Issues*:

While the phage are commonly maintained in experimental populations of bacteria, the communities are dominated by resistant bacteria (upon which the phage cannot grow) and their densities are limited by resources, rather than these viruses.

Temperate phage exist and are not replaced by lytic mutants

Nature does not have problems, Nature only has solutions.
(Andre Lwoff)

Coexistence of *E. coli* B and phage T7 in Chemostats

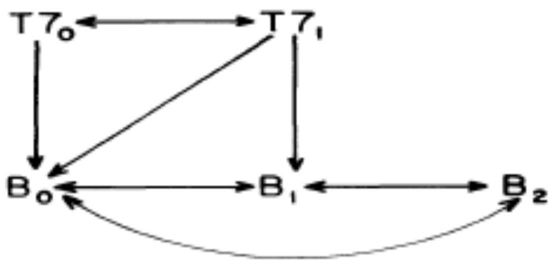


FIG. 1. Host range and resistance relationships for the T7-*Escherichia coli* B system. Horizontal arrows indicate possible directions of mutation. Vertical and diagonal arrows denote predator-prey associations. Subscripts indicate order of the various clones; "0" wild type, "1" the first order, and "2" the second order. B_2 is doubly resistant, B_1 resistant only to $T7_0$, and B_0 is sensitive to both phage.

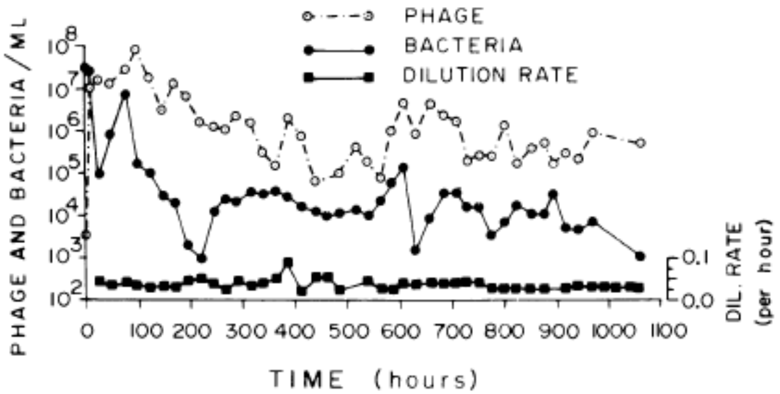


FIG. 2. A phage-limited (type A) T7-*Escherichia coli* B community in glucose minimal continuous culture. Mean dilution rate 0.036 ± 0.012/h.

372 LIN CHAO

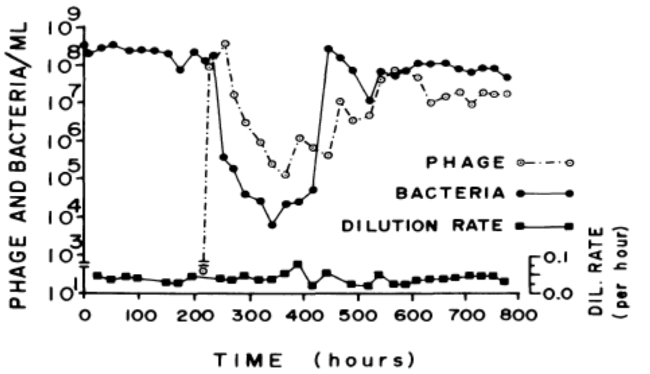
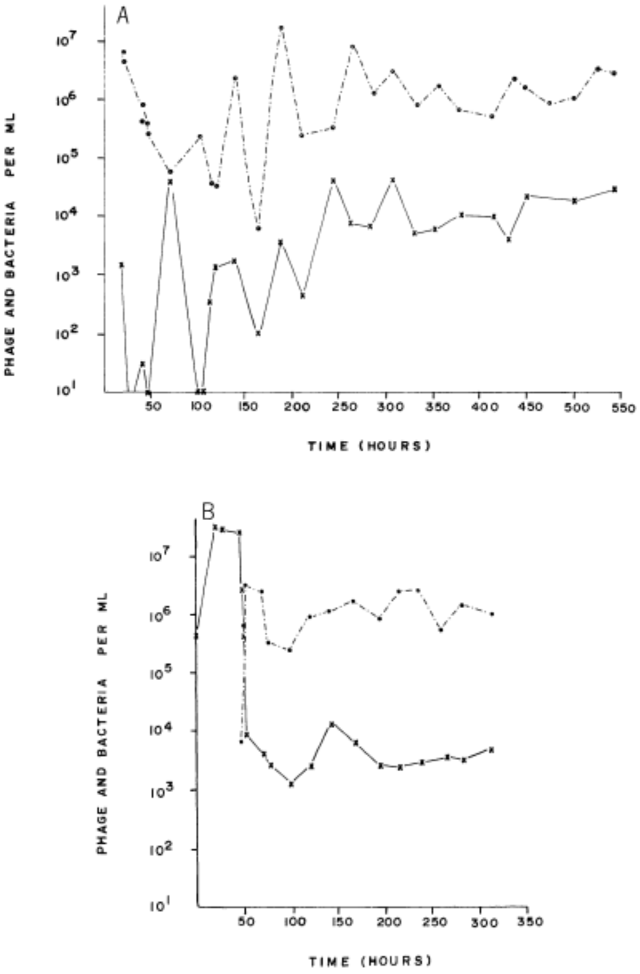


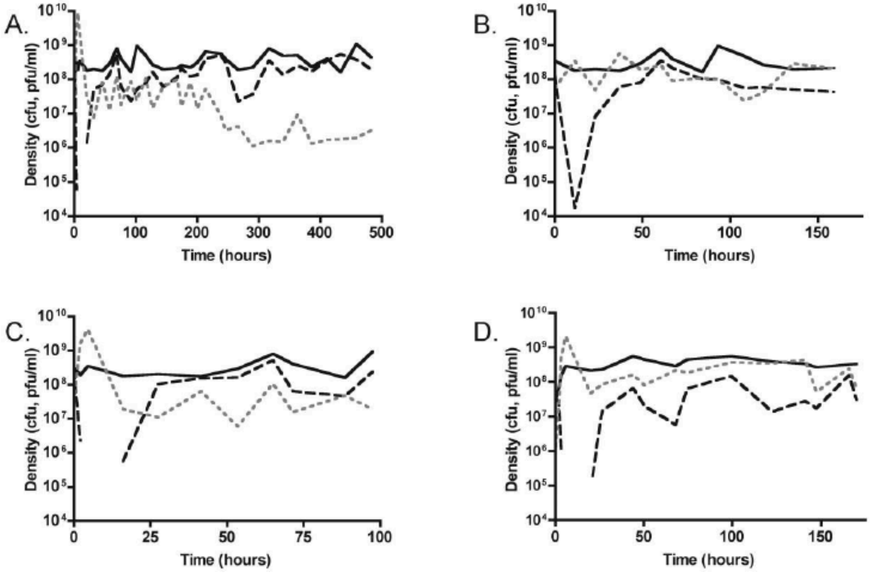
FIG. 3. A resource-limited (type B) T7-*Escherichia coli* B community in glucose minimal continuous culture. Mean dilution rate 0.040 ± 0.012/h.

Chao, L., B. R. Levin, and F. M. Stewart. 1977. A complex community in a simple habitat: an experimental study with bacteria and phage. Ecology 58:369-378.

E. coli B and Phage T2 In Chemostats



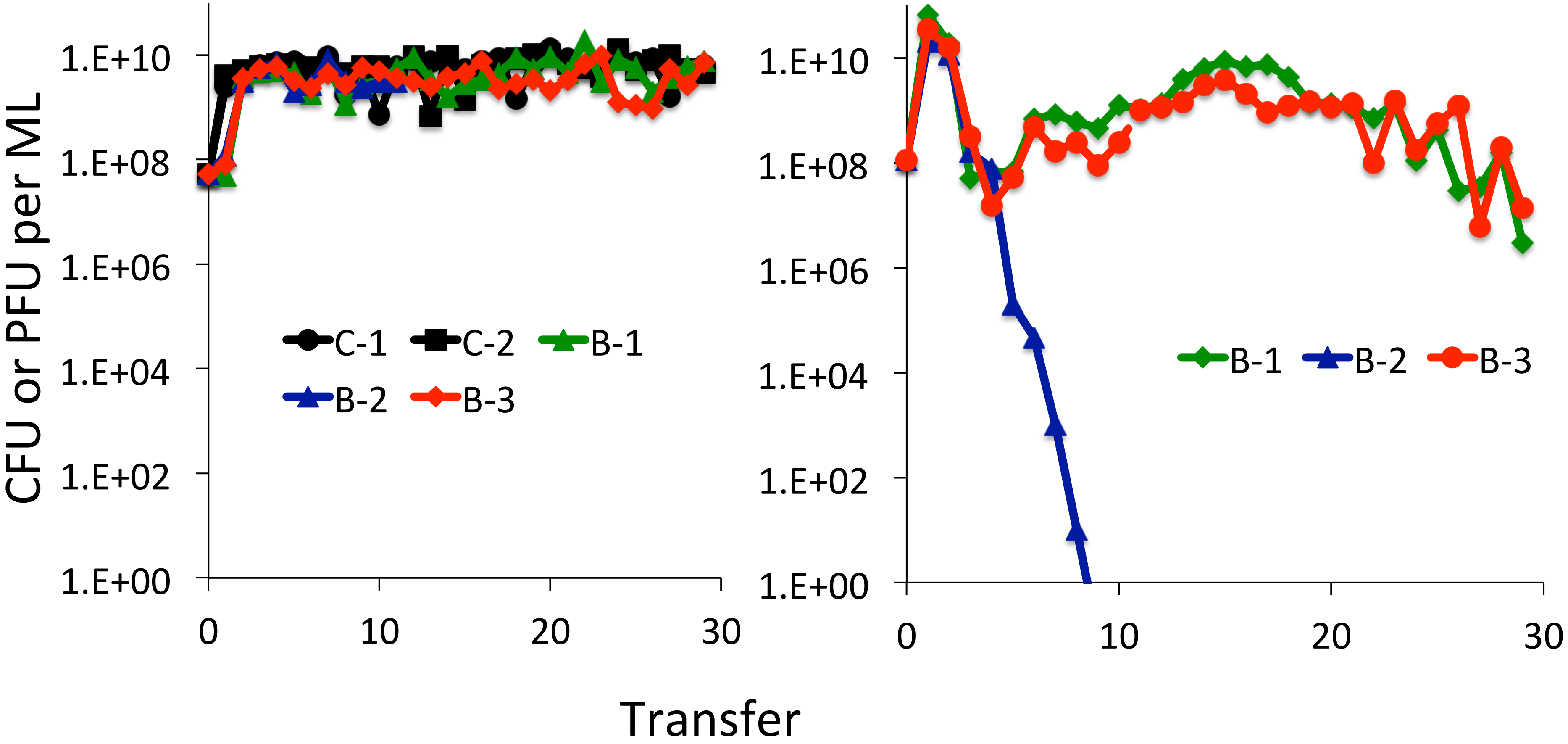
Vibrio cholerae N1961 and Peru 2 Phage in Chemostats



Levin, B. R., F. M. Stewart, and L. Chao. 1977. Resource - limited growth, competition , and predation: a model and experimental studies with bacteria and bacteriophage. American Naturalist 977:3-24.

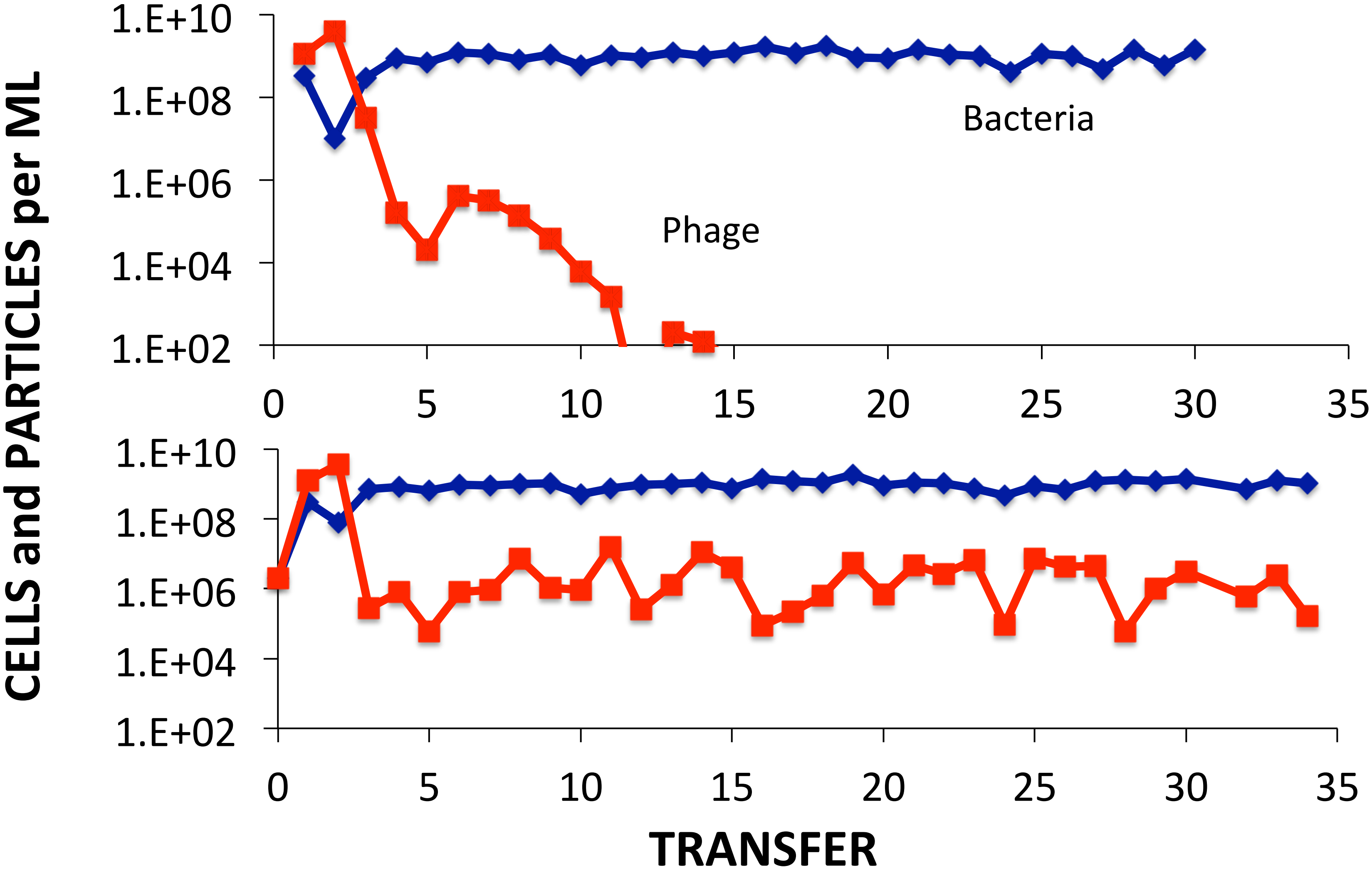
Wei, Y, A. Kirby and B.R. Levin 2011. The Population and Evolutionary Dynamics of *Vibrio cholerae* and its Bacteriophage: Conditions for Maintaining Phage-Limited Communities (The American Naturalist. 178:715-725

Pseu*domonas aeruginosa* PA14 and Phage NP3 in LB Serial Transfer Culture



Waqas Chaudhry Data

Streptococcus thermophilus and phage 2972 in Milk Serial Transfer Culture (a CRISPR-Cas Study)

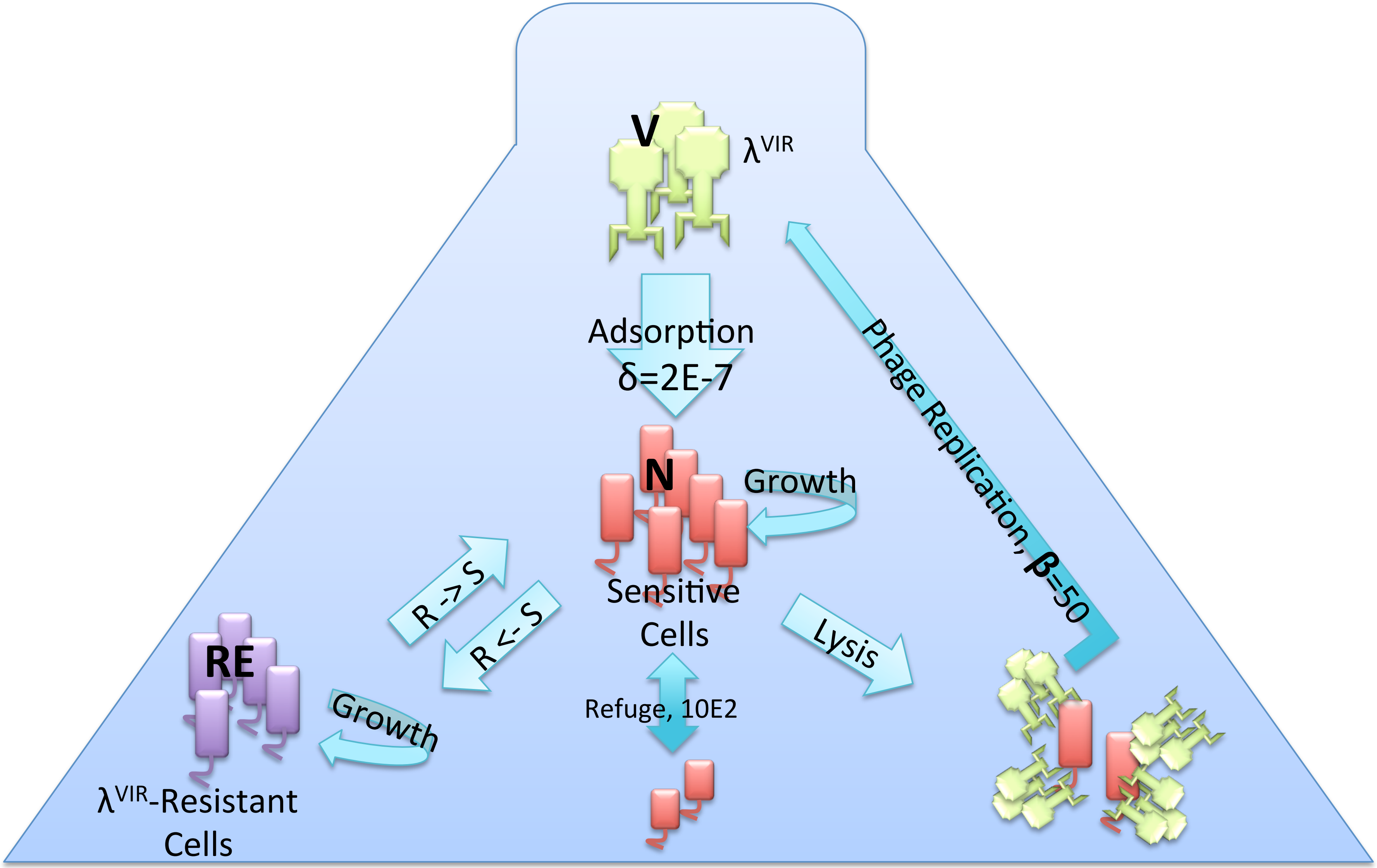


Weissman, J, H. Holmes, R. Barrengou, S Monod, W. Fagan, B. Levin and P. Johnson Immune Loss as a Driver of Coexistence During Host-Phage Coevolution

The Question

How are lytic phage maintained in communities of bacteria dominated by bacteria upon which they can’t replicate?

Model of the Population Dynamics of Lytic Phage with Sensitive and Resistant Bacteria



Model of the Population Dynamics of Lytic Phage with Sensitive and Resistant Bacteria

$$\frac{dR}{dt} = -e \cdot \psi(R) \cdot (vn \cdot N + vre \cdot RE)$$

Resource uptake

$$\frac{dN}{dt} = vn \cdot \psi(R) \cdot N \qquad - \delta \cdot N \cdot V \cdot \psi(R) \qquad - \mu n \cdot N \psi(r) \qquad + \qquad \mu r \cdot RE \cdot \psi(R)$$

Growth *phage adsorption* $N \rightarrow RE$ $RE \rightarrow N$

$$\frac{dRE}{dt} = vre \cdot \psi(R) \cdot RE \qquad - \mu r \cdot RE \cdot \psi(R) \qquad + \mu n \cdot N \cdot \psi(R)$$

Growth $RE \rightarrow N$ $N \rightarrow RE$

$$\frac{dV}{dt} = \delta \cdot N \cdot V \cdot (\beta - 1) \cdot \psi(R)$$

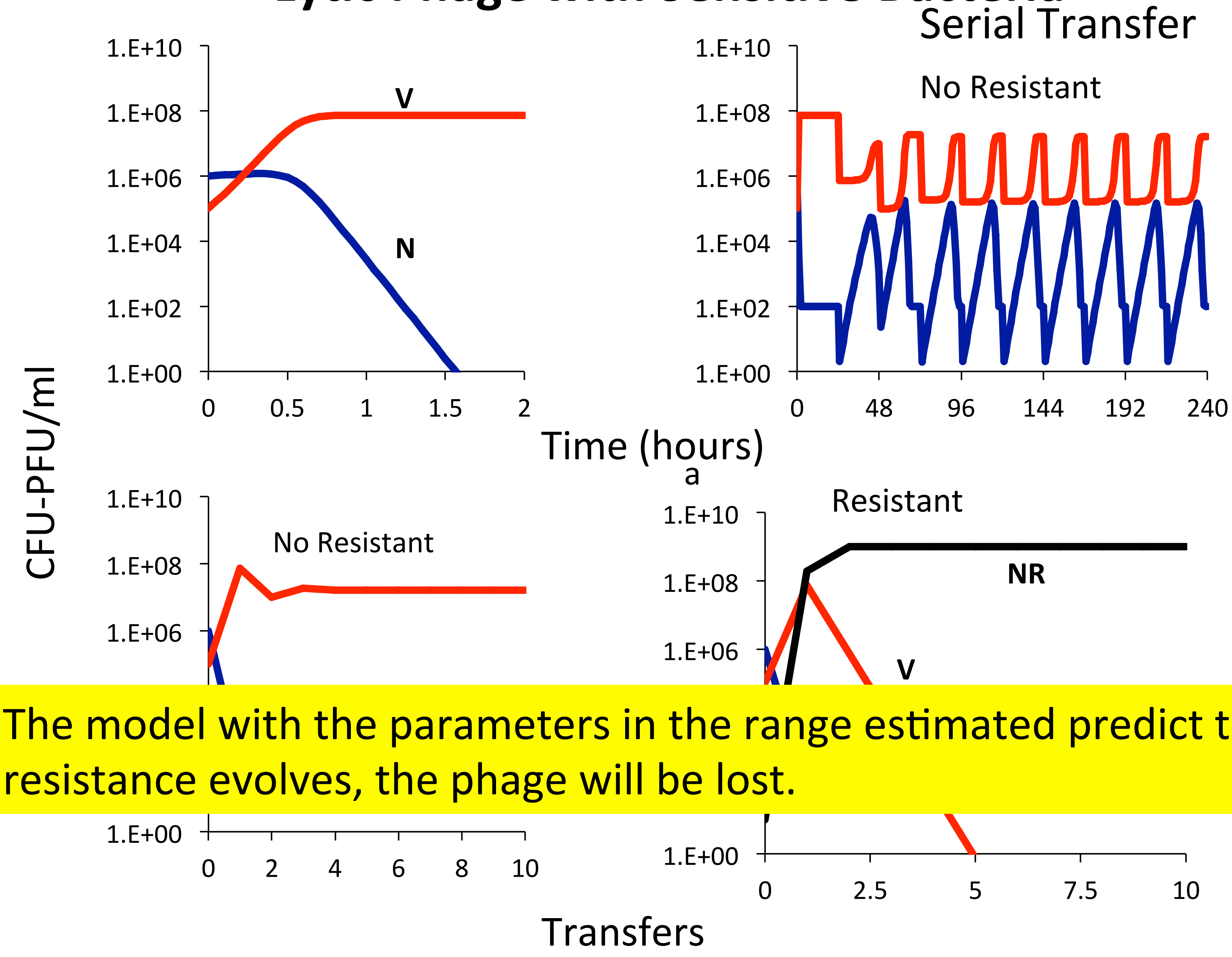
Phage replication

$$Where\ \psi(R) = \frac{R}{(R+k)}$$

vn, vr – Maximum growth rates (1 per hour)
e- Conversion efficiency ($2x10^{-7}$)
δ –Adsorption rate constant ($2x10^{-7}$)
B- Burst size (50)
un, ur – Transition N→R, R→N (Variable)
k – Monod Constant 1

For the simulations, a fraction, d=0.01 of the bacteria and phage at 24 hours are transferred to fresh cultures with R_{MAX} µg/ml of medium. The simulations are programmed in Berkeley Madonna^{TM} (copies available, www.eclf.net).

Lytic Phage with Sensitive Bacteria



The model with the parameters in the range estimated predict that if resistance evolves, the phage will be lost.

Experimental System

Temperate Lambda, λ^{KAN} - Lysogens are resistant to kanamycin

Lambda Vir λ^{VIR} - Can replicate on lysogens and non-lysogens

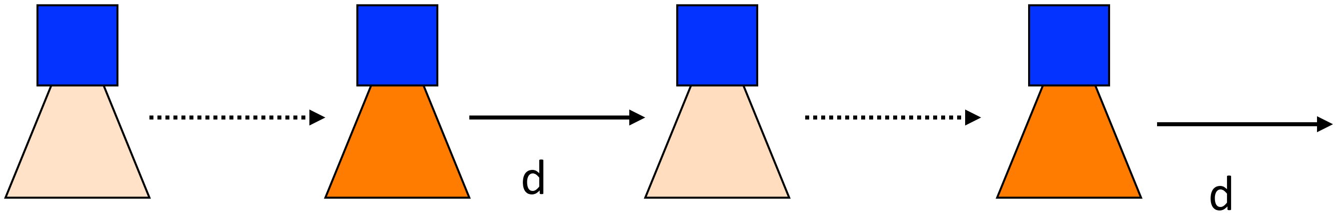
E. coli K12 (MG1655)

  Str-Streptomycin resistant to Streptomycin (*rpsL*)

  Various mutants resistant to λ^{VIR} and λ^{KAN}

Medium M9 Maltose (500 μg/ml) or LB (Lysogeny Broth)

Serial Transfer – 10 ml medium, 100μl transferred every 24 hours



Caveat – Testing for resistance

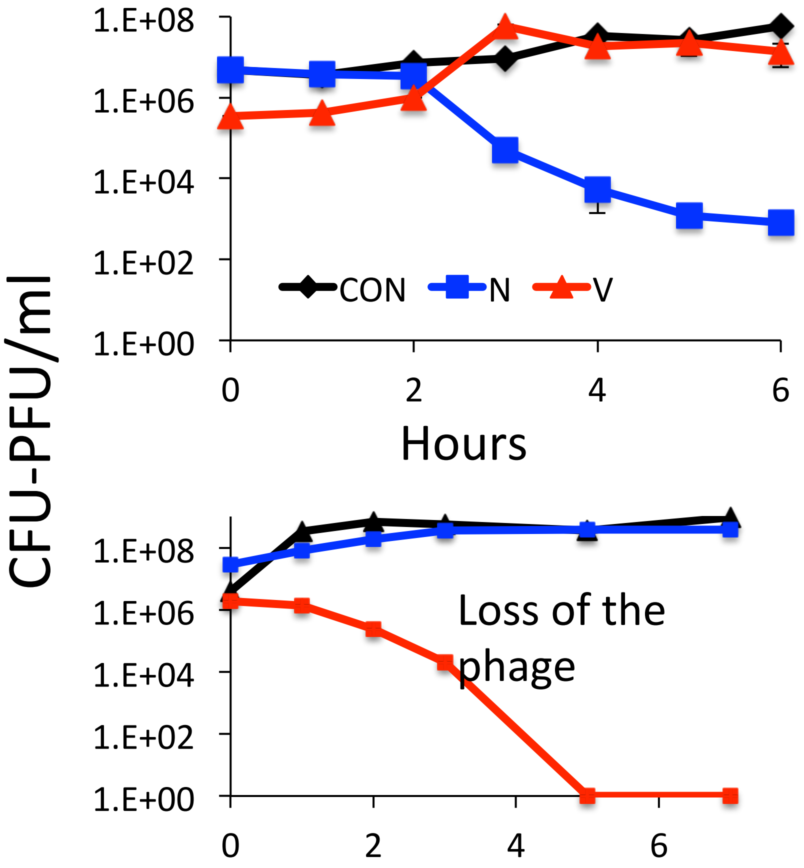
% of cells sensitive to lambda virulent phage in the lawn

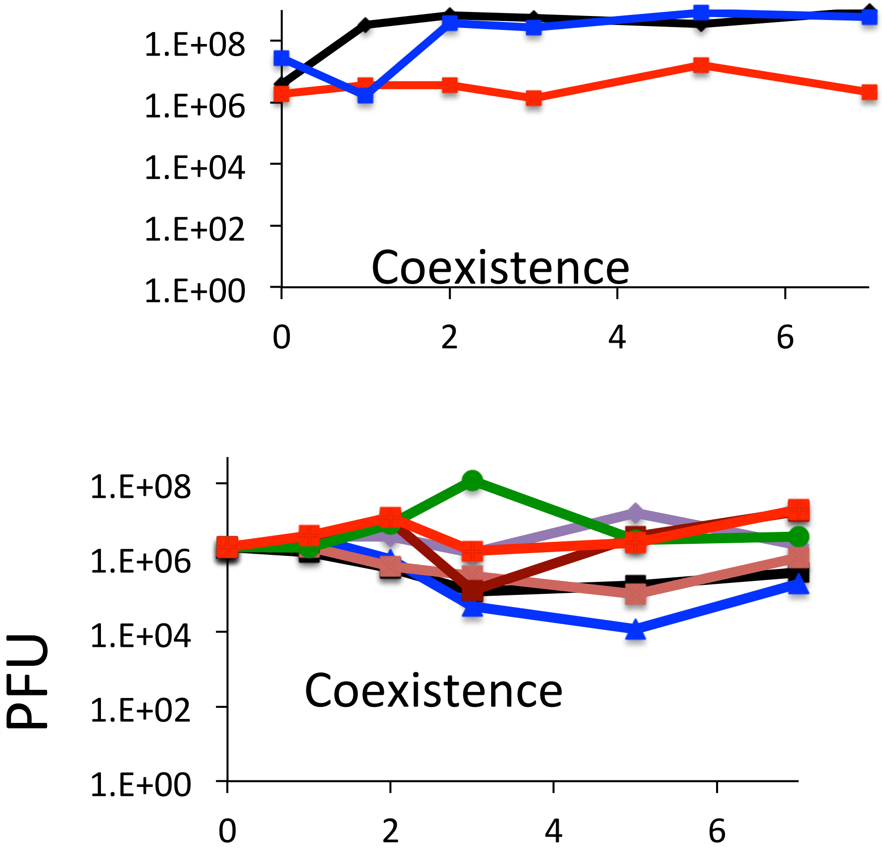
100 % 10 % 1 % 0.1 % 0 %

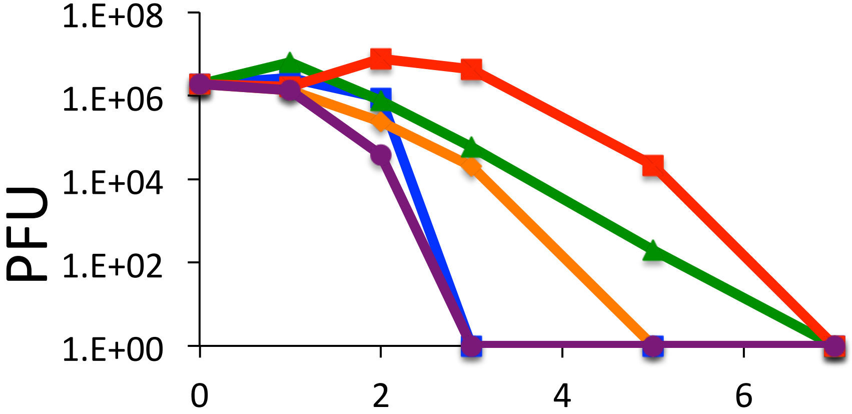
Spot test assay: Lambda virulent phage (20 µL of 5 X10^9 CFU/mL) spotted on the soft agar lawn having different percent of lambda virulent phage **sensitive** *E. coli K12* to the lambda virulent phage **resistant** *E.coli K12* malT$^-$ ompF$^-$ bacterial strains.

Observation: The lawn which has 100 % sensitive cells produce clear zone. The lawn which has 10 % of sensitive cells produce less turbid zone as compare to lawns which have 1 or less than 1% of sensitive cells.

Lytic Phage with Sensitive Bacteria in M9 Maltose







The phage can be maintained as a minority population or lost within a few transfers

Hypotheses

(a)- Phage resistance engenders a fitness cost, which allows for maintenance of the sensitive cells upon which the phage replicate (1).

(b)- There is a refuge on the walls of the flask upon which sensitive bacteria continue to persist (2)

(c)- Resistance is not absolute, there are few functional receptor sites on the seemingly resistant bacteria (3)

(d) Sensitive bacteria are continually produced from resistant cell (4, 5)

(1) Levin, B. R., F. M. Stewart, and L. Chao. 1977. Resource - limited growth, competition , and predation: a model and experimental studies with bacteria and bacteriophage. American Naturalist 977:3-24

(2) Schrag SJ, Mittler JE (1996) Host-parasite coexistence: the role of spatial refuges in stabilizing bacteria-phage interactions. Amer Natur 148: 348–377.

(3) Braun C, Hofnung M. (1978) Explanations accounting for transduction by bacteriophage lambda in maltose negative bacteriophage lambda resistant mutants of Escherichia coli K-12. Mol Gen Genet. 16;159(2):143-9.

(4) Delbruck, M. (1946), Bacterial viruses or bacteriophages. Biological reviews, 21: 30–40.

(5) Meyer et al 2012. Repeatability and contingency in the evolution of a key innovation in phage lambda. Science 2012. 27;335 (6067):428-32.

Hypotheses

(a)- Phage resistance engenders a fitness cost, which allows for maintenance of the sensitive cells upon which the phage replicate (1).

(b)- There is a refuge on the walls of the flask upon which sensitive bacteria continue to persist (2)

(c)- Resistance is not absolute, there are few functional receptor sites on the seemingly resistant bacteria (3)

(d) Sensitive bacteria are continually produced from resistant cell (4, 5)

“Leaky Resistance”

(1) Levin, B. R., F. M. Stewart, and L. Chao. 1977. Resource - limited growth, competition , and predation: a model and experimental studies with bacteria and bacteriophage. American Naturalist 977:3-24

(2) Schrag SJ, Mittler JE (1996) Host-parasite coexistence: the role of spatial refuges in stabilizing bacteria-phage interactions. Amer Natur 148: 348–377.

(3) Braun C, Hofnung M. (1978) Explanations accounting for transduction by bacteriophage lambda in maltose negative bacteriophage lambda resistant mutants of Escherichia coli K-12. Mol Gen Genet. 16;159(2):143-9.

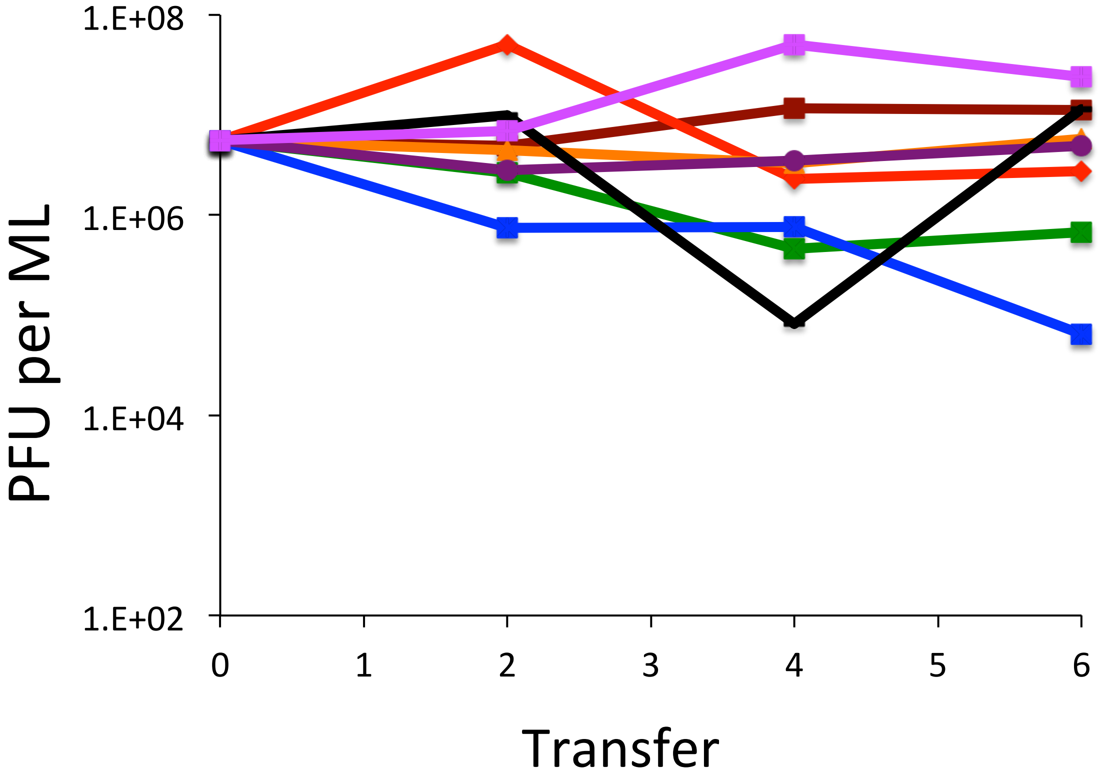
(4) Delbruck, M. (1946), Bacterial viruses or bacteriophages. Biological reviews, 21: 30–40.

(5) Meyer et al 2012. Repeatability and contingency in the evolution of a key innovation in phage lambda. Science 2012. 27;335 (6067):428-32.

Test of the Leaky Hypothesis

Proposition – If resistant cells are the unique population of the bacteria and there is a sufficient rate of transition, RE→N, μr, the phage will be maintained .

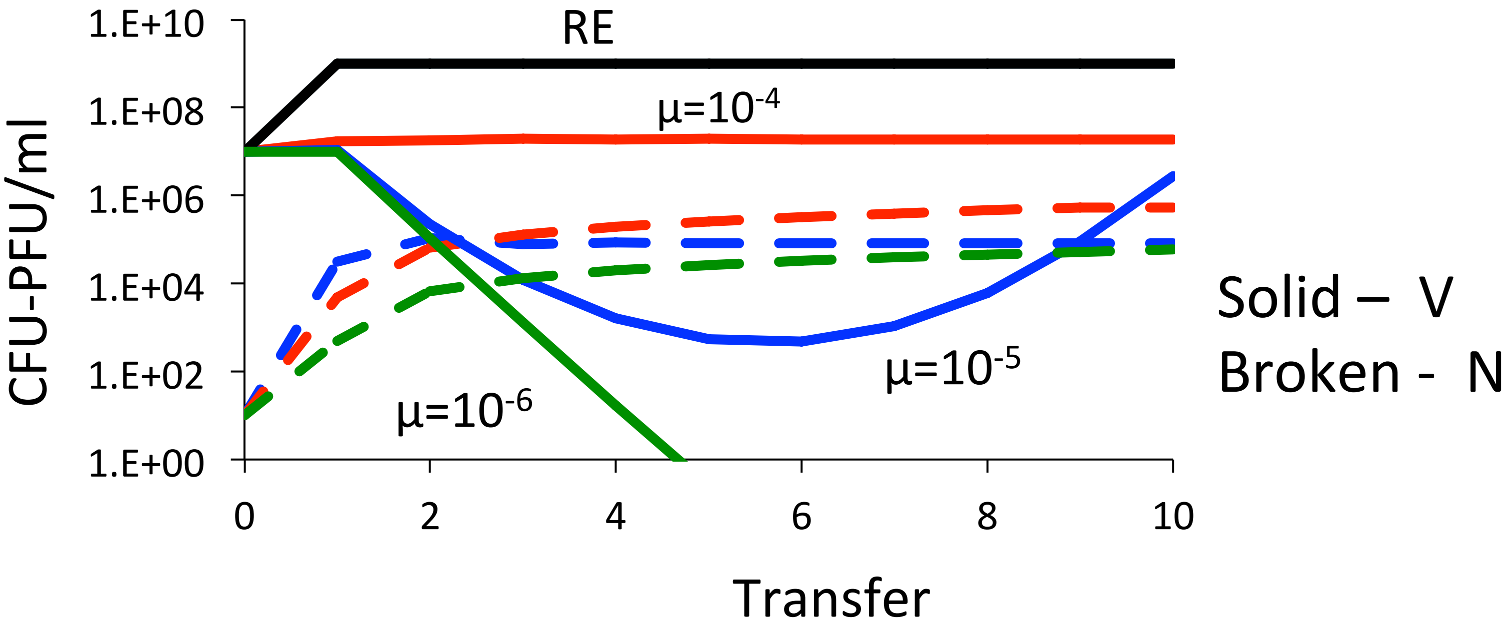
Test – Isolated 11 independent in LB and initiated serial transfer cultures in LB with ~ 10^7 MG1655 and 5×10^6 λ^{VIR}



8 maintained the phage,
3 lost the phage by the 3rd transfer

What is the rate of transition RE→N necessary to maintain the phage?

RE → N and N-→ RE at a rate μ



With the model and the parameters estimated, the transition rate has to be on the order of 10^{-4} to 10^{-5} per cell per hour or greater

How do we Estimate the Rate of Transition from RE → N, μr?

Directly – testing individual colonies for sensitivity – uncool and labor and toothpick intensive

Lysogeny and models to the rescue - Infect resistant cells with λ^{Kan} and estimate the number of lysogens (Kan-R) by plating on Kanamycin agar. The temperate phage would only be able to infect bacteria that are phenotypically or genetically sensitive to λ^{VIR}.

Model – Dynamics of Temperate Phage

$$\frac{dR}{dt} = \psi(R)e(vnN + vlNL + vreRE)$$

$$\frac{dN}{dt} = vn\psi(R)N - \delta\psi(R)NP + \mu rRE - \mu nN$$

$$\frac{dNL}{dt} = vl\psi(R)NL + \lambda\delta NP - xNL$$

$$\frac{dRE}{dt} = vre\psi(R) - \mu rRE + \mu nN$$

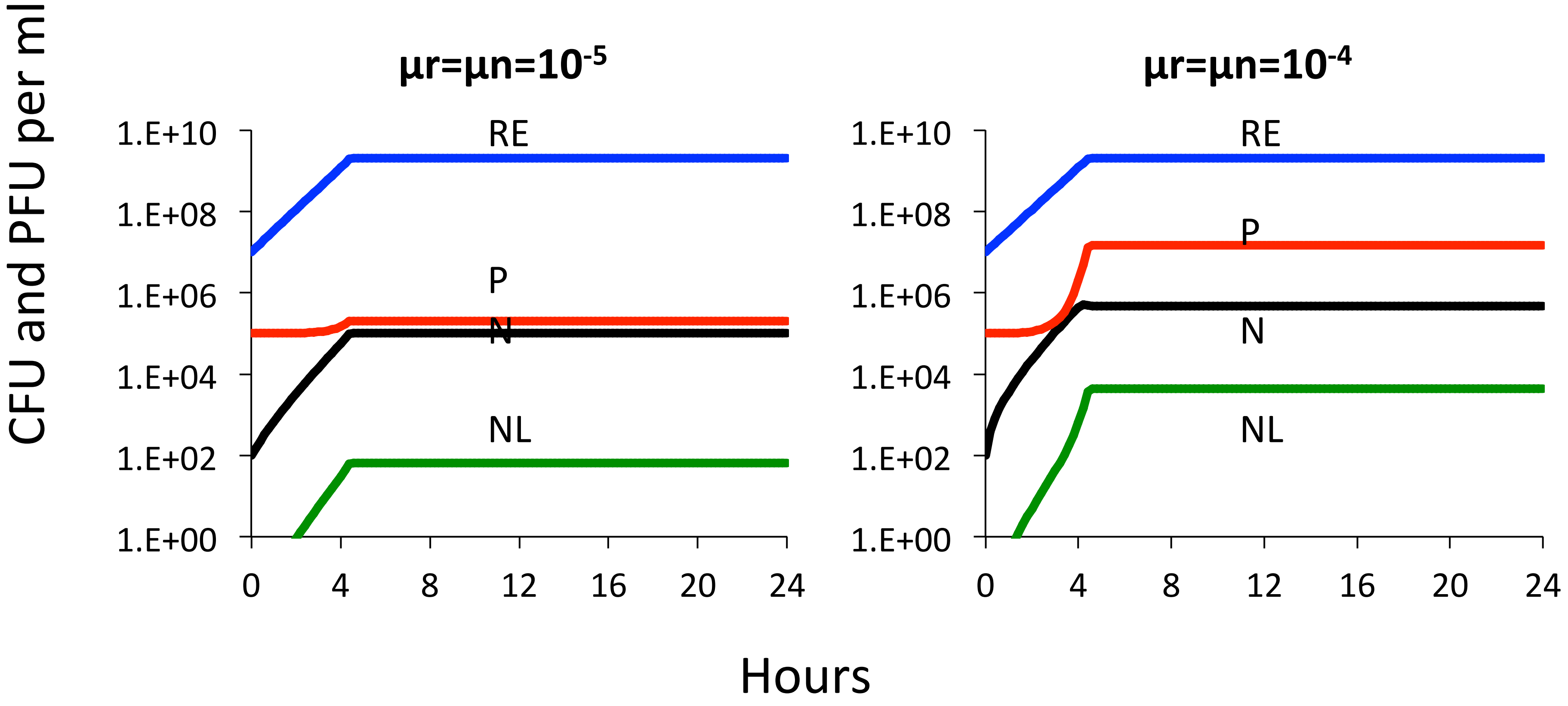
$$\frac{dP}{dt} = \delta\psi(R)NP\beta - \delta\psi(R)PNL + x\psi(R)NL\beta$$

R - Resource
N- Sensitive non-lysogens
NL – Lysogens
RE- Resistant Non-lysogens
P – Free Temperate Phage

vn, vl,vre –Max Growth Rates (1.0)
δ – Adsorption rate constant ($2x10^{-7}$)
β – Burst size (50)
λ – Lysogeny probability (0.01)
x – Induction rate (0.001)
μr, μn - Transition rates (variable)

From the dynamics of lysogen, NL, formation in cultures with free temperate phage, P, and phage resistant cells, RE, we should be able to estimate the rate of transition, NE-→N, μr.

Formation of Lysogens in Populations of Resistant Bacteria



The model predicts that lysogens will be observed if the transition rare NE→N, μr is on the order of 10^{-5} per cell per hour or greater.

Phenotypic properties of 11 Independent λ^{VIR} Resistant Mutant

Strain	Lysogens produced on λ^{VIR} resistant	Maintenance of λ^{VIR}	Lysogens Sensitive to λ^{VIR}
MG1655	+	-	5/5
W1	+	+	3/5
W2	+	+	5/5
W3	+	+	4/5
W4	+	-	0/5
W5	+	-	0/5
W6	+	-	0/5
W7	+	+	0/5
W9	+	+	5/5
W10	+	+	0/5
W11	+	+	0/5
W12	+	-	5/5

W8 has been eliminated because upon retesting, it was found to be sensitive to λ^{VIR}

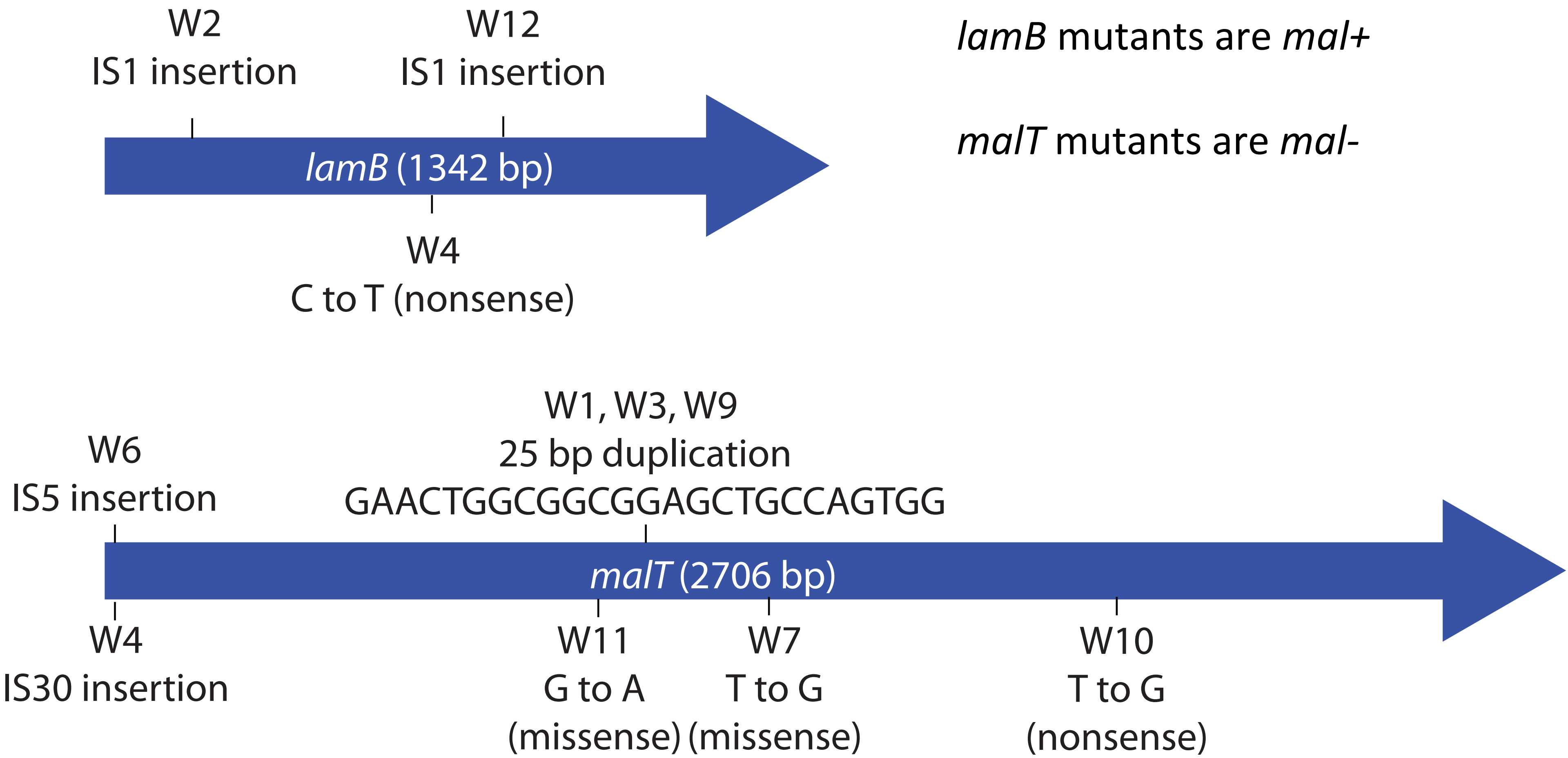
Dem low throughput, last millennium Evolutionary Biologists ain't got no respect. Dey didn't even sequence da genomes. Da y wan me ta break der legs?

Tribute for the Mechanism Mafia

Andre Lwoff, 1992 - 1994

"The triumvirate (Stent, Watson and Cairns) responsible for this volume (Phage and the Origins of Molecular Biology, 1968) has asked the presumptive authors to "describe some significant contribution they have made to molecular biology. A scientist should never attempt to judge the value of their own achievements, whether significant or not, but especially when not. This is the Golden Rule of intellectual hygiene. And I would have been embarrassed if the triumvirs had not made their own choice and decided that I had to deal with the prophage. **Prophage is a remarkable entity indeed, a molecule I should say, for I have to be molecular. Who is not?"**

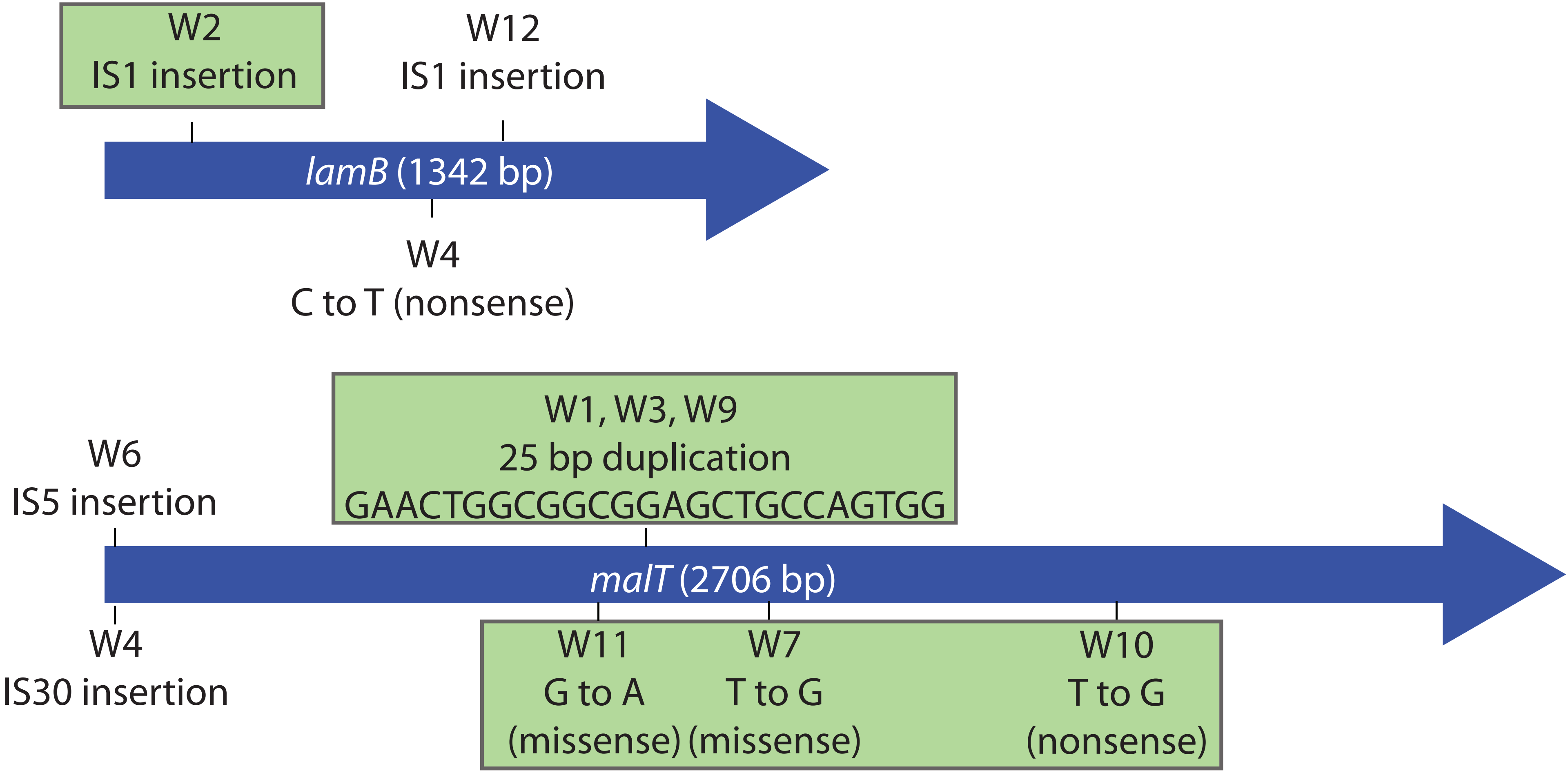
Mutations causing resistance



lamB mutants are *mal+*

malT mutants are *mal-*

Mutations allowing for phage maintenance



W2
IS1 insertion

W12
IS1 insertion

lamB (1342 bp)

W4
C to T (nonsense)

W6
IS5 insertion

W1, W3, W9
25 bp duplication
GAACTGGCGGCGGAGCTGCCAGTGG

malT (2706 bp)

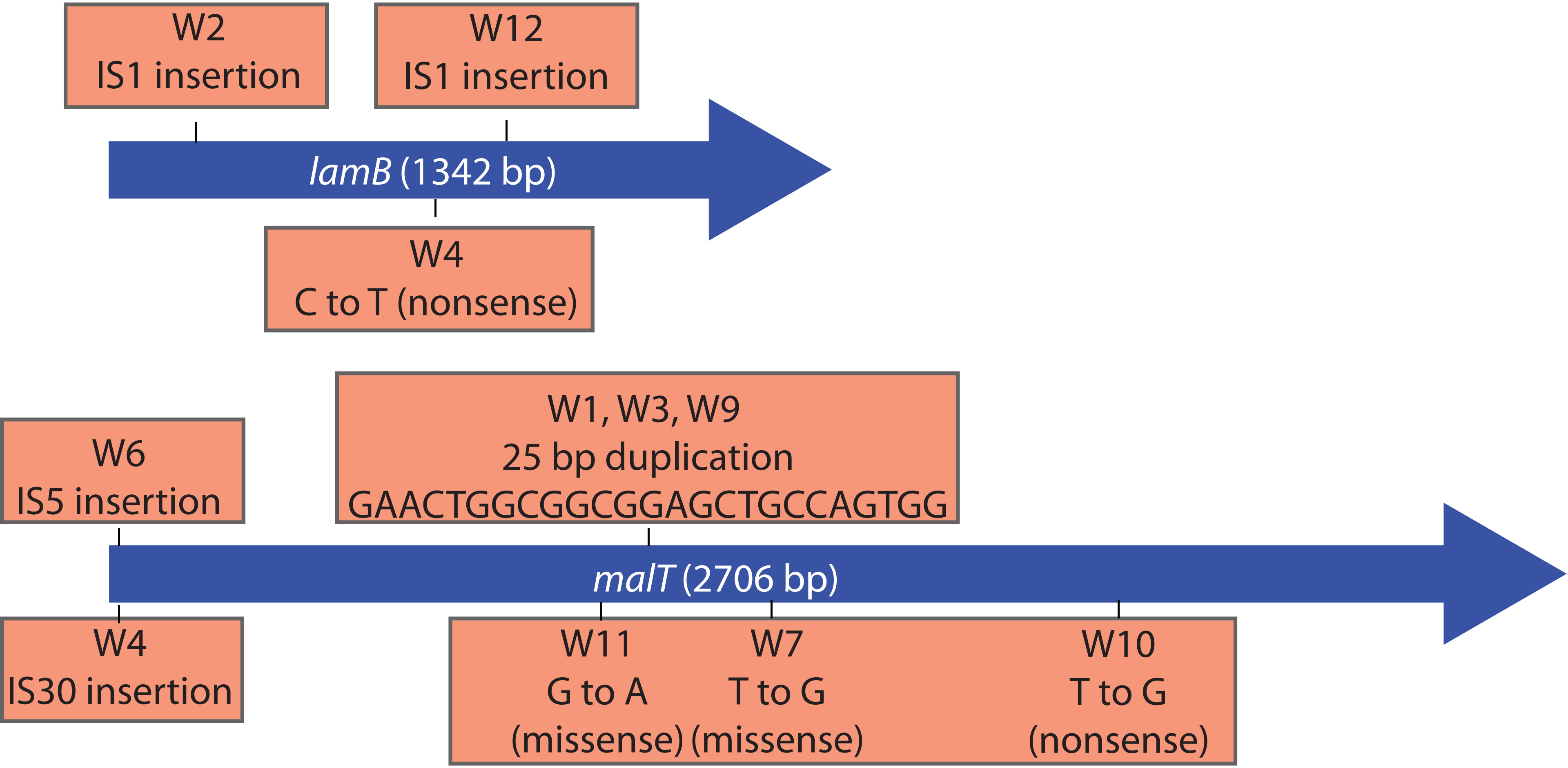
W4
IS30 insertion

W11
G to A
(missense)

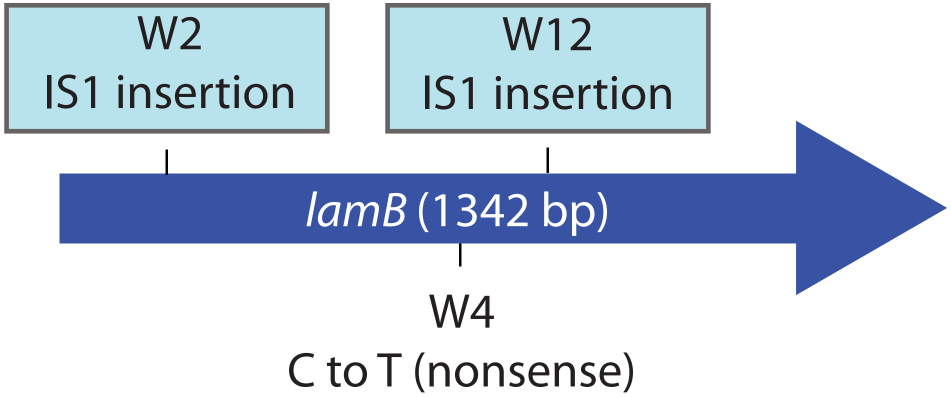
W7
T to G
(missense)

W10
T to G
(nonsense)

All mutants can produce lysogens after 72 hours of incubation (tested by λ^{KAN})



Some lysogens reverted back to sensitivity (tested by λ^{vir})



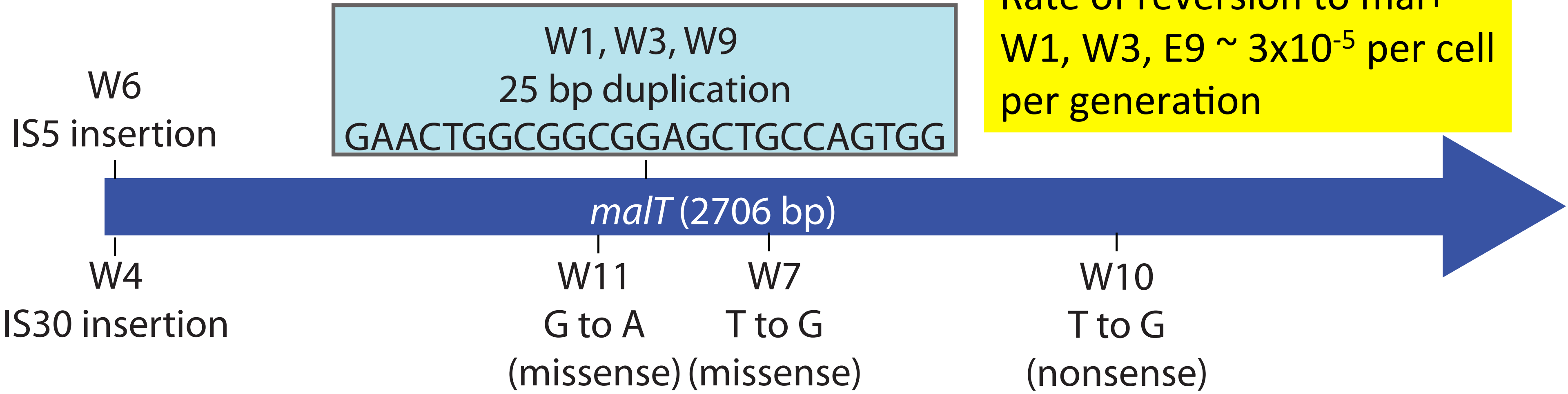
W2
IS1 insertion

W12
IS1 insertion

lamB (1342 bp)

W4
C to T (nonsense)

Lysogens that tested as sensitive also lost the mutation causing resistance and thus genetically reverted back to wild-type



W6
IS5 insertion

W1, W3, W9
25 bp duplication
GAACTGGCGGAGCTGCCAGTGG

malT (2706 bp)

W4
IS30 insertion

W11
G to A
(missense)

W7
T to G
(missense)

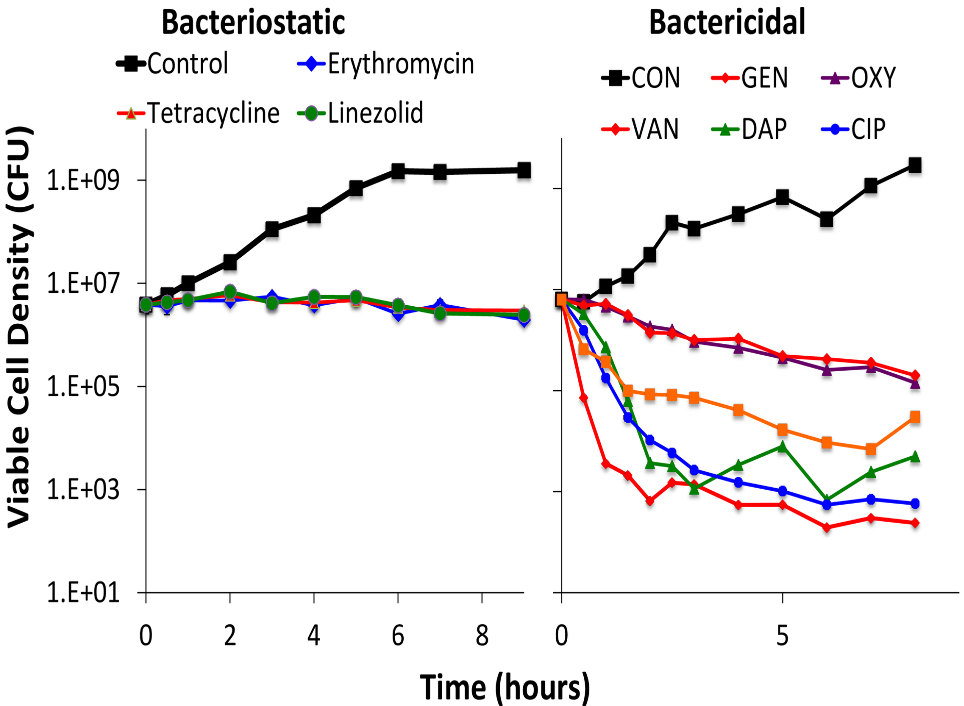
W10
T to G
(nonsense)

Rate of reversion to mal+ W1, W3, E9 ~ $3x10^{-5}$ per cell per generation

Conclusion

Some of the transition from Resistant to Sensitive is through true reversion, the high rate of which can be attributed to the excision of insertion sequences.

Some of the transition from Resistant to Sensitive is phenotypic – presumably *malT* regulatory gene "noise"



Persistence

Balaban, N.Q., et al., *A problem of persistence: still more questions than answers?* Nat Rev Microbiol, 2013. **11**(8): p. 587-91.

Levin, B.R. J. Concepcion Acevedo, K. Udekwu 2014. Persistence: a copacetic and parsimonious hypothesis for non-inherited resistance to antibiotics. Current Opinion in Microbiology 21: 18-21

The Alternative Hypotheses

Hypotheses are to be tested, not championed

1- Phage resistance engenders a fitness cost, which allows for maintenance of the sensitive cells upon which the phage replicate.

In accord with our model, for the phage to be maintained the the fitness cost resistance, as measured by the maximum growth rate would have to be more than 35% less than that of the phage sensitive bacteria. By growth rate and pairwise competition experiments there is little if any fitness difference between the λ^{VIR} resistant and sensitive *E. coli.*

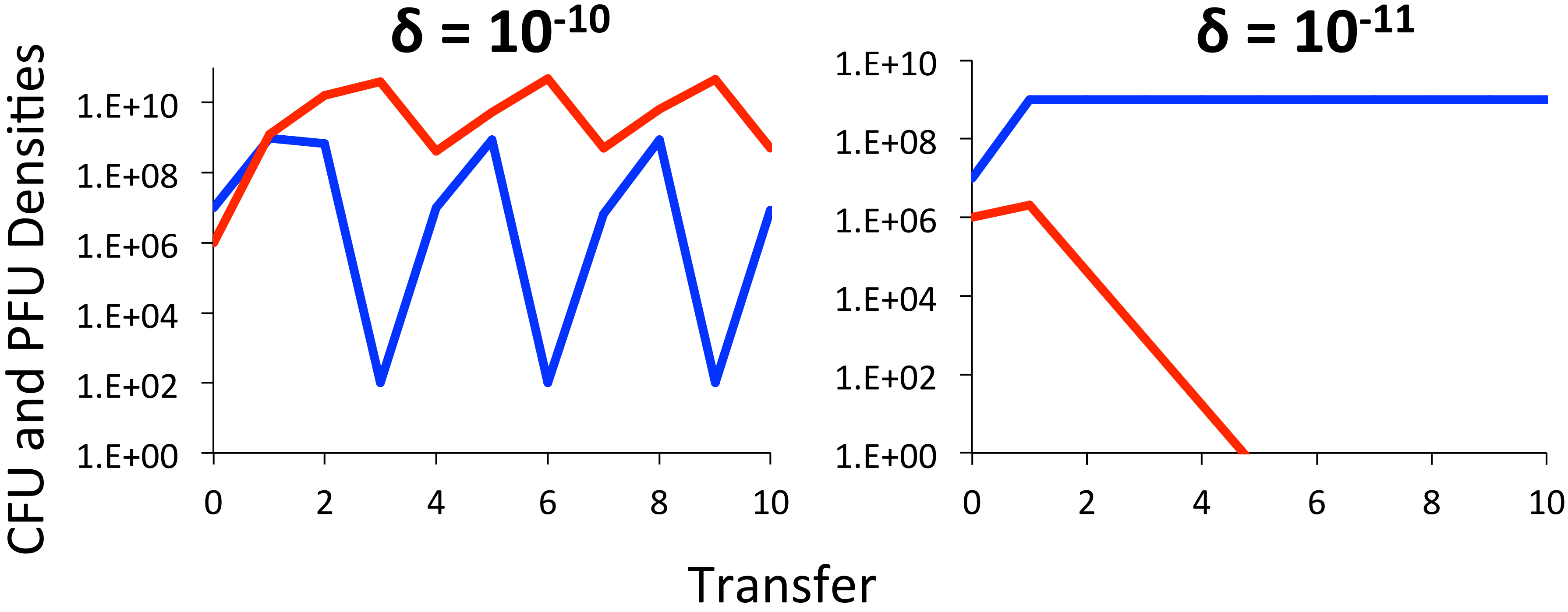
2- There is a refuge on the walls of the flask upon which sensitive bacteria continue to persist

This mechanism may contribute to the maintenance of the phage, but is unlikely to have a major role. The phage are maintained on initially monoclonal populations of bacteria that are resistant. There would not be enough sensitive cells to colonize the walls and thereby maintain the phage.

The Alternative Hypotheses

3- Resistance is not absolute, there are few functional receptor sites on the seemingly resistant bacteria

In accord with this hypothesis, the average rate of adsorption, δ would be low. Were this rate low enough for the density of bacteria to be at or near phage-free levels, in accord with our model either the dominant population would be phage, or the phage could not be maintained in serial transfer.



$\delta = 10^{-10}$

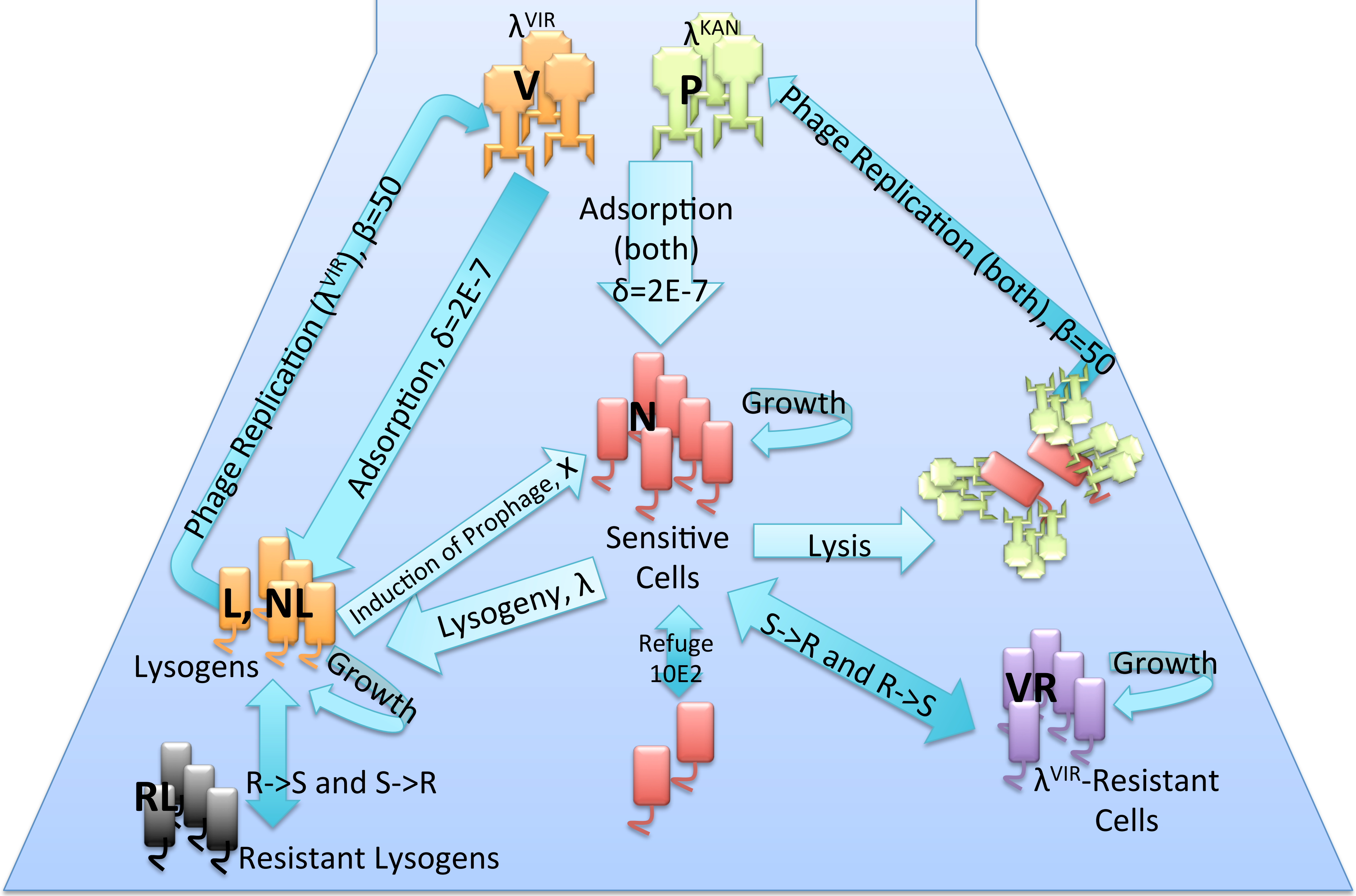
$\delta = 10^{-11}$

Temperate Phage alone

The Question

Under what conditions will temperate phage become established and be maintained in bacterial populations?

Model of the Dynamics with Temperate Phage



λ^{VIR}

V

λ^{KAN}

P

Adsorption (both) δ=2E-7

Phage Replication (both), β=50

Phage Replication (λ^{VIR}), β=50

Adsorption, δ=2E-7

N

Growth

Sensitive Cells

Lysis

Induction of Prophage, χ

Lysogeny, λ

L, NL

Lysogens

Growth

Refuge 10E2

S->R and R->S

VR

Growth

λ^{VIR}-Resistant Cells

R->S and S->R

RL,

Resistant Lysogens

Model of the Dynamics with Temperate Phage

$$\frac{dR}{dt} = -\psi(R)\cdot e\cdot(vn\cdot N + vl\cdot L + vnl\cdot NL + vrl\cdot RL + vre\cdot RE$$

$$\frac{dN}{dt} = vn\cdot\psi(R)\cdot N - \delta\cdot N\cdot(P+V)\cdot\psi(R) + \mu r\cdot RE\cdot\psi(R) - \mu n\cdot N\cdot\psi(R) + \tau\cdot L\cdot\psi(R)$$

$$\frac{dL}{dt} = vl\cdot\psi(R)\cdot L - \delta\cdot V\cdot L\cdot\psi(R) - \tau\cdot L\cdot\psi(R) - x\cdot L\cdot\psi(R) + \mu r\cdot RL\cdot\psi(R)$$

$$\frac{dNL}{dt} = vn\cdot\psi(R)\cdot NL + \delta\cdot NP\cdot\lambda\cdot\psi(R) - x\cdot NL\cdot\psi(R) - \tau\cdot NL\cdot\psi(R)$$

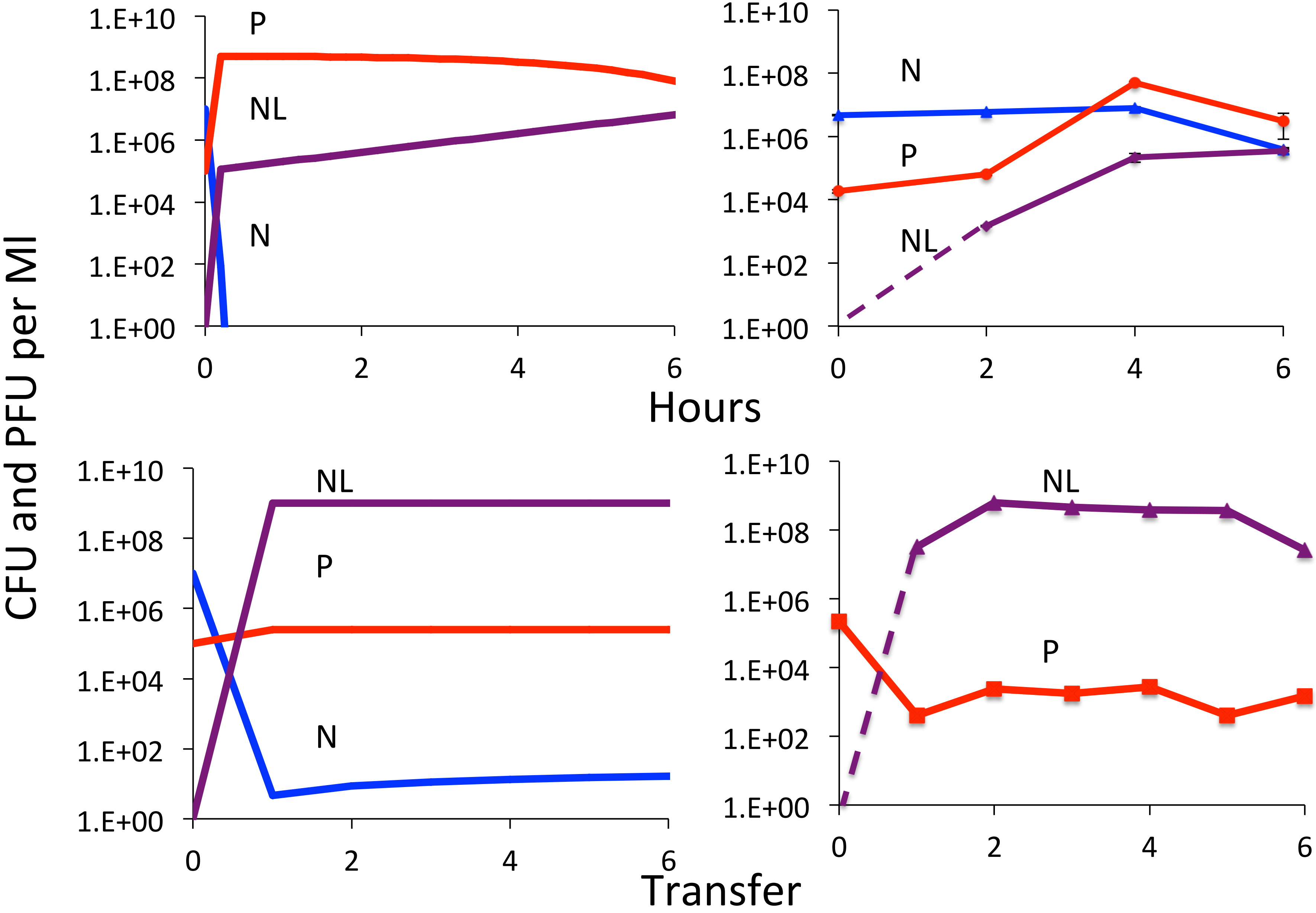
$$\frac{dRL}{dt} = vrl\cdot\psi(R)\cdot RL - x\cdot RL\cdot\psi(R) - \delta\cdot V\cdot NL\cdot\psi(R) + \mu r\cdot RL\cdot\psi(R) + \mu n\cdot N\cdot\psi(R)$$

$$\frac{dRE}{dt} = vre\cdot\psi(R) - \mu r\cdot RE\cdot\psi(R) + \mu n\cdot N\cdot\psi(R)$$

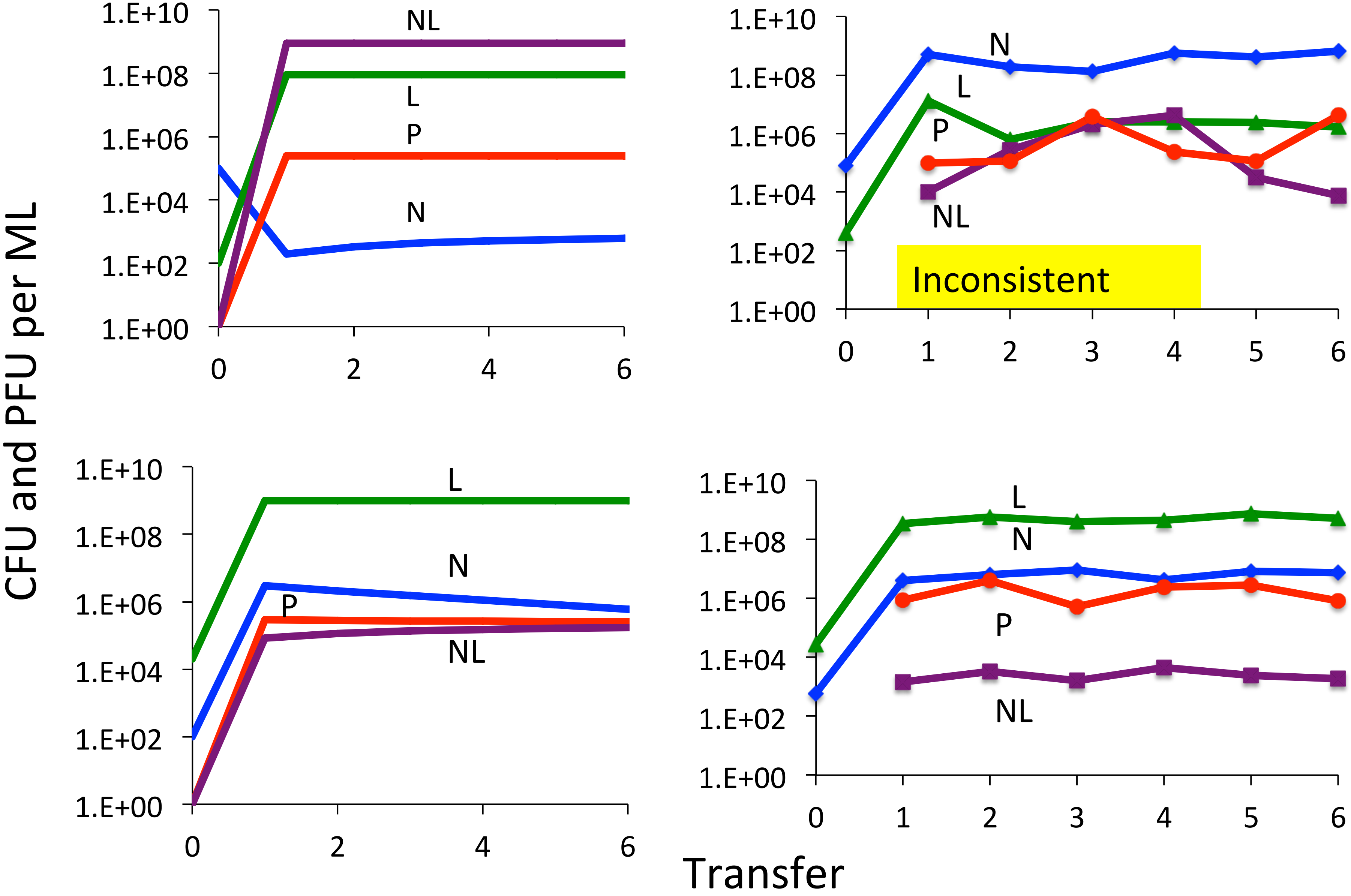
$$\frac{dP}{dt} = \delta\cdot P\cdot N(1-\lambda)\cdot\beta\cdot\psi(R) - \delta\cdot P\cdot(L+NL)\cdot\psi(R) + x\cdot(L+NL)\cdot\beta$$

$$\frac{dV}{dt} = \delta\cdot V\cdot(N+L+NL)\cdot\beta\cdot\psi(R)$$

Temperate Phage and Sensitive Bacteria



Lysogens Invading STR-R λ^{VIR} Sensitive Cells



Conclusion

Under broad conditions, either as free viruses or as lysogens, temperate phage will invade and become established in populations of bacteria sensitive to their action. The model, however, is cannot fully account for the dynamics

Temperate and Lytic Phage Together

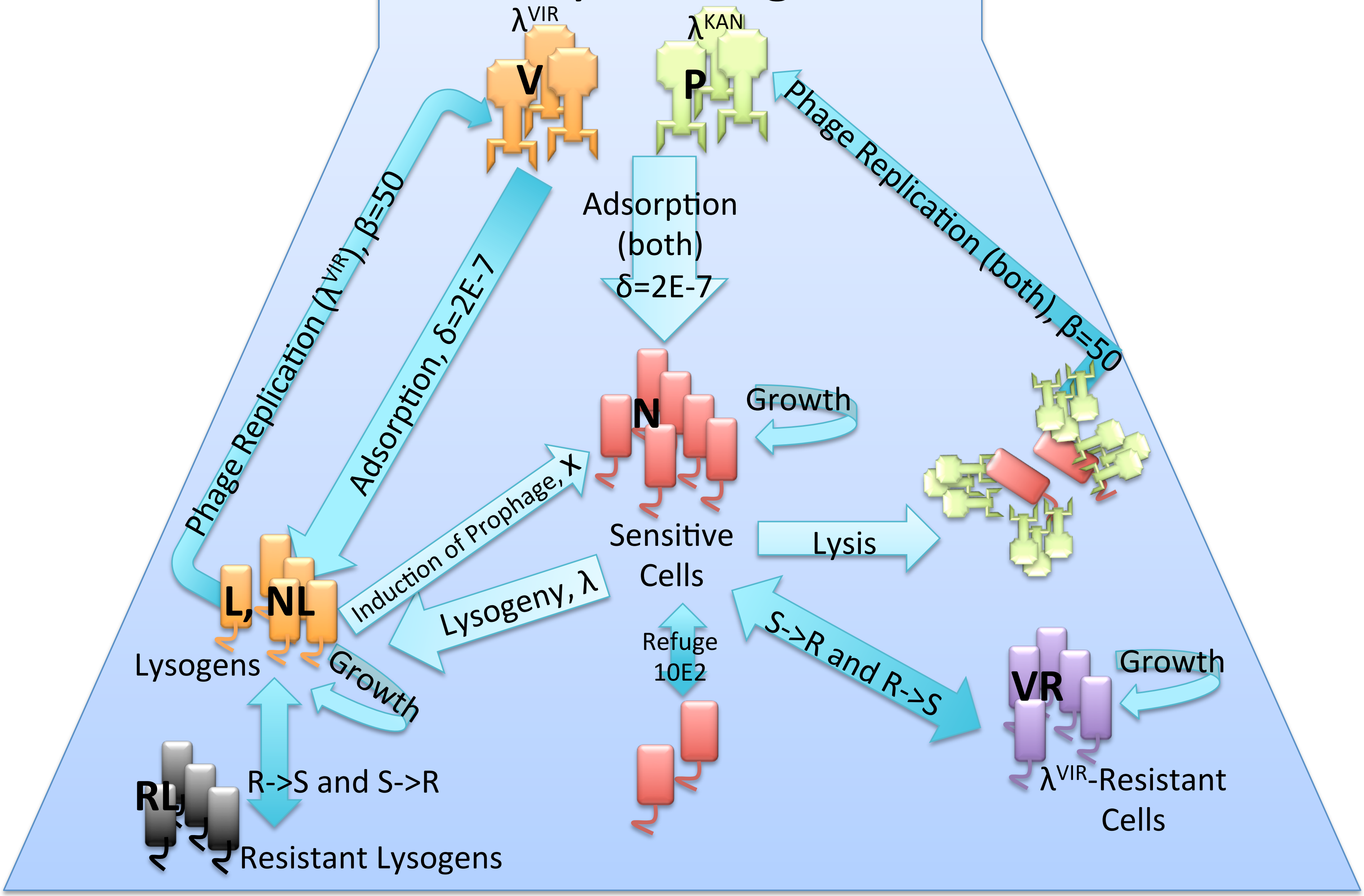
Potential Outcomes

Temperate exclude lytic
Temperance prevails

Lytic exclude Temperate
Virulence prevails

Lytic and Temperate Coexist
Equity prevails

Model of the Dynamics with Temperate and Lytic Phage



λ^{VIR}

V

λ^{KAN}

P

Adsorption (both) δ=2E-7

Phage Replication (λ^{VIR}), β=50

Adsorption, δ=2E-7

Phage Replication (both), β=50

N

Growth

Induction of Prophage, χ

Sensitive Cells

Lysis

Lysogeny, λ

L, NL

Lysogens

Growth

Refuge 10E2

S->R and R->S

VR

Growth

λ^{VIR}-Resistant Cells

RL

R->S and S->R

Resistant Lysogens

Model of Temperate and Lytic Phage

$$\frac{dR}{dt} = -\psi(R) \cdot e \cdot (vn \cdot N + vl \cdot L + vnl \cdot NL + vrl \cdot RL + vre \cdot RE$$

$$\frac{dN}{dt} = vn \cdot \psi(R) \cdot N - \delta \cdot N \cdot (P + V) \cdot \psi(R) + \mu r \cdot RE \cdot \psi(R) - \mu n \cdot N \cdot \psi(R) + \tau \cdot L \cdot \psi(R)$$

$$\frac{dL}{dt} = vl \cdot \psi(R) \cdot L - \delta \cdot V \cdot L \cdot \psi(R) - \tau \cdot L \cdot \psi(R) - x \cdot L \cdot \psi(R) + \mu r \cdot RL \cdot \psi(R)$$

$$\frac{dNL}{dt} = vn \cdot \psi(R) \cdot NL + \delta \cdot NP \cdot \lambda \cdot \psi(R) - x \cdot NL \cdot \psi(R) - \tau \cdot NL \cdot \psi(R)$$

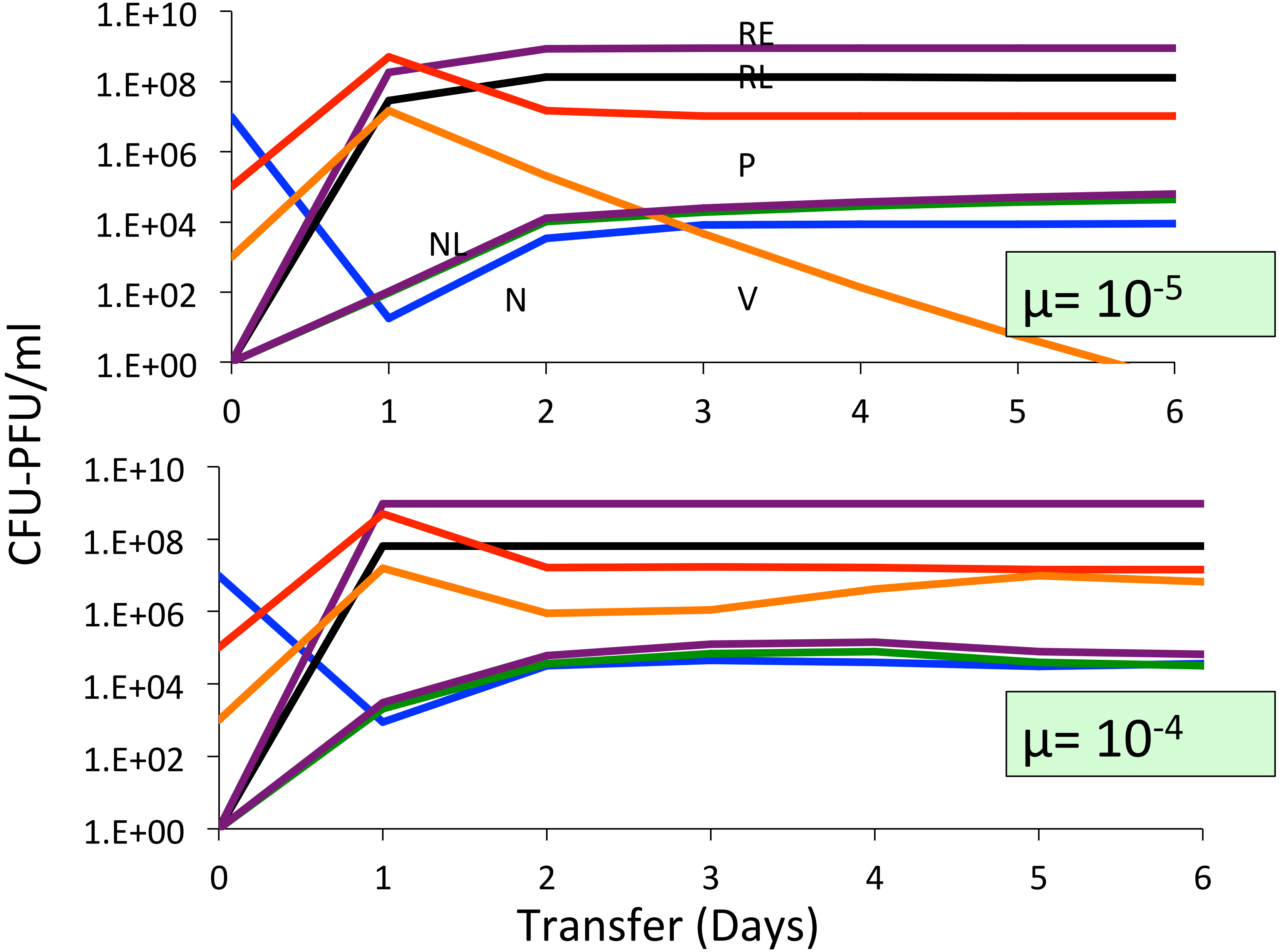
$$\frac{dRL}{dt} = vrl \cdot \psi(R) \cdot RL - x \cdot RL \cdot \psi(R) - \delta \cdot V \cdot NL \cdot \psi(R) + \mu r \cdot RL \cdot \psi(R) + \mu n \cdot N \cdot \psi(R)$$

$$\frac{dRE}{dt} = vre \cdot \psi(R) - \mu r \cdot RE \cdot \psi(R) + \mu n \cdot N \cdot \psi(R)$$

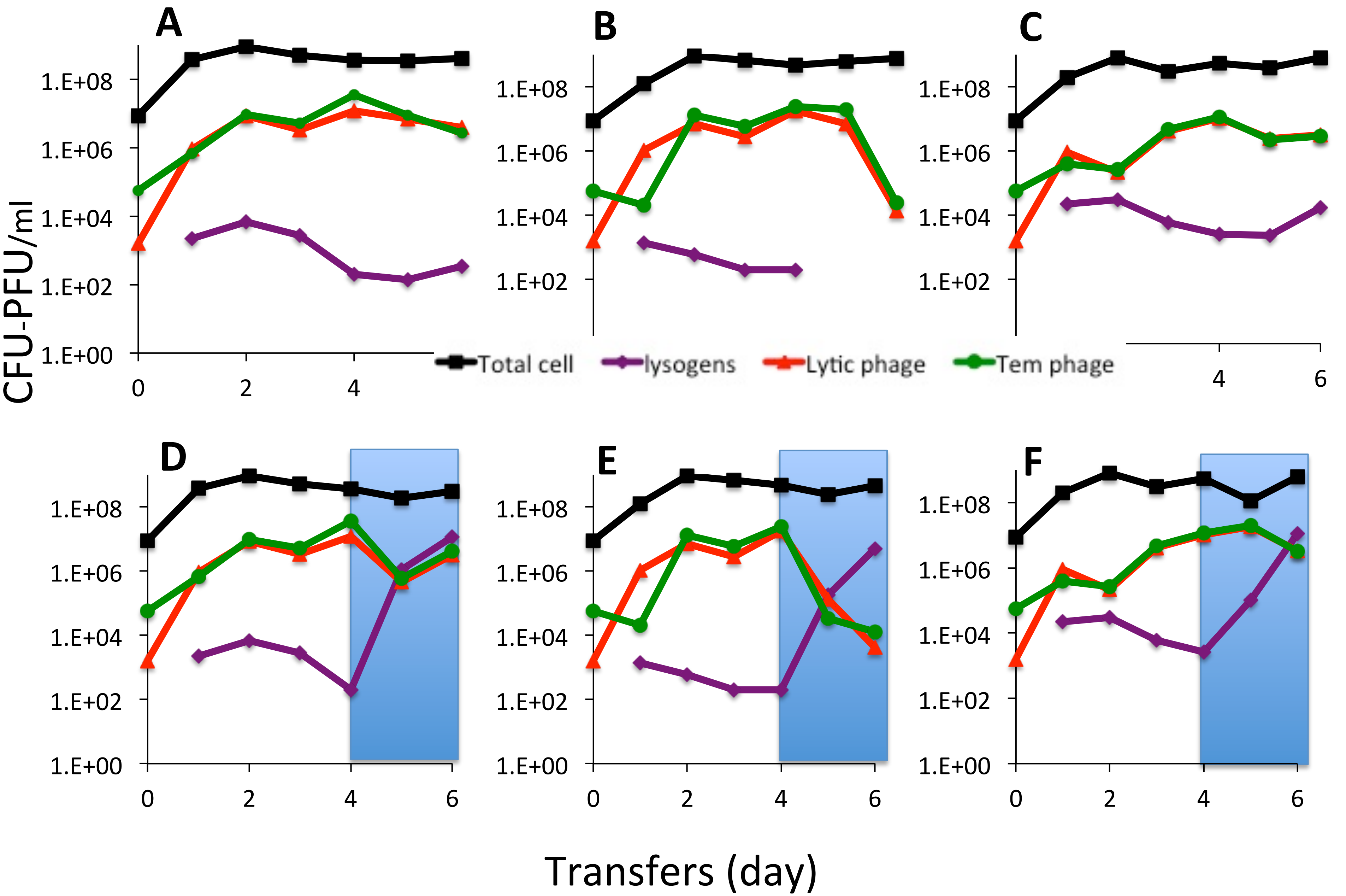
$$\frac{dP}{dt} = \delta \cdot P \cdot N(1 - \lambda) \cdot \beta \cdot \psi(R) - \delta \cdot P \cdot (L + NL) \cdot \psi(R) + x \cdot (L + NL) \cdot \beta$$

$$\frac{dV}{dt} = \delta \cdot V \cdot (N + L + NL) \cdot \beta \cdot \psi(R)$$

Temperate and Lytic Phage Together



Temperate and Lytic Phage Together



Conclusion

In accord with our models and experiments, temperate and lytic phage can and will co-exist.

Equity Prevails
At least in computer simulations and the flasks of laboratory culture

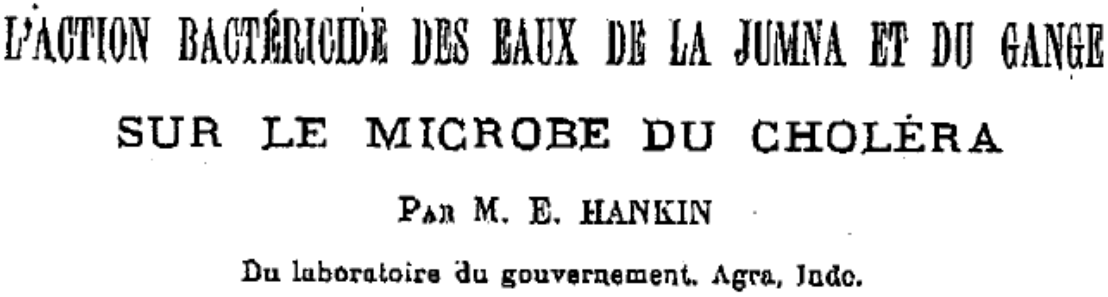
Phage and the Epidemiology of Cholera

Paolo Ocampo

Yan Wei

Wei, Y, P. Ocampo, B.R. Levin (2010) An Experimental Study of the Population and Evolutionary Dynamics of *Vibrio cholerae* O1 and the Bacteriophage JSF4 Proc. Roy. Soc. B 277: 3247-3245

Ernest Hanbury Hankin
1865 - 1939



L'ACTION BACTÉRICIDE DES EAUX DE LA JUMNA ET DU GANGE SUR LE MICROBE DU CHOLÉRA

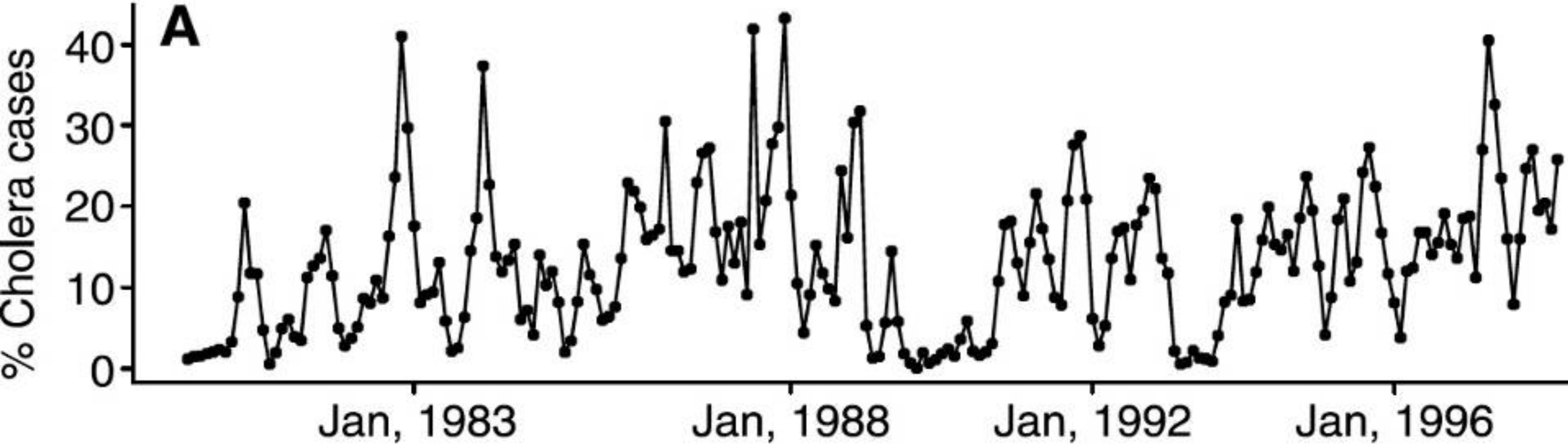
PAR M. E. HANKIN

Du laboratoire du gouvernement, Agra, Inde.

ANNALES DE L'INSTITUT PASTEUR (1896).

Although the scientific interest of the preceding results maybe limited by the fact that I have not yet discovered the nature and origin of the antiseptic substance present in the waters of the Ganga and Jamuna rivers, what appears interesting is that they explain why cholera does not travel downstream rivers in India.

What determines when a cholera outbreak occurs?

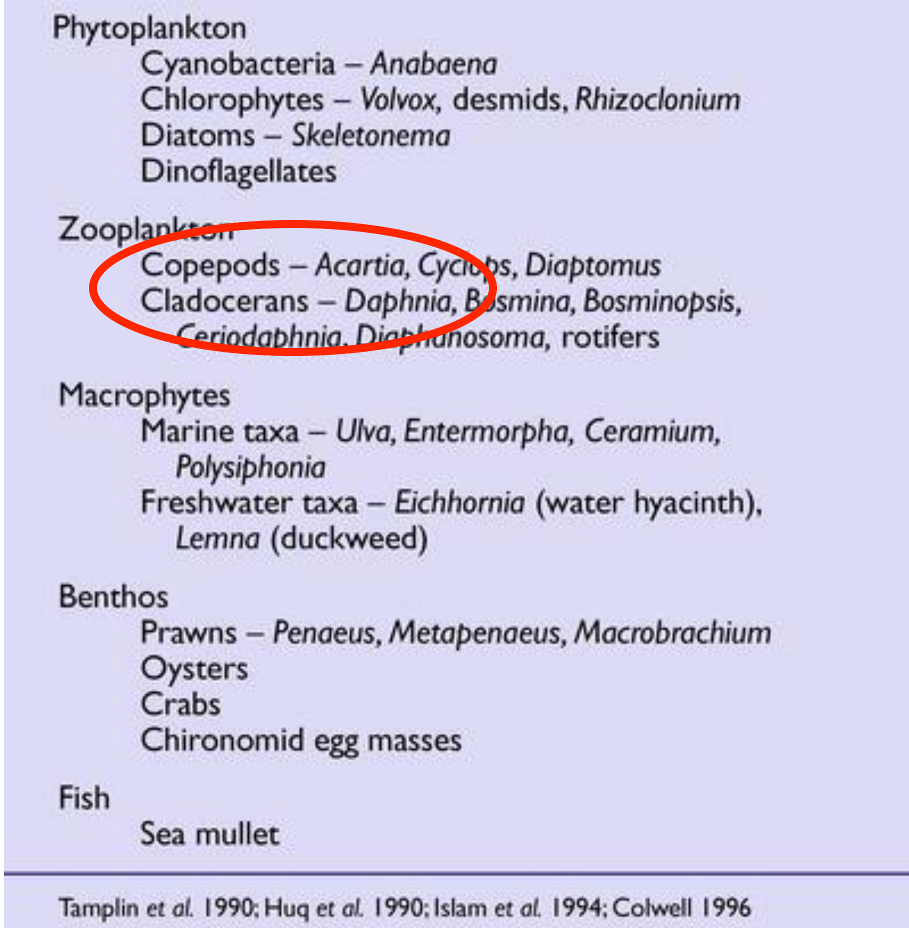


Abiotic Factors contributing to outbreaks (Temperature, rainfall, etc.)
Biotic Factors contributing to outbreaks (Algae, zooplankton, bacteriophages, etc.)

Biotic factors influencing cholera outbreaks

V. cholera has two habitats where it interacts with other organisms:

- Human intestine
- **Aquatic Environment (*V. cholerae* is associated with various plankton)**



Phytoplankton
- Cyanobacteria – *Anabaena*
- Chlorophytes – *Volvox*, desmids, *Rhizoclonium*
- Diatoms – *Skeletonema*
- Dinoflagellates

Zooplankton
- Copepods – *Acartia, Cyclops, Diaptomus*
- Cladocerans – *Daphnia, Bosmina, Bosminopsis, Ceriodaphnia, Diaphanosoma*, rotifers

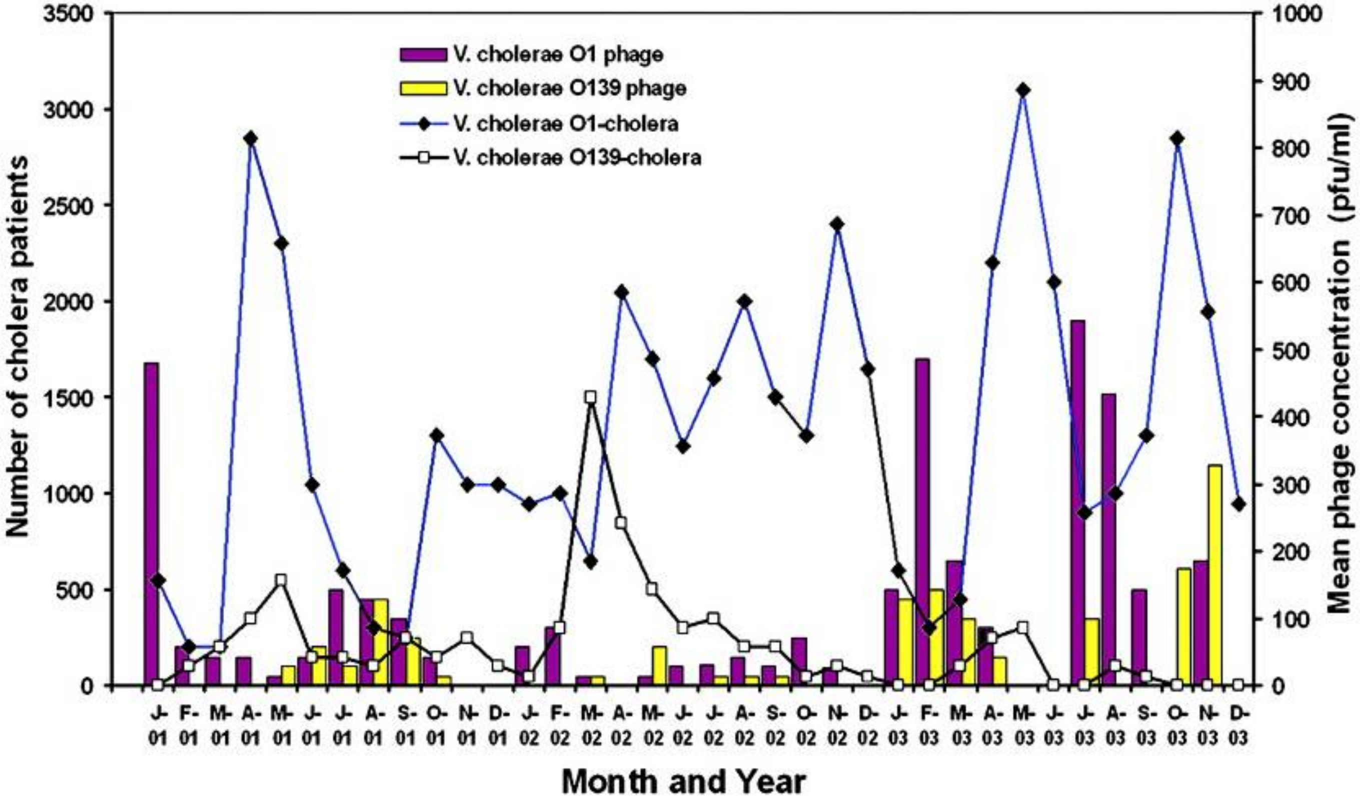
Macrophytes
- Marine taxa – *Ulva, Entermorpha, Ceramium, Polysiphonia*
- Freshwater taxa – *Eichhornia* (water hyacinth), *Lemna* (duckweed)

Benthos
- Prawns – *Penaeus, Metapenaeus, Macrobrachium*
- Oysters
- Crabs
- Chironomid egg masses

Fish
- Sea mullet

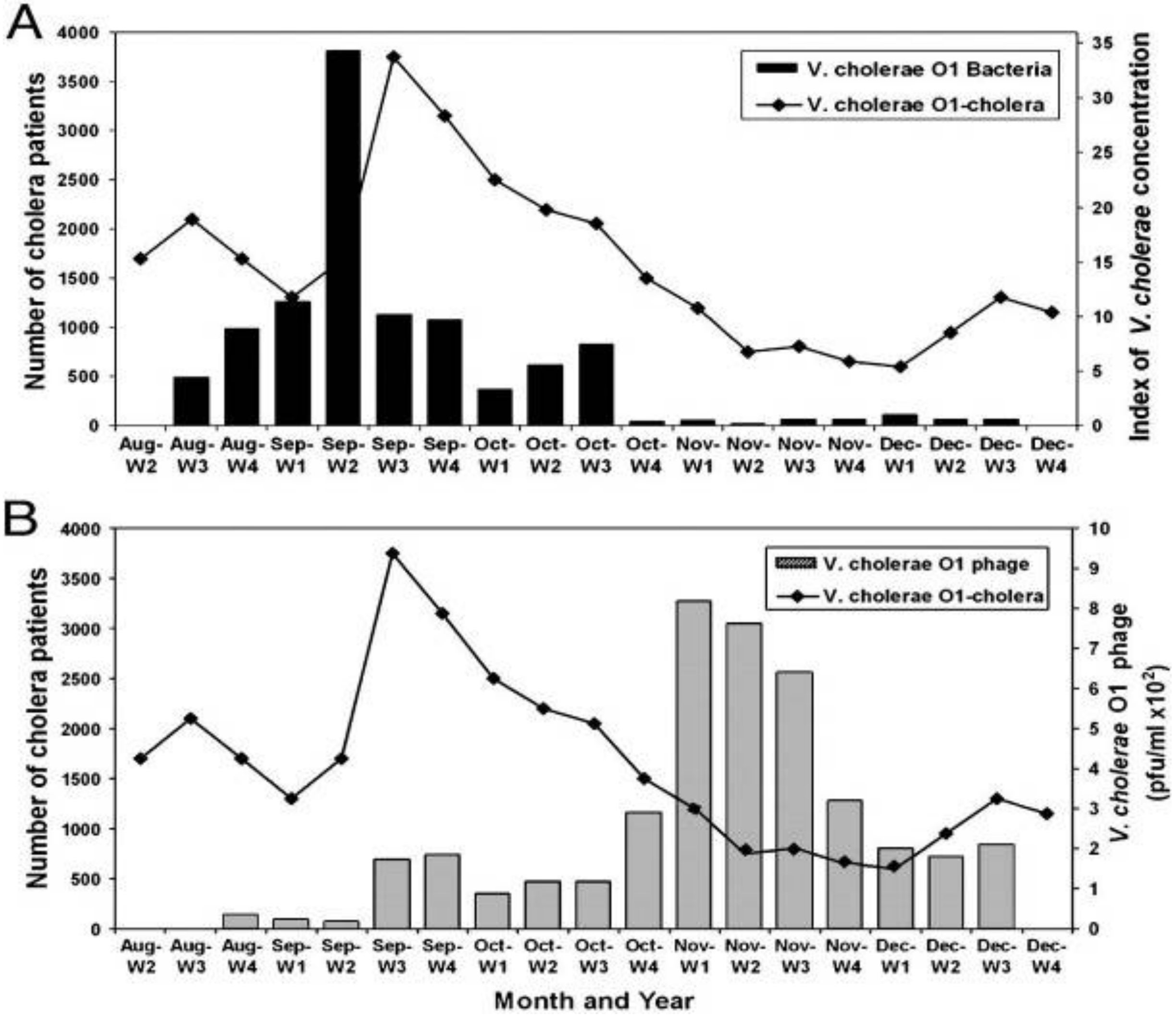
Tamplin *et al.* 1990; Huq *et al.* 1990; Islam *et al.* 1994; Colwell 1996

The waxing and waning of cholera cases in Dhaka Bangladesh (2001 - 2003) is correlated with the changing densities of vibrio-specific lytic phage



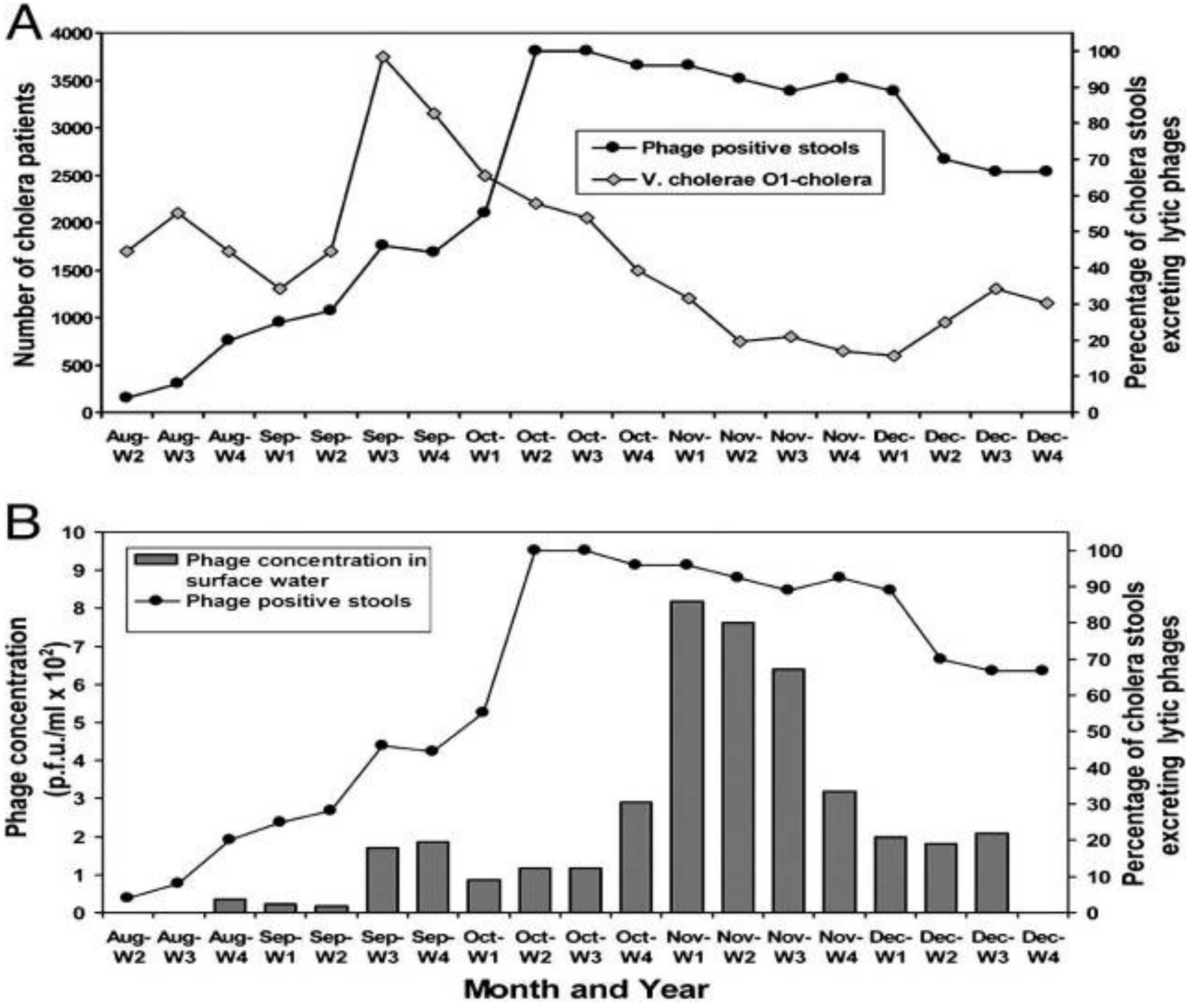
Faruque, Shah M. et al. (2005) Proc. Natl. Acad. Sci. USA 102, 1702-1707

Dynamics of the changes in the density of *V. cholerae* O1 and vibriophage in surface water during an outbreak of cholera in Bangladesh



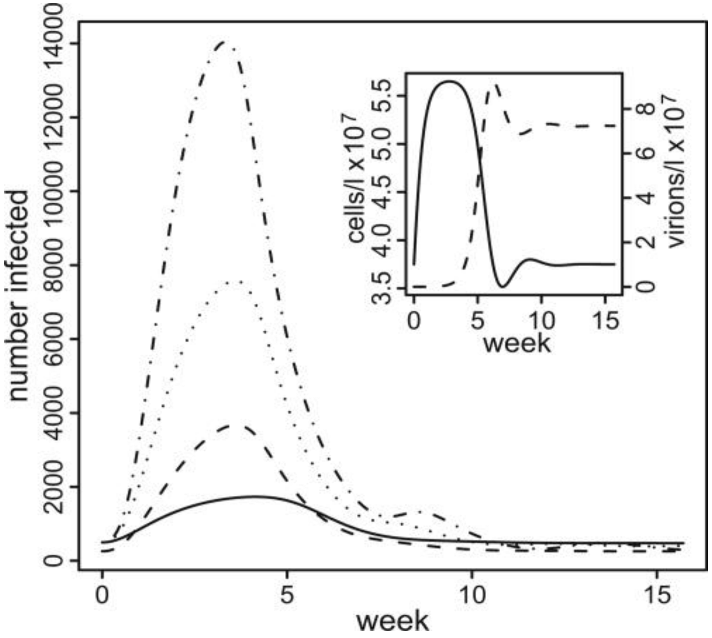
Faruque, Shah M. et al. (2005) Proc. Natl. Acad. Sci. USA 102, 6119-6124

Correlation of Vibriophage excretion by cholera patients with the environmental prevalence of the same phage



Faruque, Shah M. et al. (2005) Proc. Natl. Acad. Sci. USA 102, 6119-6124

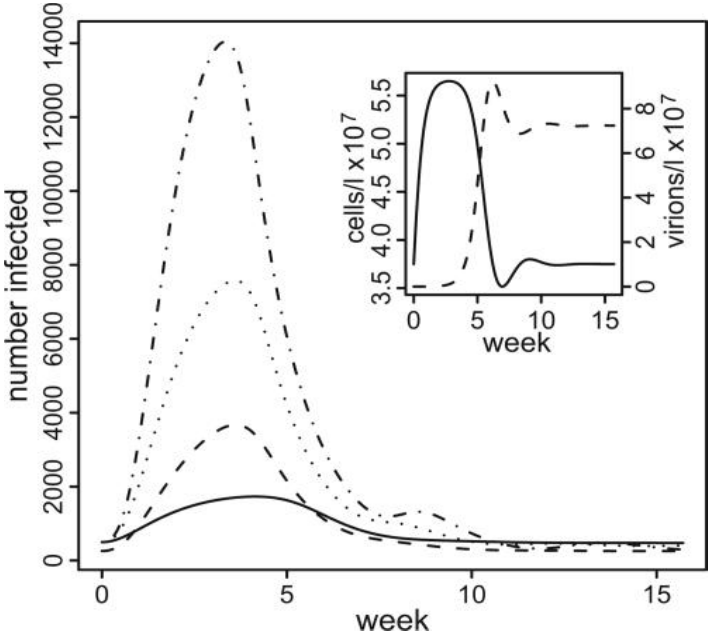
In theory, phage can regulate the waning of outbreaks of cholera



Mark Jensen

Jensen, M. S. Faruque, J.J. Mekalanos B. R. Levin, Modeling the role of bacteriophage in the control of cholera outbreaks (2006) ***Proc. Nat. Acad. Sci. US*** **103:4652-4657**

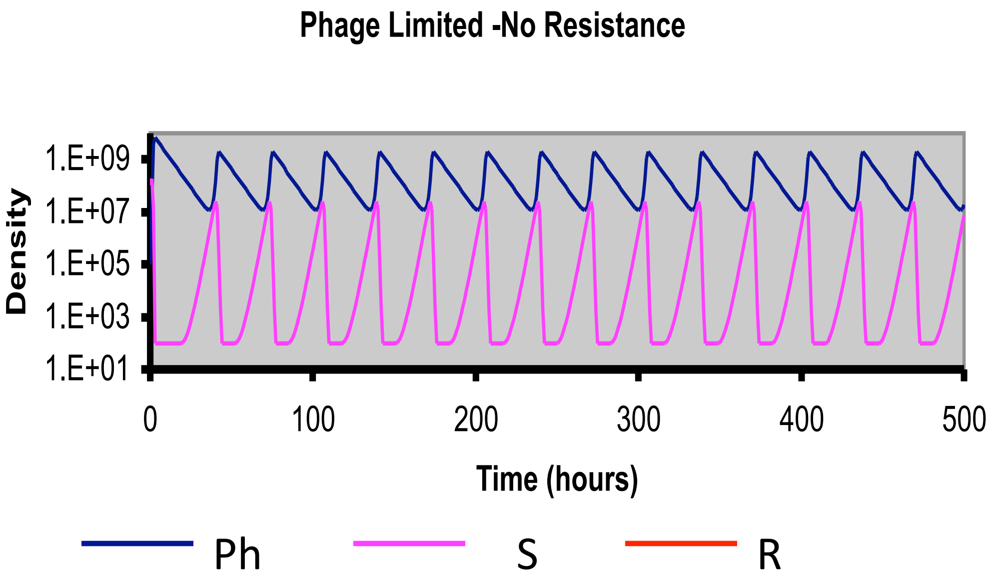
In theory, phage can regulate the waning of outbreaks of cholera



Central to this result is the condition that the population of bacteria are and continue to be limited by the phage.

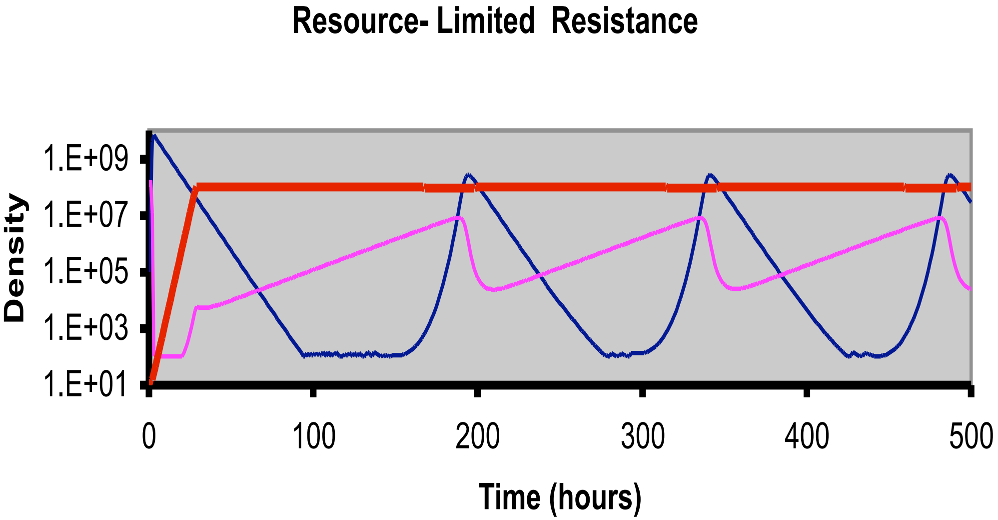
Jensen, M. S. Faruque, J.J. Mekalanos B. R. Levin, Modeling the role of bacteriophage in the control of cholera outbreaks (2006) *Proc. Nat. Acad. Sci. US* 103:4652-4657

Chemostat Model of Phage Dynamics



Phage Limited- bacteria population density controlled by phage dynamics

Resource Limited- controlled by abundance of resource

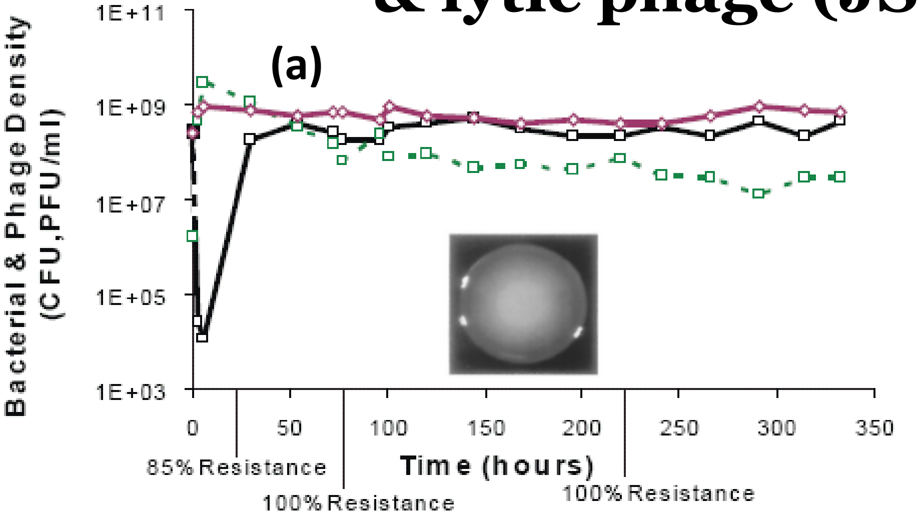


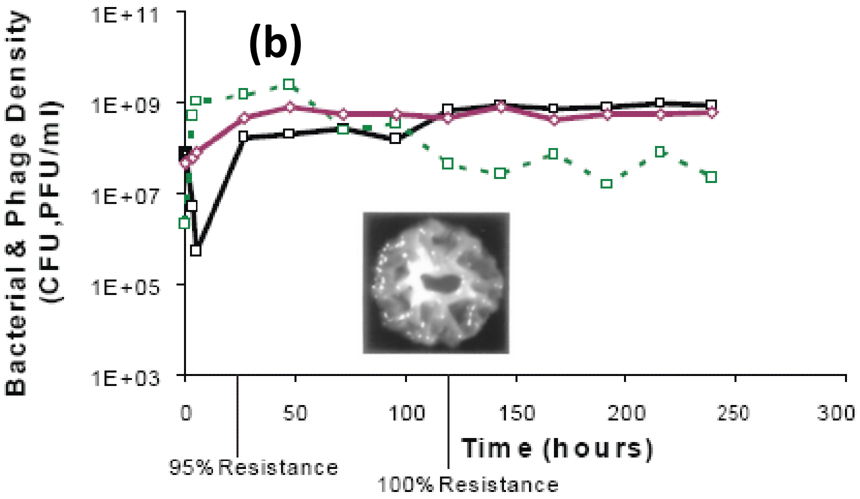
Levin, Stewart and Chao (1977) Am Nat 111: 3-24

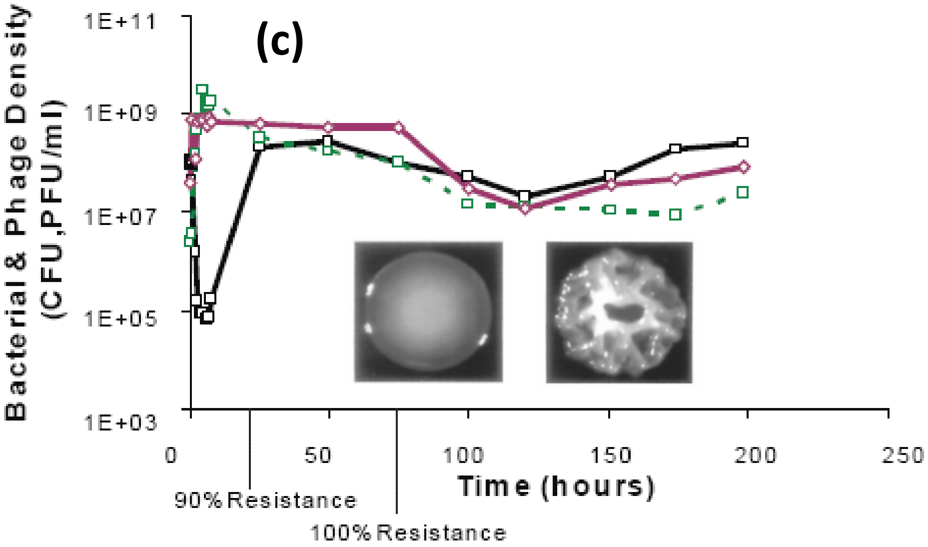
Hypothesis:

Lytic phage can limit the density of *V. cholerae* in laboratory culture.

Population dynamics of *V. cholerae* El Tor O1 (N16961) & lytic phage (JSF4) in chemostats







(a) Chemostat inoculated with wild-type *V. cholerae* N16961, w=0.07-0.12 (

(b) b) Chemostat inoculated with rugose variant of *V. cholerae* N16961, w=0.24,

(c) (c) Chemostat inoculated with smooth wild-type *V. cholerae* N16961. The phage JSF4 was introduced after the rugose variants in this chemostat reached a level of 9.4% of total bacteria, w=0.21

In vitro the phage JSF4 is unable to regulate the densities of V. cholerae

- High rate of mutation to resistance (10^{-7} per cell per generation)
- Population become limited by resource rather than phage
- No coevolution was observed (phage did not evolve to infect resistant bacteria)

Implication

These *in vitro* results are inconsistent with the hypothesis that phage predation limits the density of *V. cholerae* in the field.

Observations from Bangladesh *

- While phage replicate in humans and laboratory mice infected with sensitive *V. cholerae*, resistant mutants are not recovered
- Resistant mutants were rare among environmental isolates of *V. cholerae*

Why can’t they find phage resistant V. cholerae mutants *in vivo* and in the field?

*Shah Faruque personal communication

Hypotheses

- Phage resistant mutants have very low fitness and cannot persist in the environment.

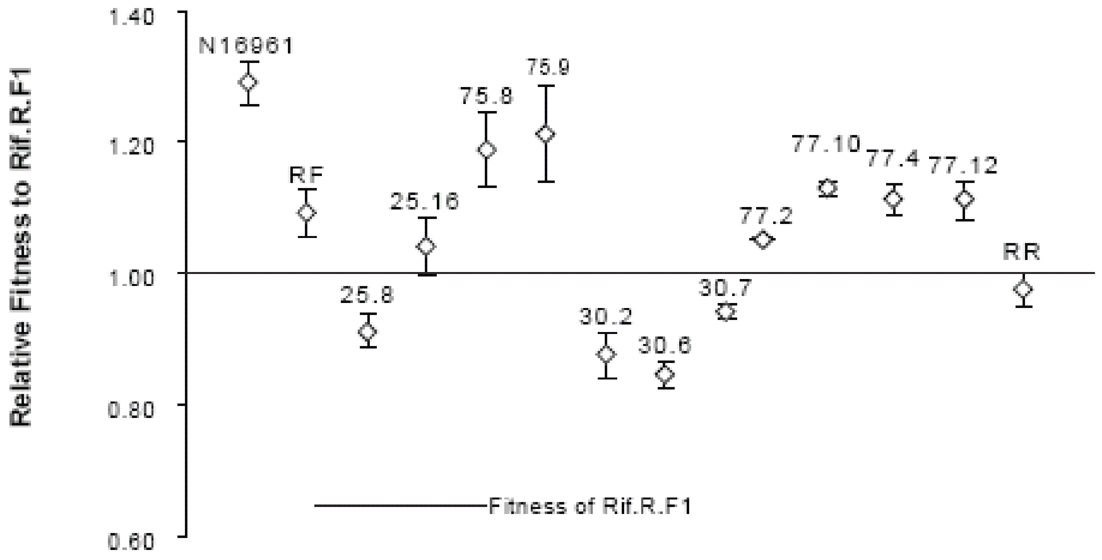
- Phage resistant mutants cannot replicate in the *V. cholerae* infected hosts (are avirulent).

Tests

Determine if phenotype or virulence of phage resistant bacteria are different from sensitive wildtype.

- Growth (Fitness)
- Motility
- Auto-agglutination
- Phenotypic switch from smooth to rugose
- Colonization on Daphnia
- Virulence

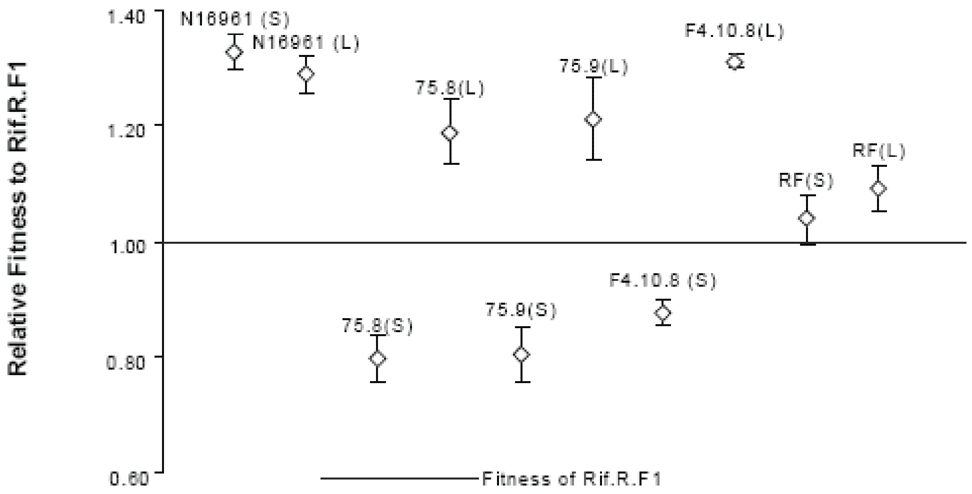
Fitness of JSF4 resistant N19161 (Liquid culture)



Relative Malthusian fitness of a Rif resistant JSF4 sensitive common competitor in pairwise competition with wild type, smooth N16961 and 13 JSF4 resistant mutants. The horizontal broken line denotes the fitness of the Rif resistant mutant assigned a value of 1.0. The error bars are the standard deviations of at least 3 independent pair-wise competition experiments.

In liquid culture, most but not all JSF4 resistant *V. cholerae* have a marked disadvantage relative to wildtype

Fitness of JSF4 resistant N19161 (Surface culture)



Relative fitness of different *V. cholerae* N16961 in competition with Rif resistant wild-type N16961 in liquid and in surface culture. (S), competition on surface, (L): competition in liquid: The 75.8, 75.9 : JSF4 resistant mutants are non-motile; RF: theJSF4 resistant mutants has reduced motility (50% of wild type N16961) F4.10.8 is a non-motile JSF4 sensitive isolate.

In surface culture all JSF4 resistant cells have a marked fitness disadvantage relative to wild type.

Phenotypic differences between JSF4 senstive and resistant *V. cholerae*


(a) Motility

(b) Agglutination

Figure 3 Motility, swimming and sinking classes of JSF4 sensitive and resistance *V. cholerae* N16961. (a). Motility (b). Behavior in still broth

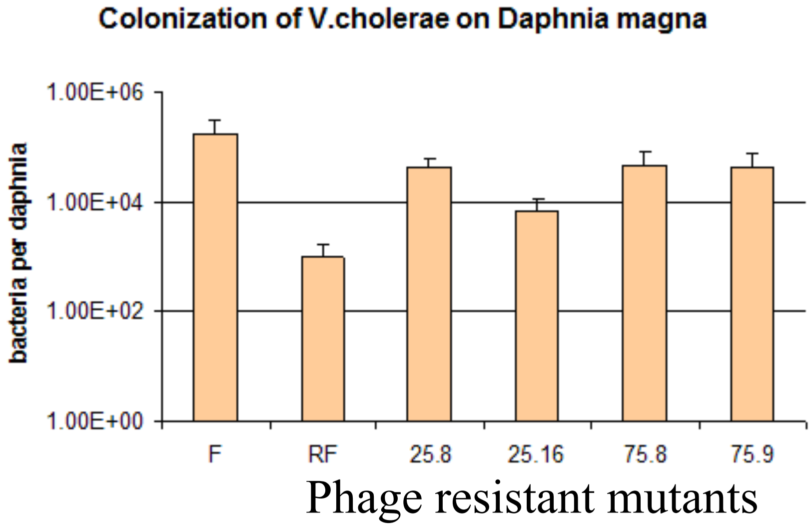
a.wild-type *V. cholerae* N16961

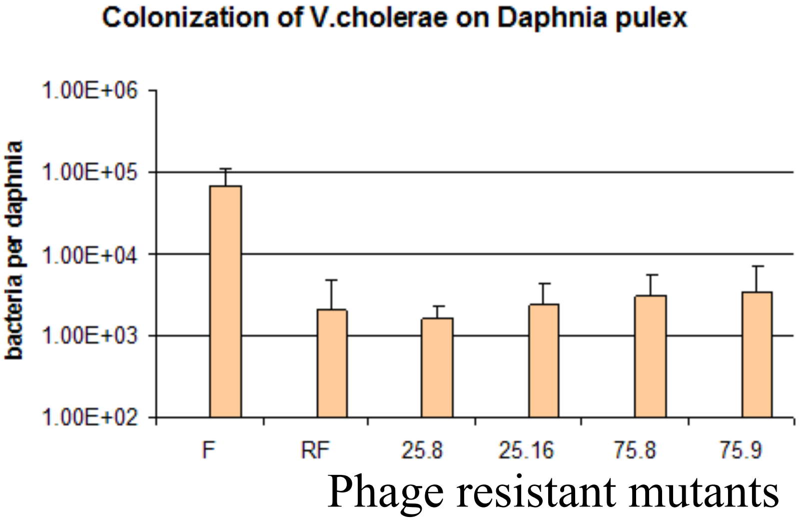
b.RF, JSF4^r *V. cholerae* N16961

c.75.9, JSF4^r resistant *V. cholerae* N16961

Colonization of *V. cholerae* N16961 on *Daphnia pulex* and *Daphina mangna*

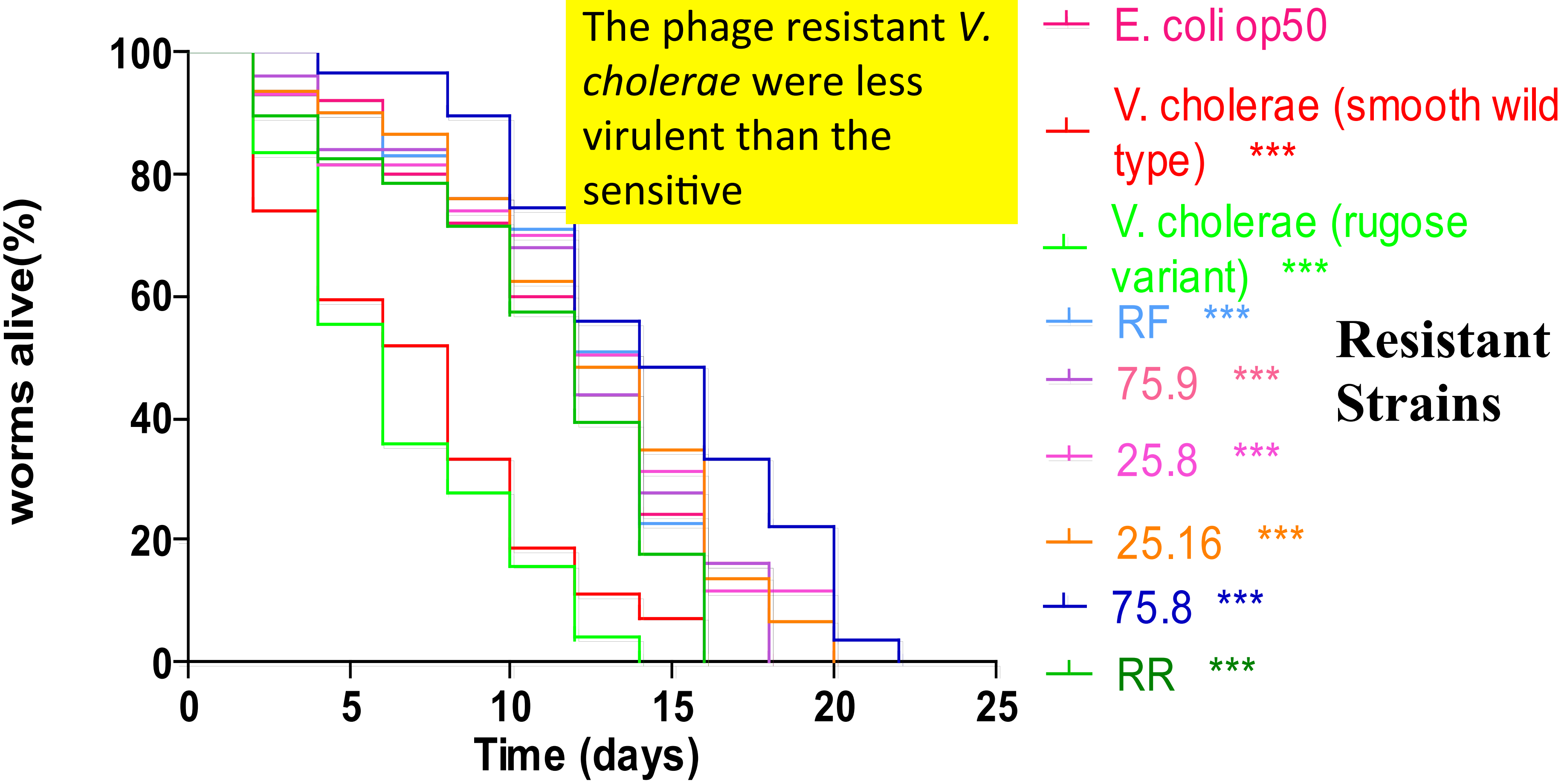
The phage resistant mutants are somewhat less effective at colonizing Daphnia than sensitive (F)





Virulence of sensitive and resistant - *C. elegans* Model

Survival of *C. elegans* growing on *E. coli* and phage sensitive and resistant *V. cholerae*



The phage resistant *V. cholerae* were less virulent than the sensitive

Resistant Strains

Thanks Steven W L'Hernault for kindly giving us *C.elegans* to play with!

Eureka (pretty good)

The results of these experiments are consistent with the hypothesis that lytic phage, like JSF4, could indeed contribute to the waning of cholera outbreaks

We postulate that although resistant mutants are anticipated to evolve rapidly, their fitness and virulence is so impaired that they are non-players in the ecology of V. cholerae.