Mathematical insights into RNA interference

Konstantin Blyuss

Department of Mathematics
University of Sussex

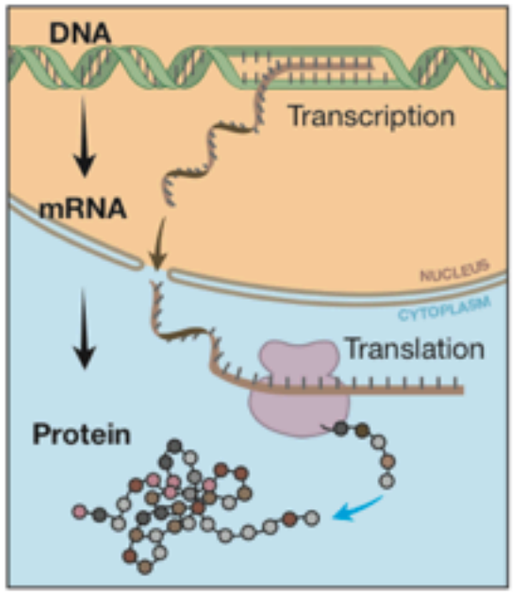
E-mail: k.blyuss@sussex.ac.uk

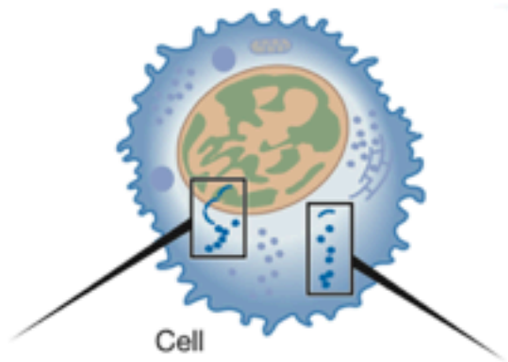
Outline

- Mechanism and history of RNAi
- RNA interference in plants
- Cross-infection with two viruses
- Dynamics of RNA silencing
- Conclusions and open problems

RNA interference (RNAi)

1. The central dogma

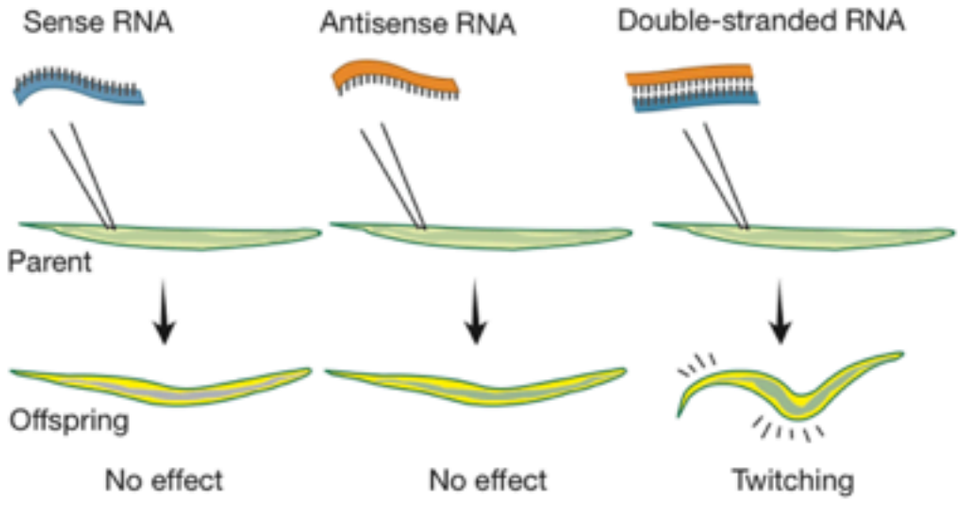




Our genome operates by sending information from double-stranded DNA in the nucleus, via single-stranded mRNA, to guide the synthesis of proteins in the cytoplasm.

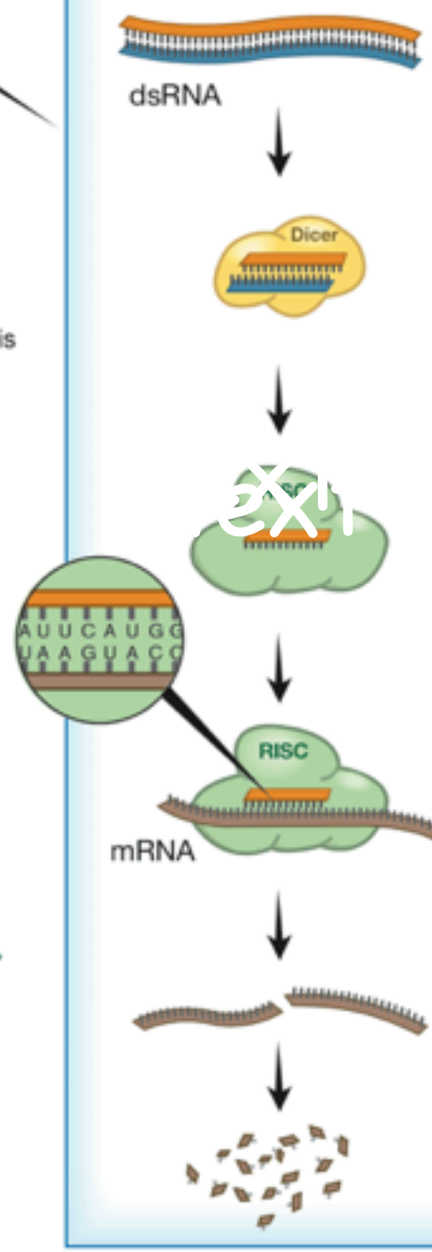
2. The experiment

RNA carrying the code for a muscle protein is injected into the worm *C. elegans*. Single-stranded RNA has no effect. But when double-stranded RNA is injected, the worm starts twitching in a similar way to worms carrying a defective gene for the muscle protein.



3. The RNAi mechanism

RNA interference (RNAi) is an important biological mechanism in the regulation of gene expression.



Double-stranded RNA (dsRNA) binds to the protein Dicer …

… which cleaves dsRNA into smaller fragments.

One of the RNA strands is loaded into a RISC complex…

…and links the complex to the mRNA strand by basepairing.

mRNA is cleaved and destroyed. No protein can be synthesized.

www.nobel.org

RNA interference (RNAi)

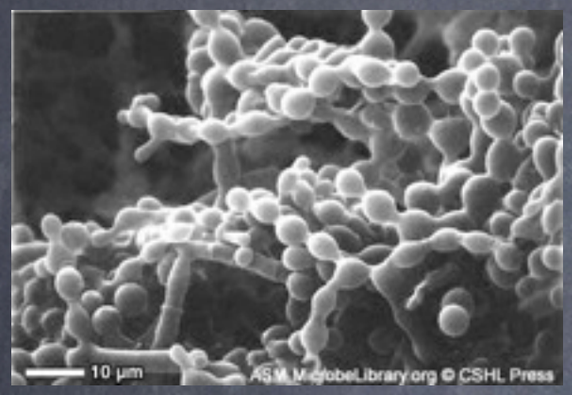
- RNAi regulates expression of genes
- RNAi mediates resistance to pathogens

  first line of viral defence
- RNAi is used in experiments to knock-out specific genes

RNAi: history

co-suppression in petunias

C. Napoli, C. Lemieux, R. Jorgensen (1990)



quelling in mold N. crassa

C. Cogoni, G. Macino (1992)

C. elegans

S. Guo, K.J. Kemphues (1995)

A. Fire et al. (1998)

siRNA Hamilton & Baulcombe (1999)

RISC S.M. Hammond et al. (2000)

Dicer: E. Bernstein et al. (2001)

RNAi: history

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

* *Carnegie Institution of Washington, Department of Embryology, 115 West University Parkway, Baltimore, Maryland 21210, USA*
† *Biology Graduate Program, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA*
‡ *Program in Molecular Medicine, Department of Cell Biology, University of Massachusetts Cancer Center, Two Biotech Suite 213, 373 Plantation Street, Worcester, Massachusetts 01605, USA*

NATURE | VOL 391 | 19 FEBRUARY 1998

Craig Mello

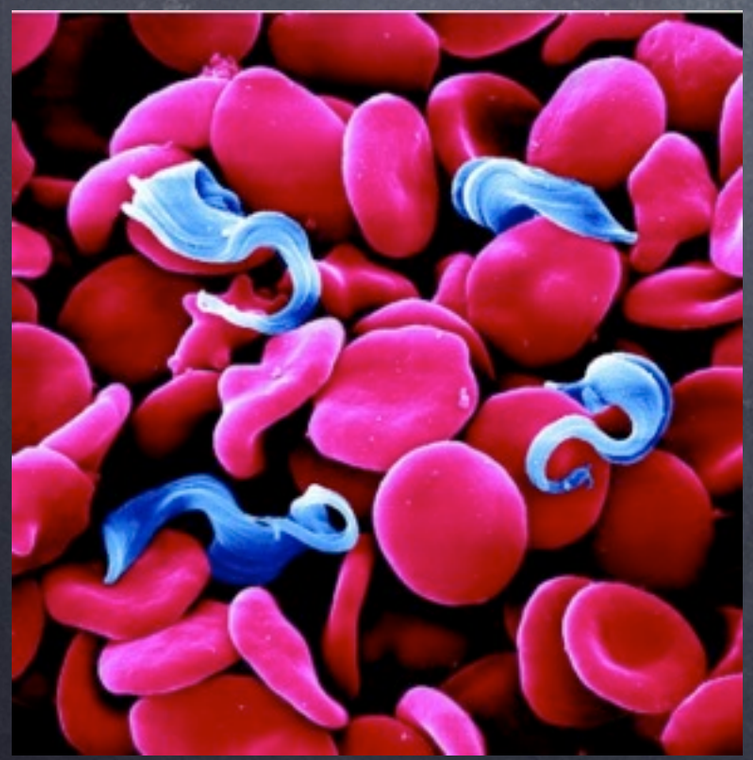
Andrew Fire

2006 Nobel Prize in Physiology and Medicine:

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

RNAi: history

RNAi was later discovered in many other species

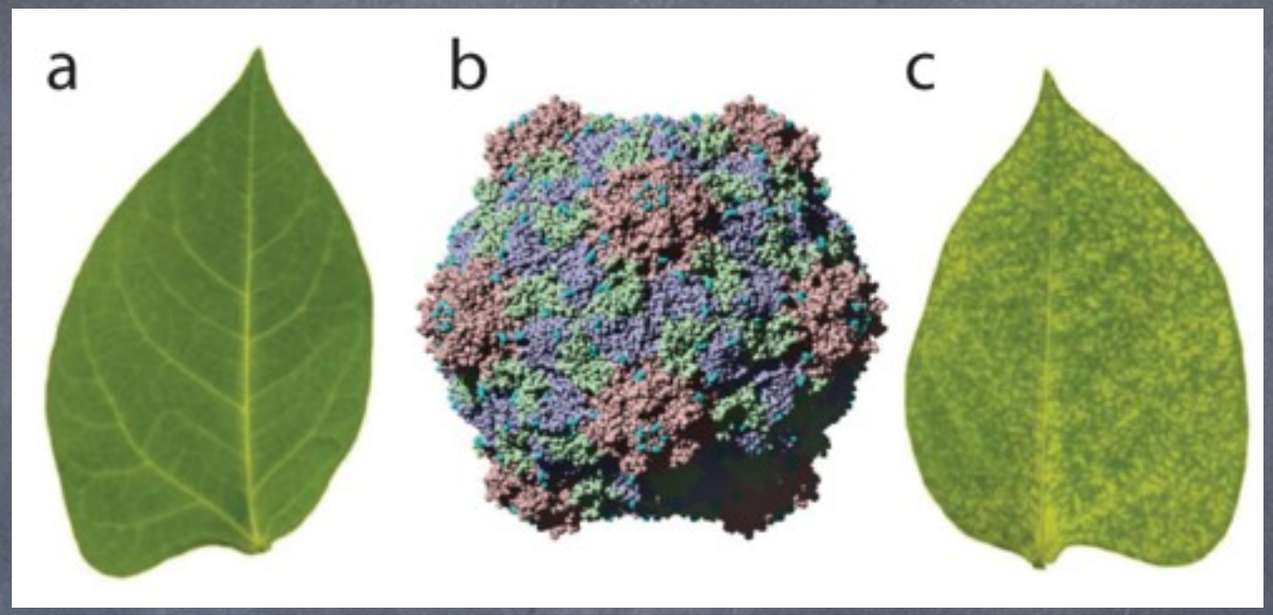


Trypanosoma brucei

but NOT

Trypanosoma cruzi, Leishmania major, S. cerivisiae,...

RNAi in plants

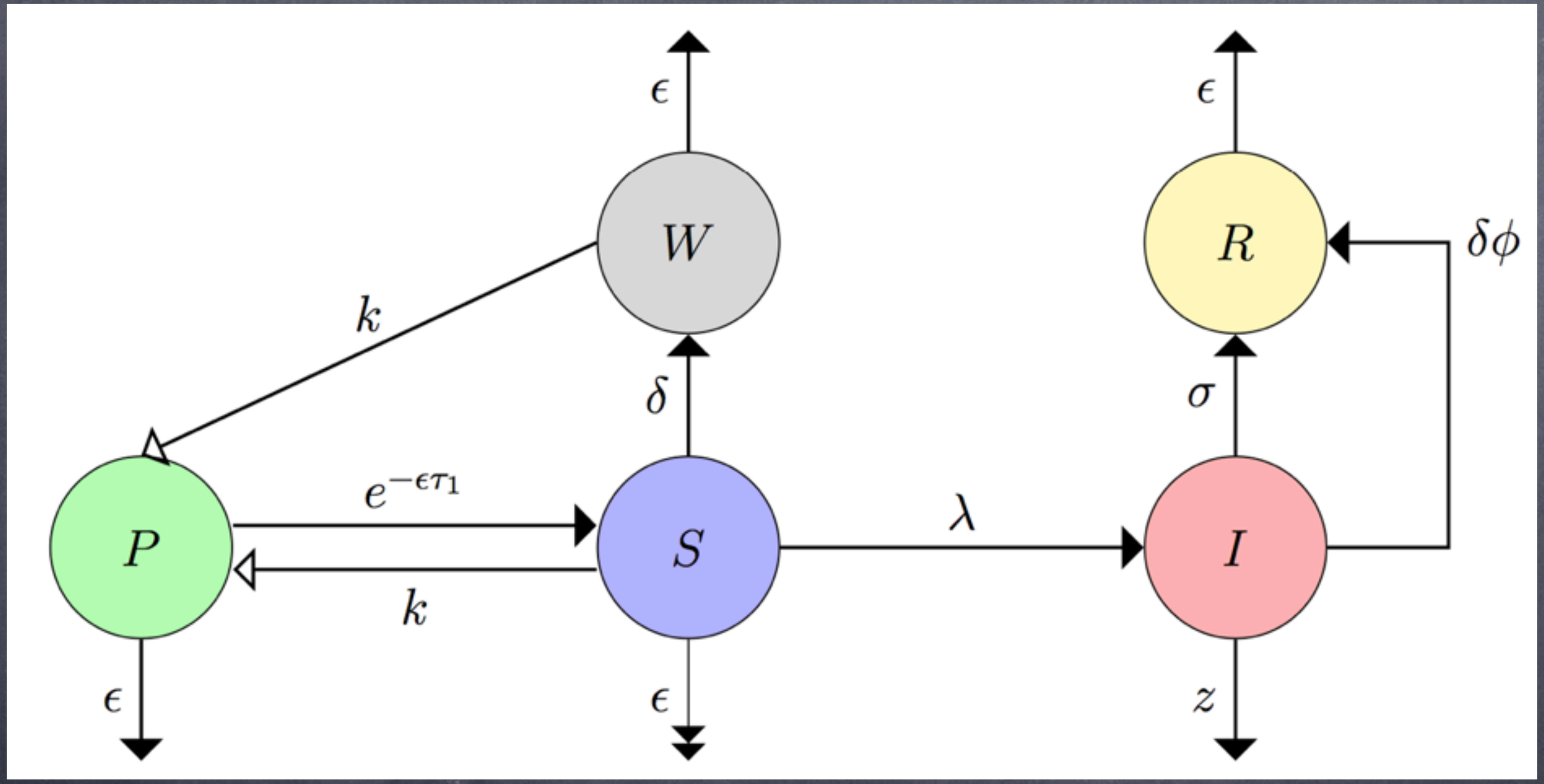


- Plant viruses - a major problem for agriculture
- $60 bn losses worldwide annually

Modelling assumptions

- New proliferating cells are immune to infection, they cannot express RNAi
- Mature cells are susceptible to infection and can induce RNAi
- Infected cells recover through RISC-mediated cleavage or RNA-directed DNA methylation
- Warned cells are mature cells that have received RNA silencing signal

Model diagram



G. Neofytou, Y.N. Kyrychko, KB, J. Theor. Biol. 389, 28-39 (2016)

Model of the plant immune response

$$\frac{dS}{dt} = k[S(t-\tau_1) + W(t-\tau_1)]e^{-\epsilon\tau_1} - S(t)[\lambda I(t) + \delta I(t-\tau_2) + \epsilon S(t)],$$

$$\frac{dI}{dt} = I(t)[\lambda S(t) - (z+\sigma) - \delta\phi I(t-\tau_2)],$$

$$\frac{dW}{dt} = \delta S(t) I(t-\tau_2) - \epsilon W(t).$$

S(t) - susceptible cells

I(t) - infected cells

W(t) - warned cells

τ_1 - maturation delay

τ_2 - silencing propagation delay

G. Neofytou, Y.N. Kyrychko, KB, J. Theor. Biol. 389, 28-39 (2016)

Model of the plant immune response

- The model is well-posed

Theorem 2.1. *Let the initial data be* $S(s) = S_0(s) > 0$, $W(s) = W_0(s) \geq 0$ *for all* $s \in [-\tau_1, 0]$, *and* $I(s) = I_0(s) \geq 0$ *for all* $s \in [-\tau_2, 0)$ *with* $I(0) > 0$. *Then solutions* $S(t), I(t)$ *and* $W(t)$ *of the system (2) are non-negative for all* $t > 0$.

- Trivial steady state E_0=(0,0,0) always unstable

characteristic equation:

$$(\mu + \epsilon)(\mu + \sigma + z)(ke^{-\epsilon\tau_1}e^{-\mu\tau_1} - \mu) = 0.$$

two stable eigenvalues plus

$$\mu = ke^{-\epsilon\tau_1}e^{-\mu\tau_1}$$

always a positive real root

Steady states

- Disease-free steady state

$$E_1 = (\epsilon^{-1} K(\tau_1), 0, 0), \qquad K(\tau_1) = k e^{-\epsilon \tau_1}$$

Theorem 4.1. *Let the disease-free steady state be given by* $E_1 = \left(\frac{K(\tau_1)}{\epsilon}, 0, 0\right)$ *and denote* $k_{\min} = \frac{\epsilon(\sigma + z)}{\lambda}$. *Then, we have the following*

(a) *Given* $k < k_{\min}$, E_1 *is linearly asymptotically stable for all* $\tau_1 \geq 0$.

(b) *Given* $k \geq k_{\min}$ *and* $\tau_{min} = \frac{\ln(k) - \ln(k_{\min})}{\epsilon}$, E_1 *is linearly asymptotically stable for* $\tau_1 > \tau_{min}$, *unstable for* $\tau < \tau_{min}$ *and undergoes a steady-state bifurcation at* $\tau_1 = \tau_{min}$.

Steady states

- Endemic steady state $E_2=(S^*,I^*,W^*)$

$$S^* = S(\tau_1) = \frac{K(\tau_1)}{\epsilon} - \frac{[\delta K(\tau_1) - \epsilon(\lambda+\delta)][\lambda K(\tau_1) - \epsilon(z+\sigma)]}{\epsilon[\epsilon\lambda^2 - \delta\lambda(K(\tau_1)-\epsilon) + \delta\phi\epsilon^2]},$$

$$I^* = I(\tau_1) = \frac{\epsilon[\lambda K(\tau_1) - \epsilon(z+\sigma)]}{\epsilon\lambda^2 - \delta\lambda[K(\tau_1)-\epsilon] + \delta\phi\epsilon^2},$$

$$W^* = W(\tau_1) = \frac{\delta\left[\epsilon\delta\phi K(\tau_1) - (z+\sigma)(\delta K(\tau_1) - \epsilon(\lambda+\delta)\right]\left[\lambda K(\tau_1) - \epsilon(z+\sigma)\right]}{[\epsilon\lambda^2 - \delta\lambda(K(\tau_1)-\epsilon) + \delta\phi\epsilon^2]^2}.$$

- Feasible when

$$\frac{\ln(k) - \ln(C_{\max})}{\epsilon} < \tau_1 < \frac{\ln(k) - \ln(C_{\min})}{\epsilon}$$

where

$$C = \left\{ \frac{\epsilon(z+\sigma)}{\lambda}, \frac{\epsilon(\lambda^2+\delta\lambda+\delta\phi\epsilon)}{\delta\lambda} \right\}$$

$C_{min}=\min(C)$, $C_{max}=\max(C)$

Stability of E_2

- Trivial maturity $\tau_1 = 0$
- Characteristic equation

$$\mu^3 + (a_1 e^{-\mu\tau_2} + a_2)\mu^2 + (b_1 e^{-\mu\tau_2} + b_2)\mu + (c_1 e^{-\mu\tau_2} + c_2) = 0$$

where

$$\begin{aligned}
a_1 &= \delta\,\phi I^*, \quad a_2 = (\lambda+\delta)\,I^* + (2\,S^*+1)\,\epsilon - k,\\
b_1 &= \delta\phi\,(\delta+\lambda)\,I^{*2} + [(\epsilon - k + 2\,\epsilon\,S^*)\,\phi + \lambda\,S^*]\,\delta\,I^*,\\
b_2 &= [(\lambda+\delta)\,\epsilon - \delta\,k + \lambda^2 S^*]\,I^* - k\epsilon + 2\,\epsilon^2 S^*,\\
c_1 &= -\delta I^*\,[[k\delta - \epsilon\,(\delta+\lambda)]\,\phi\,I^* + (k\lambda - \epsilon(2\epsilon\phi + \lambda))\,S^* + k\phi\,\epsilon]\,,\\
c_2 &= \lambda^2\epsilon\,S^* I^*.
\end{aligned}$$

Stability of E_2

- When $\tau_2=0$, Routh-Hurwitz conditions for stability

$$a_1 + a_2 > 0,\ c_1 + c_2 > 0,\ (a_1 + a_2)(b_1 + b_2) > c_1 + c_2$$

- For $\tau_2>0$, Hopf frequency is a root of

$$w^6 + ({a_2}^2 - {a_1}^2 - 2b_2)w^4 + (2c_1a_1 - 2c_2a_2 + {b_2}^2 - {b_1}^2)w^2 + {c_2}^2 - {c_1}^2 = 0$$

- Critical time delay

$${\tau_2}^{(j)}(n) = \frac{1}{w_n}\left[\tan^{-1}\left(\frac{a_1{w_n}^5+(b_1a_2-c_1-a_1b_2){w_n}^3+(c_1b_2-b_1c_2)w_n}{(b_1-a_1a_2){w_n}^4+(c_1a_2+a_1c_2-b_1b_2){w_n}^2-c_1c_2}\right)+(j-1)\pi\right]$$

$$n = 1, 2, 3; \quad j \in \mathbb{N}$$

$${\tau_2}^* = {\tau_2}^{(j_0)}(n_0) = \min_{1\le n\le 3,\ j\ge 1}\{{\tau_2}^{(j)}(n)\}, w_0 = w_{n0}$$

Stability of E_2

- When τ_2=0, the characteristic equation for stability takes the form

$$\mu^3+\left[a_1(\tau_1)e^{-\mu\tau_1}+a_2(\tau_1)\right]\mu^2+\left[b_1(\tau_1)e^{-\mu\tau_1}+b_2(\tau_1)\right]\mu+c_1(\tau_1)e^{-\mu\tau_1}+c_2(\tau_1)=0$$

- When $\tau_1=\tau_2=\tau$, the characteristic equation for stability takes the form

$$\mu^3+\left[a_1(\tau)e^{-\mu\tau}+a_2(\tau)\right]\mu^2+\left[b_1(\tau)e^{-\mu\tau}+b_2(\tau)e^{-2\mu\tau}+b_3(\tau)\right]\mu+c_1(\tau)e^{-\mu\tau}+c_2(\tau)e^{-2\mu\tau}+c_3(\tau)=0$$

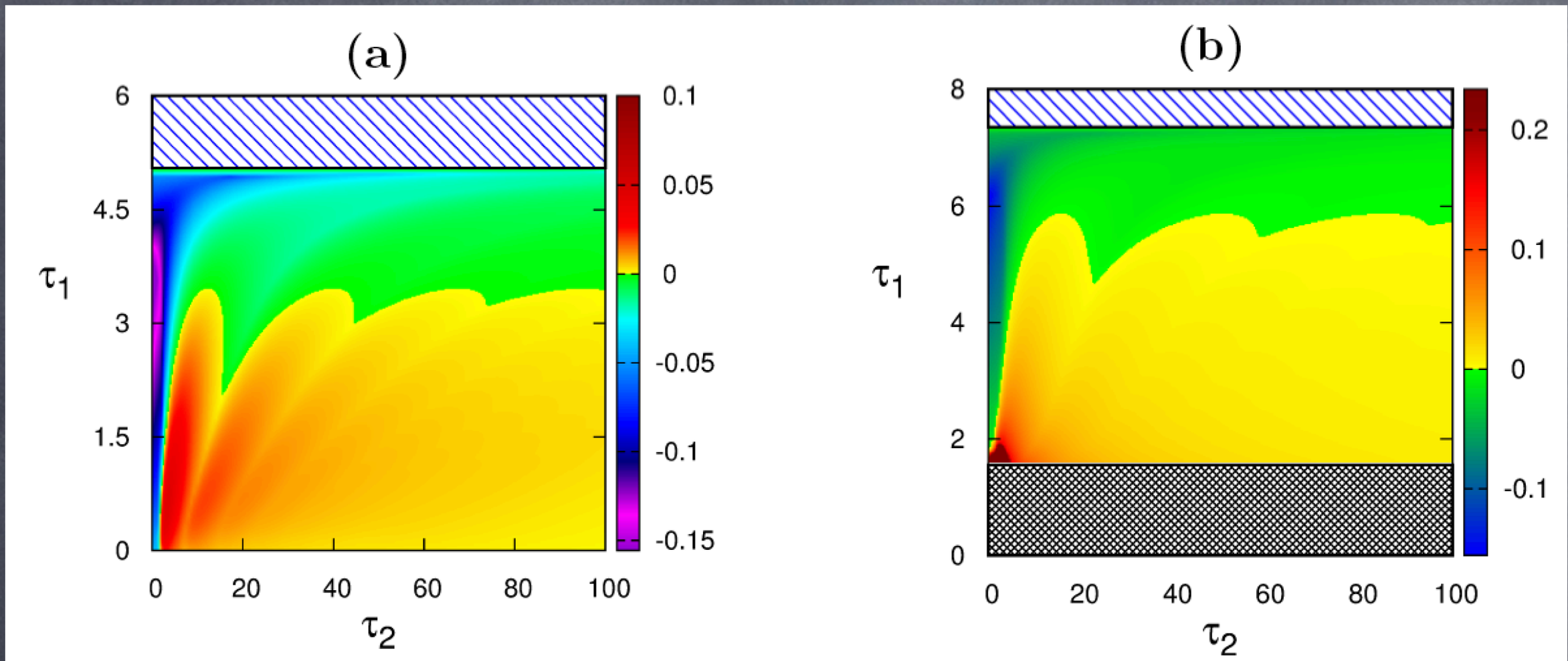
- It does not prove possible to find closed form analytical results for the critical time delay

Parameter values

Table 1: Table of parameters

Symbol	Definition	Baseline values (estimated)
λ	Rate of infection	1.5
k	Growth rate	1
σ	Recovery rate	0.5
δ	Propagation rate of silencing signal	0.5
ϕ	Recovery rate	1
ϵ	Natural death rate of cells	0.3
z	Death rate of infected cells	0.6
τ_1	Maturity time of young/proliferating tissue	1
τ_2	Acquired immunity delay	1

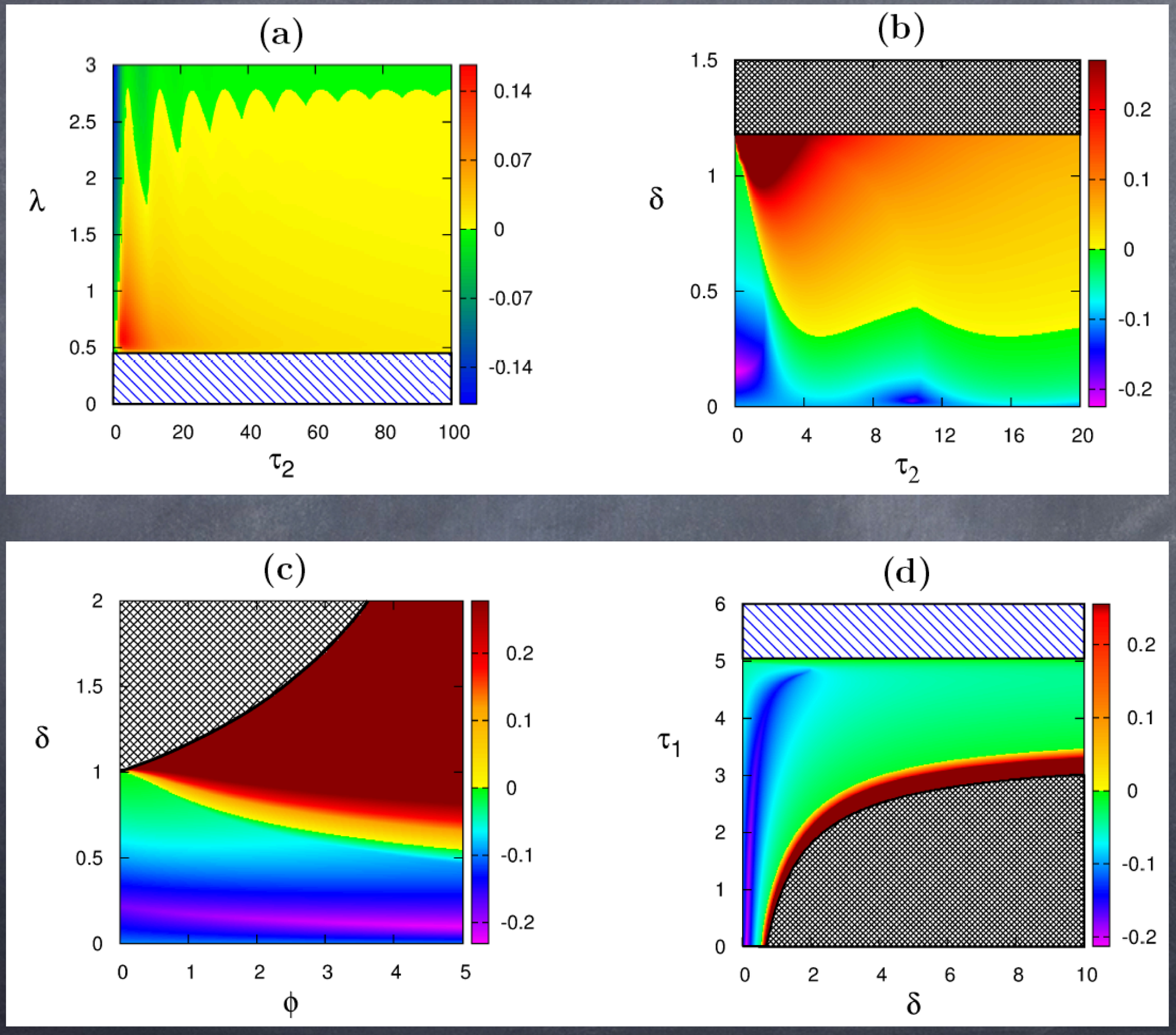
Stability chart



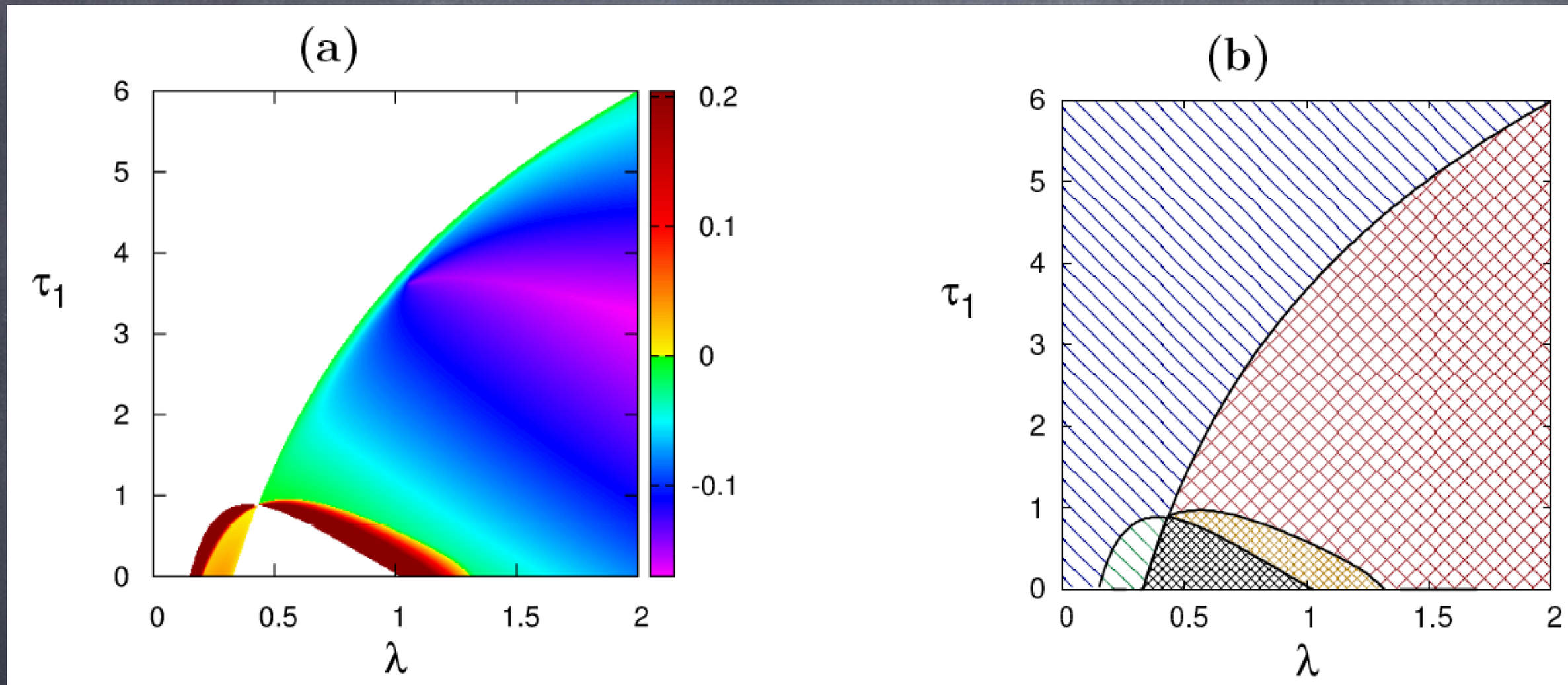
(a) k=1

(b) k=2

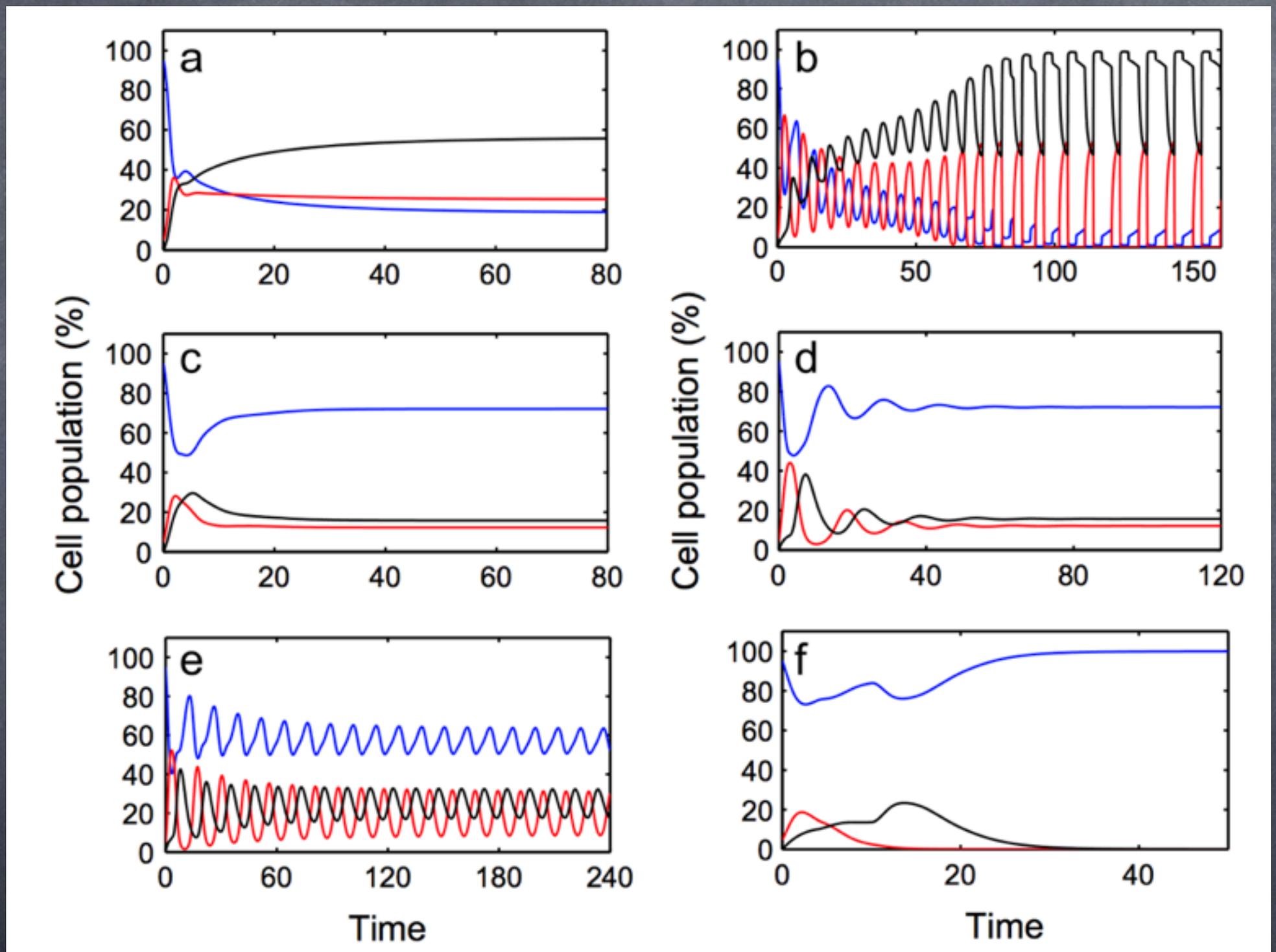
Stability chart



Stability chart

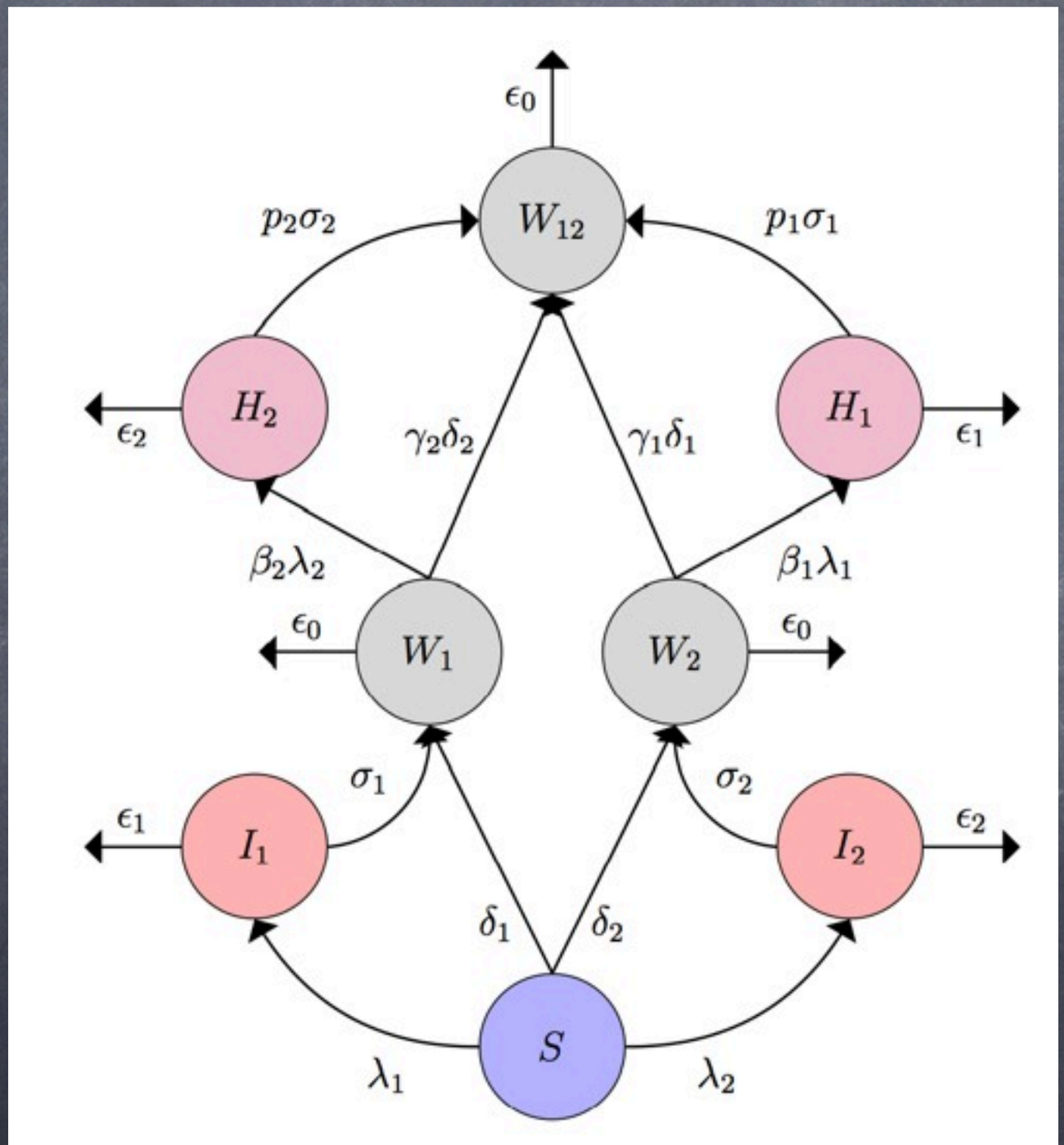


Numerical simulations



G. Neofytou, Y.N. Kyrychko, KB, J. Theor. Biol. 389, 28-39 (2016)

Modelling interactions between plant viruses



Two-virus model

$$\frac{dS}{dt} = r\widehat{S}\left(1 - \frac{N}{K}\right) - S\left[(\lambda_1 + \delta_1)I_1 + (\lambda_2 + \delta_2)I_2 + a_2\lambda_2 H_2 + a_1\lambda_1 H_1\right],$$

$$\frac{dI_1}{dt} = I_1(\lambda_1 S - \sigma_1 - \epsilon_1) + a_1\lambda_1 H_1 S,$$

$$\frac{dI_2}{dt} = I_2(\lambda_2 S - \sigma_2 - \epsilon_2) + a_2\lambda_2 H_2 S,$$

$$\frac{dW_1}{dt} = I_1(\sigma_1 + \delta_1 S) - W_1\left[\epsilon_0 + (\beta_2\lambda_2 + \gamma_2\delta_2)I_2 + \beta_2 a_2\lambda_2 H_2\right],$$

$$\frac{dW_2}{dt} = I_2(\sigma_2 + \delta_2 S) - W_2\left[\epsilon_0 + (\beta_1\lambda_1 + \gamma_1\delta_1)I_1 + \beta_1 a_1\lambda_1 H_1\right],$$

$$\frac{dH_1}{dt} = W_2(\beta_1\lambda_1 I_1 + \beta_1 a_1\lambda_1 H_1) - H_1(\epsilon_1 + p_1\sigma_1),$$

$$\frac{dH_2}{dt} = W_1(\beta_2\lambda_2 I_2 + \beta_2 a_2\lambda_2 H_2) - H_2(\epsilon_2 + p_2\sigma_2),$$

$$\frac{dW_{12}}{dt} = p_1\sigma_1 H_1 + p_2\sigma_2 H_2 + \gamma_2\delta_2 I_2 W_1 + \gamma_1\delta_1 I_1 W_2 - \epsilon_0 W_{12},$$

G. Neofytou, Y.N. Kyrychko, KB, submitted (2016)

Rescaled two-virus model

$$\frac{du_1}{d\tau} = \widehat{u}_1(1-\widehat{N}) - u_1\left[(L_1+d_1)\,u_2 + (L_2+d_2)\,u_3 + a_1L_1u_6 + a_2L_2u_7\right],$$

$$\frac{du_2}{d\tau} = L_1\left(a_1u_6+u_2\right)u_1 - u_2\left(e_1+s_1\right),$$

$$\frac{du_3}{d\tau} = L_2\left(a_2u_7+u_3\right)u_1 - u_3\left(e_2+s_2\right),$$

$$\frac{du_4}{d\tau} = u_2\left(d_1u_1+s_1\right) - u_4\left[(\beta_2L_2+\gamma_2d_2)\,u_3 + \beta_2a_2L_2u_7 + e_0\right],$$

$$\frac{du_5}{d\tau} = u_3\left(d_2u_1+s_2\right) - u_5\left[(\beta_1L_1+\gamma_1d_1)\,u_2 + \beta_1a_1L_1u_6 + e_0\right],$$

$$\frac{du_6}{d\tau} = \beta_1L_1\left(a_1u_6+u_2\right)u_5 - u_6\left(p_1s_1+e_1\right),$$

$$\frac{du_7}{d\tau} = \beta_2L_2\left(a_2u_7+u_3\right)u_4 - u_7\left(p_2s_2+e_2\right),$$

$$\frac{du_8}{d\tau} = \gamma_1d_1u_2u_5 + \gamma_2d_2u_3u_4 + p_1s_1u_6 + p_2s_2u_7 - e_0u_8,$$

Steady states

- Trivial steady state E_0=(0,0,0,0,0,0,0,0)
- Disease-free steady state E_{DF}=(1,0,0,0,0,0,0,0)
- Two one-virus steady states

$$E_1 = (\widetilde{u}_1^*, u_2^*, 0, u_4^*, 0, 0, 0)$$

$$E_2 = (u_1^*, 0, u_3^*, 0, u_5^*, 0, 0)$$

- Endemic steady state

$$S = (u_1^*, u_2^*, u_3^*, u_4^*, u_5^*, u_6^*, u_7^*, u_8^*)$$

Steady states

- Introduce basic reproduction numbers

$$\mathcal{R}_{01} = \frac{L_1}{e_1 + s_1}, \quad \mathcal{R}_{02} = \frac{L_2}{e_2 + s_2}, \quad \mathcal{R}_0 = \max\{\mathcal{R}_{01}, \mathcal{R}_{02}\}$$

- E_1 is feasible for $R_{01}>1$
- E_2 is feasible for $R_{02}>1$
- Disease-free state is stable for $R_0<1$

Stability of the steady states

Theorem. *The one-virus steady state* $E_2 = (u_1^*, 0, u_3^*, 0, u_5^*, 0, 0)$ *with* $u_1^* = (e_2 + s_2)/L_2$, u_3^* *and* u_5^* *given by*

$$u_1^* = \frac{e_2 + s_2}{L_2}, \quad u_3^* = \frac{-c_1(u_1^*) - \sqrt{c_1^2(u_1^*) - 4c_2(u_1^*)c_0(u_1^*)}}{2c_2(u_1^*)}, \quad u_5^* = A(u_1^*)u_3^*,$$

with

$$A(u_1^*) = \frac{d_2 u_1^* + s_2}{e_0}, \quad B = L_2 + d_2, \quad c_0(u_1^*) = u_1^*(1 - u_1^*),$$

$$c_1(u_1^*) = A(u_1^*) - u_1^*[2A(u_1^*) + B + 1], \quad c_2(u_1^*) = -A(u_1^*)[A(u_1^*) + 1].$$

Let x_{30}, x_{31}, x_{32} *and* u_B *be defined by*

$$x_{21} = s_1(p_1 + 1) + 2e_1 - L_1(a_1\beta_1 u_5^* + u_1^*),$$

$$x_{20} = (p_1 s_1 + e_1)(e_1 + s_1 - L_1 u_1^*) - L_1 a_1 \beta_1 (e_1 + s_1) u_5^*,$$

$$x_{32} = 2u_1^* + (L_2 + d_2 + 1)\, u_3^* + 2u_5^* + e_0 - 1,$$

$$x_{31} = d_1(u_3^*)^2 + [(L_2 + d_2)[u_1^*(L_2 + 1) + u_5 + e_0] + d_2(u_1^* + u_5^* - 1) + e_0]\, u_3^* + e_0(2u_1^* + 2u_5^* - 1),$$

$$x_{30} = L_2 u_3^* [d_2 u_1^* (2(u_1^* + u_5^*) + u_3^* + e_0 - 1) + u_1^* e_0 (L_2 + 1) + s_2 (2u_1^* + u_3^* - 1)] + L_2 u_3^* + u_5^* (e_0 + 2s_2).$$

and

$$u_B = \frac{s_1 p_1 + e_1}{L_1 a_1 \beta_1} \frac{s_1 + e_1 - L_1 u_1^*}{e_1 + s_1}.$$

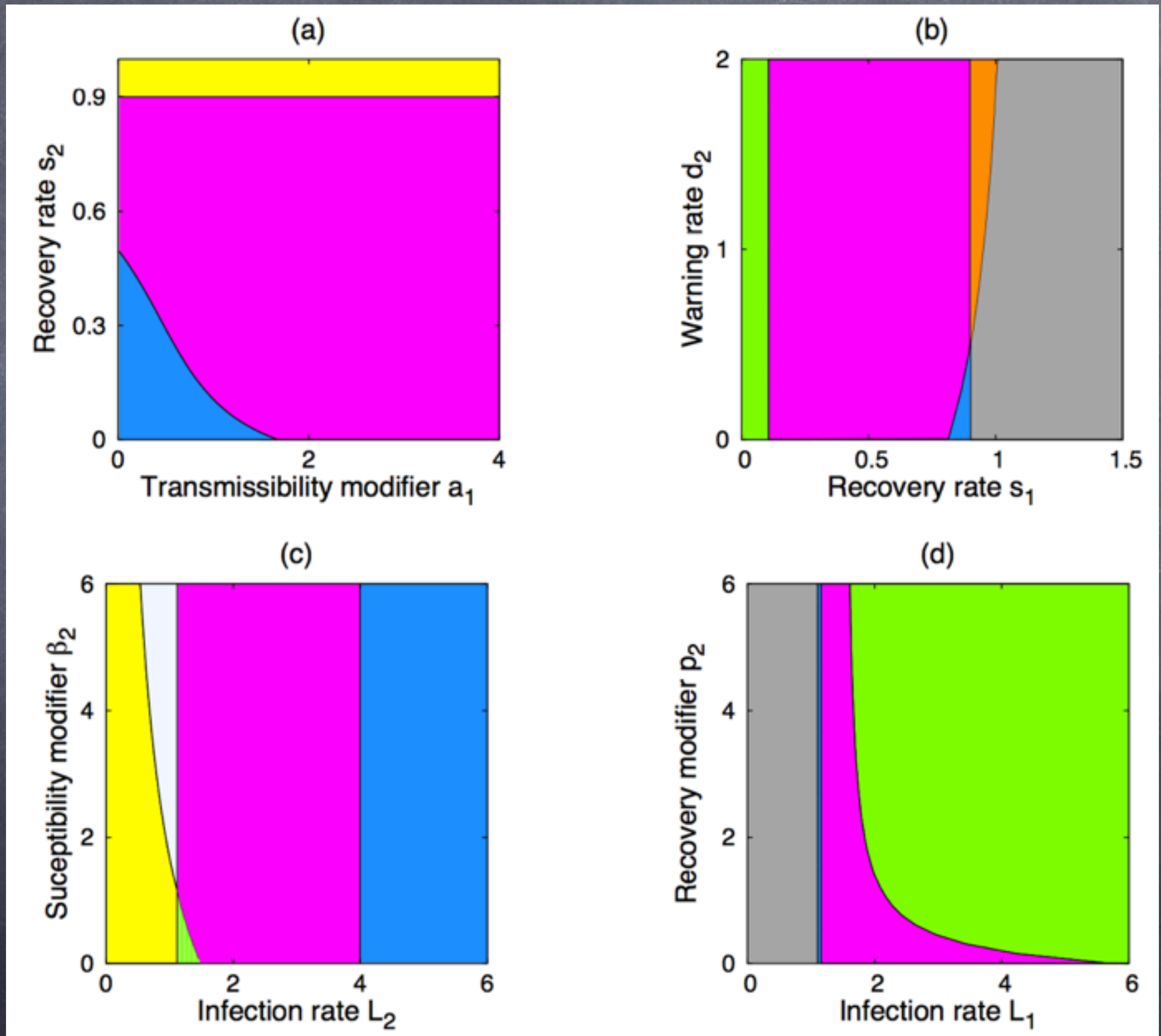
Then the steady state E_2 *is linearly asymptotically stable if and only if the following conditions hold:*

(i) $0 < u_5^* < u_B$,

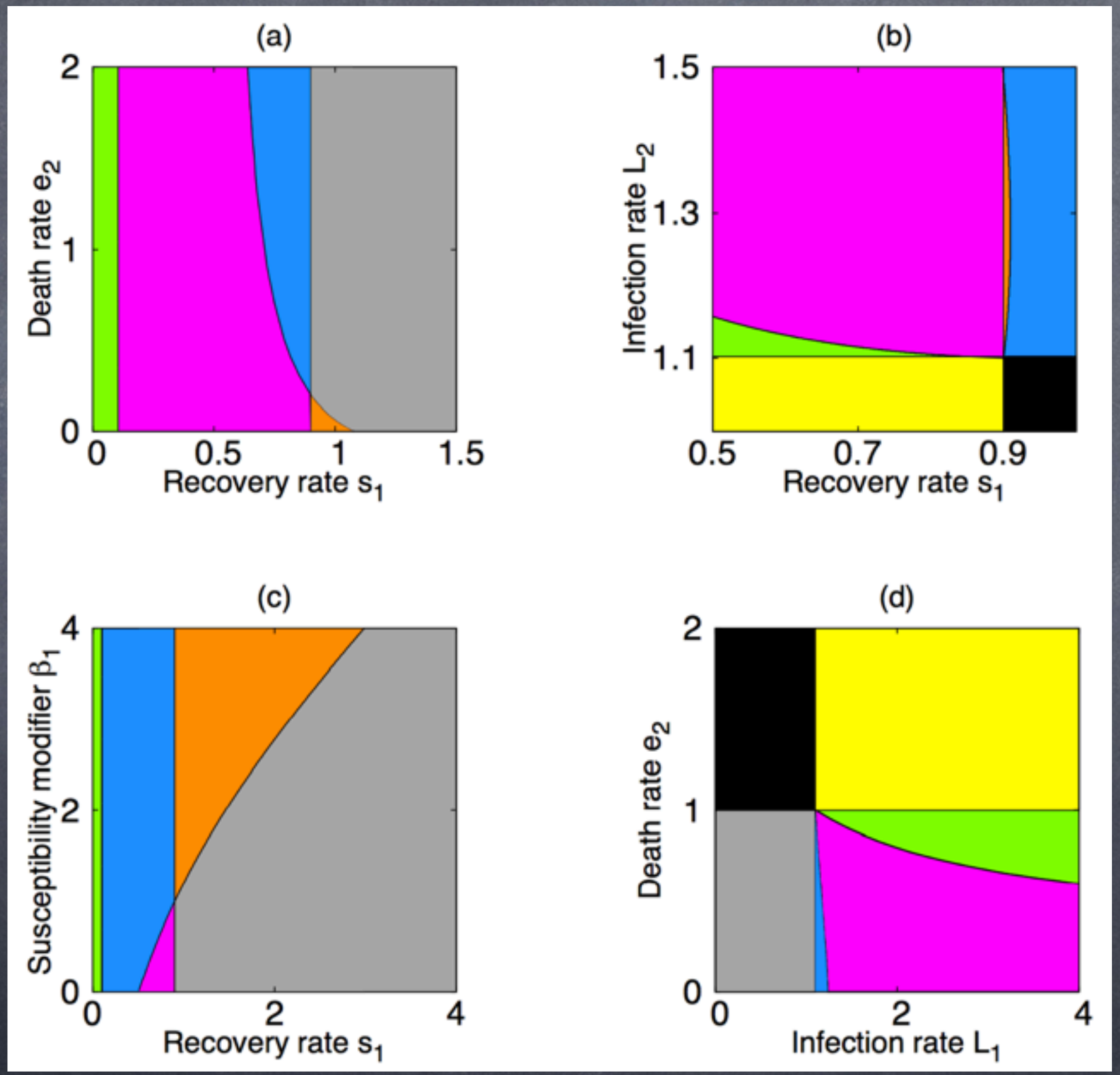
(ii) $x_{30} > 0$, $x_{31} > 0$, $x_{32} > 0$,

(iii) $x_{32}x_{31} > x_{30}$.

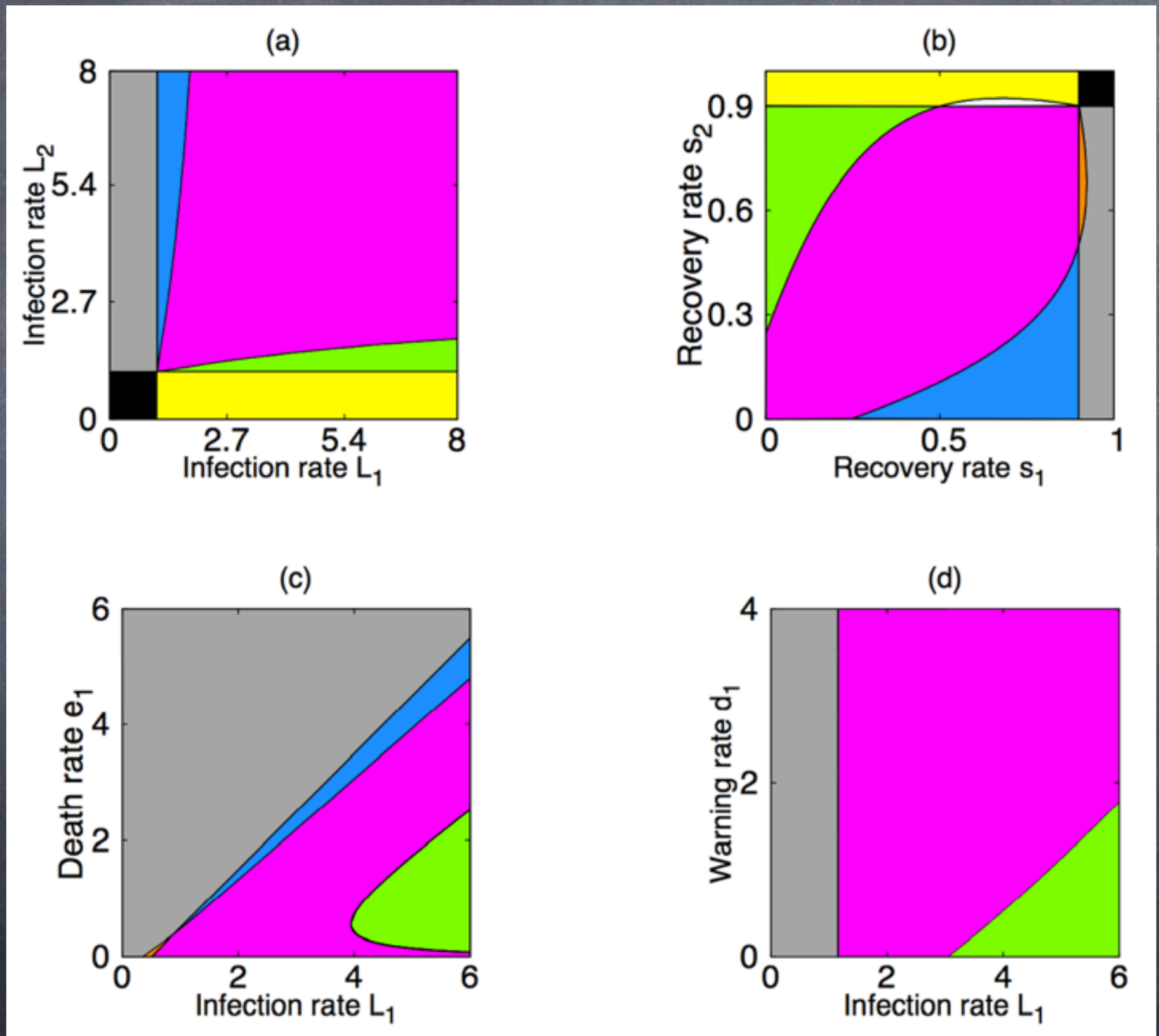
Stability of the steady states



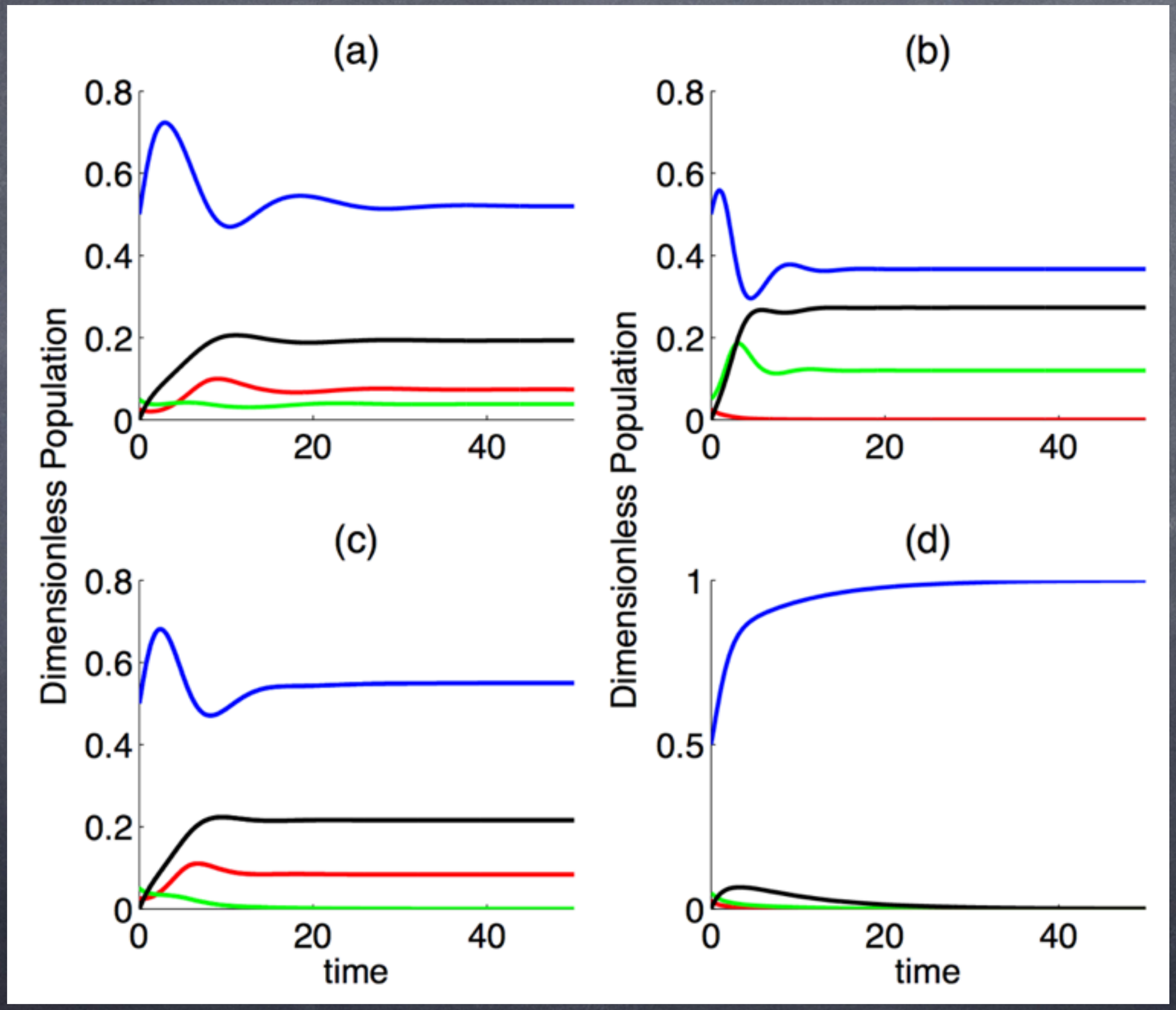
Stability of the steady states



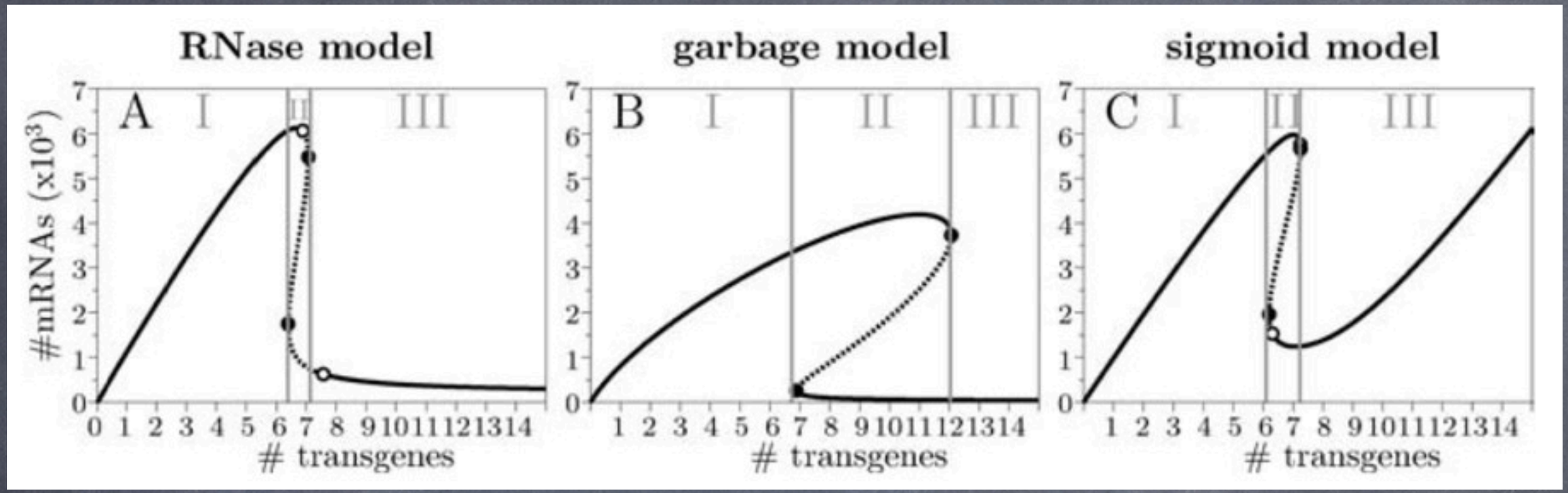
Stability of the steady states



Numerical simulations



Mechanism of RNA silencing



$$\frac{dM}{dt} = i - d_m M - pM - bSM - g_2 SM,$$
$$\frac{dD}{dt} = pM - aD + g_2 SM,$$
$$\frac{dS}{dt} = anD - \frac{\mathbf{d_r S}}{1 + \mathbf{kS}} - d_s S - bSM - g_2 SM,$$
$$\frac{dG}{dt} = bSM - d_g G.$$

$$\frac{dM}{dt} = i - d_m M - pM - bSM - g_2 SM,$$
$$\frac{dD}{dt} = pM - aD + g_2 SM + \mathbf{g_3 SG},$$
$$\frac{dS}{dt} = anD - d_s S - bSM - g_2 SM - \mathbf{g_3 SG},$$
$$\frac{dG}{dt} = bSM - d_g G - \mathbf{g_3 SG}.$$

$$\frac{dM}{dt} = i - d_m M - pM - bSM,$$
$$\frac{dD}{dt} = pM - aD + \frac{\mathbf{g_1 G^2}}{1 + \mathbf{kG^2}},$$
$$\frac{dS}{dt} = anD - d_s S - bSM,$$
$$\frac{dG}{dt} = bSM - d_g G - \frac{\mathbf{g_1 G^2}}{1 + \mathbf{kG^2}}.$$

M.A.C. Groenenboom et al., PLoS Comp. Biol. 1, e21 (2005)

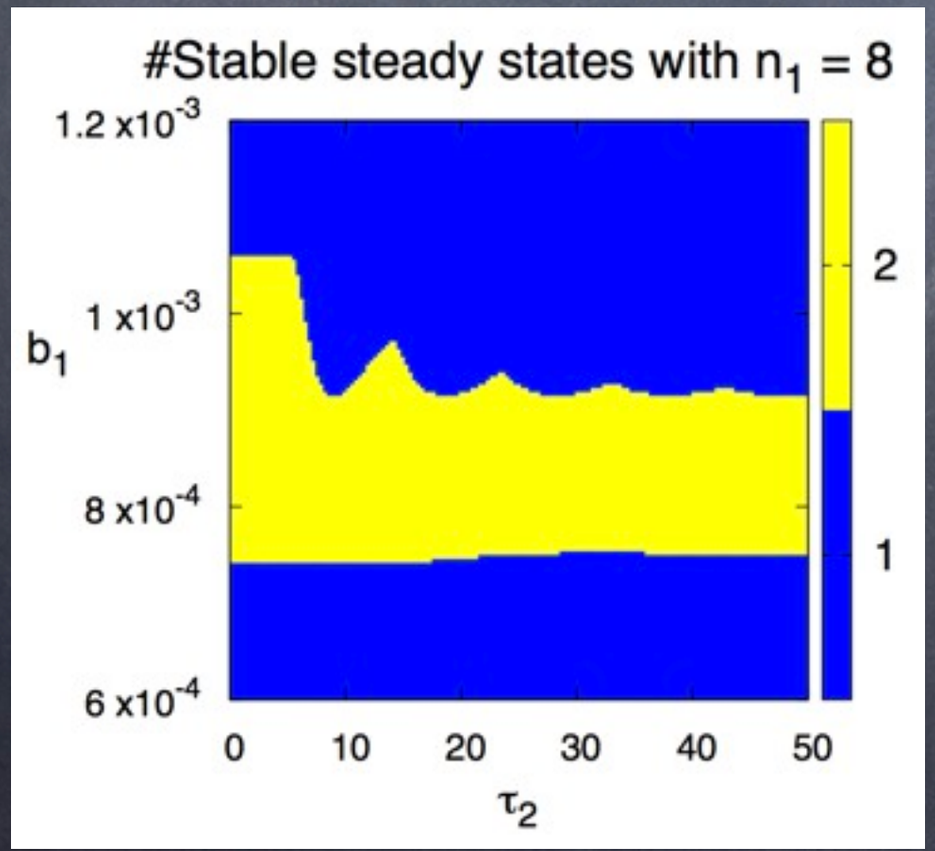
Mechanism of RNA silencing

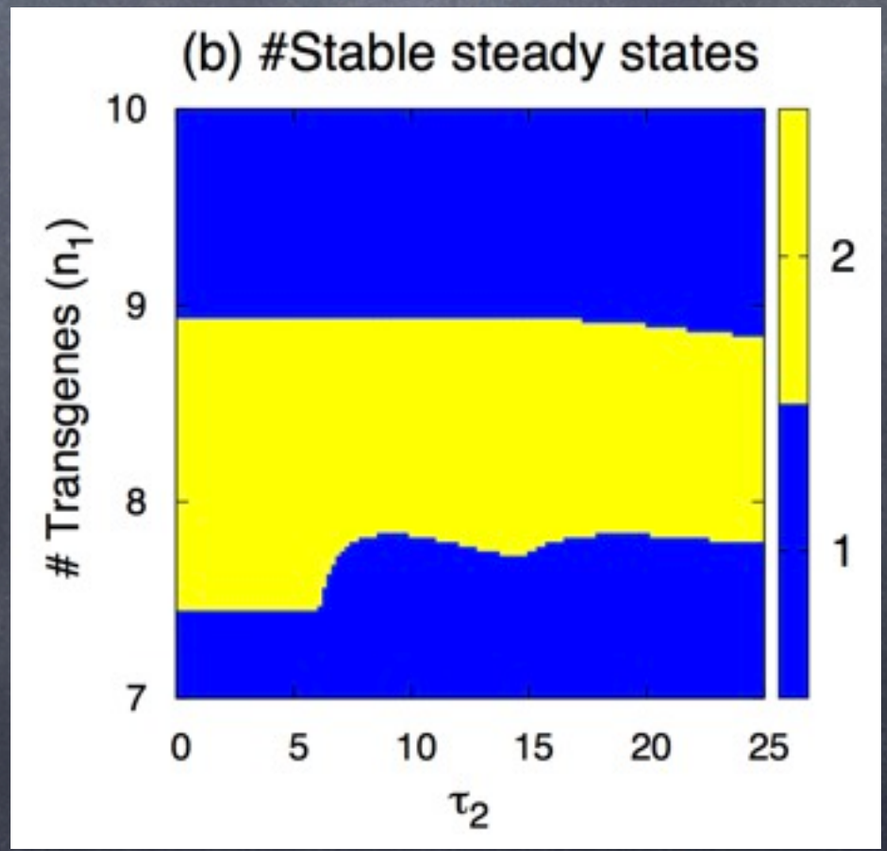
$$\frac{dM}{dt} = n_1 h - d_m M(t) - pM(t) - b_1 S(t)M(t) - b_2 S(t)M(t)$$

$$\frac{dD}{dt} = pM(t) - aD(t) + b_2 S(t-\tau_1)M(t-\tau_1) + b_3 S(t-\tau_2)G(t-\tau_2)$$

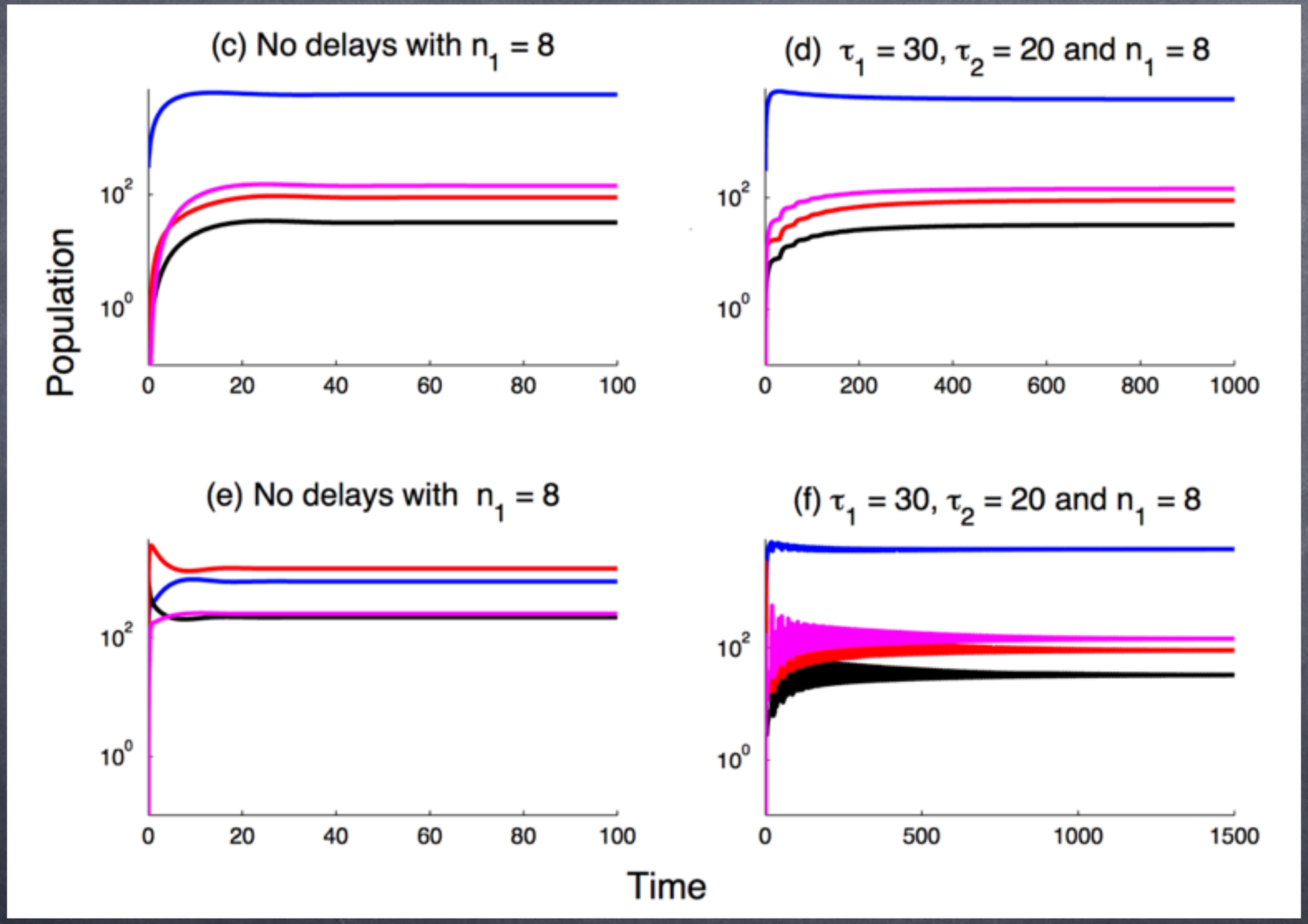
$$\frac{dS}{dt} = n_2 aD(t) - d_s S(t) - b_1 S(t)M(t) - b_2 S(t)M(t) - b_3 S(t)G(t)$$

$$\frac{dG}{dt} = n_3 b_1 S(t)M(t) - d_g G(t) - b_3 S(t)G(t)$$





Mechanism of RNA silencing

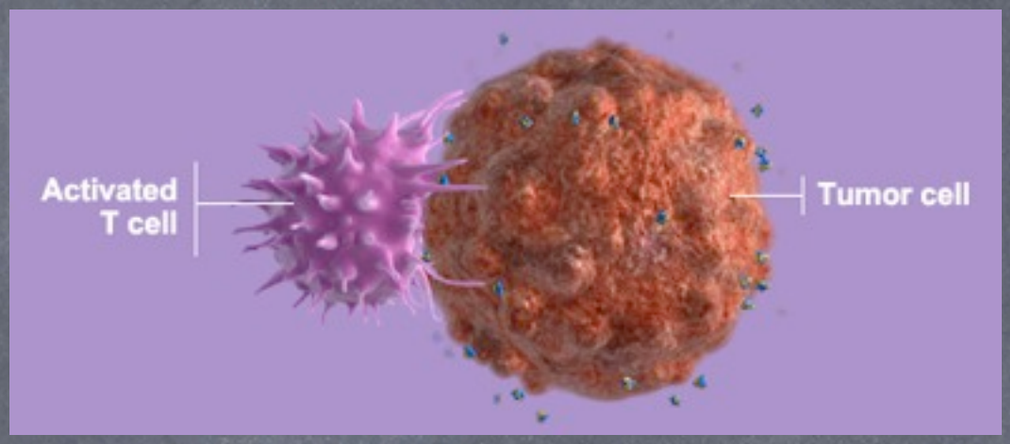


Discussion

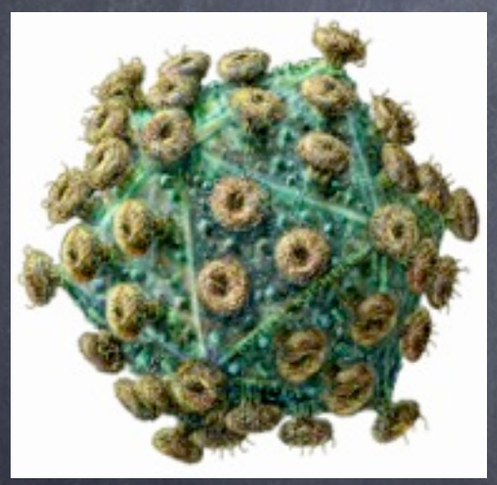
- Models provide significant insights and intuition into the dynamics of RNAi
- Very important role is played by time delays
- Spatial propagation of the silencing signal
- Explicit dynamics of virus particles
- Details of virus life cycle
- Co-evolutionary dynamics

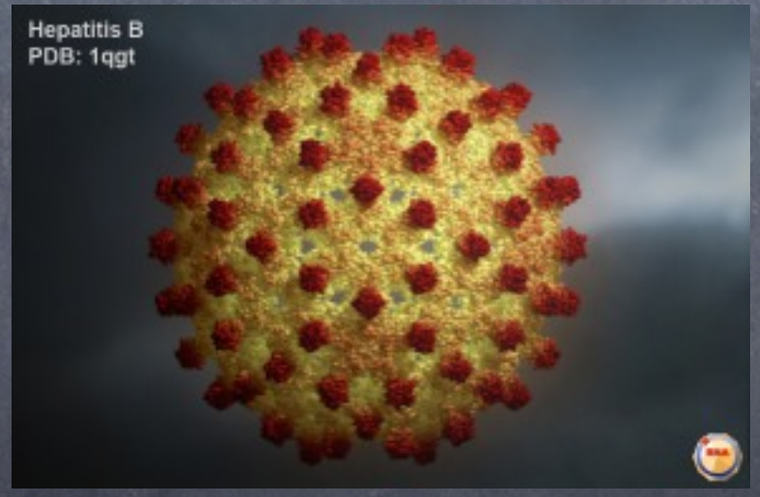
Applications: medical treatments

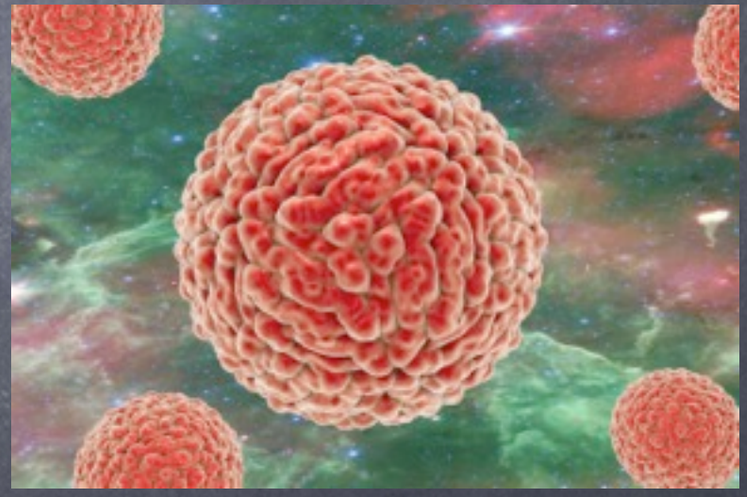
- Immunotherapy of cancer



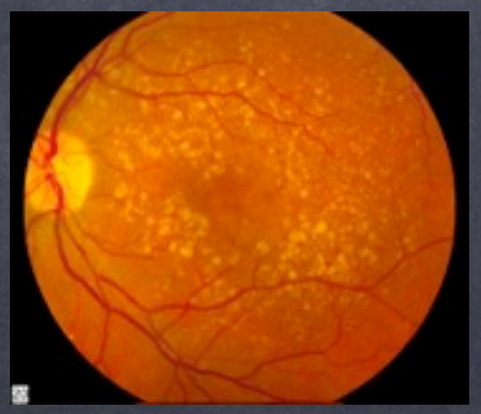
- Infectious diseases: HIV, HBV …. Zika?







- Macular degeneration



Applications: biotechnology

- Cell biology

  knock-out of specific genes

- Food industry

  decreased carcinogens in tobacco plants

  insecticides

  transgenic plants

Arctic Apple

gene silencing reduces expression of polyphenol oxidase

Thank you !