A Generalized Model of the Repressilator

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Abstract: The repressilator is a regulatory cycle of n genes where each gene represses its successor in the cycle: $1 \dashv 2 \dashv \ldots \dashv n \dashv 1$. The system is modelled by ODEs for an arbitrary number of identical genes and arbitrarily strong repressor binding. A detailed mathematical analysis of the dynamical behavior is provided for two model systems: (i) a repressilator with leaky transcription and single-step cooperative repressor binding, and (ii) a repressilator with auto-activation and cooperative regulator binding. Genes are assumed to be present in constant amounts, transcription and translation are modelled by single-step kinetics, and mRNAs as well as proteins are assumed to be degraded by first order reactions. Several dynamical patterns are observed: Multiple steady states, periodic and aperiodic oscillations corresponding to limit cycles and heteroclinic cycles, respectively. The results of computer simulations are complemented by a detailed and complete stability analysis of all equilibria and of the heteroclinic cycle.

Key words: Gene regulatory network, negative feedback loop, repressilator, stability analysis, Hopf bifurcation, heteroclinic cycle

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1 Introduction

The seminal work of Jacob, Monod, and Changeaux [1, 2] on the regulation of gene expression in the *lac* operon initiated early studies on gene regulation through repression by specific proteins which demonstrated the possibility of oscillations in some special systems with few genes [3]. Numerical integration of differential equations with delay were used to model cyclic repression systems of the type $1 \dashv 2 \dashv \ldots \dashv n \dashv 1$[1] [4, 5] and showed that cycles with odd numbers of genes exhibit oscillations over a wide range of conditions. Later work presented stability analysis of equilibria in cyclic repressor systems [6, 7, 8], and eventually the existence of oscillations resp. multiple stable steady states has been proven for cycles with odd resp. even numbers of genes [9]. The mathematical analysis of such feedback loops culminated in the establishment of a Poincaré-Bendixson theorem [10]. For a comprehensive summary of biological feedback loops we refer to the monograph by Thomas and D'Ari [11].

A milestone in understanding gene regulation by repression has been reached by the experimental preparation of a three membered negative feedback loop, called the "repressilator", on a plasmid by Elowitz and Leibler [12]: Expressed in *E.Coli* bacteria, the repressilator gave indeed rise to oscillations in living cells. From now on, the availability of an experimental system provides an excellent tool for testing predictions derived from theoretical models.

This attempt to revisit gene regulation models has been encouraged by the availability of efficient and fast numerical tools that can be applied jointly with mathematical analysis to gain detailed insight into dynamical systems. A study of two gene systems is given in a separate paper [13]. Here we present an analysis of repressilator systems with an arbitrary number of identical genes and with arbitrarily strong repressor binding. As usual, we consider the concentrations of mRNAs and proteins explicitly and assume finite lifetimes for all macromolecules as expressed by first order degradation reactions. Genes are assumed to be present in constant amounts, and transcription and translation are assumed to be slow compared to the binding reactions of proteins to genes. However, we are able to relax the usual assumption of excess protein regulators and compute the free and total protein concentrations from the binding equilibria.

Two repressilator systems are considered in detail: (i) a repressilator with leaky transcription and cooperative repressor binding, and (ii) a repressilator with auto-activation and cooperative regulator binding. In the first system (which is essentially the mathematical model used in [7, 8, 9, 12]) the oscillations for odd numbers of genes are periodic, arising from a stable limit cycle. In the second system (which has not been considered before) there may additionally be aperiodic oscillations resulting from an attracting heteroclinic cycle. We shall present

[1] As common in the biochemical literature we denote inhibition by "$\dashv$".

explicit stability criteria for all equilibria. For the interior equilibrium this allows us to precisely locate the Hopf bifurcation that generates the limit cycle. For the second system we also derive a precise stability criterion for the heteroclinic cycle.

The paper is organized as follows: First, we derive the kinetic equations for the two model systems, and then we work out the details of our analysis. Next, we summarize the most important results, and finally we draw our conclusions.

2 Mathematical model

For our analysis of gene regulatory systems, we make the following assumptions: (a) Genes are present in constant amounts. (b) Proteins bind to the regulatory regions of the genes and either enhance or inhibit their expression. Binding reactions are in equilibrium, i.e. binding is faster than transcription and translation. (c) Transcription and translation are operating under saturated conditions, i.e. polymerases and ribosomes, as well as nucleotides and amino acids abound. (d) mRNAs and free proteins are degraded by first order reactions.

Under these assumptions, the crucial processes in a gene regulatory network (binding, transcription, translation, and degradation) can be described by a system of ODEs:

$$\dot{\bar{p}}_i = k_i^{TL}\, r_i - d_i^P\, p_i \tag{1a}$$

$$\dot{r}_i = k_i^{TS}\, a_i - d_i^R\, r_i \tag{1b}$$

where:

$$a_i = a_i(p) \tag{1c}$$

$$\bar{p}_i = \bar{p}_i(p) \tag{1d}$$

The concentration of the mRNA transcribed from gene i is denoted by r_i, the total concentration of the translated protein is denoted by $\bar{p}_i$, and the respective free concentration is denoted by p_i. The transcriptional activity of gene i is given by a_i. The rate constants for transcription and translation are named k_i^{TS} and k_i^{TL}, and the rate constants for mRNA and protein degradation are named d_i^R and d_i^P.

Due to regulator binding, the transcriptional activities as well as the total protein concentrations are functions of the free protein concentrations.[2]

[2] The transcriptional activity of a gene can be seen as the "concentration" of the gene being transcribed (at a certain level). Gene numbers are discrete and each gene has different levels of transcription (e.g. zero, low, and high). A continuous function for the transcriptional activity of a gene can be seen as a time average over time spans, which are long compared to regulator binding, but short compared to transcription and translation.

In particular, we are interested in the "repressilator", a cyclic system of n genes, where each gene is repressed by its predecessor in the cycle. More precisely, the transcription of a certain gene is repressed by the product of the preceding gene. In their mathematical model of the repressilator, Elowitz and Leibler [12] make the additional assumption of identical genes. That is:

$$k_i^{TL} = k^{TL}, \quad d_i^P = d^P \tag{2}$$

$$k_i^{TS} = k^{TS}, \quad d_i^R = d^R \tag{3}$$

We retain this assuption and consider two special cases of repressilator systems:

(i) System "RepLeaky"
(The repressilator with leaky transcription)

In this system, genes are transcribed at a low rate, if they are repressed (leaky transcription), whereas genes are transcribed at a high rate, if they are not repressed. Repressor binding is assumed to be cooperative.

(ii) System "RepAuto"
(The repressilator with auto-activation)

In this system, genes are not transcribed, if they are repressed. Moreover, the transcription of a certain gene can only be activated by its own product (auto-activation). In other words, genes are transcribed if they are both not repressed and auto-activated. Repressor and activator binding may affect each other, i.e. regulator binding is assumed to be cooperative.

In the following, we determine the explicit form of the transcriptional activity as well as the relation between total and free protein concentrations for the two systems.

2.1 System "RepLeaky"

In the repressilator with leaky transcription and cooperative repressor binding, each gene G_i can be bound by up to m products P_{i-1} of the preceding gene. The gene-repressor complex $\mathsf{C}_i^{(m)}$ is formed:

$$\mathsf{G}_i + m\,\mathsf{P}_{i-1} \rightleftharpoons \mathsf{C}_i^{(m)} \tag{4}$$

A variety of reaction mechanisms can yield this overall reaction, e.g. successive binding:

$$\mathsf{G} + m\,\mathsf{P} \rightleftharpoons \mathsf{C}^{(1)} + (m-1)\,\mathsf{P} \rightleftharpoons \mathsf{C}^{(2)} + (m-2)\,\mathsf{P} \rightleftharpoons \ldots \rightleftharpoons \mathsf{C}^{(m)} \tag{5}$$

or multimer binding:

$$m\,\mathbf{P} \rightleftharpoons \mathbf{P}_2 + (m-2)\,\mathbf{P} \rightleftharpoons \mathbf{P}_3 + (m-3)\,\mathbf{P} \rightleftharpoons \ldots \rightleftharpoons \mathbf{P}_m \tag{6}$$

$$\mathbf{G} + \mathbf{P}_m \rightleftharpoons \mathbf{C}^{(m)} \tag{7}$$

(For better readability, we omitted the gene index.)

For any reaction mechanism, the binding function can be derived by using fast equilibrium kinetics. In any case, the binding function is a rational function of the free protein concentration,

$$c^{(m)}(p) = \bar{g}\,\frac{A(p)}{B(p)}, \tag{8}$$

where $\bar{g}$ is the (constant) total gene concentration, A and B are polynomials of degree m in variable p.

As an example, we derive the binding function for the simplest reaction mechanism: single-step binding. In this case, the reaction mechanism is given by the overall reaction (4). We find

$$c_i^{(m)} = \frac{g_i\,p_{i-1}^m}{K}, \tag{9}$$

where K is the dissociation constant. Mass conservation for genes amounts to:

$$\bar{g} = g_i + c_i^{(m)} \tag{10}$$

Using Eqs. (9) and (10), we obtain the desired binding function:

$$c_i^{(m)} = \bar{g}\,\frac{p_{i-1}^m}{K + p_{i-1}^m} \tag{11}$$

This binding function can also be regarded as purely empirical. In this view, the exponent m need not be an integer and is replaced by the Hill coefficient h. Instead of the dissociation constant K a constant $\tilde{K}$ is used, which has the dimension of a concentration. In the following, we use the empirical binding function

$$c_i^{(m)} = \bar{g}\,s(\frac{p_{i-1}}{\tilde{K}}), \tag{12}$$

where:

$$s(x) = \frac{x^h}{1 + x^h} \tag{13}$$

We still have to determine the transcriptional activity and the relation between total and free protein concentrations.

The transcriptional activity a_i depends linearly on the free gene concentration g_i and this linear relation is determined by the following two limit cases:

$$g_i = \bar{g} \quad \Rightarrow \quad a_i = \bar{g} \tag{14}$$

$$g_i = 0 \quad \Rightarrow \quad a_i = \delta\, \bar{g} \tag{15}$$

The leakiness $\delta \ll 1$ is the ratio of repressed to unrepressed transcription. As a consequence, the transcriptional activity is determined by:

$$a_i = (1-\delta)\, g_i + \delta\, \bar{g} \tag{16}$$

In the case of single-step binding, mass conservation for proteins amounts to:

$$\bar{p}_i = p_i + m\, c_{i+1}^{(m)} \tag{17}$$

Using Eqs. (12), (16), and (17), we obtain the desired relations:

$$a_i = \bar{g} \left[(1-\delta)\, (1 - s(\frac{p_{i-1}}{\tilde{K}})) + \delta \right] \tag{18}$$

$$\bar{p}_i = p_i + m\, \bar{g}\, s(\frac{p_i}{\tilde{K}}) \tag{19}$$

Combining our findings, we are able to give a concise presentation of system "RepLeaky". Before doing so, we rescale time by the mRNA lifetime $1/d^R$, and introduce the ratio β of degradation rates and the ratio σ of production rates to degradation rates:

$$\beta = \frac{d^P}{d^R}, \quad \sigma = \frac{k^{TL}\, k^{TS}}{d^P\, d^R} \tag{20}$$

Next, we rescale protein concentrations by the constant $\tilde{K}$, and adjust mRNA concentrations to protein concentrations:

$$x = \frac{p}{\tilde{K}}, \quad y = \frac{r}{\tilde{K}}\, \frac{k^{TL}}{d^P} \tag{21}$$

Finally, we introduce the binding parameter γ and the combined parameter α:

$$\gamma = m\, \frac{\bar{g}}{\tilde{K}}, \quad \alpha = \gamma\, \sigma \tag{22}$$

As a result, the ODEs describing the crucial processes in system "RepLeaky" can be written as:

$$\dot{\tilde{x}}_i = \beta\, (y_i - x_i) \tag{23a}$$

$$\dot{y}_i = \alpha\, f(x_{i-1}) - y_i \tag{23b}$$

where:

$$f(x_{i-1}) = (1-\delta)(1-s(x_{i-1})) + \delta \tag{23c}$$

$$\bar{x}_i = x_i + \gamma\, s(x_i) \tag{23d}$$

(Rescaled) total and free protein concentrations are denoted by $\bar{x}_i$ and x_i, respectively, whereas mRNA concentrations are denoted by y_i. For the elimination of total protein concentrations, see section 2.3.

Remark: The mathematical models for the repressilator used in [7, 8, 9, 12] correspond to the limit case $\gamma = 0$.

2.2 System "RepAuto"

In the repressilator with auto-activation, each gene G_i can be bound by its own product P_i and by the product P_{i-1} of the preceding gene. The gene-activator complex C_i^A and the gene-repressor complex C_i^R are formed:

$$\mathsf{G}_i + \mathsf{P}_i \rightleftharpoons \mathsf{C}_i^A \tag{24}$$

$$\mathsf{G}_i + \mathsf{P}_{i-1} \rightleftharpoons \mathsf{C}_i^R \tag{25}$$

In case activator and repressor share the same binding site, the system is completely determined by the above reactions. In case activator and repressor bind to different sites, we also have to consider the gene complex C_i^{AR} containing both regulators:

$$\mathsf{C}_i^A + \mathsf{P}_{i-1} \rightleftharpoons \mathsf{C}_i^{AR} \tag{26}$$

$$\mathsf{C}_i^R + \mathsf{P}_i \rightleftharpoons \mathsf{C}_i^{AR} \tag{27}$$

Genes are transcribed only if they are both auto-activated and not repressed. Hence, the transcriptional activity is given by the concentration of the gene-activator complex:

$$a_i = c_i^A \tag{28}$$

Under the equilibrium assumption, mass-action kinetics applied to reactions (24) and (25) yields the following relations for the concentrations involved:

$$c_i^A = \frac{g_i\, p_i}{K^A} \tag{29}$$

$$c_i^R = \frac{g_i\, p_{i-1}}{K^R} \tag{30}$$

The dissociation constants for auto-activator and repressor binding are denoted by K^A and K^R, respectively. In the case of two binding sites, we obtain additional

relations from reactions (26) and (27):

$$c_i^{AR} = \frac{c_i^A \, p_{i-1}}{K^{AR}} = \frac{c_i^R \, p_i}{K^{RA}} \tag{31}$$

The dissociation constants for auto-activator and repressor binding fulfill the well-known condition for cyclic reactions:

$$K^A K^{AR} = K^R K^{RA} \tag{32}$$

Until further notice, we consider the two cases separately.

2.2.1 One binding site

In the simpler case of one shared binding site, mass conservation for genes and proteins implies the following equations:

$$\bar{g} = g_i + c_i^A + c_i^R \tag{33}$$

$$\bar{p}_i = p_i + c_i^A + c_{i+1}^R \tag{34}$$

Using Eqs. (29), (30), and (33), we are able to determine the concentration of the gene-activator:

$$c_i^A = \bar{g} \, \frac{p_i}{K^A} \, (1 + \frac{p_i}{K^A} + \frac{p_{i-1}}{K^R})^{-1} \tag{35}$$

(And similarly for the gene-repressor complex c_i^R.) Inserting into Eq. (34), we obtain a system of nonlinear equations for relating total and free protein concentrations:

$$\bar{p}_i = p_i \left[1 + \frac{\bar{g}}{K^A} \, (1 + \frac{p_i}{K^A} + \frac{p_{i-1}}{K^R})^{-1} + \frac{\bar{g}}{K^R} \, (1 + \frac{p_{i+1}}{K^A} + \frac{p_i}{K^R})^{-1} \right] \tag{36}$$

2.2.2 Two binding sites

In the case of different sites for activator and repressor binding, mass conservation for genes and proteins implies more complicated equations:

$$\bar{g} = g_i + c_i^A + c_i^R + c_i^{AR} \tag{37}$$

$$\bar{p}_i = p_i + c_i^A + c_i^{AR} + c_{i+1}^R + c_{i+1}^{AR} \tag{38}$$

Still, using Eqs. (29), (30), (31), and (37), we are able to determine the concentration of the gene-activator complex:

$$c_i^A = \bar{g} \, \frac{p_i}{K^A} \left(1 + \frac{p_i}{K^A} + \frac{p_{i-1}}{K^R} + \frac{p_i \, p_{i-1}}{K^A K^{AR}} \right)^{-1} \tag{39}$$

(And similarly for the complexes c_i^R and c_i^{AR}.) Inserting into Eq. (38), we again obtain a system of nonlinear equations for relating total and free protein concentrations:

$$\begin{aligned}\bar{p}_i = p_i \Big[& 1 + \frac{\bar{g}}{K^A} \left(1 + \frac{p_{i-1}}{K^{AR}}\right) \left(1 + \frac{p_i}{K^A} + \frac{p_{i-1}}{K^R} + \frac{p_i\, p_{i-1}}{K^A K^{AR}}\right)^{-1} \\ & + \frac{\bar{g}}{K^R} \left(1 + \frac{p_{i+1}}{K^{RA}}\right) \left(1 + \frac{p_{i+1}}{K^A} + \frac{p_i}{K^R} + \frac{p_{i+1}\, p_i}{K^A K^{AR}}\right)^{-1} \Big]\end{aligned} \tag{40}$$

In the special case of two independent binding sites, i.e.

$$\frac{K^A}{K^{RA}} = \frac{K^R}{K^{AR}} = 1, \tag{41}$$

we obtain decoupled non-linear equations:

$$\bar{p}_i = p_i \left[1 + \frac{\bar{g}}{K^A} \left(1 + \frac{p_i}{K^A}\right)^{-1} + \frac{\bar{g}}{K^R} \left(1 + \frac{p_i}{K^R}\right)^{-1} \right] \tag{42}$$

Moreover, we note that the case of one binding site can be obtained from the case of two binding sites by taking the following limit:

$$\frac{K^A}{K^{RA}} = \frac{K^R}{K^{AR}} \to 0 \tag{43}$$

Hence, we will treat the system with one binding site as a special case of the system with two binding sites in the following.

Combining our findings, we are able to give a concise presentation of system "RepAuto". Before doing so, we rescale the system. Like in system "RepLeaky", we rescale time by the mRNA lifetime $1/d^R$, and introduce the parameters β and σ given by Eq. (20). Next, we rescale protein concentrations by the dissociation constant K_A, and adjust mRNA concentrations to protein concentrations:

$$x = \frac{p}{K^A}, \quad y = \frac{r}{K^A} \frac{k^{TL}}{d^P} \tag{44}$$

Finally, we introduce the repressor strength ρ, the cooperativity κ, and again the binding parameter γ and the combined parameter α:

$$\rho = \frac{K^A}{K^R}, \quad \kappa = \frac{K^A}{K^{RA}} = \frac{K^R}{K^{AR}} \tag{45}$$

$$\gamma = \frac{\bar{g}}{K^A}, \quad \alpha = \gamma\, \sigma \tag{46}$$

As a result, the ODEs describing the crucial processes in system "RepAuto" can be written as:

$$\dot{\bar{x}}_i = \beta\, (y_i - x_i) \tag{47a}$$

$$\dot{y}_i = \alpha\, f(x_i, x_{i-1}) - y_i \tag{47b}$$

where:

$$f(x_i, x_{i-1}) = \frac{x_i}{1 + x_i + \rho\, x_{i-1} + \kappa\, \rho\, x_i\, x_{i-1}} \tag{47c}$$

$$\bar{x}_i = x_i \left[1 + \gamma \left(\frac{1 + \kappa\, \rho\, x_{i-1}}{1 + x_i + \rho\, x_{i-1} + \kappa\, \rho\, x_i\, x_{i-1}} + \frac{\rho\, (1 + \kappa\, x_{i+1})}{1 + x_{i+1} + \rho\, x_i + \kappa\, \rho\, x_{i+1}\, x_i} \right)\right] \tag{47d}$$

(Rescaled) total and free protein concentrations are denoted by $\bar{x}_i$ and x_i, respectively, whereas mRNA concentrations are denoted by y_i. For the elimination of total protein concentrations, see section 2.3.

By setting $\kappa = 1$, we obtain the special case of two independent binding sites:

$$\bar{x}_i = x_i \left[1 + \gamma \left(\frac{1}{1 + x_i} + \frac{\rho}{1 + \rho\, x_i} \right)\right] \tag{48}$$

By setting $\kappa = 0$, we obtain the case of one binding site.

2.3 The elimination of total protein concentrations

Both in system "RepLeaky" and system "RepAuto", the ODEs are defined for total protein and mRNA concentrations. Still, they also contain free protein concentrations. In vector notation we have:

$$\begin{pmatrix} \dot{\bar{x}} \\ \dot{y} \end{pmatrix} = \begin{pmatrix} \beta\, (y - x) \\ \alpha\, F(x) - y \end{pmatrix} \tag{49}$$

where:

$$F(x)_{\,i} = \begin{cases} f(x_{i-1}) & \text{in system "RepLeaky", see Eq. (23b)} \\ f(x_i, x_{i-1}) & \text{in system "RepAuto", see Eq. (47b)} \end{cases} \tag{50}$$

Instead of expressing free by total protein concentrations via (a system of) nonlinear equations (which is impossible analytically), we express total by free protein flows via a linear transformation. Therefore we differentiate the mass conservation relation for proteins:

$$\dot{\bar{x}} = \frac{\partial \bar{x}}{\partial x}\, \dot{x} = M(x)\, \dot{x} \tag{51}$$

Using the inverse of the linear transformation, we obtain the desired ODEs for free protein and mRNA concentrations:

$$\boxed{\begin{pmatrix} \dot{x} \\ \dot{y} \end{pmatrix} = \begin{pmatrix} \beta\, M(x)^{-1}\, (y - x) \\ \alpha\, F(x) - y \end{pmatrix}} \tag{52}$$

In system "RepLeaky", the transformation matrix $M(x)$ is diagonal, and hence easily invertible:

$$M(x)_{i,j} = \begin{cases} 1 + \gamma \, \dfrac{h \, x_i^{h-1}}{(1 + x_i^h)^2} & \text{if} \quad j = i \\ 0 & \text{otherwise} \end{cases} \tag{53}$$

In system "RepAuto", the transformation matrix $M(x)$ is cyclically tridiagonal:

$$M(x)_{i,j} = \begin{cases} 1 + \gamma \Bigg(\dfrac{(1 + \kappa \, \rho \, x_{i-1}) \, (1 + \rho \, x_{i-1})}{(1 + x_i + \rho \, x_{i-1} + \kappa \, \rho \, x_i \, x_{i-1})^2} \\ \qquad + \dfrac{\rho \, (1 + \kappa \, x_{i+1}) \, (1 + x_{i+1})}{(1 + x_{i+1} + \rho \, x_i + \kappa \, \rho \, x_{i+1} \, x_i)^2} \Bigg) & \text{if} \quad j = i \\ \gamma \, \dfrac{\rho \, (\kappa - 1) \, x_i}{(1 + x_i + \rho \, x_{i-1} + \kappa \, \rho \, x_i \, x_{i-1})^2} & \text{if} \quad j = i - 1 \\ \gamma \, \dfrac{\rho \, (\kappa - 1) \, x_i}{(1 + x_{i+1} + \rho \, x_i + \kappa \, \rho \, x_{i+1} \, x_i)^2} & \text{if} \quad j = i + 1 \\ 0 & \text{otherwise} \end{cases} \tag{54}$$

We can prove the following claim:

Claim 2.1 *In system "RepAuto", the matrix $M(x)$ is invertible.*

Proof. The matrix $M(x)$ satisfies $M_{i,i} > |M_{i-1,i}| + |M_{i+1,i}|$. That is, $M(x)$ is diagonally dominant. Hence, $M(x)$ is invertible. Moreover, the map x to $\bar{x}$ is one-to-one by a global inverse function theorem. □

For weak regulator binding, $\gamma \ll 1$, total and free protein concentrations become equal, $\bar{x}_i = x_i$, and the linear transformation becomes the identity, $M(x) = I$. Consequently, we obtain:

$$\begin{pmatrix} \dot{x} \\ \dot{y} \end{pmatrix} = \begin{pmatrix} \beta \, (y - x) \\ \alpha \, F(x) - y \end{pmatrix} \tag{55}$$

This simplified system can be found in traditional treatments of gene regulatory networks (e.g. the repressilator). We note, that the equilibria of the exact system (52) and the simplified system (55) are the same.

3 Detailed analysis

The equilibria of system (52) are given by:

$$x = y = \alpha \, F(x) \tag{56}$$

The Jacobian matrix at an equilibrium amounts to:

$$J(x) = \frac{\partial(\dot{x}, \dot{y})}{\partial(x, y)} = \begin{pmatrix} -\beta\, M(x)^{-1} & \beta\, M(x)^{-1} \\ S(x) & -I \end{pmatrix} \tag{57}$$

where:

$$S(x) = \alpha\, \frac{\partial F(x)}{\partial x} \tag{58}$$

Using the determinant formula for block matrices with commuting blocks, the eigenvalues of the Jacobian matrix at an equilibrium can be determined as follows:

$$\begin{aligned} 0 &= |J(x) - \lambda\, I| \\ &= \begin{vmatrix} -\beta\, M(x)^{-1} - \lambda\, I & \beta\, M(x)^{-1} \\ S(x) & -(1+\lambda)\, I \end{vmatrix} \\ &= \left|\beta\,(1+\lambda)\, M(x)^{-1} + \lambda\,(1+\lambda)\, I - \beta\, S(x)\, M(x)^{-1}\right| \\ &= |T(x)\, M(x)^{-1}| \end{aligned} \tag{59}$$

where:

$$T(x) = \beta\,(1+\lambda)\, I + \lambda\,(1+\lambda)\, M(x) - \beta\, S(x) \tag{60}$$

As a result, the characteristic equation of an equilibrium is given by:

$$|T(x)| = 0 \tag{61}$$

3.1 System "RepLeaky"

System "RepLeaky", as given by Eqs. (23), is an instance of system (52). It is specified by the vector $F(x)$ and the matrix $M(x)$:

$$F(x)_i = f(x_{i-1}) = (1-\delta)\,(1 - s(x_{i-1})) + \delta \tag{62}$$

$$M(x)_{i,j} = \begin{cases} 1 + \gamma\, s'(x_i) & \text{if} \quad j = i \\ 0 & \text{otherwise} \end{cases} \tag{63}$$

where:

$$s(x) = \frac{x^h}{1 + x^h} \tag{64}$$

For the following stability analysis, we also state the matrix $S(x)$:

$$\begin{aligned} S(x)_{i,j} &= \alpha\, \frac{\partial F(x)_i}{\partial x_j} = \alpha\, \frac{\partial f(x_{i-1})}{\partial x_j} = \alpha\, f'(x_{i-1})\, \delta_{i-1,j} \\ &= \begin{cases} -\alpha\,(1-\delta)\, s'(x_{i-1}) & \text{if} \quad j = i-1 \\ 0 & \text{otherwise} \end{cases} \end{aligned} \tag{65}$$

3.1.1 Equilibria

The equilibria of system "RepLeaky" are given by $x_i = y_i = \alpha\, f(x_{i-1})$. The cyclic nature of the repressilator implies the following fixed-point equation:

$$(\alpha\, f)^n(x_i) = x_i \tag{66}$$

For $x \geq 0$, the sigmoid function $s(x)$ is positive, bounded and monotonic. Via the function $f(x)$, these properties are transfered to the n^{th} iterate $(\alpha\, f)^n(x)$.

For n odd, the n^{th} iterate is monotonically *decreasing*. Consequently, there is exactly one fixed point x_c, which is the fixed point of the first iterate, i.e. $\alpha\, f(x_c) = x_c$. The corresponding central equilibrium E_c is given by $x_i = y_i = x_c$. That is, at E_c all genes are equally regulated. (For a stability analysis of the central equilibrium E_c, see section 3.1.2.)

For n even, the n^{th} iterate is monotonically *increasing*. Again, there is the fixed point x_c and the corresponding central equilibrium E_c. (In case $|\alpha\, f'(x_c)| < 1$, the central equilibrium E_c is stable, whereas it is unstable otherwise. See section 3.1.2.) In case $|\alpha\, f'(x_c)| > 1$, there are two more fixed points x_d and x_u (with $x_d < x_c < x_u$), which are fixed points of the second iterate, i.e. $(\alpha\, f)^2(x_d) = x_d$ and likewise for $x_u = \alpha\, f(x_d)$. Moreover, we claim that there are no further fixed points. (See claim 3.1 at the end of this section.) The corresponding equilibria E_{odd} resp. E_{even} are given by $x_i = y_i = x_u$ for i odd (resp. even), and $x_i = y_i = x_d$ for i even (resp. odd). That is, at E_{odd} all odd genes are upregulated and all even genes are downregulated. At E_{even} we find the reverse situation. (The equilibria E_{odd} and E_{even} are stable. See section 3.1.2.)

Interestingly, the critical quantity $|\alpha\, f'(x_c)|$ has the following property:

Fact 3.1 *For given $\delta > 0$ and h, the quantity $S_c = |\alpha\, f'(x_c)|$ has a maximum (as a function of α):*

$$S_{max} = h\,\frac{1-\delta^{1/2}}{1+\delta^{1/2}} \quad at \quad \alpha_{max} = \delta^{-(h+1)/2h} \tag{67}$$

For $\delta = 0$, S_c is monotonically increasing (as a function of α) with supremum $S_{sup} = h$.

Fig. 1 shows the first and second iterate of $\alpha\, f(x)$ for different values of α, as well as the resulting fixed points. Fig. 2 shows the fixed points of the second iterate as a function of α.

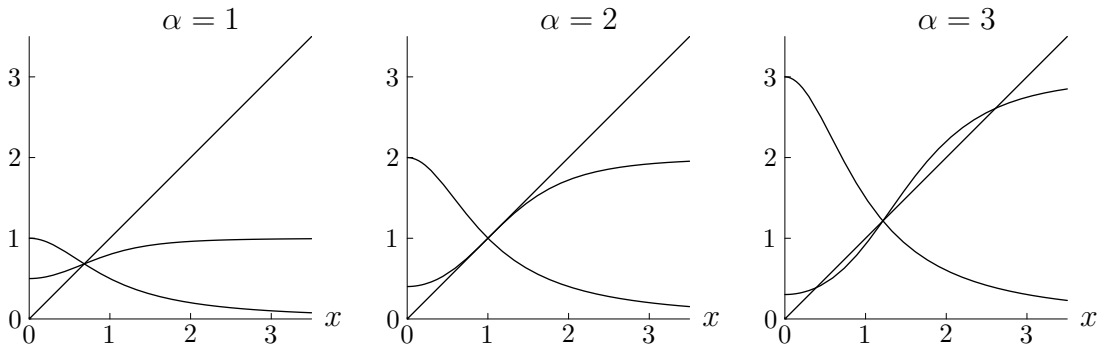

Figure 1: System "RepLeaky". The first and second iterate of the function $\alpha\, f(x)$ for different values of α, as well as the resulting fixed points. ($\delta = 10^{-3}$, $h = 2$, $\alpha = 1, 2, 3$.) The fixed point x_c of the first iterate is always a fixed point of the second iterate. In case $|\alpha\, f'(x_c)| > 1$, there are two more fixed points x_d and x_u.

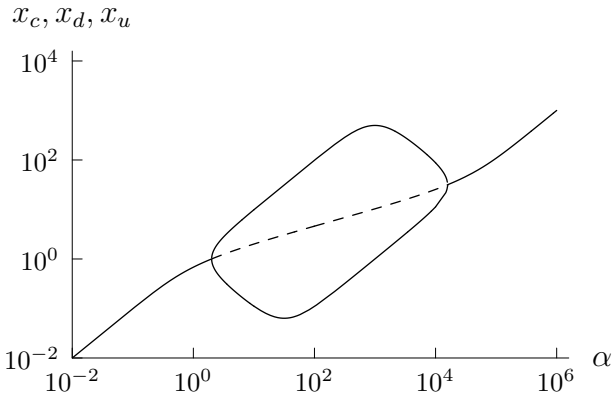

Figure 2: System "RepLeaky". The fixed points of the second iterate of the function $\alpha\, f(x)$ as a function of α. ($\delta = 10^{-3}$, $h = 2$.) In case $|\alpha\, f'(x_c)| < 1$, the fixed point x_c is the only fixed point and it is stable. In case $|\alpha\, f'(x_c)| > 1$, the fixed point x_c is unstable (indicated by the dashed line), and there are two more fixed points x_d and x_u, which are stable.

For n even, we have claimed that the n^{th} iterate of the function $\alpha\, f(x)$ has either one or three fixed points. Finally, we prove this claim:

Claim 3.1 *For n even, $(\alpha\, f)^n(x)$ has either one or three fixed points.*

Proof. We show that $(\alpha\, f)^n(x)$ has only one inflection point. First of all, we calculate the Schwarzian Derivative of $s(x)$. From the general definition

$$\mathrm{SD}(f) = \frac{f'''(x)}{f'(x)} - \frac{3}{2}\left(\frac{f''(x)}{f'(x)}\right)^2 \tag{68}$$

we obtain:

$$\mathrm{SD}(s) = -\frac{h^2 - 1}{2\, x^2} \tag{69}$$

Clearly, the Schwarzian Derivative of $s(x)$ is negative. Via the function $f(x)$, this property is transfered to the n^{th} iterate $(\alpha\, f)^n(x)$. (Since $\mathrm{SD}(f) < 0$ and $\mathrm{SD}(g) < 0$ implies $\mathrm{SD}(f \circ g) < 0$.) Additionally, the derivative of the n^{th} iterate is positive. As a consequence, any extremum of this derivative is a maximum. (From the definition of the Schwarzian Derivative.) Obviously, there can be only one such maximum, i.e. only one inflection point of the n^{th} iterate. □

3.1.2 Stability analysis of the central equilibrium

At the central equilibrium E_c, the matrix $T(x)$ is circulant:

$$T_{i,j} = \begin{cases} T_\Delta = \beta\,(1+\lambda) + \lambda\,(1+\lambda)\,M_c & \text{if} \quad j = i \\ T_- = \beta\, S_c & \text{if} \quad j = i-1 \\ 0 & \text{otherwise} \end{cases} \tag{70}$$

where:

$$S_c = -S_{i,i-1} = -\alpha\, f'(x_c) = \alpha\,(1-\delta)\, s'(x_c) \tag{71}$$

$$M_c = M_{i,i} = 1 + \gamma\, s'(x_c) \tag{72}$$

Remark: The quantity S_c has already been defined in Fact 3.1. Obviously, the two definitions are equivalent.

The characteristic equation amounts to:

$$|T| = (T_\Delta)^n + (-1)^{n-1}\,(T_-)^n = 0 \tag{73}$$

We extract the n^{th} root (using the roots of unity $z_k = e^{i2\pi k/n}$) and solve the resulting quadratic equations for the eigenvalues:

$$\begin{aligned} 0 &= T_\Delta + T_-\, z_k \\ &= \beta\,(1+\lambda) + \lambda\,(1+\lambda)\,M_c + \beta\, S_c\, z_k \\ &= (1+\lambda)\,(\beta + \lambda\, M_c) + \beta\, S_c\, z_k \\ &= (1+\lambda)\,(\beta/M_c + \lambda) + (\beta/M_c)\, S_c\, z_k \end{aligned} \tag{74}$$

$$\lambda_{k,\pm} = -\frac{1+\beta/M_c}{2} \pm \sqrt{\left(\frac{1+\beta/M_c}{2}\right)^2 - (\beta/M_c)\,(1+S_c\, z_k)} \tag{75}$$

The eigenvalue with the largest real-part is given by:

$$\lambda_{\max} = -\frac{1+\beta/M_c}{2} + \sqrt{\left(\frac{1+\beta/M_c}{2}\right)^2 + (\beta/M_c)\,(S_c\, z - 1)} \tag{76}$$

where:

$$z = \begin{cases} 1 & n \text{ even} \\ e^{i\pi/n} & n \text{ odd} \end{cases} \tag{77}$$

There is a crucial difference whether n is even or odd.

For n even, $\lambda_{\max}$ is real-valued and its sign is determined by the sign of $S_c - 1$. Consequently, the stability of the central equilibrium E_c is determined by:

$$\Re(\lambda_{\max}) < 0 \quad \Leftrightarrow \quad S_c < 1 \tag{78}$$

If $S_c < 1$, the central equilibrium E_c is asymptotically stable and it is the only equilibrium. If $S_c > 1$, the central equilibrium E_c is unstable and there are two more equilibria, E_{odd} and E_{even}, which are asymptotically stable. At $S_c = 1$, the system undergoes a supercritical pitchfork bifurcation.

The results from Smith [9, 14] allow us to make assertions about the global dynamics.

Theorem 3.1 *For n even, system "RepLeaky" has the following property: (i) If $S_c < 1$, then the central equilibrium E_c is globally asymptotically stable. (ii) If $S_c > 1$, then almost all orbits converge either to E_{odd} or to E_{even}.*

Proof. For $\gamma = 0$, the exact system (52) reduces to the simplified system (55). This system has the desired property, as was shown in [9, Theorem 2.1] by applying Hirsch's theory of monotone flows. The crucial idea is that for n even, one can divide all $2n$ variables x_i, y_i into two disjoint groups, the odd-numbered and the even-numbered, where within-group interactions are positive and between-group interactions are negative. Hence, the simplified system (55) generates a strongly monotone flow with respect to a certain cone in $\mathbb{R}^{2n}_+$ [14]. For $\gamma > 0$, the same argument applies: the exact system (52) is still a monotone system with respect to this ordering, since the transformation matrix (53) does not change the interaction pattern. $M(x)$ is a diagonal matrix, with positive entries on the diagonal. Indeed, for the within-group and between-group interactions we have the following inequalities:

$$\frac{\partial \dot{x}_i}{\partial y_i} = \beta / M_{ii}(x_i) > 0 \quad \text{and} \quad \frac{\partial \dot{y}_i}{\partial x_{i-1}} = \alpha\, f'(x_{i-1}) < 0. \tag{79}$$

All other off-diagonal entries in the Jacobian matrix are zero. □

For n odd, the central equilibrium E_c is the only equilibrium. Its stability is determined by:

$$\Re(\lambda_{\max}) < 0 \quad \Leftrightarrow \quad \frac{\beta/M_c}{(1+\beta/M_c)^2} < \frac{1 - S_c \cos(\pi/n)}{S_c^2 \sin^2(\pi/n)} \tag{80}$$

The stability criterion depends on n, S_c (i.e. on δ, h, and α), and β/M_c (i.e. also on γ). Still, there are sufficient conditions for stability resp. instability of the central equilibrium E_c, which do not depend on β or γ:

$$S_c < \frac{2}{1+\cos(\pi/n)} \quad \text{resp.} \quad S_c > \frac{1}{\cos(\pi/n)} \tag{81}$$

When the central equilibrium E_c looses stability, a periodic orbit appears. The system undergoes a Hopf bifurcation. Near a bifurcation, the (scaled) angular frequency of the oscillations amounts to:

$$\Im(\lambda_{\max}) = \frac{\beta/M_c}{1+\beta/M_c}\, S_c\, \sin(\pi/n) \tag{82}$$

The results from Mallet-Paret and Smith [10] allow us to make assertions about the global dynamics.

Theorem 3.2 *For n odd, system "RepLeaky" has the following property: (i) Every orbit converges to E_c or to a periodic orbit. (ii) If E_c is unstable, then there exists a periodic attractor.*

Proof. Written in the coordinates $(z_1, z_2, z_3, z_4, \dots) = (y_1, x_1, y_2, x_2, \dots)$, system (52) is a cyclic feedback system, since $\dot{z}_i$ depends on z_{i-1} and z_i only. The inequalities (79) translate into $\partial \dot{z}_i/\partial z_{i-1}$ being positive for i even and negative for i odd. Hence, system (52) is a monotone cyclic feedback system. The assertions follow then from the Poincaré–Bendixson theory for such systems [10]. □

3.2 System "RepAuto"

System "RepAuto", as given by Eqs. (47), is an instance of system (52). It is specified by the vector $F(x)$ and the matrix $M(x)$:

$$F(x)_i = f(x_i, x_{i-1}) = \frac{x_i}{1 + x_i + \rho\, x_{i-1} + \kappa\, \rho\, x_i\, x_{i-1}} \tag{83}$$

$M(x)$ is defined in Eq. (54). For the following stability analysis, we also state the matrix $S(x)$:

$$S(x)_{i,j} = \alpha\, \frac{\partial F(x)_i}{\partial x_j} = \begin{cases} \alpha\, \dfrac{1+\rho\, x_{i-1}}{(1 + x_i + \rho\, x_{i-1} + \kappa\, \rho\, x_i\, x_{i-1})^2} & \text{if} \quad j = i \\ \alpha\, \dfrac{-\rho\, x_i\, (1+\kappa\, x_i)}{(1 + x_i + \rho\, x_{i-1} + \kappa\, \rho\, x_i\, x_{i-1})^2} & \text{if} \quad j = i-1 \\ 0 & \text{otherwise} \end{cases} \tag{84}$$

3.2.1 Equilibria

The equilibria of system "RepAuto" are given by $x_i = y_i = \alpha\, f(x_i, x_{i-1})$, or equivalently by:

$$x_i = 0 \quad \vee \quad x_i = g(x_{i-1}) \tag{85}$$

where:

$$g(x) = \frac{\alpha - 1 - \rho\, x}{1 + \kappa\, \rho\, x} \tag{86}$$

In case $x_i > 0$ for all i, the cyclic nature of the repressilator implies the following fixed-point equation:

$$g^n(x_i) = x_i \tag{87}$$

On the interval $[0, (\alpha{-}1)/\rho]$, the function $g(x)$ is positive and bounded. Moreover, $g(x)$ is monotonic and has no inflection point. On a suitable subinterval, also the n^{th} iterate $g^n(x)$ has these properties. As a consequence, the n^{th} iterate has exactly one fixed point x_c, which is the fixed point of the first iterate, i.e. $g(x_c) = x_c$. Equivalently, $\alpha\, f(x_c, x_c) = x_c$, and explicitly:

$$\alpha = 1 + x_c + \rho\, x_c + \kappa\, \rho\, x_c^2 \tag{88}$$

The corresponding central equilibrium E_c is given by $x_i = y_i = x_c > 0$.

In case $x_i = 0$ for some i, the remaining $x_j \neq 0$ can be determined iteratively. The corresponding equilibrium lies on the boundary of the state space $\mathbb{R}_+^{2n}$.

In general, the equilibria depend on the parameters α, ρ, and κ:

For $\alpha < 1$, the only equilibrium is the origin O. (For $\alpha < 1$, the origin O is stable.)

For $\alpha > 1$, we find the following equilibria: (i) the central equilibrium E_c and (ii) a set of boundary equilibria.

For $\rho < 1$, the support $\mathcal{S}$ of any boundary equilibrium is a proper subset of $\{1, 2, \ldots, n\}$. For any such set $\mathcal{S}$, there is a unique equilibrium $E_{\mathcal{S}}$ with support $\mathcal{S}$ given by $x_i = y_i = 0$ for $i \notin \mathcal{S}$ and $x_i = y_i = g(x_{i-1})$ for $i \in \mathcal{S}$. Altogether, there are $2^n - 1$ boundary equilibria. (For $\rho < 1$, the central equilibrium E_c is asymptotically stable, whereas all boundary equilibria are unstable. See sections 3.2.2 and 3.2.3.)

For $\rho > 1$, the support $\mathcal{S}$ of any boundary equilibrium is a sparse subset of $\{1, 2, \ldots, n\}$ in the sense that it does not contain successive elements (modulo n). Hence, $|\mathcal{S}| \leq n/2$. For any such set $\mathcal{S}$, there is a unique equilibrium $E_{\mathcal{S}}$ with support $\mathcal{S}$ given by $x_i = y_i = 0$ for $i \notin \mathcal{S}$ and $x_i = y_i = \alpha - 1$ for $i \in \mathcal{S}$. Interestingly, there are L_n boundary equilibria, where L_n is the n^{th} Lucas number, i.e. $L_1 = 1$, $L_2 = 3$, and $L_n = L_{n-1} + L_{n-2}$ for $n \geq 3$. For $\rho > 1$, there is again a crucial difference, whether n is even or odd:

For n even, the maximal support $|\mathcal{S}| = n/2$ arises for $\mathcal{S}_{\text{odd}} = \{1, 3, \ldots, n-1\}$ and $\mathcal{S}_{\text{even}} = \{2, 4, \ldots, n\}$. The corresponding boundary equilibria E_{odd} and E_{even} are asymptotically stable, whereas all other equilibria, including the central equilibrium E_c, are unstable. See sections 3.2.2 and 3.2.3. For the simplified system (55) (i.e. $\gamma = 0$), the theory of monotone flows applies again and, as in Theorem 3.1, almost all orbits converge either to E_{odd} or to E_{even}.

For n odd, all boundary equilibria are unstable. There are three possible attractors: (i) the central equilibrium E_c, (ii) a periodic attractor in the interior (a limit cycle), and (iii) an "aperiodic" attractor on the boundary (a heteroclinic cycle connecting unstable equilibria). See sections 3.2.2, 3.2.3, and 3.2.4.

3.2.2 Stability analysis of the central equilibrium

At the central equilibrium E_c, the matrix $T(x)$ is circulant:

$$T_{i,j} = \begin{cases} T_\Delta = \beta\,(1+\lambda) + \lambda\,(1+\lambda)\,M_\Delta - \beta\,S_\Delta & \text{if} \quad j = i \\ T_- = \lambda\,(1+\lambda)\,M_\pm + \beta\,S_- & \text{if} \quad j = i-1 \\ T_+ = \lambda\,(1+\lambda)\,M_\pm & \text{if} \quad j = i+1 \\ 0 & \text{otherwise} \end{cases} \tag{89}$$

where:

$$S_\Delta = S_{i,i} = \frac{1+\rho\,x_c}{\alpha} \tag{90}$$

$$S_- = -S_{i,i-1} = \frac{\rho\,x_c\,(1+\kappa\,x_c)}{\alpha} \tag{91}$$

$$M_\Delta = M_{i,i} = 1 + \gamma\,\frac{(1+\kappa\,\rho\,x_c)\,(1+\rho\,x_c) + \rho\,(1+\kappa\,x_c)\,(1+x_c)}{\alpha^2} \tag{92}$$

$$M_\pm = M_{i,i-1} = M_{i,i+1} = \gamma\,\frac{\rho\,(\kappa-1)\,x_c}{\alpha^2} \tag{93}$$

Using the formula for circulant determinants (with the roots of unity $z_k = e^{i2\pi k/n}$), we get a factorization of the characteristic equation:

$$|T| = \prod_{k=0}^{n-1} (T_\Delta + T_-\,z_k + T_+\,z_k^{-1}) = 0 \tag{94}$$

Each factor yields a quadratic equation for the eigenvalues:

$$\begin{aligned} 0 &= T_\Delta + T_- \, z_k + T_+ \, z_k^{-1} \\ &= \beta \, (1+\lambda) + \lambda \, (1+\lambda) \, M_\Delta - \beta \, S_\Delta \\ &\quad + (\lambda \, (1+\lambda) \, M_\pm + \beta \, S_-) \, z_k \\ &\quad + \lambda \, (1+\lambda) \, M_\pm \, z_k^{-1} \\ &= \beta \, (1+\lambda) + \lambda \, (1+\lambda) \, (M_\Delta + M_\pm \, (w_k + w_k^{-1})) - \beta \, (S_\Delta - S_- \, z_k) \\ &= \beta \, (1+\lambda) + \lambda \, (1+\lambda) \, M_k - \beta \, S_k \\ &= (1+\lambda) \, (\beta + \lambda \, M_k) - \beta \, S_k \\ &= (1+\lambda) \, (\beta/M_k + \lambda) - (\beta/M_k) \, S_k \end{aligned} \tag{95}$$

$$\lambda_{k,\pm} = -\frac{1+\beta/M_k}{2} \pm \sqrt{\left(\frac{1+\beta/M_k}{2}\right)^2 + (\beta/M_k) \, (S_k - 1)} \tag{96}$$

where:

$$S_k = S_\Delta - S_- \, z_k \tag{97}$$

$$M_k = M_\Delta + M_\pm \, 2 \, \cos(2\pi k/n) \tag{98}$$

In general, we have to consider the real-part of all eigenvalues. Since $\Re(\lambda_{k,-}) = \Re(\lambda_{n-k,+})$ (except for n even and $k = n/2$, where we find $\lambda_{k,-} < \lambda_{k,+}$), we can restrict ourselves to the positive branch of the square root:

$$\lambda_k \equiv \lambda_{k,+} \tag{99}$$

Again, there is a crucial difference whether n is even or odd.

For n even, we obtain the following criterion for the stability of the central equilibrium E_c:

$$\Re(\lambda_{\text{even}}) < 0 \quad \Leftrightarrow \quad \forall k : \Re(\lambda_k) < 0 \tag{100}$$

where:

$$\lambda_{\text{even}} = \lambda_{n/2} \tag{101}$$

The critical eigenvalue λ_{even} arises for the branch $k = n/2$. The corresponding value S_{even} is given by:

$$S_{\text{even}} = S_{n/2} = S_\Delta + S_- = 1 + \frac{(\rho - 1) \, x_c}{\alpha} \tag{102}$$

Clearly, λ_{even} is real-valued, and its sign is determined by the sign of $S_{\text{even}} - 1 = (\rho - 1) \, x_c/\alpha$. Consequently, the stability of the central equilibrium E_c is determined by the parameter ρ only:

$$\Re(\lambda_{\text{even}}) < 0 \quad \Leftrightarrow \quad S_{\text{even}} < 1 \quad \Leftrightarrow \quad \rho < 1 \tag{103}$$

For $\rho < 1$, the central equilibrium E_c is asymptotically stable. (And all boundary equilibria are unstable.) For $\rho > 1$, the central equilibrium E_c is unstable. (And the two boundary equilibria E_{odd} and E_{even} are asymptotically stable.) At $\rho = 1$, the system undergoes a highly degenerate bifurcation, since also the number of boundary equilibria changes drastically, from $2^n - 1$ for $\rho < 1$ to L_n for $\rho > 1$.

Remark: For the simplified system (55), i.e. for $\gamma = 0$, the theory of monotone flows applies again. Consequently, Theorem 3.1 holds also for system "RepAuto".

For n odd, we obtain the following criterion for the stability of the central equilibrium E_c (see Claim 3.2 at the end of this section):

$$\Re(\lambda_{\text{odd}}) < 0 \quad \Leftrightarrow \quad \forall k : \Re(\lambda_k) < 0 \tag{104}$$

where:

$$\lambda_{\text{odd}} = \lambda_{(n+1)/2} \tag{105}$$

The critical eigenvalue λ_{odd} arises for the branch $k = (n+1)/2$ (or equivalently for the branch $k = (n-1)/2$). The corresponding values S_{odd} and M_{odd} are given by:

$$S_{\text{odd}} = S_{(n+1)/2} = S_\Delta + S_- \, e^{i\pi/n} \tag{106}$$

$$M_{\text{odd}} = M_{(n+1)/2} = M_\Delta - M_\pm \, 2 \, \cos(\pi/n) \tag{107}$$

Using these values, we can restate the stability criterion:

$$\Re(\lambda_{\text{odd}}) < 0 \quad \Leftrightarrow \quad \frac{\beta/M_{\text{odd}}}{(1+\beta/M_{\text{odd}})^2} < \frac{1 - \Re(S_{\text{odd}})}{(\Im(S_{\text{odd}}))^2} \tag{108}$$

The stability criterion depends on S_{odd} (i.e. on n, α, ρ, and κ), and β/M_{odd} (i.e. also on γ). Still, we can specify sufficient conditions for the stability of the central equilibrium E_c, which depend on S_{odd}, but not on β/M_{odd}.

$$\Re(\sqrt{S_{\text{odd}}}) < 1 \quad \Rightarrow \quad \Re(\lambda_{\text{odd}}) < 0 \tag{109}$$

$$\Re(S_{\text{odd}}) > 1 \quad \Rightarrow \quad \Re(\lambda_{\text{odd}}) > 0 \tag{110}$$

For given n, there are regions in parameter space in which the stability of the central equilibrium E_c only depends on α, ρ and κ. These regions of definite stability (or instability) are separated by a region of indefinite stability, i.e. a region in which the stability also depends on β and γ.

The region of definite stability, $\Re(\sqrt{S_{\text{odd}}}) < 1$, is bounded by an implicit function of n, α, ρ, and κ. In contrast, the region of definite instability can be given explicitly:

$$\Re(S_{\text{odd}}) > 1 \quad \Leftrightarrow \quad \frac{(\rho - 1/\cos(\pi/n))\,(\rho - \cos(\pi/n))}{\rho} > \frac{(1-\cos(\pi/n))^2}{\cos(\pi/n)}\,\kappa\,(\alpha - 1)$$
$$\wedge \quad \rho > 1/\cos(\pi/n) \tag{111}$$

Fig. 3 shows the stability diagram of the central equilibrium E_c for the smallest number of genes ($n = 3$) and different regulator binding, in particular for one binding site ($\kappa = 0$) and two independent binding sites ($\kappa = 1$).

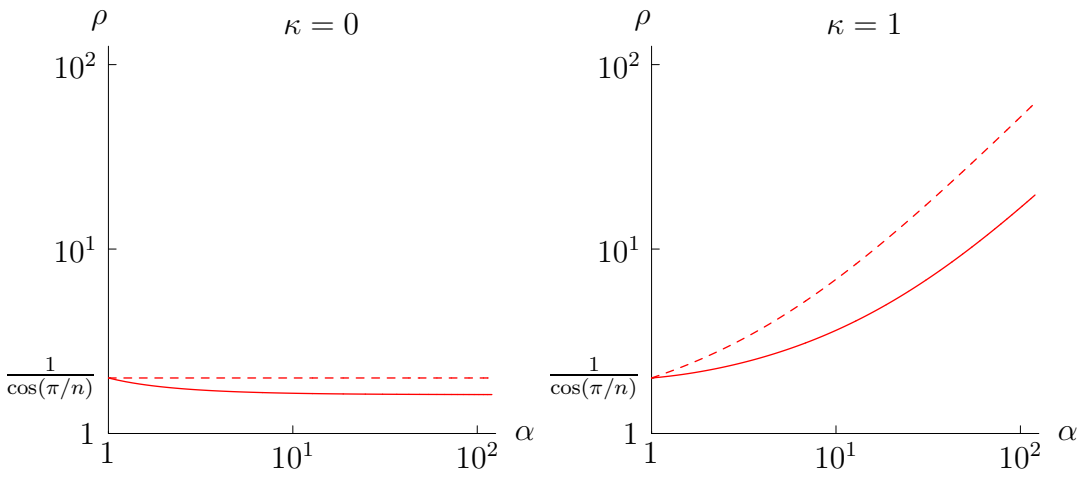

Figure 3: System "RepAuto" for n odd. Stability diagram of the central equilibrium for the smallest number of genes ($n = 3$) and different regulator binding, in particular for one binding site ($\kappa = 0$) and two independent binding sites ($\kappa = 1$). There are regions of definite stability (below the solid line) and definite instability (above the dashed line), i.e. regions in which the stability only depends on α and ρ (for given n and κ). In the intermediate region, the stability of the central equilibrium also depends on β and γ.

When the central equilibrium E_c looses stability, a periodic orbit appears. The system undergoes a Hopf bifurcation. Near a bifurcation, the (scaled) angular frequency of the oscillations amounts to:

$$\Im(\lambda_{\max}) = \frac{\beta/M_{\text{odd}}}{1+\beta/M_{\text{odd}}}\,\Im(S_{\text{odd}}) \tag{112}$$

For n odd, we have claimed that the stability of the central equilibrium E_c is determined by the eigenvalue $\lambda_{\text{odd}} = \lambda_{(n+1)/2}$. Finally, we prove this claim.

Claim 3.2 *For n odd, the stability of the central equilibrium E_c is determined by the eigenvalue $\lambda_{odd} = \lambda_{(n+1)/2}$:*

$$\Re(\lambda_{odd}) < 0 \quad \Leftrightarrow \quad \forall k : \Re(\lambda_k) < 0 \tag{113}$$

Proof. In general, the eigenvalues λ_k depend both on S_k and β/M_k. Still, by analyzing the dependency on β/M_k, we obtain the following conditions depending only on S_k:

$$\Re(\sqrt{S_k}) < 1 \quad \Rightarrow \quad \Re(\lambda_k) < 0 \tag{114}$$

$$\Re(S_k) > 1 \quad \Rightarrow \quad \Re(\lambda_k) > 0 \tag{115}$$

Suppose $\Re(\lambda_{\text{odd}}) < 0$. Then $\Re(S_{\text{odd}}) < 1$ by Eq. (115). Together with $0 < S_- < 1$ and trigonometric identities, this yields $\Re(\sqrt{S_k}) < 1$ for all $k \neq (n \pm 1)/2$, and consequently $\Re(\lambda_k) < 0$ by Eq. (114). □

3.2.3 Stability analysis of boundary equilibria

For a boundary equilibrium with support $\mathcal{S}$, the matrix $T(x)$ contains rows with only one non-zero entry (the diagonal entry). Every $i \notin \mathcal{S}$ (i.e. $x_i = 0$) yields:

$$S_{i,i} = \frac{\alpha}{1 + \rho\, x_{i-1}} \tag{116}$$

$$S_{i,i-1} = 0 \tag{117}$$

$$M_{i,i} = 1 + \gamma \left(\frac{1 + \kappa\, \rho\, x_{i-1}}{1 + \rho\, x_{i-1}} + \frac{\rho\,(1 + \kappa\, x_{i+1})}{1 + x_{i+1}} \right) \tag{118}$$

$$M_{i,i-1} = M_{i,i+1} = 0 \tag{119}$$

Consequently, every $i \notin \mathcal{S}$ (and every $i \in \mathcal{S}$ with $i - 1 \notin \mathcal{S}$, $i + 1 \notin \mathcal{S}$) produces a simple factor in the characteristic equation $|T| = 0$:

$$T_{i,i} = \beta\,(1 + \lambda) + \lambda\,(1 + \lambda)\, M_{i,i} - \beta\, S_{i,i} = 0 \tag{120}$$

$$\lambda_{i,\pm} = -\frac{1 + \beta/M_{i,i}}{2} \pm \sqrt{\left(\frac{1 + \beta/M_{i,i}}{2} \right)^2 + (\beta/M_{i,i})\,(S_{i,i} - 1)} \tag{121}$$

Clearly, the resulting eigenvalues are real-valued. Since $\lambda_{i,-} < \lambda_{i,+}$, we can restrict ourselves to the positive branch of the square root:

$$\lambda_i \equiv \lambda_{i,+} \tag{122}$$

The sign of λ_i is determined by the sign of $S_{i,i} - 1$. The value of $S_{i,i}$ depends on x_{i-1} and x_i. See Eq. (84).

For $\rho < 1$, we only consider $i \notin \mathcal{S}$ (i.e. $x_i = 0$) and obtain the following cases:

x_{i-1}	x_i	$S_{i,i}$
0	0	α
$0 < x_{i-1} \leq \alpha - 1$	0	$\geq \frac{\alpha}{1+\rho\,(\alpha-1)}$

(123)

In any case, we obtain $S_{i,i} - 1 > 0$ and consequently $\lambda_i > 0$. Hence, all boundary equilibria are unstable.

For $\rho > 1$, we additionally consider $i \in \mathcal{S}$ with $i-1 \notin \mathcal{S}$, $i+1 \notin \mathcal{S}$ (i.e. $x_i = \alpha - 1$, $x_{i-1} = x_{i+1} = 0$) to deal with all cases:

x_{i-1}	x_i	$S_{i,i}$
0	0	α
$\alpha - 1$	0	$\frac{\alpha}{1+\rho\,(\alpha-1)}$
0	$\alpha - 1$	$\frac{1}{\alpha}$

(124)

Only a pair $(x_{i-1}, x_i) = (0, 0)$ yields $S_{i,i} - 1 > 0$ and consequently $\lambda_i > 0$. For n odd, every equilibrium contains such a pair, hence all boundary equilibria are unstable. For n even, the two equilibria E_{odd} and E_{even} with support $\mathcal{S}_{\text{odd}} = \{1, 3, \ldots, n-1\}$ and $\mathcal{S}_{\text{even}} = \{2, 4, \ldots, n\}$ do not contain such a pair. Hence, they are asymptotically stable. All other boundary equilibria are unstable.

3.2.4 Stability analysis of the heteroclinic cycle

For $\alpha > 1$ and $\rho > 1$, there are heteroclinic connections between unstable equilibria on the boundary. We assume $\gamma = 0$ in the following.

Claim 3.3 *For $\alpha > 1$ and $\rho > 1$, system "RepAuto" (with $\gamma = 0$) has the following property: All orbits on the boundary converge to an equilibrium.*

Proof. If initially $x_{i-1} = y_{i-1} = 0$, but $x_i + y_i > 0$, then $x_i(t) \to \alpha - 1$ and $y_i(t) \to \alpha - 1$. Once $x_i(t)$ is close enough to $\alpha - 1$, even if the next species $i + 1$ is initially present, repression leads to its removal. In particular, $x_i > (\alpha - 1)/\rho$ yields

$$\dot{y}_{i+1} < x_{i+1} - y_{i+1} \tag{125}$$

and:

$$(x_{i+1} + \beta\, y_{i+1})^{\cdot} < 0 \tag{126}$$

Hence, $x_{i+1}(t) \to 0$ and $y_{i+1}(t) \to 0$.

If the next species is initially present, it will again converge to its equilibrium values $x_{i+2} = y_{i+2} = \alpha - 1$ and repress the next, etc. Hence, the orbit converges to some boundary equilibrium. If only one species (say $i - 1$) is missing initially, then the limit equilibrium has support $\{i, i + 2, \ldots, i + 2\lfloor \frac{n}{2} \rfloor - 2\}$. □

This implies the existence of heteroclinic connections and cycles on the boundary.

For $n \geq 3$ (and for each $i = 1, \ldots, n$) the above proof shows that all orbits in the 4-dimensional boundary face containing only species i and $i + 1$ converge to E_i. The closure of this boundary face contains two more equilibria, the repellor O

and the saddle E_{i+1}. In particular, the one dimensional unstable manifold of E_{i+1} converges to E_i. Hence there is a connection $E_{i+1} \to E_i$ in this 4-dimensional boundary face. All these connecting orbits together form a heteroclinic cycle $E_1 \to E_n \to E_{n-1} \to \cdots \to E_2 \to E_1$. For $n \geq 4$ these connecting orbits and hence the full heteroclinic cycle are unstable within the (maximal invariant set of the) boundary. This follows from the last sentence in the above proof.

However, for each odd n there is a heteroclinic cycle that is asymptotically stable within the boundary. For $n = 3$ it is $E_1 \to E_3 \to E_2 \to E_1$, whereas for $n = 5$: $E_{13} \to E_{35} \to E_{52} \to E_{24} \to E_{41} \to E_{13}$, and for $n = 7$: $E_{135} \to E_{357} \to E_{572} \to E_{724} \to E_{246} \to E_{461} \to E_{613} \to E_{135}$. For general odd n, this heteroclinic cycle connects the n equilibria $E_{i,i+2,i+4,\ldots,i+n-3}$, whose support has the pattern $*0*0\cdots*00$ (cyclically modulo n). The latter equilibrium is asymptotically stable within the $2n-2$ dimensional boundary face where species $i+n-1 = i-1$ is missing, as shown above. So $i+n-1$ is the only unstable direction at $E_{i,i+2,i+4,\ldots,i+n-3}$, and in the face consisting of species $i, i+2, i+4, \ldots, i+n-3, i+n-1$, all orbits converge to the 'next' equilibrium $E_{i+2,i+4,\ldots,i+n-3,i+n-1}$.

For even $n \geq 6$ there are similar additional heteroclinic cycles, but they are all unstable. Indeed, there are orbits nearby converging to the equilibria E_{even} and E_{odd}, as shown above.

Theorem 3.3 *For n odd, $\alpha > 1$ and $\rho > 1$, system "RepAuto" (with $\gamma = 0$) has a heteroclinic cycle connecting the n equilibria of support size $\frac{n-1}{2}$. (i) The system is permanent if $\lambda + \frac{n-1}{2}\mu > 0$. (ii) The heteroclinic cycle is asymptotically stable if $\lambda + \frac{n-1}{2}\mu < 0$, where:*

$$\lambda = -\frac{1+\beta}{2} + \sqrt{\left(\frac{1+\beta}{2}\right)^2 + \beta(\alpha - 1)} > 0 \tag{127}$$

$$\mu = -\frac{1+\beta}{2} + \sqrt{\left(\frac{1+\beta}{2}\right)^2 + \beta\left(\frac{\alpha}{1+\rho(\alpha-1)} - 1\right)} < 0 \tag{128}$$

Proof. See end of this section.

Near $\lambda + \frac{n-1}{2}\mu = 0$, where the heteroclinic cycle changes stability, a new invariant set is created in a heteroclinic bifurcation. We expect that this is a periodic orbit of large period. A proof of this as well as a stability analysis of the bifurcating orbit requires the computation and analysis of the Poincaré map and is outside the scope of this paper, but see [15, p. 226-227] for a similar (but simpler) situation.

The stability criterion $\lambda + \frac{n-1}{2}\mu < 0$ is symmetric around the plane $\beta = 1$ (on a logarithmic scale for β). By considering the limit cases $\beta \to \infty$ and $\beta = 1$, we obtain sufficient conditions for the stability of the heteroclinic cycle, which

depend on n, α, and ρ, but not on β.

$$(\lambda + \tfrac{n-1}{2}\,\mu)_{\beta\to\infty} < 0 \quad \Rightarrow \quad \lambda + \tfrac{n-1}{2}\,\mu < 0 \tag{129}$$

$$(\lambda + \tfrac{n-1}{2}\,\mu)_{\beta=1} > 0 \quad \Rightarrow \quad \lambda + \tfrac{n-1}{2}\,\mu > 0 \tag{130}$$

For given n, there are regions in parameter space in which the stability of the heteroclinic cycle only depends on α and ρ. These regions of definite stability (or instability) are separated by a region of indefinite stability, i.e. a region in which the stability also depends on β.

The regions of definite stability (or instability) can be given explicitly:

$$(\lambda + \tfrac{n-1}{2}\,\mu)_{\beta\to\infty} < 0 \quad \Leftrightarrow \quad \rho > \frac{\frac{n+1}{2}}{\frac{n+1}{2} - \alpha} \quad \wedge \quad \alpha < \tfrac{n+1}{2} \tag{131}$$

$$(\lambda + \tfrac{n-1}{2}\,\mu)_{\beta=1} > 0 \quad \Leftrightarrow \quad \rho < \frac{\frac{n+1}{2}\,(\frac{n+1}{2} + \frac{n-3}{2}\,\sqrt{\alpha})}{(\frac{n+1}{2} - \sqrt{\alpha})^2\,(1+\sqrt{\alpha})} \quad \vee \quad \alpha > (\tfrac{n+1}{2})^2 \tag{132}$$

Fig. 4 shows the stability diagram of the heteroclinic cycle for the smallest number of genes ($n = 3$) and weak regulator binding ($\gamma = 0$).

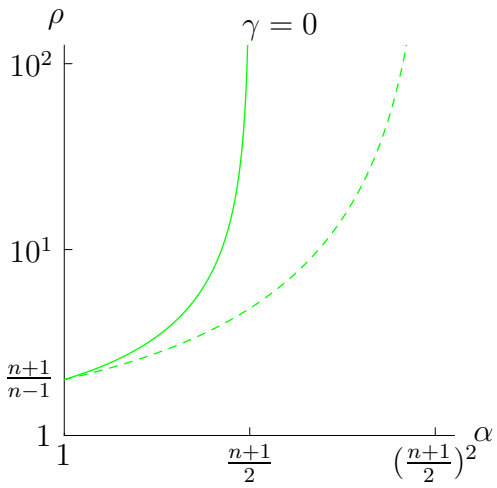

Figure 4: System "RepAuto" for n odd. Stability diagram of the heteroclinic cycle for the smallest number of genes ($n = 3$) and weak regulator binding ($\gamma = 0$). There are regions of definite stability (above the solid line) and definite instability (below the dashed line), i.e. regions in which the stability only depends on α and ρ (for given n). In the intermediate region, the stability of the heteroclinic cycle also depends on β.

We expect that Theorem 3.3 continues to hold also for $\gamma > 0$. However, at present we do not have a proof of Claim 3.3 in that case. The remaining part of the proof goes through as below. Only the stability criterion for the heteroclinic cycle is a bit more complicated for $n \geq 5$.

Conjecture 3.1 *For n odd, $\alpha > 1$ and $\rho > 1$, system "RepAuto" has a heteroclinic cycle connecting the n equilibria of support size $\frac{n-1}{2}$. (i) The system is permanent if $\lambda + \mu + \frac{n-3}{2}\,\mu' > 0$. (ii) The heteroclinic cycle is attracting if $\lambda + \mu + \frac{n-3}{2}\,\mu' < 0$, where:*

$$\lambda = \lambda_i(0, 0, *) \tag{133}$$
$$\mu = \lambda_i(*, 0, 0) \tag{134}$$
$$\mu' = \lambda_i(*, 0, *) \tag{135}$$

The stability criterion of the heteroclinic cycle, given in Conjecture 3.1, depends on certain eigenvalues of a boundary equilibrium. In the following, we determine these eigenvalues.

For $\rho > 1$, the support $\mathcal{S}$ of any boundary equilibrium is sparse, i.e. either $i \notin \mathcal{S}$ or $i \in \mathcal{S}$ with $i-1 \notin \mathcal{S}$, $i+1 \notin \mathcal{S}$. Consequently, all eigenvalues of a boundary equilibrium are given by Eq. (121). Again, we restrict ourselves to the positive branch of the square root, given by Eq. (122). Via $S_{i,i}$ and $M_{i,i}$, the eigenvalues λ_i depend on x_{i-1}, x_i, and x_{i+1}:

$$\begin{aligned}\lambda_i &= \lambda_i(x_{i-1}, x_i, x_{i+1}) \\ &= -\frac{1+\beta/M_{i,i}}{2} + \sqrt{\left(\frac{1+\beta/M_{i,i}}{2}\right)^2 + (\beta/M_{i,i})\,(S_{i,i}-1)}\end{aligned} \tag{136}$$

For Conjecture 3.1 we have to consider the following cases (where we use the symbol $*$ for the value $\alpha - 1$):

x_{i-1}	x_i	x_{i+1}	$S_{i,i}$	$M_{i,i}$	λ_i
0	0	$*$	α	$1+\gamma\,(1+\rho\,\frac{1+\kappa\,(\alpha-1)}{\alpha})$	λ
$*$	0	0	$\frac{\alpha}{1+\rho\,(\alpha-1)}$	$1+\gamma\,(\frac{1+\kappa\,\rho\,(\alpha-1)}{1+\rho\,(\alpha-1)}+\rho)$	μ
$*$	0	$*$		$1+\gamma\,(\frac{1+\kappa\,\rho\,(\alpha-1)}{1+\rho\,(\alpha-1)}+\rho\,\frac{1+\kappa\,(\alpha-1)}{\alpha})$	μ'

(137)

We conclude this section with the proof of Theorem 3.3.

Proof of Theorem 3.3

Let $\mathcal{S}$ be the support of a boundary equilibrium, and $\mathcal{S}+1$ the set of all successors of elements in $\mathcal{S}$. At the boundary equilibrium $E_{\mathcal{S}}$, each $i \in \mathcal{S}+1$ is a repressed species and hence its decline is governed by the linearized system

$$\dot{x}_i = \beta\,(y_i - x_i), \quad \dot{y}_i = \frac{\alpha}{1+\rho\,(\alpha-1)}\,x_i - y_i$$

whose leading eigenvalue μ is negative. Let $(1, u_\mu)$ (with $u_\mu > 0$) be the corresponding left eigenvector, so that

$$(x_i + u_\mu\, y_i)^{\cdot} = \mu\,(x_i + u_\mu\, y_i) \tag{138}$$

holds near $E_{\mathcal{S}}$.

If both i and $i-1$ are not in $\mathcal{S}$, then species i is not repressed, and hence its invasion is governed by the linearized system

$$\dot{x}_i = \beta\,(y_i - x_i), \quad \dot{y}_i = \alpha\, x_i - y_i$$

whose leading eigenvalue λ is positive. Let $(1, u_\lambda)$ (with $u_\lambda > 0$) be the corresponding left eigenvector, so that

$$(x_i + u_\lambda\, y_i)^{\cdot} = \lambda\,(x_i + u_\lambda\, y_i) \tag{139}$$

holds near $E_{\mathcal{S}}$.

Let $z = (x, y)$. We use the function

$$P(z) = \prod_{i=1}^{n} (x_i + c_i(z)\, y_i) \tag{140}$$

as an average Liapunov function [15, 16].

We choose the functions $c_i(z) > 0$ in a smooth way, such that $c_i(z) = u_\mu$ for z close to any boundary equilibrium $E_{\mathcal{S}}$ with $i-1 \in \mathcal{S}$ and $c_i(z) = u_\lambda$ for z close to any boundary equilibrium $E_{\mathcal{S}}$ with $i-1 \notin \mathcal{S}$.

The function $\frac{\dot{P}(z)}{P(z)}$ is bounded below and (138) and (139) imply that near $E_{\mathcal{S}}$

$$\frac{\dot{P}(z)}{P(z)} = \sum_{i=1}^{n} \frac{(x_i + c_i(z)\, y_i)^{\cdot}}{(x_i + c_i(z)\, y_i)} = \lambda\,(n - 2\,|\mathcal{S}|) + \mu\,|\mathcal{S}| + O(|z - E_{\mathcal{S}}|) \tag{141}$$

Since $|\mathcal{S}| \leq \frac{n-1}{2}$, the coefficient of λ is at least 1. Hence, if $\lambda + \frac{n-1}{2}\,\mu > 0$ then $\frac{\dot{P}(z)}{P(z)} > 0$ near all boundary equilibria, and the system is permanent.

On the other hand, if $\lambda + \frac{n-1}{2}\,\mu < 0$ then $\frac{\dot{P}(z)}{P(z)} < 0$ near the equilibria $E_{\mathcal{S}}$ with $|\mathcal{S}| = \frac{n-1}{2}$, i.e. all the equilibria in the heteroclinic cycle described above. Since this heteroclinic cycle is asymptotically stable within the maximal invariant set of the boundary of the state space $\mathbb{R}^{2n}_+$, it is asymptotically stable also for the full system. □

4 Main results

In this section we summarize the most important results derived in the previous section. In particular, we present detailed stability diagrams for the two systems under consideration.

4.1 System "RepLeaky"

For system "RepLeaky", the classification depends on the number of genes n, the leakiness δ, the Hill coefficient h, the combined parameter α, the degradation ratio β, and the binding parameter γ.

There is a central equilibrium E_c given by $x_i = y_i = x_c > 0$, where x_c solves the equation $x_c = \alpha\,[(1-\delta)(1-s(x_c)) + \delta]$ defined by the sigmoid function $s(x) = x^h/(1+x^h)$.

There is a crucial difference whether n is even or odd.

For n even, the central equilibrium E_c is globally asymptotically stable if $S_c < 1$, where $S_c = \alpha\,(1-\delta)\,s'(x_c)$. If $S_c > 1$, then almost all orbits converge to the equilibria $E_{\rm odd}$ and $E_{\rm even}$, where only the odd (resp. even) numbered genes are transcribed at a high rate. For given δ and h, a sufficient condition for the stability of the central equilibrium E_c is given by $S_{\max} < 1$, where $S_{\max} = h\,(1-\delta^{1/2})/(1+\delta^{1/2})$.

For n odd, the global attractor is either (i) the central equilibrium E_c or (ii) a periodic attractor. The stability of the central equilibrium E_c is determined by

$$\frac{\beta/M_c}{(1+\beta/M_c)^2} < \frac{1 - S_c\cos(\pi/n)}{S_c^2\sin^2(\pi/n)}, \tag{142}$$

where $M_c = 1 + \gamma\,s'(x_c)$. For given n, δ and h, a sufficient condition for the stability of the central equilibrium E_c is given by $S_{\max} < 2/(1+\cos(\pi/n))$. In particular, for $n = 3$ and $\delta = 0$ the central equilibrium E_c is stable if $h < 4/3$.

The transition from (i) to (ii) occurs via a Hopf bifurcation.

Fig. 5 presents the stability diagram of the central equilibrium E_c for the smallest number of genes ($n = 3$), weak or strong repressor binding ($\gamma = 0$ or $\gamma = 10^3$), leaky or non-leaky transcription ($\delta = 10^{-3}$ or $\delta = 0$), and different Hill coefficients ($h = 1.5, 2, 2.5$). Clearly, the stability diagram depends on α and β. For weak repressor binding, $\gamma \ll 1$, the stability diagram is symmetric around the plane $\beta = 1$ (on a logarithmic scale for β).

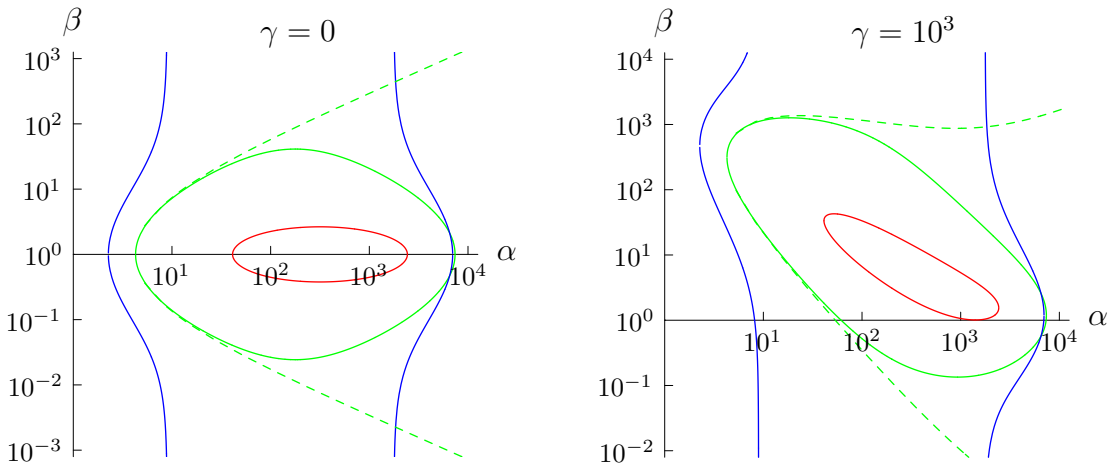

Figure 5: System "RepLeaky" for n odd. Stability diagram of the central equilibrium for the smallest number of genes ($n = 3$) and either weak or strong repressor binding ($\gamma = 0$ or $\gamma = 10^3$). Stability boundaries enclose the regions of instability (the regimes of oscillation). Solid (dashed) lines denote leaky (non-leaky) transcription, whereas colors denote different Hill coefficients. (Solid red, green, or blue lines correspond to $\delta = 10^{-3}$ and $h = 1.5$, 2, or 2.5. Dashed green lines correspond to $\delta = 0$ and $h = 2$.)

4.2 System "RepAuto"

For system "RepAuto", the classification depends on the number of genes n, the repressor strength ρ, the cooperativity κ, the combined parameter α, the degradation ratio β, and the binding parameter γ.

For $\alpha < 1$, the only equilibrium is the origin O, and it is the global attractor.

For $\alpha > 1$, there is the central equilibrium E_c given by $x_i = y_i = x_c > 0$, where x_c solves the equation $\alpha = 1 + x_c + \rho\, x_c + \kappa\, \rho\, x_c^2$. Additionally, there are equilibria on the boundary.

For $\rho < 1$, the central equilibrium E_c is asymptotically stable (presumably the global attractor). There are $2^n - 1$ boundary equilibria all of which are unstable.

For $\rho > 1$, there are L_n boundary equilibria, where L_n is the n^{th} Lucas number. There is a crucial difference whether n is even or odd.

For n even, there are two asymptotically stable boundary equilibria, E_{odd} and E_{even}, where only the odd (resp. even) numbered genes are transcribed. All other equilibria, including E_c, are unstable.

In contrast, for n odd, all boundary equilibria are unstable. There are three possible attractors: (i) the central equilibrium E_c, (ii) a periodic attractor in the interior (a limit cycle), and (iii) an "aperiodic" attractor on the boundary (a heteroclinic cycle connecting unstable equilibria).

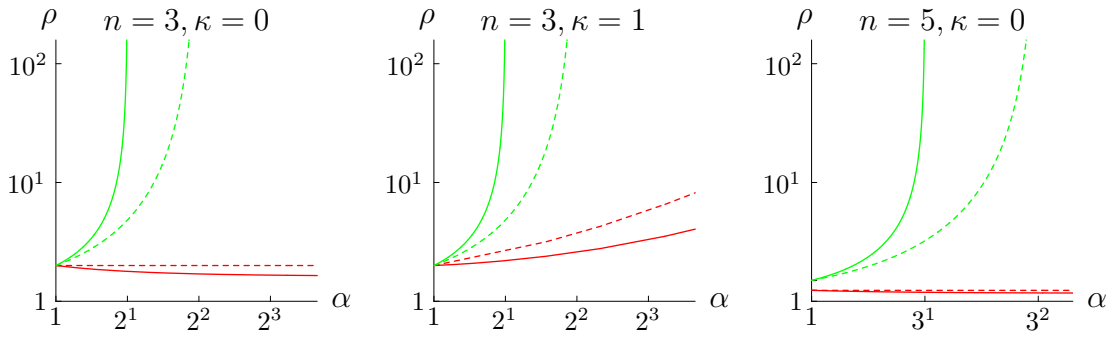

Figure 6: System "RepAuto" for n odd. Bifurcation diagrams in the (α, ρ)–plane for different numbers of genes ($n = 3$ or $n = 5$) and different types of regulator binding ($\kappa = 0$ or $\kappa = 1$). For $\alpha < 1$, the only attractor of system "RepAuto" is the origin. For $\alpha > 1$, there are three possible attractors: the central equilibrium, a limit cycle, and a heteroclinic cycle. The diagram shows the stability boundaries of the central equilibrium (red) and the heteroclinic cycle (green). The central equilibrium is stable below the solid red line (and unstable above the dashed red line), whereas the heteroclinic cycle is stable above the solid green line (and unstable below the dashed green line). Between the dashed lines there is a stable limit cycle. Between solid and dashed lines the stability (of the central equilibrium or the heteroclinic cycle) also depends on β and γ.

The transition from (i) to (ii) occurs via a Hopf bifurcation. In the transition from (ii) to (iii), the limit cycle approaches the boundary and its period grows to infinity.

The main novel behavior in "RepAuto", as compared to the traditional model "RepLeaky", is case (iii). It is known as May-Leonard behavior [15].

For given n and κ, the crucial parameters are α and ρ. Fig. 6 presents the bifurcation diagrams for different numbers of genes ($n = 3$ or $n = 5$) and different types of regulator binding ($\kappa = 0$ or $\kappa = 1$). (Recall that $\kappa = 0$ corresponds to one binding site, whereas $\kappa = 1$ corresponds to two independent binding sites.)

For $n = 3$ the stability boundaries of the central equilibrium and the heteroclinic cycle intersect at $\alpha = 1$, whereas they do not intersect at $\alpha = 1$ for $n \geq 5$. We also note that with increasing n the stability boundaries of the heteroclinic cycle are stretched to the right, whereas with increasing κ the stability boundaries of the central equilibrium are rotated counterclockwise.

For high cooperativity, $\kappa \gg 1$, the central equilibrium and the heteroclinic cycle can be stable at the same time. To illustrate this fact, Fig. 7 presents the bifurcation diagram for the smallest number of genes ($n = 3$), high cooperativity ($\kappa = 10$), and high degradation ratio ($\beta \to \infty$). (The latter is assumed for reasons of simplicity.)

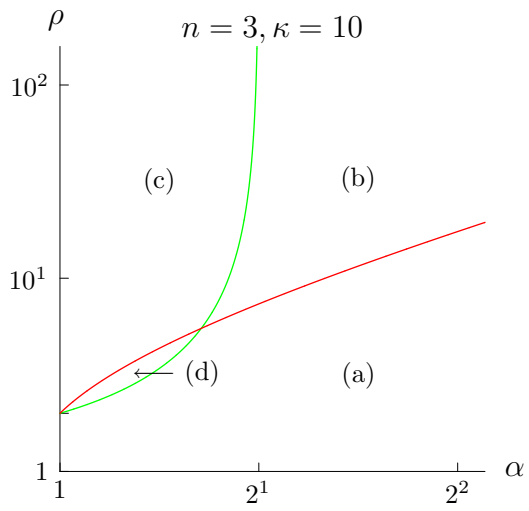

Figure 7: System "RepAuto" for n odd. Bifurcation diagram in the (α, ρ)-plane for the smallest number of genes ($n = 3$), high cooperativity ($\kappa = 10$), and high degradation ratio ($\beta \to \infty$). For $\alpha < 1$, the only attractor of system "RepAuto" is the origin. For $\alpha > 1$, there are three possible attractors: the central equilibrium, a limit cycle, and a heteroclinic cycle. The diagram shows the stability boundaries of the central equilibrium (red) and the heteroclinic cycle (green). Below the red line the central equilibrium is stable, and above the green line the heteroclinic cycle is stable. As a consequence, in region (a) there is a stable central equilibrium and an unstable heteroclinic cycle, whereas in region (c) there is a stable heteroclinic cycle and an unstable central equilibrium. In region (b) there is a stable limit cycle. Finally, in region (d) both the central equilibrium and the heteroclinic cycle are stable.

5 Conclusions

Gene regulatory networks with a cyclic (and symmetric) topology, e.g. the repressilator, can be easily analyzed for an arbitrary number of genes. Our study confirms the previously derived result [9] and earlier suggested finding [5] that odd numbers of genes in repression cycles may give rise to oscillations, whereas even numbers of genes lead to multiple stable equilibria. The additional consideration of arbitrarily strong regulator binding is more involved, since it requires a distinction between total and free protein concentrations. However, mass conservation yields a (linear) relation between total and free protein flows, and total protein concentrations can be eliminated. As in the simplified system with weak regulator binding, the resulting kinetic equations are formulated for free protein and mRNA concentrations. Moreover, the equilibria of the exact and the simplified system are the same. Only the stability of potential attractors is affected by the binding strength of the regulators.

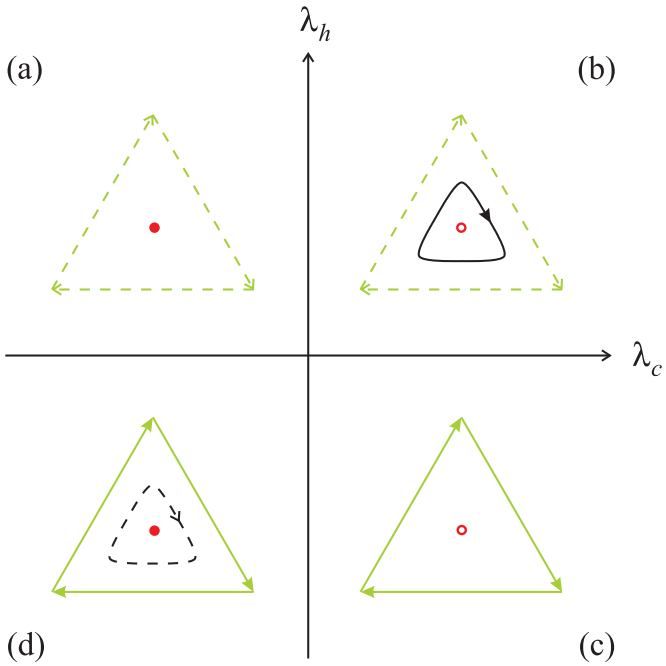

Figure 8: System "RepAuto" for n odd. A sketch of the four dynamical scenarios for the smallest number of genes ($n = 3$) and high cooperativity ($\kappa \gg 1$). There are three possible attractors (denoted by colors): the central equilibrium (red), the limit cycle (black), and the heteroclinic cycle (green). Stable orbits are shown as filled circles or solid lines, unstable orbits as empty circles or dashed lines. The "eigenvalue" of the central equilibrium, $\lambda_c = \Re(\lambda_{\text{odd}})$, and the "eigenvalue" of the heteroclinic orbit, $\lambda_h = \lambda + \mu$, are indicated on the coordinate axes. In case (a) there is a stable central equilibrium and an unstable heteroclinic cycle, whereas in case (c) there is a stable heteroclinic cycle and an unstable central equilibrium. In case (b) both eigenvalues are positive and there is a stable limit cycle. Finally, in case (d) both eigenvalues are negative and there is a stable central equilibrium and a stable heteroclinic cycle together with an unstable limit cycle.

For odd numbers of genes n, the repressilator systems presented here in detail exhibit three potential attractors: (i) the central equilibrium, (ii) a limit cycle, and (for system "RepAuto") (iii) a heteroclinic cycle connecting n unstable equilibria on the boundary of the state space. For $n = 3$, the heteroclinic cycle connects the single species equilibria $E_1 \to E_3 \to E_2 \to E_1$. Note that the flow on the heteroclinic cycle is opposite to the repression cycle $1 \dashv 2 \dashv 3 \dashv 1$.

Every single species equilibrium E_i in the heteroclinic cycle is a saddle: It is stable against invasion of species $i+1$ (modulo 3), but unstable against invasion of species $i-1$ (modulo 3). The results presented in the previous section allow for a simple visualization of the repressilator dynamics in a plane spanned by the crucial "eigenvalue" of the central equilibrium, $\lambda_c = \Re(\lambda_{\text{odd}})$, and the "eigenvalue" of the heteroclinic cycle, $\lambda_h = \lambda + \mu$. See Figure 8.

For different signs of the two eigenvalues, λ_c and λ_h, we have either a stable central equilibrium or a stable heteroclinic orbit. If both eigenvalues are positive, the central equilibrium and the heteroclinic orbit are unstable, and there is a stable limit cycle. For two negative eigenvalues, both the central equilibrium and the heteroclinic orbit are stable. In the ranges of marginal stability close to $\lambda_c = 0$ or $\lambda_h = 0$ more complex dynamical situations may arise.

The most important biological implication of the existence of a heteroclinic cycle in system "RepAuto" is the occurrence of an upper limit for the repressor binding constant with respect to oscillations. Let us again consider the case $n = 3$. If repressor binding exceeds the critical value reported here, oscillations die out and the system approaches a state in which only one gene of the repressilator is active and the other two are silenced. For the symmetric system discussed here, each of the three genes has the same probability to stay active. However, in reality the three binding constants are different and so are the probabilities for not becoming silenced. Hence, one gene is preferentially chosen.

Acknowledgements

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