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DDE MODEL OF GENE EXPRESSION: A CONTINUUM APPROACH

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ABSTRACT

This paper presents an analytical study of the stability of the steady state solutions of a gene regulatory network with time delay. The system is modeled as a continuous network and takes the form of a nonlinear delay differential-integral equation coupled to an ordinary differential equation. Two examples are given in which the critical delay causing instability is computed.

INTRODUCTION

Understanding the interactions between genes and their protein products is an important part of experimental and theoretical biology. Recent experiments [10, 29] and theoretical techniques [20, 33, 34] have been developed to understand the dynamics of gene regulatory networks. From a theoretical point of view, the gene network structure is an abstraction of the system's chemical dynamics, and it includes how protein products affect the expression of other genes and their associated proteins. If the network involves only a few genes then its dynamical behavior could be studied directly [7, 10]. On the other hand, if the network is formed of hundreds or thousands of genes then its experimental or theoretical study may be highly difficult [4, 24]. Nevertheless, research trends show that the study of these complex dynamical networks is a natural step in genomic research [32].

Several mathematical models of gene regulatory networks have been developed over the last couple of decades (for an extensive review see [13,15,28]). Some of the most common modeling techniques involve the use of graphs [17, 21], Boolean networks [3, 25, 26], Bayesian networks [8], Petri nets [9, 19], reverse engineering methods [30], and coupled differential equations (linear [16], nonlinear [5, 12, 22], partial [31], stochastic [11, 27, 36], and delayed [1, 6, 33]). Here we are interested in models where the natural lags or delays play an important role in the system's dynamics [18, 23, 33, 34]. These delays arise naturally from transcription, translation, degradation, and other cellular processes. If the time delays are of the order of the system's time scale, then taking them into account can potentially change the system's dynamics.

In this work, we study the steady state solutions and the stability of two different models of a gene network with time delay. Both of these models are characterized by a system of two coupled equations: an ordinary differential equation and a delay differential-integral equation. The first model considers uniform weighting, where each ribosome produces a given quantity of protein which is then shared equally amongst all gene sites. The second model is characterized by an exponential weighting, where each protein product is shared unequally, with nearby gene units being repressed to a greater extent than more distant genes. Both of these cases exhibit a steady state, which is stable when there is no delay. Linear analysis then reveals that a critical delay exists, where the steady state becomes unstable. Closed form expressions for the critical delay T_{cr} and associated frequency ω are thus found. We then confirm our results for the exponential weighting case by discretizing the continuous system into an N-dimensional system and showing that the discrete critical delays approach the continuous T_{cr} as N becomes large.

BIOLOGICAL BACKGROUND

Transcription and translation are the main processes by which a cell expresses the instructions encoded in its genes. Transcription is the first step in gene expression and it includes the identical replication of a gene into messenger RNA (mRNA). The second step is the translation process, where the information in the mRNA is translated into a protein with a specific amino acid sequence. The latter process is accomplished by a well-known protein-manufacturing machine called a ribosome. Once the protein is created, it unbinds from the ribosome and carries out its cellular function. From these processes mRNA and protein concentrations arise naturally as the main intracellular regu-

latory agents for gene expression.

There are several mechanisms that the cell uses to regulate the levels of mRNA and protein concentrations. An example is the cell's ability to increase or decrease the concentration of enzymes that degrade proteins. Another important regulatory mechanism is the cell's capacity to turn on and off the transcription process of a specific gene. The latter can be accomplished by means of feedback inhibition, where the expression of a gene is regulated by its own protein product. This feedback mechanism arises when the protein product returns to the nucleus and stops the transcription of its own mRNA by binding to the gene's promoter site. Previous findings [18,23] show that there are time delays associated with this feedback mechanism. These delays arise naturally as transcriptional delays (time it takes the gene to get copied into mRNA) and translational delays (time it takes the ribosome to translate mRNA into protein). Furthermore, recent studies have shown that it suffices to consider only the transcriptional time delay to have an accurate dynamic model [18,23,33]. These transcriptional delay models can be represented by the following pair of equations:

$$\frac{dm}{dt} = -\mu_m m(t) + H(p(t-T)) \tag{1}$$

$$\frac{dp}{dt} = \alpha_p m(t) - \mu_p p(t) \tag{2}$$

where the time dependent variables are the mRNA concentration, $m(t)$, and its associated protein concentration, $p(t)$, and where the constants μ_m and μ_p are the decay rates of the mRNA and protein molecules, α_p is the rate of production of new protein molecules per mRNA molecule, and $H(p(t-T))$ is a Hill function representing the rate of *delayed* production of new mRNA molecules. We assume that $H(p(t-T))$ is a decreasing function of the concentration of protein present at a previous time $p(t-T)$, where T represents the transcriptional time delay.

Recent findings reveal how the dynamics of the system depends on the model parameters [33]. For simplicity, in this paper we assume that μ_m=μ_p=μ and α_p=1.

MATHEMATICAL MODEL

In this work we investigate a model of gene expression in which the protein product of a given gene not only represses its own mRNA production, but also represses the mRNA production of other nearby genes. We tag a given gene with a variable $x \in [0,1]$, and we generalize the system (1),(2) to be of the form:

$$\dot{m} = -\mu m + \int_0^1 K(x-\bar{x}) H(p_d(\bar{x}))\, d\bar{x} \tag{3}$$

$$\dot{p} = m - \mu p \tag{4}$$

where $m = m(x,t)$, $p = p(x,t)$, and $p_d(\bar{x}) = p(\bar{x}, t-T)$. Here $K(x-\bar{x})$ is a weighting function.

In this paper we consider two special cases of Eqs.(3),(4):

CASE 1: UNIFORM WEIGHTING

This case is characterized by the choice $K(x-\bar{x}) = 1$. Here each ribosome produces a given quantity of protein which is shared equally amongst all gene sites. For the rate of production of mRNA $H(p_d(\bar{x}))$ we choose the following Hill function [23,33]:

$$H(p_d(\bar{x})) = \frac{1}{1+\left(\frac{p_d(\bar{x})}{p_0(\bar{x})}\right)^n} \tag{5}$$

where $p_d(\bar{x}) = p(\bar{x}, t-T)$ is the delayed protein concentration at location $\bar{x}$, and where $p_0(\bar{x})$ is a reference concentration of protein at $\bar{x}$, and n is a parameter [23]. The resulting system is of the form:

$$\dot{m} = -\mu m + \int_0^1 \frac{1}{1+\left(\frac{p_d(\bar{x})}{p_0(\bar{x})}\right)^n} d\bar{x} \tag{6}$$

$$\dot{p} = m - \mu p \tag{7}$$

CASE 2: EXPONENTIAL WEIGHTING

This case is characterized by the choice $K(x-\bar{x}) = e^{-|x-\bar{x}|}$. Here each protein product is shared unequally, with nearby gene sites being repressed to a greater extent than more distant ones. For mathematical simplicity we choose the rate of production of mRNA $H(p_d(\bar{x}))$ to be given by a linear function of p_d:

$$H(p_d(\bar{x})) = 1 - p_d(\bar{x}) \tag{8}$$

The resulting system is of the form:

$$\dot{m} = -\mu m + \int_0^1 e^{-|x-\bar{x}|}(1-p_d(\bar{x}))\, d\bar{x} \tag{9}$$

$$\dot{p} = m - \mu p \tag{10}$$

STEADY STATE SOLUTIONS

In this section we consider the steady state behavior of the system (3),(4). Setting $\dot{p}$=$\dot{m}$=0 we see that at steady state m^*=μp^* and p_d^*=p^*, where a * represents the steady state solution.

CASE 1: UNIFORM WEIGHTING

At steady state, Eqs.(6),(7) give

$$\mu^2 p^*(x) = \int_0^1 \frac{1}{1+\left(\frac{p^*(\bar{x})}{p_0(\bar{x})}\right)^n} d\bar{x} \tag{11}$$

Since the RHS of Eq.(11) is independent of x, we see that $p^*(x)$=p^* is a constant. Because of the difficulty in evaluating the integral in Eq.(11) for a general function $p_0(\bar{x})$, numerical integration is required in order to obtain an approximate value

for p^*. In order to illustrate the process we choose a tractable function $p_0(\bar{x}) = 1+\bar{x}$, together with $n = 3$ and $\mu = 0.2$, in which case Eq.(11) gives $p^* = 2.9876$.

CASE 2: EXPONENTIAL WEIGHTING

At steady state, Eqs.(9),(10) give

$$\mu^2 p^*(x) \;=\; \int_0^1 e^{-|x-\bar{x}|} H(p^*(\bar{x}))\, d\bar{x} \tag{12}$$

which may be written in the form:

$$\mu^2 p^*(x) \;=\; e^{-x}\int_0^x e^{\bar{x}} H(p^*(\bar{x}))\, d\bar{x} \;+\; e^{x}\int_x^1 e^{-\bar{x}} H(p^*(\bar{x}))\, d\bar{x} \tag{13}$$

Differentiating Eq.(13) twice [14] we obtain the equivalent second order ODE for the steady state solution p^*=$p^*(x)$:

$$\frac{d^2p^*}{dx^2} - p^* \;=\; -\frac{2}{\mu^2} H(p^*) \tag{14}$$

where the boundary conditions are given by

$$p^*(0) = \frac{1}{\mu^2}\int_0^1 e^{-\bar{x}} H(p^*(\bar{x}))\, d\bar{x} \tag{15}$$

$$p^*(1) = \frac{1}{e\mu^2}\int_0^1 e^{\bar{x}} H(p^*(\bar{x}))\, d\bar{x}. \tag{16}$$

For the choice of $H(p(\bar{x}))$ given by Eq.(8), Eq.(14) becomes

$$\frac{d^2p^*}{dx^2} - \gamma\, p^* \;=\; 1-\gamma \tag{17}$$

where

$$\gamma \;=\; 1+\frac{2}{\mu^2} \;>\; 0 \tag{18}$$

Thus

$$p^*(x) \;=\; c_1 \sinh\sqrt{\gamma}x \;+\; c_2 \cosh\sqrt{\gamma}x \;+\; \frac{2}{\mu^2\gamma} \tag{19}$$

where c_1 and c_2 are determined by substituting Eq.(19) into (15) and (16):

$$c_1 = \left(1-e^{\sqrt{\gamma}}\right) K \tag{20}$$

$$c_2 = \left(1+e^{\sqrt{\gamma}}\right) K \tag{21}$$

where

$$K = \frac{1-\sqrt{\gamma}-\left(1+\sqrt{\gamma}\right)e^{\sqrt{\gamma}}}{\gamma\left[\left(\mu^2\sqrt{\gamma}+\mu^2+1\right)e^{2\sqrt{\gamma}}+\mu^2\sqrt{\gamma}-\mu^2-1\right]} \tag{22}$$

For example, in the case that $\mu = 0.2$, we obtain

$$p^*(x) \;=\; 0.12040\sinh\sqrt{51}x \;-\; 0.12059\cosh\sqrt{51}x \;+\; \frac{50}{51} \tag{23}$$

See Fig.1.

STABILITY OF STEADY STATE

To study the stability of the steady state solution $(m^*(x), p^*(x))$, we set $p(x,t)$=$p^*(x)$+$\eta(x,t)$ and $m(x,t)$=$m^*(x)$+$\xi(x,t)$ and linearize the resulting equations in $\eta(x,t)$ and $\xi(x,t)$.

CASE 1: UNIFORM WEIGHTING

Here the steady state solution p^* is constant in x. Eqs.(6),(7) give

$$\dot{\xi} = -\mu\xi - \int_0^1 K_1(\bar{x})\,\eta_d(\bar{x})\, d\bar{x} \tag{24}$$

$$\dot{\eta} = \xi - \mu\eta \tag{25}$$

where

$$K_1(\bar{x}) = \frac{n\beta}{(1+\beta)^2\, p^*}\,, \quad \text{where } \beta = \beta(\bar{x}) = \left(\frac{p^*}{p_0(\bar{x})}\right)^n. \tag{26}$$

To study the stability of the origin we assume solutions of the form

$$\xi(x,t) \;=\; A(x)\, e^{\lambda t}\,, \qquad \eta(x,t) \;=\; B(x)\, e^{\lambda t} \tag{27}$$

and substitute them into Eqs.(24) and (25). Solving for $B(x)$ yields the following integral equation

$$rB(x) = \int_0^1 K_1(\bar{x})\, B(\bar{x})\, d\bar{x} \tag{28}$$

where

$$r = -e^{\lambda T}(\lambda+\mu)^2 \tag{29}$$

To solve Eq.(28), we note that the RHS is independent of x, which tells us that $B(x)$=B is constant. Eliminating B from Eq.(28), we obtain

$$r = \int_0^1 K_1(\bar{x})\, d\bar{x} \tag{30}$$

Here $K_1(\bar{x})$ is given by Eq.(26), so that r is known. We are left with the problem of determining λ from Eq.(29) when r is known. This problem is common to both the present case

of uniform weighting as well as to the case of exponential weighting. To avoid repeating the treatment, we handle this problem in the Appendix. There are two important situations: (i) when $T = 0$, in which case λ determines the stability of the system with no delay, and (ii) when $T = T_{cr}$, where the delay T_{cr} corresponds to pure imaginary λ and corresponds to a change in stability.

(i) When $T = 0$, Eq.(57) in the Appendix gives

$$\lambda = -\mu \pm \sqrt{-\int_0^1 K_1(\bar{x})\, d\bar{x}} \tag{31}$$

which shows that the system with no delay is stable since $K_1(\bar{x})>0$ from (26).

(ii) When $T = T_{cr}$, Eqs.(62),(61) in the Appendix give

$$T_{cr} = \frac{1}{\omega} \arctan\left(\frac{2\omega\mu}{\omega^2 - \mu^2}\right) \tag{32}$$

$$\omega = \sqrt{-\mu^2 + \int_0^1 K_1(\bar{x})\, d\bar{x}} \tag{33}$$

We continue the example given in the previous section, namely, $p_0(\bar{x}) = 1 + \bar{x}$, $n = 3$ and $\mu = 0.2$, which yielded the steady state $p^* = 2.9876$. By substituting Eq.(26) into (33) we obtain $\omega = 0.24977$ which we substitute into (32) to obtain the critical delay $T_{cr} = 5.40638$, where the steady state becomes unstable.

CASE 2: EXPONENTIAL WEIGHTING

In this case the steady state $p^*(x)$ satisfies the ODE (14). To study its stability, we linearize Eqs.(9) and (10), which give

$$\xi_t = -\mu\xi - \int_0^1 e^{-|x-\bar{x}|}\, \eta_d(\bar{x})\, d\bar{x} \tag{34}$$

$$\eta_t = \xi - \mu\eta \tag{35}$$

If $\xi(x,t)$=$\phi(x)e^{\lambda t}$ and $\eta(x,t)$=$\psi(x)e^{\lambda t}$ then Eqs.(34) and (35) become

$$-e^{\lambda T}(\lambda+\mu)\phi(x) = \int_0^1 e^{-|x-\bar{x}|}\, \psi(\bar{x})\, d\bar{x} \tag{36}$$

$$(\lambda+\mu)\psi(x) = \phi(x) \tag{37}$$

Substituting Eq.(37) into (36) gives

$$r\psi(x) = \int_0^1 e^{-|x-\bar{x}|}\, \psi(\bar{x})\, d\bar{x} \tag{38}$$

where r is given by Eq.(54). Next we transform the integral equation (38) to the following equivalent second order ODE [14]

$$\frac{d^2\psi}{dx^2} + \left(\frac{2}{r} - 1\right)\psi = 0 \tag{39}$$

which will have solutions of the form

$$\psi(x) = c_1 \sin(\rho x) + c_2 \cos(\rho x) \tag{40}$$

where c_1 and c_2 are constants and $\rho = \sqrt{\frac{2}{r} - 1}$. The endpoint boundary conditions of the second order ODE (39) are obtained from Eq.(38) as follows

$$\psi(0) = \frac{\rho^2+1}{2} \int_0^1 e^{-\bar{x}}\psi(\bar{x})\, d\bar{x} \tag{41}$$

$$\psi(1) = \frac{\rho^2+1}{2e} \int_0^1 e^{\bar{x}}\psi(\bar{x})\, d\bar{x}. \tag{42}$$

Substituting Eq.(40) into (41) and (42) gives a system of equations on the constants c_1 and c_2 which yields the following condition on ρ for nontrivial solutions

$$\begin{vmatrix} \rho\sin\rho - \cos\rho - e & -\sin\rho - \rho\cos\rho + e\rho \\ e\rho\sin\rho - e\cos\rho - 1 & -e\sin\rho - e\rho\cos\rho + \rho \end{vmatrix} = 0 \tag{43}$$

or equivalently

$$\left(\rho^2 - 1\right)\sin\rho - 2\rho\cos\rho = 0 \tag{44}$$

Eq.(44) has an infinite number of roots, the first three of which are $\rho = 1.30654, 3.67319, 6.58462, \cdots$ which give the following corresponding values for $r = 2/(1+\rho^2) = 0.73881, 0.13800, 0.04509, \cdots$. Now that we know r, we may use the results in the Appendix to determine stability of the steady state. Returning to eq.(40), we find

$$c_2 = \rho c_1 \tag{45}$$

See Fig.2.

(i) When $T = 0$, Eq.(57) in the Appendix gives $\lambda = -\mu \pm \sqrt{-r}$ which, in view of the fact that all the values of r are positive, shows that the system with no delay is stable.

(ii) When $T = T_{cr}$, Eqs.(61) and (62) in the Appendix give expressions for ω and T_{cr}. Since we are interested in the smallest value for T_{cr}, we take $r = 0.73881$, which gives, for $\mu = 0.2$, the values $\omega = 0.83595$ and $T_{cr} = 0.56184$.

In order to check this result, we replace the continuous variables $\xi(x,t)$ and $\eta(x,t)$ in Eqs.(34),(35) by a discrete set of N+1 variables $\xi_i(t)$ and $\eta_i(t)$. This corresponds to a model of N+1 coupled gene units, and replaces the integral in Eq.(34) by a sum of N+1 terms. As we now demonstrate, analysis of this system shows that $T_{cr} \to 0.56184$ as N goes to infinity, for $\mu = 0.2$,

in agreement with the foregoing analysis. We start by discretizing the continuous system, Eqs.(34),(35), into an (2N+2)-dimensional system given by

$$\dot{\xi}_i = -\mu\xi_i - \frac{1}{N+1}\sum_{j=0}^{N} e^{-|i-j|/N}\eta_j(t-T) \tag{46}$$

$$\dot{\eta}_i = \xi_i - \mu\eta_i \tag{47}$$

where $i = 0, 1, \ldots, N$. Next we assume solutions of the form

$$\xi_i = \phi_i e^{\lambda t} \tag{48}$$

$$\eta_i = \psi_i e^{\lambda t} \tag{49}$$

and substitute them into (46),(47) to obtain

$$-e^{\lambda T}(\lambda+\mu)\phi_i = \frac{1}{N+1}\sum_{j=0}^{N} e^{-|i-j|/N}\psi_j \tag{50}$$

$$(\lambda+\mu)\psi_i = \phi_i \tag{51}$$

eliminating ϕ_i we obtain

$$c\psi_i = \sum_{j=0}^{N} e^{-|i-j|/N}\psi_j \tag{52}$$

where $c = (N+1)r$ and r is given by (54). For nontrivial solutions, the system (52) of N+1 algebraic equations, must satisfy $\det(K - cI) = 0$ where K is the (N+1)×(N+1) matrix K=[K_{ij}]=[exp($-|i-j|/N$)] and c is its associated eigenvalue. Since K is a symmetric matrix, all of its eigenvalues are real and thus c is a real number. Numerical evaluation of these eigenvalues c shows that they are all positive. The stability results for the steady state are summarized as follows:

(i) When $T = 0$, we see from Eq.(57) in the Appendix with $r = c/(N+1)$ that the steady state in the system with no delay is stable.

(ii) When $T = T_{cr}$, we choose the smallest value of c for a given truncation size N, and use Eqs.(61) and (62) in the Appendix to obtain values for ω and T_{cr} where we take $r = c/(N+1)$. Table 1 shows results for $\mu = 0.2$ for various values of N.

Table 1. NUMERICAL RESULTS FOR $\mu = 0.2$

N	c	ω	T_{cr}
1	1.3678	0.8024	0.6089
2	2.0612	0.8044	0.6059
3	2.7844	0.8100	0.5977
5	4.2494	0.8175	0.5870
7	5.7215	0.8216	0.5813
10	7.9338	0.8253	0.5761
15	11.6246	0.8285	0.5718
30	22.7034	0.8320	0.5671
50	37.4783	0.8336	0.5649
100	74.4173	0.8348	0.5634
200	148.2960	0.8353	0.5627
300	222.1740	0.8355	0.5623

CONCLUSIONS

In this paper we investigated the steady state solutions of a continuous gene regulatory network model. The model takes the form of an ordinary differential equation coupled to a delay differential-integral equation having time, t, and gene location, x, as independent variables. The study was divided into two cases: uniform weighting and exponential weighting. For the uniform weighting case we showed that the steady state is not only constant in time but in space as well. This allowed us to solve the associated eigenvalue problem and prove that the system is stable when there is no delay. Subsequently, we showed that the system becomes unstable for a critical delay and found closed form expressions for the critical delay and associated frequency. For the exponential weighting case, we found that the steady state solution depends on gene location. This was accomplished by transforming the steady state integral equation into a second order differential equation. By solving the differential equation we found a closed form expression for the x-dependent steady state. Stability analysis then revealed that the nondelayed system is stable and expressions for the critical delay and associated frequency were found. We confirmed our results by means of a numerical approximation where the continuous system was discretized, which resulted in an N-dimensional system with delay. Numerical evaluations for different N were performed and good agreement was found with the continuous counterpart as N became large.

The model assumes that the rate at which mRNA is produced at a given site x depends on the concentration of protein at all sites $0 \le x \le 1$. Analysis of the model shows that the presence of delay produces an instability in the steady state leading to periodic behavior. The present model differs from previous models [33], [34] in that the steady state here can have spatial dependence, cf.eqs.(19),(23). In the real cell, the number of DNA sites and ribosomes are large but finite, whereas our system models them as being continuous, i.e., infinite in number. However we checked our continuum model against a finite N-dimensional approximation and saw that the two converged as $N \to \infty$.

The present work is a first step in studying the periodic response of this model of a gene regulatory network. Current work

involves extending this analysis to include nonlinear terms so that the amplitude of oscillation can be predicted as a function of delay.

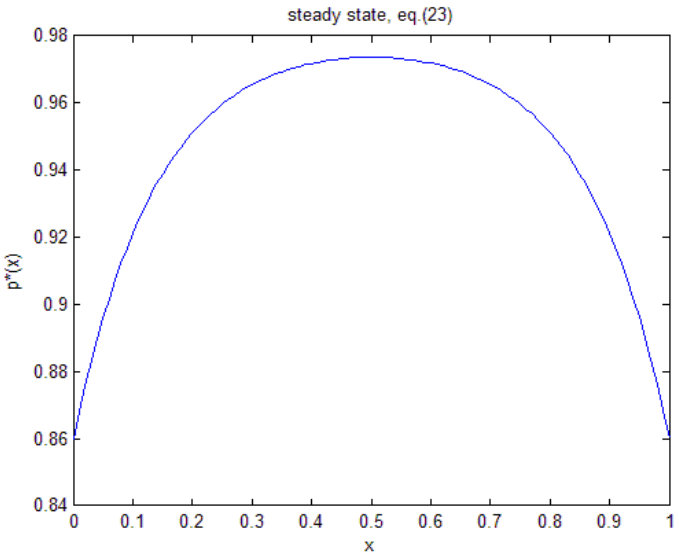

Figure 1. Steady state for CASE 2, $p^*(x)$ vs. x, eq.(23).

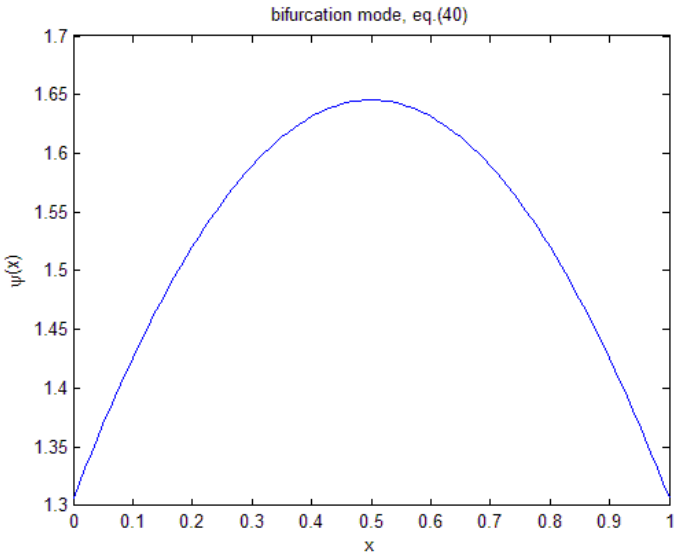

Figure 2. Bifurcation mode shape from linearized stability analysis, $\psi(x)$ vs. x, eqs.(40),(45) with c_1=1.

APPENDIX

In Eqs.(28) and (38) we have the following eigenvalue problem

$$r f(x) = \int_0^1 K(x,\bar{x}) f(\bar{x}) d\bar{x} \tag{53}$$

where $K(x,\bar{x})$ is a *symmetric* integral kernel, $f(x)$ is the eigenfunction, and r is the associated eigenvalue given by

$$r = -e^{\lambda T}(\lambda+\mu)^2 \tag{54}$$

Note that r is real since the RHS of (53) contains a symmetric kernel and thus is a self-adjoint operator of the form

$$L(\cdot) = \int_0^1 K(x,\bar{x})(\cdot)\, d\bar{x} \tag{55}$$

which has real eigenvalues.

Now given r we wish to determine λ in two special situations: (i) when $T = 0$, and (ii) when $T = T_{cr}$ and λ is pure imaginary, corresponding to a change in stability.

(i) When $T = 0$, Eq.(54) becomes

$$r = -(\lambda+\mu)^2 \tag{56}$$

and gives

$$\lambda = -\mu \pm \sqrt{-r} \tag{57}$$

If $r > 0$ then the $\mathrm{Re}(\lambda) = -\mu < 0$ (for positive μ), and we have stability of the system with no delay.

(ii) When $T = T_{cr}$ and $\lambda = i\omega$, Eq.(54) becomes

$$r = -e^{i\omega T_{cr}}(i\omega+\mu)^2 \tag{58}$$

which gives the two real equations

$$r = 2\mu\omega \sin\omega T_{cr} + (\omega^2-\mu^2)\cos\omega T_{cr} \tag{59}$$

$$0 = (\omega^2-\mu^2)\sin\omega T_{cr} - 2\mu\omega\cos\omega T_{cr} \tag{60}$$

Solving Eqs.(59),(60) for $\sin\omega T_{cr}$ and $\cos\omega T_{cr}$, and using the identity $\sin^2+\cos^2$=1 we obtain

$$\omega = \sqrt{r-\mu^2} \tag{61}$$

Dividing the expressions for $\sin\omega T_{cr}$ and $\cos\omega T_{cr}$ and solving for T_{cr} we also obtain

$$T_{cr} = \frac{1}{\omega}\arctan\left(\frac{2\mu\omega}{\omega^2-\mu^2}\right) \tag{62}$$

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