

## Heather Hardway - Research Statement

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### Introduction

I am currently investigating systems of nonlinear reaction-diffusion equations related to the study of gene networks in early fly development. Through a combination of numeric and analytic techniques, I have focused on systems capable of producing robust output under various perturbations, the premise being that a key characteristic of these biological systems is their ability to produce viable, proportioned offspring under extreme fluctuations in their environment and genetic material. If a model has any chance to accurately mirror any part of this biological system, it must possess similar notions of robustness.

### History

The history of developmental biology is closely linked with the fruit fly, *Drosophila melanogaster*. Over the past century, the *Drosophila* gene network has become one of the most studied and well understood. This is especially true of the genes involved in the anterior-posterior and dorsal-ventral axis specification, the first step in spatially organizing the developing embryo. Critical to this understanding was the concept of a morphogen, which acts to spatially distinguish cell types by forming a concentration gradient that controls the expression of other genes depending on the level of morphogen at that point.

Due to the unusual development of *Drosophila*, morphogens play the primary role in which the embryo spatially organizes, critical to the development and location of all structures and organs. While models were used before to elucidate the complex network of interactions between genes [12], the paper of Houchmandzadeh et al in 2002 [6] introduced a new feature of the system: noise filtering. The morphogen Hunchback was shown to be highly correlated with respect to embryo length and forms a sharp border at precisely the midpoint of the embryo, despite receiving noisy positional information from its upstream regulator, Bicoid. A flood of papers followed, including both mathematical and experimental papers, with various conclusions as to the nature of the Bicoid-Hunchback system [1],[7],[2],[9]. While a consensus has not been agreed upon, my research has focused on finding and analyzing mathematical models for gene networks capable of such robust output.

### Results

#### Numeric Results

As part of a VIGRE research group, we began exploring systems of reaction-diffusion equations that could produce data similar to the results of Houch-

mandzadeh [6]. Using the basic model developed in [12], we used the following equations, where  $p_1, p_2$  represent Bicoid and Hunchback respectively:

$$\begin{aligned}
p_1(x) &= e^{-kx} \\
\frac{\partial p_2}{\partial t} &= D_2 \frac{\partial^2 p_2}{\partial x^2} - \lambda_2 p_2 + R_2 g\left(\sum_{i=1}^n c_{i2} p_i + \rho_2\right) \\
&\vdots \\
\frac{\partial p_n}{\partial t} &= D_n \frac{\partial^2 p_n}{\partial x^2} - \lambda_n p_n + R_n g\left(\sum_{i=1}^n c_{in} p_i + \rho_n\right) \tag{1} \\
0 &\leq x \leq 2, t \geq 0 \\
p_i(x, 0) &= 0, i = 2, 3, \dots, n \\
\frac{\partial p_i}{\partial x}(0, t) &= \frac{\partial p_i}{\partial x}(2, t) = 0, i = 2, 3, \dots, n
\end{aligned}$$

$g$  is a bounded sigmoidal function,  $D_i, R_i, \lambda_i, \rho_i, c_{ij}, i = 2, 3, \dots, n, j = 1, 2, \dots, n, k$  are parameters. One of our undergraduate participants, Tim Burke, developed a parameter search based on a genetic algorithm and steepest descent method to find parameter sets that produced  $p_2$  gradients robust to changes in  $p_1$  (for the details of how we defined robust output and the results, see [5]).

The results were that in all robust systems, there were the following restrictions on the gene network:

1. The smallest robust network of this type consisted of three proteins,  $n = 3$
2. The interaction between  $p_2$  and  $p_3$  was opposite, meaning if  $p_2$  activated  $p_3$ ,  $p_3$  must inhibit  $p_2$  and vice versa; in terms of the parameters, this is equivalent to  $c_{23}c_{32} < 0$ ;
3.  $p_3$  must diffuse much faster than  $p_2$ , i.e.  $D_2 \ll D_3$
4.  $p_1$  can be eliminated from the system and the same robust properties persist if appropriate nonzero initial conditions are used for  $p_2, p_3$

While the conditions 2) and 3) are indicative of a Turing instability, this was not the case in any system. Many systems exhibited bistability, however there was not a consistent phase-portrait for the  $p_2 - p_3$  ODE system. Future work will include a detailed analysis of the robust systems found.

Extending the model to include a fourth protein,  $p_4$ , all previous restrictions found were still true of all robust systems in the larger network. What, if any, benefit is there having additional proteins in the network? To answer this question, I began looking at ways in which  $p_3, p_4$  could be combined into a ‘‘lumped’’ protein and still recreate the same robust dynamics. In addition, I give ways in which a redundant network can be created, which could be an obvious benefit of having a larger network. While there is still much work to be done, the results will shortly be available in a preprint [4].

Last, I found that the robust three-protein systems had reached (or were close to reaching) a steady state profile. With this information, nondimensionalizing the system reduced the number of parameters in the equations. However, even after this reduction, many of the parameters were highly correlated (linearly) with each other, with so many as to reduce the number of free parameters to two. After these reductions were introduced, I explored the regions where the two free parameters produced robust systems. There exist large open sets that satisfy the criteria. This is sharply contrasted with the near impossibility of randomly picking parameters with this property. Again, much is left to be explored, but in addition to reducing the number of free parameters, this method reduced the eight different network topologies to one essential structure. Again, these results will be available soon [4].

### Analytic Results

The results of the parameter search implied that the simplest example of a robust gene network must consist of two proteins dependent on space and time ( $p_2$  and  $p_3$  above) with nonzero initial conditions (in the case when  $p_1$  was eliminated). Therefore, I focused on analyzing systems of the following type:

$$\begin{aligned}
\frac{\partial a}{\partial t} &= D_a \frac{\partial^2 a}{\partial x^2} - \lambda_a a + g(c_{aa}a + c_{ba}b + \rho_a) \\
\frac{\partial b}{\partial t} &= D_b \frac{\partial^2 b}{\partial x^2} - \lambda_b b + g(c_{ab}a + c_{bb}b + \rho_b) \\
0 &\leq x \leq 1, t \geq 0 \\
\frac{\partial a}{\partial x} \Big|_{x=0,1} &= \frac{\partial b}{\partial x} \Big|_{x=0,1} = 0
\end{aligned} \tag{2}$$

Moreover, numeric results also indicated that for a system to be robust at a particular time, the system was at, or near, steady-state. Therefore, I also focused on the long-term dynamics of these systems, addressing the following:

1. Do solutions approach a steady-state?
2. How many steady-state solutions exist, and what are their stabilities and qualitative features (constant, symmetric, multiple peaked, etc.)?

To address these questions, I first considered a simplification of the system. Consider the symmetric case of parameters, when  $c_{aa} = c_{ab}, c_{ba} = c_{bb}, \rho_a = \rho_b, \lambda_a = \lambda_b$ . It can be easily shown that the spatial average of  $(a - b)$  goes to zero as  $t \rightarrow \infty$ . Using  $D_a \ll D_b$ , consider a second reduction to the “shadow system”, where  $D_b \rightarrow \infty$  [11]. Now the system is reduced to a single integro-differential equation:

$$a_t = D_a a_{xx} - \lambda_a a + g(a - \bar{a}) \tag{3}$$

where  $g(z) = \arctan(z)/\pi$ ,  $\bar{a} := \int_0^1 a(x)dx$ ,  $a_x|_{x=0,1} = 0$ .

**Theorem 1.** *For equation (3):*

1. *A Lyapunov functional exists, indicating solutions approach steady-state as  $t \rightarrow \infty$*
2. *At steady state,*
  - (a)  *$a = 0$  is always a solution, but undergoes a pitchfork bifurcation as  $D_a$  is decreased and two nonhomogeneous steady state solutions emerge; subsequent pitchfork bifurcations occur as  $D_a$  is further decreased*
  - (b)  *$\bar{a} = 0$  and  $a$  is even or odd*
  - (c) *Once bifurcation of the zero solution occurs, the monotone solutions become stable and remain so as  $D_a$  decreased*

In this case, the understanding of the steady-state dynamic is nearly complete. Using asymptotic methods, I give an explicit approximation for solutions near the first bifurcation point and estimations of the error for all time. Replacing  $g$  with a heaviside or piecewise-linear function gives explicit solutions to the steady state problem.

However, this reduced system does not match the dynamics for the original system for all choices of  $D_a, D_b$ .

**Theorem 2.** *Let  $c_{aa} = c_{ab}, c_{ba} = c_{bb}, \rho_a = \rho_b, \lambda_a = \lambda_b$ . There exists regions for  $D_a, D_b$  so that for the linearization of (2) around the zero solution, only the  $n$ -th eigenvalue, corresponding to the eigenfunction  $\phi_n(x) = \cos(n\pi x)$ , is positive.*

Thus, near this bifurcation point, solutions are approximated by  $\phi_n(x)$  and the system has a globally stable  $n$ -striped solution. However, these parameter regions get extremely small as  $n$  increases, so the more realistic parameter choices still result in the single-striped (monotone) solution being stable.

For very small  $D_a$ , solutions have a sharp transition layer and approach step-functions (in  $L^\infty$  sense). The point of this transition can be made at any point in space, dependent upon parameters. In particular, it can be made at the midpoint. Therefore, with these choices, the single-stripe solution can be made extremely sharp, with the transition layer at the midpoint of space, and it is globally stable. Numerically, even when a spatially inhomogenous function is added to the interaction function, such as the exponential used in the parameter search, these properties are preserved.

While my focus has been on simple networks, I have shown they can mimic many of the robust properties of the Bicoid-Hunchback dynamic in *Drosophila*. I hope to continue exploring these systems, as well as some unexpected nonlinear phenomena described below.

## Future Projects

Out of this parameter search, we discovered many different systems that were capable of robustly defining the midpoint of an embryo. Unexpectedly, we uncovered three non-linear phenomena that has either not been characterized or well studied.

1. In the first type, we found systems that begin developing as traveling fronts, but once the front edge nears the midpoint, the front decreases speed until it eventually stops. This phenomena was studied by Mori et al [10] and given the name “wave-pinning”, but under two restrictive conditions: conservation of mass and a specific form for the interaction. At least numerically, we have evidence that neither is necessary. In our example, mass is not conserved, but is bounded. We speculate this may be enough in general, but further research is needed to determine necessary conditions needed on the interaction terms.
2. In the second type, I found systems in which the stationary front could be destabilized, creating a time-periodic back and forth movement of the boundary. This dynamic was described by Y.-X. Li as “tango waves” [8], but under the condition of a spatial inhomogeneity appearing in the equations. As found in our paper, the spatial inhomogeneity was not necessary, but rather such dynamics are possible in a simple two protein activator-inhibitor system. I am currently collaborating with Y.-X. Li on understanding the dynamics under the simplification when the nonlinear interaction is a step function.
3. In the last example, the local systems posses a stable limit cycle (in time). In the full PDE system, the spatially constant time periodic solution remains stable to very small perturbations, but when large spatially inhomogeneous initial conditions are used, the transient dynamics mirror the time periodic behavior, but eventually solutions stabilize to a spatially nonconstant, time independent front. The new technology developed for real-time gradient visualization in *Drosophila* has already revealed complex behavior of the Bicoid gradient [3], there remains the possibility such behavior is occurring in this system. In addition, I would be interested to know if such phenomena has been documented in ecological systems and work on adapting the model.

In the most general, I am interested in nonlinear dynamics and pattern formation and the applications to biological problems. This includes models for much different systems, ecological and neural networks, as well as different kinds of models, discrete, hybrid, advection-reaction-diffusion, and integro-differential equations. I would also like to continue joint collaborations with biologists. While I worked closely with a biology graduate student on [5], we were not able to come up with reasonable experiments to test the validity of our claims. I hope to be able to incorporate results of experimental research in modifying models in the future and think that this is a necessary step if there is any hope to elucidate

the nature of the biological system through modeling. I would also be thrilled to learn experimental techniques as well, and learn how to conduct and design experiments. My long-term goals are to secure myself in the mathematical biology field by expanding my skills in both mathematics and biology.

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