Simulating Mouse Limb Development With A Reaction Diffusion System

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The development of complex patterns over time from seemingly homogenous initial conditions has long been a topic of interest in biology. In his seminal 1952 paper, Alan Turing found that instability in reaction diffusion equations can give rise to pattern formation. With slight variations, reaction diffusion equations have successfully reproduced patterns such as leopard spots or cheetah stripes similar to those found in nature [1]. While the complete mechanism behind digit patterning in tetrapod limbs is unknown, reaction diffusion systems have been able to reproduce many key features of digit patterning [2], such as the observed proximodistal progression from one bone (humerus) to two bones (radius/ulna) to five digits. Because computer simulations of limb development run much faster than their *in vitro* counterparts, developing a limb model *in silico* provides an efficient preliminary way to explore the developing limb which helps determine which experiments will be fruitful *in vitro*. This summer, we were able to use an existing model of limb development with modified parameters to reproduce the standard 1-2-5 (humerus-radius/ulna-fingers) progression of bones in mammalian limbs. This model can now be used to test the importance of apical ectodermal ridge (AER) shape in determining the number of digits produced, as well as removal experiments in which the AER is removed from the limb bud before development is complete.

We followed in the footsteps of [2] and attempted to simulate the development of a simplified system obtained via adiabatic elimination of the variables representing fibronectin density and the differentiated cell types which produce TGF-β. We attempted to compute the solution to this simplified system on a moving rectangular domain; however, upon performing a linear stability analysis for the system, we determined that the system and parameters provided in [2] would not lead to the desired patterning. Thus we decided to model the system

$$\begin{aligned} \partial c_a/\partial t &= D_a \Delta c_a + U(c_a) - k_a c_a c_i \\ \partial c_i/\partial t &= D_i \Delta c_i + V(c_a) - k_a c_a c_i \end{aligned} \tag{1}$$

in [3], where c_a is the concentration of the activator morphogen (TGF-β) and c_i is the concentration of the inhibitor, fibroblast growth factor (FGF). The terms $D_a \Delta_a c_a$ (resp. $D_i \Delta_i c_i$) represent the diffusion of the activator (resp. inhibitor) throughout the limb. The functions U (resp. V) control the production rates of c_a (resp c_i), and $k_a c_a c_i$ represents the reaction rate of the activator and inhibitor. The functions U and V depend crucially on a reaction kinetic parameter γ related to feedback strength of the activator morphogen. This system of equations is obtained from the full system of 8 equations in [2] by assuming that cell identity is established before cellular rearrangement (the morphostatic mechanism), rather than simultaneously (the morphodynamic mechanism). Whereas Zhu et. al. use a finite element method to compute the solutions to the system on moving domains with complex shapes, we used a 4th order Runge-Kutta finite difference method on a moving rectangular domain with a parabolic right-hand boundary. The moving domain was achieved by defining a movable active zone of specified length in which we computed solutions to system (1). Once

a grid point was no longer part of the active zone, it became a member of a "frozen zone" in which the computed values of the function remained constant throughout the rest of the simulation. Many of the biological parameters in the system have presumably been chosen particularly over time by evolution. Where these parameters were not known, it was necessary to work backwards until a suitable set of parameters was found that produced the desired limb configuration.

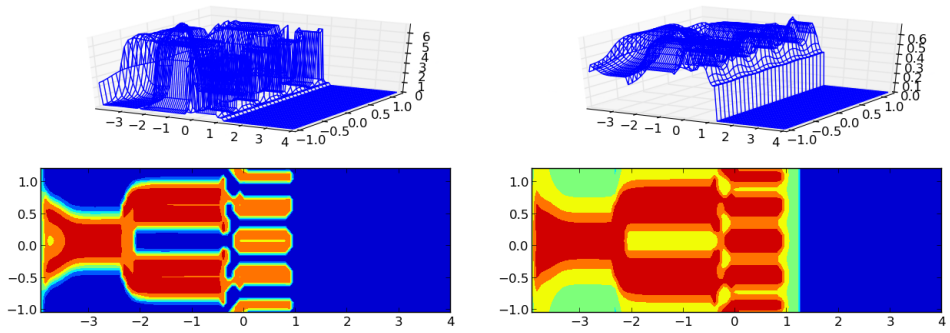

Figure 1: Simulation of system (1) on a moving rectangular domain with parabolic right-hand boundary representing the portion of the limb bud equidistant from the dorsal and ventral surfaces

Another fascinating aspect of limb development is its robustness with regard to deletions or mutations in some of the genes which express morphogens crucial for pattern formation. This is due in part to genetic redundancy from gene duplication and the ability of heat shock protein 90 (Hsp90) to correctly fold large proteins even if amino acid substitutions have occurred. However, there is also robustness built into the mechanism of limb development itself, such that if one signalling pathway is prematurely interrupted, the limb still develops normally. Therefore, if a reaction diffusion model is to truly reproduce limb development, it must be subject to the same robustness. To this end, we carried out a sensitivity analysis to determine how simultaneously changing the domain width and γ affected the number of digits produced by our code. These two parameters in particular were chosen because after some experimentation, we determined that they have the greatest impact on the number of digits produced. While it would be desirable to perform a linear stability analysis, the parabolic shape of the right side of our domain makes solutions to the linearized form of system (1) difficult to compute. As a curved right-hand boundary was crucial for pattern formation, it was not reasonable to approximate our domain by a rectangle for linear stability analysis.

As expected, increasing the width of the domain increased the number of digits produced, as did increasing γ. It was found that bifurcations (with respect to digit number) could be achieved with changes on the order of 1 in the width of the domain, and changes on the order of 1000 in γ. Furthermore, as γ increases, the system appears to become more sensitive to changes in the width of the domain.

The next step in the process is to compare the observed sensitivity to domain width with findings *in vitro* and determine if the reaction kinetic parameters chosen align well with real biological time scales. The primary obstacle to progress in computer simulations is the

limitation of current computational power. Even with efficient finite element algorithms to handle complicated moving domains, simulations are still done in 2 spatial dimensions [3]. It may be worthwhile to focus on certain geometric or topological invariants of the solution space to a system in 3 dimensions (if these can be determined without explicitly computing the solutions). For example, given a limb, one could imagine that the number of bones in the limb could be computed by determining the homology of the complement of the bones in the limb. Hence it may be faster to determine the homology of the complement of the set of values of the solution to a system above a certain threshold in a given domain. Finally, continued biological experiments which help determine the relative importance of certain morphogens will also help refine approximations to the true mechanism so that improvements can be made without a significant increase in computational time.

References

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