

# Developmental Evolution: Getting Robust About Robustness

## Dispatch

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**In the context of development, a process is robust if it can proceed normally despite the enormous capacity for perturbation inherent in all biological systems. A new mode of theoretical modeling of genetic networks holds great promise for increasing our understanding of both the quantitative mechanisms of robustness and its evolutionary impact.**

Theoretical quantitative genetics, one of the most successful of all fields of biological enquiry, seems to have run into somewhat of a dead end when it comes to the integration of development and evolution. Robustness and the dynamics of development are two particularly fundamental problems that have proven difficult to approach with classical methods [1]. There are signs though that fresh ideas [2,3] and fresh empirical approaches [4] are opening up a productive new research program that may see tighter interaction between mathematical and experimental biology.

The developmental problem essentially boils down to the fact that static statistical models cannot capture the full complexity of dynamic interactions between genes and the environment. Quantitative geneticists like to study the relationship between genetic polymorphism and phenotypic variation. Genotypes are constants, and phenotypes are captured at a single point in time, usually well after all of the interesting developmental processes have concluded. The null hypothesis is no association, and departures are fit initially as additive contributions and then, if necessary, dominance and interaction terms are introduced. For those not accustomed to quantitative genetic reasoning — namely, most molecular and cellular biologists — the arcane algebra can seem distant and irrelevant. Development, after all, is assumed to be complex and dominated by interactions due to phenomena such as redundancy, feedback and synergism. How do we reconcile these world views?

One place where it may be productive to integrate them is in terms of our understanding of robustness, or developmental and physiological stability. Robustness is gaining increasing attention as the flip side of diversification, and as a process at the heart of disease processes from psychological disturbance to diabetes to aging in general. The incredible observation is that, despite the fact that it takes 20,000 genes to make a complex multicellular organism, and that these have to work together in environments as diverse as the jungles of Mauritius or suburban

Detroit, development works. Arms and legs are the same length in each individual, livers and hearts are the appropriate size, and the brain wires itself correctly, all in the face of considerable potential for perturbation.

Theoreticians have approached robustness with abstract models of gene networks that very often highlight the inherent potential for the creation of order out of the chaos of apparently random networks of interactions. Stuart Kauffman's [5] work on Boolean networks is highly cited in this regard, but Andreas Wagner [6] has also produced an intriguing model that results in the evolution of increasing developmental stability. His notion is that, if we require the genes to produce a phenotype that remains stable over time, then stabilizing selection will ensure that increasingly stable networks of interactions evolve. Salazar-Ciudad, Newman and Solé [7,8] have also published a promising class of development-inspired models that contrast the potential inherent in particular types of network to produce stable patterns, and demonstrated the capacity of such networks to evolve under selection. The problem with these models is that they explain perhaps too much without explaining anything specific.

A general theory of robustness is nice, but it does not tell us how segmental stripes actually stabilize in insect germbands, or how lateral inhibition really works. A couple of years ago, Garrett Odell, George von Dassow and colleagues published a model that addressed the first of these questions [2], and in new work published in this issue of *Current Biology* [3], they turn their attention to the second. Their general strategy is to model the structure of well-characterized developmental genetic networks as a system of partial differential equations which, given some reasonable starting parameters, are asked to converge on a specified pattern of gene expression in a cluster of cells.

The equations are implemented in a relatively user-friendly Java-based 'genetic network construction software' package named *Ingeneue*, which Odell and colleagues have made available at <http://www.ingeneue.org/>. The model details are well explained and justified in the online supplement. As biochemists and developmental biologists really have no idea of what precise values the parameters representing the half-life of mRNAs or proteins, protein-DNA binding constants or levels of cooperativity behind sigmoidal responses take, the idea is to ask first whether any parameter sets can be found that lead to a desired outcome, and then to explore how sensitive the model networks are to parameter variation. As the authors put it, they are simply doing *in silico* what biochemists try to reconstitute *in vitro*, systematically altering one parameter at a time in an effort to understand the properties of complex real-world systems.

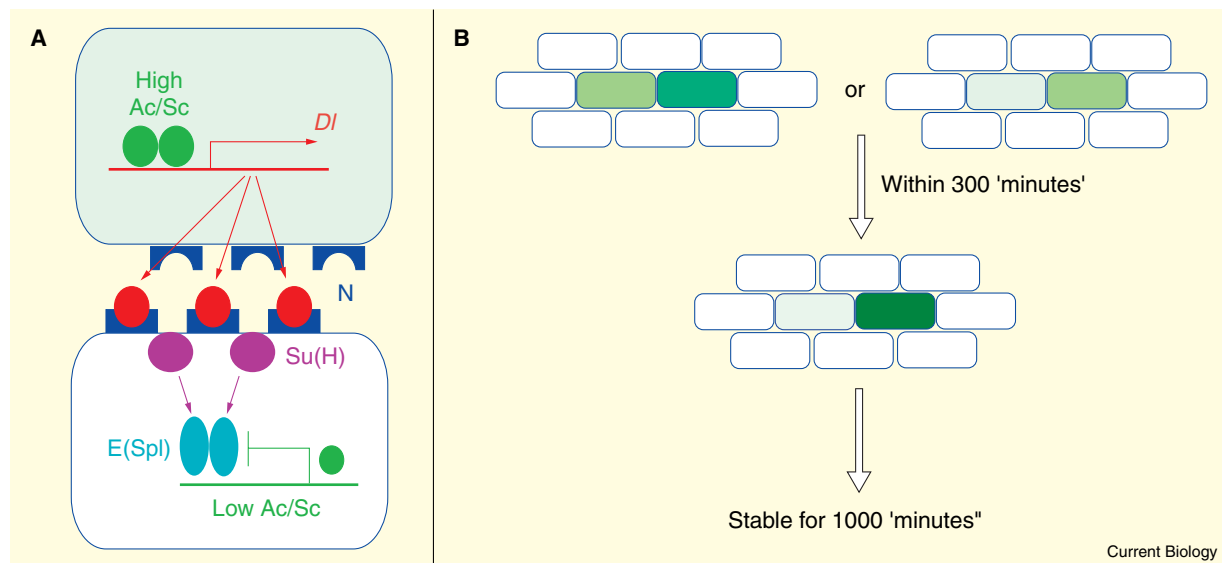


Figure 1 Modeling of lateral inhibition.

(A) A schematic outline of the core elements of the standard model of lateral inhibition. An initial slight difference in level of transcription factor Achaete/Scute (*Ac/Sc*) in one cell leads to greater production of Dorsal (*DI*), which binds Notch (*N*) receptors on the adjacent cell. This activates *enhancer of split* (*E(Spl)*) through the transcription factor Suppress of Hairy (*Su(H)*), downregulating *Ac/Sc* transcription. Augmented and reduced models that perform more efficiently are described in [3]. (B) The two-cell model for successful lateral inhibition requires that a two-fold difference in *Ac* and *Sc* mRNA in two cells within a non-expressing field of cells (top left) be converted into an over 60-fold difference in protein concentration within 300 minutes (below; with the lower cell almost off), and retained that way for 1000 minutes. To eliminate the possibility that the response was merely due to a threshold of initial concentration each successful solution was rerun with the lower concentration in the initial trial as the higher concentration in a second trial (top right).

Perhaps the two key results observed in their first model of the establishment of segment polarity in *Drosophila* embryos [2] were that an astonishingly wide range of parameter values will support the stable appearance of stripes of gene expression, and that these parameter values are robust to a variety of initial conditions. The newer study [3] confirms these results in the context of the Notch-mediated lateral inhibition network which establishes the correct pattern of *achaete* and *scute* expression in neurogenic fields [9]. Meir *et al.* [3] actually asked their equations to perform three different spatial patterning functions: simply separating the fates of a pair of adjacent cells (as shown in Figure 1); getting just the central cell in a field of seven cells to form a sensory organ precursor; and asking a double line of cells to refine to a single line, as occurs in the wing margin during fly development. One quarter of the parameter solutions to the third of these problems also supported solutions to the other two problems, immediately highlighting the potential for the evolution of pleiotropic functions.

The new work nicely demonstrates the potential of pathway-directed models to illuminate, or at least to generate hypotheses about, developmental mechanisms. Meir *et al.* [3] actually tested three different models of lateral inhibition: a standard model based on most of the well-characterized known interactions; a reduced model lacking an intracellular negative feedback loop; and an augmented model that included two inhibitory interactions that had only been hinted at in the literature. All three versions work to some extent, but the standard model was consider-

ably less efficient in so far as far fewer random parameter combinations yielded solutions.

Some clever manipulations led Meir *et al.* [3] to conclude that the *E(spl)* gene acts as a homeostat in the circuit: it is not required for the most basic patterning function, and in fact acts counter to the tendency of the network to amplify an initial discontinuity between cells, but in so doing adds stability to the network by preventing it from responding to noisy fluctuations in initial signal intensity. Building on this thought, they weigh in on a controversy over the role of lateral inhibition in neurogenesis [10] by speculating that prepatterning is more often than not sufficiently well tuned not to require lateral inhibition, but that the full circuit is structured to buffer those few percent of cases where some random developmental noise would otherwise perturb neurogenesis.

Perhaps the most thought-provoking results from an evolutionary perspective are those relating to a series of perturbations designed to test the 'shape of the working region' of parameter space. One approach was an attempt to mimic recombination among parameter solutions. Only a few percent of random recombinants reconstituted effective networks, though the percentage could be increased dramatically by restricting the parents to the subset of solutions occupied by a high density of similar parameter values. A more convincing approach was to determine how far individual parameters could be mutated to new values without disrupting the solution. In some cases, new solutions were found with over an order a magnitude change in each of the 69 param-

ters of the augmented model. Many of these new solutions will not recombine, however, so in a sense are reproductively isolated, almost as if they were members of new species.

I say almost, as there are still a couple of results that are required before we can conclude that this approach really captures the evolution of robustness. The most immediate is to place parameter values in a population genetic context, in which they become alleles coming and going and mating and mixing according to established laws of meiotic transmission, drift and selection. The most difficult may be to place the models themselves in a quantitative genetic context. Cells make all or nothing decisions, and sometimes adding up the proportions of incorrect decisions will generate a kind of continuous variation. But more generally quantitative variation is a normal component of development, and it is thought that genetic systems evolve to minimize the variability without eliminating it [11]. How they do so remains a challenge. These new models provide a hopeful strategy for getting at the causes and consequences of robustness.

It follows that ever more detailed dissection of developmental systems will help theoreticians to explain the mathematical properties of such systems. One of the best characterized of all developmental switches is the conversion of the gradient of Bicoid morphogen into a series of segmental stripes, but there is much quantification to be done here as well. Houchmanzadeh, Wieschaus and Leibler [4] have recently found that variability in the Bicoid gradient is filtered out in the course of establishment of the precise location of the anterior *hunchback* expression boundary. The location of this boundary does vary across a range of mutant backgrounds, but only by a couple of percent of egg length within a genotype — except in the presence of some mutant *staufer* alleles, in which case the variability increases dramatically. How and why some *staufer* alleles have this destabilizing effect is anyone's guess at the current time, but the point is that we now have the genetic and theoretical tools to with which to dissect robustness. Upon such dissection, we can then build robust models of the role of canalization, homeostasis, and developmental drift in orchestrating organic evolution [12].

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