Case Sudy 2: Turing's model of chemical morphogenesis

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April 6, 2005

1 Background

Alan Turing (1912-1954) has been described as the founder of computer science. He was a mathematician whose ideas about computability ushered in the computer age. (For some background, go to http://www.turing.org.uk/turing/.) Turing was also a master modeler. The *Turing machine* provides the basic unit of electronic computation, a modular "computer" from which all computing machines can in effect be assembled.

Toward the premature end of his life, Turing turned his attention to biology. He was fascinated by the complexity of early development, wherein aggregates of seemingly identical cells begin to differentiate and sort themselves into arrangements of tissue that will eventually make up the living organism. He asked a very precise question about how this process could begin, and then provided a model in which this question could be answered. In the course of this work he made one of the first uses of an electronic computer to solve differential equations. In the present case study we shall look at Turing's model; the topics we have already studied provide an introduction to the methods used by Turing in his study.

We shall describe the model as one of *chemical morphogenesis*. The "chemical" is appropriate because Turing recognized that living cells could be viewed as biochemical "factories", synthesizing proteins and regulating their use. (Turing developed his ideas about the same time that Watson and Crick were constructing their model of DNA, although I do not know if he was aware of their work). "Morphogenesis" is appropriate as a term to describe the emergence of structure and form through the process of biological development. We shall therefore study the mathematics of chemical reactions and the manner in which these reactions can interact cooperatively in aggregates of living cells.

Turing's ideas were not immediately accepted by biologists, since the model was based on a number of hypothesis that had not been tested in the laboratory. It is only in recent years that it has been found that some aspects of Turing's

model describe reasonable well some of the experimental results in developmental biology. We shall not go into the associated biological studies in any detail, but instead focus on the mathematical content of Turing's original model and what he was trying to accomplish with it.

Our study is organized as follows: we first summarize what Turing set out to do, then give some background on the use of differential equations to represent chemical reactions, and the mathematical description of diffusion between cells. Finally, we introduce and study Turing's model in the case of "one-dimensional tissue", which is a line or ring of cells where morphogenesis takes place.

2 Summary of Turing's ideas

Turing postulated that a living cell could be described by vector (surely a vector of very large dimension) $\mathbf{x}(t)$ whose components are concentrations of biochemicals. During the process of development, he argued that the manner in which these biochemcials interact must involve not just autonomous, isolated cells, but rather some sort of intercellular communication. He reasoned that the most likely candidate was diffusion, because he was interested in the stage of development well before the appearance of a nervous system and wave-like neural communication.

Early development of, say, an amphibian such as a frog is initiated by fertilization of the egg and a sequence of cell divisions leading to an aggregate of cells called the *blastula*. At some point the blastula acquires an axis of symmetry and one speaks at that time of the animal and vegetal poles. So it appears that early on the cells of the blastula cease to be identical but acquire differing characteristics which will ultimately lead to different fates in the developed organism. Some will become part of the ectoderm, some will become liver cells, some heart cells, etc. This process of *differentiation* of a group of cells, became the focus of Turings's interest. Turing reasoned that in the eariest stages of cell division, essentially identical sub-units were being created. But eventually this homogeneous state is broken and differentiated cells, or *patterns of differentiation*, are observed. A frog acquires a heart and a leopard its spots. It was this was the puzzle of the emergence of pattern from homogeneity that intrigued him and led to his model.

Turing first introduced the idea of an *isolated cell*. (I will not follow exactly Turing's terminology but will try to be faithful to his ideas.) An isolated cell is described by a vector $\mathbf{x}(t)$, which is assumed to satisfy an ODE of the form

$$\frac{d\mathbf{x}}{dt} = \mathbf{F}(\mathbf{x}). \tag{1}$$

Note that this is a vector ODE, a system of N equations for the N chemical components that define the state of a cell. The system is the saem for every cell. The idea is that before the cells of tissue are allowed to interact (communicate), they are regarded as identical objects. By identical, we mean not only that $\mathbf{F}$ is the same for all cells, but that also the *solution* of (1) that results is the

essentially the same for every cell. That is, the phase space of the solution $\mathbf{x}(t)$, of (1) must look the same for every cell. We call this solution the *resting state*. Suppose, for example, that the resting state of all cells is the same unique *equilibrium point* $\mathbf{x}_e$, so that $\mathbf{F}(\mathbf{x}_e) = 0$. Then, when the cell is in the resting state, all the chemical concentrations are a constant values given by the components of $\mathbf{x}_e$. In order for the isolated cell to stay in this resting state, it must be possible to perturb $\mathbf{x}$ slightly from its resting state and be sure that again $\mathbf{x} \to 0$ as $t \to \infty$. You will recognize that what we are saying here is that the resting state $\mathbf{x}_e$ should be *stable*, So this is what Turing postulated, that isolated cells are identical and are always found in stable resting states.

We remark that it is not necessary that the resting state be a equilibrium point $\mathbf{x}_e$ where $\mathbf{F}(\mathbf{x}) = 0$. For example, the resting state could well be a stable periodic cycle, described by a closed loop in phase space and with some fixed period T, so that in the resting state $\mathbf{x}(t+T) = \mathbf{x}(t)$ for all t. The cycle would then have to be stable in that the cell eventually returns to the same cycle after it is perturbed (i.e. $\mathbf{x}(t)$ changed by a small amount at some point in the cycle).

Next, Turing allows cells to "communicate" via diffusion of the chemical of $\mathbf{x}$, in a manner we shall formulate below. The question he then asks is, is the"tissue", i.e. the group of cells communicating via diffusion, still going to remain in the *homogeneous resting state*? That is, will the cells individually stay in their resting states even though they can communicate via diffusion? Turing's hunch was that, depending upon the chemical reactions and the nature of the diffusion, it could well be that the tissue was *unstable to pattern formation* even though the isolated cells were stable at the resting state. We use the term *pattern* here to mean that chemical concentrations vary from cell to cell, not that the $\mathbf{F}$s are different.

Thus Turing set out to show that he could find a set of reactions between N chemicals such that the isolated cells have stable rest states, but that a *diffusive instability* occurs in the tissue aggregate of diffusively communicating cells. This was found to be possible, and this is what we would like do demonstrate in this case study.

3 The chemical ODEs

Suppose that a chemical A reacts with a chemical B to form a compound C. The reaction occurs when molecules of A at a certain concentration react with molecules of B at their concentration. The reaction is usually written $A + B \to C$. These reactions proceed by pairwise interaction of molecules, and it is observed that the rate of formation of C is proportional to the product of the concentrations of A and B in grams per mole or in numbers of molecules per volume of solute. A *rate constant* is then added to the symbol of the reaction to indicate the constant of proportionality. Thus we have

$$A + B \underset{k}{\to} C. \tag{2}$$

The mathematical description is given by several ODEs. The first is

$$\frac{dC}{dt} = kAB. \tag{3}$$

This says that the concentration of C obeys the law just described, called the *law of mass action*. There are two further equations which describe how A and B behave. These are

$$\frac{dA}{dt} = -kAB, \quad \frac{dB}{dt} = -kAB. \tag{4}$$

In essence these three equations say that when M molecules of A combine with M molecules of B to produce M molecules of C, we take away M units for A and B and add M units of C, all a t a rate proportional to the instantaneous concentrations of A and B.

It is of interest to know that we can solve these equations to determine mathematically the course of the reaction. Note that

$$\frac{d(A+C)}{dt} = \frac{d(B+C)}{dt} = 0. \tag{5}$$

Thus if A_0, B_0, C_0 denote the concentrations at $t = 0$, we set $C_0 = 0$ (since C is to be made by the reaction) and have

$$A + C = A_0, \quad B + C = B_0. \tag{6}$$

Using these last expressions in (3), we have the following ODE problem for C:

$$\frac{dC}{dt} = k[A_0 - C][B_0 - C], \;\; C(0) = 0. \tag{7}$$

Since A and B are being used in equal amounts, the reaction will end when the smaller of A_0, B_0 gets used up. Note that if we set $u = C - B_0$ then the problem becomes $du/dt = ku(u_0 - u), u_0 = A_0 - B_0$, which is a logistic differential equation, which we know how to solve with the initial condition $u(0) = -B_0$.

We give another example of chemical ODEs, consider the reactions

$$E + S \underset{\leftarrow}{\overset{k_{+1}}{\underset{k_{-1}}{\rightleftarrows}}} C \overset{k_{+2}}{\rightarrow} E + P. \tag{8}$$

These reactions describe an enzyme E which acts as a catalyst for a reaction which yields a product P. An intermediary in this reaction is the complex C. The various rates are indicated. Here are the associated ODEs:

$$\frac{dE}{dt} = -k_{+1}ES + k_{-1}C + k_{+2}C, \; \frac{dS}{dt} = -k_{+1}ES + k_{-1}C, \tag{9}$$

$$\frac{dC}{dt} = k_{+1}ES - k_{-1}C - k_{+2}C, \; \frac{ds}{dt} = -k_{+1}ES + k_{-1}C, \; \frac{dP}{dt} = k_{+2}C. \tag{10}$$

The point here is to see how biochemical reactions can give rise to nonlinear equations analogous to those we have seen in other contexts, e.g. population biology. The fact that quadratic nonlinearities have appeared here is due to the fact that reactions tend to be bimolecular with overwhelming probability. There are however various feedback mechanisms which can give rise to more complex nonlinearities, and it makes sense to consider the possibility that we have essentially arbitrary nonlinearities possible in Turing's model, although of course the importance of the model would rest on its being realizable in a biochemical context.

4 Analysis of the stability of the resting state

We shall consider Turing's model for $N = 2$, that is for two chemicals. $\mathbf{x} = (x_1, x_2)$, the resting state being assumed to be a unique equilibrium point $\mathbf{x}_e$. The linearized problem near $\mathbf{x}$ leads to the following equation for the perturbations $\delta x_1(t), \delta x_2(t)$:

$$\frac{d\delta x_1}{dt} = a_{11}\delta x_1 + a_{12}\delta x_2,\ \frac{d\delta x_2}{dt} = a_{21}\delta x_1 + a_{22}\delta x_2. \tag{11}$$

Here

$$A = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix} = \begin{pmatrix} \frac{\partial F_1}{\partial x_1} & \frac{\partial F_1}{\partial x_2} \\ \frac{\partial F_2}{\partial x_1} & \frac{\partial F_2}{\partial x_2} \end{pmatrix} \Big|_{\mathbf{x}=\mathbf{x}_e}. \tag{12}$$

The eigenvalue equation, assuming the perturbations are proportional to $e^{\lambda t}$, is

$$Det(A - \lambda I) = 0 = \lambda^2 - T\lambda + D,\ T = a_{11} + a_{22},\ D = a_{11}a_{22} - a_{12}a_{21}. \tag{13}$$

Thus

$$\lambda\frac{1}{2}(T \pm \sqrt{T^2 - 4D}). \tag{14}$$

We must have the real parts of both roots negative for stability of the resting state. This is clearly not possible if $D < 0$. If $D > 0$ it is still necessary that $T < 0$ and the two conditions $D > 0, T < 0$ are sufficient for stability. Thus we must have

$$a_{11} + a_{22} < 0,\ \ a_{11}a_{22} - a_{12}a_{21} > 0. \tag{15}$$

What Turing asks in the present example is then the following question. Let the state of a cell be determined by the concentrations of the two chemicals $x_1(t), x_2(t)$. Let the reactions and the unique resting state be such that the conditions (15) are satisfied. Then, if we allow the cells to "communicate", will the resulting tissue remain stable, in the sense that all cells remain in their original resting states?

5 Cell-cell communication by diffusion

We represent a cell as a small cube of side Δ, with the chemical2 x_1, x_2 distributed homogeneously within. If two cells are adjacent and in contact, known

biological processes can allow chemicals to pass from one cell to the other. Turing assumed that this occured according to *Fick's law*. Fick's law states that the *flux* of chemical from one cell to the other is proportional to the difference of the chemical concentrations in the two cells, the flow being from the higher to the lower concentration.

We now want to consider a line of cells labeled by the index i. Thus $\mathbf{x}^{(i)}$ will be the state of the ith cell. Then the flux of x_1 into the ith cell well be

$$f_1 = K_1\Delta^2(x_1^{(i-1)} - x_1^{(i)}) + K_1\Delta^2(x_1^{(i+1)} - x_1^{(i)}) = K_1\Delta^2(x_1^{(i-1)} - 2x_1^{(i)} + x_1^{(i+1)}). \tag{16}$$

Similarly, the second chemical, which will in general diffuse at a different rate, will have flux f_2 into the ith cell, where

$$f_2 = K_2\Delta^2(x_2^{(i-1)} - 2x_2^{(i)} + x_2^{(i+1)}), \tag{17}$$

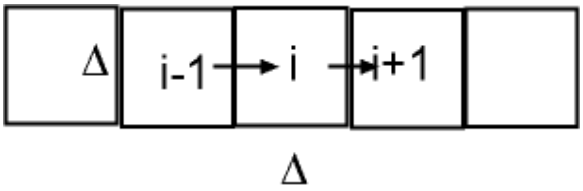
see the figure below. Note that we have included the area Δ^2 of the face across which transport is taking place in the definition of the constants of proportionality $K_1, K_2 > 0$. Thus μ_1 would represent the number of molecules per second crossing the cell interface, per unit of area, and per unit of concentration difference between the two cells.

If we now include cell-cell communication, we see that the states of all of the cells is determined by the following system of equations,

$$\frac{d\mathbf{x}^{(i)}}{dt} = F(\mathbf{x}^{(i)}) + M \cdot (\mathbf{x}^{(i-1)} - 2\mathbf{x}^{(i)} + \mathbf{x}^{(i+1)}), i = 1, 2, \ldots N_{cells}, \tag{18}$$

where

$$M = \begin{pmatrix} \Delta^2 K_1 & 0 \\ 0 & \Delta^2 K_2 \end{pmatrix}. \tag{19}$$



Since we are considering N_{cells} in a row, it is convenient to arrange them in a circle, so that cell $i = -1$ and cell $i = N_{cells}$ are equivalent, as are cells $i = N_{cells} + 1$ and $i = 1$. Then (18) is a closed system of N_{cells} equations for the same number of knowns $\mathbf{x}^{(i)}$. It is important to note that the *homogeneous resting state* $\mathbf{x}^{(i)} = \mathbf{x}_e$ is an exact solution of this system, because all of the fluxes vanish in this state.

Turing studied this system on a primitive computer for various definitions of $\mathbf{F}$ based upon chemical reactions satisfying (15) (in the case N=2). As we have explained, the purpose was so see if the homogeneous resting state was still stable in the presence of diffusive communication between cells.

We want to use analysis to study Turing's question, and so will replace the discrete cell system by a continuous one, in which the index i is related to a position s in the ring by $s = \Delta i$. The flux into the cell labeled x is then

$$f(s) = M \cdot (\mathbf{x}(s-\Delta) - 2\mathbf{x}(s) + \mathbf{x}(s+\Delta) \approx M \cdot \frac{\Delta^2}{2}\frac{d^2\mathbf{x}}{ds^2}(s). \tag{20}$$

Here we have expanded each term in its Taylor series through terms in Δ^2, e.g.

$$\mathbf{x}(s+\Delta) = \mathbf{x}(s) + \frac{d\mathbf{x}}{ds}(s)\Delta + \frac{d^2\mathbf{x}}{ds^2}(s)\Delta^2/2 + \dots. \tag{21}$$

In assuming Δ small and carrying out this expansion, we are effectively passing to the limit $\Delta \to 0$, with simultaneously $N_{cells} \to \infty$, in such a way that $N_{cells}\Delta \to L$, L being the circumference of the ring. (We also see below that $K_j\Delta^4$ should tend to a finite limit.)

Thus we want to examine the linear stability of the continuous PDE

$$\frac{\partial \mathbf{x}}{\partial t}(t,s) = F(\mathbf{x}(t,s)) + M \cdot \frac{\partial^2 \mathbf{x}}{\partial^2 s}(s,t), \tag{22}$$

where

$$M = \begin{pmatrix} \mu_1 & 0 \\ 0 & \mu_2 \end{pmatrix},\ \mu_j = K_j\Delta^4/2, j = 1,2. \tag{23}$$

The linearization is to be about the homogeneous rest state of the cells. The *diffusion coefficients* μ_1, μ_2 are assumed finite and positive.

6 Analysis of the linearized system

In order to linearize (23), only need to linearize $F(\mathbf{x})$. If we do that, and drop the δ from $\delta\mathbf{x}$, we get the system

$$\frac{\partial \mathbf{x}}{\partial t} = A \cdot \mathbf{x} + M \cdot \frac{\partial^2 \mathbf{x}}{\partial s^2}, \tag{24}$$

where

$$A = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & A_{22} \end{pmatrix} \tag{25}$$

is a matrix satisfying (15). We will follow Turing in considering only *pattern waves*, i.e solutions of (24) of the form

$$\mathbf{x} = e^{\sigma t + iks}\mathbf{x}_0, \tag{26}$$

where $\mathbf{x}_0$ is a constant vector. If, for some μ_1, μ_2, k there exist solutions of this form such that the real part of σ is positive, then the *tissue* is unstable to pattern waves even though the isolated cells are stable. When this occurs it is called a *diffusive instability*. In essence what Turing was after was to demonstrate the existence of diffusive instabilities.

Since $\frac{\partial^2}{\partial s^2}e^{iks} = -k^2 e^{iks}$, and $\frac{\partial}{\partial t}e^{\sigma t} = \sigma e^{\sigma t}$, we see that the pattern wave solutions are determined by a nontrivial solution of the equation $B \cdot \mathbf{x}_0 = 0$ where

$$B = A - \begin{pmatrix} \mu_1 k^2 + \sigma & 0 \\ 0 & \mu_2 k^2 + \sigma \end{pmatrix}. \tag{27}$$

For a nontrivial solution the determinant must vanish:

$$Det \begin{pmatrix} \mu_1 k^2 + \sigma - a_{11} & -a_{12} \\ -a_{21} & \mu_2 k^2 + \sigma - a_{22} \end{pmatrix}. \tag{28}$$

We can rewrite this determinant in the following form:

$$\sigma^2 - \bar{T}\sigma + \bar{D} = 0, \tag{29}$$

where

$$\bar{T} = a_{11} + a_{22} - k^2(\mu_1 + \mu_2) = T - k^2(\mu_1 + \mu_2), \tag{30}$$

$$\begin{aligned} \bar{D} &= a_{11}a_{22} - a_{12}a_{21} - \mu_1 k^2 a_{22} - \mu_2 k^2 a_{11} + \mu_1\mu_2 k^4 \\ &= D - \mu_1 k^2 a_{22} - \mu_2 k^2 a_{11} + \mu_1\mu_2 k^4. \end{aligned} \tag{31}$$

Now comes a straightforward but slightly tricky argument which will tell us when a diffusive instability can be possible. We have a quadratic for σ, just as we had a quadratic for λ in the study of stability of the isolated cell. By that earlier argument, the necessary and sufficient condition for an instability is that, now referring to the present problem, that $\bar{T} < 0$ and $\bar{D} > 0$. Given that $T < 0$ by assumption, and that $\mu_1, \mu_2 > 0$, we see that $\bar{T} < 0$ Hence we only need to show that with diffusion we can make $D < 0$. But this clearly requires that at one of the numbers a_{11}, a_{22} be positive. Since $T < 0$ the other of this pair must be *negative*. But since $a_{11}a_{22} > a_{12}a_{21}$, it follows that $a_{12}a_{22} < 0$.

It follows that *necessary conditions for a diffusive instability are the both* $a_{11}a_{22}$ *and* $a_{12}a_{22}$ *be negative.*

We now examine when, given that these necessary conditions are satisfied, a diffusive instability actually occurs. Now $D > 0$, and we may suppose that $a_{11} < 0$. So that we can deal with positive numbers we set $\bar{a}_{11} = -a_{11} > 0$. Then we see that to make $\bar{D}$ negative we must have

$$a_{22}\mu_1 - \bar{a}_{11}\mu_2 > 0. \tag{32}$$

This is the basic inequality behind the existence of a diffusive instability, and it is worth some discussion. Since we are dealing with the case $a_{11} < 0$, we note that the entry b_{11} in the matrix B given by (27) is negative, indicating that the chemical x_1 will decay to the rest state in the absence of chemical x_2. So we shall call x_2 and *activator chemical* and correspondingly x_1 as an inhibitor chemical. Now according to (32)

$$\frac{\mu_2}{a_{22}} < \frac{\mu_1}{\bar{a}_{11}}. \tag{33}$$

Also $T < 0$ implies $\bar{a}_{11} > a_{22}$. Taken with (33), this last inequality implies

$$\mu_2 < \mu_1. \tag{34}$$

Thus we find that for a diffusive instability *the inhibitor chemical should diffuse more rapidly that the activator chemical*. (Here, "diffuse faster" means that the corresponding μ is larger.) Thus we get some insight into how nature might biologically structure the communication between cells in terms of activator and inhibitor substances. Turing terms these activator-inhibitor chemicals *morphogens*. The existence of morphogens in nature has been controversial for many years, and only recently have these substances been discovered in biological systems, with functions very close in concept to those envisaged by Turing.

With these inequalities satisfied we can now see specifically how a pattern can form from a diffusive instability. We know that we need to make $\bar{D} = D - \mu_1 k^2 a_{22} + \mu_2 k^2 \bar{a}_{11} + \mu_1 \mu_2 k^4$ negative, where $D > 0$. This is a quadratic in k^2 of the form $D - c_1 k^2 + c_2 k^4,\ c_1, c_2 > 0$. The minimum occurs at

$$k = k_{min} = \sqrt{\frac{a_{22}\mu_1 - \bar{a}_{11}\mu_2}{2\mu_1\mu_2}}. \tag{35}$$

At that minimum

$$\bar{D} = D - \frac{(a_{22}\mu_1 - \bar{a}_{11}\mu_2)^2}{4\mu_1\mu_2}. \tag{36}$$

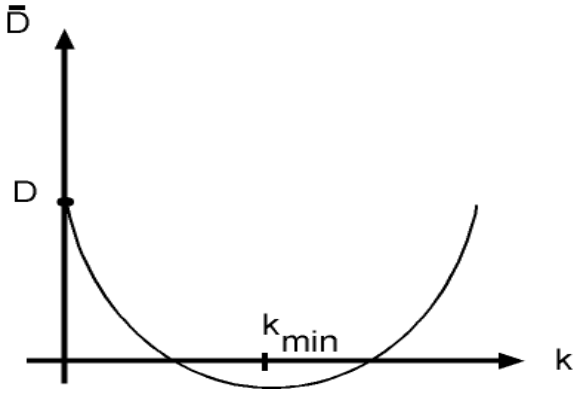
Since we must make $\bar{D} < 0$ we must have

$$a_{22}\mu_1 - \bar{a}_{11}\mu_2 > 2\sqrt{\mu_1\mu_2 D}. \tag{37}$$

This last result gives us a complete set of conditions for the occurrence of a pattern instability.

Let us apply this now to our ring of cells. The pattern must now be periodic in s, and so k must be an integral multiple of $2\pi/L$, where L is the circumference of the ring. The smallest k that will allow a pattern is therefor $2\pi/L$. Thus the condition for the instability of the first realizable pattern is that (37) be satisfied ins such but also that the values of k for which this holds include the value $2\pi/L$, see the figure below.

We mention that there have been many elaborations of Turing's idea applied to problems of mathematical biology, see the book by Murrray referenced below, and in recent years Turing's morphogens, or at least substances with function similar to shatg Turing proposed, have been isolated in the laboratory.



An example: Consider two chemicals (x, y) with reaction equations

$$\frac{dx}{dt} = 1/2 - x + x^2 y, \quad \frac{dy}{dt} = 1 - x^2 y. \tag{38}$$

(The constants on the right represent sources of the chemicalss, something we have not considered until now. We will not try to relate these equations to specific chemical reactions, however.) One sees that the unique equilibrium is $(x_e, y_e) = (3/2, 4/9)$. The corresponding matrix of the linearized isolated cell at this equilibrium is

$$A = \begin{pmatrix} -1 + 2xy & x^2 \\ -2xy & -x^2 \end{pmatrix}_{(x,y)=(x_e,y_e)} = \begin{pmatrix} 1/3 & 9/4 \\ -4/3 & -9/4 \end{pmatrix}. \tag{39}$$

Here $a_{22} = -9/4$ indicates that y is the inhibitor. We see that

$$\bar{D} = 9/4 + \frac{9}{2}\mu_x k^2 - \frac{1}{3}\mu_u k^2. \tag{40}$$

If $\mu_x = 1$, then the condition for a diffusive or pattern instability is

$$-\frac{9}{4} + \frac{1}{3}\mu_y > 3\sqrt{\mu_y}. \tag{41}$$

This occurs when $\sqrt{\mu_y} > \frac{9+\sqrt{108}}{3}$.

References

[1] Turing, Alan (1952) *The Chemical Basis of Morphogenesis* Phil. Trans. R. Soc. London B 237 pp 37-72.

[2] Murray, J.D. (2003) *Mathematical Biology*, 3rd edition, Vol. II, chap 2. Springer-Verlag.