FLOWER COLOR AND PATTERNING IN THE GENUS *HEMEROCALLIS* AND ITS HYBRIDS: A MATHEMATICAL MODEL AND EXPERIMENTAL ANALYSIS

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The development of patterns in flowers has been examined by many over the years. The seminal work of Alan Turing in 1952 laid out a method to model such coloration by looking at the process as a distributed communications between cells with feedback. This paper uses the Turing model combined with the current knowledge of gene expression and secondary pathways. We look at the patterns as classified by Petit as a canonical baseline and from the Petit Patterns we apply the Turing-Murray model to validate that the patterns can be duplicated by well defined and reproducible genetic control mechanisms.

The production of patterns in flowers has been an intriguing process with limited explanation. In this paper we propose a model for analyzing that process, for experimentally verifying the process, and for being able to reverse engineer the process via control of genetic pathways. There has been a great deal of discussion in the Hemerocallis community regarding the genetics of the flower. There are two issues: (i) what are the true species and how are they defined, (ii) what causes the coloration and does one have a genetic explanation for them. In this paper we continue our work on the second question. There has been earlier works by Norton who proposed asset of genes, without any evidence, that control colors. This was a classic Mendellian approach. The Norton model fails to deal with the known pathways generating the colorants such as anthocyanin and totally fails to relate that to the now know gene enzyme pathway controlling products. This paper provides an integral approach which is experimentally verifiable to explain and obtain patterned flowers. As such our approach herein uses in each step experimentally verified or verifiable procedures to explain patterning.

We begin with a summary of the work performed to date.

1. Genes and Gene Control: The understanding of the gene and its functions began with the publication of the Watson and Crick paper in April 1953. In August 1952 the Royal Society published a paper by Alan Turing entitled the Chemical Basis for Morphogenesis. In this paper Turing proposed a solution to the color pattern problem but unfortunately he had to hypothesize chemical reactions which no one at the time was yet aware. In the 1953 paper by Watson and Crick, the authors proposed a structure for DNA and they also proposed the mechanism for DNA making RNA and then proteins. This was the beginning. After some 55 years we now know a great deal about the mechanics of this system.

2. Plant Color and Anthocyanins: Plant colors, especially the flowers, are controlled by such secondary chemicals as anthocyanins. The anthocyanins absorb from the white incident light and reflect the colors we perceive in the flower. The secondary colorants such as the anthocyanins result from secondary chemical pathways which are driven by enzymes, proteins, produced by genes in the cell. The recent work by Mol et al, Jaakola, and Winkle-Shirley has provided reviews which provide up to data understanding of these processes. Each cell has its own secondary colorant process and the resulting concentrations of the colorants create a cell by cell color. Each cell may therefore result in differing concentrations of colorants and these concentrations are controlled by the genetic pathways in the cell, and are also affected by the flow of genetic pathway proteins which may have arrived from adjacent cells as well. The recent work by Mol (1998, 1999) Winkel Shirley (2001) and the work of Milgrom provide details on these processes. Likewise that of Durbin provides a connection with the evolution of the plants based on color structure. Harborne (1958, 2001) has provided a detailed basis to analyze the anthocyanins by spectral methods and we shall use these in this analysis. The other pathways such as the carotenoids have been recently summarized by Naik. A summary of all pathways has also recently been done by Yu and also by Holton et al. as well as the recent book by Lee. We rely heavily on the Mol and Winkel Shirley work.

3. Enzyme Reactions and Control: Proteins generate by the plant genes are the enzymes which effect the production and production rates of the secondary pathway colorants. Recent review papers by Baici shows the variants of models which can be applied to the dynamics here. Enzymes take part in the reactions by acting as facilitators. The more enzymes present the more the reaction moves forward. The enzymes may be produced locally in the specific cell or they may flow into the cell from adjacent cells. In addition the work by Murrells also provides a strong basis for modeling the reaction kinetics in this approach.

4. Intercellular Flow of Proteins: Plant cells have intercellular communications paths which differ greatly from those of animals. The paths are facilitated by the plasmodesmata. Recent studies have greatly elucidated the operations of these elements of plants and have shown that they are an integral part in the control of the overall genetic pathways and secondary pathways in plants. The recent work summarizing this field by Haywood et al, Cilia et al and Oparka and Zambryski display the extensive knowledge of the plasmodesmata and its role in the control in gene expression across a large matrix of plant cells. As we shall see latter, this mechanism for intercellular transfer can be viewed as the basis for a diffusion process between cells.

5. Patterning in Plants: In 1952 Alan Turing, in the last year and a half of his life, was focusing on biological models and moving away from his seminal efforts in encryption and computers. It was Turing who in the Second World War managed to break many of the German codes on Ultra and who also

created the paradigm for computers which we use today. In his last efforts before his untimely suicide Turing looked at the problem of patterning in plants and animals. This was done at the same time Watson and Crick were working on the gene and DNA. Turing had no detailed model to work with, he had no gene, and he had just a gestalt, if you will, to model this issue. Today we have the details of the model to fill in the gaps in the Turing model.

The Turing model was quite simple. It stated that there was some chemical, and a concentration of that chemical, call it C, which was the determinant of a color. Consider the case of a zebra and its hair. If C were above a certain level the hair was black and if below that level the hair was white. As Turing states in the abstract of the paper:

"It is suggested that a system of chemical substances, called morphogens, reacting together and diffusing through a tissue, is adequate to account for the main phenomena of morphogenesis. Such a system, although it may originally be quite homogeneous, may later develop a pattern or structure due to an instability of the homogeneous equilibrium, which is triggered off by random disturbances. Such reaction-diffusion systems are considered in some detail in the case of an isolated ring of cells, a mathematically convenient, though biologically unusual system. The investigation is chiefly concerned with the onset of instability. It is found that there are six essentially different forms which this may take. In the most interesting form stationary waves appear on the ring. It is suggested that this might account, for instance, for the tentacle patterns on Hydra and for whorled leaves. A system of reactions and diffusion on a sphere is also considered. Such a system appears to account for gastrulation. Another reaction system in two dimensions gives rise to patterns reminiscent of dappling. It is also suggested that stationary waves in two dimensions could account for the phenomena of phyllotaxis.

The purpose of this paper is to discuss a possible mechanism by which the genes of a zygote may determine the anatomical structure of the resulting organism. The theory does not make any new hypotheses; it merely suggests that certain well-known physical laws are sufficient to account for many of the facts. The full understanding of the paper requires a good knowledge of mathematics, some biology, and some elementary chemistry. Since readers cannot be expected to be experts in all of these subjects, a number of elementary facts are explained, which can be found in text-books, but whose omission would make the paper difficult reading."

Now, Turing reasoned that this chemical, what he called the morphogen, could be generated and could flow out to other cells and in from other cells. Thus focusing on one cell he could create a model across space and time to lay out the concentration of this chemical. He simply postulated that the rate of change of this chemical in time was equal to two factors; first the use of the chemical in the cell, such as a catalyst in a reaction or even part of the reaction, and second, the flow in or out of the cell. The following equation is a statement of Turing's observation.

$$\frac{\partial C_1(x,t)}{\partial t} = F_1(C_1, C_2, x, t) + \lambda_1 \nabla^2 C_1(x,t)$$

This is a nonlinear diffusion equation. It allows one to determine the concentration of say a protein as a function of time and space across a cell matrix. It requires two fundamental experimental parameters: first, the factors in the rate portion of the equation frequently found in reaction kinetics and second a diffusion constant determined by intercell transport most likely via the plasmodesmata. Recent works by Benson and Benson et al have added additional structure to the approach as well as the text by Murray establishing a full framework for the analysis of the model.

In the model we develop herein, we look at the concentrations of activator and repressor protein concentrations generated by activator and repressor genes. We employ the Murray-Turing model which states that the gene controlling a specific anthocyanin pathway is either on or off depending upon the concentration of activator or repressor. If activator concentration exceeds the repressor concentration then the gene functions and drives the secondary pathways thus producing the desired anthocyanin. In a linearized model of this process all one needs is linearized reaction coefficients and diffusion constants. The result is a two dimensional wave of activator and repressor concentrations and the result of these two waves is a set of fluctuating color patterns. If we were to then do this for multiple anthocyanin pathways we would then determine a set of multiple patterns which have been described by Petit.

6. Genetic Expression and Secondary Pathways, an Integrative View: The next step in developing this approach is to have an experimentally established model for the control of secondary pathways. We know from prior work that the secondary pathways exist and that they produce anthocyanins. The anthocyanins are the basis for plant color. We have established elsewhere that one can determine concentrations of anthocyanins observationally from a cell by cell spectroscopy (see McGarty, 2008). Namely there is an inversion process which permits the estimation of concentration densities from spectroscopic data.

The gene expression modeled developed in McGarty (2007) shows how the genes which create the protein which is the enzyme regulating each of the colorants pathways are themselves controlled by repressor and/or activator genes. By means of microarray analysis it is possible to both identify those genes and to determine the degrees of coupling between them as well.

Thus from the work of McGarty (2007) we have a model that connects genes to secondary pathways and moreover allows the connection to be quantified.

7. Patterning and a Canonical Model: Patterns in flowers have been a driving factor for many hybridizers who seek to have unique plants for sale. Ted Petit is a Hemerocallis breeder who is well known for his patterned flowers. He has recently published an article which has place many of the patterns in some reasonable analytical form (see Petit, 2007). The canonical forms proposed by Petit, we refer to them as "Petit Patterns", cover a wide gamut of the flower structures observed in Hemerocallis. One can then takes the Petit Patterns and using the Turing Space analysis discussed above and performs an experimental verification.

Turing did not have many of the elements we have developed above. He did not even know of the gene as we now know it, for he died less than a year after Watson and Crick published their famous paper. In fact, Turing went on trial for his admitted indiscretions merely days after he published his paper. At the time of his work many saw him as attempting to describe how daisies have so complex a petal set and the like. One may wonder what he would have thought of the problem as posed by Petit.

7. The selection of Hemerocallis as a target genus is based upon the fact that a great deal is known about the species but more importantly the hybridization of the genus over the past hundred years provides a great genetic pool to track the development of color and patterns. The original work on the genus was written by Stout in the 1930s and was reissued in the late 1980s. There is a great deal of field and analysis work performed in Japan, Korean and some in China as well. The work by Munson depicts the status of the genus as it has been hybridized until the early 1990s. Kang and Chung have performed many studies looking at the genus from a systematics perspective as well as a genetic perspective. Erhardt has proposed a key which he alleges is better than that of Stout. Unfortunately Erhardt provides no justification for his phylogenetic key whereas the Stout key was used solely for identification and had no systematics function. Tompkins has extended the analysis of the genus by using AFLPs and his analysis appears to support some but clearly not all of the alleged Erhardt phylogenetic analysis.

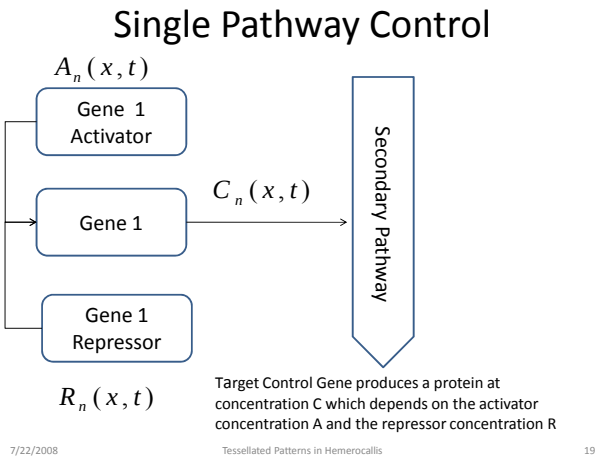
We first provide an overview and canonical model of the genetic pathways and secondary pathways which control color. The canonical model is shown below. The model is based upon the following experimentally observed facts:

1. There exist secondary pathways which convert primitive compounds into complex compounds which are the basis for color in plants. These compounds may be anthocyanins or many other similar secondary compounds.

2. There exists a gene which acts upon a secondary pathway as an enzyme in a catalytic manner. The concentration of the gene acting on the secondary pathway may increase the conversion in that pathway resulting in higher concentrations of the element activated. The greater the concentration of one secondary product as compared to another the more they viewed color may change. Secondary elements act as additive colorants in the Newtonian sense. They do not act as pigments which are subtractive. Care must be taken in calling the secondary elements pigments since their behavior in a cumulative manner is additive rather than subtractive as one finds in classic pigments in the world of painting.

3. There exist Activator and Repressor genes which can modulate the production of the target gene used to control, the secondary pathway. These Activator and Repressor genes may or may not themselves be so controlled. For the purpose of simplicity we assume at most one dominant Activator and Repressor gene.

We graphically depict this model below.



The processes in plant genes are generally identical to those in animal and thus human genes. The figure below shows a typical gene structure along with key sites. This structure shows the gene activator site which is where activator proteins can bind to start or enhance the expression of the gene.

MATERIALS AND METHODS

We have obtained samples from many hybrids of the genus Hemerocallis and have sectioned the petals. The sectioned petals were obtained using standard microtome techniques and we focused on taking sections which were indicative of the bands of color variation.

We then mounted the sections on slides and used no other means of preparation.

We then used a Pentax 200D to capture all images in the RAW mode. We performed this for both the slide data as well as the inflorescence data using RAW format and doing so within a six hour period. The choice of the six hours was to ensure that the anthocyanins did not degrade. All petal and sepal samples were kept refrigerated and in the dark during the waiting period.

The capture resolution used was 8.6 Mb of resolution. Comparisons were also made with jpg capture modes and no significant difference was obtained.

We also took sections of slides of the flower and of the cells and using Photoshop CS3 determined the color content on a cell by cell basis using the CS3 spectrophotometer facility. The CS3 spectrophotometer allows one to determine RBG components on selected sets of pixels. This we did on a cell by cell basis across the samples shown and others obtained. We then compared these to similar sample across the sepals and petals of the flowers as captured in situ.

We used several calibration techniques to obtain both absolute and relative calibration.

RESULTS

The results of the analysis of the flowers were as follows. In Table 1 we depict three early Stout hybrids with both flower and cell. The flowers show that there is an abrupt change at the gross level in color. The cell data reinforces that fact. The

cell either contains an anthocyanin for red or it does not. The change is an immediate and abrupt change in color.

The following Table summarizes several of the secondary pathway products and their colors. Note that the anthocyanidins present in a reddish manner, flavonols as cream and carotenoids as oranges. There are variations but we have shown how these can be normalized and measured, as well as separated when combined.

Class	*Agent*	*Color*[1]
Anthocyanidin		
	Pelargonidin	orange-red
	Cyanidin	purplish-red
	Delphinidin	bluish-purple
	Peonidin	rosy red
	Petunidin	purple
Flavonol		
	Kaempferol	ivory cream
	Quercetin	cream
	Myricetin	cream
	Luteolin	yellowish
	Agipenin	cream
Carotenoids		
	Carotene	orange
	Lycopene	orange-red

First, consider the Mikado flower. We collected a sample across the petal coloration from yellow to red. The sample is shown in the Table. The boundary between the two colors is seen at the cell level as an abrupt boundary, there is no slow degradation. Cells in the red region are clearly that there is a generation of peonidin or cyanidin in the red region. The specific identification of the specific anthocyanin or the complex may be determined by using the techniques detailed in McGarty, MIT, 2008, "Color". Namely one may use an inversion method to determine relative concentrations subject to appropriate normalizations. This can be achieved using RBG measurements alone.

The boundary effect is frankly a bit abrupt and it appears to be a validation of the Turing mechanism, namely that one sees that the pelargonidin pathway is either on or off. The spatial wave motion that one would anticipate with the Turing model is perceive in the macro analysis of the flower. If one looks closely at Mikado one can see the red and the flow to yellow at the edges. The anomaly is the yellow band along the central rib of the petal. One would suspect that there is a change in the diffusion coefficients at the cell level at this point. More detailed analysis of the cell structure must be performed to ascertain this.

[1] See Taiz p. 334 for the Anthocyanidin color and Bernhardt for the Flavonol and carotene.

We performed a more details analysis with Theron with samples taken from the throat region where there is a transition from red to the gold throated area. Again as can be seen in the Table the boundary is abrupt.

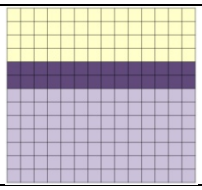
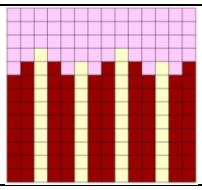
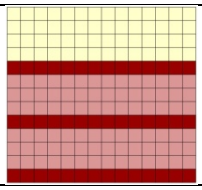
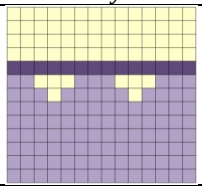
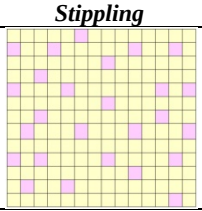
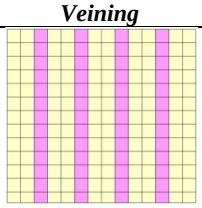
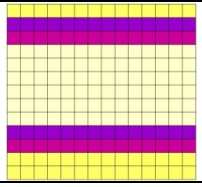
DISCUSSION

Patterns occur in Hemerocallis hybrids in a variety of manner and shape. Consider the following example of a 1941 hybrid produce by Stout, called Buckeye: We see what appears as an early eye pattern. This pattern had never appeared before. It shows a building of red, heavy on petals and light on sepals and it is bursting forth from the throat. It then ends abruptly. The question we pose is why? And how did this pattern result. It is patterns like these that we see a great deal of in the current generations of Hemerocallis hybridizing.

Petit, in the Daylily Journal of Summer 2007, described a multiple set of patterns that breeders were producing. He found the following to hold: The patterns take on a canonical set of forms. The forms are generally quite similar, perhaps because of common breeding practices or perhaps because of some underlying genetic makeup. These forms can be characterized canonical by a defining set of multiple overlays of tessellated secondary pathway expressions. Petit Patterns can therefore be explained and predicted. The patters described by Petit can be characterized as in the Table below.

Characteristic	*Turing Model*
Appliqué Throats	Unknown mechanism
Mascara Eyes or Bands	Demonstrates multiple layers of low spatial frequency outward growth of color.
Inward Streaks	If flower grows outward then the flow of control is unstable across new rows of growth.
Concentric Circles or Bands	If flower grows outward then the flow of control is unstable between new rows of growth.
Washed Eyezones	Ultra High intercellular instability, with almost localized oscillations allowing high spatial frequency of color change.
Stippling	High intercellular instability, with almost localized oscillations allowing high spatial frequency of color change.
Metallic Eyes	Unknown mechanism
Veining	Demonstrates multiple layers of low spatial frequency lateral growth of color.
Rainbow Edges and Midribs	Unknown mechanism

We can expand these descriptions if we create a collection of cells, say plant cells, and then color them to match the described and exampled patterns. We do this in the Table below. We also have shown in the Figure containing recent hybrids the actual physical embodiment of many of these patterns.

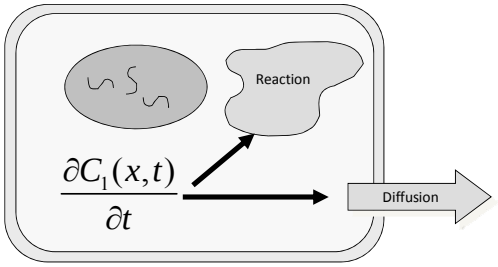
Mascara	*Inward Streaks*
	
Concentric Circles	***Washed Eyezones***
	
Stippling	***Veining***
	
Rainbow Edges	
	

What the Petit Patterns resemble is what we typically in oscillations occurring with the wave motion of second order spatial partial differential equations. By looking at the Petit Patterns as one with cells of a finite color contrast, constant hue, we can see that we can model this by means of such a second order equation but with nonlinearity in the output. That is we may have certain concentrations of enzymes but the dominant color could be controlled by the enzyme controlling the fastest or dominant secondary path. We now will explain this in the context of the linked genetic channel.

In this section we use the concepts of the Petit Patters and then combine them with the Turing model of morphogenesis to develop a verifiable and manipualatable system as regards to patters of the typ2 shown by Petit. We have shown that cells transmit to one another via the plasmodesmata. They communicate proteins and other concentrates from cell to cell. These thousands of small pipe ways create a diffusion process between the cells. We show a typical example in the figure below. The following is a detailed description of what Turing proposed in his model.

[Rate of change of concentration] = [Reaction Kinetics] + [Diffusion]

C_1 = concentration of protein or secondary c_1 in the cell



$$\frac{\partial C_1(x,t)}{\partial t} = F_1(C_1, C_2, x, t) + \lambda_1 \nabla^2 C_1(x,t)$$

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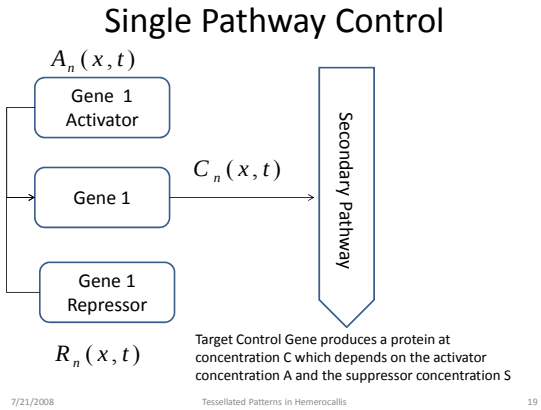
The following depicts the gene control paths that we will focus upon. The target gene produces a protein which enzymatically activates the secondary pathway producing the colorant. The target gene is activated by a gene which produces protein A and is repressed by a gene producing protein R. These proteins control the generation of C. In addition these proteins flow back and forth across the cell boundaries building up and decaying, as if in waves, and when the A exceed then we have activation and when R exceeds A we get repression.



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The simplest model we have can then be stated as follows:

$$C_n(x,t)=\begin{cases}C_n(x,t) \text{ if } A_n(x,t)>S_n(x,t)\\ 0 \text{ if } A_n(x,t)<S_n(x,t)\end{cases}$$

$$\frac{\partial A_n(x,t)}{\partial t}=F_1(A_n,R_n,x,t)+\lambda_1\nabla^2 A_n(x,t)$$

$$\frac{\partial R_n(x,t)}{\partial t}=F_1(A_n,R_n,x,t)+\lambda_R\nabla^2 R_n(x,t)$$

The Turing Space is that space of a set of parameters, generally related to the enzyme (protein) reactions of the activator-suppressor genes which permit instabilities in the control mechanism of the Target Gene protein to the secondary pathway. Turing in 1952 showed that diffusion of the activator-suppressor proteins can cause instabilities, rather than the more common stable solutions. We now develop the following:

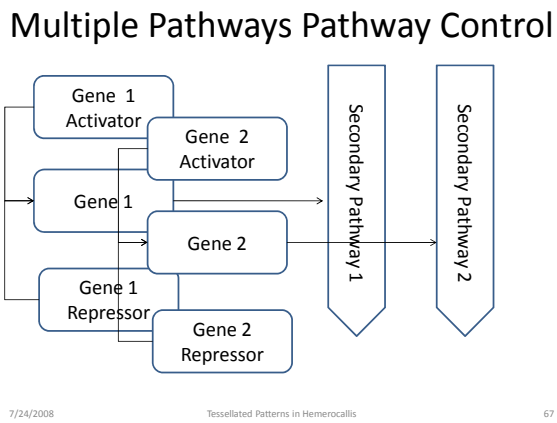
A model for the enzyme reactions in a competitive environment has been employed. A method to solve for the Turing space the diffusion model A model to apply the results to a single anthocyanin The ability to apply to multiple anthocyanin The ability to determine the analysis and the synthesis problem The Turing model has been discussed earlier. What Turing proposed was that there was some chemical whose concentration made something one way or another. That this something then diffused throughout the organism in some manner and if it was greater in one part than a threshold the morphology was one way and if less the morphology was another. He had no underlying basis in the current understanding of genetics to put details to his models. We now have that detail.

We know that if we have an activator protein on a secondary pathway then that protein will cause the pathway to become active and create the secondary product, an anthocyanin. The more of that protein we have, the greater it concentration, the more secondary product we can get. This is P is the controlling protein concentration, we have:

$$\frac{\partial P_n(x,t)}{\partial t}=H(P_n,A_n,R_n,x,t)+D_P\nabla^2 P_n(x,t)$$

We show such pathways below. In the above pathway we have a complex but modellable set of interactions. They are characterized by:

When there are multiple A-R interactions then they add and the net result is an overlapping of the anthocyanin pathway products. The overlays can be shown to create the typical patters in the Petit list. The model allows for an analysis of any tessellated product and also provides a basis for determining what products are achievable as well as how to achieve them, at least at the genetic level. Now we want to build on this model. First we must look at the dynamics of the activator and repressor genes and then we look at the dynamics of the controlling enzyme. Remember that the activator suppressor genes produce products which control the colorant gene. Let us now look at a single cell and look at the tempero-spatial dynamics of the concentrations of the products of the activator and repressor genes, A and S respectively. We assume we have a model as shown below



Where in this model we have sets of genes and each has activators and repressors. Each gene may activate a separate pathway as we have shown. First we write the model for the controlling enzyme:

$$\frac{\partial P_n(x,t)}{\partial t}=H(P_n,A_n,R_n,x,t)+D_P\nabla^2 P_n(x,t)$$

In the above we show the concentration for the controlling enzyme in a cell for path n. It has a function H which results from a Michaelis-Menten pathway mechanism which we described earlier. From the Michaelis-Menten analysis before we have, if we assume some separate A, R process:

$$H(P,S,SP)=[+k_1PS-(k_{-1}+k_2)C]Q(A,R)$$

where we had defined PS and C as before and where Q is a function of A and R which either turns on or off the process creating the P reactant. That is if A>R we have a reaction and otherwise we do not.

Thus P is also affected by concentrations of activator and repressor genes, A and R respectively, but in a binary manner. Second, now we write the general model for the activator and repressor product concentrations. As we have just discussed, the pathway activating protein is either on or off. If on we can then calculate its intensity and if off it is irrelevant. For the activator we have:

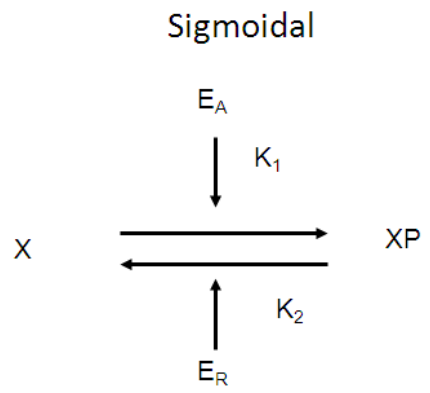
$$\frac{\partial A_n(x,t)}{\partial t}=F(A_n,R_n,x,t)+D_A\nabla^2 A_n(x,t)$$

and for the suppressor we have:

$$\frac{\partial R_n(x,t)}{\partial t}=G(A_n,R_n,x,t)+D_R\nabla^2 R_n(x,t)$$

Here we have A and R as the relative concentrations of the products of the Activator and Repressor genes. The F and G functions are the mass balance functions for this mix and the additional loss or gain come from the diffusion term. Here we assume that A and R may diffuse at different rates and this fact is key to the oscillations in space and in turn to the tessellation. We now need a model for the interaction functions. We choose the model provided by Conrad and Tyson in Szallasi et al which is termed the phosphorlation-dephosphorlation model or the sigmoidal model. We show its network below. Here we use the enzyme approach with one enzyme, the activator moving the production of the product enzyme and another the repressor enzyme driving the process backward. As we have done with the enzyme case we assume limited amounts and thus we have the

denominators in the equation.



This is the mathematical model we have deployed again using the same reference. This as we have said assumes that we have some form of enzymatic limiting reaction.

$$\frac{dx}{dt} = -\frac{k_1 e_A x}{K_{m1} + x} + \frac{k_2 e_S (x_T - x))}{K_{m2} + x_T - x}$$

The above are also normalized concentrations. We rely upon the recent summary by Baici and the work of McMurray, Schnell as well as Szallasi and his co-authors. The generalized solutions we have are as follows, each normalized as we had done for the enzyme reaction. The equations are for the activator, repressor and product enzymes respectively.

$$\frac{\partial a}{\partial t} = f(a,r) + D_a \frac{\partial^2 a}{\partial x^2}$$

The same set of models for the repressor concentrations can now be developed except that we have a different diffusion constant.

$$\frac{\partial r}{\partial t} = g(a,r) + D_r \frac{\partial^2 r}{\partial x^2}$$

Finally for the controlling enzyme product:

$$\frac{\partial p}{\partial t} = h(p,a,r) + D_p \frac{\partial^2 p}{\partial x^2}$$

We can now linearize the system as follows about a point:

$$w(x,t) = \begin{bmatrix} a(x,t) - a_0(x,t) \\ s(x,t) - s_0(x.t) \\ p(x,t) - p_0(x,t) \end{bmatrix}$$

This will yield the following linear model:

$$\frac{\partial w}{\partial t} = Aw + K\nabla^2 w$$

where A is a 3x3 matrix and we have K also a 3x3 matrix. and where we have linearized the system to read as follows:

$$A = \begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{pmatrix} = \begin{bmatrix} \frac{\partial f}{\partial a} .. \frac{\partial f}{\partial r} .. \frac{\partial f}{\partial p} \\ \frac{\partial g}{\partial a} .. \frac{\partial g}{\partial r} .. \frac{\partial g}{\partial p} \\ \frac{\partial h}{\partial a} .. \frac{\partial h}{\partial r} .. \frac{\partial h}{\partial p} \end{bmatrix}_{a_0, s_0} = \begin{bmatrix} f_a \cdots f_r .. f_p \\ g_a \cdots g_r .. g_p \\ h_a \ldots h_r .. h_p \end{bmatrix}$$

Murray shows that the following five properties are necessary and sufficient to determine the Turing Space for any reaction kinetics. These follow the stability requirements:

$$\frac{\partial f}{\partial a} + \frac{\partial g}{\partial s} < 0 \quad \frac{\partial f}{\partial a}\frac{\partial g}{\partial s} - \frac{\partial f}{\partial s}\frac{\partial g}{\partial a} > 0 \quad \gamma\frac{\partial f}{\partial a} + \frac{\partial g}{\partial s} > 0$$

$$\left(\delta\frac{\partial f}{\partial a} + \frac{\partial g}{\partial s}\right) - 4\delta\left(\frac{\partial f}{\partial a}\frac{\partial g}{\partial s} - \frac{\partial f}{\partial s}\frac{\partial g}{\partial a}\right) > 0$$

Now we can solve these equations and the concentrations for a and r are shown as follows:

$$a(x,t) = a(x) = \sum_{n=1}^{\infty} \eta_k \cos\left(2\pi\frac{x}{\kappa_a}\right) + \mu_k \sin\left(2\pi\frac{x}{\kappa_a}\right)$$

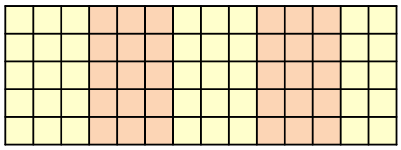
$$r(x,t) = r(x) = \sum_{n=1}^{\infty} \theta_k \cos\left(2\pi\frac{x}{\kappa_r}\right) + \xi_k \sin\left(2\pi\frac{x}{\kappa_r}\right)$$

$$d(x,t) = d(x) = a(x) - r(x)$$

$$D(x) = 1\mathrm{sgn}(d(x))$$

The k values of the wavelength are determined by the eigen values of the A matrix. What this model shows is that A and R has wavelike behavior for each anthocyanin dependent upon the diffusion coefficient for the specific proteins through the plasmodesmata. We now show several example of the solution to these equations. The following Table presents solutions determined via the approach in Murray. There have been many others over the years who have obtained similar results.

Example 1: p. 392 Murray, low spatial frequency instabilities across the cells showing wide striped variation. This assumed a single unstable secondary pathway which was on or off. It assumed also another stable secondary pathway.



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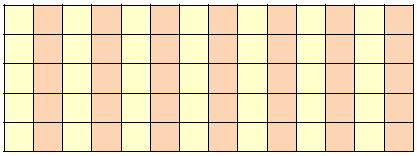
Example 2: p. 392 Murray, high spatial frequency instabilities across the cells showing wide striped variation. This also assumed a single unstable secondary pathway which was on or off. It assumed also another stable secondary pathway.



We can now consider a simple model. This is a one dimensions, x axis only, model but it proves the point. Consider the following. We have two waves with the following amplitudes and wave numbers. Specifically we chose: k_A=0.1 and k_B = 0.2 and the coefficient of A to be 0.5 and the coefficient of R to be 0.2 The pattern is shown below. It shows a flip between pink and yellow. There is not direct relationship between these colors and any specific anthocyanin. This example can then be spread to two dimensions readily as is shown in Murray.

The above is a Pattern using 1 dimensional Turing Equation. Other more complex patterns can be readily generated. It should be remembered that in this pattern we assumed an activator and repressor gene and that if the concentration of one was greater than the other we generated one anthocyanin or the other. Thus the bi-color banding can be seen as above. In effect this is the coherence pattern seen in interference optics.

We may now consider two simple models for an analytical result. The first model is a linearized and uncoupled model and the second is linearized and couples. In both models we look solely at the concentrations of the A and R proteins.

Example 1: Linearized and Uncoupled Model, we assume that the reaction rate is dependent solely upon the active concentration and that there is a linear diffusion process. We let A(x) and R(x) be the concentrations of the activator and repressors as a function of distance. Thus we have:

$$\pi_A A(x) + D_A \frac{\partial^2 A(x)}{\partial x^2} = 0$$

or

$$\kappa_A A(x) + \frac{\partial^2 A(x)}{\partial x^2} = 0$$

$$\kappa_A = \frac{\lambda_A}{D_A}$$

and

$$\kappa_R R(x) + \frac{\partial^2 R(x)}{\partial x^2} = 0$$

This is the simple model for two waves of concentrations having a spatial frequency of the square root of the appropriate κ and that the waves of concentrations are independent, ascending and descending. Clearly they must be between zero and a maximum value.

Example 2: Linearized and Coupled, we now assume that the reactions, although linearized are coupled. Consider the following model,

$$\lambda_{A,A} A(x) + \lambda_{A,R} R(x) + D_A \frac{\partial^2 A(x)}{\partial x^2} = 0$$

$$\lambda_{R,A} A(x) + \lambda_{R,R} R(x) + D_R \frac{\partial^2 R(x)}{\partial x^2} = 0$$

or

$$\kappa_{A,A} A(x) + \kappa_{A,R} R(x) + \frac{\partial^2 A(x)}{\partial x^2} = 0$$

$$\kappa_{R,A} A(x) + \kappa_{R,R} R(x) + \frac{\partial^2 R(x)}{\partial x^2} = 0$$

Now this is a bit more complicated that the first example and it can be solved analytically but we can make a few simplifications and see what the impact of some limited coupling would be. We can now write the above as follows:

$$\begin{bmatrix} \frac{\partial A}{\partial x} \\ \frac{\partial^2 A}{\partial x^2} \\ \frac{\partial R}{\partial x} \\ \frac{\partial^2 R}{\partial x^2} \end{bmatrix} = \begin{bmatrix} 0.....1.....0.....0 \\ \kappa_{A,A}.0.....\kappa_{A,B}.0 \\ 0.....0.....0.....1 \\ \kappa_{B,A}.0.....\kappa_{B,B}.0 \end{bmatrix} \begin{bmatrix} A \\ \frac{\partial A}{\partial x} \\ R \\ \frac{\partial R}{\partial x} \end{bmatrix} \backslash$$

This is readily solvable and depending on the relative values one may see that the coupling merely perturbs the spatial frequency obtained from the first example.

From an experimental perspective two final observations may be made' First, one may determine the constants of reaction and diffusion by examining the pattering of the specific flower. We have demonstrated that elsewhere (see McGarty, MIT, 2008). Second, one may reverse the problem and seek to affect a specific pattern. That pattern will then stipulate a set of diffusion equations and in turn a set of constants. These can now be genetically engineered (again see McGarty, MIT, 2008). Thus the analysis and models developed herein take what Turing had developed more than fifty years ago, and apply specifics for the patterning of flowers.

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	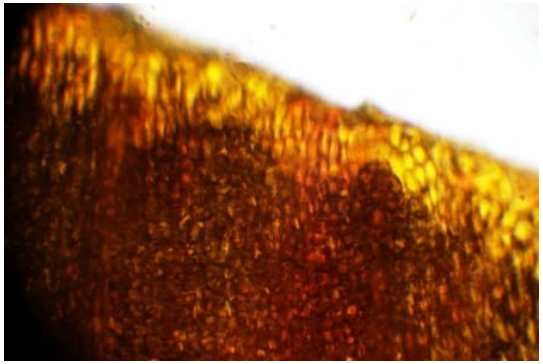	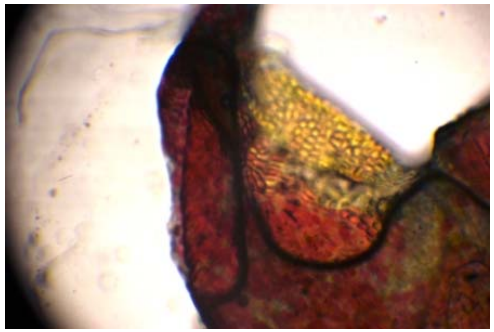
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	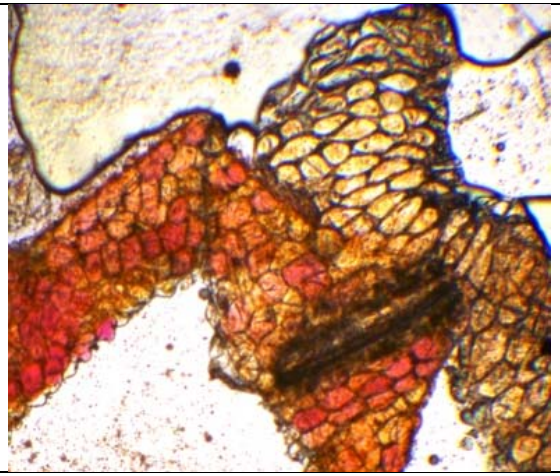	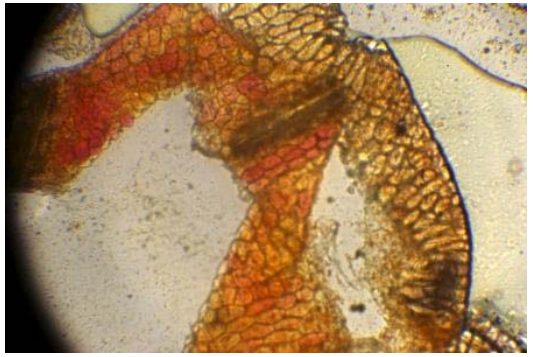
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	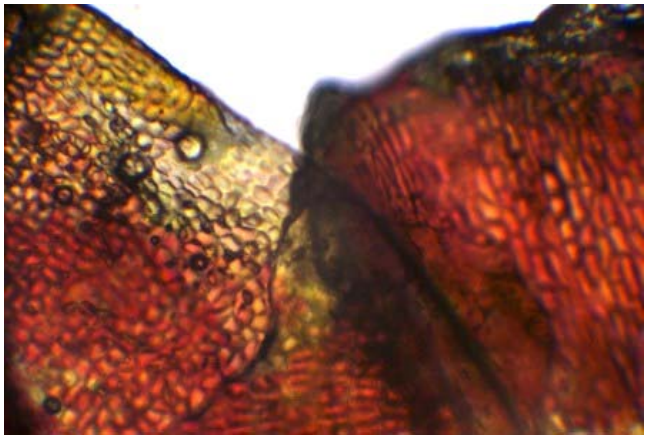	
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EXAMPLES OF HYBRID PATTERNING