Flower Tessellation Patterning and a Genetic Pathway Explanation[1]

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Abstract

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.

Contents

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1 INTRODUCTION

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 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.

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 Basic show the variants of models which can be applied to the dynamics here. Enzymes take part in the reactions by acting as facilitators. The more enzyme 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.

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 e 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 the nonlinear diffusion equation. It allows one to solve for a concentration, C, as a function of time and space. It requires two things. First is the diffusion coefficient to and from cells and second the functional relationship which shows how the chemical is used within a cell.

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. We summarize these steps in the Table below.

Fact	Basis
Genes produce proteins which in turn act as catalysts.	Classic Watson-Crick model at Cambridge in 1953.
There are secondary pathways which are controlled by the protein catalysts and these pathways produce compounds which create the color.	Winkle-Shirley studies in 1990s.
There are activator and suppressor genes which modulate the Target genes protein production rate.	Watson and the Cold Spring Harbor Group
The concentration of the Target control protein is controlled by (i) the enzyme reaction mechanism of the secondary pathway and (ii) diffusion from and to the cell.	Michaelis-Menten models of enzymes and Fisher model of diffusion in cells 1937.
The Turing-Murray model shows that there exist modes of protein concentration production which can result in the switching on and off of a specific secondary pathway in a multidimensional manner creating patterns.	Turing study in 1952 at Manchester and detailed studies by Murray at Oxford.
Petit has classified patterned flowers in a somewhat canonical form, the patterns exist.	Petit 2006.
The process of tessellated patterns from one pathway can be combined with other pathways at different spatial frequencies in two dimensions resulting in Petit patterns.	McGarty at MIT 2007

2 GENES AND SECONDARY PATHWAYS

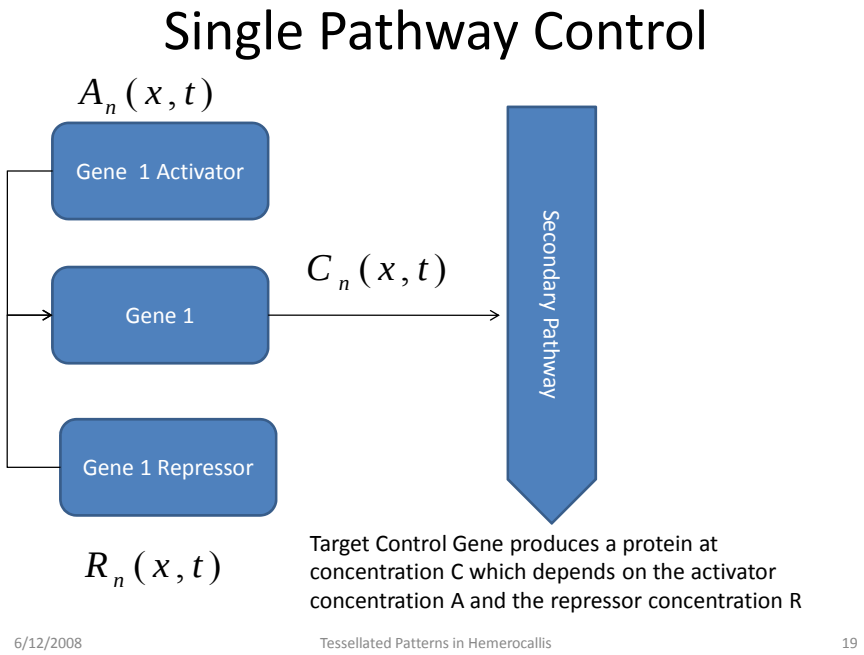
In this section we provide an overview of the genetic pathways and secondary pathways which control color. The canonical model is shown below. The model is based upon the following experimentally observed facts:

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.

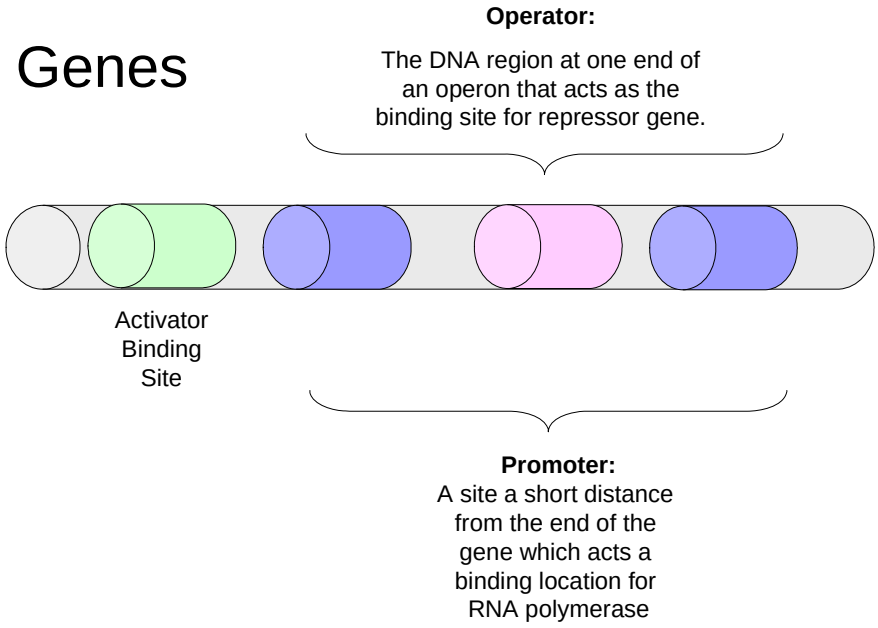
We graphically depict this model below.



2.1 Genes

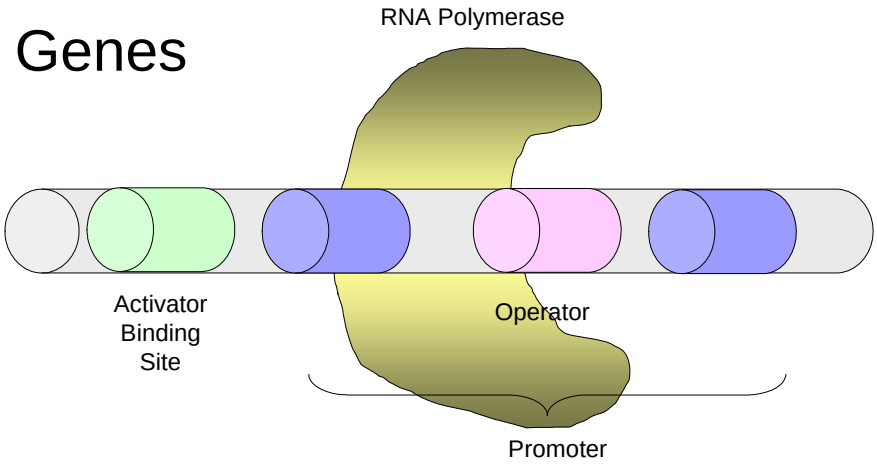
The processes in plant genes are generally identical to those in animal and thus human genes. The figure below shows a typical gene structure along with key sites. This structure shows the gene activator site which is where activator proteins can bind to start or enhance the expression

of the gene. The operator sits and the overall promoter sequence are shown down from the activator site.[2]



Genes express themselves with the assistance of RNA polymerase. The RNA polymerase is key in that it binds to the DNA and then opens it up to allow for the transcription creating the mRNA required for the translation process. In the figure below we show this process.

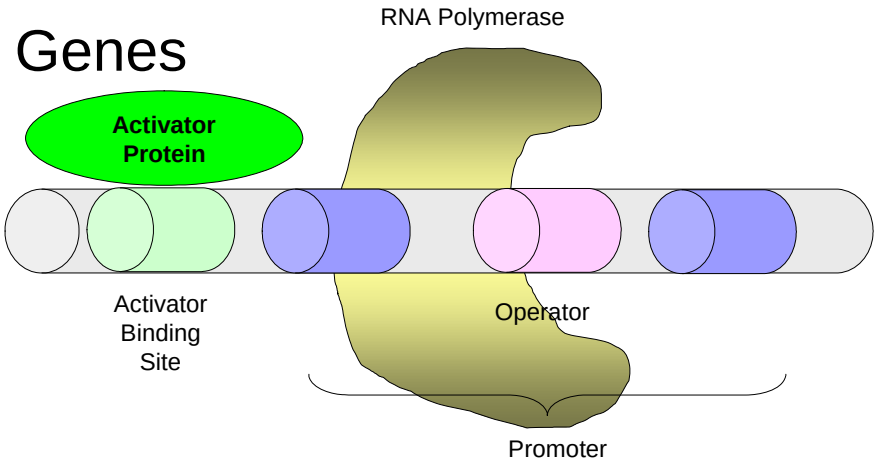
[2] This is detailed in Watson et al. Also see Griffiths et al.



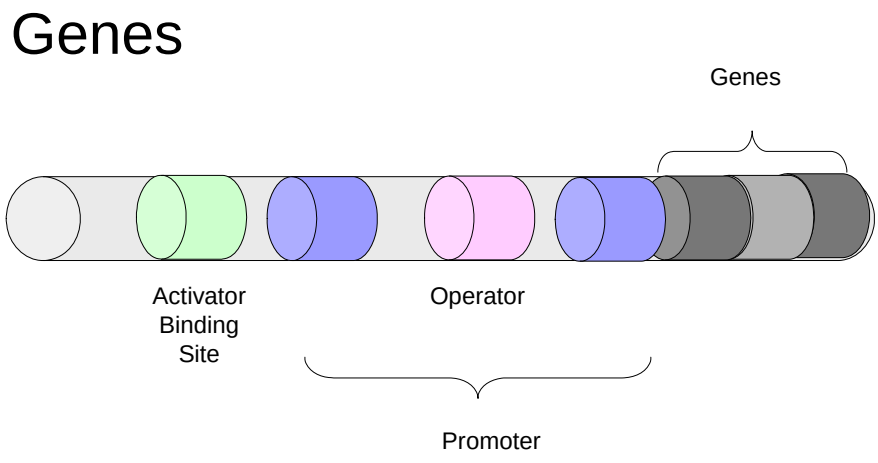
We will now focus on two actions which control the gene expression; activators and suppressors.

2.1.1 Activators

Activators are proteins which when attached to the gene assist in the expression of the gene. An activator is a protein resulting from another gene which can assist and facilitate the expression of a gene. Remember we want to look at the ensemble view, not the time view. Thus we assume that the RNA polymerase is continuously acting to produce proteins and that there is a continuous flow at some level of the activators. The cell process from the time view is shown below. An activator binds facilitates the RNA polymerase binding which in turn produces the mRNA and then in turn the proteins via the translation process.



If there is an activator then the gene can be readily expressed. The RNA polymerase then binds, creates the mRNA and this in turn produces the related protein. Activators stimulate this process. The Figure below depicts the location of the gene downstream from the activator and the promoter.



Now it is important to understand the activator from a time perspective and then from the ensemble perspective.

1. Activators are proteins generated by other genes in the cell.
2. Activators bind to the DNA and facilitate the production of the gene, which in turn produces another protein.
3. Activators can bind, release and then rebind. Each time they do that they produce another mRNA and that in turns produces another protein molecule.
4. From a time perspective, it is activator, produces gene reading, produces mRNA, and produces protein.
5. From an ensemble perspective we have a concentration of activator proteins and then we get a concentration of result proteins.

This then leads to a simple model:

$$P_o = \text{Output Protein Concentration}$$
$$P_i = \text{Input Protein Concentration}$$
$$P_o = A_{o,i} P_i$$

But there is also a dynamic model which we can state; to some degree this model is a hybrid of the time and ensemble approach. The model states:

$$\frac{dP_o}{dt} = f\left(P_o(t), P_i(t), t\right)$$
$$P_o(0) = P_o^0$$
$$P_i(0) = P_i^0$$

Now we must remember that this simple "two protein, two gene" model is just a simplification. In reality we may have dozens of not hundreds of genes in this process. Now consider a simple linear model for this two gene system:

$$P_i(t) = P_i^0 \exp(-\lambda_i t)$$

$$\frac{dP_o(t)}{dt} = A_{o,i} P_i(t) + A_{o,o} P_o(t)$$

We can solve this differential equation. It is:

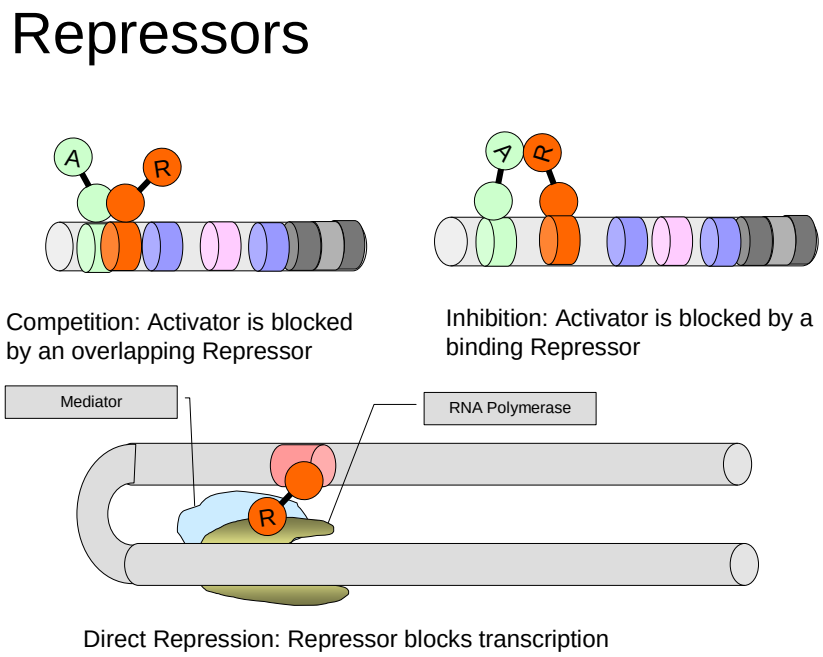
$$P_o(t) = k_{o,i} P_i(0) \left[\frac{\exp(-\lambda_i t) - \exp(-k_{o,o} t)}{\lambda_i - k_{o,o}} \right]$$

where;

$$A_{o,o} = -k_{o,o}$$
$$A_{o,i} = +k_{o,i}$$

2.1.2 Repressors

In contrast to activators we also have genes which are suppressors. Three methods of suppressor action are shown below. A suppressor does the opposite of an activator. It suppresses the expression of a gene. The same logic will follow the repressor as was with activators. We again also want to view this from an ensemble perspective.



As we did with the activator, we see a repressor stops the generation of the protein. This it is nothing more than a negative driver to protein generation.

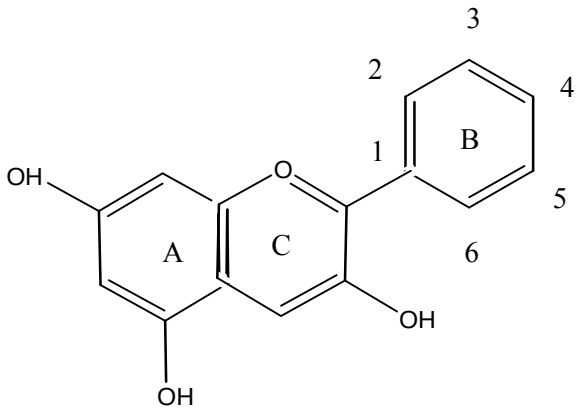
2.2 Secondary Products

We first review the secondary products. We start with the anthocyanins since they are frequently the most dominant. Then we provide an overview of the pathway process. Recent review papers by Mol et al as well as Winkel-Shirley provide the details on the gene controls on these pathways.

2.2.1 Anthocyanins

Let us consider our first pathway. This is the pathway which creates anthocyanins.[3] The anthocyanin molecule is shown below. Note on the B ring we have six sites to which we can attach differing molecular chains. This will be an important element when we see the different configurations and their implications.

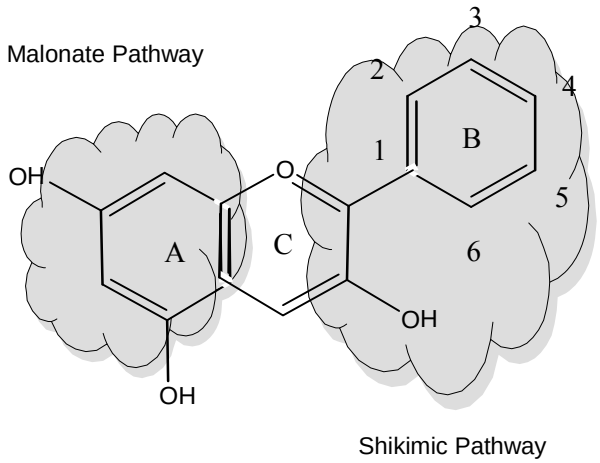
Anthocyanidin



The anthocyanin or anthocyanidin molecules come from two different pathways. In the figure below we have taken the basic resulting molecule and have shown that there are two elements; one is from the shikimic pathway and the other from the malonate pathway. This means that we have to understand both pathways to understand the ultimate abundance of the product.

[3] See the papers by Mol and also by Winkel-Shirley. They are excellent in the characterization of the pathways. Also the papers by Holton and the one by Jaakola are quite useful here as well.

Anthocyanidin



Before continuing we want to look at what the results would look like if we have different substitutes on the B ring. In the Table below we show that the terminations on the 3, 4 or 5 elements yield different results. The results give pelargonadin, cyanidin, delphinidin, peonidin, and petunidin. Each obviously named after their related flower and each resulting in an anthocyanin of a different color.

Anthocyanidin	*Substituents*	*Color*
Pelargonadin	4'-OH	orange-red
Cyanidin	3'-OH, 4'-OH	purplish red
Delphinidin	3'-OH, 4'-OH, 5'-OH	bluish purple
Peonidin	3'-OCH_3, 4'-OH	rosy red
Petunidin	3'-OCH_3, 4'-OH, 5'-OCH_3	purple

2.2.2 Other Color Elements

Anthocyanins are not the only elements which are secondary products which produce color. There are three classes of chemicals which give rise to color; anthocyanins, flavones or flavonols, and carotenoids. The Table below depicts the different elements and their colors. The approach we took above for the anthocyanins can be taken for the flavones and carotenoids as well. It should be noted that there may not be a unique solution here but there are several

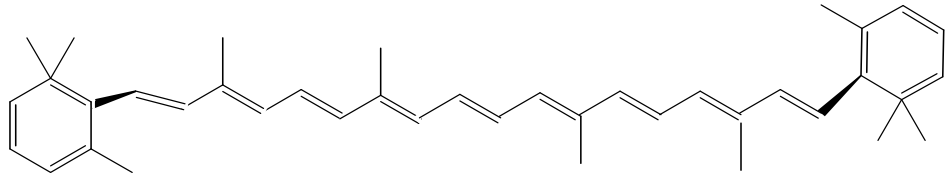
possible but they can be narrowed down by actual determination of one to three elements as baseline.

Class	*Agent*	*Color*[4]
Anthocyanidin		
	Pelargonidin	orange-red
	Cyanidin	purplish-red
	Delphinidin	bluish-purple
	Peonidin	rosy red
	Petunidin	purple
	Malvinidin	
Flavonol		
	Kaempferol	ivory cream
	Quercetin	cream
	Myricetin	cream
	Isorhamnetin	
	Larycitrin	
	Syringetin	
	Luteolin	yellowish
	Agipenin	Cream
Carotenoids		
	Carotene	orange
	Lycopene	Orange-red

We now summarize the other element classes.

2.2.2.1 Carotenoids

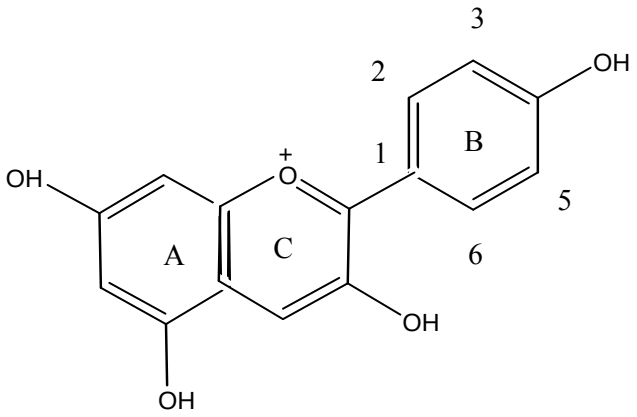
Carotenoids are what is quite common in the carrot, the orange hew we see in that root. Its molecular structure is shown below, this is beta carotene.



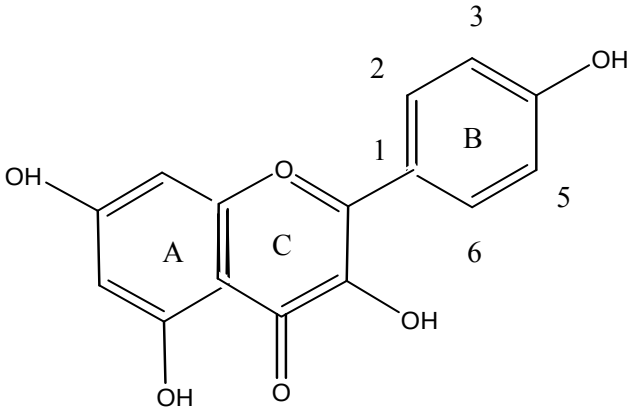
[4] See Taiz p. 334 for the anthocyanidin color and Bernhardt for the flavonol and carotene.

2.2.2.2 Flavones

The flavonols or flavones are quite similar to anthocyanin. Their structure is shown below. Note that we have compared it to that of anthocyanin.



Anthocyanidin



Flavonol

We can also show how closely they relate in substitutions and colors. This is shown in the Table below.

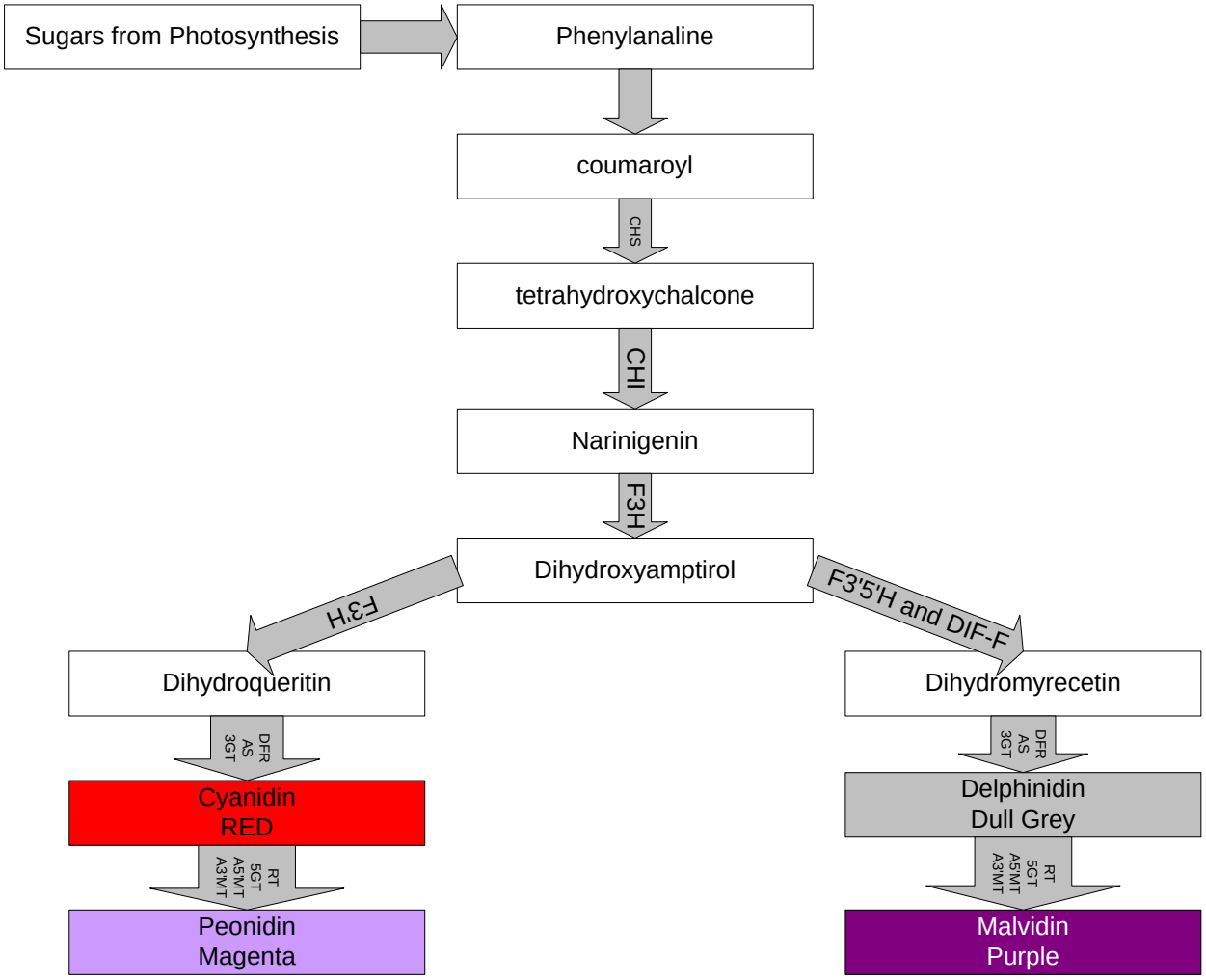
Flavonol	Anthocyanidin	Substitution	
		3’	5’
Kaempferol	Pelargonidin	H	H
Quercetin	Cyanidin	OH	H
Myricetin	Delphinidin	OH	OH
Isorhamnetin	Peonidin	OCH_3	H
Larycitrin	Petunidin	OCH_3	OH
Syringetin	Malvinidin	OCH_3	OCH_3

2.3 Pathways

In this section we present the pathways for the three classes we have described above. We first present an overview of the pathway and then we present the details of the pathway and the enzymes used in each step. The key observation is that we must have enzymes to go from step to step in the pathways and that if any one enzyme is missing we cannot proceed on that path, and further the path with the small amount of enzyme becomes the limiting path. Thus, we do not have a one to one map here. The production of any one anthocyanin, for example, if limited by the lowest produced enzyme, and the other enzymes may be present in abundance.

2.3.1 Anthocyanin Pathway

The anthocyanin pathway with the controlling enzymes is shown below. The enzymes are presented in the arrows linking each step in this pathway. This pathway shows the start as a sugar element and then goes to phenyanaline and then down through the chain to one of the four indicated anthocyanins. Also depicted are the gene products, enzymes, which modulate these pathways.

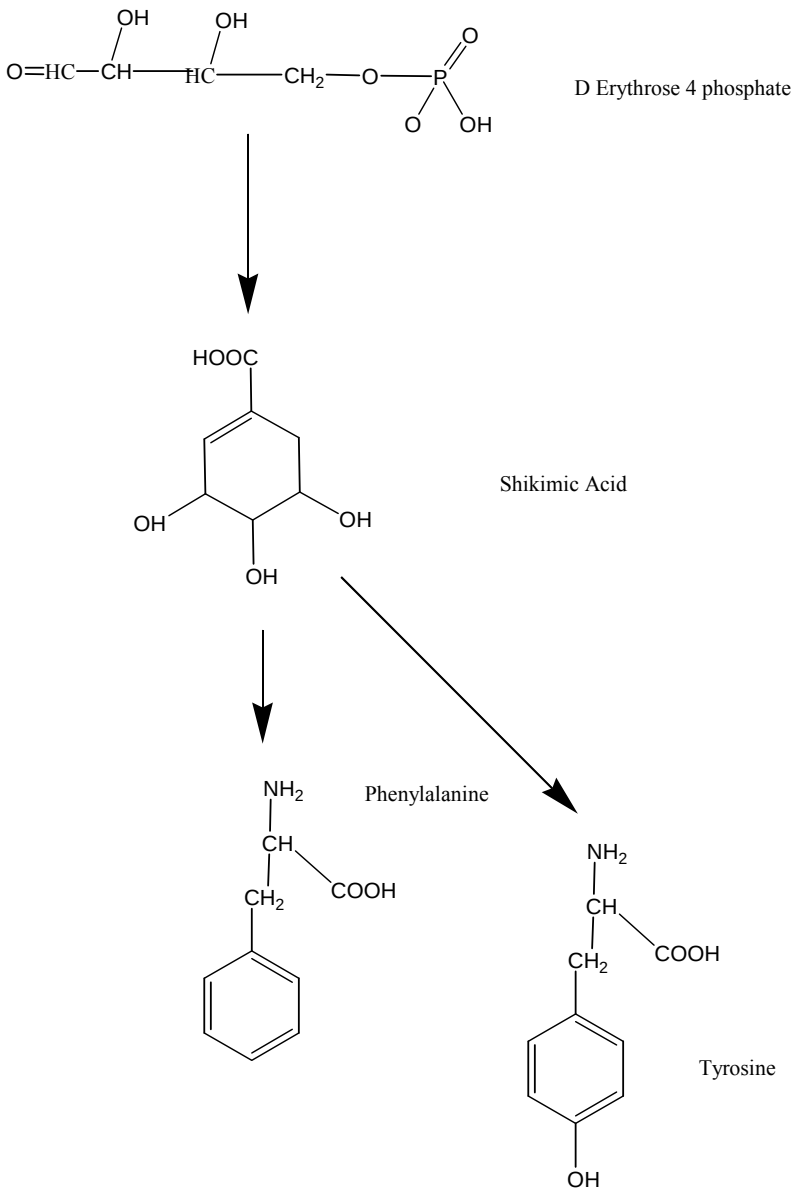


Note that at each step there is an enzyme element. The genetic loci for cloned flavonoid enzymes in Arabidopsis are shown in the following Table.[5]

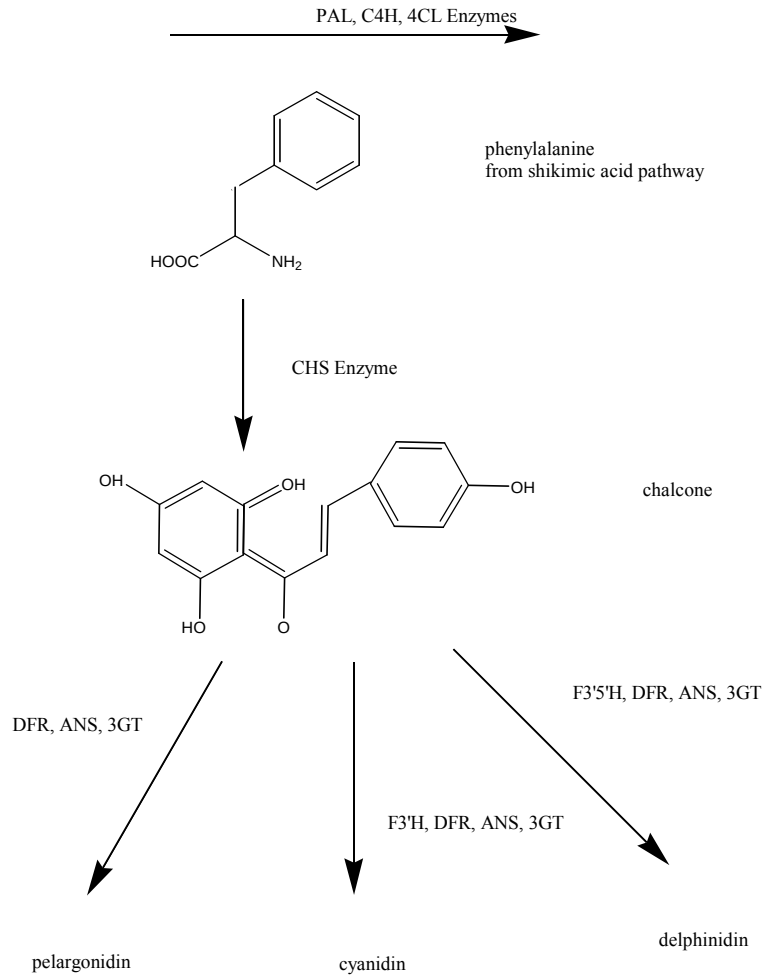
[5] See Similar information for maize, petunia, and snapdragon is described by Holton and Cornish (1995). b Based on the AGI map, 11/12/00; numbers in parentheses refer to P1 or bacterial artificial chromosome clones on which these sequences reside. c Transposon- tagged mutant for FLS1 (Wisman et al., 1998).

Enzyme	Locus	Chromosome	Map Position
CHS	tt4	5	7,050 kb (MAC12)
CHI	tt5	3	21,000 kb (T15C9)
F3H	tt6	3	19,600 kb (F24M12)
F39H	tt7	5	4,400 kb (F13G24)
FLS	fls1<Enc	5	FLS1: 4,700 kb (MAH20) FLS2-5;: 32,150 kb (MBK5) FLS6: 24,350 kb (MRH10)
DFR	tt3	5	23,800 kb (MJB21)
LDOX	tt19	4	16,900 kb (F7H19)
LCR	ban,ast d	1	26,800 kb (T13M11)

The pathway for the conversion of the sugar erythrose to penylanaline is shown in the reaction below. This accounts for the upper part of the pathway which we have shown. It uses the Shikimic pathways which we have shown in the initial discussion on the pathways.



The conversion details from pehnyanaline through chalcone to the anthocyanins are shown in the reaction below. We have reiterated by transition the enzymes which facilitate each step in this process.



What these process point out can be summarized as follows:

1. There are common pathways which are operational in all plants for the generation of the pigments.

2. Enzymes used as activators modulate the amount of production of the enzymes.

3. The products of these pathways, the anthocyanins, are driven by the concentration of the facilitating enzymes.

Secondary products always have this type of production process. As we look at a cell, from a system point of view we see facilitating proteins and secondary products. The concentration of the secondaries is proportional, in some general way, to the concentration of the facilitating proteins. However we see there are many facilitating proteins which may make this a more complex analysis, however doable.

2.3.2 Carotenoid Pathway

We have shown the carotenoids as above. The carotenoid pathway is shown in Taiz et al p. 321. The pathways and the facilitating enzymes in many ways appear identical to the anthocyanin pathway and the facilitating enzymes.

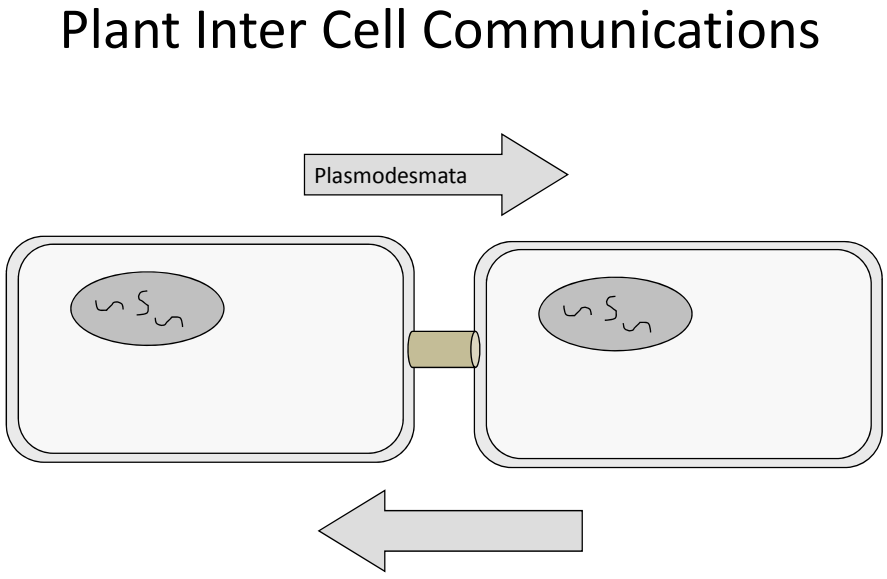
2.3.3 Flavonol Pathway

The flavonol pathway is identical to that of the anthocyanin. See Winkel-Shirley for details.

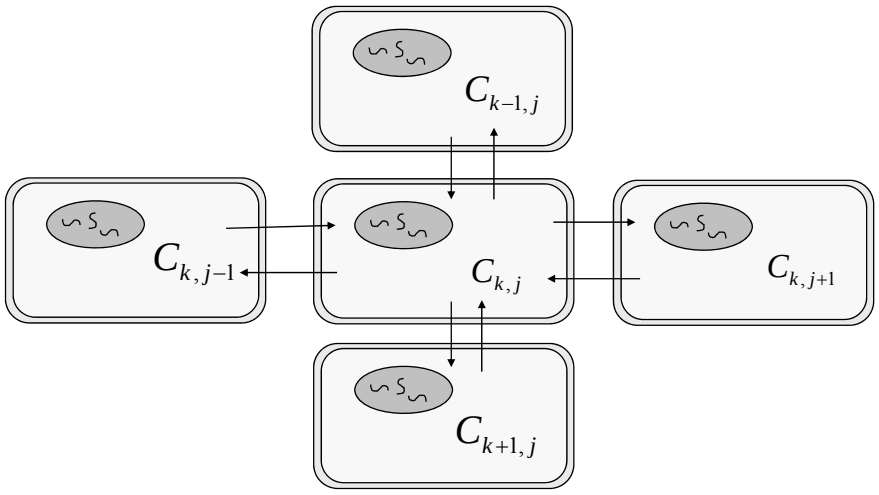
3 PLANT CELLS AND INTERCELL COMMUNICATIONS

The ability of plants cells to communicate between and amongst each other is a very unique feature. The cells do so via an organelle called the plasmodesmata. There are hundreds if not thousands of these small tubules between the cells and the results of the cell metabolism can enter into and out of these small tubules. The tubules are modulateable so that the flow rates can be changed. In effect, the cellulose wall is a barrier and the plasmodesmata are the points for cell content diffusion.

3.1 Plasmodesmata



Thus between all adjacent cells there is a complex flow of cell products, including both proteins and secondary products, via the plasmodesmata. This is shown below.



3.2 Flow Rates and Models

4 REACTION KINETICS

Reaction kinetics is a powerful set of chemical dynamics that make the cell function. In this section we review several of the models for reaction kinetics including the Michaelis-Menten model[6]. To start we know that there is a change in a concentration C of some substance and that the concentration may be resulting from one or several competing processes. The following is a general statement of that result;

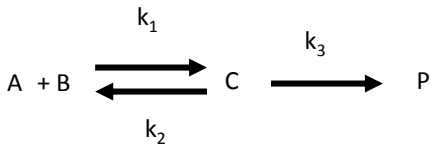
$$\frac{dC}{dt} = [Synthesis] - [Degradation] - [Phosphorylation]$$
$$+ [Dephos] - [Binding] + [Release] + etc$$

4.1 Enzyme Reactions

[6] See p. 111 Murray.

Now we must provide some specifics to this model to determine its form in detail. The Figure below depicts a typical reaction. This shows how the rate of reaction occurs.

Basic Reaction I



C is the intermediate. Assume that the first reaction is in equilibrium. Assume first reaction is so fast and the second is so slow that first is in equilibrium. Then:

$$K = \frac{[C]}{[A][B]}; \quad and \quad K = \frac{k_1}{k_2}$$

$$\frac{d[P]}{dt} = k_3[C] = k_3 K[A][B] = k[A][B] \quad where \quad k = \frac{k_1 k_3}{k_2}$$

Now if we assume that we have the same reaction but there exists a constraint on production, the change in the reaction dynamics is as shown below.

$\beta] - k_2[C] - k_3[C]$

Basic Reaction II

look at the steady state, derivative equals zero;

Now consider same reaction, but do NOT assume that the first reaction provides an unlimited amount and assume that there is some form of competition. Then we have:

We will use these basic concepts in the following. We begin with the Michaelis-Menten model and its reactions...

4.2 Michaelis- Menten Model

Let us begin with a simple enzyme reaction. We start with a source S and a product P with an enzyme E. The reaction is as fo**l**lows:

We first note that this reaction is what controls the production of an anthocyanin in the secondary pathway. Namely E is the enzyme and P the resulting product. However, one can also state that the same reaction would occur when the activator and repressor genes produce their proteins and they then modulate the up or down production of the target gene, the one producing E. The intertwining of all of these control and modulation processes is essential if we are to understand the coloring of the flower. Thus, we need to understand this reaction since when we look at plant colors we are effectively looking at anthocyanin concentrations, and more importantly the target protein, enzyme or gene productions controlling the secondary pathways. If we were to focus on say the protein E, then the more E the more P. Conversely the less E the less P. This domain is where we must look. In most enzyme reactions we have always assumed that E was excessive.

That E could not be exhausted and that given the available E the reaction as above proceeds. This may not be the case here, thus the range of evaluating the solution must be somewhat expansive.

Note that in an enzyme reaction the enzyme E facilitates the reaction and does not end up in any way being part of the product. In fact the enzyme has remained intact at the end of the reaction. Thus the enzyme concentration between the free enzyme and bound portion remains constant. That will be a critical fact in modeling this reaction.

We denote the following as measure of concentrations for this reaction:

s=[S], e=[E], p=[P], c=[SE]

for the respective concentrations. We can now, from the law of mass action, write four equations for the four concentrations. They are as follows[7]:

$$\frac{ds}{dt} = -k_1 es + k_{-1} c$$
$$\frac{de}{dt} = -k_1 es + (k_{-1} + k_2) c$$
$$\frac{dc}{dt} = k_1 es - (k_{-1} + k_2) c$$
$$\frac{dp}{dt} = k_2 c$$

we assume that the initial conditions are as follows:

$$s(0) = s_0, e(0) = e_0, c(0) = 0, p(0) = 0$$

From the above differential equations we note the following:

1. p(t) can be calcula6ted if c(t) has been calculated

2. If we add the equations for e and c we find that:

$$\frac{de(t)}{dt} + \frac{dc(t)}{dt} = 0$$

Thus we are left with two differential equations:

[7] See Murray p. 310.

$$\frac{ds}{dt} = -k_1 s + (k_1 + k_{-1})c$$
$$\frac{dc}{dt} = k_1 e_0 s - (k_1 + k_{-1} + k_2)c$$

Remember in this notation c is the bound enzyme and source combination, namely SE in the center of the total reaction. Now we want to solve these two equations for s and c. This will yields the results also for e and p since they are as defined as above. We follow Murray by now normalizing the equations. We also note that Murray calculates the change in S not the change in E. Our focus is the change in E over time and space, from that we can obtain the change in S as well. We note from above that we could just as well use the two equations:

$$\frac{ds}{dt} = -k_1 s + (k_1 + k_{-1})c$$
$$\frac{de}{dt} = -k_1 e_0 s + (k_1 + k_{-1} + k_2)c$$

and then solve for s and e. Now for the normalizations we define:

$$\tau = k_1 e_0 t, u(\tau) = \frac{s(t)}{s_0}, v(\tau) = \frac{c(t)}{c_0}$$
$$\lambda = \frac{k_2}{k_1 s_0}, K = \frac{k_{-1} + k_2}{k_1 s_0}, \varepsilon = \frac{e_0}{s_0}$$

Note that we have use ε as the ratio of initial enzyme to initial source. The use of this will become clear since we are assuming that this is small number since the enzymes concentration is small as compared to the source. This assumption must be looked at closely for this reaction. Then we obtain the following set of equations:

$$\frac{du}{d\tau} = -u + (u + K - \lambda)v$$
$$\varepsilon \frac{dv}{dt} = u - (u + K)v$$
$$u(0) = 1$$
$$v(0) = 0$$

These are nonlinear differential equations which we must solve. However the equations have a singularity which must be dealt with before proceeding. If we want to make the ratio ε small then we need to redefine certain factors. We do this as follows:

$$\sigma = \frac{\tau}{\varepsilon}$$
$$u(\tau, \varepsilon) = U(\sigma, \varepsilon)$$
$$v(\tau, \varepsilon) = V(\sigma, \varepsilon)$$

which yields:

$$\frac{dU}{d\sigma} = -\varepsilon U + \varepsilon(U + K - \lambda)V$$
$$\frac{dV}{d\sigma} = U - (U + K)V$$
$$U(0) = 1$$
$$V(0) = 0$$

This transformation eliminates the singularity about 0. The steady state can be shown as follows:

$$[ES] = \frac{k_1}{k_2 + k_3}[E][S]$$

now let the total enzyme be as follows:

$$[E] + [ES] = [E]_0$$

since only small amount enzyme added, the free free substrate
is almost the total substrate, eg S, then;

$$[ES] = \frac{k_1[E]_0[S]}{k_2 + k_3 + k_1[S]}$$

and we can show that the steady state implies:

$$\frac{d[P]}{dt} = k[E]_0 \text{ where we have } k = \frac{k_3[S]}{K_M + [S]}$$

and we define:

$K_M \frac{k_2 + k_3}{k_1}$ *as Michaelis constant*

The above defines the Michaelis-Menten uptake formula.

There are many other types of reactions and we have discussed them elsewhere. The issue we want to focus on here is that the enzyme is in a reaction of the form where we have a definable

time change of enzyme based upon a definable model. That is if [C] is the concentration of an enzyme involved in an enzymatic model we will have a reaction of the type:

$$\frac{\partial[C]}{\partial t} = F([C], t)$$

where F is definable by the reaction. The function F may also consider concentrations of the reactant source and product materials as well. We will use this model in the next section/

5 PETIT PATTERNS

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.

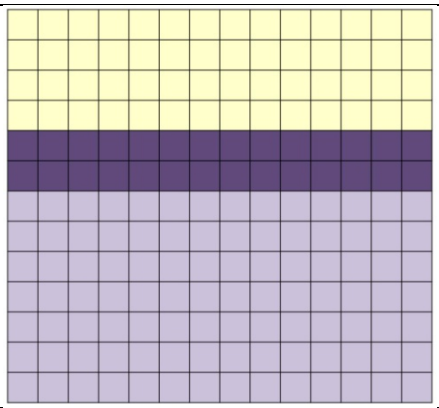
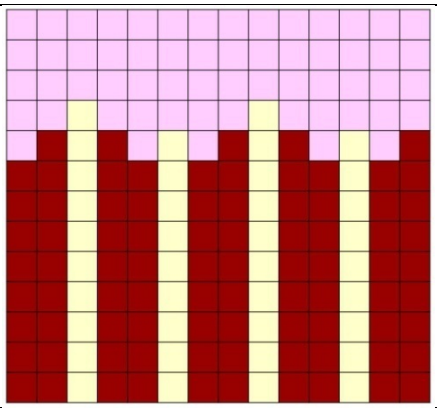
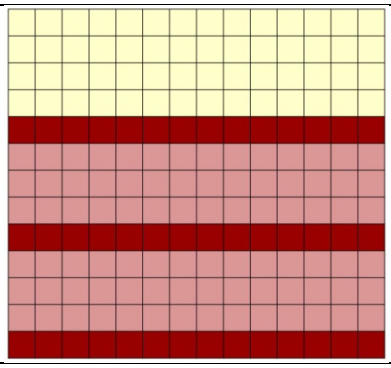
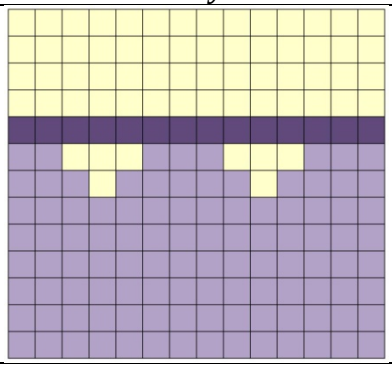
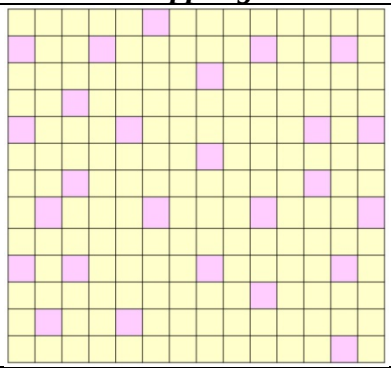
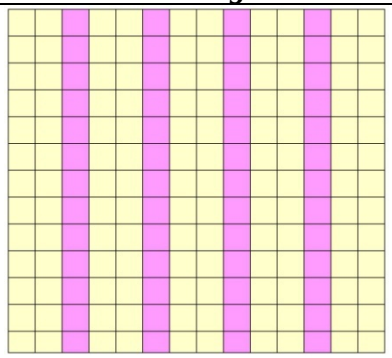
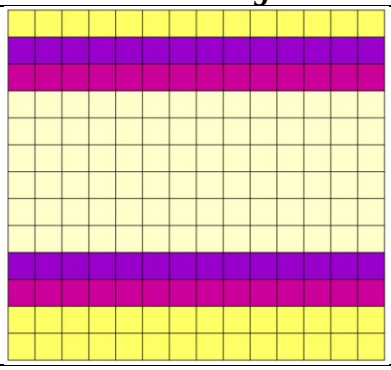
Ted Petit, in the Daylily Journal of Summer 2007, described a multiple set of patterns that breeders were producing. He found the following to hold:

- 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	
Narrow Formed	Not Applicable
Others	Not Applicable

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.

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.

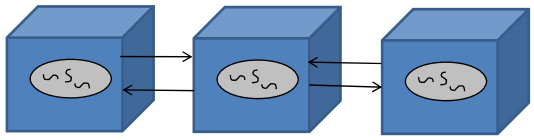
6 TURING-MURRAY MODEL

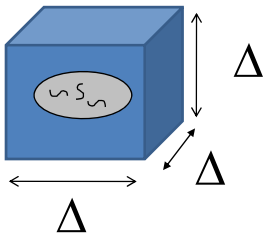
In this section we use the concepts of the Petit Patters and then combine them with the Turing model of morphogenesis to develop a verifiable and manipualatable system as regards to patters of the typ2 shown by Petit.

6.1 Patterning

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.

C is concentration of enzyme in the cell and the cell is at position x. Thus we note it as C(x). Adjacent cells diffuse their concentrations across into other cells and the target cell diffuses its out as well. The diffusion is proportional to the concentration C and a constant per unit cell wall area.



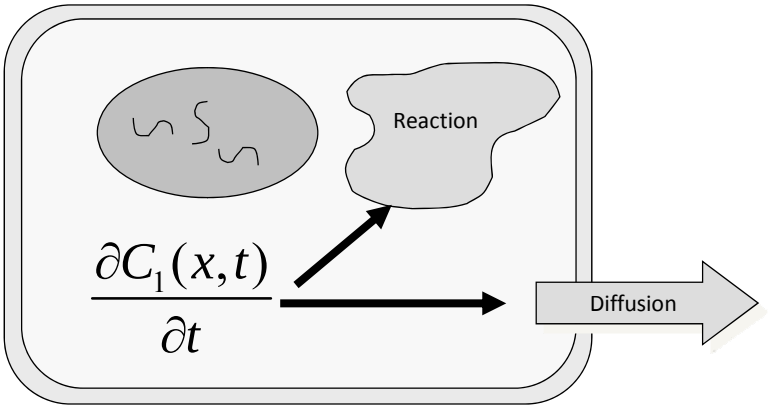


$$\frac{\delta C(x)}{\delta t} = F(C(x))$$
$$+kC(x-\Delta)+kC(x+\Delta)$$
$$-kC(x)$$
= Amount Lost to Reaction + Amount Flowing In - Amount Flowing Out

The following is a detailed description of what Turing proposed in his model.

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

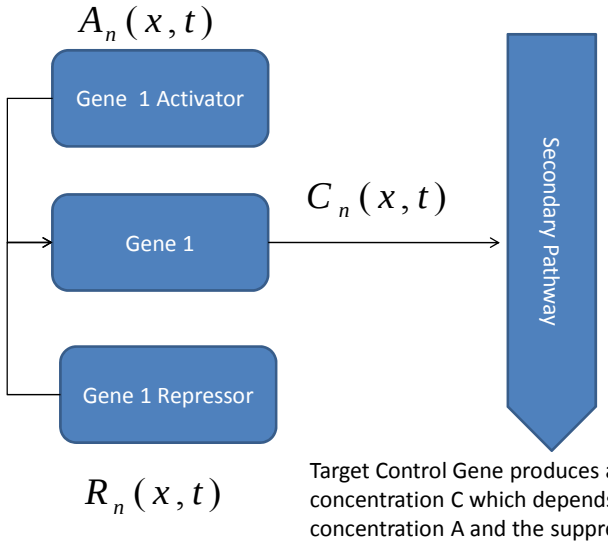
C_1 = concentration of protein or secondary c_1 in the cell



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

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.

Single Pathway Control



Target Control Gene produces a protein at concentration C which depends on the activator concentration A and the suppressor concentration S

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

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

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

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

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:

1. A model for the enzyme reactions in a competitive environment.
2. A method to solve for the Turing space the diffusion model
3. A model to apply the results to a single anthocyanin
4. The ability to apply to multiple anthocyanin
5. The ability to determine the analysis and the synthesis problem

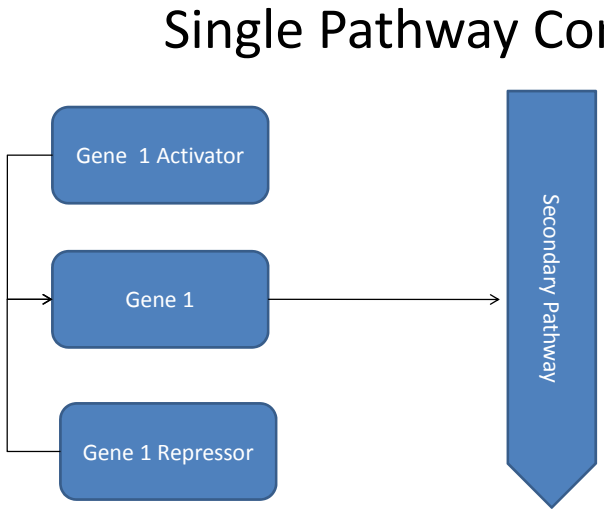
6.2 Turing-Murray Tessellation

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.



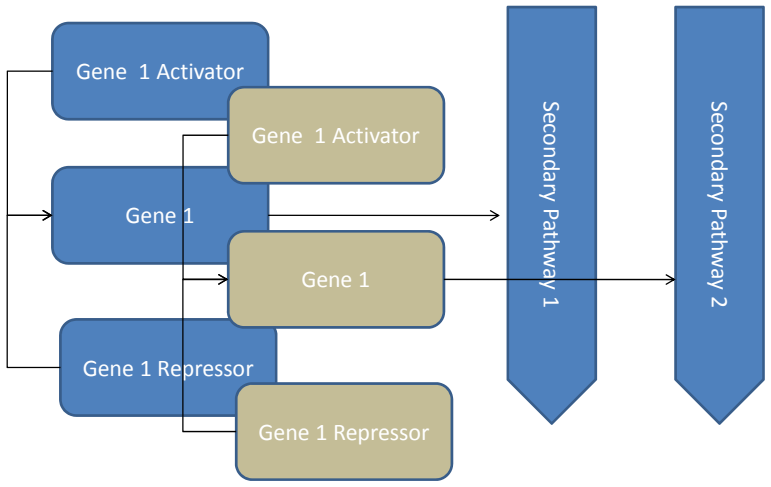
In the above pathway we have a complex but modellable set of interactions. They are characterized by:

- 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.

6.3 The Model

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:

$$H(P,S,SP) = [+k_1 PS - (k_{-1} + k_2)C]Q(A,R)$$

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:

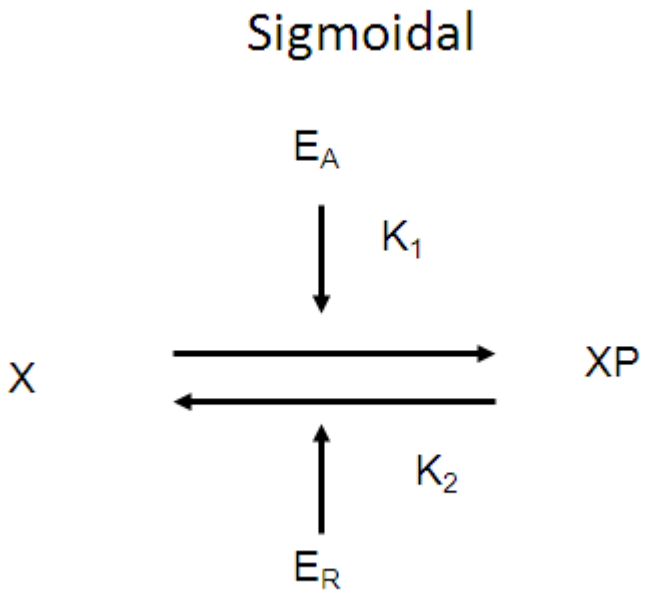
$$\frac{\partial A_n(x,t)}{\partial t} = F(A_n, R_n, x, t) + D_A \nabla^2 A_n(x,t)$$

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.

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

The above are also normalized concentrations.

6.4 Linearized Solutions

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.

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

The same now for the repressor except we have a different diffusion constant.

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

Finally for the controlling enzyme product:

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

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:

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

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:

$$\frac{\partial f}{\partial a}+\frac{\partial g}{\partial s}<0$$

$$\frac{\partial f}{\partial a}\frac{\partial g}{\partial s}-\frac{\partial f}{\partial s}\frac{\partial g}{\partial a}>0$$

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

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

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

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

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

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

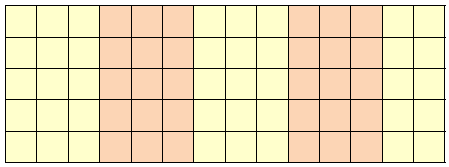
$$D(x)=1\text{sgn}(d(x))$$

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.

6.5 Pattern Examples

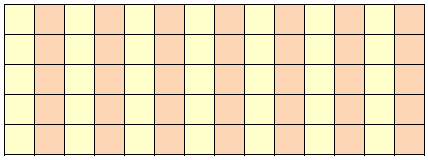
We now show several example of the solution to these equations. The following Table presents solutions determined via the approach in Murray. There have been many others over the years who have obtained similar results.

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.



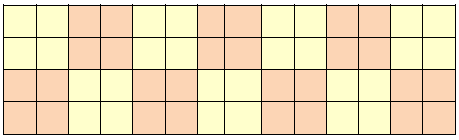
6/11/2008 Tessellated Patterns in Hemerocallis 63

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.



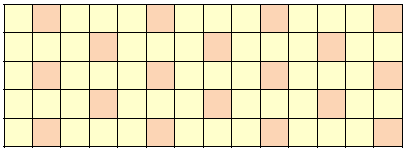
6/11/2008 Tessellated Patterns in Hemerocallis 64

Example 3: p. 393 Murray, low spatial frequency instabilities across the cells showing wide checkered variation. This also assumed a single unstable secondary pathway which was on or off. It assumed also another stable secondary pathway.



6/11/2008 Tessellated Patterns in Hemerocallis 65

Example 4: p. 393 Murray, high spatial frequency instabilities in two dimensions 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.



6/11/2008 Tessellated Patterns in Hemerocallis 66

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.

Figure 1 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 seen above. In effect this is the coherence pattern seen in interference optics.

7 CONCLUSIONS

This paper has demonstrated the Turing model is applicable to explaining the Petit Patterns. The Petit Patterns being generally recognizable pattern set provides a basis for validation of the model. The model has been assembles on experimentally verified and verifiable techniques and processes in the flower growth.

There are several issues worth exploring:

1. **Dynamic Growth Behavior of the Flower**: We have not included the dynamic growth factor of the flower. Namely when do these changes occur in the stage of development of a flower, when do the anthocyanins develop and is there a temporal factor which we should include. This is an interesting problem because it again goes back to the morphogenetic issues first raised by Turing.

2. **Analysis of Multiple Paths:** We have performed this analysis on multiple paths. That is a path for peonidin and a path for Malvinidin. We have also done this for two dimensional paths as well. The full complement of the Petit patterns is attainable and we believe that there are more patterns as well.

3. **Methods for Inversion from Pattern to Paths:** In our previous work we showed that is possible to go from the output to the input, the inversion problem, Namely from the colors produced we can find the gene paths producing them. These techniques can be directly applied to patterning.

4. **Experimental Analysis Methods:** In a prior paper we have shown how to experimentally separate the effects of different anthocyanins and thus reduce each problem to a single anthocyanin analysis or inversion problem. We have been using these techniques extensively.

5. **Methods for Genetically Engineering Specific Patterns:** The inversion problem is but one step. The design problem is another, in that the design problem stipulates a desired patter and the asks what should be the genetic makeup to effect that pattern. The techniques provided here and in previous papers details that effect.

We believe that this paper provides a stepping stone for additional work in this area.

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