ACHIEVING THE "BLUE DAYLILY": GENETIC CAPABILITIES FOR COLOR IN THE GENUS HEMEROCALLIS

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The color of flowers is a complex issue which has received a great deal of study for well over a hundred years. The presence of anthocyanins, carotenoids, and flavones has allowed us to understand where the color comes from, and in fact the complex quantum effects in the spin bands of these molecules are what we see when we observe color. The environment in which these molecules exist also changes the colors we perceive in a complex quantum environment. In this paper we examine the question of the ability to engineer a blue daylily. This begs two questions. First what do we mean by blue, and of course how do we measure blue to be certain we have what we want. Second, given the chemicals yielding the colors in a flower, what sets of combinations of such chemicals do we need to get the desired color. The third question implicitly asked is how can we achieve those concentrations, and we have left that answer for discussion in our other papers on the subject. Thus this paper seeks to define blue and to ascertain if such a definition can be achieved in nature with the pallets provided.

1 INTRODUCTION

There is a saying that states that if you cannot measure it and you cannot predict it then you do not know it. Macroeconomics is an example of something we can measure but cannot predict and thus one questions any knowledge of the subject, one need look no further than the day to day economy and the general chaos in suggestions and recommendations. Plant colors have often been descriptive in that we understand that anthocyanins yield colors but we all too often cannot measure the color and we definitely cannot not predict what will happen when we deal with either hybridizing or more so with genetic engineering of the plant. In fact to even use the term engineering implies both measurement and predictions. In this paper we address the determination of plant colors both in the context of measurement and in the context of predictions. Namely we seek to engineer color. We use the concept of the blue daylily as our model and seek to ask if such a plant is possible.

The approach we take is as follows:

First we review the fundamental physics of color. Simply it is the excitation of electrons into higher states by incident photons. However the simplicity is often not so apparent and thus the absorption is thus often spread over a band of wavelengths. In addition the center wavelength of the absorption is also very dependent upon the environment including such factors as the local pH as well as other compounds which may be in the cell.

Second we consider the issues related to the absorption or transmission of light through plant cells. This is a complex issue and we consider a simplified model. The complexities are often fortunately secondary factors in the dynamics of color perception but there are times when they may become dominant. We discuss those issues. Thus there are several factors which relate to the physics of color creation:

1. The physics of transmitting white light to a surface and then the reflection of that light off the surface.
2. The physics of white light being absorbed differentially at different wavelengths so that what is sent back has been shaped as a result of the absorption.
3. The physics of the combination of transmission, reflection, and reception
4. The chemistry of the specific molecules which absorb
5. The chemistry and physics of the specific molecules which absorb when they may be modified in an environmental change; temperature, pH, sunlight etc
6. The genetics of the control of the secondary pathways controlling the generation of the various color creating molecules. This latter elements we shall discuss later in the paper.

Third we then consider color from a colorimetry perspective, namely what do humans see when the see a color. Thus when we seek to have a blue flower what are we seeing, it is not a simple line spectrum but the compilation of the transmitted light, the absorption or transmission and the responsiveness of the retina in its ability to transform what is received into a stimulus which is perceived. Blue is ultimately in the eye of the beholder. Yet there must be some consistent and repeatable method to characterize color. That we discuss and we focus on the RGB format used in video monitors and in digital photography.

Fourth we then review why flowers are certain colors building on the physics and the colorimetry we have developed. The main factor is the presence of anthocyanins, carotenoids and flavones. We then address the following issues:

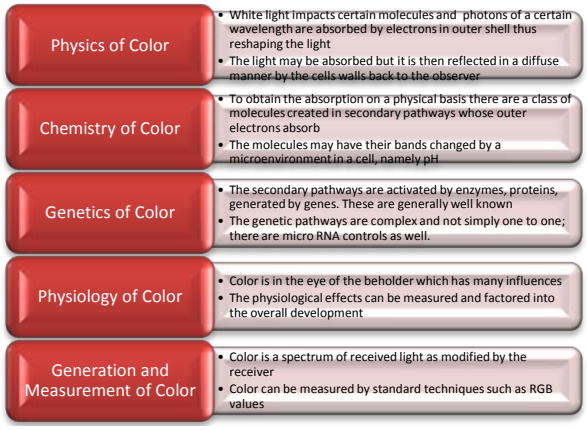
1. Why are flowers a certain color?
2. What are the various mechanisms which generate/control color.
3. What are the genetics of flower color?
4. How are the genetics modified by the environment?

Fifth we seek to define what is meant by blue. In the RGB world blue is simple 0,0,255. A blue dot. Yet how do we relate that to the anthocyanins and the colorimetry. We develop a model for that characterization and that relationship. We then examine in more detail the following:

1. What do we mean by blue?
2. Is there a genetic/environmental combination where one can achieve a specific/desired color, say blue?

Finally we develop a method by which we can seek to achieve as close to a blue color as we can by means of the existing plant pigments. Thus by knowing from our prior work what the genetic pathways are which generate the anthocyanins and their relative concentrations we can then determine the genetic makeup required to achieve a specific color. This is the embodiment of the reverse problem. We incidentally demonstrate the impossibility of achieving the ideal blue in terms of the 0,0,255 color. In fact we argue that blue as we would anticipate it is truly in the eye of the beholder.

We summarize the details of this paper in the following chart.

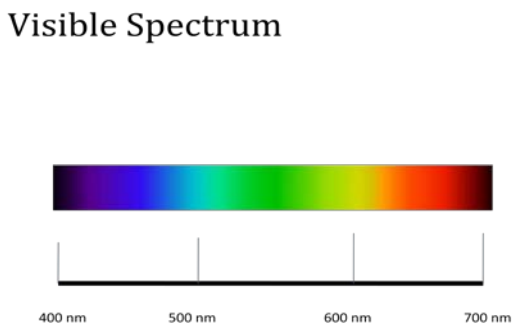


2 COLOR CHARACTERIZATION

We begin by examining colorimetry, the observation and interpretation of color by the human. This is fundamentally the field of colorimetry. Namely how do we measure color. How do we come to an agreement what blue is or red or any other color and how do we transmit that in a repeatable manner to other humans. Inherent in this approach is an assumption of human repeatability. Namely what I see and what you see are the same now and in the future. Thus if I were to select a blue sweater for you and I knew what blue you liked than if I bought it in September then in December when I gave it to you we both would have the same sensation of blue, as was the dyes in the sweater. Needless to say this is a non trivial problem.

2.1 The Visible Spectrum and the Human

Let us first start with the visible spectrum. We describe it as below. We assign to each wavelength a certain color. In reality that is not what we see. We see through our eyes and the rods and cones on the retina respond in a more integrative manner.



What we see is an integrative effect on three different color stimulus sensors in the eye. In an attempt to simulate this physiological effect and at the same time simplify the definition of a specific color, the Tristimulus approach was taken, as described by Berns, and this provides functions which vary over wavelength on a given spectrum so that when integrated these three integrated and weighted values define the color, namely color becomes a quantitative triplet.

The initial approach is to use red, green and blue stimulus functions but for certain reasons they become complex and require negative values. In a substitution mode the Tristimulus values are converted to the XYZ domain, with a simple conversion available.

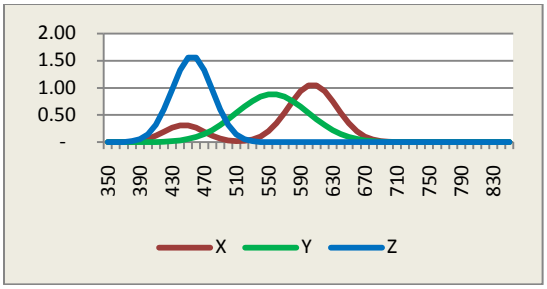
A simple way to solve this integrative color problem has been to use functions obtained via an agreed to experimental process then combined with the Tristimulus functions. That is, there are three functions, x, y, z, which are wavelength dependent. If we were to multiple and then integrate the received color intensity I times the x, y, or z over all wavelengths and then determine three constants, X, Y, and Z, then we say two colors are the same if the X, Y and Z are the same. We take some spectrum of light, say $I(\lambda)$, and then we weight it by these functions, integrate and obtain a number. Here we define them as:

$I(\lambda) =$ *the spectrum of a specific sample*

Define

$$X = \int I(\lambda)\bar{x}(\lambda)d\lambda$$
$$Y = \int I(\lambda)\bar{y}(\lambda)d\lambda$$
$$Z = \int I(\lambda)\bar{z}(\lambda)d\lambda$$

The following chart depicts these XYZ functions.



Also we should note that these functions are chosen so that:

$$\int x(\lambda)d\lambda = \int y(\lambda)d\lambda = \int z(\lambda)d\lambda = 1$$

Furthermore we can normalize the XYZ values so that they sum to one. We do this as follows:

$$x = \frac{X}{X+Y+Z}$$
$$y = \frac{Y}{X+Y+Z}$$
$$z = \frac{Z}{X+Y+Z}$$

and

$$x + y + z = 1$$

The reason for this is that if we have say x and y, then by definition we have z. Now in a similar manner we can define a r, g, and b set of constants which we would then may use in a computer monitor for example. The rgb, or equally the RGB values were the initial Tristimulus functions but the corresponding

$$\begin{bmatrix} r \\ g \\ b \end{bmatrix} = A \begin{bmatrix} x \\ y \\ z \end{bmatrix}$$

A such that sums of r,g,b and x,y,z are unitary

We in a similar manner define r, g, b, as the normalized values of R, G, and B defined as below.

$I(\lambda) =$ *the spectrum of a specific sample*

Define

$$R = \int I(\lambda)r(\lambda)d\lambda$$
$$G = \int I(\lambda)\bar{g}(\lambda)d\lambda$$
$$B = \int I(\lambda)b(\lambda)d\lambda$$

where we define rgb as:

$$r = \frac{R}{R+G+B}$$
$$g = \frac{G}{R+G+B}$$
$$b = \frac{B}{R+G+B}$$

and

$$r + g + b = 1$$

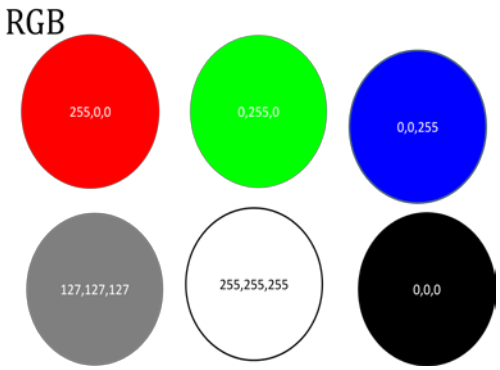
The color matching primaries of rgb show negative values because the negative was the way the CIE arbitrarily represented an excess positive contribution required to be added to a primary to achieve the desired spectrum response while keeping the elements normalized. Also as with the xyz values we have"

$$\int \bar{r}(\lambda)d\lambda = \int \bar{g}(\lambda)d\lambda = \int \bar{b}(\lambda)d\lambda = 1$$

One final point, we have colors which are additive and those which are subtractive. Additive Colors combine to form white. Traditionally adding lights is additive whereas adding colorants, pigments or dyes, is subtractive. Subtractive Colors form black. Subtractive mixing involves the removal, subtraction, of light from the mix. Removing all light ultimately results in black. Absorption only is called simple subtractive mixing whereas combining this with scattering is complex subtractive mixing.

2.2 RGB Computer Standard

In computer use we have the RGB system where this is the red, green. blue color emitters on the screen. This has carried over to a great many similar systems such a digital photography. We define certain color by their RGB numbers as shown below:



Note that in the RGB world we have blue as 0,0,255 and we have black as 0,0,0. That is no light at all is black. Full light on each emitter is white. Halfway is grey. This is the world of computers and computer related sensing of color. The RGB values are determined from the spectrum and the r, g, b functions which weight that spectrum. The quantized levels of 0 to 255 are then also determined by a quantization metric which we shall discuss now.

To convert from rgb to xyz we use the following[1]:

$$\begin{bmatrix} x \\ y \\ z \end{bmatrix} = M \begin{bmatrix} r \\ g \\ b \end{bmatrix}$$

where:

M=

3.2405	(1.5371)	(0.4985)
(0.9693)	1.8760	0.0416
0.0556	(0.2040)	1.0572

whereas to convert from xyz to rgb we use the following:

$$\begin{bmatrix} r \\ g \\ b \end{bmatrix} = M^{-1} \begin{bmatrix} x \\ y \\ z \end{bmatrix}$$

where:

M^{-1}=

0.41	0.36	0.18
0.21	0.72	0.07
0.02	0.12	0.95

Now to convert from rgb to RGB quantized from 0 to 255 we used another function described by Berns[2]:

$$R,G,B = \left[1.02 \left(\frac{d_{r,g,b}}{255} \right) - 0.02 \right]^{2.4}$$

or

$$d_{r,g,b} = \left[\frac{255}{1.02} \right] \left[0.02 + \left(R,G,B \right)^{1/2.4} \right]$$

Thus given the RGB value we can invert the above and determine the RGB quantized values, called here the d values. Note that the above uses the RGB and not the normalized rgb values. Also not that we will use the xyz Tristimulus values when calculating since it is easier to do so in spreadsheet forms.

The following depicts some values of this function for n, where n is r, g, or b, and the corresponding 0 to 255 value.

[1] Reinhard p 416.

[2] Berns p 169.

n	N
0.05	76.75
0.10	100.78
0.15	118.41
0.20	132.85
0.25	145.31
0.30	156.38
0.35	166.42
0.40	175.66
0.45	184.24
0.50	192.29
0.55	199.88
0.60	207.07
0.65	213.92
0.70	220.48
0.75	226.76
0.80	232.80
0.85	238.63
0.90	244.26
0.95	249.71
1.00	255.00

Thus by the means discussed in this section we can take any color spectrum and define it in RGB values from 0 to 255. The principle conclusion then is that almost all humans will agree on a color in this triplet form, (R,G,B).

3 FUNDAMENTAL PRINCIPLES OF COLOR

We start with a short discussion of the fundamentals of the physics of color. Color has two distinct dimensions; the physical, namely what causes color in the real world of photons and spectra, and the biological, namely what causes the perception of color in the human so that we can all agree on what we mean by a certain color. The human aspects are often subjective yet can be agreed upon in a consistent manner, all one has to do is look at the glossy magazines and the video which surrounds humans every day.

3.1 Photons and Absorption

Understanding light means dealing with the fundamental back and forth of waves and particles. When considering absorption we deal initially with particles since absorption is a complex quantum phenomenon.

Let us begin with a few simple facts:

1. The photon is basic element of light
2. Light is composed of photons of many wavelengths and thus many colors
3. Photons can be absorbed by molecules by means of exciting electrons in the outer rings
4. The photon is then absorbed and the light is then changed by loss of that particular "color"
5. What is seen then is what it sent back to the eye, and that process is key

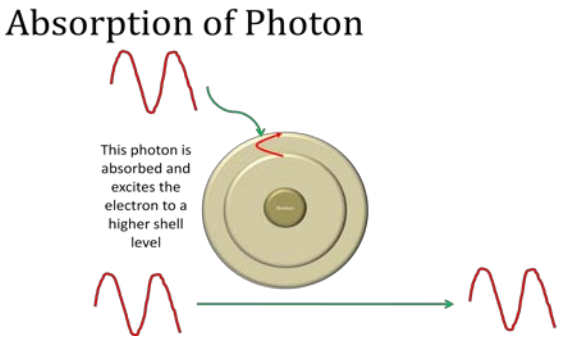
The photon has certain key physical properties which we should remain familiar with. The energy in a photon is:

1. $E=hf=h/\lambda$; f is the frequency, λ the wavelength and h Planck's constant ($h=6.625 \times 10^{-34}$ joule-sec)
2. also $c=\lambda f$ where c is speed of light
3. Light is composed of many photons of all levels of energy
4. White light has an equality of "density" of photons at all λ or f.

Similarly we have for light and photons:

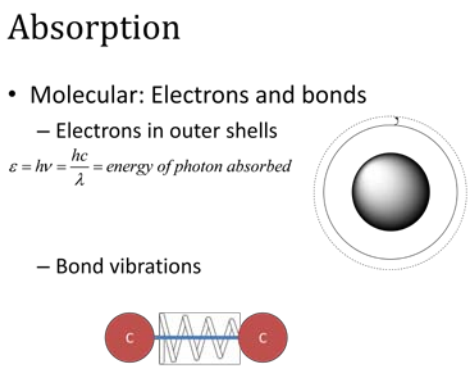
1. Optical visible spectrum wavelength (λ) is between 380 to 780 nm, $380\ 10^{-12}$ to $780\ 10^{-12}$ m or $0.38\ 10^{8}$ m to $0.78\ 10^{15}$ m
2. $c=3\ 10^{15}$ m/sec
3. f (c/λ) is from $7.9\ 10^{-34}$ Hz to $3.8\ 10^{15}$ Hz
4. E (hf, $h=6.625 \times 10^{-19}$ joule-sec) is from $52.4\ 10^{-19}$ to $25.2\ 10^{-19}$ joule

In simple terms a photon in a stream of photons at some wavelength impacts an electron and if and only if the energy of the photon, directly related to its wavelength, is identical to the band energy allowable by the electron to change bands, or spin state, then it may be absorbed and taken from the stream. We depict that below.



If the photon is not of the exact energy it passes through and is not absorbed. A second mechanism for absorption is exciting bond vibrations and especially the double bonds we see in many of the anthocyanin pigments. This

resonance absorption is another means for removing or absorbing electrons. We depict this below.



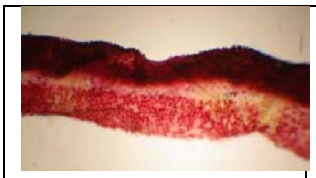
3.2 Cellular Effects and Absorption

The propagation of light through cells, especially plant cells, is often a complex process. We will attempt to simplify it and avoid some of the difficulties which for the most part are secondary effects. The absorption we discussed above is the absorption of a photon by a single electron. There are many such electrons and the absorption is occurring across a wide band of wavelengths not just the optical band. We focus here solely on optical absorption.

We will consider two specific phenomenon; transmission and reflection. Transmission is the passing of light from one side of the cells to the other and what we examine for its spectral characteristics is the light on the side after it has passed. In reflection we look at what has been transmitted a bit but then reflected. A perfect reflector such as a mirror will send back what was transmitted, with no change in the spectrum. In contrast a plant cell can reflect light off the walls of the cell or other such reflecting surfaces while at the same time absorbing the light as it passes through to the reflecting surface and then as it returns back to the surface and out beyond the cell again.

In the following figure we show an example of transmission and reflection. The first is a microscope analysis looking at a thin slice of the petal with "white" light coming from below. The light goes through s small layers of cells which absorb certain bands
Reflection

The second is an example of the same petal but as see in the light of day. The light impinges upon a top layer of cells and gets reflected back up to the viewer passing through layers of cells and color absorbent molecules. Note the difference in color. In the transmission there is a thin layer of cells, one or two, whereas in the reflection there is a deep layer of cells.



This is a transmission view, namely light is on the opposite side as compared to reflection view. The top layer is what we see on top of petal. This is H Halloween Cat	This is a reflection view, namely light impinges on top surface and begins to penetrate cell layers. This is H. Halloween Cat

Now we can look at a bit more detail in the cell. But first a simple analysis of the absorption phenomenon in gross terms. We assume that light can be measured by means of a power spectrum, and furthermore, white light has a spectrum which is flat, unit amplitude, over the visible band. This white light is then shined upon a collection of plant cells, we assume that such cells have a single anthocyanin, namely cyanidin, in the cell, and that there is either a reflection or transmission which permits a power spectrum measurement on the receiving side. We assume at this point that there is no human involved.

The chart below depicts the bands and the color and their complements.

Colored Organic Materials

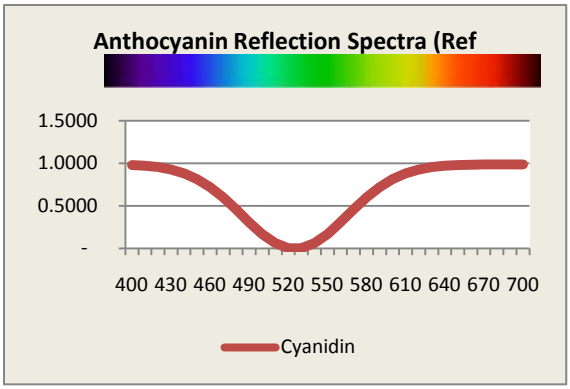
- Light of a specific wavelength is perceived as the "color" on the chart
- If the wavelength is absorbed from white light its is perceived as the complement.
- Absorptions are generally π=>π*
- If the absorption band is narrow or sharp the color will be bright, brilliant and clean. A broad band will yield a muddy color.
- See: Streitwieser, Organ Chem (1992), pp 1224-1225.

Band nm	Color as perceived with the wavelength	Complement perceived with wavelength removed
400-430	violet	green-yellow
430-480	blue	yellow
480-490	green-blue	orange
490-510	blue-green	red
510-530	green	purple
530-570	yellow-green	violet
570-580	yellow	blue
580-600	orange	green-blue
600-680	red	blue-green
680-750	purple	green

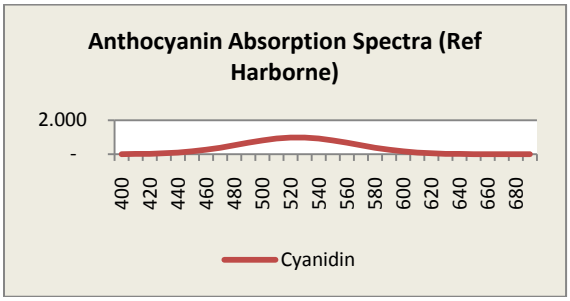
3.3 Absorption Spectrum

We thus measure the received spectrum as below. Recall that the incident light is unity in its power spectrum from 400 nm to 700 nm, and that we receive the spectrum as

shown below. The maximum is unity and the minimum occurs at a certain wavelength and it has an effective width which may be defined by say a 50% point on either side. We will get into the detail on this later in the discussion.



We now assume that we can then invert this to define an absorption curve, as shown below. The absorption curve may be relative, namely the amount absorbed will depend on thickness of the path, absorption characteristics and density of the substance. We shall return to that later. Thus the absorption curve shown below has a peak which may be normalized and it is a result of the absorption as shown above.



Now to the absorption issue. There is a blending of the wave and particle issue of light which are concerned here. At the particle level, the photons hitting the absorbing molecules tend to moves electrons to higher energy levels and it is this quantum issue which results in the loss of photons from the white light. The wave issue relate to the transmission and subsequently to possible reflection of light within the cell. In the case of transmission we look at transmission through cell walls, through the water in a cell and the absorption of light by the molecules.

The amount of absorption is a key issue. The thicker the sample, that is the more cells involved, and the more dense the absorbing molecules per cell, will drive the absorption. Consider an incident energy I(λ) where λ is the wavelength. It enters a cell and the amount coming out on an incremental basis is determined by:

$$I(\lambda, x+\delta) = I(\lambda, x) - \alpha(\lambda) I(\lambda, x) dx$$

or

$$\frac{dI(\lambda, x)}{dx} = -\alpha(\lambda) I(\lambda, x)$$

yielding

$$I(\lambda, x) = I(\lambda, x_0) \exp(-\alpha(\lambda)(x - x_0))$$

This is the Bouger, Beer, Lambert law of absorption[3]. Simply the total absorption depends on the absorption coefficient and the depth of the absorption layer. Thus the absorption curve we have shown must be reflective of that and in fact the curve should be the curve of α(λ) and not the power or energy level. Namely we should be concerned about α(λ) and not about the received signal. This absorption term is thus derived as a constant independent of depth and dependent only on wavelength.

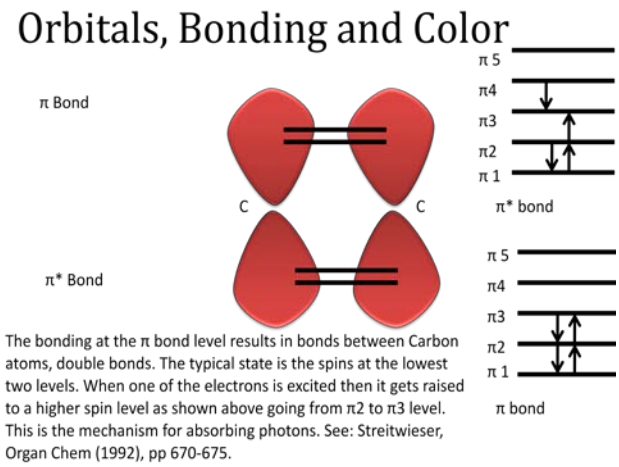
Thus the total absorption at any wavelength will depend upon total depth, whether it is one way or two way depth, direct or reflected.

It is the differential absorption which yields different colors. With Cyanidin, we take out the central part of the spectrum and we yield the end, with emphasis on reds, and thus we see red. What the absorber removes is what is take out and the remainder is what we see. However as we shall show what we see is also dependent upon our visual system and that is perceptual.

As Streitwieser et al state it is the π -> π* transitions which give rise to the absorptions[4]. Specifically the longer the chain of conjugation the longer the wavelength at which the maxim absorption will occur. A narrow band gives sharp colors and a broad band gives muddy colors. As Streitwiser also states the colors will depend upon the pH of the medium. Both the cornflower and the poppy have the same cyanidin, cyanin, and one is blue and the other red. The blue results from a potassium salt and the red is absent the potassium. Three anthocyanidins are important; cyanidin (crimson to blue red), delphinidin (grape) and pelargonidin (geranium flowers).

[3] Reinhard, p 89, Berns, p 157.

[4] See Streitwieser et al pp 671-675, 1225-1227.



Streitwieser gives a calculation for a Beer's law coefficient for $(CH_3)_2C{=}CHCOCH_3$[5]. Namely he states:

$$\alpha = \frac{\log \frac{I_0}{I}}{cd} = 12,600\ L\ mole^{-1}\ cm^{-1}$$

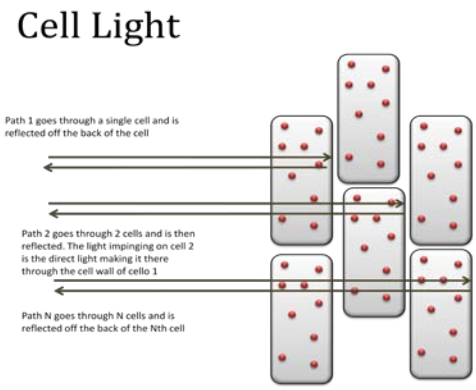
where c is concentration and d the thickness. This is for the 235 nm absorption peak.

3.4 A Simple Cell Model

We now consider a simple cell model. The example is shown below. Here we depict a simple reflection in cells. This is a complex problem to analyze in full detail. The refractive index of the fluid in the cell may be between 1.3 and 1.6 so than rays entering in at an angle are bent accordingly. The top surface may reflect light back, it may absorb light, and it may transmit light. Then the cell may allow light through to get absorbed and then either reflected or transmitted at the inner surface of the cell. This process continues layer after layer.

What we see is the light reflected back to us from the cell, with the absorption from the anthocyanins which are in the water in the cell vacuole. The following is a simplified description of what happens. Lee (p 93) presents an excellent description of some of these effects along with reference to work done a century ago analyzing them. We can look at the analysis in detail, which frankly has almost endless complexity, or in a more gross manner, an engineering manner, which characterizes these gross effects in empirical constants. We chose the latter approach.

[5] Streitwieser, p 674.



We now want to summarize for both reflection and transmission the basic characteristics of these models.

3.4.1 Reflective

A Reflective spectrum is given by the equation below. Here we have a reflection from N cells deep and at each we have a distance which attenuates the spectrum as well. We also assume that there is a single reflection.

$$P_N(\lambda) = \sum_{n=1}^{N} I(\lambda)\exp(-2d_n\alpha(\lambda))[\sum_{m=1}^{n} T_m(\lambda)]R_n(\lambda)$$

where

d_n *is the total depth at which the reflection occurs, note that we have a 2d factor to account for the input and output*

α *accounts for the wavelength dependent absorption factor*

T the transmission gain (<1) at the cell walls to the reflection point

R the reflection gain (<1) at the reflecting cell wall.

We can now assume there are multiple reflections at different layers. Namely we can describe the result as follows:

$$P(\lambda) = \sum_{N=1}^{M} \sigma_N(\lambda) P_N(\lambda)$$

where

$$\sum_{N=1}^{M} \sigma_N(\lambda) = 1$$

and

σ *is the fraction of reflections just from the depth N and M is the total number of possible reflections*

3.4.2 Transmission

We have a similarly equation for transmission but now the light is passed through the cell way and T rather than R applies. Also the distance must reflect the signal at the transmitted distance.

$$P(\lambda) = \sum_{n=1}^{N} I(\lambda)\exp(-d_n\alpha(\lambda))\sum_{m=n}^{N} T_m(\lambda)$$

3.5 Variation With Concentration

The analysis to this point assumes a single anthocyanin and a full absorption via the transmission or reflection. We now consider what occurs with a single anthocyanin as we vary the concentration and thus do not have full absorption.

Assume we have n absorbers per cell, and that each absorber has absorption characteristics as we have shown above. Now is the number n per cell is high enough and if the reflection is via the top cell alone then we can be concerned with full absorption as we have shown before. If however n is not large and then we have limited absorption per cell then even if we go through k layers and back we may not have complete absorption. How does that change the color?

Consider a simple example. We have three cell layers and at the back end we have a cell reflectance of 75% and transmittance of 25%. Thus 75% of the light goes through one cell and back up again with absorption occurring in that cell alone.

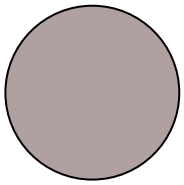
$$R(\lambda) = (0.75)I(\lambda)[\exp(-2\alpha(\lambda)cd)$$
$$+(0.75*0.25)\exp(-4\alpha(\lambda)cd)$$
$$+(0.75*0.25*0.25)*\exp(-6\alpha(\lambda)cd)]$$

As we had assumed before I, the incident light spectrum, is constant across the band since it is white light and initially we assumed we has total absorption in the top cell. We also assumed we have perfect reflection from that top cell backwards. Thus at the max point of absorption we had zero reflection. As we vary concentrations that no longer applies.

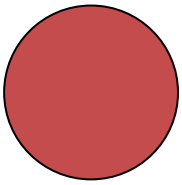
We can now make the following modification.

$$R(\lambda) = (0.75)I\exp(-2\alpha(\lambda)cd)$$
$$[(1+(0.25)\exp(-2\alpha(\lambda)cd)+(0.25*0.25)*\exp(-4\alpha(\lambda)cd)]$$
or
$$R(\lambda) = 0.75I\exp(-2\alpha(\lambda)cd)G(\lambda,c,d)$$
where
$$G(\lambda,c,d) = \sum_{n=0}^{N}\left[0.25\exp(-2\alpha(\lambda)cd)\right]^n$$

We can now show variations of RGB values depending on peak wavelength of absorption and percent total loss with total reflectance received. Percent goes from 0% to 100% at peak and 470 to 550 nm. These are the RGB values for absorption spectra with peaks going from 470 nm to 550 nm and with absorption going from 0% to 100%. We start with a color 176, 160, 160. That color looks like:



Then we can see how it changes:



Red					
214	470	500	520	540	550
0%	176	176	176	176	176
10.0%	181	181	181	179	177
20.0%	186	187	185	182	179
30.0%	191	193	191	185	181
40.0%	196	199	196	188	182
50.0%	202	205	201	192	184
60.0%	208	212	207	195	186
70.0%	215	218	213	200	189
80.0%	222	226	220	204	191
90.0%	229	234	227	209	194
100.0%	238	242	234	214	197

Green					
47	470	500	520	540	550
0%	160	160	160	160	160
10.0%	160	157	156	156	156
20.0%	160	154	151	151	152
30.0%	159	150	146	146	147
40.0%	159	146	140	140	142
50.0%	159	142	133	133	136
60.0%	159	137	125	125	129
70.0%	159	130	115	114	120
80.0%	159	123	103	101	109
90.0%	159	114	85	82	95
100.0%	158	103	55	47	73

Blue					
188	470	500	520	540	550
0%	160	160	160	160	160
10.0%	160	157	156	156	156
20.0%	160	154	151	151	152
30.0%	159	150	146	146	147
40.0%	159	146	140	140	142
50.0%	159	142	133	133	136
60.0%	159	137	125	125	129
70.0%	159	130	115	114	120
80.0%	159	123	103	101	109
90.0%	159	114	85	82	95
100.0%	158	103	55	47	73

Then when we get 100% at 550 we end up with:

This is an amazing change from the incident grey.

It should be noted that 100% absorption is not often seen before reflection. Absorption reflected in color may be a much smaller percent. To attain 100% absorption one would need a deep and dense solution of a specific anthocyanin with no reflections until the bottom surface and then a total reflection from there.

4 THE ANTHOCYANINS

We have just shown that there are a wide variety of coloration in the daylily. In a little over a hundred years we have taken the dozen or so species and intermixed them and as a result have created a very complex set of flowers with characteristics which differ dramatically from the species.[6]

The species have managed to maintain their separate identities over thousands of years but in a small fraction of time we have been able to introduce multiple forms and colors. To understand this process we first have to understand where the colors come from. How do we get purple from a plant which is red, yellow, orange and possibly even brown? How are the colors made and how do we get from there to where we are today.

The first step in understanding that process is to understand the pathways that lead to color production in a single cell. Then we can address the issue of multiple cells and finally how the cells communicate. How do we get an eyezone for example. Why if a cell is whit do we go so abruptly to a purple eyezone. What is the mechanism for this process? We begin the exploration of this issue with a analysis of the underlying pathways.

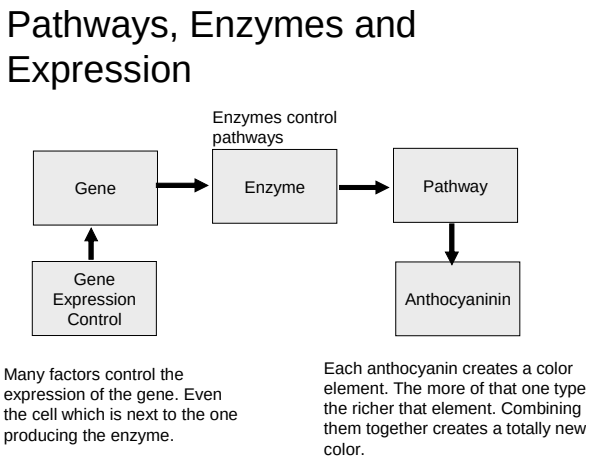
4.1 Pathways and Enzymes

Pathways are nothing more than a set of chemical reactions which get us from some primitive chemical to a more complex but useful chemical structure.[7] In fact the pathways may be just a set of processes going from any one chemical structure to another independent of the nature of the starting and starting chemical. Some pathways are linear going from a beginning to an end and some are circular taking us from the beginning and back again; the Krebs cycle is an example. What makes the pathway work? Just three elements are required: (i) the underlying chemical constituents, (ii) some form of energy, (iii) generally some form of facilitation such a catalyst and in our analyses this is an enzyme.

The general flow structure we look at is shown below. In our view, not the only such view but one convenient for the development of our argument, we have the pathway but it facilitated by an enzyme, a protein. The protein is generated by a gene. And the gene is activated by some other element, generally another protein. In our case shown below the output is some anthocyanin. The more of the enzyme, namely the more the gene expresses itself the more anthocyanin we get. Thus if we can get the gene to express then we get more of that specific anthocyanin, more pelargonidin for example. We defer to the next section how we get this gene to express so strongly.

[6] See Lensaw and Ghabrial for an excellent discussion of the tulip. In contrast to the daylily, the tulip craze of the seventeenth century was a dramatic bubble, and the irony was that most of the color variations were induced by viruses.

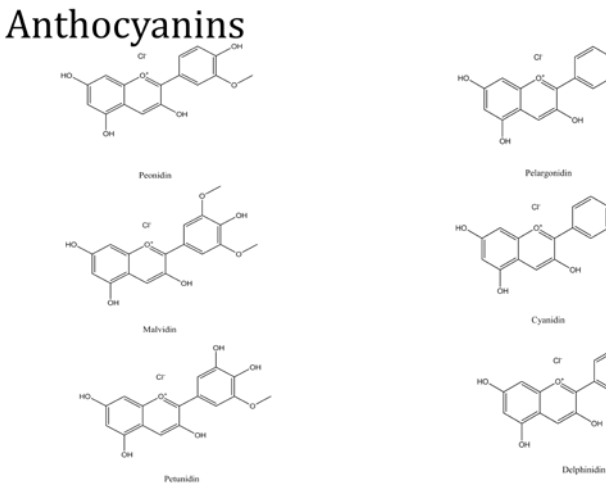
[7] See Taiz for an excellent overview. Dey is also a superb and current reference. The older references by Goodwin are useful but they fail to account for the genetic effects.



The opposite is also true. Namely if we can suppress the gene then we can get less and even possibly no anthocyanin from the pathway. This is the first step in the development of an overall system model.

4.2 Anthocyanins

Let us consider our first pathway. This is the pathway which creates anthocyanins.[8] The anthocyanin molecules 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.



The anthocyanin or anthocyanidin molecules comes 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.

4.3 Other Color Elements

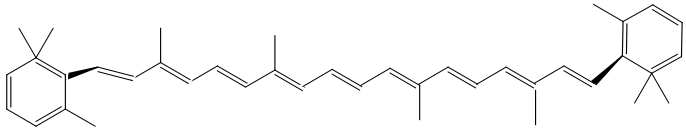
Anthocyanins are not the only elements which are secondary products which produce color. There are three classes of chemicals which give rise to color; anthocyanins, flavones or flavonols, and carotenoids. The Table below depicts the different elements and their colors. The approach we took above for the anthocyanins can be take 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*[9]
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.

4.4 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.



[8] 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.

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

4.5 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.

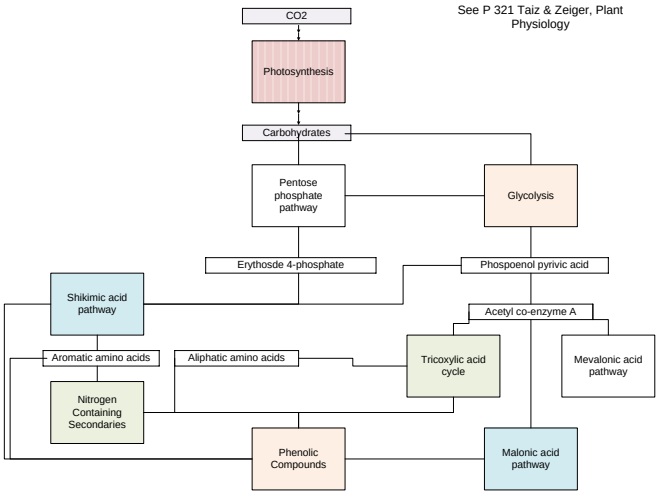
We can also show how closely they relate in substitutions and colors. This is shown in the Table below.

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

4.6 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.

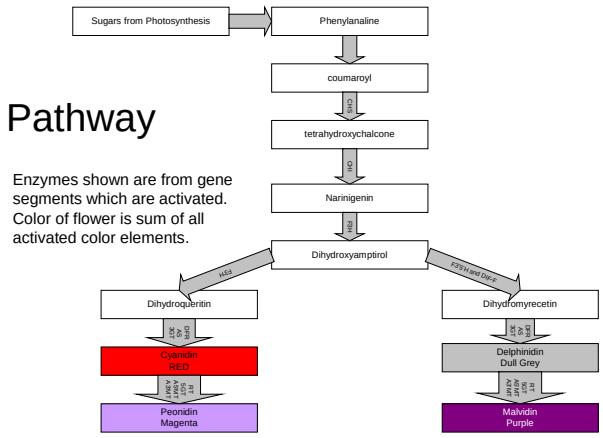
The following is the overall pathway for all elements.



The above shows how we start from CO2 and then go through a variety of other pathways. We will review those pathways in some detail since it is the enzyme control in them which is key.

4.6.1 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 pathways 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.



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.[10]

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.

[10] 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).

The conversion details from pehnyanaline through chalcone to the anthocyanins is 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. Specifically we can write:

$$z_k = \min(B_j x_j; \forall j \in \Theta)$$

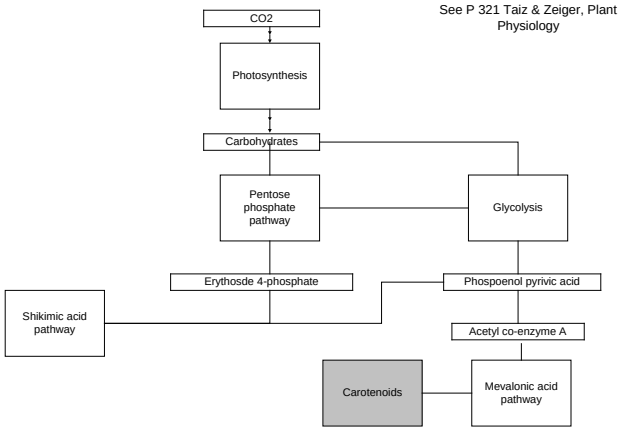
Namely the concentration of the secondary product, the z element, is proportional to the minimal concentration of the facilitating enzymes, namely the set θ.

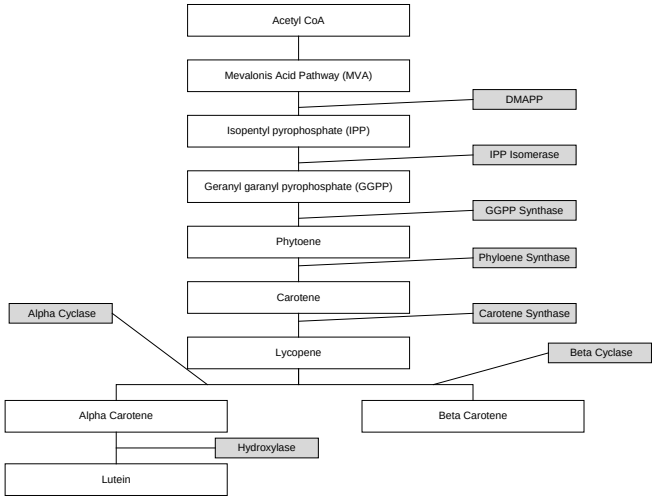
Secondary products always have this type of production process. As we look at a cell, from a system point of view we see facilitating proteins and secondary products. The concentration of the secondaries are proportional, in some general way, to the concentration of the facilitating proteins. However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

4.6.2 Carotenoid Pathway

We have shown the carotenoids as above. The carotenoid pathway is shown below. We have demonstrated this in general terms earlier but in this case below we see the specific details.

We show below the pathways and the facilitating enzymes. In many ways it appears identical to the anthocyanin pathway and the facilitating enzymes.





4.6.3 Flavonol Pathway

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

4.7 Anthocyanin Absorption

The absorption of light by anthocyanins has been discussed but there are several points worth relating. The first is that the absorption characteristics vary greatly depending upon secondary environmental factors. The absorption curve below by Harborne shows the great variation as we change

pH. This is a significant variation. The same changes have been seen in the anthocyanins.

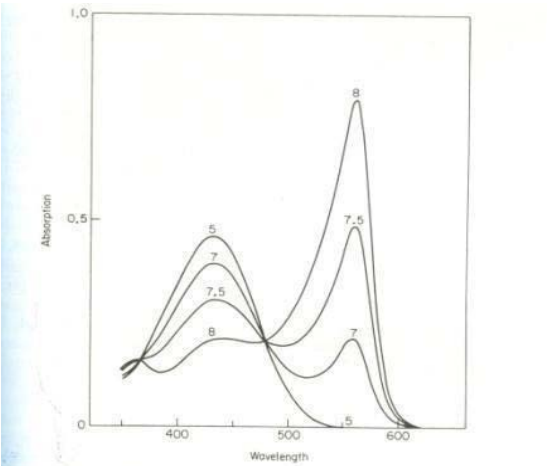
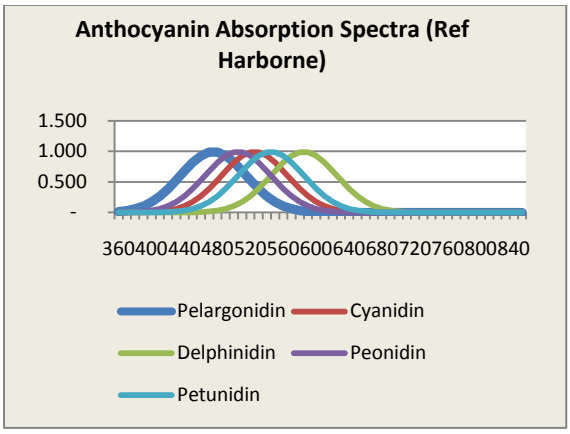
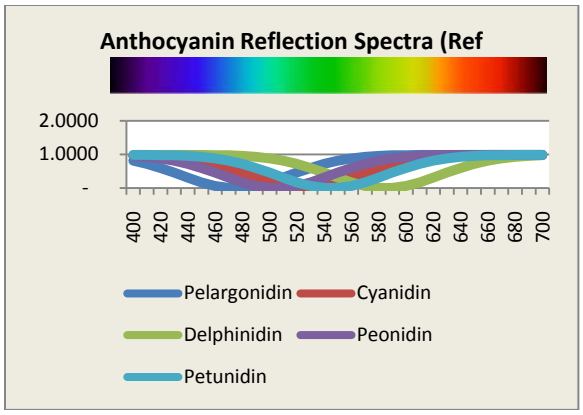


FIG. 12. The absorption spectrum of phenol red at different pH values.

We have taken the data from Harborne for the anthocyanins and have assembled it to be used to demonstrate the absorption curves. It must be emphasized that there is substantial variability here. We have modeled this as Gaussian curves with means at the peak and using the Harborne limits for determining the standard deviations. With these numbers we obtain the relative absorption,



In reality we see the transmission or reflectance and from that infer the absorption. Thus the curve below assume 100% absorption at peak. That may very well be extreme as we discuss later.



The following Tables represent the xyz value for the anthocyanins followed by the rgb values.

	x	y	z
Petunidin	0.3572	0.1819	0.4609
Peonidin	0.4463	0.2805	0.2732
Delphinidin	0.2248	0.2279	0.5474
Cyanidin	0.4096	0.2148	0.3757
Pelargonidin	0.4710	0.4058	0.1232

	r	g	b
Petunidin	0.6482	0.0142	0.4700
Peonidin	0.8789	0.1049	0.2564
Delphinidin	0.1053	0.2324	0.5447
Cyanidin	0.8099	0.0215	0.3761
Pelargonidin	0.8411	0.3098	0.0737

Anthocyanin	L max	E 400/ E max (%)	Lest	SD est
Pelargonidin	520	39	470	40
Cyanidin	535	19	520	40
Delphinidin	544	16	550	40
Peonidin	532	25	500	40
Petunidin	543	17	540	40

Finally we demonstrate the RGB values on 0,255 space for each of the anthocyanins.

Anthocyanin	R	G	B	Color	Center	SD
Petunidin	214	47	188	purple	540	40
Peonidin	242	103	147	rosy red	500	40
Delphinidin	103	141	199	bluish purple	580	40
Cyanidin	234	55	171	purplish red	520	40
Pelargonidin	238	158	89	orange red	470	40

The images below demonstrate the RGB colors we would perceive on a video monitor for each of the anthocyanins.

We will use the above models but it is essential to address the anthocyanin absorption characteristic with in situ environmental conditions. What we have presented here is reasonable for an analytical tool but lack predictability in a broad environment.

5 FLOWER COLORS: GENES TO PERCEPTION

In this section we briefly review the molecular genetics of a plant cell. We do not get into any significant details but merely review the elements which we can use letter in developing the mathematical models for plant regulation. As we have shown in the previous section, plant colors are the result of the expression of three types of secondary plant cell products; anthocyanins, flavones and carotenoids. We have focused mainly on the anthocyanins but have shown the details on all three. What we focused on is that the production of any one of these is a result of a specific pathway and that the production in that pathway is controlled by a set of enzymes. The enzymes are proteins produced within the cell. The proteins are the result of the expression of a set of genes.

In this section we now by reviewing the current understanding of plant cell micro genetics show that the proteins are expressed by the normal process understood since Watson and Cricks seminal work and that there are factors which and activate their production, indeed enhance their production, or repress their production. These are the activators or repressor proteins. The activator and repressor proteins are in effect other genes expressing themselves. We will combine the last section with the results in this section to effect a dynamic system model for plant color generation in the next section.

What will be critical to understand here is that we just want to place the process of activators and repressors in context. We discuss in the next section what our overall design approach will be; that of an engineering model development and not a detailed understanding at the cell level. Frankly, we are not interested in the lower level detail, only gross modeling of cells, genes, and their proteins. They will become the inputs, outputs and control mechanisms of our design approach.

In this section we develop a systems approach to the problem of color analysis and synthesis. This work is based upon the recent work of Szallasi and others. However this also builds upon the work in McGarty (1971) which focused a systems approach to the overall identification problem.

5.1 The Approach

The approach we take in this paper is an engineering approach rather than a biological approach.[11] Our interest is in developing a model or sets of models which allow us by a verifiable means to show how the genes react and interact to produce the plant colors. We can compare this to the engineering approach to circuit design of transistor circuits versus the science of understanding the semiconductor from the point of view of detailed quantum mechanical models. The biologist in our approach is akin to the physicists and engineers who approach the cell from the bottom up, trying to understand all of the intricate processes and steps that lead at the micro level to the developments we look at herein. In our approach it is akin to the engineer knowing that there is some function inside the semiconductors which may clearly be important but the engineer's interest is in designing and analyzing the transistor as a circuit element. Thus for an engineer, if we increase a current here we get a decrease or an increase at some other point.

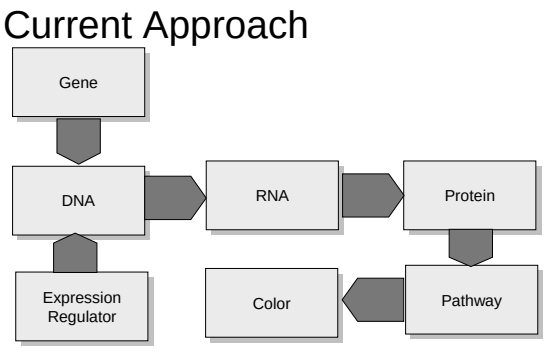
The engineer creates a world view of a macro set of processes and models the details of the biologists in our case with a few set of equations which show the results of increases and decreases. This model must then be valid table and verifiable. One must be able to make measurements to show that the processes predicted indeed occur, to a reasonable degree of accuracy. Then one can analyze a genetic circuit and then in addition one can design a genetic circuit. We then can understand where the colors come from and possibly engineer the genes to develop and deliver on colors we desire.

5.2 Current Paradigm

The basic control paradigm is contained in the following Figure. The expression regulator may be an activator or suppressor. It may be a result of a gene expression in the

[11] There has been a significant set of development recently in analyzing genetic data from a systems perspective. In this paper we have taken such an approach. The recent work by such authors as Perkins et al, Vohradsky, Hatzimanikatis et al, and the recent book by Szallasi are seminal. However, there is an issue here also or world view and what does one really want from the analysis. The bench scientists looks to understand all the details of the underlying processes. The engineer seeks to understand enough to model the process and to do so with a reasonable degree of accuracy but the ultimate goal for the engineer is control of the process and generation of new processes.

cell itself or quite possibly as we shall discuss fed through from another cell. There are many of these regulatory cycles and they are all interconnected. This basic paradigm is one of hundreds or thousands of such interconnected flows.



In developing our models we will use this construct. However, we can frequently focus on natural clusters of related genes. They may be a dozen or more such related genes in each cluster and possibly hundred of such clusters. Although cells and their proteins may affect all other cells, only a few of the genes regulated have a significant level of regulation. The low levels of "regulation" we shall consider just as noise.

The following depicts the basic principles which apply in controlling the secondary pathways. They work as follows:

There are secondary pathways which convert simple chemicals such as CO2 into complex molecules like anthocyanins.

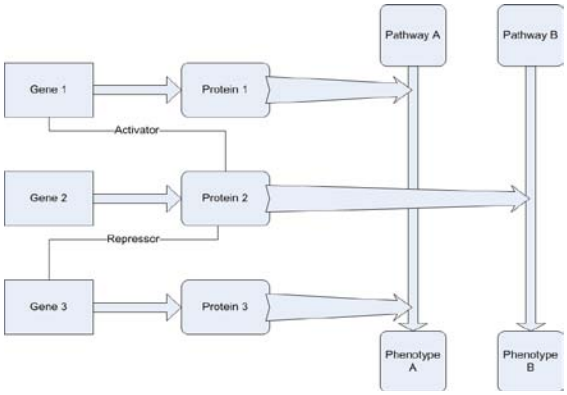
The secondary pathways are activated by proteins, enzymes if you will, that make the pathway convert one set of molecules into another.

Genes are activated or repressed by a variety of factors, often other genes but many times by such factors as micro RNAs which can halt gene activity or in some cases activate it.

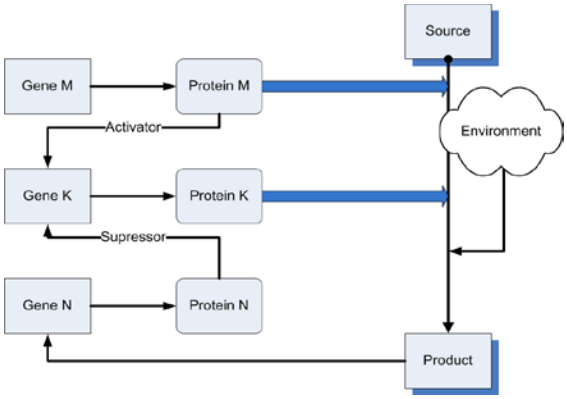
Gene produce proteins and these proteins become catalysts in certain steps in the secondary pathways. The genes continue to produce at rates which yield certain concentrations of the end product of the secondary pathways. The concentration of the end product is an important result.

The colors we perceive, as defined by RGB or some other well accepted metric are the result of terminal concentration levels of a mix of secondary elements.

Other factors such as the pH and the presence of tertiary mineral or metallic ions may alter the absorption spectrum of the pigments and thus a shift in the resulting color may result.

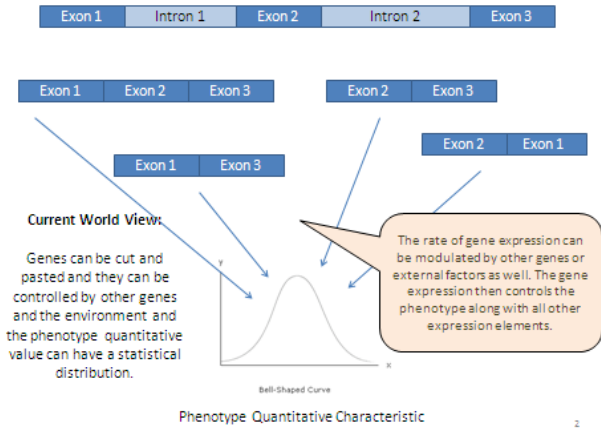


We characterize this environmental factor in the diagram below.

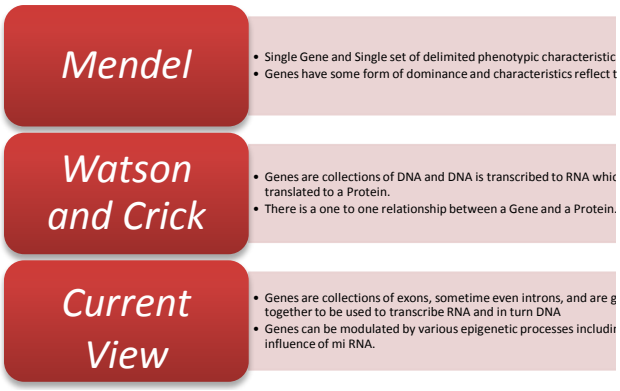


The impact of other genes, si RNAs, mi RNAs and other controlling factors in the cell may as of yet not been fully determined. However we generally know the gene and protein combination and we generally know the protein and secondary product relationship.

The following depict some of the other factors which affect the gene environment.



Thus when we look at the development of a blue daylily in a genetic sense we must abandon the old world view held by Mendel. There is not a gene for yellow, orange and the like and the genes are not like Mendel on separate chromosomes. Mush of the early guesses on the genetics of the daylily were guesses at best and much even to the present in the lay literature ignores the fundamental genetics of even Watson and Crick. We argue here and elsewhere that the genetics is a highly complex issue and that even genes on separate chromosomes may affect other genes as activator, suppressors or even as catalysts in other pathways. We summarize this below.



6 ATTAINING BLUENESS

We now address the issue of how do we get blue. The solution falls into two questions; what do we mean by blue and then how close can we get assuming everything works to getting blue. The first question we have established the groundwork for and the second question we can answer in a manner which makes certain assumptions regarding the genetics. The question left unanswered is how to we engineer a daylily or any flower in general to achieve the color mix that yields the answer we seek. What we provide here is not how to get genes to do what they must but a simpler question but one which must be answered first; what must the genes do to achieve the color required. The next question of how does one get those genes to do that task is left to be answered separately.

6.1 What is Blue?

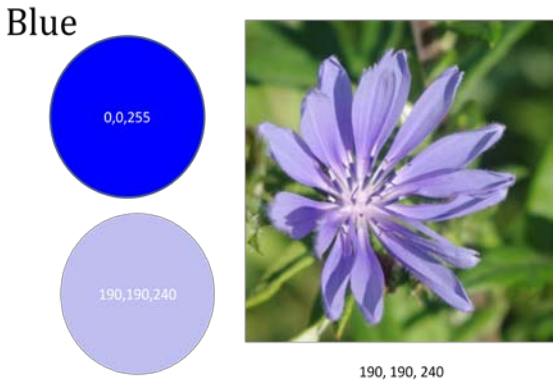
How do we define blue? Several ways are available to us:

1. Definitional, in an RGB system; it is 0,0,255. This is plain blue, it is the blue we see on the computer screen. As we shall show it is a blue which is unachievable in any real plant.

2. Observational; see a cornflower and we can determine that it is blue and then we can seek that color; we can do the same with a morning glory blue, etc. This approach is one where we look first to nature, then accept a blue that nature has already produced, get the RGB of that blue and then see if we can get close with a daylily. This approach is the one which starts by saying observationally; "That is the blue I want" and then going to our method of quantifying that in an RGB world.

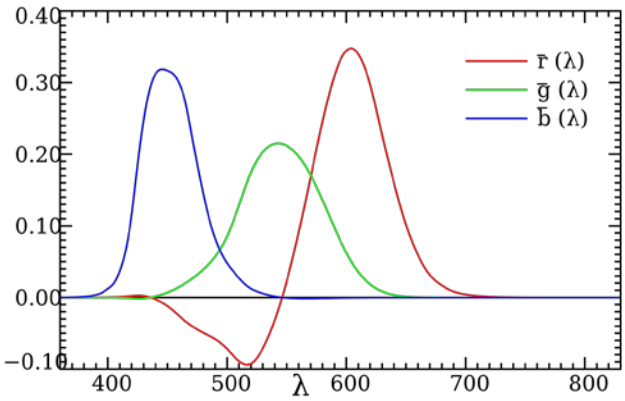
The prior sections all considered single anthocyanins and varied with complete absorption at peak to variations in absorptions due to thickness and/or concentrations. We now consider the effect of mixing anthocyanins in an attempt to get a blue. Let us look at the cornflower, a blue we see along the road side at mid to late summer.

We show below the cornflower blue and the blue of the standard computer screen. The 0,0,255 blue is a strong blue with no R or G component. We know that the RGB elements are a result of the xyz or even the rgb elements from the transmission or reflection spectrum. Consider the rgb elements. r has a negative portion with a much larger positive portion.



Now the rgb functions are shown below. To see what RGB are, we must integrate the received spectrum across these functions. But to achieve pure blue we must have zero integrals in R and G. Clearly if we try to eliminate G we

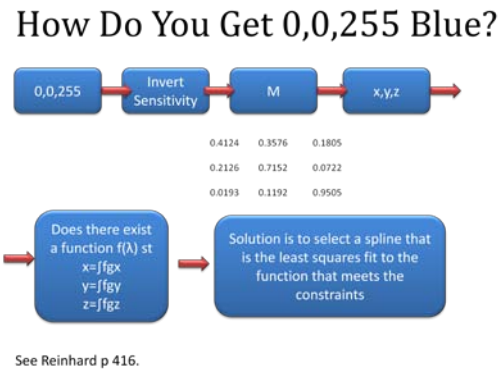
reduce part of B and we must also eliminate all of R as well. Now looking at any of the anthocyanin profiles there is clearly o way to eliminate, reduce to 0, any R and G contribution. The anthocyanins all have wide tails on receive. They all result in some R or G. Thus an immediate conclusion is that it is physically impossible to achieve a 0,0,255 RGB blue. We can mix any possible number of anthocyanins in any possible set of concentrations and the tails will always result in some R and G.



6.2 How to Get Close to a Blue

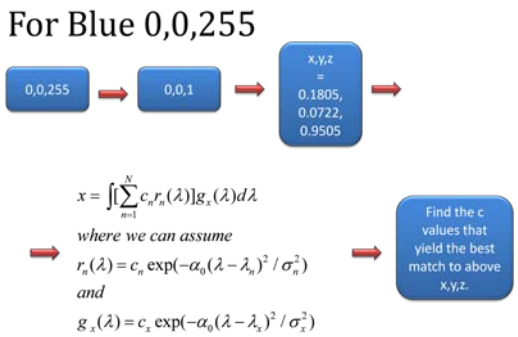
How close can we get to blue. Let us say our goal is 0,0,255. What methods do we have at our disposal to see if there is some mix of anthocyanins that will get us near and what would that look like. Remember we are not answering the question of what must the genes look like, just assuming the genes can be manipulated to do what is required, what is required?

We pose the problem as below. We start with 0,0,255 and then convert it to xyz values via the inverse. At that point we ask what must the concentrations be to get close.



See Reinhard p 416.

+The specific approach is as follows:



Now we can make the following assumptions:

1. Let us assume that we have a received signal, from a reflection or transmission, of the sum of all received signals where we have allocated the absorptions to separate segments. Namely we assume that the received signal is:

$$r_{tot}(\lambda) = r_{transmitted}(\lambda) - \sum_{n=1}^{N} r_{absorb,n}(\lambda)$$

where

$$\sum_{n=1}^{N} r_{absorb,n}(\lambda) = r_{transmitted} \sum_{n=1}^{N} \exp(-a_n(\lambda) c_n d) =$$

$$r_{transmitted} - r_{transmitted} \sum_{n-1}^{N} a_n(\lambda) c_n d$$

if the attenuation per absorber is small

2. Thus for XYZ we can write:

$$X = \int r_{transmitted}(\lambda) x(\lambda) d\lambda$$

$$= \int r_{transmitted} \sum_{n-1}^{N} a_n(\lambda) c_n dx(\lambda) d\lambda$$

$$= r_{transmitted} \sum_{n-1}^{N} \int a_n(\lambda) c_n dx(\lambda) d\lambda$$

$$= \sum_{n=1}^{N} c_n \beta_n$$

where

$$\beta_n = k \int a_n(\lambda) x(\lambda) d\lambda$$

Thus we have concentrations of each anthocyanin, or other absorber, and the integrals of the absorption profile for each with the xyz Tristimulus functions.

3. Now we leave much of the detail to Appendix A but we will summarize the approach. We now can get XYZ values and then we can get RGB from the M transform matrix.

That is we can state what the RGB values would be as a linear function of the concentrations. Recall that this was the result of the assumption of low attenuation which is the case if we transmit through a thin layer of cells or more so if we see reflection from the internal surface of the top cell layers. If this were not the case then we could still follow the method but the results would be non linear yet solvable by standard methods.

4. Now what are we seeking. We can get the RGB values for any concentration as mentioned above. If we want a desired RGB value we then ask what is the set of concentrations which yield that value or close to it.

5. Suppose we want a specific RGB value. We call that E, the expected value, a triplet number from 0 to 255. We then convert that to RGB in non digital state and we then use M inverse to obtain the E in XYZ space. We call these the desired e values. Now we want to select the c values to give us the best match to the e values. That is:

$$x=\sum_{n=1}^{N} c_n \beta^x{}_n$$

$$y=\sum_{n=1}^{N} c_n \beta^y{}_n$$

$$z=\sum_{n=1}^{N} c_n \beta^z{}_n$$

find c to minimize:

$$min\left[(e_x-\sum_{n=1}^{N} c_n \beta^x{}_n)^2+(e_y-\sum_{n=1}^{N} c_n \beta^y{}_n)^2+(e_z-\sum_{n=1}^{N} c_n \beta^z{}_n)^2\right]$$

where for all c

$0<c<1$

This is the optimality condition which yields the c values which get us as close to the desired e values.

6. From this, by minimizing the mean square difference by selecting the c values, we obtain a simple vector solution of:

$$c=\begin{bmatrix} c_1 \\ \dots \\ c_N \end{bmatrix}=B^{-1}d$$

This yields a unique solution if B has an inverse, or if not we can determine it with a pseudo inverse. In addition with a solution we can determine the color resulting and determine how close we could come. This also is how close we could ever come to that desired state.

7 CONCLUSIONS

This paper dealt with the simple question; is it possible to ever get a blue daylily. Our conclusions are simple as well:

1. No one has seemed to define blue. If you ever hope to achieve something you must define it so that we can all agree that you have gotten there. In all the writings on the blue daylily no one ever bothered to define it.

2. If we define blue in an RGB world, and specifically as 0,0,255, the blue we see on a computer screen, then the answer is simple that you can never get a blue daylily.

3. If we choose a flower in nature that has a blue we like, say a cornflower, then we can measure what the blue is, and then we have developed a simple methodology to show what the concentrations of the available anthocyanins should be to attain that. This of course assumes we have stable anthocyanin environments.

4. Knowing the genetic makeup of the control of the secondary pathways we have previously demonstrated that it is possible to genetically engineer a daylily to attain a desired yet achievable set of concentrations and thus a desirable color, almost.

None of the above admits the existential question, whither the blue daylily.

8 APPENDIX A

In this appendix we detail the analysis which we have used in the paper.

First recall that x, as well as y and z are determined by integrals of the form:

$$I(\lambda)= \textit{ the spectrum of a specific sample}$$

Define

$$X=\int I(\lambda)\bar{x}(\lambda)d\lambda$$

$$Y=\int I(\lambda)\bar{y}(\lambda)d\lambda$$

$$Z=\int I(\lambda)\bar{z}(\lambda)d\lambda$$

Now we assume for simplicity that we have anthocyanins and that we seek the absorption or transmission spectral characteristics. We also model the xyz functions with Gaussian curves as well as the absorption function with a Gaussian curve. Thus we have:

$$x = \int [\sum_{n=1}^{N} c_n \exp(-\alpha_0(\lambda-\lambda_n)^2/\sigma_n^2)] c_x \exp(-\alpha_0(\lambda-\lambda_x)^2/\sigma_x^2) d\lambda$$

but

$$\exp(-\alpha(\lambda-\lambda_0)^2/\sigma_0^2)\exp(-\alpha(\lambda-\lambda_1)^2/\sigma_1^2) =$$

$$\exp(-\alpha\left[\frac{(\lambda-\lambda_0)^2}{\sigma_0^2} - \frac{(\lambda-\lambda_1)^2}{\sigma_1^2}\right]) =$$

$$\exp(-\alpha\frac{a\lambda^2+b\lambda+c}{\sigma_0^2\sigma_1^2})$$

such that we can write

$$x = \sum_{n=1}^{N} c_n \beta_n$$

where

$$\beta_n = \int c_x \exp(-\alpha\frac{a_n\lambda^2+b_n\lambda+c_n}{\sigma_0^2\sigma_1^2}) d\lambda$$

This then allows us to write our problem as follows. We now have xyz values, or equivalently rgb or RGB values as well. We also know we want a specific RGB value. Thus we want a certain concentration of an anthocyanin to yield that value. Yet we have many anthocyanins possibly so we seek the combination which yields the nest fit. This we express as follows:

$$x = \sum_{n=1}^{N} c_n \beta^x{}_n$$

$$y = \sum_{n=1}^{N} c_n \beta^y{}_n$$

$$z = \sum_{n=1}^{N} c_n \beta^z{}_n$$

find c to minimize:

$$min\left[(e_x - \sum_{n=1}^{N} c_n \beta^x{}_n)^2 + (e_y - \sum_{n=1}^{N} c_n \beta^y{}_n)^2 + (e_z - \sum_{n=1}^{N} c_n \beta^z{}_n)^2\right]$$

where for all c

0<c<1

Here the e's are the expected values we are trying to match, the c's are the concentrations of the respective anthocyanins and the β's are the constants which we calculated based upon the xyz Tristimulus weights and the anthocyanin absorption spectrum.

Now we consider a specific example. Suppose we seek to match the cone flower. We can determine the RGB values in 255 space and then work backward to determine the e values. We express that specific problem below.

Using the 5 pigments try to find concentrations of each to reach cornflower blue:
Find the c_i

$$\min((0.52844 - \sum_{n=1}^{5} c_n \beta_n^x)^2 + (0.50888 - \sum_{n=1}^{5} c_n \beta_n^y)^2 + (0.90292 - \sum_{n=1}^{5} c_n \beta_n^z)^2)$$

where β are from the matrix.

Following through on the above equation leads ut to the following:

Minimize by differentiating on the c_n values, namely:

For each c_i we have:

$$((-0.52844\beta_i^x + 2c_i\beta_i^x\beta_i^x + 2\beta_i^x \sum_{n=1,n\neq i}^{5} c_n\beta_n^x) +$$

$$(-0.50888\beta_i^y + 2c_i\beta_i^y\beta_i^y + 2\beta_i^y \sum_{n=1,n\neq i}^{5} c_n\beta_n^y) +$$

$$(-0.90292\beta_i^z + 2c_i\beta_i^z\beta_i^z + 2\beta_i^z \sum_{n=1,n\neq i}^{5} c_n\beta_n^z)) = 0$$

where β are from the matrix.

This can be combined to show:

Bc=d and thus $c=B^{-1}d$,

subject to constraint 0<c<1

where

$$c = \begin{bmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \\ c_5 \end{bmatrix} \text{ and}$$

$$d = \begin{bmatrix} ... \\ 0.52844\beta_i^x + 0.50888\beta_i^y + 0.90292\beta_i^z \\ ... \end{bmatrix}$$

and B likewise defined as above

Note there we have reduced it to a simple matrix inversion. We seek the c values, the relative concentrations and we know the target or expected e values. The solution is simple, we need the B matrix. We work through the details below:

$$((-0.52844\beta_i^x + 2c_i\beta_i^x\beta_i^x + 2\beta_i^x \sum_{n=1,n\neq i}^{5} c_n\beta_n^x) +$$

$$(-0.50888\beta_i^y + 2c_i\beta_i^y\beta_i^y + 2\beta_i^y \sum_{n=1,n\neq i}^{5} c_n\beta_n^y) +$$

$$(-0.90292\beta_i^z + 2c_i\beta_i^z\beta_i^z + 2\beta_i^z \sum_{n=1,n\neq i}^{5} c_n\beta_n^z)) = 0$$

$$c = \begin{bmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \\ c_5 \end{bmatrix} \text{ and } d = \begin{bmatrix} \dots \\ 0.52844\beta_i^x + 0.50888\beta_i^y + 0.90292\beta_i^z \\ \dots \end{bmatrix}$$

and B likewise defined as above.

$$2c_i\beta_i^x\beta_i^x + 2\beta_i^x \sum_{n=1,n\neq i}^{5} c_n\beta_n^x + 2c_i\beta_i^y\beta_i^y + 2\beta_i^y \sum_{n=1,n\neq i}^{5} c_n\beta_n^y + 2c_i\beta_i^z\beta_i^z + 2\beta_i^z \sum_{n=1,n\neq i}^{5} c_n\beta_n^z$$

or

$$2c_1\beta_1^x\beta_1^x + 2\beta_1^x \sum_{n=1,n\neq 1}^{5} c_n\beta_n^x + 2c_1\beta_1^y\beta_1^y + 2\beta_1^y \sum_{n=1,n\neq 1}^{5} c_n\beta_n^y + 2c_1\beta_1^z\beta_1^z + 2\beta_1^z \sum_{n=1,n\neq 1}^{5} c_n\beta_n^z$$

etc

$$\begin{bmatrix} 2\left(\beta_1^{x2} + \beta_1^{y2} + \beta_1^{z2}\right),\ 2(\beta_1^x\beta_2^x + \beta_1^y\beta_2^y + \beta_1^z\beta_2^z), \dots, 2(\beta_1^x\beta_5^x + \beta_1^y\beta_5^y + \beta_1^z\beta_5^z) \\ \dots \\ 2(\beta_5^x\beta_1^x + \beta_5^y\beta_1^y + \beta_5^z\beta_1^z),\ 2(\beta_5^x\beta_2^x + \beta_5^y\beta_2^y + \beta_5^z\beta_2^z), \dots, 2\left(\beta_5^{x2} + \beta_5^{y2} + \beta_5^{z2}\right) \end{bmatrix}$$

This demonstrates the straightforward solution we have proposed.

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