Eye Color and its Inheritance

Color generally provides a **readily visible and reliable attribute, characteristic, trait or quality to easily differentiate or distinguish one individual or group from another**. We often describe people by the color of their eyes, skin and hair, although it is important to remember not only the **value** but the **limitations** of this description as the Reverend Martin Luther King pointed out on the steps of the Lincoln Memorial on August 28, 1968, when he professed the sentiment described the Book of Samuel[1]: "*I have a dream that my four little children will one day live in a nation where they will not be judged by the color of their skin but by the content of their character."* Out of the seven traits **Gregor Mendel** chose with intention to observe in culinary peas (*Pisum sativum*), three had to do with color. (Two traits had to do with shape, one had to do with length and one had to do with position).

Gregor Mendel was born Johann Mendel but changed his name to Gregor Mendel on October 9, 1843, after he joined the Order of Saint Augustine to become an Augustinian monk. The monastery in Brünn (Brno, Czech Republic) that Mendel joined was run by an Abbot and scientist named Cyrill Napp, who had a great interest in breeding fruit trees and

[1] 1 Samuel 16:7. But the LORD said to Samuel, "Do not consider his appearance or his height, for I have rejected him. The LORD does not look at the things people look at. People look at the outward appearance, but the LORD looks at the heart."

understanding the scientific basis of inheritance. In fact, many of the monks in the Monastery were also scientists.

Mendel read a book by **Carl von Gärtner** entitled, *Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreiche* (Research and Observations on the Production of Hybrids in the Vegetable Kingdom). The book documented the results of thousands of **hybridization** or **artificial fertilization** experiments that he performed on useful and ornamental plants. Gärtner (1849) showed that sometimes the progeny looked more or less like one parent while other times the progeny looked like intermediates between the two parents. The results did not seem to be generalizable, but that was okay since and Gärtner was concerned with documenting the proposition that **plants were sexual organisms** whose parts could be differentiated into male and female and that pollen was absolutely necessary for the formation of seeds. Mendel, on the other hand, was interested in using artificial fertilization to find a **law of nature** that **explained inheritance**.

In 1865, after eight years of experimentation, Mendel presented his results and his interpretations of his work at a meeting of the Brünn Natural History Society. He began by stating "*Experience of artificial fertilisation, such as is effected with ornamental plants in order to obtain new variations in colour, has led to the experiments which will here be discussed*."

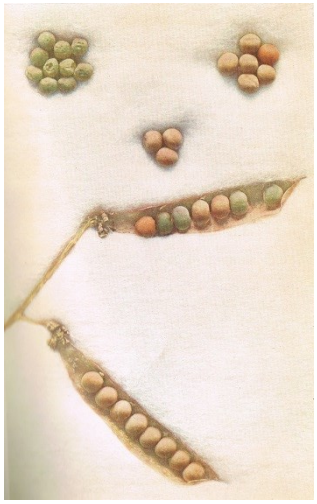
Mendel went on to say, "*among all the numerous experiments made* [by others]*, not one has been carried out to such an extent and in such a way as to*

*make it possible to determine the **number of different forms** under which the offspring of hybrids appear, or to arrange these forms with certainty according to their separate generations, or definitely to ascertain their **statistical relations**. It requires indeed some courage to undertake a labour of such far-reaching extent; this appears, however, to be the only right way by which we can finally reach the solution of a question the importance of which cannot be overestimated in connection with the history of the evolution of organic forms."*

Knowing the importance of the question was only the beginning. Mendel had to find the right experimental material to answer the question. Mendel went on to say, "***The value and utility of any experiment are determined by the fitness of the material to the purpose for which it is used**, and thus in the case before us it cannot be immaterial what plants are subjected to experiment and in what manner such experiments are conducted. The selection of the plant group which shall serve for experiments of this kind must be made with all possible care if it be desired to avoid from the outset every risk of questionable results. The experimental plants must necessarily — 1. Possess **constant differentiating characters**…."*

Color was a **constant differentiating character**. Mendel performed his experiments using hybrids of peas that varied in **one or two constant differentiating characters**. Because of the shape of the pea flowers, in the absence of bees, pistils of the hybrids were naturally pollinated by pollen from the anthers of the same flower—allowing Mendel to know, with near certainty, who the parents were.

According to Mendel, "*The object of the experiment was to* ***observe these variations*** *in the case of* ***each pair of differentiating characters****, and to* ***deduce the law*** *according to which they appear in the successive generations."*



Mendel chose the "*difference in the colour of the seed cotyledons*" to be the constant differentiating character. "*The cotyledon of the ripe seeds is either pale yellow, bright yellow and orange coloured, or it possesses a more or less intense green tint. This difference of colour is easily seen in the seeds….*"

Mendel noted that for each of the seven traits he selected to study, "*the hybrid-character resembles that of one of the parental forms so closely that the other either escapes observation completely or cannot be detected with certainty. This circumstance is of great importance in the determination and classification of the forms under which the offspring of the hybrids appear. Henceforth in this paper those* ***characters which are transmitted entire, or almost unchanged*** *in the hybridisation, and therefore in themselves constitute the characters of the hybrid, are termed the* ***dominant****, and those which become* ***latent*** *in the process* ***recessive****.*"

Mendel then presented his results:

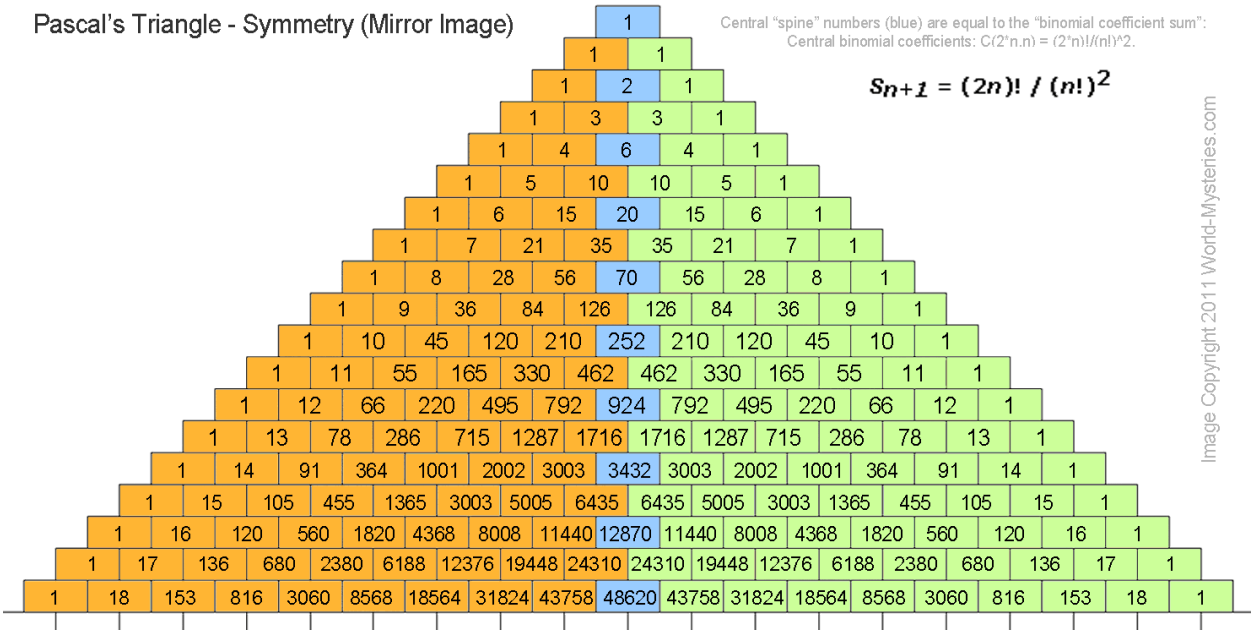
Colour of cotyledon. — 258 plants yielded 8,023 seeds, 6,022 yellow, and 2,001 green; their ratio, therefore, is as 3.01 to 1."

Mendel then **averaged** and **rounded off** to the nearest integer the results from the various experiments to find the essence of the data: "*If now the results of the whole of the experiments be brought together, there is found, as between the*

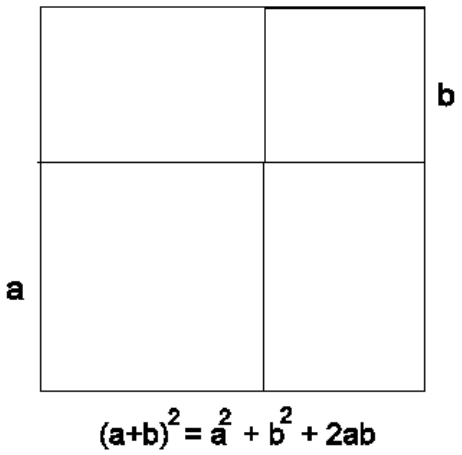
number of forms with the dominant and recessive characters, an average ratio of 2.98 to 1or ***3 to 1****.*"

Mendel then found a way to **reduce the characters to letters** and then **deduce the algebraic model that describes and explains the results***: "If A be taken as denoting one of the two constant characters, for instance the dominant, a, the recessive, and Aa the hybrid form in which both are conjoined, the expression A + 2Aa + a shows the terms in the series for the progeny of the hybrids of two differentiating characters."*

Mendel was good at numbers. We will also get familiar with the power of numbers. Note that 1*A* + *2Aa* + 1*a* looks like the third row of the triangle created by polymaths such as Omar Khayyám (1070) and Blaise Pascal (1653) to visualize the relationships between numbers—especially the numbers involved in a binomial distribution (where there are two possible outcomes like heads or tails and *bi* means 2).



Pascal's triangle is amazing! The rows (0, 1, 2, 3…n) give the coefficients of the binomial expansion, the sum of the values in a row give the value of 2^n, and the powers of 11 can be read off directly from each row. The Binomial Theorem, which relates the parts to the whole, goes back to **Euclid** (Elements II:4) who wrote "*if a straight line be cut at random, the square on the whole is equal to the squares on the segments and twice the rectangle of the segments*." This can be stated algebraically like so:

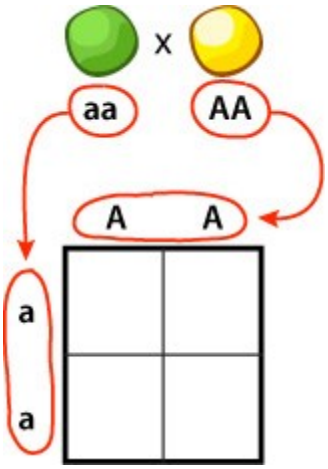


$$(a+b)^2 = 1a^2 + 2ab + 1b^2$$

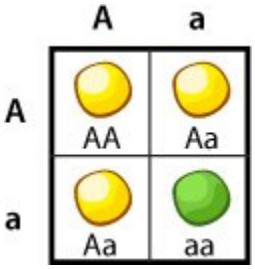
Pascal's triangle gives the coefficients of the binomial expansion. Imagine that we were flipping two coins ($n = 2$) and that a is heads and b is tails and there is equal probability of each coin landing on heads or tails. This is known as a Bernoulli trial. Out of the $2^n = 4$ possible combinations, there is one way to get two heads (a^2), one way to get two tails (b^2) and two ways of getting one head and one tail (ab).

Now imagine along with Mendel that the coins are color genes that come from the mother and father (which makes $n = 2$). The gene from the mother can land head up (A) or tail up (a) and the gene from the father can land head up (A) or tail up (a). Again, there are $2^n = 4$ possible combinations with one way to get AA, one way to get aa, and two ways to get Aa. This made sense mathematically, but how did Mendel make sense of it biologically?

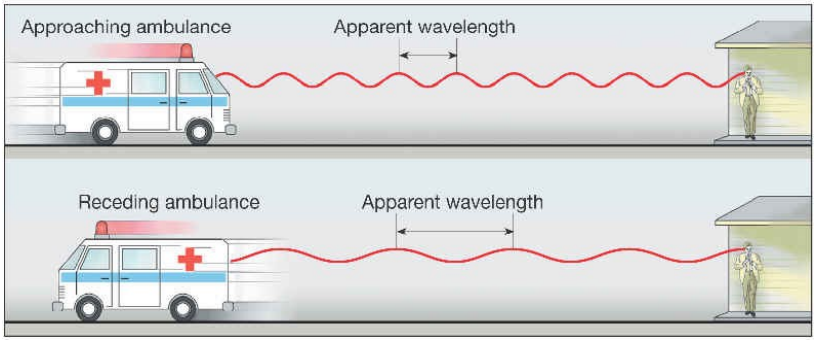
Building on Gärtner's conclusions, Mendel assumed that the anthers produce **sperm-containing pollen** just like male animals produce sperm and the pistils produce **egg-containing ovules** just like female animals produce eggs. If this were true and the sperm and egg both carried the hereditary information to make the zygote, the sperm and egg would each have to have half the information contained in the zygote. Otherwise, the amount of information would double at each generation. Mendel assumed that the **gametes** had only one form of each gene.



If the **characters** transmitted from one generation to the next by the sperm and egg were **particulate and elastic**, they would be transmitted unchanged from one generation to the next. According to Mendel's **Law of Segregation**, during the formation of gametes, the two factors (e.g. *A* and *a*) that coexist in the parent plant, would **segregate**, each gamete getting only one (e.g. *A* or *a*). Reginald Punnett devised a method, known as the **Punnett Square** to keep track of the segregated factors in the gametes and the predicted characters in the offspring. Assuming that a pea plant of type *Aa* produced pollen that had either *A* or *a* and ovules that had either *A* or *a*, then the ratio of progeny would be 3:1. The **dominant character** would be that one that appears in 75% of the progeny and the **recessive character** would be that one that appears in 25%. In today's parlance, the **phenotype** or outward appearance of the progeny is explained by the **genotype** or internal content of genes.



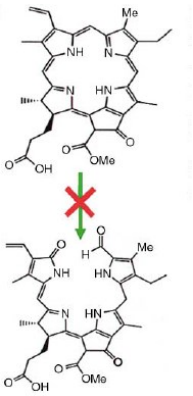
Mendel made so much progress, in part, because he complemented good **experimental design** with **mathematical modeling** and **analysis** in order to understand the **nature of inheritance**. Mendel had learned the power of numbers and the importance of mathematical modeling from his physics teacher, **Christian Doppler**, the man who mathematically modeled and predicted the change in the color of light emitted by moving bodies.



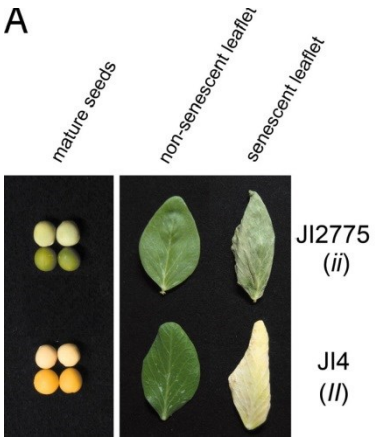
Interestingly, Mendel's work lay buried until it was rediscovered by three botanists in 1900. Perhaps Mendel's work was not taken seriously until 1900; because his **laws of inheritance** emphasized atomistic, **discrete** or "digital" traits and neglected the vast number of **continuous** or "analog" phenotypic differences that clearly exist between individuals and could easily be explained by **blending**. In 1900, the atomistic, discrete, **quantum of energy**, which would later be known as the **photon,** was discovered by **Max Planck**. The onset of the twentieth century was a time for appreciating the possibility that **continuous** appearances can result from summing many small **discrete** events. That is, the biologists and physicists arrived at the conclusion that was already well-known to chemists who build up molecules, both on paper and in test tubes from atomic parts. Chemists were the rock stars of the time as a result of the dye and drug industry that could transform black coal tar to mauve, perfume, aspirin and heroin.

Just over a century after the rediscovery of Mendel, Armstead et al. (2006,2007) and Sato et al. (2007) found the gene itself that causes the seeds to remain **green** instead of turning yellow. The dominant version of the gene codes

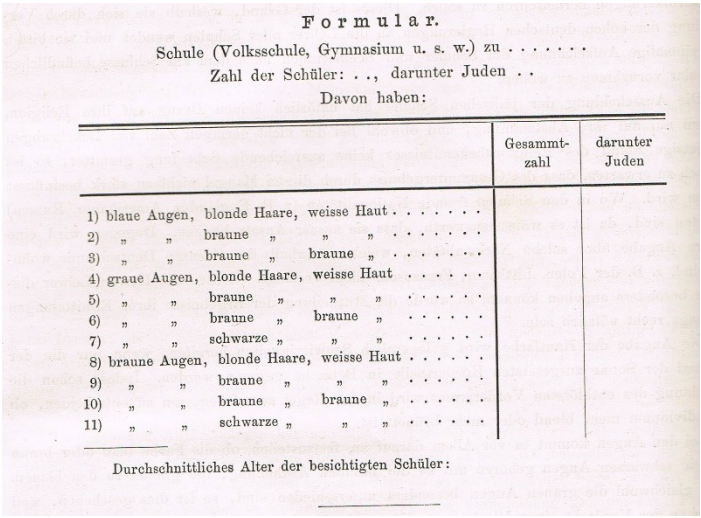
for an **enzyme** involved in the breakdown (**catabolism**) of **chlorophyll**. The seeds with the **dominant** character are **yellow** because the chlorophyll, which is green, is broken down before the seed dries. The **breakdown of chlorophyll** allows for the **mobilization** of the nitrogen that was originally part of the chlorophyll to the developing seedling where it can be used to make amino acids and proteins. In the **green** seeds that exhibit the **recessive** trait, the enzyme does not function, chlorophyll is not broken down, and the nitrogen of chlorophyll is not mobilized to the developing seedling.



The **gene** that determines the color of the seeds also determines the **color of the senescing leaves**. The leaves in the plant with the **dominant** gene turn **yellow** during **senescence** so that the **nitrogen** in its **chlorophyll** can be **mobilized** to the growing part of the plant, especially the seeds. The leaves in the plant with the **recessive** gene remain **green** so that the **nitrogen** is not be **mobilized** to the growing parts of the plant.



Before the rediscovery of Mendel, the inheritance of physical characteristics were of interest to anthropologists. Rudolf **Virchow** (1886), who is famous for saying, "*Omne vivum ex ovo*," published a survey on the color of hair and eyes of school children in Germany. He found that only twenty percent of the Jewish (*Juden*) children had blond hair



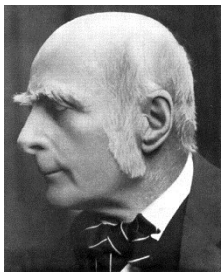
Formular.

Schule (Volksschule, Gymnasium u. s. w.) zu . . . . . .
Zahl der Schüler: . ., darunter Juden . .
Davon haben:

	Gesammtzahl	darunter Juden
1) blaue Augen, blonde Haare, weisse Haut		
2) „ „ braune „ „ „		
3) „ „ braune „ braune „		
4) graue Augen, blonde Haare, weisse Haut		
5) „ „ braune „ „ „		
6) „ „ braune „ braune „		
7) „ „ schwarze „ „ „		
8) braune Augen, blonde Haare, weisse Haut		
9) „ „ braune „ „ „		
10) „ „ braune „ braune „		
11) „ „ schwarze „ „ „		

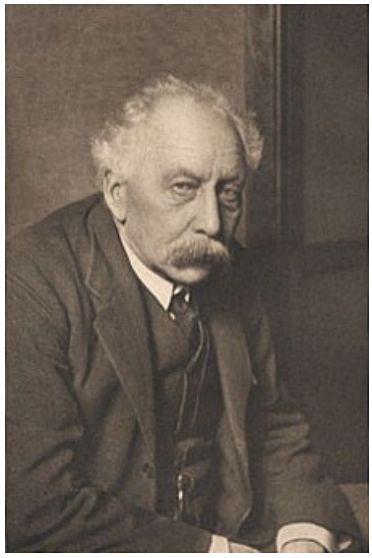
Durchschnittliches Alter der besichtigten Schüler:

and blue eyes, while fifty percent of the non-Jewish children had blond hair and blue eyes. There was clearly a **relationship between eye color and heredity**.



Francis Galton (1889), a cousin of Charles Darwin, had already stated in his book, *Natural Inheritance*, that eye-color was **non-blending** but inherited as an **either-or trait**, blue or brown.

In 1902, **William Bateson** wrote in his "*Mendel's Principles of Heredity. A Defence*" "*Soon every science that deals with animals and plants will be teeming with discovery, made possible by Mendel's work. The breeder, whether of plants or of animals, no longer trudging in the old paths of tradition, will be second only to the **chemist** in resource and in foresight. Each conception of life in which heredity bears a part—and which of them is exempt?—must change before the coming rush of facts."* Could Mendel's Laws apply to cabbages and kings, animals and humans?



In 1907, following the rediscovery of Mendel, **Charles Hurst** examined the **eye colors** of 139 pairs of parents and their 383 offspring in Burbage, Leicestershire, England, where he owned a plant nursery, and determined that mating of **blue-eyed** (simplex) parents resulted in **blue-eyed** children and the mating of **brown-eyed** (duplex) parents resulted in **brown-eyed** and **blue-eyed** children in a **ratio of 3:1**. Consequently Hurst concluded that eye color was a **Mendelian trait** where **brown eyes** are **dominant** and **blue eyes** are **recessive**.

In America, also in 1907, **Gertrude and Charles Davenport** published a paper entitled, *Heredity of eye-color in man*. The Davenports wondered, "*Is human eye-color inherited in Mendelian fashion? The importance of knowing whether it is depends on the fact that, if Mendelian, the result of any combination of eye-colors of the parents upon the eye-color of the offspring can be, within certain limits, predicted*." The Davenports passed out survey cards asking about the eye colors of grandparents, parents and children. They passed out 132 survey cards to principals of schools and friends. They tallied the results and concluded, "*The practical applications of these results to human marriage are as follows: Two blue-eyed parents will have only blue-eyed children….*"

Charles Davenport (1911) tabulated the various studies of eye color in his book entitled, *Heredity in Relation to Eugenics*.

THE INHERITANCE OF FAMILY TRAITS 31

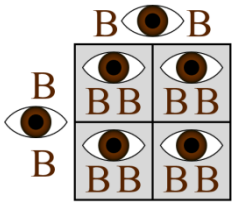
TABLE IV

		HURST		DAVEN-PORT		HOLMES & LOOMIS		TOTAL		P'ORTION	
One Parent	*Other Parent*	*Blue*	*Pig't*	*Blue*	*Pig't*	*Blue*	*Pig't*	*Blue*	*Pig't*	*Blue*	*Pig't*
pure blue	pure blue	101	0	77	0[1]	51	1	229	1	99.5	0.5
pigmented (Pp)	blue	137	121	428	506	89	85	654	712	48.0	52.0
pigmented (PP)	blue	0	66	0	70			0	136	0	100
pigmented (Pp)	pigmented (Pp)	18	45	98[2]	169	5	34	121	248	33	67
pigmented (PP)	pigmented (Pp)	0	195	0	99			0	294	0	100

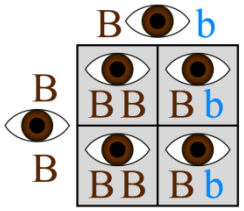
Assume people with brown eyes that have two dominant factors are *BB*, people with brown eyes that have one dominant factor are *Bb* or *bB*, and people

with blue eyes that have no dominant factors are *bb*. People with **two identical factors** (*BB* or *bb*) are **homozygous** for the character and people with **two different factors** (*Bb* or *bB*) are **heterozygous**. The following **Punnett Squares** describe the predicted proportion of each eye color in children of various parents **if only one gene,** with **dichotomous** forms, is involved in eye color.

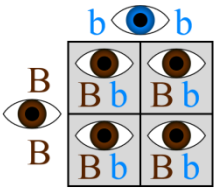
If only one gene is involved in eye color, when two homozygous brown-eyed parents mate, all the children will be homozygous and will have brown eyes. The ratio of the phenotypes and genotypes will be 4:0 and 4:0:0, respectively.



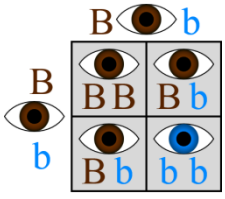
If only one gene is involved in eye color, when one homozygous and one heterozygous brown-eyed parents mate, all the children will have brown eyes, but two will be homozygous and two will be heterozygous. The ratio of the phenotypes and genotypes will be 4:0 and 2:2:0, respectively.



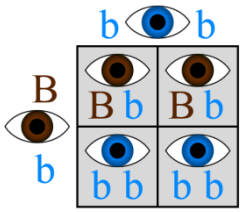
If only one gene is involved in eye color, when one homozygous brown-eyed parent and one homozygous blue-eyed parent mates, all the children will be heterozygous and will have brown eyes. The ratio of the phenotypes and genotypes will be 4:0 and 0:4:0, respectively.



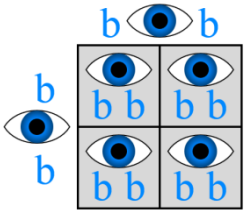
If only one gene is involved in eye color, when two heterozygous brown-eyed parents mate, the ratio of the phenotypes of brown eyes to blue eyes will be 3:1. The genotypes will be 1:2:1.



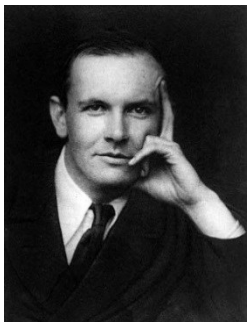
If only one gene is involved in eye color, when one heterozygous brown-eyed parent mates with a homozygous blue-eyed parent, the ratio of the phenotypes and genotypes will be 1:1 and 0:2:2, respectively.



If only one gene is involved in eye color, when two homozygous blue-eyed parents mate, all the children will be homozygous and will have blue eyes. The ratio of the phenotypes and genotypes will be 0:4 and 0:0:4, respectively.



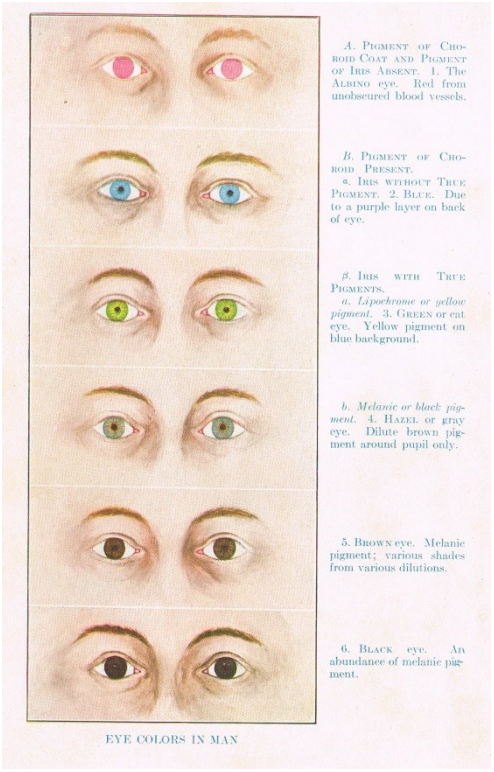
Davenport (1911) analyzed the data in the table: "***When both parents have pure blue eyes all of the children will have pure blue eyes (the discordant case is probably due to an error)*,** and he added, "***We have heard of two blue-eyed parents regretting that they had no brown-eyed children. They wished for the impossible***."



Likewise **Arthur Darbishire** (1912) wrote in *Breeding and the Mendelian Discovery*, "*The offspring of the union of two persons with simplex* [blue] *eyes, whatever their ancestry is,* ***will never have brown eyes****. At any rate, no exceptions to this rule have yet been observed and recorded."*

Reginald Punnett (1911) wrote in *Mendelism*, "…***no individuals of the brown class are to be looked for among the offspring of blues mated together***."

There you have it, **eye color in humans**, at least as far as the **experts** were concerned, could be **reduced to a single gene** where **brown was the dominant form** and **blue was the recessive form** of the gene. That was the story and they were sticking to it. The geneticists abstracted out of the data that one single gene was important for eye color but what about that one brown-eyed child of two blue-eyed parents? Could it be possible that there was no error in the data, that the wife was not unfaithful, that the milkman was not to blame for the **outlier**? Could it be that the outlier was meaningful and the geneticists who tended towards **reductionism** simplified the story too much? There is a difference between **simple** and **simplistic** (too simple).



EYE COLORS IN MAN

Helene Boas, an anthropologist wrote in 1919, "*These conclusions are not convincing because the investigator has been forced to substitute a hypothetical eye-color for the color actually recorded or to "doubt whether the term is used with precision" in order that his results may conform to the Mendelian formula."* Indeed, Boas found, that "*there are here among the children of two blue-eyed parents 12 per cent with brown eyes*." But what does an anthropologist, who crosses disiplinary lines and contradicts the experts, know about Mendel?

Let's look at the pictures of eyes that you took this week. Is eye color a simple, brown *v* blue, **discrete**, digital and dichotomous trait? If not, is it likely that more than one gene is involved? Is eye color so **continuous** that **many** genes may be involved or **relatively discrete** so that only a **few** genes would be involved? What if some of the factors have **incomplete dominance** and there is more of a **blending effect**, like Carl Gärtner saw in many of his horticultural hybrids?

Eye Colors
Strum and Frudakis (2004) Trends in Genetics 20:327

How many Mendelian genes with alternative, dichotomous, dominant or recessive characters might you guess have a major effect on eye color?

Number of Mendelian dichotomous Genes	**Number of Phenotypes**
1	$2^1 = 2$
2	$2^2 = 4$
3	$2^3 = 8$
4	$2^4 = 16$
n	2^n

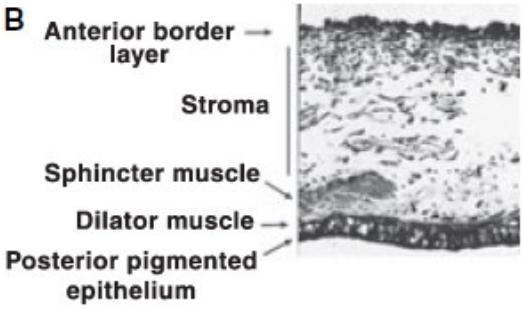
How is reality best described? Are eyes ***either*** brown or blue? Is there a **continuous variation** in color? Are there a **few or several distinct categories** of color? Scientists must consider more than one version of reality simultaneously: The reductionist and the wholistic—otherwise the assumption may incorrectly give the expected conclusion.

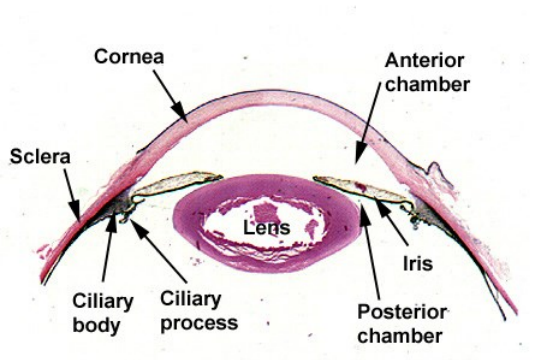
We can **assume** that only one gene is involved in eye color and then we consider brown-eyed children of blue-eyed parents as **outliers** and **conclude** that

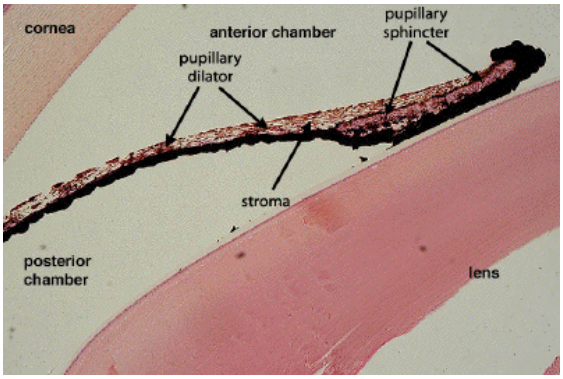
eye color is a one gene dichotomous trait. This has been the **textbook version**. We can also say that in general, one gene for blue eyes explains the great majority of the variation in eye color, but there are other factors, as yet unknown. In part our position will depend on the **value** we put on the **individual** or the **mathematics** as the **fundamental reality**. Which version of reality is truer? Is it unreasonable or reasonable to be a dualist where reality may be characterized in two ways?

Other examples of the dual nature of reality exist. Most people can be described as males with XY chromosomes or females with XX chromosomes; however 0.005% of males and females have XX and XY chromosomes, respectively. Does this mean that male/female is not a valid dichotomy and that male/female and XY/XX is not a one-to-one correlation; or does it mean that in general the male/female classification is a good categorization, but we must leave room for individual differences that do not fit into a dichotomous classification system?

Before we look at the genetic cause of eye color, let's look at the **anatomy of the iris**. The iris is composed of **three layers** that influence eye color. From front to back, they are called, the **anterior layer of the pigmented stroma**, the internal tissue of the **stroma**, and the **posterior layer of the iris pigmented epithelium (IPE)**.





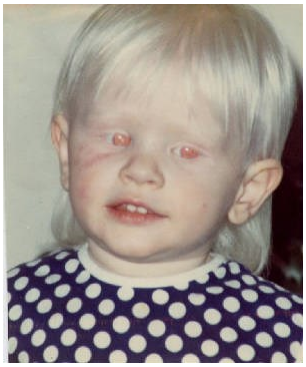


Layer of Iris	**Pink eye**	**Blue eye**	**Brown eye**
Anterior layer of stroma (absorption):			melanin
Internal tissue of stroma (scatter/absorption):	collagen	collagen	collagen melanin
Posterior layer of IPE (absorption):		eumelanin	eumelanin

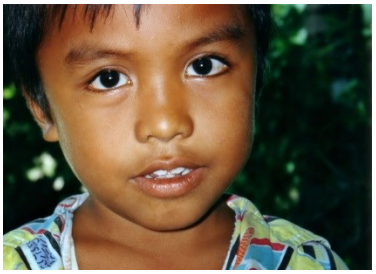
People with no melanin in the stroma ***but*** with melanin in the posterior layer of the iris pigmented epithelium have blue eyes:



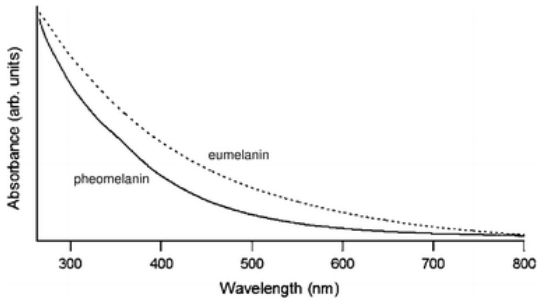
A person with melanin ***neither*** in the stroma ***nor*** in the posterior layer of the iris pigmented epithelium will have pink eyes. The additional light that bounces around the eye causes **photosensitivity** and leads to **glare** or multiple reflections, which reduces visual acuity.



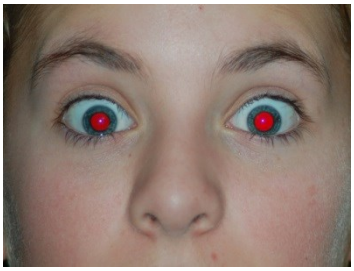
People with melanin in the stroma ***and*** in the posterior layer of the iris pigmented epithelium have brown eyes:



In **brown eyes**, the anterior layer of the pigmented stroma contains a **pigmen**t, known as **melanin** that **absorbs** the majority of the incoming light. The **melanin** may be either **eumelanin**, which absorb nearly all colors of light and reflects back little light and thus appears black or brown, or **phaeomelanin** that absorbs nearly all the colors of light but reflects back a little more reddish-pink light than eumelanin does. The more melanin in this layer, the less light is transmitted through the colored part of the iris to the internal layer of the stroma. Blue-eyed and pink-eyed people do not have any melanin in the anterior layer, so all the incoming light is transmitted to the internal tissue of the stroma. Eumelanin and pheomelanin may also occur in the internal tissue of the stroma, providing even more variety in eye color.



An eye of any color will appear red (***red eye***) if it is illuminated with so much light (i.e. from a flash) that the light is not absorbed by the melanin in the pigmented layer in the retina so that the light is reflected from the blood vessels in the eye and out through the pupil.



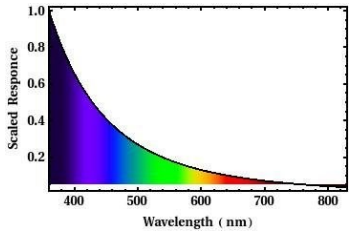
The internal tissue of the **stroma** is composed of transparent **fibers** made of the **protein collagen.** Some of the **collagen fibers** circle the pupil and some radiate from it. The **collagen fibers** are connected to the sphincter and dilator muscles, which close and dilate the pupil, respectively. The collagen fiber is much smaller than the **wavelength of a photon** or perhaps the **photon** itself (my research). Consequently, when white light strikes the collagen molecules, it gets **scattered** in a manner such that the intensity *I* of light of that color that is scattered is inversely proportional to the fourth power of the wavelength (or inversely proportional to the square of the cross-sectional area ($\frac{\lambda^2}{4\pi}$) of the photon):

$$I \, \alpha \, \frac{1}{\lambda^4}$$

The light is **scattered** by the collagen fibers in all directions, which includes the direction that the light came in. When we look at someone with blue eyes, we see the light that is scattered back out of the eye.

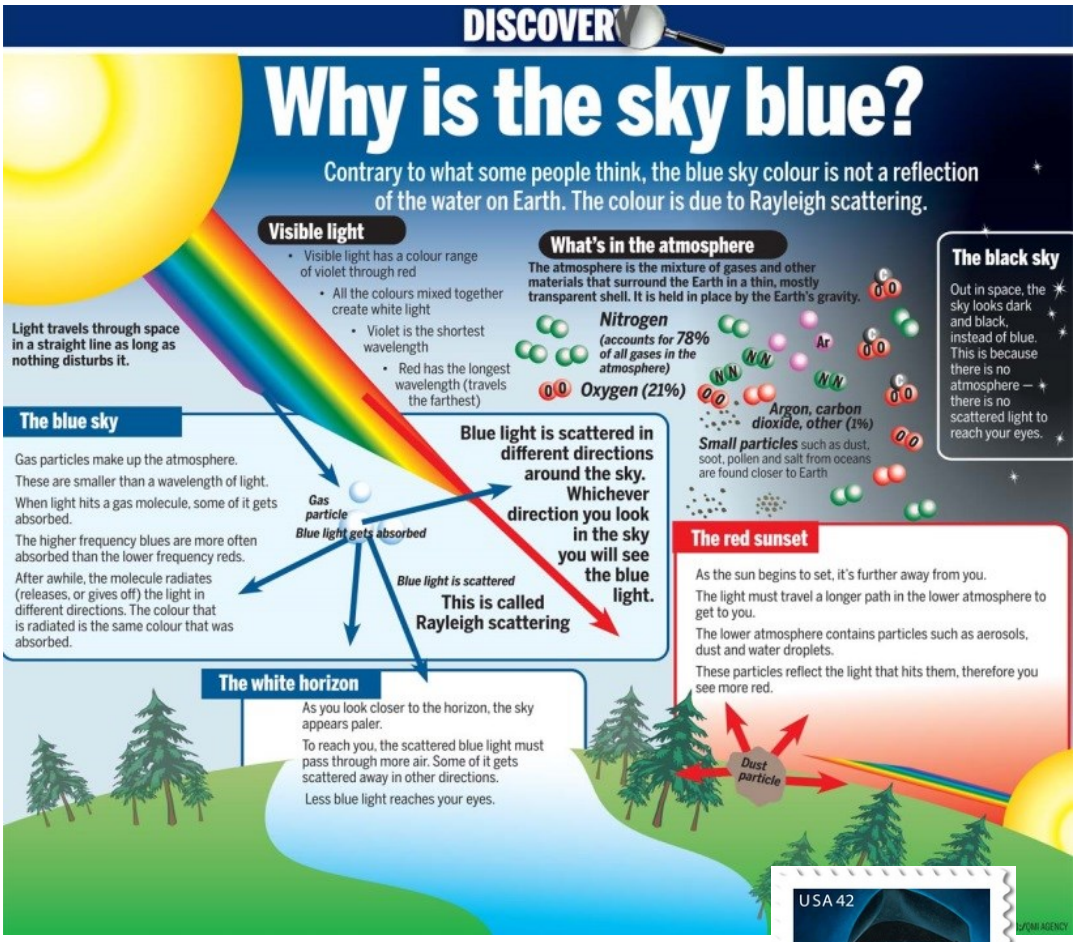
Blue light has a wavelength of about 400 nm, green light has a wavelength of about 500 nm and red light has a wavelength of about 600 nm:

Color	Amount Scattered	Relative Amount Scattered
Blue	3.9×10^{-11}	4.9
Green	1.6×10^{-11}	2
Red	0.8×10^{-11}	1



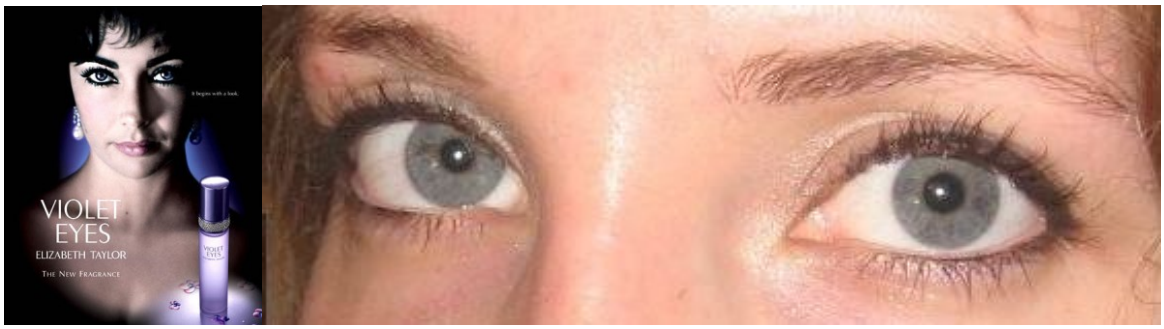
The **blue light** component of the light is **scattered** almost 5 times more than the **red light** component is. That is, the red light component of the light goes right by the collagen molecules into the posterior layer of the iris pigmented epithelium almost five times better than the blue light component does. The green light component acts intermediately.

The scattering of light from molecules smaller than the wavelength of light is called **Rayleigh scattering**, after Lord Rayleigh, who wanted to describe and explain **why the sky is blue**. Eyes are blue because the incoming light is scattered back out from the eye of the observed person to the eye of the beholder.



Aside: A **mirror** (1 cm) **reflects** all the colors (violet, indigo, blue, green, yellow, orange and red; or ROYGBIV) without discrimination. Imagine making a mirror smaller and smaller until it is as small as a gas molecule (about 0.3 nm) like N_2 or O_2 that scatters sunlight to make the sky blue. **Collagen fibers** are smaller than a mirror and larger than gas molecules. Perhaps light eyes such as **violet** and **gray** eyes result from collagen fibers that are smaller or larger than those in blue eyes. Perhaps violet-eyed people have the smallest collagen molecules and scatter violet

light; blue-eyed people have intermediate-sized collagen molecules and scatter violet and blue light; and gray-eyed people have the largest collagen fibers and scatter violet, blue, green, and red light. The following color simulation allows you to construct gray from blue green and red.
http://phet.colorado.edu/en/simulation/color-vision



In **blue eyes** and **brown eyes**, the **innermost or posterior layer of the iris pigment epithelium** contains eumelanin. The eumelanin absorbs almost all of the light that reaches it. People with pink eyes ***do not*** have eumelanin in this layer, so the light is transmitted all the way to the blood vessels in the choroid and red light is reflected out from the eye. The combination of red reflected from the blood vessels and blue scattered from the stroma, makes magenta.

The **pupil** is a **hole in the iris** that contains neither melanin nor collagen, so all the light that enters the pupil is transmitted to the retina.

The pigment in the stroma is produced in cells called **melanocytes**. The color of the iris is determined, not by the number of melanocytes, but by the number of the **melanosomes** in the melanocytes in the stroma of the eye (Imesch et al., 1997). Brown eyes have more melanin in the anterior layer of the iris than do blue eyes.

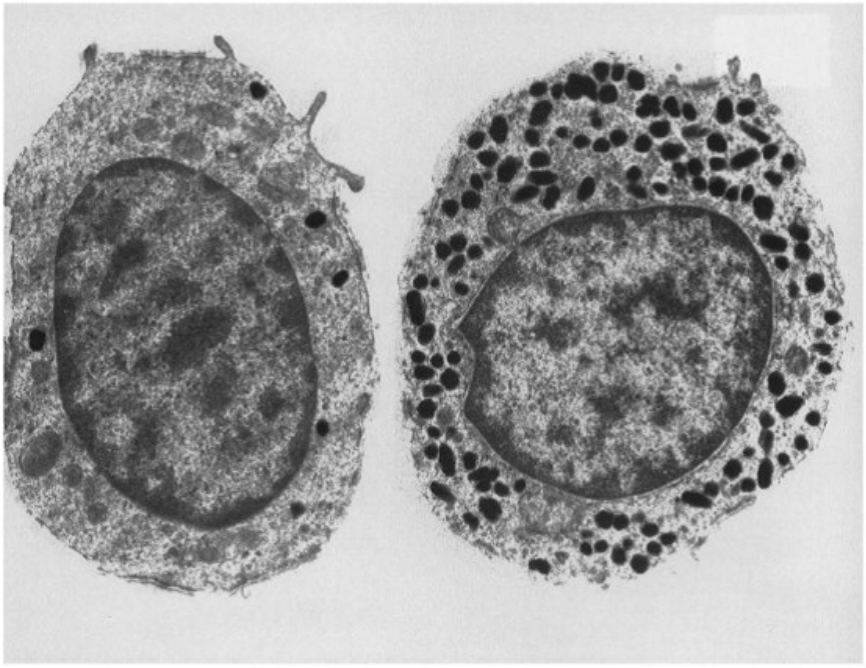
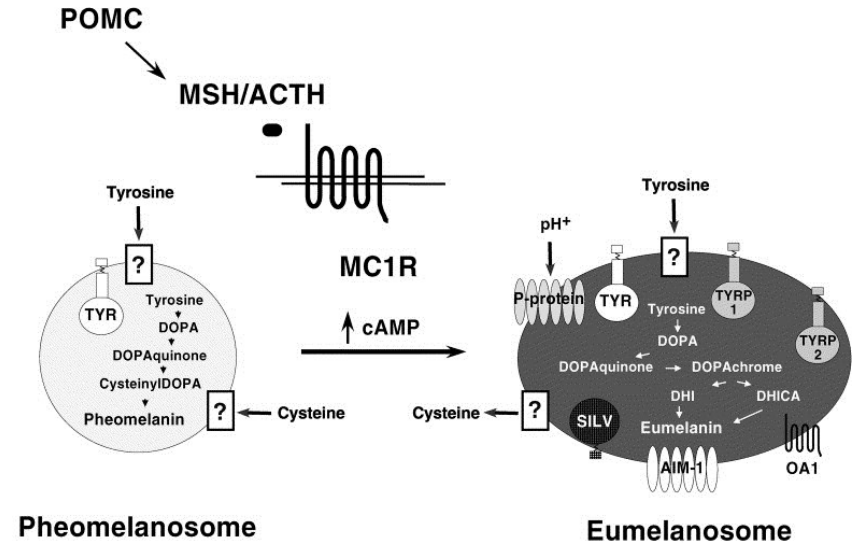
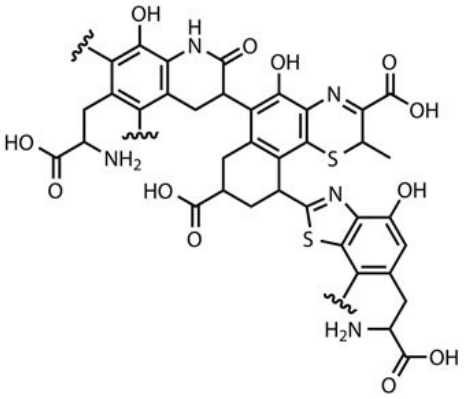


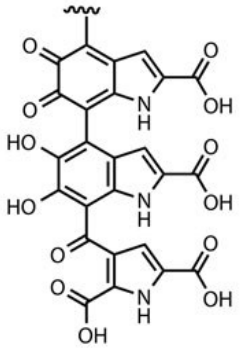
Fig. 2. Electron microscopic photograph at 4000× of a superficial stromal iris melanocyte containing only a few melanin granules from an eye with a blue iris on the left and an example of melanocyte from a brown eye on the right.

Brown eyes can vary from black to amber. The type of melanin, **eumelanin** and **pheomelanin** in the melanosomes determines the shade of brown (Prota et al., 1998). Eumelanin produces **darker brown eyes** and pheomelanin produces **lighter brown eyes**. Note, both eumelanin and phaeomelanin **absorb ultraviolet light** very well. As we will discuss later in the semester, while ultraviolet light is required to make vitamin D, too much ultraviolet light can do damage to cells.

Melanin is formed in the melanosomes of the melanocytes from the amino acid tyrosine in a pathway that involves **several enzymes**, each of which is encoded by a **gene**.

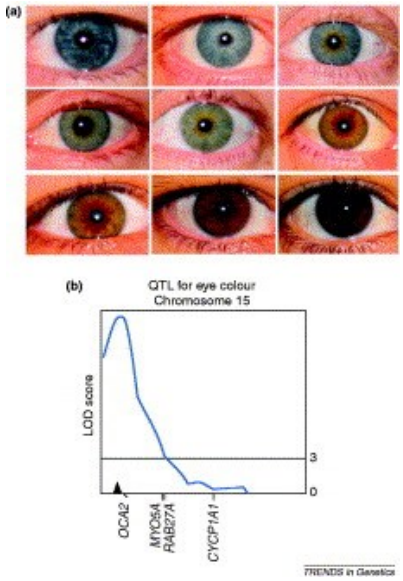




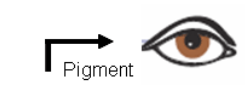


Ocularcutaneous albinism is the lack or reduction of melanin pigment in the eyes and skin. A variation in the ocularcutaneous albinism gene that codes for tyrosinase (OCA1), the enzyme that initiates the transformation of tyrosine to melanin, results in the complete lack of production of melanin. A person with this form of the gene will be an albino with blue or pink eyes.

A variation in in another **ocularcutaneous albinism** gene (**OCA2**) is correlated with fair skin, light hair and blue eyes. Three of the **variations** or **single nucleotide polymorphisms** (SNPs) in the DNA of the OCA2 gene are statistically **correlated** with blue eye color. So it seems that some versions of the OCA2 gene lead to brown eyes and others lead to blue eyes. There is no direct causal chain between OCA2 and eye color since the function of the OCA2 gene is unknown. However it **is believed** to be an integral

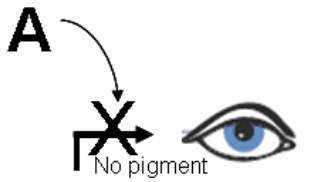


membrane protein involved in the **transport of tyrosine** into the **melanosome** where it can act as a precursor for melanin synthesis.



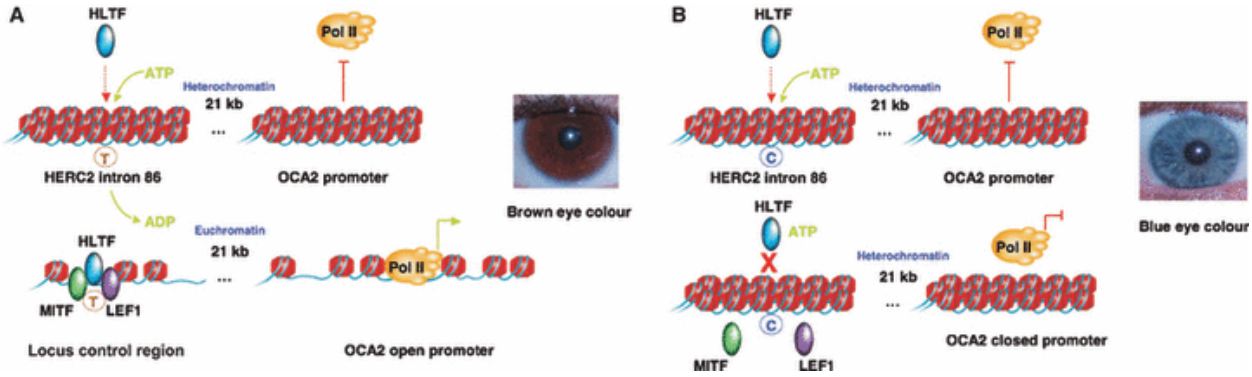
OCA2

OCA2



OCA2

There is another gene called **HERC2** that is adjacent to **OCA2**. One version of the HERC2 gene DNA sequence (TGACA(T/C)TTAAT) where there is a base change from T to C is highly **correlated** with blue eyes. The following **model** has been proposed by Sturm and Larsson (2009) to explain the interaction of the two genes: The T form of the HERC2 gene binds a helicase-like transcription factor (HLTF) that opens up the OCA2 gene so that RNA polymerase II can bind to OCA2 and the OCA2 gene **can be expressed**. Consequently, the protein for tyrosine transport into the melanosomes is produced. The variation of HERC2 that is correlated with blue eyes (C) prevents the binding of a helicase-like transcription factor (HLTF) and consequently, RNA polymerase II never binds to OCA2 and the OCA2 gene is **never expressed.**



Assume that the form of OCA2 that would lead to brown eyes if only one gene is involved is dominant and is given by B, and the recessive form of OCA2

that would lead to blue eyes if only one gene was involved is given by b. Assume that the form of HERC2 that would allow the expression of OCA2 and would lead to brown eyes is dominant and given by A and the form of HERC2 that would prevent the expression of OCA2 and would lead to blue eyes is recessive and given by a.

If blue eyes were determined by these two genes, there are many possible ways of having blue eyes: AAbb, Aabb, aabb, aaBB, and aaBb. For example, a person with the blue-eyed version of HERC2 (aa) and the brown-eyed version of OCA2 (BB or Bb), would have blue eyes. Here are a couple of examples:

Aabb x aabb

	ab
Ab	Aabb (blue)
ab	aabb (blue)

AAbb x aaBB

	aB
Ab	AaBb (brown)

aaBb x Aabb

	Ab	ab
aB	AaBb (brown)	aaBb (blue)
ab	Aabb (blue)	aabb (blue)

etc.

Since there are a number of variations in each gene and the proportion of each variation is unknown, we cannot determine the exact probability that two blue-eyed parents can have a brown-eyed child. **Experience tells us that it is rare; our analysis of the inheritance of two interacting genes tells us that it is not impossible**. Studies of the irises of identical twins show that approximately 58-78% of the iris characters have a genetic basis.

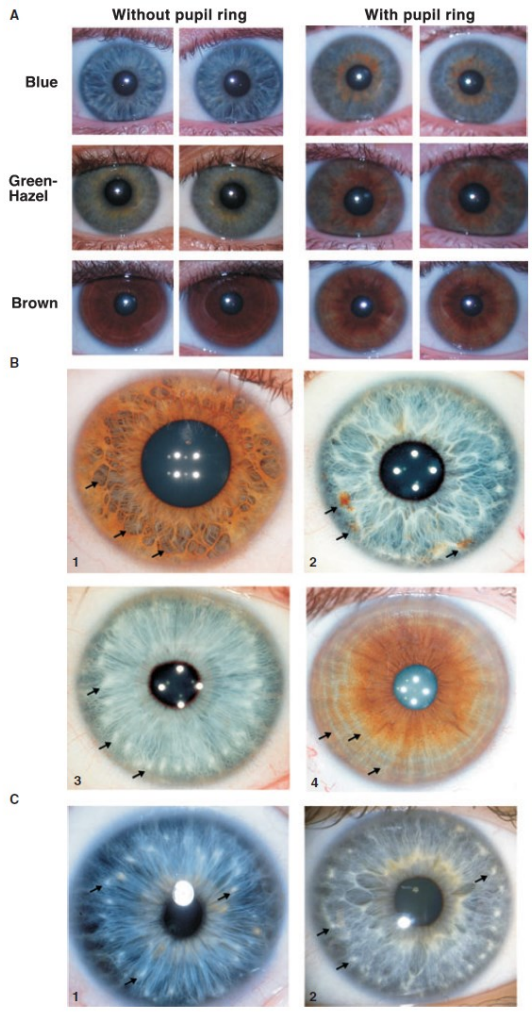
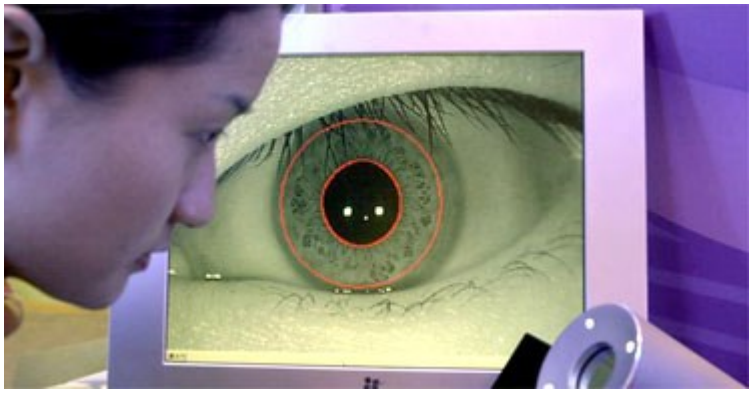


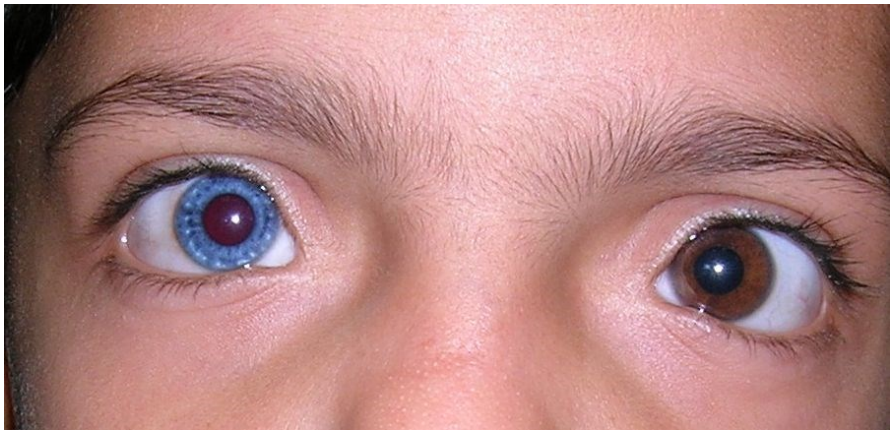
Figure 2. Human iris colour classification and patterns. (A) Three major classes of eye colour are shown as Blue, Green-Hazel, Brown with and without a brown peripupillary ring. (B) Patterns found within the iris highlighted by arrows: 1. Fuchs' Crypts, mild stroma atrophy; 2. Nevi, dots of accumulated melanin; 3. Wolfflin nodules, dots of accumulated collagen fibrils; 4. Contraction Furrows, fold marks in thicker irises due to iris contraction and dilation. (C) Patterns found within the iris highlighted by arrows: 1. Brushfield spots, observed in Downs syndrome; 2. Wolfflin nodules, observed in normal controls.

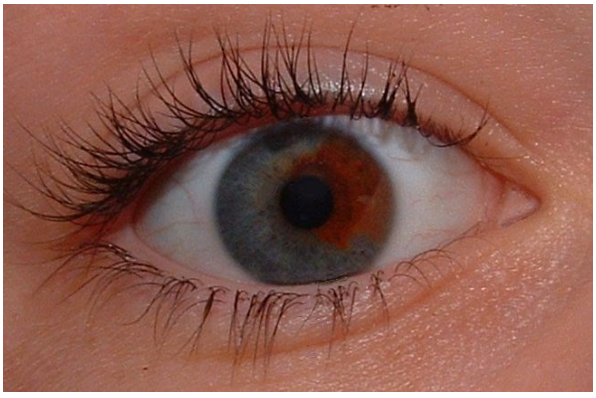
Is it possible that the environment has an effect on eye color? The only data I have that the environment affects eye color is the existence of color contact lenses. A **phenocopy** is someone who has a certain genotype but looks like he or she has a different one.

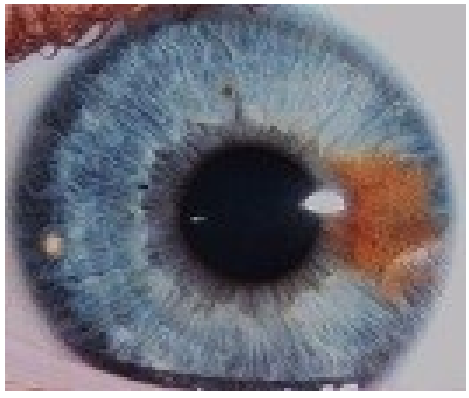
The human iris shows as many as 240 different features that can contribute to our individuality. In this respect, it is no surprise that the **Iris Recognition Immigration System** (IRIS) is used for individual identification.



Because the detailed development of the iris depends on initial conditions in the embryonic mesoderm and ectoderm from which it develops, the phenotypic expression of the two irises possessed by one individual can be are different. This is known as **heterochromia**. Heterochromia can also exist in one iris.





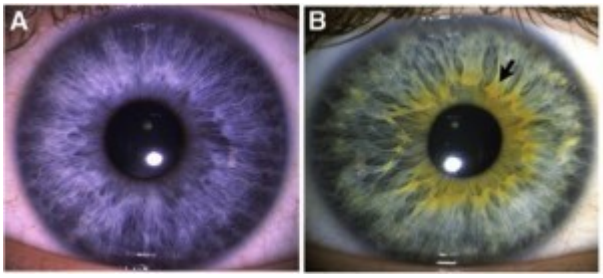


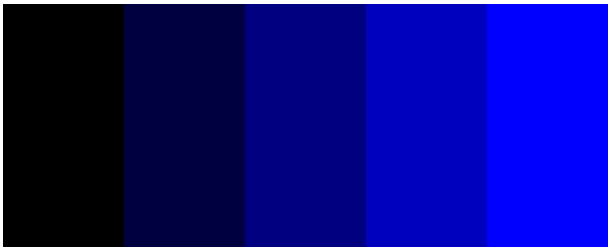
Mendelian genetics was used as a basis of paternity tests. While blue eyes were considered to a simple Mendelian character, one must remember that blue-eyed babies may become brown eyed children due to the developmental control of melanin production in Caucasian children (Beckman et al., 1960).

TABLE 32. *Qualitative characters. Summary of the results of the analysis of the family data.*

Character	Nothing contradicts simple inheritance	Inherited though not in a simple way	Sex variation	Age variation	Bilateral variation
Eye color	+		−	+	
Hair color	−	+	−	+	
„ form	−	+	−	+	
„ whorl	+		−	−	
Frontal hair	−	+	+	+	
Hair between the eyebrows	−	+	+	+	
Mid-digital hair	−	+	−	+	−
DARWIN's tubercle	−	−	−	−	−
Ear lobes	−	+	+	+	−
Tongue-rolling	+		−	−	
Chin groove	−	+	+	+	
Eye openings	−	+	−	+	
Folds on the upper eyelids	−	+	−	+	−
Extensibility of the proximal thumb joint	−	+	−	+	+
BONNEVIE's fingerprint formulae	−	−	−	−	
Length of the second toe	−	+	−	+	−
Tasting	+		−	−	

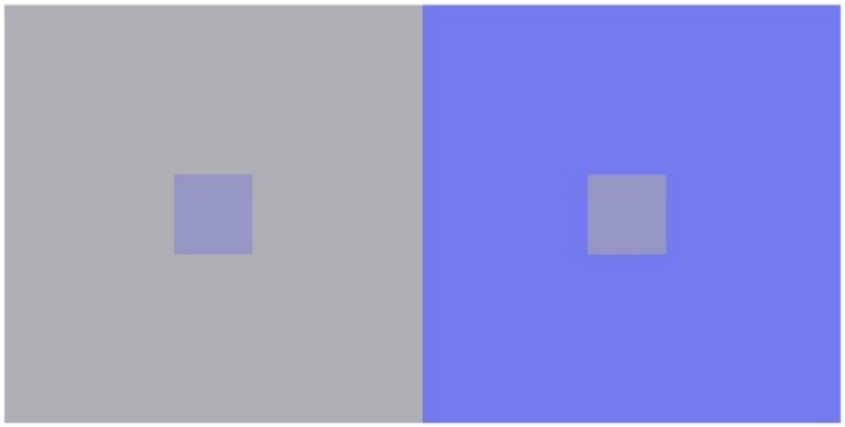
Because our brain judges colors relative to the surrounding or nearby color, the distribution of melanin in an iris may have a great effect on the perceived color of the iris.

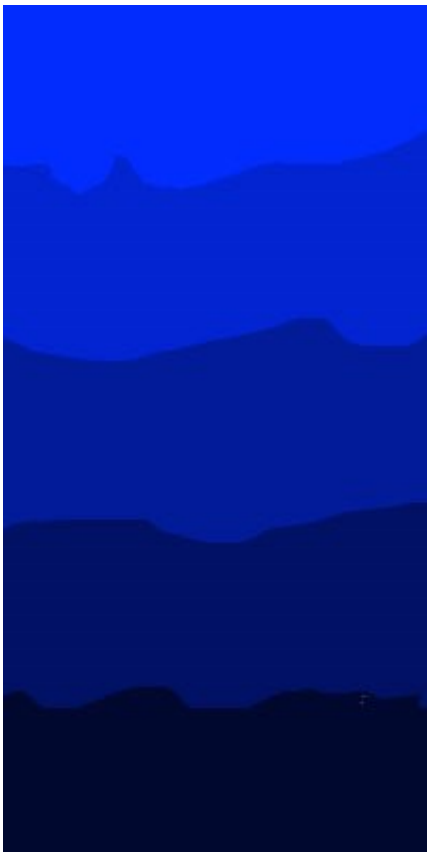




Look at the **Chevreul illusion**. Each rectangle is a homogenous, yet the part next to the lighter rectangle looks darker. The Chevreul illusion works for uneven shapes too, as long as the color family is the same.

This color saturation illusion shows you that nearby colors influence your perception of a given color. While two small squares appear to be different colors, they are the same color.

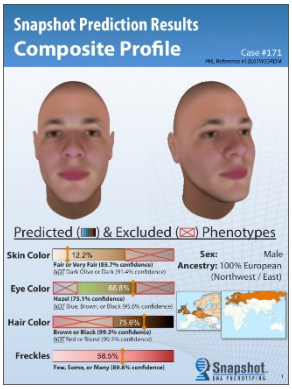


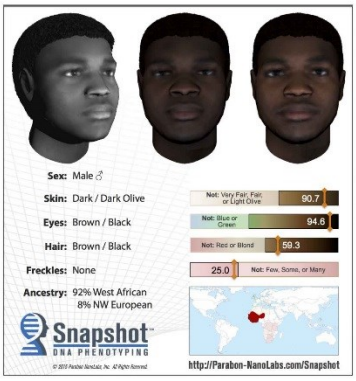


Both irises in the figure below are the same shade of gray, but the red color surrounding the iris makes the iris on the left look blue. But after masking the iris on the left, it looks like the same shade of gray as the one on the right.

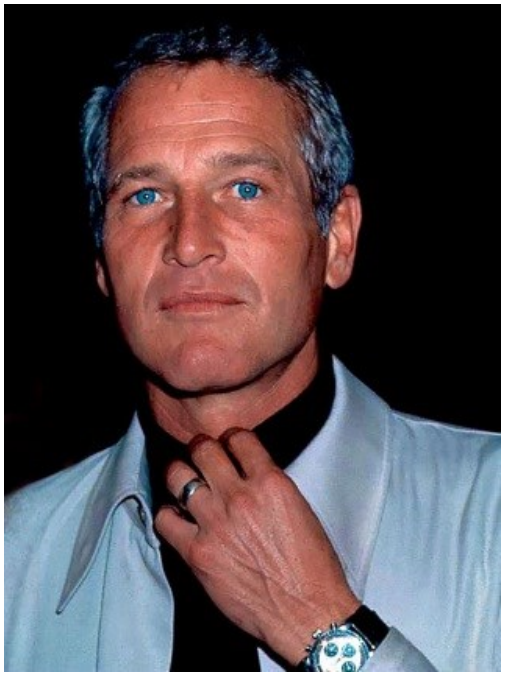
We have found that **eye color is not absolutely a single Mendelian trait,** but a **polygenetic trait**. Indeed there are even more than two genes that are correlated with eye color. You may expect this for two reasons: One, that there are more than two categories of blue-brown eye color and two, that there are a number of enzymes that are involved in melanin synthesis. **Each enzyme is encoded by a gene and each gene may be regulated by other genes**, like the OCA2 gene is regulated by the HERC2 gene. Moreover **any gene may exhibit incomplete dominance**. In addition, since only 58-78% of iris characteristics can be explained genetically in identical twins, **the environment** or **chance related to the position of iris cells derived from different cell populations in the embryo** may contribute to iris color.

Law enforcement is interested in predicting the eye color of the person who left behind DNA at a crime scene. Currently it is possible to predict the eye color of an individual with greater than 90% accuracy for brown and blue using six single nucleotide polymorphisms (SNPs). http://snapshot.parabon-nanolabs.com/





Melanin production in the iris begins during gestation in African American, Hispanic and Asian babies. Melanin production is usually delayed up to a year after birth in the irises of Caucasian infants so Caucasian babies are usually born with blue eyes. Within a year melanin production begins if the final color of the eye is going to be brown. Melanin production begins earlier in Caucasian girls than Caucasian boys. Some, like Paul Newman, keep their baby blue eyes.



Aside: Speaking of children, not all kids are so lucky. According to Dr. Abraham C., Nazi physician **Josef Mengele** noticed that a few seven-year old boys in Auschwitz "*had one odd characteristic: they were blond and had brown eyes, so Mengele was trying to find a way to color their eyes blue.*" Mengele tried to find a way to change their eye color and **injected their eyes with methylene blue**. While the treatment caused severe pain and inflammation, "*their eyes of course did not change."* Robert Jay Lifton (1986) wrote in his book *The Nazi Doctor*, "*But the methylene blue injections are of a different order, not in their cruelty (which was usual) but in their extraordinary scientific naïveté—or, one might more accurately say, their scientific corruption."*

Mengele shared the eyes he obtained from the "*inferior human material*" at Auschwitz with Karin Magnussen, a scientist who worked on eye pigmentation at the Kaiser Wilhelm Institute (KWI) for Anthropology, Human Genetics, and Eugenics, which was directed by Mengele's mentor Otmar von Verschuer. Lifton wrote that Verschuer spoke of the "enormously interesting specimens" of different-colored eyes Mengele had sent him, and seemed "surprised and upset" when he was told him they had come from Gypsies that Mengele had ordered killed because of this abnormality. "*In Verschuer's attitude we encounter a hypocritical academic accessory to Mengele's characteristic pattern of killing for science*."

Interested in eye color, Reginald Punnett (1911) wrote, "*A discussion of eye-colour suggests reflections of another kind. It is difficult to believe that the markedly different states of pigmentation which occur in the same species are not associated with deep-seated chemical differences influencing the character and*

bent of the individual. May not differences in pigmentation be coupled with and so become in some measure a guide to mental and temperamental characteristics? In the National Portrait Gallery in London the pictures of celebrated men and women are largely grouped according to the vocations in which they have succeeded. The observant will probably have noticed that there is a tendency for a given type of eye-colour to predominate in some of the larger groups. It is rare to find anything but a blue among the soldiers and sailors, while among the actors, preachers, and orators the dark eye is prominent, although for the population as a whole it is far scarcer than the light. ***The facts are suggestive, and it is not impossible that future research may reveal an intimate connection between peculiarities of pigmentation and peculiarities of the mind."***

Unbelievably to me, this kind of speculation has not stopped. Today there are many studies done by psychologists to understand how "*early expressing genes in the iris, are linked to brain development, and thereby potentially can contribute to identify networks of genes that influences different behavioural tendencies (Larsson et al., 2007)."* Some titles of published papers include, Eye color predicts alcohol use in two archival samples (Personality and Individual Differences 31 (2001) 535-539); Associations between iris characteristics and personality in adulthood (Biological Psychology 75 (2007) 165-175); and Eye color predicts but does not directly influence perceived dominance in men (Personality and Individual Differences 49 (2010) 59-64). Another study asks, Why do blue-eyed men prefer women with the same eye color (Behav Ecol Sociobiol 61 (2007) 371-384)?

Others ask about the evolutionary advantage of blue eyes in Northern climates where they are more common. According to Sturm and Larsson (2009),

the ability to overcome seasonal affective disorder (SAD) is linked to lighter eye color. "*Perhaps those with blue eyes may have been able to withstand the dark, depressing days of the Neolithic European winters better than those with brown eye colour*."

Prosthetic iris replacements made of colored silicone are now available in the United States (http://www.brightocular.com/) and laser surgery to convert brown eyes to blue is being developed (http://www.stromamedical.com/page/patient-info-faq). I think that there is something nice about keeping the eye color we were born with!