INTEGRATED DNA TECHNOLOGIES

Mendelian Genetics

Contents

Introduction

Today, when the word "genetics" is mentioned the mind is at once occupied with terms like cloning, PCR, the genome project, and genomics. Just a few decades ago, however, the word genetics conjured up a very different set of terms including crossing, segregation, Punnett square, and binomial expansion. It is not that these terms have disappeared or have been replaced since, it is, rather, that genetics moved full force into the molecular era in the late 1970s and, in the beginning of the twenty-first century, has passed on to the post-genome era. So much has genetics expanded and diversified that it is no longer adequate to study just genetics. To properly identify an area of study today requires the use of modifiers such as molecular genetics, quantitative genetics, behavioral genetics, plant genetics, human genetics, medical genetics, anthropological genetics, biochemical genetics, functional genomics, pharmacogenomics, and so on. In the minds of some who can still remember when you could take a genetics course and have the whole field covered in a single book that a person of average strength could actually carry to class, the unmodified term "genetics" refers to "Mendelian Genetics," the transmission of whole traits from one generation to the next.

While such a reduction may appear to be a quibble, it does reflect the historical truth that, prior to the elucidation of the genetic code in 1966, the development of DNA sequencing in the late 1970s and the discovery of the polymerase chain reaction in the early 1980s, all of genetics was in some way Mendelian Genetics concerned with the transmission of whole traits in families, pure lines, or breeding stocks. Here, a brief history of genetics up to the dawn of the molecular era is presented with a focus on Mendel and the laws of transmission genetics he discovered.

Heredity Before Mendel

The basic concept of heredity is at least as old as civilization itself. It was no coincidence that animals and plants produced offspring very similar to the parents and that reproduction was usually restricted to members of the same general group. In the ancient world it was clear that there was a process in which both parents made some form of contribution for it was observed that exact copies were never made, there was always some slight variation to be seen. Indeed, as long ago as the time of the Babylonians, farmers were aware that desirable traits could be manipulated by carefully selecting which specific parent animals or plants were allowed to reproduce. Records left by the ancient Egyptians clearly indicate that they practiced cross pollination of plants as a means of improving crops. And yet, while the practical benefits of hereditary manipulation were recognized by the ancients, there are no records prior to those of the Greeks that suggest their thoughts concerning the mechanism of heredity.

Pythagoras wrote some 2,500 years ago that semen was the product of fluids collected from the entire body and that there was a complete being preformed in the semen that was transferred intact to the female. This preformation theory was accepted, with various modifications, for more than two thousand years. Only occasionally did the notion that the female was simply the receptacle and had no role in determining traits appear to bother anyone. One such objection was raised by Empedocles about a century after Pythagoras when he proposed that there was, in fact, a blending of male and female that created an embryo and that the result was a combination of traits. This, and other objections, were shelved until well into The Reformation because of the pronouncement of Aristotle who held that while the female did make a contribution it was in the form of undifferentiated matter upon which the male imprinted life and form. Aristotle believed that semen was purified blood that carried the essence of the offspring to the less pure matter contributed by the female. Writing this in his great treatise *On the Generation of the Animals* in the 4th century BC, Aristotle had simply applied his view that all matter was formless until acted upon by an essence to the realm of biology. With the imprimatur of Aristotle firmly affixed, this was how things stood until the 17th century as there were no means available with which a skeptic could truly determine how heredity worked.

In the late 17th and early 18th centuries the English physician William Harvey (1578-1657) and the Dutch biologist Anton van Leeuwenhoek (1632-1723) independently discovered with the aid of new technology that female animals produced eggs, that embryos were formed by the union of egg and sperm, and that the embryo underwent a subsequent development that was similar regardless of the animal being investigated. This, of course, meant that Empedocles' notion was closer to the truth but there were detractors. Another Dutch scientist, Jan Swammerdam (1637-1680), proposed that what van Leeuwenhoek was actually seeing in his microscope when he looked at sperm was in fact a tiny, pre-formed being, a homunculus, that entered the egg and used it for its source of nourishment as it grew. About a century later a Swiss scientist called Charles

Bonnet (1720-1793) reversed the role completely by suggesting that it was the eggs that held the homunculus and that each succeeding generation was similarly housed within in the manner of an endless succession of Russian matryoshka dolls (Figure 1).

Figure 1. A classic set of Russian nesting "matryoshka" dolls in which each dolls is housed, fully formed, within the next larger doll in series. This is the view of heredity suggested by Bonnet in the 17th century. One objection never addressed was what happened when the last doll (homunculus) was reached.

Alternative theories were advanced by the French scientist Pierre Louis Moreau de Maupertuis (1698-1759) and the German anatomist Kaspar Friedrich Wolff (1733-1794). Both dismissed the notion that there were preformed homunculi carried by either sperm or egg and proposed instead that the actual reproductive material consisted of particles contributed by each parent and carried by both sperm and egg into union as the embryo. Both envisioned these particles as jointly determining not only form but sex as well. Maupertuis even held that some particles from one parent could exert a stronger influence than those from the other. However, both of these theories were advanced in the absence of actual experimental evidence.

Had they known of the work of a German botanist named Joseph Gottlieb Koelreuter (1733-1806), both Maupertuis and Wolff would have had the experimental evidence they needed. Koelreuter originally studied medicine at Tübingen but became interested in natural history at the Academy of Sciences in St. Petersberg. There, he studied the structure of flowers and the mechanisms of pollination. In 1763 he was appointed Professor of Natural History at Karlsruhe and also Director of the Gardens. He began to carry out experiments in cross pollination in the tobacco plant that he carefully recorded and later published. The most important discovery made by Koelreuter was sexual differentiation in plants that led to his demonstrations that traits in offspring were equally determined by the parents.

Unfortunately, apart from a very few attempts to reproduce his results, Koelreuter's work was largely ignored except by those who dismissed it as completely wrong. He had found that the characters exhibited by the first generation of a cross (called the F1 generation) would lie intermediate between those of the parents in many cases but that the next generation (called the F2) would display a range of types including those of the original parents. Koelreuter was dismayed by this because he viewed the F1 blending as evidence of natural harmony and perfection and the F2 results as a breakdown of this

harmony. The explanation of these disturbing breakdowns was soon to be discovered by an obscure Silesian (Austrian) monk named Johann Gregor Mendel (1822-1884).

Mendel and the Laws of Heredity

Gregor Mendel was born in the Silesian village of Heizendorf (now called Hynčice) one of five children. Originally named Johann, he was renamed Gregor in 1843. Mendel demonstrated his intellectual abilities at an early age and was sent at age eleven to the Piarist High School in Leipnik and then to the Gymnasium at Troppau (now called Opava). He completed his education there in 1840 and moved on to the University in Olmütz (Olomouc). After a brief illness he was advised to enter the priesthood in the monastery in Brno (Figure 2). Here, he entered a world in which, unlike the conventional view of a monastery, he was immersed in a well respected seat of scientific learning. Many of the members of the Augustinian order at Brno held professorships in the local university or left to assume similar positions at other universities. Thus he was able to continue on an academic track. In 1851 he was sent to the University in Vienna where he was influenced by a number of great minds who were leaders in their fields. The most influential of these to Mendel was Franz Unger (1800-1870), Professor of Plant Physiology. However, in addition to his studies with Unger in which he learned of the work of influential biologists such as Carl Naegeli (1817-1891) and Matthias Schleiden (1804-1881), Mendel learned the value of precise observation and the importance of statistical evaluation from the physicists in Vienna, notably Christian Doppler (1803-1853) and Andreas von Ettinghuasen (1796-1878).

During his years in Vienna Mendel, by virtue of his relationship with Unger, was well aware of a raging controversy in which Unger figured prominently. One of the dominant views in biology at the time was the fixity of species. That is, species were set and constant and, therefore, could not change and certainly could not evolve. Unger was a vocal proponent of the view that variants would arise in natural populations and that slight variants gave rise to varieties and sub-species while large variants would result in new species. So controversial was this view at the time that Unger was almost dismissed from the faculty in Vienna in 1856. One of the motivations ascribed to Mendel for beginning his plant hybridization experiments in the first place was to resolve this issue [1].

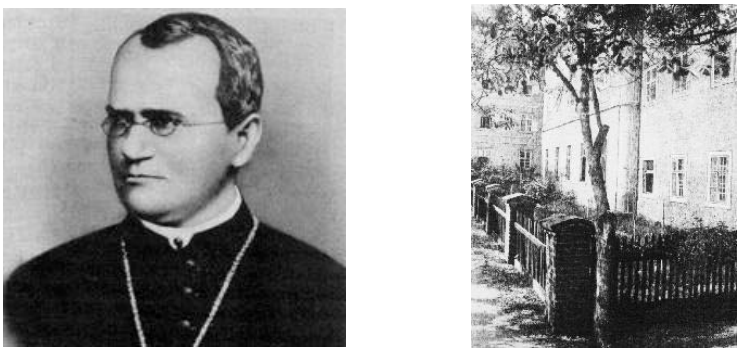

Figure 2. On the left is a portrait of Gregor Mendel from 1880 and on the right is a photograph of the gardens at the Brno monastery taken in the 1920s.

Regardless of his motivation, Mendel had set himself a monumental task. He was determined to catalog all of the different forms that hybrids could take and to carry out a statistical analysis of these forms. The experimental system he chose was the common garden pea, *Pisum sativum* (Figure 3). He began his crosses in earnest in the summer of 1856 and over the course of the next fifteen years he identified several traits in his plants that appeared to breed "true" and used them in his crosses. In all, he made tens of thousands of observations of which only a few are well known. However, it is these few well known traits that led to the formulation of what are now called Mendel's Laws of Heredity.

Figure 3. A traditional rendering of the pea plant from a botany text of the time.

Mendel wrote of his experiments under the title *Versuche über Pflanzen-Hybriden* (Experiments in plant hybridization) published in *Verh. Naturf. Ver. in Brunn, Abhandlungen* (Proceedings of the Brunn Society for Natural History) in 1866.

In his paper, Mendel laid out his experimental procedures and noted that the traits he had selected to use, among others, related to the difference in the form of the ripe seeds, to the difference in the color of the seed albumin, and to the difference in the form of the ripe pods (Mendel, 1866 translation in Peters, 1959). Mendel noted the number of plants used for each cross and the forms of the hybrids. He then noted the circumstances and results of the next generation (the F2) of crosses. For example, Mendel noted for albumen color 258 plants yielded 8,023 seeds, 6,022 yellow, 2,001 green; their ratio, therefore, is as 3.01 to 1. He noted the results of various combinations of traits such as round and wrinkled seeds with yellow and green albumin.

Among his observations was that, in the single trait crosses, one of the two forms of the trait would appear in the F1 generation intact and, therefore, "those characters which are transmitted entire, or almost unchanged in the hybridization, and therefore in themselves constitute the characters of the hybrid, are termed the *dominant*, and those which become latent in the process *recessive*." Among his traits round seeds were dominant over wrinkled, yellow albumen was dominant over green, and smooth pods were dominant over rough. This led him to formulate his First Law, "… hybrids form seeds having one or the other of the two differentiating characters, and of these one-half develop again the hybrid form, while the other half yield plants which remain constant and receive the dominant or the recessive characters [respectively] in equal numbers." This is now called **segregation**. Take the character of albumin color, yellow is dominant over green. If Y is the symbol for yellow and y is the symbol for green, then, starting with pure lines in the parental generation;

P1: YY x yy
F1: Yy
F2: YY yY Yy yy

The F1 will all be yellow and the F2 will display the 3 to 1 ratio of yellow to green. The outward, physical appearance is called the **phenotype** (literally, the form that is shown). The "particles" that create the phenotype are now known to be genes and, therefore, each phenotype has an underlying **genotype**. (Note: the terms gene and genotype did not exist in Mendel's day, these terms were coined later by the Danish geneticist Wilhelm Johannsen, 1857-1927). Segregation refers to the separating of the particles in the F1 cross. A convenient means of keeping track of the segregating particles no matter how many there are was developed by and named for the English geneticist Reginald Crundell Punnett (1875-1967). Called the **Punnett Square**, the two forms of the gene (called **alleles**) are segregated by parent to permit an easy tabulation of the resulting offspring's genotypes:

		Male Parent	
		Y	**y**
Female Parent	**Y**	**YY**	**Yy**
	y	**Yy**	**yy**

Parents: all yellow Yy

Offspring: 1 yellow YY, 2 yellow Yy, 1 green yy

Mendel went on to consider various traits in combination. He observed that, "the hybrids in which several essentially different characters are combined exhibit the terms of a series of combinations, in which the developmental series for each pair of differentiating characters are united." Further, "the relation of each pair of different characters in hybrid union is independent of the other differences in the two original parent stocks." This is Mendel's Second Law of Heredity called **Independent Assortment**. Taking yellow and green albumen together with round and wrinkled seeds, if the pure lines are yellow (YY) and round (RR) and green (yy) and wrinkled (rr):

P1: YYRR x yyrr
F1: YyRr
F2: YYRR yYRR YyRR yyRR
YYRr yYRr YyrR yyrr

Again, using the Punnett Square and assorting the two traits independently,

		Male Parent			
		YR	**yR**	**Yr**	**yr**
Female Parent	**YR**	**YYRr**	**YyRR**	**YYRr**	**YyRr**
	yR	**YyRR**	**yyRR**	**YyRr**	**yyRr**
	Yr	**YYRr**	**YyRr**	**YYrr**	**Yyrr**
	yr	**YyRr**	**yyRr**	**Yyrr**	**yyrr**

Parents: all yellow, round

Offspring: 9 yellow, round; 3 yellow, wrinkled; 3 green, round; 1 yellow, wrinkled

Many examples of using of the Punnett Square to work out various crosses and combinations of traits are presented in the **Supplemental Material** at the end of this tutorial.

The "Rediscovery" of Mendel

Despite the fact that copies of the issue of the Proceedings in which Mendel's work appeared were sent to numerous institutions such as the Royal Society and the Linnean Society as part of a regular mailing list, apart from a few letters exchanged with contemporary scientists, notably Carl Naegeli, the paper and its results went completely unnoticed until 1900. During the latter part of the 18th century, scientists were grappling not only with concepts of heredity but also with incorporating them into Darwin's model of evolution. Notable among these scientists were the Dutch botanists Hugo de Vries (1848-1935) and Carl Correns (1864-1933), Austrian botanist Erich von Tschermak (1871-1962), and English biologist William Bateson (1861-1926). Correns, de Vries, and von Tschermak were all independently working along the same lines as Mendel and were reaching the same general conclusions at the close of the 18th century. Then, in 1900, each became aware of Mendel's paper and de Vries sent a copy of a report on his own work to Bateson that contained a mention of Mendel. Bateson searched out the original publication of Mendel's paper and an English translation appeared in 1901. An excellent account of the facts surrounding the rediscovery of Mendel is provided by Olby (1966).

Most historians of science set the year 1900 as the birth of genetics because that is the year that Mendel's paper was "rediscovered." Much of what we regard as standard terminology and concepts were developed in the first few years after the translation of Mendel's paper appeared. Bateson himself coined the term genetics, Johannsen defined and refined the terms gene, genotype, and phenotype, and the essential blending of Mendelian inheritance and Darwinian evolution was well under way. One of the lesser-known stories about the rediscovery of Mendel's work was that some, including Bateson, believed that Mendel had enunciated three laws of heredity. In addition to segregation and independent assortment, many regarded the phenomenon of **dominance** as a hereditary law at the beginning. It was viewed as an inherent property of traits and that it was immutable. Evolutionary geneticists grappled with the idea that dominance was just another trait subject to Darwinian selection until, in 1928, Sir Ronald Fisher (1890-1962) published his view that dominance could be modified by modest levels of selection. Fisher reiterated and expanded upon this in his monumental

1930 treatise *The Genetical Theory of Natural Selection*. Instead of settling the debate over the nature of dominance, Fisher's work sparked a debate about the nature and role of selection with the great American population geneticist Sewell Wright (1889-1988) that had dominance as the center piece and lasted well into the 1980s with many of Fisher's students and colleagues carrying on after his death [2]. The story of the evolution of dominance is a fascinating tale in its own right as it involved nearly all of the giants of twentieth century genetics, years of arduous field and laboratory breeding work, and some of the most elegant mathematics theoretical population genetics has to offer [3, 4, 5, 6, 7, 8].

The Misuse of Mendel

The rediscovery of Mendel's laws of segregation and independent assortment set genetics on a sound theoretical footing in the early 20th century. Among those that used that footing to build up a solid edifice of genetic science many have already been mentioned such as Johanson, Correns, and Punnett. Another group that deserves special mention all worked in the same laboratory at Columbia University in New York. Under the guidance of the great American geneticist Thomas Hunt Morgan (1866-1945), a group of students that included Herman Joseph Muller (1890-1967), Calvin B. Bridges (1889-1938), and Alfred H. Sturtevant (1891-1970), studied the transmission of phenotypes cataloged by them in the fruit fly *Drosophila melanogaster*. From this work emerged most of the founding principles of modern genetics including chromosomal linkage and mutation [9].

So powerful were the discoveries of the early years of the 20th century and so compelling were the models built to explain them, that some carried genetic principles to an unfortunate and, ultimately, tragic extreme. A number of scientists and non-scientists alike saw the elegant simplicity of Mendel as the answer to everything. Ignoring the complications and the exceptions that were piling up as experiments in Mendelian genetics became more sophisticated and the traits being studied more complex, some seized upon very simple models as all that were needed to explain even the most convoluted biological characteristics. Nowhere was this more evident than in the rapidly expanding discipline of human genetics.

Attracted by the allure of simplicity, some of the attempts to explain complex human traits with basic Mendelian principles are humorous when viewed from a 21st century perspective. Many of the texts of the period contained family histories that purported to demonstrate simple Mendelian inheritance of artistic ability or musical ability. One extensive pedigree displayed evidence for the inheritance of ship building skill over several generation of a Norwegian family. Another prominently showed that three generations of band directors followed a basic Mendelian pattern. It is often common even today for people to casually note that doctors or lawyers "run in certain families" and, while no one today would seriously believe that medicine or law or music or even ship building is determined by a single Mendelian gene, such comments were taken very

seriously in the early 20th century. In fact, such belief was strong enough for a field of scientific inquiry to arise that sought to enhance traits deemed to be beneficial and to eliminate traits held to be deleterious. This science was called eugenics.

Eugenics comes from the Greek roots for "good" and "origin" or "generation." The term was first used to refer to good breeding through selective heredity around 1883. By the 1920s the eugenics movement in the United States and Europe was gaining wide acceptance and was being championed by the respected American geneticist Charles Davenport (1866-1944). Eugenics was being portrayed as a sound mathematical science based upon Mendel's law that could produce superior offspring via selective mating. Eugenicists held that desirable traits should be encouraged and numerous societies like the Race Betterment Foundation were established. Contests were held and prizes were awarded to "good families" at fairs and other events (Figure 4).

The other side of the eugenics movement was much darker. The goal of promoting the inheritance of "good" traits was being mirrored by the goal of preventing the inheritance of "bad" traits. Complex human traits like alcoholism, feeblemindedness, criminality, and even poverty were attributed to a simple model of Mendelian transmission. Prevention in the United States took the form of designating certain countries and groups as being prone to these traits and banning immigration. In addition, there was a massive program of involuntary sterilization of those already here. As late as 1942 the ethics of "euthanizing" children with disabilities was seriously debated in the pages of a major medical journal. In all, thousands of American citizens and immigrants were sterilized by court order.

Figure 4. This family was awarded a prize in a eugenics contest at a 1923 Kansas fair. Thousands of similar examples of "good breeding" were recognized during the heyday of the eugenics movement. Source: PBS Science Odyssey.

In Europe the eugenics movement gained equal acceptance but its power was nowhere exceeded than in Germany when it became an official policy of the Nazi Party. There, its precepts were taken to the ultimate extreme when the Nazi Party came to power in the 1930s. Soon, the list of traits to be eliminated grew quite long and "undesirables" were being rounded up and sent to camps. Selective human breeding programs, called the "liebensborn," were established and "stocked" with young women who, by the criteria

established under the Nazis, displayed the desired traits. Eventually the Nazis took this movement to the “final solution” of the question of the unfit and the concentration camps became death camps (Figure 5).

Figure 5. A photo taken at the liberation of one of the many Nazi death camps discovered as WWII came to an end. In all, the Nazis exterminated more than seven million people of whom six million were Jews.

Mendel in the Modern World

The laws of heredity established by Mendel form the backbone of modern genetics. Nowhere is this more evident than in the ongoing search for genes that cause diseases in humans, animals and plants. The sophisticated, contemporary methods for mapping and, ultimately, identifying individual genes that either increase risk for developing diseases or actually cause them is firmly rooted in Mendelian genetics. Genetic linkage analysis is based upon the co-transmission of genetic material that is physically linked together on the same region of a chromosome. The mathematics of linkage analysis works because of segregation and independent assortment. A genetic marker that displays independent assortment in families relative to a trait of interest such as cystic fibrosis, Huntington’s Disease, Breast Cancer, or Alzheimer’s Disease cannot be physically linked to that trait whereas a marker that segregates along with the trait is likely to be near the gene that causes the illness. Through this method literally hundreds of human, animal and plant genes have been mapped, cloned, and studied. Indeed, while the various genome sequencing projects, including the Human Genome Project, have made this search far easier than it was just a few years ago, the initial genetic maps that were used as the guides for ordering the sequences were made using mathematical and laboratory techniques, like linkage, that are grounded in the application of Mendel’s Laws.

References and Resources

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Supplemental Material

Exercises in Mendelian Genetics
A range of hands-on exercises that can be used to present various aspects of basic Mendelian genetics are presented here. Most of these are straight-forward pencil and paper problems. In addition, some on-line resources of materials are listed.

I. Basic Mendelian Segregation
a. Complete Dominance

Phenotype: flower color

Alleles: R (red), r (white); R is dominant

Genotypes: RR, Rr = red; rr = white RR and rr are the **homozygotes** and Rr is the **heterozygote.**

Parental Pure Line Cross (P1): RR  x rr 

F1 generation: all Rr heterozygotes 

F1 cross: Rr  x Rr 

Gametes:

	R	r
R	 RR	 Rr
R	 Rr	 rr

In his paper Mendel reported that he observed 705 red flowers and 244 white flowers on a total of 929 F2 plants. His observed **phenotypic ratio** was 3.15 to 1.

Consider the problem of **backcrossing**. Given the following F2 backcrosses and the resulting F3 phenotype ratios, what are the genotypes of the F2 parents?

F2:	Red x Red	Red x White	Red x Red
F3	712 Red	505 Red; 490 White	740 Red; 260 White

<u>b. Partial or Incomplete Dominance</u>

Sometimes you can see a difference between the phenotype of the F1 hybrids and the two parental pure lines. This occurs when dominance is incomplete. The phenotype of the heterozygote F1 may lie in between that of the parental lines.

Phenotype: flower color

Alleles: R (red), r (white); R is dominant

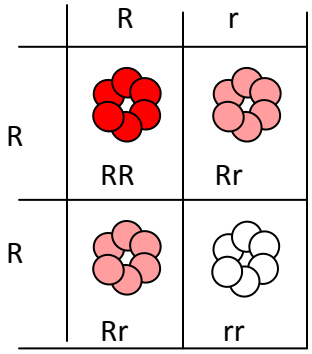
Genotypes: RR, Rr = red; rr = white RR and rr are the **homozygotes** and Rr is the **heterozygote.**

Parental Pure Line Cross (P1): RR  x rr 

F1 generation: all Rr heterozygotes 

F1 cross: Rr  x Rr 

Gametes:

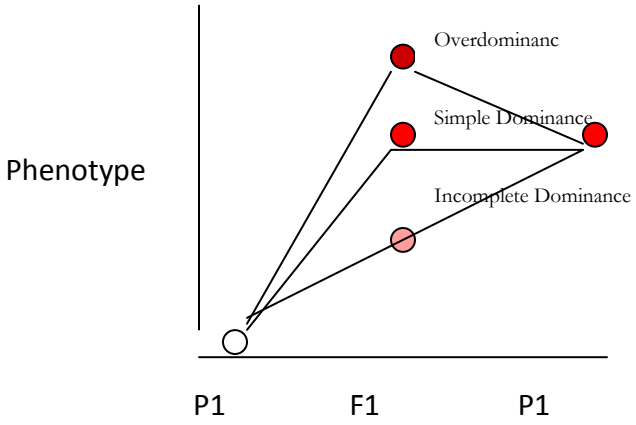


It is possible to know exactly what the F2 genotypes are now. Of course this is very easy when the F1 is a clear intermediate phenotype. Many times the heterozygote will not be so obvious. Take the case:

Parental Pure Line Cross (P1): RR  rr 

F1 generation: all Rr heterozygotes 

Here, the F1 actually has a completely different color from the parental lines. The actual relationships that are possible with dominance, even in simple crosses, range from various degrees of partial dominance, through complete dominance, and on to what is called **overdominance** such as shown here where the F1 heterozygote is a deeper red than even the homozygous parent. Clearly, this is an oversimplification but the range of potential dominance effects is shown graphically below.

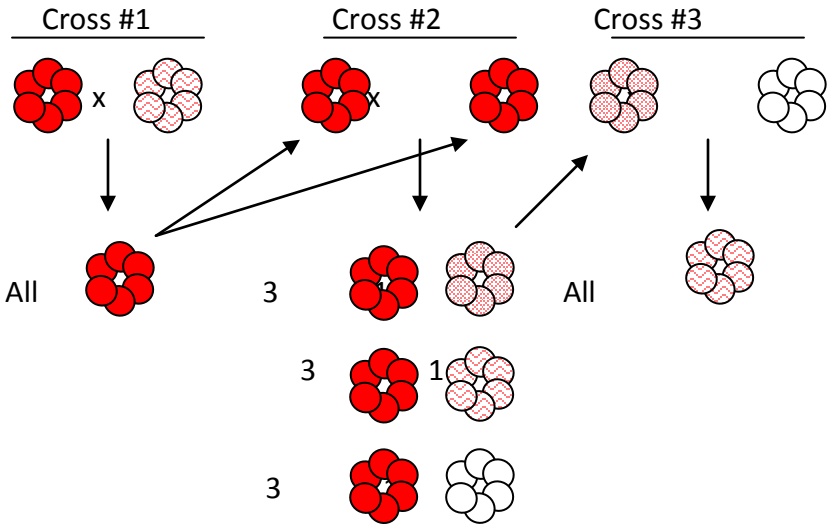


It is important to note here that an observation that looks like a departure from simple dominance may not be simple at all. In the case of the F1 in which the flower color is a deeper red than the pure line parent red, overdominance is only one possible explanation. Other possibilities include a mutation creating a new allele (which will be presented next) and **epistasis**, the phenomenon that occurs when the genotype of one trait interacts to change the phenotype of another (which will be presented later).

c. Multiple Alleles

Now, consider the case in which the gene that encodes a trait has not two but three alleles. Again using a flower color example, pure lines of red and white flowers are crossed and a third color appears on one plant. Now there are  and

and you want to know where the new color comes from. Set up the following crosses:



Now there are four phenotypes but the genetics can now be worked out. Numbering the alleles, call the pure red 1,1 and the pure white 3,3. The new phenotype can be called 2,- for the time being since the dominance relationships are not known.

In Cross #1 all of the flowers are red so 1 must be dominant. New mutants are always hheterozygous, so the genotypes of Cross #1 must be 1,1 x 2,3 and the F1 must be an equal mixture of 1,2 and 1,3. Cross #2 is fairly simple to work out. In the F1 of Cross #1 the genotypes are a mixture of 1,2 and 1,3. This means that there are three possible F1 crosses in Cross #2: (1,2 x 1,2); (1,2 x 1,3) ; and (1,3 x 1,3). The results of these crosses are:

1,2 x 1,2	1,2 x 1,3	1,3 x 1,3
↓	↓	↓
1,1 ; 1,2 ; 2,2	1,1 ; 1,3 ; 2,3	1,1 ; 1,3 ; 3,3

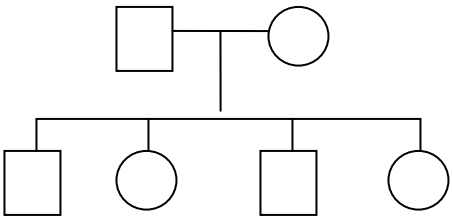
Thus, Cross #3 must be 2,2 x 3,3 since there are no white flowers the genotypes can only be 2,3. Therefore,

1 is dominant over 2 and 3 and 2 is co-dominant with 3.

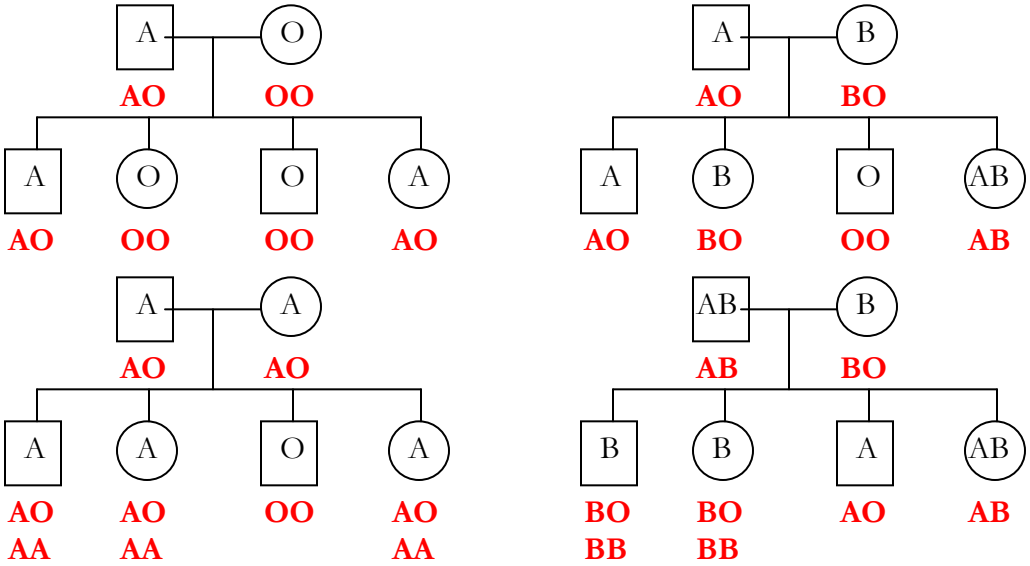
If this seems complicated, it is. However, this is what Karl Landsteiner (1868-1943) had to work out when he discovered the human ABO blood groups in 1900. He observed that mixing human blood samples together gave a pattern of reactions that could only be explained if there were three alleles. He called them A, B, and O and found that tracing the reaction patterns did conform to Mendel's laws if;

1. A and B were both dominant over O
2. A and B were co-dominant with each other

Landsteiner could not set up crosses so he did the next best thing. He traced the blood group reaction patterns in families. This method is known as **pedigree analysis**. Consider the following basic family pedigree:

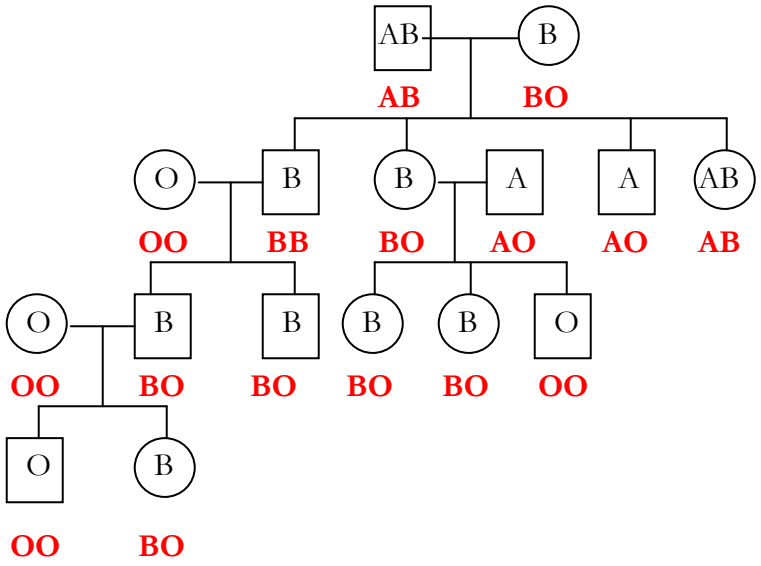


By convention, squares denote males and circles denote females. This is a simple nuclear family with two parents and four children. Taking this basic pedigree and the two conclusions of Landsteiner regarding the ABO blood groups, fill in the genotypes based on the following phenotype patterns;



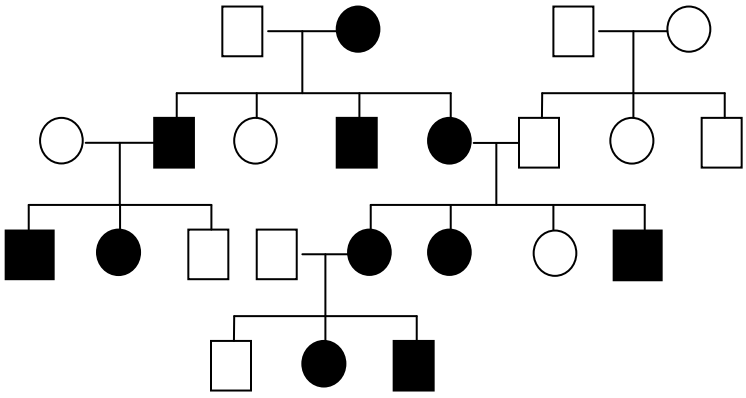
Note that the genotypes of a number of the children can not be determined precisely. When this occurs, additional information needs to be obtained by **extending** the pedigrees vertically and horizontally. An example of such an extended pedigree for the

ABO blood groups is shown below. It is easy to fill in each genotype uniquely by working through the pedigree using Landsteiner's observations.

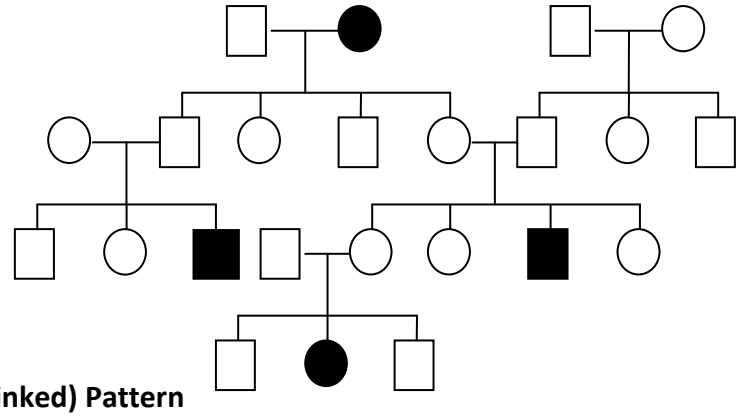


Observing traits such as simple genetic diseases in extended pedigrees was the way in which the inheritance of many traits was discovered. The patterns formed in extended pedigrees are often all that is needed to determine whether a trait is dominant, recessive, or sex-linked. Examples of these patterns are shown below:

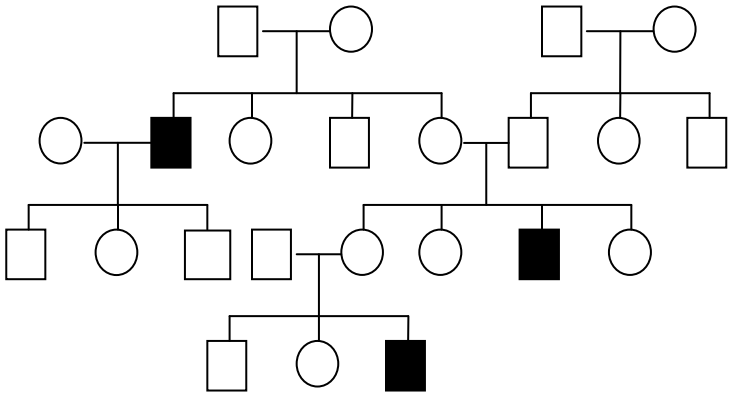
Dominant Inheritance Pattern



Recessive Inheritance Pattern

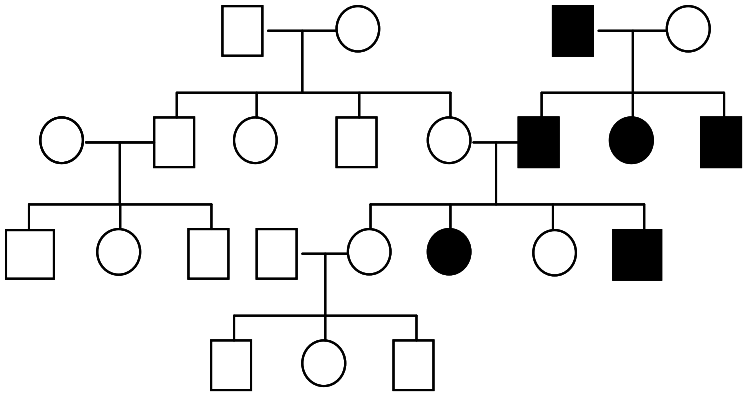


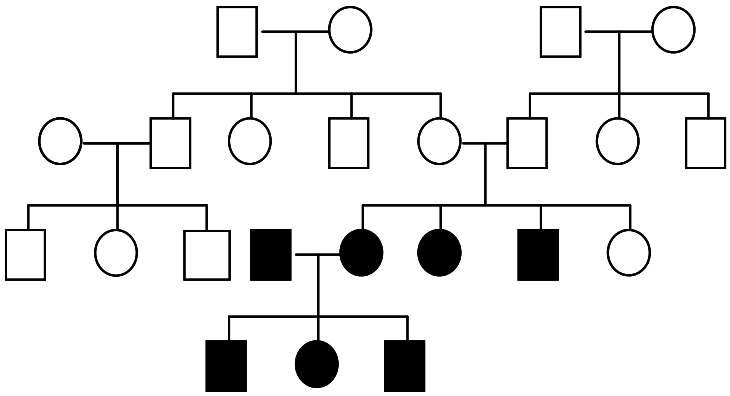
Sex-linked (X-linked) Pattern

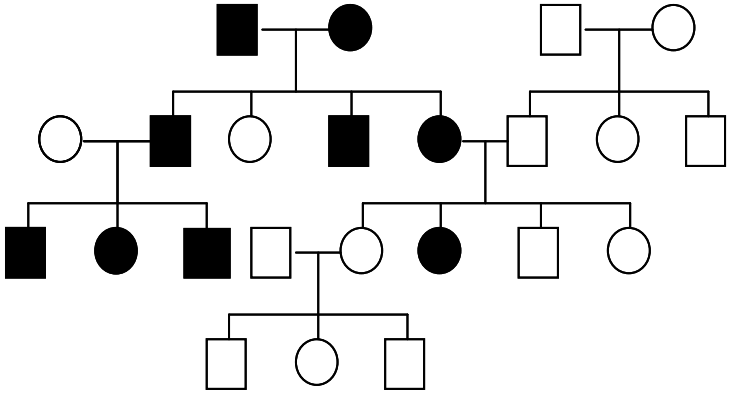


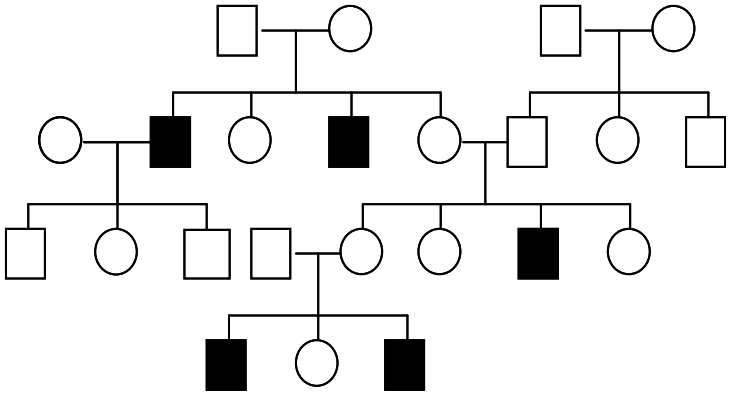
The differences among the three pedigrees are related to the density of the affected individuals (the black symbols) and the sex distribution. A dominant trait will exhibit a higher density than a recessive trait since the dominant allele only requires one copy , that is, heterozygotes will be affected, while a recessive trait must be homozygous for the trait to be expressed in the phenotype. In the sex-linked, or X-linked, pattern, expression is limited to males only.

What pattern of inheritance is represented in the following four pedigrees?









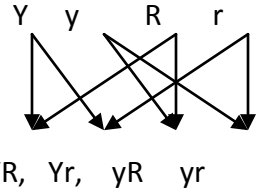
II. Independent Assortment

Mendel's careful recording of single trait crosses clearly established the ubiquitous nature of allelic segregation. Fortunately, he did not stop there. Considering two traits at atime is called **dihybrid crossing**. As an example, take Mendel's observation of the two traits of yellow and green albumin and round and wrinkled seed. Noting that yellow (Y) is dominant over green (y) and that round (R) is dominant over wrinkled (r), pure lines for both traits simultaneously will have the genotypes YYRR and yyrr. If these lines are then crossed as the P1 generation, the F1 will all be yellow and round, or YyRr. In subsequent F2 dihybrids Mendel observed phenotypic ratios approximating the following distribution:

9 yellow, round
3 yellow, wrinkled
3 green, round
1 green, wrinkled

Mendel reasoned that this pattern could only be produced if the two traits were acting independently. We can see this easily using a Punnett Square for the F1 cross.

F1 gametes:



F2 Genotypes:

	YR	Yr	yR	yr
YR	YYRR	YYRr	YyRR	YyRr
Yr	YYRr	YYrr	YyRr	Yyrr
yR	YyRR	YyRr	yyRR	yyRr
yr	YyRr	Yyrr	yyRr	yyrr

Keeping the dominance relationships in mind, the 9:3:3:1 dihybrid phenotype ratio can be found in the 16 squares.:

9 yellow and round: YYRR, 2 YYRr, 2 YyRR, 4 YyRr

3 yellow and wrinkled: YYrr, 2 yYrr

3 green and round: yyRR, 2 yyRr

1 green and wrinkled: yyrr

Of course, this can be extended to a **trihybrid cross**. Add blue (B) and white (b) flowers to the cross shown above:

Parental pure lines : YYRRBB and yyrrbb

F1 trihybrids; YyRrBb

If all three traits assort independently there will be eight possible gametes:

YRB, YRb, YrB, Yrb, yRB, yRb, yrB, yrb

And the Punnett Square will have 64 cells:

	YRB	YRb	YrB	Yrb	yRB	yRb	yrB	yrb
YRB	YYRRBB	YYRRBb	YYRrBB	YYRrBb	YyRRBB	YyRRBb	YyRrBB	YyRrBb
YRb	YYRRBb	YYRRbb	YYRrBb	YYRrbb	YyRRBb	YyRRbb	YyRrBb	YyRrbb
YrB	YYrRBB	YYRrBb	YYrrBB	YYrrBb	YyRrBB	YyRrBb	YyrrBB	YyrrBb
Yrb	YYRrBb	YYRrbb	YYrrBb	YYrrbb	YyRrBb	YyRrbb	YyrrBb	Yyrrbb
yRB	YyRRBB	YyRRBb	YyRrBB	YyRrBb	yyRRBB	yyRRBb	yyRrBB	yyRrBb
yRb	YyRRBb	YyRRbb	YyRrBb	YyRrbb	yyRRBb	yyRRbb	yyRrBb	yyRrbb
yrB	YyRrBB	YyRrBb	YyrrBB	YyrrBb	yyRrBB	yyRrBb	yyrrBB	yyrrBb
yrb	YyRrBb	YyRrbb	YyrrBb	Yyrrbb	yyRrBb	yyRrbb	yyrrBb	yyrrbb

The phenotype ratio is:

Yellow, round, blue: 27
Yellow, round, white: 9
Yellow, wirnkled, blue: 9
Yellow, wrinkled, white: 3
Green, round, blue: 9
Green, round, white: 3
Green, wrinkled, blue: 3

Green, wrinkled, white: 1

Note that, because each of the three traits is assorting independently, each individual phenotype ratio is 3 : 1; i.e., 48 yellow to 16 green; 48 round to 16 wrinkled; 48 blue to 16 white!

There is an additional mathematical treatment that can be demonstrated with each of the crosses that have been worked out. Segregation of alleles means that there are known probabilities associated with gamete formation. For any monohybrid cross, for example, the probability that a **AA** parent will donate an **A** allele to an offspring is 1.00 and the probability that a **AA** parent will donate an **a** allele is 0.00. If the parent is **Aa** these probabilities are both 0.50. Thus, if one parent is **AA** and the other parent is **aa**, the chances that their off spring will be either **AA** or **aa** is 0.00 and the probability that their offspring will be **Aa** is 1.00.

Extending this to a dihybrid cross, if the parental genotypes are **AABb** and **AaBb**, the probability of each possible off spring genotype can be easily calculated by multiplying the individual probabilities of the independent genotypes:

Genotype	Probability
AABB	0.125 (0.5 x 0.25)
AABb	0.250 (0.5 x 0.5)
AAbb	0.125 (0.5 x 0.25)
AaBB	0.125 (0.5 x 0.25)
AaBb	0.250 (0.5 x 0.5)
Aabb	0.125 (0.5 x 0.25)
aaBB	0.000 (0.0 x 0.25)
aaBb	0.000 (0.0 x 0.5)
aabb	0.000 (0.0 x 0.25)

The total probability will always add up to 1.000. This exercise can be extended to include any number of traits so long as they assort independently. For example, given the parental genotypes **AABbccDd** and **AabbCcdd** in a **tetrahybrid cross**, what is the probability that an offspring will be **AAbbCcDd**? (0.0625 from 0.5 x 0.5 x 0.5 x 0.5) What is the probability that another offspring will be **AabbccDD**? (0.000 from 0.5 x 0.5 x 0.5 x 0.0)

III. Epistasis

The final issue to be raised here is that of **epistasis**. This phenomenon is defined as the influence of one genotype on another. For example, in the trihybrid cross shown above, the phenotype ratios will be distorted if the genotype for flower color influences that for seed shape such that a homozygous white (bb) makes all seeds round regardless of their

genotype. In such a case, any occurrence of bb and rr will result in round seeds and the phenotype ratios will become:

Yellow, round, blue:	27
Yellow, round, white:	12
Yellow, wirnkled, blue:	9
Yellow, wrinkled, white:	0
Green, round, blue:	9
Green, round, white:	4
Green, wrinkled, blue:	3
Green, wrinkled, white:	0

What would the ratios be homozygous white made yellow albumin green but only if the albumin genotype was heterozygous Yy?

Yellow, round, blue:	27
Yellow, round, white:	3
Yellow, wirnkled, blue:	9
Yellow, wrinkled, white:	1
Green, round, blue:	9
Green, round, white:	9
Green, wrinkled, blue:	3
Green, wrinkled, white:	3

These are effects that distort the phenotype ratios overall but note that the phenotype ratios for the unaffected traits remain at expectation; i.e., the ratio of yellow to green albumin in the first example is still 48 : 16 and the ratio of round to wrinkled seeds is still 48 : 16 in the second example.

Resources

There are many sources for additional questions and problems available on the web. A few of these are listed below.

http://people.whitman.edu/~hutchidw/Mendelanswers.html

http://web.mit.edu/esgbio/www/mg/problems.html

http://www.biology.arizona.edu/mendelian_genetics/mendelian_genetics.html

In addition, a nice coin toss exercise for Mendelian genetics can be found at,

http://www.wsu.edu/~omoto/papers/cointoss.html

