The Genetics of Individual Variation[1]

Campbell Chapter 14

Introduction

By and large the physical sciences dismiss individual variation and in so doing can produce very generalized, mathematically precise results. Physics, for example, deals with the behavior of ideal bodies so that the color, shape and composition of a falling body according to the law of gravity can be dismissed as irrelevant. **In the life sciences** such **variation** cannot be dismissed; in fact it **is often the phenomenon that must be explained**. Living individuals differ in genetics, environmental influences and history. Hence, to explain the behavior of a living individual, its unique characteristics must be taken into account.

Individual variation is a critical component of Darwin's theory of natural selection: without individual variation there is simply nothing to select!

Differences between individuals can be due to either

- differences in heredity -- or --
- differences in environmental influences

e. g., brown eyes that are inherited vs. black eyes that are acquired through some trauma (a little humor here – I am of course talking about two different traits). In this discussion we will focus on the production of heritable variation, but keep in mind that not all examples of individual variation are under genetic control. We owe much of our understanding of the genetic basis of individual variation to Gregor Mendel and so will start by examining his important contribution to biology.

In fact, the phenotype is always due to the interaction between genes and environment. We will discuss this interaction in a later class.

Mendel's study of heredity

Gregor Mendel (1822-1884) was an Augustinian monk whose experiments with plant breeding conducted at the monastery of St. Thomas in Brunn, Austria between 1854 and 1869 formed the basis of modern genetics. Unfortunately, Mendel's results, published in 1865 and 1869 in the *Proceedings of the Society of Natural History of Brunn*, went unnoticed until 1900 when three independent investigators, **Carl Correns** (Germany), **Hugo De Vries** (Holland) and **Erich Von Tschermak** (Austria), conducted similar experiments and reached the same conclusions as had Mendel. All three found in researching the literature on the subject that Mendel was the first to describe rules for the transmission of genetically controlled traits and so gave him all the credit. This rediscovery and confirmation of Mendel's work started the field of genetics.

Mendel was not the first to conduct breeding experiments, but he was the first to discover rules governing heredity. His success was due to his choice of experimental plant (the garden pea, *Pisum sativum*) and his knowledge of mathematics, especially statistics. The garden pea reproduces by self-fertilization. This makes it relatively easy to inbreed[2] a line of plants with the eventual result being "genetically pure" (non-variable) lines. Furthermore, garden peas possess a number of **discontinuous traits,** i. e., aspects of the **phenotype** that exist in only a small number (usually 2) of discrete character states with no intermediates between them. With discontinuous traits, each organism's phenotype shows only one of the possible **character states**.

Mendel chose seven discontinuous traits to study:

- seed shape (either round or wrinkled),
- seed color (yellow or green),
- pod shape (smooth or wrinkled),
- pod color (green or yellow),
- flower color (red or white),
- flower position (axial or terminal) and
- vine length (tall or short).

By crossbreeding genetically pure line individuals (true-breeding) with contrasting character states and observing the phenotypes of their offspring, he was able to determine the pattern of inheritance. He was able to do this when so many others had failed because, since these character states are discontinuous (either-or expressions) with no intermediates, he could quantify his results by simply counting the two different phenotypes among the offspring of each experimental cross. Then, his knowledge of statistics enabled him to recognize that his results followed exact proportions or ratios, even though small sample size caused variations in observed ratios from the theoretical or expected ratios he was able to recognize.

Mendel's study provides an excellent example of the inducto-deductive method used in science and we will now examine how he handled each of this method's

[2] Inbreeding is the crossing of genetically very similar individuals. Generation after generation crossing of individuals with themselves is an extremely effective form of inbreeding. One of the results of inbreeding is to remove most types of genetic variation -- the result is "true-breeding" strains with respect to particular traits. All true breeding means is that if these individuals are crossed with other members of the same true-breeding line, the offspring will look like the parents with respect to the trait of interest.

components. Also, notice that although statistics is certainly not an "anti-discipline" of genetics, his knowledge of statistics (a new branch of mathematics at the time) greatly aided his work.

Induction

Mendel started his work by allowing individuals with contrasting character states to self-fertilize through many generations until he was convinced that their traits were pure. Such strains of individuals are termed **"true breeding"** with respect to the trait in question. Whenever a true breeding individual is crossed to other true-breeders of its type, the result is that the offspring have the same phenotype as the parents (assuming the environment was functionally the same).

Note: many species are "self-fertile" -- they not only contain both sexes on the same individual **but their "male" and "female" gametes can fertilize each other and produce vigorous fertile offspring**. Please note however that not all species that have both sexes in the same individual are self-fertile; many are not. When both sexes are found on the same individual, the species is termed **"monecious"** (one house). When sexes are separate, such as in all vertebrates, the species is **diecious**.

Mendel then selected two lines that were true breeding for different character states of the same trait. An example would be to cross true breeding "tall" with true breeding short" vine heights. These two individuals constituted the **parental or P generation**. **Crossbreeding** was effected by removing the pollen producing stamens from both plants and then using the pollen of one to fertilize the ovules of the other.

This is also an example of a **reciprocal cross** -- it is where two monecious individuals are crossed with each other.

The offspring produced by this cross constituted the **first filial (or F_1)** generation. He then crossbred these individuals or simply let them self-fertilize to produce the **second filial (F_2)** generation. Mendel's results can be illustrated by the phenotypic expression of seed shape when he crossed individuals from a pure line of plants with round seeds with individuals from a pure line of plants with wrinkled seeds.

P generation: round x wrinkled

F_1 generation	all offspring had round seeds
F_2 generation	3:1 ratio of offspring with round and wrinkled seeds (3 round : 1 wrinkled)

When he performed this experiment with the six other traits, he obtained the exact same results. Thus, he recognized **a pattern: a 3:1 phenotypic ratio in the F_2 generation**. Remember that the purpose of induction is to generalize patterns based on a limited number of observations. Mendel extended his experimental results (based only on the observation of seven traits in a single plant species) through **inductive reasoning** to claim that **all discontinuous traits** no matter what animal or plant species is studied **would follow the same pattern**. The next step was to find a reason why this pattern obtained, i. e., to develop a hypothesis to explain this result.

Hypothesis

To explain this pattern **Mendel hypothesized that each discontinuous phenotypic trait in an individual was controlled by two hereditary factors**. The alternative hypothesis that each trait was controlled by one factor was not a viable explanation because it could not explain the presence of wrinkled seeds in the F_2 generation. Since all F_1 plants produced only round seeds, then the factor controlling the wrinkled condition would have to have been lost. But, by postulating two hereditary factors behind each trait, Mendel could explain the reappearance of wrinkled-seeded plants in the second filial generation.

> Notice that he could have postulated more than two hereditary factors in each individual but he used the principle of parsimony.

To account for the observation that all F_1 individuals had round seeds, Mendel suggested that the factor for wrinkled seeds was masked by the factor for round seeds and so the wrinkled seed factor was **recessive** to the **dominant** round seed factor. Whereas dominant factors can influence the phenotype when only one is present, recessive factors must be paired together to produce a phenotypic effect.

It must be emphasized that these hereditary factors (= genes or more precisely, **alleles**) cannot be observed directly; their existence is only inferred from phenotypes produced in breeding experiments. Mendel explained the hereditary make-up (**genotypes = factor pairs**) of his experimental plants as follows:

P generation: round (RR) x wrinkled (rr)

F_1 generation	all round (Rr)
F_2 generation	3 round (1 RR + 2 Rr) : 1 wrinkled (rr)

To distinguish the three different genotypes in a **monohybrid cross** (a cross between two individuals who are heterozygous for a single trait) the following terminology is used: **R** = dominant **allele**, **r** = recessive **allele**, **RR** = **homozygous dominant genotype**, **Rr** = **heterozygous genotype,** and **rr** = **homozygous recessive genotype**.

Mendel's Law of Segregation

If, as Mendel hypothesized, each discontinuous phenotype is controlled by two alleles, it follows then that only one is passed on to an offspring by each parent, otherwise the number of alleles would double with each generation. This corollary of Mendel's hypothesis is known as his **law of segregation** that states that the two alleles that together form a genotype *segregate* into sister gametes when gametes are formed during reproduction. Note that for a single trait **gametes** are designated by a **single letter** (an allele) due to the law of segregation, but **genotypes** contain **two letters** because Mendel hypothesized that genotypes or two hereditary factors control a single phenotypic trait.

Mendel's Use of Deduction

For a hypothesis to be a scientific hypothesis, it must generate testable predictions through the process of deduction. Remember that a prediction is a fact that is deduced from the hypothesis and must be true if the hypothesis is true.

Predictions Deduced from Mendel's Hypothesis

The fact that only phenotypes can be observed provided Mendel with the opportunity to make **two predictions about the unseen genotypes in the F_2 generation**. The two predictions that follow from Mendel's hypothesis are:

1. Underlying the dominant phenotypes in the F_2 generation are **two different genotypes**: RR and Rr.
2. The ratio of these two predicted genotypes is 1 RR to 2Rr. Thus, **the 3:1 phenotypic ratio is accompanied by a 1:2:1 genotypic ratio**.

According to his law of segregation, the two different factors (R and r) in the F_1 heterozygotes segregated into sister gametes with the result that males produced two types of pollen grains and females produced two types of eggs in their ovules. Since fertilization is a random process, each type of pollen grain has an equal chance of fertilizing each of the two types of eggs (here again is an example of how Mendel's knowledge of mathematics helped him interpret results). Consequently, the F_2 generation should contain three genotypes in the following ratio: one RR; two Rr; one rr. This expected result is illustrated below with a **Punnett Square**. A Punnett square is simply a device for listing all possible interactions. It is a nice tool to start your understanding of genetics with, but we will see that it soon becomes cumbersome and we will abandon it. However, for the moment:

Female Gametes (from ovule)	Male Gametes (from pollen) **R**	**r**
R	RR (round)	Rr (round)
r	Rr (round)	rr (wrinkled)

Hypothesis testing through experimentation

Mendel's two predictions could be tested in either of two ways. He could **allow all F_2 individuals to self-fertilize, or** he could **backcross each one with a homozygous recessive plant (test cross)**.

A **backcross** occurs when an F_1 or later individual (F_2 in this case) is crossed with a parent; a **test cross** is a special type of backcross where the individual is crossed to a homozygous recessive individual.

The phenotypic results of the test cross would reveal the presence of the three different genotypes even though the genotypes could not themselves be observed. **Each genotype would produce a different set of offspring that would be phenotypically recognizable**. The test cross results are presented below:

- RR x rr = all round (Rr)
- Rr x rr = both round and wrinkled offspring (50 % Rr; 50% rr)
- rr x rr = all wrinkled (rr)

Assignment: By allowing the F_2 plants to self-fertilize Mendel could also have distinguished the three different genotypes. **Show how this can be done by depicting both genotypes and phenotypes.** Use Punnett squares. Hint: remember that dominant phenotype (round) are composed of two different types of plant, RR and Rr. Do the results differ in any way from those of the test cross shown above? ***Be ready to discuss this one in class – you will not turn it in!***

Abandoning the Punnett Square: Notice that each cross that we have learned has probabilities associated with it. We can also view what happens in the production of gametes and syngamy as a series of probabilities.

OK, let's see if you understand how this works. Let's say that an individual has genotype RR.
According to Mendel's model where the individual has two copies of a given gene and gametes have only one -- how many distinct types of gametes will the RR individual make?
What is the probability (a number between 0 and 1) of a gamete with R? With r?
Do the same for an individual who is rr.

You should have decided that:
In the first case all gametes were R so the probability of an R gamete is 1.0 and the probability of a r gamete is zero (ignoring the infinitesimally small chance of mutation).
The same is true for rr -- all gametes are r (prob. = 1.0).

Now, **let's say we cross Rr males with an rr females.**
What is the chance of an R gamete from the males?
An r gamete from the males?
An R gamete from a females?
An r gamete from a females?

Answers (in order) 0.5, 0.5, 0 and 1.0

Now, if there were thousands of matings between individuals with these genotypes, what is the chance that an R sperm and r egg come together?
To answer this, we should realize that gametes generally get together purely at random. In other words, the genes present in one gamete in no way bias the chance that it will get together with other specific gametes[3].

We need to invoke an important law of statistics: the law of independence to find the solution to our problem. The <u>**law of independence**</u> states that <u>when two or more events have **no influence on each other**, then the **aggregate chance that both events will happen is equal to the product of the probabilities of each of the independent events happening on its own**</u>**.**

So, the chance of R from heterozygous (male) parent = 0.5
And the chance of r from the homozygous parent = 1.0

chance of Rr from these two = 0.5 * 1 = 0.5

The same calculation could be made for rr.
What if <u>both</u> parents are Rr? What is the chance of the recessive homozygote?

[3] This is a famous assumption and it is true in nearly every case. But as usual in biology, there are some rare but very interesting exceptions.

Chance of R = 0.5; r = 0.5 for both parents

Chance of rr = 0.5 * 0.5 = 0.25

Let's try another – this time with parents Rr and Rr.
What is the chance of getting the homozygous dominant phenotype? This is a bit harder because there are two genotypes that cause this phenotype: RR and Rr

Let's start with getting RR offspring from two Rr parents.
The chance of R = 0.5 for both parents
So, the chance of RR = 0.5 * 0.5 = 0.25.

How about Rr? Notice that there are two ways to get this one: in one case the R donor is the first parent, in the other case the R donor is the second parent --*i.e.*,

R * r = 0.5 *0.5 = 0.25
AND
r * R = 0.5 * 0.5 = 0.25

with the overall chance being 0.25 + 0.25 = 0.5

Notice that where there are two different ways, each with its own probability, for doing something (in this case, two ways of producing Rr offspring) we add the probabilities to get the overall chance.

So, the chance of getting a **R_ (read as dominant phenotype)** individual is chance of RR + chance of Rr = 0.25 + 0.5 = 0.75

Notice now that we have our 3 R_ : 1 rr ratio!

This method may seem overly cumbersome and in the expanded version I have given you, it is! We will see in the next class that when working with two or more genes, we normally use some shortcuts that involve memorizing the probabilities of different phenotypes or genotype from different crosses.

In an Rr X Rr cross, what is the chance of the following genotypes: RR and rr? -- *no Punnett squares, please.*

Other Forms of Heredity

1. Particulate vs. blending inheritance: Mendel hypothesized a form of particulate inheritance with the hereditary units consisting of free-floating particles. This is in stark contrast to the prevailing idea of his day that inheritance followed a blending pattern, i.e., the parental traits blended together to form the offspring traits. An example of **<u>blending inheritance</u>** would be the production of pink-flowered offspring from a cross between a red-flowered plant and a white-flowered one. Mendel's concept of dominance enabled the particles (alleles) to retain their integrity and be passed on intact to offspring. **A particulate inheritance theory was essential for Darwin's theory of natural selection**, <u>otherwise any selectively favored phenotype would be blended away as soon as the selected individual reproduced with one of a different phenotype</u>. Thus, **Mendel's theory of inheritance provided the essential requirement for Darwin's theory of evolution**: <u>an explanation of heredity based on particulate genes</u>, even though that was not recognized at the time.

On the other hand, there are many examples of inheritance that appear to be blending. This was why the concept existed in the first place. In the example of pink, red and white flowers above, the system would work like this:

P true breeding red ($C^R C^R$) X true breeding white ($C^W C^W$)

F_1 all pink ($C^R C^W$)

This sort of arrangement is called "**<u>incomplete dominance"</u>** or "lack of dominance". The crucial factor is that the phenotype of the heterozygote appears to be somewhat intermediate to that of the two homozygotes. Note that neither allele at the "color" locus is dominant over the other. But notice that unlike earlier non-particulate models of inheritance that the genetic factors do not blend. If these F1 individuals are crossed with each other we get:

1 red ($C^R C^R$) 2 pink ($C^R C^W$) 1 white ($C^W C^W$)

So the factors still exist -- as we now know to be true for DNA -- inheritance of two factors does not cause them to mix together permanently into some new form.

<u>2. Co-dominance:</u> In co-dominance, heterozygotes express the phenotypes associated with both alleles. The most famous human example of this is ABO blood types. The locus is named "I" and three alleles are possible – I^A (the "A" phenotype allele), I^B (B phenotype allele), and I (the "O" phenotype allele). The I^A and I^B alleles are associated with the production of certain proteins called antigens (more about this later this semester) in the membranes of blood cells. A person with genotype $I^A I^A$ expresses the "A" antigen only; a person with $I^B I^B$ expresses the "B" antigen only and someone who is genotype $I^A I^B$ expresses roughly equal amounts of both "A" and "B" antigens (phenotype "AB"). Thus, co-dominance – both traits are expressed in the heterozygote. In this case, allele i is recessive to both I^A and I^B and so I^Ai has type "A" and I^Bi has type "B" and ii is type "O".

3. Qualitative vs. quantitative inheritance: Mendel was successful because he chose **discontinuous traits** for his analysis and these traits are qualitatively different, i. e., either/or traits with no intermediate conditions. Many traits, however, are quantitative rather than qualitative and **vary continuously** within a population. Such traits, e.g., height and weight, tend to follow a bell-shaped curve when the number of individuals is plotted against the range of the variable trait (see histograms in the first class notes). With continuous or quantitative variation there will always be a phenotype in between any two chosen for comparison.

Can Mendel's theory of particulate inheritance explain quantitative variation, or is it limited to discontinuous variation? The answer is that **Mendel's theory explains both qualitative (discontinuous) and quantitative (continuous) patterns of individual variation**. With continuous variation, however, many different genes influence the same trait, not just one as Mendel proposed. Hence, crossing individuals with different character states of a continuously varying phenotype will not produce the discrete ratios that enabled Mendel to discover his laws of segregation and independent assortment. The genetics of continuous variation is not different from that of discontinuous variation - it is just far more complex. In many cases it involves having many copies of the same locus, each of which, however, is independent of the other. Height, for example, is commonly inherited this way. In other cases, it involves loci that make entirely different proteins but that interact in different ways to produce phenotypic effects. You'll learn more about these modes of inheritance in a genetics class.

Explain dominance/recessiveness in terms of differences in the products of structural genes and/or differences in regulation. In fact, are traits such as flower color likely to be produced entirely by the action of one gene? You should be able to answer these questions with a little thought about what you learned earlier about genes and gene regulation.