

LABORATORY 5

Mendelian Genetics

Despite what you may have heard, you're *not* what you eat. Rather, you are a product of the genetic code you inherited from your parents, and its interactions with the environment. The study of inheritance—at the molecular, organismal, and evolutionary levels—comprises the field of **Genetics**.

By now you should be familiar with the work of **Gregor Mendel**, the Austrian abbot sometimes known as the “Father of Modern Genetics”. If you have not already done so, be sure to read the pertinent sections in your textbook on Mendel and his discoveries about inheritance.

In today's lab, you will examine genetic data from several organisms, including yourself. You will also be responsible for completing the genetics problems at the end of the exercise, and turning them in to your laboratory instructor.

I. The Terminology of Genetics

Mendel had no idea that inside the nucleus of each eukaryotic cell, units of inheritance (genes) consist of lengths of DNA wrapped around protein “spools” and neatly spiraled to form **chromosomes**. Each chromosome is, in essence, a long strand of genes. Before we go any further, let's review the terminology of modern genetics.

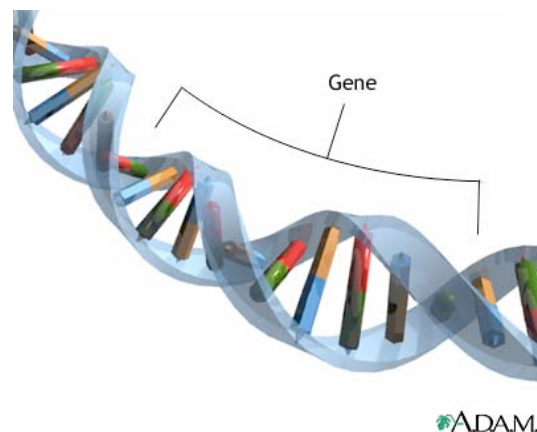


Figure 1. A short strand of DNA. The translucent bars on either side are the sugar-phosphate “backbone” of the molecule. The “rungs” of the twisted “ladder” are the nucleotide bases. Each “rung” is formed by either (T) hydrogen-bonded to adenine (A) or guanine (G) hydrogen-bonded to cytosine (C).

A. Vocabulary

gene - a section of DNA that (usually) encodes the structure of a **protein** (= **polypeptide**).

genome - the full complement of ONE COPY of all the genes in a given organism.

- **diploid** cells have two complete copies of the genome (example: somatic (body) cells). In a diploid organism, one copy of the genome comes from each parent.
- **haploid** cells have one copy of the genome (example: germ cells, such as sperm or ovum)

genotype - the genetic makeup of an organism for a particular trait

phenotype - the physical appearance/makeup of a trait

ploidy - the term used to describe the number of genome copies in a cell's nucleus.

- The number of chromosomes in ONE COPY of the genome in any given cell is **n**.
- The cell's **ploidy** is the number of chromosomes sets in the cell. For example:
 - The ploidy of a diploid cell is **2n**. (two copies of the genome)
 - The ploidy of a haploid cell is **n**. (one copy of the genome)
 - The ploidy of triploid is **3n**. (three copies of the genome)
 - ...and so on.

chromosome – a strand of deoxyribonucleic acid (DNA) organized around histone proteins located in the nucleus of eukaryotic cells. A chromosome carries genetic information in the form of genes.

locus - the physical location of a gene on a chromosome

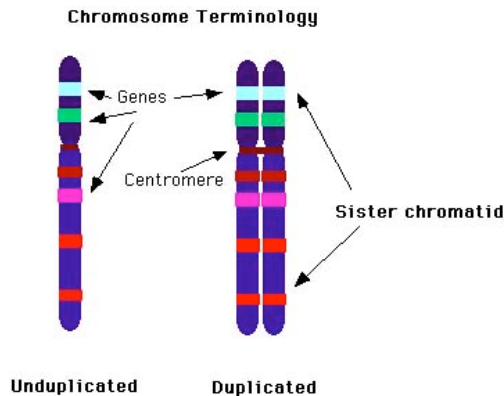


Figure 2. A chromosome consists of DNA spooled on histone “spools” and neatly coiled and spiraled to form the finished chromosome. Each gene is located at specific, physical location on a chromosome known as its **locus**. When duplicated in preparation for cell division, a chromosome becomes two identical **sister chromatids**.

allele - one of several different *versions* of a gene, each coding for a different protein product of the gene (This sometimes results in a different phenotype).

- **dominant allele** - an allele that masks the expression of another, alternate allele
- **recessive allele** - an allele whose phenotypic effect can be masked by a dominant allele
- **wild type allele** - the most commonly found/expressed allele in a population
- **mutant allele** - any allele that is changed (mutated) from wild type.

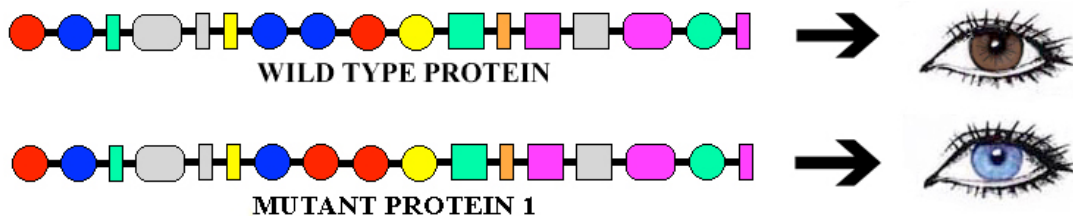


Figure 3. A mutation is any change in a gene. In some cases, this change will alter the protein encoded by the DNA, resulting in a changed phenotype. The form of a trait found most often in a natural population is known as the “wild type” of that trait. Any form other than wild type that is caused by a mutation is known as a “mutant” form of the trait.

homozygous - organism has two of the same allele for a particular gene locus

heterozygous - organism has two different alleles for a particular gene

monogenic trait - trait controlled by only one gene (e.g. - tongue rolling)

polygenic trait - trait affected by many different genes (e.g. - human stature/height)

B. Two types of chromosomes

The sex of an individual organism can be determined in a number of different ways, depending on its species. You will learn more about this in your upper division genetics course. For now, we will concentrate on mammals, in which sex is determined by the complement of two **sex chromosomes**, the X and the Y. As you probably already know, female mammals have two X chromosomes, and males have an X and a Y. While the X chromosome is relatively large and carries many genes, the Y chromosome is comparatively quite small. Most of the genes it carries are involved in the determination of maleness.

Genes located on either the X or the Y chromosome are said to be **sex-linked** (or, more specifically, **X-linked** or **Y-linked**). Because males have only one X chromosome, any X-linked alleles they carry on that X chromosome will be expressed. They do not have another homologous allele on the Y to mask it. Hence, males are more likely than females to express X-linked recessive traits.

Chromosomes other than the X and Y chromosome are known as **autosomes**. Traits encoded by genes located on autosomes are known as **autosomal traits**.

II. Simple autosomal human traits

Relatively few phenotypic traits are controlled by a single gene. However, it was only by studying these monogenic traits that Mendel was able to discover the basic workings of inheritance. He studied such things as flower petal color and fruit characteristics in the edible pea (*Pisum sativum*). But we can now extrapolate Mendelian genetics to some monogenic traits expressed by *Homo sapiens*.

The following section describes some easily recognizable human traits. Some are entirely monogenic, and some are influenced by factors other than a single gene. Check yourself for each trait, and use the table provided at the end of this section to record your phenotype for each one. (In some cases you may find it necessary to have a partner confirm your opinion as to whether a suspected trait is being fully expressed.)

Your laboratory instructor will keep a record of your lab section's distribution of traits. After each test below, let your lab instructor know your phenotype and sex so that your data can be included in the table, which will be projected for the entire class to see.

A. Ability to taste PTC.

The ability to taste certain substances varies from person to person. For example, the ability to taste phenylthiocarbamide (PTC) is genetically determined and is inherited as a simple autosomal dominant trait:

TT	=	homozygous taster
Tt	=	heterozygous taster
tt	=	homozygous non-taster

1. Data Collection

Go to the PTC taste station located on the back table. There you will find two sets of nearly identical test paper strips. The only difference between them is that one set is saturated with PTC (phenylthiocarbamide), whereas the other ("control") is not.

The ability to taste PTC is determined by a single gene locus, with the ability to taste the chemical encoded by the dominant allele. We will designate the dominant allele of this gene as **T**, and the recessive allele as **t**.

1. Place a strip of control paper on your tongue. Note the taste sensation.
2. Place a strip of PTC-impregnated paper on your tongue. If you are a non-taster, the PTC and control test papers will taste very much the same. If you are a taster, you will notice a bitter taste.
3. Enter these data (be sure to indicate your gender) on the blackboard table.
4. Turn to Table 2 and enter your phenotype and possible genotypes in the Personal Genetic Data Record provided for you.

2. Data analysis, Predictions, and Problem-solving

1. What are your possible genotypes? _____
2. What are the possible genotypes of your parents? _____

If you are a taster, you cannot know your genotype unless one of your parents was a non-taster. If you are a taster, and one of your parents is a non-taster, what would be your genotype? _____

If you are a taster, and both of your parents are tasters, what would be your possible genotypes? _____

Given your genotype, what are the possible alleles that your gametes might carry for this trait? (Remember that only one allele of each gene is carried in a normal gamete.)

Now let's pretend that you have found the love of your life, get married and want to have children. You can predict the probability of having taster or non-taster children by using a simple matrix known as a **Punnett Square**. For example, in a mating between two individuals heterozygous for a gene segregating a dominant (X) and a recessive (x) allele, you would set up the Punnett Square as follows:

	X	x
X	XX	Xx
x	Xx	xx

The single Xs outside the square represent the alleles of each parent, and the paired Xs inside the square represent the diploid condition (at that locus) of any possible offspring.

As you can see from this hypothetical example, a mating (also known as a **cross**) between two heterozygotes for any given trait should produce a genotypic ratio of 25% homozygous dominant, 50% heterozygous, and 25% homozygous recessive offspring (1:2:1). The phenotypic ratio would be 75% tasters to 25% non-tasters (3:1).

The square can be used to calculate any combination of parental phenotypes. So now you can give it a try with your own PTC-tasting phenotype.

A **hybrid** is defined as something that has been made by combining two different elements. In the case of genetics, these two different elements are different alleles of a given gene. A cross like the one above, **in which both parents are heterozygous** for the trait of interest, is known as a **monohybrid cross**. (Note that BOTH parents must be heterozygous for the cross to be called monohybrid. A mating between XX and Xx is NOT a monohybrid cross.)

3. If your mate were a non-taster, predict the possible genotypes and phenotypes of your offspring with one or more Punnett Squares, as necessary.

4. It's a long way from gene to phenotype. Explain of how the recessive allele could lead to the inability to detect the presence of a specific molecule. Remember that genes code for the manufacture of proteins.

5. If the ability to taste PTC is inherited as a simple autosomal dominant trait, can you make a well-supported prediction about what ratio of tasters to non-tasters you should see in your lab section? Why or why not? _____

6. What is the ratio of tasters to non-tasters in your lab section? _____

7. Since the PTC-tasting trait is autosomal, what would you predict about the expression of the trait in males vs. females? _____

8. Is your prediction borne out by the ratio in your lab section? Explain. _____

While PTC does not occur naturally in foods, chemically similar compounds do. It has been found that food preferences are correlated with the ability to taste (or not

taste) PTC (Forrai and Bánkóvi, 1984). Now that you know what PTC tastes like (even if it's only from the expressions of disgust on your taster lab partner's face), what might be the evolutionary/adaptive significance of this trait?

Now that we've told you that PTC-tasting is a monogenic trait, we must be more honest with you. Recent research has suggested that there may be at least two loci involved in the expression of this trait (Drayna, et al. 2003). Further research may some day explain how this might explain why some tasters have an extremely strong, negative reaction to PTC, while others report a relatively mild bitter taste.

B. The genetics of blood type in *Homo sapiens*.

1. The A-B-o blood groups

An **antigen** is a foreign substance that stimulates an immune response in an organism whose body it enters. An **antibody** is a protein produced by the organism to bind the antigen and help aid the immune system in destroying it.

When considering human blood types, we refer to specific proteins located on the cell membranes of the red blood cells (erythrocytes) as antigens. This is because the immune system of a healthy human will produce antibodies against those specific proteins (antigens) that it does not manufacture. This is central to understanding the genetics of the A-B-O blood system.

The human blood types we call **A**, **B**, **AB** and **O** are phenotypes reflecting the expression of a single gene locus with three different possible alleles, **I^A**, **I^B** or **i^O**.

I^A represents the allele coding for production of antigen A

I^B represents the allele coding for production of antigen B

i^O represents the allele coding for *neither* antigen A nor B

Thus, a person carrying the I^A allele produces a specific protein, **antigen A**, located on the cell membranes of his/her erythrocytes (red blood cells or "RBC's"). Similarly, a person carrying the I^B allele produces **antigen B** on his/her erythrocytes. A person homozygous for the i^O allele does not produce either of these antigens, and has blood type O.

Every human has two copies of the A-B-O antigen gene. The I^A and I^B alleles are codominant, which means that both are expressed on the membrane in a heterozygote. Because a person carrying either an I^A or I^B allele will always express that protein, a heterozygote with genotype I^A i^O will have type A blood, and a person with genotype I^B i^O will have type B blood. Hence, the O allele is recessive to both the A and the B alleles.

Because persons with type O blood manufacture neither A nor B antigens, their immune systems will produce antibodies against both. If a type O person were to be infused with type A, B or AB blood, s/he would produce anti-A and anti-B antibodies in

his/her plasma. As the antibodies bound to the foreign antigens, clotting and other **anaphylactic** (allergic) reactions would ensue in a potentially fatal cascade.

Conversely, because it contains neither antigen A nor B, type o blood can be introduced into the bloodstreams of persons with types A, B or AB blood without eliciting an A-B-o immune response. For this reason, individuals with type o blood are called **universal donors**, even if they faint at the sight of a needle. Similarly, because the plasma of type AB individuals will produce neither anti-A nor anti-B antibodies, type AB individuals are called **universal recipients**, and can receive blood of any A-B-o type.

Table 1 shows A-B-O genotypes and phenotypes. Fill in the last column.

Table 1. A summary of ABO human blood type phenotypes and genotypes.

blood phenotype	antigens on RBC	plasma antibodies	possible genotypes	can receive blood type(s)
A	A	anti-B	$I^A I^A$; $I^A i^o$	
B	B	anti-A	$I^B I^B$; $I^B i^o$	
AB	AB	none	$I^A I^B$	
o	none	anti-A, anti-B	$i^o i^o$	

a. Data collection

In this age of deadly blood-transmitted diseases, we have decided to avoid the risk entirely by using Space Age Technology™: Fake Blood! It's even better than the stuff you get at Halloween in little squeeze tubes. Not only do the various fake blood types agglutinate like real blood in the presence of the proper "antiserum"--but you can even look at the simulated "cells" under the microscope and count erythrocytes (red), two types of leukocytes (blue "lymphocytes" and yellow "neutrophils") and platelets (grey/black) in the approximate ratios they actually occur in real blood. (Cell counting isn't part of this lab exercise, but you are welcome to have a look if you are interested.)

You will work in teams of four. Your TA will prepare four different plates of unknown "blood." Using the various antisera, you will determine the blood type of your sample.

If your mystery donor's "blood" is type A, then the anti-A serum (which contains anti-A "antibodies") will cause agglutination (clotting). If it is type B, then the anti-B serum will cause agglutination. If it's type AB, then both anti-A and anti-B sera will cause agglutination, and if in type O, you should observe no agglutination.

When you have determined your unknown's blood type (A, B or O), record the data below. Enter the phenotype and possible genotypes in Table 2. DO NOT DISCARD YOUR SAMPLE. YOU WILL NEED IT FOR PART 2.

b. Data analysis

1. What genotypes (and blood groups) could the parents of your unknown donor have?

2. List all the possible blood genotypes of your donor's offspring if his/her mate were:

- $I^A I^A$ _____
- $I^A i^o$ _____
- $I^B I^B$ _____
- $I^B i^o$ _____
- $I^A I^B$ _____
- $i^o i^o$ _____

Stanford University has an excellent web site showing the distribution of blood types worldwide. You can view their maps here: http://anthro.palomar.edu/vary/vary_3.htm

Consider that while these populations belong to a single species, *Homo sapiens*, the phenotypic frequencies differ rather markedly across populations. What possible reasons could explain this variation? List some hypotheses here:

Choose one of your hypotheses, make a prediction about what you would expect to see should you perform a particular experiment to test your hypothesis, and then describe that experiment here:

2. The Rhesus factor

The Rhesus factor system includes about eight different types of antigens which may occur on the surface of the erythrocytes. The most important of these is known as **antigen D**. The allele for the production of antigen D (**I^D**) is dominant to the allele for

absence of the antigen (i^d). Thus, Rh-positive persons are either heterozygous or homozygous dominant, and Rh-negative persons are homozygous recessive.

As with the A-B-o antigens, an Rh-negative person will produce anti-D antibodies if Rh-positive erythrocytes are introduced into his/her bloodstream. The most familiar Rh incompatibility disorder is *erythroblastosis fetalis*, which may occur when an Rh-negative woman carries an Rh-positive child.

During pregnancy, a few fetal erythrocytes may cross the placenta into the maternal bloodstream. If the mother is Rh-negative, her immune system will become sensitized to the fetal antigen, treating it as an invader, and manufacture anti-Rh antibodies. These can cross the placenta back into the fetal bloodstream and cause **hemolysis**, the destruction and rupture of the baby's red blood cells.

One of the breakdown products of hemoglobin is bilirubin. The circulation of this waste product can cause the infant to appear jaundiced (yellow), and the level of jaundice can indicate the degree of damage.

The immune response is not instantaneous. A woman carrying her first child may not manufacture antibodies that harm the child. But any subsequent children will face the mother's sensitized immune system, and be subject to *erythroblastosis fetalis*.

In developed countries, medications containing special immunoglobulins are available to prevent sensitization of the maternal immune system.

a. Data collection

Using the artificial Rh antiserum available at the demo station, determine whether your unknown donor is Rh+ or Rh-. When you have finished typing your blood, enter the data in Table 2.

b. Data analysis

1. What are the possible Rh genotypes of your donor's parents? _____

2. What would be the genotypes and phenotypes of your donor's offspring if his/her mate were. . .
|D|D _____
|D|i _____
|i|i _____
3. Which of these matings (if any) might cause concern for possible *erythroblastosis fetalis*? _____

C. Other Human Traits

Following is a series of easily determined human phenotypic characters that are believed to be inherited as simple autosomal traits. You and your lab partner will catalog your phenotypes and record them. You may find that even dominant traits are not all expressed to the same degree in every individual. When this is the case, the trait is said to exhibit variable **expressivity**. Variable expressivity can be caused by interactions with other gene loci, as well as by environmental influences during development or at any stage of the life cycle.

See Figure 4 for illustrations of a few autosomal human traits. Determine your phenotype and possible genotypes for each, and enter the data in Table 2.

1. Hairy digits

The presence or absence of hair on the backs of the fingers between the first and second joints is determined by a simple autosomal trait in humans. Examine your fingers to ascertain your phenotype ("hairy" or "hairless" digits). The presence of even a single hair indicates the presence of at least one dominant "hairy" gene. This trait does exhibit variable expressivity, so you might have to really look for those hairs in some individuals.

2. Widow's Peak

A Widow's Peak is the term applied to a hair margin that forms a point, vampire-like, over the center of the forehead. The gene for this character is dominant over the gene expressing a straight or curved hairline. Which do you have? How sharp are your canines? Do you sparkle annoyingly when you play baseball?

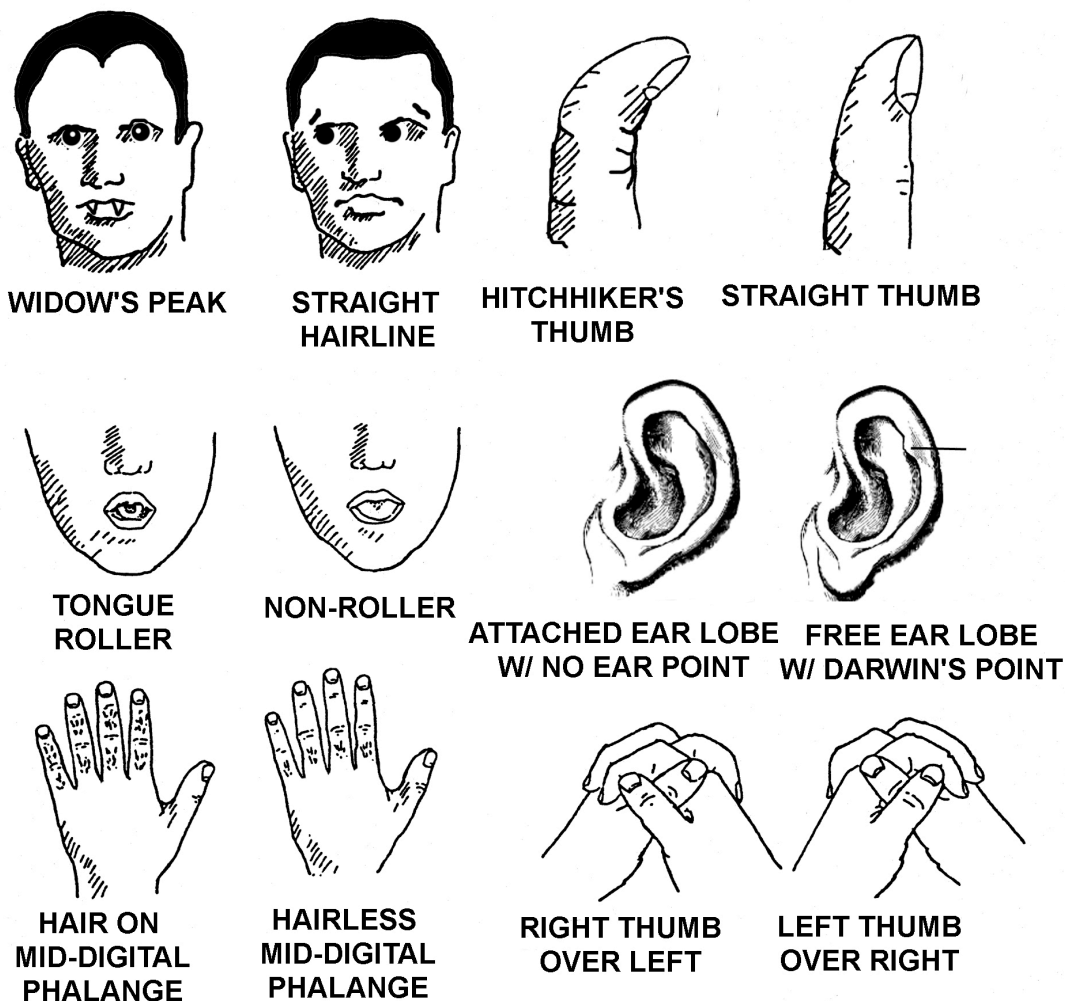


Figure 4. Some human phenotypic characters controlled by autosomal genes

3. Tongue rolling

Determine whether or not you have the ability to roll your tongue into a longitudinal U-shaped trough, running from the tip to the rear of your tongue. (Other weird

permutations of which you might be capable do not count, but you can entertain your lab partner with them, anyway.) You must be able to accomplish this mighty feat without the aid of your teeth, fingers or other appendages. (No one wants to see that.)

If you carry the dominant gene ("roller"), you will be able to roll your tongue with little difficulty. If you are homozygous recessive, no amount of facial contortion, bodily twisting or prayer will allow you to roll your tongue. Once you have finished humiliating yourself, enter your phenotype on the blackboard, being sure to indicate your sex.

(NOTE: A dominant gene is also responsible for the ability of a person to fold his/her backwards at the tip until it touches the flat part of the tongue. Fortunately, this trait is reported to be quite rare.)

4. Ear Lobe Morphology

Although the extent to which ear lobes hang free varies (the trait is probably controlled by more than one gene locus, and is thus **polygenic**), the pendulous lobe is thought to be dominant over the lobe type that is attached all the way to the ear's base at the neck. Unless you have a mirror (or stalked eyes), you may have to rely on your lab partner's judgment to determine which phenotype you express.

5. Freckles

Freckles are localized masses of pigment occurring in relatively high density on the skin, between areas of normal pigmentation. Anyone can have freckles, as long as they have the dominant allele of the MC1R (melanocortin-1 receptor) gene. Sunlight (specifically, ultraviolet-B radiation) triggers pigment-containing cells known as **melanocytes** to increase production of the brown pigment, **melanin**.

Although freckles are not considered a pathology (disease or disorder), people expressing freckles usually have less ability to produce protective melanin than people without copious freckles, and are thus more susceptible to DNA damage in the skin due to ultraviolet radiation in sunlight. So if you express a lot of freckles, slap on that SPF 100, and keep yourself covered.

Note that freckles are not the same as "moles," which are dark areas of pigment, often slightly raised above the rest of the skin.



Figure 5. Freckles.

6. Hitch-hiker's Thumb

"Distal hyperextensibility of the thumb" can be determined by bending the joint of the thumb back as far as possible. If the top segment of thumb bends backward, you have a very useful "hitch-hiker's thumb." This capacity is the result of a recessive gene, and you should never use it on a deserted road at night.

7. Bent Little Finger.

Hold your hands together, palms facing you, so that the little fingers are touching along their outer edges. If the top joint of each little finger bends in the direction of the fourth finger of the same hand, you express the dominant trait. Straight little fingers extending parallel to one another all the way out to the tips is recessive. (Figure 6)

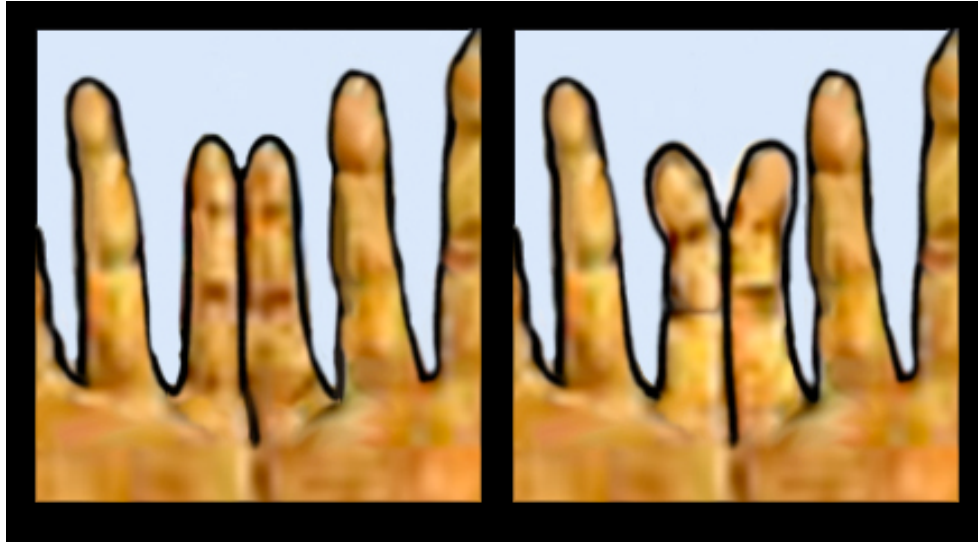


Figure 6. Pinky fingers that run straight are recessive to pinkies that bend dramatically inwards towards the fourth finger.

8. Cleft Chin

The mandible (lower jaw) of a mammal fetus starts out right and left halves, fusing during development. If the jawbones fail to completely fuse to the tip of the chin, this bone structure is reflected on the outer tissues, as well. The resulting phenotype is called a **cleft chin** (“cleft” means “split”). The trait is usually more obvious in males than in females, but in both sexes, the cleft is the result of a dominant allele’s action.

Not everyone who has a cleft chin has a very distinct one, and there are different morphologies of the cleft chin, from the classic Y-shaped split (sometimes called “butt chin” by those who don’t have one and are jealous) to a circular dimple. Such variation suggests that there may be more than one gene involved in the final phenotypic product. The full development of cleft chin phenotype is yet to be fully understood.

9. Interlocking fingers.

Quickly! Before even thinking about what you’re doing, fold your hands together, fingers interlocking. Now look down at your thumbs. If the left thumb is riding on top of the right thumb, you are expressing a dominant gene. If the right thumb is riding on top of the left thumb, you are a recessive mutant.

Although the nine traits discussed above are considered monogenic, most are more complex than that. There can be slight variability in the expression of each of these traits, suggesting that other genes as well as environmental influence during development can influence the final phenotype. Many human traits you may have been told are monogenic are turning out to be far more complex. These are discussed in the next section.

III. More Complex Human Traits

Even traits that are set in motion by a single gene can be influenced during their development by factors are complex as interactions with products of other genes, and environmental influences such as hormone exposure during fetal development or later stages of the life cycle. Consider your own phenotype for each of the following traits, and try to determine what genetic and environmental influences might have been at work as they gradually developed to become part of you.

1. Hair color

Mammals produce two main types of melanin pigment that color the skin and hair. **Eumelanins** produce brown to black coloration, while **phaeomelanin** is responsible for reddish and yellowish hues. As many as twelve different gene loci are known to affect variation in human hair color, probably by affecting the degree of deposition of each pigment in the hair shaft. Cells known as **melanocytes** are responsible for pigment deposition in the non-living hair shaft, and it is these cells that provide pigment in the skin and some other tissues, as well.

Eumelanin concentration generally determines how dark the hair color is. Blonde hair indicates a low deposition of melanin, and the different shades of brown result from increasing amounts of the brown eumelanin in the hair shaft. A darker version of eumelanin is responsible for black hair when it is present in the hair shaft in very high concentrations. As the hair follicles produce less eumelanin with age, the hair begins to turn gray, and finally white.

Phaeomelanin is responsible for red hair, or reddish tinge to blonde or brown hair. All humans have at least some phaeomelanin in their hair, though some individuals have so little that it is not possible to notice with the naked eye.

The ratio of eumelanin to phaeomelanin in a given individual is of medical interest, as research has shown that the lower the ratio, the higher the individual's sensitivity to UV-B, the potentially harmful region of the spectrum in sunlight.

2. Eye color

Like hair color, eye color is determined by the deposition of different concentrations of melanin in the various layers of the iris. Once thought to be a result of simple dominance/recessiveness at one or two gene loci, eye color is now known to be far more complicated than that. It is a polygenic trait influenced by possibly a dozen different genes. Some of these encode pigment production, others the transport of those pigments, and still others the exact location and concentration of deposition.

At least three gene loci involved in the development of human eye color have been identified. EYCL1, located on chromosome 19, is involved in the coloration of green/blue eyes, EYCL2 in brown eyes, and EYCL3 in brown/blue eye coloration. The action of these genes is, in turn, affected by numerous other gene products encoded at other loci.

The most common eye color in humans is brown, which should be considered the wild type for our species. Lighter variants are produced by lower concentrations of melanin in the iris tissues, with blue eyes indicating little, if any melanin present. The blue color is due to refraction of light by the iris tissues themselves, and is not a pigment. Different concentrations of melanin in the iris will change the light-colored blue iris to green, hazel, grey, etc.

Eye color is so complex that it is now considered nearly impossible to predict the eye color of a child simply by examining the eye color of its parents. So if you're a brown-eyed child with light-eyed parents, you can relax.

3. Finger Length Ratio

Place your fingers flat on your lab table. Now observe your index finger (2D) and ring finger (4D). Are they the same length? Is the index finger shorter (low 2D:4D ratio)? Is the index finger longer (high 2D: 4D ratio)?

The second digit (2D) to fourth digit (4D) ratio (Figure 7) is believed to be a sex-influenced trait. It is autosomal, but its expression depends on the sex of the individual, and specifically upon the level of androgens (male hormones) to which the individual was exposed during a critical time in fetal development. Although the ratio appears to have predictable heritability, its exact expression varies among individuals with the same genotype for the trait. This is another example of **variable expressivity**, this time influenced by sex hormones.

Males are more likely to have a low 2D:4D ratio (index finger shorter) than females, who tend to have index and ring fingers of the same length, or with a slightly longer index finger. A great deal of fascinating research has shown that the 2D: 4D ratio can be a predictor the effects of fetal testosterone exposure on the brain and future behaviors.

Use the millimeter rules available on the lab tables to measure your 2D:4D ratio. And don't panic if it's not what you expect. Just start to read the vast literature on this topic, and prepare to be amazed.

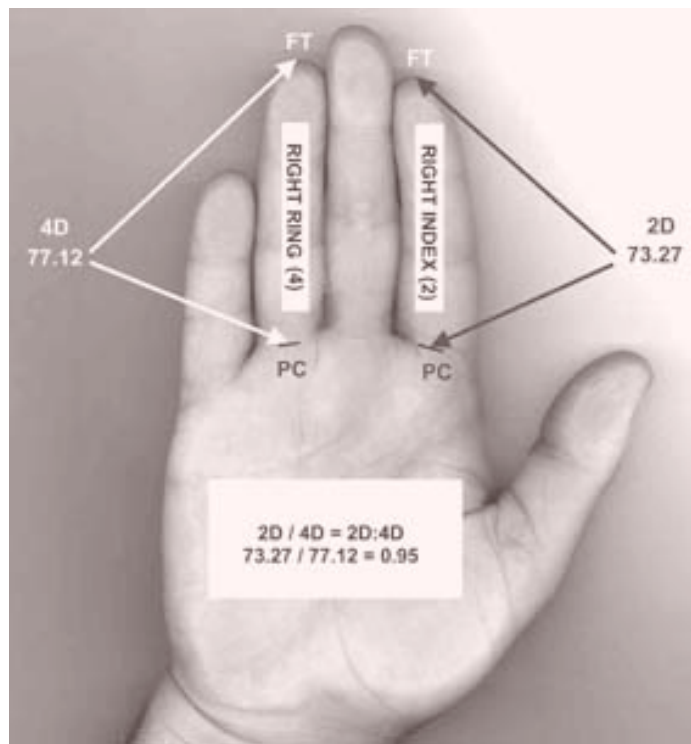


Figure 7. The ratio of the length of the second digit (2D) to the fourth digit (4D) is controlled not only by genes, but also by *in utero* fetal exposure to testosterone.

Table 2. A personal record of your phenotypes and possible genotypes. As you ascertain your phenotypic expression of each trait, circle (or fill in, if necessary) the appropriate choices. Save this for yourself and all of your descendants.

trait	your phenotype	your possible genotypes
PTC tasting ability	taster nontaster	TT Tt tt
Blood type (A-B-o)	A B AB o	I ^A I ^A I ^A i ^o I ^B I ^B I ^B i ^o I ^A I ^B i ^o i ^o
Blood type (Rh factor)	Rh+ Rh-	I ^D I ^D I ^D i ^d i ^d i ^d
digital hair	hairy hairless	HH Hh hh
tongue rolling	roller nonroller	RR Rr rr
widow's peak	yes no	WW Ww ww
ear lobe morphology	free attached	FF Ff ff
Darwin's ear point	yes no	EE Ee ee
freckles	yes no	FF Ff ff
cleft chin	yes no	CC Cc cc
bent little finger	yes no	BB Bb bb
hitch-hiker's thumb	no yes	HH Hh hh
interlocking fingers	L over R R over L	RR Rr rr

II. The Genetics of Corn (*Zea mays*)

In the back of the room you will find three bins brimming with Thanksgiving-type corn. A cob of corn is actually a cohort of offspring from a corn plant. Each kernel is an individual fruit and seed containing the genetic instructions to make one new corn plant.

In flowering plants such as corn, two identical sperm take part in reproduction. One sperm unites with the egg to produce the **embryo**. The other unites with two **polar nuclei** (genetically identical to the egg) to form the nutritive **endosperm**, which will feed the seedling before it can make its own food via photosynthesis. Both the embryo and the endosperm exhibit phenotypic traits they have inherited from both parent plants.

Each of our corn cobs represents a population of the offspring produced by one of three different sets of parents. Although the transparent hull of each corn grain is composed partly of maternal tissue, phenotypic traits exhibited by the offspring's endosperm are visible through it. By counting the phenotypic traits exhibited by grains on a cob of corn, one can sample a relatively large number of offspring and determine whether the principles of Mendelian genetics hold true.

A. Trait #1: Aleurone pigmentation

The endosperm in corn grains is found directly under the seed coat, and is composed of an outer, single-celled, proteinaceous layer called the **aleurone** and an inner layer of **starches and sugars**. The presence of purple anthocyanin pigments (**Y**) in the aleurone layer is dominant to the lack of such pigments (**y**). A grain with a genotype of **YY** or **Yy** will express the purple phenotype, whereas a grain with the **yy** genotype will appear yellow to white, because the translucent aleurone allows the color of the underlying starch layer to show through. You will use Corn Groups 1, 20 and 64 to examine the inheritance pattern of this trait.

B. Trait #2: Endosperm starch to sugar ratio

The presence of a high ratio of starches to sugars (**S**) is dominant to the presence of a high ratio of sugars to starches (**s**) in the inner endosperm layer. Fortunately for the corn-counting biology student, these phenotypes are easily sorted in a dry corn cob: starchy grains (**SS** or **Ss**) are smooth when dry, and sugary grains (**ss**) are very wrinkled when dry.

You will use only Corn Group 64 to examine the inheritance pattern of this trait.

a. Data collection

On the back table are three bins of corn cobs from three different parental crosses. Choose one cob from each bin and take it to your lab table for population counting and sorting of genetic traits. **DO NOT REMOVE PLASTIC SEAL OR KERNELS OF CORN FROM THE CORN COBS.** Count phenotypes through the seal, and leave the cobs intact.

A cross between two individuals who are both hybrid for a single trait of interest is known as a **monohybrid cross**. A cross between two individuals who are both hybrid for two traits of interest is known as a **dihybrid cross**. The prefix of a hybrid cross depends on the number of traits being examined (there are trihybrid, tetrahybrid, and even dodecahybrid crosses!), but remember that in such a cross **BOTH PARENTS MUST BE HYBRID FOR ALL TRAITS UNDER CONSIDERATION**.

A **test cross** is performed when the breeder wishes to know the genotype of an individual expressing the dominant allele. Since this individual could be either homozygous dominant or heterozygous, breeding it with an individual who is homozygous recessive and counting the offspring phenotypes will reveal the genotype of the dominant-expressing individual.

The corn groups you will be studying are produced by the following crosses:

CG 1 cobs are the offspring of a **monohybrid cross** ($Yy \times Yy$).

CG 20 cobs are the offspring of a **test cross** ($Yy \times yy$).

CG 64 cobs are the offspring of a **dihybrid cross** ($YySs \times YySs$).

You are now a curious geneticist, testing hypotheses concerning expected phenotypic ratios for each of the three sets of offspring. Given what you know about Mendelian genetics, construct a null hypothesis concerning phenotypic ratios for each of the corn groups. A sample hypothesis for the first group has been constructed for you. Construct hypotheses for the other two corn groups.

CG 1 *The ratio of purple: yellow corn kernels will not be significantly different from that predicted for a monohybrid cross between parents with genotypes Yy (i.e. - 3:1).*

CG 20 _____

CG 64 _____

Tabulate your findings below.

CG 1 purple _____ yellow _____
CG 20 purple _____ yellow _____
CG 64 purple smooth _____ yellow smooth _____
 purple wrinkled _____ yellow wrinkled _____

As you know all too well by now, a valid test of your null hypotheses, requires more than a mere visual inspection of the data. The investigator can make no claims about significance without first performing a statistical test on his/her data.

When you have finished counting and tabulating your corn grains, you will perform a simple, non-parametric test that will allow you to determine whether your corn cohort exhibits any significant deviation from the expected ratios. If they do, it is up to you to hypothesize WHY.

b. Data analysis

Remember: a scientifically observed result is said to be statistically significant if there is less than or equal to a 5% ($P \leq 0.05$) probability that the phenomenon is due simply to random chance. Conversely, this means that there is a 95% chance that the phenomenon is due to some factor *other* than chance.

To determine whether the variation in phenotype frequency of the corn you counted is significantly different from the frequencies expected from each of the three parental genotypes, you will use the Chi square test.

The Chi square test

Before we count the offspring of CG 1, we must ask whether the ratio of corn phenotypes we actually count is likely to occur in a *random* sample of corn kernels produced by a monohybrid cross. If our count is not significantly different from the expected, we have no reason to reject our null hypothesis. However, if our number is significantly different from the expected, we conclude that there is very little probability of obtaining such a ratio by random chance. Some other factor must be at work here--one we must try to explain in terms of other genetic events such as gene linkage, lethal genes, etc.

We will use a simple, non-parametric test called the Chi square (C^2) to determine whether our observed (counted) ratios of corn phenotypes are significantly different from the expected (from the known parental genotypes) ratios. The formula for this test is as follows

$$X^2 = \sum \frac{(o - e)^2}{e}$$

in which

- **o** = the *observed* (counted) number of grains of a particular phenotype
- **e** = the *expected* number of grains of a particular phenotype.
- Σ represents the summation of X^2 values over every phenotypic category (1 through n)

EXAMPLE: If you knew the parents of 100 kernels of corn on a cob were a monohybrid cross for the purple vs. yellow trait, your knowledge of Mendelian genetics would lead you to *expect* 75 purple kernels and 25 yellow kernels. Your null hypothesis would be that the ratio of purple to yellow corn kernels *should not differ* from a 3:1 ratio. Your *observed*, ratio, however, is 70 purple: 30 yellow. Is this deviation *significant*, or simply due to *random chance*?

If we calculate our χ^2 value for the above example, we obtain:

For the purple kernels:

$$\chi^2 = \frac{(70 - 75)^2}{75} = 0.333 \dots$$

For the yellow kernels:

$$\chi^2 = \frac{(30 - 25)^2}{25} = 1.0$$

Summing (Σ) the two χ^2 values, we obtain a χ^2 statistic of 1.333.

1. To accept or reject our null hypothesis, we must now determine the degrees of freedom. For the Chi Square test, **df = n - 1**, where **n** = the number of phenotypic categories possible. In our case, n = 2 (purple and yellow). Calculate the df for our example problem.
2. In the far left vertical column of the table of Chi Square Critical Values (Table 3) locate the df you obtained for the sample problem.
3. Now go *across* the appropriate df row until you find a χ^2 value closest to the value we obtained in our sample problem. Our χ^2 statistic was 1.333. If you look across the df = 1 row, you will find that this χ^2 value lies between 1.323 and 2.706 on the table.
4. Next, look *vertically* above the two χ^2 values closest to our example's χ^2 value to locate the P values associated with the χ^2 at one degree of freedom. The P value of our sample statistic lies between 0.25 and 0.1, or
0.25 > P > 0.1
5. Literally, this means that the probability that our *observed* corn phenotype ratio's deviation from the *expected* corn phenotype ratio is due simply to chance is between 10 and 25%. That's a fairly *big* probability that chance is the culprit for our deviation.
6. **Recall that for P to be *significant*, it must be less than or equal to 0.05 (5%).** As you can see, our example does *not* support rejection of H_0 . A ratio of 70 : 30 purple to yellow corn grains is not a significant deviation from the expected 75 : 25 ratio, and is probably due to chance and random sampling error.

Run the χ^2 test on each of your three corn cob cohorts to determine whether their phenotypic ratios deviate significantly from the expected. (Note: be careful when you calculate *df* for CG 64) Test all three of your hypotheses, accept or reject each one, and explain your results.

Table 3. A partial table of critical values of the Chi-square distribution.

df \ p	0.995	0.975	0.9	0.5	0.1	0.05	0.025	0.01	0.005	df
1	.000	.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879	1
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597	2
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838	3
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860	4
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750	5
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548	6
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278	7
8	1.344	2.180	3.490	7.344	13.362	15.507	17.535	20.090	21.955	8
9	1.735	2.700	4.168	8.343	14.684	16.919	19.023	21.666	23.589	9
10	2.156	3.247	4.865	9.342	15.987	18.307	20.483	23.209	25.188	10
11	2.603	3.816	5.578	10.341	17.275	19.675	21.920	24.725	26.757	11
12	3.074	4.404	6.304	11.340	18.549	21.026	23.337	26.217	28.300	12
13	3.565	5.009	7.042	12.340	19.812	22.362	24.736	27.688	29.819	13
14	4.075	5.629	7.790	13.339	21.064	23.685	26.119	29.141	31.319	14
15	4.601	6.262	8.547	14.339	22.307	24.996	27.488	30.578	32.801	15

YOUR LABORATORY INSTRUCTOR WILL TELL YOU WHAT YOU ARE TO TURN IN FOR THIS LAB EXERCISE. YOU ARE ALSO RESPONSIBLE FOR THE GENETICS PROBLEMS ON THE FOLLOWING PAGES. YOUR LAB INSTRUCTOR WILL TELL YOU WHEN THEY ARE TO BE TURNED IN.

Literature Cited

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Forrai G, and Bánkóvi G. (1984). "Taste perception for phenylthiocarbamide and food choice - a Hungarian twin study". *Acta Physiol Hung* **64** (1): 33–40.

Vincensi, M, d'Ischia, M, Napolitano, A., Procaccini, E., Riccio, G., Monfrecola, G., Santoianni, P., Prota, G. (1998) Phaeomelanin versus eumelanin as a chemical indicator of ultraviolet sensitivity in fair-skinned subjects at high risk for melanoma: a pilot study. *Melanoma Research* Feb 8 (1): 53-58.

GENETICS PROBLEMS

These problems will give you further experience on how to use information you have gathered from lecture, lab, and your textbook. If you become confused while working any of the problems, ask your instructor for help. Your completed questions are to be turned in one week from the time you do this genetics lab. Show all your work.

1. Most humans (and vertebrates, in general) are genetically "programmed" to produce a brown skin pigment known as melanin. Albinism--the inability to produce melanin--is inherited in humans as an autosomal recessive trait. An individual with genotype AA or Aa will have normally pigmented skin, whereas an individual with genotype aa will produce no melanin. The albino's skin is very pale white, and the (highly vascularized) irises of the eyes appear red because no melanin obscures the red reflectance of blood. Predict the frequencies of all genotypes and phenotypes expected to result from the cross of an albino male with a heterozygous female.

Parental genotypes: male:_____ female:_____

2. Do the same for a mating of two heterozygotes.
3. What can you say about the genetic constitution of the parents of an albino child?
4. Given what you know about human A-B-O blood types, what parental cross would yield an F₁ consisting of 50% type A, 25% type AB, and 25% type B?

5. As a genetic advisor to a hospital you are confronted with the following problem: two women had babies in the hospital at about the same time on the same day. Mrs. Cartman took home a boy (Eric), and Mrs. Simpson took home a girl (Lisa). However, in her post-partum daze, Mrs. Simpson thought she recalled the delivery room nurses commenting on her child as being a boy.

The blood types of all concerned parents were determined as follows:

Mr. Cartman = B	Mrs. Cartman = AB	Eric = A
Mr. Simpson = B	Mrs. Simpson = O	Lisa = O

Has there been an interchange of babies? Support your conclusion or be fired.

6. The polled (hornless) condition in cattle (H) is dominant over the horned condition (h) and is autosomal. A polled bull (Spike) is bred to three different cows with the following results:
- Faye is horned. She produces a polled calf.
 - Holly is horned. She produces a horned calf.
 - Clover is polled. She produces a horned calf.

What are the genotypes of each animal?

Spike: _____ Faye: _____ Holly: _____ Clover: _____

What offspring would you expect from each of these matings? Punnett squares:

Spike x Faye:

Spike x Holly:

Spike x Clover:

NOTE: Although you will not need the information to work problems 7 - 9, be sure to review text readings on mosaic expression to fully understand the genetics involved.

7. In cats, yellow fur (B) is dominant to black fur (b), and the gene is located on the X chromosome. The heterozygous "tortoise-shell" or "calico" condition is exhibited only by the female, and is a result of mosaic expression. What offspring (genotypes, phenotypes and frequencies) would you expect from the cross of a black male with a yellow female?

8. What kinds of offspring would you expect to result from the cross of a black male with a tortoise-shell female?

9. Is it possible to cross two tortoise-shell animals? Explain.

10. In guinea pigs rough hair (R) is dominant with respect to smooth hair (r), and black hair (B) is dominant to white (b). These genes are not linked (i.e., the locus for hair color is not on the same chromosome as the locus for hair texture), nor are they sex-linked. Cross a guinea pig heterozygous for hair texture and color, with a white-smooth-haired guinea pig. Describe the predicted F₁ in terms of phenotype and genotype frequencies.

11. Over several years, a pair of guinea pigs produced the following offspring
25% Rough-black 25% Rough-White
25% Smooth-black 25% Smooth-White
Describe the phenotypes and genotypes of each parent.

12. Alas! Your prize potato patch is infested with greedy, potato-munching leperchauns! Not to be confused with the more attractive and popular "leprechaun" (*Homunculus patrickii*), the "leperchaun" (*Homunculus odoratus*) has a strongly disagreeable odor, and extremities that fall off messily when the organism is handled. This is an effective defense mechanism.

At your friendly neighborhood BillyBob's Bargain Barn of Biological Control, you purchase a pair of special, mutant ferrets--each of which is true breeding. This strain of ferret is highly prized by potato farmers because of two simple, autosomal traits affecting (1) coat color and (2) appetite for leperchauns.

Coat color has two alleles. Black fur (B) is dominant to the more desirable brown fur (b), which camouflages the ferrets as they lie in ambush in the potato patch. Diet preference in these voracious carnivores also has two alleles. Unfortunately, the allele which drives the ferrets to chase and devour leperchauns (p) is recessive to the gene which drives them to chase and devour your ankles (P).

Your pair consists of a true-breeding black, ankle-biting male and a true-breeding brown, leperchaun-eating female. What type of offspring do you expect to obtain in the F₁?

What about the F₂?

13. If a black, ankle-biting ferret is selected from the F₂ progeny above, what is the probability that it will breed true in succeeding generations?

14. Over several months, a black, ankle-biting ferret and a brown, ankle-biting ferret yield the following phenotypic ratios in their offspring:

18 black, leperchaun-eating	23	brown, leperchaun eating
64 black, ankle-biting	59	brown, ankle-biting

What are the genotypes of the parents?

Pedigree analysis

15. When investigators study human genetics, they often must examine "family trees," or **pedigrees**. The following page shows two different pedigrees, each following the expression of a single genetic trait. To figure out the type of inheritance involved, consider each generation separately, and determine phenotypic ratios in each set of offspring. If you can "work" the following human pedigrees, you will have mastered several fundamental aspects of Mendelian genetics!

Let us make the assumption that only four types of inheritance are involved in the pedigrees described.

autosomal dominant
autosomal recessive

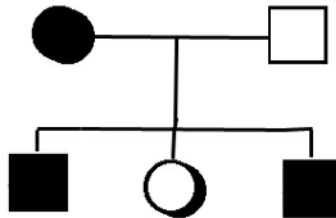
X-linked dominant
X-linked recessive

The symbols used are:

□ = male ○ = female

When the symbol is filled in, the individual expresses the trait. When the symbol is empty, the individual does not express the trait.

For example, the figure below represents a marriage in which the female exhibits the trait, but the male does not. The symbols on below the "parental" symbols represent the children of this marriage. In this case, both sons exhibit the trait, but the daughter does not.

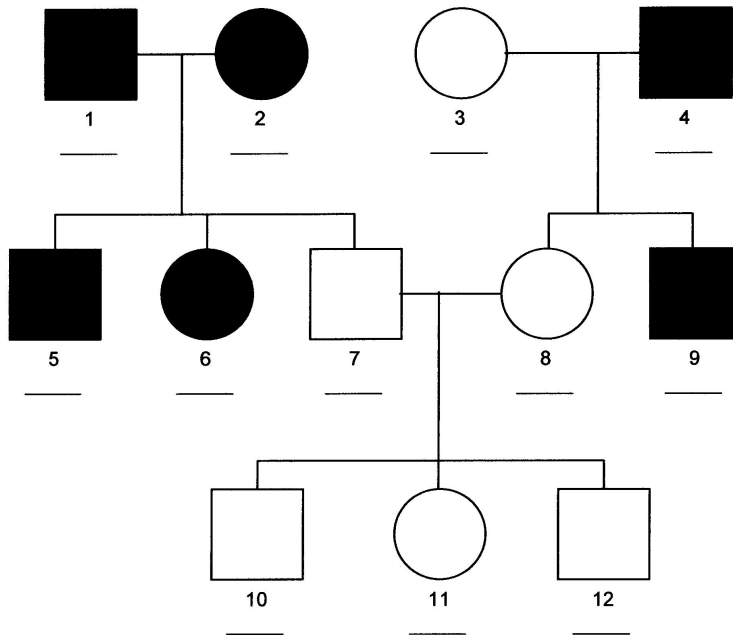


Pedigrees are constructed by a process of elimination. Begin by writing down all possible genotypes for each individual. Rule out each type of inheritance as it becomes evident that, from the information given, a certain type of inheritance is not possible. If a certain type of inheritance "fits" the pedigree, it means only that that type of inheritance is *possible* in the given situation--not that you are absolutely correct.

On the next page you will find two pedigrees, each typical of a particular type of inheritance (autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive, Y-linked dominant, or Y-linked recessive). Examine the pedigree, and fill in the most likely genotypes for each individual on the family tree. Finally, given what you have hypothesized about the family, write the type of inheritance in the space provided below each pedigree.

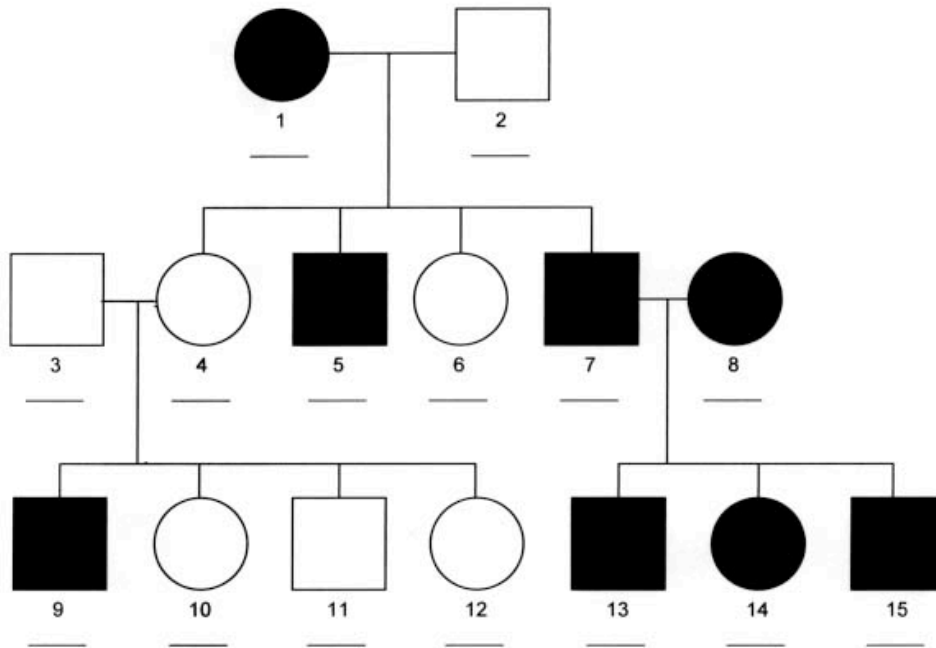
Pedigree analysis: The family trees

Pedigree I.



Type of inheritance: _____

Pedigree II



Type of inheritance: _____