



The *Drosophila* Genome

Lab 4.

**GIANT CHROMOSOMES
AND MENDEL'S LAWS**



AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

Polytene (Giant chromosomes) from *Drosophila* larval Salivary Glands

Introduction:

Many larval and some adult tissues of insects in the family Diptera are characterized by nuclei with giant chromosomes. These chromosomes develop by multiple replications of the chromosomes within each cell during development. Each nucleus will contain hundreds of copies of each chromosome. Cells are considered polyploid if they have more than two copies of each chromosome. If the chromosomes align perfectly forming large cables of chromosomes they are polytene.



In *Drosophila melanogaster*, chromosomes of the larval salivary gland contain about 1024 copies of the DNA, or ten doublings from the normal $2n$ condition, of each of the three chromosomes..

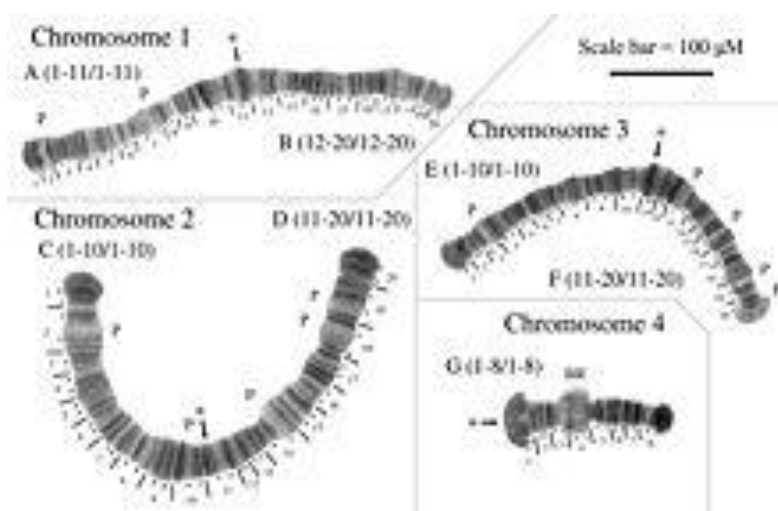
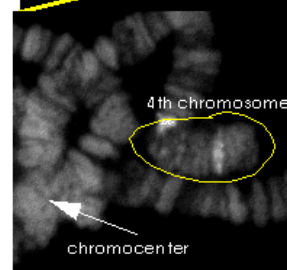
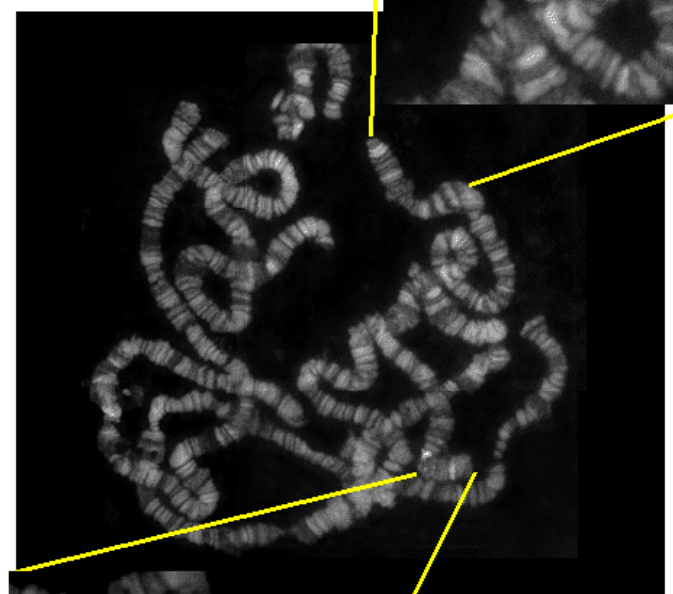
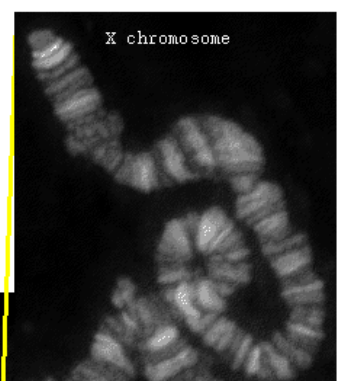
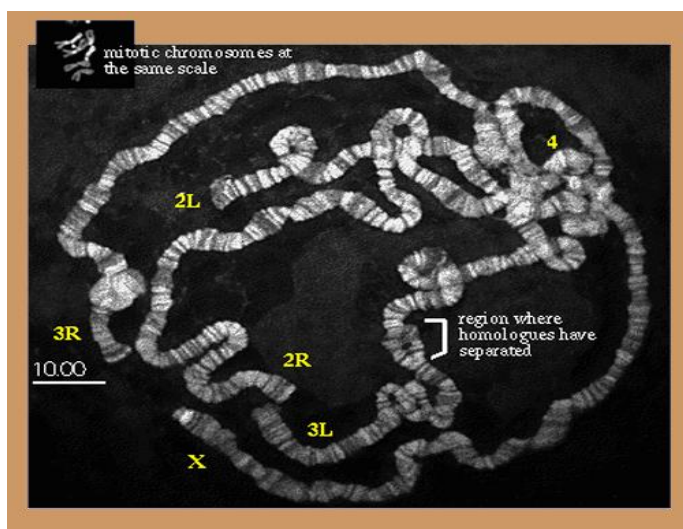
The pattern of condensed regions (heterochromatin 85 %), and transcribed regions (euchromatin 15 %) gives a series of about 5000 light and dark bands when the chromosomes are stained with orcein. In general, the DNA in each band codes for a single function, although there are exceptions to this observation. *Drosophila* has given us substantial insight into DNA function and gene organization.

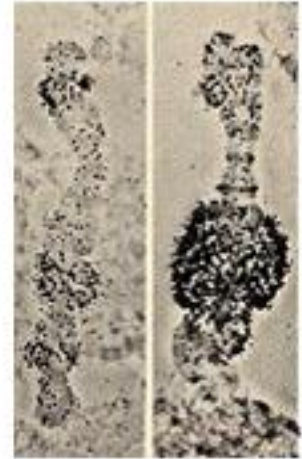
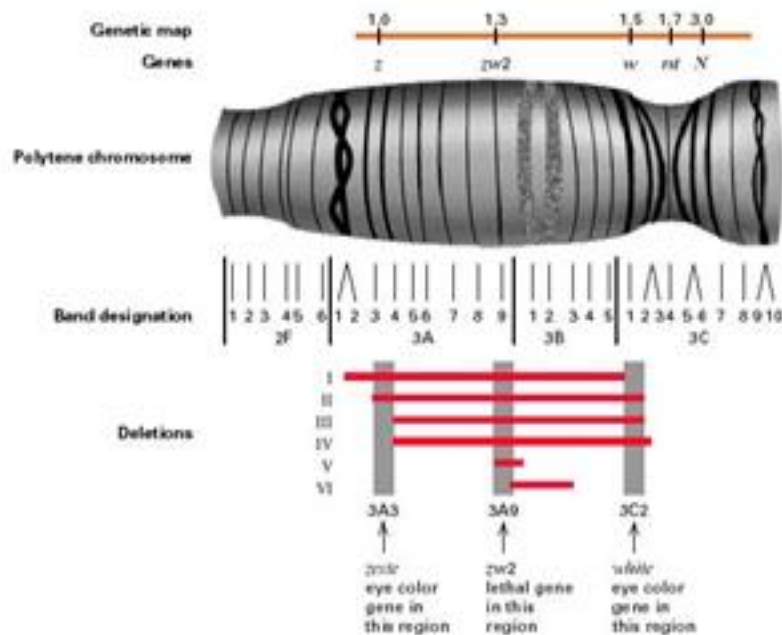
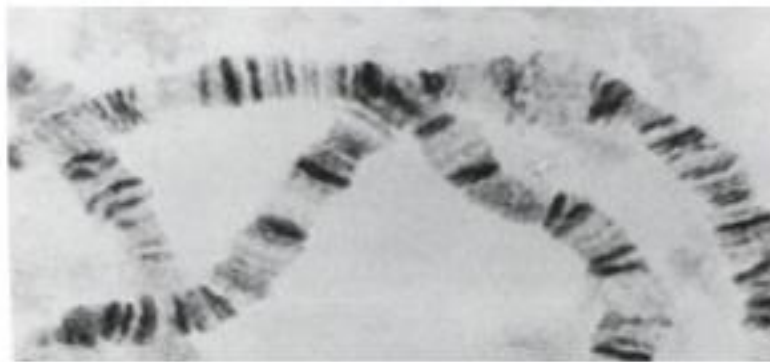
At certain times during development, some bands undergo a reversible modification to form structures known as "puffs." Puffs are localized expansions of the polytene chromosome structures. **Puffs are sites of synthesis of RNA** and result from the activation of the gene (or genes) contained within a particular band. This means that changes in gene activity can be observed microscopically as changes in polytene chromosome puffing pattern. The size of the puff reflects the number of copies of that

gene that are being transcribed - larger puffs have a larger percentage of the 1024 chromatids being simultaneously copied into mRNA. The specific RNAs are translated into a set of polypeptides.

What is the function of polyteny?

- The probable answer: **gene amplification**. Having multiple copies of genes permits a high level of gene expression; that is, abundant transcription and translation to produce the gene products. This would account of polyteny being associated with large, metabolically active cells (like salivary glands).
- Polytene chromosomes are subdivided into some 5,000 **dark bands** separated by **light interbands**. Genes are located in both, but those in the interband regions seem to be more active.
- Polytene chromosomes also have regions — called "puffs" — that are swollen and appear to have a looser structure. The pattern of puffing within a cell varies over time. For example, each time an insect larva prepares to molt, a definite, predictable sequence of puffing occurs.





Procedure for removing *Drosophila* salivary glands

Objective: To stain and identify the polytene chromosomes of the salivary glands of *Drosophila melanogaster*.

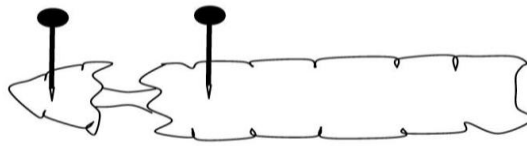
Materials

For each student group: compound microscope - insect pins - microscope slides - cover glasses - stock supply of *Drosophila* larvae - dropping bottle of aceto-orcein stain - dropping bottle of 45% acetic acid solution

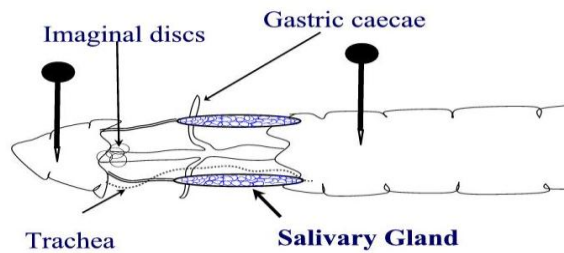
Procedures: Removing the salivary glands:

1. Remove a large larva from the stock of *D. fly*. Larger larvae are easier to dissect. However, select an active larva and one that has not started to pupate.

2. Using the stereomicroscope, dissect the larva by placing one teasing needle on the posterior aspect of the larva and the other needle at the anterior end, near the black mouth parts.



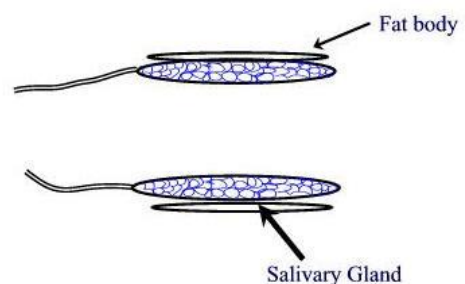
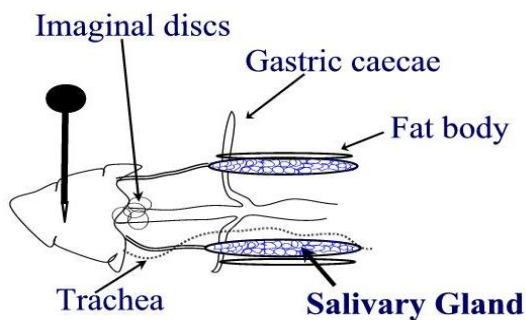
3. Carefully pull outward with the anterior needle.



If you pull gently, the glands will be easy to find between the anterior and posterior sections. If the gut and other tissues just form a lump. Once you see the salivary glands, dissect away the abdomen.

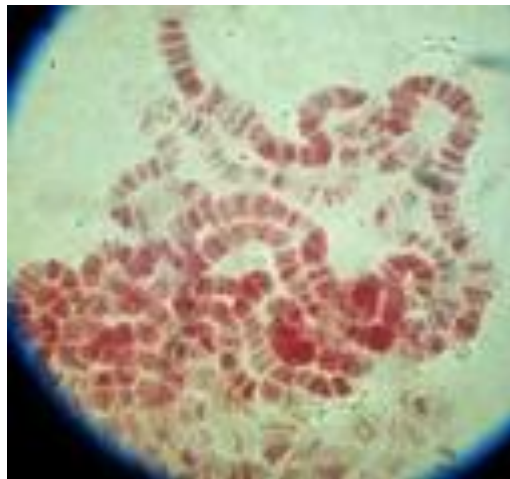
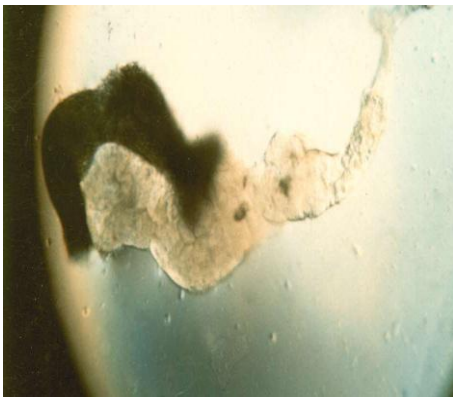
4. There are two transparent salivary glands located anteriorly in the larva. The glands are characterized by a granular, bead-like appearance. A narrow, white ribbon of fat surrounds the glands and should be torn away. **Do not allow the glands to dry.**

5. Discard all of the larva except for the salivary glands.



Staining and Observing:

1. Place 2 drops of aceto-orcein stain on the salivary glands, and let it stand for 10 minutes.
2. Place a cover slip over the glands, and using your thumb and a paper towel, push down on the slide. The pressure applied will squash the glands, rupture the nuclear membrane, and free the chromosomes.
3. Using a compound microscope, observe the slide under low and high magnification.
4. Make the slide permanent by brushing along the edges of the cover slip with clear nail polish.



Mendel's Lasws

By the 1890's, the invention of better microscopes allowed biologists to discover the basic facts of cell division and sexual reproduction. The focus of genetics research then shifted to understanding what really happens in the transmission of hereditary traits from parents to children.

A number of hypotheses were suggested to explain heredity, but Gregor Mendel, a little known Central European monk, was the only one who got it more or less right. His ideas had been published in 1866 but largely went unrecognized until 1900, which was long after his death. His early adult life was spent in relative obscurity doing basic genetics research and teaching high school mathematics, physics. In his later years, he became the abbot of his monastery and put aside his scientific work.

















While Mendel's research was with plants, the basic underlying principles of heredity that he discovered also apply to people and other animals because the mechanisms of heredity are essentially the same for all complex life forms.

Through the selective cross-breeding of common pea plants (*Pisum sativum*) over many generations, Mendel discovered that certain traits show up in offspring without any blending of parent characteristics. For instance, the pea flowers are either purple or white--intermediate colors do not appear in the offspring of cross-pollinated pea plants.

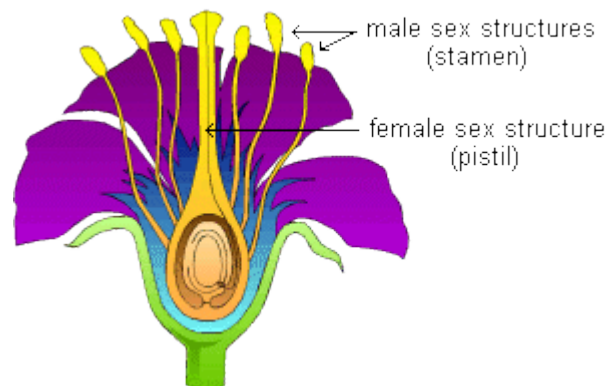
Mendel observed seven traits that are easily recognized and apparently only occur in one of two forms:

- | | |
|--|---|
| 1. flower color is purple or white | 5. seed color is yellow or green |
| 2. flower position is axil or terminal | 6. pod shape is inflated or constricted |
| 3. stem length is long or short | 7. pod color is yellow or green |
| 4. seed shape is round or wrinkled | |

Seed		Flower	Pod		Stem	
Form	Cotyledons	Color	Form	Color	Place	Size
						
Grey & Round	Yellow	White	Full	Yellow	Axial pods, Flowers along	Long (6-7ft)
						
White & Wrinkled	Green	Violet	Constricted	Green	Terminal pods, Flowers top	Short (1ft)
1	2	3	4	5	6	7

Mendel picked common garden pea plants for the focus of his research because they can be grown easily in large numbers and their reproduction can be manipulated. Pea plants have both male and female reproductive organs. As a result, they can either self-pollinate themselves or cross-pollinate with another plant.

Reproductive structures of flowers



Dominant and Recessive Traits

There are two types of genes for each trait: Dominant, and Recessive. The combination determines the trait to be expressed

At the time of conception, sperm cell fuses with the ovum to create a new cell called Zygote with 46 (23 pairs) chromosomes. On each pair of Chromosomes, there are two genes that determine the inherited trait. This gene pair is called allele. If the two pair of allele are identical (one pair from each parent), the off spring will express that trait.

Normally, there are two types of genes for each trait: Dominant, and Recessive. Dominant genes when paired with recessive gene

determines the trait to be expressed. Recessive gene is not expressed when paired with dominant gene. Recessive genes are only expressed when paired with another recessive gene.

Genes associated with dominant traits have more complete information than the recessive traits. The dominant trait genes always trump the recessive trait genes.

Mendel summarized his findings in two laws; the **Law of Segregation** and the **Law of Independent Assortment**.

Law of Segregation (The "First Law")

The Law of Segregation states that when any individual produces gametes, the copies of a gene separate so that each gamete receives only one copy. In meiosis the paternal and maternal chromosomes get separated and the alleles with the traits of a character are segregated into two different gametes.

Law of Independent Assortment (The "Second Law")

The Law of Independent Assortment, also known as "Inheritance Law", states that alleles of different genes assort independently of one another during gamete formation.

With all of the seven pea plant traits that Mendel examined, one form appeared **dominant** over the other. Which is to say, it masked the presence of the other allele. For example, when the genotype for pea seed color is YG (heterozygous), the phenotype is yellow. However, the dominant yellow allele does not alter the **recessive** green one in any way. Both alleles can be passed on to the next generation unchanged.

You must first learn the terminology of genetics before you can apply it to solving genetics problems:

P1 (parental): The original individuals in a breeding experiment.

A hybrid: Is the result of breeding two different variations of a trait.

F1 (First filial): The offspring are the first generation of the parents.

F2 generation: When the first filial individuals are allowed to breed at random, they produce the second generation.

Homozygous: If two alleles are identical, the organism is called a homozygote and is said to be homozygous

Heterozygous: If instead the two alleles are different, the organism is a heterozygote and is heterozygous.

The genotype: The genetic makeup of an organism, either at a single locus or over all its genes collectively.

The phenotype: The molecular, physical, behavioral affects of a genotype of an organism.

At heterozygous gene loci, the two alleles interact to produce the phenotype. The simplest form of allele interaction is the one described by Mendel, now called Mendelian, in which the appearance/phenotype caused by one allele is apparent, called dominant, and the appearance/phenotype caused by the other allele is not apparent, called recessive.

Monohybrid breeding (cross): If we mix one trait it will always resulted in a 3:1 ratio between dominant and recessive phenotypes.

Dihybrid breeding (cross): If we mix two traits it will show 9:3:3:1 ratios But the 9:3:3:1 means that each of the two genes are independently inherited with a 3:1 phenotypic ratio. Mendel concluded that different traits are inherited independently of each other.

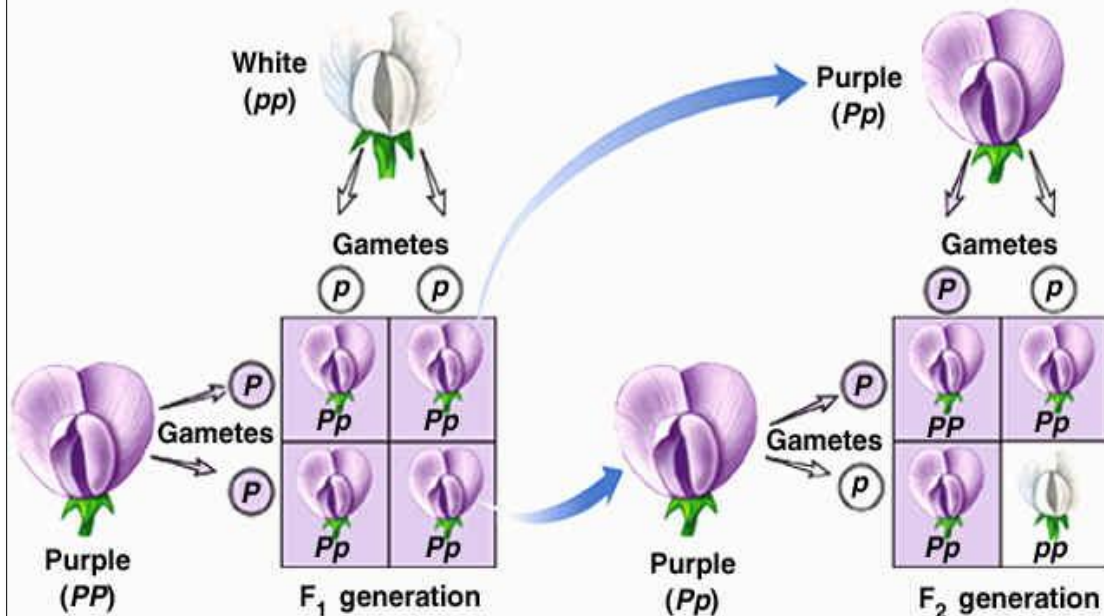
Some of Mendel crosses:

In this series of crosses between true-breeding purple and white flowered plants (the P1 generation), the offspring (F1) all have purple flowers. When those are allowed to breed, flowers of their offspring (the F2 generation) will be 75% purple and 25% white. Of these purple flowered plants, 50% of them will not be true breeding, because they are heterozygous, that is they have one allele for purple flowers (P) and one for white flowers (p). The true-

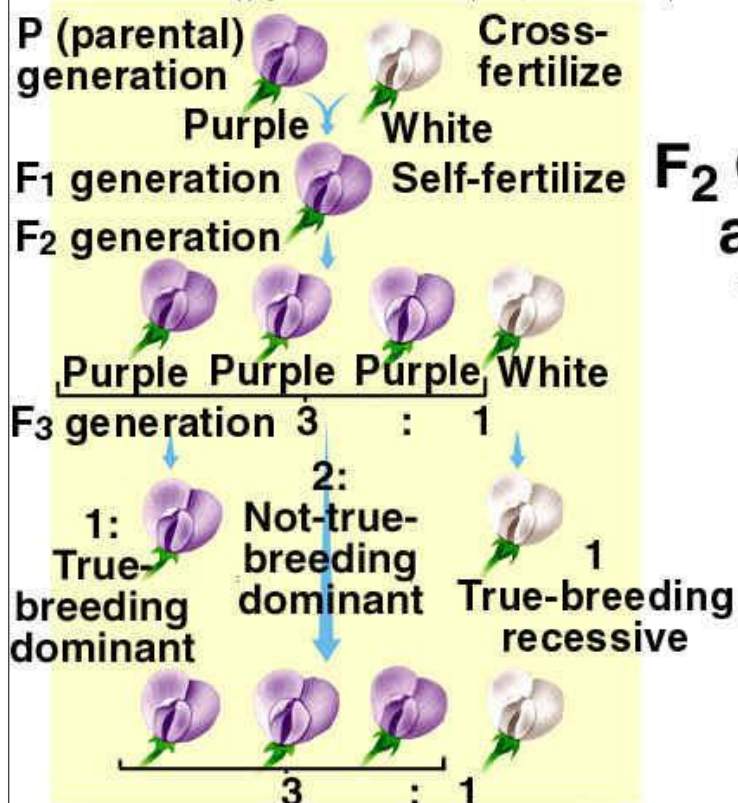
breeding purple flowered plants are homozygous dominant (PP), while the white flowered plants are homozygous recessive (pp).

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Mendel's Cross of Pea Plants for Color



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**F₂ Generation—
a Disguised
1:2:1 Ratio**

Testing Mendel results:

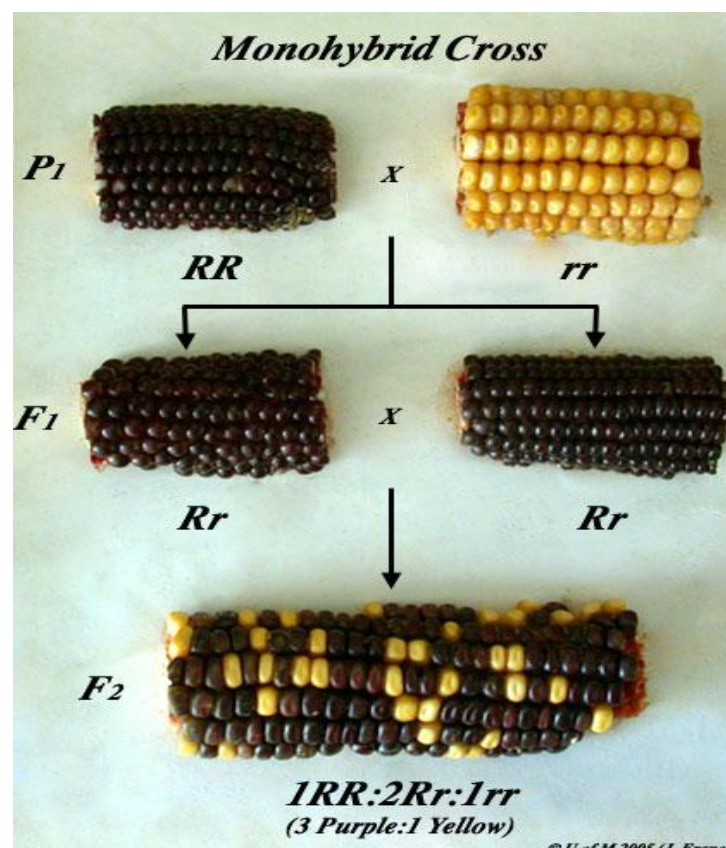
In this course you will test the result that Mendel achieved from his experiments and you will apply the result on the *Drosophila* fly generation in the coming labs. You will study F₂ corn ears from a monohybrid cross or dihybrid cross and count the maize (corn ears) seeds either.

Corn Genetics

Many models are useful for illustrating basic Mendelian genetics. Whether you use the fruit fly, corn, sweet pea or some other system the basic principles are applicable to all diploid systems including humans. We will be using corn to illustrate some of these principles. Mature corn plants produce ears that contain hundreds of seeds or kernels. Each seed is formed by the fertilization of an egg by a male gamete. Therefore, each kernel on an ear of corn can grow into a whole new plant. A complete ear represents a compact population of offspring which may be sampled.

Monohybrid Cross

The cob above was taken from a corn plant which was a F₂ plant resulting from an original parental cross between a homozygous purple corn seeds plant and a homozygous yellow corn seeds plant. The allele R(purple) is dominant over the recessive allele r (yellow).



Testing a Monohybrid Cross

1. Count the number of purple and yellow kernels in five of the rows on your ear of corn and record the number on the chart. Be sure to use the same five rows for each calculation.
2. Make a table and put your data on.
3. Calculate the percentage between the individual traits.



Testing a Monohybrid test cross

1. Count the number of purple and yellow kernels in five of the rows on your ear of corn and record the number on the chart. Be sure to use the same five rows for each calculation.
2. Make a table and put your data on.
3. Calculate the percentage between the individual traits.

