Lab 4 Genetics

Giant chromosomes and Mendel's Laws

Polytene (Giant chromosoms)

- 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.

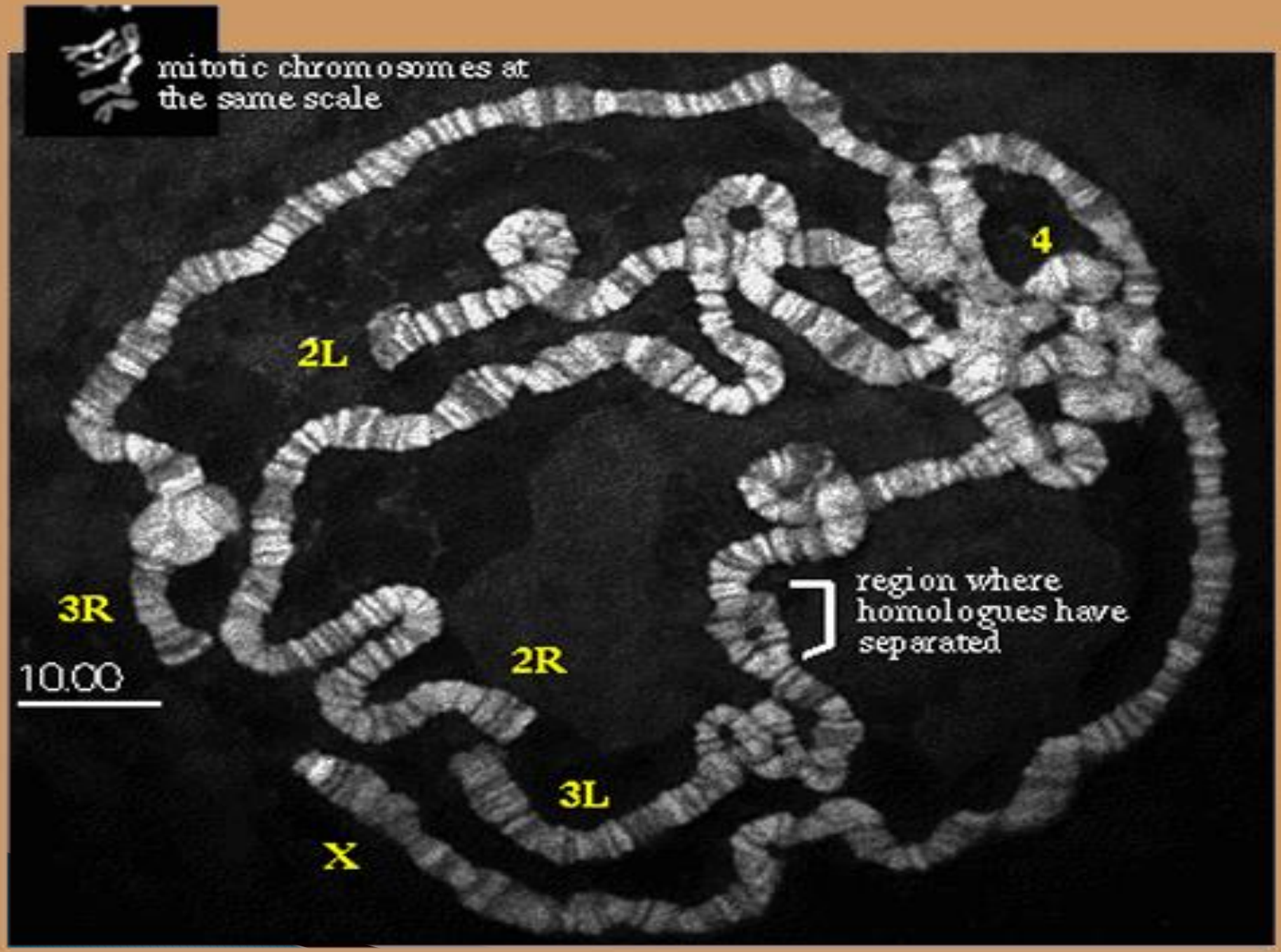
Polytene (Giant chromosoms) from drosophila larval Salivary Glands

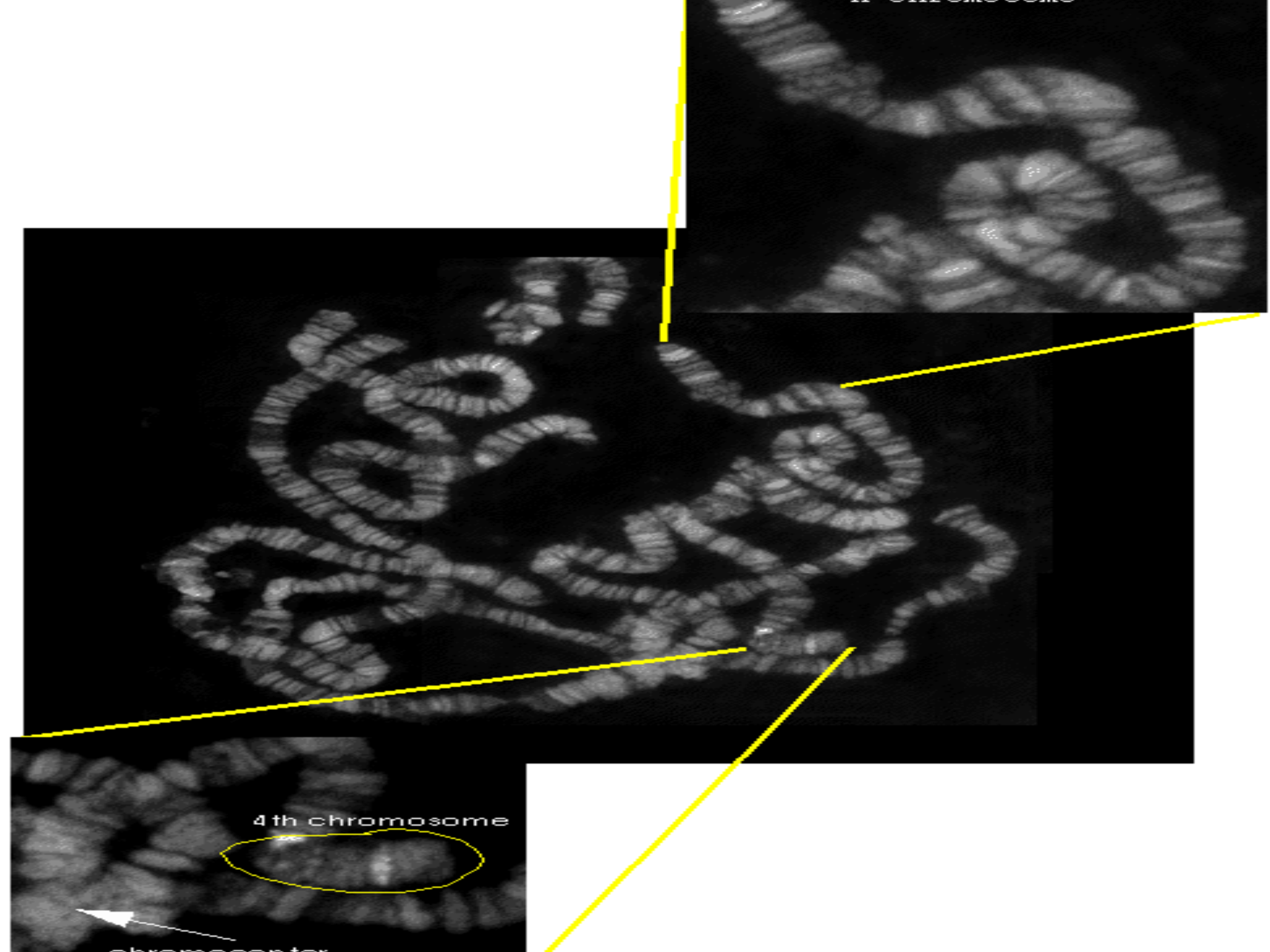
- Each of Drosophila's 4 pairs of chromosomes has undergone 10 rounds of DNA replication **(DNA amplification).**
- The chromosomes of the larval salivary gland contain about 1024 copies of the DNA, or ten doublings from the normal 2n.
- The maternal and paternal homologs — as well as all their duplicates — are aligned in exact register with each other, and all chromosomes attach to each other in a region called **chromocenter.**
- So each chromosome consists of a cable containing 2048 identical strands of DNA.
- These are so large that they can be seen during interphase; even with a low-power light microscope.

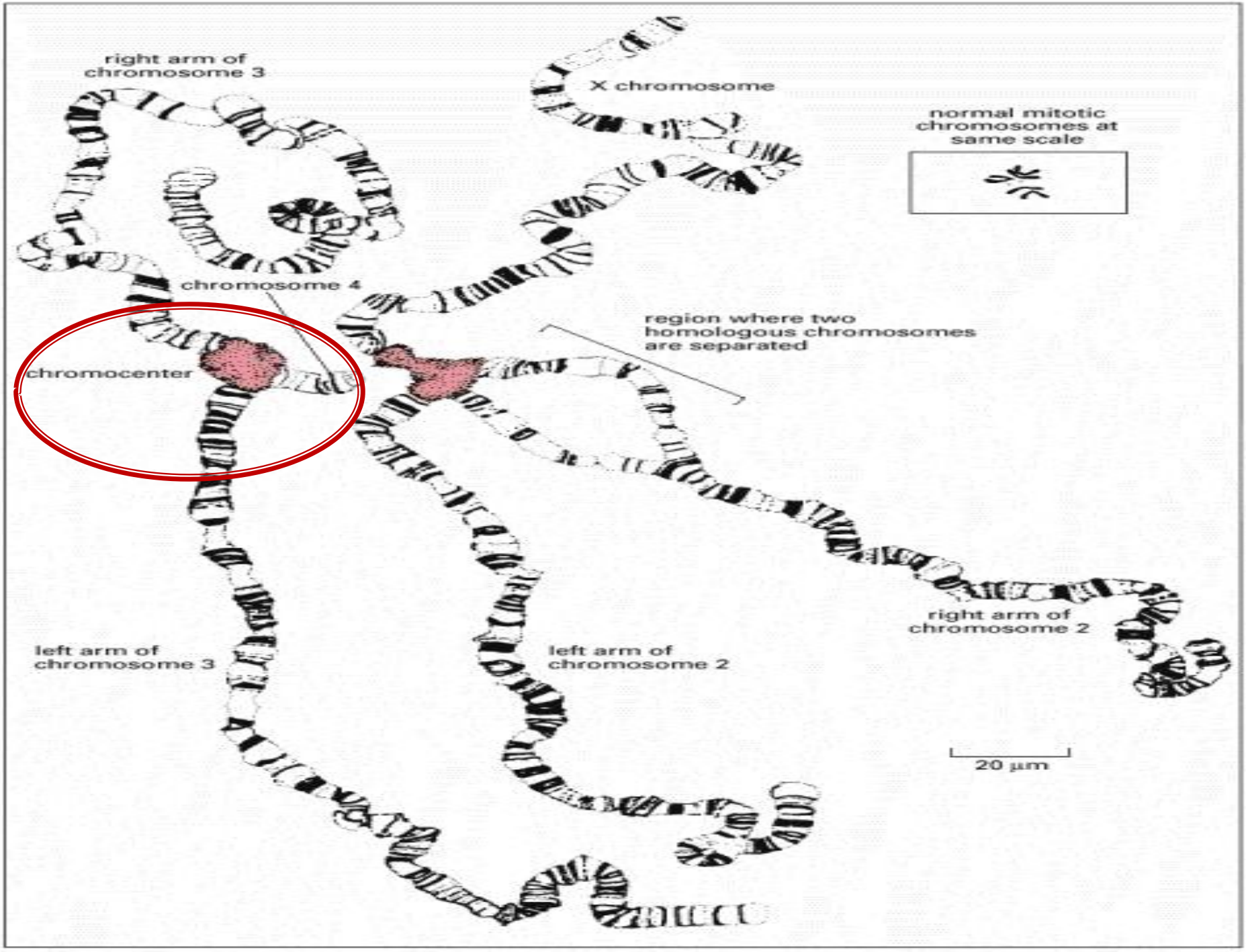
What is the function of polyteny?

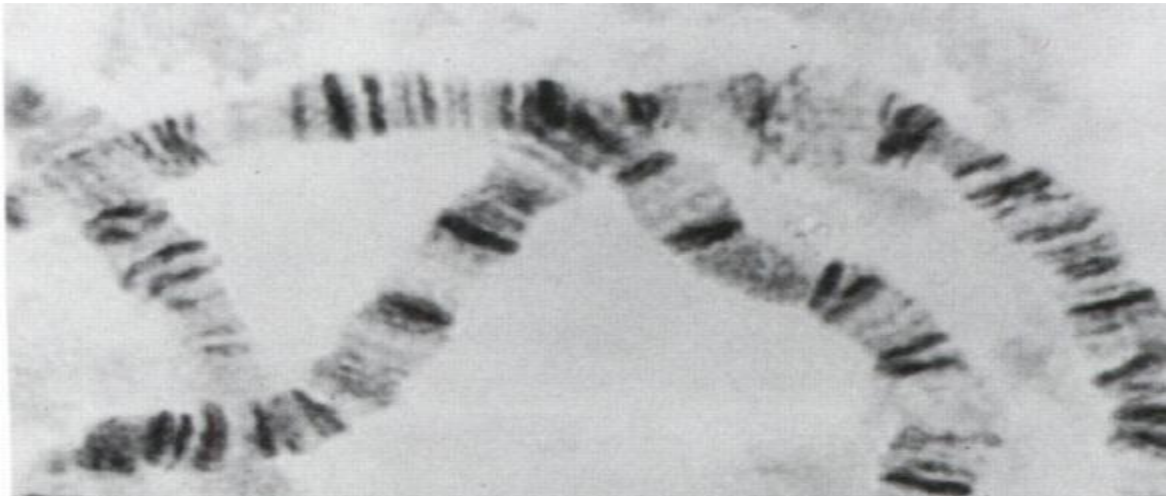
- The probable answer: **gene amplification.** Having multiple copies of genes permits a high level of gene expression; that is, abundant transcription and translation to produce the gene products. This would account of polyteny being associated with large, metabolically active cells (like salivary glands).

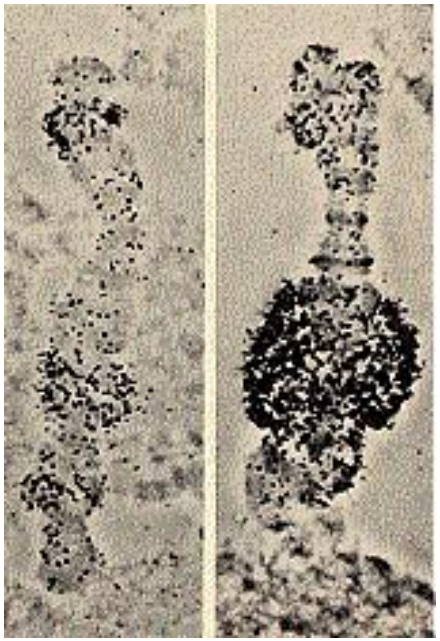
- Polytene chromosomes is apattern of condensed regions (heterochromatin 85 ٪), and transcribed regions (euchromatin 15 ٪) gives a series of about 5000 **light** and **dark** bands.
- Genes are located in both, but those in the interband regions seem to be more active.
- **Chromosome puffs** are diffuse uncoiled (swollen and appear to have a looser structure). regions of the polytene chromosome that are sites of RNA transcription.
- 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.

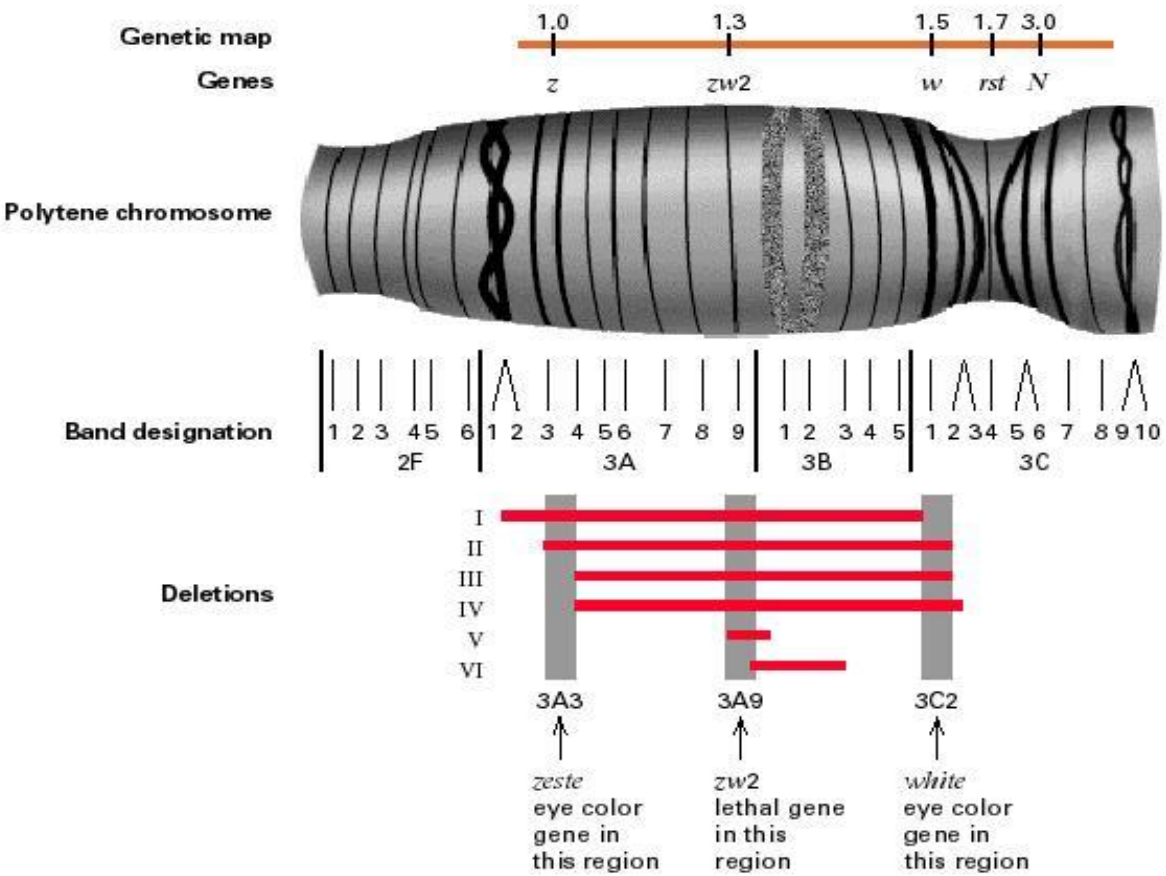

mitotic chromosomes at
the same scale
4
2L
region where
homologues have
separated
3R
2R
10.00
3L
X


4th chromosome


right arm of chromosome 3
X chromosome
normal mitotic chromosomes at same scale
chromosome 4
region where two homologous chromosomes are separated
chromocenter
right arm of chromosome 2
left arm of chromosome 3
left arm of chromosome 2
20 µm






Genetic map
1.0
1.3
1.5
1.7
3.0
Genes
z
zw2
w
rst
N
Polytene chromosome
Band designation
1 2 3 4 5 6 1 2 3 4 5 6 7 8 9 1 2 3 4 5 1 2 3 4 5 6 7 8 9 10
2F
3A
3B
3C
Deletions
I
II
III
IV
V
VI
3A3
3A9
3C2
zeste
eye color
gene in
this region
zw2
lethal gene
in this
region
white
eye color
gene in
this region

Drosophila larval

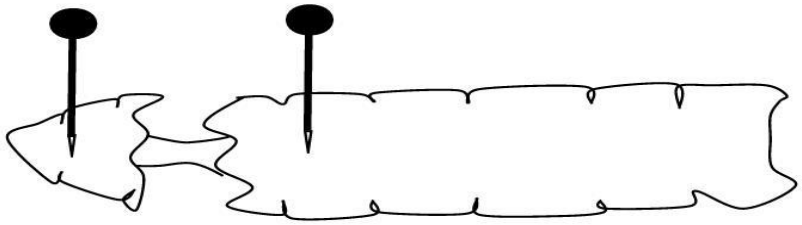
Procedures

Removing the salivary glands:

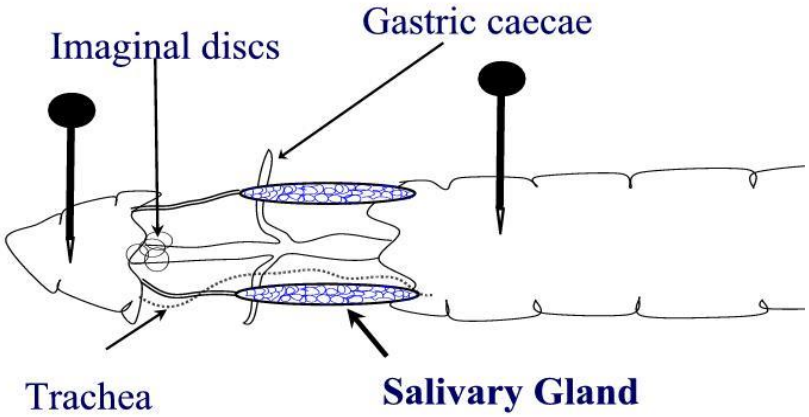
1. Remove a large larva (third instar) from the stock of D. fly.

- Larger larvae are easier to dissect. However, select an active larva and one that has not started to pupate.

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

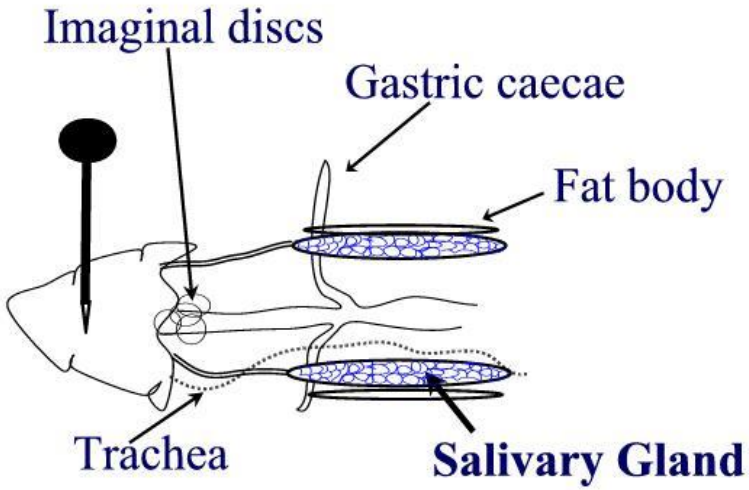


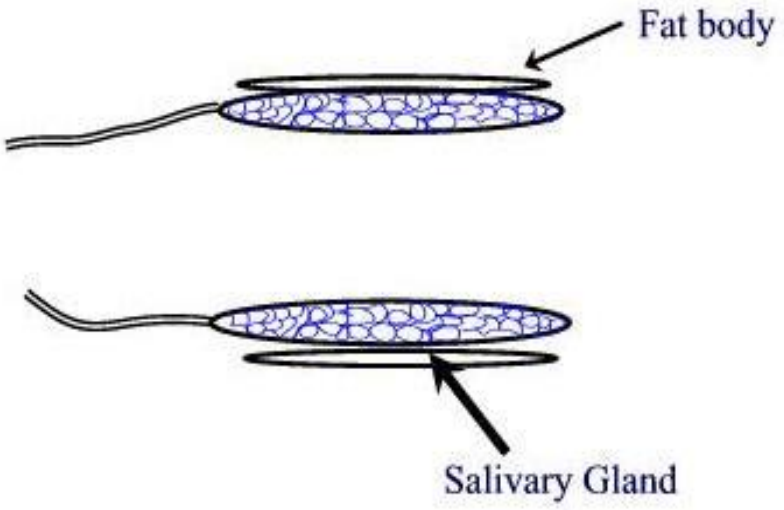
3.Carefully pull outward with the anterior needle.



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.

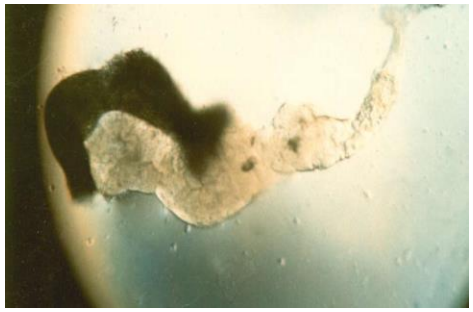
5.Discard all of the larva except for the salivary glands.

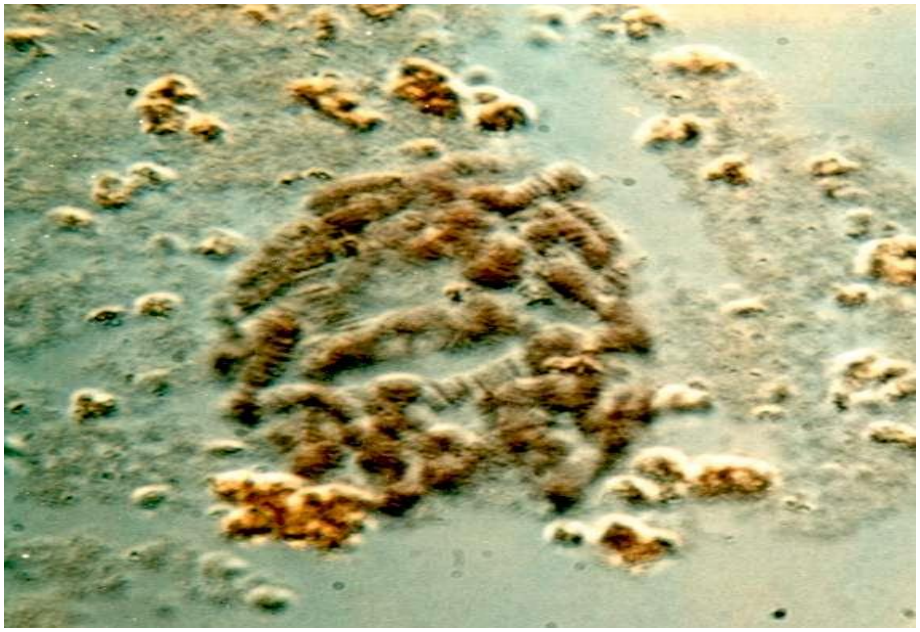




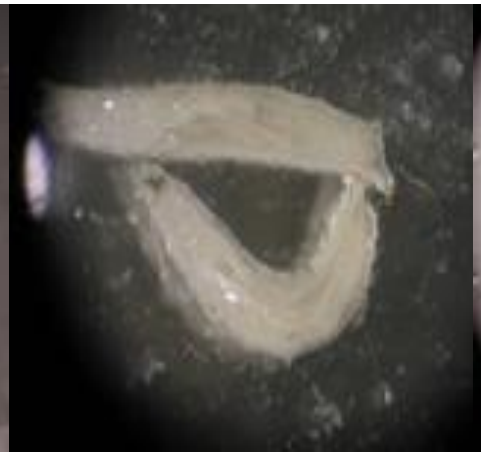
Staining and Observing

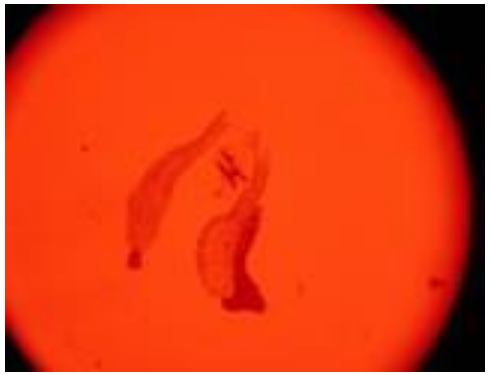
- Place 2 drops of aceto-orcein stain on the salivary glands, and let it stand for 10 minutes.
- Place a cover slip over the glands, and using your thumb and a paper towel, push down on the slide. The pressure applied will squash the glands, rupture the nuclear membrane, and free the chromosomes.
- Using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

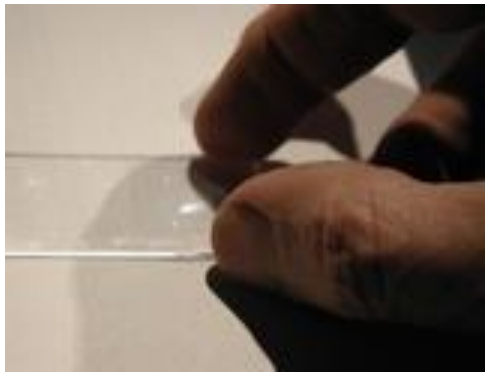
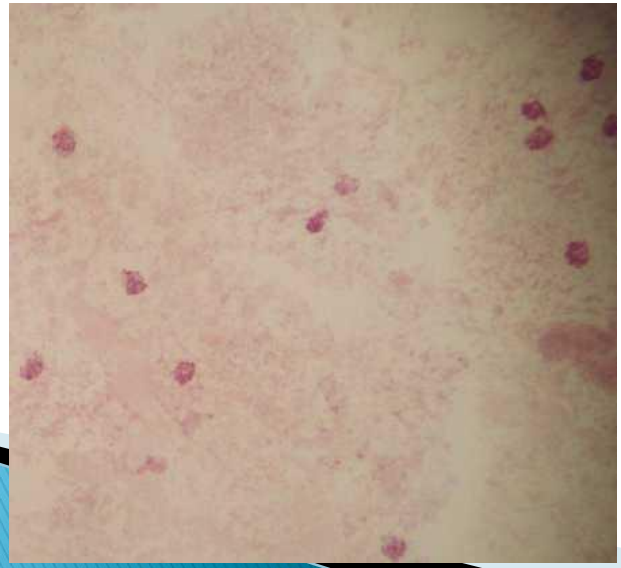
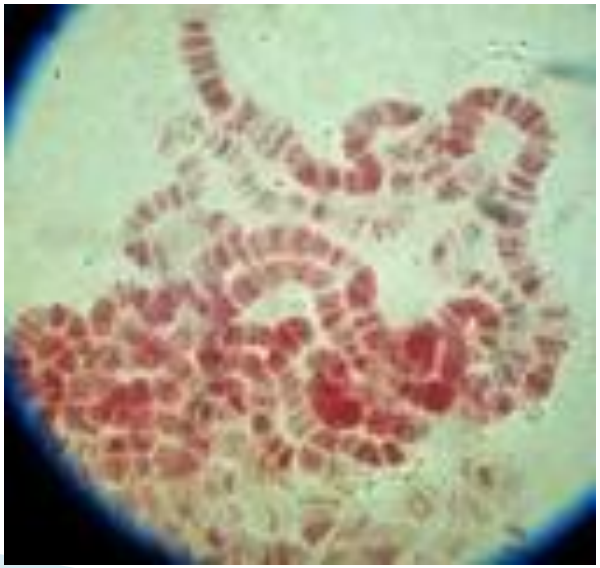










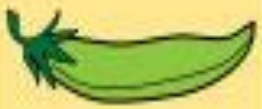




Mendel's Lasws

- 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.
- He underlie 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.
- Mendel use the selective cross-breeding of common pea plants (*Pisum sativum*).
- Mendel observed seven traits that are easily recognized and apparently only occur in one of two forms.

The seven traits

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

Character	Dominant trait	Recessive trait
Seed shape	 Spherical	 Wrinkled
Seed color	 Yellow	 Green
Flower color	 Purple	 White
Pod shape	 Inflated	 Constricted
Pod color	 Green	 Yellow

Character	Dominant trait	Recessive trait
Flower position	 Axial	 Terminal
Stem height	 Tall	 Dwarf

Mendel's Lasws

- **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.

- **Law of Independent Assortment (The "Second Law")**

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

Terminology:

1-P (Parental) : Original individuals in a breeding experiment .

2- F1 (First generation): First generation of the parents

3- F2 (Second generation): When the first generation individuals are allowed to breed at random , they produce the second generation .

4-Genotype:Genetic makeup of an organism either at a single locus or over all its genes collectively .

5-Phenotype:Molecular , Physical , Behavioral affects of genotype .

6- Homozygous :If two alleles are identical . Ex: (Pure : RR (dominant) or rr (recessive).

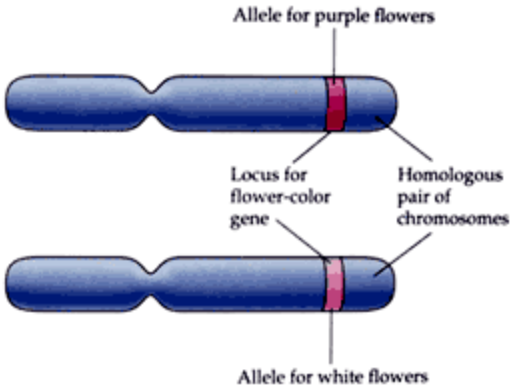
7-Heterozygous:Two alleles are different . Ex: (Mix : Rr (dominant))

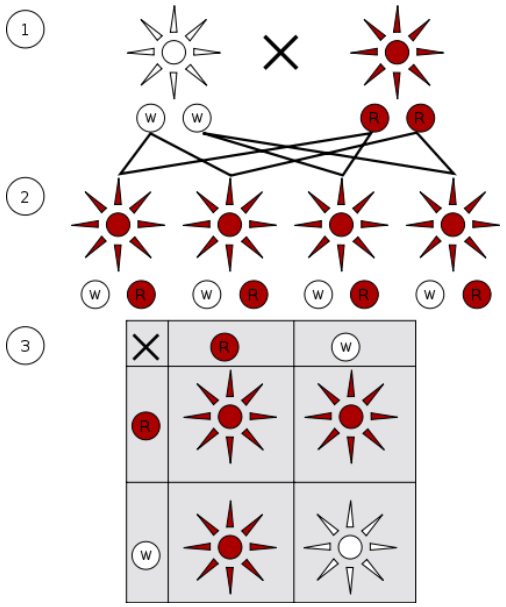
Terminology:

1- A (Hybrid تهجين):Result of breeding different variations of a trait .

2- Mono-hybrid cross: If we mix one trait from original parental between a homozygous purple corn (RR) that allele is dominant with a homozygous yellow corn (rr) that allele is recessive it will always resulted in **F2** generation 3dominant phenotype :1recessive phenotype ratio

Allele: An allele is an alternative form of a gene (one member of a pair) that is located at a specific position on a specific chromosome.





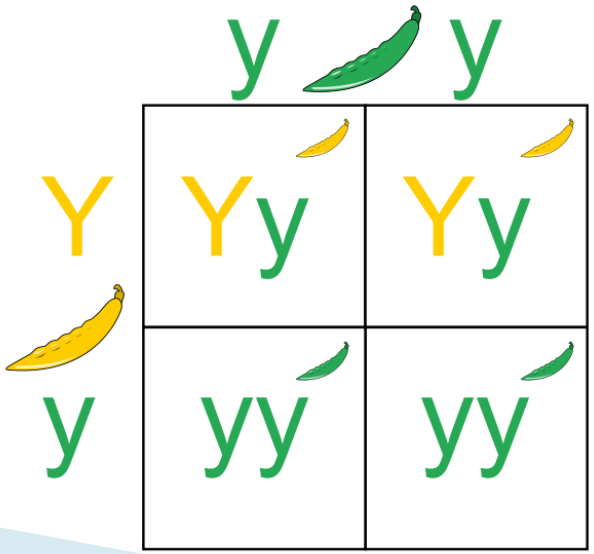
parents

F_1 generation
(all tall)

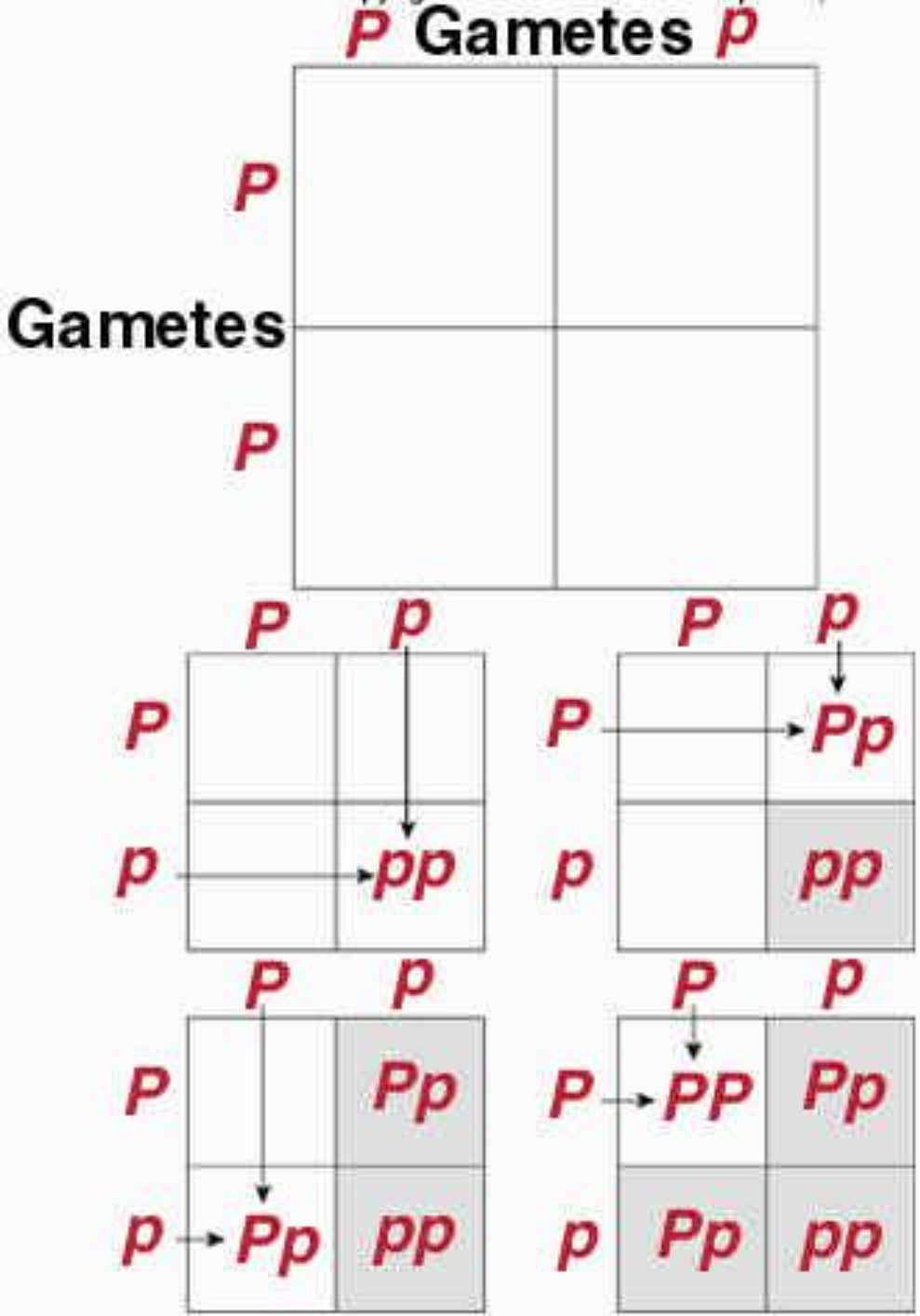
F_2 generation
(3 tall : 1 dwarf)

3- Mono-hybrid test cross : A cross between an individual exhibiting the dominant phenotype of a trait and an individual that is homozygous recessive for that trait in order to determine the genotype of the dominant individual.

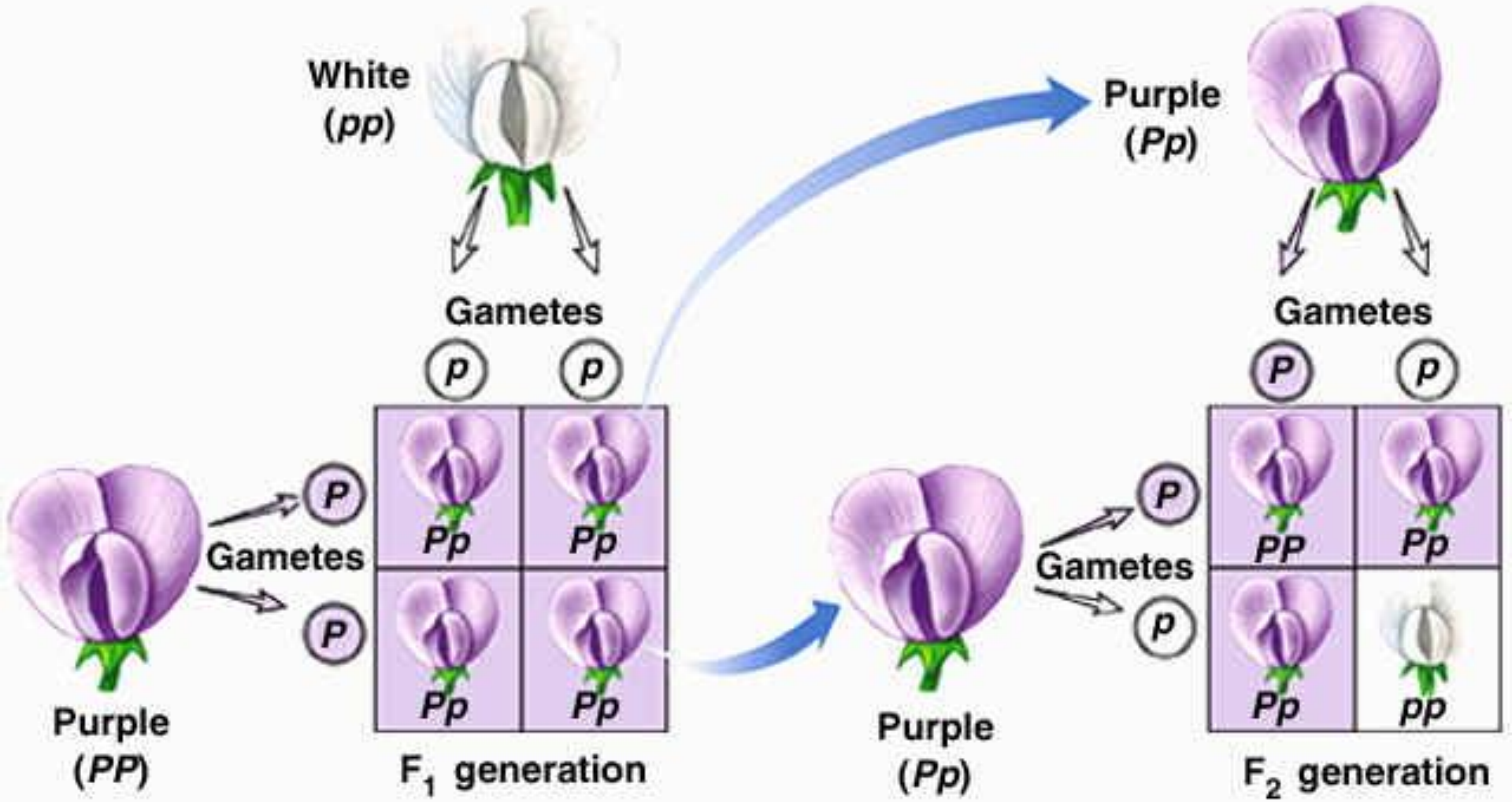
count the number of (purple and yellow) in 5 of rows corn and record the number on the table then calculate the % between the individual traits . it will always resulted in **F2** generation 1:1 ratio between dominant phenotype .

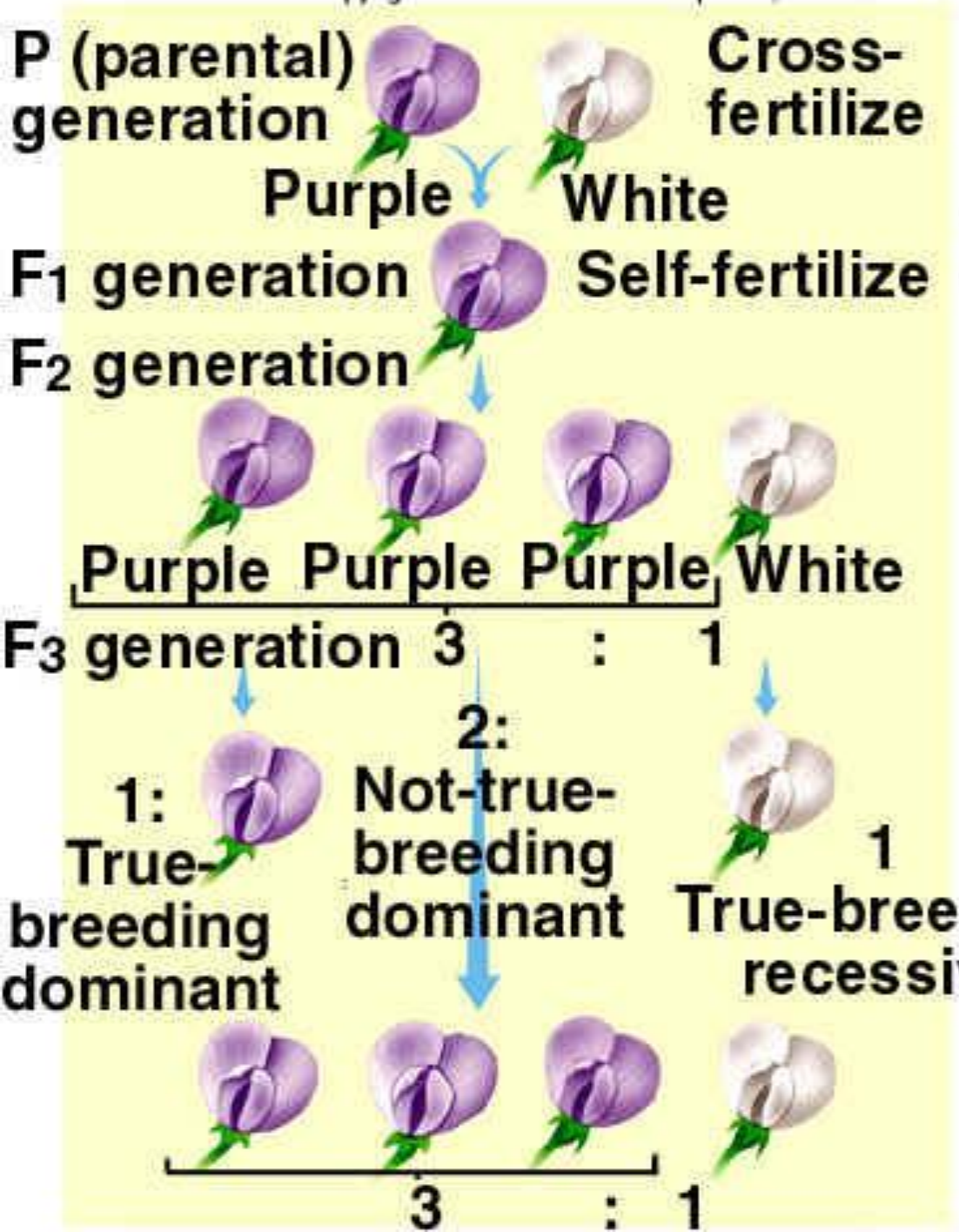


Punnett Square



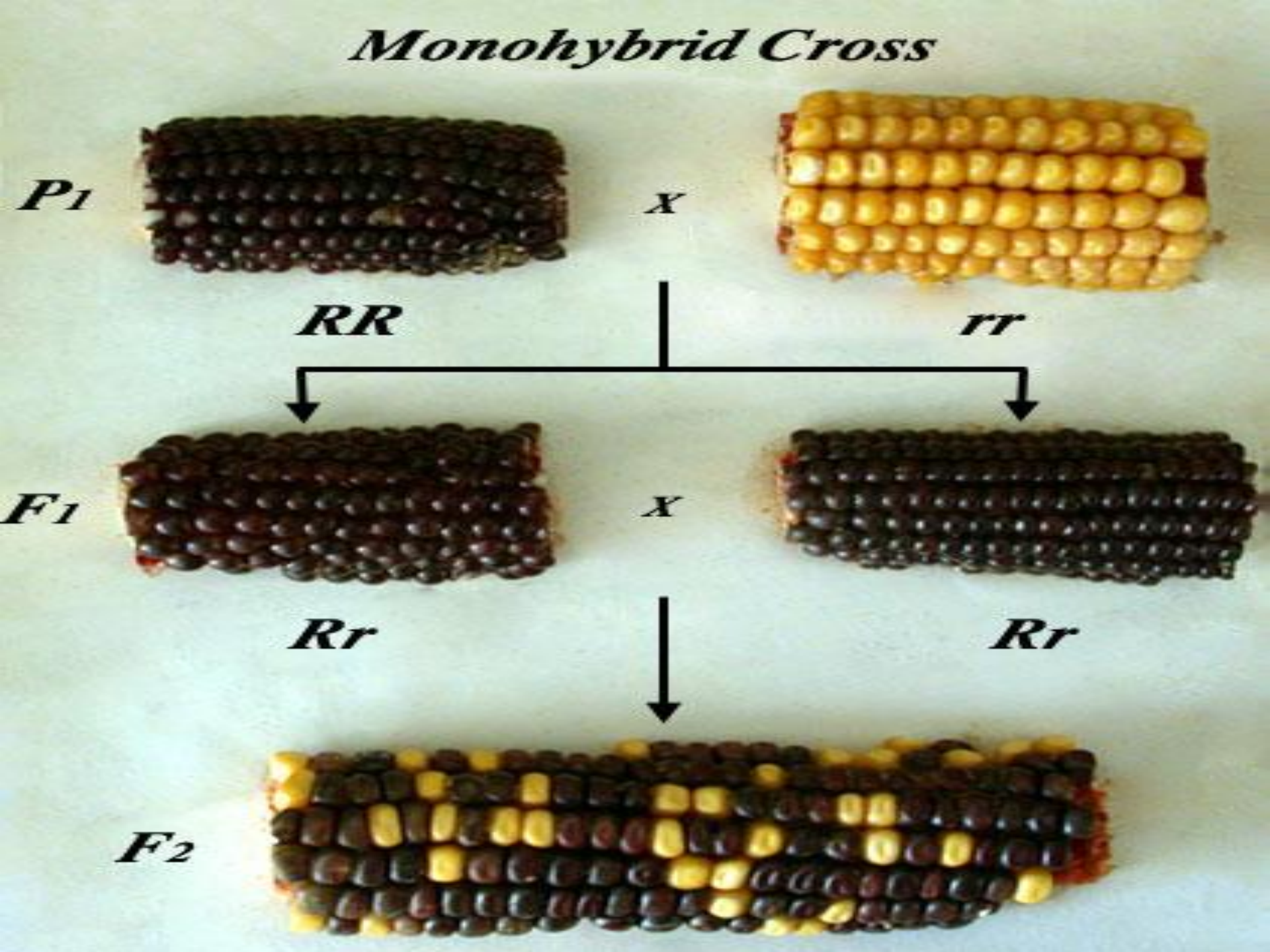
Mendel's Cross of Pea Plants for Color





F_2 Generation—a Disguised 1:2:1 Ratio

Monohybrid Cross



Mono-hybrid cross

1- Test cross (Ratio= 1:1)

2-Cross (Ratio =3:1)

Row	Black (Purple) (RR)	Yellow (rr)
1 2 3 4 5		
Total	/ Divided on small N.	/ Divided on small N.

A Monohybrid Cross

A Monohybrid test cross

Experimental

The "First Law in Drosophila Fly

Meting between Parents (1Wild male ++ with 2female of vestigial wings (vg) , then wait) :
(F1 = Wild type ,

F2= 3 wild type + 1 Vg type)

	++	vg
Male		
Female		
Total	/D . S	/D . S

Obs.	
1-++	
2- vg	

TESTCROSS

- Consider the case in which a testcross is performed with a white coat male guinea pig and a black coat female of unknown genotype.

Scenario A: The black female is homozygous

Scenario B: The black female is heterozgous.

Test cross- an unknown genotype is crossed with a homozygous recessive individual