PREPARARTION OF LINKAGE IN *DROSOPHILA* CROSSES

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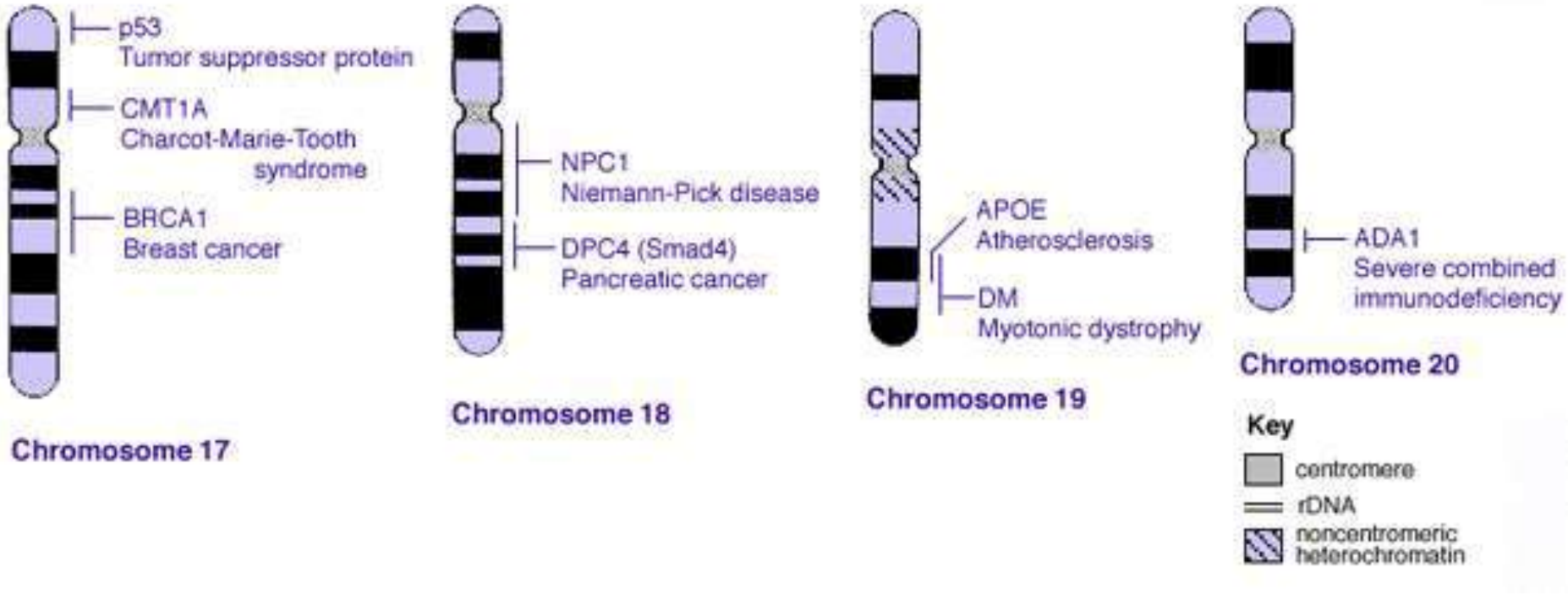
Introduction

- Genes that are close together in the same chromosome tend to remain together in inheritance, a phenomenon known as linkage and such genes are said to be linked. ***~Thomus Hunt Morgan***
- Some gametes are produced with different combinations of allele than those in the parental chromosomes because of crossing over which results in recombination between the homologous chromosomes.
- The greater the frequency of recombination between the two alleles, the greater the physical distance between them.
- The probability of recombination between any two genes serves as a measure of genetic distance between the genes that allows the construction of genetic map.
- Genetic map is a diagram of a chromosome showing the relative positions of the genes, which is also known as linage map.

Linked genes



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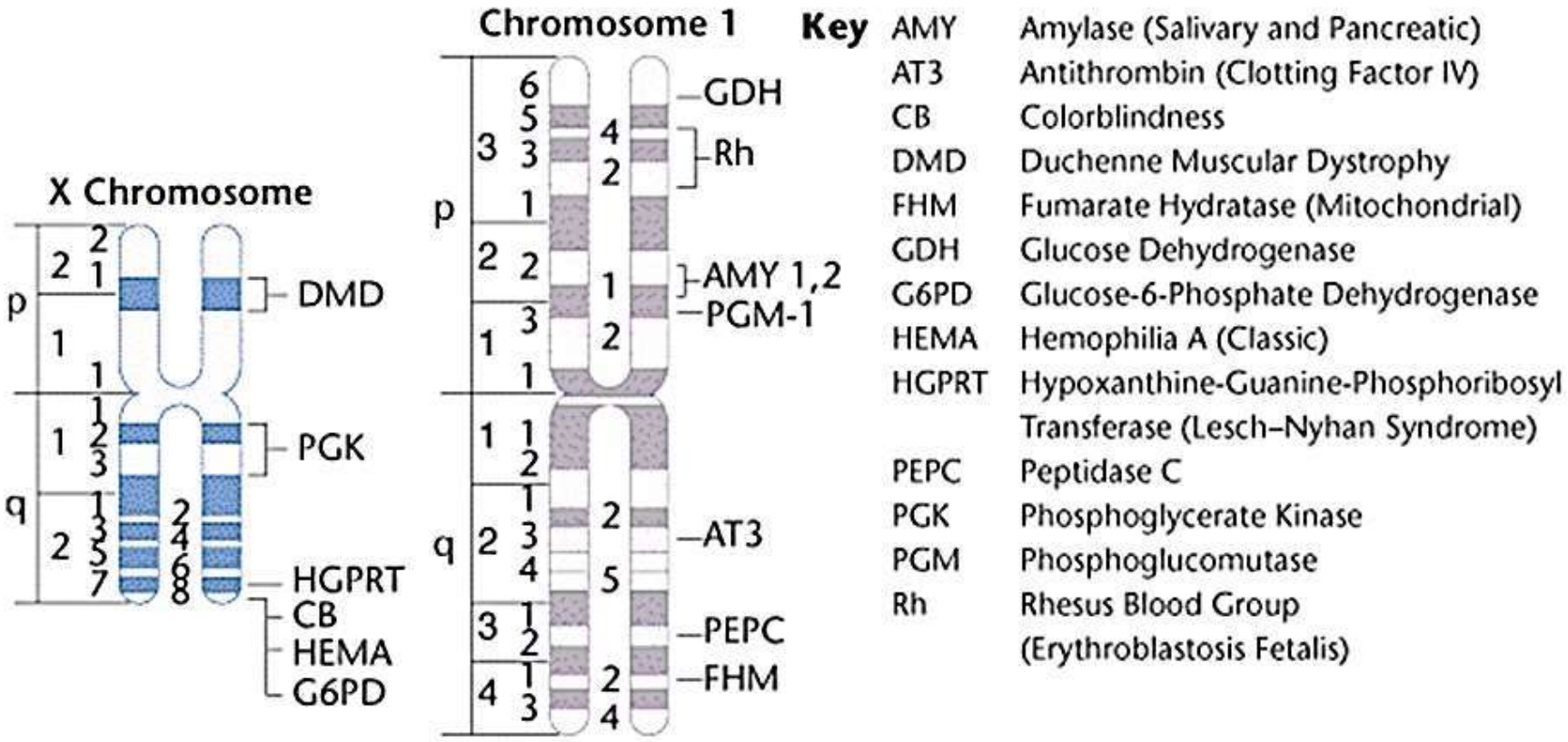
Linked genes



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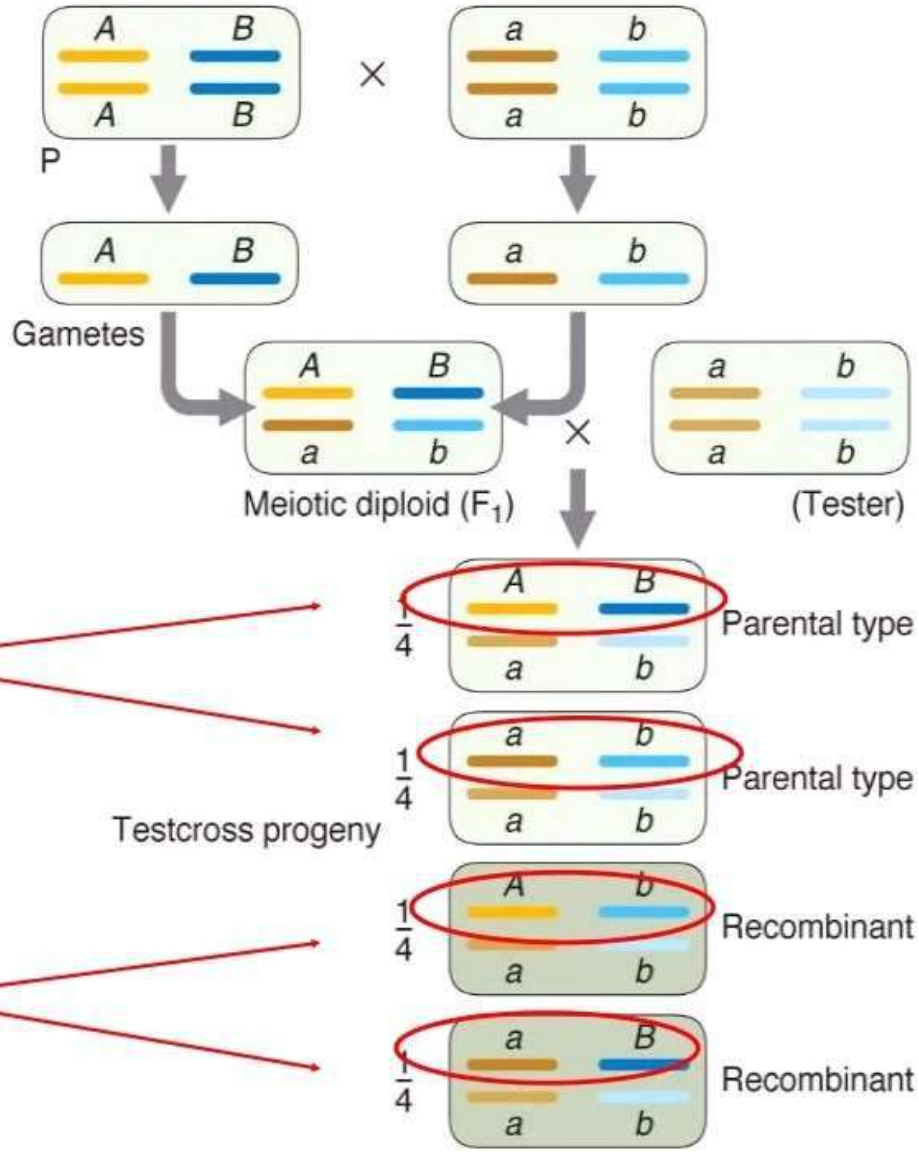
Test Cross



Half look like they got a set of the parents chromosomes...

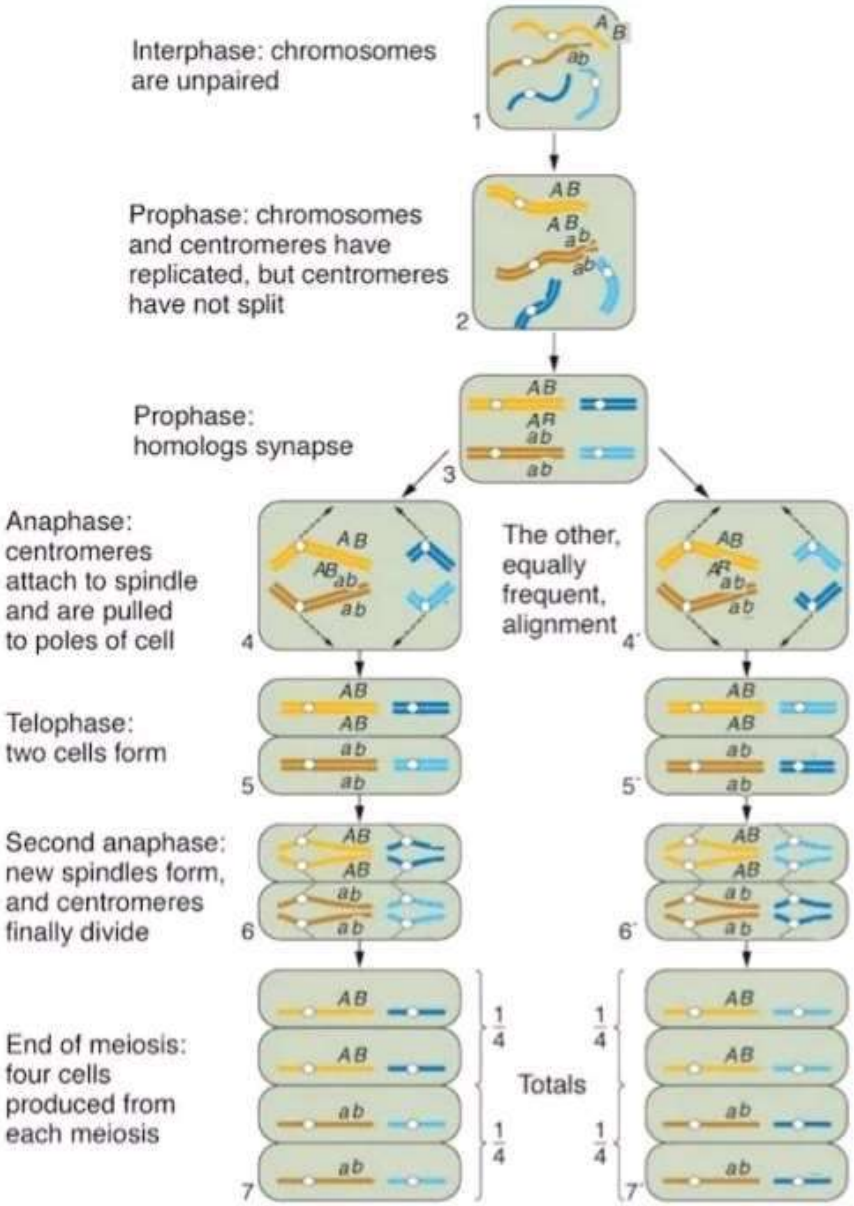
And half look like they got a mix of both parents chromosomes...

Source: slideshare.com

Now suppose both gene A and B were next to each other on the same chromosome.

(linked genes)

What happens to the ratios in this diagram?



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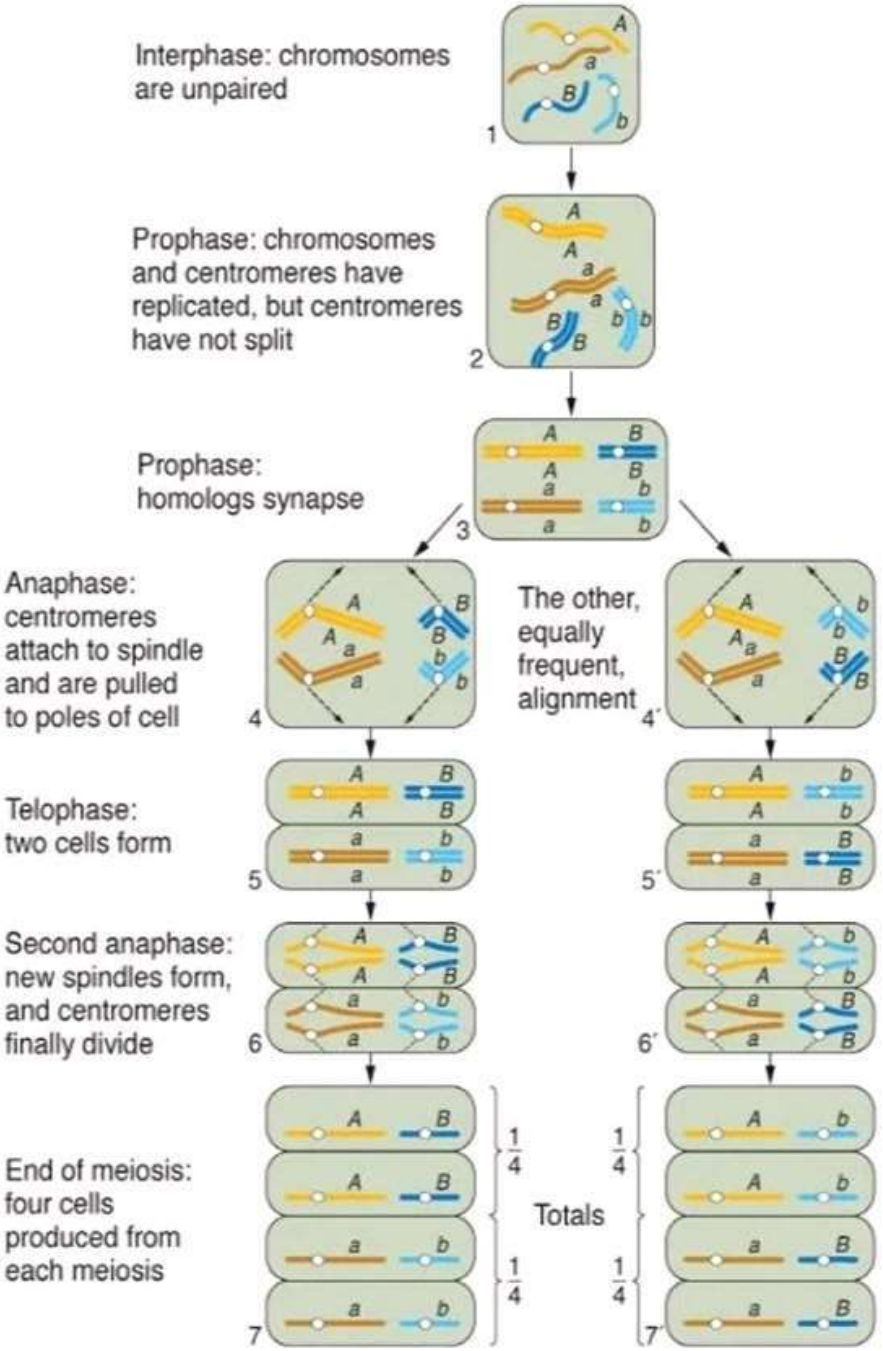
If two genes are on different chromosomes...

(Accoding to Mendel)



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Following meiosis

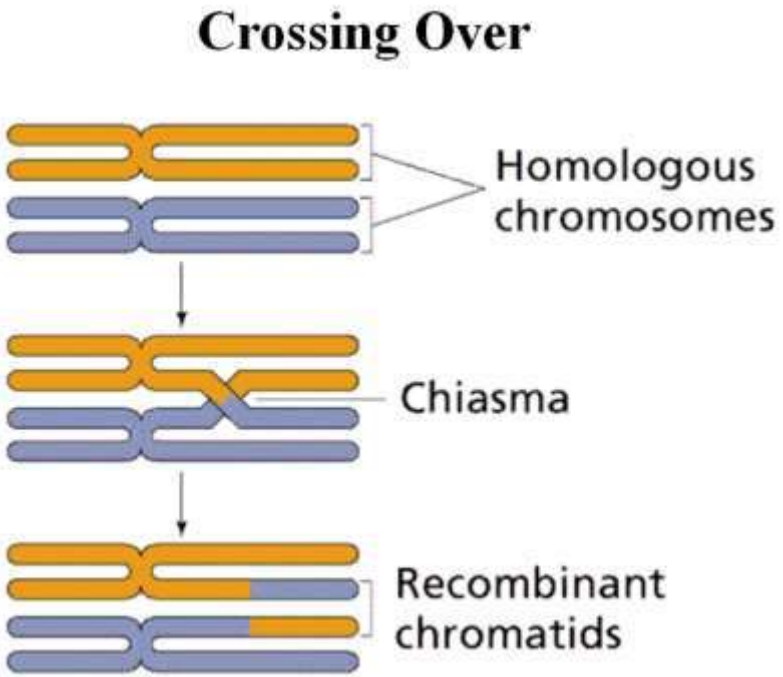
- In meiosis there is a linkage which may lead to crossing over and recombination of chromosome.
- This leads to following chromosomal types:

Parental chromosomal types

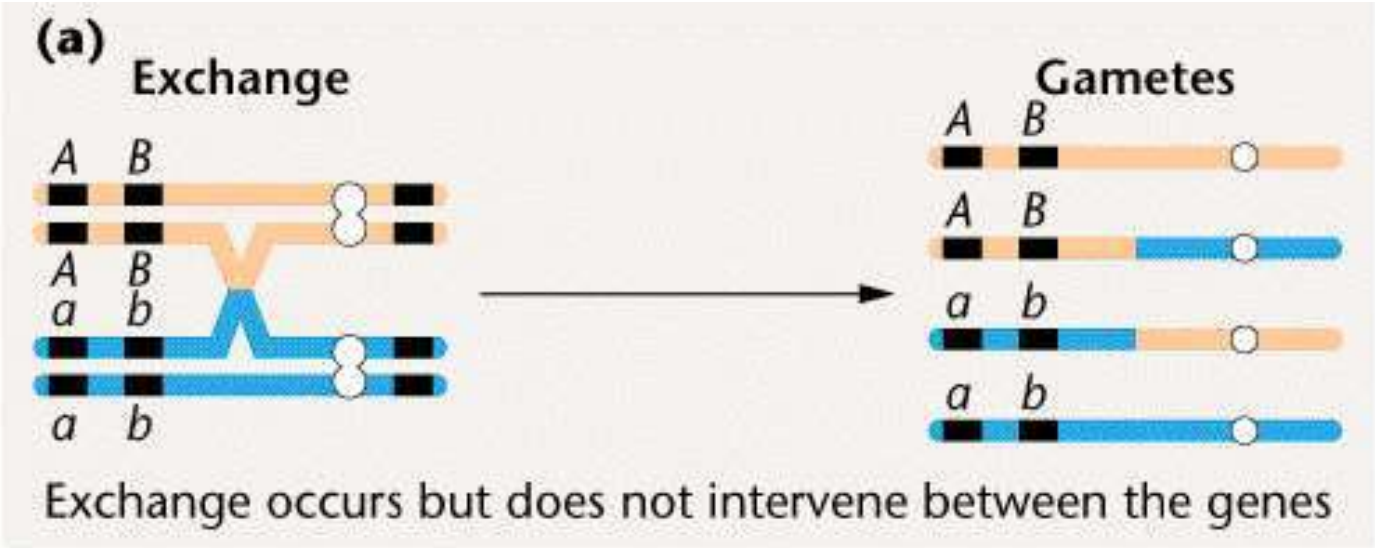
- Complete linkage and crossing over.
- Seldom occurs.

Non-parental chromosomal types

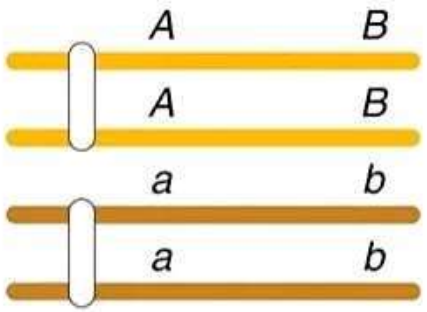
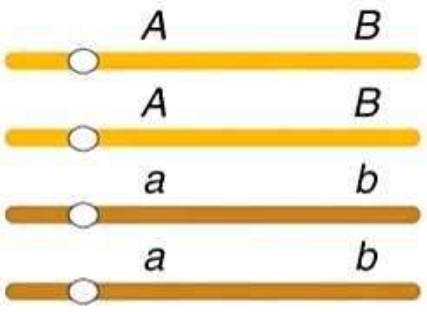
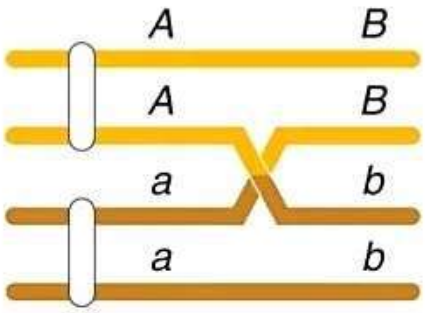
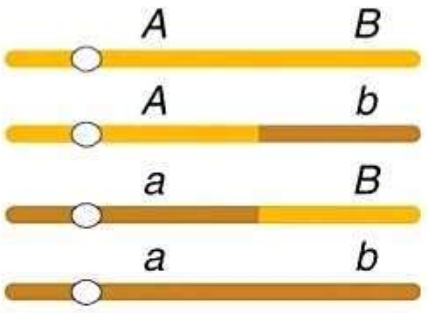
- Result from crossing over.
- Recombination of alleles occurs.



Following meiosis

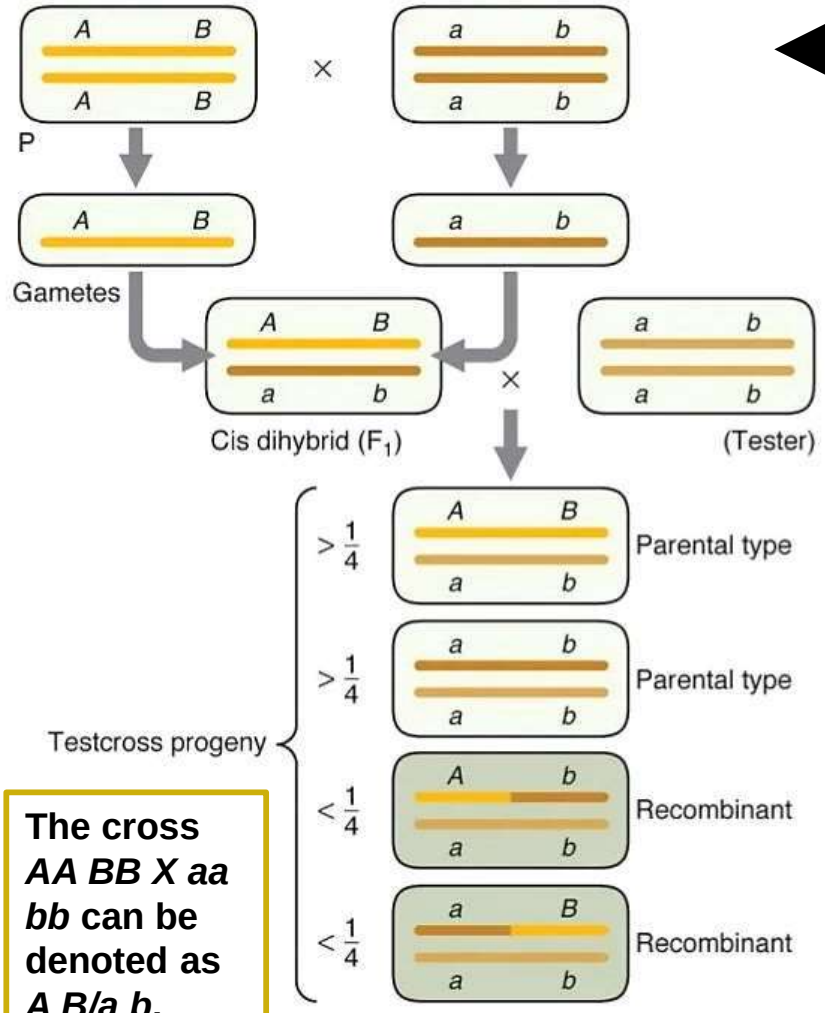


Following meiosis

	Meiotic chromosomes	Meiotic products	
Meioses with no crossover between the genes			Parental Parental Parental Parental
Meioses with a crossover between the genes			Parental Recombinant Recombinant Parental

Source: slideshare.com

Following meiosis



The cross *AA BB X aa bb* can be denoted as *A B/a b*.

- In this cross ***AA BB X aa bb*** , the ***A*** and ***B*** alleles and the ***a*** and ***b*** alleles are said to be in the coupling or *cis* configuration.
- Here, among the four possible gametes, the ***A B*** and ***a b*** types are called parental combinations (same configuration as in the parental chromosomes), and the gametes ***A b*** and ***a B*** types are called recombinants.

- In another cross ***A a/b B,*** alleles **A** and ***B*** are said to be in the repulsion or trans configuration.
- Now the both the gamete types are reversed; the ***A b*** and ***a B*** are the parental combinations, and ***A B*** and ***a b*** types are the recombinants,.

Types of Linkage

There are two types of linkages; which are as follows:

1. **Linked genes**
   - → Genes on same chromosomes and tends to stay together during the formation of gametes.
   - → Doesn't assort independently.
   - → Have recombination frequency of less than 50%.

1. **Unlinked genes**
   - → Genes on non-homologous chromosomes or far apart on same chromosome.
   - → Assort independently.
   - → Have recombination frequency of 50% or more.

Coupling and repulsion hypothesis: Put forth by Bateson & Punnett to explain the lack of independent assortment, but failed to explain. Later T. H. Morgan explained it.

Coupling and Repulsion Hypothesis

Put forth by Bateson & Punnett to explain the lack of independent assortment, but failed to explain. Later T. H. Morgan explained it.

1. **Coupling phase:**
   - → When two linked genes on each chromosome are the same types.
   - → Is also known as *Cis* phase.
   - → Parental types, i.e., both dominant A B or both recessive a b.
2. **Repulsion phase**
   - → When linked genes on each chromosome are different type.
   - → Is also known as *trans* phase.
   - → Recombinant types, i.e., A b or a B.
   - → Recombination of linked genes occurs in same frequency.

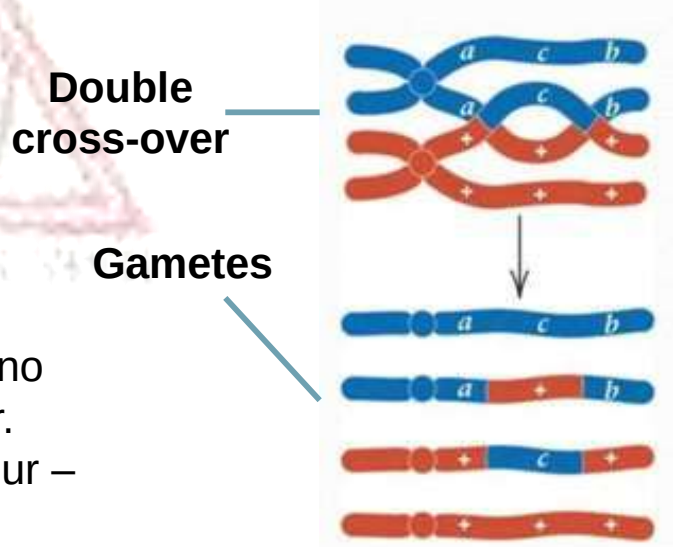
Crossing Over

- Exchange of DNA (genes) between paired homologous chromosomes (one from each parent) is known as crossing over.
- Crossing over occurring outside the regions between two genes can't alter their arrangement – *pass on to next as such*.
- The result of double crossing over between paired genes is indistinguishable from independent assortment of the genes.
- Crossing over involving 3 pairs of alleles specify gene order, i.e., linear sequence of genes.

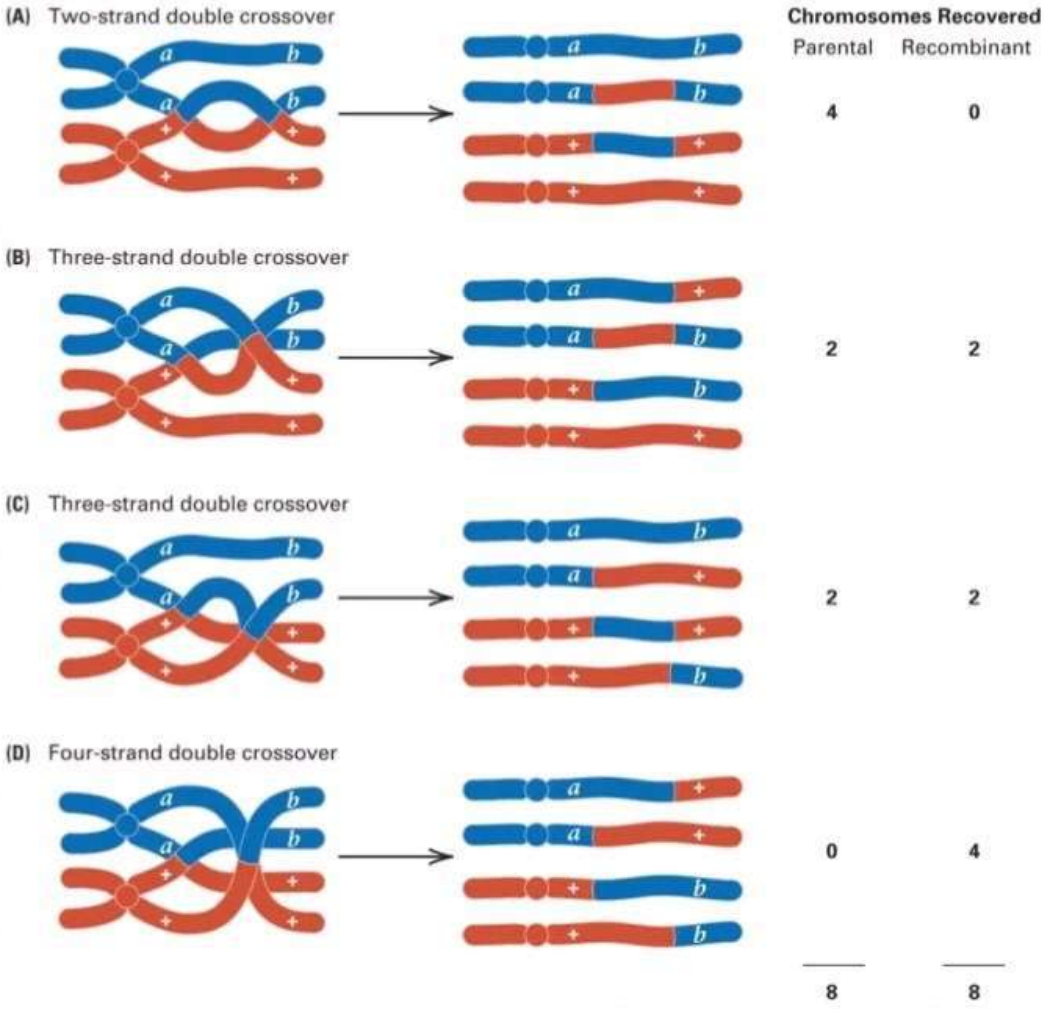
Fate of two genes following meiosis

There might be three situations:

1. If present on separate chromosomes, genes are segregated independently.
2. If both genes on same pair of chromosomes and no crossing over occur – genes always stay together.
3. If both genes on same pair and crossing over occur – produces recombinant gametes.



Crossing Over



Gene Mapping

- Genetic map or linkage map is the representation of linkages of genes in the chromosomes.
- **Alfred Sturtvent** was the person who first constructed linkage map successfully in Drosophila using gene recombination frequency.

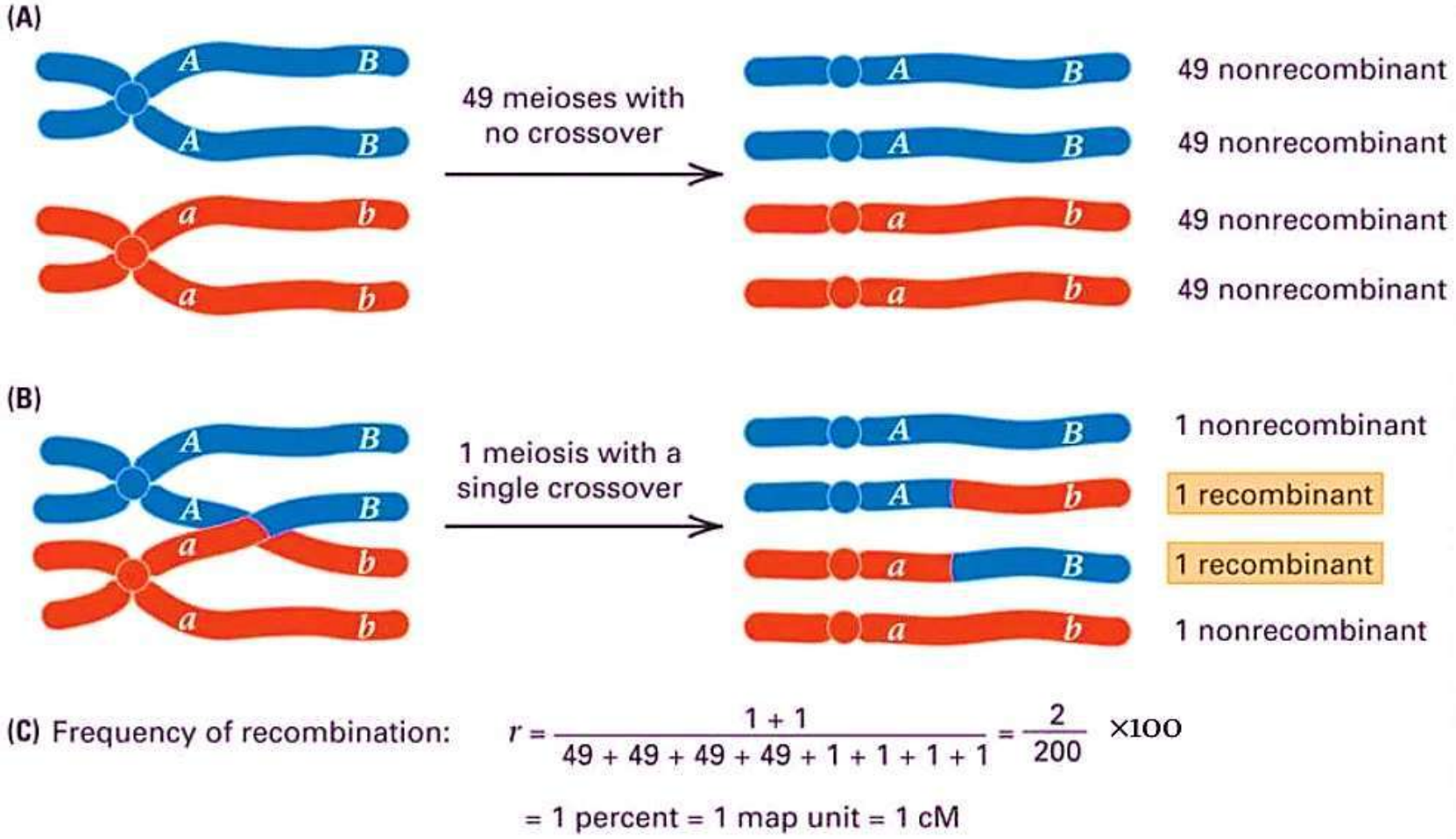
$$\text{Gene recombination frequency} = \frac{\text{Total number of recombinants}}{\text{Total number of offspring}} \times 100 \text{ map units}$$

Map unit *is also called as* ***recombination unit*** *or* ***Centimorgan.***

It shows ***1% crossover*** *or* ***recombination frequency.***

Map unit is different from physical distance of the genes.

Gene Mapping



(C) Frequency of recombination: $r = \frac{1+1}{49+49+49+49+1+1+1+1} = \frac{2}{200} \times 100$

= 1 percent = 1 map unit = 1 cM

Gene Mapping for Two Point Test Cross

- It is a test cross between two genes.
- It is used to determine the loci of two genes.

Steps in gene mapping of two point test cross

1. Gene are linked or unlinked.
2. Parental combination.
3. Calculation of recombination frequency.
4. Construction of genetic map.

Gene Mapping for Two Point Test Cross

In Drosophila melanogaster two point test cross:

Wild body color, Vestigial wings (**Eevv**) X Ebony body color, Long wing (**eeVV**)

↓

F1: all fruit flies with Wild body color & Long wing (**EeVv) {Gametes: Ev & eV} - P**

↓

F1: (**EeVv**) crossed with Ebony vestigial winged fruit fly: **{Gametes: EV & ev} - R**

↓

F2: Four genotypes

1. Wild body color; Longed winged –---- 120
2. Wild body color; Vestigial winged –- 3420
3. Ebony body color, Longed winged – 3334
4. Ebony body color; Vestigial winged -- 126

Rec. Gametes: 120 + 126 = 246

Therefore, $RF = \frac{246}{7000} x\ 100 = 3.5\%$

Therefore,

Distance between E,e & V, v loci is 3.5 map unit

MAP is

Gene Mapping for Three Point Test Cross

- It is a test cross between three genes.
- It is used to determine the loci of three genes.

Steps in gene mapping of three point test cross

1. Gene are linked or unlinked.
2. Parental combination.
3. Calculation of recombination frequency.
4. Gene ordering
5. Construction of genetic map.

Gene Mapping for Two Point Test Cross

In Drosophila melanogaster three point test cross:

P: v b p / v b p female X V B P / V B P Male

↓

F1: v b p / V B P

Test cross: v b p / V B P (F1 female) X v b p / v b p male

↓

F2: Test Cross Offspring

1. V b p ---------- Parental type ---------- 1779
2. V B P ---------- Parental type ---------- 1654
3. V b p ---------- Recombinant for **v** relative to **b** & **p** ------- 252
4. V B P ---------- Recombinant for **v** relative to **b** & **p** -------- 241
5. V b P ---------- Recombinant for **b** relative to **v** & **P** -------- 131
6. v B p ---------- Recombinant for **b** relative to **v** & **P** --------- 118
7. v b P ---------- Recombinant for **p** relative to **v** & **b** --------- 13
8. V B p ---------- Recombinant for **p** relative to **v** & **b** --------- 9

4197

Double Cross-over

Gene Mapping for Two Point Test Cross

In Drosophila melanogaster three point test cross:

Test cross: v b p / V B P (F1 female) X v b p / v b p male

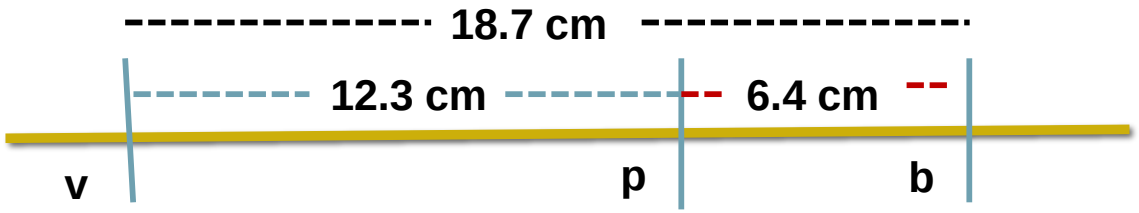
$$Distance\ between\ v\ \&\ b = \frac{252 + 241 + 131 + 118 + 13 + 13 + 9 + 9}{4197} \times 100 = 18.7\ mu$$

$$Distance\ between\ v\ \&\ p = \frac{252 + 241 + 13 + 9}{4197} \times 100 = \frac{515}{4197} \times 100 = 12.3\ mu$$

$$Distance\ between\ b\ \&\ p = \frac{131 + 118 + 13 + 9}{4197} \times 100 = \frac{271}{4197} \times 100 = 6.4\ mu$$

Therefore, genetic map will be

Total = 18.7 mu



Interference and Coincidence

Chromosome interference: Crossovers in one region decrease the probability of a second crossover close by. Interference can be measured by coefficient of coincidence.

$$Coefficient\ of\ coincidence = \frac{Observed\ number\ of\ double\ recombinants}{Expected\ number}$$

$$Expected\ double\ cross\ over = Expected\ double\ cross\ over\ frequency \times Total\ number\ of\ progenies$$

$$Interferenec = 1^{-Coefficient\ of\ coincidence}$$

Interference and Coincidence

If the coefficient of coincidence is:

- ✓ =0; then interference is complete and no double crossovers are observed.
- ✓ 0-1; partial interference
- ✓ =1; there is no interference and expected double crossovers are observed.

For 3 point cross from previous slide (slide no. 21)

We would expect probability of two recombination as;

RF between v & p = 0.123, & RF between b & p = 0.064

Therefore, probability of recombination would be 0.123 x 0.064 = 0.008

It means for every 4197 offspring, 4197 x 0.008 = 33.57 would be double recombinants.

We actually observed only 13 + 9 = 22 double recombinants in this cross.

Therefore, $\boldsymbol{Coefficient\ of\ coincidence = \frac{Observed\ value}{Expected\ value} = \frac{22}{33.57} = 0.655}$

&, $\boldsymbol{Interference = 1^{-iCoefficinet\ of\ coincidence} = 1^{-0.65}}$ **= 0.35**

Preparation of linkage map of *Drosophila*

Objective

Preparartion of genetic map of test cross of Drosophila melanogaster in the laboratory by determining map distance between two genes located on the X chromosome, (e.g. ey; eyeless and eb; ebony body color) in *Drosophila melanogaster.*

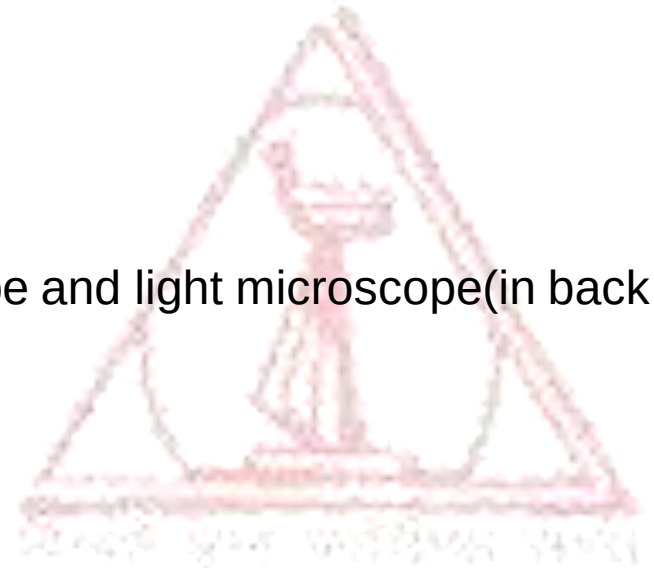
Principle

If we cross pure breed in parental generation (+/+, +/+), it will give heterozygotes (+/ey +/eb) as F1s by following Mendelian inheritance. If these F1s are then crossed with the genotype (ey/ey, eb/eb), genes are assort independently and F2s will results into the following four phenotypic categories in equal numbers, eyeless, ebony: eyeless, wild: wild, ebony: wild, wild. When we observed number of offspring in these class, we get two of classes have fewer number of offspring, while two of classes are enriched. The classes which have fewer number of classes are recombinants. It shows genes of enriched class are linked, while recombinants class are not linked. By deriving the genetic map distance between genes with the following formula, a genetic map can be easily made: $ADistance\ between\ genes = \frac{Number\ of\ recombinant\ offspring}{Total\ number\ of\ offspring} \times 100\ map\ units$

Laboratory Procedure

Equipment

- Fly manual
- Index cards
- Sorting tray
- Camel hair brush
- FlyNap
- Dissecting microscope and light microscope(in back cabinet)
- Fly morgue.

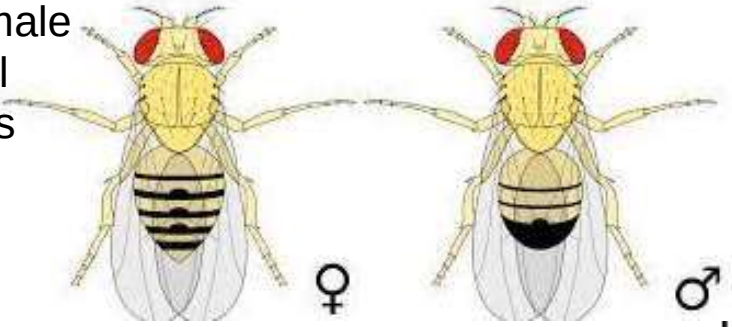


Preparation of linkage map of *Drosophila*

Identification of male and female Fruit Flies

There are three criteria that helps in easy identification of sexes in Fruit Flies (*Drosophila melanogaster*), which are as follows:

1. ***Sex comb:*** Male flies have sex combs, while female flies do not have sex combs. It consists of a row of about 10 stout bristles.
2. ***Abdominal structure:*** Male has heavy pigmentation with two pigmented bands anterior at dorsal surface , while female has only five pigmented bands along the entire abdomen. The posterior end is rounded in male, while pointed in female for ovipositing.
3. ***Genital organ:*** The genital organs are visible form ventral side in both the sexes. The penis of the male is surrounded by dark and heavily bristled genital arches, while the vaginal opening of the female is covered by a light ovipositor plate.



Sex combs in *D. melanogaster*

Preparation of linkage map of *Drosophila*

Identification of mutants in Fruit Flies

A mutant fly differs from a normal (or wild type) fly in one or more heritable characteristics which is due to change in the normal DNA sequence or its location.

- A list of common mutations with the drawings and photographs are provided in the lab which help in the identification of mutant characters.
- A name and symbol are designated for each mutation; Recessive mutations are expressed in lower case letters, while the name and symbols of dominant mutations are expressed in capital letters – Mendelian nomenclature.
- In modern nomenclature, a symbol with a + superscript designates the wild-type allele of a mutant gene (*most mutant alleles are recessive and designated by lower case letter*).
- A fly carrying one identifiable mutation is called a single mutant. Those exhibiting two, three, or multiple mutant traits are double, triple, or multiple mutants, respectively.
- Morphological mutations usually involve color, size, or shape of the eyes, bristles, wings, or body that can be easily recognized with the naked eye, however, many others can only be identified by a careful comparison between wild-type and mutant flies under a dissecting microscope

Preparation of linkage map of *Drososphila*

Culturing and crossing Fruit Flies

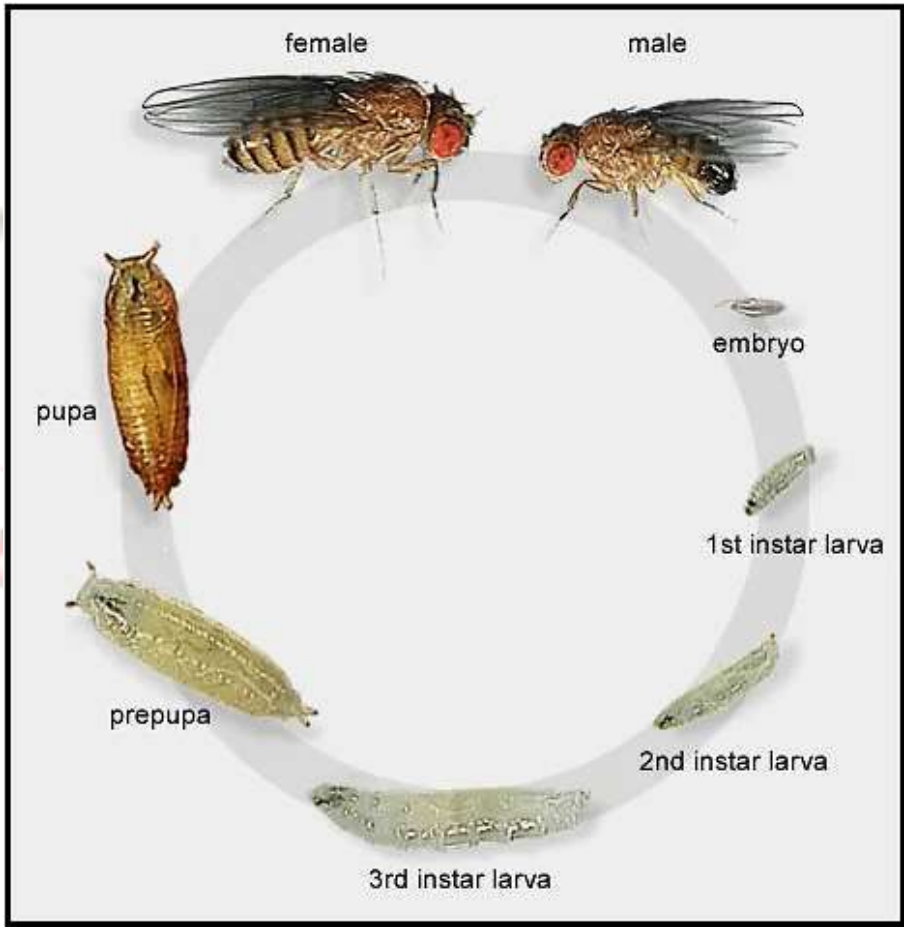
- In the laboratory, fruit flies are raised in culture bottles containing cooked food which mostly contain molasses-cornmeal medium for most experiments. A few granules of yeast is added to the medium in each bottle to promote the active growth of the flies.
- Culture is incubated at 25°C (Flies cultured at high temperature like 31°C or higher for several hours may become sterile).
- In making a cross, 3 to 5 pairs of selected male and female flies are placed in a fresh medium bottle. Before it, the sex and phenotype of each fly used in the cross should be carefully checked and recorded, and bottle should be carefully labeled.
- After emerging from their pupal case (eclosion) adults remain virgin for at least 10 hours, during which time the sexes can be separated and held until crosses are made.
- Virgin females are required in many cases to make a cross.

Preparation of linkage map of *Drosophila*

Life cycle of Fruit Flies

Day 0: Female lays eggs
Day 1: Eggs hatch
Day 2: First instar (one day in length)
Day 3: Second instar (one day in length)
Day 5: Third and final instar (two days in length)
Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying
Day 11-12: Eclosion (adults emerge from the pupa case).

- Females become sexually mature 8-10 hours after eclosion.
- The generation time of D. melanogaster varies with temperature (above cycle is for 22°C). At lower temp, it may take about twice as long to develop.



Preparation of linkage map of *Drosophila*

Recombination frequency of Fruit Flies

- After making cross of F1 x F1, all possible gene combinations, or genotype of progenies are organized in a Punnett square, or used rules of probability.
- After organizing on a Punnett square, recombination frequency is calculated for every gene by applying following formula:

$$\boldsymbol{Gene\ recombination\ frequency} = \frac{\boldsymbol{Total\ number\ of\ recombinants}}{\boldsymbol{Total\ number\ of offspring}} \times \boldsymbol{100\ map\ units}$$

- Through recombination frequency, we get a genetic map distance of alleles, which can be used in making genetic map for particular genes on the chromosome.

Preparation of linkage map of *Drososphila*

Recombination frequency of Fruit Flies

Few traits of Drosophila melanogaster are listed below:

Trait	Dominant Form	Recessive Form
Hairline Shape	widow's peak present	widow's peak absent
Earlobe Form	free	attached
Ability to Roll Tongue	present	absent
Freckling	present	absent
Number of Digits	more than 5 (polydactyly)	five
Pigmentation	present	absent (albinism)
Red Blood Cell Shape	disk-shaped	sickled (sickle cell anemia)
Ability to taste PTC	tastes bitter	no taste
Response to eating asparagus	urine smells	no smell

Sample Problems

1. Given the crossover frequency of each of the genes on the chart, construct a chromosome map.

Gene	Frequency of Crossover
A-C	30%
B-C	45%
B-D	40%
A-D	25%

2. In Drosophila, bar shaped eyes (B), scalloped wings (S), Cross veinless wings (W), and Eye Color (C) are located on the X chromosome. The recombination frequency of each gene is indicated on the table. Construct a chromosome map.

Gene	Frequency of Crossover
W-B	2.5%
W-C	3.0%
B-C	5.5%
B-S	5.5%
W-S	8.0%
C-S	11.0%


SUCCESS ISN'T ABOUT GREATNESS. IT'S ABOUT CONSISTENCY. CONSISTENT HARD WORK LEADS TO SUCCESS. GREATNESS WILL COME