Gene Mapping

- Gene mapping involves determining the locations of genes within specific chromosomes.
- Two methods of gene mapping are used:
  - genetic mapping
  - physical mapping
- Genetic mapping is used *to determine the relative position of genes within a chromosome. This is measured by whether or not two genes are "linked".*
- *If both* genes are inherited together they are considered linked.
- By determining which genes are linked, the relative positions of genes can be worked out.

1

Physical mapping

- *involves determining the exact position of a specific gene within a* chromosome.
- There are multiple techniques for accomplishing this, including creating cell hybrids for mapping out DNA in specific chromosomes.

2

Genetic map

- The genes on a chromosome can be represented as a single linear structure that goes from one end of the chromosome to the other.
- A **genetic map** is a representation of the genes on a chromosome arranged in linear order
- Also called a **linkage map**.
- **Genetic distance** is measured by frequency of crossing over between loci on the same chromosome (distances between loci expressed as percent recombination or (recombination frequency RF)
  - **map units (mu)**,
  - **centimorgans(cM)**.
- One **map unit** = one **centimorgan** (**cM**) = 1% recombination between loci.

3

Linkage, Recombination and Eukaryotic Gene Mapping

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Basic Eukaryotic Chromosome Mapping

Key Concepts

1. Two genes close together on the same chromosome pair do not assort independently at meiosis.
2. Recombination produces genotypes with new combinations of parental alleles.
3. A pair of homologous chromosomes can exchange segments by crossing-over.
4. Recombination results from either independent assortment or crossing-over.
5. Gene loci on a chromosome can be mapped by measuring the frequencies of recombinants produced by crossing-over.
6. Interlocus map distances based on recombination measurements are roughly additive.
7. The occurrence of a crossover can influence the occurrence of a second crossover in an adjacent region.

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GENE MAPPING IN EUKARYOTES

- **Discovery of genetic linkage**
- **Gene recombination and chromosomal exchange**
- **Constructing genetic maps**
- **Tetrad analysis**
- **Gene conversion**
- **Mitotic recombination**

6

Types of segregation

A. Independent Assortment

B. Complete Linkage

C. Partial Linkage

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A. Independent assortment

- Alleles of two loci are unlinked
- Segregation follows Mendel's fourth postulate (independent assortment)
- In a cross involving more than one gene, the different genes assort independently of each other ie , they are transmitted independently of each other
- Independent assortment is true for:
  - Genes on separate chromosomes.
  - Genes on the same chromosome (linked genes) if they are far apart.

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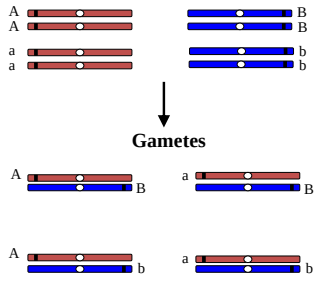
Independent assortment – Genes on different chromosomes



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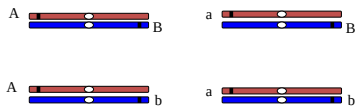
A dihybrid produces 4 types of gametes in equal frequencies

a. Two gametes are parental types ie AB and ab(non-recombinants)
b. Two gametes are non-parental types ie Ab ana aB (recombinants) types)

c. The parental (non-recombinant) gametes and non-parental (recombinant gametes occur in equal frequencies.
Ie 50% Ps AND 50% NPs

- **This is <u>interchromosomal recombination</u>**



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B. Complete linkage

1) Alleles of two loci are always transmitted together
2) Segregation does not follow Mendel's fourth postulate
3) A dihybrid produces only 2 types of gametes, both are parental types

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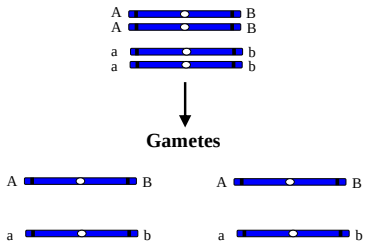
Linkage: Two genes on same chromosome segregate together



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Some genes on the same chromosome assort together more often than not

- In dihybrid crosses F_1 gametes occur in the ratio 1:1:1:1
- Any departures from a 1:1:1:1 ratio of F_1 gametes indicate that the two genes are on the same chromosome ie they are linked

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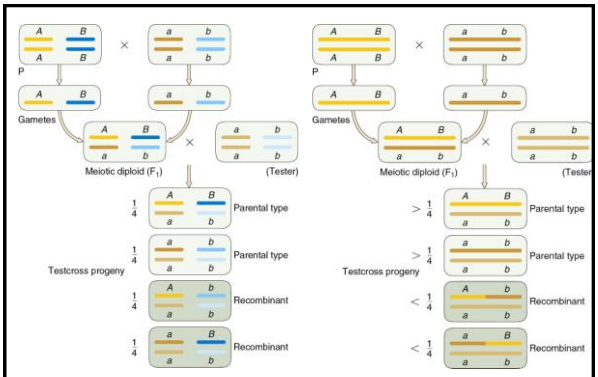
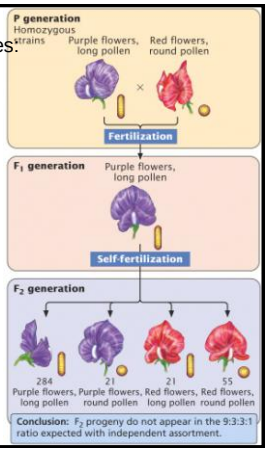


Note: Recombination significantly less than 50% means the genes are **linked**. **50% = independent assortment**

Observation of linkage for two genes:



The discovery of Linkage: William Bateson and R.C. Punnett

- Two or more genes located on the same chromosome
- Early 1900's: While studying sweet peas, William Bateson and R.C. Punnett identified a deviation from expected
- Mendelian ratios:
- They coined the terms coupling and repulsion:

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LINKAGE: When 2 genes are located on the same chromosome and they do not sort independently.

Coupling vs. Repulsion:

Coupling confirmation pr vg

pr+ vg+

linkage of 2 dominant or 2 recessive alleles.

Repulsion confirmation pr vg+

pr+ vg

linkage of a dominant allele with a recessive allele.

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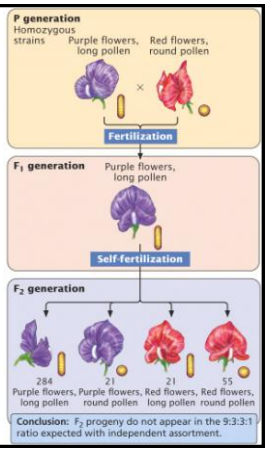
Observation of linkage for two genes in a dihybrid cross:

•Where purple colour is dominant over red; long pollen grain dominant over round pollen grain)

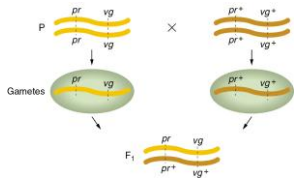
•9:3:3:1 ratio expected among the F_2 progeny

•Any deviations imply the genes are linked



Thomas Hunt Morgan

- postulated that a single chromosome contained a gene for each trait.
- (He used Drosophila to show this)



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How does linkage affect Mendelian segregation patterns?

- **Discovery of genetic linkage in *Drosophila* (Thomas H. Morgan, ~1911)**
- **Morgan determined that the gene for white-eyes (*w*) and a gene for miniature wings (*m*) occur on the X-chromosome.**
- **Cross female white-miniature (*wm*/*wm*) & wild type male (*w+m+*/Y):**

– *wm* x *w+m+* ⇒ *w+m+* & *wm*
– *wm* (Y) wm (Y)

- **F1 ⇒ wild type (*w+m+*/*wm*) females and white-eyed miniature wing (*wm*/ Y) males.**

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Discovery of genetic linkage in *Drosophila* (Thomas H. Morgan, ~1911)

F_1 x F_1 ⇒ $\frac{w+m+}{wm}$ **x** $\frac{wm}{(Y)}$

F_2 "parental" genotypes and phenotypes (same allele states as F_1):

$\frac{w+m+}{wm}$ **wild-type female (*n* = 439)**

$\frac{w+m+}{(Y)}$ **wild-type male (*n* = 352)**

$\frac{wm}{wm}$ **white-eye/miniature female (*n* = 359)**

$\frac{wm}{(Y)}$ **white-eye/miniature male (*n* = 391)**

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Morgan also observed F_2 non-parental genotypes and phenotypes:

$\frac{w+m}{wm}$ **wild-type eye/miniature female (*n* = 235)**

$\frac{w+m}{(Y)}$ **wild-type eye/miniature male (*n* = 210)**

$\frac{wm+}{wm}$ **white-eye/wild-type wing female (*n* = 218)**

$\frac{wm+}{(Y)}$ **white-eye/wild-type wing male (*n* = 237)**

F_1 x F_1 ⇒ $\frac{w+m}{wm+}$ **x** $\frac{wm}{(Y)}$

- ✓ **Non-parental combinations of linked genes are called recombinants.**
- ✓ **50% recombinant phenotypes are expected if independent assortment occurs.**
- ✓ **Morgan observed 900/2,441 (36.9%) recombinant phenotypes and concluded that the two genes must be linked.**

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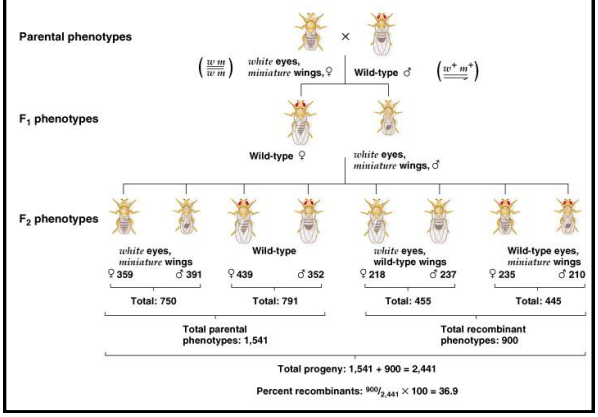

Symbolism:

Commas are placed between genes that are linked
Semicolons are placed between genes that are unlinked (assort independently)

- If two loci are linked, the alleles that are on the same chromosome are described as **coupled** or in **Cis configuration (coupling) ie in a dihybrid, both domiannt alleles are on one homolog**
- alleles on opposite homologous chromosomes are in **repulsion or in Trans configuration (repulsion), in a dihybrid, one dominant allele is on each homolog**

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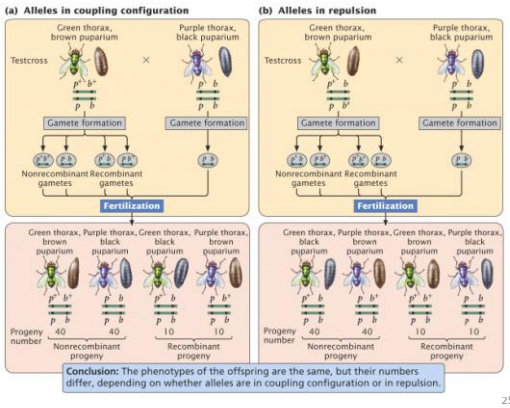

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Morgan also observed and hypothesized:

1. **Parental phenotypes always were more common in many other types of crosses, and recombinant phenotypes occurred less frequently.**
2. **During meiosis, some alleles assort together because they lie adjacent to each other on the same chromosome.**
3. **The closer two genes are on the chromosome, the more likely they are to remain together during meiosis.**
4. **Recombinants are produced by crossing-over.**

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C. Partial (incomplete) linkage

- **1) Alleles of two loci are usually (greater than 50%) transmitted together**
  2) Segregation does not follow Mendel's fourth postulate
  3) A dihybrid produces 4 types of gametes; however,
  a. The two parental types occur more frequently than the non-parental types
  b. This is intrachromosomal recombiantion

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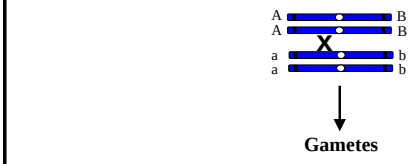
Crossing over and linkage leads to separation of linked genes



Parental A B a b

Recombinant a B A b

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For recombination to occur between two linked genes:

1) a crossover must occur between them
2) The probability that a crossover will occur between two linked genes is directly proportional to the distance between them.
1) Therefore, the frequency of recombination can be used as an indicator of the distance between genes.

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Recombination results when crossing-over during meiosis separates linked genes

- 1909 – F. Janssens observed chiasmata, regions in which nonsister chromatids of homologous chromosomes cross over each other
- T.H. Morgan suggested these were sites of chromosome breakage and change resulting in genetic recombination

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- The farther apart two loci are, the more likely that a crossover will occur between them.
- Conversely, if two loci are close together, a crossover is less likely to occur between them.
- Recombination can only be detected between two loci, both of which are heterozygous.

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- If two loci are very far apart, two or more crossovers may occur.
  - 1. Even numbers of crossovers restore the original combinations of alleles and are counted as zero crossovers.
  - 2. Odd numbers of crossovers create recombinant allelic combinations and are counted as one crossover.
  - 3. A recombination rate of 50% corresponds to independent assortment. Therefore, only distances less than 50 map units can be measured directly. Greater distances can be constructed by adding up distances between closer loci.

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- E. Closely linked genes show association of alleles within families but not necessarily within populations.
- Crossing over generates random **haplotype** combinations within populations.
- If the loci are very close together, equilibrium among the possible combinations may take many generations.

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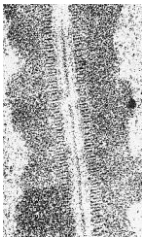
Recombination: Crossing-over



Synaptonemal complex Chiasmata

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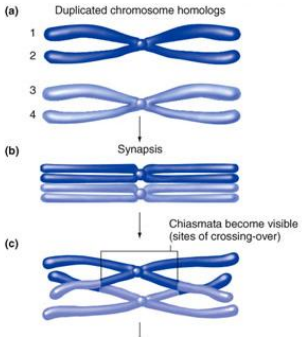
Chiasmata mark the sites of recombination



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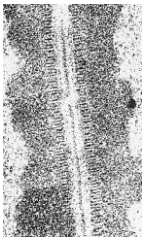
Recombination: Crossing-over



Synaptonemal complex Chiasmata

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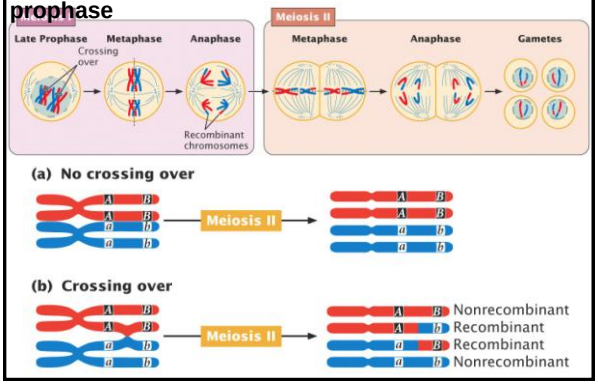
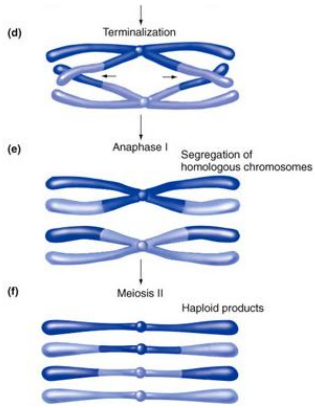
Crossing-over occurs in Prophase I:
-is initiated in zygotene; completed by the end of prophase





Chiasmata mark the sites of recombination

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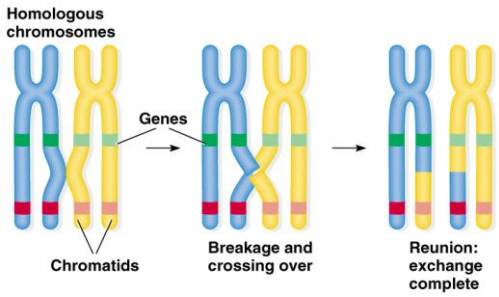
Mechanism of crossing-over gives rise to recombinant (non-parental) genotypes and phenotypes for linked genes.



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Some basics terminology about crossing-over:

1. **Crossing over occurs in prophase I of meiosis I.**
2. **Chiasma (pl. chiasmata) is the site where crossing-over occurs.**
3. **Crossing-over is a reciprocal exchange of DNA, involving breaking and rejoining of homologous chromatids.**
4. **Crossing-over leads to recombination between linked genes and produces novel genetic variation.**

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Recombination:

- Any meiotic process that generates a haploid product with a genotype that differs from both the haploid genotypes that constituted the meiotic diploid cell.

Produced by:

-Normal **independent assortment** of chromosomes

-**Crossing-over** of non-sister chromatids.

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RECOMBINATION ANALYSIS

- ***Recombination analysis is a technique used to determine how frequently* a crossover occurs *between two genes during meiosis***
- ***Used to determine***
  - *distance* ***between* genes on the chromosome**
  - *Linkage relationship* ***between genes ie genetic map***

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SOME DEFINITIONS

- A. **Chiasma** (chiasmata = plural), what you see with a microscope
- B. **Exchange** (crossing-over)- the process
- C. **Crossover chromatid** (crossover), the product
- D. **Recombinant chromatid** (recombinant); product with a new combinations of genes

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To do recombination analysis

- You need:

1)A heterozygote for two genes known to be on the same chromosome.

2) A homozygous recessive to test cross it to (so that every genotype will have a unique phenotype).

3) Enough offspring for accurate counts of non-crossover and crossover progeny.

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Genes that show independent assortment (RF=50%) may be:

- on different chromosomes
- far apart on the same chromosome.

Recombination analysis cannot didistinguish between these.

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LINKAGE GROUPS

- Genes on the same chromosome are described as **linked** or **syntenic**.
- Recombination analysis reveals **linkage groups** (groups of genes located close together on the same chromosome).
- Each linkage group represents a chromosomele no of linkage groups = haploid chromosome no
- Eg Humans have 24 **linkage groups**, corresponding to the 22 autosomes, plus X and Y chromosomes; *Drosophila 4 linkage groups*
- Groups of genes that are widely separated on a chromosome may show independent assortment; however, all such groups can eventually be tied together by mapping additional loci between them.

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SUMMARY

- **Linkage and meiotic recombination**
  - **Genes linked together on the same chromosome usually assort together**
  - **Linked genes may become separated through crossing over**
- **Mapping**
  - **The frequency with which genes become separated reflects the physical distance between them**
- **Mitotic recombination**
  - **Rarely, recombination occurs during meiosis**
  - **In eukaryotes mitotic recombination produces genetic mosaics**

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GENE MAP

- **indicates which genes occur on a chromosome,**
- **their order on the chromosome and their**
- **distance apart as calculated by recombination frequencies.**
- **Two genes located on the same chromosome are linked (syntenic).**

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Significance of a Genetic Map

- A genetic map tells us:
- (1) Which genes are on a particular
- chromosome
- (2) The order in which the genes occur
- on the chromosome
- (3) An indication of the relative (but not
- absolute) distances between genes.

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Position Effects

- Crossing over is inhibited near the centromere.
- Would the calculated distance be the same? less? greater?

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How to determine physical distances between genes?

- Deletion Mapping
- Somatic -cell Hybridization
- In situ Hybridization
- Mapping by DNA

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Somatic Cell Hybridization

- Cells of two different species are fused – the
- heterokaryon initially has 2*n* chromosomes from both cells.
- After the nuclei fuse, there is a tendency to randomly lose chromosomes during subsequent mitotic cell divisions.
- Result – A series of clones with different combinations of the original double set of chromosomes.

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