Human & Molecular Genetics

Molecular Genetics

A+ Notes
UNLOCK KNOWLEDGE

HMG 399 | Lecture 7 | Dr. Asmaa Abu Obaid

Linkage Analysis

Genetic Studies and Approaches · Mapping Complex Disease Genes Using Linkage

INSIDE THESE NOTES

01 **Chromosomes & Alleles**
Karyotype, homologous chromosomes, homozygous vs heterozygous

02 **Meiosis**
Diploid to haploid, meiosis I and II, the stages

03 **Genetic Variation**
Independent assortment, crossing over, random fertilization, synapsis

04 **Linkage & Linkage Disequilibrium**
Linked vs unlinked genes, LD, causes, measuring LD, recombinant gametes

05 **Recombination Frequency & Maps**
Fruit fly example, RF formula, linkage maps, centimorgans, GWAS

THE ULTIMATE SOURCE
For an A+

SETS OF CHROMOSOMES IN HUMAN CELLS

Chromosomes & Homologs

01

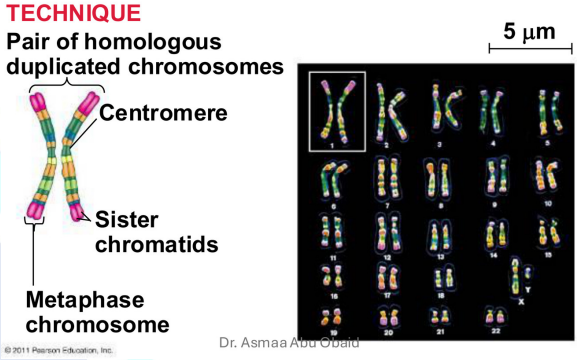
23 pairs | Karyotype | Homologs | Alleles

1. Human **somatic cells** (any cell other than a gamete) have **23 pairs** of chromosomes.
2. A **karyotype** is an ordered display of the pairs of chromosomes from a cell, arranged in pairs starting with the longest.
3. The two chromosomes in each pair are called **homologous chromosomes**, or homologs.
4. Homologs are the **same length and shape** (centromere position and staining pattern) and carry genes controlling the **same inherited characters.**

APPLICATION

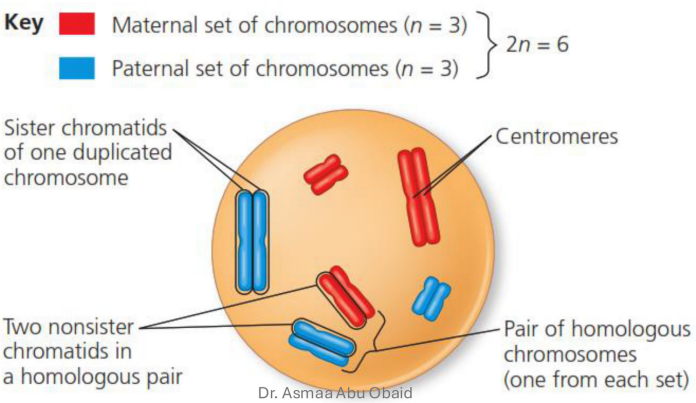


TECHNIQUE



Preparing a karyotype; pair of homologous duplicated chromosomes

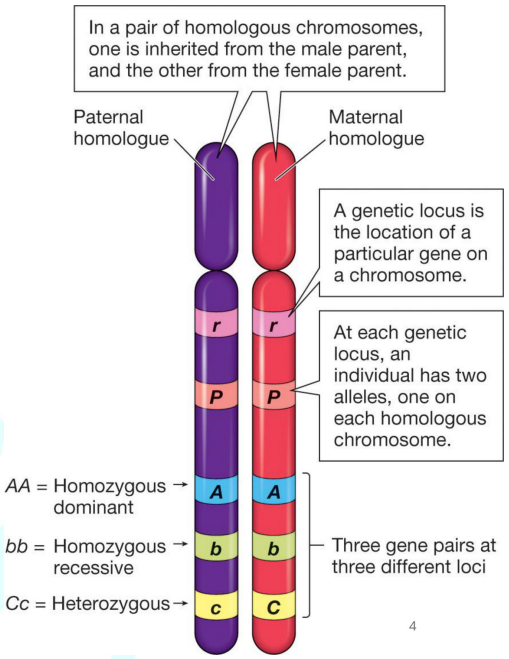
▼ **Figure 13.4 Describing chromosomes.** A cell from an organism with a diploid number of 6 ($2n = 6$) is depicted here following chromosome duplication and condensation. Each of the six duplicated chromosomes consists of two sister chromatids associated closely along their lengths. Each homologous pair is composed of one chromosome from the maternal set (red) and one from the paternal set (blue). Each set is made up of three chromosomes in this example (long, medium, and short). Together, one maternal and one paternal chromatid in a pair of homologous chromosomes are called nonsister chromatids.



Describing chromosomes (Figure 13.4)

Homologous Chromosomes

Homologous chromosomes have genes controlling the **same trait at the same position.**



A GENE OCCURS IN DUPLICATE

Maternal copy from the mother

Paternal copy from the father

Many genes exist in several variant forms in a large population. They are called **alleles.**

AN INDIVIDUAL MAY HAVE

Homozygous

Identical alleles for a specific gene on **both homologues.**

VS

Heterozygous

A maternal allele that **differs** from the corresponding paternal allele.

Example: a gene coding for **short fingers** on one homologue and a gene coding for **long fingers** at the same location on the other.

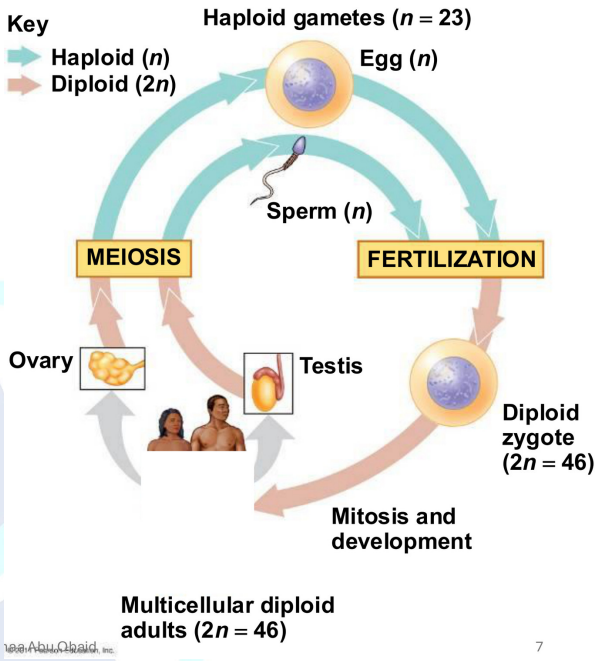
SEXUAL LIFE CYCLES

Meiosis

02

Haploid gametes | Diploid zygote | Meiosis I | Meiosis II

- At sexual maturity, the ovaries and testes produce **haploid gametes.**
- Gametes are the **only human cells produced by meiosis,** rather than mitosis.
- Meiosis results in **one set of chromosomes** in each gamete.
- Fertilization and meiosis **alternate** in sexual life cycles to maintain chromosome number.



DIPLOID TO HAPLOID

Meiosis reduces the number of chromosome sets

Like mitosis, meiosis is **preceded by the replication** of chromosomes.

Takes place in **two sets of cell divisions**, called meiosis I and meiosis II.

Results in **four daughter cells**, rather than the two in mitosis.

Each daughter cell has only **half as many chromosomes** as the parent cell.

The Stages of Meiosis

After chromosomes duplicate, **two divisions** follow.

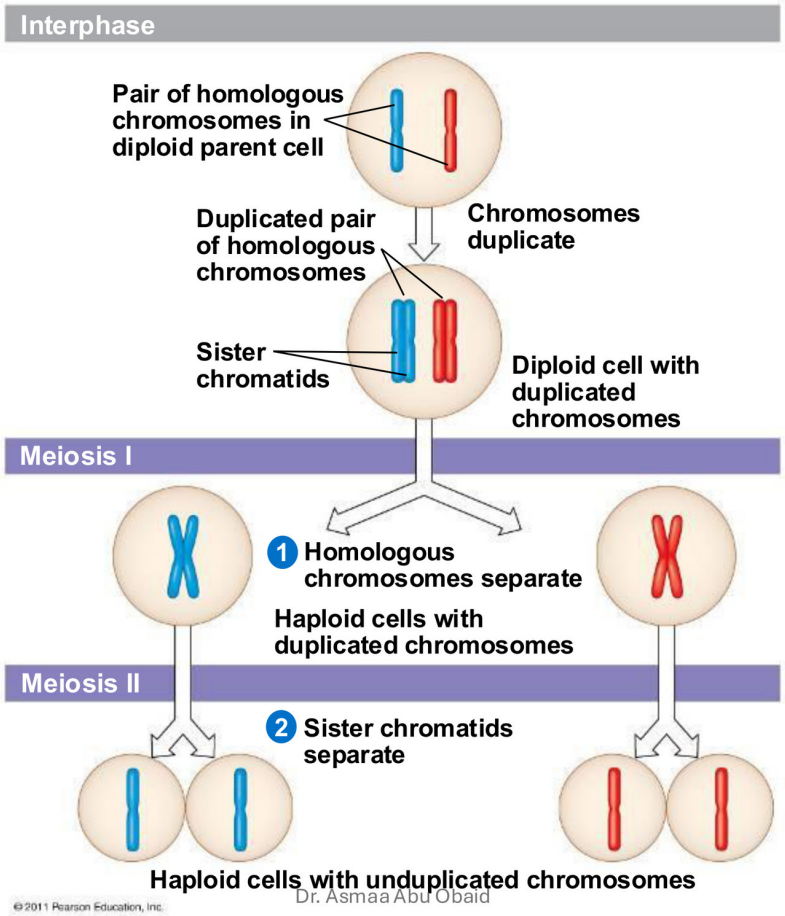
MEIOSIS I Reductional division

Homologs pair up and separate, giving **two haploid daughter cells** with replicated chromosomes.

MEIOSIS II Equational division

Sister chromatids separate. The result is four haploid daughter cells with unreplicated chromosomes.

Crossing over or recombination occurs between the two homologous chromosomes (two non-sister chromatids) during **meiosis I.**



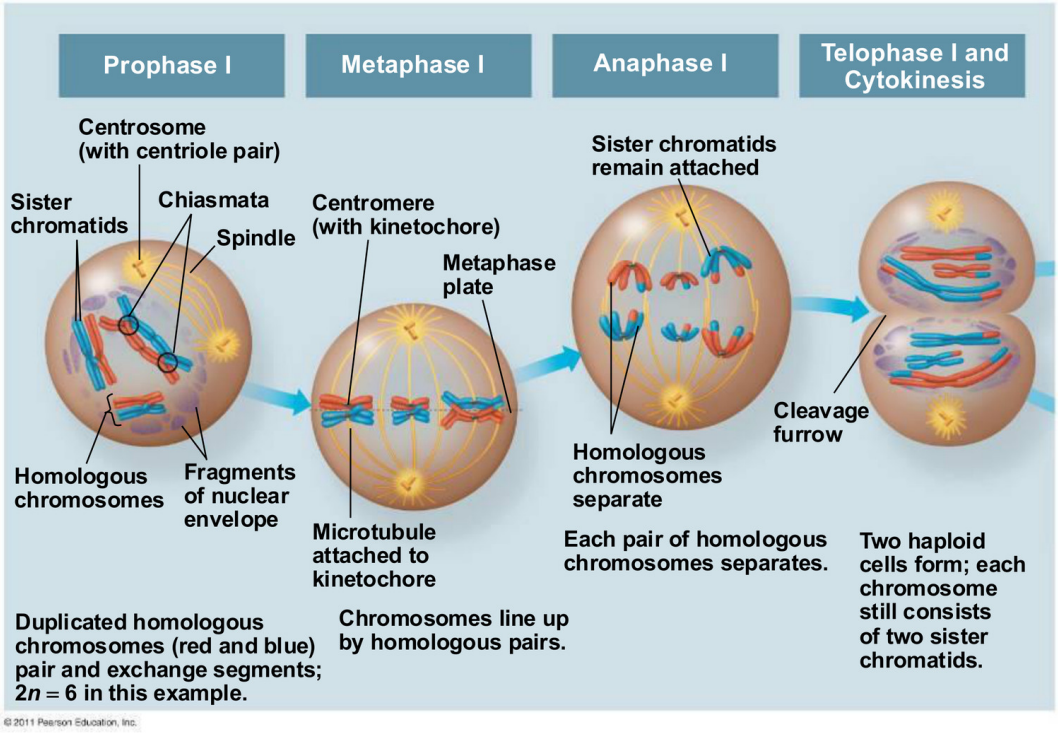
Dr. Asmaa Abu Obaid

Overview of meiosis (Figure 13.7)

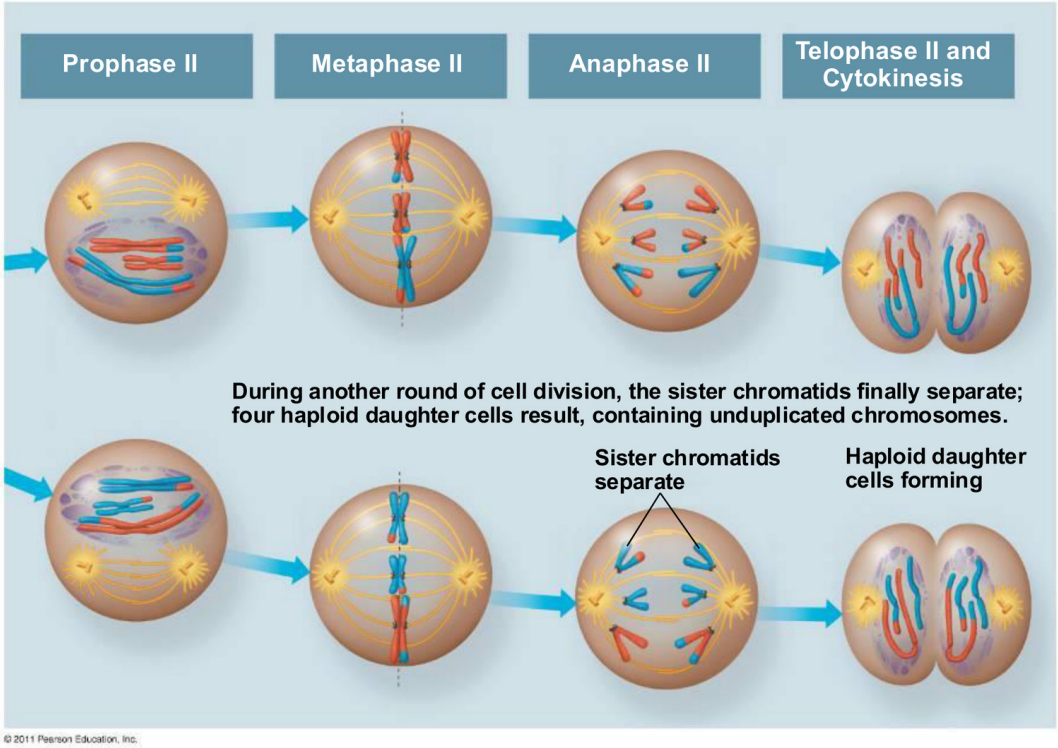
02 MEIOSIS

Meiosis I and Meiosis II, Step by Step

FIGURE 13.8



Meiosis I: homologous chromosomes pair, exchange segments and separate; two haploid cells form



Meiosis II: sister chromatids separate; four haploid daughter cells result

03 GENETIC VARIATION

SEXUAL LIFE CYCLES AND EVOLUTION

Origins of Genetic Variation

03

Mutations | Alleles | Reshuffling

SOURCE	RESULT	VARIATION
Mutations: the original source of genetic diversity	Mutations create different versions of genes (alleles)	Reshuffling of alleles in sexual reproduction

The behavior of chromosomes during **meiosis and fertilization** is responsible for most of the variation that arises in each generation.

THREE MECHANISMS CONTRIBUTE TO GENETIC VARIATION

1 Independent assortment

Each pair of chromosomes sorts maternal and paternal homologs into daughter cells **independently of the other pairs.** For humans (n = 23): more than **8 million (2^{23})** combinations.

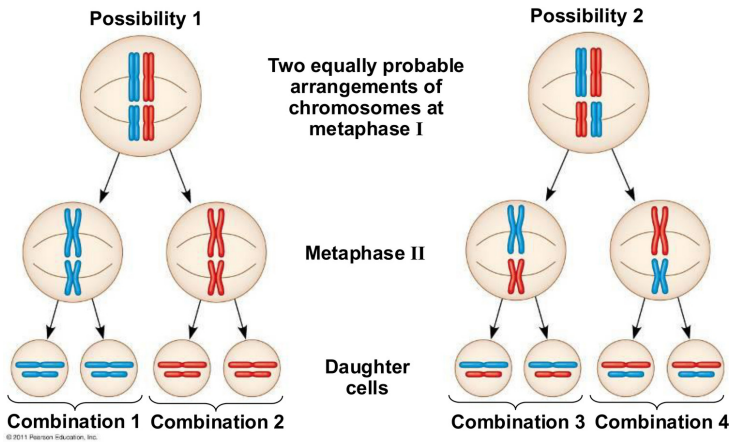
2 Crossing over

Produces **recombinant chromosomes**, combining DNA inherited from each parent into a single chromosome.

3 Random fertilization

Any sperm can fuse with any ovum. Two gametes (each with 8.4 million combinations) give a zygote with any of about **70 trillion** diploid combinations.

Crossing over adds even more variation: **each zygote has a unique genetic identity.**

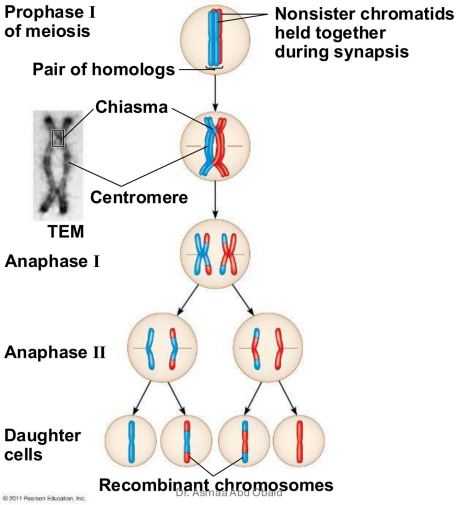


Independent assortment: two equally probable arrangements at metaphase I

03 GENETIC VARIATION

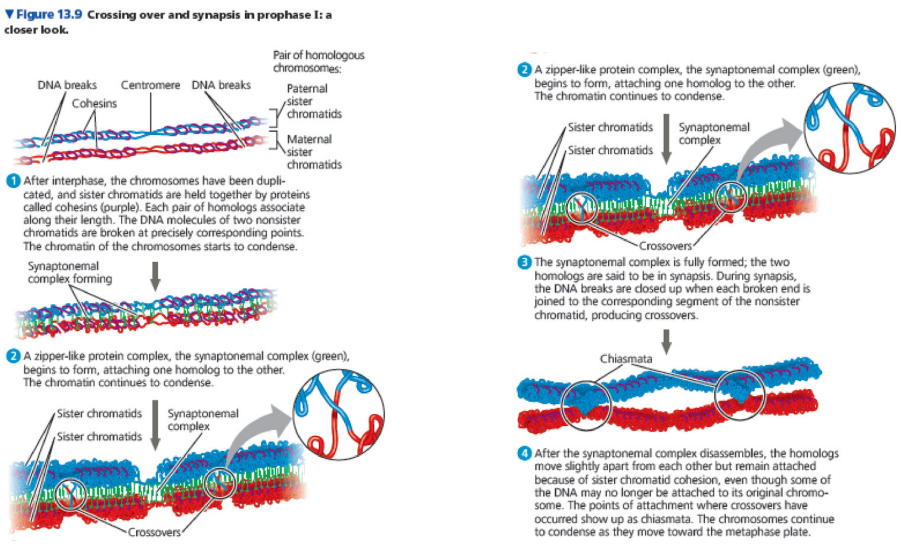
Crossing Over and Synapsis During Prophase I

1. Prophase I typically occupies **more than 90%** of the time required for meiosis.
2. Chromosomes begin to condense; sister chromatids are held together by proteins called **cohesins.**
3. Homologous chromosomes loosely pair up, **aligned gene by gene.**
4. Nonsister chromatids are broken by specific proteins at **precisely matching points.**



THEN

- A zipper-like structure called the **synaptonemal complex** holds one homolog tightly to the other.
- In **synapsis**, DNA breaks are closed up so each broken end is joined to the corresponding segment of the nonsister chromatid.
- In **crossing over**, nonsister chromatids exchange DNA segments, visible as **chiasmata** after the synaptonemal complex disassembles.
- Homologs stay attached because sister chromatids are still held together by **sister chromatid cohesion.**
- Each pair forms a **tetrad** (four chromatids), usually with one or more chiasmata, X-shaped regions where crossing over occurred.



Crossing over and synapsis in prophase I

RECOMBINATION AND MAPS

Linkage

04

Recombination | Interlocus distance | Linked | Unlinked

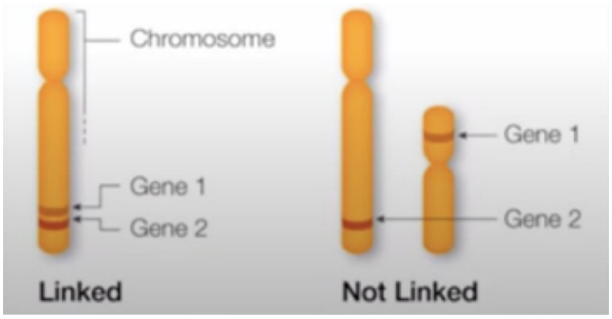
1. In theory, the alleles at all loci of one chromosome should be **transmitted as a unit** during gamete formation. However, in many instances this does not occur.
2. When homologs are synapsed, a **reciprocal exchange** of chromosome segments may take place.
3. This crossing over results in the reshuffling, or **recombination,** of alleles between homologs and always occurs during the **tetrad stage.**
4. The frequency of crossing over between two loci is **proportional to the distance between them** (the interlocus distance).
5. This correlation allows us to construct **chromosome maps**, which indicate the relative locations of genes.

Linked genes

Genes in different loci on the same chromosome.

Unlinked genes

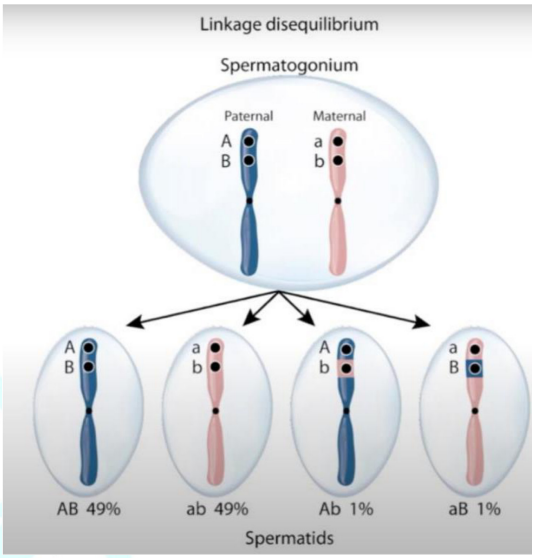
Genes on different (non-homologous) chromosomes; also used for genes on the same chromosome inherited independently (independent assortment).



Linkage Disequilibrium (LD)

DEFINITION

The **non-random association of alleles** at different loci in a given population.



- Certain alleles are **inherited together more frequently** than expected from their individual frequencies.
- Gives insights into the **genetic architecture** of traits and the **history of populations**; crucial in mapping disease-associated loci.

PUNNETT SQUARE (GAMETES): ONE INDIVIDUAL HETEROZYGOUS FOR TWO GENES

	A	A
B	AB (25%)	aB (25%)
b	Ab (25%)	ab (25%)

Independent assortment: four possible gametes, **25% chance each.**

	A	A
B	**AB (45%)**	aB (5%)
b	Ab (5%)	**ab (45%)**

Linkage disequilibrium: some combinations appear **more often** than expected.

04 LINKAGE & LD

Causes of LD

WHY ALLELES STAY TOGETHER

1 Physical proximity

Genes or markers close together on a chromosome tend to be inherited together because **recombination between them is less frequent.**

2 Population history

Influenced by **population bottlenecks, founder effects, and admixture.**

3 Natural selection

Certain allele combinations may confer an **advantage**, so they are passed on together more frequently.

Measuring LD

LD is often measured by two statistics: **D′ and r^2.**

D′

Ranges from 0 to 1; 1 indicates complete LD (alleles are always inherited together)

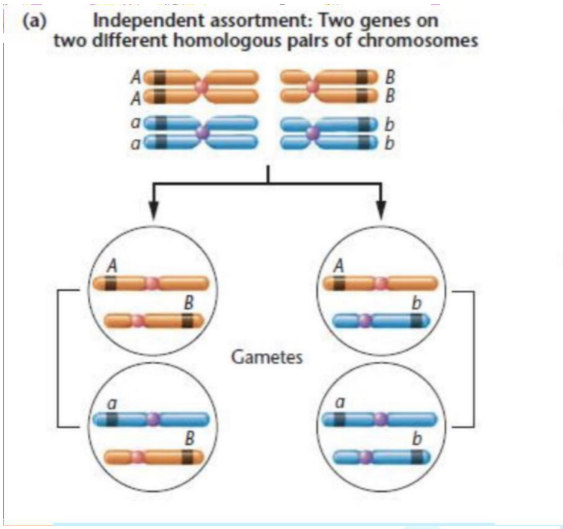
r^2

Measures the correlation between alleles at two loci; also ranges from 0 to 1

Linkage equilibrium — occurs when linkage disequilibrium is at zero.

Linkage disequilibrium — the non-random association of alleles at different loci.

No Linkage vs Crossing Over Between Linked Genes



(a) Independent assortment: two genes on two different homologous pairs

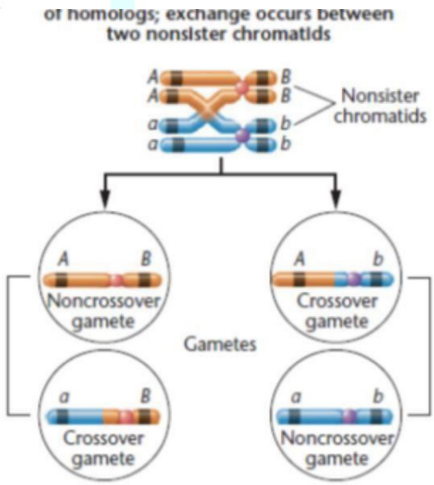
NO LINKAGE **Exhibited when**

Independent assortment of two pairs of chromosomes, each containing one heterozygous gene pair.

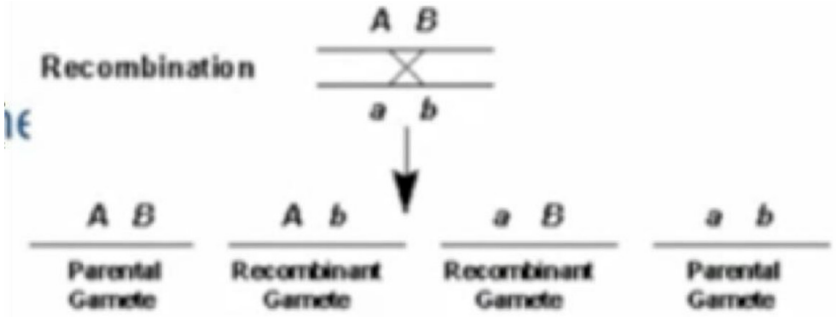
A large number of meiotic events produce **four genetically different gametes in equal proportions**, each with a different combination of alleles.

CROSSOVER **Between two linked genes**

- Involves only **two nonsister chromatids** of the four in the tetrad.
- Generates **recombinant, or crossover, gametes** : two new allele combinations.



(c) Linkage: exchange between two nonsister chromatids



Linked genes with crossing over: parental and recombinant gametes

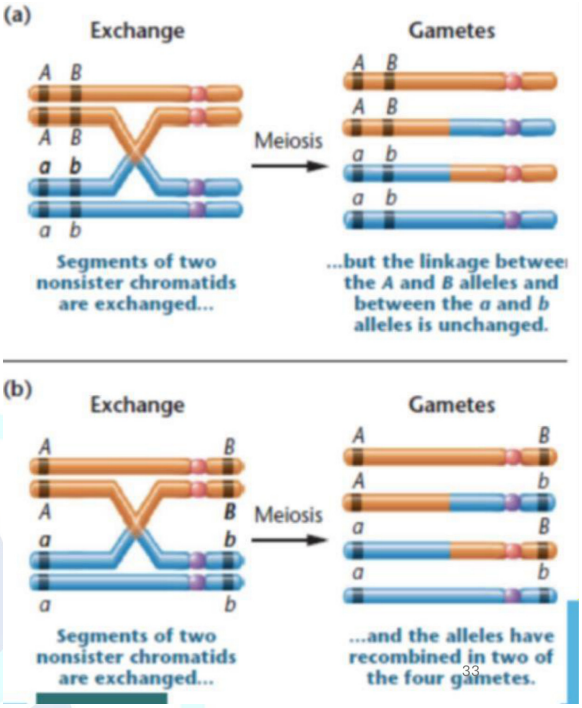
04 LINKAGE & LD

Why Does Distance Between Two Loci Matter?

CROSSING OVER & RECOMBINATION

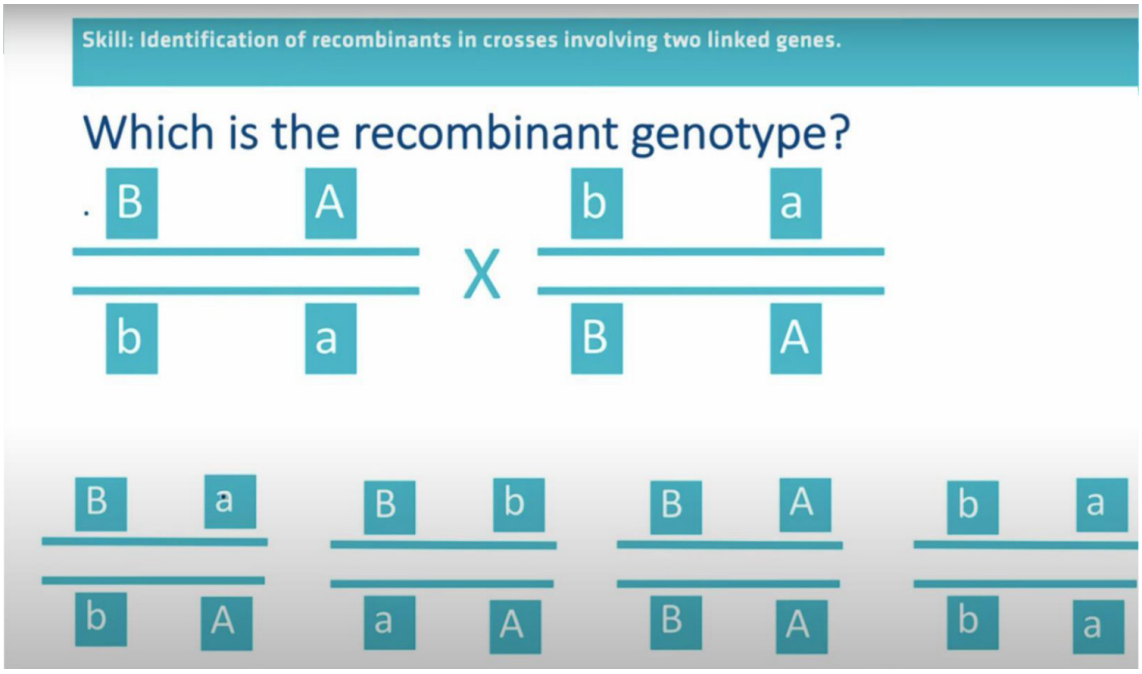
1. When a single crossover occurs between two nonsister chromatids, the **other two chromatids** of the tetrad are not involved and enter the gamete unchanged.

2. Even if a single crossover occurs **100 percent of the time** between two linked genes, recombination is observed in only **50 percent** of the potential gametes.



SKILL

Identification of recombinants in crosses involving two linked genes



Which is the recombinant genotype?

05 RECOMBINATION FREQUENCY & MAPS

GENETIC LINKAGE AND MAPPING

Recombination Frequency

05

RF | Centimorgan | Linked < 50% | Unlinked = 50%

- When genes are on **different chromosomes or far apart** on the same chromosome, they assort independently and are **unlinked.**
- When genes are **close together** on the same chromosome, they are **linked**: alleles already together on one chromosome are inherited as a unit more frequently than not.
- We can see if two genes are linked, and how tightly, by using data from genetic crosses to calculate the **recombination frequency.**
- Finding recombination frequencies for many gene pairs lets us make **linkage maps** showing the order and relative distances of genes.

KEY DEFINITIONS

Recombination fraction is a **measure of the distance** between two loci.

Two loci showing **1% recombination** are **1 centimorgan (cM)** apart; 1 map unit = 1 cM.

Unlinked = 50%

Genes that undergo independent assortment have an RF of **50 percent**: on nonhomologous chromosomes or far apart on the same chromosome.

VS

Linked < 50%

Genes with recombination frequencies **less than 50 percent** are on the same chromosome.

05 RECOMBINATION FREQUENCY & MAPS

1 · Finding Recombination Frequency

Are two genes in the fruit fly (*Drosophila*) linked, and if so, how tightly?

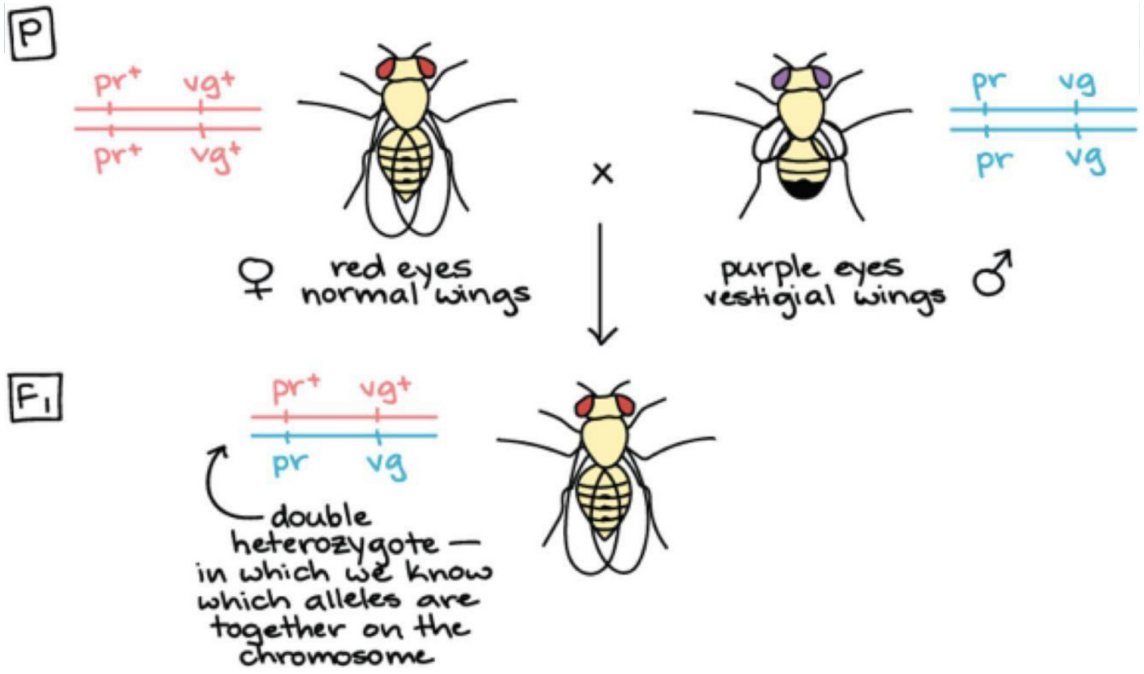
purple gene

Dominant **pr$^+$** allele: normal, red eyes.
Recessive **pr** allele: purple eyes.

vestigial gene

Dominant **vg$^+$** allele: normal, long wings.
Recessive **vg** allele: short, "vestigial" wings.

To measure RF we need a fly that is heterozygous for both genes **and** in which we know exactly which alleles are together on the chromosome. We start by crossing **two homozygous flies:**



P cross gives an F1 double heterozygote

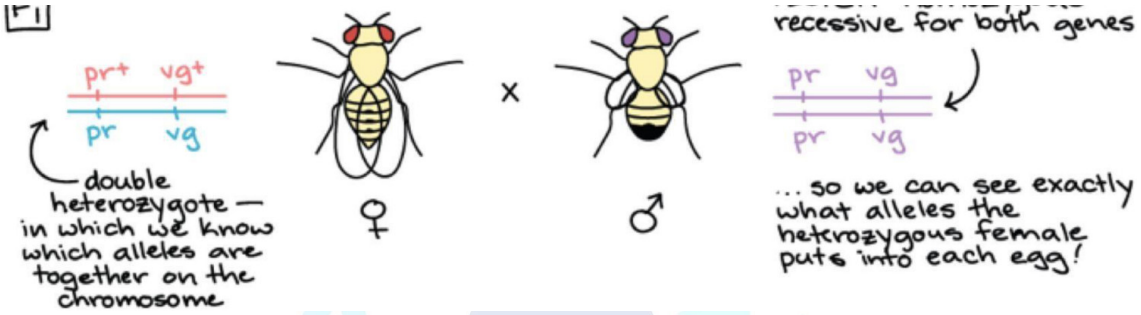
This gives a fly that's heterozygous for *purple* and *vestigial*, in which we know which alleles are together. It is simpler to **use its gametes in a cross** and see what the offspring look like than to look at the gametes directly.

05 RECOMBINATION FREQUENCY & MAPS

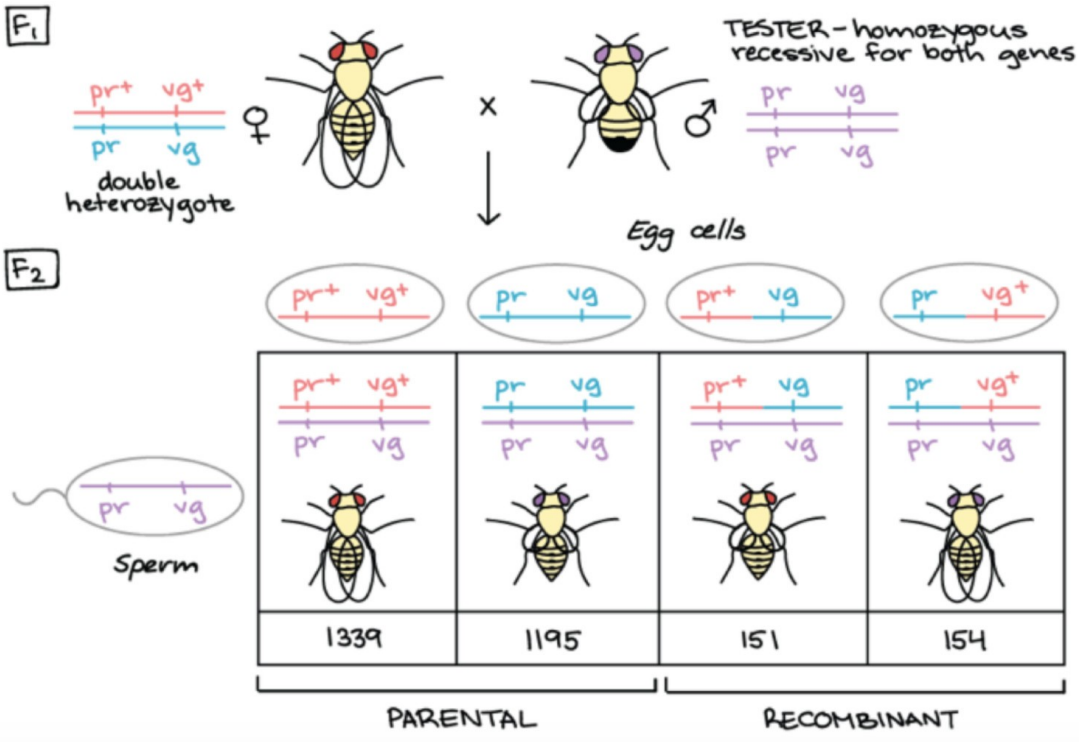
The Test Cross

TESTER FLY

Cross the double heterozygous fly with a **tester**, a fly homozygous recessive for all genes of interest (*pr* and *vg*). The tester ensures that the alleles from the non-tester parent **fully determine the phenotype**, so we can directly "read" the genotype of each gamete from the offspring's appearance.



The double heterozygous female makes **four types of eggs**, each combining with a sperm from the tester male, giving **four phenotypic classes** of offspring:



05 RECOMBINATION FREQUENCY & MAPS

Calculating the Recombination Frequency

The four classes of offspring are **not produced in equal numbers**, which tells us *purple* and *vestigial* are linked: parental configurations are **over-represented**, recombinant configurations are **under-represented.**

THE FORMULA

Recombination frequency (RF) = **Recombinants ÷ Total offspring** × 100%

1339	1195	151	154
Parental	Parental	Recombinant	Recombinant

Recombinant classes: **red-eyed, vestigial-winged** and **purple-eyed, long-winged** flies. We know they inherited a chromosome that had undergone recombination, and they are the **underrepresented** classes.

RF = (151 + 154) ÷ (1339 + 1195 + 151 + 154) × 100% = **10.7%**

The recombination frequency between the *purple* and *vestigial* genes is **10.7%.**

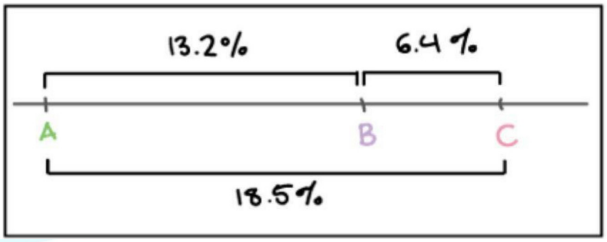
05 RECOMBINATION FREQUENCY & MAPS

2 · Recombination Frequency and Linkage Maps

- RF is **not a direct measure** of physical distance, but an **estimate**: a larger RF suggests genes are farther apart; a smaller RF, closer together.
- RFs have been used historically to build **linkage maps**, chromosomal maps based on recombination frequencies; these helped establish that chromosomes are **linear** and each gene has its own place.
- RF **"maxes out" at 50%** (genes unlinked); for genes further apart, we add RFs of multiple pairs, "building up" a map.

ORDERING THREE GENES: A, B AND C

PAIR	RF
A–B	13.2%
B–C	6.4%
A–C	**18.5%**



The pair with the **largest RF** flanks the third gene, so the order is **A – B – C.**

Adding genes D, E and F builds up **linkage maps of entire chromosomes**, with distances in centimorgans or map units.

1 centimorgan or **1 map unit** = 1% recombination frequency

05 RECOMBINATION FREQUENCY & MAPS

Morgan, Sturtevant and Linkage Maps

KEY POINTS

MORGAN **Thomas Hunt Morgan**

Expanded the understanding of linkage. He observed that crossing over between linked genes differs, suggesting crossover frequency might indicate gene distance. The **centimorgan** is named in his honour.

STURTEVANT **Alfred Sturtevant**

A student of Morgan, he **first developed linkage maps.**

- A **linkage map** (genetic map) shows the position of known genes or markers relative to each other in terms of **recombination frequency**, not physical distance.
- It is based on frequencies of recombination between markers during crossover of homologous chromosomes.
- The **greater the recombination frequency,** the further apart two markers are assumed to be; the lower, the smaller the physical distance.

Genome-Wide Association Studies (GWAS)

WHAT **A powerful method**

Identifies genetic variations associated with complex diseases by **scanning the genomes** of large groups of individuals.

HOW **No family data needed**

Uses **case-control or cohort** studies with thousands of participants to find common variants, usually **SNPs**, more frequent in people with a disease.

LD **Leverages LD**

Nearby SNPs act as **markers** for disease-associated regions, since closely located SNPs often inherit together.

FINDINGS **Discoveries**

Numerous loci for **diabetes, heart disease, and cancer**; reveals the genetic architecture of complex traits and biological pathways.

To Summarise...

CHROMOSOMES 23 pairs

Homologs & alleles

- Karyotype orders the pairs
- Maternal + paternal copy
- Homozygous vs heterozygous

MEIOSIS 2n → n

Two divisions

- Meiosis I: homologs separate
- Meiosis II: sister chromatids separate
- Four haploid daughter cells

VARIATION 3 ways

Sources of variation

- Independent assortment: 2^{23}
- Crossing over: recombinant chromosomes
- Random fertilization: ~70 trillion

LD $D' \cdot r^2$

Linkage disequilibrium

- Non-random allele association
- Proximity, history, selection
- Both statistics range 0 to 1

RF 10.7%

Recombination frequency

- Recombinants ÷ total × 100%
- Unlinked = 50%; linked < 50%
- purple–vestigial example

MAPS 1 cM = 1%

Linkage maps & GWAS

- Largest RF flanks the third gene
- Morgan and Sturtevant
- GWAS uses LD and SNPs