

GENETICS · STUDY NOTES

Linkage & Recombination

Mechanism, Genetic Mapping & Cytological Basis

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1. Introduction

Mendel's Law of Independent Assortment holds true only for genes located on **different chromosomes**, or far apart on the same chromosome. When two or more genes lie close together on the **same chromosome**, they tend to be transmitted together from parent to offspring rather than assorting independently. This phenomenon was first recognised by Thomas Hunt Morgan (1910) while working with *Drosophila melanogaster*.

Linkage: The tendency of genes located on the same chromosome to be inherited together, as a unit, because they do not assort independently during meiosis.

Recombination: The process that generates offspring with combinations of alleles different from either parent — either through crossing over between linked genes, or through independent assortment of genes on different chromosome pairs.

Genes on the same chromosome are not inherited together with absolute fidelity, however — the physical exchange of segments between homologous chromosomes during meiosis, called **crossing over**, breaks up parental combinations and creates new (recombinant) ones. Linkage and recombination are therefore two sides of the same coin: linkage keeps genes together, while recombination measures how often that grouping is broken.

2. Types of Linkage

2.1 Complete Linkage

Genes are inherited together with no exchange between them at all — crossing over does not occur between the loci concerned. Only the two **parental (non-recombinant)** gamete types are produced. This is rare and is mostly seen in organisms/sexes with achiasmate meiosis, e.g. the **male *Drosophila*** (no crossing over in males) and the **female silkworm, *Bombyx mori*** (no crossing over in females).

2.2 Incomplete Linkage

Genes are located on the same chromosome but far enough apart that crossing over occurs between them in a fraction of meioses. This produces a majority of parental-type gametes and a minority of **recombinant** gametes. Most linked genes in nature show incomplete linkage, and the recombination frequency is always **less than 50%**.



Fig. 1. Complete linkage yields only parental-type gametes, while incomplete linkage yields mostly parental types plus a minority of recombinant types produced by crossing over.

3. Morgan's Experimental Evidence

Morgan crossed *Drosophila* heterozygous for two X-linked genes — body colour (grey, **B**, dominant; black, **b**, recessive) and wing size (normal, **V**, dominant; vestigial, **v**, recessive). When the F_1 dihybrid females were testcrossed to homozygous recessive males, the progeny did **not** appear in the 1:1:1:1 ratio expected from independent assortment.

OBSERVATION

Parental-type progeny (grey-normal and black-vestigial) vastly outnumbered the recombinant types (grey-vestigial and black-normal). Morgan concluded that the two genes lie on the same chromosome and are usually inherited together, with recombinants arising only from occasional crossing over.

4. Linkage Groups

All the genes located on a single chromosome constitute one **linkage group**. Since genes are distributed among the chromosomes of a genome, the **number of linkage groups equals the haploid chromosome number (n)** of the species.

Organism	Haploid number (n)	Linkage groups
<i>Drosophila melanogaster</i>	4	4
<i>Pisum sativum</i> (garden pea)	7	7
<i>Zea mays</i> (maize)	10	10
<i>Homo sapiens</i>	23	23

5. Crossing Over — the Mechanism

Crossing over is the reciprocal exchange of chromosomal segments between non-sister chromatids of homologous chromosomes. It takes place during **meiosis I**, specifically at the **pachytene sub-stage of prophase I**, after homologous chromosomes have paired closely by **synapsis** (mediated by the synaptonemal complex) to form a **bivalent / tetrad** of four chromatids.

1. **Leptotene**: chromosomes condense and appear as single, thin threads.
2. **Zygotene**: homologous chromosomes pair up (synapsis) via the synaptonemal complex.
3. **Pachytene**: each bivalent now visibly consists of four chromatids (a tetrad); **recombination nodules** mediate breakage and reciprocal exchange between two non-sister chromatids.
4. **Diplotene**: the synaptonemal complex dissolves and homologs begin to separate, remaining attached at the points of exchange, which are now visible as X-shaped structures called **chiasmata**.
5. **Diakinesis**: chiasmata move toward the chromosome ends (terminalisation) as the bivalents condense further.

Because only **two of the four chromatids** in a tetrad take part in any single crossover event, one crossover produces two unchanged parental chromatids and two new recombinant chromatids — so the maximum recombination frequency from a single region is **50%**.

Pachytene tetrad — chiasma formation and outcome

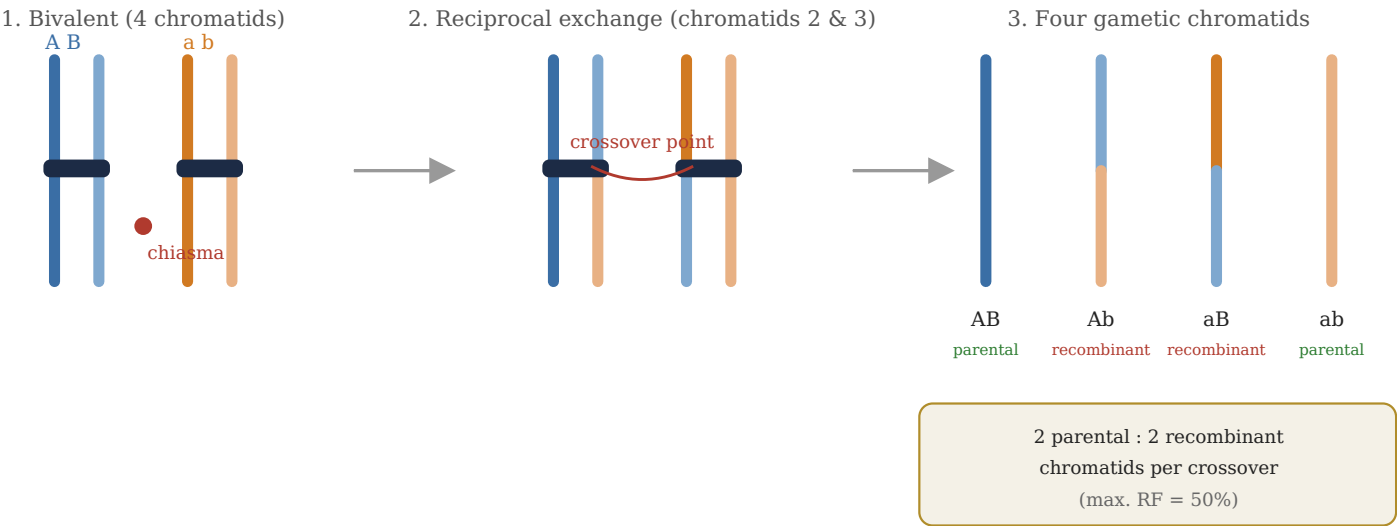


Fig. 2. During pachytene, two non-sister chromatids of a bivalent break and reciprocally rejoin at a chiasma. The result is two unchanged parental chromatids (AB, ab) and two new recombinant chromatids (Ab, aB).

KEY POINT

Chiasmata are the cytological (visible) expression of crossing over — the two terms are related but not identical: crossing over is the molecular/genetic event, chiasma is what you actually see under the microscope at diplotene-diakinesis.

6. Recombination Frequency & Genetic Mapping

A. H. Sturtevant (1913), a student of Morgan, reasoned that the **frequency of recombination between two linked genes is roughly proportional to the physical distance separating them** on the chromosome — genes far apart have more opportunity for a crossover to occur between them than genes lying close together. This principle allowed him to construct the first ever genetic (linkage) map, of the *Drosophila* X chromosome.

$$\text{Recombination Frequency (RF)} = \left(\frac{\text{Number of recombinant progeny}}{\text{Total progeny}} \right) \times 100$$

Recombination frequency is measured using a **two-point testcross**: a dihybrid (heterozygous at both loci) is crossed with a double-recessive homozygote, and the resulting phenotype classes are scored as parental or recombinant.

Map unit (m.u.) / centimorgan (cM): the unit of genetic distance; **1 map unit = 1% recombination frequency** between two genes. Named after T. H. Morgan.

As the distance between two genes increases, RF rises — but only up to a ceiling of **50%**, at which point genes appear to assort independently even though they lie on the same chromosome (they are said to be "genetically unlinked" despite being physically linked). This is why genetic map distances become progressively less accurate over long distances (multiple crossovers can mask each other), and map units are not perfectly additive over large regions.

Linear genetic map from recombination frequencies

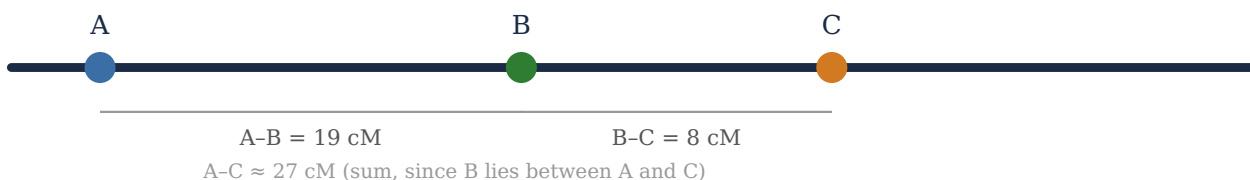


Fig. 3. Genes are ordered along a chromosome using pairwise recombination frequencies; the gene distances (in cM) are approximately additive when the middle gene lies between the two outer genes.

7. Three-Point Testcross

A three-point testcross uses a trihybrid, heterozygous at three linked loci, crossed with a homozygous recessive tester. It is more efficient than three separate two-point crosses because it also reveals **double crossovers** and lets the gene order be determined directly.

The eight testcross progeny classes fall into four pairs of reciprocal phenotypes, ranked by frequency:

- **Parental (non-crossover) types** — most frequent, matching the original gamete combinations.
- **Single crossover, region I** — moderate frequency.
- **Single crossover, region II** — moderate frequency.
- **Double crossover (DCO) types** — least frequent, since two independent crossover events must occur in the same meiosis.

FINDING THE GENE ORDER

Compare the double-crossover class with the parental class: the gene whose allele appears "switched" relative to its neighbours identifies the **middle gene** of the three — because only the middle gene changes its linkage partner when two crossovers occur on either side of it.

8. Interference & Coefficient of Coincidence

If crossovers in adjacent regions occurred independently of each other, the expected double-crossover frequency would equal the product of the two single-crossover frequencies. In practice, a crossover in one region often reduces the likelihood of a second crossover nearby — a phenomenon called **interference**.

$$\begin{aligned} \text{Coefficient of Coincidence (C)} &= \frac{\text{Observed DCO frequency}}{\text{Expected DCO frequency}} \\ \text{Interference (I)} &= 1 - C \end{aligned}$$

A coefficient of coincidence of 1 indicates no interference; values less than 1 (positive interference, the usual case) indicate that observed double crossovers are fewer than expected.

9. Cytological Proof of Crossing Over

Genetic mapping demonstrated recombination statistically, but did not directly prove that a physical exchange of chromosome material occurs. Two classic experiments supplied that direct cytological evidence, both published in 1931:

9.1 Creighton & McClintock (Maize)

Harriet Creighton and Barbara McClintock worked with a chromosome 9 of maize that carried two visible cytological markers — a dense knob at one end and a translocated segment at the other — as well as two genetic markers, **C/c** (coloured/colourless kernel) and **Wx/wx** (starchy/waxy endosperm). Whenever the genetic markers recombined, the cytological markers recombined along with them — proving that genetic recombination corresponds exactly to a physical exchange of chromosome segments.

9.2 Curt Stern (*Drosophila*)

Working independently in the same year, Stern used X chromosomes of *Drosophila* carrying distinctive cytological rearrangements together with linked genetic markers, and obtained the same result in animals — new combinations of genetic markers appeared only together with the corresponding exchange of cytologically visible chromosome parts.

10. Factors Affecting Crossing Over

Factor	Effect
Distance between genes	Directly proportional — greater distance, higher chance of crossing over
Sex	Absent in male <i>Drosophila</i> and female silkworm (achiasmate meiosis)
Age	Frequency generally declines with advancing age of the organism
Temperature	Extremes of temperature can increase crossover frequency
Chemicals / radiation	X-rays and certain chemicals can increase recombination
Chromosomal aberrations	Inversions and translocations suppress crossing over locally by disrupting synapsis

11. Significance of Linkage & Recombination

- **Genetic variation:** recombination generates new allele combinations each generation, providing raw material for natural selection.
- **Genetic/linkage mapping:** the basis of gene mapping, marker-assisted breeding, and localising disease genes relative to known markers.
- **Plant & animal breeding:** desirable linked traits can be selected together; undesirable linkages can potentially be broken by selecting rare recombinants.
- **Evolution:** recombination breaks up unfavourable gene combinations and allows favourable mutations arising on different chromosomes to be brought together.