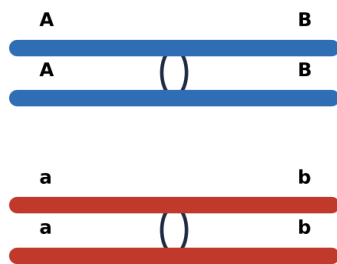


Crossing Over

DNA Hybridisation & the Holliday Model

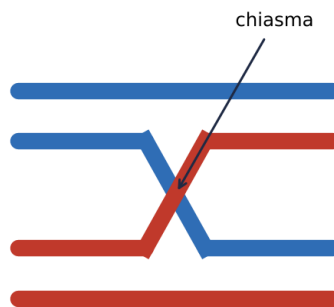
Definition · Types · Mechanism · Cytological proof · Map distance · Hybrid DNA · Holliday junction · Gene conversion · Numericals · Exam questions · MCQs

1. Tetrad (pachytene)



sister chromatids · homologues paired

2. Chiasma (2 non-sisters cross)



3. Products after meiosis

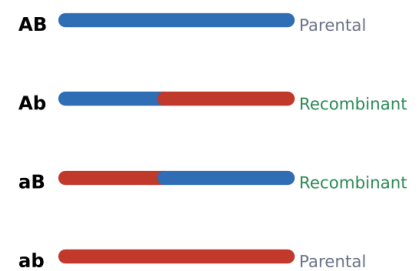


Fig. 1 — Crossing over between non-sister chromatids produces two parental and two recombinant chromatids.

Definition: Crossing over is the exchange of corresponding segments between **non-sister chromatids** of homologous chromosomes during **pachytene of prophase I**, producing new combinations of linked genes (recombinants). Term coined by **Morgan & Cattell (1912)**; the visible cross-shaped point is the **chiasma** (Janssens, 1909).

01 · Key features & terms

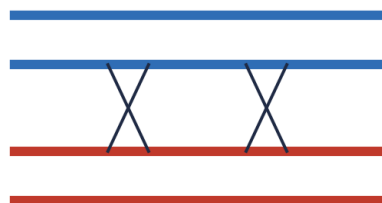
- Occurs between **non-sister** chromatids of a homologous pair, at the **four-strand (tetrad) stage** — after DNA replication.
- Takes place at **pachytene**; chiasmata become visible in **diplotene** and move to the ends (**terminalisation**) in diakinesis.
- Exchange is **reciprocal** and precise — no gain or loss of genes.
- Each bivalent usually has **at least one** chiasma (obligate crossover), needed for proper segregation.
- Frequency is proportional to the distance between genes — the basis of **genetic mapping**.

Term	Meaning
Synapsis	Pairing of homologues in zygotene, held by the synaptonemal complex (Moses, 1956)
Recombination nodules	Protein bodies on the synaptonemal complex marking crossover sites
Chiasma (pl. chiasmata)	Visible point of contact where non-sister chromatids have exchanged
Terminalisation	Movement of chiasmata towards chromosome ends during diplotene-diakinesis
Recombinant	Chromatid / gamete carrying a new combination of alleles
Centimorgan (cM)	Map unit: 1% recombination frequency = 1 cM

02 · Types of crossing over

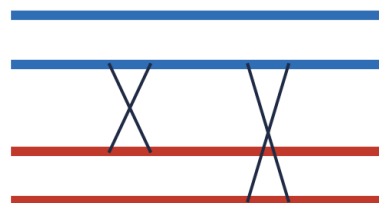
Basis	Type	Notes
Cell type	Germinal (meiotic)	In germ cells during meiosis; common; heritable
	Somatic (mitotic)	In body cells; rare; discovered by Curt Stern (1936) in <i>Drosophila</i> — 'twin spots' of yellow and singed bristles
Number of chiasmata	Single	One chiasma between two genes
	Double	Two chiasmata; may involve 2, 3 or 4 strands (Fig. 2)
	Multiple	More than two chiasmata in a long chromosome

Two-strand DCO



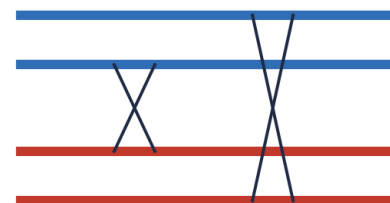
ends: 0 of 4 recombinant

Three-strand DCO



ends: 2 of 4 recombinant

Four-strand DCO



ends: 4 of 4 recombinant

Fig. 2 — Double crossovers (DCO) between end genes. On average, DCO give 50% recombinants — so DCO are missed in two-point crosses.

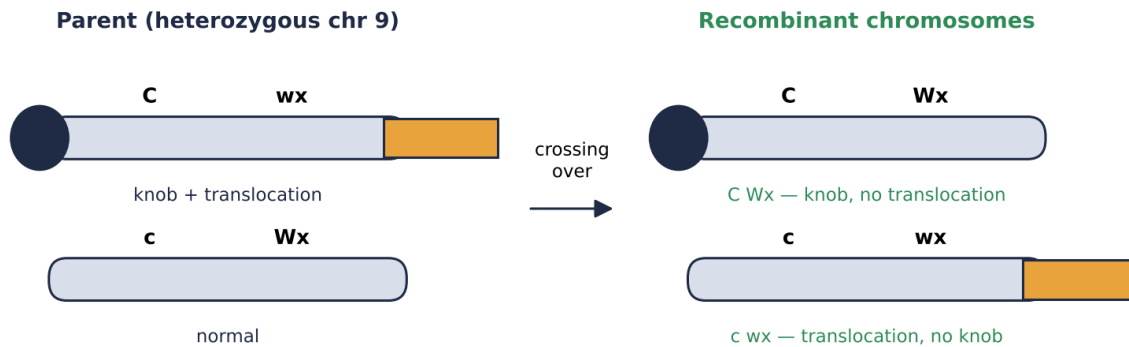
03 · Mechanism (cytological sequence) & theories

- **1. Synapsis** (zygotene) — homologues pair gene-by-gene via the synaptonemal complex.
- **2. Tetrad formation** — each homologue already has two chromatids, so the bivalent has four.
- **3. Crossing over** (pachytene) — endonuclease breaks non-sister chromatids at corresponding points, segments exchange, and ligase rejoins them (**breakage and reunion**).
- **4. Terminalisation** (diplotene → diakinesis) — homologues repel; chiasmata slide to the ends; separation at anaphase I.

Theory	Proposed by	Idea
Chiasma-type theory	Janssens (1909)	Chiasma formation causes crossing over; the visible chiasma = site of exchange (now accepted)
Classical (two-plane) theory	Sax & others	Crossing over results from chiasma breakage during separation — chiasma is cause, not result
Copy-choice theory	Belling (1928/1931)	New strand copies part of one template, then switches to the other — no breakage. Rejected: cannot explain 4-strand crossing over or reciprocity
Breakage and reunion	Darlington (1937)	Chromatids break and rejoin with exchange — confirmed by Meselson & Weigle (1961), Taylor (1965)

04 · Cytological & molecular proof

(a) Creighton & McClintock (1931), maize. One chromosome 9 carried a **knob** at one end and a **translocated piece of chromosome 8** at the other, with alleles **C** (coloured) and **wx** (waxy). Its homologue was normal with **c** and **Wx**. Every genetic recombinant (C Wx or c wx) also showed a new cytological combination (knob without translocation, or translocation without knob) — proving a **physical exchange** of chromosome segments.



Knob = cytological marker at one end; translocated segment (from chr 8) at the other. New genetic combinations always appeared with new cytological combinations → physical exchange of chromosome parts (Creighton & McClintock, 1931).

Fig. 3 — Creighton & McClintock: genetic and cytological markers recombine together.

(b) Curt Stern (1931), Drosophila. Used X chromosomes with visibly distinct shapes (one broken, one with a Y fragment attached) carrying *car* (carnation eye) and *B* (Bar). Genetic recombinants always carried morphologically recombinant X chromosomes — the same conclusion in animals.

(c) Meselson & Weigle (1961), λ phage. Phages labelled with heavy isotopes (^{13}C , ^{15}N) were crossed with light phages; recombinants had intermediate density — they contained DNA from both parents, proving **breakage and reunion** at the molecular level.

05 · Frequency, interference & genetic mapping

Recombination frequency (RF) = (number of recombinants ÷ total progeny) × 100. 1% RF = 1 map unit = 1 cM (Sturtevant, 1913 — first genetic map of *Drosophila* X).

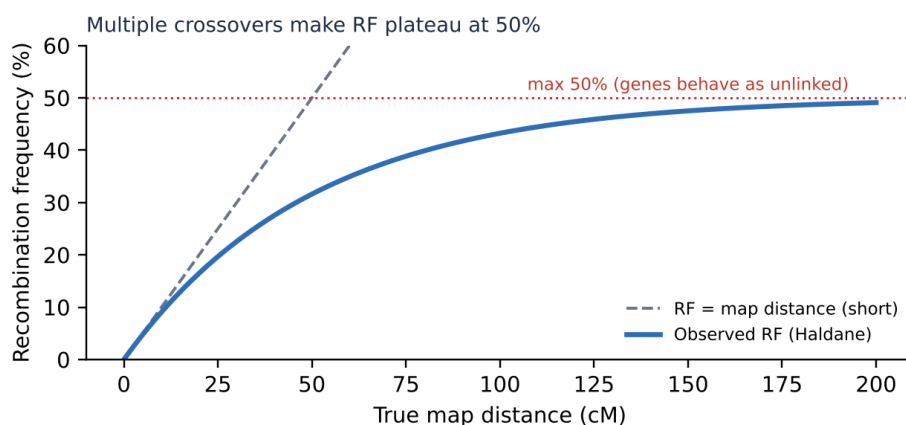


Fig. 4 — RF underestimates long distances because double crossovers are hidden.

Interference: one crossover reduces the chance of another nearby. **Coefficient of coincidence (CoC)** = observed DCO ÷ expected DCO; **Interference (I)** = 1 – CoC. I = 1 → complete interference; I = 0 → none.

Factor	Effect on crossing over
Distance between genes	Greater distance → more crossing over
Near centromere / heterochromatin	Reduced (centromere effect)
Sex	Absent in male <i>Drosophila</i> and female silkworm (heterogametic sex); differs between sexes in humans
Age	Decreases with age in female <i>Drosophila</i>
Temperature	Increases above and below ~22 °C in <i>Drosophila</i>
Radiation (X-rays) & chemicals	Increase it; mitomycin-C induces somatic crossing over
Inversions	Suppress recombinants (crossover suppressors)
Nutrition (Ca ²⁺ , Mg ²⁺)	Calcium/magnesium levels alter frequency

Significance: creates new gene combinations (variation for evolution and plant/animal breeding); proves genes are linearly arranged; allows construction of linkage maps; helps locate disease genes; essential for correct chromosome segregation in meiosis.

06 · DNA hybridisation (hybrid / heteroduplex DNA)

DNA hybridisation is the base-pairing of two single-stranded nucleic acid molecules from **different sources** to form a double-stranded **hybrid (heteroduplex)**. It depends on **complementarity** — the more similar the sequences, the more stable the hybrid.

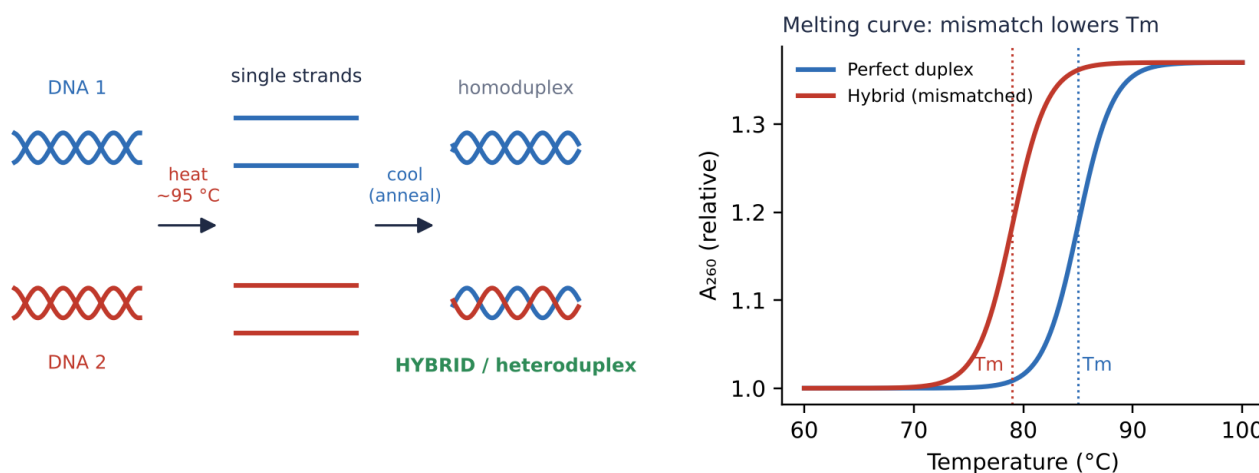


Fig. 5 — Denaturation and renaturation; a mismatched hybrid melts at a lower T_m.

- **Denaturation:** heat (~90–95 °C), high pH or formamide breaks hydrogen bonds → single strands; absorbance at 260 nm rises (**hyperchromic effect**, ~37%).
- **T_m (melting temperature):** temperature at which 50% of DNA is single-stranded. Rises with **G-C content** (3 H-bonds) and salt; ~1 °C fall per 1% mismatch.
- **Renaturation (annealing):** slow cooling lets complementary strands find each other (Marmur & Doty, 1961). Rate described by **C₀t curves** (Britten & Kohne, 1968) — repetitive DNA reanneals fastest.
- **Stringency:** high temperature / low salt allows only perfect matches; low stringency tolerates mismatches.

Application	How hybridisation is used
Southern / Northern blotting	Labelled probe hybridises to DNA (Southern, 1975) or RNA on a membrane

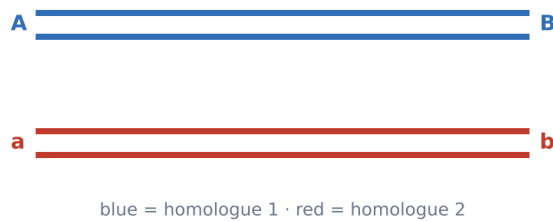
Application	How hybridisation is used
FISH	Fluorescent probe hybridises to chromosomes to locate genes
Colony / plaque hybridisation	Screens libraries for a clone carrying a gene
Microarrays	Thousands of probes measure gene expression
DNA-DNA hybridisation in taxonomy	ΔT_m between species estimates relatedness (Sibley & Ahlquist — bird phylogeny)
R-loop / heteroduplex mapping	Electron microscopy reveals introns and deletions as single-stranded loops
Genetic recombination	Hybrid DNA forms naturally in the Holliday model (next section)

Link to recombination: In recombination, **heteroduplex DNA** = a stretch of duplex with one strand from each parental homologue. If the homologues carry different alleles there, the hybrid has a **mismatch** — the root of gene conversion.

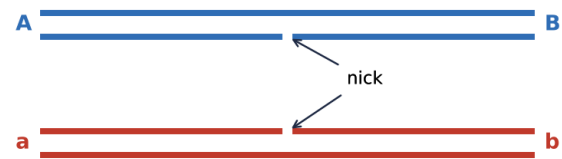
07 · The Holliday model (1964)

Proposed by **Robin Holliday (1964)** to explain gene conversion in fungi. It is the first **molecular model of homologous recombination**; its central intermediate, the four-stranded **Holliday junction (HJ)**, has since been seen directly by electron microscopy (**Potter & Dressler, 1976**, 'Chi forms').

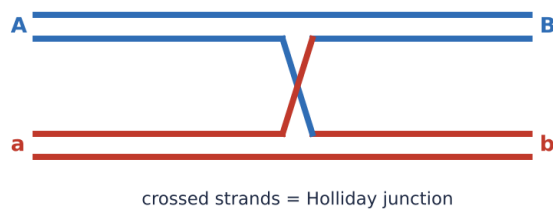
(a) Two homologous duplexes align



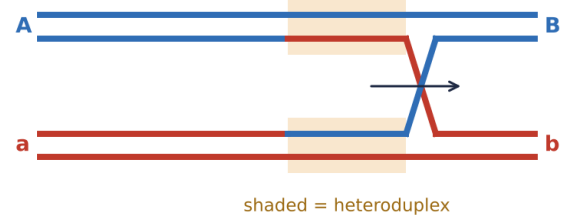
(b) Endonuclease nicks one strand of each, same position & polarity



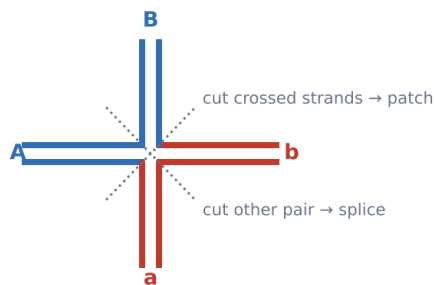
(c) Strand invasion + ligation
→ Holliday junction



(d) Branch migration → heteroduplex
(hybrid DNA) in both duplexes



(e) Rotation / isomerisation
(open-square Chi form)



(f) Resolution by resolvase (RuvC)

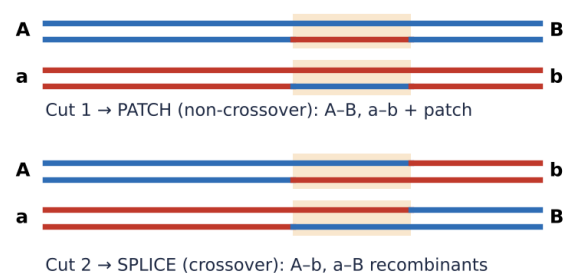


Fig. 6 — Steps of the Holliday model. Blue and red = the two homologous DNA duplexes.

Step-by-step:

- 1. Alignment** — two homologous double helices line up side by side.
- 2. Nicking** — an endonuclease cuts one strand of each duplex at the **same position** and with the **same polarity**.
- 3. Strand invasion** — the free ends unwind and each invades the other duplex, pairing with its complementary strand.
- 4. Ligation** — ligase seals the ends, forming a cross-shaped **Holliday junction**.
- 5. Branch migration** — the junction slides along the DNA, unwinding and rewinding strands; this forms **heteroduplex (hybrid) DNA** in both molecules.
- 6. Isomerisation** — the junction rotates 180° (open-square 'Chi' form), so either pair of strands can become the crossing pair.
- 7. Resolution** — a resolvase cuts one pair of strands; ligase seals them.
 - Cut the **crossed (inner) strands** → **patch**: flanking markers stay parental (A-B, a-b) with a short heteroduplex patch.
 - Cut the **other (outer) strands** → **splice**: flanking markers are recombinant (A-b, a-B) with heteroduplex — a true crossover.

Patch recombinant	Splice recombinant
Non-crossover for flanking markers	Crossover for flanking markers
A-B and a-b	A-b and a-B
Only a heteroduplex 'patch' remains	Heteroduplex at the junction of the exchange
Can still show gene conversion	Shows gene conversion + reciprocal exchange

Step	E. coli protein	Eukaryotic equivalent
Initiating break	RecBCD (stops at Chi site 5'-GCTGGTGG-3')	Spo11 (meiotic DSB), MRN complex
Strand invasion / pairing	RecA + SSB	Rad51, Dmc1 (meiosis), RPA
Branch migration	RuvA (binds HJ) + RuvB (ATPase motor)	BLM, RAD54
Resolution	RuvC resolvase	GEN1, MUS81-EME1, SLX1-SLX4
Sealing	DNA ligase	DNA ligase I

Merits: explains reciprocal exchange, heteroduplex formation, gene conversion and post-meiotic segregation. **Limitations:** requires two simultaneous nicks at identical sites (unlikely); predicts **symmetric** heteroduplex in both chromatids, but fungi often show it in only one; does not explain how recombination starts.

Later model	Key change
Meselson-Radding (1975)	Single nick; one strand invades → asymmetric heteroduplex first, later symmetric
Double-strand break repair, DSBR (Szostak, Orr-Weaver, Rothstein & Stahl, 1983)	Starts with a double-strand break ; 5'→3' resection; D-loop; DNA synthesis; double Holliday junction — now the accepted meiotic model
SDSA (synthesis-dependent strand annealing)	Invading strand is displaced after synthesis → non-crossover products only

08 · Gene conversion & mismatch repair

Gene conversion is the non-reciprocal transfer of genetic information, so that one allele is 'converted' into the other, giving **non-Mendelian ratios**. It was first detected by **Lindegren (1953)** in yeast and studied in *Neurospora*, *Sordaria* and *Ascomolus*, where all eight spores of an ascus can be scored.

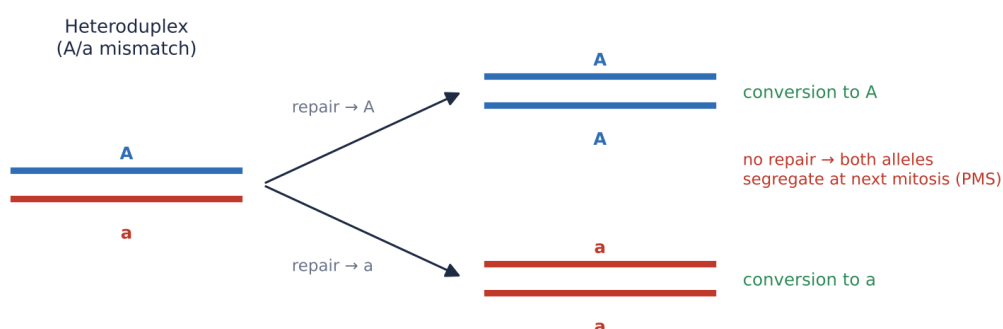


Fig. 7 — Mismatch repair of a heteroduplex decides which allele is kept.

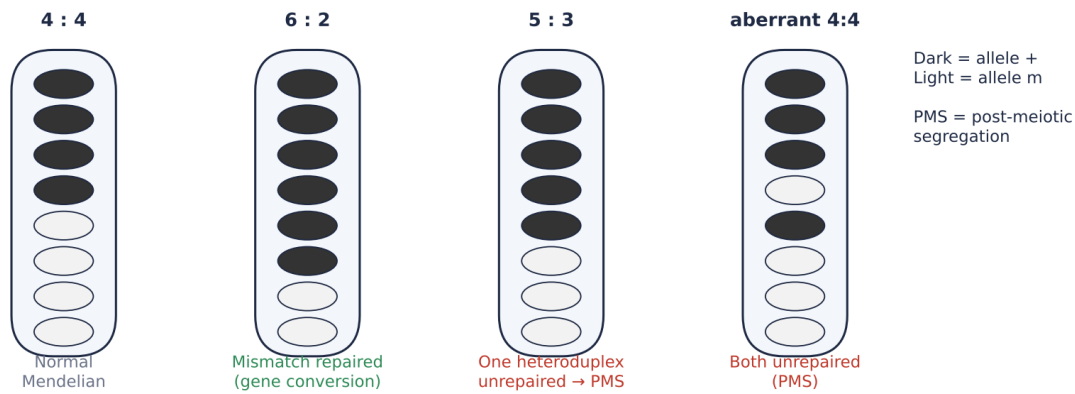


Fig. 8 — Ascus ratios: 4:4 normal; 6:2 / 2:6 = conversion; 5:3 / aberrant 4:4 = post-meiotic segregation (PMS).

How the Holliday model explains it: branch migration across an A/a site makes heteroduplex.

Mismatch repair excises one strand and copies the other, converting a→A or A→a. If one heteroduplex is repaired, a **6:2 ratio** results; if none is repaired, the mismatch separates at the next mitosis, giving a **5:3 ratio** (post-meiotic segregation). Gene conversion is often associated with crossing over of outside markers, which is strong support for a common heteroduplex intermediate.

09 · Worked numericals

Q1. Two-point test cross: AaBb × aabb gave AB 420, ab 400, Ab 90, aB 90. Find RF and map distance.

Solution: Recombinants = 90 + 90 = 180; total = 1000. RF = $180/1000 \times 100 = 18\%$ → genes are **18 cM** apart. Parental types (AB, ab) are the majority, confirming the cis (coupling) arrangement.

Q2. Coefficient of coincidence: Genes a-b are 10 cM apart and b-c 20 cM. In 1000 progeny, 10 double crossovers were observed. Find CoC and interference.

Solution: Expected DCO = $0.10 \times 0.20 \times 1000 = 20$. CoC = $10/20 = 0.5$; Interference = $1 - 0.5 = 0.5$ (50% of expected DCOs suppressed).

Q3. Map distance with DCO: In a three-point cross: single crossovers region I = 80, region II = 150, DCO = 10, total = 1000. Find a-b and b-c distances.

Solution: a-b = $(80 + 10)/1000 \times 100 = 9 \text{ cM}$; b-c = $(150 + 10)/1000 \times 100 = 16 \text{ cM}$; a-c = 25 cM. DCOs are counted in **both** intervals.

Q4. Chiasma to RF: If chiasmata form between genes A and B in 30% of meiocytes, what is the RF?

Solution: Each chiasma involves only 2 of the 4 chromatids, so RF = $\frac{1}{2} \times 30\% = 15\%$ (15 cM). Rule: RF = $\frac{1}{2} \times$ chiasma frequency.

Q5. Holliday resolution: A Holliday junction forms between markers A and B (homologue 1) and a and b (homologue 2). What products arise from each resolution?

Solution: Cutting the crossed strands gives **patch** products A-B and a-b (non-crossover). Cutting the outer strands gives **splice** products A-b and a-B (crossover). Both carry heteroduplex DNA, so either can show gene conversion of a marker inside the hybrid region.

10 · End-semester exam questions

Short answer (2-5 marks)

- Define crossing over. At which stage of meiosis does it occur?
- Distinguish between crossing over and chiasma.
- What is somatic crossing over? Who discovered it?
- Give the cytological proof of crossing over (Creighton & McClintock).
- What is coefficient of coincidence? How is it related to interference?
- What is DNA hybridisation? Define T_m and hyperchromic effect.
- What is a Holliday junction? Name the enzymes that move and resolve it.
- Distinguish patch and splice recombinants.
- What is gene conversion? How is a 5:3 ascus produced?
- Why is recombination frequency never more than 50%?

Long answer (8-15 marks)

1. Describe the mechanism of crossing over. Add a note on its theories and significance.
2. Describe the Holliday model of recombination with labelled diagrams. How does it explain gene conversion?
3. What is DNA hybridisation? Describe the principle, factors affecting it and its applications in molecular biology.
4. Give cytological and molecular evidence that crossing over involves physical exchange of chromosome segments.
5. Explain the molecular basis of homologous recombination, comparing the Holliday, Meselson-Radding and double-strand break repair models.
6. Discuss the factors affecting crossing over. Explain interference and how it is measured.

11 · Case-based MCQs

1. A test cross shows 85% parental and 15% recombinant offspring. The genes are:
(A) Unlinked (B) 15 cM apart, linked (C) 85 cM apart (D) On different chromosomes
2. Crossing over in meiosis occurs between:
(A) Non-sister chromatids of homologues (B) Sister chromatids (C) Non-homologous chromosomes (D) Centromeres
3. Creighton and McClintock proved crossing over using:
(A) *Drosophila* X chromosomes (B) Yeast asci (C) λ phage density labels (D) Maize chromosome 9 with knob and translocation
4. In the Holliday model, heteroduplex DNA is produced mainly by:
(A) Ligation (B) Replication (C) Nicking (D) Branch migration
5. Cutting the crossed strands of a Holliday junction gives:
(A) Patch (non-crossover) products (B) Deletions (C) Splice (crossover) products (D) No products
6. The *E. coli* protein that catalyses strand invasion is:
(A) Spo11 (B) RecA (C) DNA ligase (D) RuvC
7. A *Sordaria* ascus shows 6 black : 2 white spores. This indicates:
(A) Gene conversion (B) Normal segregation (C) Post-meiotic segregation (D) Mutation

8. A DNA hybrid between two species melts 8 °C below the native duplex. This suggests:
 (A) About 8% sequence mismatch (B) Higher GC content (C) No relationship (D) Identical DNA
9. Observed DCO = 5, expected DCO = 20. Interference is:
 (A) 0.75 (B) 4 (C) 1.25 (D) 0.25
10. Which model proposes that meiotic recombination starts with a double-strand break?
 (A) Meselson–Radding (1975) (B) Holliday (1964) (C) Belling (1931) (D) Szostak et al. (1983)

Answer key: 1 B · 2 A · 3 D · 4 D · 5 A · 6 B · 7 A · 8 A · 9 A · 10 D

12 · One-page revision

Topic	Must-remember point
Crossing over	Non-sister chromatids · pachytene · 4-strand stage · reciprocal
Chiasma	Visible site of exchange (Janssens, 1909) · terminalisation in diakinesis
Proofs	Creighton & McClintock 1931 (maize) · Stern 1931 (Drosophila) · Meselson & Weigle 1961 (λ phage)
Mapping	1% RF = 1 cM · max RF 50% · CoC = obs/exp DCO · I = 1 – CoC
DNA hybridisation	Denature → anneal → hybrid · T _m ↑ with GC & salt · uses: blots, FISH, phylogeny
Holliday model	1964 · nick → invasion → HJ → branch migration → isomerisation → resolution
Resolution	Crossed strands cut → patch; outer strands cut → splice
Enzymes	RecBCD, RecA, RuvA/B, RuvC · Spo11, Rad51/Dmc1
Gene conversion	Mismatch repair of heteroduplex · 6:2 = conversion · 5:3 = PMS
Modern model	Szostak 1983 DSB — double Holliday junction