

Linkage

with Three-Point Test Cross Mapping — fully worked examples

Definition · Coupling & repulsion · Complete vs incomplete linkage · Theories · Factors · Map distance · Three-point cross step-by-step · 3 solved problems · Practice set · Exam questions · MCQs

Definition: Linkage is the tendency of genes located close together on the **same chromosome** to be inherited together, so they do not assort independently. The term was given by **T. H. Morgan (1910)** from his work on *Drosophila*; the phenomenon was first noticed by **Bateson & Punnett (1906)** in sweet pea.

01 · Discovery & linkage groups

Bateson & Punnett (1906), sweet pea: crossing purple-flowered, long-pollen plants with red-flowered, round-pollen plants gave an F₂ that departed strongly from 9:3:3:1 — too many parental types and too few new combinations. They called this **coupling** (dominants entering together stay together) and **repulsion** (dominant and recessive entering together stay together), but could not explain it.

Morgan (1910-1911): explained it as genes lying on the same chromosome — the **chromosome theory of linkage**. All genes on one chromosome form a **linkage group**, and the number of linkage groups equals the **haploid chromosome number**.

Organism	2n	Linkage groups
<i>Drosophila melanogaster</i>	8	4
Pea (<i>Pisum sativum</i>)	14	7
Maize (<i>Zea mays</i>)	20	10
Human	46	23 (24 in males: X and Y differ)
<i>Neurospora</i>	n = 7	7

02 · Coupling and repulsion phases

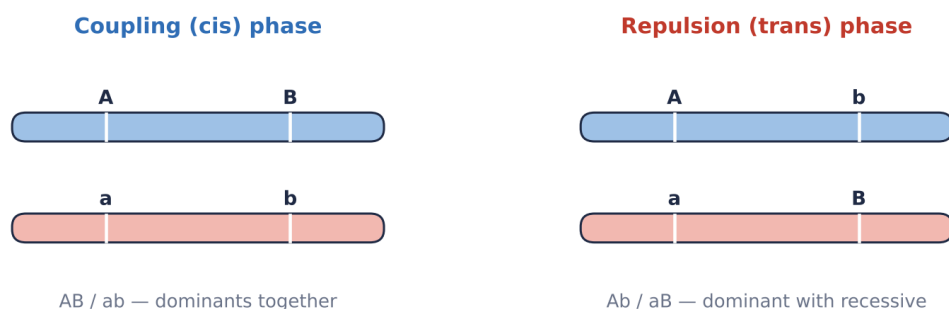


Fig. 1 — Arrangement of two linked genes in a double heterozygote.

The phase decides **which classes are parental**. In coupling (AB/ab) the parentals are AB and ab; in repulsion (Ab/aB) they are Ab and aB. Always identify the parental classes as the **most frequent** in a

test cross.

03 · Complete vs incomplete linkage

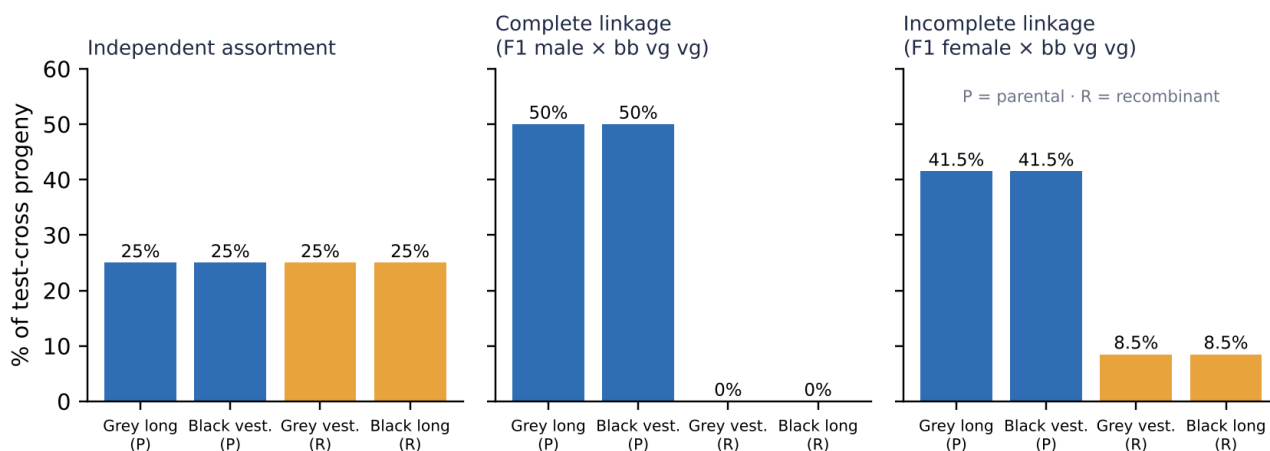


Fig. 2 — Morgan's *Drosophila* test crosses: grey body, long wing ($b^+ vg^+$) × black body, vestigial wing ($b vg$).

Complete linkage	Incomplete linkage
Genes so close that crossing over never separates them	Crossing over occasionally separates them
Only parental types in test cross (1:1)	Parentals > 50%, recombinants < 50%
e.g. F1 male <i>Drosophila</i> (no crossing over in males); female silkworm	e.g. F1 female <i>Drosophila</i> : 83% parental, 17% recombinant
Rare in nature	Common

Maize example (Hutchinson, 1922): coloured, full seed (C Sh) × colourless, shrunken (c sh). F1 test-crossed with c sh/c sh gave **96.4% parental** (C Sh and c sh) and **3.6% recombinant** (C sh and c Sh). So C and Sh are 3.6 cM apart.

04 · Theories of linkage

Theory	Proposed by	Explanation
Reduplication (differential multiplication) hypothesis	Bateson & Punnett (1911)	Gametic cells multiply unequally after segregation, e.g. 7:1:1:7 in coupling and 1:7:7:1 in repulsion. Rejected — no cytological basis
Chromosome theory of linkage	Morgan & Castle (1911)	Linked genes lie in a linear order on the same chromosome; closer genes show stronger linkage. Crossing over between them gives recombinants — accepted

Factor	Effect on linkage
Distance between genes	Closer genes → stronger linkage (fewer crossovers)
Age	Linkage strength increases with age in female <i>Drosophila</i> (crossing over falls)
Temperature	Extreme temperatures reduce linkage (more crossing over)
X-rays, chemicals	Reduce linkage by increasing crossing over
Sex	Complete linkage in male <i>Drosophila</i> and female silkworm
Inversions / centromere	Strengthen linkage by suppressing crossing over

Linkage	Independent assortment
Genes on the same chromosome	Genes on different chromosomes (or > 50 cM apart)
Parental combinations exceed 50%	All combinations equal (25% each in test cross)
Dihybrid test cross deviates from 1:1:1:1	Test cross gives 1:1:1:1
Reduces variation; keeps useful combinations together	Increases variation

Significance: keeps favourable gene combinations together; allows construction of chromosome maps; helps plant and animal breeders (undesirable linked genes must be broken by crossing over); used to locate disease genes by linkage with markers; explains deviations from Mendel's ratios.

05 · Recombination frequency & two-point mapping

Recombination frequency (RF) = (number of recombinant progeny ÷ total progeny) × 100. **1% RF = 1 map unit = 1 centimorgan (cM)**. A. H. Sturtevant (1913) used this to build the first genetic map (Drosophila X chromosome).

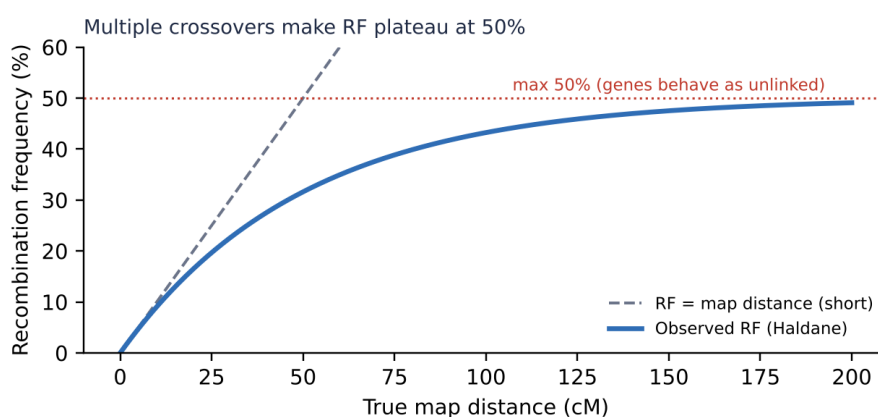


Fig. 3 — RF never exceeds 50%; long distances are underestimated because double crossovers go undetected.

Limitations of two-point crosses: (i) each cross gives only one distance; (ii) gene order is uncertain when distances are similar; (iii) **double crossovers are missed**, so long distances are underestimated. The **three-point test cross** fixes all three.

06 · Three-point test cross — method

A **trihybrid** (heterozygous for three linked genes) is crossed with a **triple-recessive tester**. The tester contributes only recessive alleles, so each progeny phenotype directly shows the gamete made by the trihybrid. Eight phenotypic classes appear, in **four reciprocal pairs** of roughly equal size.

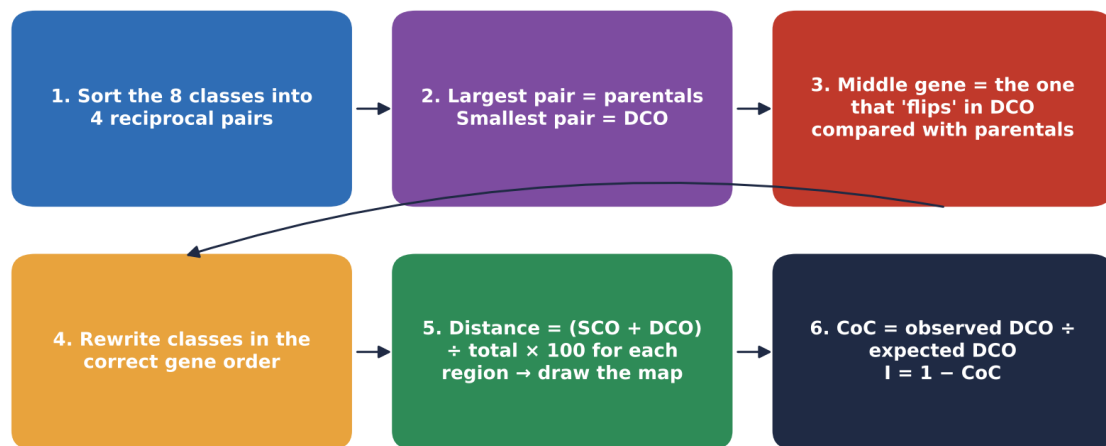


Fig. 4 — The six-step recipe used in every three-point problem.

Class pair	Size	Origin
Parental (non-crossover)	Largest	No crossover between the genes
Single crossover, region I	Intermediate	Crossover between gene 1 and middle gene
Single crossover, region II	Intermediate	Crossover between middle gene and gene 3
Double crossover (DCO)	Smallest	One crossover in each region

Key rule: Expected DCO frequency = RF(region I) × RF(region II). Because DCO need two simultaneous events, they are always the rarest class — that is how you spot them.

07 · Solved Example 1 — *Drosophila* (v, cv, ct)

Question. A female *Drosophila* heterozygous for *vermillion* eyes (v), *crossveinless* wings (cv) and *cut* wings (ct) was crossed with a triple-recessive male (v cv ct / v cv ct). The progeny were:



Tester gives only recessive gametes → each progeny phenotype directly reveals the F1 female's gamete

Phenotype (gamete from female)	Number
v cv ⁺ ct ⁺	580
v ⁺ cv ct	592
v cv ct ⁺	45
v ⁺ cv ⁺ ct	40
v cv ct	89
v ⁺ cv ⁺ ct ⁺	94
v cv ⁺ ct	3

Phenotype (gamete from female)	Number
$v^+ cv ct^+$	5
Total	1448

Determine (a) the gene order, (b) map distances, (c) the linkage map, (d) coefficient of coincidence and interference.

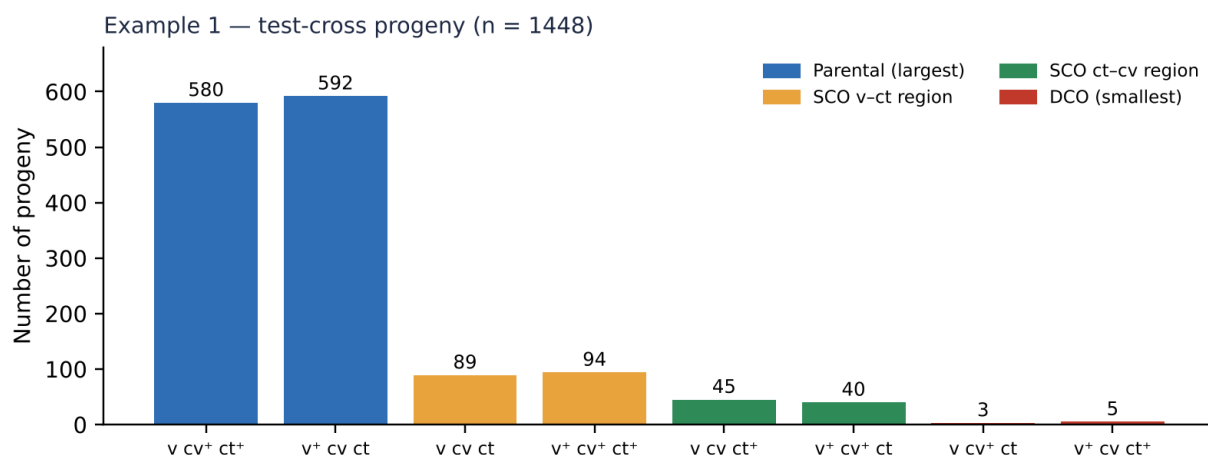
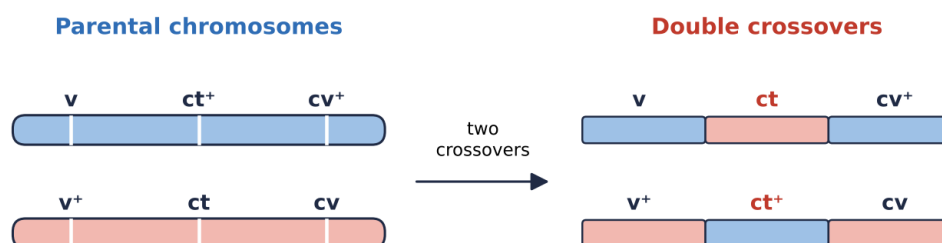


Fig. 5 — The four reciprocal pairs sorted by size.

Step 1-2: Identify classes. Parentals (largest): $v cv^+ ct^+$ (580) and $v^+ cv ct$ (592). DCO (smallest): $v cv^+ ct$ (3) and $v^+ cv ct^+$ (5).

Step 3: Gene order. Compare the DCO $v cv^+ ct$ with the parental $v cv^+ ct^+$: only **ct** differs. Hence **ct is in the middle** → order **v - ct - cv**.



Only the MIDDLE gene changes partners in a DCO. Compare DCO ($v cv^+ ct$, $v^+ cv ct^+$) with parentals ($v cv^+ ct^+$, $v^+ cv ct$): the gene that 'flips' is ct → order is **v - ct - cv**.

Fig. 6 — The middle gene is the one that switches in a double crossover.

Step 4: Rewrite in correct order (v ct cv) and assign regions:

Class (v ct cv order)	No.	Type	Crossover in
$v ct^+ cv^+ / v^+ ct cv$	$580 + 592 = 1172$	Parental	—
$v ct cv / v^+ ct^+ cv^+$	$89 + 94 = 183$	SCO	Region I (v-ct)
$v ct^+ cv / v^+ ct cv^+$	$45 + 40 = 85$	SCO	Region II (ct-cv)
$v ct cv^+ / v^+ ct^+ cv$	$3 + 5 = 8$	DCO	Both regions

Step 5: Map distances (DCO are added to both regions):

- $v - ct = (183 + 8) \div 1448 \times 100 = 191 \div 1448 \times 100 = \mathbf{13.2 \text{ cM}}$
- $ct - cv = (85 + 8) \div 1448 \times 100 = 93 \div 1448 \times 100 = \mathbf{6.4 \text{ cM}}$

- $v - cv = 13.2 + 6.4 = \mathbf{19.6 \text{ cM}}$. (A direct two-point count gives only $(183 + 85) \div 1448 = 18.5\%$ — the DCO are missed, showing why the three-point cross is more accurate.)

Linkage map — Example 1 (v - cv total = 19.6 cM)



Step 6: Interference.

- Expected DCO = $0.132 \times 0.064 \times 1448 = \mathbf{12.2}$
- Observed DCO = **8**
- CoC = $8 \div 12.2 = \mathbf{0.65}$
- Interference $I = 1 - 0.65 = \mathbf{0.35} \rightarrow 35\%$ of expected double crossovers did not occur.

08 · Solved Example 2 — gene order not given (p, q, r)

Question. A trihybrid of unknown phase was test-crossed with $p \ q \ r / p \ q \ r$. Progeny (written in $p \ q \ r$ order, not necessarily the true order):

Class	No.
$p \ q^+ \ r$	352
$p^+ \ q \ r^+$	340
$p \ q \ r$	55
$p^+ \ q^+ \ r^+$	53
$p \ q^+ \ r^+$	96
$p^+ \ q \ r$	92
$p^+ \ q^+ \ r$	7
$p \ q \ r^+$	5
Total	1000

Parentals: $p \ q^+ \ r$ (352), $p^+ \ q \ r^+$ (340) — so the F1 was $p \ q^+ \ r / p^+ \ q \ r^+$ (mixed phase). **DCO:** $p^+ \ q^+ \ r$ (7), $p \ q \ r^+$ (5).

Gene order: DCO $p \ q \ r^+$ vs parental $p^+ \ q \ r^+$ → only **p** differs → **p is the middle gene**. Order: **q - p - r**.

Rewritten (q p r)	No.	Type
$q^+ \ p \ r / q \ p^+ \ r^+$	$352 + 340 = 692$	Parental
$q \ p \ r / q^+ \ p^+ \ r^+$	$55 + 53 = 108$	SCO region I (q-p)
$q^+ \ p \ r^+ / q \ p^+ \ r$	$96 + 92 = 188$	SCO region II (p-r)
$q^+ \ p^+ \ r / q \ p \ r^+$	$7 + 5 = 12$	DCO

- $q - p = (108 + 12) \div 1000 \times 100 = \mathbf{12 \text{ cM}}$
- $p - r = (188 + 12) \div 1000 \times 100 = \mathbf{20 \text{ cM}}$
- Expected DCO = $0.12 \times 0.20 \times 1000 = 24$; CoC = $12 \div 24 = \mathbf{0.5}$; $I = \mathbf{0.5}$

Linkage map — Example 2 (q-r total = 32 cM)



09 · Solved Example 3 — predicting progeny from a map

Given map — Example 3



Question. Genes a, b, c are arranged as shown (a-b = 10 cM, b-c = 20 cM) with interference = 0.4. A trihybrid **A B C / a b c** is test-crossed and 2000 progeny are scored. Predict each class.

- Expected DCO = $0.10 \times 0.20 \times 2000 = 40$. CoC = $1 - 0.4 = 0.6 \rightarrow$ observed DCO = $0.6 \times 40 = \mathbf{24}$ (12 A b C + 12 a B c).
- Region I total = 10% of 2000 = 200, which includes DCO $\rightarrow$ SCO I = $200 - 24 = \mathbf{176}$ (88 A b c + 88 a B C).
- Region II total = 20% of 2000 = 400 $\rightarrow$ SCO II = $400 - 24 = \mathbf{376}$ (188 A B c + 188 a b C).
- Parentals = $2000 - 24 - 176 - 376 = \mathbf{1424}$ (712 A B C + 712 a b c).

Class	Expected number
A B C / a b c (parental)	712 / 712
A b c / a B C (SCO I)	88 / 88
A B c / a b C (SCO II)	188 / 188
A b C / a B c (DCO)	12 / 12
Total	2000

10 · Practice problems (answers below)

P1. A test cross of an x y z trihybrid gave: + + + 382; x y z 377; x⁺ y z 46; x y⁺ z⁺ 45; x y⁺ z 70; x⁺ y z⁺ 71; x y z⁺ 5; x⁺ y⁺ z 4 (total 1000). Find the gene order, distances, CoC and interference.

P2. In a test cross, 1000 progeny include 60 double crossovers. If the two regions are 20 cM and 25 cM, find the coefficient of coincidence. What does the value tell you?

P3. A two-point cross gives AB 46%, ab 44%, Ab 5%, aB 5%. Are A and B linked? State the phase and the distance.

P4. Genes are ordered d - e - f with d-e = 15 cM and e-f = 10 cM and no interference. In 1000 test-cross progeny, how many double crossovers are expected?

Answers: **P1:** parentals +++ / x y z (coupling); DCO x y z⁺ and x⁺ y⁺ z $\rightarrow$ z is middle; order **x - z - y**; x-z = $(46+45+9)/1000 = \mathbf{10 \text{ cM}}$; z-y = $(70+71+9)/1000 = \mathbf{15 \text{ cM}}$; expected DCO 15, CoC = $9/15 = \mathbf{0.6}$, I = **0.4**.

P2: expected DCO = $0.20 \times 0.25 \times 1000 = 50$; CoC = $60/50 = \mathbf{1.2} \rightarrow$ more DCO than expected = **negative interference** (I = -0.2).

P3: linked (parentals 90%), **coupling** phase, RF = 10% $\rightarrow \mathbf{10 \text{ cM}}$.

P4: $0.15 \times 0.10 \times 1000 = \mathbf{15}$.

11 · End-semester exam questions

Short answer (2-5 marks)

- Define linkage and linkage group. How many linkage groups are there in humans and in *Drosophila*?
- Distinguish between coupling and repulsion phases.
- Differentiate complete and incomplete linkage with examples.
- What is the reduplication hypothesis? Why was it rejected?
- Why is there no crossing over in male *Drosophila*? What is its genetic consequence?
- Define centimorgan. Why can RF not exceed 50%?
- How is the middle gene identified in a three-point test cross?
- Define coefficient of coincidence and interference. What is negative interference?

Long answer (8-15 marks)

1. What is linkage? Describe Morgan's experiments on complete and incomplete linkage in *Drosophila*.
2. Discuss the theories of linkage and the factors affecting linkage. Add a note on its significance.
3. Explain the three-point test cross. How does it overcome the limitations of the two-point cross?
4. Solve a three-point test-cross problem (as in Examples 1-2): gene order, map distances, map and interference.

12 · Case-based MCQs

1. A dihybrid test cross gives 1:1:1:1. The genes are most likely:
(A) Completely linked (B) Unlinked or far apart (C) Sex-linked (D) Tightly linked
2. An F1 male *Drosophila* $b^+vg^+/b\ vg \times b\ vg/b\ vg$ gives only parental types because:
(A) Black is lethal (B) Vestigial is dominant (C) Crossing over does not occur in males (D) The genes are on different chromosomes
3. Number of linkage groups in maize ($2n = 20$) is:
(A) 10 (B) 5 (C) 40 (D) 20
4. In a three-point test cross the two smallest classes represent:
(A) Mutants (B) Parentals (C) Double crossovers (D) Single crossovers in region I
5. Parentals: A b C and a B c; DCO: A B C and a b c. The middle gene is:
(A) B (B) A (C) C (D) Cannot tell
6. Regions of 10 cM and 30 cM, 1000 progeny, 15 DCO observed. Interference is:
(A) 2 (B) 0.3 (C) 0.15 (D) 0.5
7. RF between two genes is 8%. Their map distance is:
(A) 80 cM (B) 0.08 cM (C) 8 cM (D) 92 cM
8. A direct two-point count between the outer genes gives a smaller value than the sum of the two intervals because:
(A) Double crossovers are missed (B) Of interference only (C) Of mutation (D) Of linkage drag
9. A trihybrid in repulsion has parental classes:
(A) The two most frequent classes, mixing dominant and recessive (B) Any two classes (C) The smallest classes (D) Always +++ and abc

10. Who constructed the first genetic linkage map?

(A) Mendel (B) Bateson (C) Morgan (D) Sturtevant

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

13 · Formula sheet

Quantity	Formula
Recombination frequency	$RF (\%) = \text{recombinants} \div \text{total} \times 100$
Map distance	$1\% RF = 1 \text{ cM}$
Distance in 3-point cross	$(SCO \text{ in region} + \text{all DCO}) \div \text{total} \times 100$
Expected DCO	$RF_I \times RF_{II} \times \text{total}$
Coefficient of coincidence	$CoC = \text{observed DCO} \div \text{expected DCO}$
Interference	$I = 1 - CoC$ ($I < 0 \rightarrow$ negative interference)
RF from chiasmata	$RF = \frac{1}{2} \times \% \text{ meiocytes with a chiasma between the genes}$
Middle gene	The allele that switches between parental and DCO classes