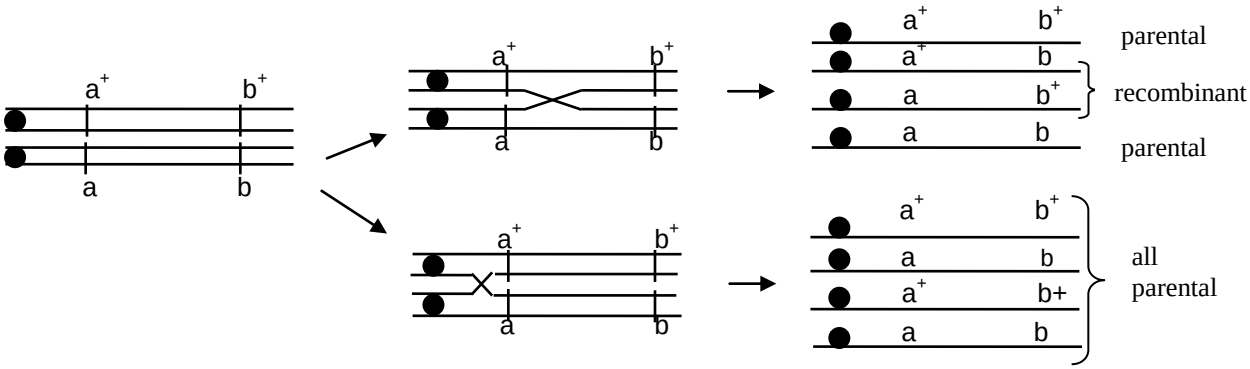
Lecture 6 Gene mapping by 3-point crosses

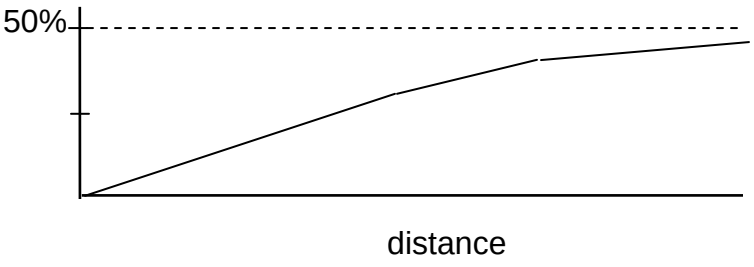
1. Two-point testcross (a brief review of Lecture 5)
2. Three-point testcross
3. Double cross-overs
4. Interference
5. X-chromosome mapping in humans

Two-point testcross

RF between linked loci is less than 50% because cross-overs frequently occur outside the distance between the loci in question:



Frequency (and map distance) between the loci is related to their physical distance, and the graph asymptotically approaches the 50% level. When the frequency is about 50%, the two genes are either on separate chromosomes, or they are on the same chromosome but very far from each other.



Within the region of linearity, the frequency of the recombinant offspring can be used as a tool to determine distances between the genes on a chromosome.

P: $\frac{a+\ \ b+}{a+\ \ b+}$ x $\frac{a\ \ b}{a\ \ b}$ a+, b+ : dominant wild type;
a, b : recessive mutant characters

F1 $\frac{a+\ \ b+}{a\ \ b}$

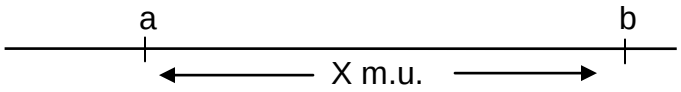
To study recombination products in F1, we perform a test cross:

an F1 female $\frac{a+\ \ b+}{a\ \ b}$ is crossed to a recessive homozygous male $\frac{a\ \ b}{a\ \ b}$

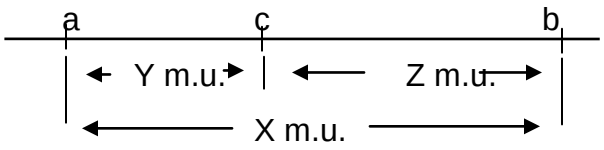
Progeny of four genotypes and phenotypes classes is obtained, as determined by the four classes of the mother's gametes:

Genotypes:			Phenotypes:
$\frac{a+\ \ b+}{a\ \ b}$ $\frac{a\ \ b}{a\ \ b}$	parental	$a^+\ \ b^+$; $a\ \ b$ (100-X)%	parental
$\frac{a+\ \ b}{a\ \ b}$ $\frac{a\ \ b+}{a\ \ b}$	recombinant	$a^+\ \ b$; $a\ \ b^+$ X%	recombinant

Therefore, on a linkage map the loci *a* and *b* are separated by X m.u.



We can do a similar procedure for loci *a* and *c*, and loci *b* and *c*, determine the recombination frequencies and draw a final linkage map for the three loci:



Three-point test cross

Instead of using three two-point crosses, we can use a single three-point cross.

The following example is from the textbook:

Mutant alleles *v,* determines vermilion eyes (bright red)

cv, crossveinless wings

ct, cut wing edges

P: $v^+/ v^+ \cdot cv / cv \cdot ct / ct$ X $v / v \cdot cv^+/ cv^+ \cdot ct^+ / ct^+$ (in the arbitrary order!)

F1: ♀ $v^+ / v \cdot cv / cv^+ \cdot ct / ct^+$ is test-crossed with triple recessive homozygous male

X ♂ $v / v \cdot cv / cv \cdot ct / ct$

Female gametes determine eight phenotypic classes:

v	cv+	ct+	580	Parental, no CO
v+	cv	ct	592	
v	cv	ct+	45	SCO between ct and cv
v+	cv+	ct	40	
v	cv	ct	89	SCO between v and ct
v+	cv+	ct+	94	
v	cv+	ct	3	DCO
v+	cv	ct+	5	

Total 1448

How to derive a linkage map for these three loci?

1. First, we identify the parental types. They are not necessarily completely wild type or mutant:

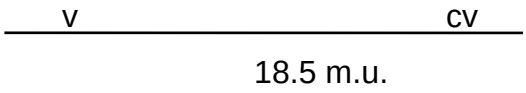
v cv+ ct+ and v+ cv ct

2. Now let's select any two loci, for example *v* and *cv*. Recombinants for these two loci should have a nonparental configuration: v cv and v+ cv+.

Parental: v cv+ Recombinant: v cv

v+ cv v+ cv+

Let's identify and count the corresponding phenotypic classes: 45 + 40 + 89 + 94 = 268. Recombination frequency in this case is the same as the frequency of recombinant progeny: 268/ 1448 = 18.5 %



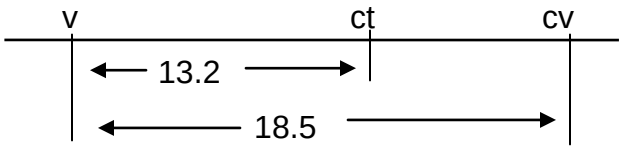
2. Now select the other two, e.g. *v* and *ct*.

Parentals are: v ct+ Recombinants are: v ct

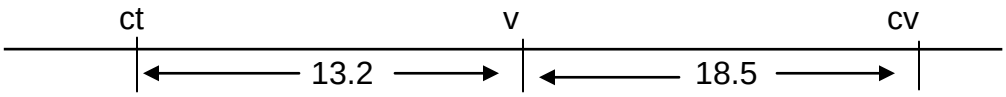
v+ ct v+ ct+.

Count their frequency: 89 + 94 + 3 + 5 /1448 = 0.132%. RF (v, ct) = 13.2%.

Two map options are available:

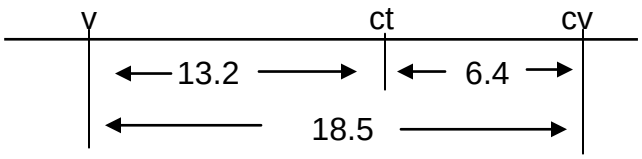


or



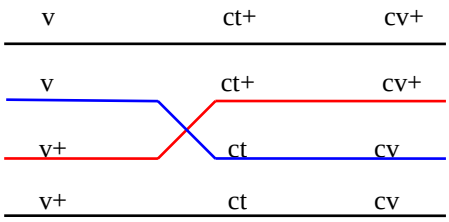
3. Let's look at the third pair of loci: *ct* and *cv*. Recombinant are ct+ cv and ct cv+: 45 + 40 + 3 + 5 /1448 = 6.4%.

Thus, we have to choose the first option:

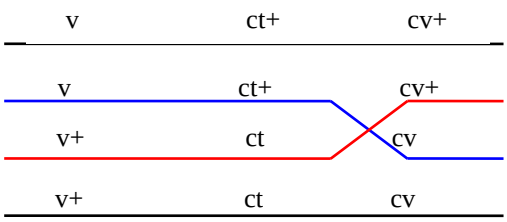


However, paradoxically there is a discrepancy: 13.2 + 6.4 = 19.6 > 18.5. What's wrong? We must have missed some recombination events when we considered recombinant progeny between the two most distant loci - *v* and *cv*.

Now we know configuration of alleles in the F1 heterozygote. We can figure out what happens when there is a cross-over between any two loci:

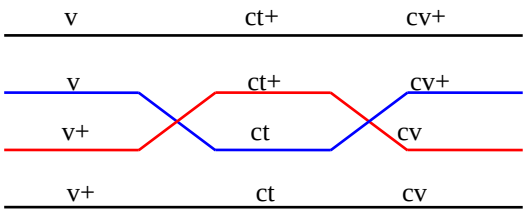


This generates the recombinant classes v ct cv and v^+ ct^+ cv^+.



This generates the recombinant classes v ct^+ cv and v^+ ct cv^+ .

But what if a double cross-over occurs between the loci v and cv?



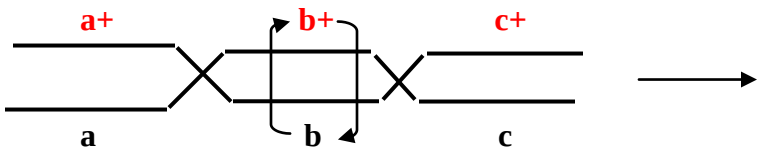
This generates the recombinant classes v ct cv^+ and v^+ ct^+ cv. They look like parental type with regards to v and cv loci, we did not count them originally.

Recombination frequency determined between two distant loci without accounting for double cross-overs is an underestimate.

Corrected map distance needs to take into account the double cross-over progeny twice:

RF (v,cv) = 45 + 40 + 89 + 94 + 2x3 + 2x5 / 1448 = 0.196 or 19.6%

In order to deduce a gene order, one only needs to identify a 'flip-flop' locus:



a+ b c+

a b+ c

Parental classes (arbitrary order)

v cv+ ct+

v+ cv ct

flip-flop

Double CO classes (arbitrary order)

v cv+ ct

v+ cv ct+

The REAL gene order:

v--------ct--------cv

which is the same as:

cv------ct--------v

Interference

When two cross-overs occur on the same chromosome they may interfere with each other. Expected frequency of double cross-overs is determined by product rule:

Exp. DCO (v, cv) = CO (v, ct) x CO (ct, cv) = 0.132 x 0.064 = 0.0084

Expected number of the double cross-over progeny:

0.0084 x 1448 = 12.

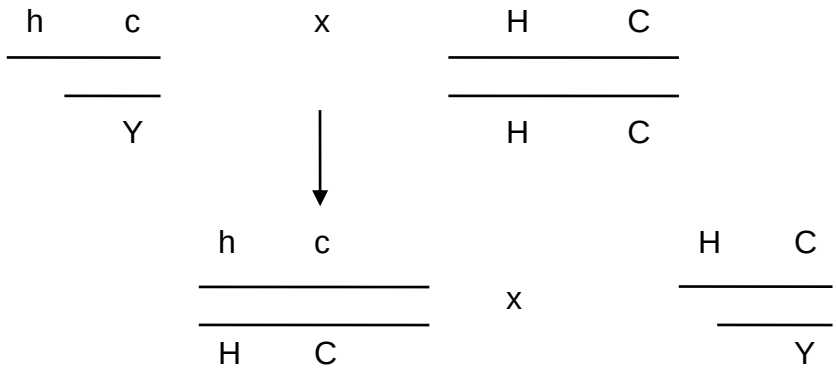
But we saw only 8 double crossover progeny. Coefficient of coincidence is determined as the ratio of observed DCO and expected DCO, in our case 8/12.

Interference (I) = (1 – coefficient of coincidence) x 100% = (1- 8/12) x 100% = 33%

When there are no observed cross-overs, interference is 100%, and when the number of observed DCO equals the number of expected DCO, I = 0%.

Linkage mapping of X-chromosome in humans

Especially amenable for linkage mapping is the X-chromosome, because it is hemizygous in males.



Male offspring of the double heterozygous female is then considered:

h c
H C
} parental type progeny

h C
H c
} recombinant progeny