Genetics Lecture Notes

7.03 2005

Lectures 6 – 9

Lecture 6

Until now our analysis of genes has focused on gene function as determined by phenotype differences brought about by different alleles or by a direct test of function – the complementation test.

For the next five lectures our analysis will be concerned with the tests of gene position starting with the position of genes on chromosomes and finally mapping point mutations at the resolution of single nucleotide pairs.

We've taken it for granted that genes reside on chromosomes, but how do we know this? Let's review the properties of gene segregation.

Consider a cross between individuals that differ in two different traits:

A/A, B/B × a/a, b/b

The gametes from one parent will be **A**, **B** and from the other parent **a, b**

These gametes will then give an F_1 generation of all **A/a, B/b**

Crosses between F_1 individuals will give an F_2 generation with a 9 : 3 : 3 : 1 phenotypic ratio.

A better way to look at segregation is by a test cross of the F_1 heterozygote to a homozygous recessive individual.

A/a, B/b × a/a, b/b

The possible gamete genotypes from the F_1 will be:

A, B	**a, b**	**A, b**	**a, B**
(parental)	(parental)	(recombianant)	(recombinant)

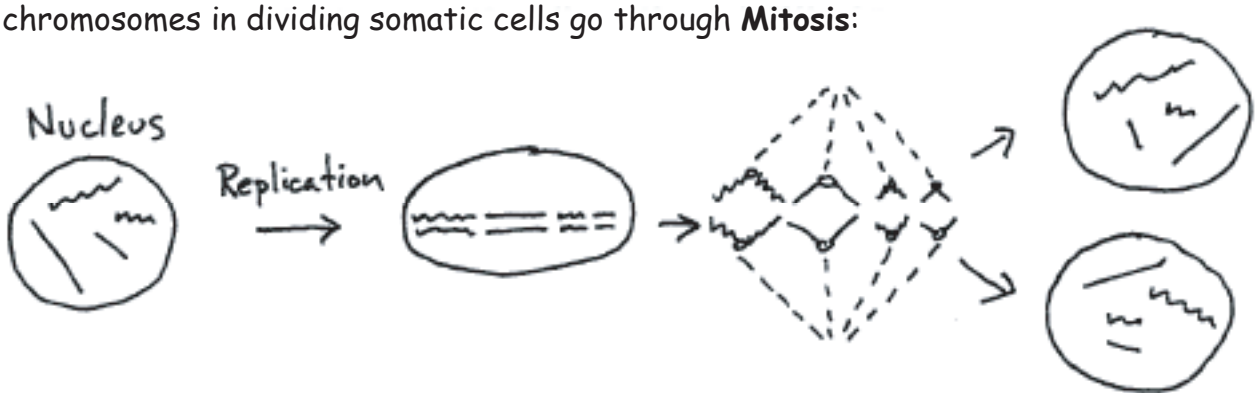
The corresponding genotypes of the offspring in the testcross will be:

A/a, B/b **a/a, b/b** **A/a, b/b** **a/a, B/b**

Note that each genotype will have a unique and identifiable phenotype

Each offspring receives either one or the other parental allele: **gene segregation**.
For most gene pairs, the frequency of each of the four classes of gametes is the same indicating that the two genes segregate independently: **independent assortment**.
At the turn of the century microscopes allowed people to watch chromosomes in the nuclei of dividing cells. (Human cells, for example, contain 46 chromosomes.)

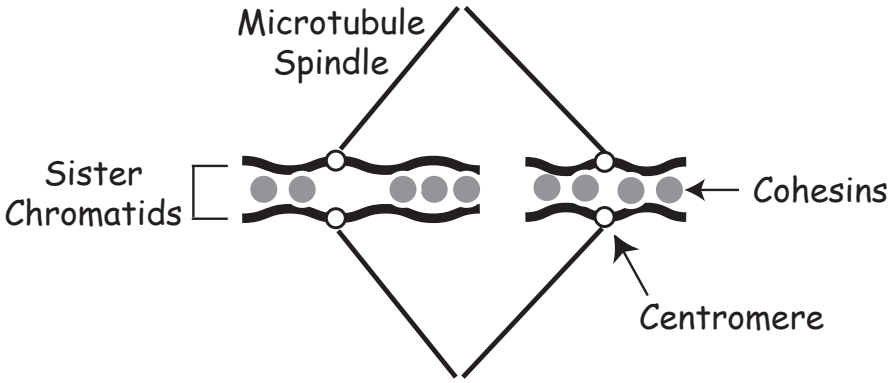
The chromosomes in dividing somatic cells go through **Mitosis**:



The net result of mitosis is to distribute a replica of each chromosome into the two daughter cells.

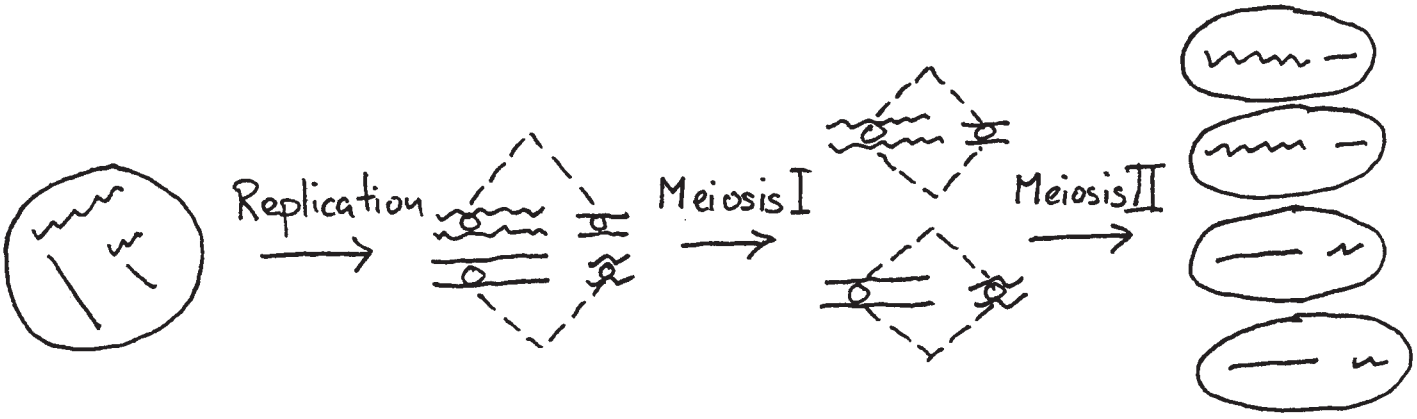
The stages of mitosis are as follows:

i) **Interphase**; DNA replication
ii) **Prophase**; Chromosomes condense and centromeres attach to microtubule spindle
iii) **Metaphase**; Chromosomes align
iv) **Anaphase**; Sister chromatids move apart
v) **Telophase**; Nuclei reform



The cell has evolved a simple mechanical mechanism to insure that after mitosis each daughter cell has received exactly one copy of each chromosome. (Failure of proper chromosome segregation is known as nondisjunction). The steps in the mechanism are as follows: 1) After DNA replication two daughter chromosomes known as sister chromatids are held together by special proteins known as cohesins. 2) As chromosomes align in metaphase microtubule spindles attach to centromeres on each chromatid. 3) Once all of the chromatids are attached to spindles a protease known as separase becomes active (Actually unattached chromatids produce a signal to keep separase inactive and only when every chromatid pair is under tension generated by spindles pulling in opposite directions is the inhibitory signal turned off.) 4) Finally, active separase cleaves the cohesin proteins detaching sister chromatids and allowing them be pulled apart by the spindle to be distributed to different daughter cells.

Cells in production of germ cells such as pollen undergo a very different kind of division, **Meiosis**.



Meiosis differs from mitosis in two fundamental respects: 1) in meiosis there are two rounds of chromosome segregation for one round of synthesis so each germ cell receives only one of the two homologous chromosomes and 2) in meiosis the homologs pair with one another then move to opposite poles.

Chromosomes behave in meiosis the same way that Mendel showed genes to behave. Each germ cell receives only one of the two homologs, a behavior that is analogous to gene segregation.

The relative alignment of chromosomes is arbitrary which is analogous to independent assortment of genes.

To show that genes are on chromosomes what was needed was a chromosome that could be identified in the microscope and that carried an allele for a trait that could also be followed. The proof for chromosome theory would then depend on correlating the segregation of the trait with segregation of the chromosome.

T.H. Morgan proved chromosome theory in 1910 using Drosophila.

Flies normally have brick-red eyes. The first white-eyed mutant was found by Morgan's wife, Lillian, who worked in the lab.

white ♂ x red ♀ (wild type)

↓

F_1: all red

↓

F_2: 3 red : 1 white (but only males)

Thus, the white mutation behaves like a recessive allele, but there was something unusual about the white mutation because only the male flies in the F_2 have white eyes.

white ♂ x red ♀ (heterozygote from F_1)

↓

1 red : 1 white

In this case equal numbers of males and females have white eyes. Again this is consistent with white being a recessive trait. The most informative cross is the reciprocal of the first cross.

white ♀ x red ♂ (wild type)

↓

red ♀, white ♂

Ignoring the sex of the flies, it looks as if a wild type ♂ is heterozygous: the wild type allele is always passed on to the daughters and the white allele is passed on to sons.

The explanation is that eye color gene is on the sex determining chromosome X. Males only have one copy of the X chromosome and daughters always get one copy of the X from the mother and one copy from the father.

♀ (XX) x ♂ (XY)

XX XY

X^w = white allele on X, X^+ = red allele on X

X^wX^w x X^+Y

↓

X^wX^+ X^wY

red ♀ white ♂

Thus, the trait for red eyes is always inherited along with the X chromosome from the father. The absence of red (giving white eyes) always goes with the Y chromosome.

Lecture 7

When we talk about gene position the term **locus** is used to designate the chromosomal location of a gene.

What we are going to do is to map genes relative to one another. To begin, we need two genes on the same chromosome. Last lecture we saw how you could tell whether a gene is on the X chromosome by how alleles of the gene are inherited differently by males and females.

Consider two mutations on the X chromosome of Drosophila; crossveinless and white eye.

Genotype	Phenotype
X^{cv+w+} y	wild-type
X^{cv-w+} y	crossveinless wings
X^{cv+w-} y	white eye

X^{cv-w+} y x X^{cv+w-} X^{cv+w-} (true breeding)

All of the daughters from this cross will have two different X-chromosomes, which differ at two loci: X^{cv+w-} X^{cv-w+}

We want to follow these X chromosomes into the next generation so after a cross we look at male flies.

parental classes: X^{cv+w-} y and X^{cv-w+} y

crossover classes: X^{cv-w-} y and X^{cv+w+} y
(crossveinless, white) (wild type)

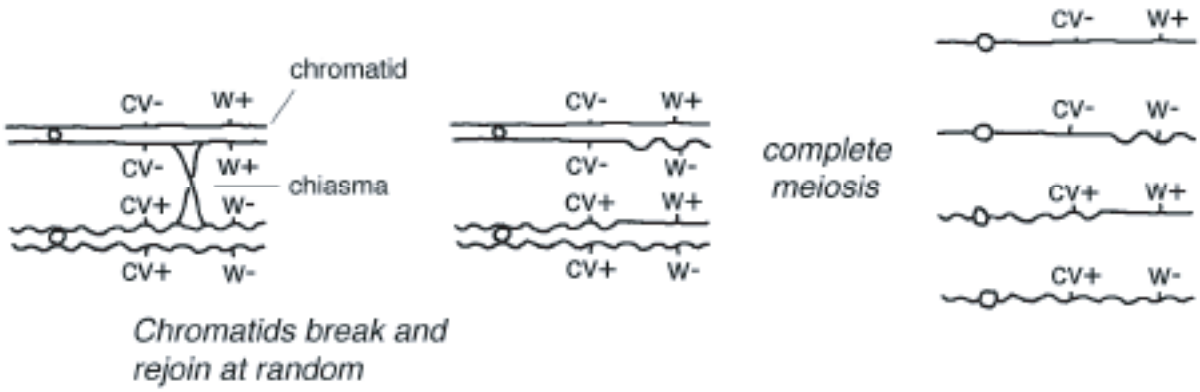
In the crossover classes the alleles appear to have separated and moved from one X to the other. Genes on the same chromosome often do not assort independently. Such behavior is known as **Linkage**.

unlinked — crossover classes appear at same frequency as parental classes.
(Note that traits that show independent assortment are unliked)

weakly linked – crossover classes appear often but less often than parental classes.

tightly linked — crossover classes appear rarely or never.

To see what's really going on we need to look at the chromatids in prophase of meiosis in the mother.



Crossovers between homologous chromosomes occur more or less at random during meiosis. To give you a rough idea of how frequent these crossovers are, in several different well studied organisms (Yeast, Drosophila, and humans) there is about one crossover per chromosome arm per meiosis. The geneticist uses these random crossovers as a tool to measure distance. Distance can be obtained because crossovers between two points that are close together will rarely occur whereas crossovers between points that are far apart will occur frequently.

Definition of genetic distance:

$$\text{map distance (m.u. or cM)} = 100 \times \frac{\text{crossover gametes}}{\text{total gametes}}$$

♀ $\frac{cv^-\ W^+}{CV^+\ w^-}$ × ♂ (Note new notation)

(In order to detect both dominant and recessive alleles, we look at males only)

$cv^-\ W^+$	430
$CV^+\ w^-$	450
$cv^-\ w^-$	52
$CV^+\ W^+$	68

Number of crossover gametes = 120 Total gametes = 1,000

$$\text{Distance} = 100 \times \frac{120}{1000} = 12 \text{ cM}$$

It is important to note that once a map distance between two genetic markers has been established this distance can be used to calculate the expected numbers of each type of progeny. For example, if you know that two mutations are 12 cM apart then you should expect that 6% of the progeny from a cross will be of each recombinant class.

Things get interesting when we make several pairwise crosses between genes on the same chromosome.

We can use this data to construct a **Genetic Map**

Genetic maps have the following properties:
i) Distance is proportional to frequency of crossover classes (this approximation actually only holds for relatively short distances of less than about 20 cM)

ii) Distances are approximately additive: mapped points fall on a line.

iii) Maps are internally consistent and concise.

(The first genetic map was constructed in 1911 by Alfred Sturtevant when he was a sophomore student in Morgan's lab)

It is important to remember that genetic distances are measured using a property of meiosis (genetic recombination) that varies from one organism to another. The relationship between genetic distance and actual physical distance can be summarized in this way:

Genetic distance = physical distance x recombination rate

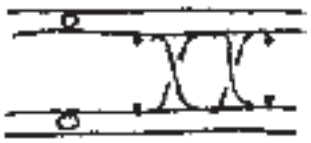
The actual relationship between genetic distance in cM and physical distance in base pairs (bp) depends on the recombination rate and is different for different organisms.

For example: Human: 1.3 cM/Mbp Yeast: 360 cM/Mbp

Sometimes recombination rates in the male and female of a species are different. In Drosophila there is no recombination in the male so the genetic distance between markers on the same chromosome are always zero when examined by meiosis in the male. In humans the recombination rate (and therefore map distances) in the female are twice that of the male.

Another issue that is often misunderstood concerns the map distances of genes that are far apart on the same chromosome.

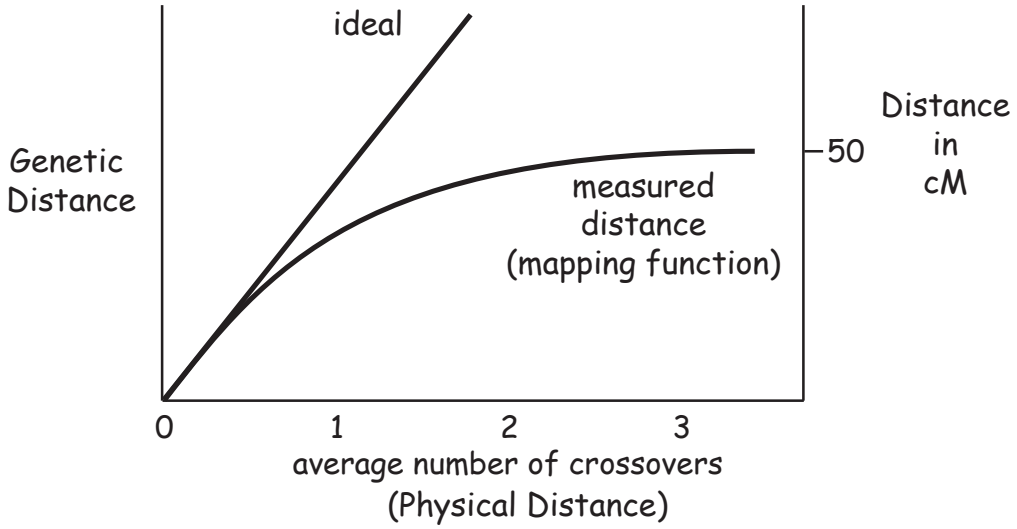
The physical length of a genetic interval is proportional to the frequency of crossovers that occur in that interval during meiosis but in a cross we are not actually counting crossovers rather we are counting the number of recombinant progeny that are produced. The number of recombinants provide a good approximation of distance for short intervals but as the interval length increases, multiple crossovers are possible making the relationship between frequency of recombinants and crossovers not linear.



Crossovers between same chromatids	Recombinant progeny
1	yes
2	no
3	yes
4	no

This produces a relationship between physical distance and measured distance that is known as a **Mapping Function.**

Here the ideal (linear) mapping function is is compared to the actual function for two markers.



If the measured distance in a cross is statistically indistinguishable from 50 cM then we say that the genes are unlinked. This doesn't mean that distances greater than 50 cM can not be obtained. By adding intervals, longer distances that are meaningful can be obtained. For example, if all the intervals between linked genes in the human genome are added together the total length of the genome (in males) is 2,500 cM.

Lecture 8

In the last lecture we discussed how to measure the distance between two genes on the X chromosome. To do this we used the trick of looking only at male progeny so the genotype of the X chromosome could be scored directly since these flies only carry one copy of the X. For autosomes, we can have the same ability to score all recombinant classes by crossing to a homozygous recessive individual (ie a test-cross).

Consider the recessive traits vestigial wings and short bristles that are specified by two different genes on the same autosome (non-sex chromosome).

$$\text{♀}\ \frac{vg\quad sh}{vg\quad sh} \times \text{♂}\ \frac{+\quad +}{+\quad +}$$

$$\downarrow$$

All F_1: $\dfrac{vg\quad sh}{+\quad +}$

The F_1 flies are heterozygous for both genes so we are in position to see how often crossovers between these chromosomes occur in meiosis by doing a test-cross.

$$\text{♀}\ \frac{vg\quad sh}{+\quad +} \times \text{♂}\ \frac{vg\quad sh}{vg\quad sh}$$

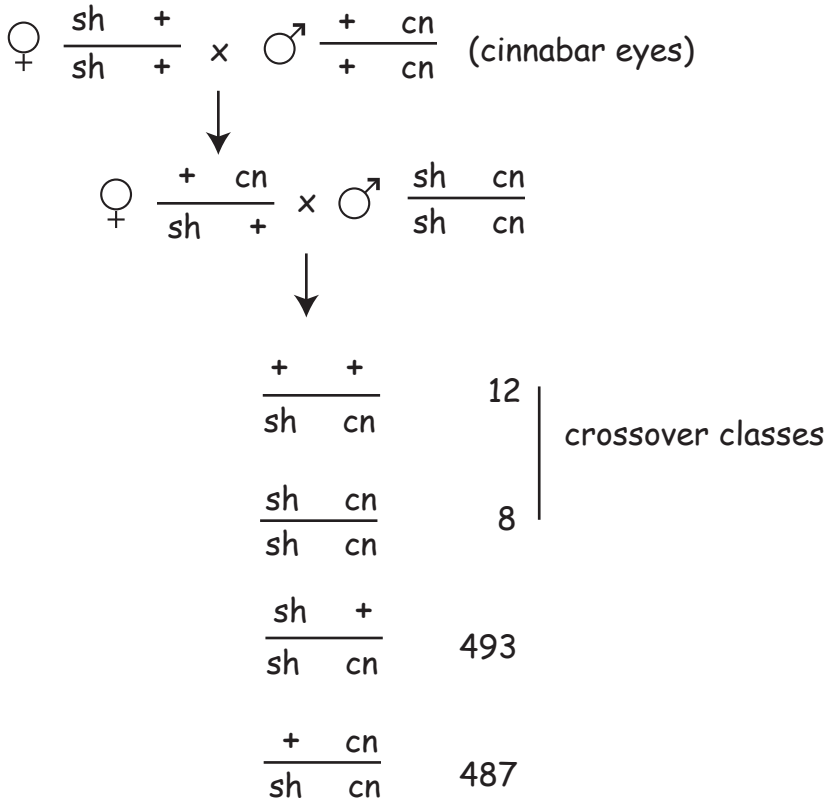
$$\downarrow$$

(Note that the progeny have distinct phenotypes)

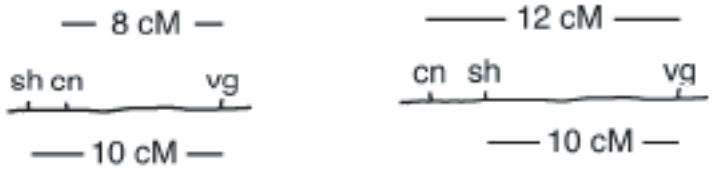
$\dfrac{vg\quad sh}{vg\quad sh}$	458	
$\dfrac{+\quad +}{vg\quad sh}$	442	
$\dfrac{+\quad sh}{vg\quad sh}$	47	(crossover classes)
$\dfrac{vg\quad +}{vg\quad sh}$	53	

The distance between vg and sh = $100 \times \dfrac{100}{1000}$ = 10 cM

Now we'll do a second cross. Note that the key is to set up a parent that is heterozygous at two loci.



Distance between sh and cn = 2 cM



There are two possible orders. We could resolve them by measuring the cn to vg distance, which should be either 8 cM or 12 cM depending on the order. However, it's difficult in practice to get a statistically significant measurement that would cleanly distinguish between these possibilities.

A better way to find the order is to set up all three heterozygous markers at the same time and to look at the frequencies of the eight different gamete genotypes.

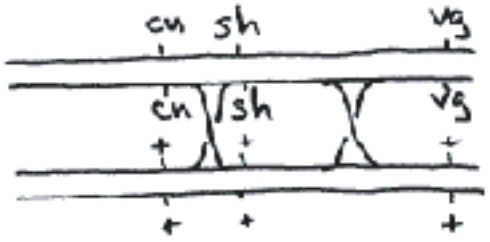
This is known as a 3 factor cross

$$\text{♀}\ \frac{cn\ \ sh\ \ vg}{+\ \ +\ \ +} \times \text{♂}\ \frac{cn\ \ sh\ \ vg}{cn\ \ sh\ \ vg}$$

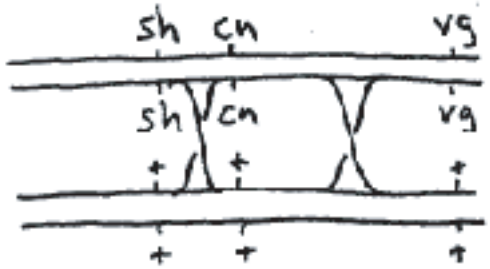
cn sh vg	900
+ + +	912
cn + +	2
+ sh vg	1
cn sh +	75
+ + vg	70
cn + vg	18
+ sh +	22

These are all of the possible combinations. One pair of these gamete classes must be the result of double crossovers. This class will be very rare ($0.1 \times 0.02 = 2 \times 10^{-3}$). By finding the rare class we have a qualitative test to determine gene order.

The double crossover classes for the two possible orders are:



cn + vg
or
+ sh +
(If this were the order, these would be the rare classes)



sh + vg
or
+ cn +
(Since these are the rare classes we know this to be the order)

There is a simple system for evaluating 3-factor crosses:

1) Group recombinant classes into reciprocal pairs.

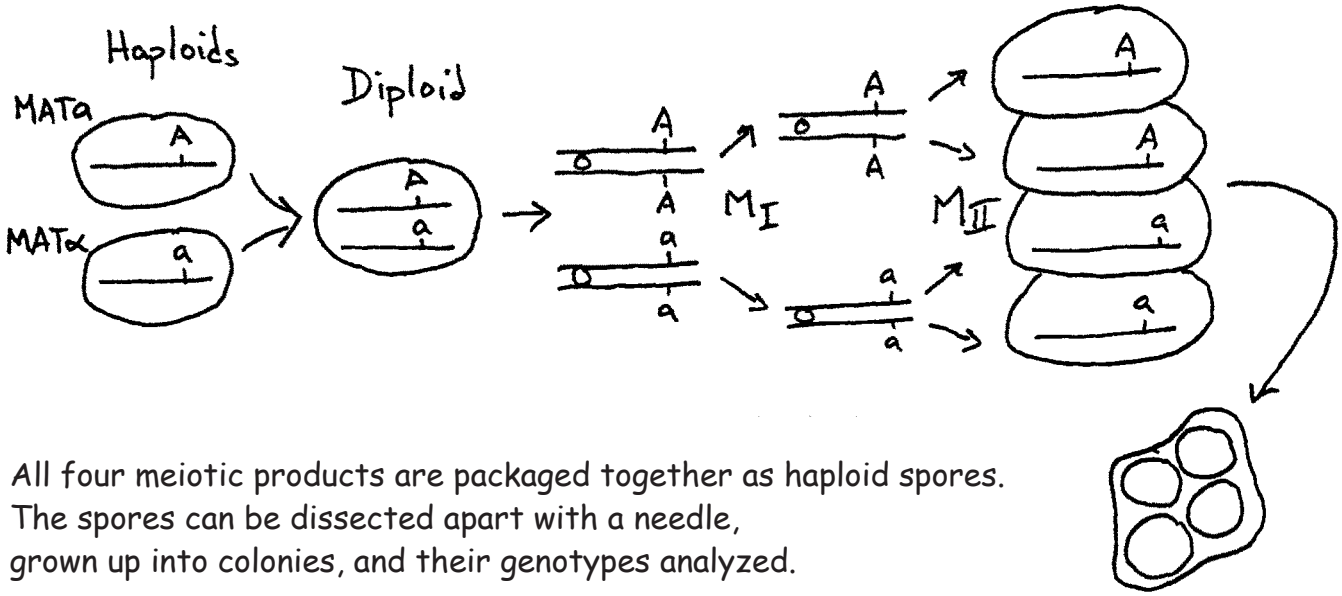
2) The most frequent pair is the parental classes.

3) Derive the gene order from the least frequent pair, which are the double crossover classes.

4) The single crossover frequency for the two intervals can be obtained by adding the frequency of each of the single crossover class pairs to the frequency of the double crossover class pair. (In the present example the double crossovers are so rare that their inclusion doesn't matter).

Lecture 9

Meiosis in yeast is particularly easy to study.



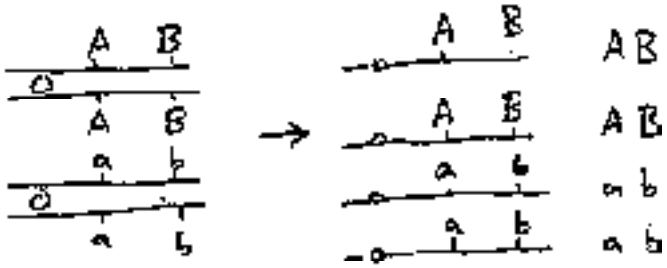
All four meiotic products are packaged together as haploid spores.
The spores can be dissected apart with a needle,
grown up into colonies, and their genotypes analyzed.

4 spores = tetrad

The ability to look at the genotypes of all four gametes gives us extra information about the meiosis that is not obtainable in diploid organisms (eg. mice, flies and peas) where only one of the meiotic products is selected at random.

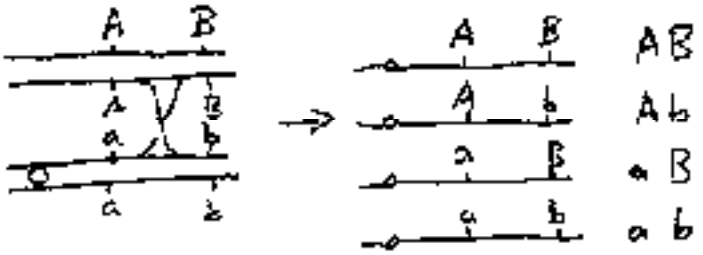
Consider two linked genes in a cross: A B x a b $\xrightarrow{\text{mate}}$ $\frac{A\ B}{a\ b}$ (diploid) $\xrightarrow{\text{sporulate}}$ Tetrad

Meiosis with
no crossovers



PD
(Parental ditype)

Meiosis with one
crossover



T
(Tetratype)

If the genes are close together multiple crossovers in this region will be very rare and only PD and T type tetrads will be seen.

The overall aim of tetrad analysis is to express distance as a function of tetrad types.

First, we apply the formula for genetic distance:

$$\text{Distance in cM} = 100 \times \frac{\text{crossover gametes}}{\text{total gametes}}$$

There are two crossover gametes in each T tetrad.

□ = number of tetrads

$$\text{Distance in cM} = 100 \times \frac{2\,T}{4\,□} = 100 \times \frac{T}{2\,□}$$

(this holds true only for tightly linked genes ie no double crossovers)

For genes that are far apart association of A with B is random.
There are six equally likely arrangements of B alleles with A alleles.

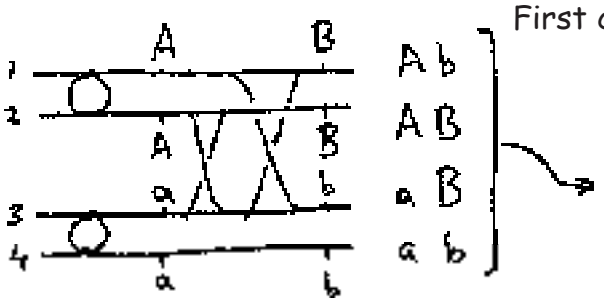
A	B	b	B	b	b	B
A	B	b	b	B	B	b
a	b	B	B	b	B	b
a	b	B	b	B	b	B
	PD		T	T	T	T
		NPD				

(Nonparental ditype)

Thus for unlinked loci:

PD		T		NPD
1	:	4	:	1

Now we will see how to use tetrad analysis to make a more accurate mapping function that will take the hidden double crossovers into account.



First crossover	Second crossover	Type
2,3	2,3	PD
2,3	1,4	NPD
2,3	1,3	T
2,3	2,4	T

NPD is unique designator of double crossovers that we can use to keep track of other double crossovers that look like single crossovers or no crossovers.

All four classes are equally likely, therefore:

Total double crossovers = 4 NPD, T tetrads that are doubles not singles = 2 NPD

To make a better mapping function we will take into account both single and double crossovers.

	number of tetrads	"crossover gametes"	
double crossovers	4 NPD	4	(By counting all of the spores in these tetrads as crossover gametes we have a more accurate mapping function)
single crossovers	T– 2 NPD	2	

$$\text{Distance in cM} = 100 \times \frac{2(T - 2NPD) + 4(4NPD)}{4\square}$$

$\square$ = number of tetrads

$$= 100 \times \frac{T - 2NPD + 8\ NPD}{2\square}$$

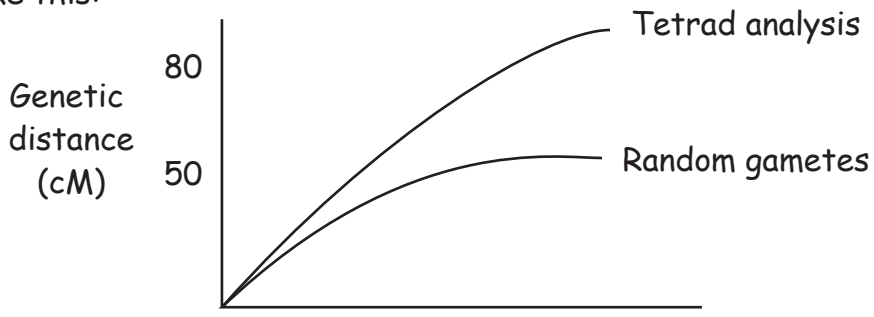
$$= 100 \times \frac{T + 6\ NPD}{2\square}$$

Example: 100 tetrads give: 75 PD, 20 T, 5 NPD

Applying the formula for linkage in tetrads we get: $100 \times \frac{20 + 6{\cdot}5}{200} = 25$ cM

If we were just to count crossover gametes: $100 \times \frac{40 + 4{\cdot}5}{400} = 15$ cM

A comparison of the mapping functions for tetrad analysis and random gametes looks something like this:



The mapping function for tetrad analysis is pretty accurate for distances $\square$40 cM