

Lecture 5

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 six 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 two different traits.

$$\mathbf{A/A, B/B} \times \mathbf{a/a, b/b}$$

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

These gametes will then give an F₁ generation of all **A/a, B/b**

Crosses between F₁ individuals will give an F₂ generation with a 9 : 3 : 3 : 1 phenotypic ratio as shown before.

A better way to look at segregation is by a test cross of the F₁ heterozygote to a homozygous recessive individual.

$$\mathbf{A/a, B/b} \times \mathbf{a/a, b/b}$$

The possible gamete genotypes from the F₁ will be:

A, b	a, b	A, B	a, B
(recomb.)	(parental)	(parental)	(recomb.)

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

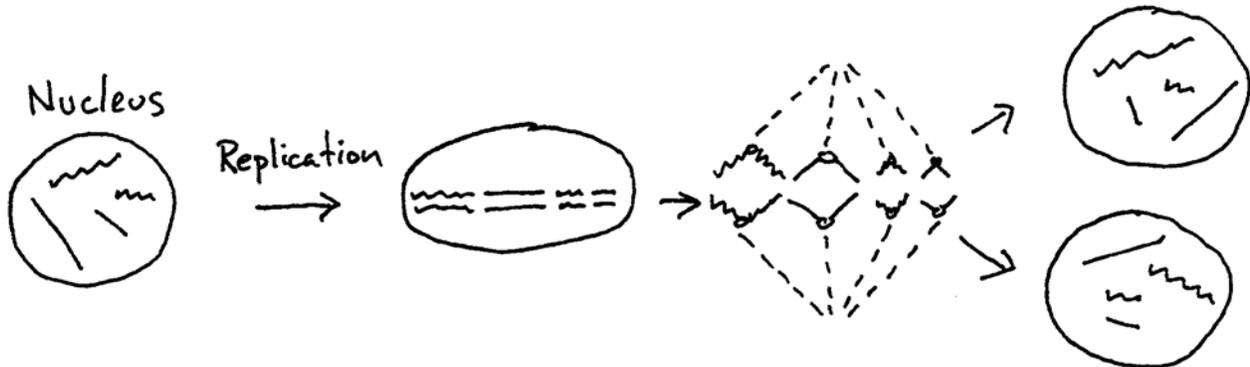
A/a, b/b	a/a, b/b	A/a, B/b	a/a, B/b
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Each offspring receives either one or the other parental allele: **gene segregation**.

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).

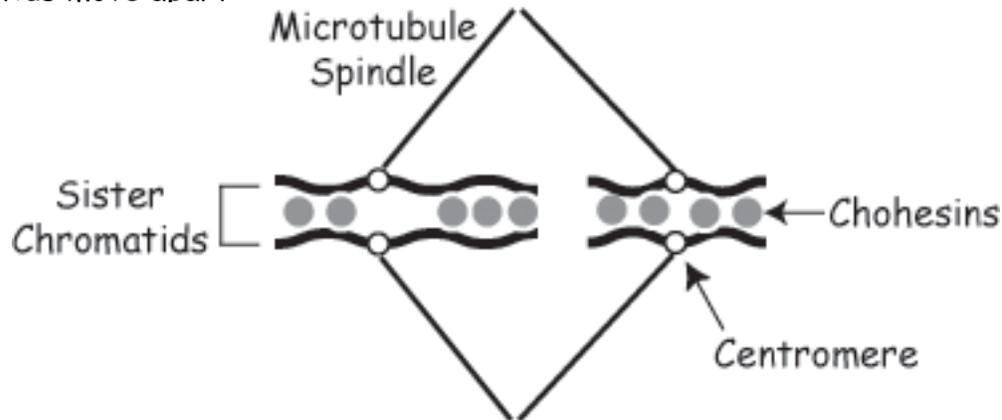
The chromosomes in dividing somatic cells go through **Mitosis**:



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

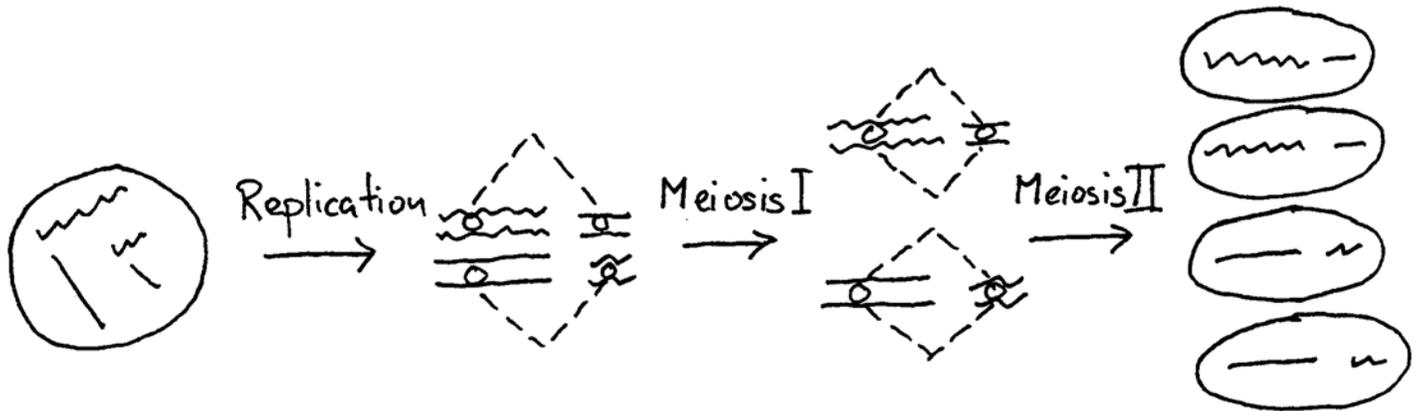
The stages of mitosis are as follows:

- 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.

What was needed to show that genes are on chromosomes 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)
 ↓
 F₁: all red
 ↓
 F₂: 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 F₂ have white eyes.

white ♂ x red ♀ (heterozygote from F₁)
 ↓

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.