

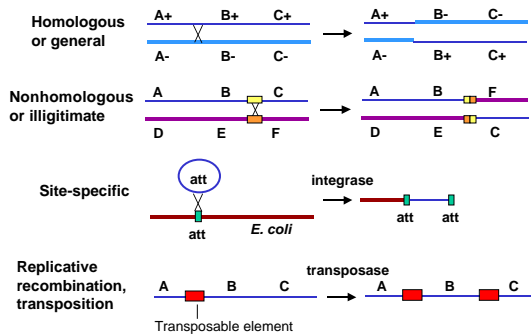
Recombination

Definitions
Models
Mechanisms

Definition of recombination

- Breaking and rejoining of two parental DNA molecules to produce new DNA molecules

Types of recombination



Recombination

- Breaking and rejoining of two parental DNA molecules to produce new DNA molecules
- **Reciprocal recombination:** new DNA molecules carry genetic information from **both** parental molecules.
- **Gene conversion:** **one way** transfer of information, resulting in an allele on one parental chromosome being changed to the allele from the other homologous chromosome

Gene Conversion



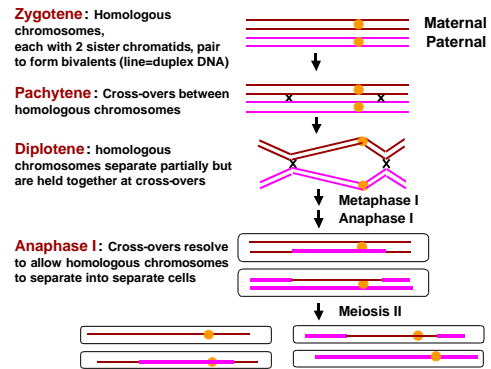
Recombination occurs when two homologous chromosomes are together

- Homologous or general recombination:
 - Bacterium with two viruses
 - Bacterium after conjugal transfer of part of a chromosome
 - At chiasmata during **meiosis** of eukaryotic cells
 - Post-replication repair via retrieval system
- Other types of recombination
 - Site specific : Integration of bacterial, viral or plasmid DNA into cellular chromosome
 - **Replicative : Transposition**

B. Meiotic recombination

- Recombination appears to be needed to keep maternal and paternal homologs of chromosomes together prior to anaphase of meiosis I
 - Zygotene: Pairing of maternal and paternal chromosomes (each has 2 sister chromatids)
 - Pachytene: Crossing over between maternal and paternal chromosomes
 - Diplotene: Centromeres of maternal and paternal chromosomes separate, but chromosomes are held together at chiasmata (cross-overs)
 - Anaphase I: Homologous chromosomes separate and move to 2 daughter cells.
- Results in ≥ 1 exchange between pairs of homologous chromosomes in each meiosis.
- Failure to keep homologous chromosomes together prior to anaphase I can lead to aberrant numbers of chromosomes, e.g. trisomy for chromosomes 15, 18, 21

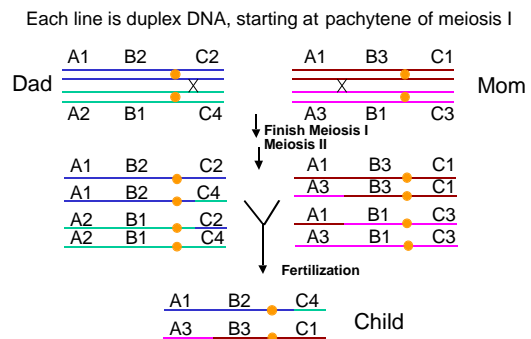
Cross-overs during meiosis I



Benefits of recombination

- Greater variety in offspring: Generates new combinations of alleles
- Negative selection can remove deleterious alleles from a population without removing the entire chromosome carrying that allele
- Essential to the physical process of meiosis, and hence sexual reproduction
 - Yeast and Drosophila mutants that block pairing are also defective in recombination, and *vice versa!!!!*

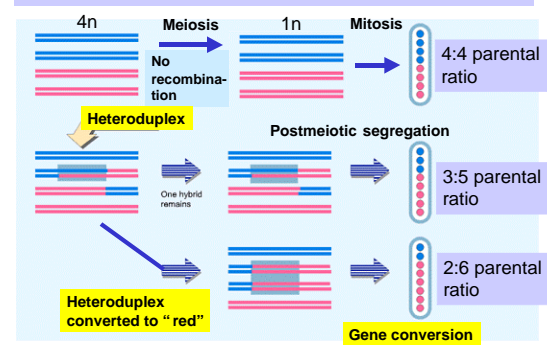
Meiotic recombination generates new combinations of alleles in offspring



Analysis of individual DNA strands during recombination in fungi

- During spore formation of some fungi, (e.g. *Ascomycetes*), the chromosomes are replicated **after meiosis**.
- Thus each DNA chain (strand) of the chromosomes produced during meiosis becomes a duplex DNA in a spore.
- The 8 spores are ordered in the ascus like the initial homologous chromosomes at the beginning of meiosis.
- Heterozygotes usually produce a **4:4 parental ratio** for spores carrying each allele

Spores formed during meiosis in *Ascomycetes* reflect the genetic composition of the parental DNA chains



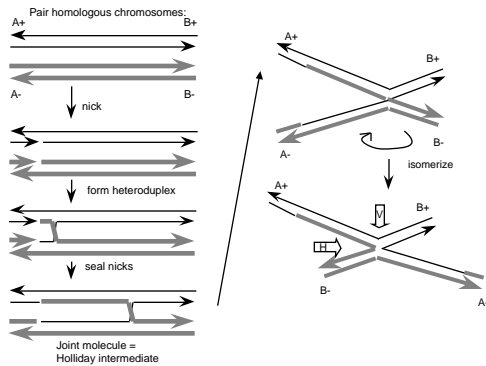
Proof of heteroduplex formation in fungi

- Deviation from a 4:4 ratio is explained by the presence of **heteroduplex DNA** after separation of homologous chromosomes during anaphase of meiotic division I.
- Replication of heteroduplex:
 - a 3:5 ratio (3 “blue”:5 “red”) indicates that a patch of heteroduplex DNA remained in one of the recombined chromosomes.
 - The two strands of the heteroduplex were separated by **post-meiotic segregation**.
- Alternatively, **gene conversion** results in a 2:6 ratio.

Holliday model for recombination

- Pairing: align homologous duplexes
- **Single strand invasion:**
 - Endonuclease nicks at corresponding regions of the same strands of homologous chromosomes
 - Ends generated by the nicks invade the other, homologous duplex
 - Ligase seals nicks to form a **joint molecule**.
 - (“**Holliday intermediate**” or “**Chi structure**”)
- Branch migration expands heteroduplex region.

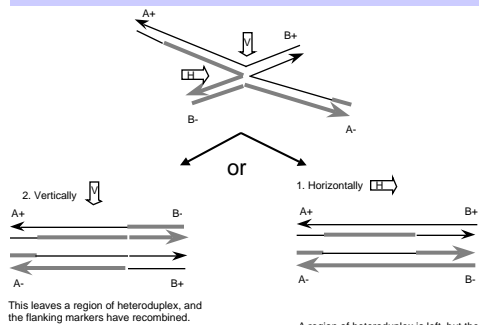
Holliday Model: single strand invasion



Resolution of joint molecules

- Can occur in one of two ways
- The Holliday junction can be nicked in the same strands that were initially nicked = “**horizontal resolution**.” This results in **NO** recombination of flanking markers.
- The Holliday junction can be nicked in the strands that were **not** initially nicked = “**vertical resolution**.” This results in **RECOMBINATION** of flanking markers.

Vertical & horizontal resolution



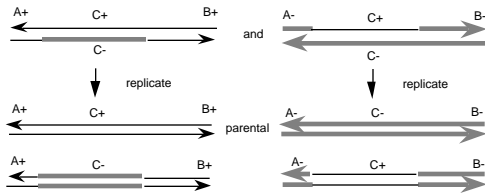
Animations of Holliday structures

Check out

<http://www.wisc.edu/genetics/Holliday/index.html>

Gene conversion can occur by replication through a heteroduplex

Products of horizontal resolution of the Holliday intermediate:



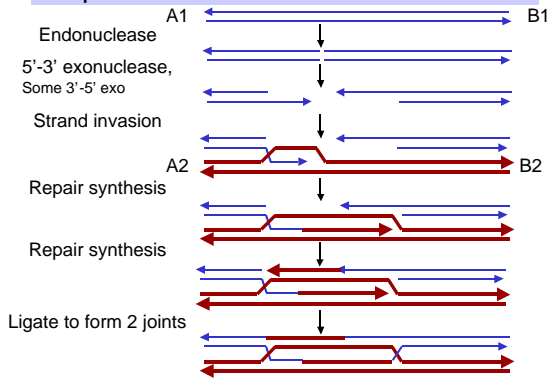
In the lower duplex, the C gene has been converted from C+ to C- with no recombination of the flanking markers.

In the lower duplex, the C gene has been converted from C- to C+ with no recombination of the flanking markers.

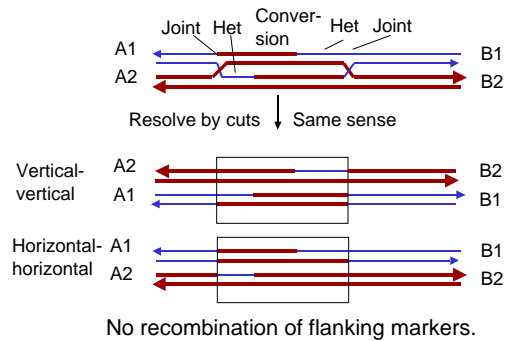
Double strand break model: Evidence

- This model provides a better explanation for recombination events in yeast:
- A **double strand break** precedes recombination.
- One DNA molecule is used preferentially as the **donor** of genetic information.
- Gapped substrates can initiate recombination and in the process be repaired (probl. 2.38)

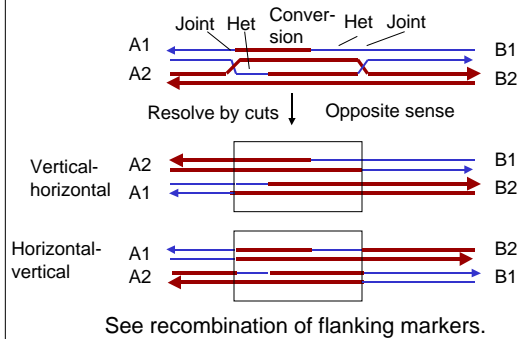
Steps in the double strand break model



Double strand break model: resolution



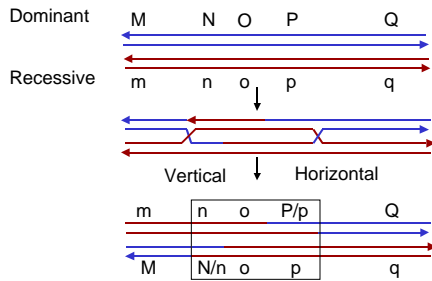
Double strand break model: Resolution #2



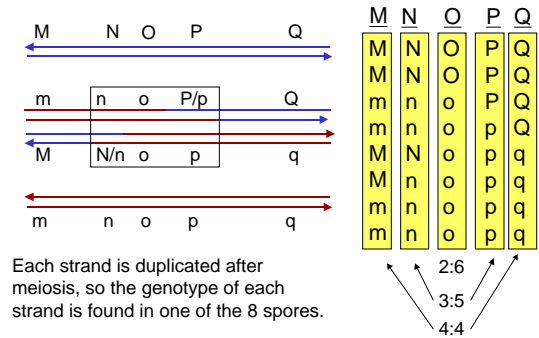
Distinguishing features of the models

- **Double strand break**
 - The original gap in the aggressor (recipient) duplex now has the sequence of the donor duplex = conversion
 - Conversion region is flanked by heteroduplex asymmetrically (on "right" on one chromosome, "left" on other)
- **Single strand invasion**
 - Each chromosome has heteroduplex covering the region of the initial site of exchange to the migrating branch: heteroduplexes are in the same place on each chromosome

Problem 2.34: Effects of recombination on phenotypes



Probl. 2.34: effects of post-meiotic segregation



Common steps in models

- Generate a single-stranded end
 - Search for homology
 - Strand invasion to form a joint molecule
 - Branch migration
 - Resolution
-
- Enzymes catalyzing each step have been isolated.