Homologous Recombination (Introductory Concepts)

Recombination is the process of DNA exchange by which all (or almost all) living organisms reshuffle their genetic decks, and create new combinations of genes. A diploid organism contains two homologues of each chromosome (or more accurately autosomes), one derived from the father and the other from the mother. An XY pair of sex chromosomes (X from the mother and Y from the father) specifies 'maleness' in humans, and an XX pair of chromosomes (one X from the mother and the other X from the father) specifies 'femaleness'.

A pair of homologous chromosomes are pretty much identical in sequence along their lengths (accounting for their homology) with occasional differences dispersed here and there. It is these differences that are crucial in generating a range of combinations of genes, depending on where DNA exchanges occur between the chromosomes. Recombination frequencies are quite high in meiosis, the process by which haploid germ line cells (sperms and eggs) are created. A sperm cell harbors the chromosome products generated by recombination events during meiosis in the father. An egg cell contains the chromosome products resulting from recombination events during meiosis in the mother. Fusion between an egg and a sperm generates a diploid cell that goes onto develop into an individual that displays the characteristics representatives of the gene combinations acquired from the two parents.

Recombination between homologous chromosomes does occur during mitosis, although the rates are lower by two to three orders of magnitude.

Homologous recombination refers to DNA exchange between two DNA partners that share extensive sequence homology, as in two homologous chromosomes, for example. This is in contrast to site-specific recombination (to be discussed later), in which DNA exchange occurs within well-defined short DNA segments. The extent of actual strand exchange during site-specific recombination does not exceed one turn (10 bp) of DNA.

Homologues recombination requires a number of proteins that act in a coordinated fashion. They mediate initiation of recombination, strand exchange, branch migration and generation of heteroduplex DNA regions, formation of four-armed DNA intermediates and resolution of these intermediates into recombinant products.

Double stranded DNA breaks are highly recombinogenic. In E. coli, an enzyme complex RecBCD can enter the DNA at a break and chew the ends to generate a single stranded extension that can invade a homologous duplex. Strand invasion is mediated by the recombination protein RecA. Genetic exchange between the DNA partners by mechanisms that we will discuss in detail in the class will generate a four-way DNA intermediate called the Holliday junction. This junction can branch migrate in a directional manner with the help of the RuvA-RuvB helicase. The junction can be resolved into recombinant products by the RuvC junction resolving enzyme.

Recombination requires two DNA partners, so it is easy to imagine how it can happen in a diploid organism. Bacteria are in general haploid, containing one copy of the chromosome (for practical

purposes). How can recombination occur in this case? Replication can produce two sister copies of the chromosome, but exchange between them cannot generate new gene combinations as the two chromosome copies are identical (unless mistakes in replication has generated mutations). However, the presence of an intact template copy of the genome is highly useful in salvaging by recombinational repair replication forks that stall/collapse when they encounter damages in DNA (strand breaks, for example). DNA exchange goes on between closely related (and even distantly related) bacteria by a process called 'transformation'. When a chunk of DNA from a dead bacterium enters a living one, we have temporarily created a diploid state for part of the genome. Hence recombination within this genome segment is possible. This sort of partial diploid is referred to as a 'merodiploid'. One can genetically construct merodiploids in E .coli by a number of ways. For example a portion of the bacterial chromosome can be introduced into a cell as part of a plasmid or an episome (the F episome in E. coli) or phage genome (transduction). E. coli cells can mate (or conjugate), and certain plasmids, or the F episome or parts of the donor chromosome can be transferred to the recipient cell. The genes from the transferred segment can be fixed in the recipient chromosome by recombination. Thus DNA transfer by conjugation, transduction and transformation can generate sets of chromosomal loci in the diploid state, setting the stage for recombination.

Exchange between DNA duplexes of different topology/geometry

When exchange occurs between two linear duplexes (linear eukaryotic chromosomes), the products are also linear molecules.

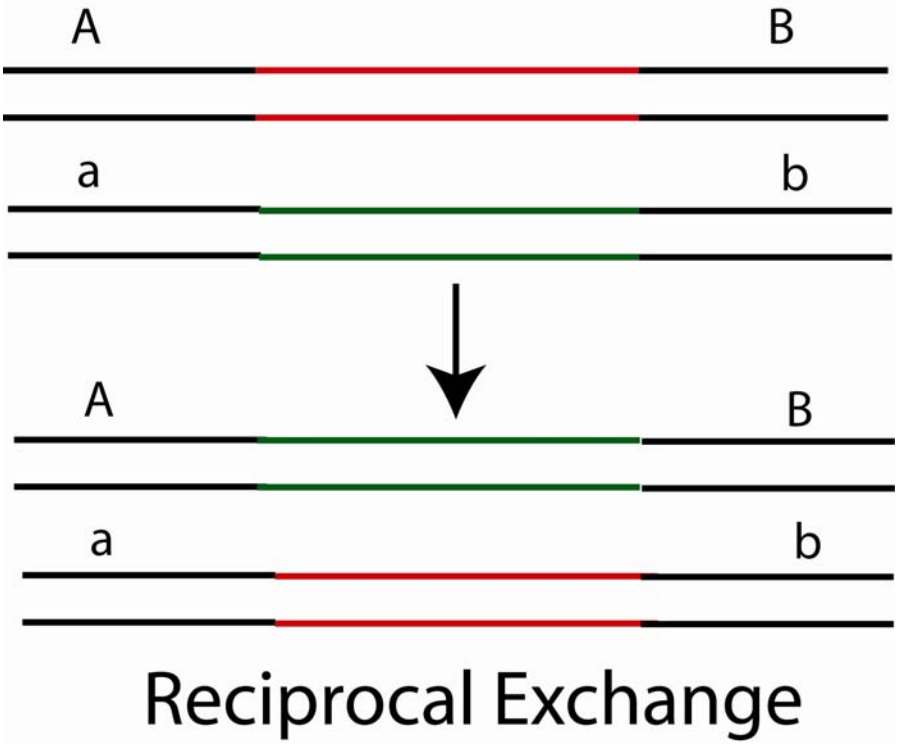
When exchange occurs between two circular molecules, the result is the fusion of the two circles into a single larger circle. When two exchanges occur between partner circles, the products remain as individual circles.

When the exchange is between a circular DNA (say, a bacterial chromosome) and a linear duplex (say a DNA segment transferred by conjugation), a single exchange event will generate a linear (or broken) chromosome, which is a lethal event (unless fixed by repair). Two exchanges will leave the chromosome in its circular viable state. Hence, in most instances of homologous recombination in bacteria, we are considering a minimum of two exchanges, or higher even number of exchanges, 4, 6, 8 etc. The probability of two simultaneous exchanges is significantly smaller than one exchange (the product of fractional probabilities), four such exchanges are much rarer, and 6 and 8 become negligible. Since bacteria divide rapidly, one can sample large populations, 10^7 to 10^8 cells in a ml of culture, so that even rare events can be detected with proper selection or screening methods.

Reciprocal Exchange

When two DNA partners exchange equal segments of genetic information mutually, the exchange is called reciprocal recombination. In the diagram below, the two DNA partners are homologous in the global sense, although locally they can be nonhomologous (indicated by red and green). In the exchange reaction shown, the green segment is replaced by red and, reciprocally, the red segment is replaced by green. We added two markers A, B on the top duplex, and equivalently a, b on the bottom duplex flanking the region of exchange. We can tell that exchange has occurred if we can separate the

recombination products (by meiosis), and examine each product by the phenotype it generates in the progeny. Thus, in the parent was red was associated with A, B and green with a, b. In the recombinants, red is associated with a, b; green is associated with A, B.



Gene Conversion (non-reciprocal Exchange)

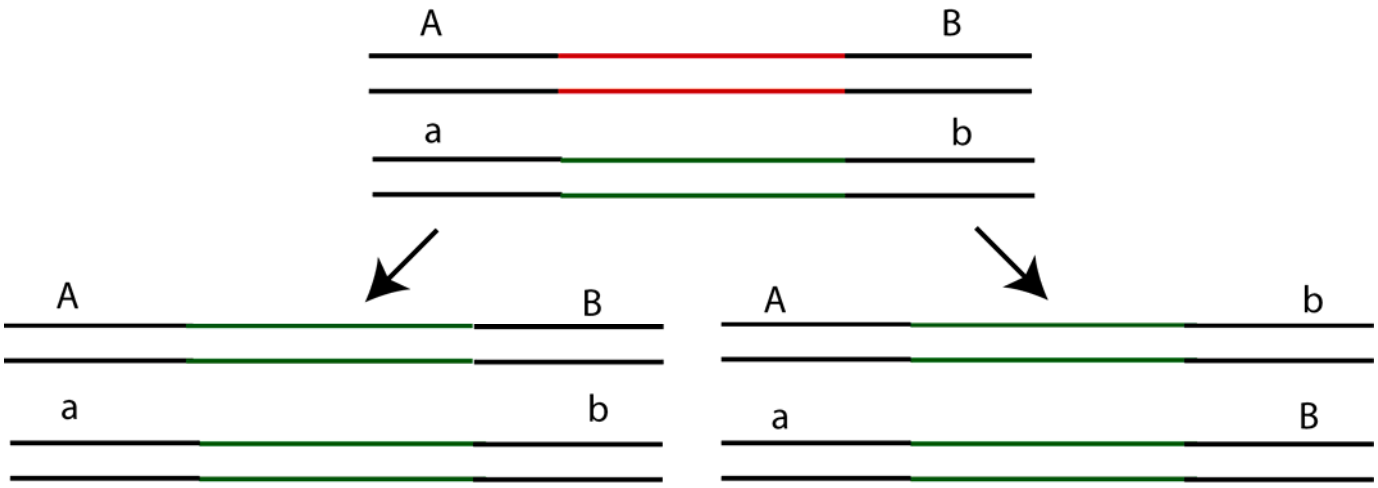
Let us say, the exchange event replaced the red on the top duplex by the green information. At the same time, the green on the bottom duplex was retained. This is a case of unidirectional information transfer non-reciprocal exchange, or gene conversion. In this case the red is gene converted to green. Equally probable is the gene conversion of green to red.

When gene conversion occurs, the flanking markers (A, B on the top duplex; a, b on the bottom duplex) can be in two possible configurations.

In one case the green segment acquired by conversion is flanked by A and B and the unconverted green is flanked by a and b. In other words, the flanking markers remain as they were in the parent duplexes. This sort of non-reciprocal exchange is called gene conversion without cross-over of flanking markers (or with the flanking markers retaining their parental configuration).

In the second case, the green segment acquired by gene conversion is flanked by A and b. The unconverted green segment is flanked by a and B. In other words, the flanking markers have exchanged themselves between the two parent duplexes. This sort of non-reciprocal exchange is called gene conversion with cross-over of flanking markers (or recombinant configuration of flanking markers.

In a broad sense, the probabilities of cross-over or no cross-over of flanking markers during gene conversion are roughly equal. This equality is most easily explained by the two ways in which a four-way junction intermediate of recombination can be resolved. We will talk more about the junction intermediate and its resolution later.



Gene Conversion

No Cross-over Cross-over

Further considerations of Recombination mechanisms

With the background concepts described above, we can begin to think about recombination mechanisms. We will discuss three basic models of recombination. The purpose of these models is to explain the sort of exchanges geneticists encounter when they do crosses, and analyze the outcomes. Since genomes are large, we cannot attempt to understand all of the genetic exchanges that have gone on in a particular experiment; we cannot even glean a tiny fraction of such events. We have to pick a manageable region of the genome and focus on events confined to that region. For example we could pick a marker M, m (you can think of M as red and m as green as in the gene conversion example discussed above) and two additional markers to the left and right of M, m. These flanking markers are equivalent to A, B and a, b (also used earlier). We can then follow what happens at M, m and also address how A, B and a, b behave when genetic exchange occurs between M and m.

In most experimental systems, it is hard to obtain the four products of a single meiotic event and analyze the genotypes of each one of these. One has to obtain the products from a large number of meiotic events and analyze them as a population, and make deductions with respect to the particular questions being tackled. Certain fungi, for example Saccharomyces yeast and Ascobolus, are particularly suited for studying meiosis, since they package the products of each meiosis in a sac called the ‘ascus’ (plural is ‘asci’). Thus, one can get information on meiotic gene exchange in two ways. One is by a global

analysis of random spores (sampling multiple meiotic events). The other is by the specific analysis of single meiotic events (tetrad analysis).

In meiosis, the two homologous chromosomes first replicate, to generate in all four duplexes. It is at this four chromosome (constituted by two sister pairs) stage that genetic exchange occurs. A particular chromosome carrying m can exchange with either of the two chromosome sisters carrying M. For simplicity, we will use illustrations that depict the exchange in one consistent way from diagram to diagram.

After the recombination intermediates have been resolved, in Saccharomyces, the four duplexes are packaged into individual cells called spores, and the four spores are enveloped by the spore sack. One can uncover the sack, separate the four spores, and allow them to germinate on growth medium. Each spore will give rise to a colony, and the phenotype of the four colonies with respect to the genetic markers being analyzed can be scored.

In Ascobolus, after recombination is completed, the four duplexes go through one more round or replication to give eight duplexes. And these are packaged into spores. Thus the ascus of Ascobolus contains eight instead of four spores. And these are comprised of four sister spores.

Let us first consider the Saccharomyces case. Then, we will see how we can relate this to the Ascobolus case.

Saccharomyces

A. If there is no exchange within M, m, two duplexes will carry M, the other two will carry m. This is the normal segregation pattern for the M, m. Hence the four spores will generate two colonies with the M phenotype and two with the m phenotype. This is the normal 2:2 segregation.

One could have exchange between M and m and still get apparent normal 2:2 segregation if additional events (such as mismatch correction) have reversed the consequences of the exchange.

B. If the ratio differs from the 2:2 normal pattern, we know that M and m have taken part in genetic exchange. There are three kinds of aberrant pattern: (i) 2:2 aberrant; (ii) 2 and 1/2:1 and ½; and 3:1.

In the 2:2 aberrant pattern, the four colonies generated by the four spores from a meiosis give rise to one M colony and one m colony, with the other two colonies displaying a half-sectored phenotype. That is one half of each colony shows the M phenotype; the other half shows the m phenotype. Thus, if you count the M colonies, the total is 1 + ½ + ½ =2.0. Similarly, the total count for the m colonies is also 1 + ½ + ½ = 2.0. Hence the segregation of the M and m markers is still, 2:2, but this is not the normal 2:2. Hence the description ‘aberrant 2:2’.

C. In the 2 and ½: 1 and ½ case, three colonies are normal, two being M (or m) and one being m (or M). The fourth colony is half-sectored for M and m.

D. In the 3:1 segregation, three colonies how the M (or m) phenotype, and one shows the m (or M) phenotype.

Ascobolus

Recall that, in Ascobolus, the four duplexes formed by meiosis go through one more round of replication before being package into spores. Every spore in the Saccharomyces case corresponds to two sister spores in Ascobolus.

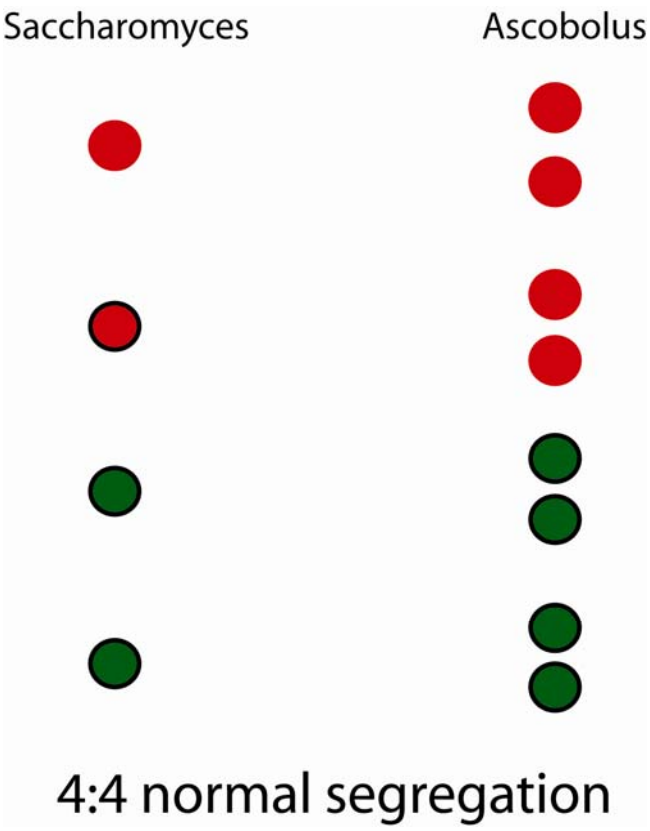
Hence the normal segregation in Ascobolus terms would be 4: 4 for the markers M and m. That is, two pairs of sister spores will be M; and the other two pairs of sister spores will be m. Each spore will be identical in phenotype (M or m) to its sister.

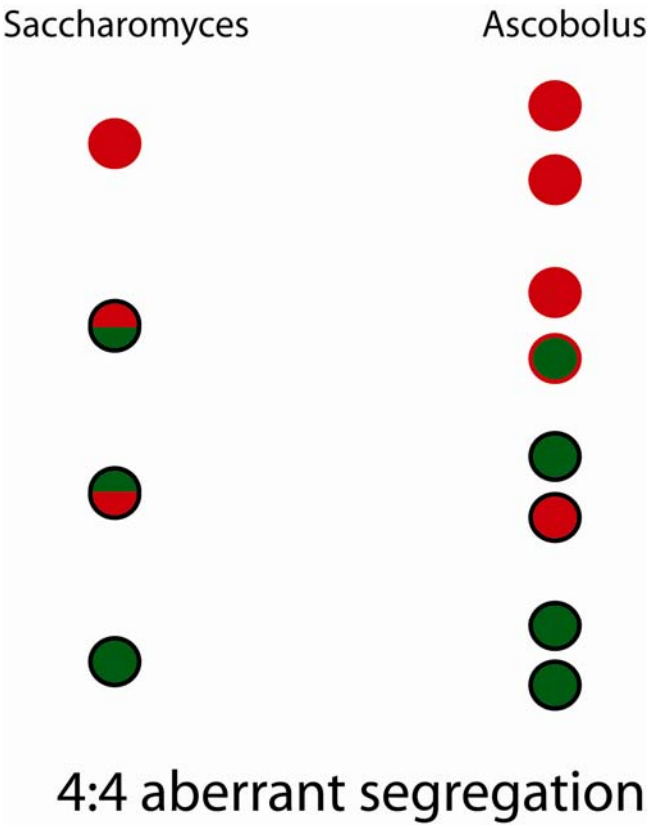
The three aberrant segregation patterns will be (i) 4:4 aberrant; (ii) 5 to 3; and 6:2.

In the 4:4 aberrant case, there will be two pairs of sister spores that show 'aberrant' phenotype. For each pair, the sisters will be non-identical; one will be M, and the other m.

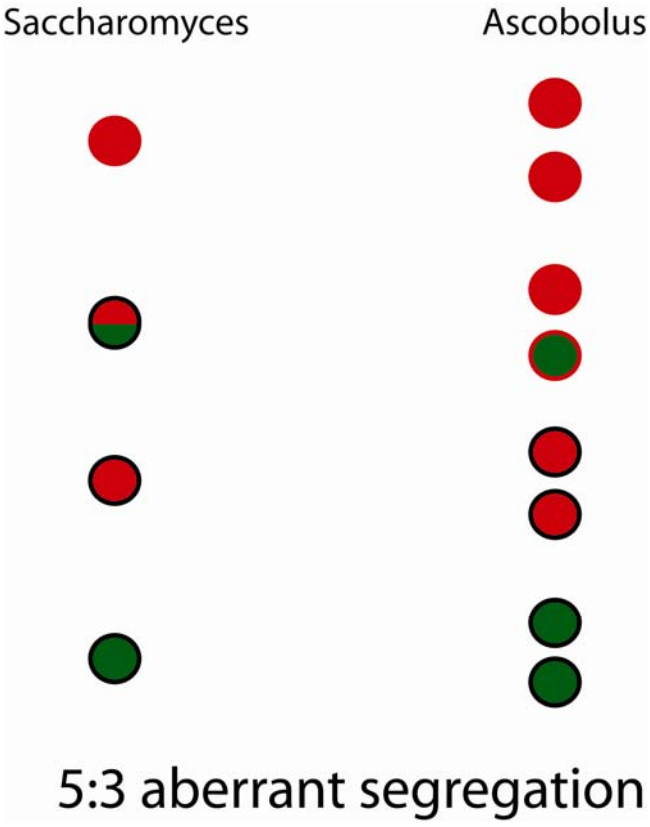
In the 5:3 case, there will be one pair of sister spores that are non-identical, one being M and the other m.

In the 6:2 case, sister spores are identical. However, there are three pairs with one phenotype (M or m) and only one the other (m or M).

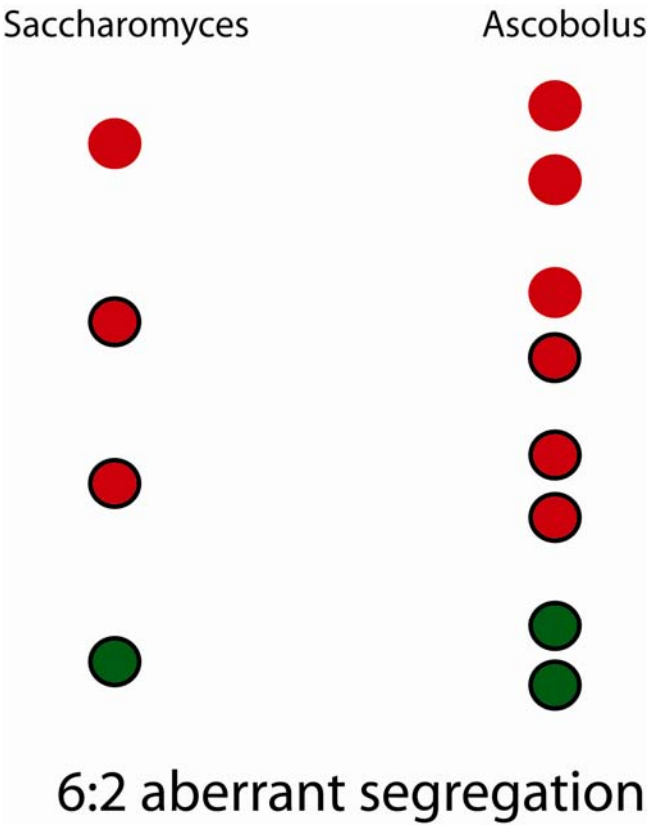




4:4 aberrant segregation



5:3 aberrant segregation



Models of recombination

Models of recombination, that attempt to explain the mechanism by which genetic exchange occurs, must be able to account for the deviations from the normal 4:4 pattern that a marker (M, m) would follow in the absence of genetic exchange within it. In addition, they should also account for the two possible configurations, parental or cross-over configurations of markers (A, B and a, b) flanking M, m.

We shall discuss the possible models in terms of how they deal with aberrant segregation patterns.