Advanced Gene Mapping in Eukaryotes

PRINCIPAL POINTS

- Tetrad analysis is a mapping technique that can be used to map the genes of certain haploid eukaryotic organisms in which the products of a single meiosis—the meiotic tetrad—are contained within a single structure. In these situations, the map distance between genes is computed by analyzing the relative proportion of tetrad types, rather than by analyzing individual progeny.

- In organisms in which the meiotic tetrads are arranged linearly, it is easy to map the distance of a gene from its centromere.

- Crossing-over can also occur during mitosis, although at a frequency much lower than during meiosis. As in meiosis, mitotic crossing-over occurs at a four-strand stage.

- For organisms amenable to such analysis, such as the fungus Aspergillus nidulans, mitotic recombination can be used to determine gene order and map distances between genes.

- Due to both ethical and practical issues, human genes cannot be mapped by making crosses and analyzing progeny. A number of approaches are used to determine the linkage relationships between human genes, including analyzing pedigree data recombinationally and physically locating genes on chromosomes by molecularly aided methods.

SPECIAL TECHNIQUES ARE AVAILABLE FOR constructing linkage maps for eukaryotic organisms beyond the standard genetic mapping crosses. In this chapter you will learn about these techniques. Then, in the iActivity, you can apply what you’ve learned and further explore one of the advanced mapping techniques.
In the previous chapter, we considered the classical principles for mapping genes in eukaryotes by means of recombination analysis. We saw that the outcome of crosses can be used to construct genetic maps, with distances between genes given in map units (centimorgans), and we also saw that map distances are useful in predicting the outcome of other crosses. In the current chapter, we discuss the determination of map distance by tetrad analysis in certain appropriate haploid organisms and the rare incidence of crossing-over in mitosis, and we give an overview of some of the traditional methods used in the construction of genetic linkage maps of the human genome.

**Tetrad Analysis in Certain Haploid Eukaryotes**

Tetrad analysis is a special mapping technique that can be used to map the genes of haploid eukaryotic organisms in which the products of a single meiosis—the meiotic tetrad—are contained within a single structure. The eukaryotic organisms in which this phenomenon occurs are either fungi or single-celled algae, all of which are found predominantly in the haploid state. During sexual reproduction in these organisms, a diploid is formed (transiently in some) which then undergoes meiosis to produce spores that germinate to produce the next haploid generation. In particular, tetrad analysis is used with the yeast Saccharomyces cerevisiae, the orange bread mold Neurospora crassa, and the single-celled alga Chlamydomonas reinhardtii. Tetrad analysis allows the researcher to study the details of meiotic events that would be impossible to study in any other system.

By analyzing the phenotypes of the meiotic tetrads, geneticists can infer the genotypes of each member of the tetrad directly. That is, because haploid organisms have only one copy of each gene, the phenotype is the direct result of the allele that is present. In other words, dominance and recessiveness issues do not come into play as they do in diploid organisms. We will outline this technique shortly.

Before we discuss the principles of tetrad analysis, let us learn a little about how meiotic tetrads arise in the life cycles of organisms upon which tetrad analysis can be performed. The life cycle of baker’s yeast (also called budding yeast), Saccharomyces cerevisiae, is diagrammed in Figure 7.1. Yeast has two mating types: MATα and MATα (MAT = mating type). The haploid cells of this organism reproduce mitotically (the vegetative life cycle), with each new cell arising from the parental cell by budding. Fusion of haploid MATα and MATα cells produces a diploid cell that is stable...
and that also reproduces by budding. Diploid MAT\textalpha/MAT\textalpha cells sporulate; that is, they go through meiosis. The four haploid meiotic products of a diploid cell (the meiotic tetrad) are called ascospores and are contained within a roughly spherical ascus. Two of the ascospores are of mating type MAT\textalpha, and two are of mating type MAT\textalpha. When the ascus is ripe, the ascospores are released, and they germinate to produce haploid cells that, on a solid medium, grow and divide to produce a colony. In yeast, the four ascospores are arranged randomly within the ascus in what is called an unordered tetrad.

Like yeast, *Chlamydomonas reinhardtii* has haploid vegetative cells. Each individual is a single green algal cell that can swim freely as a result of the motion of its two flagella. (See Figure 1.7), p. 10). When nitrogen is limited, the cells change morphologically to become gametes so that mating is possible. There are two mating types, designated plus (mt\textsuperscript{+}) and minus (mt\textsuperscript{−}). Only gametes of opposite mating types can fuse to produce diploid zygotes. After a maturation process, the zygote enters meiosis. The four haploid meiotic products are contained within a sac as an unordered tetrad, and there are two \textit{mt\textsuperscript{+}} and two \textit{mt\textsuperscript{−}} cells. When these cells are released, they are free swimming, and, by mitosis, they give rise to clones of those meiotic products.

The haploid fungus *Neurospora crassa* (Figure 7.2) has a life cycle somewhat similar to that of *C. reinhardtii*. *Neurospora crassa* is a mycelial-form fungus, meaning that it spreads over its growth medium in a weblike pattern. The mycelium produces asexual spores called conidia; their orange color (resulting from carotenoid pigments) gives the fungus its common name. *Neurospora* can be propagated vegetatively (asexually) by inoculating pieces of the mycelial growth or the asexual spores (conidia) on a suitable growth medium to give rise to a new mycelium. *Neurospora crassa* can also reproduce by sexual means. There are two morphologically identical mating types: A and a. The sexual cycle is initiated by mixing A and a mating type strains on nitrogen-limiting medium. Under these conditions, cells of the two mating types fuse, followed by the fusion of two haploid nuclei to produce an

---

**Figure 7.2**

**Life cycle of the haploid, mycelial-form fungus Neurospora crassa.** (Parts not to scale.)
A diploid nucleus, which immediately undergoes meiosis and produces four haploid nuclei (two A and two a) within an elongating sac called an ascus. A subsequent mitotic division results in a linear arrangement of eight haploid nuclei around which spore walls form to produce eight sexual ascospores (four A and four a). This linear arrangement of the spores in the ascus is called an ordered tetrad. Importantly, the order of the four spore pairs within an ascus reflects exactly the orientation of the four chromatids of each tetrad at the metaphase plate in meiosis I. The spores can be isolated in the same order as they are in the ascus, or they can be isolated randomly from the ascus. Germination of an ascospore begins the formation of a new haploid mycelium.

Using Random-Spore Analysis to Map Genes in Haploid Eukaryotes
In the three haploid eukaryotes we have discussed, the meiotic products can be collected after they have been released from the ascus (in yeast and Neurospora) or sac (in Chlamydomonas). In the case of the fungi (yeast and Neurospora), spores are germinated and the resulting cultures are analyzed. The free-swimming meiotic products of Chlamydomonas are analyzed directly. The haploid nature of the mature stage of all three organisms simplifies the analysis, because this stage is exactly equivalent to that of the gametes produced after meiosis in a diploid eukaryote, so genotypes can be determined directly from the phenotypes. In short, we can make two-point and three-point crosses to map genes on a chromosome, using the same approach as the one we used for a diploid eukaryote.

Calculating Gene–Centromere Distance in Organisms by UsingOrderedTetrads
As mentioned earlier, Neurospora produces ordered tetrads. In actuality, there are eight spores in each linear ascus, produced when the four meiotic products undergo one more mitotic division before the ascus is mature. The mitotic division represents the replication of the DNA molecules that have passed through the same meiosis. For the purposes of our genetic discussions, the eight spores can be considered as four pairs. The most interesting thing about ordered tetrads is that their genetic content directly reflects the orientation of the four chromatids of each chromosome pair in the diploid zygote nucleus at metaphase I. This fact allows us to map the distance between genes and their centromeres. Locating the centromeres on the genetic maps of chromosomes makes the maps more complete. In more complex organisms, centromeres are located primarily through cytological studies (which often are not possible in haploid eukaryotes because their chromosomes are too small).

In this example, we will map the position of the mating-type locus of Neurospora in relation to its centromere. Mating type is a function of whichever allele, A or a, is present at a locus in linkage group I. If a Neurospora strain of mating type A is crossed with one of mating type a, a diploid zygote of genotype A/a results. Figure 7.3 shows the various ways in which this zygote can give rise to the four meiotic products—the four pairs of ascospores in an ascus. For the purposes of illustration, the symbol • is used to indicate the centromere of the A parent and the symbol ○ is used to indicate the centromere of the a parent. (In reality, there is no difference between the two.)

If no crossing-over occurs between the mating-type locus and the centromere, the resulting ascospores have the genotypes shown in Figure 7.3a. Centromeres do not separate until just before the second meiotic division, so the spores in the top half of the ascus always have the centromere from one parent (the • centromere in this case), and the spores in the bottom half of the ascus always have the centromere from the other parent (○ here).

Since the two types of centromeres segregate to different nuclear areas after the first meiotic division, we say that they show first-division segregation. Also, because no crossing-over occurs between the mating-type locus and the centromere in Figure 7.3a, each allelic pair also shows first-division segregation. That is, after meiosis I, both copies of the A allele are at one pole, and both copies of the a allele are at the other pole; the A and a alleles have segregated into different nuclear areas. So after completion of meiosis II and the ensuing mitosis, there is a 4:4 segregation of the A and a alleles in terms of their positions in the ascus. More specifically, all eight spores in an ascus that show first-division segregation of alleles are parental types: The A allele is on the chromosome with a • centromere, and the a allele is on the chromosome with the ○ centromere. Furthermore, because it is equally likely that the four chromatids in the diploid zygote will be rotated 180 degrees, we expect equal numbers of first-division segregation ascii in which the four • A spores are in the bottom half and the four ○ a spores are in the top half.

To determine the map distance between a gene and its centromere, we measure the crossover frequency between the two chromosomal sites. When a single crossover between the mating-type locus and the centromere occurs, one of four possible ascus types is produced (Figure 7.3b). These four types are generated in equal frequencies because they reflect the four possible orientations of the four chromatids in the diploid zygote at metaphase I. Each has first-division segregation of the centromere; that is, segregation occurred during meiosis I. By contrast, A and a in Figure 7.3b are both present in each of the two nuclear areas after the first division; they do not segregate into separate nuclei until the second division. This situation is called second-division segregation of the alleles; that is, segregation of A from a occurs during meiosis II. Here, the pattern of gene segregation depends on which chromatids are involved in the crossover event. The 2:2:2:2 (AAaaAAaa and aaAAaaAA) and 2:4:2 (AAaaaAAa and aaAAAaaa) second-division segregation patterns are
Figure 7.3
Determination of gene–centromere distance of the mating-type locus in Neurospora. (a) Production of an ascus from a diploid zygote in which no crossing-over occurred between the centromere and the mating-type locus, first-division segregation for the mating-type alleles. (b) Production of asci after a single crossover occurs between the mating-type locus and its centromere. Chromosomes are shown after the crossover is complete. The asci show second-division segregation for the mating-type locus, and the four types of asci are produced in equal proportions.

a) No crossover

<table>
<thead>
<tr>
<th>Diploid cell</th>
<th>Developing ascus</th>
<th>Mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st meiotic division</td>
<td>2nd meiotic division</td>
<td>First-division segregation patterns of genes</td>
</tr>
<tr>
<td>2:2:2:2</td>
<td>4:4</td>
<td></td>
</tr>
</tbody>
</table>

b) Crossover between gene and centromere (four ways)

<table>
<thead>
<tr>
<th>Diploid cell</th>
<th>Developing ascus</th>
<th>Mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st meiotic division</td>
<td>2nd meiotic division</td>
<td>Second-division segregation patterns of genes</td>
</tr>
<tr>
<td>2:4:2</td>
<td>2:2:2:2</td>
<td>2:2:2:2</td>
</tr>
</tbody>
</table>

2N zygote nucleus
readily distinguishable from the 4:4 (AAAAaaaa and aaaaAAAA) first-division segregation pattern.

By analyzing ordered tetrads, we can count the number of asci that show second-division segregation for a particular gene marker. For the mating-type locus, about 14 percent of the asci show second-division segregation. This value must then be converted to a map distance via the formula

$$\text{gene–centromere distance} = \frac{\text{percent of second-division tetrads}}{2}$$

That is, if we consider the centromere to be a gene marker, then the nonrecombinant parental types are ● A and ○ a, and the recombinant types are ● a and ○ A. In a first-division segregation ascus (Figure 7.3a) all the spores are parentals (i.e., nonrecombinants), whereas in a second-division segregation ascus (Figure 7.3b) half are parentals and half are recombinants. Therefore, to convert the tetrad data to crossover or recombination data, we divide the percentage of second-division segregation asci (14 percent) by 2. Thus, the mating-type gene is 7 μ from the centromere of linkage group I.

In essence, the computation of gene–centromere distance is a special case of mapping the distance between two genes. If ordered tetrads are isolated, then not only can genes be mapped one to another, but each can be mapped to its centromere.

**KEYNOTE**

In some eukaryotic organisms, the products of a meiosis are arranged in a specialized structure in a way that reflects the orientation of the four chromatids of each homologous pair of chromosomes at metaphase I. The ordered tetrads allow us easily to map the distance of a gene from its centromere, a task that is very difficult or impossible with standard genetic-mapping procedures. Where no crossover occurs between the gene and the centromere, the result is a first-division segregation tetrad in which one parental type is found in half the ordered tetrad and the other parental type is found in the other half, giving a 2:2 segregation pattern. When a single crossover occurs between the gene and its centromere, several different tetrad segregation patterns are found, and each exemplifies second-division segregation. The gene–centromere map distance is computed as the percentage of second-division tetrads divided by 2.

**Using Tetrads Analysis to Map Two Linked Genes**

Let us see how we can analyze gene linkage relationships by analyzing unordered tetrads.

By making an appropriate cross, a diploid is constructed that is heterozygous for both genes. Then, after meiosis,
the resulting tetrads are analyzed. Consider the cross $a^+b^+ \times a b$. Figure 7.4 shows the three different tetrad types that result. A **parental ditype** (PD) tetrad contains only two types of meiotic products, both of which are of the parental type (hence the name). A **tetrateype** (T) tetrad contains two parentals (one of each type, here $a^+ b^+$ and $a b$) and two recombinants (one of each type, here $a^+ b$ and $a b^+$). A **nonparental ditype** (NPD) tetrad contains two types of meiotic products, both of which are of the non-parental (recombinant) types, here $a^+ b$ and $a b^+$. (Notice that, in each tetrad type, there is a 2:2 segregation of alleles.)

Tetrad analysis determines whether two genes are linked. This determination is based on the ways each tetrad type is produced when genes are unlinked and when genes are linked.

Figure 7.5 shows how the three tetrad types are produced when two genes are on different chromosomes. The PD and NPD tetrads result from events in which no crossovers are involved; the metaphase plate orientation of the four chromatids for the two chromosomes determines whether a PD or an NPD tetrad results. Since the two sets of four chromatids align at the metaphase plate indepen-

---

**Figure 7.5**

*Origin of tetrad types for a cross $a b \times a^+ b^+$ in which the two genes are located on different, independently assorting chromosomes.*

<table>
<thead>
<tr>
<th>No crossover: One metaphase alignment</th>
<th>Products</th>
<th>Resulting tetrad types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 1</td>
<td>Chromosome 2</td>
<td>Chromosome 1</td>
</tr>
<tr>
<td>$a$</td>
<td>$b$</td>
<td>$a^+$</td>
</tr>
<tr>
<td>$a$</td>
<td>$b$</td>
<td>$a^+$</td>
</tr>
</tbody>
</table>

*Parental ditype (PD) (4 parentals)*

<table>
<thead>
<tr>
<th>Alternative metaphase alignment</th>
<th>Products</th>
<th>Resulting tetrad types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 1</td>
<td>Chromosome 2</td>
<td>Chromosome 1</td>
</tr>
<tr>
<td>$a$</td>
<td>$b^+$</td>
<td>$a$</td>
</tr>
<tr>
<td>$a^+$</td>
<td>$b$</td>
<td>$a^+$</td>
</tr>
</tbody>
</table>

*Nonparental ditype (NPD) (4 recombinants)*

<table>
<thead>
<tr>
<th>Single crossover: Single crossover between $a$ gene and its centromere</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 1</td>
<td>Chromosome 2</td>
</tr>
<tr>
<td>$a$</td>
<td>$b$</td>
</tr>
<tr>
<td>$a^+$</td>
<td>$b^+$</td>
</tr>
</tbody>
</table>

*Tetrateype (T) (2 parentals, 2 recombinants)*

Sums to 50% parentals 50% recombinants
dently, the PD and NPD orientations occur with approximately equal frequency. Thus, if two genes are unlinked, the frequency of PD tetrad equals the frequency of NPD tetrad. T tetrad are produced when there is, for example, a single crossover between one of the genes and the centromere on that chromosome. The sum of all possible progeny types equals 50 percent parental types and 50 percent recombinant types when the two genes are unlinked.

Figure 7.6 shows the origins of each tetrad type when two genes are linked on the same chromosome.

**Figure 7.6**

Origin of tetrad types for a cross $a^+ b^+ 	imes a b$ in which both genes are located on the same chromosome. (a) No crossover. (b) Single crossover. (c–e) Three types of double crossovers.
A PD tetrad results if no crossing-over occurs between the genes (Figure 7.6a). A single crossover (Figure 7.6b) produces two parental and two recombinant chromatids and, hence, a T tetrad. For double crossovers, the chromatid strands involved must be considered. In a two-strand double crossover (Figure 7.6c) the two crossover events involve the same two chromatids, resulting in a PD tetrad. Three-strand double crossovers (Figure 7.6d) involve three of the four chromatids. In either of the two ways in which this event can occur, two recombinant and two parental progeny types are produced in each tetrad, which is a T tetrad. Finally, in four-strand double crossovers (Figure 7.6e), each crossover event involves two distinct chromatids, so all four chromatids of the tetrad are involved. This rare event is the only way in which NPD tetrads are produced. In sum, PD tetrads are produced either when there is no crossing-over—the most frequent event—or when there is a two-strand double crossover, whereas NPD tetrads are produced only by four-strand double crossovers (which are 1/4 of all the possible double crossovers and therefore are rare). Accordingly, two genes are considered to be linked if the frequency of PD tetrads is far greater than these frequency of NPD tetrads (i.e., PD ≫ NPD).

Once we know that two genes are linked, and once we have data on the relative numbers of each type of meiotic tetrad, the distance between the two genes can be computed by using a modification of the basic mapping formula:

\[
\frac{\text{number of recombinants}}{\text{total number of progeny}} \times 100
\]

In tetrad analysis, we analyze types of tetrads rather than individual progeny. To convert the basic mapping formula into tetrad terms, the recombination frequency between genes \(a\) and \(b\) becomes

\[
\frac{1/2T + \text{NPD}}{\text{total tetrads}} \times 100
\]

In essence, we are looking at tetrads with recombinants and determining the proportion of spores in those tetrads that are recombinant. So, in the formula, the 1/2 T and the NPD represent the recombinants from the cross; the other 1/2 T and the PD represent the nonrecombinants (the parents). Thus, the formula does indeed compute the percentage of recombinants. For instance, if there are 200 tetrads with 140 PD, 48 T, and 12 NPD, then the recombination frequency between the genes is

\[
\frac{1/2(48) + 12}{200} \times 100 = 18\%
\]

If more than two genes are linked in a cross, the data can best be analyzed by considering two genes at a time and by classifying each tetrad into PD, NPD, and T for each pair.

---

**KEYNOTE**

In organisms in which all products of meiosis are contained within a single structure, the analysis of the relative proportion of tetrad types provides another way to compute the map distance between genes. If PD = NPD, the two genes are unlinked, whereas if PD > NPD, the two genes are linked. The general formula when two linked genes are being mapped is

\[
\frac{1/2T + \text{NPD}}{\text{total tetrads}} \times 100
\]

---

**Mitotic Recombination**

**Discovery of Mitotic Recombination**

Crossing-over occurs during mitosis as well as during meiosis. Mitotic crossing-over (mitotic recombination) produces a progeny cell with a combination of genes which differs from that of the diploid parental cell that entered the mitotic cycle. Mitotic crossing-over occurs at a stage similar to the four-strand stage of meiosis. This stage forms only rarely, so the incidence of mitotic crossing-over between linked genes is much lower than the incidence of meiotic crossing-over.

Mitotic crossing-over was first observed by Curt Stern in 1936 in crosses involving *Drosophila* strains carrying recessive sex-linked mutations that cause short, twisty bristles (singed, \(sn\)), instead of the normal long, curved bristles, and yellow body color (\(y\)), instead of the normal grey body color. In flies with a wild-type grey body color, all bristles are black; in yellow-bodied (mutant) flies, the bristles are yellow. From a cross of homozygous \(sn^y/+\) \(sn^y/+\) females (grey bodies, singed bristles) with \(sn^+/yw\) (yellow bodies, normal bristles), Stern found, as expected, that the female \(F_1\) progeny were mostly wild type in appearance: They had grey bodies and normal bristles (genotype \(sn^+/yw\)). Some females, however, had patches of yellow or singed bristles that were not explained by regular gene segregation (Figure 7.7a,b). The origin of these flies could have been explained by chromosome nondisjunction or by chromosomal loss. Other females had twin spots—two adjacent regions of mutant bristles, one showing the yellow phenotype and the other showing the singed phenotype—a mosaic phenotype (Figure 7.7c). For the rest of the animal, the phenotype was wild type. Stern reasoned that, because the two parts of a twin spot were always adjacent, the spots must be the reciprocal products of the same genetic event. The best explanation was that they were generated by a mitotic crossing-over event, an event that occurs rarely.

The production of twin spots by mitotic crossing-over is shown in Figure 7.8. This figure also serves to illustrate the principles underlying the production of
genetic recombinants by mitotic crossing-over. The genotype of the F1 flies was sn y+/sn y+, with the mutant alleles in repulsion. As the flies develop, in some cells mitotic tetrads rarely form after chromosome duplication. In these tetrads, mitotic crossing-over can occur. For our example, consider mitotic crossing-over either between the centromere and the sn locus (Figure 7.8, left) or between the sn and the y locus (Figure 7.8, right). The chromatids are numbered, so we can track them to the progeny cells. After crossing-over has occurred, the chromatid pairs separate and become oriented on the mitotic metaphase plate in one of two possible ways, each of which is equally likely.

For the crossover between the centromere and the sn locus (see Figure 7.8, left), in one orientation chromatids 1 and 3 will segregate to one daughter nucleus and chromatids 2 and 4 will segregate to the other daughter nucleus. The former nucleus is genotypically homozygous sn+ y+/sn+ y+ and the latter is homozygous sn y+/sn y+. When these cells divide, they produce a yellow patch of tissue and a singed patch of tissue—a twin spot has been produced. The surrounding tissue, not involved in any mitotic crossing-over, will be wild type in phenotype because it is sn+ y+/sn+ y+. In the other orientation, chromatids 1 and 4 segregate to one nucleus and chromatids 2 and 3 to the other nucleus, to give genotypes sn+ y/y+sn+ y and sn y+/sn+ y, respectively. Both have a wild-type phenotype.

In the case of crossing-over between sn and y (see Figure 7.8, right), we can consider the two possible mitotic metaphase orientations and chromatid segregation patterns similarly. In one orientation, segregation of chromatids 1 and 3 gives sn+ y+/sn y+ cells, which are wild type in phenotype. In the other orientation, segregation of chromatids 1 and 4 gives sn+ y/y+sn y+ cells and segregation of chromatids 2 and 3 gives sn+ y+/sn y+ cells; both cell types have a wild-type phenotype.

In general, mitotic crossing-over makes all genes distal to the crossover point (i.e., between the crossover and the end of the chromosome arm) homozygous if the chromatid pairs align appropriately at the metaphase plate. Such alignment occurs one-half of the time. This generation of homozygosity applies only to the genes on the same chromosome arm as the crossover—that is, to those genes from the centromere outward. A mitotic crossover on one arm of the chromosome has no effect on genes on the other arm of the chromosome.

### KEYNOTE

Crossing-over can occur during mitosis as well as during meiosis, although it occurs much more rarely during mitosis. As in meiosis, mitotic crossing-over occurs at a four-strand stage. Single crossovers during mitosis can be detected in a heterozygote because loci distal to the crossover and on the same chromosome arm may become homozygous when passed to the same daughter cell.

### Mitotic Recombination in the Fungus Aspergillus nidulans

Mitotic recombination has been studied most extensively in fungi. Here we will look at some experiments carried out with the fungus *Aspergillus nidulans*; the experiments showed that mitotic recombination analysis can be used to construct genetic maps. Meiotic recombination analysis is not feasible with *Aspergillus* because the organism selfs; hence, controlled crosses cannot be made.

The fungus *Aspergillus nidulans* is a mycelial-form fungus like *Neurospora*, and its colonies are greenish because of the color of the asexual spores. *Aspergillus* is a suitable organism for genetic studies that use mitotic recombination because (1) the asexual spores of this fungus are uninucleate—they have a single nucleus; (2) the phenotype of each asexual spore is controlled by the genotype of the nucleus it carries; and (3) two haploid strains can be fused by mixing them together.

As in meiotic recombination analysis, in mitotic recombination analysis a strain must be constructed that is heterozygous for the genes to be studied. In *Aspergillus*, this is done by fusing two haploid strains that differ in nuclear genotypes. The result of such fusion is a mycelium in which the two nuclear types coexist and divide mitotically within the same cytoplasm. Cells of this type are called heterokaryons (literally, "different nuclei").
Figure 7.8
Production of the twin spot and the single yellow spot shown in Figure 7.7 by mitotic crossing-over.

Mitotic Recombination
Chapter 7  Advanced Gene Mapping in Eukaryotes

For example, consider the following two haploid strains:

Strain 1:  \( w^+ ad^+ pro^+ paba^+ y^+ bi \)
Strain 2:  \( w^+ ad^+ pro^+ paba y bi^+ \)

The alleles \( ad, pro, paba, \) and \( bi \) are recessive, and they specify that adenine, proline, para-aminobenzoic acid, and biotin, respectively, must be added to the growth medium in order for the strain carrying those mutant alleles to survive. Thus, either parent strain alone cannot grow without the appropriate supplements. However, a heterokaryon resulting from the fusion of the two strains requires no growth supplements, since all four genes are then heterozygous.

The recessive \( w \) and \( y \) alleles control the color of the asexual spores and hence the overall color of the colony. A strain with genotype \( w^+ y^+ \) is green, a \( w^+ y \) strain is white, a \( w^+ y^+ \) strain is yellow, and a \( w y \) strain is white because of epistatic effects. (See Chapter 4, pp. 89–94.) The heterokaryon of strain 1 and strain 2, however, is not green, because the color of the uninucleate spores (and hence the colony coloration) is controlled by the genotype of the nucleus contained in each spore. Therefore, the heterokaryon has a mixture of mostly yellow and white spores and has a mottled appearance.

Rarely, two haploid nuclei in the heterokaryon will fuse to produce a diploid nucleus, in a process called diploidization. The spores from these diploid cells will be diploid also and will be green because of their \( w^+ y^+ y \) genotype. The diploid spores (easily distinguished from haploid cells because they are larger) may then be isolated and cultured for study. The diploid cultures derived from these spores require no growth supplements, because wild-type alleles for all the nutritional requirement genes are present: \( w ad^+ pro^+ paba^+ y^+ bi \).

When a diploid Aspergillus spore with the foregoing genotype is used to inoculate a solid growth medium, a predominantly green colony will be produced, with haploid or diploid, and white or yellow (or both), sectors occurring rarely (Figure 7.9). (Green haploid sectors are also produced, but they cannot be distinguished easily from the parental diploid colony.) Whether the sector is haploid or diploid is shown by the spore size.

The haploid sectors are produced by **haploidization**—the formation of haploid nuclei from a diploid nucleus. That is, the diploid nuclei are unstable and eventually divide by mitosis (without any chromosome duplication) to produce haploid progeny nuclei, called haploid segregants. Let us consider the haploid white sectors in order to exemplify the information these haploids can give us. About half the white haploid sectors have the genotype \( w ad^+ pro^+ paba^+ y^+ bi \), and half have the genotype \( w ad^+ pro^+ paba y bi^+ \). With the exception of the common white allele, these two genotypes are reciprocals.

The 50:50 segregation of the two sets of five alleles indicates that they are located on a different chromosome from that carrying the \( w \) gene. Thus, in some haploid white sectors a chromosome with \( ad^+ pro^+ paba y bi^+ \) has segregated, while in others its homolog with \( ad^+ pro^+ paba^+ y^+ bi \) alleles has segregated. The interpretation of the haploid white-sector data is that the six gene loci are located on two nonhomologous chromosomes; the white gene is on one chromosome, and the other five genes are on the other. The yellow haploid sectors could be analyzed in a similar way. It is not possible to determine gene order by analyzing haploid segregants, although the correct gene order is given in the following diagram:

\[
\begin{align*}
&\text{\( w \)} &\text{\( + \)} &\text{\( \text{pro} \)} &\text{\( + \)} &\text{\( \text{bi} \)} \\
&\text{\( ad \)} &\text{\( + \)} &\text{\( \text{paba} \)} &\text{\( y \)} &\text{\( + \)}
\end{align*}
\]

Once the information about which genes are linked on which chromosomes is known, the next stage of mitotic analysis is to establish gene order and determine map distances between genes on the same chromosome. To do this, we must study the diploid white and yellow segregants.

The diploid segregants are produced by mitotic crossing-over. Since this event is very rare, only single crossovers need to be considered. As was discussed earlier, a crossing-over event in mitosis makes all those genes distal to the crossing-over point (on the same chromosome arm) homozygous. Thus, any recessive alleles that are heterozygous in the diploid cell may become
homozygous recessive as a result of the crossover, and the recessive phenotype will be seen.

One way in which a diploid yellow sector can arise is diagrammed in Figure 7.10. A mitotic crossover between the pro and paba genes has produced a segregant that is homozygous for the y allele and hence is yellow. The same crossover produces a twin spot that is homozygous y’y’, but since it is green, it is not detected in the overall green color of the colony. The crossover diagrammed has made all genes distal to that point homozygous. Therefore, the yellow segregant is also paba/paba and requires para-aminobenzoic acid in order to grow. The homozygosity for the bi’ allele goes undetected.

One other crossover that could produce a diploid yellow sector is a crossover between the paba and y loci (Figure 7.11). In this case, the yellow sector is still

---

**Figure 7.10**

Possible mitotic crossing-over event between the pro and paba loci that can give rise to a diploid yellow sector in the green diploid Aspergillus strain of Figure 7.9.
In the example, of genes become homozygous in the various segregants. Distal marker, it is then a simple matter to see which sets marking the chromosome from the centromere out to the recombination are isolated. With a number of genes segregants homozygous for that gene as a result of mitotic away from the centromere is chosen, and then diploid order for each chromosome arm. A gene marker that is far point. These facts give us a way to determine the gene results.

Possible movements of chromosomes at anaphase

After diploid segregants, which are produced by mitotic recombination, are obtained, they can be counted. Such quantitative data can be used to compute map distance between the genes; that is, the mitotic recombination frequency between genes can be computed from the formula used in meiotic recombination studies. The map distance between the paba and y genes, for example, is given by the percentage of yellow segregants that result from crossing-over in the paba–y region. Those particular segregants are still heterozygous for paba and pro.

Systems that achieve genetic recombination by means other than the regular alternation of meiosis and fertilization are called parasexual systems. In fungi (such as Aspergillus), the parasexual cycle consists of the following sequence of events: the formation of a heterokaryon; the rare fusion of haploid nuclei with different genotypes within the heterokaryon, to produce a heterozygous diploid nucleus; mitotic crossing-over within that diploid nucleus; and the subsequent haploidization of the diploid nucleus.

Retinoblastoma, a Human Tumor That Can Be Caused by Mitotic Recombination

Retinoblastoma is a childhood cancer of the eye (OMIM 18020); a patient with retinoblastoma is shown in Figure 22.9, and the cancer is described in more detail in Chapter 22, pp. 619–622. There are two forms of retinoblastoma. In sporadic (or nonhereditary) retinoblastoma, the development of an eye tumor is a spontaneous event in a patient from a family with no history of the disease. In these cases, a unilateral tumor will develop; that is, the tumor is in one eye only. In hereditary retinoblastoma, the susceptibility to develop the eye tumors is inherited. Patients with this form of retinoblastoma typically develop multiple eye tumors involving both eyes (bilateral tumors), usually at an earlier age than is the case for unilateral tumor formation in sporadic retinoblastoma patients.

Mutations in a gene called RB are responsible for retinoblastoma. In patients with hereditary retinoblastoma, the tumor cells always have mutations in both RB genes, while in normal cells from the same individuals, one of the two RB genes has the mutation. Thus, a second mutation is all that is needed in the normal cells to change it to a tumor cell. Using the molecularly cloned RB gene for molecular analysis of hereditary retinoblastoma patients, researchers have found that, in

heterozygous for paba, since mitotic crossing-over produces homozygotes only for genes distal to the crossover point. These facts give us a way to determine the gene order for each chromosome arm. A gene marker that is far away from the centromere is chosen, and then diploid segregants homozygous for that gene as a result of mitotic recombination are isolated. With a number of genes marking the chromosome from the centromere out to the distal marker, it is then a simple matter to see which sets of genes become homozygous in the various segregants. In the example, y and paba y genotypes were found in the various yellow sectors. From the mechanics of mitotic recombination, the order of genes is centromere–paba–y. We cannot assign a position to those genes that become homozygous wild type, so their relative positions must be determined from other mitotic recombination experiments.
a significant fraction of cases, the second mutation produces a mutated allele that is identical to the inherited mutated allele. This means that the normal wild-type copy of the retinoblastoma gene is somehow replaced by a duplicated copy of the homologous chromosome region carrying the mutant allele. While there are several mechanisms that could account for this phenomenon, one possibility is that mitotic recombination is the cause.

**KEYNOTE**

The parasexual cycle describes genetic systems that achieve genetic recombination by means other than the regular alternation of meiosis and fertilization. The parasexual cycle in fungi such as *Aspergillus* consists of (1) the formation of a heterokaryon by mycelial fusion and then fusion of the two haploid nuclei to give a diploid nucleus; (2) mitotic crossing-over within the diploid nucleus; and (3) haploidization of the diploid nuclei without meiosis, a process that produces haploid nuclei into which one or the other parental chromosome has segregated randomly. Using the parasexual cycle, gene order and map distances between genes can be calculated.

**Mapping Human Genes**

For practical and ethical reasons, with humans it is not possible to do genetic-mapping experiments of the kind performed on other organisms. Nonetheless, we have had a strong interest in mapping genes in human chromosomes, since there are so many known diseases and traits that have a genetic basis. (Of course, with the human genome completely sequenced, we now have the data in hand to identify every human gene and to learn the location of each gene with precision on the chromosomes.) In Chapters 2 and 3, we saw that pedigree analysis could be used to determine the mode by which a particular genetic trait is inherited. In this way, many genes have been localized to the X chromosome. However, pedigree analysis cannot show on which chromosome a particular autosomal gene is located. In this section, we discuss some of the methods used to map human genes.

**Mapping Human Genes by Recombination Analysis**

It is not possible to set up appropriate testcrosses for human genetic mapping by recombination analysis. Only in a very few cases have multigenerational pedigrees included individuals with segregating genotypes that were appropriate to permit any analysis of linkage between autosomal genes. Recombination analysis in humans is simpler for X-linked genes, however, because the hemizygosity of the X chromosome in males provides a rich source of useful genotypic pairings in pedigrees.

Consider the following theoretical example (Figure 7.12): A male with two rare X-linked recessive alleles \(a\) and \(b\) marries a woman who expresses neither of the traits involved. Since the traits are rare, it is likely that the woman is homozygous for the wild-type allele of each gene; that is, she is \(a^+ b^+/a^+ b^+\). A female offspring from these parents would be doubly heterozygous \(a^+ b^+/a b\) because of the hemizygosity of the X chromosome. Thus, analysis of the male progenies from pairings such as this \((a^+ b^+/a b \times a^+ b^+/Y)\) in a large number of pedigrees will produce a value for the frequency of recombination between the two loci involved, and an estimate of genetic map distance can be obtained.

---

**Figure 7.12**

Calculation of recombination frequency for two X-linked human genes by analyzing the male progenies of a woman doubly heterozygous for the two genes.
Chapter 7  Advanced Gene Mapping in Eukaryotes

Using this approach, researchers mapped a number of genes along the human X chromosome. For example, it was found that the distance between the green weakness gene, g (the recessive allele responsible for a form of color blindness), and the hemophilia A gene, h, was 8 map units. Clearly, though, this approach has a limited application.

**lod Score Method for Analyzing Linkage of Human Genes**

The rarity of suitable pedigrees also makes it hard to test for linkage and to calculate map distance between genetic markers. So, in most cases, a statistical test known as the lod (logarithm of odds) score method, invented by mathematical geneticist Newton Morton in 1955, is used to test for possible linkage between two loci. The lod score method is usually done by computer programs that use pooled data from a number of pedigrees. A full discussion of the method is beyond the scope of this text, so only a brief presentation is given here.

The lod score method compares the probability of obtaining the pedigree results if two markers are linked with a certain amount of recombination between them to the probability that the results would have been obtained if there was no linkage (i.e., 50% recombination) between the markers. The results are expressed as the \( \log_{10} \) of the ratio of the two probabilities. By convention, a hypothesis of linkage between two genes is accepted if the lod score at a particular recombination frequency is +3 or more, because a score of +3 means that the odds are \( 10^3 \) to 1 (1,000:1) in favor of linkage between two genes or markers (the \( \log_{10} \) of 1,000 is +3). Similarly, a hypothesis of linkage between two genes is rejected when the lod score reaches −2 or less because a score of −2 means that the odds are \( 10^2 \) to 1 (100:1) against the two genes or markers being linked.

Once linkage is established between genetic markers, the map distance is computed from the recombination frequency giving the highest lod score. (The higher the lod score, the closer to each other are the two genes.) This is done by solving lod scores for a range of proposed map units. For the human genome, 1 mu corresponds, on average, to approximately 1 million base pairs (1 megabase, or 1 Mb).

**High-Density Genetic Maps of the Human Genome**

Creating a genetic map for two genes is the simplest genetic mapping analysis one can do. Building a genetic map with mapping information for two genes at a time is not very efficient for a very large genome, however. Even using a handful of gene loci in genetic-mapping experiments makes the assembly of gene maps quite slow—and, for humans, there are simply not enough known genes to give chromosome maps with closely spaced genes. Fortunately, another type of genetic marker was discovered that elevated genetic mapping in humans to a new level. DNA markers are molecular markers in which DNA regions in the genome differ sufficiently between individuals so that those regions can be detected easily and rapidly by molecular analysis of DNA. (See Chapter 17, p. 438.) Briefly, the methods of mapping are the ones outlined earlier for human pedigree analysis, except that hundreds of loci are involved. Each DNA marker corresponds to a particular sequence at a site in the genome. If more than one type of sequence is found in the population at the site, then the DNA marker is polymorphic; in essence, we have alleles of a locus that differ in a molecular phenotype, rather than a phenotype such as eye color or plant height. Therefore, to analyze a pedigree for the segregation of DNA markers, all individuals in the pedigree must be analyzed—typed—for the particular DNA alleles present at each locus and the results analyzed by sophisticated computer algorithms to determine linkage relationships.

Making genetic maps of a large number of DNA markers is too much for a single research lab, because of the combinatorics of manipulations needed. For example, to type 5,000 DNA marker loci in 500 individuals would require performing 2,500,000 typing tests, each involving molecular techniques, and then entering 2,500,000 results into a database. So, typically for such analyses, geneticists set up a collaboration among many laboratories to do the work and, most importantly, to have the consortium work on the same set of DNA samples from the same set of individuals. The set of DNA samples used in this type of analysis is called a mapping panel.

One of the initial goals of the Human Genome Project (HGP; described in more detail in Chapter 18) was a genetic map with a density of at least one genetic marker per million base pairs of the genome by 1998. For this key genetic-mapping study, a mapping panel was used from a human DNA collection held at the Centre d’Étude du Polymorphisme Humain (CEPH), a research center in Paris, France. This panel is from 517 individuals representing 40 three-generation families. Eight of the families were used for most of the mapping analysis in the HGP study. The result was a high-density genetic map completed in 1994, with 5,264 of a particular type of DNA marker localized to 2,335 chromosomal loci (Figure 7.13). (The reason the two numbers do not match is because some markers are too close together to be separated definitively.) In this map, the average density was one marker per 599 kb (kilobase pairs = 1,000 base pairs) for all chromosomes considered together, with a range from one marker per 495 kb to one marker per 767 kb.
Figure 7.13
A high-density genetic map with 5,264 microsatellites localized to 2,335 chromosomal loci. (From Dib et al. 1996. Nature 380:152–154.)

KEYNOTE
Genetic maps of genomes are constructed with the use of recombination data from genetic crosses in the case of experimental organisms or from pedigree analysis in the case of humans. Both gene markers and DNA markers are used in genetic-mapping analysis.

Physical Mapping of Human Genes
An alternative to mapping genes via crosses and the analysis of recombinants is to use physical methods to locate genes on chromosomes. This is physical mapping of genes in the genome, and a variety of approaches may be employed. We consider two examples here.

In fluorescent in situ hybridization (FISH) mapping, individual eukaryotic chromosomes are colored fluorescently at the locations of specific genes or DNA sequences. Human metaphase chromosomes on a microscope slide are treated to cause the two DNA strands of each DNA molecule in the chromosomes to separate, but stay in the same physical location. Specific DNA sequences corresponding to a gene or a DNA marker are molecularly cloned and tagged with fluorescent chemicals. The tagged single-stranded DNA sequences—the DNA probes—are added to the chromosomes, where they pair to the single-stranded chromosomal DNA sequence that they match. This pairing process is called hybridization (described in more detail in Chapter 16, pp. 433–434). In this way, the chromosome sites corresponding to the probe are identified by the fluorescent emissions of the tag. By utilizing chemicals that fluoresce at different wavelengths, it is possible to use a number of different probes in the same experiment. Computer imaging analysis of the sample examined under a fluorescence microscope then identifies the locations where the probes have bound.

Figure 7.14 shows the results of FISH with six different DNA probes. The probe colors are not the true colors from the fluorescence, but are generated by the computer. The complete chromosomes are visualized by staining them with a chemical that colors all the DNA (blue in this case). Each chromosome to which a probe has hybridized has two dots, because the DNA in metaphase chromosomes is already duplicated in preparation for cell division. For example, the yellow dots identify an uncharacterized DNA sequence on chromosome 5, and the red dots identify the Duchenne muscular dystrophy gene on the X chromosome.
Another physical mapping approach involves determining gene linkage through **radiation hybrids (RH)**. A radiation hybrid (RH) is a rodent (hamster, rat, mouse) cell line that carries a small fragment of the genome of another organism, such as a human. The genome fragments are produced by irradiating human cells with X rays to cause random breakage of the DNA (Figure 7.15). The size of the fragments decreases as the dosage of X rays increases. The irradiation kills the human cells, but the chromosome fragments can be “rescued” by fusing the irradiated cells with rodent cells.

For RHs irradiated with human fragments the human fragment typically is a few megabase pairs long. The human DNA in the RH is then analyzed for the genetic markers it carries. The principle of RH mapping is straightforward: The closer two markers are, the greater is the probability that those markers will be on the same DNA fragment and therefore end up in the same RH. Both gene markers and DNA markers can be used in RH mapping. A detailed RH map of the human genome was published in 1997.

**KEYNOTE**

Gene markers and DNA markers can be mapped on human chromosomes by physical mapping approaches. A variety of such approaches is available, including FISH and radiation hybrid mapping.

**Summary**

In this chapter, we learned how to map genes in certain haploid microorganisms by using tetrad analysis. In some of those microorganisms, the meiotic tetrads are ordered in a way that reflects the orientation of the four chromatids of each homologous pair of chromosomes at metaphase I. Ordered tetrads make it possible to map a gene’s location relative to its centromere.

Next, we learned about the rare incidence of crossing-over in mitosis and how mitotic recombination may be used in certain organism to map genes. Finally, we learned about gene mapping in humans, in which controlled crosses obviously cannot be conducted. Instead, recombination analysis is done by means of multigenerational pedigrees, and genes may be pinpointed to chromosomal locations via physical mapping approaches.

**Analytical Approaches to Solving Genetics Problems**

**Q7.1** A *Neurospora* strain that required both adenine (*ad*) and tryptophan (*trp*) for growth was mated to a wild-type strain (*ad*+ *trp*+), and this cross produced seven types of ordered tetrads in the following frequencies:

| Spore Pair 1: | ad | trp | ad | + | ad | trp | ad | trp |
| Spore Pair 2: | ad | trp | ad | + | + | + | + | + |
| Spore Pair 3: | + | + | + | trp | + | trp | + | ad |
| Spore Pair 4: | + | + | + | trp | + | + | + | + |
| Type | (1) | 63 | (2) | 15 | (3) | 3 | (4) | 9 |
Analytical Approaches to Solving Genetics Problems

Spore Pair 1:  ad trp  ad +  ad trp
Spore Pair 2:  ++  + trp  ++
Spore Pair 3:  ad trp  ad +  ad trp
Spore Pair 4:  ++  + trp  ad +

Type  (5) 3  (6) 1  (7) 6

a. Determine the gene–centromere distance for the two genes.

b. From the data given, calculate the map distance between the two genes.

A7.1

a. The gene–centromere distance is given by the formula

\[
\text{percent second-division tetrads} = x \text{ mu from centromere}
\]

For the ad gene, tetrads 4, 5, and 6 show second-division segregation; the total number of such tetrads is 13. There are 100 tetrads, so the ad gene is \((13/100)\% = 13\text{ mu from its centromere. For the trp gene tetrads, 3, 5, 6, and 7 show second-division segregation, and the total number of such tetrads is 25, indicating that the trp gene is 12.5 mu from its centromere.}

b. The linkage relationship between the genes can be determined by analyzing the relative number of parental ditype (PD), nonparental ditype (NPD), and tetratype (T) tetrads. Tetrads 1 and 5 are PD, 2 and 6 are NPD, and 3, 4, and 7 are T. If two genes are unlinked, the frequency of the PD tetrads will approximately equal the frequency of the NPD tetrads. If two genes are linked, the frequency of the PD tetrads will greatly exceed that of the NPD tetrads. Here, the latter case prevails, so the two genes must be linked. The map distance between two genes is given by the general formula

\[
\frac{1/2 \ T + \ NPD}{\text{total}} \times 100
\]

For this example, the number of T tetrads is 30 and the number of NPD tetrads is 4. Thus, the map distance between ad and trp is

\[
\frac{1/2 (30) + 4}{100} \times 100 = 19 \text{ mu}
\]

Hence, we have the following map, with c indicating the centromere:

Q7.2 In Aspergillus, forced diploids were constructed between a wild-type strain and a strain containing the mutant genes y (yellow), w (white), pro (proline requirement), met (methionine requirement), and ad (adenine requirement). All these genes are known to be on a single chromosome.

Homzygous yellow and homozygous white segregants were isolated and analyzed for the presence of the other gene markers. The following phenotypic results were obtained:

- y/y segregants:  \( w^* \ pro^* \ met^* \ ad^* \) 15
- w/w segregants:  \( y^* \ pro^* \ met \ ad \) 6
- w/w segregants:  \( y^* \ pro^* \ met^* \ ad \) 12

Draw a map of the chromosome, giving the order of the genes and the position of the centromere.

A7.2 The segregants are all diploid. In mitotic recombination, a single crossover renders all gene loci distal to that point homozygous. In this regard, the crossover events in one chromosome arm are independent of those in the other chromosome arm. Therefore, we must inspect the data with these concepts in mind.

There are two classes of y/y segregants: wild-type segregants and proline-requiring segregants, which are homozygous for the pro gene. Thus, of the four loci other than yellow, only pro is in the same chromosome arm as y. Furthermore, since not all the y/y segregants are pro in phenotype, the pro locus must be closer to the centromere than the y locus is, as shown in the following map:

\[
\text{y} \quad \text{pro} \quad \text{met} \quad \text{w} \quad \text{ad}
\]

A single crossover between pro and y will give y/y segregants that are wild-type for all other genes, whereas a single crossover between the centromere and pro will give homozygosity for both pro and y—hence the pro requirement.

Similar logic can be applied to the w/w segregants. Again, there are two classes. Both are also phenotypically ad, indicating that the ad locus is further from the centromere than the w locus. Consequently, every time w becomes homozygous, so does ad. The remaining gene to be located is met. Some of the w/w segregants are met* and some are met, so the met gene is closer to the centromere than the w gene is. The reasoning here is analogous to that for the placement of the pro gene in the other arm. Taking all the conclusions together, we have the following gene order:

\[
\text{y} \quad \text{pro} \quad \text{met} \quad \text{w} \quad \text{ad}
\]
Questions and Problems

7.1 A cross was made between a pantothenate-requiring (*pan*) strain and a lysine-requiring (*lys*) strain of *Neurospora crassa*, and 750 random ascospores were analyzed for their ability to grow on a minimal medium (a medium lacking pantothenate and lysine). Thirty colonies subsequently grew. Map the *pan* and *lys* loci.

7.2 Four different albino strains of *Neurospora* were each crossed to the wild type. All crosses resulted in half wild-type and half albino progeny. Crosses were made between the first strain and the other three, with the following results:

- 1 x 2: 975 albino, 25 wild type
- 1 x 3: 1,000 albino
- 1 x 4: 750 albino, 250 wild type

Which mutations represent different genes, and which genes are linked? How did you arrive at your conclusions?

*7.3* Genes *met* and *thi* are linked in *Neurospora crassa*; we want to locate *arg* with respect to *met* and *thi*. From the cross *arg + + x + thi met*, the following random ascospore isolates were obtained:

| arg thi met | arg + + | 26 |
| arg thi + | 17 | + thi + | 4 |
| arg + met | 3 | + + met | 14 |
| + thi met | 56 | + + + | 29 |

Map the three genes.

7.4 Double exchanges between two loci can be of several types, called two-strand, three-strand, and four-strand doubles.

a. Four recombinant gametes would be produced from a tetrad in which the first of two exchanges is depicted in the following figure:

```
        a+    b+
       / \   / \  
a   ---\-+   \-+   b
   /   \     /   
a    b    a   b
```

Draw in the second exchange.

b. In the following figure, draw in the second exchange so that four nonrecombinant gametes would result:

```
        a+    b+
       / \   / \  
a   ---\-+   \-+   b
   /   \     /   
a    b    a    b
```

7.5 A cross between a pink (*p*) yeast strain of mating type *a* and a cream strain (*p*) of mating type *a* produced the following tetrads:

| 18 | *p* a | *p* a | *p* a |
| 8  | *p* a | *p* a | *p* a |
| 20 | *p* a | *p* a | *p* a |

On the basis of these results, are the *p* and mating-type genes on separate chromosomes?

7.6 The following asci were obtained from the cross:

| leu + x + rib | leu + rib + rib | leu + + + |
| 110 | 45 | 6 | 39 |

Draw the linkage map and determine the map distance.

*7.7* The genes *a*, *b*, and *c* are on the same chromosome arm in *Neurospora crassa*. The following ordered asci were obtained from the cross *a b x + + c*:

| 45 | 5 | 146 | 1 |
| a b + | a b + | a b + | a b + |
| + b c | a + + | a b + | a b + |
| a + + | b c | + + c | a b c |
| + + c | + + c | + + c | + + c |

Determine the correct gene order and calculate all gene–gene and gene–centromere distances.

*7.8* Under transmitted light, spores of wild-type (+) Neurospora appear black, while spores of an albino mutant (*al*) appear white.

a. Assume that there is no chromatid interference—that is, that crossing-over occurs equally frequently between any of the four chromatids during meiosis. What patterns of ordered asci do you expect to see, and in what frequencies, if there is exactly one crossover between *al* and its centromere in every meiosis?

b. Under the preceding conditions, what is the map distance between *al* and its centromere? Are *al* and its centromere linked or unlinked?

*7.9* The frequency of mitotic recombination in experimental organisms can be increased by exposing them to low levels of ionizing radiation (such as X rays) during development. Hans Becker used this method to examine the patterns of clones produced by mitotic recombination in the...
Questions and Problems

Drosophila retina. (Drosophila has a compound eye consisting of many repetitive units called ommatidia.) What type of spots would be produced in the Drosophila retina if you irradiated a developing Drosophila female obtained from crossing a white-eyed male with a cherry-eyed female? (See Table 4.2 [p. 86] for a description of the w and wⁿ att alleles.)

7.10 A diploid strain of Aspergillus nidulans (forced between wild type and a multiple mutant) that was heterozygous for the recessive mutations y (yellow), w (white), ad (adenine), sm (small), phe (phenylalanine), and pu (putrescine) produced haploid segregants. Forty-one haploid white and yellow segregants were tested and were found to have the following genotypes and numbers:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>y w pu ad sm phe</td>
<td>7</td>
</tr>
<tr>
<td>y w pu ad + +</td>
<td>11</td>
</tr>
<tr>
<td>y + + + sm phe</td>
<td>16</td>
</tr>
<tr>
<td>y + + + + +</td>
<td>7</td>
</tr>
</tbody>
</table>

What are the linkage relationships of these genes?

7.11 A heterokaryon was established in the fungus Aspergillus nidulans between a met⁻ trp⁻ auxotroph and a leu⁻ nic⁻ auxotroph. A diploid strain was selected from this heterokaryon. From the diploid strain, the following eight haploid strains were obtained: white and yellow segregants were tested and were found to have the following genotypes and numbers:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>y w pu ad sm phe</td>
<td>7</td>
</tr>
<tr>
<td>y w pu ad + +</td>
<td>11</td>
</tr>
</tbody>
</table>

Which, if any, of these four marker genes are linked, and which are unlinked?

*7.12 A (green) diploid of Aspergillus nidulans is heterozygous for each of the following recessive mutant genes: sm, pu, phe, bi, w (white), y (yellow), and ad. Analysis of white and yellow haploid segregants from this diploid indicated several classes with the following genotypes:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>y w pu ad sm phe</td>
<td>7</td>
</tr>
<tr>
<td>y w pu ad + +</td>
<td>11</td>
</tr>
<tr>
<td>y + + + sm phe</td>
<td>16</td>
</tr>
<tr>
<td>y + + + + +</td>
<td>7</td>
</tr>
</tbody>
</table>

How many linkage groups are involved, and which genes are on which linkage group?

7.13 A (green) diploid of Aspergillus nidulans is heterozygous for the recessive mutant gene ad and heterozygous for the following recessive mutant genes: paba, ribo, y (yellow), an, bi, pro, and su-ad. Those recessive alleles which are on the same chromosome are in coupling. The su-ad allele is a recessive suppressor of the ad allele: The +/su-ad genotype does not suppress the adenine requirement of the ad/diploid, whereas the su-ad/su-ad genotype does suppress that requirement. Therefore, the parental diploid requires adenine for growth. From this diploid, two classes of segregants were selected: yellow and adenine independent. The following table lists the types of segregants obtained:

<table>
<thead>
<tr>
<th>Segregant Type Selected</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine-independent</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>ribo</td>
</tr>
<tr>
<td></td>
<td>ribo an</td>
</tr>
<tr>
<td></td>
<td>ribo an pro</td>
</tr>
</tbody>
</table>

Analyze these results as completely as possible to determine the location of the centromere and the relative locations of the genes.

7.14 High-density genetic maps can be generated through the use of mapping panels with a set of DNA markers and lod score methods. The same DNA markers can be mapped by means of radiation hybrid methods.

a. In what ways will maps generated by these two methods be identical, and in what ways will they differ?

b. Much or most of the entire genomic sequence has been obtained for a number of complex eukaryotes, including humans, mice, and the plant Arabidopsis thaliana. What is the value of high-density genetic maps in the genetic analysis of organisms whose genome has been sequenced?

*7.15 Two panels of radiation hybrids were produced by irradiating human tissue culture cells and then fusing them with hamster tissue culture cells. The differing properties of the two panels are shown in the following table (1 Mb = 10⁶ bp of DNA):

<table>
<thead>
<tr>
<th>X-ray dosage used to generate cell hybrids</th>
<th>Panel GB4</th>
<th>Panel G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cell lines established</td>
<td>93</td>
<td>83</td>
</tr>
<tr>
<td>Average retention of human genome per hybrid</td>
<td>32%</td>
<td>16%</td>
</tr>
<tr>
<td>Average human DNA fragment size</td>
<td>25 Mb</td>
<td>2.4 Mb</td>
</tr>
<tr>
<td>Effective map resolution</td>
<td>1 Mb</td>
<td>0.25 Mb</td>
</tr>
</tbody>
</table>
a. A haploid human genome has about $3 \times 10^9$ bp of DNA. About how many different human DNA segments are present, on average, in the hybrid cells of each panel?

b. Two human markers are found together in some, but not all, cell hybrids. Are they necessarily linked?

c. How do these panels differ in their advantages with respect to mapping genes and markers?

d. DNA markers A, B, and C derive from a single chromosomal region. Their presence or absence is assessed in DNA isolated from the hybrids of each panel, with the following results:

<table>
<thead>
<tr>
<th>Marker(s) Present</th>
<th>Panel GB4</th>
<th>Panel G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A only</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>B only</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>C only</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>A and B only</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>A and C only</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>B and C only</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A, B and C</td>
<td>27</td>
<td>0</td>
</tr>
</tbody>
</table>

Why do the two panels give such different results? What reasonable hypothesis can you generate concerning the arrangement of these three markers?

7.16 As discussed in Chapter 3, XO individuals have Turner syndrome. Some individuals who display a Turner phenotype are mosaic individuals with 45,X/46,XX or 46,XY/45,X karyotypes. It is clinically important to address mosaicism in Turner individuals, as some types of mosaics have an increased risk of gonadal cancer.

7.17 Some dogs love water, while others avoid it. A dog that loved water was mated to a dog that avoided it, and their F1 progeny were interbred to give an F2. The parental, F1, and F2 generations were evaluated by DNA typing, and the lod-score method was used to assess linkage between DNA markers and genes for water affection (waf genes). Suppose that the following data were obtained for one marker, where \( \theta \) gives the value of the recombination frequency between the marker and a waf gene used in calculating the lod score:

<table>
<thead>
<tr>
<th>( \theta )</th>
<th>lod Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.05</td>
<td>-12.51</td>
</tr>
<tr>
<td>0.10</td>
<td>-2.34</td>
</tr>
<tr>
<td>0.15</td>
<td>-1.32</td>
</tr>
<tr>
<td>0.20</td>
<td>2.66</td>
</tr>
<tr>
<td>0.25</td>
<td>4.01</td>
</tr>
<tr>
<td>0.30</td>
<td>3.21</td>
</tr>
<tr>
<td>0.35</td>
<td>2.14</td>
</tr>
<tr>
<td>0.40</td>
<td>1.56</td>
</tr>
<tr>
<td>0.50</td>
<td>0</td>
</tr>
</tbody>
</table>

Graph these lod scores and evaluate whether the marker is linked to a waf gene. If it is, estimate the physical distance between the marker and the gene.