6

LINKAGE AND MAPPING IN EUKARYOTES

STUDY OBJECTIVES

1. To learn about analytical techniques for locating the relative positions of genes on chromosomes in diploid eukaryotic organisms 110
2. To learn about analytical techniques for locating the relative positions of genes on chromosomes in ascomycete fungi 122
3. To learn about analytical techniques for locating the relative positions of genes on human chromosomes 132

STUDY OUTLINE

Scanning electron micrograph (false color) of a fruit fly, *Drosophila melanogaster.*

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After Sutton suggested the chromosomal theory of inheritance in 1903, evidence accumulated that genes were located on chromosomes. For example, Morgan showed by an analysis of inheritance patterns that the white-eye locus in Drosophila is located on the X chromosome. Given that any organism has many more genes than chromosomes, it follows that each chromosome has many loci. Since chromosomes in eukaryotes are linear, it also follows that genes are arranged in a linear fashion on chromosomes, like beads on a string. Sturtevant first demonstrated this in 1913. In this chapter, we look at analytical techniques for mapping chromosomes—techniques for determining the relationship between different genes on the same chromosome. These techniques are powerful tools that allow us to find out about the physical relationships of genes on chromosomes without ever having to see a gene or a chromosome. We determine that genes are on the same chromosome when the genes fail to undergo independent assortment, and then we use recombination frequencies to determine the distance between genes.

If loci were locked together permanently on a chromosome, allelic combinations would always be the same. However, at meiosis, crossing over allows the alleles of associated loci to show some measure of independence. A geneticist can use crossing over between loci to determine how close one locus actually is to another on a chromosome and thus to map an entire chromosome and eventually the entire **genome** (genetic complement) of an organism.

Loci carried on the same chromosome are said to be linked to each other. There are as many **linkage groups** (l) as there are autosomes in the haploid set plus sex chromosomes. *Drosophila* has five linkage groups ($2n$ = 8; l = 3 autosomes + X + Y), whereas human beings have twenty-four linkage groups ($2n$ = 46; l = 22 autosomes + X + Y). Prokaryotes and viruses, which usually have a single chromosome, are discussed in chapter 7.

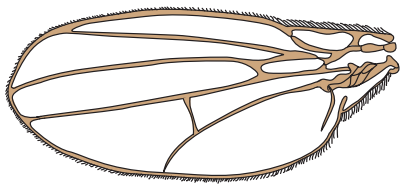
Historically, classical mapping techniques, as described in this chapter and the next, gave researchers their only tools to determine the relationships of particular genes and their chromosomes. When geneticists know the locations of specific genes, they can study them in relation to each other and begin to develop a comprehensive catalogue of the genome of an organism. Knowing the location of a gene also helps in isolating the gene and studying its function and structure. And mapping the genes of different types of organisms (diploid, haploid, eukaryotic, prokaryotic) gives geneticists insight into genetic processes. More recently, recombinant DNA technology has allowed researchers to sequence whole genomes, including the human and fruit fly genomes; this means they now know the exact locations of all the genes on all the chromosomes of these organisms (see chapter 13). Geneticists are now creating massive databases containing this information, much of it available for free or by subscription on the World Wide Web. Until investigators mine all this information for all organisms of interest, they will still use analytical techniques in the laboratory and field to locate genes on chromosomes.

DIPLOID MAPPING

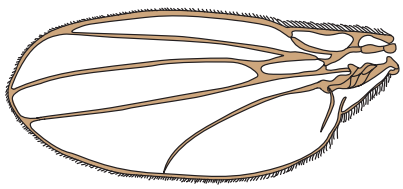
Two-Point Cross

In *Drosophila,* the recessive band gene (*bn*) causes a dark transverse band on the thorax, and the detached gene (*det*) causes the crossveins of the wings to be either detached or absent (fig. 6.1). A banded fly was crossed with a detached fly to produce wild-type, dihybrid offspring in the F_1 generation. F_1 females were then testcrossed to banded, detached males (fig. 6.2). (There is no crossing over in male fruit flies; in experiments designed to detect linkage, heterozygous females—in which crossing over occurs—are usually crossed with homozygous recessive males.) If the loci were assorting independently, we would expect a 1:1:1:1 ratio of the four possible phenotypes. However, of the first one thousand offspring examined, experimenters recorded a ratio of 2:483: 512:3.

Several points emerge from the data in figure 6.2. First, no simple ratio is apparent. If we divide by two, we get a ratio of 1:241:256:1.5. Although the first and last categories seem about equal, as do the middle two, no simple numerical relation seems to exist between the middle and end categories. Second, the two cate-



Wild-type



Detached

Figure 6.1 Wild-type (det^+) and detached (*det*) crossveins in *Drosophila*.

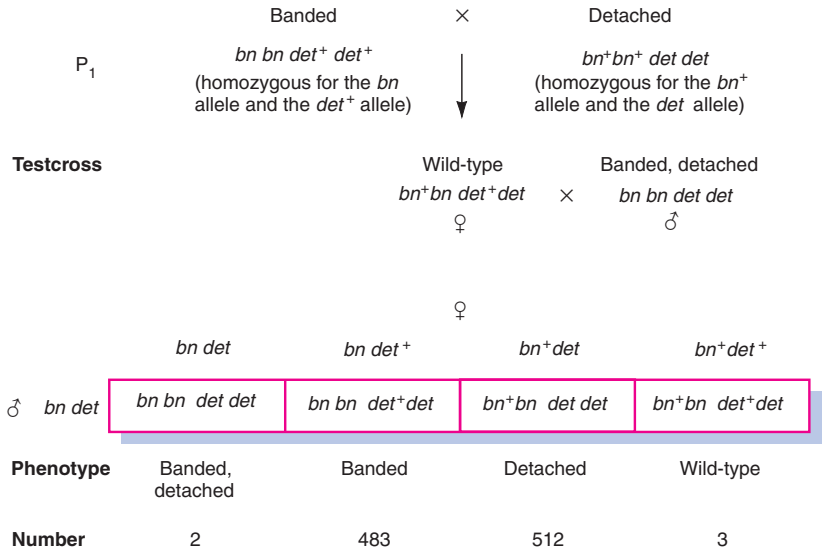


Figure 6.2 Testcrossing a dihybrid *Drosophila.*

gories in very high frequency have the same phenotypes as the original parents in the cross (P_1 of fig. 6.2). That is, banded flies and detached flies were the original parents as well as the great majority of the testcross offspring. We call these phenotypic categories **parentals,** or **nonrecombinants.** On the other hand, the testcross offspring in low frequency combine the phenotypes of the two original parents (P_1). These two categories are referred to as **nonparentals,** or **recombinants.** The simplest explanation for these results is that the banded and detached loci are located near each other on the same chromosome (they are a linkage group), and therefore they move together as associated alleles during meiosis.

We can analyze the original cross by drawing the loci as points on a chromosome (fig. 6.3). This shows that 99.5% of the testcross offspring (the nonrecombinants) come about through the simple linkage of the two loci. The remaining 0.5% (the recombinants) must have arisen through a crossover of homologues, from a chiasma at meiosis, between the two loci (fig. 6.4). Note that since it is not possible to tell from these crosses which chromosome the loci are actually on or where the centromere is in relation to the loci, the centromeres are not included in the figures. The crossover event is viewed as a breakage and reunion of two chromatids lying adjacent to each other during prophase I of meiosis. Later in this chapter, we find cytological proof for this; in chapter 12, we explore the molecular mechanisms of this breakage and reunion process.

From the testcross in figure 6.3, we see that 99.5% of the gametes produced by the dihybrid are nonrecombinant, whereas only 0.5% are recombinant. This very small frequency of recombinant offspring indicates that the two loci lie very close to each other on their particular chromosome. In fact, we can use the recombination percentages of gametes, and therefore of testcross offspring, as estimates of distance between loci on a chromosome: 1% recombinant offspring is referred to as one **map unit** (or one **centimorgan,** in honor of geneticist T. H. Morgan, the first geneticist to win the Nobel Prize; box 6.1). Although a map unit is not a physical distance, it is a relative distance that makes it possible to know the order of and relative separation between loci on a chromosome. In this case, the two loci are 0.5 map units apart. (From sequencing various chromosomal segments—see chapter 13—we have learned that the relationship between centimorgans and DNA base pairs is highly variable, depending on species, sex, and region of the chromosome. For example, in human beings, 1 centimorgan can vary between 100,000 and 10,000,000 base pairs. In the fission yeast, *Schizosaccharomyces pombe,* 1 centimorgan is only about 6,000 base pairs.)

The arrangement of the *bn* and *det* alleles in the dihybrid of figure 6.3 is termed the **trans** configuration, meaning "across," because the two mutants are across from each other, as are the two wild-type alleles. The alternative arrangement, in which one chromosome carries both mutants and the other chromosome carries both wild-type alleles (fig. 6.5), is referred to as the **cis** configuration. (Two other terms, **repulsion** and **coupling,** have the same meanings as *trans* and *cis,* respectively.)

A cross involving two loci is usually referred to as a **two-point cross;** it gives us a powerful tool for dissecting the makeup of a chromosome. The next step in our

BOX 6.1

Historical Perspectives

The Nobel Prize

On 10 December each year, the king of Sweden awards the Nobel Prizes at the Stockholm Concert Hall. The date is the anniversary of Alfred Nobel's death. Awards are given annually in physics, chemistry, medicine and physiology, literature, economics, and peace. In 2000, each award was worth $900,000, although an award sometimes goes to two or three recipients. The prestige is priceless.

Winners of the Nobel Prize are chosen according to the will of Alfred Nobel, a wealthy Swedish inventor and industrialist, who held over three hundred patents when he died in 1896 at the age of sixty-three. Nobel developed a detonator and processes for detonation of nitroglycerine, a substance invented by Italian chemist Ascanio Sobrero in 1847. In the form Nobel developed, the explosive was patented as dynamite. Nobel also invented several other forms of explosives. He was a benefactor of Sobrero, hiring him as a consultant and paying his wife a pension after Sobrero died.

Nobel believed that dynamite would be so destructive that it would serve as a deterrent to war. Later, realizing that this would not come to pass, he instructed that his fortune be invested and the interest used to fund the awards. The first prizes were awarded in 1901. Each award consists of a diploma, medal, and check.

American, British, German, French, and Swedish citizens have earned the most prizes (table 1). Table 2 features some highlights of Nobel laureate achievements in genetics.

The Nobel medal. The medal is half a pound of 23-karat gold, measures about 2 1/2 inches across, and has Nobel's face and the dates of his birth and death on the front. The diplomas that accompany the awards are individually designed. (Reproduced by permission of the Nobel Foundation.)

Table 1 Distribution of Nobel Awards to the Top Five Recipient Nations (Including 2000 Winners)

	Physics	Chemistry	Medicine and Physiology	Peace	Literature	Economics	Total
United States	77	46	88	20	9	30	270
Britain	20	24	25	9	8	5	91
Germany	18	27	15	4	6	1	71
France	12	7	7	8	12	1	47
Sweden	4	4	7	5	7	2	29

Table 2 Some Nobel Laureates in Genetics (Medicine and Physiology; Chemistry)

Name	Year	Nationality	Cited for
Thomas Hunt Morgan	1933	USA	Discovery of how chromosomes govern heredity
Hermann J. Muller	1946	USA	X-ray inducement of mutations
George W. Beadle	1958	USA	Genetic regulation of biosynthetic pathways
Edward L. Tatum	1958	USA	
Joshua Lederberg	1958	USA	Bacterial genetics
Severo Ochoa	1959	USA	Discovery of enzymes that synthesize nucleic acids
Arthur Kornberg	1959	USA	
Francis H. C. Crick	1962	British	Discovery of the structure of DNA
James D. Watson	1962	USA	
Maurice Wilkins	1962	British	
François Jacob	1965	French	Regulation of enzyme biosynthesis
André Lwoff	1965	French	
Jacques Monod	1965	French	
Peyton Rous	1966	USA	Tumor viruses
Robert W. Holley	1968	USA	Unraveling of the genetic code
H. Gobind Khorana	1968	USA	
Marshall W. Nirenberg	1968	USA	
Max Delbrück	1969	USA	Viral genetics
Alfred Hershey	1969	USA	
Salvador Luria	1969	USA	
Renato Dulbecco	1975	USA	Tumor viruses
Howard Temin	1975	USA	Discovery of reverse transcriptase
David Baltimore	1975	USA	
Werner Arber	1978	Swiss	Discovery and use of restriction endonucleases
Hamilton Smith	1978	USA	
Daniel Nathans	1978	USA	
Walter Gilbert	1980	USA	Techniques of sequencing DNA
Frederick Sanger	1980	British	
Paul Berg	1980	USA	Pioneer work in recombinant DNA
Baruj Benacerraf	1980	USA	Genetics of immune reactions
Jean Dausset	1980	French	
George Snell	1980	USA	
Aaron Klug	1982	British	Crystallographic work on protein-nucleic acid complexes
Barbara McClintock	1983	USA	Transposable genetic elements
Cesar Milstein	1984	British/Argentine	Immunogenetics
Georges Koehler	1984	German	
Niels K. Jerne	1984	British/Danish	
Susumu Tonegawa	1987	Japanese	Antibody diversity
J. Michael Bishop	1989	USA	Proto-oncogenes
Harold E. Varmus	1989	USA	
Thomas R. Cech	1989	USA	Enzymatic properties of RNA
Sidney Altman	1989	Canada	
Kary Mullis	1993	USA	Polymerase chain reaction
Michael Smith	1993	Canada	Site-directed mutagenesis
Richard Roberts	1993	British	Discovery of intervening sequences in RNA
Phillip Sharp	1993	USA	
E. B. Lewis	1995	USA	Genes control development

continued

BOX 6.1 CONTINUED

Table 2 *continued*

Name	Year	Nationality	Cited for
Christiane Nüsslein-Volhard	1995	German	
Eric Wieschaus	1995	USA	
Stanley B. Prusiner	1997	USA	Discovery of prions
Günter Blobel	1999	German	Signal recognition during protein synthesis

analysis is to look at three loci simultaneously so that we can determine their relative order on the chromosome. More important, we can also analyze the effects of multiple crossovers, which cannot be detected in a two-point cross, on map distances. Two crossovers between two loci can cause the chromosome to look as if no crossovers took place, causing us to underestimate map distances. Thus we need a third locus, between the first two, to detect multiple crossover events.

Three-Point Cross

Analysis of three loci, each segregating two alleles, is referred to as a **three-point cross.** We will examine wing morphology, body color, and eye color in *Drosophila.* Black body (*b*), purple eyes (*pr*), and curved wings (*c*) are all recessive genes. Since the most efficient way to study linkage is through the testcross of a multihybrid, we will study these three loci by means of the crosses shown in

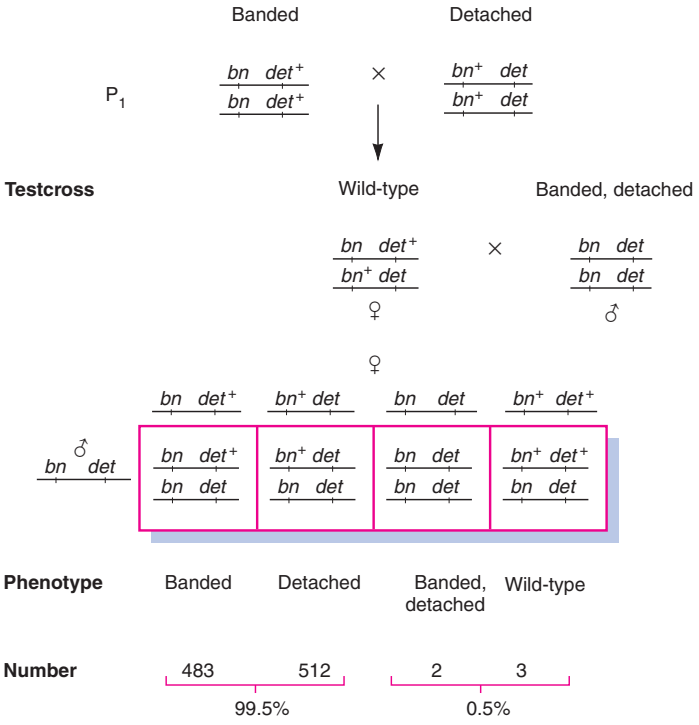


Figure 6.3 Chromosomal arrangement of the two loci in the crosses of figure 6.2. A line arbitrarily represents the chromosomes on which these loci are actually situated.

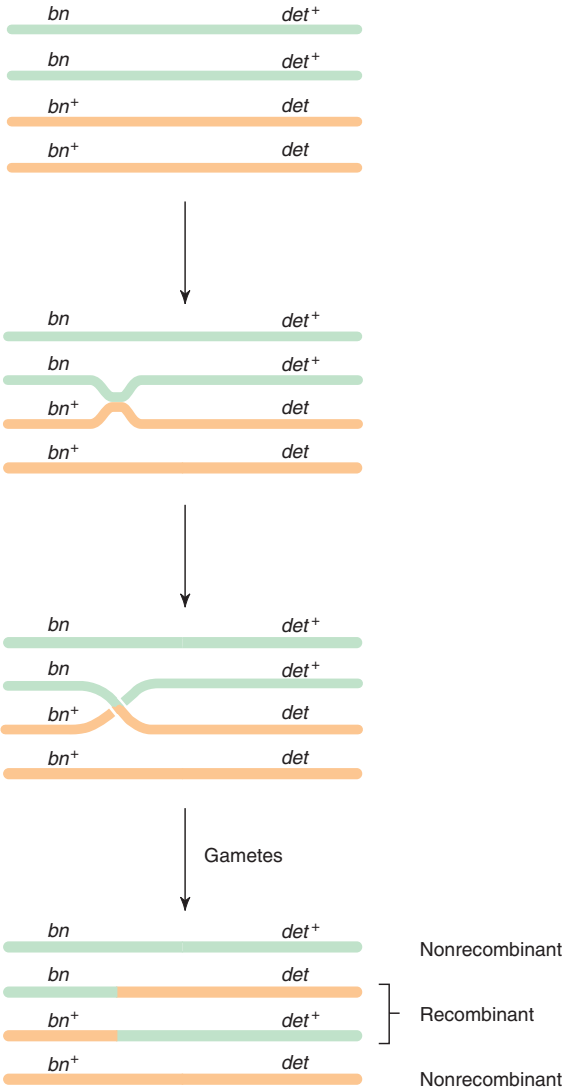


Figure 6.4 Crossover of homologues during meiosis between the *bn* and *det* loci in the tetrad of the dihybrid female.

figure 6.6. One point in this figure should be clarified. Since the organisms are diploid, they have two alleles at each locus. Geneticists use various means to present this situation. For example, the recessive homozygote can be pictured as

1. *bb prpr cc*
2. *b/b pr/pr c/c* or $\frac{b}{b}\ \frac{pr}{pr}\ \frac{c}{c}$
3. *b pr c/b pr c* or $\frac{b\ pr\ c}{b\ pr\ c}$

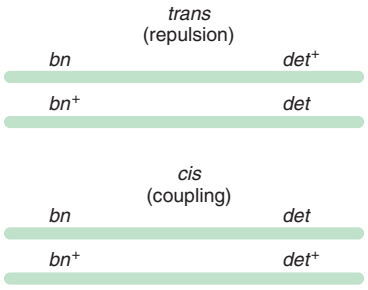


Figure 6.5 *Trans* (repulsion) and *cis* (coupling) arrangements of dihybrid chromosomes.

A slash (also called a rule line) is used to separate alleles on homologous chromosomes. Thus (*1*) is used tentatively, when we do not know the linkage arrangement of the loci, (*2*) is used to indicate that the three loci are on different chromosomes, and (*3*) indicates that all three loci are on the same chromosome.

In figure 6.6, the trihybrid organism is testcrossed. If independent assortment is at work, the eight types of resulting gametes should appear with equal frequencies, and thus the eight phenotypic classes would each make up one-eighth of the offspring. However, if there were *complete linkage,* so that the loci are so close together on the same chromosome that virtually no crossing over takes place, we would expect the trihybrid to produce only two gamete types in equal frequency and to yield two phenotypic classes identical to the original parents. This would occur because, under complete linkage, the trihybrid would produce only two chromosomal types in gametes: the *b pr c* type from one parent and the b^+ pr^+ c^+ type from the other. Crossing over between linked loci would produce eight phenotypic classes in various proportions depending on the distances between loci. The actual data appear in table 6.1.

The data in the table are arranged in reciprocal classes. Two classes are reciprocal if between them they contain each mutant phenotype just once. Wild-type and black, purple, curved classes are thus reciprocal, as are the purple, curved and the black classes. Reciprocal classes occur in roughly equal numbers: 5,701 and 5,617; 388 and 367; 1,412 and 1,383; and 60 and 72. As we shall see, a single meiotic recombinational event produces reciprocal classes. Wild-type and black, purple, curved are the two nonrecombinant classes. The purple, curved class of 388 is grouped with the black class of 367. These two would be the products of a crossover between the *b* and the *pr* loci if we assume that the three loci are linked and that the gene order is *b pr c* (fig. 6.7). The next two classes, of 1,412 and 1,383 flies, would result from a crossover between *pr* and *c,* and the last set, 60 and 72, would result from two crossovers, one between *b* and *pr* and the other between

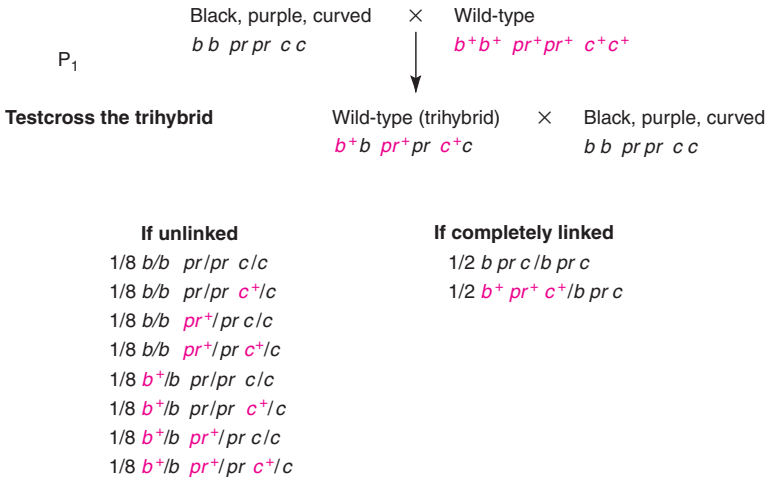


Figure 6.6 Possible results in the testcross progeny of the *b pr c* trihybrid.

pr and *c* (fig. 6.8). Groupings according to these recombinant events are shown at the right in table 6.1.

In the final column of table 6.1, recombination between *b* and *c* is scored. Only those recombinant classes that have a new arrangement of *b* and *c* alleles, as compared with the parentals, are counted. This last column shows us what a *b–c,* two-point cross would have revealed had we been unaware of the *pr* locus in the middle.

Map Distances

The percent row in table 6.1 reveals that 5.9% (887/15,000) of the offspring in the *Drosophila* trihybrid testcross resulted from recombination between *b* and *pr,* 19.5% between *pr* and *c,* and 23.7% between *b* and *c.* These numbers allow us to form a tentative map of the loci (fig. 6.9). There is, however, a discrepancy. The distance between *b* and *c* can be calculated in two ways. By adding the two distances, *b–pr* and *pr–c,* we get 5.9 + 19.5 = 25.4 map units; yet by directly counting the recombinants (the last column of table 6.1), we get a distance of only 23.7 map units. What causes this discrepancy of 1.7 map units?

Returning to the last column of table 6.1, we observe that the double crossovers (60 and 72) are not counted, yet each actually represents two crossovers in this re-

Table 6.1 **Results of Testcrossing Female *Drosophila* Heterozygous for Black Body Color, Purple Eye Color, and Curved Wings ($b^+b\ pr^+pr\ c^+c \times bb\ prpr\ cc$)**

Phenotype	Genotype	Number	Alleles from Trihybrid Female	Number Recombinant Between		
				b and *pr*	*pr* and *c*	*b* and *c*
Wild-type	$b^+b\ pr^+pr\ c^+\ c$	5,701	$b^+\ pr^+\ c^+$			
Black, purple, curved	*bb prpr cc*	5,617	*b pr c*			
Purple, curved	b^+b *prpr cc*	388	b^+ *pr c*	388		388
Black	*bb* $pr^+pr\ c^+c$	367	*b* $pr^+\ c^+$	367		367
Curved	$b^+b\ pr^+pr$ *cc*	1,412	$b^+\ pr^+$ *c*		1,412	1,412
Black, purple	*bb prpr* c^+c	1,383	*b pr* c^+		1,383	1,383
Purple	b^+b *prpr* c^+c	60	$b^+pr\ c^+$	60	60	
Black, curved	*bb* pr^+pr *cc*	72	*b* pr^+ *c*	72	72	
Total		15,000		887	2,927	3,550
Percent				5.9	19.5	23.7

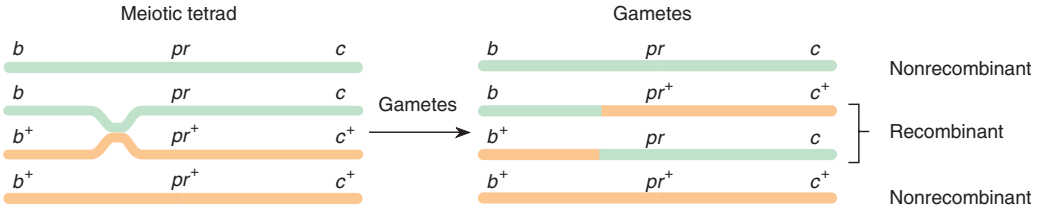


Figure 6.7 Results of a crossover between the black and purple loci in *Drosophila*.

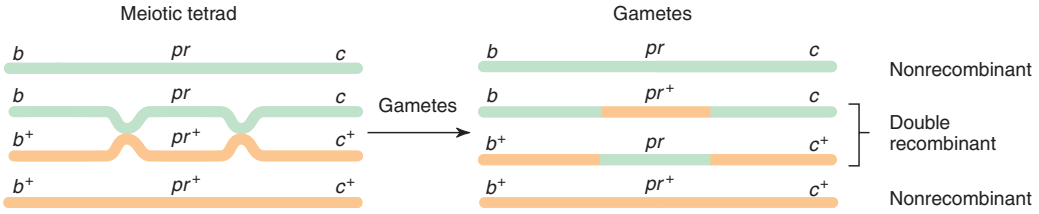


Figure 6.8 Results of a double crossover in the *b pr c region of the Drosophila* chromosome.

gion. The reason they are not counted is simply that if we observed only the end loci of this chromosomal segment, we would not detect the double crossovers; the first one of the two crossovers causes a recombination between the two end loci, whereas the second one returns these outer loci to their original configuration (see fig. 6.8). If we took the 3,550 recombinants between *b* and *c* and added in twice the total of the double recombinants, 264, we would get a total of 3,814. This is 25.4 map units, which is the more precise figure we calculated before. The farther two loci are apart on a chromosome, the more double crossovers occur between them. Double crossovers tend to mask recombinants, as in our example, so that distantly linked loci usually appear closer than they really are. Thus, the most accurate map distances are those established on very closely linked loci. In other words, summed short distances are more accurate than directly measured larger distances.

The results of the previous experiment show that we can obtain at least two map distances between any two loci: measured and actual. Measured map distance between two loci is the value obtained from a two-point cross. Actual map distance is an idealized, more accurate value obtained from summing short distances between many intervening loci. We obtain the short distances from crosses involving other loci between the original two. When we plot measured map distance against actual map distance, we obtain the curve in figure 6.10. This curve is called a **mapping function.** This graph is of both practical and theoretical value. Pragmatically, it allows

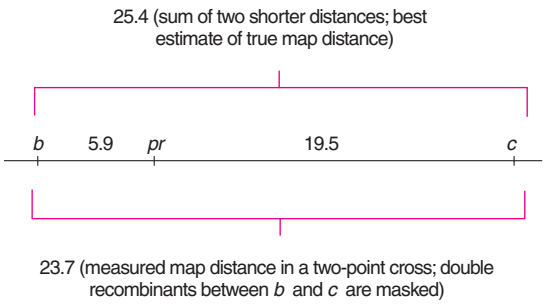


Figure 6.9 Tentative map of the black, purple, and curved chromosome in *Drosophila*. Numbers are map units (centimorgans).

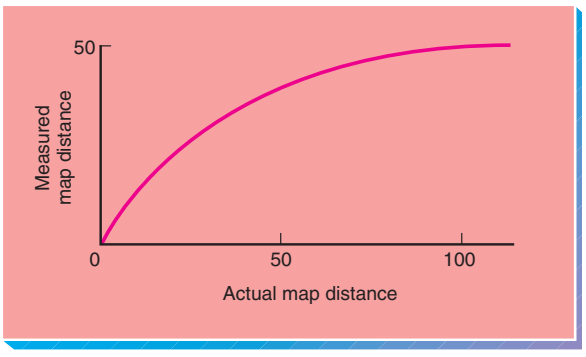


Figure 6.10 Mapping function.

us to convert a measured map distance into a more accurate one. Theoretically, it shows that measured map distance never exceeds 50 map units in any one cross. Multiple crossovers reduce the apparent distance between two loci to a maximum of 50 map units, the value that independent assortment produces (50% parentals, 50% recombinants).

Gene Order

Although we performed the previous analysis merely assuming that *pr* was in the middle, the data in table 6.1 confirm our original assumption that the gene order is *b pr c.* Of the four pairs of reciprocal phenotypic classes in table 6.1, one pair has the highest frequency (5,701 and 5,617) and one pair has the lowest (60 and 72). The pair with highest frequency is the nonrecombinant group. The one with the lowest frequency is the double recombinant group, the one in which only the middle locus has been changed from the parental arrangement. A comparison of either of the double recombinant classes with either of the nonrecombinant classes shows the gene that is in the middle and, therefore, the gene order. In other words, the data allow us to determine gene order. Since b^+ pr^+ c^+ was one of the nonrecombinant gametes, and b^+ *pr* c^+ was one of the double recombinant gametes, *pr* stands out as the changed locus, or the one in the middle. In a similar manner, comparing *b* pr^+ *c* with *b pr c* would also point to *pr* as the inside locus (or **inside marker**). So would comparing b^+ *pr* c^+ with *b pr c* or *b* pr^+ *c* with b^+ *pr* c^+. In each case, the middle locus, *pr,* displays the different pattern, whereas the allelic arrangements of the outside markers, *b* and *c,* behave in concert.

If this seems confusing, simply compare the double crossovers and nonrecombinants to find one of each in which two alleles are identical. For example, the double recombinant b^+ *pr* c^+ and the nonrecombinant b^+ pr^+ c^+ share the b^+ and c^+ alleles. The *pr* locus is mutant in one case and wild-type in the other. Hence, *pr* is the locus in the middle.

From the data in table 6.1, we can confirm the association of alleles in the trihybrid parent. That is, since the data came from testcrossing a trihybrid, the allelic configuration in that trihybrid is reflected in the nonrecombinant classes of offspring. In this case, one is the result of a b^+ pr^+ c^+ gamete, the other, of a *b pr c* gamete. Thus, the trihybrid had the genotype *b pr c*/b^+ pr^+ c^+: all alleles were in the *cis* configuration.

Coefficient of Coincidence

The next question in our analysis of this three-point cross is, are crossovers occurring independently of each other? That is, does the observed number of double recombinants equal the expected number? In the example, there were 132/15,000 double crossovers, or 0.88%. The expected number is based on the independent occurrence of crossing over in the two regions measured. That is, 5.9% of the time there is a crossover in the *b–pr* region, which we can express as a probability of occurrence of 0.059. Similarly, 19.5% of the time there is a crossover in the *pr–c* region, or a probability of occurrence of 0.195. A double crossover should occur as a product of the two probabilities: $0.059 \times 0.195 = 0.0115$. This means that 1.15% of the gametes (1.15% of 15,000 = 172.5) should be double recombinants. In our example, the observed number of double recombinant offspring is lower than expected (132 observed, 172.5 expected). This implies a **positive interference,** in which the occurrence of the first crossover reduces the chance of the second. We can express this as a **coefficient of coincidence,** defined as

$$\frac{\text{observed number of double recombinants}}{\text{expected number of double recombinants}}$$

In the example, the coefficient of coincidence is $132/172.5 = 0.77$. In other words, only 77% of the expected double crossovers occurred. Sometimes we express this reduced quantity of double crossovers as the *degree of interference,* defined as

$$\text{interference} = 1 - \text{coefficient of coincidence}$$

In our example, the interference is 23%.

It is also possible to have **negative interference,** in which we observe more double recombinants than expected. In this situation, the occurrence of one crossover seems to enhance the probability that crossovers will occur in adjacent regions.

Another Example

Let us work out one more three-point cross, in which neither the middle gene nor the *cis-trans* relationship of the alleles in the trihybrid F_1 parent is given. On the third chromosome of *Drosophila,* hairy (*h*) causes extra bristles on the body, thread (*th*) causes a thread-shaped arista (antenna tip), and rosy (*ry*) causes the eyes to be reddish brown. All three traits are recessive. Trihybrid females were testcrossed; the phenotypes from one thousand offspring are listed in table 6.2. At this point, it is possible to use the data to determine the parental genotypes (the P_1 generation, assuming that they were homozygotes), the gene order, the map distances, and the coefficient of coincidence. The table presents the data in no particular order, as an experimenter might have recorded them. Phenotypes are tabulated and, from these, the genotypes can be reconstructed. Notice that the data can be put into the form found in table 6.1;

Table 6.2 **Offspring from a Trihybrid ($h^+h\ ry^+ry\ th^+th$) Testcross ($x\ hh\ ryry\ thth$) in *Drosophila***

Phenotype	Genotype (order unknown)	Number
Thread	$h^+ry^+th/h\ ry\ th$	359
Rosy, thread	$h^+ry\ th/h\ ry\ th$	47
Hairy, rosy, thread	$h\ ry\ th/h\ ry\ th$	4
Hairy, thread	$h\ ry^+th/h\ ry\ th$	98
Rosy	$h^+ry\ th^+/h\ ry\ th$	92
Hairy, rosy	$h\ ry\ th^+/h\ ry\ th$	351
Wild-type	$h^+ry^+th^+/h\ ry\ th$	6
Hairy	$h\ ry^+th^+/h\ ry\ th$	43

Table 6.3 **Data from Table 6.2 Arranged to Show Recombinant Regions**

Trihybrid's Gamete	Number	*h–th*	*th–ry*	*h–ry*
$h^+th\ ry^+$	359			
$h\ th^+ry$	351			
$h\ th\ ry^+$	98	98		98
h^+th^+ry	92	92		92
$h^+th\ ry$	47		47	47
$h\ th^+ry^+$	43		43	43
$h\ th\ ry$	4	4	4	
$h^+th^+ry^+$	6	6	6	
Total	1,000	200	100	280

we see a large reciprocal set (359 and 351), a small reciprocal set (4 and 6), and large and small intermediate sets (98 and 92, 47 and 43).

From the data presented, is it obvious that the three loci are linked? The pattern, as just mentioned, is identical to that of the previous example, in which the three loci were linked. (What pattern would appear if two of the loci were linked and one assorted independently? See problem 6 at the end of the chapter.) Next, what is the allelic arrangement in the trihybrid parent? The offspring with the parental, or nonrecombinant, arrangements are the reciprocal pair in highest frequency. Table 6.2 shows that thread and hairy, rosy offspring are the nonrecombinants. Thus, the nonrecombinant gametes of the trihybrid F_1 parent were *h ry* th^+ and h^+ ry^+ *th,* which is the allelic arrangement of the trihybrid with the actual order still unknown—*h ry* th^+/h^+ ry^+ *th.* (What were the genotypes of the parents of this trihybrid, assuming they were homozygotes?) Continuing, which gene is in the middle? From table 6.2, we know that *h ry th* and h^+ ry^+ th^+ are the double recombinant gametes of the trihybrid parent because they occur in such low numbers. Comparison of these chromosomes with either of the nonrecombinant chromosomes (h^+ ry^+ *th* or *h ry* th^+) shows that the thread (*th*) locus is in the middle. We now know that the original trihybrid had the following chromosomal composition: *h* th^+ *ry*/h^+ *th* ry^+. The *h* and *ry* alleles are in the *cis* configuration, with *th* in the *trans* configuration.

We can now compare the chromosome from the trihybrid in each of the eight offspring categories with the parental arrangement and determine the regions that had crossovers. Table 6.3 does this. We can see that the *h–th* distance is 20 map units, the *th–ry* distance is 10 map units, and the apparent *h–ry* distance is 28 map units (fig. 6.11). As in the earlier example, the *h–ry* discrepancy is from not counting the double crossovers twice each: 280 + 2(10) = 300, which is 30 map units and the more accurate figure. Last, we wish to know what the coefficient of coincidence is. The expected occurrence of double recombinants is 0.200 × 0.100 = 0.020, or 2%. Two percent of 1,000 = 20. Thus

$$\text{coefficient of coincidence} = \frac{\text{observed number of double recombinants}}{\text{expected number of double recombinants}} = 10/20 = 0.50$$

Only 50% of the expected double crossovers occurred.

Geneticists have mapped the chromosomes of many eukaryotic organisms from three-point crosses of this type—those of *Drosophila* are probably the most extensively studied. *Drosophila* and other species of flies have giant **polytene** salivary gland chromosomes, which arise as a result of **endomitosis.** In this process,

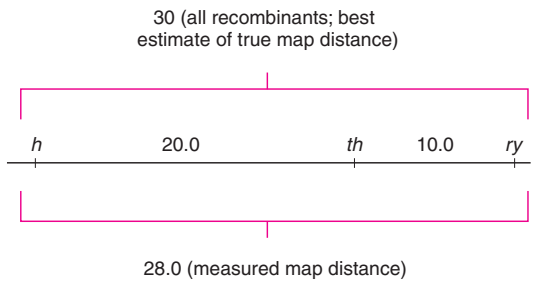


Figure 6.11 Map of the *h th ry* region of the *Drosophila* chromosome, with numerical discrepancy in distances. Numbers are map units (centimorgans).

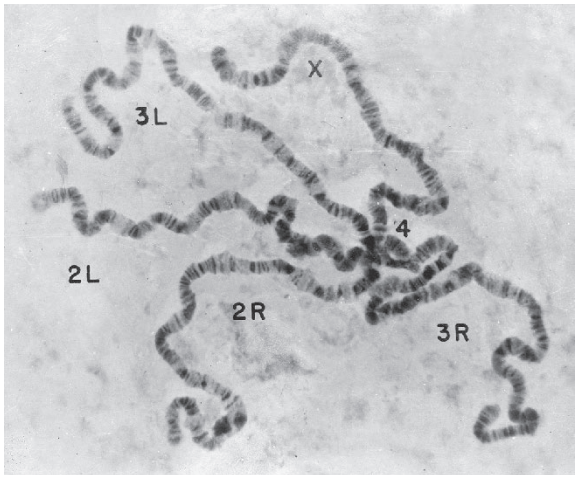


Figure 6.12 Giant salivary gland chromosomes of *Drosophila.* X, 2, 3, and 4 are the four nonhomologous chromosomes. *L* and *R* indicate the *left* and *right* arms (in relation to the centromere). The dark bands are chromomeres. (B. P. Kaufman, "Induced Chromosome Rearrangements in *Drosophila melanogaster,*" *Journal of Heredity,* 30:178–90, 1939. Reproduced by permission of Oxford University Press.)

the chromosomes replicate, but the cell does not divide. In the salivary gland of the fruit fly, homologous chromosomes synapse and then replicate to make about one thousand copies, forming very thick structures with a distinctive pattern of bands called chromomeres (fig. 6.12). Using methods chapter 8 will discuss, scientists have mapped many loci to particular bands. Part of the *Drosophila* chromosomal map is presented in figure 6.13 (see also box 6.2). Locate the loci we have mapped so far to verify the map distances.

In summary, we know that two or more loci are linked if offspring do not fall into simple Mendelian ratios. Map distances are the percentage of recombinant offspring in a testcross. With three loci, determine the parental (nonrecombinant) and double recombinant groups first. Then establish the locus in the middle, and recast the data in the correct gene order. The most accurate map distances are those obtained by summing shorter distances. Determine a coefficient of coincidence by comparing observed number of double recombinants to expected number.

Cytological Demonstration of Crossing Over

If we are correct that a chiasma during meiosis is the visible result of a physical crossover, then we should be able to demonstrate that genetic crossing over is accompanied by cytological crossing over. That is, the recombination event should entail the exchange of physical parts of homologous chromosomes. This can be demonstrated if we can distinguish between two homologous chromosomes, a technique Creighton and McClintock first used in maize (corn) and Stern first applied to *Drosophila,* both in 1931. We will look at Creighton and McClintock's experiment.

Harriet Creighton and Barbara McClintock worked with chromosome 9 in maize ($n = 10$). In one strain, they found a chromosome with abnormal ends. One end had a knob, and the other had an added piece of chromatin from another chromosome (fig. 6.14). This knobbed chromosome was thus clearly different from its normal homologue. It also carried the dominant colored (*C*) allele and the recessive waxy texture (*wx*) allele. After mapping studies showed that *C* was very close to the knob and *wx* was close to the added piece of chromatin, Creighton and McClintock made the cross shown in figure 6.14. The dihybrid plant with heteromorphic chromosomes was crossed with the normal homomorphic plant (only normal chromosomes) that had the genotype of *c Wx/c wx* (colorless and nonwaxy phenotype). If a crossover occurred during meiosis in the dihybrid in the region between *C* and *wx*, a physical crossover, visible cytologically (under the microscope), should also occur, causing the knob to become associated with an otherwise normal chromosome and the extra piece of chromosome 9 to be associated with a knobless chromosome. Four types of gametes would result (fig. 6.14).

Barbara McClintock (1902–1992). (Courtesy of Cold Spring Harbor Research Library Archives. Photographer, David Miklos.)

Harriet B. Creighton (1909–). (Courtesy of Harriet B. Creighton.)

BOX 6.2

Historical Perspectives

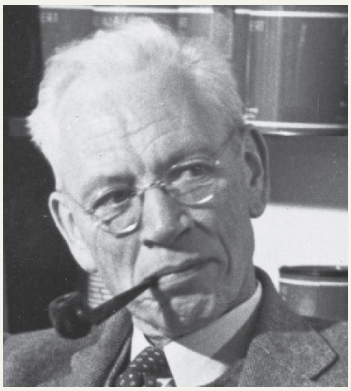
The First Chromosomal Map

The first chromosomal map ever published included just five loci on the X chromosome of *Drosophila melanogaster* (fig. 1). It was published in 1913 by Alfred H. Sturtevant, who began working in Thomas Hunt Morgan's "fly lab" while an undergraduate student at Columbia University. The fly lab included H. J. Muller, later to win a Nobel Prize, and Calvin B. Bridges, whose work on sex determination in *Drosophila* we discussed in the last chapter.

Sturtevant worked with six mutants: yellow body (*y*); white (*w*), eosin (w^e), and vermilion eyes (*v*); and miniature (*m*) and rudimentary wings (*r*). (White and eosin are actually allelic; Sturtevant found no crossing over between the two "loci.") Using crosses similar to the ones we outline in this chapter, he constructed the map shown in figure 1. The map distances we accept today are very similar to the ones he obtained.

Sturtevant's work was especially important at this point because his data supported several basic concepts, including the linear arrangement of genes, which argued for the placement of genes on chromosomes as the only linear structures in the nucleus. Sturtevant also pointed out crossover interference. His summary is clear and succinct:

> It has been found possible to arrange six sex-linked factors in *Drosophila* in a linear series, using the number of crossovers per one hundred cases as an index of the distance between any two factors. This scheme gives consistent results, in the main.
>
> A source of error in predicting the strength of association between untried factors is found in double crossing over. The occurrence of this phenomenon is demonstrated, and it is shown not to occur as often as would be expected from a purely mathematical point of view, but the conditions governing its frequency are as yet not worked out.
>
> These results . . . form a new argument in favor of the chromosome view of inheritance, since they strongly indicate that the factors investigated are arranged in a linear series, at least mathematically.



Alfred H. Sturtevant (1891–1970). (Courtesy of the Archives, California Institute of Technology.)

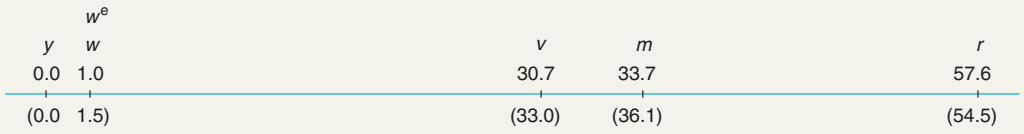


Figure 1 The first chromosomal linkage map. Five loci in *Drosophila melanogaster* are mapped to the X chromosome. The numbers in parentheses are the more accurately mapped distances recognized today. We also show today's allelic designations rather than Sturtevant's original nomenclature. (Data from Sturtevant. "The linear arrangement of six sex-linked factors in *Drosophila,* as shown by their mode of association," *Journal of Experimental Zoology,* 14:43–59, 1913.)

Of twenty-eight offspring examined, all were consistent with the predictions of the Punnett square in figure 6.14. Those of class 8 (lower right box) with the colored, waxy phenotype all had a knobbed interchange chromosome as well as a normal homologue. Those with the colorless, waxy phenotype (class 4) had a knobless interchange chromosome. All of the colored, nonwaxy phenotypes (classes 5, 6, and 7) had a knobbed, normal chromosome, which indicated that only classes 5 and 6 were in the sample. Of the two that were tested, both were *WxWx,* indicating that they were of class 5. The remaining classes (1, 2, and 3) were of the colorless, nonwaxy phenotype. All were knobless. Of those that contained only normal chromosomes, some were

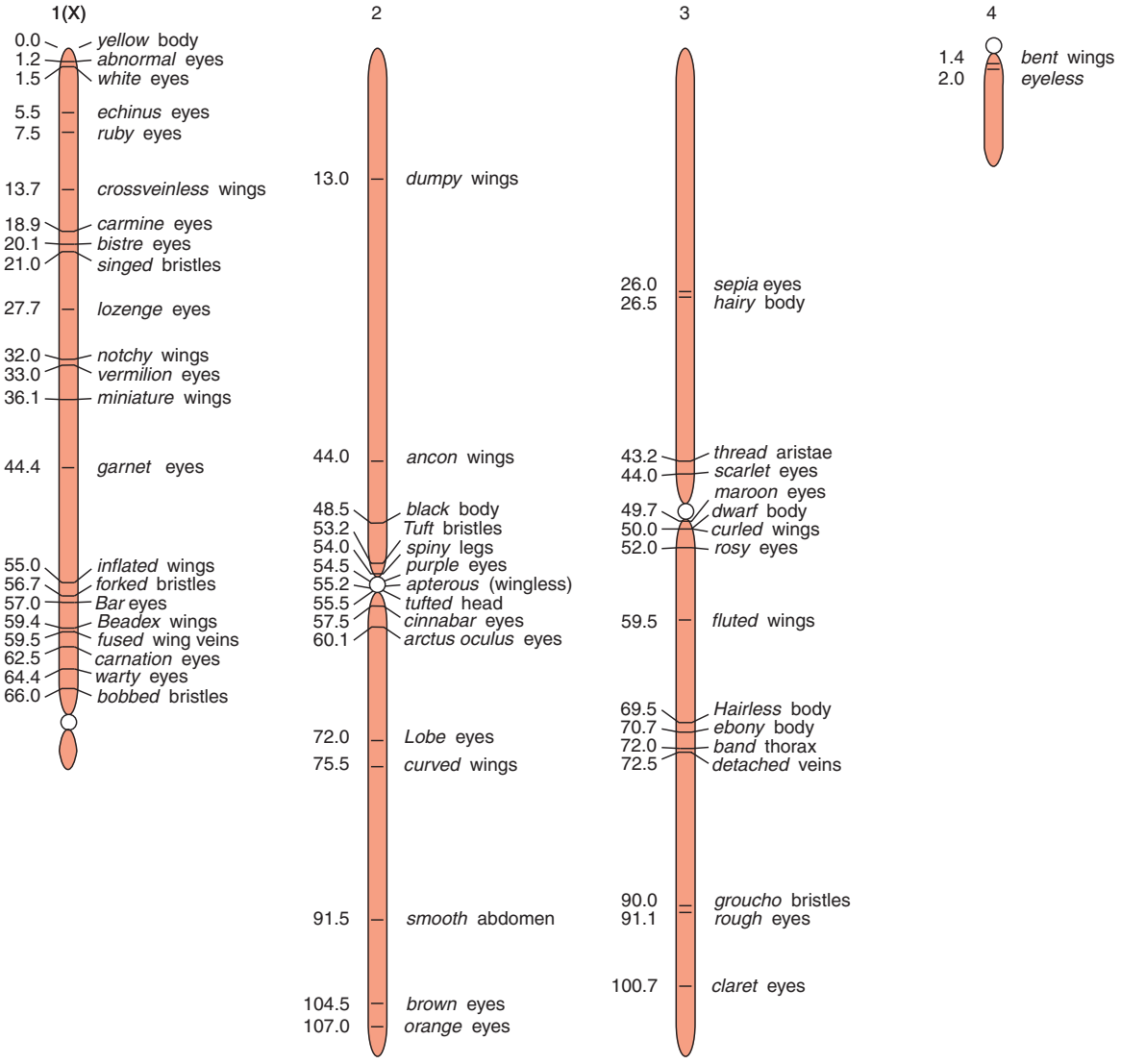


Figure 6.13 Partial map of the chromosomes of *Drosophila melanogaster.* The centromere is marked by an open circle.
(From C. Bridges, "Salavary Chromosome Maps," *Journal of Heredity,* 26:60–64, 1935. Reprinted with permission of Oxford University Press.)

WxWx (class 1) and some were heterozygotes (*Wxwx*, class 2). Of those containing interchange chromosomes, two were heterozygous, representing class 3. Two were homozygous, *WxWx*, yet interchange-normal heteromorphs. These represent a crossover in the region between the waxy locus and the extra piece of chromatin, producing a knobless-*c*-*Wx*-extra-piece chromosome. When combined with a *c*-*Wx*-normal chromosome, these would give these anomalous genotypes. The sample size was not large enough to pick up the reciprocal event. Creighton and McClintock concluded: "Pairing chromosomes, heteromorphic in two regions, have been shown to exchange parts at the same time they exchange genes assigned to these regions."

HAPLOID MAPPING (TETRAD ANALYSIS)

For *Drosophila* and other diploid eukaryotes, the genetic analysis considered earlier in this chapter is referred to as **random strand analysis.** Sperm cells, each of which carry only one chromatid of a meiotic tetrad, unite with

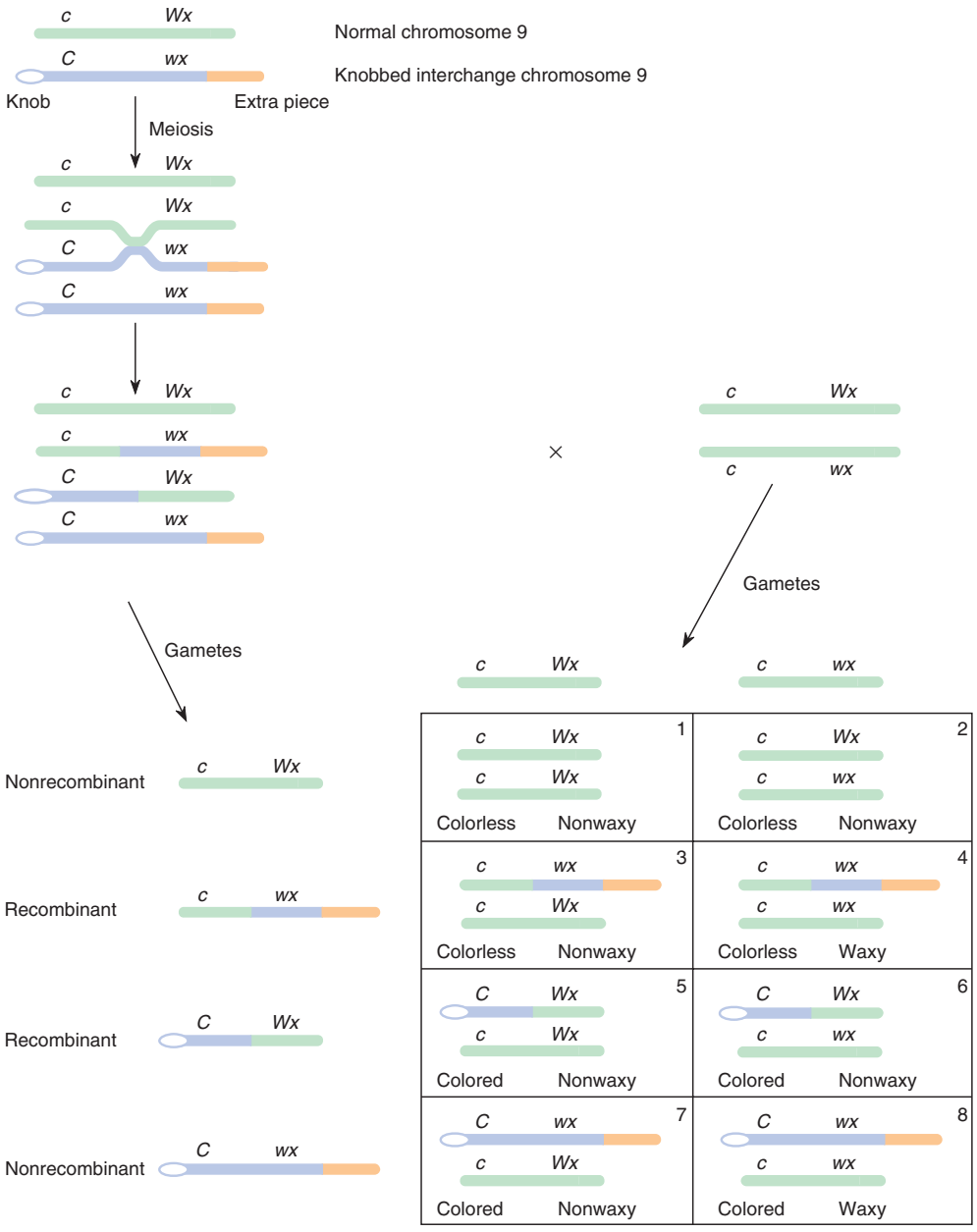


Figure 6.14 Creighton and McClintock's experiment in maize demonstrated that genetic crossover correlates with cytological crossing over.

eggs, which also carry only one chromatid from a tetrad. Thus, zygotes are the result of the random uniting of chromatids.

Fungi of the class Ascomycetes retain the four haploid products of meiosis in a sac called an **ascus.** These organisms provide a unique opportunity to look at the total products of meiosis in a tetrad. Having the four products of meiosis allowed geneticists to determine such basics as the reciprocity of crossing over and the fact that DNA replication occurs before crossing over. Different techniques are used for these analyses. We will look at two fungi, the common baker's yeast, *Saccharomyces cerevisiae,* and pink bread mold, *Neurospora crassa,* both of which retain the products of meiosis as **ascospores.**

Phenotypes of Fungi

At this point, you might wonder what phenotypes fungi such as yeast and *Neurospora* express. In general, microorganisms have phenotypes that fall into three broad categories: colony morphology, drug resistance, and nutritional requirements. Many microorganisms can be cultured in petri plates or test tubes that contain a supporting medium such as agar, to which various substances can be added (fig. 6.15). Wild-type *Neurospora,* the familiar pink bread mold, generally grows in a filamentous form, whereas yeast tends to form colonies. Various mutations exist that change colony morphology. In yeast, the *ade* gene causes the colonies to be red. In *Neurospora,* fluffy (*fl*), tuft (*tu*), dirty (*dir*), and colonial (*col4*) are all mutants of the basic growth form. In addition, wild-type *Neurospora* is sensitive to the sulfa drug sulfonamide, whereas one of its mutants (*Sfo*) actually requires sulfonamide in order to survive and grow. Yeast shows similar sensitivities to antifungal agents.

Nutritional-requirement phenotypes provide great insight not only into genetic analysis but also into the biochemical pathways of metabolism, as mentioned in chapter 2. Wild-type *Neurospora* can grow on a medium containing only sugar, a nitrogen source, some organic acids and salts, and the vitamin biotin. This is referred to as **minimal medium.** However, several different mutant types, or strains, of **Neurospora** cannot grow on this minimal medium until some essential nutrient is added. For example, one mutant strain will not grow on minimal medium, but will grow if one of the amino acids, arginine, is added (fig. 6.16). From this we can infer that the wild-type has a normal, functional enzyme in the synthetic pathway of arginine. The arginine-requiring mutant has an allele that specifies an enzyme that is incapable of converting one of the intermediates in the pathway directly into arginine or into one of the precursors to arginine. We can see that if the synthetic pathway is long, many different loci may have alleles that cause the strain to require arginine (fig. 6.17). This, in fact, happens, and the different loci are usually named arg_1, arg_2, and so on. There are numerous biosynthetic pathways in yeast and *Neurospora,* and mutants exhibit many different nutritional requirements. Mutants can be induced experimentally by radiation or by chemicals and other treatments. These, then, are the tools we use to analyze and map the chromosomes of microorganisms, including yeast and *Neurospora.* These techniques are expanded on in the next chapter.

Unordered Spores (Yeast)

Baker's, or budding, yeast, *Saccharomyces cerevisiae,* exists in both a haploid and diploid form (fig. 6.18). The haploid form usually forms under nutritional stress (starvation). When better conditions return, haploid cells of the two sexes, called **a** and α **mating types,** fuse to form the diploid. (Mating types are generally the result of a one-locus, two-allele genetic system that determines that only opposite mating types can fuse. We discuss this system in more detail in chapter 16.) The haploid is again established by meiosis under starvation conditions. In yeast, all the products of meiosis are contained in the ascus. Let us look at a mapping problem, using the *a* and *b* loci for convenience.

When an *ab* spore (or gamete) fuses with an a^+b^+ spore (or gamete), and the diploid then undergoes meiosis, the spores can be isolated and grown as haploid colonies, which are then observed for the phenotypes the two loci control. Only three patterns can occur (table 6.4).

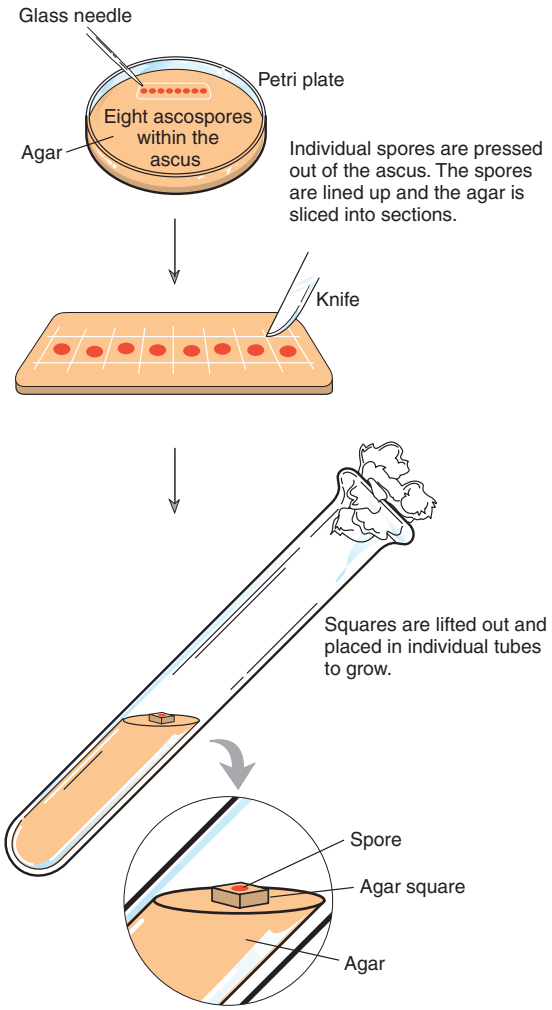


Figure 6.15 Spore isolation technique in *Neurospora.*

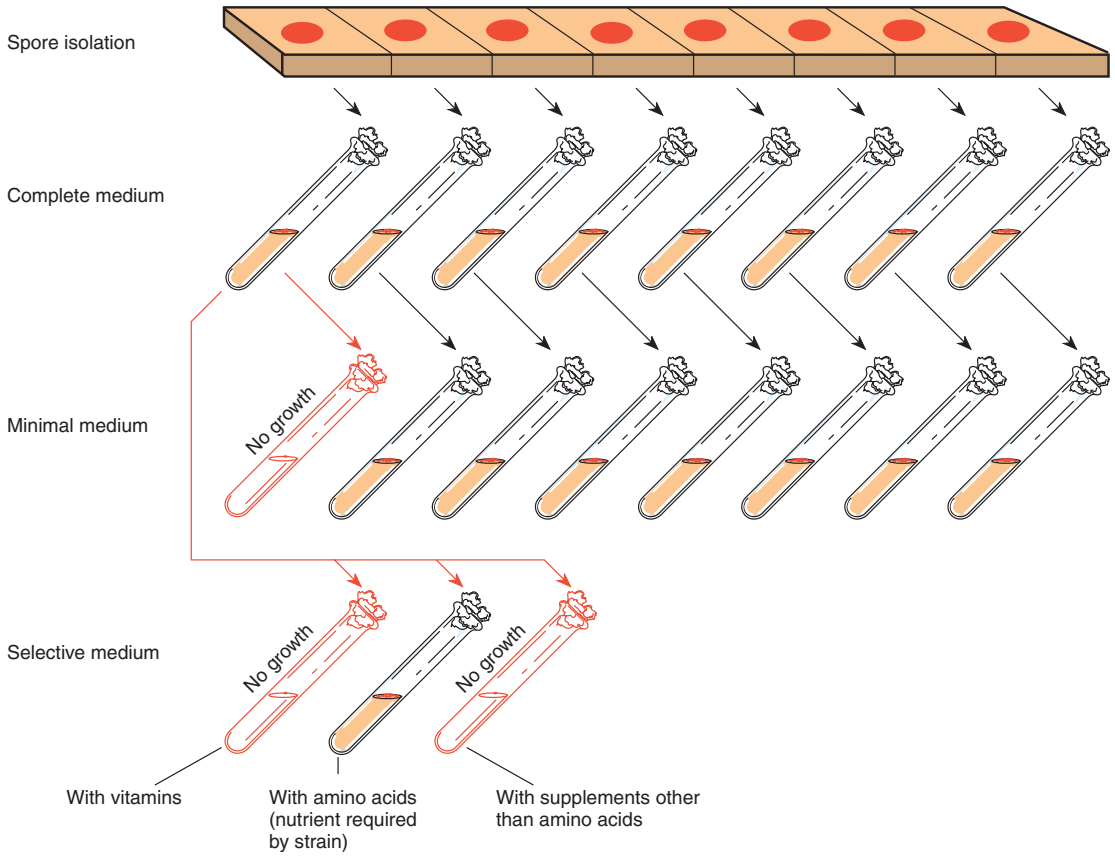


Figure 6.16 Isolation of nutritional-requirement mutants in *Neurospora*.

Class 1 has two types of spores, which are identical to the parental haploid spores. This ascus type is, therefore, referred to as a **parental ditype (PD).** The second class also has only two spore types, but they are recombinants. This ascus type is referred to as a **nonparental ditype (NPD).** The third class has all four possible spore types and is referred to as a **tetratype (TT).**

All three ascus types can be generated whether or not the two loci are linked. As figure 6.19 shows, if the loci are linked, parental ditypes come from the lack of a crossover, whereas nonparental ditypes come about from four-strand double crossovers (double crossovers involving all four chromatids). We should thus expect parental ditypes to be more numerous than nonparental ditypes for linked loci. However, if the loci are not linked, both parental and nonparental ditypes come about through independent assortment—they should occur in equal frequencies. We can therefore determine whether the loci are linked by comparing parental ditypes and nonparental ditypes. In table 6.4, the parental ditypes greatly outnumber the nonparental ditypes; the two loci are, therefore, linked. What is the map distance between the loci?

A return to figure 6.19 shows that in a nonparental ditype, all four chromatids are recombinant, whereas in a tetratype, only half the chromatids are recombinant. Remembering that 1% recombinant offspring equals 1 map unit, we can use the following formula:

$$\text{map units} = \frac{(1/2)\text{ the number of TT asci} + \text{the number of NPD asci}}{\text{total number of asci}} \times 100$$

Thus, for the data of table 6.4,

$$\text{map units} = \frac{(1/2)20 + 5}{100} \times 100 = \frac{10 + 5}{100} \times 100 = 15$$

Ordered Spores (Neurospora)

Unlike yeast, *Neurospora* has ordered spores; *Neurospora's* life cycle is shown in figure 6.20. Fertilization takes place within an immature fruiting body after a

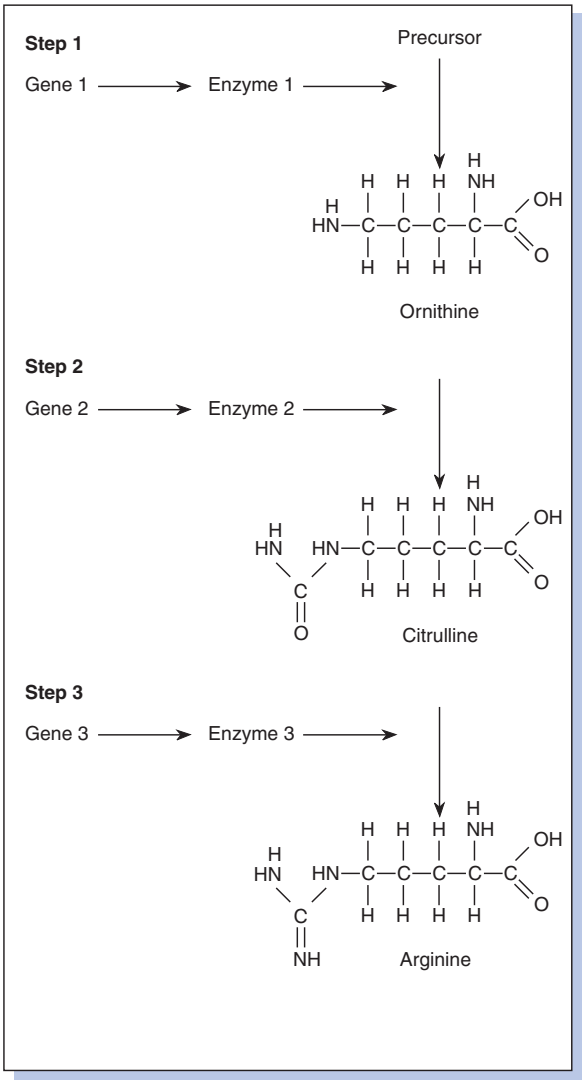


Figure 6.17 Arginine biosynthetic pathway of *Neurospora.*

Table 6.4 **The Three Ascus Types in Yeast Resulting from Meiosis in a Dihybrid, aa^+bb^+**

1 (PD)	2 (NPD)	3 (TT)
ab	ab^+	*ab*
ab	ab^+	ab^+
a^+b^+	a^+b	a^+b
a^+b^+	a^+b	a^+b^+
75	5	20

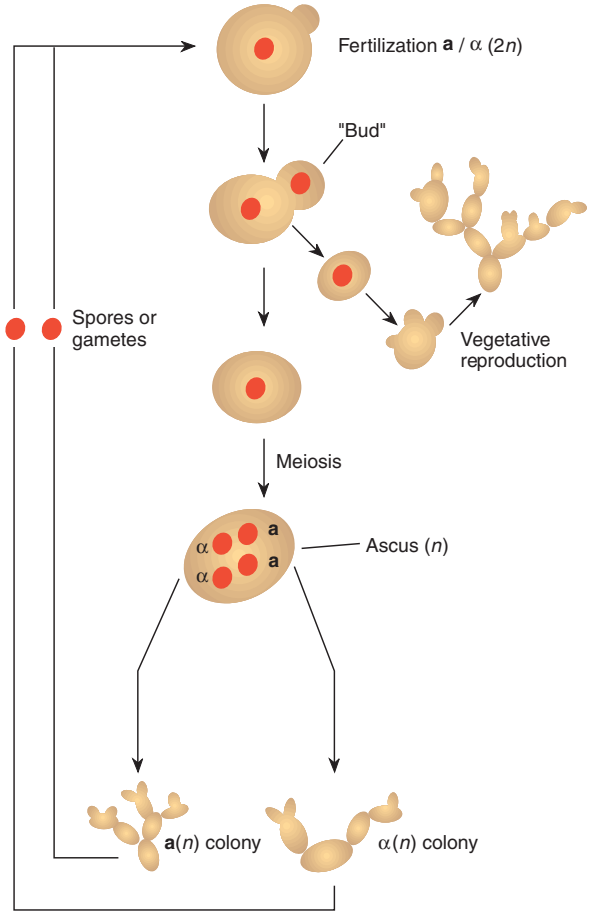


Figure 6.18 Life cycle of yeast. Mature cells are mating types **a** or α; *n* is the haploid stage; 2*n* is diploid.

spore or filament of one mating type contacts a special filament extending from the fruiting body of the opposite mating type (mating types are referred to as *A* and *a*). The zygote's nucleus undergoes meiosis without any intervening mitosis. Unlike yeast, *Neurospora* does not have a diploid phase in its life cycle. Rather, it undergoes meiosis immediately after the diploid nuclei form.

Since the *Neurospora* ascus is narrow, the meiotic spindle is forced to lie along the cell's long axis. The two nuclei then undergo the second meiotic division, which is also oriented along the long axis of the ascus. The result is that the spores are ordered according to their centromeres (fig. 6.21). That is, if we label one centromere *A* and the other *a,* for the two mating types, a tetrad at meiosis I will consist of one *A* and one *a* centromere. At the end of meiosis in *Neurospora,* the four ascospores are in the order *A A a a* or *a a A A* in regard to centromeres. (We talk more simply of centromeres rather than chromosomes or chromatids because of the complications that

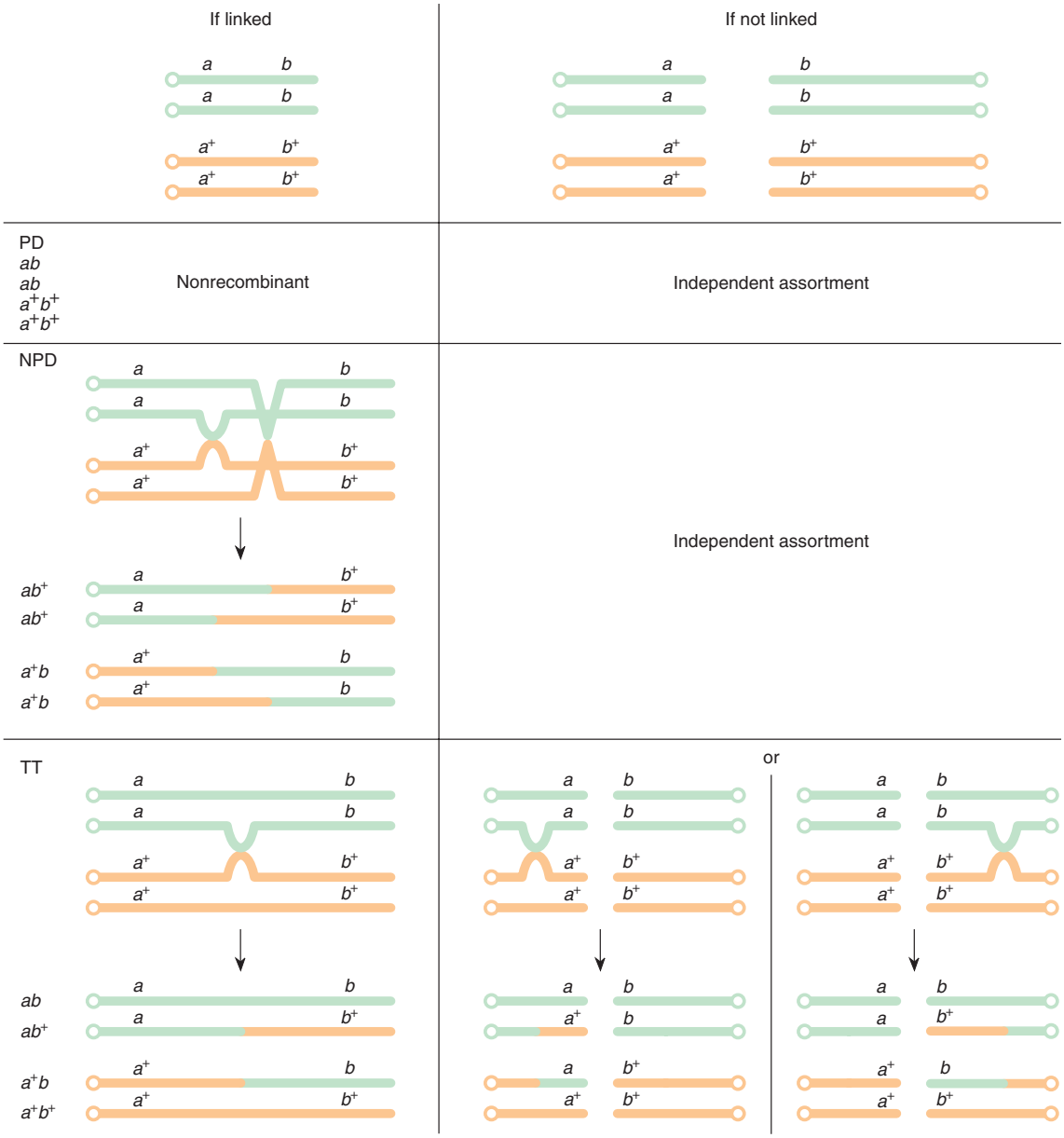


Figure 6.19 Formation of parental ditype (PD), nonparental ditype (NPD), and tetratype (TT) asci in a dihybrid yeast by linkage or independent assortment at meiosis. Open circles are centromeres.

crossing over adds. A type *A* centromere is always a type *A* centromere, whereas, due to crossing over, a chromosome attached to that centromere may be partly from the type *A* parent and partly from the type *a* parent.)

Before the ascospores mature in *Neurospora,* a mitosis takes place in each nucleus so that four pairs rather than just four spores are formed. In the absence of phenomena such as mutation or gene conversion, to be discussed later in the book, pairs are always identical (fig. 6.21). As we will see in a moment, because of the ordered spores, we can map loci in *Neurospora* in relation to their centromeres.

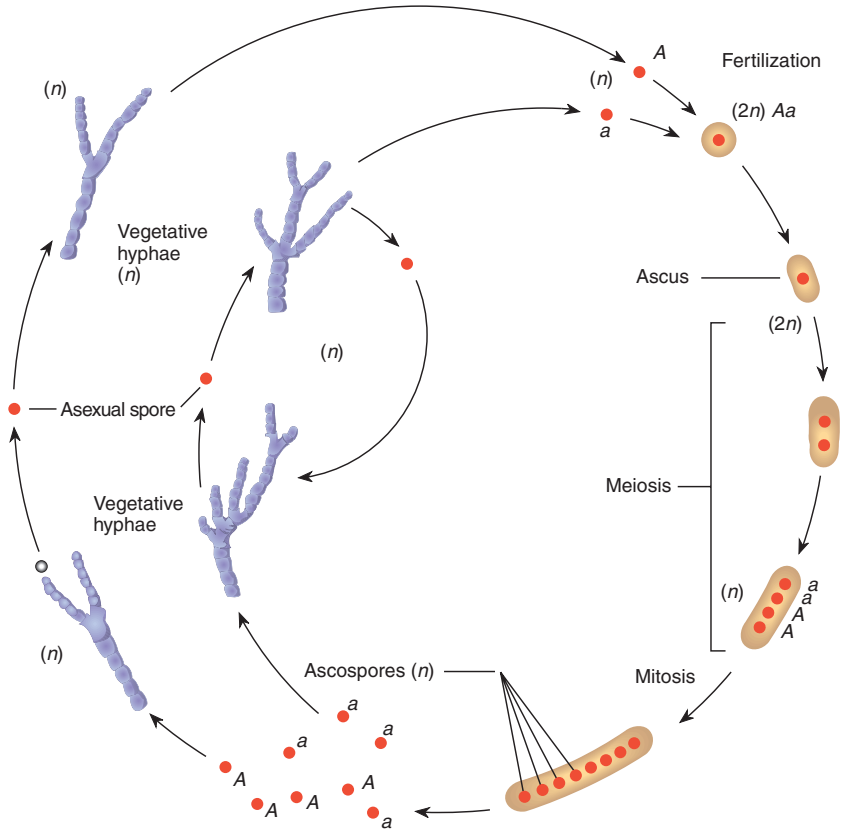


Figure 6.20 Life cycle of *Neurospora*. *A* and *a* are mating types; *n* is a haploid stage; 2*n* is diploid.

First and Second Division Segregation

Recall that there is a 4:4 segregation of the centromeres in the ascus of *Neurospora*. Two kinds of patterns appear among the loci on these chromosomes. These patterns depend on whether there was a crossover between the locus and its centromere (fig. 6.22). If there was no crossover between the locus and its centromere, the allelic pattern is the same as the centromeric pattern, which is referred to as **first-division segregation (FDS),** because the alleles separate from each other at meiosis I. If, however, a crossover has occurred between the locus and its centromere, patterns of a different type emerge (2:4:2 or 2:2:2:2), each of which is referred to as **second-division segregation (SDS).** Because the spores are ordered, the centromeres always follow a first-division segregation pattern. Hence, we should be able to map the distance of a locus to its centromere. Under the simplest circumstances (fig. 6.22), every second-division segregation configuration has four recombinant and four nonrecombinant chromatids (spores). Thus, half of the chromatids (spores) in a second-division segregation ascus are recombinant. Therefore, since 1% recombinant chromatids equal 1 map unit,

$$\text{map distance} = \frac{(1/2)\text{ the number of SDS asci}}{\text{total number of asci}} \times 100$$

An example using this calculation appears in table 6.5.

Three-point crosses in *Neurospora* can also be examined. Let us map two loci and their centromere. For simplicity, we will use the *a* and *b* loci. Dihybrids are formed from fused mycelia ($ab \times a^+b^+$), which then undergo meiosis. One thousand asci are analyzed, keeping the spore order intact. Before presenting the data, we should consider how to group them. Since each locus can show six different patterns (fig. 6.22), two loci scored together should give thirty-six possible spore arrangements (6×6). Some thought, however, tells us that many of these patterns are really random variants of each other. The tetrad in meiosis is a three-dimensional entity rather than a flat, four-rod object, as it is usually drawn. At the first meiotic division, either centromere can go to the left *or* the right, and when centromeres split at the second mei-

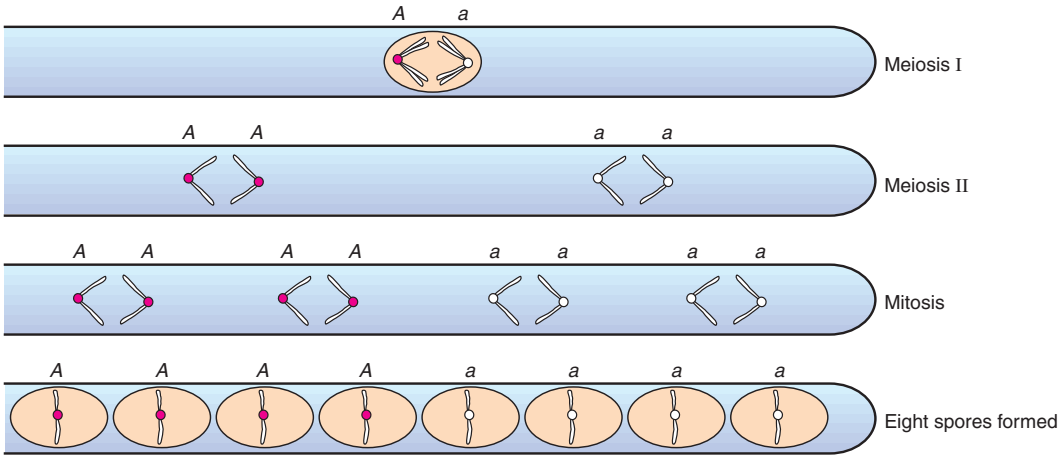


Figure 6.21 Meiosis in *Neurospora.* Although *Neurospora* has seven pairs of chromosomes at meiosis, only one pair is shown. *A* and *a,* the two mating types, represent the two centromeres of the tetrad.

otic division, movement within the future half-ascus (the four spores to the left or the four spores to the right) is also random. Thus, one genetic event can produce up to eight "different" patterns. For example, consider the arrangements figure 6.23 shows, in which a crossover occurs between the *a* and *b* loci. All eight arrangements, producing the ascus patterns of table 6.6, are equally likely. The thirty-six possible patterns then reduce to only the seven unique patterns shown in table 6.7. Note also that these asci can be grouped into the three types of asci found in yeast with unordered spores: parental ditypes, nonparental ditypes, and tetratypes. Had we not had the order of the spores from the asci, that would, in fact, be the only way we could score the asci (see the bottom of table 6.7).

Gene Order

We can now determine the distance from each locus to its centromere and the linkage arrangement of the loci if they are both linked to the same centromere. We can establish by inspection that the two loci are linked to each

Table 6.5 **Genetic Patterns Following Meiosis in an a^+a Heterozygous *Neurospora* (Ten Asci Examined)**

Spore Number	Ascus Number									
	1	2	3	4	5	6	7	8	9	10
1	a	a	a^+	a	a	a^+	a	a^+	a^+	a^+
2	a	a	a^+	a	a	a^+	a	a^+	a^+	a^+
3	a	a	a^+	a^+	a^+	a^+	a	a	a	a^+
4	a	a	a^+	a^+	a^+	a^+	a	a	a	a^+
5	a^+	a^+	a	a^+	a	a	a^+	a	a^+	a
6	a^+	a^+	a	a^+	a	a	a^+	a	a^+	a
7	a^+	a^+	a	a	a^+	a	a^+	a^+	a	a
8	a^+	a^+	a	a	a^+	a	a^+	a^+	a	a
	FDS	FDS	FDS	SDS	SDS	FDS	FDS	SDS	SDS	FDS

Note: Map distance (*a* locus to centromere) = (1/2)% SDS
= (1/2) 40%
= 20 map units

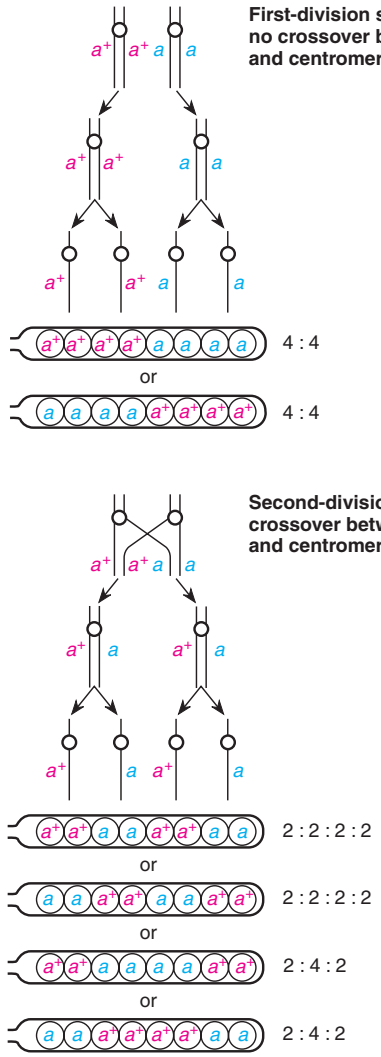


Figure 6.22 The six possible *Neurospora* ascospore patterns in respect to one locus.

other—and therefore to the same centromere—by examining classes 1 (parental ditype) and 2 (nonparental ditype) in table 6.7. If the two loci are unlinked, these two categories would represent two equally likely alternative events when no crossover takes place. Since category 1 represents almost 75% of all the asci, we can be sure the two loci are linked.

To determine the distance of each locus to the centromere, we calculate one-half the percentage of second-division segregation patterns for each locus. For the *a* locus, classes 4, 5, 6, and 7 are second-division segregation patterns. For the *b* locus, classes 3, 5, 6, and 7 are second-division segregation patterns. Therefore,

Table 6.6 **Eight of the Thirty-Six Possible Spore Patterns in *Neurospora* Scored for Two Loci, *a* and *b* (All Random Variants of the Same Genetic Event)**

Spore Number	Ascus Number 1	2	3	4	5	6	7	8
1	ab	ab^+	ab	ab^+	a^+b^+	a^+b^+	a^+b	a^+b
2	ab	ab^+	ab	ab^+	a^+b^+	a^+b^+	a^+b	a^+b
3	ab^+	ab	ab^+	ab	a^+b	a^+b	a^+b^+	a^+b^+
4	ab^+	ab	ab^+	ab	a^+b	a^+b	a^+b^+	a^+b^+
5	a^+b	a^+b	a^+b^+	a^+b^+	ab^+	ab	ab^+	ab
6	a^+b	a^+b	a^+b^+	a^+b^+	ab^+	ab	ab^+	ab
7	a^+b^+	a^+b^+	a^+b	a^+b	ab	ab^+	ab	ab^+
8	a^+b^+	a^+b^+	a^+b	a^+b	ab	ab^+	ab	ab^+

Table 6.7 **The Seven Unique Classes of Asci Resulting from Meiosis in a Dihybrid *Neurospora*, ab/a^+b^+**

Spore Number	Ascus Number 1	2	3	4	5	6	7
1	ab	ab^+	ab	ab	ab	ab^+	ab
2	ab	ab^+	ab	ab	ab	ab^+	ab
3	ab	ab^+	ab^+	a^+b	a^+b^+	a^+b	a^+b^+
4	ab	ab^+	ab^+	a^+b	a^+b^+	a^+b	a^+b^+
5	a^+b^+	a^+b	a^+b^+	a^+b^+	a^+b^+	a^+b	a^+b
6	a^+b^+	a^+b	a^+b^+	a^+b^+	a^+b^+	a^+b	a^+b
7	a^+b^+	a^+b	a^+b	ab^+	ab	ab^+	ab^+
8	a^+b^+	a^+b	a^+b	ab^+	ab	ab^+	ab^+
	729	2	101	9	150	1	8
SDS for *a* locus				9	150	1	8
SDS for *b* locus			101		150	1	8
Unordered:	PD	NPD	TT	TT	PD	NPD	TT

the distances to the centromere, in map units, for each locus are

$$\text{for locus } a\text{: } (1/2)\frac{9 + 150 + 1 + 8}{1{,}000} \times 100$$

$$= 8.4 \text{ centimorgans}$$

$$\text{for locus } b\text{: } (1/2)\frac{101 + 150 + 1 + 8}{1{,}000} \times 100$$

$$= 13.0 \text{ centimorgans}$$

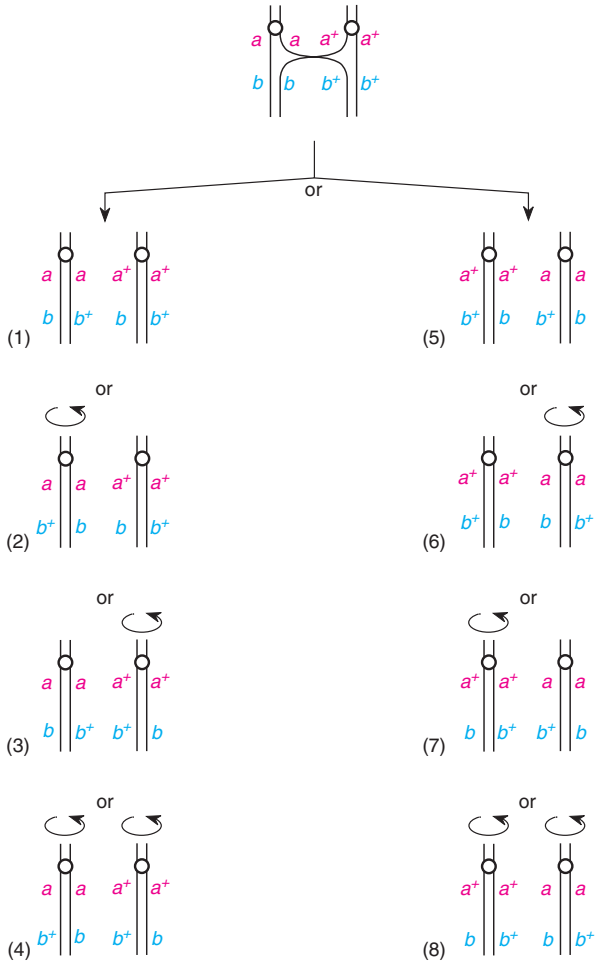


Figure 6.23 The eight random arrangements possible when a single crossover occurs between the *a* and *b* loci in *Neurospora* (see table 6.6). *Circular arrows* represent the rotation of a centromere from its position in the original configuration.

It should now be possible to describe exactly what type of crossover event produced each of the seven classes in table 6.7.

Unfortunately, these two distances do not provide a unique determination of gene order. In figure 6.24, we see that two alternatives are possible: one has a map distance between the loci of 21.4 map units; the other has 4.6 map units between loci. How do we determine which of these is correct? The simplest way is to calculate the *a*–*b* distance using the unordered spore information. That is, the map distance is

$$\text{map units} = \frac{(1/2)\text{ the number of TT asci} + \text{the number of NPD asci}}{\text{total number of asci}} \times 100$$

$$= \frac{(1/2)118 + 3}{1{,}000} \times 100 = 6.2$$

Since 6.2 map units is much closer to the *a*–*b* distance expected if both loci are on the same side of the centromere, we accept alternative 2 in figure 6.24.

A second way to choose between the alternatives in figure 6.24 is to find out what happens to the *b* locus when a crossover occurs between the *a* locus and its centromere. If the order in alternative 1 is correct, crossovers between the *a* locus and its centromere should have no effect on the *b* locus; if 2 is correct, most of the crossovers that move the *a* locus in relation to its centromere should also move the *b* locus.

Asci classes 4, 5, 6, and 7 include all the SDS patterns for the *a* locus. Of 168 asci, 150 (class 5) have similar SDS patterns for the *b* locus. Thus, 89% of the time, a crossover between the *a* locus and its centromere is also a crossover between the *b* locus and its centromere—compelling evidence in favor of alternative 2. (What form would the data take if alternative 1 were correct?)

In summary, mapping by tetrad analysis proceeds as follows. For both ordered and unordered spores, linkage is indicated by an excess of parental ditypes over nonparental ditypes. For unordered spores (yeast), the distance between two loci is one-half the number of

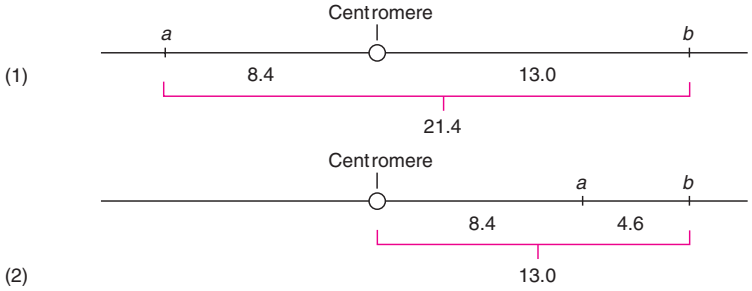


Figure 6.24 Two possible arrangements of the *a* and *b* loci and their centromere. Distances are in map units.

tetratypes plus the number of nonparental ditypes, all divided by the total number of asci, expressed as a percentage. For ordered spores (*Neurospora*), the distance from a locus to its centromere is one-half the percentage of second-division segregants. Mapping the distance between two loci is similar to the process in unordered spores.

SOMATIC (MITOTIC) CROSSING OVER

Crossing over is known to occur in somatic cells as well as during meiosis. It apparently occurs when two homologous chromatids come to lie next to each other and breakage and reunion follow, most likely as a consequence of DNA repair (see chapter 12). Unlike in meiosis, no synaptonemal complex forms. The occurrence of mitotic crossing over is relatively rare. In the fungus *Aspergillus nidulans,* mitotic crossing over occurs about once in every one hundred cell divisions.

Mitotic recombination was discovered in 1936 by Curt Stern, who noticed the occurrence of *twin spots* in fruit flies that were dihybrid for the yellow allele for body color (*y*) and the singed allele (*sn*) for bristle morphology (fig. 6.25). A twin spot could be explained by mitotic crossing over between the *sn* locus and its centromere (fig. 6.26). A crossover in the *sn–y* region would produce only a yellow spot, whereas a double crossover, one between *y* and *sn* and the other between *sn* and the centromere, would produce only a singed spot. (Verify this for yourself.) These three phenotypes were found in the relative frequencies expected. That is, given that the gene locations are drawn to scale in figure 6.26, we would expect double spots to be most common, followed by yellow spots, with singed spots rarest of all because they require a double crossover. This in fact occurred, and no other obvious explanation was consistent with these facts. Mitotic crossing over has been used in fungal genetics as a supplemental, or even a primary, method for determining linkage relations. Although gene orders are consistent between mitotic and meiotic mapping, relative distances are usually not, which is not totally unexpected. We know that neither meiotic nor mitotic crossing over is uniform along a chromosome. Apparently, the factors that cause deviation from uniformity differ in the two processes.

HUMAN CHROMOSOMAL MAPS

In theory, we can map human chromosomes as we would those of any other organism. Realistically, the problems mentioned earlier (the inability to make specific crosses coupled with the relatively small number of offspring) make these techniques of human chromosome mapping very difficult. However, some progress has been made based on pedigrees, especially in assigning genes to the X chromosome. As the pedigree analysis in the previous chapter has shown, X chromosomal traits have unique patterns of inheritance, and loci on the X chromosome are easy to identify. Currently over four hundred loci are known to be on the X chromosome. It has been estimated, by several different methods, that between fifty and one hundred thousand loci exist on human chromosomes. In later chapters, we will discuss several additional methods of human chromosomal mapping that use molecular genetic techniques.

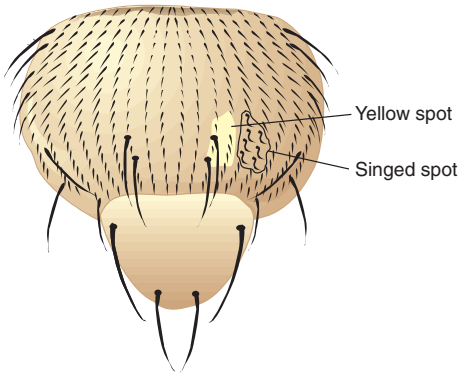
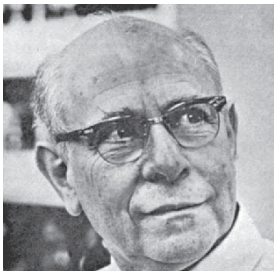


Figure 6.25 Yellow and singed twin spots on the thorax of a female *Drosophila*.



Curt Stern (1902–1981)
(Courtesy of the Science Council of Japan.)

X Linkage

After determining that a human gene is X linked, the next problem is to determine the position of the locus on the X chromosome and the map units between loci. Sometimes we can do this with the proper pedigrees, if crossing over can be ascertained. An example of this "grand-

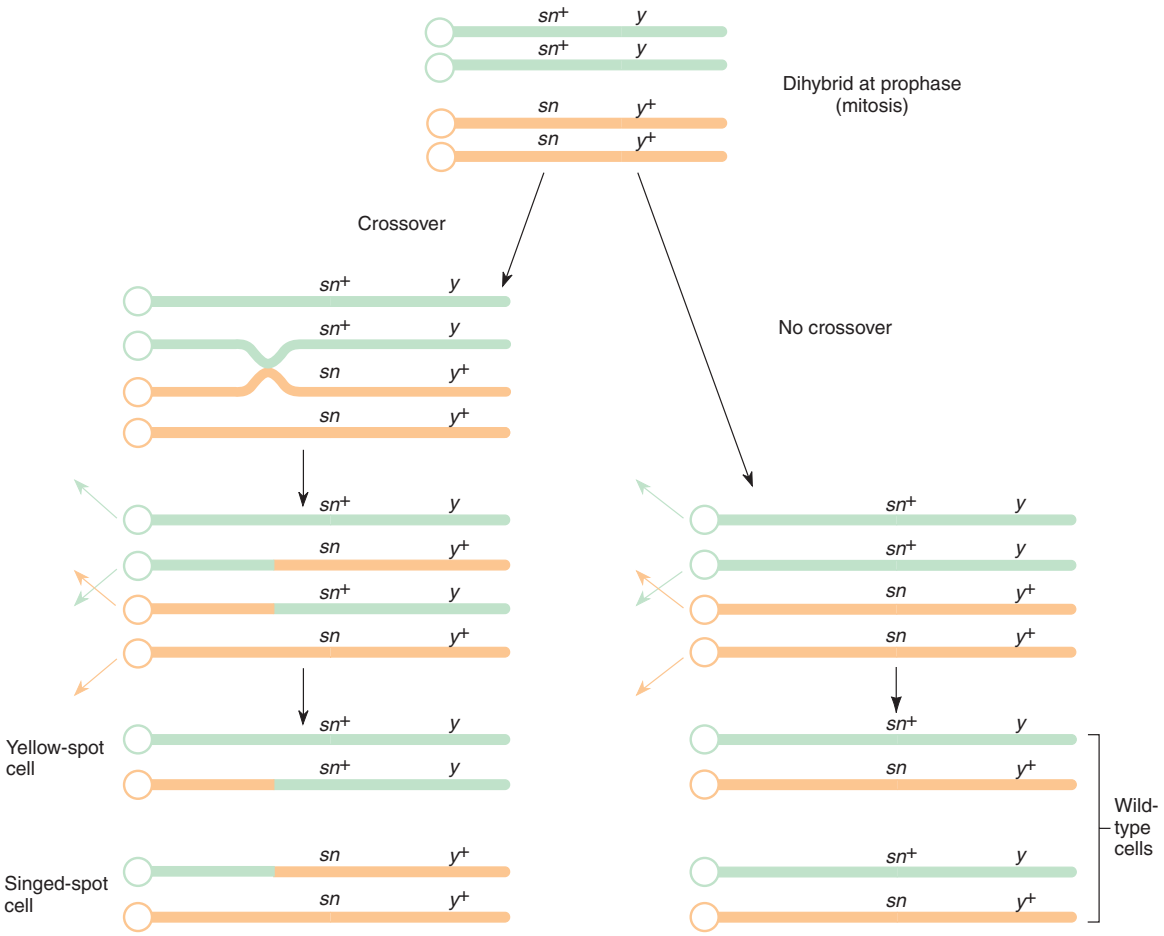


Figure 6.26 Formation of twin spots by somatic crossing over.

father method" appears in figure 6.27. In this example, a grandfather has one of the traits in question (here, color blindness). We then find that he has a grandson who is glucose-6-phosphate dehydrogenase (G-6-PD) deficient. From this we can infer that the mother (of the grandson) was dihybrid for the two alleles in the *trans* configuration. That is, she received her color-blindness allele on one of her X chromosomes from her father, and she must have received the G-6-PD-deficiency allele on the other X chromosome from her mother (why?). Thus, the two sons on the left in figure 6.27 are nonrecombinant, and the two on the right are recombinant. Theoretically, we can determine map distance by simply totaling the recombinant grandsons and dividing by the total number of grandsons. Of course, the methodology would be the same if the grandfather were both color-blind and G-6-PD deficient. The mother would then be dihybrid in the *cis* configuration, and the sons would be tabulated in the reverse manner. The point is that the grandfather's phenotype gives us information that allows us to infer that the mother was dihybrid, as well as telling us the *cis-trans* arrangement of her alleles. We can then score her sons as either recombinant or nonrecombinant.

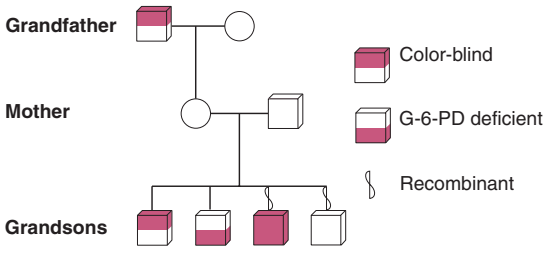


Figure 6.27 "Grandfather method" of determining crossing over between loci on the human X chromosome. G-6-PD is glucose-6-phosphate dehydrogenase.

Autosomal Linkage

From this we can see that it is relatively easy to map the X chromosome. The autosomes are another story. Since there are twenty-two autosomal linkage groups (twenty-two pairs of nonsex chromosomes), it is virtually impossible to determine from simple pedigrees which chromosome two loci are located on. Pedigrees can tell us if two loci are linked to each other, but not on which chromosome. In figure 6.28, the nail-patella syndrome includes, among other things, abnormal nail growth coupled with the absence or underdevelopment of kneecaps. It is a dominant trait. The male in generation II is dihybrid, with the *A* allele of the ABO blood type system associated with the nail-patella allele (*NPS1*) and the *B* allele with the normal nail-patella allele (*nps1*). Thus only one child in eight (III-5) is recombinant. Actually, the map distance is about 10%. In general, map distances appear greater in females than in males because more crossing over occurs in females (box 6.3).

We now turn our attention to the localization of loci to particular human chromosomes. The first locus that was definitely established to be on a particular autosome was the Duffy blood group on chromosome 1. This was ascertained in 1968 from a family that had a morphologically odd, or "uncoiled," chromosome 1. Inheritance in the Duffy blood group system followed the pattern of inheritance of the "uncoiled" chromosome. Real strides have been made since then. Two techniques, chromosomal banding and somatic-cell hybridization, have been crucial to autosomal mapping.

Chromosomal Banding

Techniques were developed around 1970 that make use of certain histochemical stains that produce repeatable banding patterns on the chromosomes. For example, Giemsa staining is one such technique; the resulting bands are called **G-bands.** More detail on these techniques is presented in chapter 15. Before these techniques, human and other mammalian chromosomes were grouped into general size categories because of the difficulty of differentiating many of them. With banding techniques came the ability to identify each human chromosome in a karyotype (see fig. 5.1).

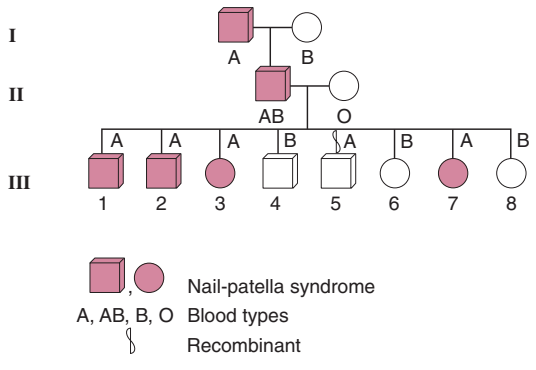


Figure 6.28 Linkage of the nail-patella syndrome and ABO loci.

Somatic-Cell Hybridization

The ability to distinguish each human chromosome is required to perform somatic-cell hybridization, in which human and mouse (or hamster) cells are fused in culture to form a hybrid. The fusion is usually mediated chemically with polyethylene glycol, which affects cell membranes; or with an inactivated virus, for example the Sendai virus, that is able to fuse to more than one cell at the same time. (The virus is able to do this because it has a lipid membrane derived from its host cells that easily fuses with new host cells. Because of this property, the virus can fuse to two cells close together, forming a cytoplasmic bridge between them that facilitates their fusion.) When two cells fuse, their nuclei are at first separate, forming a **heterokaryon,** a cell with nuclei from different sources. When the nuclei fuse, a hybrid cell is formed, and this hybrid tends to lose human chromosomes preferentially through succeeding generations. Upon stabilization, the result is a cell with one or more human chromosomes in addition to the original mouse or hamster chromosomal complement. Banding techniques allow the observer to recognize the human chromosomes. A geneticist looks for specific human phenotypes, such as enzyme products, and can then assign the phenotype to one of the human chromosomes in the cell line.

When cells are mixed together for hybridization, some cells do not hybridize. It is thus necessary to be able to select for study just those cells that are hybrids. One technique, originally devised by J. W. Littlefield in 1964, makes use of genetic differences in the way the cell lines synthesize DNA. Normally, in mammalian cells, aminopterin acts as an inhibitor of enzymes involved in DNA metabolism. Two enzymes, hypoxanthine phosphoribosyl transferase (HPRT) and thymidine kinase (TK), can bypass aminopterin inhibition by making use of secondary, or salvage, pathways in the cell. If hypoxanthine is provided, HPRT converts it to a purine, and if thymidine is provided, TK converts it to the nucleotide thymidylate. (Purines are converted to nucleotides and nucleotides are the subunits of DNA—see chapter 9.) Thus, normal cells in the absence of aminopterin synthesize DNA even if they lack HPRT activity ($HPRT^-$) or TK activity (TK^-). In the presence of aminopterin, $HPRT^-$ or TK^- cells die. However, in the presence of aminopterin, $HPRT^+$ TK^+ cells can synthesize DNA and survive. Using this information, the following selection system was developed.

Mouse cells that have the phenotype of $HPRT^+$ TK^- are mixed with human cells that have the phenotype of

BOX 6.3

Experimental Methods

Lod *Scores*

Human population geneticists can increase the accuracy of their linkage analysis by using a probability technique, developed by Newton Morton, called the ***lod* score method** (*Lo*g *Od*ds). The geneticist asks what the probability is of getting a particular pedigree assuming a particular recombination frequency (Θ), as compared with getting the same pedigree assuming independent assortment (Θ = 0.50). In other words, he or she calculates the ratio of the probability of genotypes in a family given a certain crossover frequency compared with the probability of those genotypes if the loci are unlinked. Logarithms are used for ease of calculation, and the parameter is called *z*, the *lod* score. Using this method, a researcher can try different crossover frequencies until the one giving the highest *lod* score is found.

For example, take the pedigree in figure 6.28. The father in generation II can have one of two allelic arrangements: *A NPS1/B nps1* or *A/B NPS1/nps1*. The former assumes linkage, whereas the latter does not. Our initial estimate of recombination, assuming linkage, was (1/8) × 100, or 12.5 map units. We now need to calculate the ratio of two probabilities:

$$z = \log \frac{\text{probability of birth sequence assuming 12.5 map units}}{\text{probability of birth sequence assuming independent assortment}}$$

Assuming 12.5 map units (or a probability of 0.125 of a crossover; Θ = 0.125), the probability of child III-1 is 0.4375. This child would be a nonrecombinant, so his probability of having the nail-patella syndrome and type A blood is half the probability of no crossover during meiosis, or (1 − 0.125)/2. We divide by two because there are two nonrecombinant types. This is the same probability for all children except III-5, whose probability of occurrence is 0.125/2 = 0.0625, since he is a recombinant. Thus, the numerator of the previous equation is $(0.4375)^7(0.0625)$.

If the two loci are not linked, then any genotype has a probability of 1/4, or 0.25. Thus, the sequence of the eight children has the probability of $(0.25)^8$. This is the denominator of the equation. Thus,

$$z = \log \frac{(0.4375)^7(0.0625)}{(0.25)^8}$$

$$z = \log [12.566] = 1.099$$

Any *lod* score greater than zero favors linkage. A *lod* score less than zero suggests that Θ has been underestimated. A *lod* of 3.0 or greater (10^3 or one thousand times more likely than independent assortment) is considered a strong likelihood of linkage. Thus, in our example, we have an indication of linkage with a recombination frequency of 0.125. Now we can calculate *lod* scores assuming other values of recombination, as table 1 does. You can see that the recombination frequency as calculated, 0.125 (12.5 map units), gives the highest *lod* score.

Newton E. Morton (1929–).
(Courtesy of Dr. Newton E. Morton.)

Table 1 ***Lod* Scores for the Cross in Figure 6.28**

Recombination Frequency (Θ)	*Lod* Score
0.05	0.951
0.10	1.088
0.125	1.099
0.15	1.090
0.20	1.031
0.25	0.932
0.30	0.801
0.35	0.643
0.40	0.457
0.45	0.244
0.50	0.000

$HPRT^-$ TK^+ in the presence of Sendai virus or polyethylene glycol. Fusion takes place in some of the cells, and the mixture is grown in a medium containing hypoxanthine, aminopterin, and thymidine (called **HAT medium**). In the presence of aminopterin, unfused mouse cells (TK^-) and unfused human cells ($HPRT^-$) die. Hybrid cells, however, survive because they are $HPRT^+$ TK^+. Eventually, the hybrid cells end up with random numbers of human chromosomes. There is one restriction: All cell lines selected are TK^+. This HAT method (using the HAT medium) not only selects for hybrid clones, but also localizes the *TK* gene to human chromosome 17, the one human chromosome found in every successful cell line.

Table 6.8 **Assignment of the Gene for Blood Coagulating Factor III to Human Chromosome 1 Using Human-Mouse Hybrid Cell Lines**

Hybrid Cell Line Designation	Tissue/ Factor Score	Human Chromosome Present																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
WIL1	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−	−	+	−	+
WIL6	−	−	+	−	+	+	+	+	+	−	+	+	−	−	+	−	−	+	−	+	+	+	−	+
WIL7	−	−	+	+	−	+	+	−	+	−	+	+	−	+	+	−	−	+	+	−	−	+	−	+
WIL14	+	+	−	+	−	−	−	+	+	−	+	−	+	−	+	+	−	+	−	−	−	−	−	+
SIR3	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+
SIR8	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+
SIR11	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+
REW7	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+
REW15	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	−	+	+	+	+	+	+	+
DUA1A	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	*
DUA1CsAzF	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
DUA1CsAzH	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
TSL1	−	−	−	+	+	−	−	−	−	−	+	+	−	+	+	−	+	+	+	−	+	−	−	−
TSL2	−	−	+	*	−	+	+	−	−	−	+	−	+	−	−	−	−	*	+	−	+	+	−	+
TSL2CsBF	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
XTR1	+	+	−	*	−	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+
XTR2	−	−	−	*	−	+	−	−	+	−	+	−	+	+	−	−	−	−	+	−	+	+	−	*
XTR3BsAgE	+	+	−	*	−	+	+	+	+	+	+	−	−	+	+	−	−	+	+	+	−	+	−	*
XTR22	−	−	+	*	+	+	+	−	+	−	+	+	−	−	−	+	−	−	+	+	+	+	+	*
XER9	−	−	+	−	+	−	−	−	+	−	+	*	+	−	+	−	−	+	+	−	−	+	−	*
XER11	+	+	−	+	+	−	+	+	+	−	+	*	+	+	−	+	+	+	+	+	+	+	+	*
REX12	−	−	−	+	−	−	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	*
JSR29	+	+	+	+	+	+	+	*	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
JVR22	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
JWR22H	+	*	*	−	+	−	+	−	−	−	+	+	+	−	+	+	−	+	+	−	+	+	−	−
ALR2	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
ICL15	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	+	−	−
ICL15CsBF	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−
MH21	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
% Discord†		0	32	17	24	31	21	21	31	21	24	30	21	21	28	14	24	21	28	17	34	41	21	27

Source: Reprinted with permission from S.D. Carson, et al., "Tissue Factor Gene Localized to Human Chromosome 1 (after 1p21)," *Science*, 229:229–291. Copyright © 1985 American Association for the Advancement of Science.

* A translocation in which only part of the chromosome is present.

† Discord refers to cases in which the tissue factor score is plus, and the human chromosome is absent, or in which the score is minus and the chromosome is present.

After successful cell hybrids are formed, two particular tests are used to map human genes. A **synteny test** (same linkage group) determines whether two loci are in the same linkage group if the phenotypes of the two loci are either always together or always absent in various hybrid cell lines. An **assignment test** determines which chromosome a particular locus is on by the concordant appearance of the phenotype whenever that particular chromosome is in a cell line, or by the lack of the particular phenotype when a particular chromosome is absent from a cell line. The first autosomal synteny test, performed in 1970, demonstrated that the *B* locus of lactate dehydrogenase (LDH_B) was linked to the *B* locus of peptidase (PEP_B). (Both enzymes are formed from subunits

controlled by two loci each. In addition to the *B* locus, each protein has subunits controlled by an *A* locus.) Later, these loci were shown to reside on chromosome 12.

In another example, a blood-coagulating glycoprotein (a protein-polysaccharide complex) called tissue factor III was localized by assignment tests to chromosome 1. Table 6.8 shows twenty-nine human-mouse hybrid cell lines, or **clones,** the human chromosomes they contain, and their tissue factor score, the results of an assay for the presence of the coagulating factor. (Clones are cells arising from a single ancestor.) It is obvious from table 6.8 that the gene for tissue factor III is on human chromosome 1. Every time human chromosome 1 is present in a cell line, so is tissue factor III. Every time human chromosome 1 is absent, so is the tissue factor (zero discordance or 100% concordance). No other chromosome showed that pattern.

The human map as we know it now (compiled by Victor McKusick at Johns Hopkins University), containing over six thousand assigned loci of over twelve thousand known to exist, is shown in table 6.9 and figure 6.29. At

Victor A. McKusick (1921–). (Courtesy of Victor A. McKusick.)

Table 6.9 Definition of Selected Loci of the Human Chromosome Map (figure 6.29)

Locus	Protein Product	Chromosome	Locus	Protein Product	Chromosome
ABO	ABO blood group	9	*IGH*	Immunoglobulin heavy-chain gene family	14
AG	Alpha globin gene family	16	*IGK*	Immunoglobulin kappa-chain gene family	2
ALB	Albumin	4	*INS*	Insulin	11
AMY1	Amylase, salivary	1	*LDHA*	Lactate dehydrogenase A	11
AMY2	Amylase, pancreatic	1	*MDI*	Manic depressive illness	6
BCS	Breast cancer susceptibility	16	*MHC*	Major histocompatibility complex	6
C2	Complement component-2	6	*MN*	MN blood group	4
CAT	Catalase	11	*MYB*	Avian myeloblastosis virus oncogene	6
CBD	Color blindness, deutan	X	*NHCP1*	Nonhistone chromosomal protein-1	7
CBP	Color blindness, protan	X	*NPS1*	Nail-patella syndrome	9
CML	Chronic myeloid leukemia	22	*PEPA*	Peptidase A	18
DMD	Duchenne muscular dystrophy	X	*PVS*	Polio virus sensitivity	19
FES	Feline sarcoma virus oncogene	15	*Rb*	Rhesus blood group	1
FY	Duffy blood group	1	*RN5S*	5S RNA gene(s)	1
GLB1	Beta-galactosidase-1	3	*RNTMI*	Initiator methionine tRNA	6
H1	Histone-1	7	*RWS*	Ragweed sensitivity	6
HBB	Hemoglobin beta chain	11	*S1*	Surface antigen 1	11
HEMA	Classic hemophilia	X	*SIS*	Simian sarcoma virus oncogene	22
HEXA	Hexosaminidase A	15	*STA*	Stature	Y
HLA	Human leukocyte antigens	6	*TF*	Transferrin	3
HP	Haptoglobin	16	*XG*	Xg blood group	X
HYA	Y histocompatibility antigen, locus A	Y	*XRS*	X-ray sensitivity	13
IDDM	Insulin-dependent diabetes mellitus	6			
IFF	Interferon, fibroblast	9			

Note: A more complete list appears in V. A. McKusick, *Mendelian Inheritance in Man: A Catalog of Human Genes* (Baltimore: Johns Hopkins University Press, 1994).

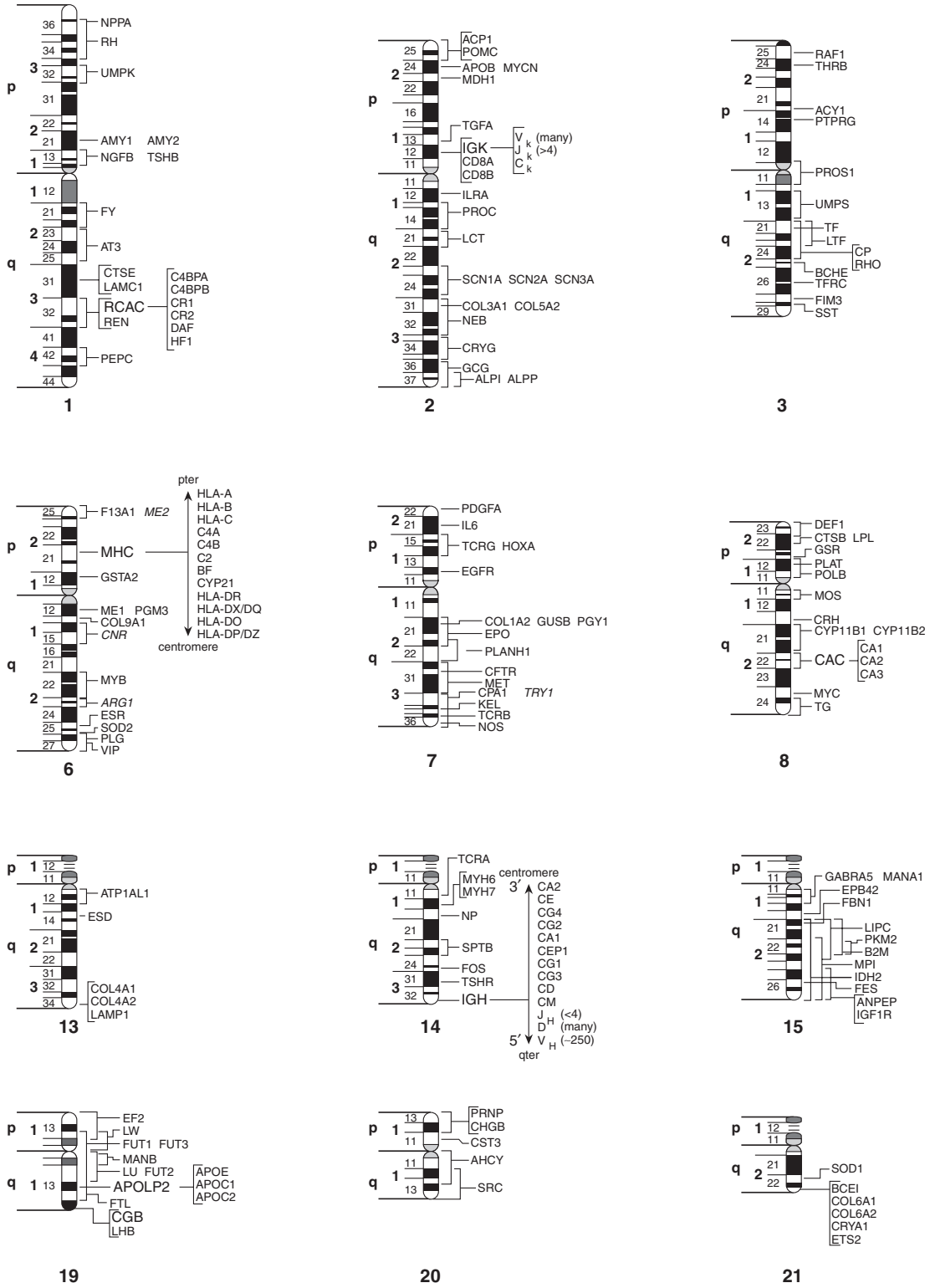
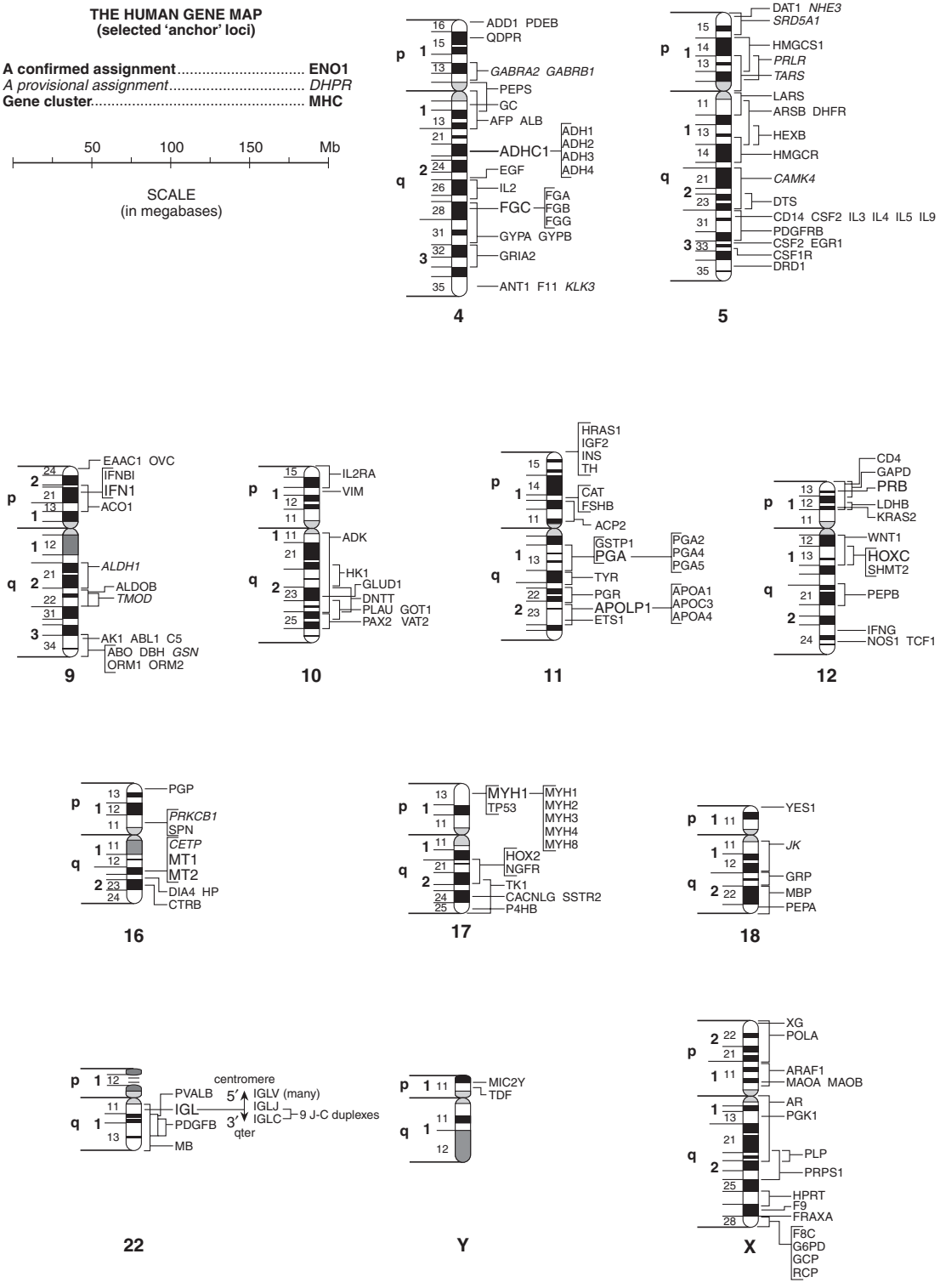


Figure 6.29 Human G-banded chromosomes with their accompanying assigned loci. The *p* and *q* refer to the short and long arms of the chromosomes, respectively. A key to the loci is given in McKusick (1994). (From Victor A. McKusick, *Mendelian inheritance in man,* 11th edition, 1994. Reprinted by permission of Johns Hopkins University Press, Baltimore, MD.)


THE HUMAN GENE MAP
(selected 'anchor' loci)
A confirmed assignment ENO1
A provisional assignment ..... DHPR
Gene cluster ..... MHC
50
100
150
Mb
SCALE
(in megabases)
4
5
9
10
11
12
16
17
18
22
Y
X

present, geneticists studying human chromosomes are hampered not by a lack of techniques but by a lack of marker loci. When a new locus is discovered, it is now relatively easy to assign it to its proper chromosome.

The problem still exists of determining exactly where a particular locus belongs on a chromosome. This is facilitated by developing particular cell lines with broken chromosomes, so that parts are either missing or have moved to other chromosomes. These processes reveal new linkage arrangements and make it possible to determine the region in which a locus is situated on a particular chromosome. In chapter 13, we describe additional techniques used to locate genes on human chromosomes, including a description of the Human Genome Project, the program that sequenced the entire human genome as well as the genomes of other model organisms.

SUMMARY

STUDY OBJECTIVE 1: To learn about analytical techniques for locating the relative positions of genes on chromosomes in diploid eukaryotic organisms 110–122

The principle of independent assortment is violated when loci lie near each other on the same chromosome. Recombination between these loci results from the crossing over of chromosomes during meiosis. The amount of recombination provides a measure of the distance between these loci. One map unit (centimorgan) equals 1% recombinant gametes. Map units can be determined by testcrossing a dihybrid and recording the percentage of recombinant offspring. If three loci are used (a three-point cross), double crossovers will be revealed. A coefficient of coincidence, the ratio of observed to expected double crossovers, can be calculated to determine if one crossover changes the probability that a second one will occur nearby.

A chiasma seen during prophase I of meiosis represents both a physical and a genetic crossing over. This can be demonstrated by using homologous chromosomes with morphological distinctions.

Because of multiple crossovers, the measured percentage recombination underestimates the true map distance, especially for loci relatively far apart; the best map estimates come from summing the distances between closely linked loci. A mapping function can be used to translate observed map distances into more accurate ones.

STUDY OBJECTIVE 2: To learn about analytical techniques for locating the relative positions of genes on chromosomes in ascomycete fungi 122–132

Organisms that retain all the products of meiosis lend themselves to chromosome mapping by haploid mapping (tetrad analysis). With unordered spores, such as in yeast, we use

$$\text{map units} = \frac{(1/2)\text{ the number of TT asci} + \text{the number of NPD asci}}{\text{total number or asci}} \times 100$$

Map units between a locus and its centromere in organisms with ordered spores, such as *Neurospora,* can be calculated as

$$\text{map units} = \frac{(1/2)\text{ the number of SDS asci}}{\text{total number of asci}} \times 100$$

Crossing over also occurs during mitosis, but at a much reduced rate. Somatic (mitotic) crossing over can be used to map loci.

STUDY OBJECTIVE 3: To learn about analytical techniques for locating the relative positions of genes on human chromosomes 132–140

Human chromosomes can be mapped. Recombination distances can be established by pedigrees, and loci can be attributed to specific chromosomes by synteny and assignment tests in hybrid cell lines.

SOLVED PROBLEMS

PROBLEM 1: A homozygous claret (*ca,* claret eye color), curled (*cu,* upcurved wings), fluted (*fl,* creased wings) fruit fly is crossed with a pure-breeding wild-type fly. The F_1 females are testcrossed with the following results:

fluted	4
claret	173
curled	26
fluted, claret	24
fluted, curled	167
claret, curled	6
fluted, claret, curled	298
wild-type	302

a. Are the loci linked?
b. If so, give the gene order, map distances, and coefficient of coincidence.

Answer: The pattern of numbers among the eight offspring classes is the pattern we are used to seeing for linkage of three loci. We can tell from the two groups in largest numbers (the nonrecombinants—fluted, claret, curled and wild-type) that the alleles are in the coupling (*cis*) arrangement. If we compare either of the nonrecombinant classes with either of the double crossover classes (fluted and claret, curled), we see that the fluted locus is in the center. For example, compare fluted, a double crossover offspring, with the wild-type, a nonrecombinant; clearly, fluted has the odd pattern. Thus the trihybrid female parent had the following arrangement of alleles:

$$\frac{ca\ fl\ cu}{ca^+\ fl^+\ cu^+}$$

A crossover in the *ca–fl* region produces claret and fluted, curled offspring, and a crossover in the *fl–cu* region produces fluted, claret and curled offspring. Counting the crossovers in each region, including the double crossovers in each, and then converting to percentages, yields a claret-to-fluted distance of 35.0 map units (173 + 167 + 6 + 4) and a fluted-to-curled distance of 6.0 map units (26 + 24 + 6 + 4). We expect $0.35 \times 0.06 \times 1{,}000 = 21$ double crossovers, but we observed only 6 + 4 = 10. Thus, the coefficient of coincidence is 10/21 = 0.48.

PROBLEM 2: The *ad5* locus in *Neurospora* is a gene for an enzyme in the synthesis pathway for the DNA base adenine. A wild-type strain ($ad5^+$) is crossed with an adenine-requiring strain, $ad5^-$. The diploid undergoes meiosis, and one hundred asci are scored for their segregation patterns with the following results:

$ad5^+$	$ad5^+$	$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	$ad5^-$	$ad5^-$	40
$ad5^-$	$ad5^-$	$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	$ad5^+$	$ad5^+$	46
$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	5
$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	3
$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	4
$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	2

What can you say about the linkage arrangements at this locus?

Answer: You can see that 14 (5 + 3 + 4 + 2) asci are of the second-division segregation type (SDS) and 86 (40 + 46) are of the first-division segregation type (FDS). To map the distance of the locus to its centromere, we divide the percentage of SDS types by 2: 14/100 = 14%; divided by 2 is 7%. Thus, the *ad5* locus is 7 map units from its centromere.

PROBLEM 3: In yeast, the *his5* locus is a gene for an enzyme in the synthesis pathway for the amino acid histidine, and the *lys11* locus is a gene for an enzyme in the synthesis pathway for the amino acid lysine. A haploid wild-type strain ($his5^+\ lys11^+$) is crossed with the double mutant ($his5^-\ lys11^-$). The diploid is allowed to undergo meiosis, and 100 asci are scored with the following results:

$his5^+\ lys11^+$	$his5^+\ lys11^+$	$his5^+\ lys11^-$
$his5^+\ lys11^+$	$his5^-\ lys11^-$	$his5^+\ lys11^-$
$his5^-\ lys11^-$	$his5^-\ lys11^+$	$his5^-\ lys11^+$
$his5^-\ lys11^-$	$his5^+\ lys11^-$	$his5^-\ lys11^+$
62	30	8

What is the linkage arrangement of these loci?

Answer: Of the 100 asci analyzed, 62 were parental ditypes (PD), 30 were tetratypes (TT), and 8 were nonparental ditypes (NPD). To map the distance between the two loci, we take the percentage of NPD (8%) plus half the percentage of TT (1/2 of 30 = 15%) = 23% or 23 centimorgans between loci.

PROBLEM 4: A particular human enzyme is present only in clone B. The human chromosomes present in clones A, B, and C appear as pluses in the following table. Determine the probable chromosomal location of the gene for the enzyme.

	Human Chromosome							
Clone	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
A	+	+	+	+	−	−	−	−
B	+	+	−	−	+	+	−	−
C	+	−	+	−	+	−	+	−

Answer: If a gene is located on a chromosome, the gene must be present in the clones with the chromosome (+). Chromosomes 1, 2, 5, 6 are present in B. If the gene in question were located on chromosome 1, the enzyme should have been present in all three clones. A similar argument holds for chromosome 2, in which the enzyme should have been present in clones A and B, and so on for the rest of the chromosomes. The only chromosome that is unique to clone B is 6. Therefore, the gene is located on chromosome 6.

EXERCISES AND PROBLEMS*

DIPLOID MAPPING

1. A homozygous groucho fly (*gro,* bristles clumped above the eyes) is crossed with a homozygous rough fly (*ro,* eye abnormality). The F_1 females are testcrossed, producing these offspring:

groucho	518
rough	471
groucho, rough	6
wild-type	5
	1,000

a. What is the linkage arrangement of these loci?
b. What offspring would result if the F_1 dihybrids were crossed among themselves instead of being testcrossed?

2. A female fruit fly with abnormal eyes (*abe*) of a brown color (*bis,* bistre) is crossed with a wild-type male. Her sons have abnormal, brown eyes; her daughters are of the wild-type. When these F_1 flies are crossed among themselves, the following offspring are produced:

	Sons	Daughters
abnormal, brown	219	197
abnormal	43	45
brown	37	35
wild-type	201	223

What is the linkage arrangement of these loci?

3. In *Drosophila,* the loci inflated (*if*, small, inflated wings) and warty *(wa*, abnormal eyes) are about 10 map units apart on the X chromosome. Construct a data set that would allow you to determine this linkage arrangement. What differences would be involved if the loci were located on an autosome?

4. A geneticist crossed female fruit flies that were heterozygous at three electrophoretic loci, each with fast and slow alleles, with males homozygous for the slow alleles. The three loci were *got1* (glutamate oxaloacetate transaminase-1), *amy* (alpha-amylase), and *sdh* (succinate dehydrogenase). The first 1,000 offspring isolated had the following genotypes:

Class 1	$got^s\ got^s\ amy^s\ amy^s\ sdh^s\ sdh^s$	441
Class 2	$got^f\ got^s\ amy^f\ amy^s\ sdh^f\ sdh^s$	421
Class 3	$got^f\ got^s\ amy^s\ amy^s\ sdh^s\ sdh^s$	11
Class 4	$got^s\ got^s\ amy^f\ amy^s\ sdh^f\ sdh^s$	14
Class 5	$got^f\ got^s\ amy^f\ amy^s\ sdh^s\ sdh^s$	58
Class 6	$got^s\ got^s\ amy^s\ amy^s\ sdh^f\ sdh^s$	53
Class 7	$got^f\ got^s\ amy^s\ amy^s\ sdh^f\ sdh^s$	1
Class 8	$got^s\ got^s\ amy^f\ amy^s\ sdh^s\ sdh^s$	1

What are the linkage arrangements of these three loci, including map units? If the three loci are linked, what is the coefficient of coincidence?

5. The following three recessive markers are known in lab mice: *h*, hotfoot; *o*, obese; and *wa*, waved. A trihybrid of unknown origin is testcrossed, producing the following offspring:

hotfoot, obese, waved	357
hotfoot, obese	74
waved	66
obese	79
wild-type	343
hotfoot, waved	61
obese, waved	11
hotfoot	9
	1,000

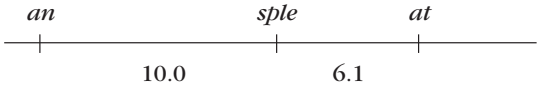
a. If the genes are linked, determine the relative order and the map distances between them.
b. What was the *cis-trans* allele arrangement in the trihybrid parent?
c. Is there any crossover interference? If yes, how much?

6. The following three recessive genes are found in corn: *bt1*, brittle endosperm; *gl17*, glossy leaf; *rgdl,* ragged seedling. A trihybrid of unknown origin is testcrossed, producing the following offspring:

brittle, glossy, ragged	236
brittle, glossy	241
ragged	219
glossy	23
wild-type	224
brittle, ragged	17
glossy, ragged	21
brittle	19
	1,000

a. If the genes are linked, determine the relative order and map distances.
b. Reconstruct the chromosomes of the trihybrid.
c. Is there any crossover interference? If yes, how much?

7. In *Drosophila,* ancon (*an*, legs and wings short), spiny legs (*sple*, irregular leg hairs), and arctus oculus (*at*, small narrow eyes) have the following linkage arrangement on chromosome 3:



*Answers to selected exercises and problems are on page A-6.

a. Devise a data set with no crossover interference that would yield these map units.
b. What data would yield the same map units but with a coefficient of coincidence of 0.60?

8. Ancon (*an*) and spiny legs (*sple*), from problem 7, are 10 map units apart on chromosome 3. Notchy (*ny,* wing tips nicked) is on the X chromosome (chromosome 1). Create a data set that would result if you were making crosses to determine the linkage arrangement of these three loci. How would you know that the notchy locus is on the X chromosome?

9. In the house mouse, the autosomal alleles Trembling and Rex (short hair) are dominant to not trembling (normal) and long hair, respectively. Heterozygous Trembling, Rex females were crossed with normal, long-haired males and yielded the following offspring:

Trembling, Rex	42
Trembling, long-haired	105
normal, Rex	109
normal, long-haired	44

a. Are the two genes linked? How do you know?
b. In the heterozygous females, were Trembling and Rex in *cis* or *trans* position? Explain.
c. Calculate the percent recombination between the two genes.

10. In corn, a trihybrid Tunicate (*T*), Glossy (*G*), Liguled (*L*) plant was crossed with a nontunicate, nonglossy, liguleless plant, producing the following offspring:

Tunicate, liguleless, Glossy	58
Tunicate, liguleless, nonglossy	15
Tunicate, Liguled, Glossy	55
Tunicate, Liguled, nonglossy	13
nontunicate, Liguled, Glossy	16
nontunicate, Liguled, nonglossy	53
nontunicate, liguleless, Glossy	14
nontunicate, liguleless, nonglossy	59

a. Determine which genes are linked.
b. Determine the genotype of the heterozygote; be sure to indicate which alleles are on which chromosome.
c. Calculate the map distances between the linked genes.

11. In *Drosophila*, kidney-shaped eye (*k*), cardinal eye (*cd*), and ebony body (*e*) are three recessive genes. If homozygous kidney, cardinal females are crossed with homozygous ebony males, the F_1 offspring are all wild-type. If heterozygous F_1 females are mated with kidney, cardinal, ebony males, the following 2,000 progeny appear:

880	kidney, cardinal
887	ebony
64	kidney, ebony
67	cardinal
49	kidney
46	ebony, cardinal
3	kidney, ebony, cardinal
4	wild-type

a. Determine the chromosomal composition of the F_1 females.
b. Derive a map of the three genes.

12. Following is a partial map of the third chromosome in *Drosophila.*

19.2 javelin bristles (*jv*)
43.2 thread arista (*th*)
66.2 Delta veins (*Dl*)
70.7 ebony body (*e*)

a. If flies heterozygous in *cis* position for javelin and ebony are mated among themselves, what phenotypic ratio do you expect in the progeny?
b. A true-breeding thread, ebony fly is crossed with a true-breeding Delta fly. An F_1 female is test-crossed to a thread, ebony male. Predict the expected progeny and their frequencies for this cross. Assume no interference.
c. Repeat *b,* but assume a coefficient of coincidence of 0.4.

13. Suppose that you have determined the order of three genes to be *a*, *c*, *b*, and that by doing two-point crosses you have determined map distances as a–$c = 10$ and c–$b = 5$. If interference is −1.5, and the three-point cross is

$$\frac{A\ C\ B}{a\ c\ b} \times \frac{a\ c\ b}{a\ c\ b}$$

what frequency of double crossovers do you expect?

HAPLOID MAPPING (TETRAD ANALYSIS)

14. Given the following cross in *Neurospora: ab* × a^+b^+, construct results showing that crossing over occurs in two of the four chromatids of a tetrad at meiosis. What would the results be if crossing over occurred during interphase before each chromosome became two chromatids? if each crossover event involved three or four chromatids?

15. A strain of yeast requiring both tyrosine (tyr^-) and arginine (arg^-) is crossed to the wild-type. After meiosis, the following ten asci are dissected. Classify each ascus as to segregational type (PD, NPD, TT). What is the linkage relationship between these two loci?

1	$arg^-\ tyr^-$	$arg^+\ tyr^+$	$arg^+\ tyr^+$	$arg^-\ tyr^-$
2	$arg^+\ tyr^+$	$arg^+\ tyr^+$	$arg^-\ tyr^-$	$arg^-\ tyr^-$
3	$arg^-\ tyr^+$	$arg^-\ tyr^+$	$arg^+\ tyr^-$	$arg^+\ tyr^-$
4	$arg^-\ tyr^-$	$arg^-\ tyr^-$	$arg^+\ tyr^+$	$arg^+\ tyr^+$
5	$arg^-\ tyr^-$	$arg^-\ tyr^+$	$arg^+\ tyr^-$	$arg^+\ tyr^+$
6	$arg^+\ tyr^+$	$arg^+\ tyr^+$	$arg^-\ tyr^-$	$arg^-\ tyr^-$
7	$arg^-\ tyr^-$	$arg^+\ tyr^+$	$arg^-\ tyr^+$	$arg^+\ tyr^-$
8	$arg^+\ tyr^+$	$arg^+\ tyr^+$	$arg^-\ tyr^-$	$arg^-\ tyr^-$
9	$arg^+\ tyr^+$	$arg^-\ tyr^-$	$arg^-\ tyr^-$	$arg^+\ tyr^+$
10	$arg^-\ tyr^-$	$arg^+\ tyr^+$	$arg^+\ tyr^+$	$arg^-\ tyr^-$

16. A certain haploid strain of yeast was deficient for the synthesis of the amino acids tryptophan (try^-) and methionine (met^-). It was crossed to the wild-type, and meiosis occurred. One dozen asci were analyzed for their tryptophan and methionine requirements. The following results, with the inevitable lost spores, were obtained:

1	$try^-\ met^-$	?	?	$try^-\ met^-$
2	?	$try^-\ met^-$	$try^+\ met^+$	$try^+\ met^+$
3	$try^-\ met^+$	$try^-\ met^-$	$try^+\ met^-$	$try^+\ met^+$
4	$try^-\ met^-$	$try^+\ met^+$	?	$try^+\ met^-$
5	$try^-\ met^+$	?	?	$try^+\ met^-$
6	$try^+\ met^+$	$try^+\ met^+$	$try^-\ met^-$	$try^-\ met^-$
7	$try^+\ met^+$	$try^+\ met^-$	?	$try^-\ met^-$
8	$try^+\ met^+$	$try^-\ met^-$	?	$try^+\ met^+$
9	$try^-\ met^+$	$try^+\ met^-$	$try^-\ met^+$	$try^+\ met^-$
10	$try^-\ met^-$	$try^+\ met^+$	$try^-\ met^-$	$try^+\ met^+$
11	$try^+\ met^+$	$try^+\ met^+$	?	?
12	?	$try^+\ met^-$	?	$try^-\ met^+$

a. Classify each ascus as to segregational type (note that some asci may not be classifiable).
b. Are the genes linked?
c. If so, how far apart are they?

17. In *Neurospora*, a haploid strain requiring arginine (arg^-) is crossed with the wild-type (arg^+). Meiosis occurs, and ten asci are dissected with the following results. Map the *arg* locus.

1	arg^+	arg^+	arg^-	arg^-	arg^+	arg^+	arg^-	arg^-
2	arg^-	arg^-	arg^+	arg^+	arg^-	arg^-	arg^+	arg^+
3	arg^+	arg^+	arg^+	arg^+	arg^-	arg^-	arg^-	arg^-
4	arg^+	arg^+	arg^+	arg^+	arg^-	arg^-	arg^-	arg^-
5	arg^-	arg^-	arg^-	arg^-	arg^+	arg^+	arg^+	arg^+
6	arg^+	arg^+	arg^-	arg^-	arg^-	arg^-	arg^+	arg^+
7	arg^-	arg^-	arg^+	arg^+	arg^+	arg^+	arg^-	arg^-
8	arg^+	arg^+	arg^+	arg^+	arg^-	arg^-	arg^-	arg^-
9	arg^-	arg^-	arg^+	arg^+	arg^+	arg^+	arg^-	arg^-
10	arg^-	arg^-	arg^-	arg^-	arg^+	arg^+	arg^+	arg^+

18. A haploid strain of *Neurospora* with fuzzy colony morphology (f) was crossed with the wild-type (f^+). Twelve asci were scored. The following results, with the inevitable lost spores were obtained:

1	?	f	f	?	?	f^+	f^+	f^+
2	f	f	f^+	f^+	f^+	f^+	f	f
3	f	?	?	?	f^+	?	?	?
4	f^+	?	?	?	f	f	f	f
5	f	f	?	?	?	f^+	?	f^+
6	?	f	f	?	?	?	?	?
7	f^+	f^+	f	f	f	f	f^+	f^+
8	f	f	f	?	?	f^+	f^+	f^+
9	f^+	?	?	?	?	f	f	?
10	f	f	f^+	f^+	f	f	f^+	f^+
11	f	f	f	f	f^+	f^+	f^+	f^+
12	f	f	?	?	?	?	f^+	f^+

a. Classify each ascus as to segregational type, and note which asci cannot be classified.
b. Map the chromosome containing the f locus with all the relevant measurements.

19. Draw ten of the remaining twenty-eight ascus patterns not included in table 6.6. To which of the seven major categories of table 6.7 does each belong?

20. In yeast, the *a* and *b* loci are 12 map units apart. Construct a data set to demonstrate this.

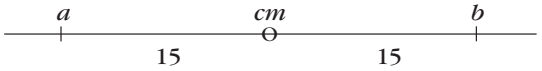
21. In *Neurospora*, the *a* locus is 12 map units from its centromere. Construct a data set to show this.

22. An *ab Neurospora* was crossed with an a^+b^+ form. Meiosis occurred, and 1,000 asci were dissected. Using the classes of table 6.7, the following data resulted:

Class 1	700	Class 5	5
Class 2	0	Class 6	5
Class 3	190	Class 7	10
Class 4	90		

What is the linkage arrangement of these loci?

23. Given the following linkage arrangement in *Neurospora*, construct a data set similar to that in table 6.7 that is consistent with it (*cm* is centromere).



24. Determine crossover events that led to each of the seven classes in table 6.7.

25. In *Neurospora,* a cross is made between ab^+ and a^+b individuals. The following one hundred ordered tetrads are obtained:

Spores	I	II	III	IV	V	VI	VII	VIII
1, 2	a^+b	a^+b	a^+b	a^+b^+	a^+b^+	a^+b	a^+b	ab^+
3, 4	a^+b	a^+b^+	a^+b^+	a^+b	a^+b	ab^+	ab^+	a^+b
5, 6	ab^+	ab	ab^+	ab	ab^+	a^+b	ab^+	a^+b
7, 8	ab^+	ab^+	ab	ab^+	ab	ab^+	a^+b	ab^+
	85	2	3	2	3	3	1	1

a. Are genes *a* and *b* linked? How do you know?
b. Calculate the gene-to-centromere distances for *a* and *b*.

26. *Neurospora* has four genes—*a*, *b*, *c*, and *d*—that control four different phenotypes. Your job is to map these genes by performing pairwise crosses. You obtain the following ordered tetrads:

	$ab^+ \times a^+b$				$bc^+ \times b^+c$		
Spores	**I**	**II**	**III**	**Spores**	**I**	**II**	**III**
1, 2	ab^+	ab	ab^+	1, 2	bc^+	b^+c^+	b^+c
3, 4	ab^+	ab	a^+b^+	3, 4	bc^+	b^+c^+	b^+c^+
5, 6	a^+b	a^+b^+	a^+b	5, 6	b^+c	bc	bc
7, 8	a^+b	a^+b^+	ab	7, 8	b^+c	bc	bc^+
	45	43	12		70	4	26

	$cd^+ \times c^+d$						
Spores	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
1, 2	cd^+	cd	cd	cd	cd^+	cd	cd^+
3, 4	cd^+	cd	cd^+	c^+d	c^+d	c^+d^+	c^+d
5, 6	c^+d	c^+d^+	c^+d^+	c^+d^+	c^+d	c^+d^+	c^+d^+
7, 8	c^+d	c^+d^+	c^+d	cd^+	cd^+	cd	cd
	42	2	30	15	5	1	5

a. Calculate the gene-to-centromere distances.
b. Which genes are linked? Explain.
c. Derive a complete map for all four genes.

27. You have isolated a new fungus and have obtained a strain that requires both arginine (arg^-) and adenine (ad^-). You cross these two strains and collect four hundred random spores that you plate on minimal medium. If twenty-five spores grow, what is the distance between these two genes?

28. Three distinct genes, *pab*, *pk*, and *ad*, were scored in a cross of *Neurospora.* From the cross $pab\ pk^+\ ad^+ \times pab^+\ pk\ ad$, the following ordered tetrads were recovered:

Spores	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
1, 2	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad$	$pab\ pk^+\ ad$
3, 4	$pab\ pk^+\ ad^+$	$pab^+pk\ ad$	$pab\ pk\ ad$	$pab\ pk^+ad$	$pab^+\ pk\ ad$	$pab^+\ pk\ ad$	$pab^+\ pk\ ad$	$pab^+pk\ ad^+$
5, 6	$pab^+pk\ ad$	$pab\ pk^+ad^+$	$pab^+pk^+ad^+$	$pab^+pk\ ad^+$	$pab\ pk\ ad$	$pab\ pk^+ad$	$pab^+pk\ ad^+$	$pab\ pk^+ad^+$
7, 8	$pab^+pk\ ad$	$pab^+pk\ ad$	$pab^+pk\ ad$	$pab^+pk\ ad$	$pab^+pk^+\ ad^+$	$pab^+pk\ ad^+$	$pab\ pk^+ad^+$	$pab^+pk\ ad$
	34	35	9	7	2	2	1	3

Based on the data, construct a map of the three genes. Be sure to indicate centromeres.

HUMAN CHROMOSOMAL MAPS

29. The Duffy blood group with alleles FY^a and FY^b was localized to chromosome 1 in human beings when an "uncoiled" chromosome was associated with it. Construct a pedigree that would verify this.

30. What pattern of scores would you expect to get, using the hybrid clones in table 6.8, for a locus on human chromosome 6? 14? X?

31. A man with X-linked color blindness and X-linked Fabry disease (alpha-galactosidase-A deficiency) mates with a normal woman and has a normal daughter. This daughter then mates with a normal man and produces ten sons (as well as eight normal daughters). Of the sons, five are normal, three are like their grandfather, one is only color-blind, and one has Fabry disease. From these data, what can you say about the relationship of these two X-linked loci?

32. In people, the ABO system (I^A, I^B, i alleles) is linked to the aldolase-B locus (*ALDOB*), a gene that functions in the liver. Deficiency, which is recessive, results in fructose intolerance. A man with blood type AB has a fructose-intolerant, type B father and a normal, type AB mother. He and a woman with blood type O and fructose intolerance have ten children. Five are type A and normal, three are fructose intolerant and type B, and two are type A and intolerant to fructose. Draw a pedigree of this family and determine the map distances involved. (Calculate a *lod* score to determine the most likely recombination frequency between the loci.)

33. Hemophilia and color-blindness are X-linked recessive traits. A normal woman whose mother was color-blind and whose father was a hemophiliac mates with a normal man whose father was color-blind. They have the following children:

4 normal daughters
1 normal son
2 color-blind sons
2 hemophiliac sons
1 color-blind, hemophiliac son

Estimate the distance between the two genes.

34. The results of an analysis of five human-mouse hybrids for five enzymes are given in table along with the human chromosomal content of each clone (+ = enzyme or chromosome present; − = absent). Deduce which chromosome carries which gene.

	Clone				
Human Enzyme	**A**	**B**	**C**	**D**	**E**
glutathione reductase	+	+	−	−	−
malate dehydrogenase	−	+	−	−	−
adenosine deaminase	−	+	−	+	+
galactokinase	−	+	+	−	−
hexosaminidase	+	−	−	+	−

	Human Chromosome																					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Clone A	−	−	−	−	+	+	+	+	−	+	−	−	−	−	+	+	−	−	−	−	+	+
Clone B	+	+	−	+	−	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−
Clone C	−	−	−	+	−	−	+	−	−	+	−	+	+	+	+	−	+	−	+	−	−	+
Clone D	+	−	+	−	+	−	−	−	−	+	−	−	−	+	+	−	−	+	+	+	+	−
Clone E	−	−	−	+	−	−	−	−	+	+	+	+	−	+	−	+	−	+	−	+	+	+

35. You have selected three mouse-human hybrid clones and analyzed them for the presence of human chromosomes. You then analyze each clone for the presence or absence of particular human enzymes (+ = presence of human chromosome or enzyme activity). Based on the following results indicate the probable chromosomal location for each enzyme.

	Human Chromosomes						
Clone	**3**	**7**	**9**	**11**	**15**	**18**	**20**
X	−	+	−	+	+	−	+
Y	+	+	−	+	−	+	−
Z	−	+	+	−	−	+	+

	Enzyme				
Clone	**A**	**B**	**C**	**D**	**E**
X	+	+	−	−	+
Y	+	−	+	+	+
Z	−	−	+	−	+

36. Three mouse-human cell lines were scored for the presence (+) or absence (−) of human chromosomes, with the results as follows:

	Human Chromosomes							
Clone	**1**	**2**	**3**	**4**	**5**	**14**	**15**	**18**
A	+	+	+	+	−	−	−	−
B	+	+	−	−	+	+	−	−
C	+	−	+	−	+	−	+	−

If a particular gene is located on chromosome 3, which clones should be positive for the enzyme from that gene?

CRITICAL THINKING QUESTIONS

1. Do three-point crosses in fruit flies capture all the multiple crossovers in a region?
2. If 4% of all tetrads have a single crossover between two loci: (a) What is the map distance between these loci if these are fruit flies? (b) What is the proportion of second-division segregants if these are *Neurospora?* (c) What is the proportion of nonparental ditypes if these are yeast?

Suggested Readings for chapter 6 are on page B-3.