LINKAGE AND CROSSING OVER

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HISTORY

T. H. Morgan

laid the foundation for chromosome mapping: demonstrated

that the gene for *white* eyes in *Drosophila* was located on the

X chromosome.

Soon afterward Morgan's students: other genes were X-linked → able to locate each of these genes on a map of the chromosome → This map was a straight line, and each gene was situated at a particular point, or locus, on it .

The structure of the map therefore implied that a chromosome was simply a linear array of genes.

T. H. Morgan

WORLD'S FIRST CHROMOSOME MAP

The procedure for mapping chromosomes was invented by Alfred H. Sturtevant, an undergraduate working in Morgan's laboratory.

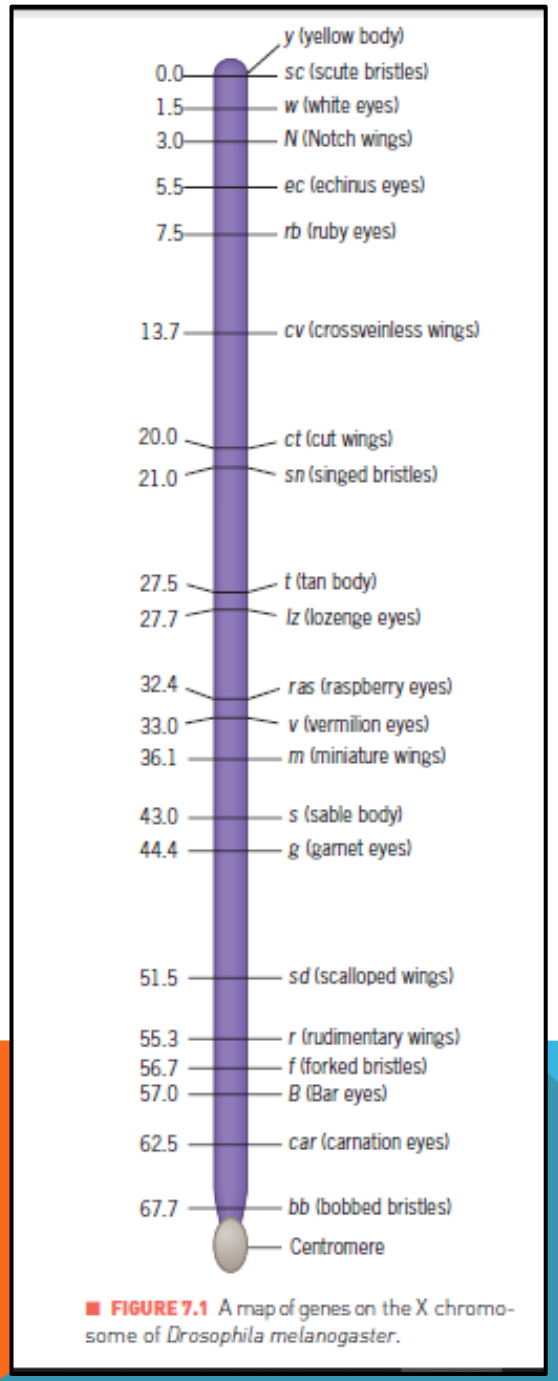
One night in 1911 Sturtevant put aside his algebra homework in order to evaluate some experimental data. Before the sun rose the next day, he had constructed the world's first chromosome map.

He relied completely on the analysis of data from experimental crosses with *Drosophila*. His method was simple and elegant, and exploited a phenomenon that regularly occurs during meiosis. This methodology laid the foundation for all subsequent efforts to study the organization of genes in chromosomes.

Linkage between genes was first discovered in experiments with sweet peas.

Alfred Henry Sturtevant

Photo courtesy of Cold Spring Harbor Laboratory Archives.



■ **FIGURE 7.1** A map of genes on the X chromosome of *Drosophila melanogaster*.

Genes that are on the same chromosome travel through meiosis together; however, alleles of chromosomally linked genes can be recombined by crossing over.

Sturtevant based his **mapping procedure** on the principle that genes on the same chromosome should be inherited together. Because such genes are physically attached to the same structure, they should travel as a unit through meiosis. This phenomenon is called **linkage.**

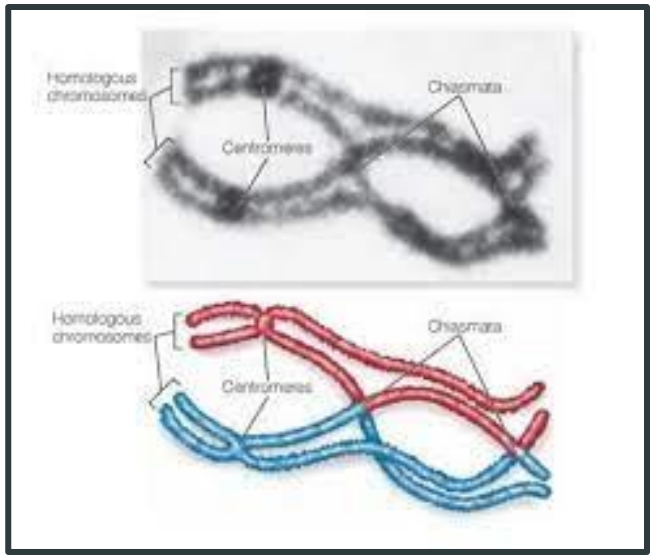
The early geneticists were unsure about the nature of linkage, but some of

them, including Morgan and his students, thought that genes were attached to one another much like **beads on a string**. Thus, these researchers clearly had a **linear model of chromosome** organization in mind.

The early geneticists also knew that **linkage was not absolute**. Their experimental data demonstrated that genes on the same chromosome could be separated as they went through meiosis and that new combinations of genes could be formed. However, this phenomenon, called **recombination,** was difficult to explain by simple genetic theory.

During meiosis, when homologous chromosomes paired, a physical exchange of material separated and recombined genes. This idea was inspired by the cytological observation that chromosomes could be seen in pairing configurations that suggested they had switched pieces with each other. At the switch points, the two homologues were crossed over, as if each had been broken and then reattached to its partner. A crossover point was called a chiasma (plural, chiasmata), from the Greek word meaning "cross."

Geneticists began to use the term *crossing over* to describe the process that created the chiasmata—that is, the actual process of exchange between paired chromosomes. They considered recombination—the separation of linked genes and the formation of new gene combinations —to be a result of the physical event of crossing over.

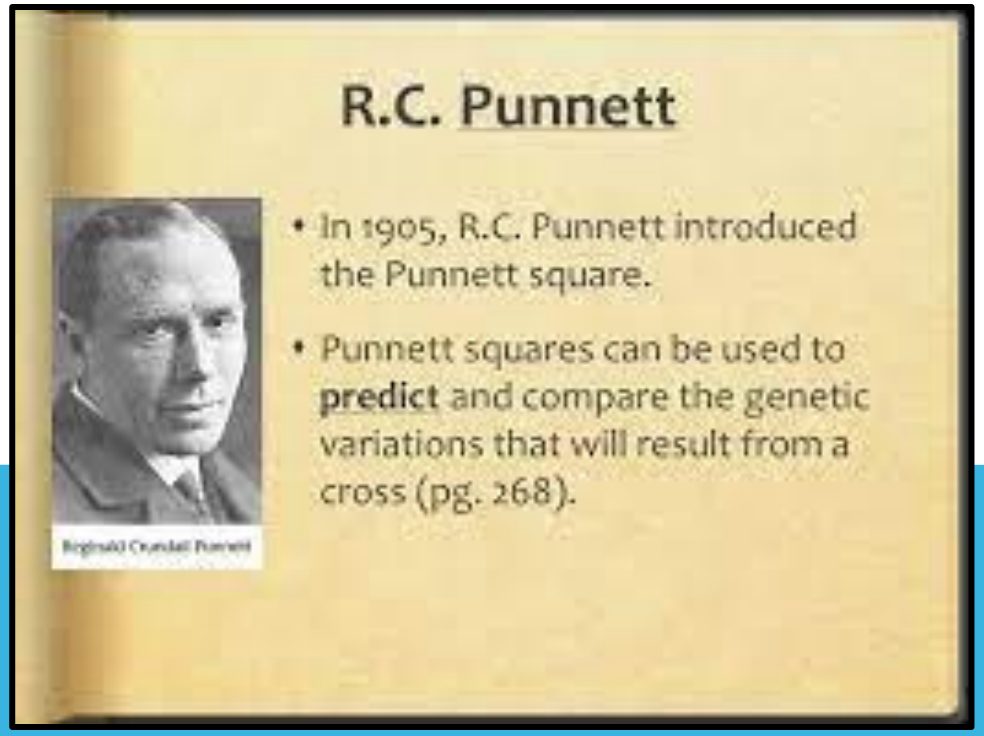


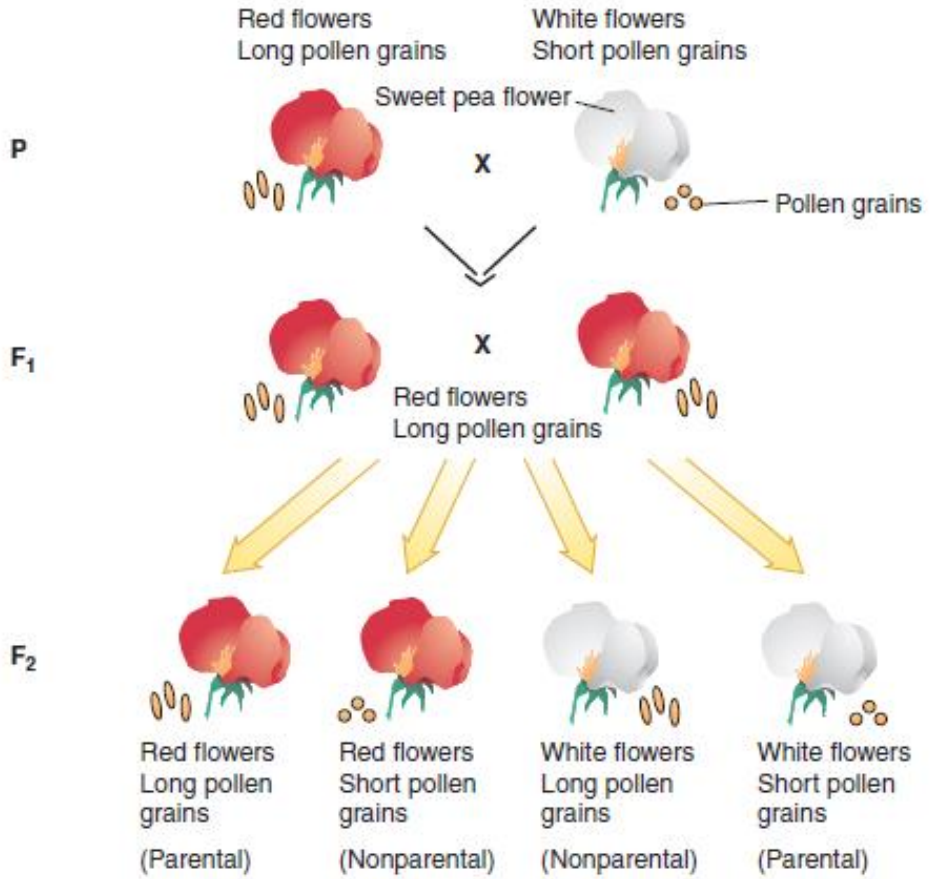
EARLY EVIDENCE FOR LINKAGE AND RECOMBINATION

- Some of the first evidence for linkage came from experiments performed by W. Bateson and R. C. Punnett (**Figure 7.2**).
- These researchers crossed varieties of sweet peas that differed in two traits, flower colour and pollen length.
- Plants with red flowers and long pollen grains were crossed to plants with white flowers and short pollen grains.

Reginald Crundall Punnett

All the F1 plants had red flowers and long pollen grains, indicating that the alleles for these two phenotypes were dominant. When the F1 plants were self-fertilized, Bateson and Punnett observed a peculiar distribution of phenotypes among the offspring. Instead of the 9:3:3:1 ratio expected for two independently assorting genes, they obtained a ratio of 24.3:1.1:1:7.1.





■ **FIGURE 7.2** Bateson and Punnett's experiment with sweet peas. The results in the F_2 indicate that the genes for flower color and pollen length do not assort independently.

TABLE 3.2

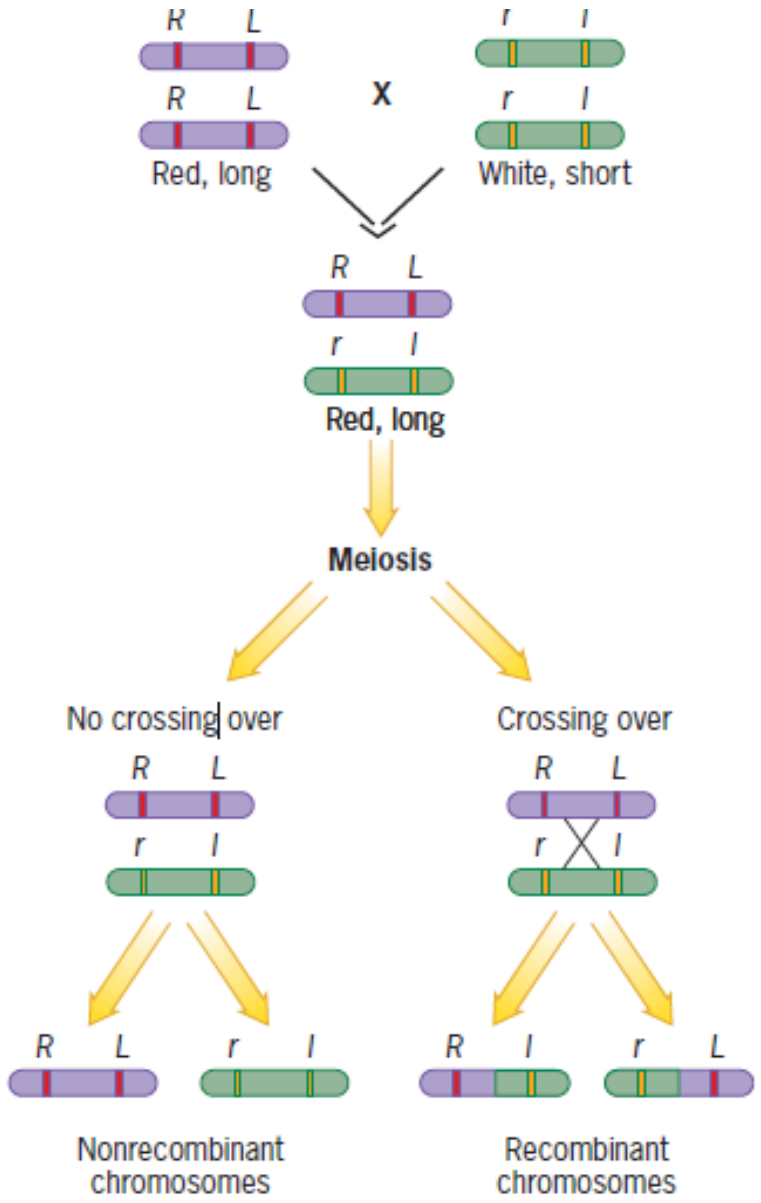
Table of Chi-Square (χ^2) 5% Critical Values[a]

Degrees of Freedom	5% Critical Value
1	3.841
2	5.991
3	7.815
4	9.488
5	11.070
6	12.592
7	14.067
8	15.507
9	16.919
10	18.307
15	24.996
20	31.410
25	37.652
30	43.773

[a]Selected entries from R. A. Fisher and Yates, 1943, *Statistical Tables for Biological, Agricultural, and Medical Research*. Oliver and Boyd, London.

- Disagreement between the observed results and the expected results (at the bottom of figure 7.2).
- Among the 803 F2 plants that were examined, the classes that resembled the original parents (called the parental classes) are significantly overrepresented and the two other (non-parental) classes are significantly underrepresented.
- For such obvious discrepancies, it hardly seems necessary to calculate a chi-square statistic to test the hypothesis that the two traits, flower colour and pollen grain length, have assorted independently. Clearly they have not. The chi-square value is enormous—much greater than 7.8, which is the critical value for a chi-square distribution with three degrees of freedom (see table 3.2).
- So, we must reject the hypothesis that the genes for flower colour and pollen grain length have assorted independently.

Bateson and Punnett devised a complicated explanation for their results, but it turned out to be wrong. The correct explanation for the lack of independent assortment in the data is that the genes for flower colour and pollen length are located on the same chromosome—that is, they are linked. This explanation is diagrammed in Figure 7.3.

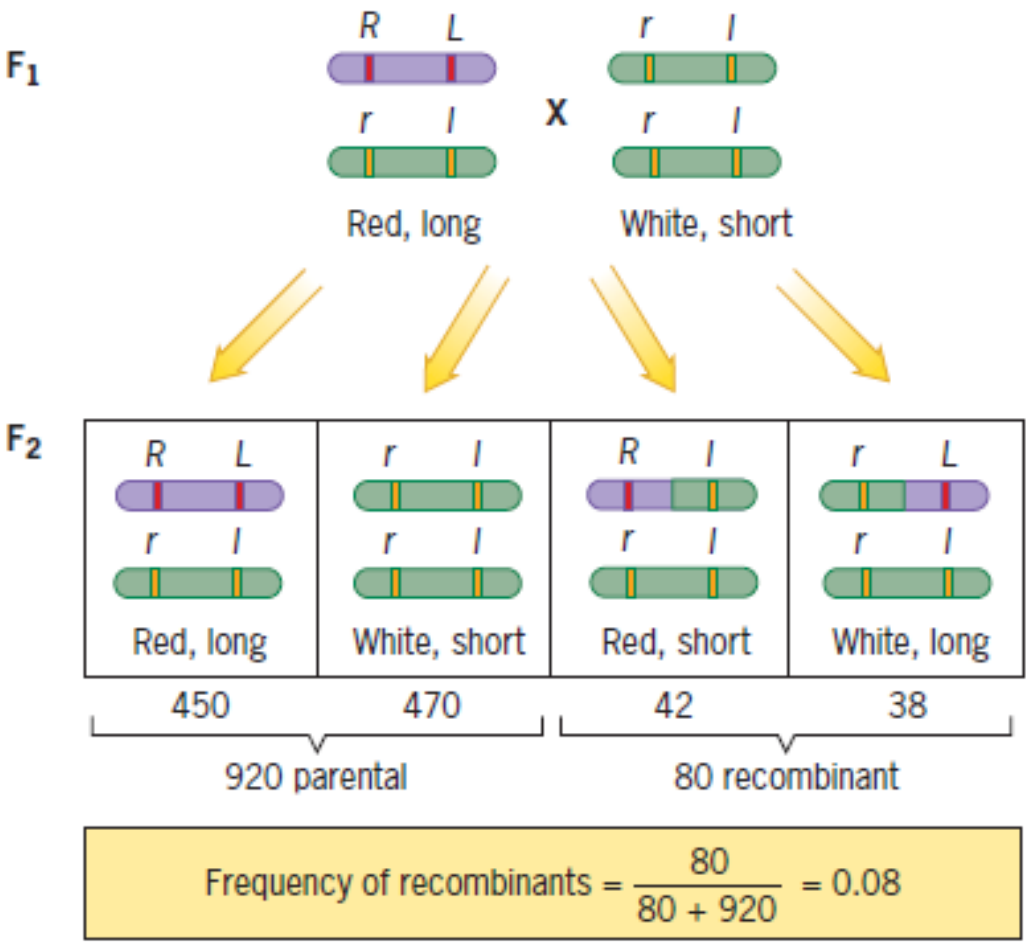


■ **FIGURE 7.3** Hypothesis of linkage between the genes for flower color and pollen length in sweet peas. In the F_1 plants the two dominant alleles, *R* and *L*, of the genes are situated on the same chromosome; their recessive alleles, *r* and *l*, are situated on the homologous chromosome.

➡ F1 Gametes

- The alleles of the flower colour gene are *R* (red) and *r* (white), and the alleles of the pollen length gene are *L* (long) and *l* (short);
- The *R* and *L* alleles are dominant. (Note here that for historical reasons, the allele symbols are derived from the dominant rather than the recessive phenotypes.)
- Because the flower colour and pollen length genes are linked, we expect the doubly heterozygous F1 plants to produce two kinds of gametes, *R L* and *r l*.
- However, once in a while a crossover will occur between the two genes and their alleles will be recombined, producing two other kinds of gametes, *R l* and *r L*.
- The frequency of these two types of recombinant gametes should depend on the frequency of crossing over between the two genes.

- Bateson and Punnett might have come up with this explanation if they had performed a testcross instead of an intercross in the F1.
- With a testcross the offspring would directly reveal the types of gametes produced by the doubly heterozygous F1 plants. **Figure 7.4** presents the analysis of such a testcross. Doubly heterozygous F1 sweet peas were crossed with plants homozygous for the recessive alleles of both genes.
- Among 1000 progeny scored, 920 resemble one or the other of the parental strains and the remaining 80 are recombinant. The frequency of the recombinant progeny produced by the heterozygous F1 plants is therefore 80/1000 0.08. Because this is a testcross, 0.08 is also the frequency of recombinant gametes produced by the heterozygous F1 plants. We can use this frequency, usually called the *recombination frequency*, to measure the intensity of linkage between genes.



■ **FIGURE 7.4** A testcross for linkage between genes in sweet peas. Because the recombinant progeny in the F_2 are 8 percent of the total, the genes for flower color and pollen length are rather tightly linked.

Genes that are tightly linked seldom recombine, whereas genes that are loosely linked recombine often.

Here the recombination frequency is fairly low. This implies that crossing over between the two genes is a rather rare event.

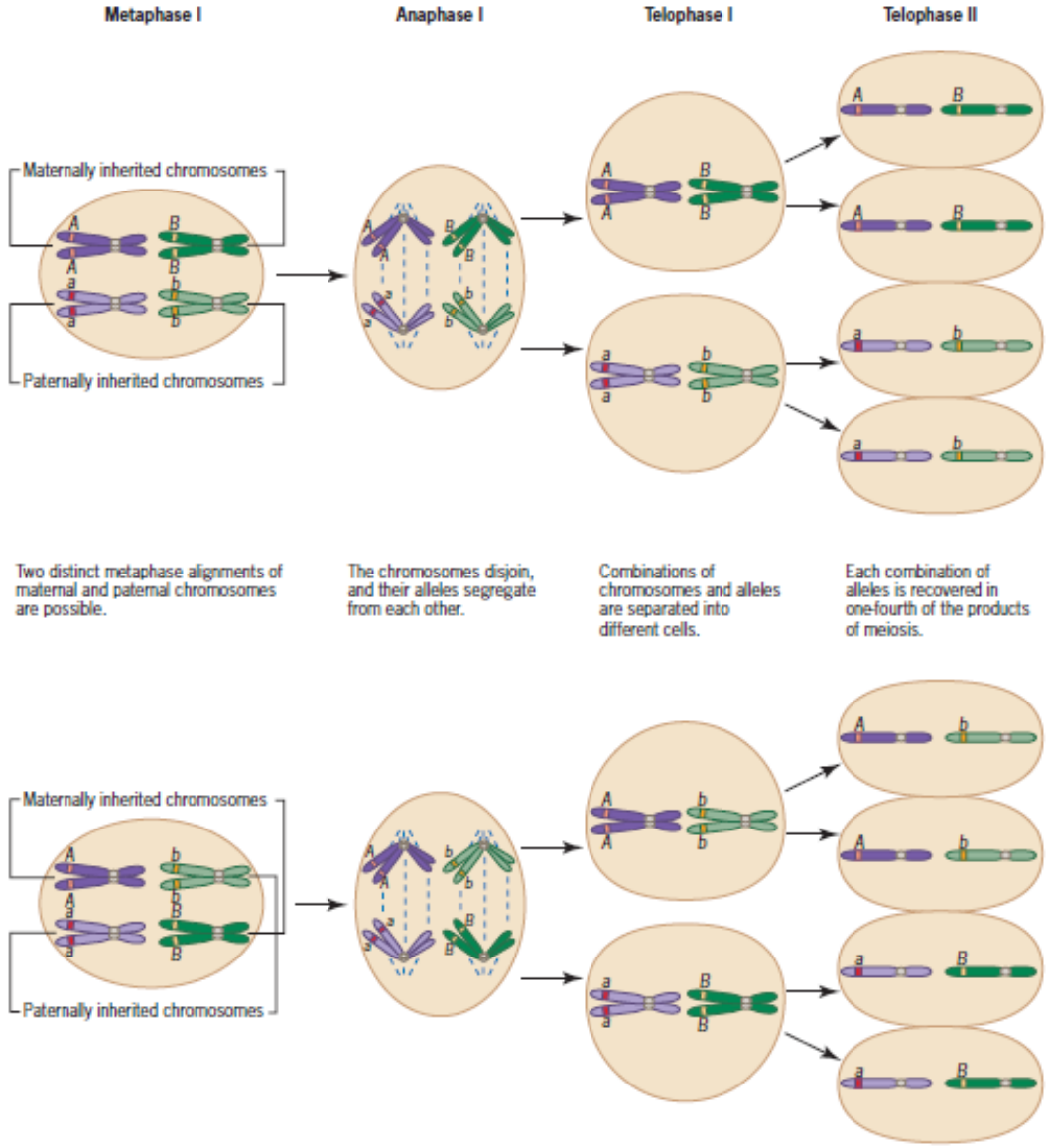
For any two genes, the recombination frequency never exceeds 50 percent. This upper limit is obtained when genes are on different chromosomes; 50 percent recombination is, in fact, what we mean when we say that the genes assort independently.

For example, let's assume that genes *A* and *B* are on different chromosomes and that an *AA BB* individual is crossed to an *aa bb* individual. From this cross the *Aa Bb* offspring are then testcrossed to the double recessive parent. Because the *A* and *B* genes assort independently, the F2 will consist of two classes (*Aa Bb* and *aa bb*) that are phenotypically like the parents in the original cross and two classes (*Aa bb* and *aa Bb*) that are phenotypically recombinant.

Furthermore, each F2 class will occur with a frequency of 25 percent (see Figure 5.7). Thus, the total frequency of recombinant progeny from a testcross involving two genes on different chromosomes will be 50 percent. A frequency of recombination less than 50 percent implies that the genes are linked on the same chromosome.

What is a test cross?

An experimental cross of an individual with dominant phenotype but unknown genotype & an organism with a homozygous recessive genotype and phenotype.

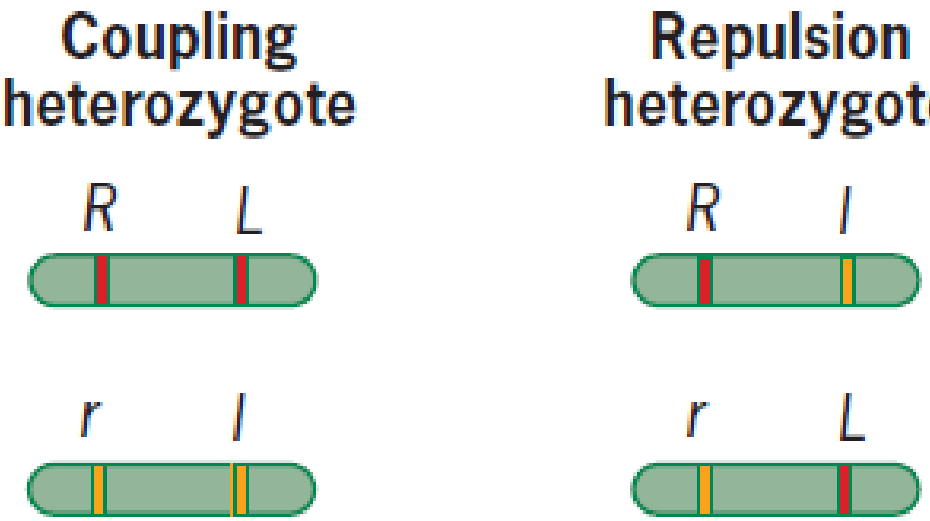


■ **FIGURE 5.7** Mendel's Principle of Independent Assortment and meiotic chromosome behavior. Alleles on different pairs of chromosomes assort independently in the anaphase of the first meiotic division because maternally and paternally inherited chromosomes have aligned randomly on the cell's equator.

Crosses involving linked genes are usually diagrammed to show the linkage phase—the way in which the alleles are arranged in heterozygous individuals (Figure 7.5). In Bateson and Punnett's sweet pea experiment, the heterozygous F1 plants received two dominant alleles, *R* and *L,* from one parent and two recessive alleles, *r* and *l*, from the other. Thus, we write the genotype of these plants *R L/r l,* where the slash (/) separates alleles inherited from different parents.

Another way of interpreting this symbolism is to say that the alleles on the left and right of the slash entered the genotype on different homologous chromosomes, one from each parent. Whenever the dominant alleles are all on one side of the slash, as in this example, the genotype has the *coupling* linkage phase. When the dominant and recessive alleles are split on both sides of the slash, as in *R l/r L*, the genotype has the *repulsion* linkage phase.

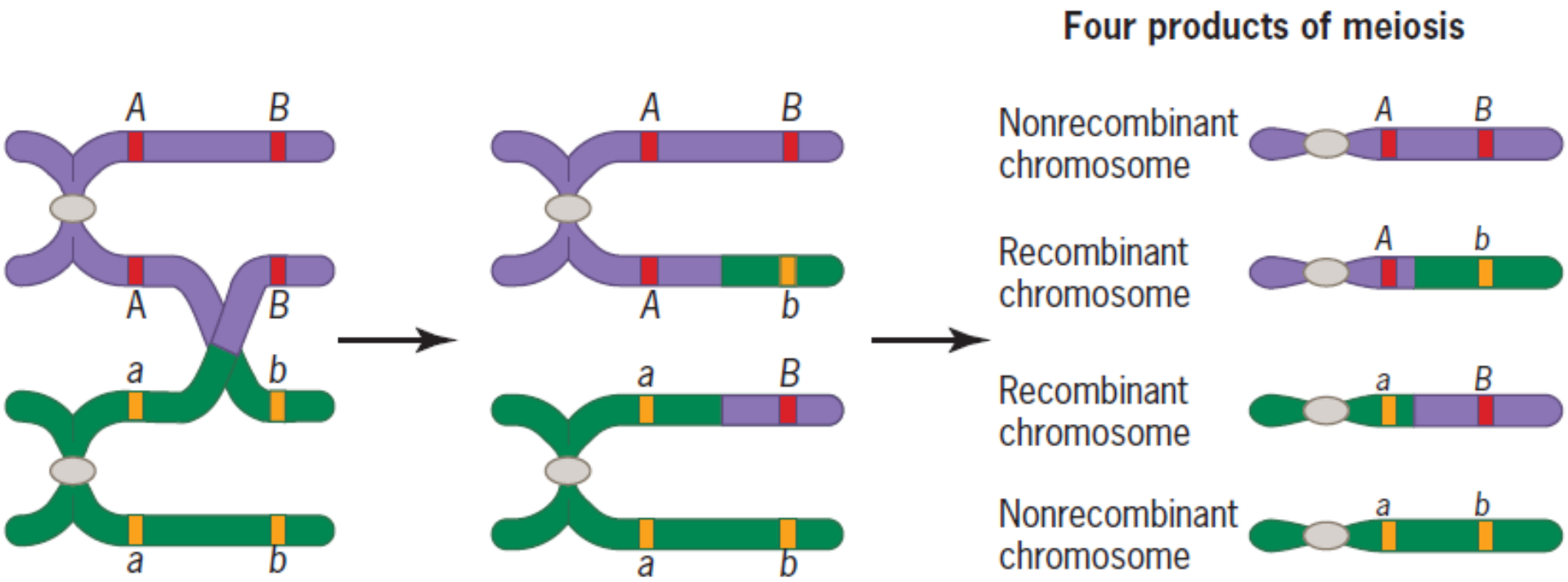
These terms provide us with a way of distinguishing between the two kinds of double heterozygotes.



■ **FIGURE 7.5** Coupling and repulsion linkage phases in double heterozygotes.

CYTOLOGICAL BASIS OF CROSSING OVER

CROSSING OVER AS BASIS OF RECOMBINATION



■ **FIGURE 7.6** Crossing over as the basis of recombination between genes. An exchange between paired chromosomes during meiosis produces recombinant chromosomes at the end of meiosis.

CROSSING OVER AS THE PHYSICAL BASIS OF RECOMBINATION

Recombinant gametes are produced as a result of crossing over between homologous chromosomes. This process involves a physical exchange between the chromosomes.

The exchange event occurs during the prophase of the first meiotic division, when duplicated chromosomes have paired.

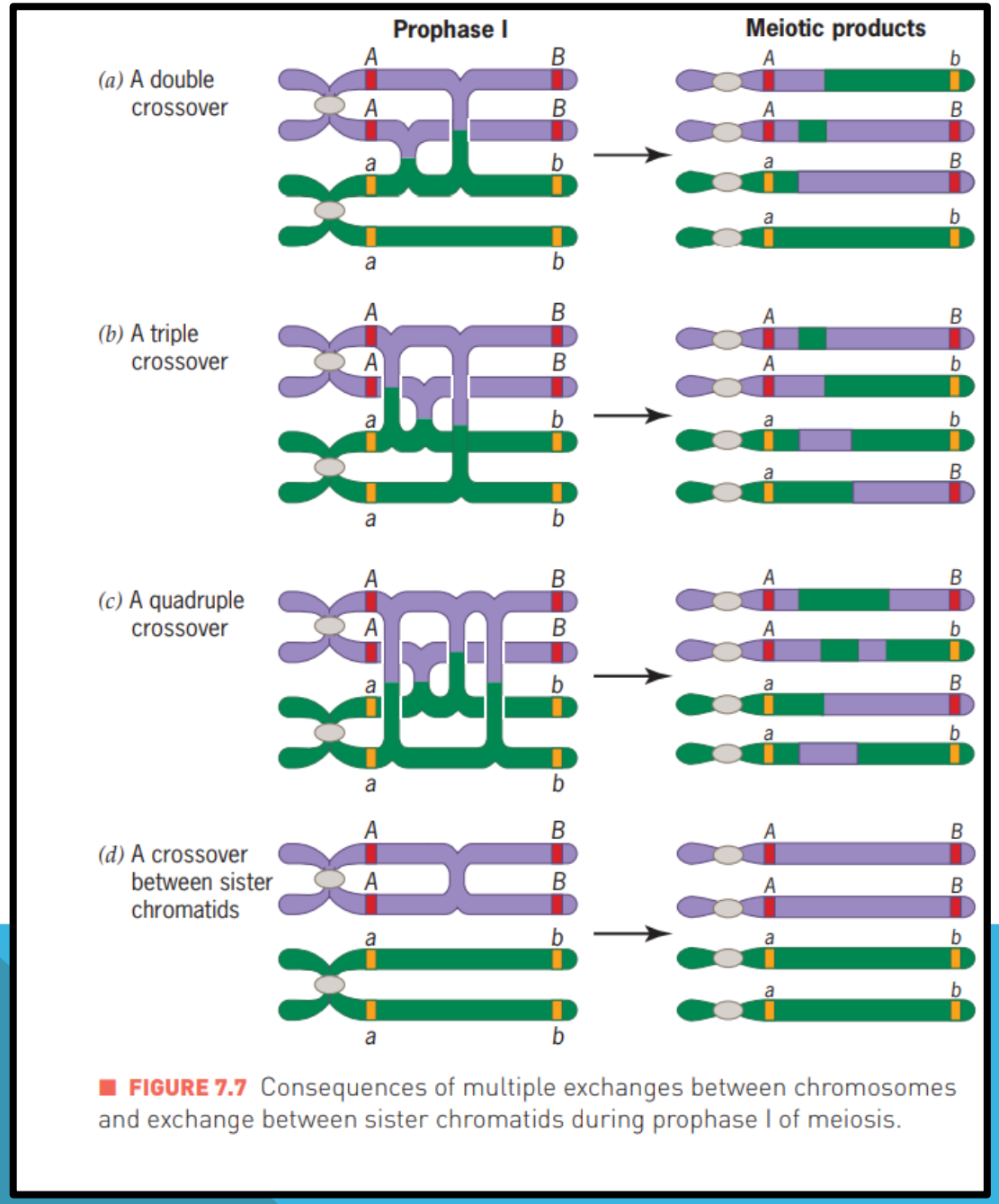
Although four homologous chromatids are present, forming what is called a tetrad, only two chromatids cross over at any one point.

Each of these chromatids breaks at the site of the crossover, and the resulting pieces reattach to produce the recombinants.

The other two chromatids are not recombinant at this site.

Each crossover event therefore produces two recombinant chromatids among a total of four.

Notice that only two chromatids are involved in an exchange at any one point. However, the other two chromatids may cross over at a different point. Thus, there is a possibility for multiple exchanges in a tetrad of chromatids



■ **FIGURE 7.7** Consequences of multiple exchanges between chromosomes and exchange between sister chromatids during prophase I of meiosis.

There may, for example, be two, three, or even four separate exchanges—customarily called double, triple, or quadruple crossovers.

An exchange between sister chromatids does not produce genetic recombinants because the sister chromatids are identical.

What is responsible for the breakage of chromatids during crossing over? The breaks are caused by enzymes acting on the DNA within the chromatids. Enzymes are also responsible for repairing these breaks—that is, for reattaching chromatid fragments to each other.

EVIDENCE THAT CROSSING OVER CAUSES RECOMBINATION

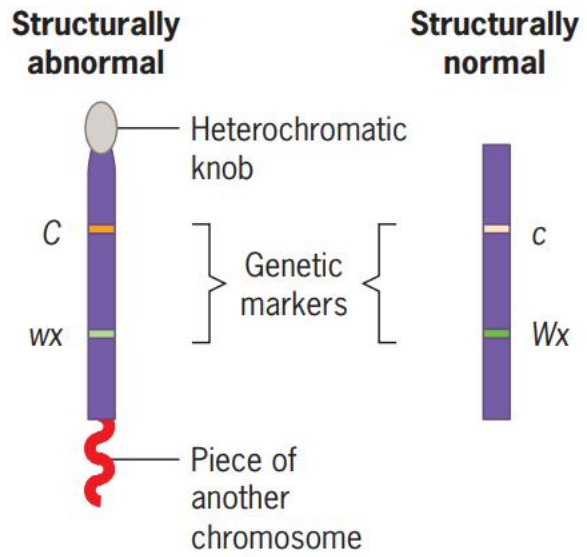
In 1931 Harriet Creighton and Barbara McClintock obtained evidence that genetic recombination was associated with a material exchange between chromosomes.

Creighton and McClintock studied homologous chromosomes in maize that were morphologically distinguishable. The goal was to determine whether physical exchange between these homologues was correlated with recombination between some of the genes they carried.

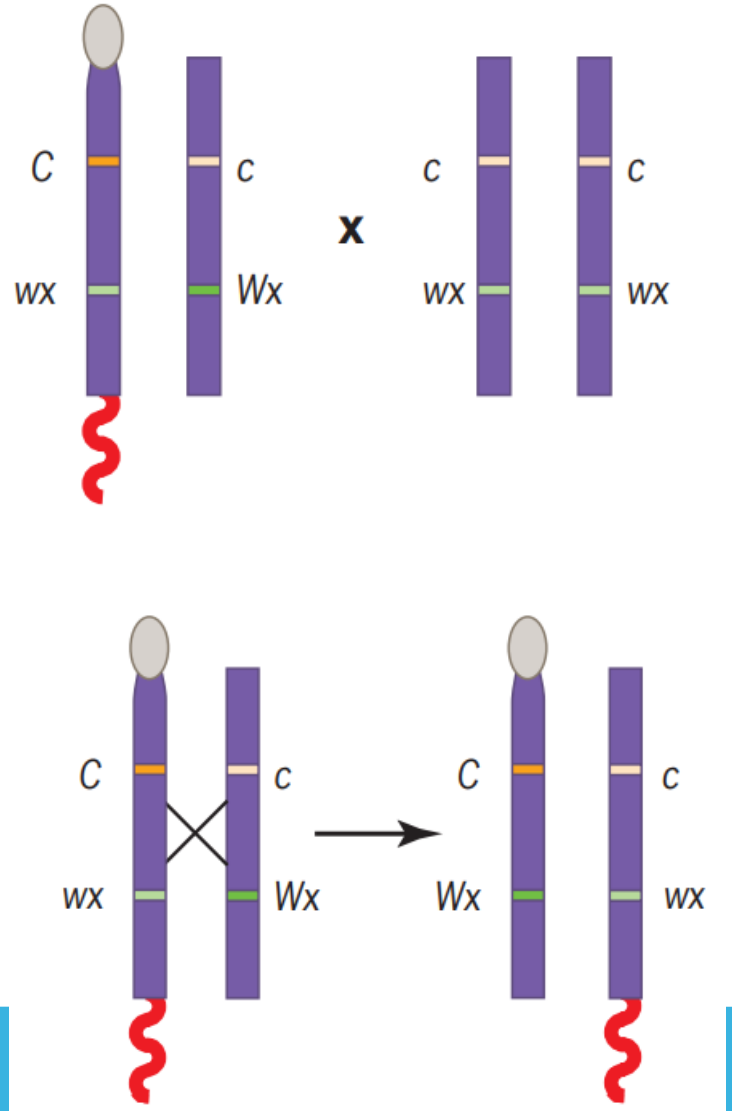
Two forms of chromosome 9 were available for analysis; one was normal, and the other had cytological aberrations at each end—a heterochromatic knob at one end and a piece of a different chromosome at the other (Figure 7.8).

These two forms of chromosome 9 were also genetically marked to detect recombination. One marker gene controlled kernel color (C, colored; c, colorless), and the other controlled kernel texture (Wx, starchy; wx, waxy).

Creighton and McClintock performed the following testcross.



■ **FIGURE 7.8** Two forms of chromosome 9 in maize used in the experiments of Creighton and McClintock.



- They then examined the recombinant progeny for evidence of exchange between the two different forms of chromosome 9.
- Their results showed that the C Wx and c wx recombinants carried a chromosome with only one of the abnormal cytological markers; the other abnormal marker had evidently been lost through an exchange with the normal chromosome 9 in the previous generation.
- These findings strongly argued that recombination was caused by a physical exchange between paired chromosomes.

CHIASMATA AND THE TIME OF CROSSING OVER

The cytological evidence for crossing over can be seen during late prophase of the first meiotic division when the chiasmata become clearly visible.

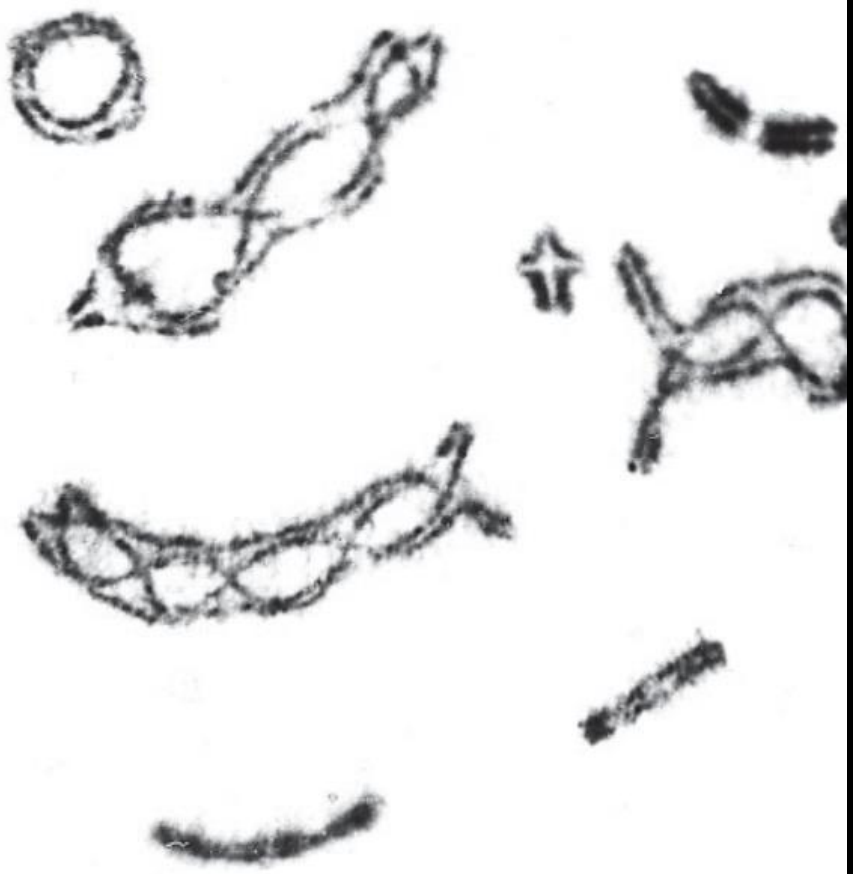
At this time paired chromosomes repel each other slightly, maintaining close contact only at the centromere and at each chiasma (Figure 7.9).

This partial separation makes it possible to count the chiasmata accurately.

As we might expect, large chromosomes typically have more chiasmata than small chromosomes.

Thus, the number of chiasmata is roughly proportional to chromosome length.

The appearance of chiasmata late in the first meiotic prophase might imply that it is then that crossing over occurs.



■ **FIGURE 7.9** Diplonema of male meiosis in the grasshopper *Chorthippus parallelus*. There are eight autosomal bivalents and an X-chromosome univalent. The four smaller bivalents each have one chiasma. The remaining bivalents have two to five chiasmata.

KEY POINTS

Linkage between genes is detected as a deviation from expectations based on Mendel's Principle of Independent Assortment.

The frequency of recombination measures the intensity of linkage. In the absence of linkage, this frequency is 50 percent; for very tight linkage, it is close to zero.

Recombination is caused by a physical exchange between paired homologous chromosomes early in prophase of the first meiotic division after chromosomes have duplicated.

At any one point along a chromosome, the process of exchange (crossing over) involves only two of the four chromatids in a meiotic tetrad.

Late in prophase I, crossovers become visible as chiasmata.

CHROMOSOME MAPPING

Linked genes can be mapped on a chromosome by studying how often their alleles recombine.

Crossing over during the prophase of the first meiotic division has two observable outcomes:

1. Formation of chiasmata in late prophase. 2. Recombination between genes on opposite sides of the crossover point.

However, the second outcome can only be seen in the next generation, when the genes on the recombinant chromosomes are expressed.

Geneticists construct chromosome maps by counting the number of crossovers that occur during meiosis. However, because the actual crossover events cannot be seen, they cannot count them directly.

Instead, they must estimate how many crossovers have taken place by counting either chiasmata or recombinant chromosomes.

Chiasmata are counted through cytological analysis, whereas recombinant chromosomes are counted through genetic analysis.

What do we mean by distance on a chromosome map?

Sturtevant's fundamental insight was to estimate the distance between points on a chromosome by counting the number of crossovers between them. Points that are far apart should have more crossovers between them than points that are close together.

The distance between two points on the genetic map of a chromosome is the average number of crossovers between them.

CROSSING OVER AS A MEASURE OF GENETIC DISTANCE

One way for us to understand this definition is to consider 100 oogonia going through meiosis (Figure 7.10).

In some cells, no crossovers will occur between sites A and B; in others, one, two, or more crossovers will occur between these loci.

At the end of meiosis, there will be 100 gametes, each containing a chromosome with either zero, one, two, or more crossovers between A and B.

We estimate the genetic map distance between these loci by calculating the average number of crossovers in this sample of chromosomes.

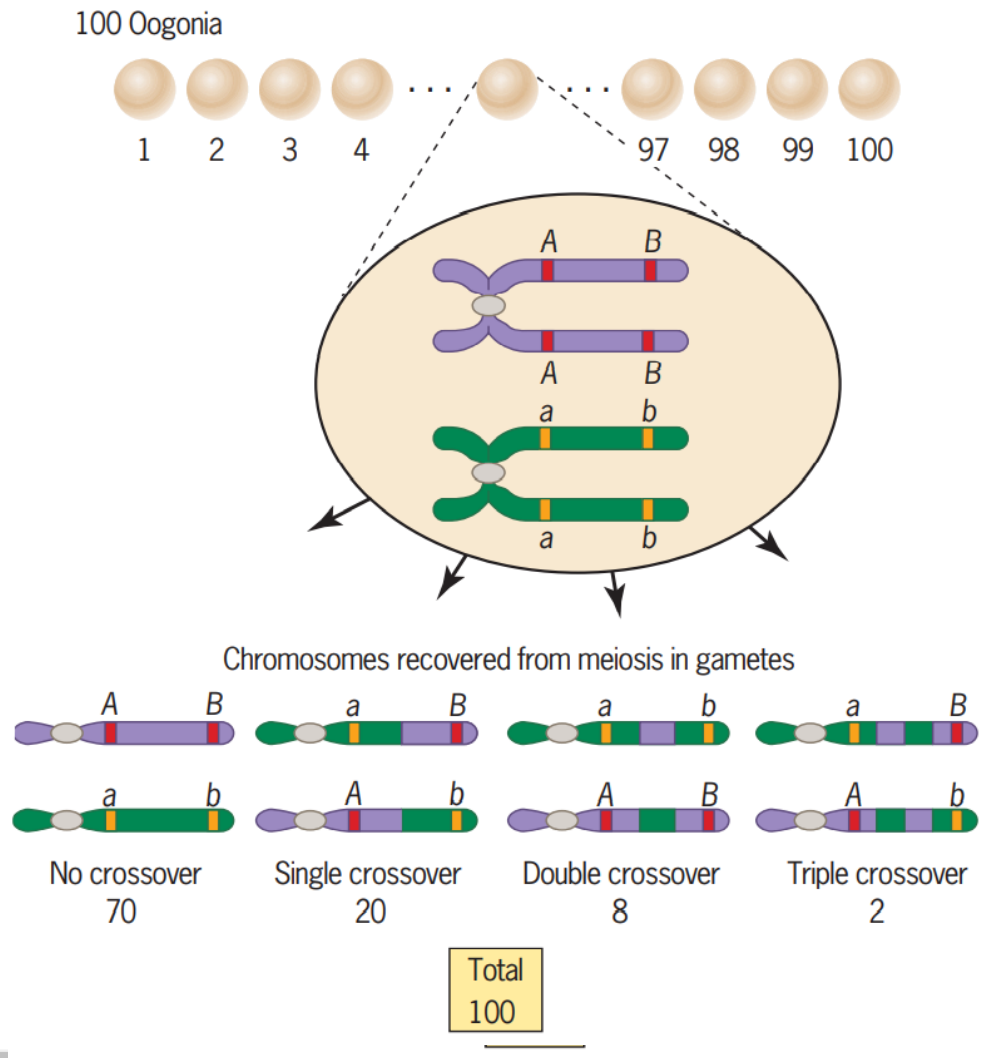
The result from the data in Figure 7.10 is 0.42.

In practice, we cannot “see” each of the exchange points on the chromosomes coming out of meiosis.

Instead, we infer their existence by observing the recombination of the alleles that flank them.

A chromosome in which alleles have recombined must have arisen by crossing over.

Counting recombinant chromosomes therefore provides a way of counting crossover exchange points.



Average number of crossovers between *A* and *B* =

$$0 \times \left(\frac{70}{100}\right) + 1 \times \left(\frac{20}{100}\right) + 2 \times \left(\frac{8}{100}\right) + 3 \times \left(\frac{2}{100}\right) = 0.42$$

■ **FIGURE 7.10** Calculating the average number of crossovers between genes on chromosomes recovered from meiosis.

RECOMBINATION MAPPING WITH A TWO-POINT TESTCROSS

To illustrate the mapping procedure, let's consider the two-point testcross in Figure 7.11.

Wild-type Drosophila females were mated to males homozygous for two autosomal mutations—vestigial (vg), which produces short wings, and black (b), which produces a black body.

All the F1 flies had long wings and gray bodies; thus, the wild-type alleles (vg^+ and B^+) are dominant.

The F1 females were then testcrossed to vestigial, black males, and the F2 progeny were sorted by phenotype and counted.

As the data show, there were four phenotypic classes, two abundant and two rare.

The abundant classes had the same phenotypes as the original parents, and the rare classes had recombinant phenotypes.

We know that the vestigial and black genes are linked because the recombinants are much fewer than 50 percent of the total progeny counted.

These genes must therefore be on the same chromosome.

To determine the distance between them, we must estimate the average number of crossovers in the gametes of the doubly heterozygous F1 females.

We can do this by calculating the frequency of recombinant F2 flies and noting that each such fly inherited a chromosome that had crossed over once between vg and b.

The average number of crossovers in the whole sample of progeny is therefore

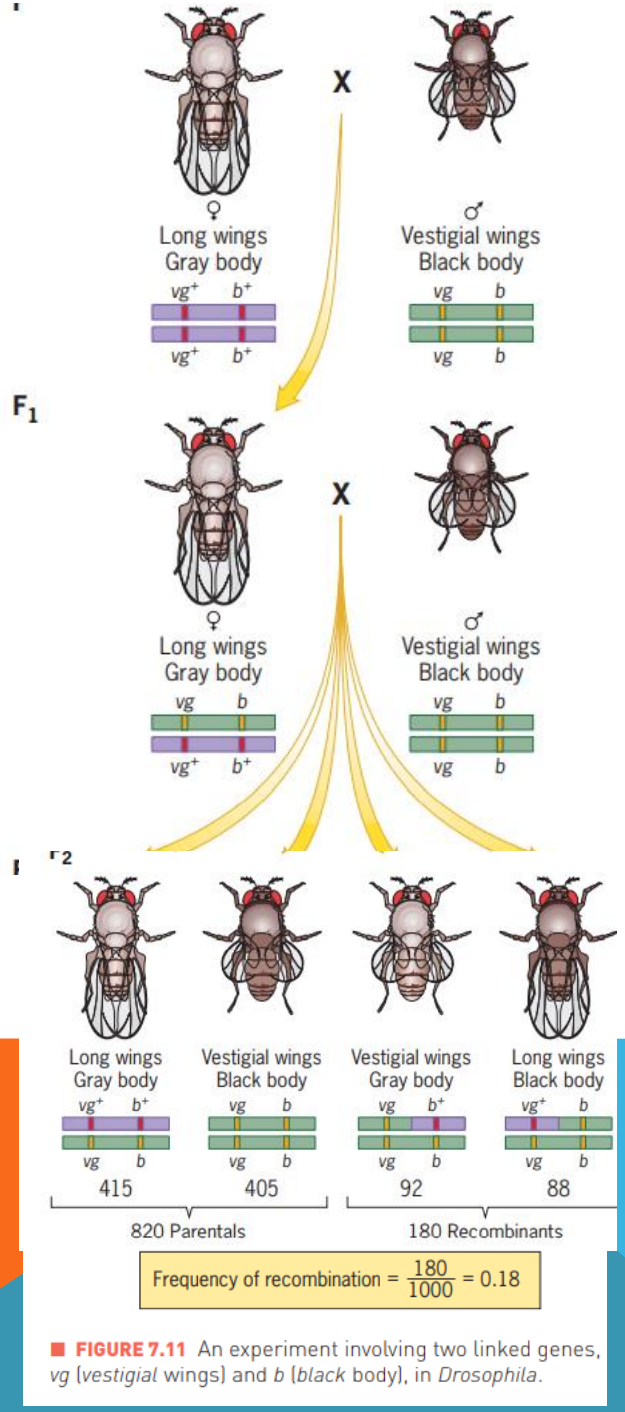
$$\underset{(0) \times 0.82}{\text{nonrecombinants}} + \underset{(1) \times 0.18}{\text{recombinants}} = 0.18$$

In this expression, the number of crossovers for each class of flies is placed in parentheses; the other number is the frequency of that class.

The non-recombinant progeny obviously do not add any crossover chromosomes to the data, but we include them in the calculation to emphasize that we must calculate the average number of crossovers by using all the data, not just those from the recombinants.

This simple analysis indicates that, on average, 18 out of 100 chromosomes recovered from meiosis had a crossover between vg and b. Thus, vg and b are separated by 18 units on the genetic map.

Sometimes geneticists call a map unit a centiMorgan, abbreviated cM, in honor of T. H. Morgan; 100 centiMorgans equal one Morgan (M). We can therefore say that vg and b are 18 cM (or 0.18 M) apart. Notice that the map distance is equal to the frequency of recombination, written as a percentage. Later we will see that when the frequency of recombination approaches 0.5, it underestimates the map distance.



■ **FIGURE 7.11** An experiment involving two linked genes, *vg* (*vestigial* wings) and *b* (*black* body), in *Drosophila*.

RECOMBINATION MAPPING WITH A THREE-POINT TESTCROSS

We can also use the recombination mapping procedure with data from testcrosses involving more than two genes.

Figure 7.12 illustrates an experiment by C. B. Bridges and T. M. Olbrycht, who crossed wild-type Drosophila males to females homozygous for three recessive X-linked mutations—scute (sc) bristles, echinus (ec) eyes, and crossveinless (cv) wings.

They then intercrossed the F1 progeny to produce F2 flies, which they classified and counted.

We note that the F1 females in this intercross carried the three recessive mutations on one of their X chromosomes and the wild-type alleles of these mutations on the other X chromosome.

Furthermore, the F1 males carried the three recessive mutations on their single X chromosome.

Thus, this intercross was equivalent to a testcross with all three genes in the F1 females present in the coupling configuration.

The F2 flies from the intercross comprised eight phenotypically distinct classes, two of them parental and six recombinant.

The parental classes were by far the most numerous.

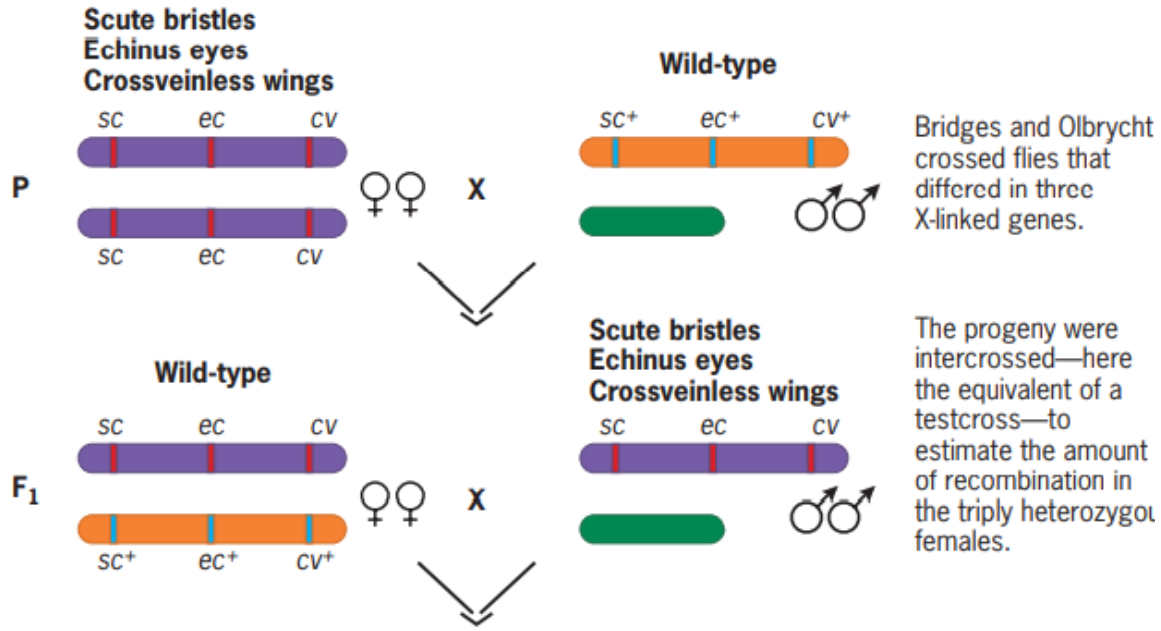
The less numerous recombinant classes each represented a different kind of crossover chromosome.

To figure out which crossovers were involved in producing each type of recombinant, we must first determine how the genes are ordered on the chromosome.

Determining the Gene Order

There are three possible gene orders:

1. sc—ec—cv
2. ec—sc—cv
3. ec—cv-sc



Class	Phenotype	Genotype of maternally inherited X chromosome			Number observed
1	Scute, echinus, crossveinless	*sc*	*ec*	*cv*	1158
2	Wild-type	*sc*+	*ec*+	*cv*+	1455
3	Scute	*sc*	*ec*+	*cv*+	163
4	Echinus, crossveinless	*sc*+	*ec*	*cv*	130
5	Scute, echinus	*sc*	*ec*	*cv*+	192
6	Crossveinless	*sc*+	*ec*+	*cv*	148
7	Scute, crossveinless	*sc*	*ec*+	*cv*	1
8	Echinus	*sc*+	*ec*	*cv*+	1
				Total:	3248

■ **FIGURE 7.12** Bridges and Olbrycht's three-point cross with the X-linked genes *sc* (*scute* bristles), *ec* (*echinus* eyes), and *cv* (*crossveinless* wings) in *Drosophila*. Data from Bridges, C. B., and Olbrycht, T. M., 1926. *Genetics* 11: 41.

HOW TO FIND THE GENE ORDER

Take a careful look at the six recombinant classes.

Four of them must have come from a single crossover in one of the two regions delimited by the genes.

The other two must have come from double crossing over—one exchange in each of the two regions.

Because a double crossover switches the gene in the middle with respect to the genetic markers on either side of it a double crossover should occur much less frequently than a single crossover.

Consequently, among the six recombinant classes, the two rare ones must represent the double crossover chromosomes.

In our data, the rare, double crossover classes are 7 (sc ec^+ cv) and 8 (sc^+ ec cv^+), each containing a single fly (Figure 7.12).

Comparing these to parental classes 1 (sc ec cv) and 2 (sc^+ ec $^+$ cv $^+$), we see that the echinus allele has been switched with respect to scute and crossveinless.

Consequently, the echinus gene must be located between the other two. The correct gene order is therefore (1) sc-ec-cv

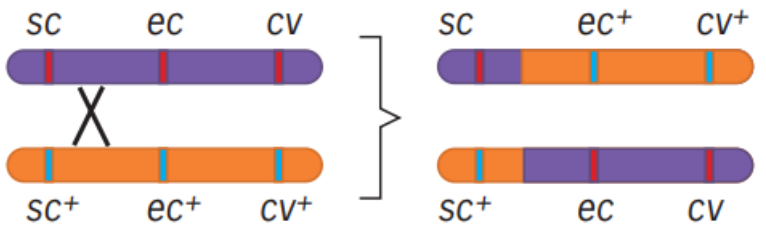
CALCULATING THE DISTANCES BETWEEN GENES

Find the gene order→determine the distances between adjacent genes.

Procedure is to compute the average number of crossovers in each chromosomal region (Figure 7.13)

- Obtain the length of the region between sc and ec by identifying the recombinant classes that involved a crossover between these genes.
- There are four such classes: 3 (sc ec+ cv+), 4 (sc+ ec cv), 7 (sc ec+ cv), and 8 (sc+ ec c+).
- Classes 3 and 4 involved a single crossover between sc and ec
- Classes 7 and 8 involved two crossovers, one between sc and ec and the other between ec and cv.

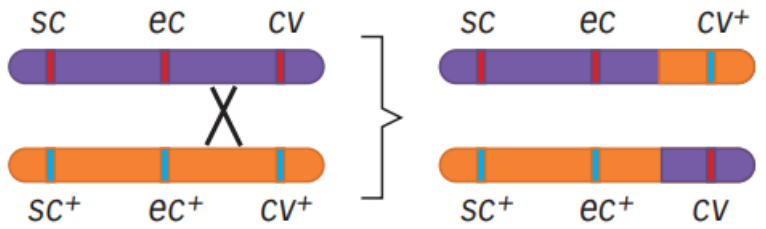
Crossovers between *sc* and *ec*



Class	Number observed
3	163
4	130
7	1
8	1
Total:	295

Map distance = $\frac{295}{3248}$ = 0.091 Morgan = 9.1 centiMorgans

Crossovers between *ec* and *cv*



Class	Number observed
5	192
6	148
7	1
8	1
Total:	342

Map distance = $\frac{342}{3248}$ = 0.105 Morgan = 10.5 centiMorgans

■ **FIGURE 7.13** Calculation of genetic map distances from Bridges and Olbrycht's data. The distance between each pair of genes is obtained by estimating the average number of crossovers.

use the frequencies of these four classes to estimate the average number of crossovers between sc and ec

(Class 3+ class 4+ class 7+ class 8)/ Total=295/3248=0.091

Thus, in every 100 chromosomes coming from meiosis in the F1 females, 9.1 had a crossover between sc and ec. The distance between these genes is therefore 9.1 map units (or, if you prefer, 9.1 centiMorgans).

sc and ec are 9.1 map units apart

- we can obtain the distance between ec and cv. Four recombinant classes involved a crossover in this region: 5 (sc ec cv+), 6 (sc+ ec+ cv), 7, and 8.
- The double recombinants are also included here because one of their two crossovers was between ec and cv. The combined frequency of these four classes is
- (Class 5+ class 6+ class 7+ class 8)/ Total=342/3248=0.105
- Consequently, ec and cv are 10.5 map units apart

Combining the data for the two regions, we obtain the map

sc —9.1— ec —10.5— cv

Map distances computed in this way are additive.

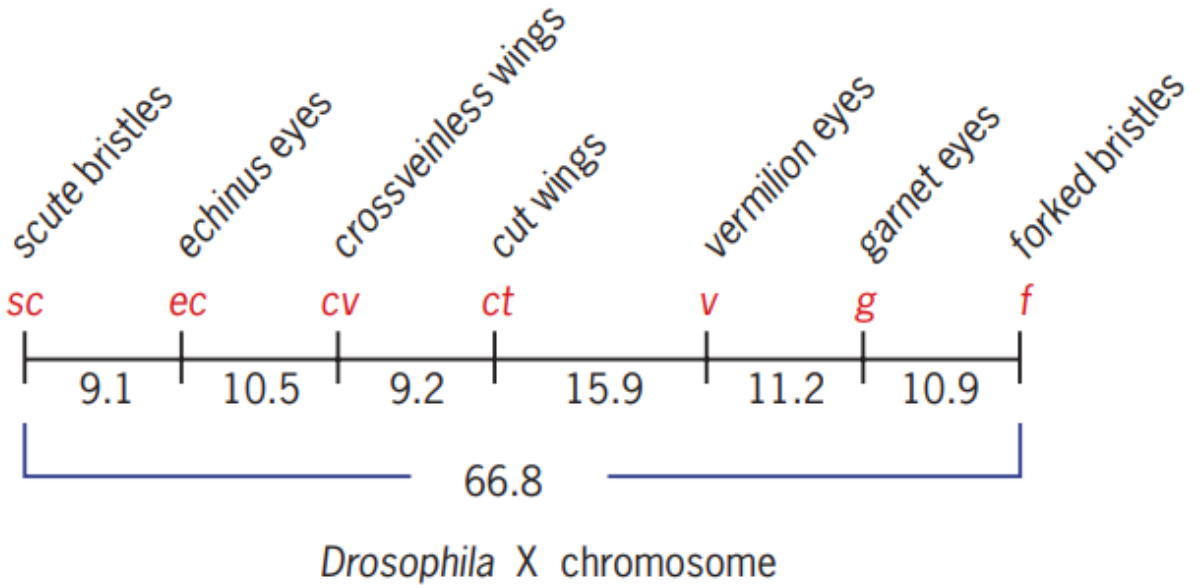
Thus, we can estimate the distance between sc and cv by summing the lengths of the two map intervals between them:

9.1 cM +10.5 cM = 19.6 cM

DIRECT CALCULATION OF MAP DISTANCE

We can also obtain this estimate by directly calculating the average number of cross-overs between these genes:

Non–crossover classes 1 and 2		Single crossover classes 3, 4, 5, and 6		Double crossover classes 7 and 8	
$(0) \times 0.805$	+	$(1) \times 0.195$	+	$(2) \times 0.0006$	$= 0.196$



GURE 7.14 Bridges and Olbrycht's map of
ı X-linked genes in *Drosophila*. Distances
iven in centiMorgans.

INTERFERENCE AND THE COEFFICIENT OF COINCIDENCE

A three-point cross has an important advantage over a two-point cross:

- It allows the detection of double crossovers,
- Permitting us to determine if exchanges in adjacent regions are independent of each other.
- For example, does a crossover in the region between sc and ec (region i on the map of the x chromosome) occur independently of a crossover in the region between ec and cv (region ii)?
- Or does one crossover inhibit the occurrence of another nearby? To answer these questions, we must calculate the expected frequency of double crossovers, based on the idea of independence.
- We can do this by multiplying the crossover frequencies for two adjacent chromosome regions.

- For example, in region I on Bridges and Olbrycht's map, the crossover frequency was (163 + 130 + 1 + 1)/ 3248 = 0.091,
- and in region II, it was (192 + 148 + 1 + 1)/3248 =0.105.
- If we assume independence, the expected frequency of double crossovers in the interval between sc and cv would therefore be 0.091 × 0.105 = 0.0095.
- We can now compare this frequency with the observed frequency, which was 2/3248 = 0.0006.
- Double crossovers between sc and cv were much less frequent than expected.
- This result suggests that one crossover inhibited the occurrence of another nearby, a phenomenon called interference.
- The extent of the interference is customarily measured by the coefficient of coincidence, c, which is the ratio of the observed frequency of double crossovers to the expected frequency:

$$c=0.0006/0.0095=0.063$$

- The level of interference, symbolized I, is calculated as I = 1 - c = 0.937

Because in this example the coefficient of coincidence is close to zero, its lowest possible value, interference was very strong (I is close to 1).

At the other extreme, a coefficient of coincidence equal to one would imply no interference at all; that is, it would imply that the crossovers occurred independently of each other.

Many studies have shown that interference is strong over map distances less than 20 cM; thus, double crossovers seldom occur in short chromosomal regions.

However, over long regions, interference weakens to the point that crossovers occur more or less independently.

The strength of interference is therefore a function of map distance.

Once a genetic map has been constructed, it is possible to use the map to predict the results of experiments.

To see how map-based predictions are made, work through the exercise in Problem-Solving Skills: Using a Genetic Map to Predict the Outcome of a Cross.

PROBLEM-SOLVING SKILLS

Using a Genetic Map to Predict the Outcome of a Cross

THE PROBLEM

The genes *r*, *s*, and *t* reside in the middle of the *Drosophila* X chromosome; *r* is 15 cM to the left of *s*, and *t* is 20 cM to the right of *s*. In this region, the coefficient of coincidence (*c*) is 0.2. A geneticist wishes to create an X chromosome that carries the recessive mutant alleles of all three genes. One stock is homozygous for *r* and *t*, and another stock is homozygous for *s*. By crossing the two stocks, the geneticist obtains females that are triple heterozygotes, $r\ s^+\ t/r^+\ s\ t^+$. These females are then crossed to wild-type males. If the geneticist examines 10,000 sons from these females, how many of them will be triple mutants, *r s t*?

FACTS AND CONCEPTS

1. For small map intervals (<20 cM), the map distance equals the frequency of a single crossover in the interval.
2. The coefficient of coincidence equals the observed frequency of double crossovers/expected frequency of double crossovers.
3. The expected frequency of double crossovers is calculated on the assumption that the two crossovers occur independently.
4. Males inherit their X chromosome from their mothers.

ANALYSIS AND SOLUTION

Triple mutant males will be produced only if a double crossover occurs in the $r\ s^+\ t/r^+\ s\ t^+$ females that were crossed to wild-type males. The frequency of such double crossovers is a function of the two map distances (15 cM and 20 cM) and the level of interference, which is measured by the coefficient of coincidence (here $c = 0.2$). Because c = observed frequency of double crossovers/expected frequency of double crossovers, we can solve for the observed frequency of double crossovers after a simple algebraic rearrangement: observed frequency of double crossovers = $c \times$ expected frequency of double crossovers. The expected frequency of double crossovers is calculated from the map distances assuming that crossovers in adjacent map intervals occur independently: $0.15 \times 0.20 = 0.03$. Thus, among 10,000 sons, 0.2×3 per cent should carry an X chromosome that had one crossover between the *r* and *s* genes and another crossover between the *s* and *t* genes. However, only half of these 60 sons—that is, 30—will carry the triply mutant X chromosome; the other 30 will be triply wild-type.

For further discussion visit the Student Companion site.

Expected frequency= 0.15 × 0.20 = 0.03

We know that c= 0.2

c= o. f. / e. f. => 0.2 = o. f. /0.03 => 0.006 = o. f.

Among 10000 sons, the no. of sons with X chromosomes with double crossovers= 10000 × 0.006 = 60

(Female having r s+ t /r+ s t+) X (male with r+ s+ t+ /-)

Result =

- Non crossovers = r s+ t /- and r+ s t+/-

single crossovers = r s t+ /- and r+ s+ t /-

- r s+ t+ /- and r+ s t /-

Double crossovers = r s t/- and r+ s+ t+/- = 60 = observed no. of such males out of 10000 sons

Sons with r s t/- = 60/2 =30

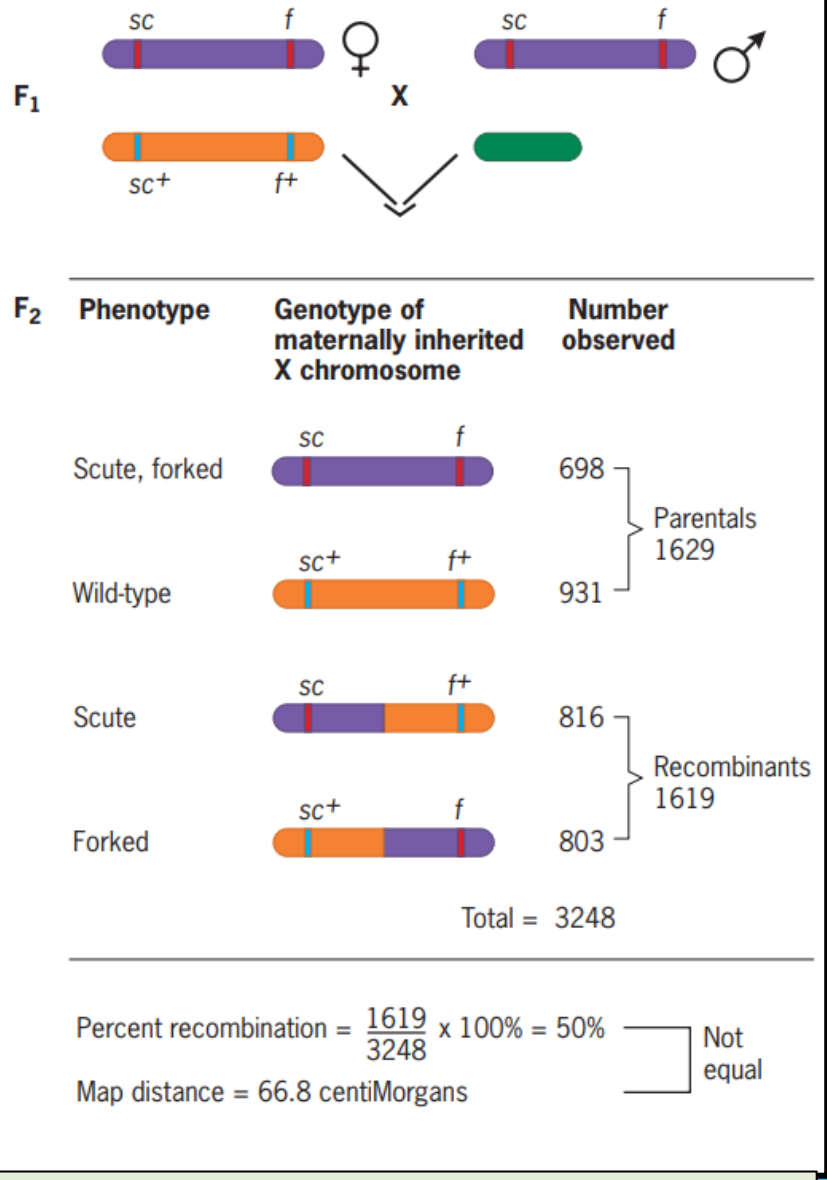


FIGURE 7.15 A discrepancy between map distance and percent recombination. The map distance between the genes sc and f is greater than the observed percent recombination between them.

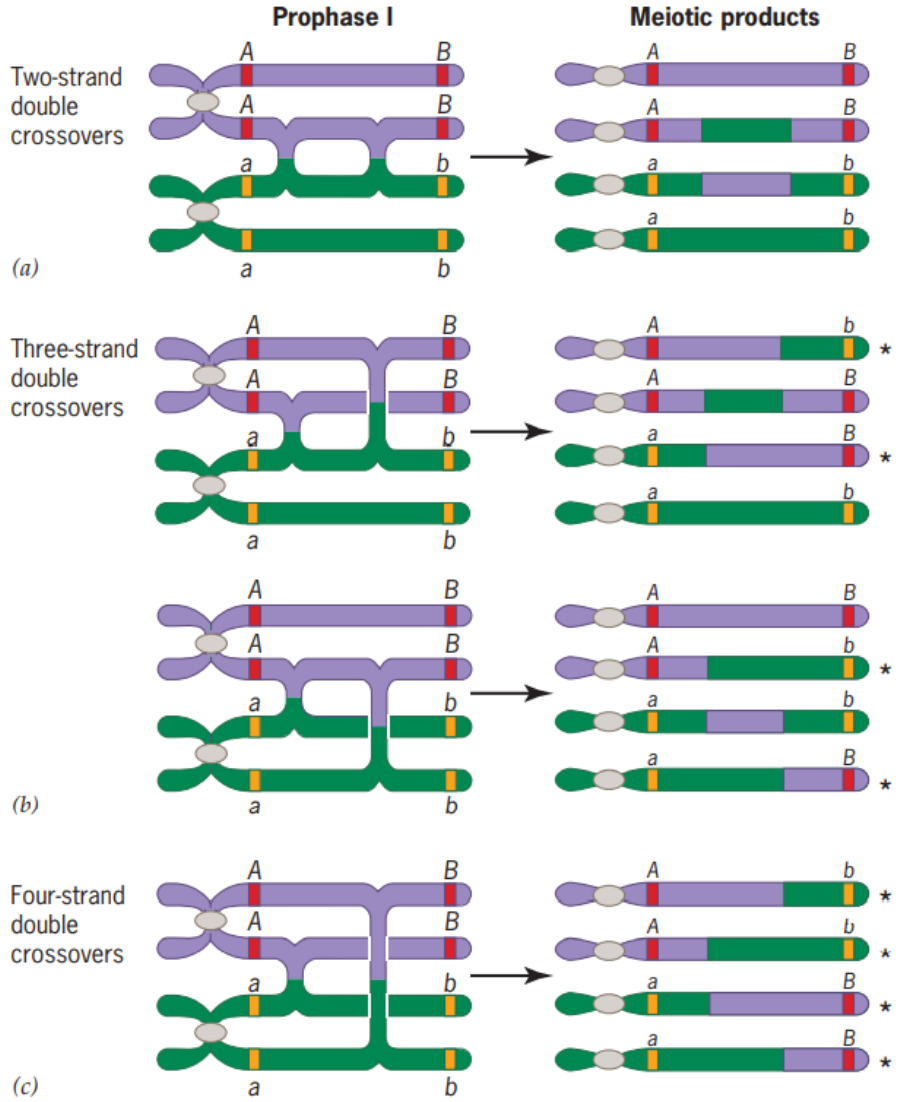
- In the preceding sections, we have considered how to construct chromosome maps from data on the recombination of genetic markers. These data allow us to infer where crossovers have occurred in a sample of chromosomes. By localizing and counting these crossovers, we can estimate the distances between genes and then place the genes on a chromosome map.
- This method works well as long as the genes are fairly close together. However, when they are far apart, the frequency of recombination may not reflect the true map distance (Figure 7.15).
- As an example, let's consider the genes at the ends of Bridges and Olbrycht's map of the X chromosome; sc, at the left end, was 66.8 cM away from f, at the right end.
- However, the frequency of recombination between sc and f was 50 percent—the maximum possible value. Using this frequency to estimate map distance, we would conclude that sc and f were 50 map units apart.
- Of course, the distance obtained by summing the lengths of the intervening regions on the map, 66.8 cM, is much greater. This example shows that the true genetic distance, which depends on the average number of crossovers on a chromosome, may be much greater than the observed recombination frequency.

Multiple crossovers may occur between widely separated genes, and some of these crossovers may not produce genetically recombinant chromosomes (Figure 7.16).

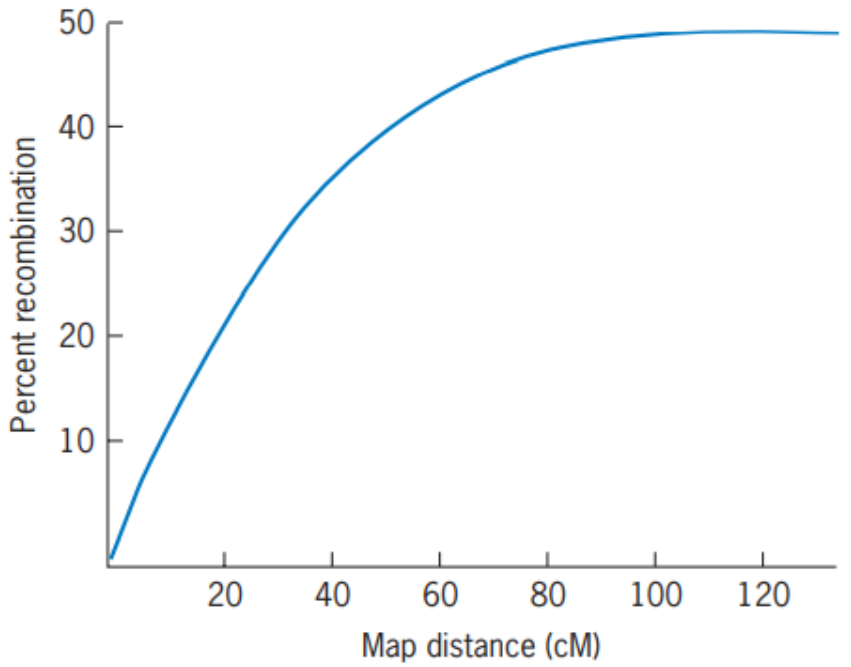
To see this, let's assume that a single crossover occurs between two chromatids in a tetrad, causing recombination of the flanking genetic markers.

If another crossover occurs between these same two chromatids, the flanking markers will be restored to their original configuration; the second crossover essentially cancels the effect of the first, converting the recombinant chromatids back into non-recombinants.

Thus, even though two crossovers have occurred in this tetrad, none of the chromatids that come from it will be recombinant for the flanking markers.



■ **FIGURE 7.16** Consequences of double crossing over between two loci. Recombinant chromosomes are denoted by an asterisk. (*a*) Two-strand double crossovers produce only nonrecombinant chromosomes. (*b*) Three-strand double crossovers produce half recombinant and half nonrecombinant chromosomes. (*c*) Four-strand double crossovers produce only recombinant chromosomes.



■ **FIGURE 7.17** Relationship between frequency of recombination and genetic map distance. For values less than 20 cM, there is approximately a linear relationship between percent recombination and map distance; for values greater than 20 cM, the percent recombination underestimates the map distance.

This second example shows that a double crossover may not contribute to the frequency of recombination, even though it contributes to the average number of exchanges on a chromosome.

A quadruple crossover would have the same effect. These and other multiple exchanges are responsible for the discrepancy between recombination frequency and genetic map distance.

In practice, this discrepancy is small for distances less than 20 cM. Over such distances, interference is strong enough to suppress almost all multiple exchanges, and the recombination frequency is a good estimator of the true genetic distance. For values greater than 20 cM, these two quantities diverge, principally because multiple exchanges become more likely. Figure 7.17 shows the mathematical relationship between recombination frequency and genetic map distance.

The genetic maps of chromosomes are based on the average number of crossovers that occur during meiosis.

Genetic map distances are estimated by calculating the frequency of recombination between genes in experimental crosses.

Recombination frequencies less than 20 percent estimate map distance directly; however, recombination frequencies greater than 20 percent underestimate map distance because multiple crossover events do not always produce recombinant chromosomes

SOMATIC-CELL HYBRIDIZATION

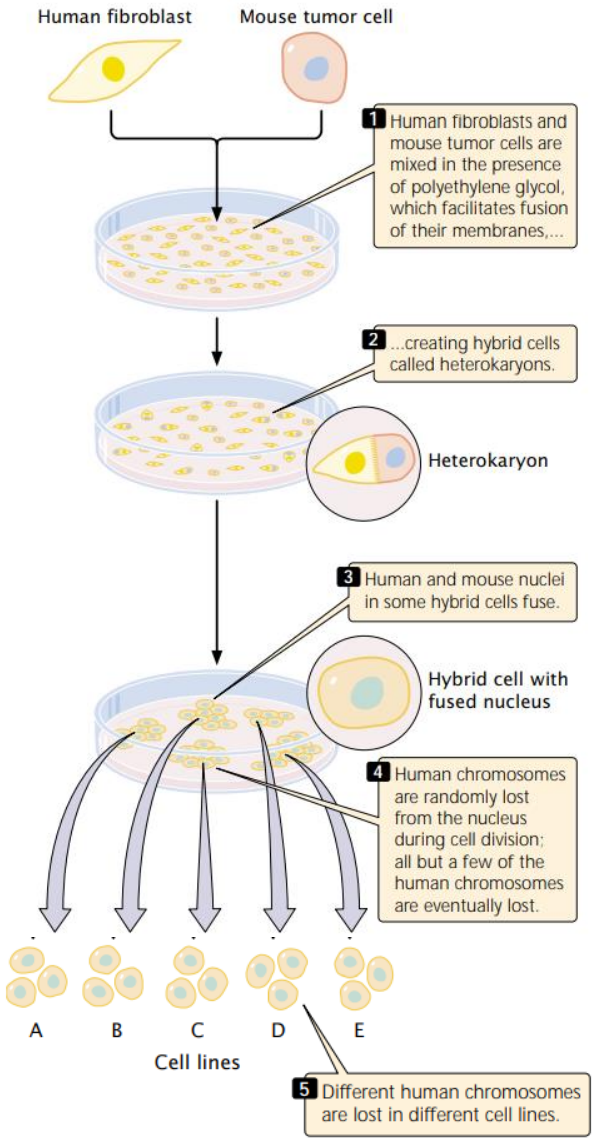
One of the methods used for positioning genes on chromosomes is somatic cell hybridization, which requires the fusion of different types of cells.

Most mature somatic (nonsex) cells can undergo only a limited number of divisions and therefore cannot be grown continuously.

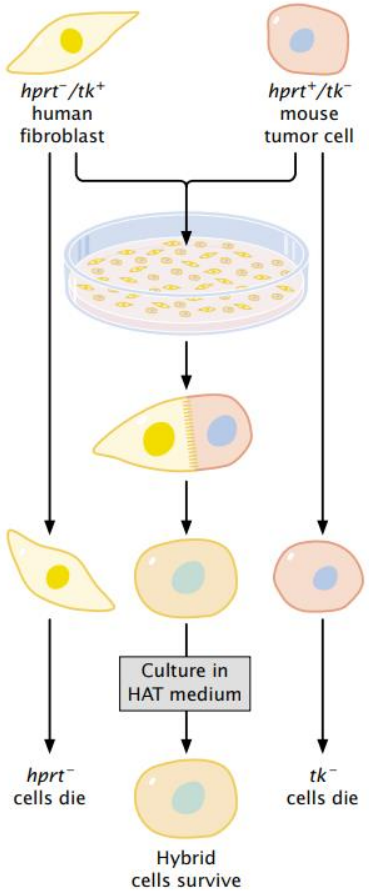
However, cells that have been altered by viruses or derived from tumors that have lost the normal constraints on cell division will divide indefinitely; these types of cells can be cultured in the laboratory and are referred to as a cell line.

Cells from two different cell lines can be fused by treating them with polyethylene glycol or other agents that alter their plasma membranes. After fusion, the cell possesses two nuclei and is called a heterokaryon.

The two nuclei of a heterokaryon eventually also fuse, generating a hybrid cell that contains chromosomes from both cell lines.



7.18 Somatic-cell hybridization can be used to determine which chromosome contains a gene of interest.



cells survive

7.19 HAT medium can be used to separate human–mouse hybrid cells from the original hybridized cells.

If human and mouse cells are mixed in the presence of polyethylene glycol, fusion results in human–mouse somatic-cell hybrids (FIGURE 7.18).

The hybrid cells tend to lose chromosomes as they divide and, for reasons that are not understood, chromosomes from one of the species are lost preferentially.

In human–mouse somatic-cell hybrids, the human chromosomes tend to be lost, whereas the mouse chromosomes are retained.

Eventually, the chromosome number stabilizes when all but a few of the human chromosomes have been lost. Chromosome loss is random and differs among cell lines. The presence of these “extra” human chromosomes in the mouse genome makes it possible to assign human genes to specific chromosomes.

In the first step of this procedure, hybrid cells must be separated from original parental cells that have not undergone hybridization.

This separation is accomplished by using a selection method that allows hybrid cells to grow while suppressing the growth of parental cells. The most commonly used method is called HAT selection (FIGURE 7.19), which stands for hypoxanthine, aminopterin, and thymidine, three chemicals that are used to select for hybrid cells.

In the presence of HAT medium, a cell must possess two enzymes to synthesize DNA: thymidine kinase (TK) and hypoxanthine-guanine phosphoribosyl transferase (HPRT). Cells that are tk- or hprt- cannot synthesize DNA and will not grow on HAT medium.

The mouse cells used in the hybridization procedure are deficient in TK, but can produce HPRT (the cells are tk- hprt+); the human cells can produce TK but are deficient for HPRT (they are tk+ hprt-). On HAT medium, the mouse cells do not survive, because they are tk-; the human cells do not survive, because they are hprt-.

Hybrid cells, on the other hand, inherit the ability to make HPRT from the mouse cell and the ability to make TK from the human cell; thus, they produce both enzymes (the cells are tk+ hprt+) and will grow on HAT medium.

To map genes using somatic-cell hybridization requires the use of a panel of different hybrid cell lines.

The cell lines of the panel differ in the human chromosomes that they have retained.

For example, one cell line might possess human chromosomes 2, 4, 7, and 8, whereas another might possess chromosomes 4, 19, and 20.

Each cell line in the panel is examined for evidence of a particular human gene.

The human gene can be detected either by looking for the protein that it produces or by looking for the gene itself with the use of molecular probes (discussed in Chapter 18).

Correlation of the presence of the gene with the presence of specific human chromosomes often allows the gene to be assigned to the correct chromosome.

For example, if a gene was detected in both of the aforementioned cell lines, the gene must be on chromosome 4, because it is the only human chromosome common to both cell lines

		Human chromosomes present																					
Cell line	Gene product present	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
A	+		+		+			+	+															
B	+	+	+		+				+	+	+	+	+	+										
C	–															+		+		+			+	
D	+		+		+		+	+	+															
E	–												+								+			
F	+				+															+	+			

7.20 **Somatic-cell hybridization is used to assign a gene to a particular human chromosome.** A panel of six cell lines, each line containing a different subset of human chromosomes, is examined for the presence of the gene product (such as an enzyme). A plus sign means that the gene product is present; a minus sign means that the gene product is missing. Four of the cell lines (A, B, D, and F) have the gene product, indicating that the gene is present on one of the chromosomes found in these cell lines. The only chromosome common to all four of these cell lines is chromosome 4, indicating that the gene is located on this chromosome.

Two genes determined to be on the same chromosome with the use of somatic-cell hybridization are said to be syntenic genes.

This term is used because syntenic genes may or may not exhibit linkage in the traditional genetic sense—remember that two genes can be located on the same chromosome but may be so far apart that they assort independently.

Syntenic refers to genes that are physically linked, regardless of whether they exhibit genetic linkage. (Synteny is sometimes also used to refer togene loci in different organisms located on a chromosome region of common evolutionary origin.)

Sometimes somatic-cell hybridization can be used to position a gene on a specific part of a chromosome.

Some hybrid cell lines carry a human chromosome with a chromosome mutation such as a deletion or a translocation.

If the gene is present in a cell line with the intact chromosome but missing from a line with a chromosome deletion, the gene must be located in the deleted region.

Similarly, if a gene is usually absent from a chromosome but consistently appears whenever a translocation (a piece of another chromosome that has broken off and attached itself to the chromosome in question) is present, it must be present on the translocated part of the chromosome.